

# **OBESITY AND BONE METABOLISM**

**Amy Lynn Evans**

Submitted for the degree of Doctor of Philosophy

Academic Unit of Bone Metabolism

Department of Human Metabolism

University of Sheffield

April 2014

## Contents

<b>SUMMARY OF THESIS .....</b>	<b>5</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>6</b>
<b>CONTRIBUTIONS.....</b>	<b>7</b>
<b>PRESENTED ABSTRACTS .....</b>	<b>9</b>
<b>ABBREVIATIONS .....</b>	<b>10</b>
<b>LIST OF TABLES.....</b>	<b>12</b>
<b>LIST OF FIGURES.....</b>	<b>12</b>
<b>CHAPTER 1: INTRODUCTION.....</b>	<b>16</b>
<b>Background.....</b>	<b>16</b>
Bone Physiology .....	16
Osteoporosis.....	19
Obesity.....	20
Osteoporosis Prevalence in Obesity.....	22
Fracture in Obesity.....	23
Determinants of Fracture in Obesity .....	25
Effects of Obesity on Bone Throughout the Life Course .....	26
<b>Limitations of the Current Literature.....</b>	<b>31</b>
<b>Thesis Overview .....</b>	<b>33</b>
<b>Long Term Research Goals .....</b>	<b>34</b>
<b>Thesis Aims .....</b>	<b>34</b>
<b>CHAPTER 2: METHODS .....</b>	<b>38</b>
<b>Study Design.....</b>	<b>38</b>
<b>Inclusion and Exclusion Criteria.....</b>	<b>39</b>
<b>Study Procedures .....</b>	<b>42</b>
Questionnaires.....	43
Anthropometry.....	43
Skeletal Imaging.....	44
Dual-Energy X-ray Absorptiometry .....	44
Quantitative Computed Tomography .....	52
High Resolution Peripheral QCT.....	55
Assessment of Adiposity by Imaging .....	62
Assessment of Muscle Mass by Imaging.....	64
Radiation Exposure.....	64
Physical Performance Assessments .....	64
Chair Stand Test.....	64
Gait and Balance Assessment.....	64
SPPB Score.....	65
Grip Strength .....	65
Assessment of Sarcopenia .....	66
Biochemistry .....	66

Assay Principles .....	67
Bone Turnover Markers .....	69
Adipokines .....	69
Further Biochemistry .....	70
Vitamin D Sub-study Biochemistry .....	71
Biochemistry Quality Control .....	74
<b>CHAPTER 3: BONE DENSITY, STRUCTURE AND STRENGTH IN OBESITY .....</b>	<b>77</b>
<b>Background .....</b>	<b>77</b>
Obesity and aBMD .....	77
Obesity and vBMD .....	77
Obesity and Bone Microarchitecture .....	79
Obesity and Bone Strength .....	79
<b>Research Questions, Aims and Hypotheses .....</b>	<b>81</b>
<b>Methods .....</b>	<b>82</b>
<b>Results .....</b>	<b>84</b>
Areal BMD in Obesity .....	84
vBMD and Bone Microarchitecture in Obesity .....	88
Bone Strength in Obesity .....	91
<b>Discussion .....</b>	<b>96</b>
<b>CHAPTER 4: POTENTIAL MECHANISMS OF ASSOCIATIONS BETWEEN OBESITY, BONE DENSITY AND BONE MICROSTRUCTURE .....</b>	<b>102</b>
<b>Background .....</b>	<b>102</b>
Mechanical Loading Effects of High Body Weight .....	102
Body Composition .....	103
Fat Mass and Lean Mass Contributions to BMD .....	103
Body Fat Distribution .....	103
Biochemical Mechanisms .....	108
Positive Biochemical Mechanisms .....	108
Negative Biochemical Mechanisms .....	113
<b>Research Questions, Aims and Hypotheses .....</b>	<b>116</b>
<b>Methods .....</b>	<b>117</b>
<b>Results .....</b>	<b>120</b>
Central Adiposity .....	120
Peripheral Adiposity .....	121
Are Associations Between Obesity and Bone Due to FM or LM? .....	122
Which Fat Compartment Has the Greatest Influence on BMD? .....	124
Which Fat Compartment Has the Greatest Influence on Bone Microstructure? ..	125
Biochemistry .....	126
Bone Turnover .....	126
Leptin and Adiponectin .....	129
Total, Free and Bioavailable E2 .....	131

Glucose, Insulin and Insulin Resistance.....	133
SHBG .....	135
Albumin .....	136
IGF-I.....	137
Calcium, PTH and 25OHD.....	138
BAP .....	139
OPG .....	140
Sclerostin.....	141
Creatinine and eGFR.....	142
Lipids .....	143
Inflammatory Cytokines .....	143
Potential Mediators of Low Bone Turnover in Obesity .....	144
<b>Discussion .....</b>	<b>146</b>
<b>CHAPTER 5: MUSCLE MASS AND PHYSICAL PERFORMANCE IN OBESITY ....</b>	<b>156</b>
<b>Background.....</b>	<b>156</b>
Muscle Mass, Muscle Strength and Physical Performance .....	157
Frailty, Sarcopenia, and Dynapenia.....	157
Sarcopenia, Bone Density, Falls and Fractures.....	158
Mechanisms Linking Obesity, Sarcopenia and Falls.....	159
<b>Research Questions and Aims .....</b>	<b>160</b>
<b>Methods.....</b>	<b>161</b>
<b>Results .....</b>	<b>164</b>
Muscle Mass in Obesity .....	164
Physical Performance in Obesity.....	164
Grip Strength .....	164
Gait Speed .....	165
Chair Stand Time.....	166
Balance .....	167
SPPB Score.....	167
Associations Between LM and Physical Performance in Obesity.....	168
Sarcopenia in Obesity.....	170
Falls in Obesity .....	170
Sarcopenia and Falls in Obesity.....	172
Potential Biochemical Mediators of Poor Physical Function in Obesity.....	173
Adiposity, Physical Performance and Falls.....	180
<b>Discussion .....</b>	<b>182</b>
<b>CHAPTER 6: VITAMIN D METABOLISM IN OBESITY .....</b>	<b>192</b>
<b>Background.....</b>	<b>192</b>
25OHD Insufficiency .....	192
25OHD in Obesity .....	193
Potential Causes of Low 25OHD in Obesity .....	194



Potential Consequences of Low 25OHD in Obesity.....	195
Low 25OHD, Muscle Mass and Physical Performance.....	196
Low 25OHD and Falls .....	197
<b>Research Question, Aims and Hypotheses .....</b>	<b>198</b>
<b>Methods.....</b>	<b>198</b>
<b>Results .....</b>	<b>202</b>
Total 25OHD in Obesity .....	202
Total 25OHD <sub>2</sub> and 25OHD <sub>3</sub> in Obesity .....	203
Free 25OHD in Obesity .....	205
Possible Causes of Low Vitamin D in Obesity .....	206
Dietary Vitamin D Intake in Obesity .....	206
Supplementation in Obesity .....	207
UVB Exposure in Obesity .....	208
Vitamin D Binding Proteins in Obesity.....	209
DBP Genotype.....	210
Consequences of Low 25OHD in Obesity.....	214
Total 1,25(OH) <sub>2</sub> D in Obesity .....	214
Free 1,25(OH) <sub>2</sub> D in Obesity .....	216
Vitamin D and PTH in Obesity .....	216
Vitamin D and Bone Turnover in Obesity .....	217
Bone Density, Microstructure and Strength by DBP Genotype .....	218
Vitamin D and Biochemical Factors in Obesity .....	222
Vitamin D and Body Fat Distribution in Obesity .....	222
Vitamin D and Physical Performance in Obesity.....	223
<b>Discussion .....</b>	<b>224</b>
<b>CHAPTER 7: DISCUSSION.....</b>	<b>239</b>
<b>Conclusion .....</b>	<b>246</b>
<b>REFERENCES .....</b>	<b>248</b>
<b>APPENDIX .....</b>	<b>284</b>

## **Summary of Thesis**

Obese adults have a lower risk of hip and vertebral fracture, but a greater risk of lower limb and proximal humerus fracture, compared to adults with a normal body mass index (BMI). Differences in fracture risk by skeletal site in obesity might be attributed to differences in bone mineral density (BMD), bone microstructure and bone strength between obese and normal BMI individuals and/or differences in physical function, possibly related to fall frequency and/or direction. The role of vitamin D in bone metabolism and physical function in obesity is unclear. The effect of obesity on bone microstructure and strength in young and older, men and women, has not been investigated in a matched case control design.

BMD, microstructure and strength were determined using novel imaging technologies. The roles of various adipose compartments and biochemical factors on BMD and microarchitecture were investigated. Physical function and vitamin D metabolism of obese and normal BMI individuals was compared.

Younger and older obese adults have higher BMD, favourable microstructure and greater bone strength, but there is a greater difference in BMD and microstructure between obese and normal BMI in older adults than in younger adults. Obese adults have greater bone strength than normal BMI adults, regardless of age. Higher BMD in obesity is associated with lower resorption, possibly mediated by circulating leptin and oestradiol which were, in turn, associated with subcutaneous abdominal adiposity. Obese adults have greater muscle mass but poorer physical performance than normal BMI adults. This might be associated with greater fall frequency and affect fall direction. Obese adults have lower total and free 25OHD and 1,25(OH)<sub>2</sub>D than normal BMI adults, likely due to greater volumetric dilution. Low 25OHD in obesity does not appear to affect BMD, microstructure or strength, or physical performance.

Despite greater BMD and bone strength, obese individuals are at greater risk of some fractures. This might be due to the effect of poor physical function on fall characteristics in obesity.

## **Acknowledgements**

Firstly, I wish to thank my Supervisors Dr Jennifer Walsh and Professor Richard Eastell. Their enthusiasm for this subject has been truly inspiring and I feel honoured to have been able to study under their supervision. Thank you for your time, patience, guidance, teaching, insight and for believing in me.

I would like to thank my colleagues within the Academic Unit of Bone Metabolism who have supported me throughout this work. I have learned so much from you all and I've built friendships that I hope will last a lifetime. Special thanks to Margaret for your practical support and encouragement, to Sarah Inglis; for getting me through the early starts and late nights in the recruitment phase as the perfect housemate-workmate, Fatma; for all of your help in the lab and for being a fantastic travel companion, Selina; thanks for showing me the ropes, your spreadsheet expertise and for making sure I had some right good fun along the way, and Lucy; thanks for always being there to celebrate the good times and picking me up during the difficult times; thank you.

I wish to thank all of the volunteers who took part in the FAB Study and to everyone who offered to participate. I will remember the stories shared for a long time to come. You made the long hours of recruitment worth it and I hope I made your research experience a positive one.

Thanks to Mr Philip Heath and the Medical Imaging Department for their support with setting up and performing the CT scans and providing assistance and company when I was analysing the CT scans.

Thank you to the National Institute for Health Research, National Osteoporosis Society, Orthopaedic Research UK, the Department of Health and R&D Systems for their financial contributions.

Thank you to my Mum and Dad, without whom, none of this would have been possible. Your support, encouragement and understanding have always enabled me to pursue my dreams, thank you.

Finally, I wish to thank Michael for all of his love, encouragement and support.

## **Contributions**

Dr Walsh wrote the original protocol for the FAB study and provided assistance with subsequent amendments. Dr Walsh was responsible for all clinical aspects of the study and confirmed the eligibility of all participants prior to scanning procedures.

I acquired ethical and Research and Development approvals for the study and was responsible for site file maintenance, submission of amendments and general study management.

I wrote the participant information sheets, consent forms and other supporting documentation. I was responsible for study recruitment; I identified GP practices willing to perform mail-outs, sent emails across the hospital trust and University of Sheffield and put up poster advertisements. I telephone screened all volunteers prior to recruitment, wrote all study invitation and appointment letters and prepared and posted the participant documentation packs.

I booked and carried out all participant visits; I took consent from participants and performed all anthropometry and muscle function tests. I was responsible for co-ordinating participant travel, expenses, screening blood requests, co-ordinating CT scans in the Medical Imaging Department, NGH and co-ordinating study visits.

I completed all paper based data collection forms and was responsible for managing the participant source notes. I completed all data entry, but was supported by Carol McGurk and Jill Thompson for data entry of the vitamin D work. Simon Bowles entered the dietary data. I coded all qualitative data from the lifestyle and UVB questionnaires. Carol McGurk and Jill Thompson performed the data checking.

All DXA and HR-pQCT scans were performed by Dr Margaret Paggiosi, Selina Bratherton and Emma Gosney. Dr Paggiosi established the scanning protocols and obtained Medical Imaging and Medical Physics approval for the study. Scan analysis and finite element analysis was performed by Dr Paggiosi and Selina Bratherton.

The CT and QCT parameters were determined and protocols set up by Mr Philip Heath and Mr David Tipper in the Medical Imaging Department, NGH. CT and QCT scans were performed by staff of the Medical Imaging Department, NGH. Analysis of the QCT spine scans was completed by Dr Paggiosi, after I had identified the reconstructed scan file. I analysed the abdominal CT scans.

Fatma Gossiel performed much of the in-house automated biochemical analysis, and I performed manual assays and ran automated analyses under the guidance of Fatma Gossiel. Screening blood work and some biochemical tests were carried out by the Clinical Chemistry and Immunology Departments, Sheffield Teaching Hospitals, Sheffield. Genotyping of vitamin D binding protein was performed by the Sheffield Diagnostic Genetics Service, Sheffield Children's Hospital Foundation NHS Trust. 25OHD<sub>2</sub> and 25OHD<sub>3</sub> were measured by Mr Brian Keevil, in the laboratory of the Institute of Human Development, University of Manchester, UK. Free 25OHD was measured by Future Diagnostics, BN Wijchen, The Netherlands.

I performed the statistical analysis for the study, but statistical support and determination of the original power calculation was provided by Dr Mike Bradburn and Dr Richard Jacques, School of Health and Related Research (SchARR), University of Sheffield. Statistical support for the vitamin D work was provided by Dr Jacques.

Dr Walsh and Professor Eastell provided guidance and direction throughout this work.

## **Presented Abstracts**

### **Oral Communications:**

Walsh JS\*, **Evans AL**, Vitamin D is low in obesity, and this is due to greater volume of distribution. European Calcified Tissue Society Meeting 2014; Prague, Czech Republic.

**Evans AL\***, Eastell R, Walsh JS. Biochemical Mechanisms of Greater Bone Density in Obese Individuals. Mellanby Centre for Bone Research, Research Day 2013, University of Sheffield, UK.

**AL Evans\***, F Gossiel, K Naylor, R Eastell, JS Walsh. Vitamin D Status In Obesity: Evaluation Of Free 25(OH)D. Bone Research Society/British Orthopaedic Research Society Meeting 2013; Oxford, UK.

**AL Evans\***, F Gossiel, K Naylor, R Eastell, JS Walsh. Vitamin D Status In Obesity: Evaluation Of Free 25(OH)D. The Rank Prize Funds Vitamin D Meeting 2013; Grasmere, UK.

**Evans AL\***, Walsh JS, Eastell R. The Effect of Obesity on Bone Density at Four Fracture Sites. National Osteoporosis Society Conference 2012; Manchester, UK.

### **Oral Poster Presentations:**

**Evans AL**, Eastell R, Walsh JS. Does bone density, bone strength, sarcopenia or dynapenia explain greater risk of fracture in obesity? European Calcified Tissue Society Meeting 2014; Prague, Czech Republic.

**Evans AL**, Eastell R, Walsh JS. Does bone density, bone strength, sarcopenia or dynapenia explain greater risk of fracture in obesity? Bone Research Society Meeting 2014; Sheffield, UK

### **Poster Presentations:**

Walsh JS\*, **Evans AL**. Vitamin D is low in obesity, and this is due to greater volume of distribution. Bone Research Society Meeting 2014; Sheffield, UK

**AL Evans**, F Gossiel, K Naylor, R Eastell, JS Walsh. Vitamin D Status In Obesity: Evaluation Of Free 25(OH)D. American Society for Bone and Mineral Research 2013 conference, Baltimore, USA .

**Evans AL**, Eastell R, Walsh JS. Greater Bone Density in Obese Individuals is Not a Result of DXA Artifact: a High Resolution Peripheral Quantitative Computed Tomography Study. CIMA in Industry Research Day 2013, Sheffield, UK.

**Evans AL**, Eastell R, Walsh JS. Greater Bone Density in Obese Individuals is Not a Result of DXA Artifact: a High Resolution Peripheral Quantitative Computed Tomography Study. American Society for Bone and Mineral Research 2012 conference, Minneapolis, USA.

Amin M\*, Manley AL, **Evans AL**, Eastell R, Walsh JS. The Effects of Gender and Obesity on Fracture Risk and Cardiovascular Risk. National Osteoporosis Society Conference 2012; Manchester, UK.

**Evans AL**, Walsh JS, Eastell R. The Effect of Obesity on Bone Density at Four Fracture Sites. University of Sheffield Medical School Research Day 2012, Sheffield,.

**Evans AL**, Eastell R, Walsh JS. Greater Bone Density in Obese Individuals is Not a Result of DXA Artifact: a High Resolution Peripheral Quantitative Computed Tomography Study. Mellanby Centre for Bone Research, Research Day 2012, University of Sheffield, UK.

## **Abbreviations**

2D / 3D	2-dimensional / 3-dimensional
μFE	Micro-finite element
aBMD	Areal bone mineral density
ALM	Appendicular lean mass
AP	Anterior-posterior
BA	Bone area
BAP	Bone alkaline phosphatase
BioE2	Bioavailable oestradiol
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMP	Bone morphogenetic protein
BRU	Bone remodelling unit
BSU	Basic structural unit
BTM	Bone turnover marker
BV/TV	Bone volume/total volume
CI	Confidence interval
CLIA	Chemiluminescent immunoassay
CK	Creatine kinase
CNS	Central nervous system
Ct.Ar	Cortical area
Ct.Pm	Cortical perimeter
Ct.Po	Cortical porosity
Ct.Po.Dm	Cortical pore diameter
Ct.Th	Cortical thickness
Ct.TMD	Cortical tissue mineral density
Ct.VM	Cortical Von Mises stress
CT	Computed tomography
CTX	β-carboxy-terminal collagen crosslinks
DBP	Vitamin D binding protein
E2	Oestradiol
ECLIA	Electrochemiluminescent immunoassay
ELISA	Enzyme linked immunoassay
DXA	Dual energy x-ray absorptiometry
fE2	Free oestradiol
FE/ FEA	Finite element / finite element analysis
FGF-23	Fibroblast growth factor 23
FM	Fat mass
FN	Femoral neck
FRAX	WHO Fracture Risk Assessment Tool
HA	Hydroxyapatite
HDL	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
HR-pQCT	High resolution peripheral quantitative computed tomography
HRP	Horseradish peroxidase
HRT	Hormone replacement therapy
HU	Hounsfield Units
HsCRP	Highly sensitive C-reactive protein
IGF-I	Insulin-like growth factor-1
IL-	Interleukin-
LDL	Low density lipoprotein cholesterol
LM	Lean mass
LS	Lumbar spine

MrOS	The Osteoporotic Fractures in Men Study
MSC	Mesenchymal stem cell
OA	Osteoarthritis
OC	Osteocalcin
OPG	Osteoprotegerin
PINP	Procollagen 1 N-terminal peptide
PAT	Peripheral adipose tissue
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PYY	Peptide-YY
QC	Quality control
QCT	Quantitative computed tomography
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor $\kappa$ B
RANKL	Receptor activator of nuclear factor $\kappa$ B ligand
SAD	Supine abdominal thickness
SAT	Subcutaneous abdominal adipose tissue
SD	Standard deviation
SHBG	Sex hormone binding globulin
SMI	Skeletal muscle index
SOF	The Study of Osteoporotic Fractures
SPPB	Short physical performance battery
T1DM/ T2DM	Type 1 Diabetes Mellitus/ Type 2 Diabetes Mellitus
Tb.vBMD	Trabecular volumetric bone mineral density
Tb.N	Trabecular number
Tb.Th	Trabecular thickness
Tb.Sp	Trabecular spacing
Tb.N/SD	Trabecular heterogeneity
Tb.VM	Trabecular Von Mises stress
TC	Total cholesterol
TH	Total hip
TG	Triglycerides
TPA	Tripropylamine
TNF	Tumor necrosis factor
Tot	Total
Tot.Ar	Total area
Tot.vBMD	Total vBMD
TotE2	Total E2
TSH	Thyroid stimulating hormone
uOC	Undercarboxylated osteocalcin
VAT	Visceral adipose tissue
VDR	Vitamin D receptor
VFA	Vertebral fracture assessment
WB	Whole body
WC	Waist circumference
WHR	Waist to hip ratio
WHO	World Health Organisation



## **List of Tables**

Table 1: FAB Study Visits .....	42
Table 2: HR-pQCT outcomes, units, definitions and abbreviations .....	59
Table 3: $\mu$ FE analysis outcomes from HR-pQCT scanning .....	62
Table 4: Primer sequences for the genotyping of DBP .....	73
Table 5: Sources of analyte variability.....	75
Table 6: Characteristics of the study population by age and BMI group; mean (SD) ...	84
Table 7: Mean percentage difference in skeletal outcomes between obese and normal BMI individuals before and after normalising for body weight .....	94
Table 8: Summary of results Chapter 3.....	95
Table 9: Potential mediators of the effect of obesity on bone density .....	115
Table 10: LM predicts aBMD at central sites, while FM predicts peripheral vBMD ....	123
Table 11: Prediction of BMD by SAT and VAT .....	124
Table 12: Prediction of BMD by gynoid FM and android FM .....	124
Table 13: Prediction of BMD by AFM and trunk FM .....	124
Table 14: Adipose compartment predictors of bone microstructure.....	125
Table 15: CTX as a predictor of BMD .....	128
Table 16: Summary of results of Chapter 4.....	146
Table 17: Summary of results Chapter 5.....	181
Table 18: Characteristics of the vitamin D sub-study sample .....	202
Table 19: Vitamin D status of the sub-study sample .....	202
Table 20: Percentage distribution of the DBP diplotypes.....	210
Table 21: Percentage distribution of the DBP genotypes .....	210
Table 22: Correlation coefficients between BMD and vitamin D metabolites .....	219
Table 23: Correlations between fat compartments and vitamin D metabolites .....	222
Table 24: Summary of results of Chapter 6.....	224

## **List of Figures**

Figure 1: Cortical and trabecular compartments of bone.....	17
Figure 2: Microarchitecture of normal and osteoporotic bone.....	19
Figure 3: Prevalence of obesity in 1980 and 2008 .....	21
Figure 4: Simplified differentiation potential of MSCs .....	28
Figure 5: Components of the DXA Scanner .....	45
Figure 6: Positioning and sub-region defining lines for the whole body DXA scan.....	47
Figure 7: Positioning for the hip DXA scan.....	47
Figure 8: Application of the bone map to a DXA hip scan .....	48
Figure 9: Positioning for the lumbar spine DXA scan .....	48
Figure 10: Lumbar spine DXA scan with global region of interest and bone map .....	49
Figure 11: Phantoms for the calibration of the DXA device .....	50
Figure 12: DXA quality control plot for bone area throughout the FAB Study .....	50
Figure 13: DXA quality control plot for BMC throughout the FAB Study .....	50
Figure 14: DXA quality control plot for BMD throughout the FAB Study .....	51
Figure 15: Scale of attenuation in Hounsfield Units of various tissues.....	53
Figure 16: Scan images of the HR-pQCT phantoms .....	56
Figure 17: Positioning for the distal radius HR-pQCT scan .....	57

Figure 18: Reference line and measurement area on a distal radius scout scan.....	57
Figure 19: Positioning for the distal tibia HR-pQCT scan .....	58
Figure 20: Positioning of the reference line on a distal tibia scout scan.....	58
Figure 21: The principle of the determination of bone strength from $\mu$ FEA.....	62
Figure 22: Manual trace to distinguish SAT and VAT on a CT abdomen scan .....	63
Figure 23: Six metre walk and narrow walk course .....	65
Figure 24: Example ELISA well-plate set-up .....	67
Figure 25: Overview of the stages of an ELISA.....	68
Figure 26: Example manual assay standard curve plot.....	74
Figure 27: Principal microstructural outcomes of HR-pQCT imaging.....	78
Figure 28: Whole body (A), total hip (B) and lumbar spine (C) aBMD .....	85
Figure 29: Whole body aBMD of normal BMI and obese individuals by group: .....	85
Figure 30: Total hip aBMD of normal BMI and obese individuals by group:.....	86
Figure 31: Lumbar spine aBMD of normal BMI and obese individuals by group:.....	86
Figure 32: aBMD of obese individuals as SD scores.....	87
Figure 33: Tibia vBMD of normal BMI and obese individuals .....	88
Figure 34: Radius vBMD of normal BMI and obese individuals .....	89
Figure 35: Tibia vBMD, microarchitecture and strength of obese individuals.....	90
Figure 36: Radius vBMD, microarchitecture and strength of obese individuals .....	91
Figure 37: vBMD of obese individuals at the lumbar spine.....	91
Figure 38: Radius estimated failure load of normal BMI and obese individuals .....	92
Figure 39: Tibia estimated failure load of normal BMI and obese individuals. ....	93
Figure 40: Anterior view of the 3D FE model of the pelvis-femur-soft tissue complex .	98
Figure 41: Location of subcutaneous and visceral fat compartments .....	104
Figure 42: The effects of leptin on bone through peripheral and central actions.....	109
Figure 43: Whole body adiposity of normal BMI and obese individuals .....	120
Figure 44: SAT, VAT and VAT:SAT ratio of normal BMI and obese individuals. <sup>1,2</sup> ....	120
Figure 45: Central adiposity of normal BMI and obese individuals .....	121
Figure 46: Peripheral adiposity of normal BMI and obese individuals.....	121
Figure 47: BTMs in normal BMI and obese individuals .....	126
Figure 48: BTMs in normal BMI and obese individuals by age and gender .....	127
Figure 49: Bone turnover coupling index in normal BMI and obese individuals .....	128
Figure 50: Leptin and adiponectin in normal BMI and obese individuals.....	129
Figure 51: Leptin and adiponectin in normal BMI and obese individuals by group.....	130
Figure 52: TotE2, fE2, bioE2 in normal BMI and obese individuals.....	131
Figure 53: TotE2, fE2 and bioE2 in normal BMI and obese individuals by group .....	132
Figure 54: Glucose, insulin and HOMA-IR of normal BMI and obese individuals.....	133
Figure 55: Glucose, insulin and HOMA-IR of normal BMI and obese individuals by group .....	134
Figure 56: SHBG in normal BMI and obese individuals.....	135
Figure 57: Albumin in normal BMI and obese individuals .....	136
Figure 58: IGF-I in normal BMI and obese individuals.....	137
Figure 59: Adjusted calcium, PTH and 25OHD in normal BMI and obese individuals	138
Figure 60: BAP in normal BMI and obese individuals.....	139
Figure 61: OPG in normal BMI and obese individuals .....	140
Figure 62: Sclerostin in normal BMI and obese individuals .....	141
Figure 63: Creatinine and eGFR in normal BMI and obese individuals .....	142
Figure 64: TC, HDL and triglycerides in normal BMI and obese individuals .....	143

Figure 65: WBLM, ALM and SMI in normal BMI and obese individuals	164
Figure 66: Gait speed in normal BMI and obese individuals	165
Figure 67: Repeated chair stand time for normal BMI and obese individuals	166
Figure 68: Balance in normal BMI and obese individuals	167
Figure 69: SPPB score of normal BMI and obese individuals	168
Figure 70: Association between LM and grip strength	169
Figure 71: Association between LM and SPPB score	169
Figure 72: Mean number of falls in normal BMI and obese individuals	170
Figure 73: Number of falls by sarcopenia or dynapenia classification	172
Figure 74: Associations between HsCRP and physical function	173
Figure 75: Associations between albumin and physical function	174
Figure 76: Associations between IGF-I and physical function	175
Figure 77: Associations between glucose and physical function	176
Figure 78: Associations between haemoglobin and physical function	177
Figure 79: Associations between HDL and physical function	178
Figure 80: Associations between CK and physical function	179
Figure 81: Simplified illustration of vitamin D metabolism	195
Figure 82: Total 25OHD in normal BMI and obese individuals	203
Figure 83: Association between total 25OHD and 25OHD <sub>3</sub>	204
Figure 84: Total 25OHD <sub>3</sub> by LC-MS/MS in normal BMI and obese individuals	204
Figure 85: Association between measured and calculated free 25OHD	205
Figure 86: Free 25OHD in normal BMI and obese individuals	206
Figure 87: Dietary vitamin D intake in normal BMI and obese individuals	207
Figure 88: Percentage of normal BMI and obese individuals taking supplements	207
Figure 89: Annual sun exposure of normal BMI and obese individuals	208
Figure 90: Sun protection factor habits of normal BMI and obese individuals	208
Figure 91: Albumin in normal BMI and obese individuals	209
Figure 92: DBP in normal BMI and obese individuals	209
Figure 93: DBP concentration by DBP Diplotype and genotype	211
Figure 94: Total 25OHD by DBP Diplotype and genotype	211
Figure 95: Free 1,25(OH) <sub>2</sub> D by DBP Diplotype and genotype	212
Figure 96: Total and free 25OHD and 1,25(OH) <sub>2</sub> D by DBP genotype	213
Figure 97: Total and free 25OHD and 1,25(OH) <sub>2</sub> D by DBP Diplotype	214
Figure 98: Association between total 1,25(OH) <sub>2</sub> D and 25OHD and 25OHD <sub>3</sub>	214
Figure 99: Total and free 1,25(OH) <sub>2</sub> D in normal BMI and obese individuals	215
Figure 100: PTH in normal BMI and obese individuals	216
Figure 101: BTMs in normal BMI and obese sub-study individuals	218
Figure 102: Associations between vitamin D and aBMD	220
Figure 103: Associations between vitamin D and vBMD	221

# **CHAPTER 1:**

## **INTRODUCTION**

## **CHAPTER 1: Introduction**

### **Background**

#### **Fracture**

A fracture is a break in the bone, which occurs when the stress applied to bone results in a strain greater than the bone's capacity to dissipate such energy. This can be a result of non-physiological loads applied to normal bone, or of physiological loads applied to abnormal bone, such as in tumour or metabolic bone disease states. Fractures are a significant economic burden and are associated with morbidity, poor physical function, poorer quality of life and greater risk of mortality (1-4). First fracture has been shown to be a risk factor for subsequent fracture (5). The identification of novel targets to improve bone health, prevent falls and ultimately prevent fractures is likely to have significant consequences at both individual and societal levels.

#### **Function of the Skeleton**

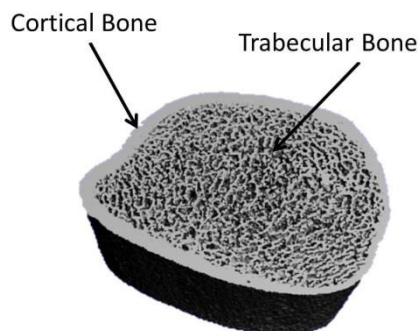
The skeleton is a multifunctional organ that provides the body with structural support, enables movement, protects internal organs, produces blood cells and facilitates the storage and release of minerals to maintain calcium homeostasis. The skeleton can be divided into two sub-skeletons; axial and appendicular. The axial skeleton consists of the skull, spinal column, sacrum, ribs and sternum, whilst the appendicular skeleton consists of the bones of the limbs and pelvic and pectoral girdles.

#### **Bone Physiology**

Bone is composed of mineral, collagen, non-collagenous proteins; including binding proteins and glycoproteins such as osteocalcin (OC), osteopontin, osteonectin and bone-sialoprotein, growth factors, glycosaminoglycans, phospholipids and water (6). Approximately 90% of bone matrix is collagenous (type I collagen). The mineral component of bone is formed from calcium and phosphate deposited as calcium-phosphate salts, which undergo mineralisation to hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ].

Bone is a highly active tissue and contains multiple cell types. Bone lining cells cover the surface of the bone and control the removal of calcium from bone and protect bone from external chemical influences. Osteoclasts resorb the bone matrix, while osteoblasts produce bone matrix and control osteoclastogenesis. Osteocytes, the most abundant bone cells, control bone formation and resorption to govern the skeletal response to mechanical forces. These cells communicate directly and also indirectly, through signalling mechanisms (6).

Bone is formed of two compartments; cortical and trabecular bone (Figure 1). Cortical bone makes up approximately 80% of the total body bone content and is found in the diaphyses of the long bones and surrounds the trabecular bone. Trabecular bone is found in the vertebrae, pelvis and metaphyses of the long bones.



**Figure 1: Cortical and trabecular compartments of bone**

### **Bone Modelling and Acquisition**

Bone modelling is responsible for changes in the shape and size of the bone, such as those observed during growth or adaptative responses to a change in mechanical loading patterns (7). Increased loading forces cause bone to deform as a result of increased strain within the bone tissue. Skeletal response to increased loading depends on the frequency, magnitude, rate and duration of these resulting strains, compared to the baseline loading condition. During modeling, bone resorption occurs without any associated formation at the site of resorption. For example, periosteal apposition accompanied by endocortical resorption can occur to increase bone size without an increase in cortical thickness, thus adapting the bone to increased loading forces, without increasing bone mass.

Young bone has been shown to be more capable of structural adaptations in response to mechanical loading than bone in older age (8). Peak bone mass is the amount of bone present at the end of skeletal maturation and is typically acquired by 25 to 30 years of age (9). Attainment of peak bone mass is predominantly genetically determined, however nutrition, physical activity and disease exposure also influence attainment (10).

## **Bone Remodelling and Loss**

Bone remodelling is the process by which bone tissue is continuously renewed in a cycle of resorption of old bone and formation of new bone. Bone is remodelled to alter its mass or microarchitecture in response to altered mechanical, nutritional and humoral signals and to maintain bone strength through the prevention of accumulated micro-damage.

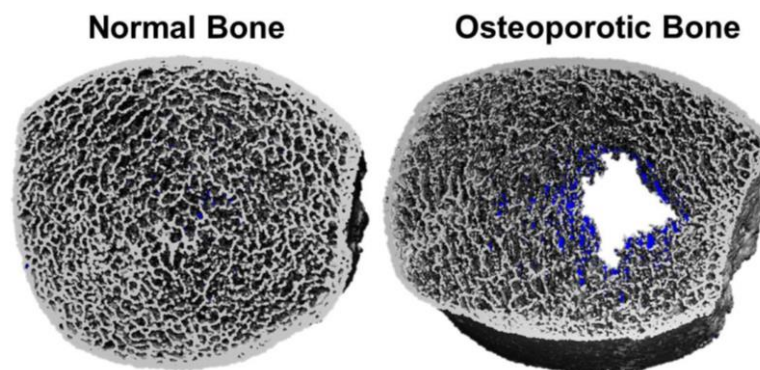
Remodelling takes place within a bone remodelling unit (BRU) which consists of osteoclasts, osteoblasts, osteocytes and bone lining cells on trabecular and Haversian bone surfaces. In the first phase of bone remodelling, activation, osteoclast precursors are activated and stimulate osteoclastogenesis. These osteoclasts resorb bone by dissolving bone mineral and breaking down the bone matrix. Osteoclasts remove approximately  $0.05 \text{ mm}^3$  of bone tissue per cycle (11), forming a resorptive cavity in the bone and releasing bone matrix components into the bone microenvironment and the circulation. This resorption takes approximately one month. In the third stage, reversal, osteoblast precursors are activated at the site of the resorption. Finally, over a period of approximately five months, osteoblasts fill the resorptive cavity by depositing newly synthesized bone matrix. Subsequently, mineralisation of this matrix occurs and a basic structural unit (BSU) of new bone is formed.

Due to the greater surface area-to-volume ratio of trabecular bone compared to cortical bone, the majority of bone remodelling occurs on the surface of bone in the trabecular compartment. In cortical bone, tunnels or cutting cones are formed by osteoclastic resorption before closing cones are formed by the osteoblast. The cortical remodelling cycle lasts approximately four months, compared to approximately seven months in trabecular bone.

After peak bone mass is attained, a very small percentage of bone is lost with each remodelling cycle, such that over one year approximately 0.5 to 1% of the total body bone mass is lost. Genetic, endocrine, nutritional and disease factors influence the amount of bone lost per cycle and may result in states where the osteoblast is increasingly unable to refill the resorption pit, resulting in a greater loss of bone per remodelling cycle. This resorption-formation imbalance also occurs with increasing age and is particularly notable at menopause. Incomplete refilling of cutting cones due to increased resorption relative to formation with increasing age, may explain greater cortical porosity and greater cortical pore cross sectional area observed with age.

## Osteoporosis

Osteoporosis is a deterioration of bone microarchitecture which results in low bone density, reduced bone strength, increased fragility and consequently increased risk of low-trauma fracture (12, 13). Osteoporosis is associated the ineffective repair of microdamage (14). Osteoporosis is defined as a bone mineral density (BMD) T-score at least 2.5 SD values below the healthy young adult mean (15). The osteoporotic deterioration of bone microarchitecture is illustrated in Figure 2. There are commonly no symptoms of osteoporosis until a fracture occurs, often as the result of a fall and so osteoporosis is frequently referred to as a 'silent epidemic'. However, once a fall and/or fracture have occurred there is often a significant increase in morbidity, including pain, loss of function, physical impairment, fatigue and a decrease in pulmonary function (1, 2, 16). Physiological implications of osteoporosis are accompanied by the predisposition to psychological and social debilitations including anxiety, depression, low self-esteem, and an overall reduction in quality of life (1, 12, 16, 17).



**Figure 2: Microarchitecture of normal and osteoporotic bone showing decreased bone volume to total volume and the loss of trabecular connectivity in osteoporotic bone**

## Prevalence of Osteoporosis and Osteoporotic Fractures

It was estimated that ten million Americans over fifty years of age had osteoporosis in 2004 and the prevalence of osteoporosis is rapidly increasing, partly due to the increasing size of the elder population (12). It is currently estimated that worldwide, one in three women and one in five men over fifty years of age will sustain an osteoporotic fracture during their lifetime (12). The annual incidence of hip fracture worldwide has been predicted to reach 8.2 million by 2050 (18). With osteoporotic fractures estimated to cost more than \$17 billion per year in the US alone, prevention of osteoporotic fractures should be a priority (18, 19).



### **Risk Factors for Osteoporosis**

Osteoporosis incidence increases with age and osteoporosis is more common in women than men (13). Postmenopausal women are at greater risk of osteoporosis, due to increased bone resorption as a result of the depletion of oestrogen. Other risk factors for osteoporosis include smoking, high alcohol intake, immobilisation, hypogonadism, chronic inflammatory diseases, glucocorticoid treatment, early menopause, family history of osteoporosis and low body weight (20).

### **Relationship Between Body Weight and Osteoporosis**

Low body weight is associated with low BMD (17, 21-23) and bone loss (24) and is an established indication for bone densitometry (25, 26). A recent systematic review showed that in addition to menopausal status, low body weight, but not other clinical risk factors typically associated with osteoporosis in older women, is an important risk factor for low BMD in women aged 40 to 60 years (27). Having a Body Mass Index (BMI) below 25kg/m<sup>2</sup> is associated with an increased risk of fracture (17, 21), particularly of hip and all osteoporotic fracture, but is protective of lower leg fracture (23, 28-30). Studies investigating the effect of anorexia nervosa on BMD have shown a high prevalence of osteopenia and osteoporosis in anorexic populations (31, 32), with fracture rates significantly greater than in healthy populations (33). However, while low body weight may contribute to such skeletal effects, anorexia nervosa is more complex and hormonal consequences may also contribute.

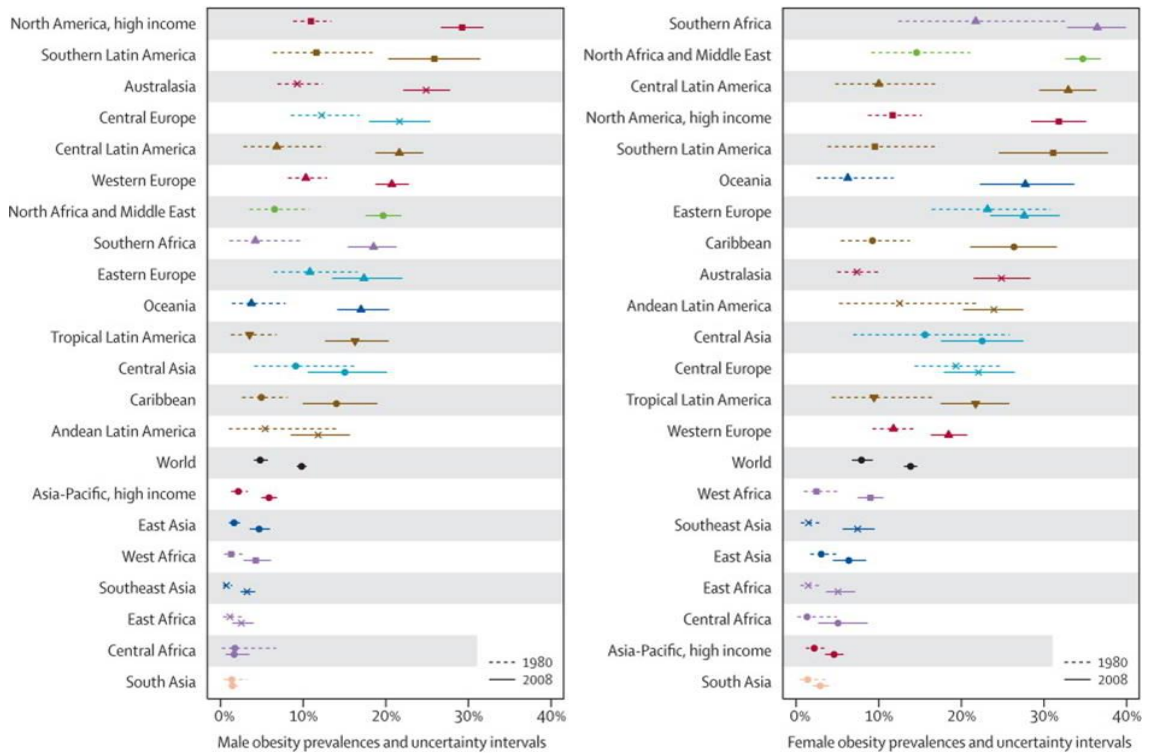
### **Obesity**

Obesity is a physical state resulting from energy imbalance, where the number of calories consumed is greater than that expended by physical activity. Obesity is characterised by excess body fat and associated with an increased risk of cardiovascular disease, hypertension, type 2 diabetes mellitus (T2DM), some cancers, liver and gall bladder disease, musculoskeletal conditions, fibromyalgia, unilateral plantar fasciitis and gout (34). Metabolic syndrome describes an amalgamation of metabolic abnormalities, typically characterised by abdominal obesity, insulin resistance, hyperglycaemia, hypertension, dyslipidaemia and hypertriglyceridemia (35).

Social and economic development and urbanisation have increased access to and dependence on high fat, energy rich, convenient foods. This has contributed to a nutritional transition, with increased consumption of high fat, high sodium, energy dense foods and away from traditional high fibre, high protein diets (36). Concurrently, technological advances have reduced physical activity demands. In combination, these

factors have contributed towards the creation of an obesogenic environment, with physiological, physical, psychological and financial consequences, and individual, societal and global implications.

The World Health Organisation (WHO) defined overweight as a BMI of 25 to 29.9 kg/m<sup>2</sup> and obesity as a BMI greater than or equal to 30 kg/m<sup>2</sup>. Global prevalence of obesity has rapidly increased and continues to increase (Figure 3) (37).



**Figure 3: Prevalence of obesity in 1980 and 2008**  
**In men (left) and women (right) (37). Reprinted from The Lancet, 377(9765), Finucane MM, et al. Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index), National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants, 557-567, Copyright (2011), with permission from Elsevier.**

The WHO estimates that around 1.2 billion people worldwide are overweight with at least 300 million of these individuals being obese (38). It is estimated that approximately 25% of adults and 15% of children in England are obese (39, 40). Based on current rates and trends in the prevalence of overweight and obesity, it has been predicted that 40% of the UK population will be obese by 2025 and more than half of the UK adult population will be obese by 2050 (39). By this time it is estimated that fewer than 10% of men and 15% of women in the UK will be categorised as having a 'healthy' BMI (18.5 to 24.9 kg/m<sup>2</sup>) (39). Consequently, the Foresight report estimated that overweight and obesity will cost the National Health Service £10 billion per year by 2050 (39).

### **Osteoporosis Prevalence in Obesity**

A recent study reported that 9.4% of obese women attending for routine bone densitometry had osteoporosis at the femoral neck (FN) compared to 52.1% of underweight individuals and 20.3% of normal weight attendees (41). In the same group of women, only 5.9% of obese individuals had a T-score  $<-2.5$  at the lumbar spine (LS) compared to 22.8% of underweight individuals and 12% of normal weight individuals (41). In the Study of Osteoporotic Fractures (SOF), only 4.6% of obese women who did not fracture between baseline and follow up 11 years later were osteoporotic (42). Elsewhere, of women with a T-score  $\geq+2.5$  at the LS, FN or total hip (TH), 43.5%, 55.6% and 73.1% respectively were obese (43).

In the SOF, of obese women who fractured between baseline and follow up, where BMD might be expected to be lower in light of the fracture, the prevalence of osteoporosis by FN T-score  $\leq-2.5$  was only 11.7%, whereas 54.4% of non-obese women who fractured were osteoporotic (42). Similarly, of individuals presenting to a fracture liaison service in the UK, the prevalence of osteoporosis was 13.4% in the obese group compared to 40.4% in the normal BMI group (44). The use of anti-osteoporosis medication in obese women with fracture was found to be significantly lower than the use in non-obese women with fracture (4).

### **Weight Loss, BMD and Bone Microarchitecture**

The existence of a relationship between body mass and BMD is also supported by weight loss studies. A large, prospective study of men found that weight loss over three decades was associated with lower hip BMD (45). Amongst men in the lowest BMI quartile at baseline who lost  $\geq 5\%$  of their body weight, there was a prevalence of osteoporosis of 31%, while amongst men in the same low BMI quartile at baseline who gained  $\geq 5\%$  of their body weight, there was only a 4% prevalence of osteoporosis, demonstrating a positive association between body mass and BMD (45). In postmenopausal women, body weight was independently associated with both BMD at baseline and the rate of BMD change during a ten year follow-up (46). A recent HR-pQCT study of women undergoing bariatric surgery showed a significant decrease in total volumetric BMD (vBMD) at the tibia at one year post-surgery (47). This was shown to be mostly due to decreases in cortical bone, with declines in cortical density and thickness and area, with no difference in trabecular vBMD or trabecular microstructural parameters (47). Despite the loss of cortical bone, there was no difference in stiffness or the distribution of load through the cortical bone (47).

## **Fracture in Obesity**

Given that low BMD is an established risk factor for fracture and that osteoporosis appears less prevalent in obesity, lower fracture risk may be expected in obesity. It has been reported that for every standard deviation decrease in body weight, the risk of incident fracture increases by 19% (48). A meta-analysis of twelve prospective cohorts found a BMI of 25 kg/m<sup>2</sup> to be the threshold below which the risk of hip and osteoporotic fracture was increased (17). Similar conclusions were reached in the SOF at a BMI of 26 kg/m<sup>2</sup> (48).

However, in the Global Longitudinal Study of Osteoporosis in Women (GLOW study), fracture prevalence in obese women at baseline was 222 per 1000 compared with a similar 227 per 1000 in non-obese women (49). At two year follow up, fracture incidence was 61.7 per 1000, again similar to the rate of 66.0 per 1000 in non-obese women (49). In the MrOS cohort, after adjustment for BMD and compared to the risk of non-vertebral fracture in men with a normal BMI, mild obesity (BMI 30 to 34.9 kg/m<sup>2</sup>) was associated with a hazard ratio of 1.29 and moderate obesity (BMI 35 to 39.9 kg/m<sup>2</sup>) associated with a hazard ratio of 1.94 (30). In a study investigating the prevalence of obesity in postmenopausal women presenting with low-trauma fracture to a fracture liaison service, an unexpectedly high proportion of obese attendees was observed (50). Recently, data collected over 5.5 years from a fracture liaison service in the UK showed that 30% of individuals with a low trauma or osteoporotic fracture were obese (44). Overall these general findings are conflicting, but indicate that obesity is perhaps less protective against fracture as might have been expected given the seemingly positive associations between obesity and BMD.

Associations between obesity and fracture risk appear to be site-dependent; protective at some sites but a risk factor at other sites, which may help to make sense of the conflicting reports of incident fracture described above. High BMI appears protective against fractures in the axial skeleton, with obesity widely shown to be protective against hip and vertebral fracture (29, 51-56). Just 9% of all postmenopausal hip fractures occurred in obese women in the UK Million Women Study, although the percentage of obese women in the cohort is unclear (53). A recent meta-analysis found just 19% of osteoporotic fractures and 13% of hip fractures occurred in obese women (55). Obesity also appears protective against wrist fracture (49, 54). Even with a lower whole body, hip and distal forearm BMD, men with abdominal obesity and metabolic syndrome had a lower incidence of vertebral and peripheral fragility fractures than men without metabolic syndrome (57).

However, despite the lower prevalence of osteoporosis in obese populations and protective effects against fracture in the axial skeleton, obesity may increase the risk of fracture at peripheral sites including the proximal humerus (23, 29, 55, 58) and lower limb (23, 52, 55). Specifically, obese individuals appear to have a greater risk of displaced ankle fractures and of Weber C fracture (above the level of the ankle joint) than Weber A or B ankle fractures (below or at the level of the ankle joint) (59, 60). Others have reported no association between BMI and the risk of ankle or wrist fracture (58) and that weight may be more strongly associated with ankle fracture in obesity than BMI or height (51).

Whether obesity is protective or a risk factor for fracture at some fracture sites is yet to be determined, with inconsistent reports at the forearm (52, 57, 58, 61-63) for example. While some have reported increased risk of rib fracture in obesity, the difficulty in confirming the presence of rib fracture and controlling for environmental factors commonly associated with rib fracture (e.g. alcohol intake) may raise questions over whether this is a true effect of adiposity on fracture risk (51, 54).

The extent of adiposity may affect fracture risk at certain sites, with some having reported lower fracture risk in overweight versus normal weight or obese groups (23). Fracture risk in obesity may also differ by gender, with obese men having been reported as at a greater risk of fracture than non-obese men (64).

As BMI is factored into the FRAX® tool, when used with or without BMD, there is a lower predicted risk of hip and major osteoporotic fracture in obese women compared to non-obese women (65). However, for predicting both hip and major osteoporotic fracture, sensitivity is lower but specificity higher in obese women compared with non-obese women (65). Compared to observed incident fracture data from the SOF, FRAX models appeared most valuable for predicting hip fracture in obese women at greatest risk of fracture (predicted values between 4 and 10%). For major osteoporotic fracture, FRAX generated predicted counts were more similar to the observed fracture counts at all predicted risk levels, but especially between 10 and 30% (65).

The economic burden of fracture in obese populations was determined in the GLOW study (4). Despite protective effects of obesity against hip and vertebral fracture, fractures elsewhere in the skeleton in obesity make a significant contribution to the burden of fracture on healthcare systems (4). Despite fracture incidence being greater in

non-obese individuals, due to the proportion of overweight and obese individuals in the overall population, the majority of fractures occur in those with a high BMI (64).

There are consistent reports of no difference in the risk of complications post fracture between obese and non-obese adults (60, 66, 67). Although smaller studies have also shown no difference between obese and non-obese individuals in length of hospitalisation after ankle fracture (60) or after high trauma fracture of the femur or tibia (66), in the GLOW study, obese women with a fracture were hospitalised for significantly longer than non-obese women (4). After fracture, obese women have a poorer functional status and health related quality of life than non-obese women as fracture related morbidity is greater in the obese (4, 68). Whether fracture related mortality is altered in obesity is unclear. Obesity has been shown to be a significant risk factor for mortality post hip fracture (68), but several large cohort studies have shown fracture related mortality to be lower in obese than normal weight groups (69-71), or inverse associations between BMI and fracture related mortality risk (72).

### **Determinants of Fracture in Obesity**

Risk factors for fracture in obesity appear to be similar to those in the non-obese population (increased age, lower BMD, history of previous fracture, family history etc.) (42, 69). However, there may be differences in skeletal and non-skeletal determinants of fracture risk in obesity, compared to non-obese individuals.

Skeletal determinants of protective effects of obesity against fracture might include greater bone density, favourable cortical and/or trabecular microstructure and greater bone strength at sites where obesity is protective against fracture.

Non-skeletal factors may also contribute to the protective effects of obesity against fracture. Obese adults may be protected against fracture at central sites due to greater soft tissue thickness which acts as 'padding' to absorb some of the impact of a fall and thus protect the underlying bone from fracture (23, 73). Obese adults may be more sedentary and participate in less physical activity, which might limit their exposure to outdoor environments which predispose to falls and fractures.

At sites where obesity is associated with greater fracture risk, there may be no difference in BMD between obese and normal BMI individuals, or lower BMD in obese individuals. Bone structure may be unfavourable and/or bone strength might be lower at sites where obesity is associated with increased fracture risk. Alternatively, if bone density, structure

and strength are favourable at sites where obesity is associated with greater fracture risk, it may be that the bone is insufficiently adapted i.e. parameters are not greater in a way that is commensurate to the greater loading effects of obesity. Importantly, the relationship between BMI and bone strength may not be linear.

Finally, non-skeletal factors may contribute to greater fracture risk at some sites in obesity. Fall patterns may be different in obese individuals compared to non-obese individuals. For example, obese people may be more likely to fall due to lower lean mass, sarcopenia, sarcopenic obesity or impaired muscle function. Obese people may fall in different directions to non-obese individuals and this may be related to the greater risk of some fractures in obesity. Pre- and mid-fall reaction times may be slower in obesity, such that there becomes the inability to break a fall by outstretching the arms, thus the wrist is protected but the proximal humerus at risk of fracture. In the general population obesity is associated with other co-morbidities such as diabetes and low vitamin D status, both of which are associated with increased fracture risk (64)

Some gender differences in fracture risk in obesity may also be contributed to by the factors above, for example gender differences in soft tissue thickness at the hips may contribute to gender-specific differences in hip fracture risk, although this has been questioned by others (64, 73). The difference in oestrogen between men and women is a possible key determinant of gender differences in fracture risk.

### **Effects of Obesity on Bone Throughout the Life Course**

Whether adiposity affects bone density, structure, strength and fracture risk, in the same way throughout the life course is unclear. The literature regarding associations between adiposity, bone structure and strength in children and adolescents is conflicting. Most of the evidence from studies of obese children and adolescents suggests negative associations between body mass, BMD, bone geometry and bone strength and that fat mass may have adverse effects on bone in childhood and adolescence (74-77).

At present it is unclear whether obesity affects bone density, structure, strength and fracture risk in younger and older adults in the same way. Age or skeletal state at the onset of obesity and also the duration of obesity may affect fracture risk, depending on the mechanism of the effect of obesity on bone density, structure and strength and may explain why some cross sectional studies report results which are inconsistent with the wider literature (78). It may be that obesity from adolescence into young adulthood is associated with beneficial effects on the attainment of peak bone mass. Obesity in later

adulthood may convey beneficial effects on the skeleton through the reduction of bone loss.

### **Obesity and Bone in Murine Models**

Murine models of high-fat-diet (HFD) and high-fat-sucrose diet (HFSD) induced obesity have been widely reported to induce bone resorption (19) and are associated with low BMD (19, 79), poor microarchitecture (19, 80, 81) and lower bone strength (80-83). Many murine studies have demonstrated short-term effects of HFDs on bone, although similar results have been observed in long-term studies (19, 84), where HFSDs exacerbated the negative effects of adiposity on bone structure and strength (85). The application of murine models to human physiology may be limited as inducing short-term obesity may not be comparable to the physiological effects of obesity observed in obese human populations, where high body mass may be the result of adiposity accumulated over a considerable duration. Additionally, most murine studies involve young animals which might serve as potential comparators for observations in children and/or adolescents, but any variation in the association between obesity and bone between younger and older groups may make such models inapplicable to adulthood.

### **Overview of Mechanistic Links Between Fat and Bone**

Numerous links between adiposity and bone have been proposed. Determining the mechanism(s) by which obesity may exert protective effects on bone might be important for the identification of novel therapeutic targets for the treatment of disorders of low bone mass, such as osteoporosis. The potential key mechanisms by which obesity may affect BMD or bone structure are described here. Several of these mechanisms will be explored and discussed in more detail in this thesis.

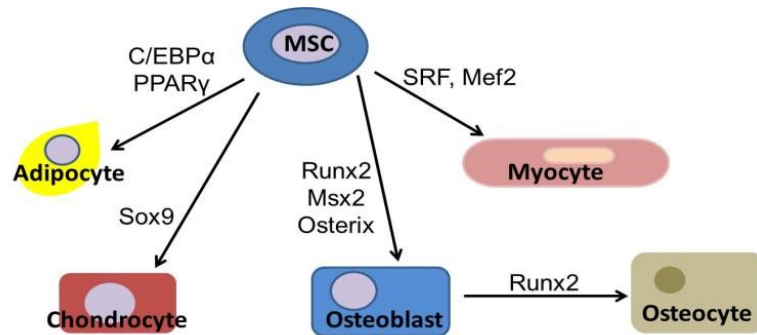
### **Mechanical Loading**

The mechanostat theory describes how a microarchitectural response is generated by bone in order to adapt to mechanical loading (86). When bone is exposed to a load beyond the level required to maintain baseline strength, bone formation is stimulated and the threshold load required for skeletal maintenance increased (87, 88). The mechanical loading effects of high body weight may therefore contribute to protective effects of obesity on BMD. However, despite supporting greater BMD in obesity, it is most likely that bones adapt to habitual loading forces rather than to infrequent greater fall forces. Consequently, fall forces may exceed the adaptation to habitual loading conditions in obesity and fracture may still result, despite greater BMD.



## Osteoblasts and Adipocytes

Mesenchymal stem cells (MSCs) are able to differentiate into chondrocytes, myocytes, osteoblasts or adipocytes as illustrated in Figure 4 (89). MSCs are “regulated by endocrine, paracrine and autocrine signals” (90) and their differentiation is mediated by oxidative tension and stress (91). As adipocytes and osteoblasts share this common origin, MSCs may play a role in the fat-bone relationship. MSCs differentiate to meet the demands of tissue growth and repair. Hence, it was hypothesised that in obesity, as demand on the skeleton to adapt to high body weight is greater, MSCs may be more likely to differentiate into osteoblasts than adipocytes and consequently, obesity is associated with greater bone mass (92). Mutually exclusive differentiation of MSCs is unlikely to affect the positive relationship between adiposity and bone, as adipocytes are able increase in size, thus adiposity can increase simultaneously with osteoblast differentiation (61).



**Figure 4: Simplified differentiation potential of MSCs along adipogenic, chondrogenic, osteogenic and myogenic lineages**

## Endocrine Links Between Fat and Bone

Numerous hormones and biochemical factors have been identified which link fat and bone through direct or indirect actions. Many of the effects exerted by hormones on the skeleton are understood to be evolutionary adaptations which act as an attempt to mediate energy metabolism (93, 94). An overview of endocrine links is given here, with a more in-depth review in Chapter 4.

## Acute Endocrine Links: Hormonal Responses to Feeding

Feeding results in an acute decrease in bone resorption, mediated by a number of hormones (95). Insulin, preptin and pancreatic peptide increase osteoblast proliferation (95), while amylin inhibits bone resorption (61, 96). Growth hormone and insulin-like growth factor-1 (IGF-I) increase following feeding and IGF-I is positively associated with bone formation and BMD (95, 96). Feeding stimulates the secretion of glucagon-like peptide 2 (GLP-2) which has been shown to reduce bone resorption without affecting bone formation (95).

## **Fat as an Endocrine Organ**

Adipose tissue is a highly active endocrine organ, producing over fifty cytokines and other related molecules (90, 97), some of which have been shown to affect bone and are summarised below.

### **Oestrogen**

Oestrogen inhibits bone resorption. Fat mass (FM) is positively associated with circulating oestrogen resulting from greater aromatisation of androgens as aromatase is expressed in fat tissue. After menopause, the production of oestrogen by aromatisation of adrenal androgens is the main source of oestrogen in older women and so FM may be a particularly important regulator of BMD in this population (98).

### **Adipokines**

Fat produced hormones, or adipokines, are produced in proportion to FM and therefore concentrations are altered in obesity. Leptin is a hormone product of the *OB* gene and plays an important role in regulating appetite and energy homeostasis via actions on the hypothalamus (90, 97, 99). Circulating leptin levels are positively associated with total FM and may be affected by sex hormones and inflammatory cytokines (97, 98). Associations between leptin and BMD are complex, with human and murine studies yielding conflicting results and leptin exerting positive and negative effects on bone metabolism, depending on whether it acts directly on bone cells or indirectly (via the hypothalamus and autonomic nervous system), respectively (98, 99). Adiponectin is reduced in obesity and inversely associated with visceral adipose tissue (VAT) (90, 96, 100). Adiponectin is involved in glucose synthesis in the liver; increasing insulin sensitivity and reducing serum insulin (96). In humans, negative associations between adiponectin and BMD have been reported (101-104). Adiponectin may stimulate RANKL and inhibit the production of osteoprotegerin (OPG) by osteoblasts, resulting in osteoclastogenesis (105, 106). Resistin is up-regulated in obesity and has been shown to increase osteoblast proliferation and osteoclastogenesis *in-vitro* (96). Visfatin levels are positively associated with adiposity and increase the production of interleukin (IL) 1 $\beta$ , IL-6 and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ). It has been proposed that visfatin may play a role in insulin metabolism (97).

### **Inflammatory Cytokines**

Obesity is considered a low grade pro-inflammatory state, associated with greater concentrations of pro-inflammatory but also some anti-inflammatory cytokines, including IL-10, IL-6, IL-8 and IL-1 receptor antagonist (IL-1Ra) and TNF- $\alpha$  (97). Pro-inflammatory

cytokines are inversely associated with BMD and positively associated with bone resorption, with *in-vitro* studies showing that inflammatory cytokines act on osteoclast precursors to up-regulate osteoclastogenesis (107, 108).

### **Vitamin D**

Vitamin D is a fat soluble pro-hormone, which functions to increase calcium absorption in the intestine. BMI is inversely associated with 25OHD (109, 110). Low 25OHD is typically associated with higher parathyroid hormone (PTH) and increased bone turnover, resulting in bone loss. However as described earlier, obesity seems to be associated with higher aBMD and this suggests that the vitamin D axis and metabolism might be altered in obesity. At present the cause(s) and consequences of low vitamin D in obesity are unclear. There may also be a role of low 25OHD in mediating the risk of falls in obesity through effects on muscle function, which could provide a link between obesity and greater risk of fractures at some sites.

### **Bone as an Endocrine Organ**

The skeleton also acts as an endocrine organ, secreting osteokines to regulate metabolism (94). Osteocalcin (OC) is “a major non-collagenous protein in the extracellular matrix” produced specifically by mature, active osteoblasts (94, 99, 111). OC regulates glucose metabolism by increasing expression of adiponectin by adipocytes and insulin by  $\beta$ -cells (93, 99) and is involved in the regulation of central adiposity (112). OC exists in two forms; undercarboxylated (uOC) and carboxylated. Greater uOC has been positively associated with vertebral and hip fracture risk (111, 113, 114). Osteoprotegerin (OPG) is released by osteoblasts and inhibits osteoclast differentiation and activity through acting as a receptor activator of RANKL; preventing the binding of RANKL to RANK (105, 115). OPG has been found to be negatively correlated with body weight, BMI, waist circumference and fasting plasma insulin and positively correlated with adiponectin (116).

### **Different compartments of fat**

Body fat distribution may play an important role in the relationship between adiposity and bone as different adipose depots express different biochemical factors and in varying concentrations. In obesity, VAT expresses more pro-inflammatory cytokines including TNF- $\alpha$  and IL-6, but less adiponectin, leptin and aromatase than subcutaneous fat (SAT) (100, 117-119). The overall effect of adiposity on bone is likely to be a balance of positive and negative influences from different adipose compartments.

## **Limitations of the Current Literature**

Associations between obesity and fracture risk are complex, with indications of age, skeletal site and gender specific differences. Interpretation of the current literature is challenging due to the majority of findings originating from observational studies or longitudinal cohorts not primarily designed to study the association between adiposity and skeletal outcomes. Such incidental findings are widely reported but obese groups are often poorly represented, resulting in low power to detect significant differences between obese and non-obese groups. Opportunistic studies frequently involve participants from heterogeneous racial backgrounds, introducing ethnic variation in bone phenotypes, FM and fat distribution (120-122).

Much of the current literature describes associations between adiposity and fracture risk in postmenopausal women or ageing men. Studying this association throughout adulthood could enable the identification of the onset of any effects of obesity on bone, and provide information on the progression of any such effects, in turn helping to identify potential mechanisms of the effect of obesity on bone. Pre- and postmenopausal women should be studied separately, as the pooling of pre- and postmenopausal women may confound findings due to the significant skeletal, soft tissue and endocrine differences between the two groups. Similarly, pooling the findings of men and women results in confounding due to variation in bone density, bone size, endocrine and soft tissue factors by gender (100).

A lack of control for potential confounding factors, such as physical activity, the use of hormone replacement therapies or the inclusion of individuals with diabetes may also distort findings. This is a further consequence of opportunistic analysis of existing data sets, avoidable by designing a study specifically to investigate the fat-bone relationship.

The overwhelming majority of previous work investigating effects of obesity on bone has involved determining areal BMD (aBMD) by dual energy x-ray absorptiometry (DXA) and inferring aBMD to be positively and proportionally associated with bone strength or lower fracture risk. As will be discussed in this thesis, there are significant limitations to the use of DXA in obesity. As bone strength is not determined by aBMD alone, but contributed to by bone structure, geometry and the material properties of bone, this limits current findings. Novel, more sophisticated technologies such as High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT) are available which enable the study of bone microarchitecture in obesity and reduce soft tissue effects. Finite element (FE)

models enable more comprehensive assessments of bone strength to be made by incorporating data on bone density, geometry and structure.

The widespread use of DXA has meant that much of the current literature is focussed on determining aBMD at the hip and lumbar spine and the results are often assumed to be applicable throughout the skeleton. Acknowledging the site-dependent associations of obesity and fracture risk, studying axial and appendicular, weight bearing and non-weight bearing skeletal sites is likely to provide a more thorough understanding of the effect of obesity on fracture risk.

A further implication of the reliance on DXA is the restricted ability to determine the effect of different soft tissue compartments on bone. Whilst FM and LM, android, gynoid and trunk regions can be distinguished by DXA, alternative depots such as subcutaneous, visceral, brown fat, bone marrow fat, inter and intra-muscular fat compartments cannot be determined. Determining the contributions of these different adipose depots to the effect of obesity on bone may be important for the identification of potential mediators of the relationship.

At present, there is a limited understanding of fracture patterns in obesity. Greater risk of some fractures in obesity may be due to increased risk of falls or specific fall kinematics associated with altered physical performance in obesity. The current literature surrounding physical performance in obesity is limited, with a paucity of studies involving obese individuals and matched non-obese controls.

Until recently, there was a longstanding lack of a consensus definition of sarcopenia which made classifying and interpreting muscle mass and function status problematic. Now that a consensus definition is in place, determining whether obese individuals are at greater risk of sarcopenia could assist with determining falls risk in obesity.

The cause of low 25OHD in obesity is unclear, as are the consequences of low 25OHD in obesity. Very few studies have investigated the effect of adiposity on free fractions of 25OHD and the effect of obesity on free 1,25(OH)<sub>2</sub>D has not been studied. No study has investigated the role that free fractions of vitamin D play in associations between vitamin D status and skeletal structure and strength. The majority of studies investigating the effect of obesity on 25OHD rely on corrections for seasonality, which could be better controlled by designing studies which recruit during specific periods of the year.

## **Thesis Overview**

This thesis seeks to understand why obese people are protected against some fractures but at greater risk of fracture at other sites and identify potential drivers of the site-specific differences in fracture risk observed in obesity. This study has been designed specifically to investigate the effect of obesity on the skeleton and risk factors for fracture in obesity. Thus the study will be sufficiently powered to detect differences in skeletal outcomes between an obese group and a normal BMI control group.

Firstly this thesis will seek to understand how obesity affects bone density. This will be achieved using conventional DXA imaging alongside state-of-the-art imaging by HR-pQCT and QCT to differentiate total, cortical and trabecular densities. This thesis will progress from studying bone density to investigate associations between obesity and bone structure using HR-pQCT and QCT. Understanding patterns of cortical and trabecular microarchitecture in obesity may give an insight into skeletal determinants of fracture in obesity previously masked by studying aBMD.

Subsequently, FE models will be used to investigate whether any observed differences in bone density and structure between obese and normal BMI individuals affect bone strength, improving on strength estimates derived from aBMD alone. Employing a range of technologies to assess bone density, structure and strength enables the investigation of effects of obesity at a range of skeletal sites; central and peripheral, weight bearing and non-weight bearing. This will eliminate assumptions that imaging outcomes at made central sites reflect those at peripheral sites.

Bone turnover markers, hormones and biochemical factors will be measured to identify potential mechanisms by which fat mediates any effect on bone outcomes. Detailed assessments of body composition will be undertaken using anthropometry, DXA and CT to determine the effects of LM, FM, SAT, VAT and subcutaneous peripheral depots on bone density and bone microstructure.

Bone is more likely to be adapted to everyday loading conditions than to loads experienced upon falling. Upon falling significant forces associated with high body weight may be exerted. Where greater bone strength is not commensurate to the loading forces applied, fracture will occur. This thesis will investigate whether obese people might fracture at sites where bone density and strength are greater compared to normal BMI individuals because they have low LM or poor physical function, which could affect fall frequency and/or fall kinematics.

Finally, this thesis will investigate vitamin D levels in obesity to better understand why low circulating 25OHD levels are observed in obese adults. This work will provide a comprehensive assessment of possible causes of low vitamin D in obesity and establish whether low vitamin D levels have any association with bone density, bone or microstructure, LM or physical performance.

### **Long Term Research Goals**

The ultimate aim of this area of research is to prevent fracture. By improving current understanding of the pathogenesis of fracture in obesity, effective and appropriate fracture prevention strategies can be established for this growing subset of the population. Identification of obese individuals at highest risk of fracture is important and there might be the need to increase awareness of the potential for fracture in obesity despite the recognised protective effect of high BMI on BMD.

Understanding how obesity affects bone density and/or structure may enable the identification of biochemical factors which mediate the association between fat and bone. Ultimately this may lead to the identification of novel therapeutic targets for the treatment of conditions characterised by low bone density, such as osteoporosis.

### **Thesis Aims**

**To identify associations between obesity and bone density, bone structure and bone strength across the skeleton (Chapter 3).**

1. To compare BMD measured by DXA and QCT at the hip and lumbar spine in normal BMI and obese individuals, to determine associations between obesity and BMD.
2. To compare BMD and bone microarchitecture at the distal tibia and distal radius, measured by HR-pQCT in normal BMI and obese individuals, to determine associations between obesity and each of these outcomes.
3. To compare bone strength determined by FEA at the hip, lumbar spine, distal radius and distal tibia, in normal BMI and obese individuals, to determine associations between obesity and bone strength.
4. To compare the magnitude of the differences in BMD, bone microarchitecture, bone geometry and bone strength at different skeletal sites to establish whether the effects of obesity on these outcomes are site-specific.

5. To determine whether the associations between obesity, BMD, bone microarchitectural parameters and bone strength appear commensurate to greater body weight in obesity.

**To investigate potential mechanisms of the associations between obesity, bone density, structure and strength (Chapter 4).**

1. To ascertain which fat compartment(s) are most strongly associated with BMD, geometry and microarchitecture, in normal BMI and obese individuals.
2. To test associations between the proposed key fat compartment, biochemical markers of bone turnover and biochemical factors to identify potential mediators of the associations between obesity, BMD, bone microarchitecture and bone strength.

**To determine whether physical performance is impaired in obesity such that despite greater BMD, greater risk of ankle and proximal humerus fracture in obesity could be explained by greater falls risk (Chapter 5).**

1. To identify whether obesity is associated with greater number of falls.
2. To compare LM, muscle strength and physical performance in normal BMI and obese individuals to determine associations between obesity and physical function.
3. To compare the prevalence of sarcopenia in the normal BMI and obese groups and ascertain any associations between sarcopenia and fall history.
4. To identify potential biochemical mediators of the association between adiposity and physical performance.

**To investigate causes and consequences of low circulating vitamin D in obesity (Chapter 6).**

5. To establish whether obese adults have low total and/or free 25OHD and 1,25(OH)<sub>2</sub>D.
6. To determine the potential cause(s) of low 25OHD in obesity.
7. To investigate associations between levels of 25OHD and BMD, bone structure and bone strength to establish whether there are skeletal consequences of low 25OHD in obesity.
8. To investigate whether total and/or free 25OHD and 1,25(OH)<sub>2</sub>D are associated with physical performance in obesity.

**To conclude whether the fracture patterns observed in obesity are attributable to skeletal inadequacy, non-skeletal determinants of fracture or both and to suggest**



**the most appropriate direction for fracture prevention to take in obesity; for example; increased utilisation of anti-osteoporotic therapies, improved fall prevention strategies or increased supplementation of vitamin D (Chapter 7).**

## **CHAPTER 2:**

## **METHODS**

## **CHAPTER 2: Methods**

### **Study Design**

The Fat and Bone Study (FAB Study) was a single centre, observational, cross-sectional, case-control study.

Cases were obese individuals (BMI greater than or equal to 30 kg/m<sup>2</sup>) and controls were individuals with a normal BMI (BMI 18.5 to 24.9 kg/m<sup>2</sup>). Cases and controls were individually and prospectively matched (each control was recruited to match a specific obese participant) by age ( $\pm 3$  years), gender, height ( $\pm 5$  cm), first part of postcode (e.g. S5) and smoking status (current or non-smoker). This was done to control for potential confounding factors by ensuring that cases and controls were as similar as possible. Participants were matched by age to control for decreases in BMD and alterations in body composition (lower LM and greater FM) which occur with age. As men have a greater BMD than women, mixed-sex pairing was avoided. Participants were matched on height as BMC by DXA is affected by skeletal size. Postcode matching was performed as socioeconomic status can affect lifestyle factors such as diet, physical activity participation and healthcare attendance (123) which could affect bone density (124-129), fracture risk (130, 131) and incidence of obesity (132). Finally, matching based on current smoking status was performed as cigarette smoking has been shown to reduce aBMD (133-135), vBMD (136), Tb.vBMD (135-137) and Ct.Th (135) and increase fracture risk (138-141).

All participants provided written informed consent prior to enrolment, in accordance with Good Clinical Practice guidelines. Ethical approval was obtained from Sheffield Research Ethics Committee.

We used data sets from a previous study of healthy women in Sheffield to estimate the variability and difference in hip BMD between normal BMI and obese pairs. The mean paired difference was 0.085 g/cm<sup>2</sup> and the standard deviation of the paired differences was 0.136. The effect size was set at 7.5% as this was likely to represent a clinically significant difference. A sample size of 240 has 80% power to detect a 7.5% difference at  $p < 0.05$  based on a paired sample t-test.

Following study completion, a sample size of 200 (mean 25 normal BMI-obese pairs per younger/older, male/female group) was obtained. As the effect size was estimated from a different population, the power calculation was repeated with data from the FAB study

to check that the FAB study had adequate power. The SD of the paired differences in total hip BMD was 0.16. For 80% power for a paired samples t-test, the standardised difference is 1.125, therefore  $(1.125 \times 0.16) / 2 = 0.09$ . The final sample size of 200 had 80% power to detect a 0.09 g/cm<sup>2</sup> difference in total hip aBMD.

## **Inclusion and Exclusion Criteria**

### **Inclusion Criteria:**

#### **Aged 25 to 40 Years or 55 to 75 Years**

Women aged 25-40 years were premenopausal, defined as having regular menstrual cycles and at least 8 menstrual cycles per year. Women aged 55-75 years were postmenopausal, defined as at least 5 years since their last menstrual period. These age groups capture individuals post-peak bone mass and exclude perimenopausal and menopausal women who undergo increased bone resorption and decreased bone mass. Similar group sizes enabled age group comparisons.

#### **BMI of 18.5 to 24.9 kg/m<sup>2</sup> or Greater Than 30 kg/m<sup>2</sup>**

The WHO BMI classifications were used to group normal BMI and obese individuals. No upper BMI limit was enforced but a maximum body weight of 159 kg was applied in accordance with guidelines from Hologic for using the DXA scan-bed.

#### **Caucasian Ethnicity**

Only Caucasian individuals were recruited to avoid confounding due to ethnic differences in skeletal acquisition, bone density, bone structure and fracture risk (15, 120, 121, 142-146). Ethnic differences in adiposity and fat distribution may also confound the results. Asian individuals have greater percentage body fat and abdominal FM than Caucasians matched for age gender and BMI, and differences in SAT and VAT exist by geographic region (122, 147, 148). White and Hispanic adults have lower SAT and higher VAT than African American and Black African adults (149-152). Relationships between FM and BMD may also vary by ethnicity (153).

#### **Sufficiently Mobile**

All participants were sufficiently mobile to undergo scanning and able to remain motionless for the duration of the scans, ensuring acquisition of acceptable quality scans from all imaging modalities within the approved radiation exposure constraints.

## **Exclusion Criteria**

### **Previous Orthopaedic Surgery, Fractures or Conditions Which Preclude Imaging**

Individuals with joint replacements or osteoarthritis at measurement sites, or other conditions which prevent the analysis or interpretation of DXA scans were ineligible. This ensured the acquisition of reliable, interpretable and complete scan data.

### **History of Long Term Immobilisation**

Defined as no weight bearing functionality for longer than three months. Immobilisation leads to decreases in total, trabecular and cortical densities and alterations in cortical and trabecular structure, with the greatest changes observed at weight bearing sites, (154-161). Suppression of bone formation and accelerated resorption is observed after 30 days of bed rest and appears more rapid in the initial phase of immobilisation (158, 162). Evidence suggests that one year post bed rest, recovery is incomplete (154, 160, 161), however time to full recovery is unclear (155, 160, 161).

### **Fracture Less Than One Year Prior to Recruitment**

As bone remodelling facilitates fracture repair, recent fractures are associated with higher bone turnover (163, 164), resulting in elevated circulating BTMs from 1 to 2 weeks post-fracture (163, 165). Most of the changes in BTMs occur within six months of fracture, after which BTMs might remain only slightly elevated up to one year post-fracture (163, 166), although only formation markers may remain elevated (165, 167, 168). Fracture can also affect bone turnover indirectly, through immobilisation or altered physical activity.

### **Current Pregnancy or Trying to Conceive**

A urinary pregnancy test was carried out for all premenopausal women prior to imaging to prevent the unethical exposure of a foetus to x-ray radiation.

### **Delivery of Last Child Less Than One Year Prior To Recruitment**

Changes in BMD occur during pregnancy in response to demands for calcium, as required for foetal growth and for the production of breast milk (169). As a result, BMD decreases during pregnancy (170, 171), with increased bone resorption and formation (172-174). At one year postpartum, adolescent mothers have been shown to have similar BMDs to those of nulliparous adolescents (175).

### **Lactation Less Than One Year Prior To Recruitment**

Bone turnover is increased during lactation, (176, 177). Three to six months after the onset of lactation, decreased BMC and altered bone structure are observed, with average decreases in BMC of five percent observed at the spine and hip (170, 178, 179). An increase in BMC occurs in later lactation (180), possibly linked to increasing oestrogen. Bone turnover decreases after six to twelve months (177, 181) and BMD returns to baseline levels by 3 to 6 months (169) or 12 months post-parturition (179).

### **Diabetes Mellitus**

Type 1 diabetes mellitus (T1DM) is associated with low BMD and higher fracture risk, possibly as a consequence of reduced bone formation and low insulin, amylin and preptin during skeletal growth (182-185). Type 2 diabetes mellitus (T2DM) is associated with higher BMD, vBMD and trabecular BMD but a greater fracture risk than in non-diabetic individuals (186-188). Greater fall risk may contribute to greater fracture risk in diabetic individuals, although greater fracture risk persists after adjustment for fall frequency (189). Accumulation of advanced glycation endpoints (AGEs) increases bone stiffness and might reduce bone strength in T2DM (190).

### **History of a Diagnosed Restrictive Eating Disorder**

Restrictive eating disorders, such as anorexia nervosa and bulimia, are associated with low BMD and increased bone marrow fat (191-193). Individuals who develop eating disorders prior to attaining peak bone mass can have skeletal consequences persisting into adulthood (194). Although weight gain and recovery may stabilise or restore BMD (195), this is not always the case (196-198).

### **Alcohol Intake of Greater Than 21 Units (168 grams) Per Week**

Whilst light consumption of alcohol may have no effect or a positive effect on BMD, heavy alcohol consumption has a deleterious effect on bone, being inversely associated with BMD, trabecular volume and cortical thickness across the skeleton (199, 200). High alcohol intake also increases the risk of falls and fractures (199, 201).

### **History of, or Current Conditions Known to Affect Bone Metabolism**

Participants with diagnosed skeletal disease, rheumatoid arthritis, chronic renal disease, endocrine disorders (e.g. hyperthyroidism, hypo- or hyper-calcaemia), polycystic ovarian syndrome, malabsorption syndromes (e.g. Inflammatory Bowel Disease, Crohns) or a history of malignancy were ineligible. Participants with markedly abnormal clinical laboratory parameters at visit 1 were excluded.

### **Use of Medications or Treatment Known to Affect Bone Metabolism**

Individuals who used hormone replacement therapy (HRT) for longer than ten years or within the year prior to recruitment were ineligible (202). Individuals using any form of hormonal contraception were ineligible. Individuals with a history of using depot medroxyprogesterone acetate for longer than six months were excluded. Participants were eligible to participate one year from stopping the combined oral contraceptive pill or four months from stopping progesterone-only contraception (mini-pill, sub-dermal implant, intrauterine device) if regular menses had resumed. Participants with a history of bisphosphonate, steroid, glucocorticoid or anticonvulsant use were ineligible, although those using inhalers for asthma were included.

### **Competitive Athlete**

Individuals participating in competitive sport at amateur or professional level or participating in moderate intensity physical activity for greater than seven hours per week were excluded. This ensured that participants were not recruited with a high BMI due to high LM and reduced confounding due to differences in physical activity levels between normal BMI and obese participants.

### **Significant Weight Loss Prior to Recruitment**

Dietary energy restriction has been shown to induce neuroendocrine changes such as reduced circulating concentrations of thyroid hormones, sex hormones and IGF-I, which accelerate bone loss (74).

### **Study Procedures**

Participants attended the Clinical Research Facility, Northern General Hospital, Sheffield for three visits (Table 1):

**Table 1: FAB Study Visits**

<b>Visit</b>	<b>Timeframe</b>	<b>Procedures</b>
1	Premenopausal women: within follicular phase	Consent, lifestyle questionnaire, height, weight, waist and hip circumference, triceps skinfold, blood sampling, physical function tests
2	Within 28 days of Visit 1	Height, weight, DXA, HR-pQCT, QCT, CT
3	September to October 2012 or April to May 2013	Diet questionnaire, UVB questionnaire, height, weight, waist and hip circumference, triceps skinfold, blood sampling

## **Questionnaires**

### **Lifestyle Questionnaire**

A lifestyle questionnaire was used to collect data on demographics, diet, supplementation, physical activity, smoking, alcohol consumption, medical history, cardiovascular risk, drug history, current medication, fracture history, family history of osteoporosis, and weight history. The questionnaire was an adapted version of the skeletal health questionnaire used in the Sheffield Metabolic Bone clinical service.

### **Dietary Vitamin D Intake**

Dietary calcium and vitamin intake were determined with DIETQ (Tinuviel Software, Warrington, UK), a food intake questionnaire with a computerised analysis programme which assesses the consumption frequency and quantity of a variety of foods. This method of dietary analysis has been validated for energy and macronutrient intake.

### **UVB Exposure Questionnaire**

Participants completed a UVB questionnaire to determine annual and summer sunlight exposure (Appendix 1). As no standard questionnaire could be identified for this purpose, a questionnaire previously cited (203) was recommended by Professor Lanham-New, University of Surrey. Sunbed use and sun protection factor habits (factor, area applied, frequency applied) were also recorded. Sunlight exposure was quantified using the rule of nines method to estimate the surface area of skin exposed to sunlight (Appendix 2). As the rule of nines method did not discriminate hand exposure without full arm exposure, a score of 0.2 was given for this purpose.

### **Anthropometry**

Height (cm) was measured to the nearest 0.1 cm using an electronic, wall-mounted stadiometer (Seca 242, Seca, Birmingham, UK). Participants were measured shoeless. Participants stood with their feet together, heels against the wall and their head was positioned so that the Frankfort Plane was horizontal. Weight (kg) was measured to the nearest 0.1 kg using an electronic balance scale (Seca, Birmingham, UK). Participants were weighed in lightweight clothing and shoeless.

### **Anthropometric Assessment of Adiposity**

#### **Body Mass Index**

Although it is unable to distinguish between FM and LM, BMI provides a quick and unobtrusive surrogate for adiposity. BMI was calculated using Quetelet's index:

$$\text{BMI} = (\text{weight (kg)} / (\text{height (m)}^2))$$



### **Waist-to-Hip Ratio (WHR)**

WHR is an indicator of trunk fat and a more accurate predictor of obesity related co-morbidity than BMI (204-208). Waist and hip circumferences were measured in cm to the nearest 0.1 cm, using a flexible tape measure. Participants stood erect with their feet approximately 30 cm apart. Waist circumference (cm) was measured horizontally, at the midpoint between the lowest rib and the uppermost aspect of the iliac crest. The measurement was made at the end of a regular expiration and directly on the skin. Hip circumference was measured horizontally at the level of the greatest protrusion of the buttocks when viewed from the side. Fatty aprons were excluded from the measurement; with the participant instructed to raise the apron above the region of interest. Hip circumference (cm) was measured over loose, lightweight clothing. Waist-to-hip ratio was calculated and used as a marker of central adiposity.

### **Triceps Skinfold Thickness**

Triceps skinfold thickness (mm) was measured using a Harpenden skinfold calliper (Baty International, West Sussex, UK) and used as a marker of peripheral subcutaneous adiposity. Skinfolds were taken on the right-hand side of the body. Skinfolds were measured as a vertical fold on the posterior midline of the upper arm, half way between the acromial and olecranon processes, with the elbow extended and the arm relaxed. The dial was read to the nearest 0.20 mm, two seconds after the grip was fully released. Time was allowed for the skin to regain normal thickness between measurements to prevent fat compression.

All anthropometric measurements were taken by a single investigator to eliminate inter-investigator variability and were measured three times and the mean value determined to reduce intra-observer error (207, 209).

### **Skeletal Imaging**

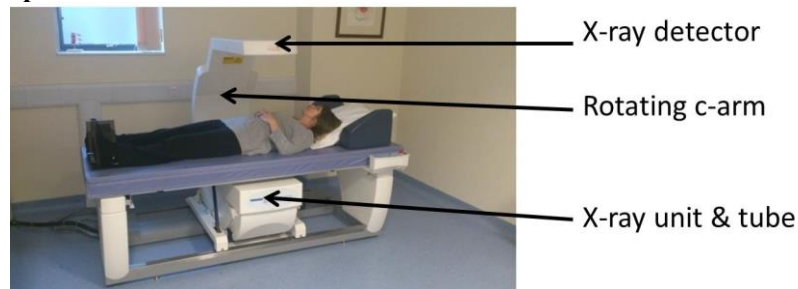
#### **Dual-Energy X-ray Absorptiometry**

DXA was used to determine aBMD ( $\text{g}/\text{cm}^2$ ) of the whole body (minus the head), total hip and lumbar spine (L1-L4). DXA is currently the standard technique for the measurement of BMD, providing a two-dimensional projection from which BMC, bone area and BMD can be determined. BMD by DXA has been shown to predict fracture risk (210-212) and for this reason is commonly used as a surrogate for bone strength.

## Principles of DXA

A DXA system is shown in Figure 5. The x-ray tube produces x-ray beams of two different energies; one high the other low, by alternating the voltage of the x-ray tube (kV switching). A fan shaped beam is generated by passing the beam through a collimator. As the beam passes through the body, some photons are absorbed and some scatter (Compton scattering) but the remaining photons pass through the body and are detected by a linear array of x-ray detectors.

Figure 5: Components of the DXA Scanner



The detector uses a scintillation detector array system to convert the attenuated emerging high and low energy x-ray beam intensities to light energies, which are detected by a photo diode and converted into electrical signals for image generation. An R-value, or attenuation coefficient, is calculated for each pixel as the ratio of the attenuation at the low keV to the attenuation at high keV, which depends on the composition and density of the tissue. Low density materials, such as air, allow more photons to pass through than high density materials, such as bone. For a mono-energetic beam passing through a homogeneous tissue, a pattern of attenuation can be represented by the following formula (213):

$$I = I_0 \exp(-\mu M)$$

Where  $I$  = the intensity of the beam after it has passed through the body,  $I_0$  = the incident beam intensity,  $\mu$  = the linear mass attenuation coefficient of the tissue ( $\text{cm}^2\text{g}^{-1}$ ) and  $M$  = the area density ( $\text{g}/\text{cm}^2$ ) (213).

As the body is not made up of a single homogeneous tissue, a series of two compartment models (bone and soft tissue) are used to calculate bone density values. Different tissues have different attenuation coefficients, with bone having a constant attenuation coefficient at a given energy. Therefore, for a given energy (213):

$$I = I_0 \exp(-\mu_B M_B + \mu_S M_S), \text{ where } B = \text{bone and } S = \text{soft tissue}$$

As the attenuation coefficient varies depending on the beam energy, separate equations are determined for the low and high-energy beams (213):

$$I^L = I^L_0 \exp(-\mu_B^L M_B - \mu_S^L M_S) \text{ and } I^H = I^H_0 \exp(-\mu_B^H M_B - \mu_S^H M_S)$$

Where L= low-energy photons and H= high-energy photons.

The area density of bone ( $M_B$ ) can then be described as (213):

$$M_B = [\text{Ln}(I^L_0 / I^L) - k \text{Ln}(I^H_0 / I^H)] / (\mu^L_B - k\mu^H_B), \text{ where } k = \mu^L_S / \mu^H_S$$

Ratio  $k$  can be derived from the patient measurement by measuring the transmitted intensity of the beam at points at which there is no bone ( $M_B = 0$ ). Once ratio  $k$  is determined, the equation can be solved to calculate the area bone density,  $M_B$ . Bone density is then calculated as the average  $M_B$  across the bone profile (213).

The software sums the number of pixels containing bone to calculate the bone area (BA) that was scanned. Using the mean BMD value and the BA, it is possible to calculate the BMC within the image (213):

$$\text{BMC (g)} = \text{BMD (g/cm}^2\text{)} \times \text{BA (cm}^2\text{)}$$

Gender specific Z and T-scores are determined to report an individual's BMD. A Z score quantifies in SDs how different an individual's BMD value is from the population mean for the individual's age, whereas a T-score quantifies how far the BMD value departs from the mean value for 'young normal' adults (age 20-39 years). Again, the difference is expressed in terms of SDs.

$$\text{Z score} = (\text{subject BMD} - \text{age matched mean}) / \text{age-matched SD}$$

$$\text{T score} = (\text{subject BMD} - \text{young normal mean}) / \text{young normal SD}$$

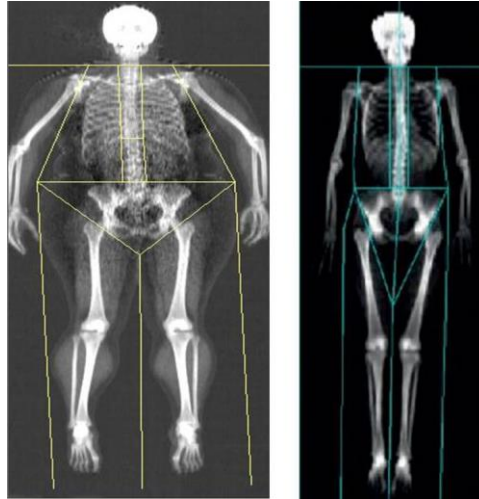
### **DXA Procedure**

Participants were scanned in the posterior-anterior (PA) projection, using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA, USA). This scanner uses a switched-pulse dual-energy (low 100kVp / high 140kVp) x-ray system with a maximum current of 10mA and has a multi-element detector array. Scans of the whole body, lumbar spine and right (or non-fractured) hip were performed.

### **DXA Procedure: Whole Body**

The subject was positioned supine, in a straight and central position on the scan table, with the head placed towards the top of the table. The operator ensured the body was within the scan line limits indicated on the scan table, with the anterior superior iliac spines equidistant from the table top to prevent rotation of the pelvis and the feet within

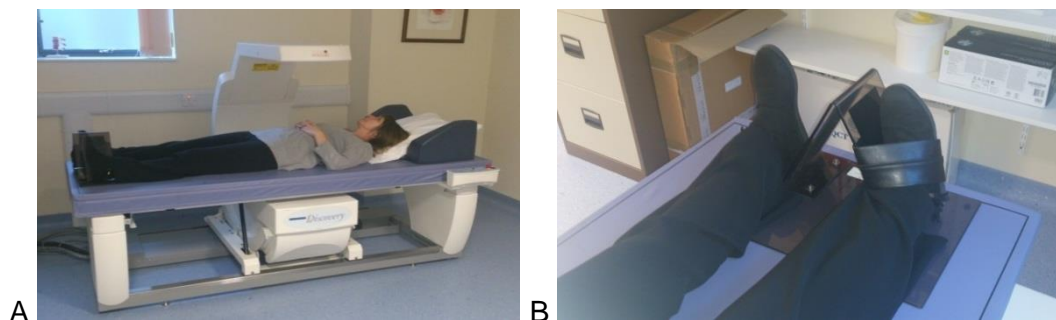
the scan limit border. Participant's arms were placed by their sides, palms flat on the table (normal BMI) or palms facing inwards (obese) and slightly separated from the thighs (Figure 6). Sub-region defining lines were positioned in accordance with the Hologic QDR User's Guide instructions (Figure 6).



**Figure 6: Positioning and sub-region defining lines for the whole body DXA scan of an obese (left) and non-obese (right) individual**

#### **DXA Procedure: Hip**

Participants adopted a supine, straight and central position on the scan table, with the head in the head positioner and feet placed either side of the hip positioner. The participant's arms were placed on the chest, away from the scan field (Figure 7A). The operator ensured the proximal femur was within the scan line limits indicated on the scan table, with the anterior superior iliac spine equidistant from the table top to prevent rotation of the pelvis. The hip was internally rotated by approximately 25° and the leg abducted (Figure 7B).

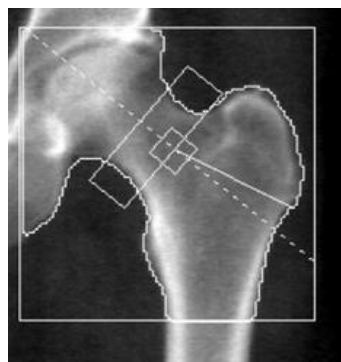


**Figure 7: Positioning for the hip DXA scan**

An express scan ensured correct positioning. From this, the operator ensured correct positioning of the image within the scan field; with at least 3 cm of femoral shaft below the lesser trochanter included and the femoral shaft straight within the scan field. The array mode was used for the final scan, which extended from at least 3 cm below the

lesser trochanter to the pelvis above the femoral head. All scans included at least 5 scan lines of adequate soft tissue around the greater trochanter and femoral head.

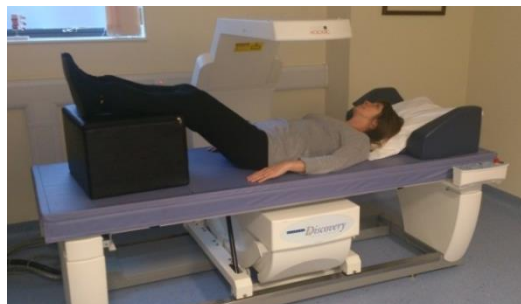
The image was then analysed. The global region of interest was positioned with the upper and right borders at least 5 scan lines away from the edge of the femoral head, the left border 5 scan lines from the edge of the greater trochanter and the bottom border 10 scan lines below the lesser trochanter. The bone map was then identified. The midline was placed on the central axis of the hip, the neck box close to the greater trochanter and the trochanteric line below the curve of the greater trochanter, with equal amounts of soft tissue included within the neck box on either side of the femoral neck (Figure 8). The Ward's triangle box was positioned automatically (Figure 8).



**Figure 8: Application of the bone map to a DXA hip scan image, indicating the midline, neck box and Ward's Triangle box**

### **DXA Procedure: Lumbar Spine**

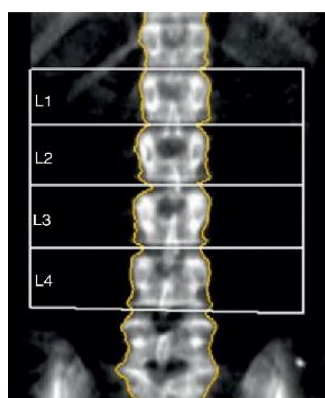
The participant was positioned in a supine, straight and central position, head in the head positioner and legs elevated over the spine scan positioning block, which was placed with the lowest side perpendicular to the table (Figure 9).



**Figure 9: Positioning for the lumbar spine DXA scan**

The operator ensured that the lumbar spine was within the scan line limits indicated on the scan table. The anterior superior iliac spine was equidistant from the table to prevent rotation of the spine. An express scan ensured correct positioning. The operator ensured the scan image was straight and central within the scan field and extended from mid-L5

to mid-T12 so as to image the full L1 to L4 region. There were equal areas of soft tissue at either side of the spine. The array mode was used for the final scan. The image was then analysed. The global region of interest was positioned with the top border within the T12-L1 intervertebral space and the bottom border within the L4-L5 intervertebral space, angled to accommodate the shape of the vertebrae. Right and left borders were not altered. The bone map was then identified with vertebral lines placed within the L1-2, L2-3 and L3-4 intervertebral spaces (Figure 10).



**Figure 10: Lumbar spine DXA scan with global region of interest and bone map**

As prior vertebral fracture may falsely increase BMD, participants aged 55 to 75 years (it was unlikely that fractures would be observed in younger participants) underwent a vertebral fracture assessment to identify prevalent vertebral fractures between T4 and L4, in the PA and lateral projections using the single energy scan mode. Scans were visually assessed using the algorithm-based qualitative definition of vertebral fracture (214). Vertebrae with evidence of fracture were excluded from the calculation of aBMD. A minimum of 2 analysable vertebrae were required for analysis of LS aBMD; participants with 3 or more fractures between L1 and L4 were excluded from the study.

### **DXA Outcomes**

Bone area (cm<sup>2</sup>), BMC (g) and mean areal BMD (g/cm<sup>2</sup>) were determined for the whole body (minus head), total hip and lumbar spine (mean L1 to L4).

### **DXA Calibration**

All scans were performed by two highly trained operators with standardised protocols for acquisition and analysis; minimising inter-operator error. The DXA scanner underwent daily quality control (QC) to ensure stability and precision, in accordance with the manufacturer's recommendations. QC was performed by scanning the Hologic device-specific anthropomorphic spine phantom, containing four semi-hydroxyapatite vertebrae of a single density (Figure 11A). The step phantom, made up of 6 fields of acrylic and

aluminium of different thicknesses and known absorptive properties was scanned weekly to calibrate the DXA scanner to enable distinction of soft tissues during the whole body scan (Figure 11B). A European Spine Phantom (QRM—Quality Assurance in Radiology and Medicine, Moehrendorf, Germany) was scanned weekly (Figure 11C).

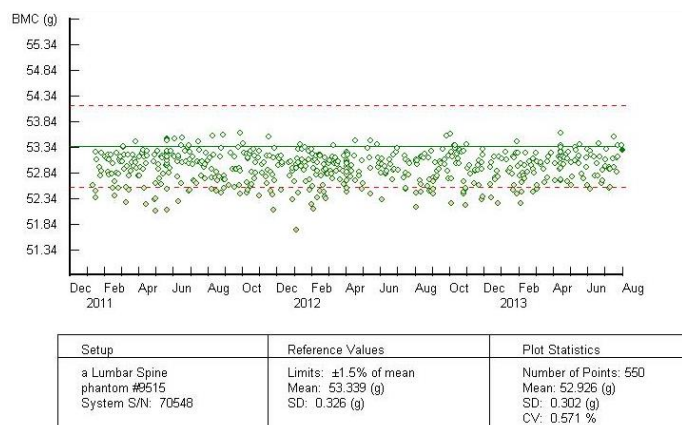


**Figure 11: Phantoms for the calibration of the DXA device**  
**Hologic anthropomorphic spine (A), step (B) and European spine (C) phantoms**

The resulting bone area (Figure 12), BMC (Figure 13) and BMD (Figure 14) values were plotted against pre-specified acceptable limit lines. The scanner is programmed to automatically inform the operator of whether the QC has passed or failed.

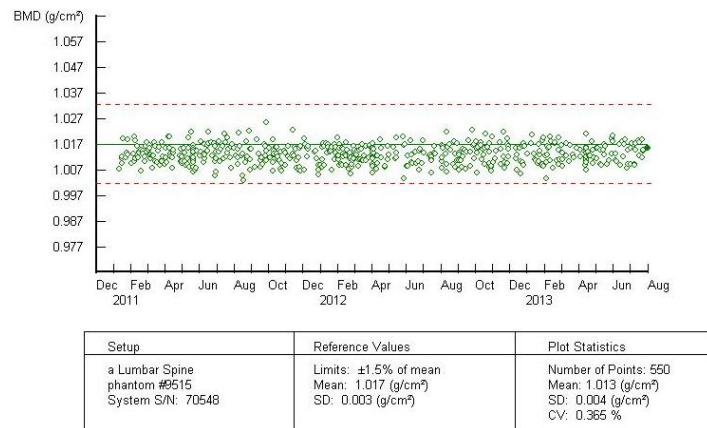


**Figure 12: DXA quality control plot for bone area throughout the FAB Study**



**Figure 13: DXA quality control plot for BMC throughout the FAB Study**





**Figure 14: DXA quality control plot for BMD throughout the FAB Study**

A previous departmental study of normal weight adults showed precision (CV) at L1-L4 to be 1.2% for area, 2.2% for BMC and 1.0% for BMD. Others have reported significant differences in precision error between normal and obese adults for LS (0.99% normal and 1.68% obese); and WB BMD (0.66 to 0.77% normal and 0.91% obese) by DXA (215, 216). When the European Spine Phantom was layered with bags of semi-solid hydrogenated vegetable oil to simulate overlying adipose tissue, precision error of the change in DXA BMD was 3.1% for the PA spine and 3.7% for TH (217). DXA precision is also lower in obese adults than those of normal BMI for measures of FM (2.98% normal BMI, 1.55% obese) and LM (1.42% normal BMI and 1.68% obese) (216).

### Limitations of DXA

The Hologic Discovery A has a scan table weight limit of 159kg, therefore participants heavier than 159 kg were not recruited. It can be difficult to position obese participants on the table, with the limited size of the scanning area leading to soft tissue being excluded from the scan. Half body scanning can be used; however this method can introduce error if the two midlines are misaligned, or where there are anatomical differences between the two sides of the body (218).

As DXA is based on a series of two compartment models, a constant level of hydration is assumed throughout the LM, which is not always true. DXA is also affected by the inhomogeneity of fat distribution (219). In pixels containing both bone and soft tissue, the composition of the soft tissue element of the pixel is estimated from 'bone free' pixels situated adjacent to the bone. By assuming the same percentage of fat to lean mass within the soft tissue portion, error can be induced. For the same reason, bone marrow fat may also affect measurements of BMD by DXA. In those with osteoporosis,



accompanied by increased bone marrow fat, DXA may “widen the difference in BMD results” when compared with normal individuals (220, 221).

As BMC is affected by bone size, this must be adjusted or controlled for to ensure accurate determination of BMD. DXA has a limited ability to assess bone geometry and the positioning of cut planes used to identify regions of interest can affect the accuracy and precision of such observations (222). Measurements of BMD are confounded by greater soft tissue thickness, which absorbs the x-ray beam such that the resulting attenuation is increased. Studies involving fat layering of phantoms have shown aBMD by DXA to be increased with increasing fat layering relative to baseline measurements (217, 223, 224).

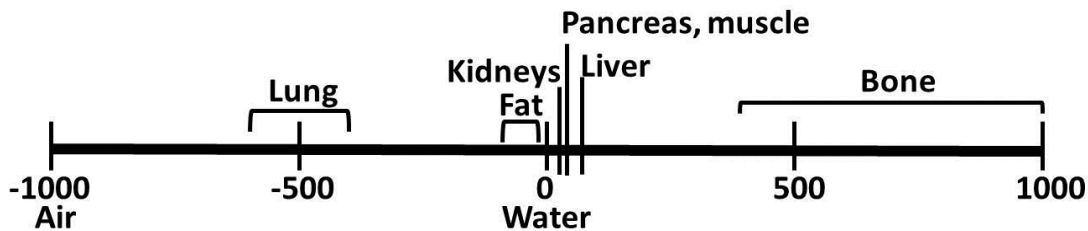
Errors in determining BMC, bone area and proximal femur geometry can arise using fan beam DXA due to magnification effects due to proximity to the x-ray source (225). In obese individuals with increased posterior soft tissue thickness, the distance of the skeleton from the x-ray source is decreased and so the distance from the apex of the fan beam is decreased, leading to magnification effects as the observed width of scanned bone increases (226). Although this does not significantly affect BMD measurements, computational input is required to correct magnification errors in BMC, area and geometry outcomes.

## **Quantitative Computed Tomography**

### **Principles of QCT**

QCT involves the use of x-rays and computational input to reconstruct a greyscale image of a section or slice of the body. X-rays are passed from the radiation source through the body and the resulting intensity is detected by a detector on the opposite side. This generates an attenuation profile, or projection, which is then reconstructed into an image by computing the spatial distribution of the attenuation onto a blank matrix (227). QCT allows the visualisation of a range of tissue densities and of within-tissue density gradations. Attenuation values within a voxel are compared to the attenuation of water and the mean attenuation within the voxel is converted into Hounsfield units (HU); an arbitrary scale to determine the derived density of tissues, where water has a HU of zero and each value represents a shade of grey on the image (Figure 15) (228). The resulting image is made up of pixels of varying gradations of HU.

$$\text{HU} = 1000 \times ((\mu \text{ tissue} - \mu \text{ water}) / \mu \text{ water}), \text{ where } \mu = \text{attenuation coefficient}$$



**Figure 15: Scale of attenuation in Hounsfield Units of various tissues adapted from (227, 229)**

The peak tube voltage (kilovolts peak (kVp)), controls the quality of the x-ray beam, and inversely affects image contrast. The tube current (mA or mAs (Ma x sec)), controls the quantity of x-ray photons produced and affects the image density. The patient dose is, therefore, directly proportional to the tube current. The noise index is chosen from several options to define the level of acceptable noise in the image. The scanner adjusts the mA according to the noise index selected, so patient dose is inversely related to image noise. Using the pre-specified noise index rather than altering the scan parameters contributing to noise ensures a consistent level of noise across the study sample (230). Slice thickness must be consistent to ensure reproducibility of repeated scan sections.

### **QCT Procedure**

Participants aged 55 to 75 years underwent a QCT scan of the right hip and lumbar spine (L1-L3). Due to the effective radiation dose to the axial skeleton, premenopausal women and men aged 25 to 40 years were excluded from QCT scanning. QCT scans were obtained using a LightSpeed VCT-XT scanner (General Electric Healthcare, Buckinghamshire, UK) in the Medical Imaging Department, Northern General Hospital, Sheffield. This scanner has 64 detector elements along the z-axis and uses a solid state, scintillation detector array system to convert the incident x-ray intensities to light energies, which are detected by a photo diode and converted into electrical signals for image generation (227). All QCT scans were performed in the axial plane, with a helical rotation and rotation time of 0.8 seconds and a table height of 155. The scan pitch, (pitch =  $d / (M \times S)$ ) or “the ratio of the table feed (d) [in mm per 360° rotation] to total slice collimation (M.S)”, where M= the number of slices and S= the slice width (227), was 0.969 for each scan. All scans had a noise index of 30.

Participants were placed in the AP position; with the upper part of a Mindways phantom (Mindways Software Inc., Austin, TX, USA) positioned level with the iliac crest. Gel bags

were used to fill any gaps under the participant as necessary. Scout scans were performed initially to ensure accurate positioning of the phantom and to select subsequent scan regions

QCT scans of the proximal femur were performed with a slice thickness of 0.625mm, beginning 3 cm above the femoral head to 3 cm below the lesser trochanter. The tube current (modulated Ma) was at a maximum 200, minimum 100, with a mean assumed tube current of 120mA and a tube voltage of 120 kilovolt peak (kVp).

QCT scans of the lumbar spine (L1-3) were attained from 5mm above the superior end plate of L1 (inclusive of the T12-L1 joint space) to 5mm below the end plate of L3 (inclusive of the L3-4 joint space). Scans had a slice thickness of 0.625mm. The tube current (modulated Ma) was at a maximum 140, minimum 80, with a mean assumed tube current of 120mA and a tube voltage of 80 kilovolt peak (kVp).

3D reconstruction and analysis of the proximal femur and lumbar spine QCT scans was performed using the Mindways QCT Pro™ software version 5.0.3 (Mindways Software, Inc., Austin, TX, USA). The results for L1-3 were averaged for each participant. The CTXA application of QCT Pro was used to determine total hip and femoral neck outcomes. Default threshold values of 100 mg/cm<sup>3</sup> and 350 mg/cm<sup>3</sup> were applied to distinguish bone from soft tissue and cortical from trabecular bone, respectively. QCT scans were analysed by a single operator blinded to BMI, although due to the nature of the analytical process, blinding could not be considered true.

### **QCT Outcomes**

QCT was used to determine L1-3 vBMD. Bone mass (g), area (cm<sup>2</sup>), volume (cm<sup>3</sup>) and vBMD (mg/cm<sup>3</sup>) were determined for the total, cortical and trabecular compartments at the TH and FN. FN angle, FN width and hip axis length were also determined.

### **QCT Calibration**

Quality assurance was performed once per month using a Mindways phantom (Mindways Software, Inc., Austin, TX, USA).

### **Advantages of QCT over DXA**

QCT enables 3D volumetric measurements to be acquired, rather than 2D areal measurements by DXA. QCT enables the assessment of trabecular and cortical bone compartments and enhances ability to determine geometric properties of bone. QCT has

been shown to be less affected by soft tissue thickness than DXA (217, 231). Being less affected by soft tissue thickness, differences in bone density by DXA and QCT imaging have been reported. Women with a BMI  $>27$  kg/m<sup>2</sup> had a mean DXA T-score 1.45 units greater than that of age and height matched controls, but the mean QCT T-score was not different between obese and non-obese groups (Weigert and Cann 1999. Cited in (224)). Therefore QCT may provide a more accurate assessment of bone density in obesity than is possible by DXA.

### **Limitations of QCT**

Generally QCT is associated with higher cost than DXA, is less widely available, and requires more specialist analysis and interpretation than DXA, restricting its use. As a result, QCT is less validated for fracture risk prediction than DXA, with a range of analysis packages and in-house FE models being used to analyse scans and estimate vBMD and bone strength. Some previous studies have shown positive associations between QCT derived vBMD and fracture risk (232-235), although adding QCT parameters to DXA based estimate of fracture risk was not advantageous (236).

The scanner gantry is of a fixed width which can lead to obese participants exceeding the field of measurement. QCT scanning involves a higher radiation dose than DXA scanning and the radiation exposure associated with QCT of the spine and proximal femur in this study was considered too high for the 25 to 40 years group and was not performed. A balance must be struck between the necessary radiation exposure and the image resolution required (228).

QCT is affected by inhomogeneity of soft tissue distribution and bone marrow fat, although to a much lesser degree than DXA. Artifact on QCT images can result from patient movement, beam hardening, scattered radiation or partial volume effects.

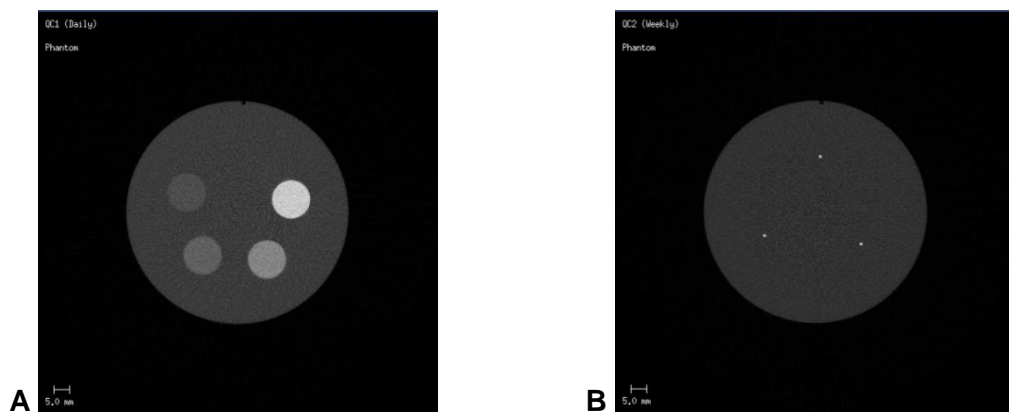
### **High Resolution Peripheral QCT**

#### **Principles of HR-pQCT**

High resolution peripheral QCT (HR-pQCT) involves the same principles as QCT, but enables high resolution scanning of the distal appendicular skeleton. A 360° rotating x-ray tube generates x-rays which are passed through a section of the distal radius or distal tibia, and detected by a static 2D detector array. This generates an attenuation profile, which is reconstructed into an image by computing the spatial distribution of the attenuation onto a blank matrix (227). This simultaneous acquisition of a series of 2D

parallel image slices, is then computed into a high resolution 3D image (isotropic resolution = 82  $\mu\text{m}$ ).

Total, cortical and trabecular densities are determined from a pre-calibration step. The scanner is calibrated using a phantom with five hydroxyapatite-resin compartments of densities from 0  $\text{mgHA}/\text{cm}^3$  (a soft tissue equivalent with no mineral content) to 800  $\text{mgHA}/\text{cm}^3$  (Figure 16). Image slices are taken of the phantom and the mean attenuation for each of the compartments calculated. From this pre-calibration data the attenuation values of the scan can be converted into measures of BMD ( $\text{mgHA}/\text{cm}^3$ ).



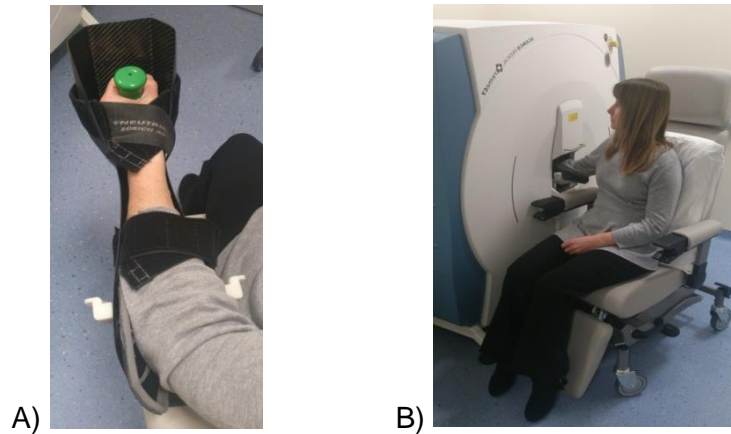
**Figure 16: Scan images of the HR-pQCT phantoms**

### **HR-pQCT Procedure**

Scans were acquired using the XtremeCT Device (Scanco Medical AG, Zurich, Switzerland) in the high resolution mode (image matrix= 1536x1536) using a source potential of 60 kVp, a tube current of 900 mA and an integration time of 100 ms. 110 slices were acquired over a scan length of 150mm, diameter of 125mm and stack height of 9.8mm. Scans were performed using the non-dominant limb, with the dominant limb used if the participant had sustained prior fracture of the non-dominant limb.

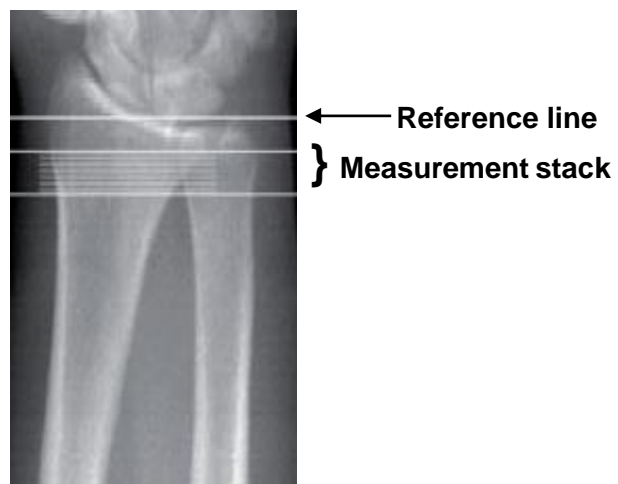
### **HR-pQCT Procedure: Distal Radius**

With the participant seated, the operator placed the hand and lower arm into the forearm cast and used an arm pad to stabilise the arm within the cast (Figure 17A). The chair was positioned so that the arm rest of the chair was level with the gantry opening and the arm was placed into the device and secured (Figure 17B). A scout scan was performed to determine the measurement area.



**Figure 17: Positioning for the distal radius HR-pQCT scan illustrating the arm within the cast (A) and positioning of the participant (B)**

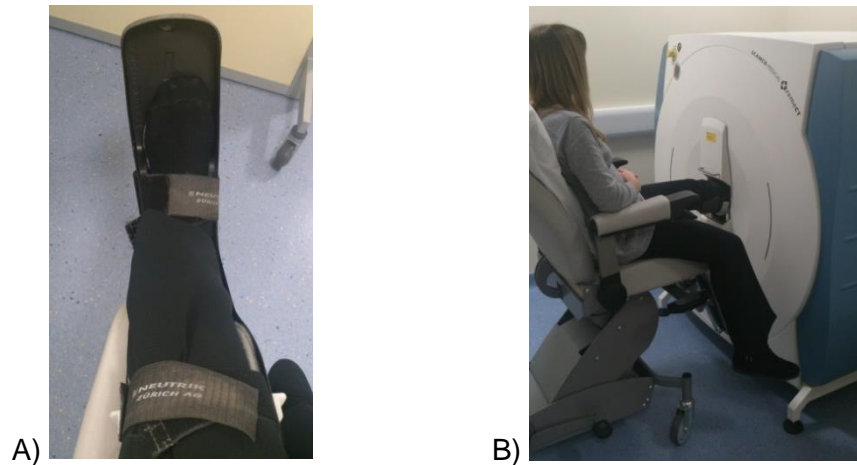
A reference line was placed on the notch on the articular surface of the distal radius on the scout image to indicate the position of the first measurement slice (9.5 mm from the reference line) (Figure 18). The participant was instructed to remain motionless and the scan was performed. Upon completion, the cast was removed and the scan quality evaluated. The operator visually inspected random slices to check consistent quality. The operator drew a contour around the cortical perimeter on the first image, before running the automatic contouring detection program which iterated the contouring process through the slice stack. Images were then analysed using the 'Evaluation 3D' option.



**Figure 18: Reference line and measurement area on a distal radius scout scan**

### **HR-pQCT Procedure: Distal Tibia**

The subject seated, the operator placed the foot and lower leg into the tibia cast and used a foot insert and pad to secure the leg within the cast (Figure 19A).



**Figure 19: Positioning for the distal tibia HR-pQCT scan illustrating the leg within the cast (A) and positioning of the participant (B)**

The leg was rested on the leg support and the chair positioned with the leg at the same height as the gantry. The leg was placed into the device and secured (Figure 19B). A scout scan was performed to determine the measurement area; with a reference line placed at the endplate of the distal tibia on the scout image to indicate the position of the first measurement slice (9.5 mm from the reference line) (Figure 20). The measurement scan and analysis was completed as per the distal radius procedure.



**Figure 20: Positioning of the reference line on a distal tibia scout scan**

### **HR-pQCT Outcomes**

Volumetric BMD, cortical and trabecular bone structures and bone microarchitectural parameters at the distal radius and distal tibia were determined. HR-pQCT outcomes are described in Table 2, supported by Figure 27.

### **HR-pQCT Calibration**

The XtremeCT device was calibrated daily using the manufacturer device-specific phantom (Scanco Medical AG, Zurich, Switzerland) to monitor the stability of the machine and pre-calibrate the scanner. Weekly measurements of the phantom were performed to monitor the assessment of bone microstructural properties.

**Table 2: HR-pQCT outcomes, units, definitions and abbreviations**

<b>Outcome</b>	<b>Units</b>	<b>Abbrev.</b>	<b>Definition</b>
Total area	mm <sup>2</sup>	Tot.Ar	Mean surface area of the cortical and trabecular compartments
Cortical area	mm <sup>2</sup>	Ct.Ar	Mean surface area of the cortical compartment
Trabecular area	mm <sup>2</sup>	Tb.Ar	Mean surface area of the trabecular compartment
Total vBMD	mgHA/cm <sup>3</sup>	Tot.vBMD	Total mineral mass divided by the total bone volume
Cortical vBMD	mgHA/cm <sup>3</sup>	Ct.vBMD	Cortical mineral mass divided by the cortical volume
Trabecular vBMD	mgHA/cm <sup>3</sup>	Tb.vBMD	Trabecular mineral mass divided by the volume inside the cortical bone
Cortical thickness	mm	Ct.Th	Mean thickness between the periosteal and endosteal surfaces
Cortical perimeter	mm	Ct.Pm	Distance covered by the perimeter of the periosteal surface
Cortical porosity	%	Ct.Po	Percentage of cortical area occupied by pores
Cortical pore diameter	µm	Ct.Po.Dm	Mean diameter of pores within the cortical bone
Cortical tissue mineral density	mgHA/cm <sup>3</sup>	Ct.TMD	Cortical mineral mass divided by the cortical volume after excluding cortical pore space
Trabecular number	mm <sup>-1</sup>	Tb.N	Mean number of trabeculae per mm within the trabecular compartment
Trabecular thickness	mm	Tb.Th	Mean thickness of trabeculae within the trabecular compartment
Trabecular spacing	mm	Tb.Sp	Mean distance between trabeculae within the trabecular compartment
Trabecular heterogeneity	mm	Tb.N.SD	SD of the intra-individual distribution of trabecular separation
Bone volume fraction	%	BV/TV	Derived by dividing Tb.vBMD by an assumed 100% mineralisation of 1200 mgHA/cm <sup>3</sup>



### **Advantages of HR-pQCT Over DXA and pQCT**

Unlike DXA, HR-pQCT enables the distinction between cortical and trabecular bone compartments, allowing the study of bone microstructure. HR-pQCT enables high resolution imaging of the non-weight bearing distal radius which is a common fracture site and of the weight bearing tibia (237). There is less soft tissue present at distal sites and therefore HR-pQCT is likely to be less affected by soft tissue confounding than DXA or axial QCT scanning. In contrast to axial QCT, radiation exposure from HR-pQCT is low, with a typical scan effective dose of 3  $\mu$ Sv (237) and areas particularly sensitive to radiation exposure (e.g. the reproductive organs) are excluded from the scan area.

### **Limitations of HR-pQCT**

It is unknown how well peripheral measurements at the distal radius and tibia reflect those of the axial skeleton. A recent study found that skeletal stiffness, density and microarchitecture at peripheral sites assessed by HR-pQCT, was significantly associated with that of the proximal femur and lumbar spine, as measured by QCT (238). Moderate correlations have also been reported elsewhere (239).

Whilst some outcomes, e.g. Tb.N, are directly measured, the majority of trabecular outcomes are derived, including Tb.Th and Tb.Sp (240). Despite HR-pQCT technology vastly improving resolution compared to previous technologies, resolution issues remain a challenge when defining the cortical and trabecular compartments, particularly when the cortical region is porous(241) . The 82 $\mu$ m resolution achieved with the HR-pQCT scanner is close to the thickness of a trabecula. Assessments of Ct.Po are also limited by the resolution of HR-pQCT, with Ct.Po assessments confined to detection of Haversian canals and larger resorption cavities resolvable by HR-pQCT (237).

Where trabeculae are between 1 to 2 voxels in thickness, soft tissue is included in the voxel volume and so partial volume effects result, lowering the threshold for the voxel to be identified as trabecular bone.

Defining the cortex from the trabeculae at the transitional zone can be difficult; particularly when the cortex is porous or when Ct.Th is low. This can be an issue both for the operator identifying the periosteal surface before the scan analysis is run and for the software during the automated analysis (237).

Movement artefact is common, and “measures of micro-architecture are more sensitive to movement artefact compared with geometric or densitometric measures” (237). A

single repeat scan was factored into the radiation protection assessment for HR-pQCT scanning to allow for a repeat scan to be taken in the event of movement on the first image.

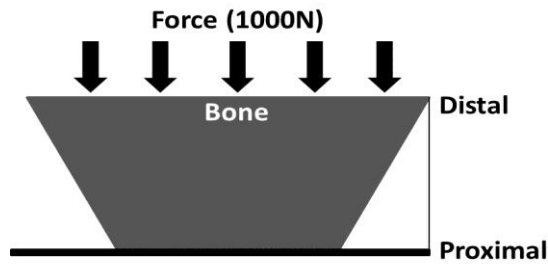
Some participants report discomfort relating to positioning within the cast or difficulty refraining from movement for the scan duration and large individuals may present with limbs greater than the width of the cast or than the width of the scanner gantry. Our department has considerable experience with positioning participants of all ages and of a range of body sizes, having performed validation studies involving the HR-pQCT device previously.

As HR-pQCT has only been commercially available since 2004, with the first publications involving the use of HR-pQCT arising in 2005 (242) there is currently limited reference population data and little data on prospective fracture rates or treatment effects relating to outcomes determined by HR-pQCT.

#### **Micro Finite Element ( $\mu$ FE) Analysis**

Bone strength was determined directly from the HR-pQCT scans using the extended  $\mu$ FE software (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland) which simulates strength-determining biomechanical tests through mathematical modelling, taking into account trabecular and cortical microarchitecture. This software is fully automated and validated for the assessment of *in-vivo* bone strength (243, 244). A compression test was used to simulate a fall from standing height onto an out-stretched hand. Each voxel was converted into equally sized elements and the elements connected by a mesh. A Young's modulus of 20 GPa and 17 GPa was applied to cortical and trabecular bone elements respectively. A Poisson's ratio of 0.3 was applied to all elements. A 1000 N load was applied in the axial direction at the distal site, while the proximal bone was constrained in all directions (Figure 21) (245). Failure was said to occur when 2% of the bone tissue was strained beyond a critical level of 3500  $\mu$ strain. This was based on a previously defined criterion by Pistoia et al. (246) but modified to reflect the elastic properties assigned in the present study in relation to those used by Pistoia et al. (245, 246). A similar model was also used to determine tibial strength.

Outcomes of the  $\mu$ FE analysis are listed in Table 3.



**Figure 21: The principle of the determination of bone strength from  $\mu$ FEA adapted from (235)**

### FEA Limitations

$\mu$ FE models which simulate direct compression at the tibia may be of limited value due to the more common sideways, twisting nature of falls leading to ankle fracture. Variation in the extent of mineralisation may affect  $\mu$ FE measurements, as inhomogeneous mineralisation is likely to affect the distribution of strains throughout the compartment, but this is not taken into consideration in the present software.

**Table 3:  $\mu$ FE analysis outcomes from HR-pQCT scanning**

<b>Outcome</b>	<b>Units</b>	<b>Abbrev.</b>	<b>Definition</b>
Stiffness	kN/mm	Stiffness	Resistance to deformation when applying a load; total reaction force divided by displacement
Estimated ultimate failure load	kN	Est.Fail.Load	Maximum load the bone can bear before fracture; when 2% of the bone is strained beyond 3500 $\mu$ strain
Mean trabecular Von Mises stress	MPa	Tb.VM	Indicates whether combined stresses in the x, y and z directions in the trabeculae will cause failure
Mean cortical Von Mises stress	MPa	Ct.VM	Indicates whether combined stresses in the x, y and z directions in the cortex will cause failure
Proximal trabecular load Proximal cortical load Distal trabecular load Distal cortical load	All %		The distribution of the load between the cortical and trabecular compartments

### Assessment of Adiposity by Imaging

#### Whole Body DXA

From the whole body DXA scan previously described, body composition with sub-region analysis software was used to determine FM (kg) of the total body, trunk, android/gynoid regions, arms and legs (from which appendicular FM was calculated). Despite advantages over anthropometrical techniques, such as a lower influence of inter and

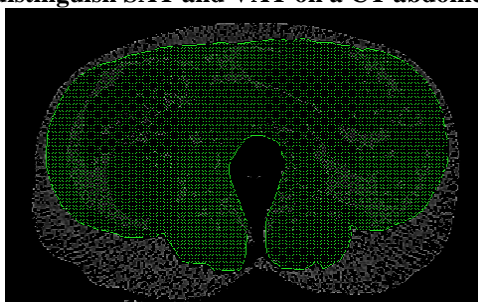
intra-operator effects, DXA measurements of FM are confounded by greater body thickness. The assessment of abdominal adiposity may be less reliable than assessments at peripheral sites due to confounding influences of other organ systems within the region of interest. A further key limitation of using DXA to estimate FM is its inability to distinguish between visceral and subcutaneous abdominal fat compartments.

### **Computed Tomography (CT)**

Five-slice CT scans were taken at the mid-level of the L3 vertebra in all participants, to estimate subcutaneous abdominal (SAT) and visceral (VAT) adipose tissue. CT scans were taken using the LightSpeed VCT-XT (General Electric Healthcare, Buckinghamshire, UK) in the Medical Imaging Department, Northern General Hospital. Participants were placed in the AP position; with a Mindways phantom (Mindways Software Inc., Austin, TX, USA) in the midline and the umbilicus central to the phantom. Gel bags were used to fill gaps under the participant as necessary. AP and lateral topograms of the abdomen were taken at the level of L3 to ensure accurate positioning of the phantom. CT scans were performed in the axial plane, with a helical rotation and rotation time of 0.8 seconds and a table height of 155. The scan pitch was 1.375 for each scan, with a noise index of 46. The tube current (modulated Ma) was at a maximum 400 mA, minimum 100 mA, with a tube voltage of 120 kilovolt peak (kVp).

Total, SAT and VAT volumes were determined using the Volume Viewer imaging software (General Electric Healthcare, Buckinghamshire, UK) on an AW Workstation (General Electric Healthcare, Buckinghamshire, UK). The mid (third) slice in the image sequence was selected with the image in the axial format. To identify adipose tissue, a threshold of -30 to -130 HU was applied. The total volume of adipose tissue was calculated using the histogram function. A manual trace function was applied inside the SAT inner border and the 'cut outside' function applied to remove SAT from the image (Figure 22). The histogram function was re-applied to quantify the remaining VAT and SAT was determined as total minus VAT.

**Figure 22: Manual trace to distinguish SAT and VAT on a CT abdomen scan**



### **Assessment of Muscle Mass by Imaging**

Whole body LM (WBLM) was determined by DXA. Appendicular LM (ALM) was calculated as the sum of the DXA determined LM of the arms and legs. Limitations to the use of DXA for determining LM include confounding by greater soft tissue thickness in obesity and the inability to account for fat infiltration of muscle.

### **Radiation Exposure**

The radiation exposure (effective dose) for each scan was:

- DXA: WB= 8.4  $\mu$ Sv, proximal femur= 8.6  $\mu$ Sv, LS= 14.9  $\mu$ Sv, VFA= 24.0  $\mu$ Sv.
- CT/QCT: Abdomen= 430  $\mu$ Sv, LS = 980  $\mu$ Sv, proximal femur = 2500  $\mu$ Sv.
- HR-pQCT: Distal radius= 6  $\mu$ Sv, distal tibia= 6  $\mu$ Sv.

The overall effective dose:

- 25 to 40 years age group = 474  $\mu$ Sv, equivalent background dose: 11 weeks.
- 55 to 75 years age group = 3978  $\mu$ Sv, equivalent background dose: 22 months.

### **Physical Performance Assessments**

Physical function was determined using a short physical performance battery (SPPB) adapted from that described by Guralnik et al. (247). Batteries typically include assessments of balance, walking speed and a chair stand test, which have been shown to associate with falls risk and morbidity (247, 248). The SPPB in this study comprised a balance assessment from a narrow walk test, walking speed assessment and a chair stand test:

#### **Chair Stand Test**

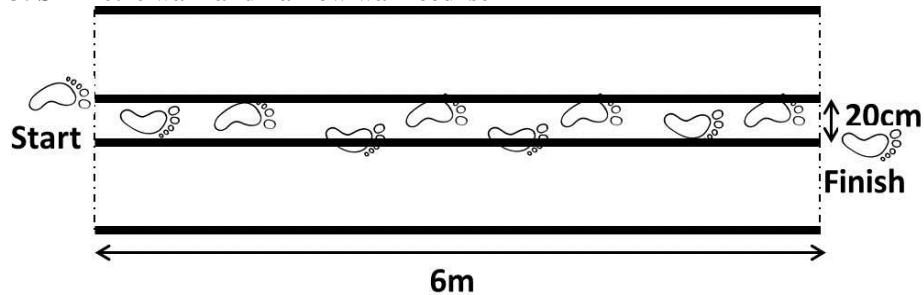
The chair stand test determines an individual's ability to stand from a chair without using their arms; reflecting lower limb strength. Participants were seated with feet on the floor, squarely in front of them, knees flexed slightly greater than 90°. Participants kept their arms folded across their chest throughout. Participants were asked to stand and sit once to determine their capability to continue to the repeated test. If able to continue, participants were instructed to stand and sit five times continuously, as quickly as possible. Time taken to complete the five stand-and-sit cycles was recorded to the nearest 0.01 seconds.

#### **Gait and Balance Assessment**

Walk tests were used to determine usual walking speed and assess balance. To assess usual walking speed (m/s), participants walked a six metre marked course (Figure 23) at

their normal pace. The number of steps and time taken, to the nearest 0.01 seconds, were recorded. This was repeated and the average determined. The course was then narrowed to 20cm wide (Figure 23). Participants were asked to walk the course, keeping their feet within the marked lines whilst looking ahead. Time taken to complete the narrow walk test was recorded to the nearest 0.01 seconds. This was repeated three times and the average time taken and number of deviations recorded.

**Figure 23: Six metre walk and narrow walk course**



### **SPPB Score**

A SPPB score was calculated out of a maximum of 12 points to provide an overall measure of physical performance. Quartile of repeated chair stand time and gait speed were determined by gender. Participants in the top quartile (Q4) were awarded 4 points, through to the poorest performing quartile (Q1) awarded 1 point. As balance was measured on an ordinal scale, participants deviating from the lines on 0 to 3 occasions were classed as 'within the lines' and awarded 4 points, participants completing the course but deviating more than 3 times were classed as 'not within the lines' and awarded 2 points and those unable to complete the assessment were awarded 0 points for that test

### **Grip Strength**

Hand grip strength was measured using a digital hand dynamometer (Saehan Corporation, Masan, Korea) to determine muscle strength. Participants were seated, feet flat on the floor, and instructed to hold the dynamometer with their upper arm in line with their body, forearm at approximately 90° and their wrist un-rotated. Participants were instructed to grip the dynamometer as tightly as possible for 5 seconds and the result recorded. No encouragement was given whilst the participant was executing the test. Grip strength was assessed 3 times on each hand, starting with the right hand. At least 30 seconds rest was given between each repetition.

### **Assessment of Sarcopenia**

Sarcopenia was defined using the European Working Group on Sarcopenia in Older Persons (EWGSOP) definition (249). Individuals were categorised as:

- 1) Not sarcopenic (Normal ALM)
- 2) Pre-sarcopenic (Low ALM)
- 3) Sarcopenic (Low ALM and either weakness or poor SPPB score)
- 4) Severely sarcopenic (Low ALM, weakness and poor SPPB score)

Low ALM was defined as an ALM corrected for height ( $ALM/(height\ (m)^2)$ ), (or Skeletal muscle index (SMI)),  $<7.23\ kg/m^2$  for men or  $<5.67\ kg/m^2$  for women (249). Weakness was defined as a maximal hand grip strength  $<30\ kg$  (men) or  $<20\ kg$  (women).

A poor SPPB score was defined as a SPPB score  $\leq 8$ .

### **Biochemistry**

Blood samples were collected at visit 1, following an overnight fast. Samples were taken between 08:00 and 10:00 to minimise the effect of inter-individual variability and circadian rhythms on circulating concentrations (250). To confirm eligibility, screening biochemical analysis was performed prior to visit 2 by the Clinical Chemistry Laboratory, Sheffield Teaching Hospitals. Participants were screened for creatinine, calcium, albumin and PTH (Cobas c701 auto-analyser, Roche Diagnostics, Mannheim, Germany), glucose (Cobas c702 auto-analyser, Roche Diagnostics, Mannheim, Germany), TSH (Cobas e602 auto-analyser, Roche Diagnostics, Mannheim, Germany) and full blood count (Sysmex XN Series, Sysmex, Norderstedt, Germany). In participants aged 55 to 75 years, screening bloods were assessed for TC, HDL, LDL and triglycerides (Cobas c701 auto-analyser, Roche Diagnostics, Mannheim, Germany). The manufacturer's reported inter assay precision is  $<2.0\%$  for each test.

### **FAB Study Biochemistry**

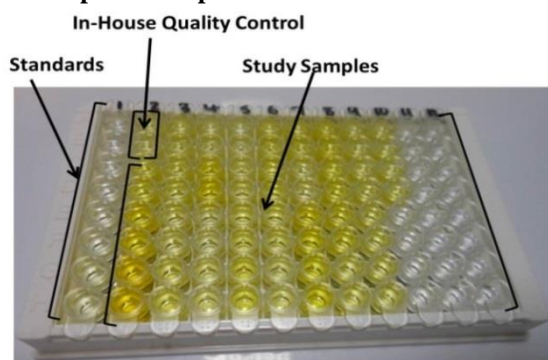
Blood samples were taken to assess hormones, BTMs and other biochemical factors which may mediate the relationship between obesity and bone. Samples were allowed to clot at room temperature for 30 minutes before being centrifuged at 3000rpm for 10 minutes. Serum samples were aliquoted and stored at  $-80^{\circ}C$  until analysis.

## Assay Principles

### Manual Sandwich Enzyme Immunoassay (ELISA)

Serum samples and standards were diluted as necessary (100 fold for leptin and adiponectin, 3 fold for OPG) and added to 96 well microplates, pre-coated with analyte-specific monoclonal antibody (Figure 24).

**Figure 24: Example ELISA well-plate set-up**



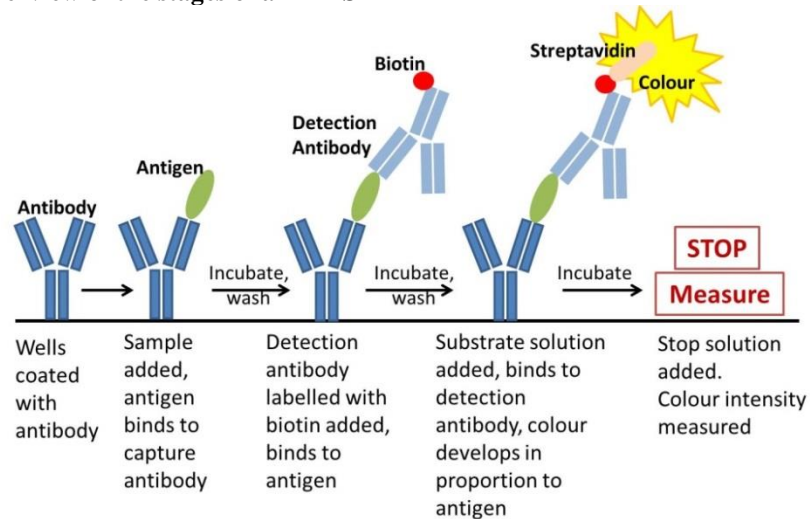
Any analyte contained in the sample binds to the antibody. Following an incubation period, the wells were washed to remove all unbound substances. An enzyme-linked antibody specific to the analyte was added (leptin and adiponectin: monoclonal antibody, OPG: biotin labelled polyclonal antibody)\*. After further incubation, the wells were washed to remove unbound antibody-enzyme reagent\*\*. Colourless substrate solution was added and the plate kept in darkness. Colour developed in proportion to the quantity of analyte contained in the sample. After a final incubation, colour development was stopped with the addition of acidic solution, and the colour changed. Final colour intensity was measured using a microplate reader. An illustrated overview of this process is given in Figure 25.

\*In the sclerostin ELISA, biotin labelled monoclonal antibody was added to the microplate with the samples, incubated, aspirated and washed before a streptavidin-HRP conjugate was added at this point.

\*\*In the OPG ELISA, streptavidin-HRP conjugate was added at this point, incubated and washed before the subsequent steps.



**Figure 25: Overview of the stages of an ELISA**



### **Chemiluminescence Immunoassay (CLIA)**

The IDS-iSYS (ImmunodiagnosticSystems, Boldon, UK) uses CLIA to detect and quantify sample analytes. The serum sample is loaded onto the autoanalyser and two antibodies are added; an anti-analyte antibody labelled with biotin and an acridinium labelled antibody. Magnetic micro-particles coated with streptavidin are added and bind to the biotin in the complex. The mixture is incubated and the magnetic particles become bound to a magnet. A wash step occurs to remove any unbound substances. Reagents are added to stimulate the acridinium conjugate to emit light, with the amount of light produced being proportional to the concentration of the analyte.

### **ElectroChemiLuminescence Immunoassay (ECLIA)**

The Cobas e411 autoanalyser (Roche Diagnostics, Mannheim, Germany) uses an ECLIA to detect small analyte concentrations. The serum sample is loaded onto the autoanalyser and two antibodies added; one labelled with biotin and the other labelled with ruthenium, which form a complex with the analyte. The mixture is incubated before microbeads coated with streptavidin are added, which interact and bind to the biotin in the complex. The mixture is aspirated into a measuring cell. When a magnetic force is applied, the microbeads bind to the surface of the oxide film-coated electrode. A solution containing tripropylamine (TPA) is added to remove any unbound substances. When a specified voltage is given by the electrode, the ruthenium and TPA excite and the TPA releases a proton which acts as a reactant to the ruthenium. As the ruthenium decays from its excited state to its lower energy basal state, it emits light. This luminescence is detected by a photomultiplier and the intensity over a period of time is subsequently quantified against a calibration curve, with the amount of light proportional to the analyte concentration.

## **Biochemical Tests**

### **Bone Turnover Markers**

Changes in BTMs reflect acute alterations in bone metabolism which may not be detectable from BMD by imaging. High bone turnover is associated with bone loss and so may help to identify individuals at high risk of fracture (251). BTMs are either by-products of collagen formation or breakdown, or cell proteins that reflect osteoclastic or osteoblastic activity. Serum and urinary CTX is proportional to the mass of the resorbed bone matrix, while serum concentrations of OC and BAP are proportional to bone formation activity assessed by histomorphometry.

Despite there being a range of BTMs available to measure, serum CTX and serum PINP were measured in this study in line with the recommendations of the International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine (252).  $\beta$ CTX is a product of type I bone collagen degradation and used as a marker of bone resorption. Collagen is degraded into long peptides of CTX and N-terminal crosslinks before further degradation into smaller molecules such as deoxypyridinoline, pyridinoline, hydroxylyzine and hydroxyproline. CTX circulates in native ( $\alpha$ ) and  $\beta$ -isomerized forms. The degradation of mature type-I collagen is determined from the  $\beta$ -isomerized form which will be measured in this study, rather than the degradation of immature collagen ( $\alpha$  form). During bone formation osteoblasts secrete procollagen to be incorporated into the bone matrix. PINP is a matrix protein derived when the N-terminal of the procollagen molecule is cleaved from the type I procollagen molecule. The PINP molecule is released into the circulation whilst the remainder of the procollagen molecule is incorporated into the bone matrix. PINP can therefore be used as a marker of bone formation. OC, a non-collagenous protein specific to osteoblast activity, and BAP, a glycoprotein found on the surface of osteoblasts, are also markers of bone formation and reflect osteoblastic activity.

CTX, PINP and OC were determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). BAP was determined by automated CLIA (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK). The inter assay CV for CTX was 2.9%, PINP 5.7%, OC 2.9% and BALP 3.9%.

### **Adipokines**

Leptin and adiponectin were determined by manual ELISA (Human Leptin Quantikine ELISA, Human Total Adiponectin Quantikine ELISA, R&D Systems, UK). The minimum

detectable dose for the leptin assay was <7.8 pg/ml. The mean minimum detectable dose for the adiponectin assay was 0.246 ng/ml. Samples above the range of detection of the adiponectin assay were diluted appropriately with calibrator diluent and the analysis repeated with the result multiplied according to the dilution factor. The inter assay CV for leptin was 3.8% and adiponectin 2.8%.

### **Further Biochemistry**

PTH, 25OHD, totE2 and insulin were determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). The inter assay CV for PTH was 2.8%, 25OHD 5.7%, totE2 5.4% and insulin 5.0%. IGF-I was measured by automated CLIA (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK). The inter assay CV for IGF-I was 4.3%. Sex hormone binding globulin (SHBG) was measured by automated ECLIA (Cobas e602, Roche Diagnostics, Mannheim, Germany). The manufacturer's inter assay precision is <5.6%. HsCRP was measured by automated nephelometry (BNII System, Siemens, Siemens Healthcare Diagnostics, Surrey, UK). The inter assay CV for HsCRP was 3.3%. IL-6 was measured by automated immunoassay (Cobas e601, Roche Diagnostics, Mannheim, Germany). The manufacturer's inter assay precision is <8.5%.

OPG was measured by manual ELISA (Biovendor, GmbH, Heidelberg, Germany). The minimum detectable dose for this OPG assay was 0.03 pmol/L. Samples above the range of detection of the OPG assay (>60 pmol/L) were diluted appropriately with calibrator diluent and the analysis repeated with the result multiplied according to the dilution factor. The inter assay CV for OPG was 1.6%. Sclerostin was measured by manual ELISA (Biomedica, Vienna, Austria). The minimum detectable dose for this sclerostin assay was 2.6 pmol/L. The inter assay CV for sclerostin was 9.1%.

### **Biochemical Calculations**

#### **Free and Bioavailable E2**

E2 circulates bound to SHBG or albumin. Free E2 (fE2) was determined using the simplified calculation cited in Rinaldi et al. (253), based on the mass action law and taking into account the affinity constants of albumin and SHBG for E2:

$$fE2 \text{ (mol/L)} = (-b + \sqrt{b^2 - 4ac}) / 2a$$

Where: N= 1+ affinity constant of albumin for E2 x albumin

a = N \* affinity constant of SHBG for E2

b = N + affinity constant of SHBG for E2 \* (SHBG - Total E2)

c = -Total E2

Affinity constant of albumin for E2 =  $4.21 \times 10^4$  L/mol

Affinity constant of SHBG for E2 =  $3.14 \times 10^8$  L/mol

Bioavailable E2 (bioE2) is that which is unbound or bound to albumin, as unlike SHBG, albumin dissociates readily to enable E2 to cross the cell membrane and bind to its receptor (253). BioE2 was calculated as:

$$\text{bioE2 (mol/L)} = (1 + \text{affinity constant of albumin for E2} \times \text{albumin}) \times \text{fE2}$$

The affinity constants used were those stated in Rinaldi et al. (253). It was not possible to use the more complex equation which takes into account competition for binding sites by testosterone, E2 and dihydrotestosterone, as no testosterone or dihydrotestosterone measurements were available.

### **Insulin Resistance**

Insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR); calculated as:

$$(\text{Fasting serum glucose (mmol/L)} \times \text{fasting serum insulin (}\mu\text{U/ml)}) / 22.5$$

### **Kidney Function**

Kidney function was estimated from calculation of estimated glomerular filtration rate (eGFR), using the Modification of Diet in Renal Disease (MDRD) equation:

$$\text{eGFR} = 175 \times (\text{serum creatinine (}\mu\text{mol/L)} \times 0.0113)^{-1.154} \times \text{age}^{-0.203} \text{ [* 0.742 if female]}$$

### **Vitamin D Sub-study Biochemistry**

At the vitamin D visit, blood samples were taken between 08:00 and 10:00 after an overnight fast. Serum and whole blood samples were kept frozen at  $-80^{\circ}\text{C}$  until analysis, with the exception of CK samples which were analysed in real-time.

### **Total 25OHD**

Total 25OHD was measured by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). The inter assay CV was 4.6%

### **25OHD<sub>2</sub> and 25OHD<sub>3</sub>**

As immunoassays may fail to discriminate between 25OHD<sub>2</sub> and 25OHD<sub>3</sub>, total 25OHD<sub>3</sub> was also measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the laboratory of the Institute of Human Development, University of Manchester. 200 $\mu\text{l}$  of sample and deuterated internal standard (d6-25OHD) were prepared using 100 $\mu\text{l}$

methanol;isopropanol (80:20) then extracted with 1ml hexane. The extracted 25OHD in the hexane supernatant was blown down and reconstituted in 150µl of 66% methanol. 37.5µl of this extract was injected onto a Waters Phenyl column attached to the mass spectrometer and eluted with an isocratic gradient over 5 minutes. Analysis was carried out in positive ion mode using the transitions m/z 401>159 and Mm/z 407>159 for 25OHD and d6-25OHD respectively. Run time injection to injection was 5.1 minutes. Between batch CV (SD) was 7.4% (2.7), 7.1% (6.5) and 6.3% (8.8) at concentrations of 37, 91, and 140 nmol/L respectively.

### **Free 25OHD**

Free 25OHD was measured by immunoassay (Future Diagnostics BV, Wijchen, Netherlands) in the laboratory of Future Diagnostics (Wijchen, Netherlands) (254). Serum samples and calibrators were pipetted into the wells of a microtiter plate coated with anti-25OHD antibody. Free 25OHD was captured by the antibody during an incubation. After washing, a biotin-labelled 25OHD analog was allowed to react with the non-occupied antibody binding sites in a second incubation. After a second wash and incubation with a streptavidin-peroxidase conjugate, bound enzyme is quantitated using a colorimetric reaction. Signal intensity is inversely proportional to the concentration of free 25OHD in the sample. The assay was calibrated against a symmetric dialysis method. The calibrator range was 0.0 to 35.0 pg/ml. The limit of the blank, from 60 replicates, was 0.7 pg/ml and the limit of detection, determined from the pooled SD from 12 measurements of five low samples, was 1.9 pg/ml, both determined according to the Clinical Laboratory and Standards Institute EP17-A guideline. Precision was determined over 20 consecutive days with two runs per day according to the CLSI- EP5 protocol. Assay CVs were <15.7%.

### **Calculated Free 25OHD**

Free 25OHD was also calculated from the concentration of DBP and albumin and their binding affinities for 25OHD using the formula (255):

$$\text{Free 25OHD} = \text{Total 25OHD} / (1 + (6 \cdot 10^5 \times \text{albumin}) + (7 \cdot 10^8 \times \text{DBP}))$$

### **Total 1,25(OH)<sub>2</sub>D**

Total 1,25(OH)<sub>2</sub>D was measured by manual immunoassay (Immunodiagnostic Systems, Boldon, UK). The inter assay CV was 6.0%.

### **Free 1,25(OH)<sub>2</sub>D**

Free 1,25(OH)<sub>2</sub>D was calculated from the concentration of DBP and albumin and their binding affinities for 1,25OHD using the formula (256):

$$\text{Free 1,25(OH)}_2\text{D} = \text{Total 1,25(OH)}_2\text{D} / (1 + (5.4 \times 10^4 \text{M}^{-1} \times \text{albumin}) + (3.7 \times 10^7 \text{M}^{-1} \times \text{DBP}))$$

### Vitamin D Binding Protein

DBP was measured by manual immunoassay (Human Vitamin DBP Quantikine ELISA, R&D Systems, UK). The inter assay CV was 3.3%.

### Vitamin D Binding Protein Genotyping

DBP genotyping was performed in the laboratories of the Sheffield Diagnostic Genetics Service, Sheffield Children's Hospital. The pyrosequencing assay was developed in house, using PSQ assay design software version 1.0.6 (Qiagen), to detect DBP rs4588 and rs7041 polymorphisms. PCR reactions contained 1µl (10 to 20 ng) of human genomic DNA, 5 pmol of forward and reverse primers, 7.5µl of OneTaq® 2X Master Mix with standard buffer (NEB) and H<sub>2</sub>O up to a final volume of 15µl. Table 4 shows the primer sequences.

**Table 4: Primer sequences for the genotyping of DBP**

Allele	PCR primers (5'→3')	Pyrosequencing primer (5'→3')
VDBP rs4588+ rs7041	F: 5'- ATCTGAAATGGCTATTATTTTG -3' R: 5' Btn- ACAGTAAAGAGGAGGTGAGTT -3'	AAAAGCTAAATTGCCTG

PCR conditions consisted of an initial denaturation at 94°C for 3 min; 47 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds and extension at 68°C for 30 seconds; followed by a final extension at 68°C for 4 minutes. PCRs were performed using a 9700 Gene Amp® PCR system (Applied Biosystems, CA). For each genotype determination, single-stranded DNA was purified from 5µl of PCR products using PyroMark Q96 Vacuum Prep Workstation (Qiagen). PCR products were bound to streptavidin Sepharose beads (GE Healthcare, UK), washed with 70% ethanol, denatured using a 0.2mol/L NaOH solution and washed again with 10mM Tris Acetate. Single stranded DNA was eluted in 12µl of sequencing buffer containing 0.3µmol/L pyrosequencing primer, denatured at 80°C for 2.5 minutes and cooled to room temperature to allow annealing of the sequencing primer. Pyrosequencing was performed (PyroMark Q96 MD , Qiagen) according to the manufacturer's instructions. Nucleotide dispensation order was: VDBP rs4588+rs7041 CATGTCACACTG. SNP analysis was carried out using the SNP analysis software provided (Qiagen).

### Biochemical Markers of Bone Turnover

CTX, PINP and OC were measured by automated ECLIA (Cobas e411, Roche Diagnostics, Germany). The inter assay CVs were: CTX 4.0%, PINP 4.1%, OC 2.2%. BAP was measured by automated CLIA (IDS-iSYS, ImmunoDiagnostic Systems, Boldon, UK). The inter assay CV for BAP was 4.5%.

### Vitamin D Sub-study Further Biochemistry

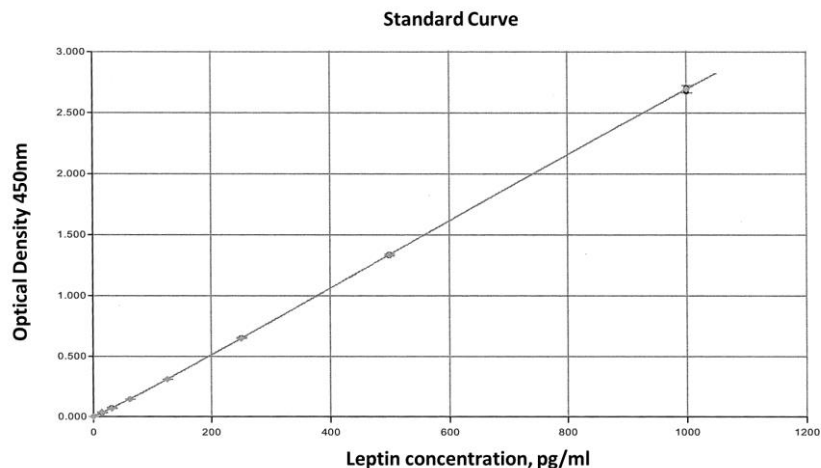
Albumin, creatinine, calcium, phosphate, PTH, CK, triglycerides, TC, HDL and LDL were measured by automated ECLIA (Cobas c701, Roche Diagnostics, Mannheim, Germany) in the Chemical Chemistry laboratory, Sheffield Teaching Hospitals. The manufacturer's reported inter assay precision was <2.0% for each test. IGF-I was measured using by automated CLIA (IDS-iSYS, ImmunoDiagnostic Systems, Boldon, UK). The inter assay CV was 3.2%. HsCRP was measured by automated nephelometry (BNII System, Siemens, Siemens Healthcare Diagnostics, Surrey, UK) in the Immunology laboratory, Sheffield Teaching Hospitals, UK. The inter assay CV was 3.1%.

In an associated study, TNF- $\alpha$ , IL-1 and IL-6 were found to be frequently below the detection limit of the available assay and therefore were not measured.

### Biochemistry Quality Control

All assays were undertaken by, or under the supervision of, an experienced technician, to minimise technician variability. For all manual assays a standard curve was produced using a range of standards from a stock solution (Figure 26). High and low QC standards were determined for OPG. Blank wells were included for sclerostin. An in-house QC sample was used for each plate. Samples were analysed in duplicates, where possible.

Figure 26: Example manual assay standard curve plot



Calibration of the Cobas e411 and IDS-iSYS was performed prior to using reagents from a new lot. Further calibration of the IDS-iSYS was performed on an analyte dependent weekly or fortnightly basis, as recommended by the manufacturer. All samples underwent a maximum of two freeze-thaw cycles. Where repeated freeze-thaw cycles were indicated as unsuitable in the manufacturer’s guidelines, a fresh sample was used.

Analyte measurement method can affect variability. Most of the auto-analysers used in the study involve a monoclonal antibody which ensures specific measurements of the analyte (251). Manual ELISAs are subject to greater operator error than automated methods. ECLIA and CLIA methods are highly sensitive, simple to perform, quick, and do not require radioisotopes (251). A further source of analyte variability is pre-analytical variability. Whilst some elements of pre-analytical variability can be easily modified, other causes are less modifiable, as shown in Table 5 (251).

**Table 5: Sources of analyte variability and attempts to control such variability in the FAB study**

	<b>Source of variability</b>	<b>How variability was controlled for</b>
<b>Modifiable</b>	Circadian variation	Samples taken between 08:00-10:00
	Menstrual variation	Samples from pre-menopausal women taken in the follicular phase
	Fasting	Overnight fasted blood samples taken
	Physical activity	Participants instructed not to participate in vigorous activity for 24 hours prior to sampling
	Seasonal variation	Not controlled for in FAB. All samples taken in two one month periods for the vitamin D sub-study
<b>Less easily modifiable</b>	Age	Normal BMI/obese pairs matched by age
	Gender	Normal BMI/obese pairs matched by gender
	Menopausal status	Older women $\geq 5$ years postmenopausal
	Diseases characterised by accelerated bone turnover	e.g. Primary hyperparathyroidism, bone metastases; participants ineligible/ excluded
	Diseases characterised by dissociation of bone turnover	e.g. Cushing’s disease, multiple myeloma; participants ineligible/ excluded
	Diseases characterised by low bone turnover	e.g. Hypoparathyroidism; participants ineligible/ excluded
	Chronic diseases associated with limited mobility	Participants ineligible/ excluded
Recent fracture	Participants with a fracture 12 months prior to recruitment ineligible	



Medications known to affect bone turnover	e.g. oral corticosteroids, aromatase inhibitors, hormonal contraceptives; participants taking such medications ineligible
Day-to-day variation	Not controlled for

---

## **CHAPTER 3:**

# **BONE DENSITY, STRUCTURE AND STRENGTH IN OBESITY**

## **CHAPTER 3: Bone Density, Structure and Strength in Obesity**

### **Background**

#### **Obesity and aBMD**

BMI is positively associated with areal bone density in younger (15, 257), older (23, 43, 45, 48, 105, 258-261) and elderly (262, 263) men and women. Body weight and BMI are positively associated with aBMD of the lumbar spine (48, 258, 259, 261), femoral neck (48, 258) distal radius (261), proximal femur and leg (23, 48, 259, 260, 263). Concordantly, a retrospective cohort study found that low body weight and low BMI were associated with osteoporosis at the lumbar spine, proximal femur, total hip, femoral neck and trochanter (48).

Despite the majority of the literature indicating a positive association between body weight and aBMD, a number of studies have reported conflicting results. Such findings commonly result from opportunistic studies with insufficient power to detect differences in aBMD between normal BMI and obese groups, or from adjustment for body composition outcomes without acknowledgment of their covariance. Whether there is a threshold BMI above which aBMD ceases to be positively associated with BMI is unclear but has been proposed in response to findings of greater LS aBMD in overweight individuals versus normal BMI controls, but lower LS aBMD in obese than overweight individuals (264).

Although obese adults may have higher aBMD than normal BMI adults, as discussed in Chapter 1, obese individuals are not exempt from fracture. There are several reports of a high prevalence of obese individuals presenting to fracture clinics even though these individuals were shown to have mean T-scores of +2.5 or greater at the lumbar spine, femoral neck or total hip (43, 44, 50). This suggests that aBMD may be a particularly poor indicator of fracture risk in obese individuals.

#### **Obesity and vBMD**

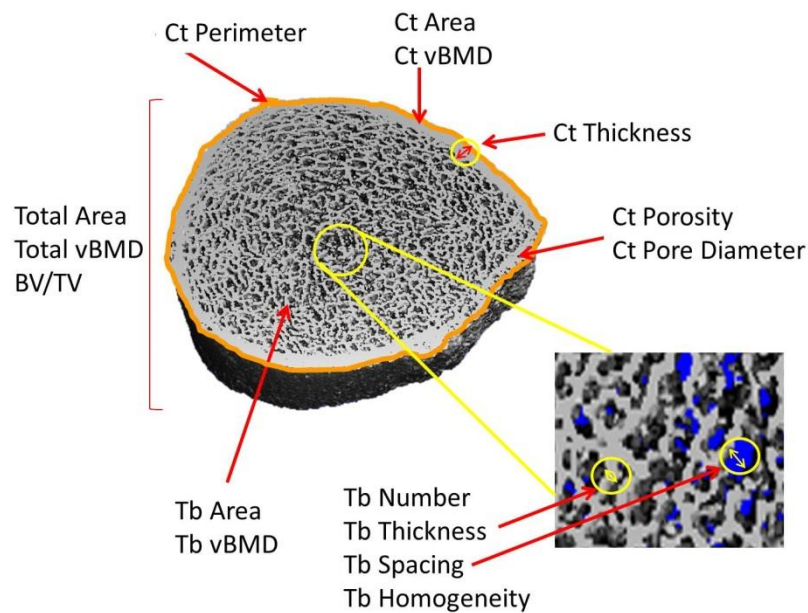
The principal outcomes of HR-pQCT imaging are illustrated in Figure 27 and described in more detail in Chapter 2.

#### **Obesity and Total vBMD**

Few studies have investigated associations between obesity and measures of vBMD. Obesity was associated with greater vBMD at the distal tibia by pQCT in pre and postmenopausal women (265) and was positively associated with vBMD by HR-pQCT

in a study of chronic kidney disease patients (266). Obese postmenopausal women in the OFELY cohort had a 13% greater vBMD at the distal radius and a 15% greater vBMD at the distal tibia than non-obese postmenopausal women (261). Another recent study found positive associations between total body FM and vBMD at the FN and LS by QCT and at the distal radius by HR-pQCT (267).

**Figure 27: Principal microstructural outcomes of HR-pQCT imaging**



Associations between BMI and total vBMD at the distal radius by HR-pQCT in young obese men were not presented by Bredella et al. (268). As there was no non-obese control group in this study, the limited BMI range may have been insufficient to detect such an association, particularly if the hypothesis of there being a threshold BMI above which increases in vBMD are disproportionate to increases in BMD holds true.

### **Obesity and Tb and Ct vBMD**

At the distal tibia, high BMI ( $BMI > 35 \text{ kg/m}^2$ ) was positively associated with Tb.vBMD in two studies, with 15% greater Tb.vBMD in the obese than the non-obese women in the OFELY cohort (261, 265). However, the two studies yielded conflicting results in terms of Ct.vBMD, with lower Ct.vBMD by pQCT observed in obese premenopausal American women (265) but a 7% greater Ct.vBMD reported in obese women from the OFELY study (261).

Positive associations between BMI and compartmental vBMD have also been reported at the distal radius, with obese women having a 14% greater Tb.vBMD and a 3% greater

Ct.vBMD, compared to non-obese women (261). Associations between BMI and Tb and Ct.vBMD at the distal radius were assumed not significant in young obese men (268).

When L4 Tb.vBMD was investigated by QCT in obese premenopausal women, no significant associations between BMI and Tb.vBMD were found, although there was no non-obese control group to refer to in this study (269).

### **Obesity and Bone Microarchitecture**

Only one study has presented bone microarchitectural findings in obesity compared to a non-obese control group. As already mentioned, greater total vBMD was observed in obesity and this was due to there being greater Ct.Th, Ct.Ar and Ct.vBMD and greater Tb.vBMD due to greater Tb.N with a lower Tb.Sp.SD (261). At the tibia, Ct.Th was not significantly greater in obesity, but trended positively. At the tibia Ct.Po was 21% lower in the obese group, whilst no differences in Ct.Po were observed at the radius (261). Tot.Ar and Tb.Ar were no different in obesity (261). Greater percentage differences in microarchitectural parameters between the obese and non-obese group were observed at the distal tibia compared to the distal radius (261).

Similar to these findings, BMI was positively associated with Tb.N and inversely associated with Tb.Sp and Tb.Th in young obese men (268). Positive associations between BMI and Tb.N and Ct.Th were reported in chronic kidney disease patients, with an inverse association between BMI and Tb.Sp (266).

A recent study examined the effect of FM and LM on HR-pQCT derived bone microarchitecture in obese individuals with metabolic syndrome (270). The study reported positive associations between LM and Tb.N and Tb.Sp at the radius, and vBMD, Tb.vBMD, BV/TV, Tb.N, Tb.Sp and Ct.Th at the distal tibia (270). No significant associations between FM and microarchitectural outcomes were observed (270). Whether associations between high BMI and bone microarchitecture are due to greater FM or greater LM in 'healthy' obese adults is unclear.

### **Obesity and Bone Strength**

Bone strength describes "the force required to produce mechanical failure under a specific loading condition" (222). Historically, bone strength has been assessed using ex-vivo, mechanical strength testing methods such as the three point bending test (271). aBMD by DXA has become a conventional surrogate for bone strength *in-vivo*, however

it is widely appreciated that bone strength is influenced by not only aBMD and bone quantity, but also by bone geometry and structure.

True assessment of bone strength would require quantification of both the structural and material properties of bone (222). Although we are unable to non-invasively quantify the material properties of bone tissue, novel *in-vivo* assessments of bone strength by FEA have been developed which take into account the density, geometry and microarchitectural components of bone. By converting each image voxel into equally sized elements connected by a mesh, applying a simulated load and observing the bone response, more accurate predictions of fracture risk can be made (222).

In pre- and postmenopausal women with a BMI  $>35\text{kg/m}^2$ , bone strength from pQCT at the distal tibia, was not higher than in normal weight women (265). Stiffness and estimated failure load were investigated in young obese men at the distal radius by HR-pQCT (268). Although there was no control group in the aforementioned study, stiffness and estimated failure load were higher than those given elsewhere for men aged 20 to 78 years (BMI  $17\text{-}39\text{kg/m}^2$ ) (272), although the comparable literature is lacking.

As only one study has presented results from HR-pQCT imaging in obese versus non-obese adults, only that data is available to infer the effect of obesity on bone strength determined by  $\mu\text{FEA}$  of HR-pQCT images. At both the distal radius and distal tibia there were no differences in load distribution, but there were lower Ct. and Tb. Von Mises Stresses (the combination of the stresses from the  $x$ ,  $y$  and  $z$  directions and all shear stresses within the cortical or trabecular compartment) and higher stiffness and estimated failure load in the obese women compared to the non-obese controls (261). Further research clearly is needed to support these findings and to understand associations between obesity and bone strength in younger women and in men. Whether differences in bone strength are proportional to differences in body weight in obese people compared to those with a normal BMI is unclear.

### **Background Summary**

In summary, obesity is associated with greater aBMD at weight bearing sites as determined by DXA and may also be associated with greater aBMD at non-weight bearing sites such as the forearm. More recent studies have begun to use HR-pQCT to assess vBMD and initial findings suggest that vBMD and compartmental vBMD are indeed greater at both weight bearing and non-weight bearing sites in obesity. Microarchitecture may be favourable in obesity, but more studies are required to better

understand such associations. Whether the extent of adiposity affects relationships between BMI and vBMD remains unclear (23). Recent work has begun to study the effect of obesity on bone in men and in both pre- and postmenopausal women, to investigate whether age, gender and/or menopausal state affect the relationship between obesity and bone. A single study is yet to address relationships between obesity, vBMD and microarchitecture by age, menopausal status and gender. It may be that obesity promotes greater BMD in younger adults and/or that obesity is protective against bone loss in older adults.

### **Research Questions, Aims and Hypotheses**

Research questions:

1. Does obesity affect BMD, bone geometry, bone microarchitecture and bone strength of the hip, lumbar spine, distal radius and distal tibia?
2. Are the effects of obesity on BMD, bone geometry, bone microarchitecture and bone strength site- specific or consistent throughout the weight-bearing and non-weight-bearing skeletal sites of the axial and appendicular skeleton?
3. Are the effects of obesity on BMD, microstructure and strength commensurate to the greater body weight?

Aims:

1. To compare BMD measured by DXA and QCT at the hip and lumbar spine in normal BMI and obese individuals, to determine associations between obesity and BMD at central sites.
2. To compare BMD and bone microarchitecture at the distal tibia and distal radius, measured by HR-pQCT in normal BMI and obese individuals, to determine associations between obesity and the peripheral skeleton.
3. To compare bone strength determined by FEA at the distal radius and distal tibia, in normal BMI and obese individuals, to determine associations between obesity and bone strength.
4. To compare the magnitude of the differences in BMD, bone microarchitecture, bone geometry and bone strength at different skeletal sites in order to establish whether associations between obesity and these outcomes are site-specific.
5. To compare differences in body weight with the differences in bone density, microstructure and strength to determine whether bones are proportionally adapted to the greater loading conditions of obesity.

Hypotheses:

1. Obesity is associated with higher aBMD and vBMD at the hip and lumbar spine, with mechanically favourable bone geometry and bone microarchitecture and resultant greater bone strength, compared to normal BMI individuals. This might explain the lower risk of vertebral and hip fracture in obese individuals compared to non-obese individuals.
2. Obesity is associated with higher vBMD at the distal tibia and distal radius, but mechanically unfavourable differences in bone geometry and bone microarchitecture, with resultant lower bone strength, compared to normal BMI individuals of similar age and height. This might explain the greater incidence of ankle fracture in obesity despite higher BMD.
3. In obese individuals, bone density, structure and strength at the lumbar spine and total hip are adapted in proportion to body weight, but this is not the case at the distal tibia. This might explain site specificity of fracture patterns in obesity.
4. Greater soft tissue thickness at central sites might contribute to site specific fracture patterns in obesity.

## **Methods**

200 individuals were recruited to the study according to the inclusion criteria detailed in Chapter 2. Participants were recruited in individually-matched, normal BMI and obese pairs, as described in Chapter 2.

Height (cm) was measured using an electronic, wall-mounted stadiometer (Seca 242, Seca, Birmingham, UK). Weight (kg) was measured to the nearest 0.1 kg using an electronic balance scale (Seca, Birmingham, UK). BMI was calculated as (weight (kg)/(height (m)<sup>2</sup>)).

Whole body, lumbar spine and total hip aBMD were determined by DXA (Discovery A, Hologic Inc., Bedford, MA, USA). Total vBMD, cortical and trabecular vBMD and bone microstructural parameters at the distal radius and distal tibia were determined by HR-pQCT (XtremeCT, Scanco Medical AG, Zurich, Switzerland) (Chapter 2).

Bone strength at the distal radius and distal tibia was estimated using micro finite element analysis (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland) (Chapter 2).

## **Statistical Analysis**

Radial images from one pair of older women were excluded due to movement artifact. Tibial images from two pairs of older women were excluded due to movement and data loss. Radial extended cortical measures outcomes from one pair of older men were excluded due to outlying results (Ct.Po =0.737, Ct.Po.Dm =2.388, compared to the sample mean of 0.033 and 0.160).

Mean (SD) age, height and BMI of the study sample were calculated by age, gender and BMI group. All variables were assessed for normality and log transformed where necessary. Paired samples t-tests were used to determine significant differences between normal BMI and obese groups, for the entire sample, by age group and by age and gender. Independent samples t-tests were used to determine significant differences between genders in the magnitude of the difference between normal BMI and obese groups.

Univariate general linear models were used to identify whether age group, gender and BMI had an effect on bone outcomes and to identify interactions between age or gender and the effect of BMI on bone outcomes.

Standard deviation scores were calculated by standardising the mean difference between normal BMI and obese groups for each variable against the standard deviation of the normal BMI, gender and age matched group. Percentage difference between normal BMI and obese individuals for each bone outcome was calculated.

To determine whether bone outcomes were proportionally adapted to greater body weight in obesity, bone outcomes were normalised to body weight and the percentage difference between the normal BMI and obese individual's normalised value was calculated. Mean percentage differences were expressed as a percentage of the mean of the normal BMI group.

Analysis was performed using IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.). Significance was accepted when  $p < 0.05$ .



## **Results**

The total sample consisted of 200 individuals. The 25 to 40 years group was made up of 18 male and 22 female pairs and the 55 to 75 years group of 30 male and 30 female pairs. Characteristics of the study population are shown in Table 6. Obese and normal BMI individuals were closely matched on age with no significant difference between normal BMI and obese groups (Table 6). Participants were well matched on height; no difference in height was observed between 55 to 75 years normal BMI and obese groups. Whilst women aged 25 to 40 years with a normal BMI were statistically significantly taller than the obese group ( $p < 0.001$ ) and younger men with a normal BMI were statistically significantly shorter than the obese men ( $p < 0.05$ ), the mean paired differences (-2.65 cm, +2.61 cm respectively) were comfortably below the  $\pm 5$  cm matching criterion and thus were not considered clinically significant. BMI was greater in the obese groups (all  $p < 0.001$ ).

**Table 6: Characteristics of the study population by age and BMI group; mean (SD)**

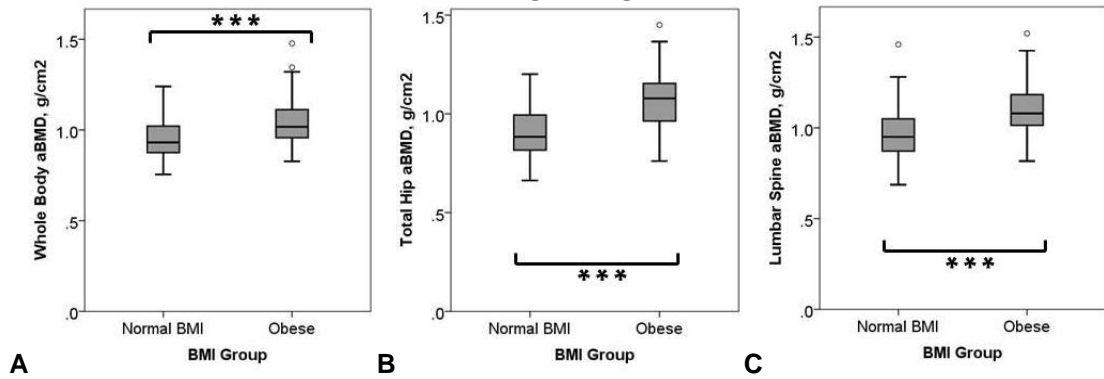
	<b>Women (n=104)</b>				<b>Men (n=96)</b>			
	<b>25 to 40 Years</b>		<b>55 to 75 Years</b>		<b>25 to 40 Years</b>		<b>55 to 75 Years</b>	
	(n=22 pairs)		(n=30 pairs)		(n=18 pairs)		(n=30 pairs)	
	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese
Age, years	34.6 (4.16)	34.8 (4.6)	65.4 (5.8)	65.1 (5.6)	33.2 (4.2)	33.9 (3.7)	65.9 (5.3)	65.5 (5.7)
Height, cm	167.1 (6.8)	164.5 (7.3)	161.1 (4.3)	160.6 (4.0)	178.4 (7.0)	180.4 (6.7)	175.5 (6.4)	176.1 (7.5)
BMI, kg/m <sup>2</sup>	22.4 (1.5)	35.4 (4.2)	22.9 (1.5)	35.9 (5.0)	23.0 (1.3)	32.9 (2.6)	23.4 (1.2)	34.6 (4.0)

### **Areal BMD in Obesity**

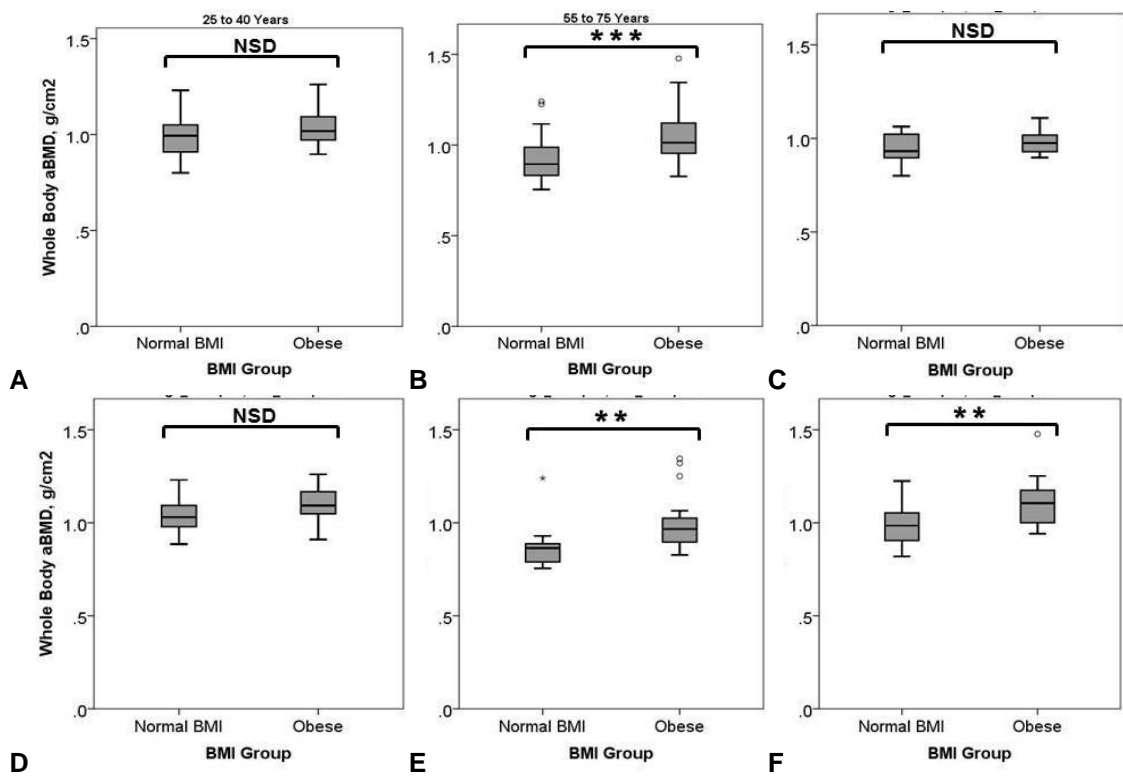
Obese individuals had a greater aBMD at the WB ( $p < 0.001$ ), TH ( $p < 0.001$ ) and LS ( $p < 0.001$ ) than normal BMI individuals (Figure 28).

Men had greater WB and TH aBMD than women (both  $p < 0.001$ ). There was no interaction between gender and the effect of BMI on WB, TH or LS aBMD. The magnitude of the difference in aBMD between normal BMI and obese groups was not significantly different between the sexes in either age group, at any site.

**Figure 28: Whole body (A), total hip (B) and lumbar spine (C) aBMD of normal BMI versus obese individuals. All ages and genders combined.<sup>1,2</sup>**

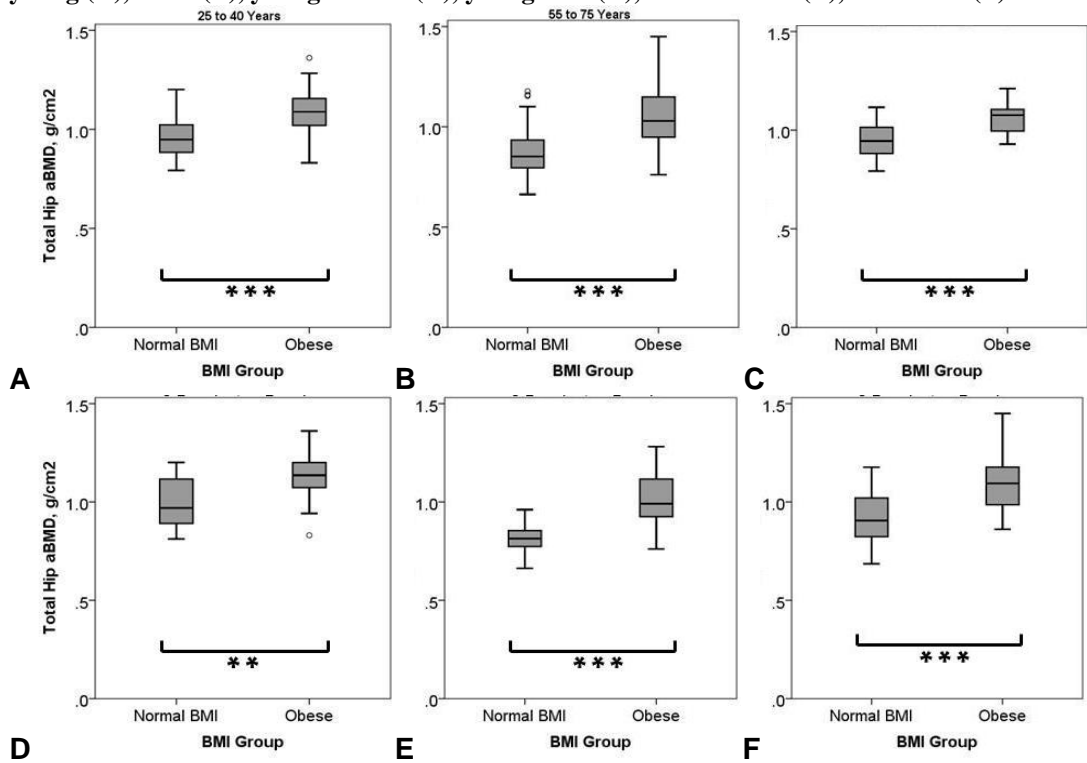


**Figure 29: Whole body aBMD of normal BMI and obese individuals by group: young (A), older (B), young women (C), young men (D), older women (E), older men (F).<sup>1,2</sup>**

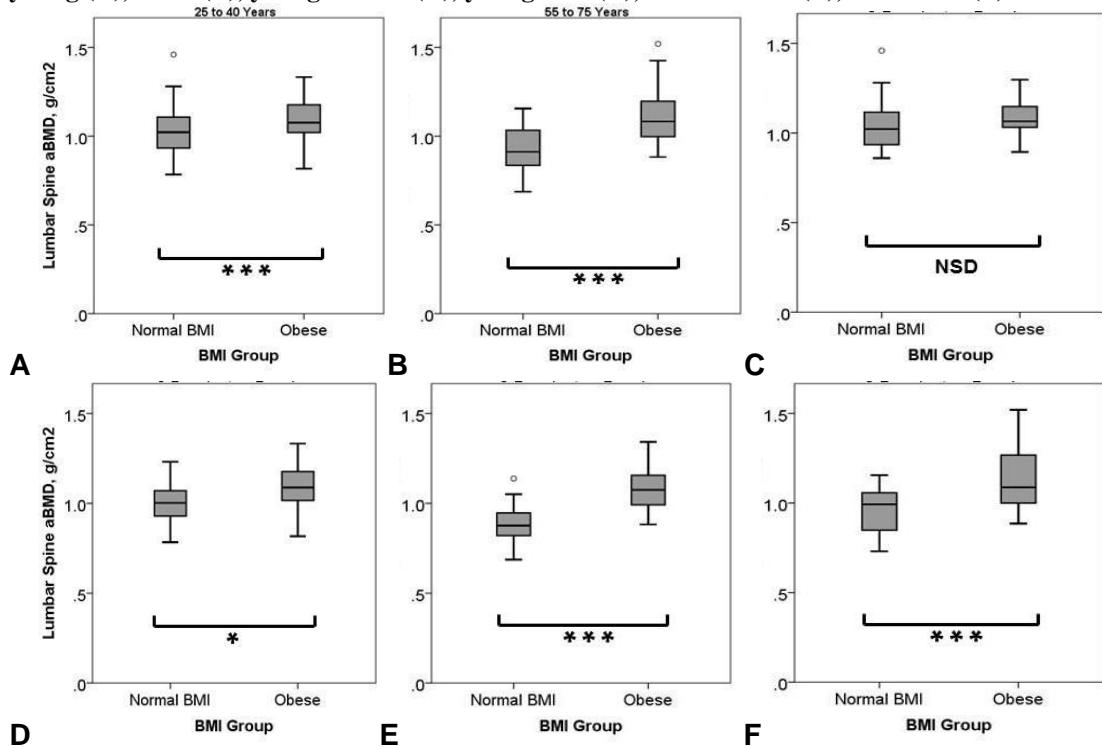


<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half times the interquartile range above or below the box boundary)  
<sup>2</sup> \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, NSD no significant difference

**Figure 30: Total hip aBMD of normal BMI and obese individuals by group: young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>**



**Figure 31: Lumbar spine aBMD of normal BMI and obese individuals by group: young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>**



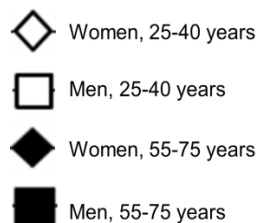
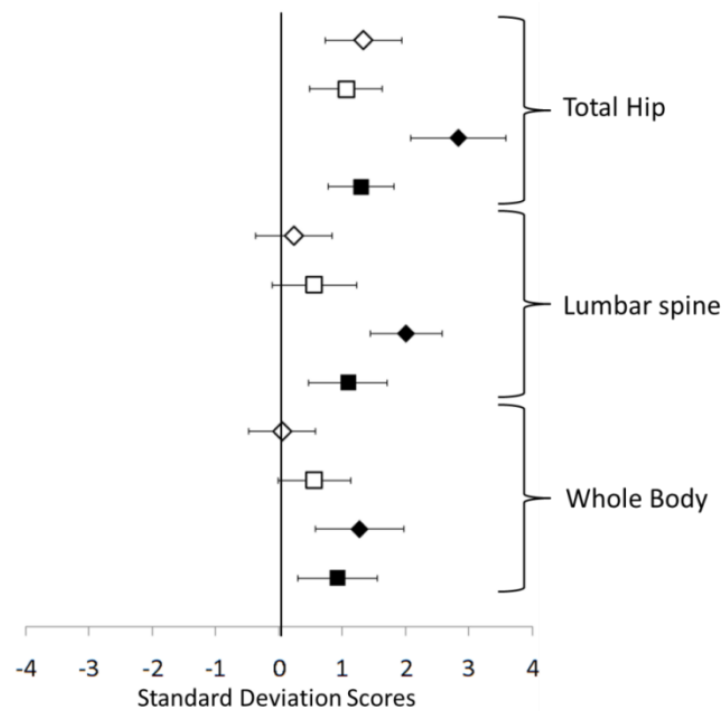
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

Young adults had greater WB, LS and TH aBMD than older adults (all  $p < 0.05$ ) (Figure 29, Figure 30, Figure 31). The effect of obesity on WB and LS aBMD was greater in older adults than young adults (both  $p < 0.01$ ). There was no difference in the effect of BMI on total hip aBMD by age group. In younger adults, aBMD was 0 to 1 SD scores greater in the obese group than in the normal BMI group (Figure 32). In older adults, aBMD was 1 to 2 SD scores greater in the obese group than in the normal BMI group (Figure 32).

**Figure 32: aBMD of obese individuals as SD scores of the mean of the normal BMI group, for total hip and lumbar spine and whole body. (Mean (95%CI)).**

Reproduced with permission: Evans AL et al. Bone Density, Microstructure and Strength in Obese and Normal Weight Men and Women in Younger and Older Adulthood, 2014 Journal of Bone and Mineral Research, John Wiley and Sons. © 2014 American Society for Bone and Mineral Research

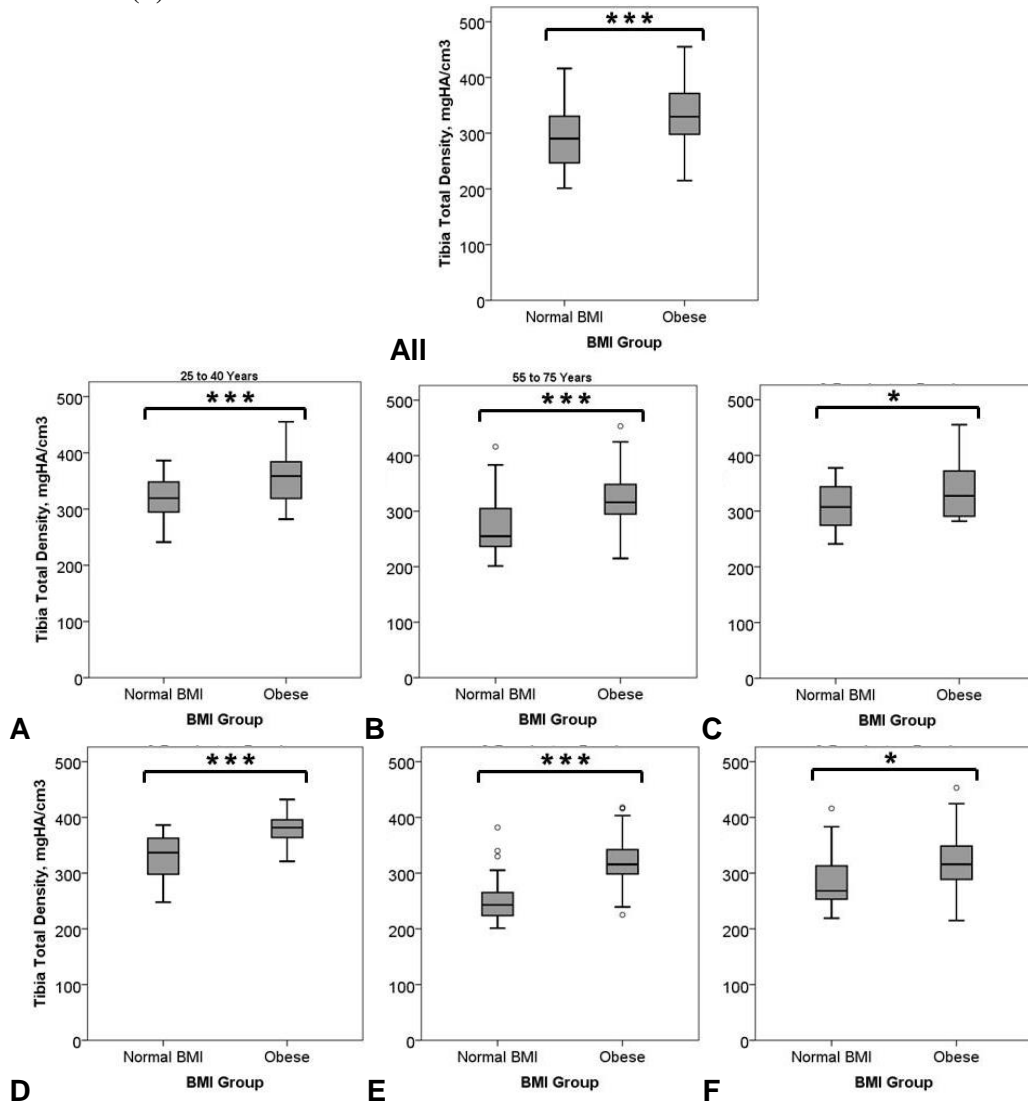


## vBMD and Bone Microarchitecture in Obesity

Obese individuals had greater vBMD at the distal tibia than normal BMI individuals ( $p < 0.001$ ) (Figure 33). Older obese adults had greater vBMD at the distal radius than older adults with a normal BMI ( $p < 0.001$ ) (Figure 34). There was a greater effect of obesity on vBMD at the distal radius in older adults than younger adults ( $p < 0.01$ ).

**Figure 33: Tibia vBMD of normal BMI and obese individuals**

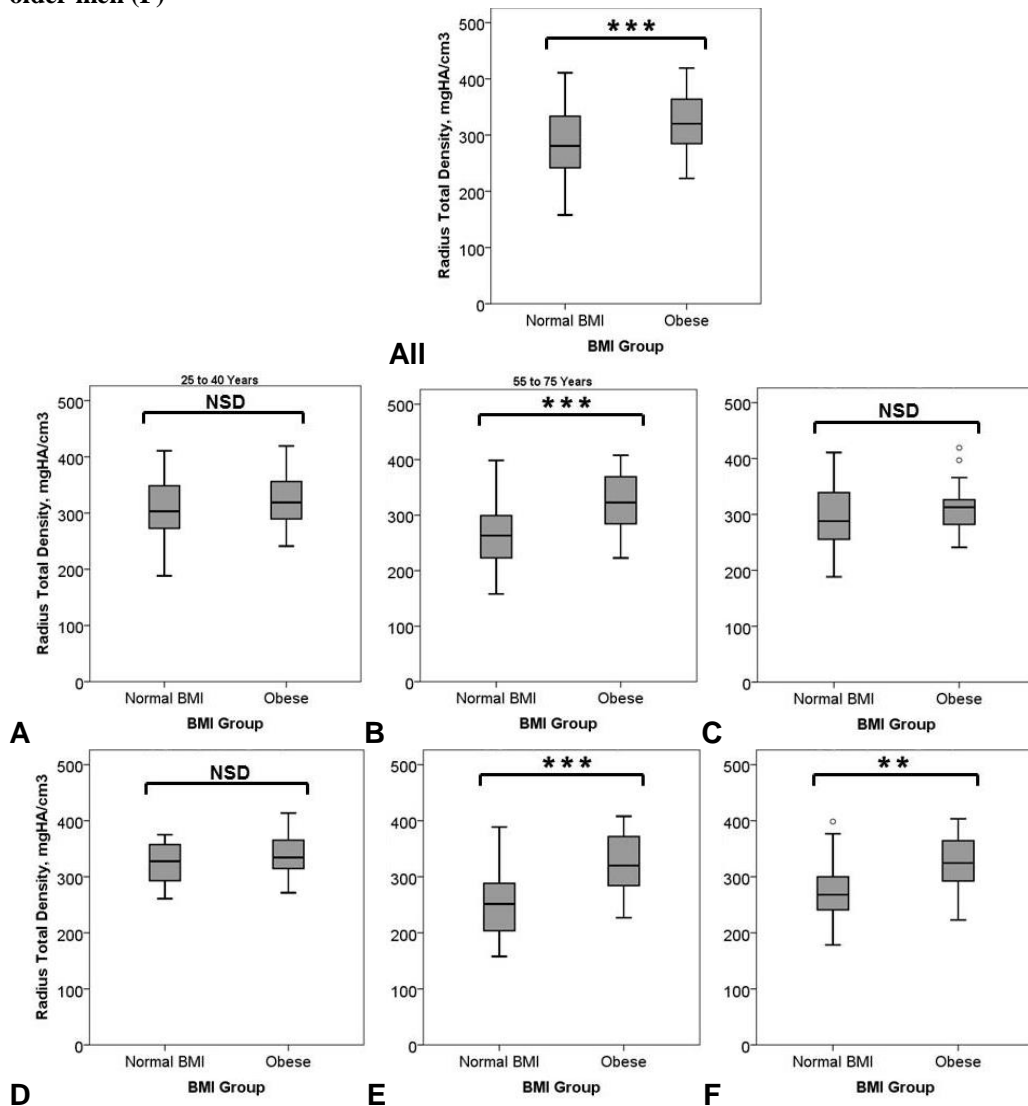
Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

**Figure 34: Radius vBMD of normal BMI and obese individuals**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E),**  
**older men (F)<sup>1,2</sup>**



Microstructure measurements showed the higher vBMD in obesity was due to greater Tb.vBMD in the young adults ( $p < 0.05$  radius,  $p < 0.001$  tibia) and greater Tb.vBMD and Ct.vBMD in the older adults (all  $p < 0.001$ ) (Figure 35, Figure 36). The higher Tb.vBMD in the obese groups was due to greater Tb.N ( $p < 0.001$  all ages, all sites) and lower Tb.Sp ( $p < 0.001$  all ages, all sites) with no difference in Tb.Th at the radius or the tibia (Figure 35, Figure 36).

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

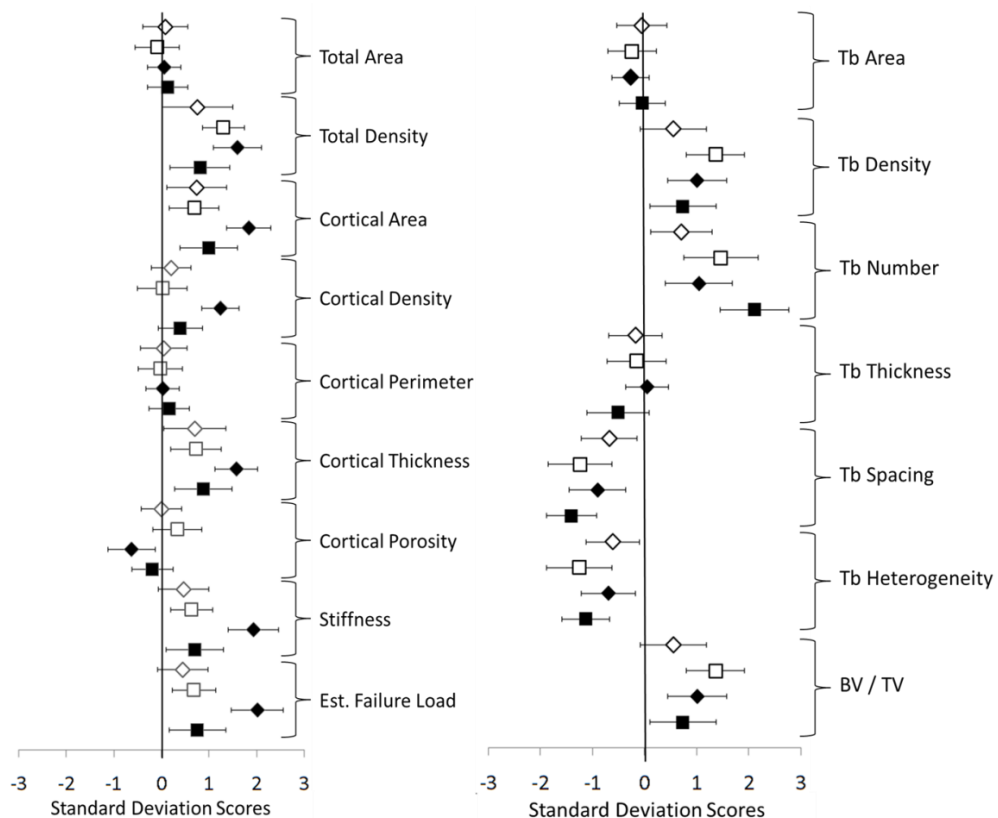
The higher Ct.vBMD in older obese adults was due to higher cortical tissue mineral density (Ct.TMD) ( $p < 0.05$  radius,  $p < 0.001$  tibia) and lower Ct.Po (NSD radius,  $p < 0.05$  tibia), whereas no differences between normal BMI and obese individuals were observed for any of these parameters in the younger adults (Figure 35, Figure 36). Obese individuals had greater Ct.Th at the distal tibia ( $p < 0.01$  younger,  $p < 0.001$  older) (Figure 35) and at the distal radius in the older adults ( $p < 0.001$ ) (Figure 35, Figure 36).

The difference in Ct.vBMD ( $p < 0.05$  radius,  $p < 0.01$  tibia) and Ct.TMD ( $p < 0.05$  radius,  $p < 0.01$  tibia) between normal BMI and obese groups was greater in older women than older men. At the tibia, the difference in Ct.Th and Ct.Ar was also greater in older women than older men (both  $p < 0.05$ ). In the younger adults, differences in cortical and trabecular properties between normal BMI and obese groups were similar by gender.

No difference was observed in bone size (Tot.Ar, Ct.Pm) between normal BMI and obese individuals (Figure 35, Figure 36).

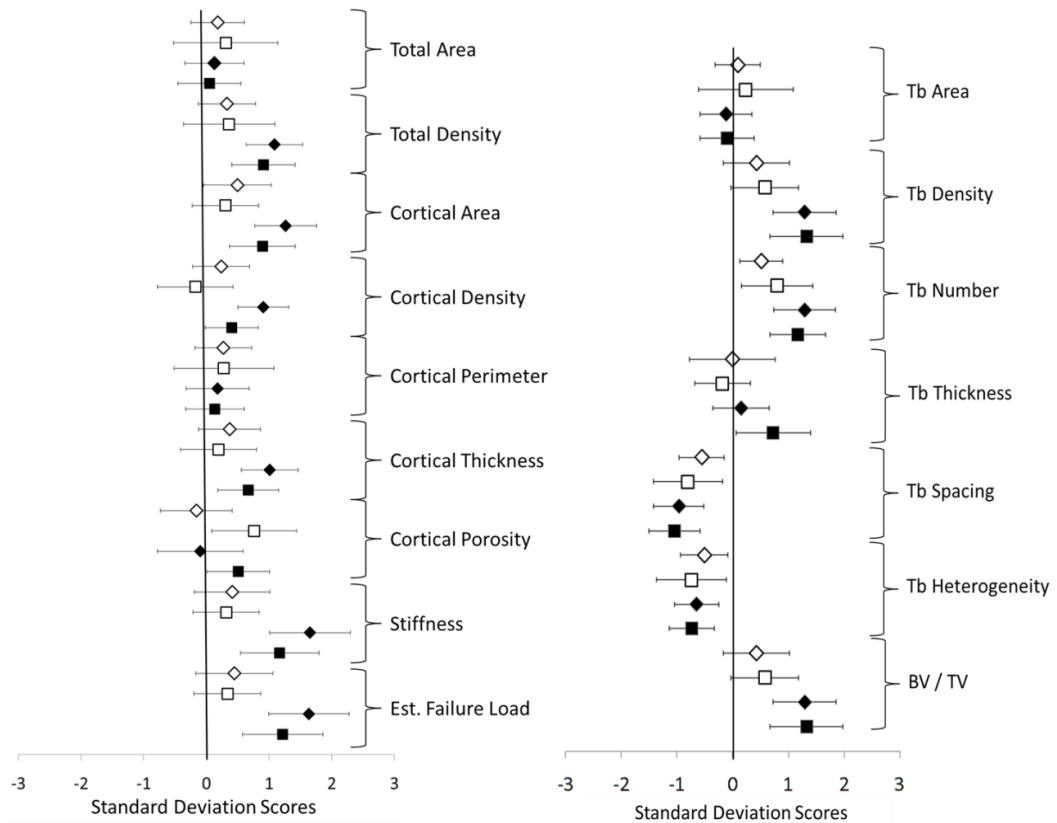
**Figure 35: Tibia vBMD, microarchitecture and strength of obese individuals as SD scores of the mean of the normal BMI group (mean (95% CI)).**

Reproduced with permission: Evans AL et al. Bone Density, Microstructure and Strength in Obese and Normal Weight Men and Women in Younger and Older Adulthood, 2014 Journal of Bone and Mineral Research, John Wiley and Sons. © 2014 American Society for Bone and Mineral Research



**Figure 36: Radius vBMD, microarchitecture and strength of obese individuals as SD scores of the mean of the normal BMI group (mean (95% CI))**

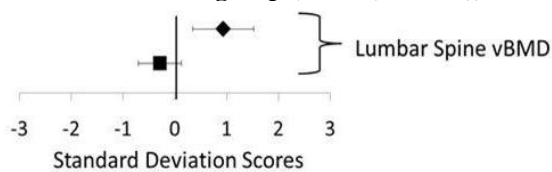
Reproduced with permission: Evans AL et al. Bone Density, Microstructure and Strength in Obese and Normal Weight Men and Women in Younger and Older Adulthood, 2014 Journal of Bone and Mineral Research, John Wiley and Sons. © 2014 American Society for Bone and Mineral Research



**Lumbar Spine vBMD by QCT**

Obese women had greater LS.vBMD than women with a normal BMI ( $p < 0.01$ ) (Figure 37). There was no effect of gender on LS.vBMD but there was an interaction between gender and the effect of obesity on LS.vBMD with a greater effect of obesity on vBMD in women (compared to a smaller *negative* effect in men) ( $p < 0.01$ ). In women, the difference in LS.vBMD between normal BMI and obese groups was significantly greater than in men ( $p < 0.01$ ).

**Figure 37: vBMD of obese individuals at the lumbar spine as SD scores of the mean of the normal BMI group (mean (95% CI))**



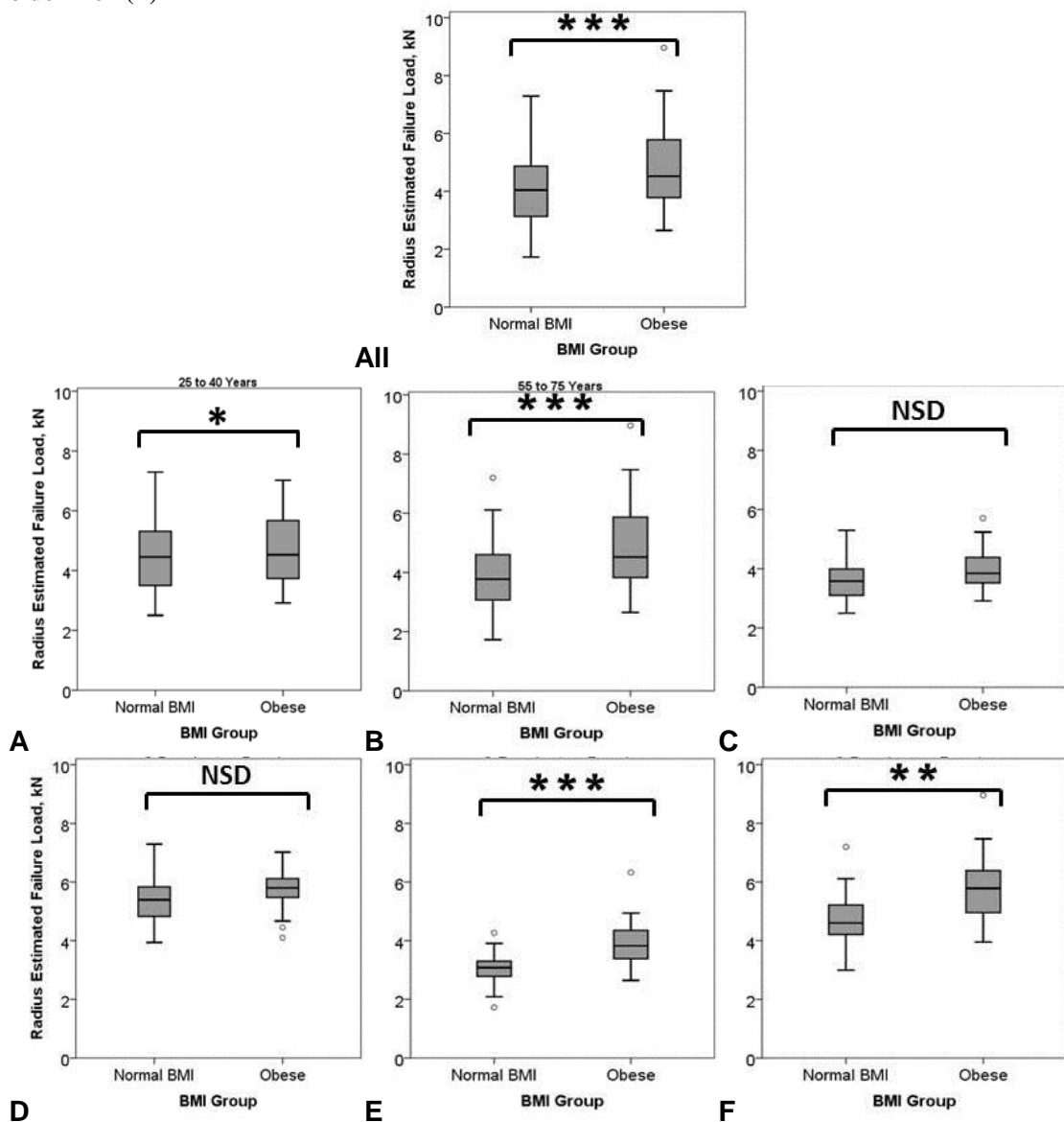
**Bone Strength in Obesity**

Obese adults had greater bone stiffness at the distal tibia ( $p < 0.01$  younger,  $p < 0.001$  older) (Figure 35) and at the distal radius in older adults ( $p < 0.001$ ) (Figure 36). In both



age groups, obesity was associated with greater bone strength determined by greater estimated failure load at the radius ( $p < 0.05$  younger,  $p < 0.001$  older) and tibia ( $p < 0.01$  younger,  $p < 0.001$  older) (Figure 35, Figure 36, Figure 38, Figure 39). There were no interactions between age or gender and the effect of obesity on bone stiffness or estimated failure load, in either age group. Therefore, although younger adults may exhibit less significant effects of obesity on bone density and microarchitecture at the distal radius, the differences observed appear to add up to an overall greater bone strength compared to normal BMI individuals.

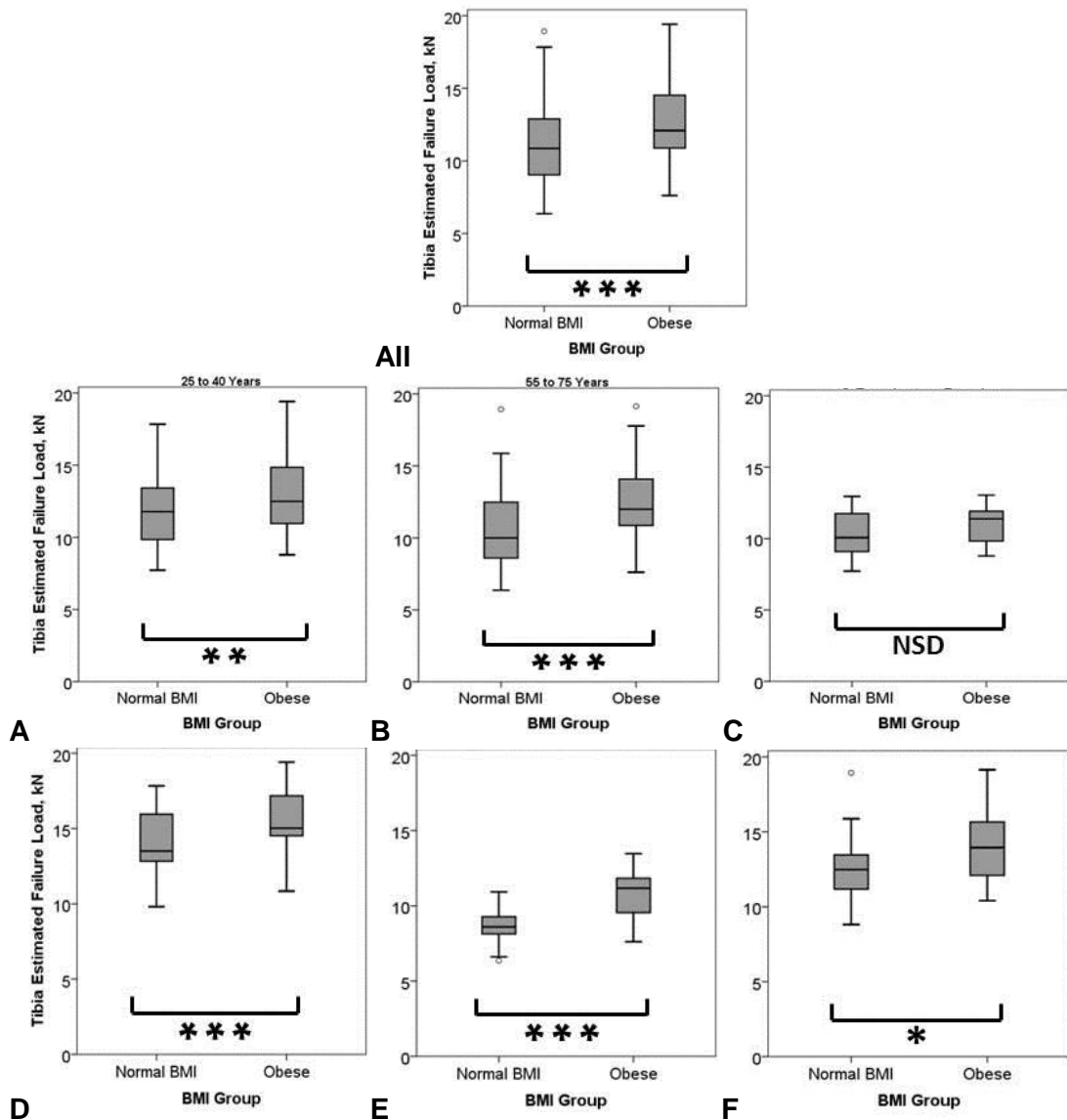
**Figure 38: Radius estimated failure load of normal BMI and obese individuals**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>**



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

**Figure 39: Tibia estimated failure load of normal BMI and obese individuals.**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E),**  
**older men (F)<sup>1,2</sup>**



### Are Greater BMD and Strength Sufficient for Greater Body Weight?

Although the results of this Chapter suggest that obese individuals may be at lower risk of fracture due to higher BMD and greater bone strength, whether the magnitude of the difference in bone density, structural parameters and bone strength ultimately conveys a protective effect given the magnitude of the difference in loading forces from high body weight is unclear. Others have suggested that BMD and bone structural outcomes might scale linearly with increasing body weight (261) and therefore linear scale comparisons

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

were made between body weight and bone outcomes. Mean percentage difference (as a percentage of the normal BMI group) between normal and obese individuals in body weight compared to mean percentage difference in aBMD, vBMD, bone microarchitectural properties and bone strength are presented in Table 7. Despite favourable bone microarchitecture in obesity, relative to the excess of weight bone parameters were lower than in normal BMI individuals. Percentage differences in bone outcomes normalised to body weight are presented in Table 7.

**Table 7: Mean percentage difference in skeletal outcomes between obese and normal BMI individuals before and after normalising for body weight**

	<b>Outcome</b>	<b>Mean % difference between obese and normal BMI adults</b>	<b>Mean % difference between obese and normal BMI adults normalised for weight</b>
	<b>Body weight</b>	<b>52.12</b>	
	WB aBMD	7.91	-26.37
	TH aBMD	19.84	-20.39
	LS aBMD	16.20	-22.72
	LS vBMD	12.87	-24.67
Radius	Tot.vBMD	19.93	-20.31
	Tb.vBMD	23.04	-18.37
	Ct.vBMD	5.47	-29.69
	Tot.Ar	4.65	-30.63
	Ct.Ar	35.53	-10.58
	Tb.Ar	3.39	-31.47
	Ct.Th	35.03	-10.65
	Ct Pm	2.31	-31.90
	Tb.N	17.60	-21.86
	Tb.Th	5.05	-29.93
	Tb.Sp	-13.66	-42.00
	Stiffness	21.42	-19.86
	Est. failure load	20.96	-20.21
	Tibia	Tot.vBMD	19.03
Tb vBMD		18.58	-21.28
Ct vBMD		4.52	-29.99
Tot.Ar		1.97	-32.04
Ct.Ar		30.36	-13.26
Tb.Ar		-0.82	-33.90
Ct.Th		30.40	-13.03
Ct Pm		0.73	-32.65
Tb.N		21.37	-19.52
Tb.Th		-1.33	-33.82
Tb.Sp		-15.65	-42.74
Stiffness		16.53	-22.64
Est. failure load		16.48	-22.70

## Summary of Results

A summary of the key messages of this Chapter is given in Table 8.

**Table 8: Summary of results Chapter 3**

**The effects of BMI and age, gender and BMI interactions on BMD, structure and strength**

Variable	Effect of BMI	Age/BMI Interaction	Gender/BMI Interaction
WB aBMD	↑ (older)	↑effect in older	-
TH aBMD	↑	-	-
LS aBMD	↑	↑effect in older	-
LS vBMD	↑(women)	NA	↑effect in women
Total vBMD	↑	-	-
Tb vBMD	↑	-	-
Ct vBMD	↑ (older)	↑effect in older	↑effect in women (older)
Ct TMD	↑ (older)	↑effect in older	↑effect in women (older)
Total area	-	-	-
Ct area	↑	↑effect in older	↑effect in women (older)
Tb area	-	-	-
Ct.Th	↑	-	↑effect in women (older)
Ct Pm	-	-	-
Ct Po	↓ (older)	↑effect in younger	-
Tb N	↑	-	-
Tb.Th	-	-	-
Tb.Sp	↓	-	-
Stiffness	↑	-	-
Est. failure load	↑	-	-
Total vBMD	↑ (older)	↑effect in older	-
Tb vBMD	↑	↑effect in older	-
Ct vBMD	↑ (older)	↑effect in older	↑effect in women (overall)
Ct TMD	↑ (older)	↑effect in older	↑effect in women (overall)
Total area	-	-	-
Ct area	↑	↑effect in older	-
Tb area	-	-	-
Ct.Th	↑ (older)	↑effect in older	-
Ct Pm	-	-	-
Ct Po	-	-	-
Tb N	↑	-	-
Tb Th	-	-	-
Tb Sp	↓	-	-
Stiffness	↑ (older)	-	-
Est. failure load	↑	-	-

## **Discussion**

The results presented in this Chapter are consistent with the existing literature which shows greater aBMD in obesity. BMI has previously been positively associated with aBMD in adults (15, 23, 43, 45, 48, 105, 257-261) and older adults (262, 263) of both genders. Body weight and BMI have been positively associated with aBMD of the lumbar spine (48, 258, 259, 261), femoral neck (48, 258), distal radius (261), proximal femur and leg (23, 48, 259, 260, 263). This is the first study to address relationships between obesity, bone microarchitecture and micro FEA derived bone strength in an individually-matched case control study of younger and older men and women.

Obese individuals have greater vBMD at the lumbar spine, distal tibia and, in older adults, at the distal radius. Greater vBMD in obese individuals is due to there being greater Tb.vBMD as a result of greater Tb.N and lower Tb.Sp without a difference in Tb.Th. The greater vBMD in older adults is also contributed to by greater Ct.vBMD as a result of greater Ct.TMD and lower Ct.Po. Obese individuals have greater Ct.Th at the distal tibia, and at the distal radius in older adults. There is no difference in bone size at the distal radius or tibia between obese and normal BMI individuals, indicating that there is no difference in periosteal apposition in obesity. As periosteal apposition typically occurs in response to greater loading conditions, it may be hypothesised that there are no effects of greater loading per se on bone in obesity and that the differences in bone density and structure observed are attributed to the altered hormonal milieu associated with greater adiposity. As there is no difference in bone size but greater Ct.Th, obese individuals may exhibit greater endosteal apposition.

As expected, there is greater aBMD and vBMD at the distal radius and distal tibia in younger adults than older adults. As shown by the interaction terms, there is a greater effect of obesity on LS and WB aBMD and distal radius vBMD in older adults. This indicates that high body weight may be of particular skeletal benefit in older age, possibly through the reduction of bone loss with age in obesity. Although obesity in younger adults appears to afford some clear skeletal benefits, the dominant effect of obesity on the skeleton appears to be to reduce bone loss in older adulthood. Whether older adults who have been obese since younger adulthood will have benefited from both mechanisms of obesity on the skeleton is unclear and warrants further study.

Sornay-Rendu et al. (2013) have reported an assessment of bone microarchitecture in obese postmenopausal women, compared with a non-obese control group (261). In agreement with the current study findings, they reported greater vBMD at the distal

radius and distal tibia in obesity. This greater vBMD resulted from greater Tb.vBMD, due to greater Tb.N and lower Tb.Sp, and greater Ct.vBMD, due to greater cortical area and Ct.Th and lower Ct porosity. Also in agreement with the results of this Chapter, the authors reported no difference in total area or Tb area in obesity (261). Sornay-Rendu et al. reported greater percentage differences in microarchitectural parameters at the distal tibia compared to the distal radius in the obese group versus the non-obese group, whereas in the present study the mean percentage differences in microarchitectural parameters were higher at the radius than the tibia (261).

Similar to our findings, BMI was positively associated with Tb.N and inversely associated with Tb.Sp in a study of young obese men (268).

A recent study examined the effect of FM and LM on HR-pQCT derived bone microarchitecture in obese individuals with metabolic syndrome (270). The study reported positive associations between LM and Tb.N and Tb.Sp at the radius, and vBMD, Tb.vBMD, BV/TV, Tb.N, Tb.Sp and Ct.Th at the distal tibia (270). No significant associations between FM and microarchitectural outcomes were observed (270). However as there was no control group, and metabolic syndrome may have effects on bone metabolism through effects on glucose metabolism in addition to the effects of greater adiposity, it is difficult to compare these findings directly with our results.

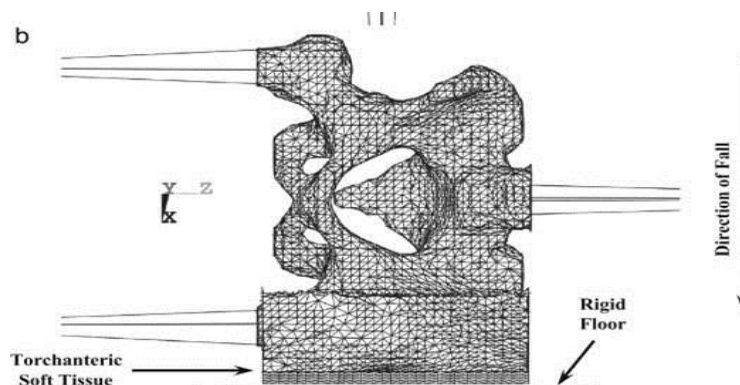
Fat distribution may affect associations between adiposity and bone microarchitecture (267-269, 273). Premenopausal women with greater central adiposity have been shown to have higher cortical porosity with lower trabecular bone volume, bone stiffness and bone formation (12). The inverse relationship between trunk fat and trabecular bone volume by bone biopsy remained significant after controlling for age and BMI (12).

As expected, aBMD at all sites measured and vBMD at the distal tibia were greater in men than in women. There was no difference in vBMD by gender at the distal radius or lumbar spine. In younger adults, gender did not alter the effect of obesity on bone microstructure at the distal radius or tibia. However, in older adults, the effect of obesity on LS.vBMD and Ct.vBMD and Ct.TMD at the radius and tibia was greater in older women than in men. Gender differences in the distribution of adiposity and/or endocrine profile could explain this difference and are an area for further investigation.

While patterns of bone density and structure are consistent between the distal tibia and distal radius in older adults, younger people show less significant effects of obesity at

the radius. However, despite this, bone strength is greater at the radius and the tibia in both older and younger obese individuals. The differences in bone density and microstructure observed are associated with greater estimated failure load at the distal radius and distal tibia. However, differences in bone parameters between obese individuals and those with a normal BMI show that bone adaptations are not commensurate to greater body weight. This was first shown by Sornay-Rendu et al., who suggested that the greater BMD observed in obesity does not appear to be sufficiently great in proportion to the greater body weight (261). Fractures often occur in obese individuals despite normal or high aBMD (49, 50). In particular, tibial vBMD and estimated failure load are greater in obese people, so lower bone density is not the cause of the increased risk of lower limb or ankle fracture observed in obesity (23, 44, 52, 55). Simple scaling such as that used by both in this Chapter and by Sornay-Rendu et al. may not be sophisticated enough to fully determine appropriate bone strength for body size. Further development of finite element models that account for body weight in the forces acting may provide a better understanding of fracture risk in obesity. Bone is more likely to adapt to the exposure to daily forces and loads, which differ from forces acting upon fall impact. Therefore it may not be surprising that obese individuals continue to fracture despite greater BMD than normal weight individuals.

Whilst findings of greater BMD at the hip and lumbar spine support reports of obesity being protective against hip and vertebral fracture, non-skeletal determinants of fracture may also contribute to fracture risk in obesity. Greater soft tissue thickness at the greater trochanter may be protective of hip fracture (73, 274, 275) and this has begun to be incorporated into novel finite element models simulating the risk of hip fracture from a sideways fall (Figure 40) (274, 275).



**Figure 40: Anterior view of the 3D FE model of the pelvis-femur-soft tissue complex by Majumder et al. (2008), (275). Permission to reproduce granted by Elsevier <http://www.elsevier.com> (license number 3345380469871)**

Non-skeletal determinants of fracture may also explain increased risk of lower limb and proximal humerus fracture in obesity. Obese individuals may be at greater risk of falls due to impaired muscular function, sarcopenic obesity, and/or fat infiltration of skeletal muscle (276-278). Different fall direction and fall forces may also increase fracture risk.

The cross-sectional design of this study must be acknowledged as a limitation. Determining adiposity by BMI may be considered a limitation as body fat distribution may be an important determinant of bone density and microarchitecture, but the obese group did have significantly higher fat mass than the normal BMI group, as shown in Figure 43. Whilst the possible confounding differences between obese and normal BMI individuals (age, body size, smoking and socioeconomic status) were controlled for as much as possible, any remaining differences may have affected the results. DXA and CT density measurements may be affected by the soft tissue thickness effects of increasing BMI, however this is unlikely to affect microarchitectural outcomes by HR-pQCT. The HR-pQCT finite element analysis model used in this study does not take into account individual loads upon falling and adopting this approach would increase the validity of the model. The current model simulates a direct compression force on the distal tibia which may not be the most suitable strength test for the prediction of ankle fracture which is affected by torsion forces and contribution of ligaments. Finally, the resolution of the HR-pQCT scanner may limit the reliability of measurements of cortical porosity and cortical pore diameter.

Results of the hip QCT scans were not presented in this Chapter. The analysis of these scans has proved troublesome, due to the need to 'trim' the soft tissue from the image without compromising the bone compartment of the image before the analysis is run. This has been difficult in the obese group where a greater thickness of soft tissue must be removed. Where soft tissue is left untrimmed, the density of the bone derived from the subsequent analysis is affected. Advice on analysing these scans has been sought, but the issue not yet resolved. Hip QCT scans will also be analysed using alternative in-house software which does not require soft tissue trimming and the results of the two analyses will be compared.

In conclusion, this Chapter has confirmed that aBMD is greater in obesity, and for the first time has demonstrated relationships between obesity, bone microarchitecture and micro finite element analysis derived bone strength in younger and older, men and women. Obese individuals have greater bone density than their normal BMI counterparts, at all sites measured. The greater density in trabecular bone is due to



greater trabecular number, but trabecular thickness does not differ between obese and normal weight people. Cortical thickness and cortical tissue mineral density were also higher in obese people, and cortical porosity was lower. Bone size at the radius and tibia did not differ between obese and normal BMI individuals. The magnitude of the difference in bone density observed between obese and normal BMI individuals using DXA was comparable to that observed using HR-pQCT, suggesting that greater bone density in obesity is a true biological effect and not solely an artefact of DXA imaging linked to greater soft tissue thickness. The greater differences between obese and normal BMI individuals in the older adults suggest obesity may be associated with reduced bone loss. Reduced bone loss might be associated with greater circulating E2 in obesity as a result of increased aromatisation of androgens which could confer a greater advantage in older adulthood where adipose tissue is the main source of E2. Alternative mechanisms might include age-related differences in the effects of mechanical loading, greater leptin, lower adiponectin and altered glucose metabolism on bone outcomes in obesity.

The identification of mechanisms responsible for greater bone density in obesity will improve our understanding of the pathophysiology of osteoporosis and could lead to new therapeutic targets. The results of this Chapter do not explain the greater risk of some fractures in obesity, as described in Chapter 1. At these sites, aBMD and/or vBMD may be poor indicators of fracture risk. Understanding why some fractures are increased in obesity may require more sophisticated models, which may lead to further insights into the site-specific mechanisms of fractures.

## **CHAPTER 4:**

# **POTENTIAL MECHANISMS OF ASSOCIATIONS BETWEEN OBESITY, BONE DENSITY AND BONE MICROSTRUCTURE**

## **CHAPTER 4: Potential Mechanisms of Associations Between Obesity, Bone Density and Bone Microstructure**

### **Background**

The work in Chapter 3 has identified differences in aBMD, vBMD, cortical and trabecular microarchitecture and bone strength between obese individuals and those with a normal BMI. What causes these differences in density and structure is unknown. The identification of potential mediators of the effect of obesity on bone outcomes might improve current understanding of why and how such differences arise. This might eventually enable the identification of potential therapeutic targets for the treatment of conditions characterised by low bone mass, such as osteoporosis.

There are multiple potential mechanisms which may contribute to positive associations between obesity and bone, some of which might also help to explain the greater differences in BMD and microstructural properties between obese and normal weight individuals in older adults, compared to younger adults. There are also differences in the hormonal milieu in obesity which might negatively affect bone density. As the overall effect of obesity on bone is most likely determined by the combination of positive and negative influences, the distribution of adipose tissue may be an important factor in the associations between biochemical influences and bone outcomes.

### **Mechanical Loading Effects of High Body Weight**

Previously it was considered that the protective effects of obesity on BMD were due to mechanical loading effects of high body weight on bone. Bone modelling occurs in response to changes in mechanical loading to maintain skeletal competence (7). Frost's mechanostat hypothesis proposed that strain magnitudes could stimulate bone modelling following tissue deformation in response to mechanical loading thresholds, so it is logical that in obesity, bone mass at weight bearing sites would be greater than in non-obese individuals (279). However, a single mechanostat does not control the entire skeleton; rather the strain magnitude required for skeletal maintenance is site-specific and varies within individual units of bone (87). This may contribute to understanding why obese adults have greater bone mass at load bearing sites which are not weight bearing, such as the radius. The magnitude of the difference in vBMD between obese and normal BMI individuals at the distal radius and distal tibia, was similar (Chapter 3). As the radius is a less frequently loaded, non-weight bearing bone, this might suggest that the strain magnitude required at the radius is lower than that of the tibia for a BMD difference of

equal magnitude. As the skeletal response to increased loading depends on the frequency, magnitude, rate and duration of the resulting strains, compared to the baseline loading conditions, duration of obesity may also be a factor. Young bone has been shown to be more capable of structural adaptations in response to mechanical loading than in bone in older age (8). Whether the contribution of mechanical loading to the effect of obesity on bone changes with age is unknown.

## **Body Composition**

### **Fat Mass and Lean Mass Contributions to BMD**

Both LM and FM contribute to body mass and affect bone density (257, 280-283). There is evidence both for (61, 284-287) and against (257, 280, 281) FM being the stronger determinant of bone density. While “passive loading from fat mass is less anabolic to bone than the active dynamic loads from muscle contraction”, the endocrine actions of FM make it a likely determinant of BMD irrespective of the loading effects high BMI (288). The relationship between LM, FM and BMD might be affected by menopausal status. In premenopausal women LM was the significant determinant of lumbar spine BMD, whereas in postmenopausal women, FM and both LM and FM were significant determinants of BMD (284, 289).

### **Body Fat Distribution**

Many studies have shown relationships between BMI or total body FM and BMD. However, total body FM is comprised of multiple fat depots, which not only differ in their anatomical location, but also in their production and expression of hormones, cytokines and inflammatory factors which can affect bone. The effect of adiposity on BMD may be highly dependent on the distribution and relative proportions of adipose tissue compartments, rather than overall adiposity.

### **Peripheral Adiposity**

Peripheral adiposity (PAT) describes subcutaneous fat deposited away from the trunk (290, 291). There is evidence to support there being a protective effect of PAT on aBMD at the whole body (292), FN (293), femur, spine and forearm (294), although others have found no association with arm aBMD (295, 296).

## Central Adiposity

### Abdominal Adiposity

Much of the available evidence supports a negative association between abdominal adiposity and BMD and bone microstructure. Total abdominal FM was inversely associated with vBMD at the lumbar spine and distal radius (267-269). At the distal radius, abdominal FM was associated with lower Tb.Th and lower bone strength, despite greater Tb.N lower Tb.Sp (268). In young women divided into tertiles based on trunk FM, although aBMD by DXA and vBMD by QCT were no different or lower in those with the highest trunk FM,  $\mu$ CT of transiliac biopsies taken from the same subjects showed inferior bone microarchitecture; with lower Tb.vol, Tb.N, Tb.Th, and stiffness and higher Ct.Po) with greater trunk FM (273). Markers of bone formation (bone formation rate, cancellous bone formation rate and mineralising surface) were also lower in the highest tertile of trunk FM (273).

### Subcutaneous Abdominal and Visceral Adiposity

Subcutaneous abdominal fat (SAT) is the fat beneath the skin of the abdomen but above the abdominal muscle wall, while visceral fat (VAT) is that which accumulates beneath the abdominal muscle wall, around the organs (Figure 41). Subcutaneous adipocytes are smaller, less metabolically active, less sensitive to lipolysis and less insulin-resistant than visceral adipocytes (297). Associations between SAT and BMD appear mostly positive (118, 119), SAT being associated with greater vBMD, Tb.vBMD and Ct.vBMD at the FN, LS, and radius and with trabecular microarchitecture at the radius (267). A recent study, however, reported no associations between L4 Tb.vBMD and SAT in obese women (269). Positive, though non-significant, correlations between SAT and Tb.N and an inverse correlation between SAT and Tb.Sp were reported in obese men (268).



**Figure 41: Location of subcutaneous and visceral fat compartments in relation to the abdominal wall on an abdominal CT image**

VAT contains more inflammatory cells and macrophages and is more vascularised and innervated than SAT (297). VAT is associated with an increased risk of obesity related complications such as insulin resistance, hyperlipidaemia and coronary heart disease

(117, 118). VAT appears to be negatively associated with BMD (118, 119, 268, 298, 299). In young obese men, inverse associations were observed between VAT and Tb.vBMD and Tb.Th, and VAT trended toward an inverse association with vBMD and Ct.Ar (268). Similarly, in young obese women, VAT was inversely associated with L4 Tb.vBMD, although VAT was not a significant predictor of Tb.vBMD (269). As a result, negative associations were found between VAT and stiffness and estimated failure load at the radius, with VAT explaining 17% and 20% of the variability in these variables, respectively (268).  $\mu$ CT of transiliac biopsies showed inverse associations between VAT and trabecular bone formation rate (273).

It has been suggested that a critical amount of VAT may be required before the deleterious effects on bone are observed (118). This seems logical when the proposed positive effects of SAT and PAT are considered; with the overall effect of high adiposity dependent on the distribution of FM throughout various compartments. Some studies have attempted to address the issue of conflicting effects of SAT and VAT compartmental effects on bone by using a VAT:SAT ratio. VAT:SAT ratio was negatively associated with FN and LS vBMD in postmenopausal women, supporting a negative effect of VAT on BMD (267).

### **Waist Circumference and Waist-to-Hip Ratio**

Waist circumference and WHR are surrogates for VAT and consequently have been inversely associated with WB, LS and TH aBMD (57, 300-303). Although other authors report positive correlations with BMD at the hip (304), spine (305) and radius (306), this may be reflective of the positive contribution of SAT to measurements of waist circumference and WHR.

### **Android and Gynoid Adiposity**

Android fat patterning, defined as increased fat deposition around the trunk, is associated with increased risk of metabolic disease compared to gynoid fat patterning, where the majority of FM is deposited in the gluteo-femoral region (290, 291, 307, 308). Android FM comprises both SAT and VAT and might have a negative effect on BMD, possibly depending on the ratio of VAT to SAT within the android region (283, 300). In postmenopausal women, android:gynoid ratio was negatively associated with total body, arm and leg BMD (283). As there was no association in premenopausal women, this relationship may depend on menopausal status (283).

## **Bone Marrow Fat**

Bone marrow fat content increases with age as fatty yellow marrow replaces functional, hematopoietic red marrow. Marrow fat has also been shown to accumulate in cortical pores (309). Adipose infiltration of the marrow compartment varies by skeletal site; beginning at peripheral sites and progressing into central sites (310). Whether the increase in bone marrow fat with age reflects marrow adipocyte hypertrophy or hyperplasia is unclear (311). Bone biopsies have shown marrow adipocyte volume to be directly associated with WBFM, trunk FM and SAT (273).

Marrow fat content is inversely associated with BMD (91, 220, 311, 312), cortical and trabecular microarchitecture (268, 313) and bone strength (91, 220, 221, 311). It is unclear whether marrow fat causes, or is a consequence of low bone density. Although “an increase in marrow fat may simply represent a compensation for trabecular thinning”, this suggests a rather passive role of marrow fat in a relationship driven by bone loss (311, 314). Marrow fat possesses characteristics of both white and brown fat, and the ability to adapt to changes in systemic energy metabolism, although with age, marrow fat exhibits mainly white fat characteristics and has a decreased ability to respond to changes in energy metabolism (314). These findings suggest that marrow fat accumulation is unlikely a passive filling of the trabecular compartment with age. It may be that inverse associations between marrow fat and BMD demonstrate a lineage switch of MSCs away from osteoblastogenesis and toward marrow adipogenesis (311). Preadipocyte factor-1 (Pref-1) may play a role in the regulation of MSC differentiation within the bone marrow, by preventing preadipocyte proliferation and differentiation to adipocytes (315, 316) and inhibiting MSCs differentiation along adipocyte and osteoblast lineages (317). Obese individuals have lower Pref-1 than those of normal weight (318). In anorexia, low BMD, high marrow adiposity and high pref-1 are observed compared controls (319, 320). As oestrogen treatment has been shown to decrease pref-1 while decreasing marrow fat and increasing BMD in anorexia, adipokines and sex hormones may affect the differentiation of MSCs and bone marrow fat content (319)

Current evidence indicates an inverse association between marrow adiposity and bone density and microstructure, although the mechanisms for such a relationship are unclear, but may involve low IGF-I expression by marrow fat (321). The marrow fat compartment might exert negative influences on BMD in obesity, similar to the hypothesised effects of VAT. Marrow fat and VAT were positively associated in obese women (321). Marrow fat accumulation in cortical pores might exacerbate cortical resorption and the trabecularisation of cortical bone.

## **Brown Fat**

Brown fat is predominantly found in neonates and rodents but also appears to be present in human adults (322-324). Whereas white fat mainly consists of lipid and has the principal role of energy storage, brown fat has a high mitochondrial content and is highly metabolically active; the main function of brown fat being to dissipate heat (325). Brown fat is located at the bottom of neck and in para-spinal and peritoneal areas, although depots change with age (322). Cold exposure can induce white fat to become brown fat and others have postulated this ability may hold anti-obesogenic therapeutic potential (326). Brown fat activity and volume are inversely associated with BMI, WBFM, percentage FM and VAT (324, 327). Brown fat is positively associated with inflammatory cytokines, but may exert a less pro-inflammatory effect than white fat (328). Brown fat volume was positively associated with BMD in young women (329, 330) and trended positively with femoral total and cortical cross sectional area (323). Misty mice, which have impaired brown fat function, have recently been shown to have lower aBMD and BMC, L5 Tb.vBMD, distal femur Tb.BV/TV, connectivity density and Tb.N, greater Tb.Sp, but no difference in Tb.Th compared to wild-type animals (331). Misty mice also have accelerated trabecular bone loss with age compared to wild type mice, due to impaired bone formation and increased bone resorption as shown by histomorphometry (331). The contribution of brown fat to associations between obesity, bone density and bone microstructure is unclear and warrants further research.

## **Gender Differences in Adiposity**

Men and women differ metabolically and hormonally, resulting in differences in the amount and distribution of body fat by gender. Women have greater total adiposity and greater gynoid adiposity than men, who have greater abdominal fat (291). Within the abdominal region, women have greater SAT and lower VAT than men and so a lower VAT:SAT ratio (291). However, obesity can induce altered fat patterning, with obese women exhibiting high VAT and obese men having large amounts of PAT (291).

Relationships between fat compartments and skeletal outcomes may vary by both age and gender. Ng et al. reported that after adjustment for age, correlations between adiposity and vBMD weakened (267). In premenopausal women, associations between adiposity and BMD were weak, while in young men, VAT was negatively associated with LS and radius total vBMD and FN Ct.vBMD (267). In older men, correlations were again weak (267). As associations were strongest in postmenopausal women, this might suggest that adiposity, namely SAT in that study, conveys a greater advantage in older



women, possibly related to increased aromatisation of androgens with high fat mass(267). While VAT:SAT ratio was negatively associated with FN vBMD in young men, the opposite was found in older men (267). Such differences highlight the need to study the effects of obesity on bone in both genders, pre- and post-menopause and by adipose depot.

### **Biochemical Mechanisms**

Although FM contributes to the loading effect of high body weight on the skeleton, adipose tissue is a highly active endocrine organ known to be involved in the production and release of more than fifty cytokines and other related molecules (90, 97), some of which can affect bone. Previous research has identified biochemical factors associated with adiposity which might positively affect bone, as well as other factors which may contribute to bone loss.

### **Positive Biochemical Mechanisms**

#### **Oestrogen**

Greater FM is associated with higher circulating oestrogen due to increased aromatisation of androgens. When oestrogen binds to its receptors on the osteoclast, suppression of RANKL-induced osteoclast differentiation occurs, accompanied by an increase in osteoclast apoptosis (332). Oestradiol (E2) is also able to increase OPG to further suppress osteoclastogenesis, while down regulating inflammatory cytokines (332). E2 maintains bone formation by inhibiting osteoblast apoptosis (332). The ability of E2 to lower resorption and maintain formation implicates it as a major protective determinant of BMD (332-334). After menopause, the conversion of androgens to E2 in adipose tissue is the main source of E2 in the body. This has led to the hypothesis that FM may be an important regulator of bone mass in older adults in particular (98).

In obese young adults, differences in the relationships between E2 and bone microstructural outcomes have been reported by gender, such that positive associations were observed for vBMD and Tb.Th in men (268), while there was no association between E2 and aBMD (334) or L4 Tb.vBMD (269) in premenopausal women. In obese women, studies have shown no association between fE2 and BMI, trunk or abdominal FM (269, 273).

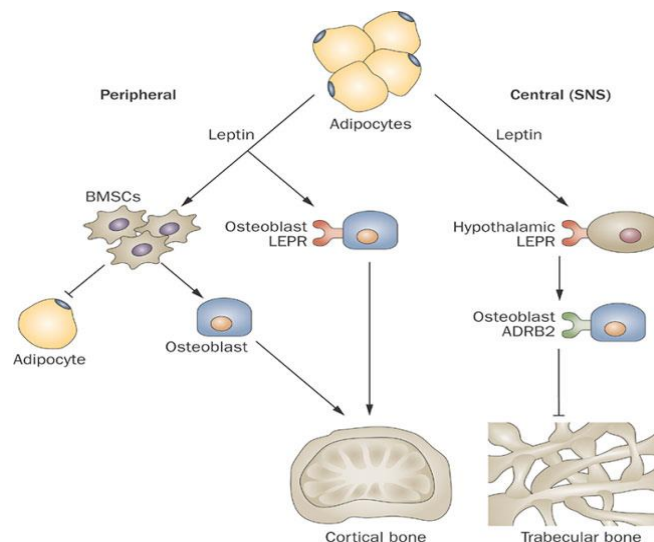
Aromatase expression is lower in VAT, indicating that body fat distribution might influence relationships between adiposity and BMD if E2 is found to be a mediator of the

relationship (100, 119). As E2 circulates bound to SHBG and albumin, both of which could be affected by adiposity (273), free or bioavailable E2 may be better predictors of BMD or bone turnover than total E2.

## Leptin

Leptin is a hormone product of the *OB* gene. Leptin is produced primarily by white adipose tissue and therefore highly associated with FM (335). Leptin plays an important role in regulating appetite and energy homeostasis via its actions on the hypothalamus (90, 97, 99). Circulating leptin concentrations are influenced by sex hormones, inflammatory cytokines and lipopolysaccharides (97, 98, 273).

*In vitro* work has shown anabolic bone responses to the direct actions of leptin on bone cells (96, 98). Leptin receptors (LEPR) are expressed on hemopoietic precursor cells, and leptin can act as a growth factor to enhance the development of hemopoietic precursor cells (98). Leptin can induce MSC proliferation and the *in vitro* administration of leptin influences MSC differentiation favouring the chondrocyte lineage over the adipocyte lineage (Figure 42) (98, 99). Inhibition of adipogenesis might be regulated via a negative feedback loop, possibly through the induction of the Mitogen-Activated Protein Kinase pathway, which also stimulates osteoblast differentiation (96, 98). As well as enhancing osteoblastic differentiation, leptin also regulates osteoclastogenesis by inhibiting RANKL (98).



**Figure 42: The effects of leptin on bone through peripheral and central actions.**

Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Rheumatology](#) (99), copyright 2009

Leptin knockout (ob/ob) mice have low femoral and whole body BMD compared to wild type mice, accompanied by greater bone marrow adiposity (336). Subcutaneous leptin treatment of ob/ob mice is associated with an increase in BMC and a decrease in the number of bone marrow adipocytes (337). A study of recombinant leptin therapy in a case of human congenital leptin deficiency showed increased bone mass, despite weight loss with treatment, indicating a positive effect of leptin on bone, independent of FM (338).

Contrary to the direct effects of leptin on bone, leptin appears to exert catabolic effects on bone when it acts via the central nervous system (CNS) (96, 98, 99). In mice, central leptin administration reduces appetite and is associated with reductions in caloric intake, body weight, adipocyte size and circulating insulin (96). Mouse models show that when leptin is administered via the CNS, leptin binds to hypothalamic LEPR, which activates the  $\beta$ 2-adrenoreceptor (ADRB2) on the osteoblast, decreasing osteoblast activity, increasing RANKL production and stimulating trabecular resorption (Figure 42) (90, 96, 99).

While “antiosteogenic leptin effects are often seen in axial elements but anabolic effects are observed in appendicular components” in mice (99, 336), the relationship between leptin and bone in humans is more consistent and positive in nature. Most studies report positive associations between leptin and aBMD (339-342). There may be differences in the relationship between leptin and bone by gender (334, 341, 342), possibly reflecting sexual dimorphism in adiposity, leptin production and/or interactions between leptin and sex hormones (343, 344). Some authors report a negative association (345-347) and others have reported no association (304, 335, 348-350) between leptin and BMD. Inconsistency between studies may be attributed to whether associations were adjusted for adiposity. After adjustment for BMI, significant associations between leptin and BMD are often weaker, reflecting the strong relationship between adiposity and leptin levels (342).

Positive associations between leptin and BMD in humans suggest that the positive peripheral effects of leptin likely outweigh the adverse central effects of leptin on bone in humans. This may partly be because leptin is primarily derived from extraosseous adipocytes, bone marrow adipocytes, chondrocytes and osteoblasts in peripheral locations and not from the CNS (96). As obesity is associated with reduced transfer of leptin across the blood-brain barrier, which might also suggest that peripheral actions of leptin on bone would predominate over any central effects in obese humans (96).

## **Adiponectin**

Adiponectin is secreted exclusively by adipocytes and is highly expressed by bone marrow adipocytes (335). Circulating adiponectin is lower in obesity and inversely associated with VAT (90, 96, 100, 273). Adiponectin is involved in glucose synthesis in the liver; increasing insulin sensitivity and reducing serum insulin (96). Adiponectin is also involved in fatty acid oxidation (97, 351) and has anti-inflammatory properties (342).

Adiponectin receptors are expressed by osteoblasts (96, 97). Upon binding to the adiponectin receptor, adiponectin may stimulate RANKL production and inhibit the production of OPG by osteoblasts, driving osteoclastogenesis (105, 106). Consequently inverse associations have been observed between adiponectin and aBMD across the skeleton, in men and women of various ages (101-104, 335, 340, 342, 348, 350, 352, 353). Adiponectin also appears to be inversely associated with total vBMD (352, 353), Tb.vBMD and Ct.vBMD (352). Others have found no significant association between adiponectin and BMD (105, 300, 347, 349, 354).

## **Bone Turnover**

Nutritional disturbances can lead to imbalances in bone formation and resorption and consequently affect bone remodelling and BMD. Having low LM or FM is associated with greater bone resorption and bone loss (263, 355). Weight loss is associated with increased bone turnover and consequently bone loss (356, 357). Therefore, high FM in obesity may be associated with lower bone turnover which is positively associated with BMD and might contribute favourable microarchitecture properties of bone. Previous studies have shown high body weight to be characterised by lower bone turnover, when assessed by markers of resorption (273, 358-361) and formation (269, 273, 358-361). Lower rates of bone loss in overweight women were proposed to be due to low bone turnover (362).

While there is some evidence to support inverse associations between bone resorption (by CTX) and BMI, WBFM and abdominal adiposity determined by trunk FM, SAT and VAT (273), associations between adiposity and bone formation (by PINP) in obesity are less clear and perhaps more dependent on the degree and distribution of adiposity. In women with a range of BMI values, PINP was negatively associated with BMI, total FM, trunk FM, SAT and VAT (273). However, in obese women, a positive association was observed between PINP and SAT, but there was no association with BMI, WBFM or PAT or VAT (269). While positive correlations were reported between PINP and Tb.vBMD at the level of L4, PINP was not a significant predictor of Tb.vBMD in young obese women

(269). Bone resorption was not measured in this study, but it may have been that bone resorption and bone turnover were lower better determinants of BMD than PINP alone.

There is insufficient evidence to conclude the effect of obesity on bone turnover, nor the effect of different fat compartments on bone turnover. Associations between adiposity and bone turnover are particularly unclear in populations other than postmenopausal women, although recent evidence suggests findings in older women may extend to premenopausal women (273). Characterising bone turnover in younger and older adults might improve current understanding of potential mechanisms driving larger differences between normal BMI and obese adults which could be mediated by reduced bone loss with advancing age.

### **Osteocalcin**

OC is a non-collagenous protein produced by mature, active osteoblasts (94, 99, 111), with OC transcription stimulated by RunX2 and vitamin D3 (363). OC plays a major role in energy metabolism by increasing energy expenditure and adipose, lipid and glucose metabolism (364, 365). OC regulates glucose metabolism by increasing the expression of adiponectin by adipocytes and insulin by  $\beta$ -cells (93, 99) and is positively associated with glucose tolerance and insulin sensitivity (366). Murine models have been studied to describe the mechanism by which OC regulates glucose metabolism. When leptin is secreted by adipocytes, sympathetic tone is stimulated which increases Esp expression in osteoblasts; reducing OC activity and altering insulin secretion from  $\beta$ -cells (99). As Esp is not expressed in humans, the applicability of this model in humans may be limited. (99).

There are two forms of OC; carboxylated and under carboxylated (uOC). uOC has a low affinity for hydroxyapatite and so to increase this affinity, glutamine acid residues of OC undergo post-translational  $\gamma$ -carboxylation into  $\gamma$ -carboxyglutamic acid in the presence of vitamin K (94, 99). The relationship between adiposity and OC and uOC may differ. Total OC has been implicated in the association between central adiposity and insulin resistance, with inverse associations between OC and BMI, waist circumference, trunk FM and VAT, which may be linked to elevated glucose and triglyceride levels in individuals with high central adiposity (112, 261, 273, 365, 367, 368). However, uOC was not associated with markers of adiposity (273).

## **Sclerostin**

Sclerostin is a glycoprotein secreted by mature osteocytes at the onset of mineralisation, which inhibits the Wnt pathway by binding with LRP; preventing bone formation (369). In mice, sclerostin knockout (SOST) animals exhibit increased bone formation and higher bone mass than wild type mice (370), whereas overexpression of the SOST gene is associated with osteopenia (371). In humans, mutations in the SOST gene cause sclerosteosis and van Buchem's disease, both of which are autosomal recessive conditions, characterised by high PINP and high bone mass amongst other clinical features (372).

Sclerostin expression is stimulated by mechanical loading, PTH and E2 (373) and levels correlate positively with adiposity (359, 369, 374). Associations between sclerostin and BTMs are inconsistent (359, 375, 376) but sclerostin may be inversely associated with OC (369). Sclerostin is positively associated with BMD (369, 374, 376-378) and may also be associated with favourable bone microarchitecture (378). Whether sclerostin has a causative role in the association between obesity and high BMD is unclear. It may be that high sclerostin with high BMI simply reflects the greater osteocyte number associated with greater bone mass.

## **Negative Biochemical Mechanisms**

High adiposity is also associated with several factors which may negatively affect BMD. Although the effect of high BMI on BMD and microstructure appears to be positive, it may be that certain adipose compartments exert negative effects on bone, such as VAT.

## **IGF-I**

IGF-I is a member of the IGF superfamily. Approximately 80% of circulating IGF-I is produced by the liver, although both skeletal and adipose tissues produce IGF-I (379). IGF-I mediates growth hormone (GH) concentrations through the GH/IGF axis, and regulates adipogenesis by impairing the activation of the Mitogen-Activated Protein Kinase pathway (379). Relationships between IGF-I and bone depend on whether the IGF-I is circulating or local; circulating IGF-I exerts mainly endocrine effects, whereas IGF-I produced by skeletal tissue exerts paracrine or autocrine effects associated with skeletal acquisition (379). IGF-I has positive effects on osteoblast activity and is positively associated with skeletal acquisition, periosteal apposition and bone mass (269, 273, 379-381). In obesity, IGF-1 is positively associated with Tb.vBMD (269), bone size and cortical geometry; predicting cortical area, perimeter and thickness (268, 382).

IGF-I is inversely associated with BMI (269). Recent studies have reported inverse associations between IGF-I and trunk FM and VAT, while no relationship was observed between IGF-I and SAT (269, 273). This suggests that low IGF-I with high BMI could be attributed to low IGF-I expression by the VAT compartment, rather than the overall abdominal compartment. Whether VAT exerts upregulates bone turnover with consequential negative effects on bone density and microstructural compartments through low IGF-I expression is unclear.

### **Pro-Inflammatory Cytokines**

Obesity is a low grade pro-inflammatory state associated with greater circulating pro-inflammatory cytokines including IL-1, IL-6, TNF- $\alpha$  and IL-8 (97). Pro-inflammatory cytokines have roles in the autoimmune and metabolic consequences of obesity, such as the regulation of glucose and lipid metabolism by IL-6 (97). IL-6 enhances the formation of C-reactive protein (CRP) by the liver which is inversely associated with adiponectin (107, 353). Inflammatory cytokines are positively associated with osteoclastogenesis (383, 384), osteoclast activity (108) and bone resorption (107, 385). IL-1, TNF- $\alpha$ , IL-6 (386, 387) and CRP (388, 389) have been inversely associated with total BMD, Tb.vBMD and trabecular microstructure (390). As a result, inflammatory cytokines are negatively associated with bone strength (391) and positively associated with fracture risk (391-393).

VAT has greater expression of pro-inflammatory cytokines than SAT, once again indicating that VAT may be a positive predictor of bone turnover and a negative predictor of BMD and bone microstructure (100, 117-119).

### **Vitamin D**

Serum 25OHD maintains calcium homeostasis by functioning to increase calcium absorption in the intestine. Low 25OHD is associated with higher PTH, increased bone turnover and bone loss (394). Low 25OHD is typically associated with low BMD (395, 396) and increased risk of fracture (394, 397-400). Associations between low 25OHD and the microarchitectural and mechanical properties of bone are unclear (268). As obese individuals have lower 25OHD, but higher bone density than normal BMI individuals, it is possible that vitamin D metabolism might be altered in obesity. Low 25OHD status in obesity may play a role the relationship between obesity and fracture risk through effects on physical performance and falls risk (401). This will be discussed

in more detail in Chapters 5 and 6. Potential causes and consequences of low 25OHD in obesity will be investigated in Chapter 6.

Although it is established that total 25OHD is inversely associated with adiposity (110, 396, 402-407), associations between 25OHD and different fat compartments are unclear (110, 404, 408, 409). It may be that the effect of low 25OHD on bone metabolism in obesity is outweighed by the greater positive influences exerted by adiposity on bone turnover and bone density.

### Summary

There is evidence to support complex crosstalk between bone, adipocytes, cytokines, osteokines and other biochemical factors in obesity. Endocrine influences and the mechanical loading effects of high body weight, might drive lower bone turnover and thus explain the greater BMD and favourable bone microarchitecture observed in obesity. A summary of the potential mechanisms by which obesity might influence bone density is given in Table 9.

**Table 9: Potential mediators of the effect of obesity on bone density**

<b>Positive Mechanisms</b>	<b>Negative Mechanisms</b>
↑Mechanical loading ↑WBFM ↑SAT ↑PAT ↑Gynoid FM	↑VAT, ↑VAT:SAT ↑Trunk FM ↑Android FM ↑Bone Marrow fat ↓Brown fat
↓Bone turnover (↓resorption, ↓formation) ↑Osteocalcin	
↑Leptin ↓Adiponectin ↑E2 ↑Glucose, ↑Insulin ↑Sclerostin	↑Inflammatory cytokines ↓IGF-I ↓25OHD

The varying metabolic profiles of distinct adipose compartments are likely to contribute to differing effects of adiposity dependent on body fat distribution. While high total body adiposity may be associated with the down-regulation of some biochemical factors which are positively associated with bone metabolism (e.g. IGF-I) and the up-regulation of factors which are negatively associated with bone metabolism (e.g. inflammatory



cytokines), the overall effect of obesity on bone turnover and density will likely result from the balance of favourable and unfavourable influences on bone tissue.

Identification of potential mediators of the effects of adiposity on bone turnover, bone density and microstructure is required to better understand how adiposity exerts positive effects on bone density and microstructure. In the short term, this knowledge may be used to focus further investigations into the effects of adiposity on bone. Longer term, identification of novel therapeutic targets for use in conditions characterised by low or deteriorating BMD, such as osteoporosis may be possible.

### **Research Questions, Aims and Hypotheses**

Research questions:

1. Do certain fat compartments, such as peripheral, subcutaneous abdominal or visceral fat, exert greater effects on BMD, bone geometry, bone microarchitecture and bone strength than other fat compartments?
2. What are the potential mediators of the effects exerted by obesity on BMD, bone geometry, bone microarchitecture and bone strength?

Aims:

1. To ascertain which fat compartment is most strongly associated with BMD, geometry and microarchitecture, in normal BMI and obese individuals.
2. To test associations between the proposed key fat compartment, biochemical markers of bone turnover and hormones to identify potential mediators of associations between obesity, BMD, bone microarchitecture and bone strength.

Hypotheses:

1. Bone turnover is decreased in obesity and as such, there is a significant difference in BTMs between obese and normal BMI individuals.
2. Peripheral, subcutaneous abdominal and gynoid fat exert a positive effect on BMD, bone geometry and bone microarchitecture.
3. Visceral, android and trunk fat exert a negative effect on BMD, bone geometry and bone microarchitecture.
4. The positive effect of peripheral, subcutaneous abdominal and gynoid fat on bone turnover will be greater than the negative effect of visceral, android and trunk fat; such that there is an inverse association between adiposity and bone turnover.

5. Associations between peripheral, subcutaneous abdominal and gynoid fat and BMD, bone geometry and bone microarchitecture are mediated by higher E2 and leptin and lower adiponectin, which lower bone resorption.
6. There is a greater difference in E2 between normal BMI and obese older women than between normal BMI and obese younger women and this is associated with the greater difference in BMD between normal BMI and obese older adults, compared to that of younger adults.
7. Associations between visceral, android and trunk fat and BMD, bone geometry and bone microarchitecture are mediated by higher inflammatory cytokines and lower IGF-I.

### **Methods**

Whole body FM, trunk FM, android FM, gynoid FM and FM on the arms and legs were determined by whole body DXA (Discovery A, Hologic Inc., Bedford, MA, USA). Appendicular FM was calculated as the sum of the FM on the arms and legs.

Waist circumference (cm) was measured at the midpoint between the lowest rib and the uppermost aspect of the iliac crest. Hip circumference (cm) was measured at the level of the greatest protrusion of the buttocks. Waist-to-hip ratio was calculated as a marker of central adiposity. Triceps skinfold thickness (mm) was measured using a Harpenden skinfold calliper (Baty International, West Sussex, UK) as a marker of PAT.

SAT and VAT was determined by five-slice CT, taken at the mid-level of the L3 vertebra (LightSpeed VCT-XT, General Electric Healthcare, Buckinghamshire, UK).

Creatinine, calcium, albumin, lipid profile (Cobas c701 auto-analyser, Roche Diagnostics, Mannheim, Germany) and glucose (Cobas c702 auto-analyser, Roche Diagnostics, Mannheim, Germany) were measured from the screening blood samples in the Clinical Chemistry Laboratory, Sheffield Teaching Hospitals .

CTX, PINP and OC were determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). BAP was determined by automated CLIA (IDS-iSYS Immunodiagnostic Systems, Boldon, UK). OPG was measured by manual ELISA (Biovendor, GmbH, Heidelberg, Germany). Sclerostin was measured by manual ELISA (Biomedica, Vienna, Austria).

Leptin and adiponectin were determined by manual ELISA (Human Leptin Quantikine ELISA, Human Total Adiponectin Quantikine ELISA, R&D Systems, UK).

PTH, 25OHD, albumin and insulin were determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). IGF-I was measured by automated CLIA (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK).

Insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR):

$$(\text{Fasting serum glucose (mmol/L)} * \text{fasting serum insulin (}\mu\text{U/ml)} / 22.5)$$

SHBG was measured by automated ECLIA (Cobas e602, auto-analyser, Roche Diagnostics, Mannheim, Germany). Total E2 was determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). Free E2 (fE2) was determined as:

$$fE2 \text{ (mol/L)} = (-b + \sqrt{(b^2 - 4ac)}) / 2a$$

Where:

$N = 1 + \text{affinity constant of albumin for E2} \times \text{albumin}$

$a = N * \text{affinity constant of SHBG for E2}$

$b = N + \text{affinity constant of SHBG for E2} * (\text{SHBG} - \text{Total E2})$

$c = -\text{Total E2}$

Affinity constant of albumin for E2 =  $4.21 \times 10^4$  L/mol

Affinity constant of SHBG for E2 =  $3.14 \times 10^8$  L/mol

BioE2 was calculated as:

$$\text{bioE2 (mol/L)} = (1 + \text{affinity constant of albumin for E2} \times \text{albumin}) * fE2$$

HsCRP was measured by automated nephelometry (BNII System, Siemens, Siemens Healthcare Diagnostics, Surrey, UK). IL-6 was measured by automated immunoassay (Cobas e601 auto-analyser, Roche Diagnostics, Mannheim, Germany).

Kidney function was estimated from calculation of estimated glomerular filtration rate (eGFR), using the MDRD equation:

$$eGFR = 175 * (\text{serum creatinine (}\mu\text{mol/L)} * 0.0113)^{-1.154} * \text{age}^{-0.203} [* 0.742 \text{ if female}]$$

## **Statistical Analysis**

Coupling Index was calculated as a SD score, by subtracting the mean of the age and gender matched normal weight group from each result and subsequently dividing by the SD of the normal weight group. Mean SD scores were then calculated. All variables were assessed for normality and log transformed where necessary. As paired t-tests were to be used to determine significant differences between normal BMI and obese groups, the absolute differences between the matched pairs were assessed for normality. Where the difference between the pairs was skewed, the raw data was log transformed and the differences re-evaluated for normality.

Paired samples t-tests were used to determine significant differences between normal BMI and obese groups, for the entire sample, by age group and by age and gender. Where transformed values remained non-normal, the Wilcoxon Signed Rank test was used. Univariate general linear models were used to identify whether age group, gender and BMI had an effect on FM or biochemical outcomes and to identify interactions between age or gender and the effect of BMI on FM or biochemical outcomes.

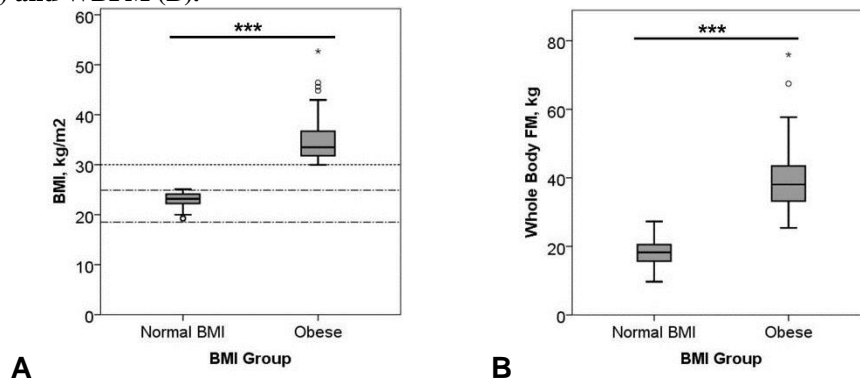
Pearson correlation coefficient was used to determine associations between FM and biochemical variables, FM and skeletal outcomes and biochemistry and skeletal outcomes. Spearman's rank correlation coefficient was used where the sample distribution remained non-normal. Multiple linear regression adjusting for age and gender was used to determine whether FM or LM predicted bone density and microstructural outcomes. WBLM and each FM variable separately were entered as dependent variables to avoid collinearity. Multiple linear regression models were constructed to determine the influence of different fat compartments on BMD by testing the working hypotheses. BMD or microstructural variables were entered as the independent variable and age, gender, and pairs of contrasting fat compartments (SAT and VAT, trunk FM and AFM, android FM and gynoid FM) were entered as dependent variables.

To determine the influence of different fat compartments on cortical and trabecular outcomes, multiple linear regression with microstructural outcome as the independent variable and age, gender, and pairs of contrasting fat compartments (SAT and VAT, trunk FM and AFM, and android FM and gynoid FM) as dependent variables was performed. Analysis was performed using IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.). Significance was accepted when  $p < 0.05$ .

## Results

All measures of adiposity except VAT:SAT ratio, were higher in the obese individuals than individuals with a normal BMI (all  $p < 0.001$ ) (Figure 43, Figure 44, Figure 45). All measures of adiposity (except VAT:SAT ratio) were higher in obese individuals when assessed by age group (all  $p < 0.001$ ) or by age and gender (all  $p < 0.001$ , except WHR in older women where  $p < 0.05$ ). There was no effect of age on BMI or WBFM. There was no effect of gender on BMI, but women had a higher WBFM than men ( $p < 0.005$ ).

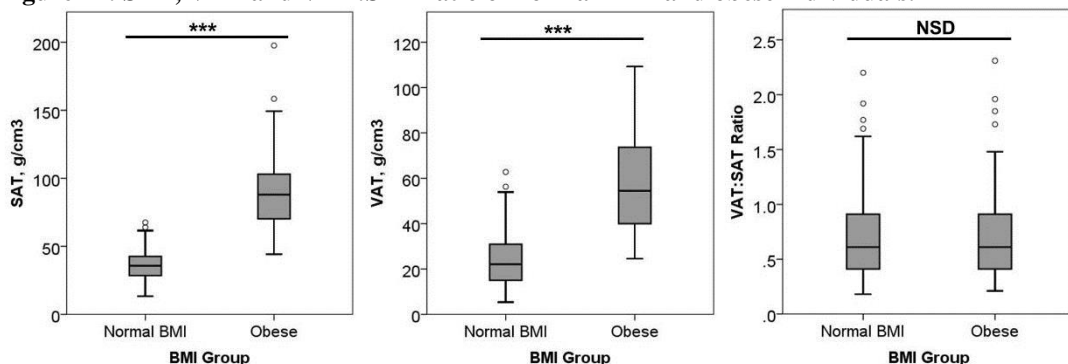
**Figure 43: Whole body adiposity of normal BMI and obese individuals by BMI (A) and WBFM (B).**<sup>1,2</sup>



## Central Adiposity

Older adults had greater trunk FM ( $p < 0.01$ ), android FM ( $p < 0.01$ ), VAT ( $p < 0.001$ ), VAT:SAT ratio ( $p < 0.001$ ), waist circumference ( $p < 0.001$ ) and WHR ( $p < 0.001$ ) than younger adults. Younger adults had greater gynoid FM than older adults ( $p < 0.001$ ). There was no effect of age on SAT or hip circumference. Age associations were unchanged by adjustment for WBFM. There was a greater effect of BMI on SAT and VAT in younger adults than older adults (SAT:  $p < 0.01$ , VAT:  $p < 0.05$ ).

**Figure 44: SAT, VAT and VAT:SAT ratio of normal BMI and obese individuals.**<sup>1,2</sup>

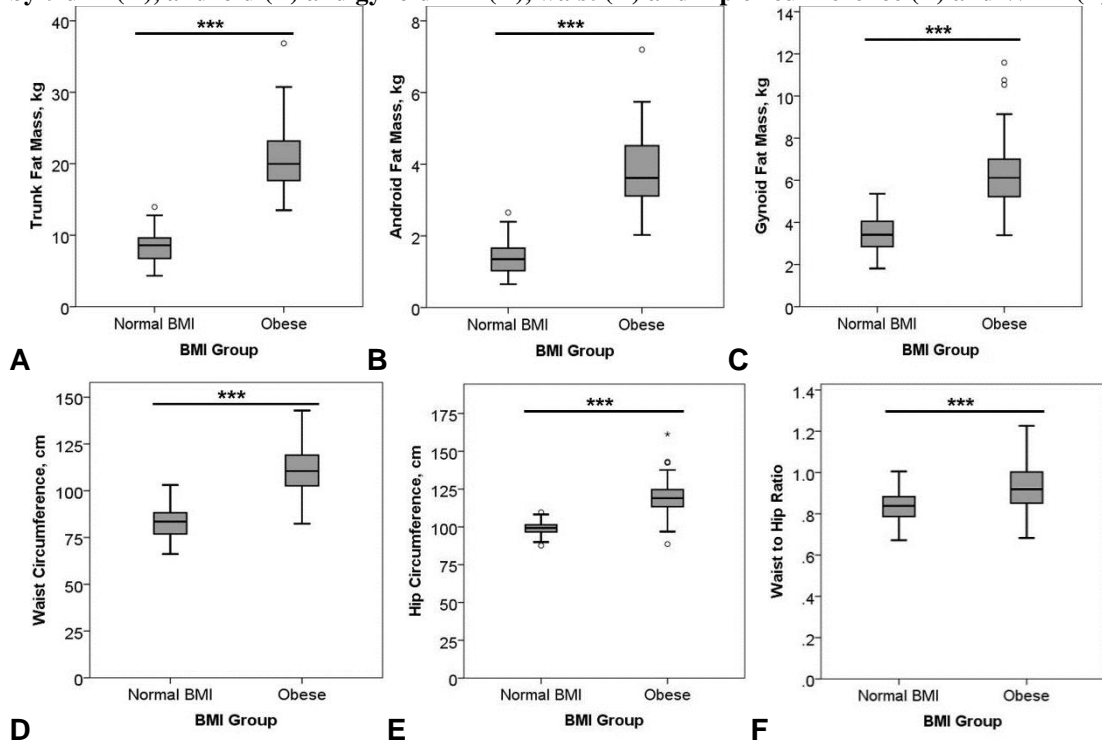


<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

Women had greater trunk FM ( $p < 0.01$ ), gynoid FM ( $p < 0.001$ ), SAT ( $p < 0.001$ ) and hip circumference ( $p < 0.01$ ). Men had greater VAT, VAT:SAT ratio, waist circumference and WHR (all  $p < 0.001$ ). There was no effect of gender on android FM. There was a greater effect of BMI on hip circumference in women than men ( $p < 0.01$ ). There was a greater effect of BMI on WHR in men (univariate GLM  $p < 0.05$ ).

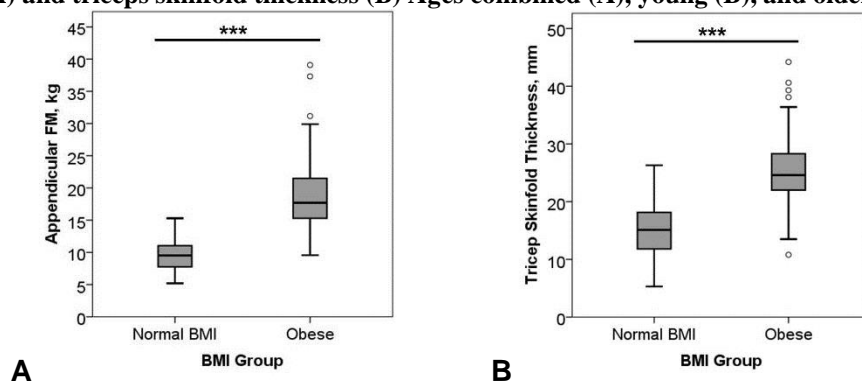
**Figure 45: Central adiposity of normal BMI and obese individuals by trunk (A), android (B) and gynoid FM (D), waist (D) and hip circumference (E) and WHR (F).<sup>1,2</sup>**



### Peripheral Adiposity

There was no effect of age on AFM or triceps skinfold thickness. Women had greater AFM and triceps skinfold thickness than men (both  $p < 0.001$ ) (Figure 46).

**Figure 46: Peripheral adiposity of normal BMI and obese individuals by AFM (A) and triceps skinfold thickness (B) Ages combined (A), young (B), and older (C).<sup>1,2</sup>**



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Are Associations Between Obesity and Bone Due to FM or LM?

A summary of the effects of WBLM and WBFM is given in Table 10.

**aBMD** at all sites was positively predicted by WBLM but not by FM variables.

As there were interactions between age and the effect of FM variables on aBMD, models were re-run by age group:

In young adults:

- WB aBMD was positively predicted by all FM variables and not by WBLM
- TH aBMD remained predicted by WBLM not FM
- LS aBMD remained predicted by WBLM not FM

In older adults:

- WB aBMD remained predicted by WBLM and not by FM variables
- TH aBMD was predicted by all FM variables and not by WBLM
- LS aBMD remained predicted by WBLM not FM, except for android FM and LM

**Tibia vBMD** was positively predicted by:

- WBFM (adjusted R square .291, beta .302  $p < 0.001$ )
- AFM (adjusted R square .283, beta .240,  $p < 0.05$ )
- Trunk FM (adjusted R square .311, beta .331,  $p < 0.001$ )
- Android FM (adjusted R square .322, beta .349,  $p < 0.001$ )
- Gynoid FM (adjusted R square .291, beta .279,  $p < 0.01$ )
- SAT (adjusted R square .315, beta .291,  $p < 0.01$ )

In each of these models, tibia vBMD was not predicted by WBLM.

Tibia vBMD was predicted by both WBLM and VAT (adjusted R square .293, beta WBLM .315,  $p < 0.01$ , beta VAT .213,  $p < 0.05$ ).

**Radius vBMD** was positively predicted by:

- WBFM (adjusted R square .133, beta .136,  $p < 0.001$ )
- AFM (adjusted R square .121, beta .297,  $p < 0.01$ )
- Triceps skinfold thickness (adjusted R square .116, beta .223,  $p < 0.01$ )
- Trunk FM (adjusted R square .139, beta .335,  $p < 0.01$ )
- Android FM (adjusted R square .146, beta .342,  $p < 0.001$ )
- Gynoid FM (adjusted R square .128, beta .322,  $p < 0.01$ )
- SAT (adjusted R square .140, beta .291,  $p < 0.01$ )
- VAT (adjusted R square .123, beta .230,  $p < 0.05$ )

In each of these models, radius vBMD was not predicted by WBLM.

As there were interactions between age and the effect of FM variables on distal radius vBMD, models were re-run by age group. Except for android and gynoid FM in older adults, where neither FM nor LM predicted vBMD, FM remained the dominant predictor of vBMD

**Lumbar spine vBMD** was positively predicted by both WBLM and FM indices, with the exception of android, gynoid and AFM where only LM predicted LS.vBMD.

**Table 10: LM predicts aBMD at central sites, while FM predicts peripheral vBMD**

Dependent variable	Independent variables	Beta	p value	Model p value	R square	Adj. R square	Age Interaction
WB aBMD	<b>WBLM</b> WBFM	<b>.824</b> -.073	<b>&lt;.001</b> .366	<.001	.496	.485	WBFM* Trunk FM* SAT** AFM* Android FM* Gynoid FM*
TH aBMD	<b>WBLM</b> WBFM	<b>.777</b> .120	<b>&lt;.001</b> .134	<.001	.515	.505	-
LS aBMD	<b>WBLM</b> WBFM	<b>.654</b> .092	<b>&lt;.001</b> .333	<.001	.303	.289	WBLM** VAT* Android FM*
Radius vBMD	WBLM <b>WBFM</b>	-.090 <b>.336</b>	.487 <b>&lt;.001</b>	<.001	.147	.133	WBFM** Trunk FM** AFM** Android FM** Gynoid FM**
Tibia vBMD	WBLM <b>WBFM</b>	.039 .302	.741 <b>&lt;.001</b>	<.001	.302	.291	-
LS vBMD	<b>WBLM</b> <b>WBFM</b>	<b>.517</b> <b>-.304</b>	<b>&lt;.05</b> <b>&lt;.05</b>	.088	.056	.031	

\*p<0.05, \*\*p<0.01, + when entered with WBFM, AFM, triceps skinfold or gynoid FM



### Which Fat Compartment Has the Greatest Influence on BMD?

Results of the linear regression models to determine which FM variable has a dominant effect on BMD are presented in Table 11, Table 12 and Table 13. aBMD was predicted by SAT, gynoid FM and AFM, whereas vBMD was predicted by SAT, android FM and trunk FM.

**Table 11: Prediction of BMD by SAT and VAT**

Dependent variable	Independent variables	Beta	p value	Model p value	R square	Adjusted R square
WB aBMD	<b>SAT</b>	<b>.356</b>	<b>&lt;.001</b>	<.001	.356	.341
	VAT	-.026	.815			
TH aBMD	<b>SAT</b>	<b>.418</b>	<b>&lt;.001</b>	<.001	.351	.336
	VAT	.096	.387			
LS aBMD	<b>SAT</b>	<b>.328</b>	<b>.004</b>	<.001	.173	.155
	VAT	.088	.480			
Radius vBMD	<b>SAT</b>	<b>.288</b>	<b>.012</b>	<.001	.158	.139
	VAT	.079	.530			
Tibia vBMD	<b>SAT</b>	<b>.342</b>	<b>.001</b>	<.001	.321	.306
	VAT	.058	.607			

**Table 12: Prediction of BMD by gynoid FM and android FM**

Dependent variable	Independent variables	Beta	p value	Model p value	R square	Adjusted R square
WB aBMD	Android FM	-.068	.588	<.001	.374	.361
	<b>Gynoid FM</b>	<b>.474</b>	<b>&lt;.001</b>			
TH aBMD	Android FM	.230	.061	<.001	.398	.386
	<b>Gynoid FM</b>	<b>.341</b>	<b>.010</b>			
LS aBMD	Android FM	.065	.636	<.001	.241	.225
	<b>Gynoid FM</b>	<b>.433</b>	<b>.004</b>			
Radius vBMD	<b>Android FM</b>	<b>.299</b>	<b>.039</b>	<.001	.164	.147
	Gynoid FM	.059	.704			
Tibia vBMD	<b>Android FM</b>	<b>.407</b>	<b>.002</b>	<.001	.334	.320
	Gynoid FM	-.015	.915			

**Table 13: Prediction of BMD by AFM and trunk FM**

Dependent variable	Independent variables	Beta	p value	Model p value	R square	Adjusted R square
WB aBMD	<b>AFM</b>	<b>.553</b>	<b>&lt;.001</b>	<.001	.383	.370
	Trunk FM	-.140	.303			
TH aBMD	<b>AFM</b>	<b>.300</b>	<b>.036</b>	<.001	.396	.384
	Trunk FM	.263	.050			
LS aBMD	AFM	.279	.087	<.001	.218	.202
	Trunk FM	.188	.217			
Radius vBMD	AFM	.015	.929	<.001	.156	.139
	<b>Trunk FM</b>	<b>.326</b>	<b>.041</b>			
Tibia vBMD	AFM	-.083	.582	<.001	.324	.310
	<b>Trunk FM</b>	<b>.452</b>	<b>.002</b>			

### Which Fat Compartment Has the Greatest Influence on Bone Microstructure?

Results of the linear regression models to determine which FM variable has a dominant effect on bone microstructure are presented in Table 14.

Bone microarchitectural outcomes (Ct.vBMD, Ct.Th, Tb.vBMD and Tb.N at the distal radius and Ct.Th, Tb.vBMD and Tb.N at the distal tibia) were best predicted by SAT (Table 14). Although bone microarchitectural outcomes were also predicted by android FM and trunk FM (Table 14), both android FM and trunk FM incorporate SAT and may therefore be acting through the dominant effect of SAT.

Due to the highly collinear nature of SAT, android FM and trunk FM it was not possible to run accurate paired models to determine the dominant FM from these variables. SAT was therefore chosen as the fat depot with the greatest influence on bone microstructure for subsequent analysis.

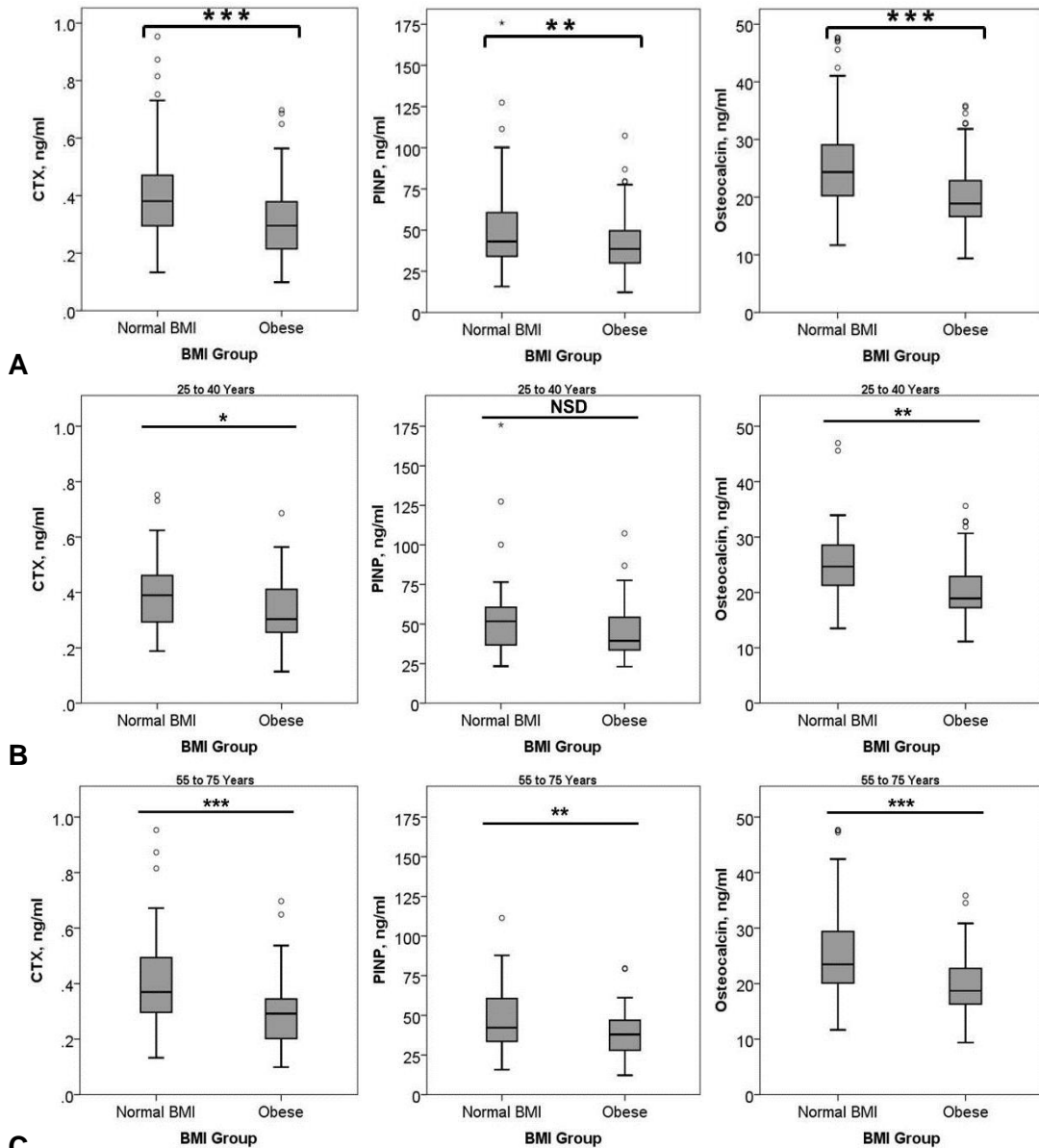
**Table 14: Adipose compartment predictors of bone microstructure**

	Dependent variable	Significant predictors	Beta	p value	Model p value	R square	Adjusted R square	
<b>Radius</b>	Ct.vBMD	SAT	.245	.035	<.001	.138	.119	
	Ct.Ar	SAT	.350	.001	<.001	.333	.318	
	Ct.Th	SAT	.303	.009	<.001	.144	.125	
	Ct.Pm		Gynoid FM	.331	.003	<.001	.578	.570
			Android FM	-.214	.038	<.001	.578	.570
			AFM	-.137	.225	<.001	.571	.562
	Tb.vBMD		SAT	.253	.014	<.001	.322	.307
			Android FM	.258	.047	<.001	.326	.312
			Trunk FM	.350	.015	<.001	.313	.299
	Tb.N		SAT	.428	<.001	<.001	.257	.241
			Gynoid FM	.309	.032	<.001	.284	.270
	<b>Tibia</b>	Ct.vBMD	-	-	-	-	-	-
Ct.Ar		SAT	.302	.001	<.001	.483	.471	
Ct.Th			SAT	.290	.004	<.001	.353	.338
			Android FM	.332	.009	<.001	.371	.358
			Trunk FM	.305	.027	<.001	.366	.353
Ct.Pm			Gynoid FM	-.349	<.001	<.001	.553	.544
			Android FM	.454	.001	<.001	.553	.544
			Trunk FM	-.321	.006	<.001	.546	.537
			AFM	.435	.001	<.001	.546	.537
Tb.vBMD			SAT	.331	.002	<.001	.242	.225
			Trunk FM	.344	.024	<.001	.232	.216
Tb.N			SAT	.604	<.001	<.001	.267	.250
			Gynoid FM	.546	<.001	<.001	.307	.292
			AFM	.624	<.001	<.001	.323	.309

## Biochemistry

### Bone Turnover

Obese individuals had lower bone turnover, with lower CTX ( $p < 0.001$ ), PINP ( $p < 0.01$ ) and OC ( $p < 0.001$ ) compared to individuals with a normal BMI. Univariate GLM showed a significant effect of BMI on CTX ( $p < 0.001$ ), PINP ( $p < 0.01$ ) and OC ( $p < 0.001$ ).

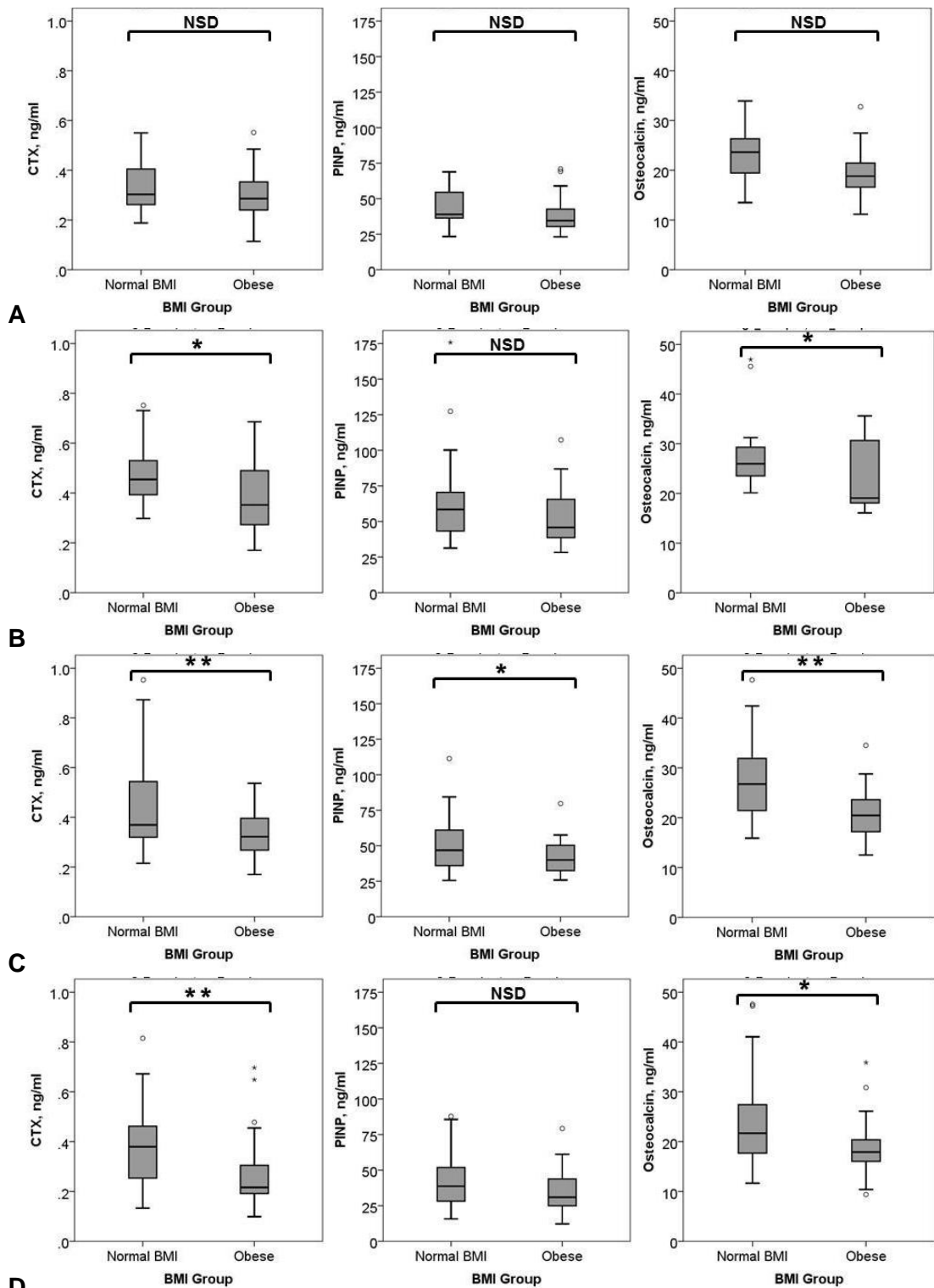


**Figure 47: BTMs in normal BMI and obese individuals** CTX (left), PINP (centre) and OC (right) by ages combined (A), young (B) and older (C) ).<sup>1,2</sup>

There was no effect of age on CTX or OC. Younger adults had higher PINP than older adults ( $p < 0.01$ ). There was no effect of gender or interactions between age or gender and the effect of BMI on CTX, PINP or OC.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference



**D**  
**Figure 48: BTMs in normal BMI and obese individuals by age and gender**  
**CTX (left), PINP (centre) and OC (right) in young women (A), young men (B), older women (C),**  
**older men (D) ).<sup>1,2</sup>**

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

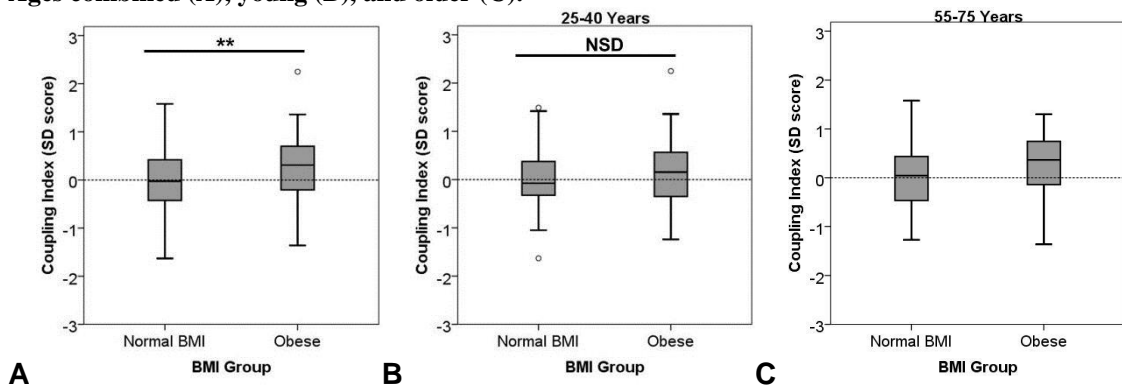
<sup>2</sup> \*p<0.05, \*\*p<0.01. \*\*\*p<0.001, NSD no significant difference

### Coupling of CTX and PINP

CTX and PINP were highly correlated ( $r=0.779$ ,  $p<0.01$ ). Obese individuals had a coupling index on average 0.24 SD scores greater than that of normal BMI individuals ( $p<0.01$ ) (Figure 49). There was no effect of gender on coupling index.

The ratio of PINP to CTX was higher in young adults than older adults ( $p<0.05$ ). Young obese adults had a coupling index on average 0.16 SD scores greater than normal BMI young adults, which was not significant. Older obese adults had a coupling index on average 0.29 SD scores higher than normal BMI older adults (paired t-test:  $p<0.01$ , GLM  $p<0.05$ ).

**Figure 49: Bone turnover coupling index in normal BMI and obese individuals**  
Ages combined (A), young (B), and older (C).<sup>1,2</sup>



Multiple linear regression adjusting for age and gender showed that CTX was the significant predictor of aBMD and vBMD at all sites measured, whereas PINP was not a significant predictor of aBMD or vBMD (Table 15).

**Table 15: CTX as a predictor of BMD**

Dependent variable	Independent variables	Beta	p value	Model p value	R square	Adj R square
WB aBMD	<b>CTX</b>	<b>-.299</b>	<b>&lt;.010</b>	<.001	.304	.289
	PINP	.031	.749			
TH aBMD	<b>CTX</b>	<b>-.399</b>	<b>&lt;.001</b>	<.001	.229	.213
	PINP	.090	.382			
LS aBMD	<b>CTX</b>	<b>-.342</b>	<b>&lt;.010</b>	<.001	.136	.188
	PINP	.017	.877			
Radius vBMD	<b>CTX</b>	<b>-.287</b>	<b>&lt;.050</b>	<.010	.087	.068
	PINP	.115	.303			
Tibia vBMD	<b>CTX</b>	<b>-.211</b>	<b>&lt;.050</b>	<.001	.239	.223
	PINP	.042	.683			

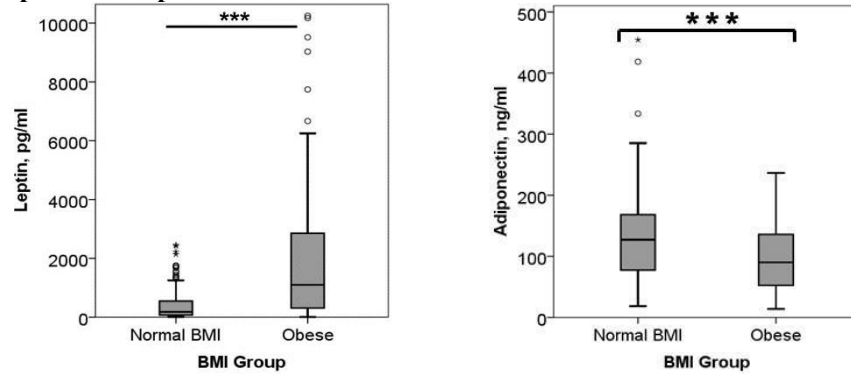
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p<0.05$ , \*\* $p<0.01$ . \*\*\* $p<0.001$ , NSD no significant difference

## Leptin and Adiponectin

Obese individuals had higher leptin ( $p < 0.001$ ) and lower adiponectin ( $p < 0.001$ ) than individuals with a normal BMI (Figure 50). Univariate GLM showed a significant effect of BMI on leptin and adiponectin ( $p < 0.001$ ).

Figure 50: Leptin and adiponectin in normal BMI and obese individuals.<sup>1,2</sup>

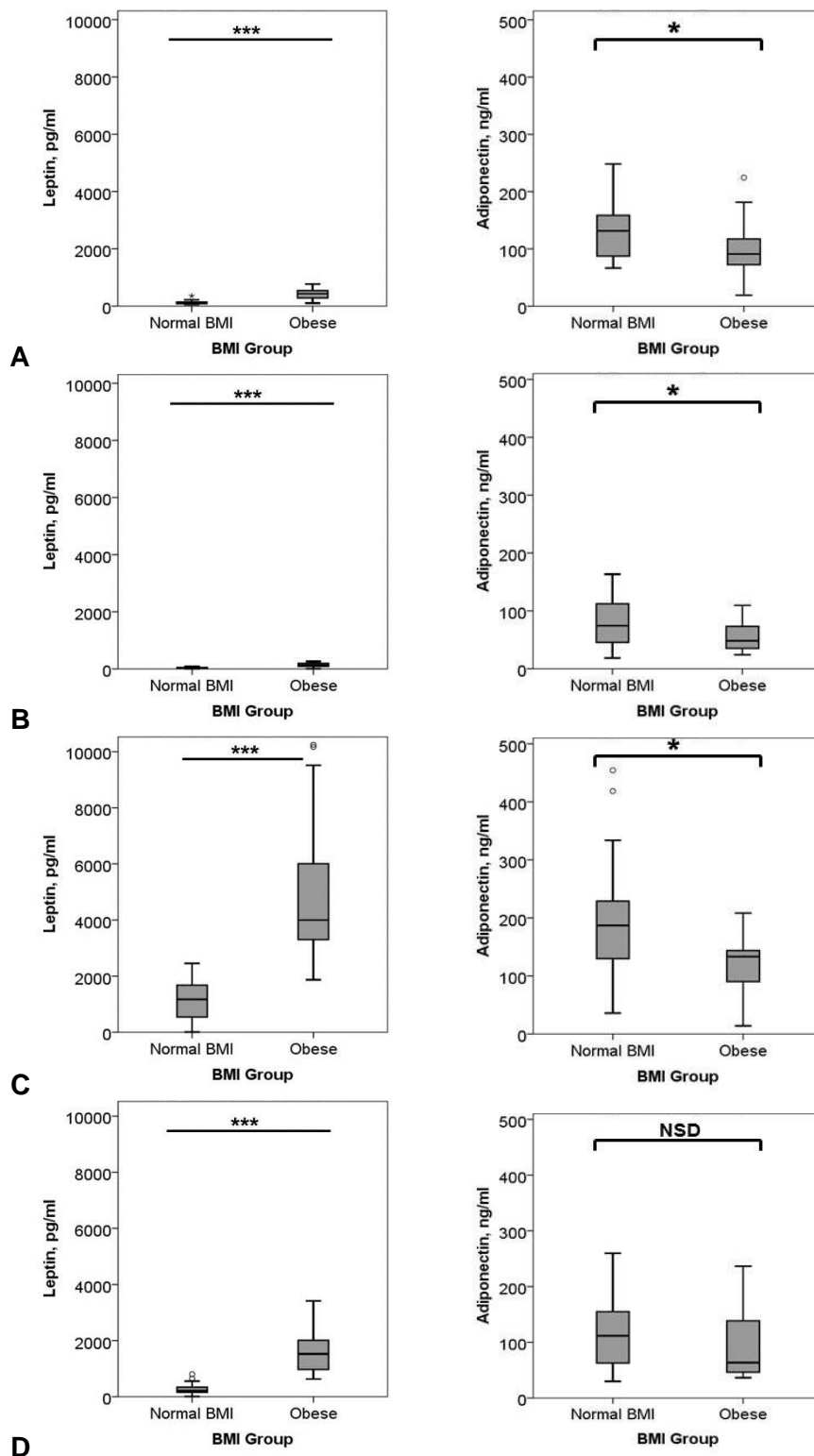


Older adults had higher leptin and adiponectin than younger adults (both  $p < 0.001$ ). Women had higher leptin and adiponectin than men (both  $p < 0.001$ ), even after adjustment for WBFM (both  $p < 0.001$ ) (Figure 51).

There was a significant interaction between age and the effect of BMI on leptin; with a greater effect of BMI on leptin in the older adults than the younger adults ( $p < 0.05$ ). There was no interaction between age or gender and BMI on adiponectin.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference



**Figure 51: Leptin and adiponectin in normal BMI and obese individuals by group by age and gender; young women (A), young men (B), older women (C), older men (D).<sup>1,2</sup>**

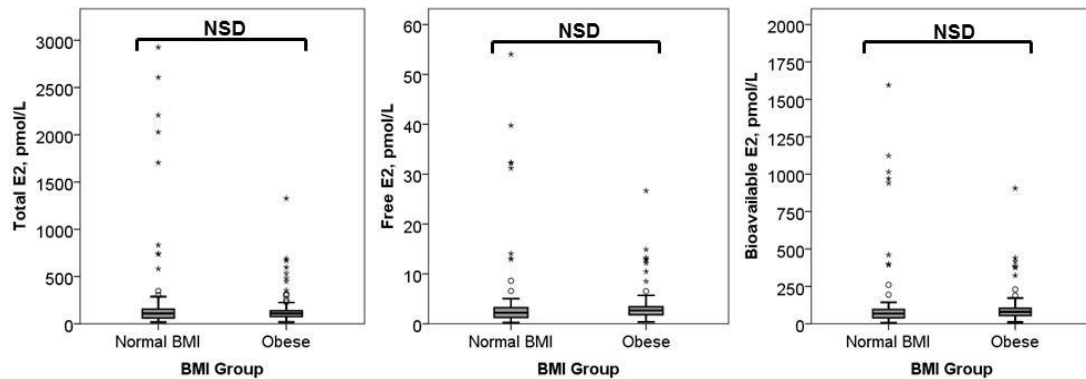
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Total, Free and Bioavailable E2

There was no difference in totE2, fE2 or bioE2 between obese individuals and those with a normal BMI (Figure 52).

Figure 52: TotE2, fE2, bioE2 in normal BMI and obese individuals.<sup>1,2</sup>



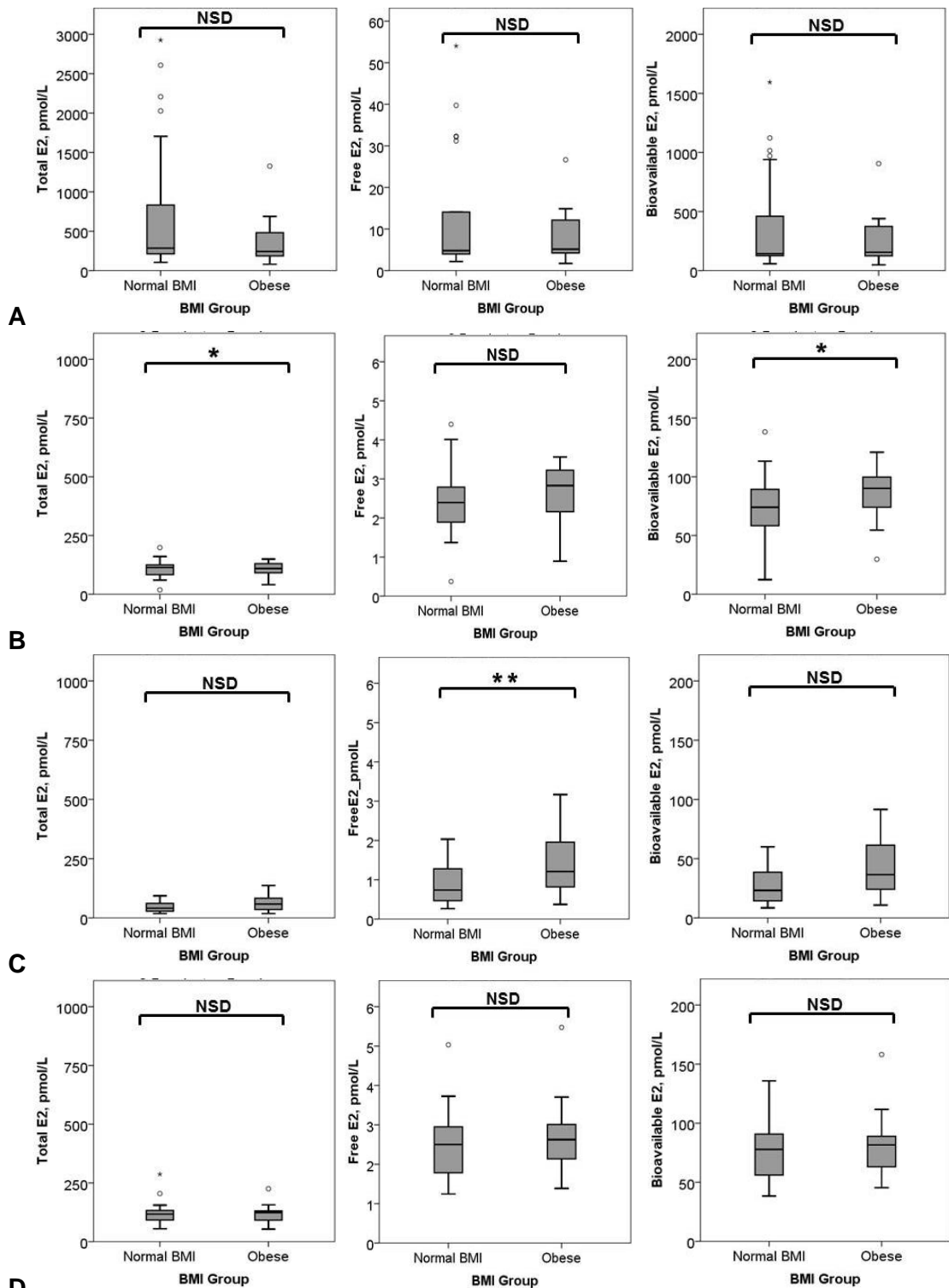
Younger adults had greater totE2, fE2 and bioE2 than older adults (all  $p < 0.001$ ). Women had higher totE2 than men ( $p < 0.05$ ), but there was no difference in fE2 or bioE2 by gender (Figure 53).

There was an interaction between age and the effect of BMI on totE2 and fE2 (univariate GLM, both  $p < 0.05$ ) but not for bioE2 ( $p = 0.052$ ), such that the effect of BMI on totE2 and fE2 was greater in older adults than younger adults.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference





**Figure 53: TotE2, fE2 and bioE2 in normal BMI and obese individuals by group young women (A), young men (B), older women (C), older men (D).<sup>1,2</sup>**

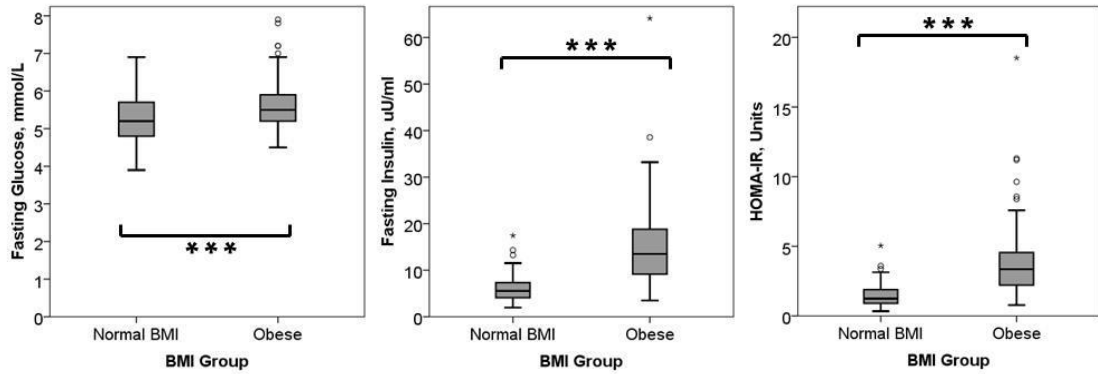
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Glucose, Insulin and Insulin Resistance

Obese individuals had higher glucose, Insulin and HOMA-IR than individuals with a normal BMI (all  $p < 0.001$ ) (Figure 54). GLM showed a significant effect of BMI on glucose, insulin and HOMA-IR (all  $p < 0.001$ ).

Figure 54: Glucose, insulin and HOMA-IR of normal BMI and obese individuals.<sup>1,2</sup>

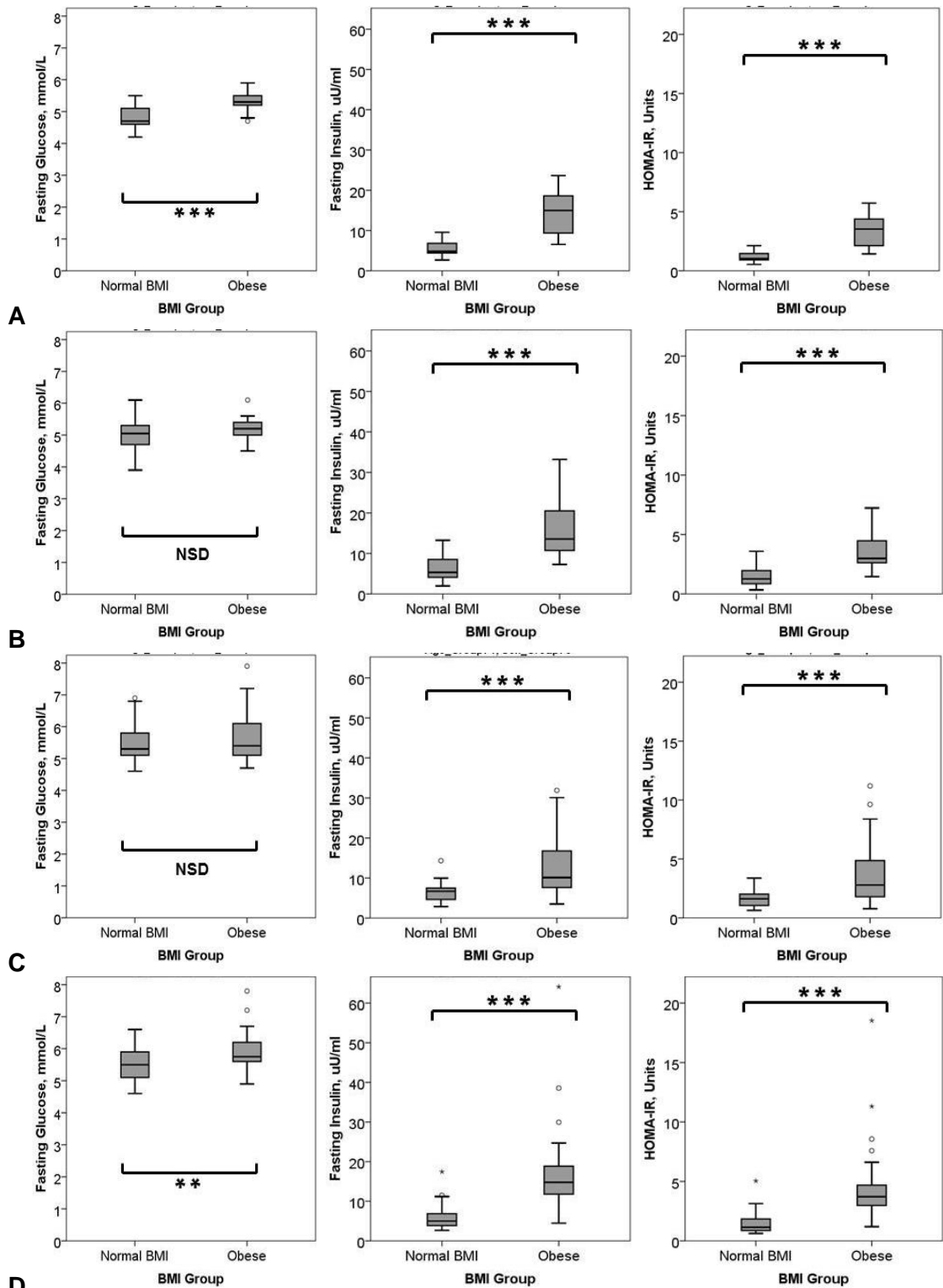


Older adults had higher glucose than younger adults ( $p < 0.001$ ), but there was no effect of age on insulin or HOMA-IR (Figure 55).

There was no effect of gender, or any interaction between age or gender and the effect of BMI, on glucose, insulin or HOMA-IR.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference



**Figure 55: Glucose, insulin and HOMA-IR of normal BMI and obese individuals by group young women (A), young men (B), older women (C), older men (D) <sup>1,2</sup>**

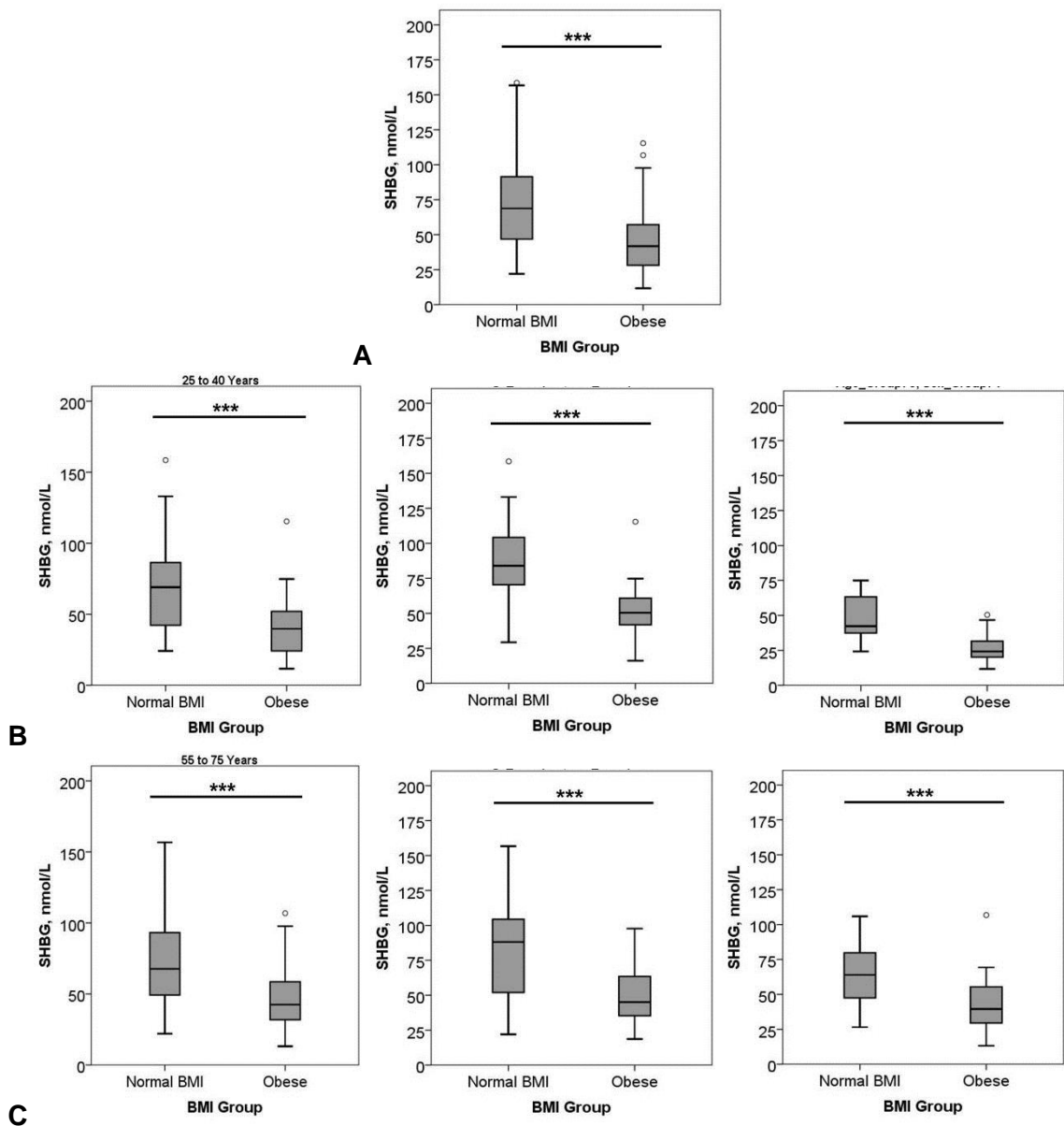
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## SHBG

Obese individuals had lower SHBG than individuals with a normal BMI ( $p < 0.001$ ) (Figure 56). Univariate GLM showed BMI had a significant effect on SHBG ( $p < 0.001$ ). There was no effect of age on SHBG levels. Women had higher SHBG than men ( $p < 0.001$ ). There was an interaction between gender and the effect of BMI on SHBG, with a greater effect of BMI on SHBG in women than in men (univariate GLM,  $p < 0.05$ ).

**Figure 56: SHBG in normal BMI and obese individuals**  
 Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right) <sup>1,2</sup>



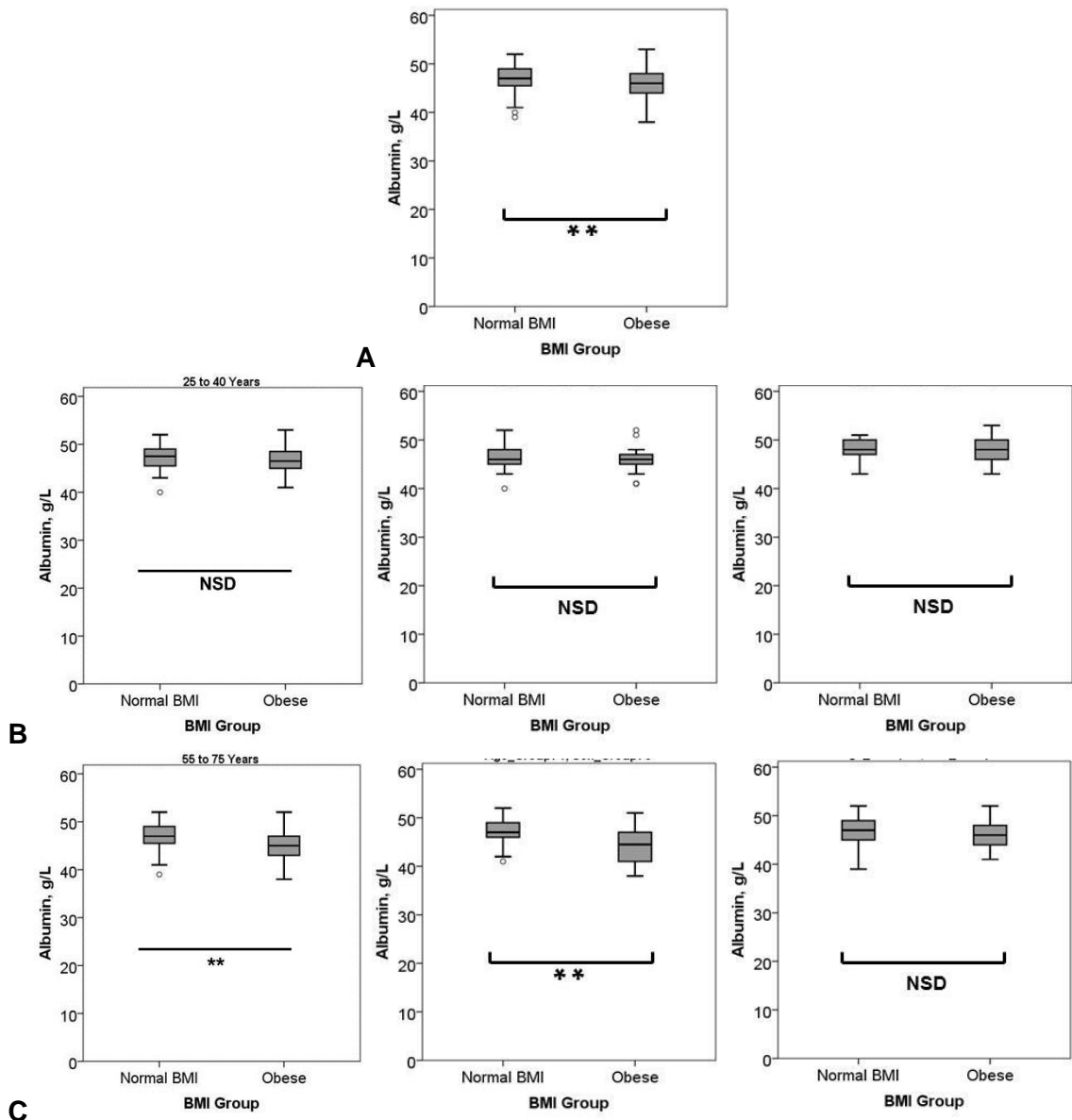
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Albumin

Obese individuals had lower albumin than individuals with a normal BMI ( $p < 0.01$ ) (Figure 57). Univariate GLM showed a significant effect of BMI on albumin ( $p < 0.05$ ). Young adults had higher albumin than older adults ( $p < 0.01$ ). Men had higher albumin than women ( $p < 0.001$ ). There were no interactions between age or gender and the effect of BMI on albumin.

**Figure 57: Albumin in normal BMI and obese individuals**  
Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right) <sup>1,2</sup>



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

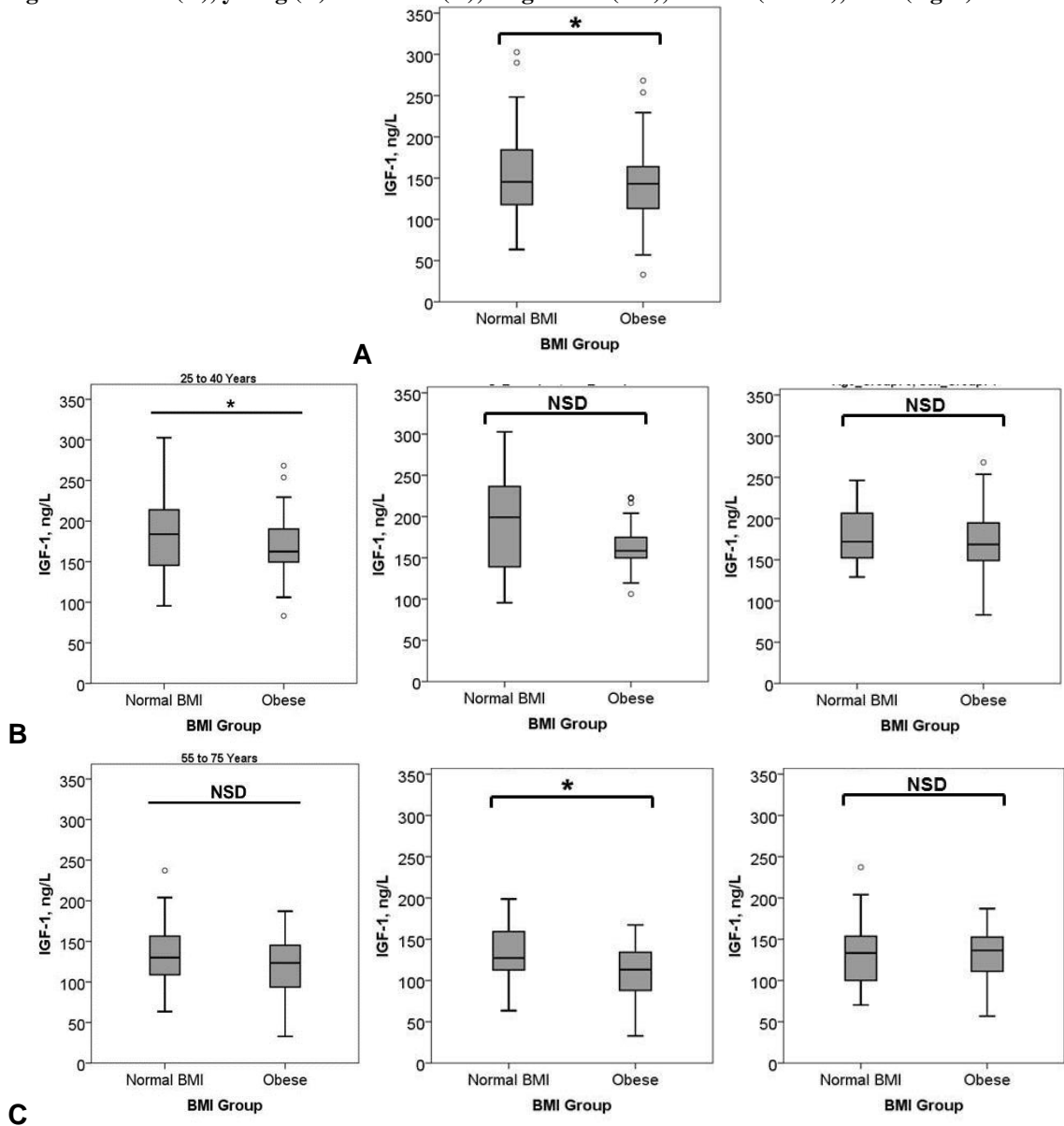
<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## IGF-I

Overall, IGF-I was lower in obese individuals than those with a normal BMI ( $p < 0.05$ ) (Figure 58). Univariate GLM showed a significant effect of BMI on IGF-I ( $p < 0.05$ ). Younger adults had higher IGF-I than older adults ( $p < 0.001$ ). There was no effect of gender, or interactions between age or gender and the effect of BMI, on IGF-I.

**Figure 58: IGF-I in normal BMI and obese individuals**

**Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right)**<sup>1,2</sup>



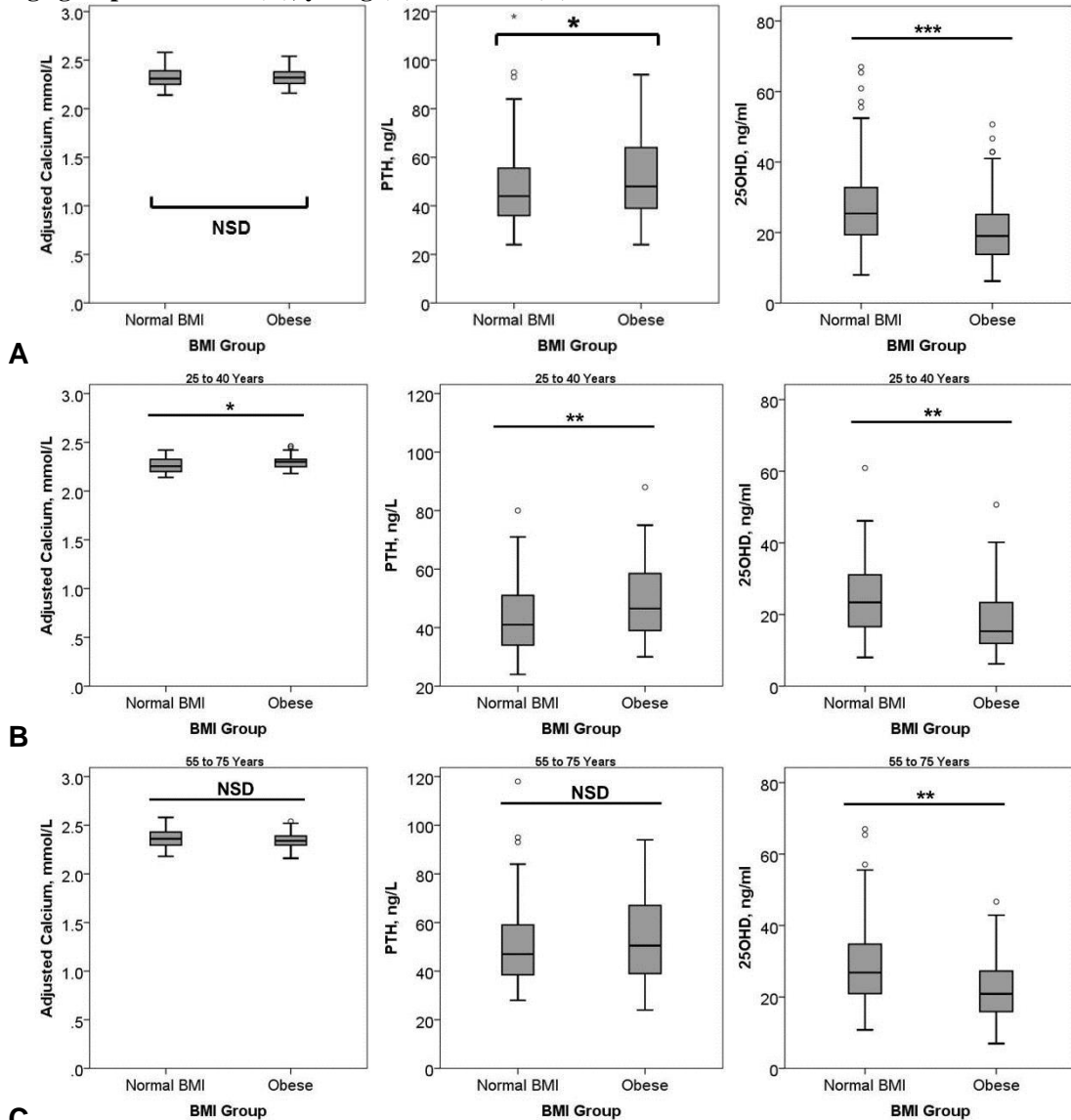
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## Calcium, PTH and 25OHD

There was no difference in adjusted calcium between obese and normal BMI individuals (Figure 59). Obese individuals had higher PTH ( $p < 0.05$ ) and lower 25OHD ( $p < 0.001$ ) than normal BMI individuals (Figure 59).

**Figure 59: Adjusted calcium, PTH and 25OHD in normal BMI and obese individuals**  
Age groups combined (A), young (B) and older (C)<sup>1,2</sup>



Older adults had higher adjusted calcium, PTH and 25OHD than young adults (calcium and PTH  $p < 0.001$ , 25OHD  $p < 0.01$ ). There was a greater effect of BMI on adjusted calcium in young adults, than older adults ( $p < 0.01$ ). There was no effect of gender on adjusted calcium, PTH or 25OHD. There was a greater effect of BMI on PTH levels in women than men ( $p < 0.05$ ).

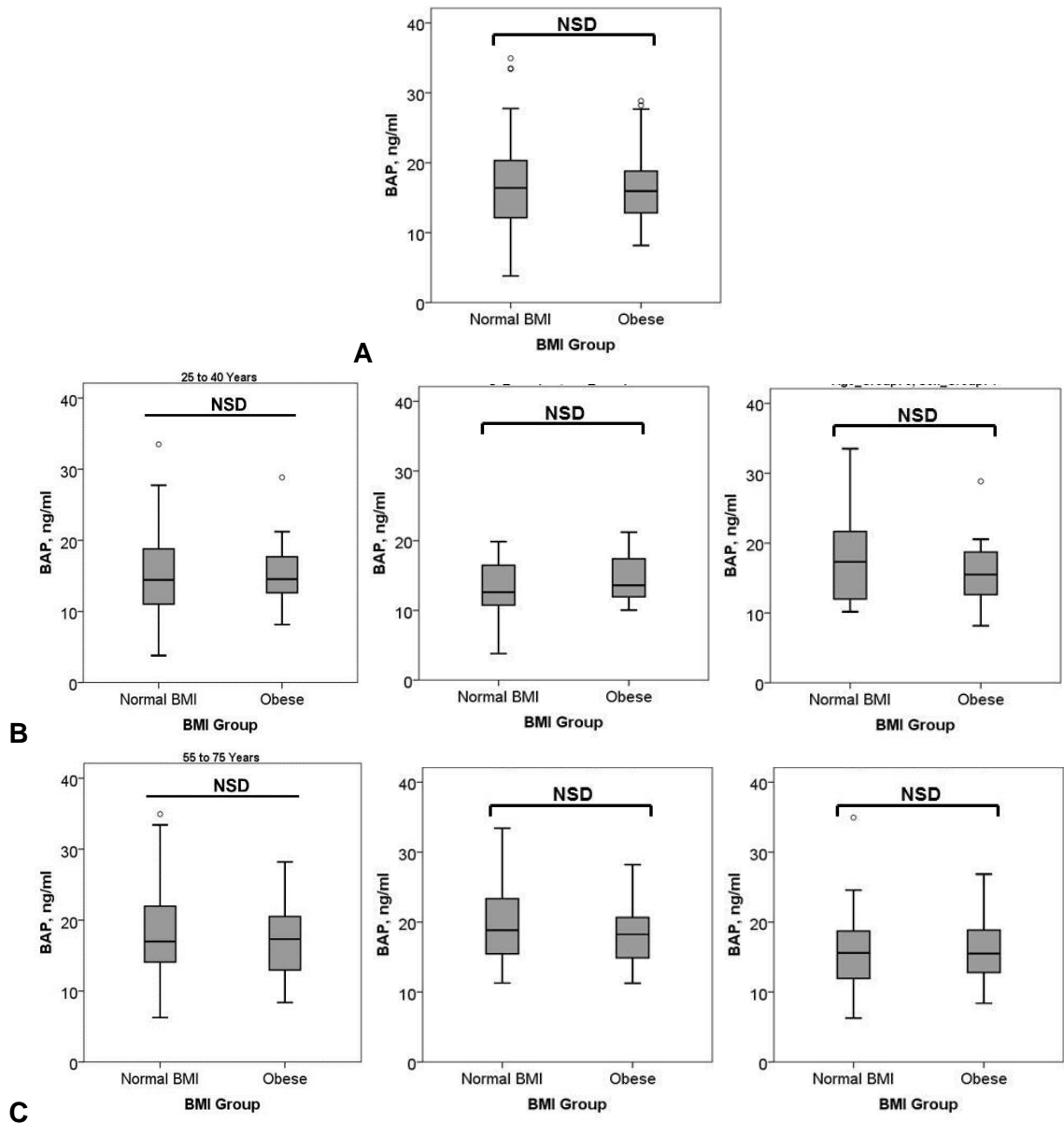
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## BAP

There was no difference in BAP between obese individuals and those with a normal BMI (Figure 60). Older adults had higher BAP than young adults ( $p < 0.01$ ). There was no effect of gender, or interactions between age or gender and the effect of BMI on BAP.

**Figure 60: BAP in normal BMI and obese individuals**  
Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right) <sup>1,2</sup>



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

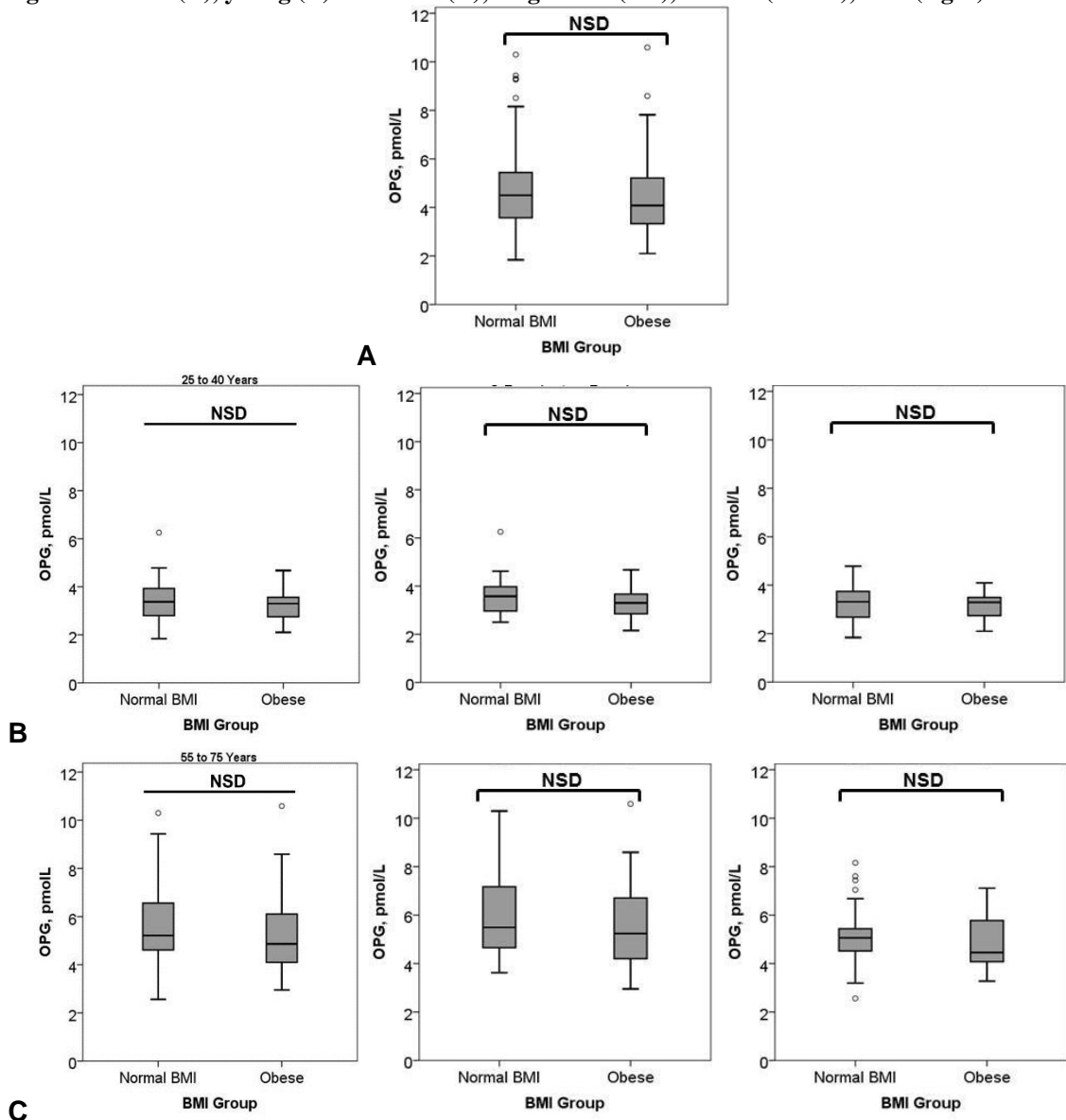
<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference



## OPG

There was no difference in OPG between obese and normal BMI individuals (Figure 61). Older adults had higher OPG than young adults ( $p < 0.001$ ). Women had higher OPG than men ( $p < 0.01$ ). There were no interactions between age or gender and the effect of BMI on OPG.

**Figure 61: OPG in normal BMI and obese individuals**  
Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right) <sup>1,2</sup>



<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

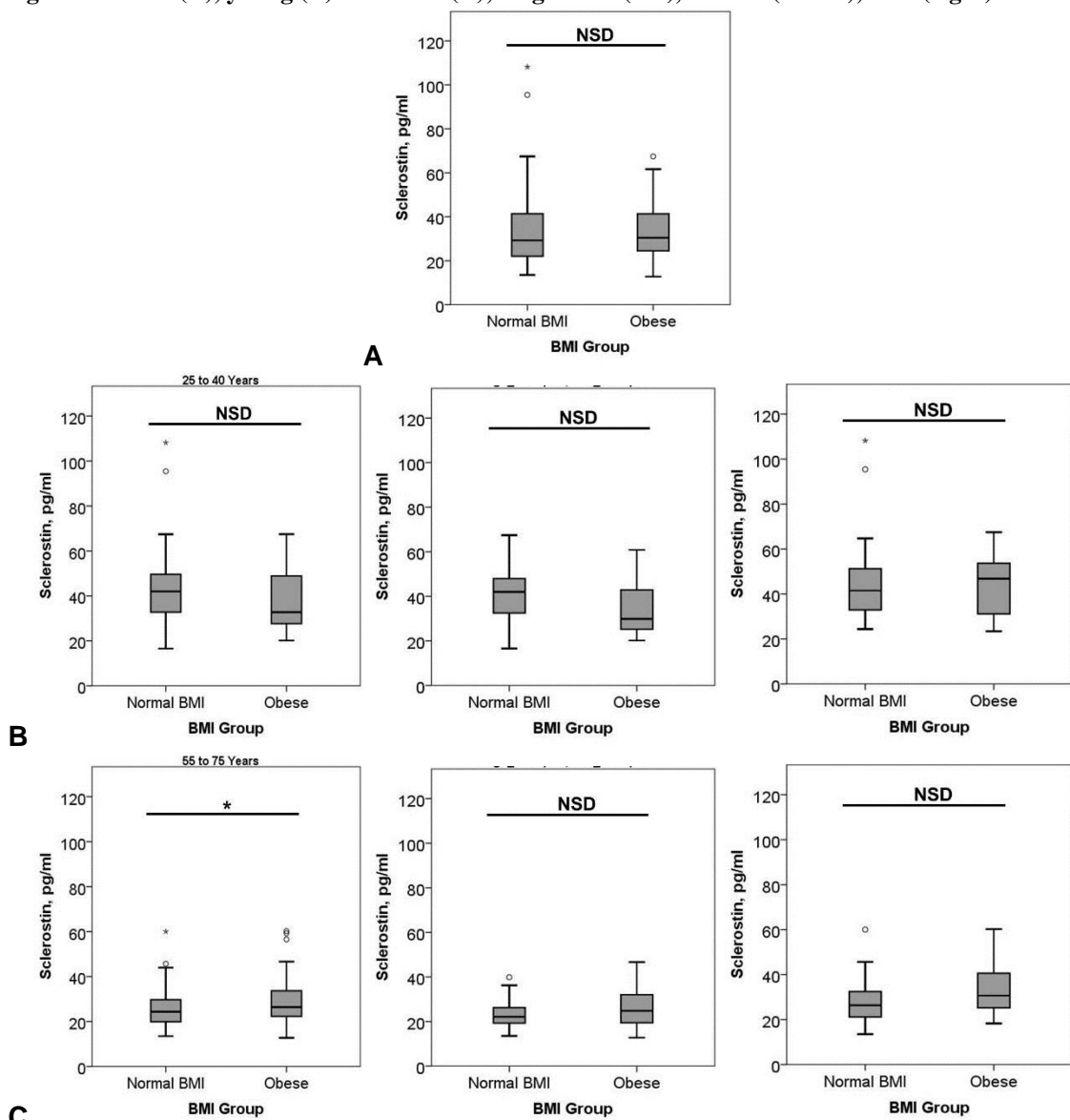
<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Sclerostin

There was no difference in sclerostin between obese and normal BMI individuals (Figure 62). Younger adults had higher sclerostin than older adults ( $p < 0.001$ ). Men had higher sclerostin than women ( $p < 0.001$ ).

Sclerostin was not associated with coupling index in young adults. In older adults, sclerostin was positively correlated with coupling index (unadjusted  $r = 0.356$ , adjusted for gender  $r = 0.368$ , both  $p < 0.001$ ).

**Figure 62: Sclerostin in normal BMI and obese individuals**  
Ages combined (A), young (B) and older (C), all genders (left), women (centre), men (right) <sup>1,2</sup>



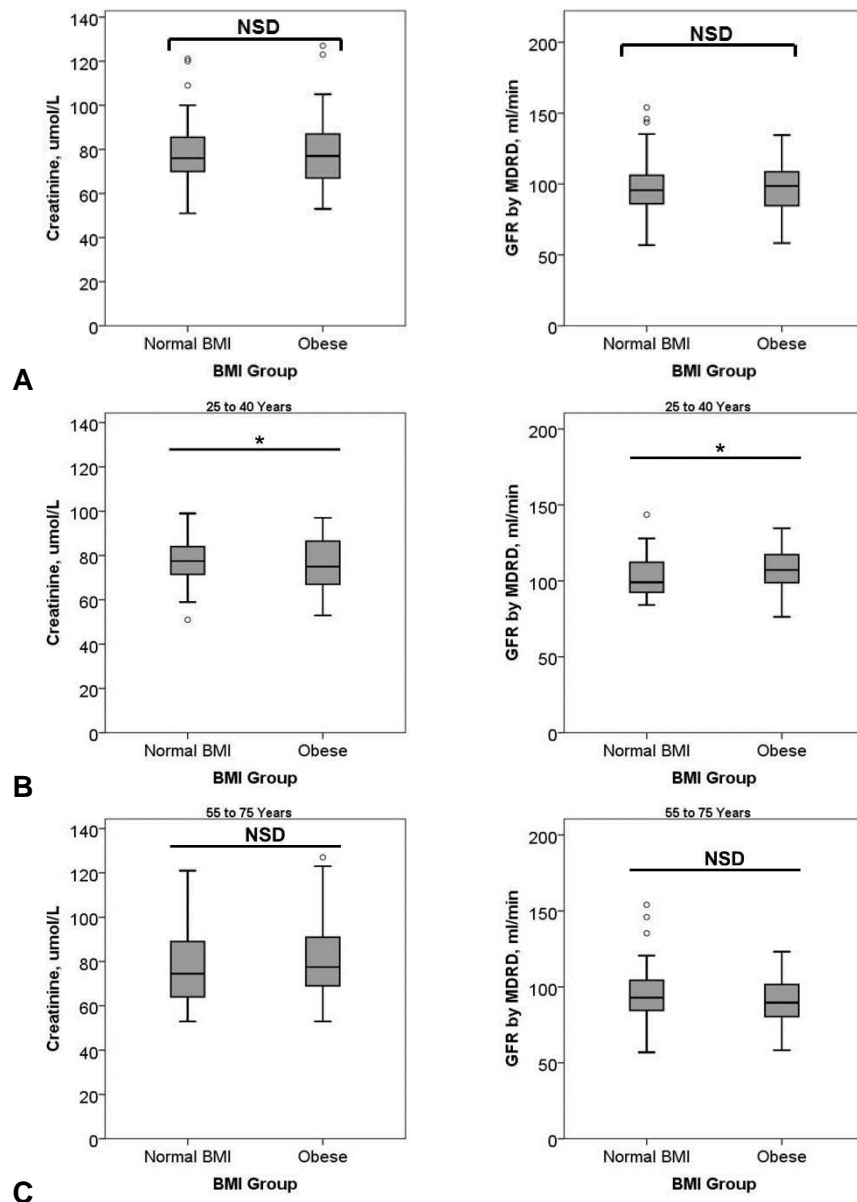
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Creatinine and eGFR

There was no difference in creatinine or eGFR between obese individuals and those with a normal BMI (Figure 63). There was no effect of age on creatinine. Men had higher creatinine than women ( $p < 0.001$ ). Younger adults had higher eGFR than older adults ( $p < 0.001$ ). There was no effect of gender on eGFR. There were no interactions between age or gender and the effect of BMI on creatinine or eGFR.

**Figure 63: Creatinine and eGFR in normal BMI and obese individuals**  
Groups combined (A), younger adults (B), older adults (C) <sup>1,2</sup>



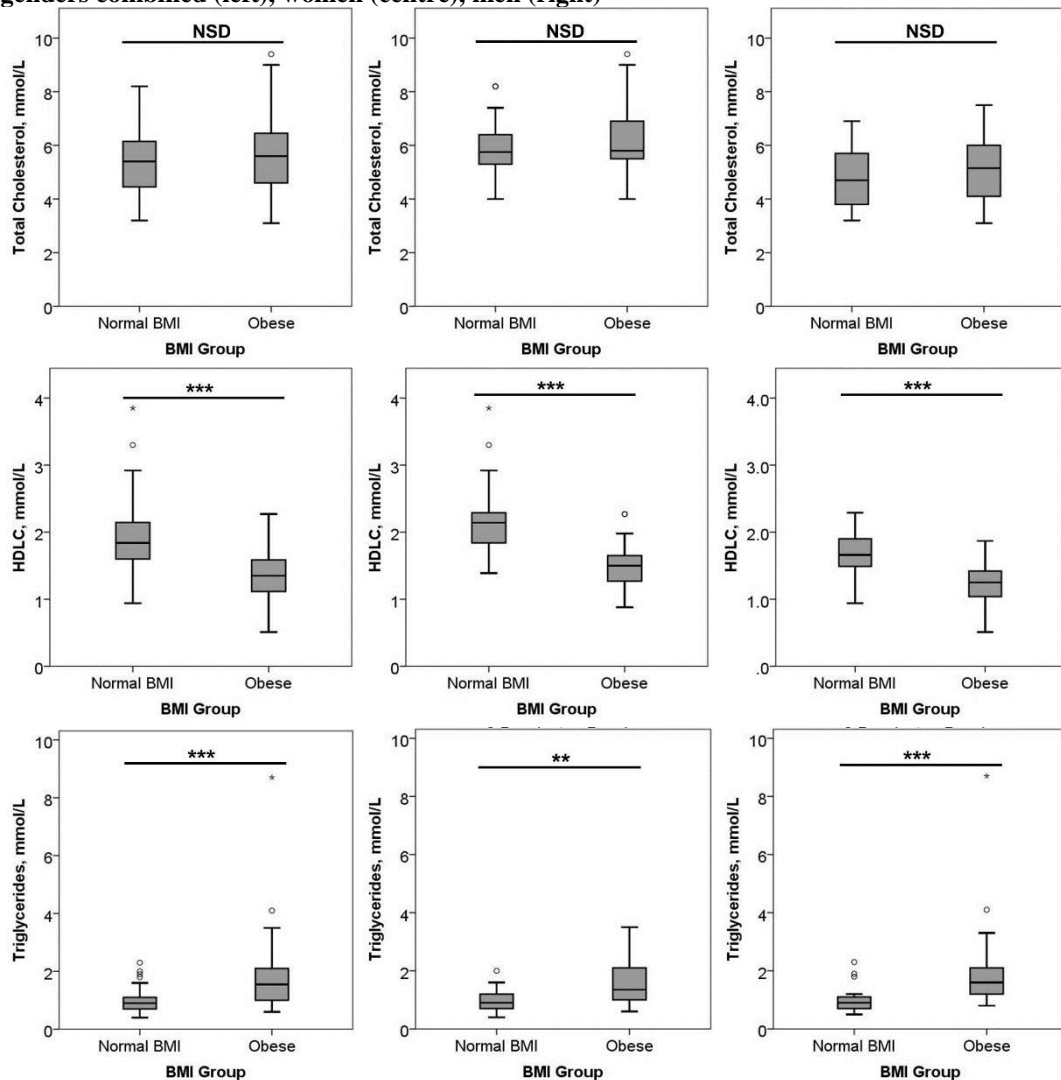
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

## Lipids

There was no difference in TC between obese individuals and individuals with a normal BMI (Figure 64). Obese individuals had lower HDL than individuals with a normal BMI ( $p < 0.001$ ) (Figure 64). Obese individuals had higher TC:HDL ratio ( $p < 0.001$ ). Obese individuals had higher triglycerides than individuals with a normal BMI ( $p < 0.001$ ) (Figure 64). Women had a higher TC and HDL than men (both  $p < 0.001$ ) but there was no effect of gender on total:HDL ratio or triglycerides.

**Figure 64: TC, HDL and triglycerides in normal BMI and obese individuals genders combined (left), women (centre), men (right)**<sup>1,2</sup>



## Inflammatory Cytokines

Obese individuals had higher HsCRP than individuals with a normal BMI ( $p < 0.001$ ). There was no effect of gender on HsCRP.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

### **Potential Mediators of Low Bone Turnover in Obesity**

As hypothesised, bone turnover appears to be lower in obesity. As previously stated, SAT was identified as the fat depot with the greatest influence on bone microstructure and so multiple linear regression models were built to a) determine the effect of SAT on bone resorption and formation and b) determine which, if any, of the biochemical factors studied, accounted for the effect of SAT on CTX and PINP. The effect of each of the key biochemical mediators proposed in Table 9 on CTX and PINP were studied, based on the hypothesis outlined for this Chapter.

### **Mediators of Low Bone Resorption in Obesity**

Adjusting for age and gender, SAT was a negative predictor of CTX (overall model  $p < 0.01$ , adjusted R square 0.078, effect of SAT: beta -0.288,  $p < 0.001$ ).

When leptin was added to this model, SAT was no longer a predictor of CTX (beta -0.053,  $p = 0.639$ ), but leptin was a negative predictor of CTX (overall model  $p < 0.001$ , adjusted R square 0.101, effect of leptin: beta -0.409,  $p < 0.05$ ).

When TotE2 was added to the CTX model, SAT remained a significant predictor of CTX (beta -0.293,  $p < 0.001$ ), and totE2 was also a significant predictor of CTX (overall model  $p < 0.001$ , adjusted R square 0.121, effect of totE2: beta -0.258,  $p < 0.01$ ). Adding fE2 or bioE2 instead of totE2 did not alter this relationship (both  $p < 0.01$ ).

When both leptin and TotE2 were added to the CTX model, SAT was no longer a significant predictor of CTX (beta -0.074,  $p = 0.505$ ), but both leptin (beta -0.380,  $p < 0.05$ ) and TotE2 (beta -0.247,  $p < 0.01$ ) were significant negative predictors of CTX (overall model  $p < 0.001$ , adjusted R square 0.140).

Adiponectin, IGF-I, glucose, insulin and HOMA-IR were not predictors of CTX.

OPG was positively correlated with leptin ( $r = 0.454$ ,  $p < 0.001$ ). To see whether leptin might lower CTX in obesity through positive associations with OPG, multiple linear regression adjusting for age and gender was performed with OPG as the dependent variable. SAT was a significant predictor of OPG (overall model  $p < 0.001$ , adjusted R square 0.479, effect of SAT: beta -0.128,  $p < 0.05$ ). When leptin was added into this model, SAT remained a significant predictor of OPG and leptin was not a predictor (overall  $p < 0.001$ , adjusted R square 0.484, effect of SAT: beta -0.233,  $p < 0.01$ ).

### **Mediators of Low Bone Formation in obesity**

Adjusting for age and gender, SAT was a negative predictor of PINP (overall model  $p < 0.001$ , adjusted R square 0.084, effect of SAT: beta -0.230,  $p < 0.01$ ) and OC (overall model  $p < 0.001$ , adjusted R square 0.106, effect of SAT: beta -0.348,  $p < 0.001$ ).

When leptin was added to the PINP or OC model, SAT was no longer a predictor of PINP (beta -0.170,  $p = 0.0140$ ) or OC (beta -0.177,  $p = 0.119$ ), and leptin was not a significant predictor of PINP (overall model  $p < 0.01$ , adjusted R square 0.081, effect of leptin: beta -0.108,  $p = 0.502$ ) or OC (overall model  $p < 0.001$ , adjusted R square 0.112, effect of leptin: beta -0.291,  $p = 0.067$ ).

When TotE2 was added to the PINP or OC model, SAT remained a significant predictor of PINP (beta -0.235,  $p < 0.001$ ) and OC (beta -0.352,  $p < 0.001$ ), and totE2 was also a negative predictor of PINP (overall model  $p < 0.001$ , adjusted R square 0.116, effect of totE2: beta -0.226,  $p < 0.01$ ) and OC (overall model  $p < 0.001$ , adjusted R square 0.131, effect of totE2: beta -0.202,  $p < 0.05$ ). Adding fE2 or bioE2 instead of totE2 did not alter this relationship (all  $p < 0.01$ )

When both leptin and TotE2 were added to the PINP model, SAT was no longer a significant predictor of PINP (beta -0.189,  $p = 0.096$ ) and leptin was not a significant predictor of PINP (beta -0.082,  $p = 0.605$ ) but TotE2 was a predictor of PINP (overall model  $p < 0.001$ , adjusted R square 0.112, effect of TotE2: beta -0.223,  $p < 0.01$ ).

When both leptin and TotE2 were added to the OC model, SAT was no longer a significant predictor of OC (beta -0.193,  $p = 0.085$ ) and leptin was not a significant predictor of OC (beta -0.268,  $p = 0.087$ ) but TotE2 was a predictor of OC (overall model  $p < 0.001$ , adjusted R square 0.135, effect of TotE2: beta -0.195,  $p < 0.05$ ).

Adiponectin, IGF-I, glucose, insulin and HOMA-IR were not predictors of PINP.

Adiponectin, IGF-I and glucose were not predictors of OC. When insulin and HOMA-IR were added (separately) to the OC model, SAT remained a significant predictor of OC (insulin model: beta -0.204, and HOMA-IR model: beta -0.224, both  $p < 0.05$ ) and insulin and HOMA-IR were also negative predictors of OC (insulin beta: -0.212, HOMA-IR beta: -0.206, both  $p < 0.05$ ).

## Summary of Results

A summary of the differences between BMI, age and gender groups for the outcomes of this Chapter is provided in Table 16. The variables identified as having a potential role in the association between obesity and BMD and microstructure are also labelled.

**Table 16: Summary of results of Chapter 4**

Variable	Effect of BMI	Effect of Age	Effect of Gender	Interactions with BMI
WBFM	↑	-	↑ in women	-
<b>SAT*</b>	↑	-	↑ <b>in women</b>	↑ <b>effect in younger</b>
VAT	↑	↑ in older	↑ in men	↑ effect in younger
VAT:SAT	-	↑ in older	↑ in men	
Trunk FM	↑	↑ in older	↑ in women	-
AFM	↑	-	↑ in women	-
Android FM	↑	↑ in older	-	-
Gynoid FM	↑	↑ in younger	↑ in women	-
<b>CTX*</b>	↓	-	-	-
PINP	↓	↑ in younger	-	-
OC	↓	-	-	-
<b>Coupling index*</b>	↑	↑ <b>in younger</b>	-	↑ <b>effect in older</b>
<b>Leptin*</b>	↑	↑ <b>in older</b>	↑ <b>in women</b>	↑ <b>effect in older</b>
Adiponectin	↓	↑ in older	↑ in women	-
<b>TotE2*</b>	-	↑ <b>in younger</b>	↑ <b>in women</b>	↑ <b>effect in older</b>
<b>fE2*</b>	-	↑ <b>in younger</b>	-	↑ <b>effect in older</b>
<b>BioE2*</b>	-	↑ <b>in younger</b>	-	-
SHBG	↓	-	↑ in women	↑ effect in women
OPG	-	↑ in older	↑ in women	-
IGF-I	↓	↑ in younger	-	-
Glucose	↑	↑ in older	-	-
Insulin	↑	-	-	-
PTH	↑	↑ in older	-	↑ effect in women
Ca	-	↑ in older	-	↑ effect in younger
25OHD	↓	↑ in older	-	-

\*Variables implicated in the predictive model as mediating the association between obesity and greater BMD and favourable microarchitecture

## Discussion

The results of this Chapter show obese individuals have lower bone turnover, mainly as a result of lower bone resorption, accompanied by lower bone formation. Lower bone resorption in obesity was the result of greater adiposity and not of greater lean mass. Subcutaneous abdominal adiposity was the key negative determinant of bone resorption, lowering resorption through greater leptin production. Leptin was higher in older adults and the greater effect of adiposity on leptin in older adults than younger adults may go some way to explaining the positive coupling index and greater effect of obesity on BMD and bone microstructure in older adults, whereas coupling index is not affected by BMI

in the young adults, in whom weaker beneficial effects are observed in bone density and structure with high BMI.

Obese individuals have lower bone turnover than individuals with a normal BMI, with lower CTX and lower PINP and OC. These findings are consistent with the existing literature which shows lower makers of resorption and formation with high BMI in premenopausal women (410), through the menopausal transition (411) and in postmenopausal women (261, 358, 410, 412, 413). Studies in men from which to draw comparisons are lacking, although a recent study of young men and women by Viljakainen et al. showed PINP, CTX, TRAP, total OC and carboxylated OC were 40%, 35%, 17%, 31% and 32% lower, respectively, than in non-obese age and gender matched controls (414). In further agreement with the results of this Chapter, Viljakainen et al. found no difference in coupling index between young obese and non-obese men and women (414). There was no effect of age on bone resorption in the present study, while younger adults had higher bone formation than older adults, possibly associated with the period of consolidation in early adulthood, despite bone turnover typically increasing with age (382).

This Chapter shows that leptin appears to be the key mediator of the effect of SAT on CTX. Consistent with the findings of the present study, Viljakainen et al. suggested that leptin may be the principal determinant of bone turnover in obese young adults (414). Goulding and Taylor reported no association between leptin and bone resorption (by Deoxypyridinoline) or formation (by OC) in postmenopausal women, although the sample size was considerably smaller than that of the present study (415).

Leptin has been shown to reduce ovariectomy-induced trabecular bone loss by maintaining Tb.BV/TV and Tb.N in rats (416). Consistent with the effect of obesity on bone structure in the present study, there was no effect of leptin on Tb.Th but periosteal bone formation was reduced (416). Reduced periosteal bone formation with increased leptin levels might account for there being no difference in bone size by Ct.Pm in obese individuals compared to normal BMI individuals. In these rats, co-administration of leptin and oestrogen decreased bone turnover to a greater extent than oestrogen administration alone, which supports the findings of the current study where both E2 and leptin contribute to lower bone resorption (416).

Leptin administration reduced bone loss in tail-suspended rats through the uncoupling of bone turnover; with a reduction in bone loss and an increase in bone formation at the



tibia compared to non-suspended animals (417). The increase in bone formation conflicts with the findings of this Chapter which show lower formation and maintenance of coupling, although coupling index in obese individuals is higher than in those with a normal BMI.

Leptin has been shown to exert anabolic effects on bone when it acts directly on bone cells, making it a plausible candidate to explain favourable bone density in obesity (96, 98). Although leptin is able to act as a growth factor to influence the proliferation of hemopoietic stem cells induce MSC differentiation along the chondrocytic lineage (418), this effect would most likely drive bone formation, whereas it appears that the mechanisms by which older obese adults have greater bone density is through reduced bone loss. Therefore the key leptin action in obesity may be the regulation of osteoclastogenesis through inhibition of RANKL, supported by increased OPG mRNA expression and decreased RANKL mRNA expression with leptin administration *in vitro* (96, 98, 416).

If leptin does lower bone resorption through upregulation of OPG, higher OPG may have been expected in obesity. In this study, there was no difference in OPG between obese and normal BMI individuals, consistent with the findings of previous work (419), although others have shown lower OPG in obesity (116, 420). Older adults had higher OPG than younger adults, as previously shown (421, 422) and in support of a greater protective effect of obesity in older adults than younger adults. As leptin did not explain the effect of SAT on OPG, other biochemical factors associated with SAT may play a role in the mediation of OPG in obesity.

Although leptin exerts negative effects on bone when it acts through the CNS, leptin is primarily derived from extraosseous adipocytes, bone marrow adipocytes, chondrocytes and osteoblasts in peripheral locations and not from the CNS (96). Thus, whilst the overall effect of leptin on bone is determined by contributions of both central and peripheral effects, the principal effect is likely to result from the direct, systemic actions on osteoclastogenesis (96). Although much of the literature involving mouse models to study the effects of leptin on bone suggests leptin has adverse effects on bone, this may be attributed to the differences in the method and concentration of leptin administration compared to physiological leptin production, as well as the physiological differences between mice and humans.

The results of the present work are consistent with a suggestion made by Thomas and Burguera that “leptin could stimulate bone growth and bone size through angiogenic effects and osteogenic activity in immature cortical bone. Later, it may decrease bone remodelling in the mature skeleton when trabecular bone turnover is high” (98). In Chapter 3 it was shown that obesity has a beneficial effect on the skeleton in young adulthood and it is plausible that the lesser effect of leptin on CTX in the young adults was due to the difference in mechanism by which leptin exerts protective effects on bone with age.

Leptin was higher in older adults than young adults, despite no difference in WBFM between groups. This is consistent with the finding of increased expression of leptin with age, independent of adiposity, in rats (423). However, Rosenbaum et al. showed leptin was higher in pre-menopausal women than postmenopausal women after correction for WBFM (424). Similarly, Moller et al. found a positive association between leptin and WBFM in young adults but no association between leptin and WBFM in older adults (425). There is also some evidence to support no effect of age on leptin concentration (426). With age, adiposity increases, accompanied by increased adipocyte size. Larger adipocytes are associated with greater expression of leptin, and subcutaneous and omental biopsies show greater *Ob* mRNA expression in individuals with a high BMI compared to normal BMI individuals (427-429).

Leptin was higher in women than in men in the present study. This is consistent with other studies which report leptin levels in women to be two to three times higher than in men (98, 334, 343, 424-427). The difference in leptin by gender is not the result of greater FM in women as this association persisted after adjustment for WBFM (343, 424, 426). The difference in leptin by gender may be a result of differences in body fat distribution in men and women (334, 343, 344). VAT expresses lower leptin than SAT women have greater SAT for a given WBFM. Greater leptin expression by SAT may contribute to the understanding of why SAT was a stronger predictor of CTX than was VAT (343, 430).

Although leptin expression and circulating levels are controlled by sex hormones, sex hormones did not explain differences in leptin by gender (343, 426) but did contribute to leptin levels in postmenopausal women (343). However, Baumgartner et al. found that sex differences in leptin were associated with testosterone in men (344) and changes in leptin with age were associated with changes in testosterone in men, whereas in women, changes in BMI not oestrogen were associated with changes in leptin (431).

Some studies have shown leptin is positively associated with aBMD (339-342), others report a negative association (345-347) and some have reported no association (304, 335, 348-350). After adjustment for BMI, significant associations between leptin and BMD typically become non-significant, as do associations between WBFM and BMD when adjusted for leptin, indicating relationships between adiposity and BMD are mediated by leptin (334, 342). The relationship between leptin and BMD may differ between men and women (334, 341, 342),

In a review of the leptin-bone relationship, Thomas reported that there is evidence of associations between leptin and aBMD and bone area by DXA to suggest that “leptin might act on the periosteal envelope of cortical bone, thereby increasing bone size” (98). This conflicts with the results of the present work, which has studied vBMD and microarchitecture to the conclusion that there is no difference in Ct.Pm or cortical area in obese individuals who have greater leptin than individuals with a normal BMI. Leptin was inversely associated with periosteal circumference in unadjusted analysis in young adult men, although this effect did not persist after adjustment for WBFM (432). Recently Wong et al. have suggested that neuropeptide YY may drive lower cortical bone formation in leptin resistance, with higher cortical apposition, particularly higher periosteal apposition, in neuropeptide YY and leptin knockout mice (433).

Recent investigations into the effect of bariatric surgery induced weight loss on bone microarchitecture have shown decreases primarily in cortical bone, with decreases in cortical density, area and thickness (47). As the peripheral action of leptin has been found to impact cortical bone, this supports a role for leptin in the maintenance of cortical bone (99). At six and eighteen months following Roux-En-Y bariatric surgery, decreases in leptin were significantly correlated with increases in OC, BAP and NTX, with the strongest association between leptin and NTX (434). Unlike the decrease in BMI, the decrease in leptin was a significant predictor of the increase in NTX (434). These findings further illustrate the role of leptin in the regulation of bone turnover, and particularly of bone resorption.

While totE2 was no different between normal BMI and obese individuals in the overall study population, totE2 appears to reduce bone resorption independent of the relationship between SAT and leptin on bone resorption. There was no difference in total, free or bioavailable E2 between obese and non-obese groups overall, or in younger adults alone. Therefore E2 was perhaps unlikely to explain differences in bone density between BMI groups in younger adults, Consistent with the findings of this Chapter,

others have reported no difference in fE2 in young obese women (269) and in young women by tertile of trunk FM (273). Despite no difference in fE2, lower bone formation was associated with greater trunk FM in young women (273)

fE2 and bioE2 were higher in obese older adults than older adults with a normal BMI. Thus, the hypothesis that greater circulating E2 in obesity as a result of increased aromatisation of androgens might protect obese postmenopausal women from bone loss, is supported by the findings of this Chapter, despite not being the predominant mechanism by which obesity drives lower bone turnover. The difference in fE2 and bioE2 between obese and normal BMI groups by age group might contribute to understanding why there was a greater effect of obesity on bone density in older adults than younger adults; with two mechanisms driving beneficial effects of adiposity on the skeleton in older age, compared to the sole effect of leptin in young adults.

There is longstanding evidence of an inverse association between oestrogen and bone resorption (332). Heshmati et al. showed oestrogen had a protective effect against bone resorption by treating postmenopausal women with Letrozole to virtually eliminate E2 in the circulation, which increased bone resorption markers without affecting formation markers (435). In postmenopausal women taking HRT, there was no difference in OC between obese and normal BMI individuals (412). Unlike normal weight women, there was no difference in bone turnover between obese women taking HRT and obese women not taking HRT, suggesting oestrogen has a protective effect against lower bone formation in obesity (412).

While fE2 and bioE2 are positively associated with Ct.vBMD (436, 437) and Ct.Th in older men (437, 438) and inversely with Ct.Po (439), fE2 was a negative predictor of bone size by cortical cross sectional area, Ct.Pm and endosteal circumference in male adolescents (436). However, Vandewalle et al. recently described greater Ct.Pm and endosteal circumference associated with higher oestrogen exposure in obese male adolescents compared to matched normal BMI controls (440). As the present work has shown no difference in bone size in younger or older adulthood, the effect of oestrogen on periosteal apposition in obesity is unclear. Age at obesity onset may affect skeletal exposure to oestrogen which might have lasting effects on bone size through adulthood. Age at onset of obesity was not collected in the present study. As Bredella et al. found a positive association between fE2 and Tb.Th in young obese adults, there being no difference in fE2 in young adults may contribute to there being no difference in Tb.Th in the present cohort (268).

There was no effect of adiponectin, IGF-I or insulin on bone resorption. IGF-I and insulin are positive determinants of skeletal acquisition (379). Bredella et al. found that IGF-I was inversely associated with total abdominal fat in obese young men and that those with high VAT trended toward having lower IGF-I than obese men with low VAT (268). Although obese adults had higher VAT in the present study, VAT:SAT ratio was not different between the two groups. This might suggest that any inverse effects of VAT on bone turnover through mechanisms such as low IGF-I or higher adiponectin, were outweighed by the greater positive effects of SAT on bone turnover.

Despite previous indications that abdominal adiposity may be detrimental to bone, the results of this Chapter show that adiposity in all compartments measured was positively associated with bone density and favourably associated with bone microarchitectural outcomes. As both trunk FM and android FM capture adiposity in both the subcutaneous abdominal and visceral compartments, this may explain why SAT was the strongest and most consistent predictor of bone microarchitecture, as it was not confounded by any effect of visceral adiposity. Furthermore, SAT was determined by CT, whereas trunk FM and android FM were determined by DXA, which is less reliable due to confounding effects of soft tissue thickness. As VAT:SAT ratio was not different in this population of obese individuals compared to the normal BMI individuals, further research into the effect of VAT:SAT imbalance on the biochemical milieu in obesity may lead to a better understanding of the effect of VAT on bone microarchitecture.

Confounding due to the use of DXA may also explain why WBLM better predicted aBMD at central sites, whereas WBFM better predicted vBMD at peripheral sites. While this could be a true physiological effect of body composition on measures of BMD at varying skeletal sites, there may be an effect of bone size on measures of aBMD by DXA, whereas vBMD by HR-pQCT is not affected by bone size. Interestingly, LS.vBMD was predicted by both LM and FM, possibly suggesting that central sites are better associated with LM but that FM is more closely associated with vBMD.

While others have reported higher sclerostin in individuals with high BMI, this was not found in the overall sample in the present study, but was found in the older adults alone (359, 369, 374). No difference in sclerostin was reported between bariatric surgery patients and controls, although the obese group was significantly shorter in stature than the controls, whereas the FAB study population are matched for height (441). Sclerostin was higher in men than women, consistent with the current literature (369, 377, 442). In

the present study, sclerostin was higher in younger adults than older adults. This is conflicting with the current literature which shows sclerostin increases with age (369, 442). Differences between studies may be attributed to variation in sclerostin immunoassays and heterogeneity of populations studied (375).

The main limitation to the work in this Chapter is the cross-sectional study design. The results therefore only indicate associations between adiposity, leptin and resorption, and it cannot be taken from this work alone that leptin is the cause of low bone resorption in obesity.

RANKL was not measured in this study due to low circulating levels being difficult to measure using available techniques. As there was no difference in OPG between obese and normal BMI individuals, it would be interesting to investigate the OPG:RANKL ratio to better understand how (and if) leptin regulates bone turnover by reducing osteoclastogenesis in obesity.

Much of the literature indicates a role for testosterone in the relationship between adiposity and bone density, however testosterone was not measured at part of the FAB study. Testosterone may play a role in regulating leptin and previous work has shown testosterone to be more strongly associated with leptin than E2, especially in men (343, 431). However, E2 has been shown to be more important for bone than testosterone in men (438, 439).

There are several other adipokines which may play a role in the relationship between adiposity and bone which were not investigated in this study. Resistin and visfatin are adipokines produced by white adipose tissue and positively associated with FM (443, 444). Resistin might be associated with osteoblast proliferation, and is expressed by circulating peripheral mononuclear cells and bone marrow which are associated with osteoclastogenesis (96, 335, 445). Visfatin is highly expressed in VAT (444), regulated by GH, inflammatory cytokines and glucocorticoids and increases production of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  (97). Visfatin may also play a role in insulin metabolism (444). Relationships between BMD and resistin and visfatin are unclear but appear to be weakly inverse or not significant (342, 446-448).

Only total leptin was measured in this study; whether free leptin is associated with lower bone resorption to an equal extent in obesity was not determined but may provide a more accurate marker of bone resorption in obesity than total leptin. Total serum adiponectin

was measured in the FAB study, and it is acknowledged that there may be differences in the effect of total serum adiponectin versus high molecular weight adiponectin on BMD (103, 105).

Contributions of bone marrow fat, brown fat and muscle fat were not assessed in this study. Based on current understanding of the function of each of these fat compartments it seems unlikely that any one of the compartments would exert a greater effect on bone density or microarchitecture than SAT, but it is recognised that each of these compartments is likely to exert some effect on the relationship between total adiposity, bone turnover and bone density. Future work should address such contributions.

Investigating whether age at onset of obesity has an effect on bone acquisition and bone size which persists through adulthood might increase current understanding of the mechanism by which obese individuals have greater bone density without a difference in bone size, compared to normal BMI individuals.

In conclusion, this Chapter has tested several hypotheses to better understand the mechanisms by which obesity might exert positive effects on bone density and bone microarchitecture. This Chapter has shown that obese individuals have lower bone turnover, mainly as a result of lower bone resorption, which is associated with lower bone formation. Lower resorption in obesity was associated with greater adiposity, best predicted by subcutaneous abdominal adiposity. Lower resorption might be driven by greater leptin production associated with the subcutaneous abdominal compartment. Oestrogen is associated with lower resorption independent of the effect of leptin. The greater effect of obesity on bone density and microstructural properties of bone in older adults might be explained by higher leptin, free E2 and bioavailable E2 levels and a greater effect of adiposity on leptin in older adults than young adults. Older adults also have a positive coupling index, whereas coupling index is not affected by BMI young adults.

## **CHAPTER 5:**

# **MUSCLE MASS AND PHYSICAL PERFORMANCE IN OBESITY**



## **CHAPTER 5: Muscle Mass and Physical Performance in Obesity**

### **Background**

Chapters 3 and 4 have shown that obese adults have greater BMD, favourable bone microarchitecture and greater bone strength than adults with a normal BMI, associated with lower bone turnover which might be driven by the effects of greater leptin and E2 in obesity. These traits are likely to contribute to the lower risk of hip and vertebral fracture in obesity. However, the greater risk of proximal humerus and ankle fracture in obesity, despite greater bone density and strength, remains unexplained. Greater risk of proximal humerus and ankle fracture in obesity could be due to greater propensity for falls, as older obese women have been shown to fall more frequently than non-obese individuals (23, 49, 261, 278). Furthermore, fall characteristics, such as fall direction, ability to break a fall and soft tissue influences on impact forces, may differ between obese and normal BMI individuals. The cause of greater fall frequency and differences in fall patterns between obese and normal BMI adults is unclear.

It could be that greater fall frequency or differences in fall patterns between obese and normal BMI individuals are related to low LM and/or impaired muscle function. Several age-related changes in muscle tissue are also common to obesity. With age, changes in muscle tissue include decreases in fibre number and fibre atrophy, conversion to type 1 fibres, denervation and excitation–contraction uncoupling (449). These changes contribute to a loss of strength and impaired physical function, potentially associated with a greater number of falls.

It is possible that age related changes in muscle composition and function are exaggerated in obese individuals due to greater fat infiltration of skeletal muscle, or myosteatosis, in obesity (450, 451). Studies have shown fat infiltration of the muscles of the lower limb to be associated with lower muscle power and strength, poor neurological and physical function and greater fracture risk (450, 452-457). Muscle fat may also be associated with the release of inflammatory factors which may impair muscle function and physical performance (457). The extent of intramuscular fat infiltration can be identified from assessments of muscle density on pQCT or MRI imaging, where lower muscle density on the image indicates greater intramuscular fat infiltration.

### **Muscle Mass, Muscle Strength and Physical Performance**

With ageing, LM and muscle strength deteriorate along different time courses, with the loss of muscle strength occurring more rapidly than the loss of LM (458). Age-related decreases in LM occur at a rate of approximately 1 to 2% per year from the fifth decade, while the loss of muscle strength occurs at a rate of approximately 1.5% per year, increasing to 3% in the sixth decade (459). Therefore, if obesity is associated with greater decreases in LM with age compared to non-obese individuals, there may be considerable loss of muscle strength. Dissociation of the loss of LM and muscle function could affect physical performance in obesity even if LM is greater or indifferent in obese individuals compared to non-obese individuals.

### **Frailty, Sarcopenia, and Dynapenia**

Individuals at risk of functional decline due to muscular deterioration can be defined in several ways. Previously, individuals may have been described as frail, characterised by the presence of three or more of the following indicators: weight loss, weakness by grip strength in the lowest 20<sup>th</sup> percentile, self-reported exhaustion, slowness by fifteen foot walk speed in the lowest 20<sup>th</sup> percentile and low physical activity (460). Several additional overlapping, conditions have been described, which differ in whether they are characterised by a loss of LM, muscle function, or both. The term sarcopenia was coined initially by Rosenberg from the Greek words sarx (flesh) and penia (loss) (461). Thus traditionally sarcopenia was considered a condition purely concerning the loss of LM. At present, no standard definition of sarcopenia exists, although this is a topic of much debate. Recently, sarcopenia has been used to describe the loss of both LM and muscle function. Dynapenia has been used to describe the loss of muscle strength, power and force not caused by neurologic or muscular disease (462). There is currently no standard definition or standard diagnostic criteria for dynapenia, but dynapenia is typically inferred from performance of a range of physical function tests, such as isometric strength and/or tests of static and dynamic performance.

It has been suggested that dynapenia may be a better predictor of physical function than the pure loss of LM, as declines in LM explain a relatively low percentage of the variability in muscle strength decline and maintaining LM does not necessarily prevent the loss of muscle strength with age (458). Loss of muscle strength and loss of appendicular LM (ALM) have been shown not to correlate (463) and to be dissociated in obesity (464); hence their distinction may be of importance. BMI has been shown to inversely predict the loss of ALM; predicting 83% of the variation in loss of ALM, whereas BMI only predicted 3% of the variance in loss of muscle strength (463). Whether obese individuals

are more appropriately categorised as sarcopenic or dynapenic is not clear, but it may be important to distinguish the two conditions, to best identify obese individuals at the greatest risk of falls.

### **Sarcopenia, Bone Density, Falls and Fractures**

Sarcopenia is positively associated with the prevalence of osteoporosis (465-468). This may be due to reduced muscle-bone interactions and lower forces exerted on bone by the muscle, as well as indirect associations, such as lower levels of physical activity.

In young obese individuals, muscle area was positively associated with L4 Tb.vBMD (269), Tb.N (268) and estimated failure load (268). LM was positively associated with aBMD, Ct.Th and bending strength but was not associated with vBMD at the FN or distal radius (469). Thigh muscle density trended positively with bone strength in young obese men, indicating lower muscle fat might have a positive effect on bone strength (268). Muscle area and density may be associated with bone formation (269). Although in Chapter 4 it was shown that WBLM was not a predictor of bone microarchitecture or vBMD, LM was a predictor of aBMD at central sites. It is possible that individual compartments of LM, such as ALM, or the size and density of individual muscle groups might affect bone density and microstructure irrespective of the effect of total body LM.

In addition to effects on BMD and bone microstructure, low LM is associated with impaired balance and an increased risk of falls (469, 470). Di Monaco et al. showed a high prevalence of sarcopenia in women with hip fracture (468). Physical performance is inversely associated with the risk of falls (471-473). Gait speed, chair stand ability and overall physical performance scores have been associated with prevalent and incident hip and vertebral fracture (471, 474). Greater intramuscular fat is also associated with fracture risk (452, 475, 476).

Not only might obese individuals with sarcopenia or dynapenia fall more frequently than non-affected individuals, but fall direction, fall forces such as torsion or compression, and fall impact could also be affected by poor physical function. Such differences in fall kinetics may contribute to the site specific fracture risk in obesity.

Differences in body fat distribution may affect balance; with abdominal adiposity able to displace the centre of mass, for example (477). Alterations in foot structure may also develop in response to high body weight and this could affect postural stability and gait (478). Obesity is associated with pes planus, a pronated foot type, greater rear-foot

eversion and foot abduction, all of which may contribute to altered postural stability and increased risk of ankle fracture, particularly Webber C ankle fracture, typically observed with greater BMI (59, 478).

It is not known whether differences in fall characteristics in obesity can be explained by poorer physical performance, sarcopenia, dynapenia or anatomical factors.

### **Mechanisms Linking Obesity, Sarcopenia and Falls**

Several biochemical factors which have been identified as markers for sarcopenia are also common to obesity. Vitamin D (479), albumin (480, 481) and IGF-I (482, 483) are positively associated with physical performance, but are all lower in obese individuals than normal BMI individuals (Chapter 4). While albumin may exert protective effects on LM through associations with inflammatory markers (480), IGF-I prevents myocyte apoptosis (482). There may be links between 25OHD and the accumulation of muscle fat (484).

Muscle tissue is the main site for glucose uptake and muscle strength is associated with higher insulin sensitivity and muscular glucose uptake (485). As circulating glucose is higher and insulin sensitivity lower in obese individuals than normal BMI individuals (Chapter 4), there may be impaired regulation of glucose metabolism by skeletal muscle in obesity, associated with lower muscle strength. The formation of advanced glycation end-products is increased in obesity (486) and linked to muscle stiffness and poorer physical function (487, 488).

There may be associations between sex hormones, sarcopenia and poor physical function (489). Such associations may be age dependent, as no significant associations were observed between free testosterone or fE2 and thigh muscle area or density in young women (269). As testosterone and E2 are abundant in young women, there may be a threshold effect before associations can be detected.

Inflammatory cytokines are up-regulated in obesity, and have been associated with sarcopenia and poor physical performance (490-492).

Obese individuals have lower HDL, which is positively associated with physical performance (493, 494). Distribution of adiposity may affect the relationship between obesity, HDL and physical performance, with lower HDL in dynapenic adults with abdominal obesity than non-abdominally obese dynapenic adults and controls (495).

Creatine kinase (CK) is an enzyme which converts creatine to phosphocreatine and circulating levels increase with muscle degeneration (496). CK has been shown to be greater in obesity, although without adjustment for LM it is unclear whether this reflects greater LM or more muscle degeneration in obesity (496).

Finally, anaemia may be more prevalent in obesity and has been associated with poor physical performance, low muscle strength and an increased risk of falls (497-501).

### **Summary**

The site-specificity of fracture risk in obesity, despite greater BMD, favourable cortical and trabecular microarchitecture and bone strength, may be attributed to falls mechanisms. Fall characteristics and greater fall risk in obesity may be attributed to impaired LM, poor physical function or both. Obese individuals exhibit multiple biochemical traits associated with impaired LM and/or poor muscle function. Fall characteristics and greater risk of falls might necessitate alternative fracture prevention strategies in obese individuals for the prevention of ankle and proximal humerus fracture, and highlight a need for active fracture prevention despite 'normal' BMD by densitometry.

In light of a recent recommendation that "a combination of serum markers, diagnostic imaging, and functional tests of muscle function would constitute an ideal biomarker panel" for sarcopenia (502), this Chapter aims to evaluate LM, physical function, and sarcopenia prevalence in an obese and normal BMI population, using biochemical markers, imaging and functional tests, to determine whether non-skeletal factors such as, but not limited to greater fall frequency, might explain fracture risk in obesity.

### **Research Questions and Aims**

Research Questions:

1. Are there differences in WBLM or ALM between normal BMI and obese individuals?
2. Do any differences in LM between normal BMI and obese individuals result in differences in muscle function and physical performance by BMI?
3. Is the prevalence of sarcopenia different in normal BMI and obese groups?
4. Do obese people fall more frequently than those with a normal BMI?

#### Aims:

1. To compare LM in normal BMI and obese individuals to determine associations between obesity and LM.
2. To compare muscle function in normal BMI and obese individuals to determine associations between obesity and physical performance.
3. To compare the prevalence of sarcopenia in normal BMI and obese adults and ascertain any associations between sarcopenia and BMD, bone structure and strength, and fall frequency.
4. To compare reported fall history in obese and normal BMI groups and determine whether fracture patterns in obesity might be explained by greater falls risk despite greater BMD.

#### **Methods**

Number of falls in the six months prior to consent and perceived difficulty completing habitual tasks such as climbing and descending stairs, dressing and grooming oneself, walking various distances etc. were self-reported in the lifestyle questionnaire described in Chapter 2.

Full details on all assays can be found in Chapter 2. CK and lipid profile were measured by automated ECLIA (Cobas c701, Roche Diagnostics, Mannheim, Germany). Haemoglobin was measured by automated analyser from the full blood count analysis (Sysmex XN Series, Sysmex, Norderstedt, Germany). Glucose was measured by automated immunoassay (Cobas c702 auto-analyser, Roche Diagnostics, Mannheim, Germany). HsCRP was measured by automated nephelometry (BNII System, Siemens, Siemens Healthcare Diagnostics, Surrey, UK). IL-6 was measured by automated immunoassay (Cobas e601 auto-analyser, Roche Diagnostics, Mannheim, Germany). Albumin and 25OHD were measured by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). IGF-I was measured by automated CLIA (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK).

SHBG was measured by automated ECLIA (Cobas e602 auto-analyser, Roche Diagnostics, Mannheim, Germany).

Total E2 was determined by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany).

Free E2 (fE2) was determined as:

$$fE2 \text{ (mol/L)} = (-b + \sqrt{b^2 - 4ac}) / 2a$$

Where:  $N = 1 + \text{affinity constant of albumin for E2} \times \text{albumin}$

$a = N \times \text{affinity constant of SHBG for E2}$

$b = N + \text{affinity constant of SHBG for E2} \times (\text{SHBG} - \text{Total E2})$

$c = -\text{Total E2}$

Affinity constant of albumin for E2 =  $4.21 \times 10^4$  L/mol

Affinity constant of SHBG for E2 =  $3.14 \times 10^8$  L/mol

Bioavailable E2 (bioE2) was calculated as:

$$\text{bioE2 (mol/L)} = (1 + \text{affinity constant of albumin for E2} \times \text{albumin}) \times fE2$$

Whole body LM was determined by DXA (Discovery A, Hologic Inc., Bedford, MA, USA) and ALM was calculated as the sum of the LM of the arms and legs (Chapter 2).

SPPB score was calculated from performance in a repeated chair stand test, six metre walk to determine gait speed, and a six metre narrow walk to determine balance. Muscle strength was determined from maximal hand grip strength (Chapter 2).

Sarcopenia was defined using the European Working Group on Sarcopenia in Older Persons (EWGSOP) definition (249). Individuals were categorised as:

- 5) Not sarcopenic (Normal ALM)
- 6) Pre-sarcopenic (Low ALM)
- 7) Sarcopenic (Low ALM and either weakness or poor SPPB score)
- 8) Severely sarcopenic (Low ALM, weakness and poor SPPB score)

Low ALM was defined as an ALM corrected for height ( $\text{ALM (kg)} / (\text{height (m)}^2)$ ) (Skeletal muscle index (SMI))  $< 7.23 \text{ kg/m}^2$  (men) or  $< 5.67 \text{ kg/m}^2$  (women) (249). Weakness was defined as a maximal hand grip strength  $< 30 \text{ kg}$  (men) or  $< 20 \text{ kg}$  (women). A poor SPPB score was defined as a SPPB score  $\leq 8$ .

## **Statistical Analysis**

One older female participant was excluded from the analysis of chair stand performance and SPPB score as she was deemed unable to complete the repeated chair stand safely due to her hip width exceeding the chair arm parameters. This was not recorded as 'unable to complete the test' as I made the decision not to proceed from the single chair stand performance, rather than the participant's failure to attempt or complete the repeated chair stand test.

All variables were assessed for normality and log transformed where necessary. As paired t-tests were to be used to determine significant differences between normal BMI and obese groups, the absolute differences between the matched pairs were assessed for normality. Where the difference between the pairs was skewed, the raw data was log transformed and the differences re-evaluated for normality.

Paired samples t-tests were used to determine significant differences in LM and physical function between normal BMI and obese groups, for the entire sample, by age group and by age and gender. Where transformed values remained non-normal (gait speed, WBLM, SMI), the Wilcoxon Signed Rank test was used. Chi-squared test was used to determine the association between BMI group and sarcopenia classification.

Univariate general linear models were used to identify whether age group, gender and BMI had an effect on LM and physical performance outcomes and to identify interactions between age or gender and the effect of BMI on LM and physical function.

Pearson correlation coefficient was used to determine associations between logged LM and logged function parameters and between logged biochemical mediators and logged function parameters. Spearman's rank correlation coefficient was used where the sample distribution remained non-normal. Multiple linear regression was used to determine the association between adiposity and physical performance, adjusting for possible confounders.

Analysis was performed using IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.). Significance was accepted when  $p < 0.05$ .

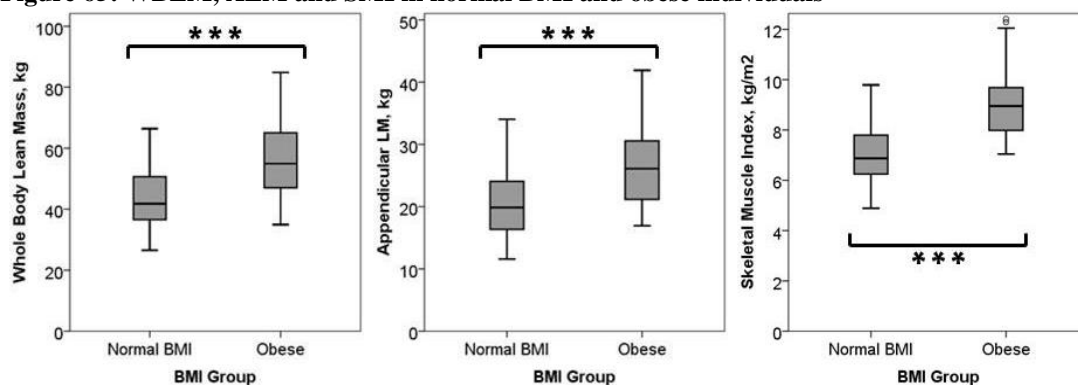


## Results

### Muscle Mass in Obesity

Obese individuals had greater WBLM, ALM and SMI than normal BMI individuals (all  $p < 0.001$ ) (Figure 65).

Figure 65: WBLM, ALM and SMI in normal BMI and obese individuals<sup>1,2</sup>



Young adults had greater WBLM, ALM and SMI than older adults (all  $p < 0.001$ ). Men had greater WBLM, ALM and SMI than women (all  $p < 0.001$ ). There were no interactions between age or gender and the effect of BMI on WBLM or ALM, but there was a greater effect of BMI on SMI in women ( $p < 0.01$ ). Multiple linear regression showed the effect of BMI on WBLM, ALM and SMI remained significant after adjusting for age and gender (all  $p < 0.001$ ).

### Physical Performance in Obesity

#### Grip Strength

There was no difference in grip strength between obese and normal BMI individuals. Younger adults had greater grip strength than older adults ( $p < 0.01$ ). Men had a greater grip strength than women ( $p < 0.001$ ). There were no interactions between age or gender and the effect of BMI on grip strength. After adjusting for age and gender, there remained no effect of BMI on grip strength.

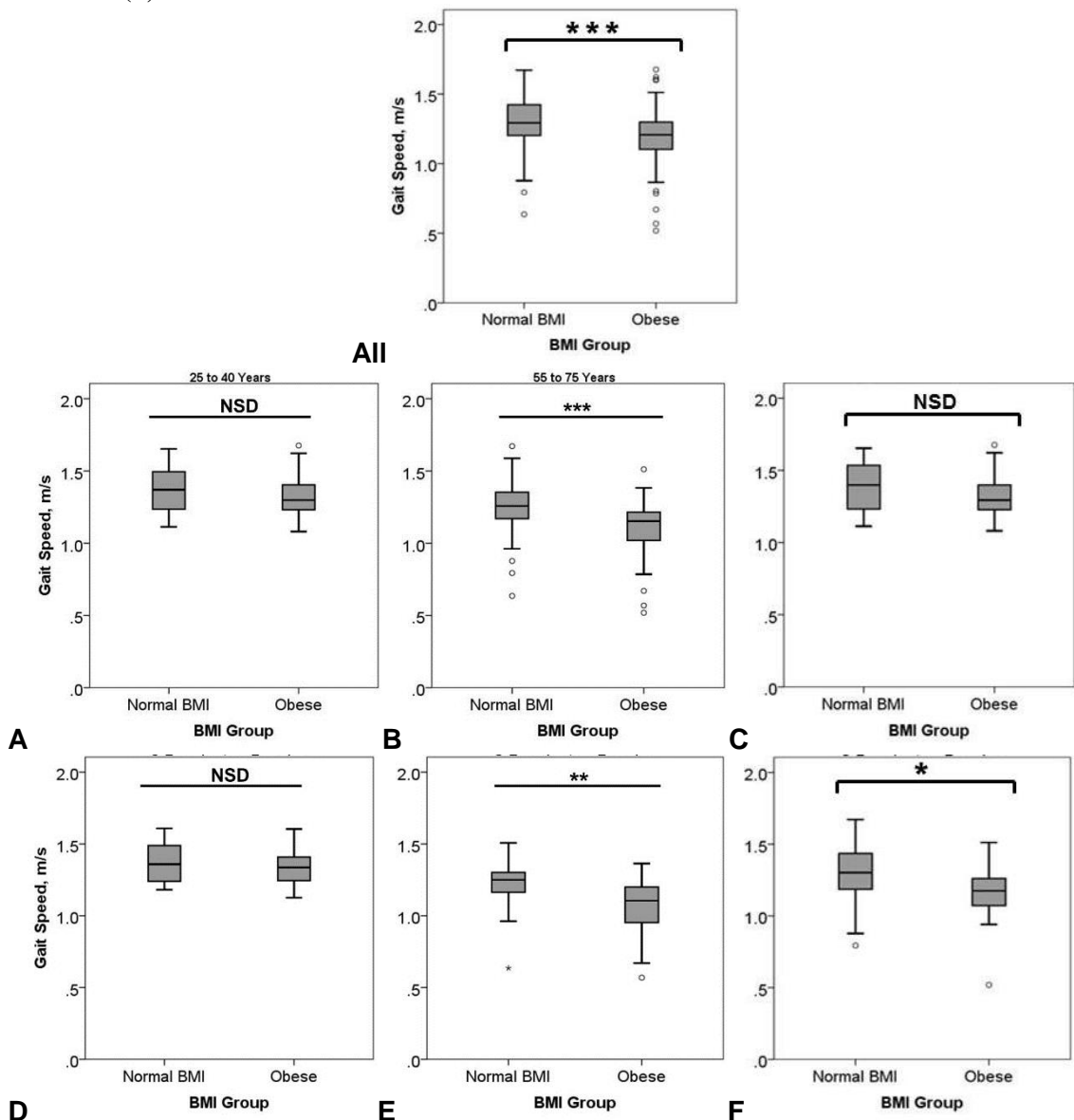
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## Gait Speed

Obese individuals had slower gait speed than normal BMI individuals ( $p < 0.001$  ages combined and older adults, younger NSD) (Figure 66). Older adults had slower gait than young adults ( $p < 0.001$ ). There was an interaction between age and BMI on gait speed such that the effect of BMI on gait speed was greater in older adults ( $p < 0.05$ ). There was no effect of gender on gait speed. Multiple linear regression showed adjusting for age, the effect of BMI on gait speed remained significant ( $p < 0.001$ ).

**Figure 66: Gait speed in normal BMI and obese individuals**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>**



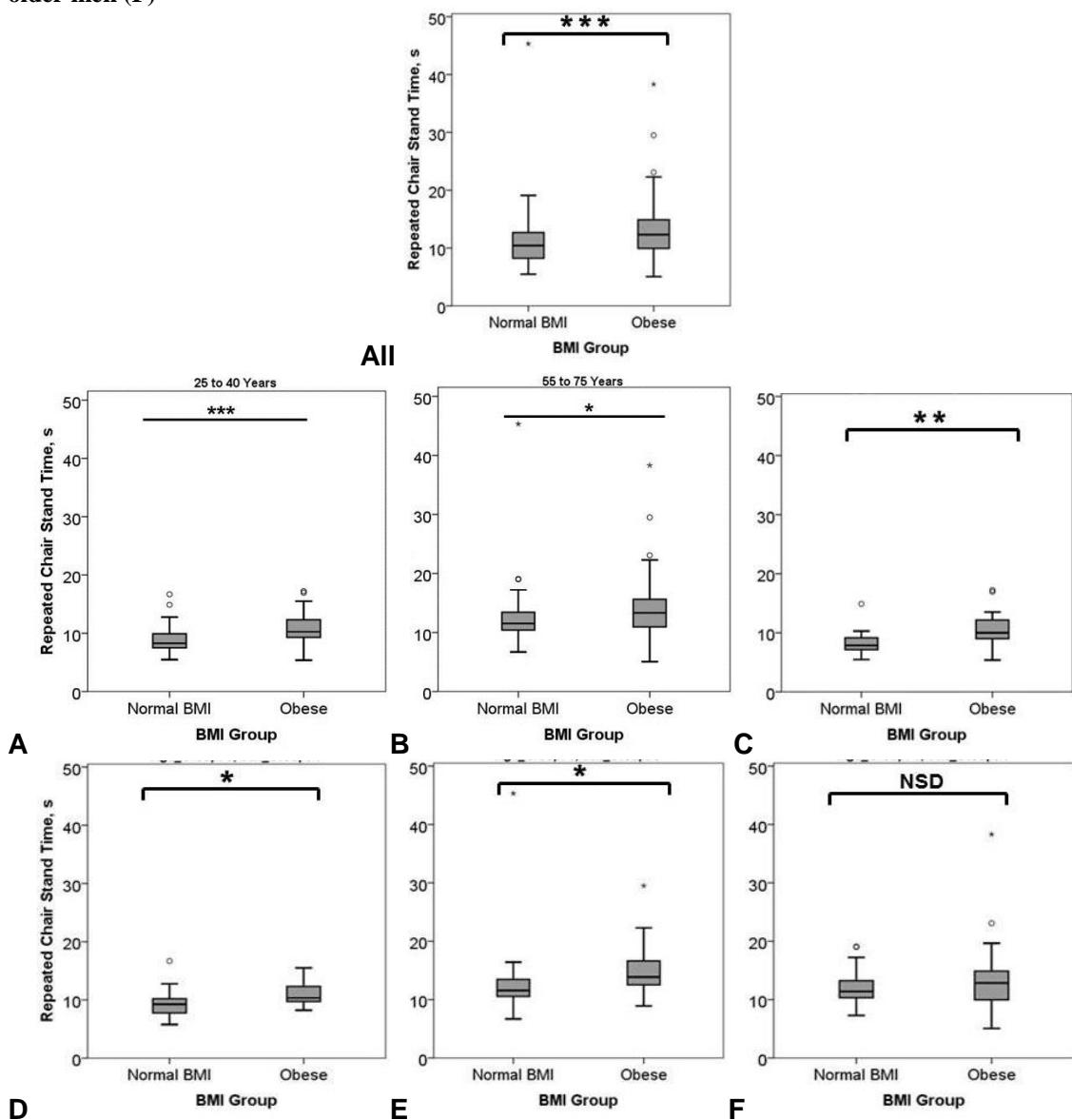
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## Chair Stand Time

Obese individuals took longer to complete the repeated chair stand test than normal BMI individuals ( $p < 0.001$  combined,  $p < 0.001$  younger,  $p < 0.05$  older) (Figure 67). Older adults took longer to complete the test than young adults ( $p < 0.001$ ). There was no effect of gender on chair stand time. There was an interaction between age and BMI on chair stand time such that the effect of BMI on chair stand time was greater in the younger adults ( $p < 0.05$ ). Multiple linear regression showed, the effect of BMI on chair stand time remained significant after adjusting for age ( $p < 0.001$ ).

**Figure 67: Repeated chair stand time for normal BMI and obese individuals**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E), older men (F)**<sup>1,2</sup>



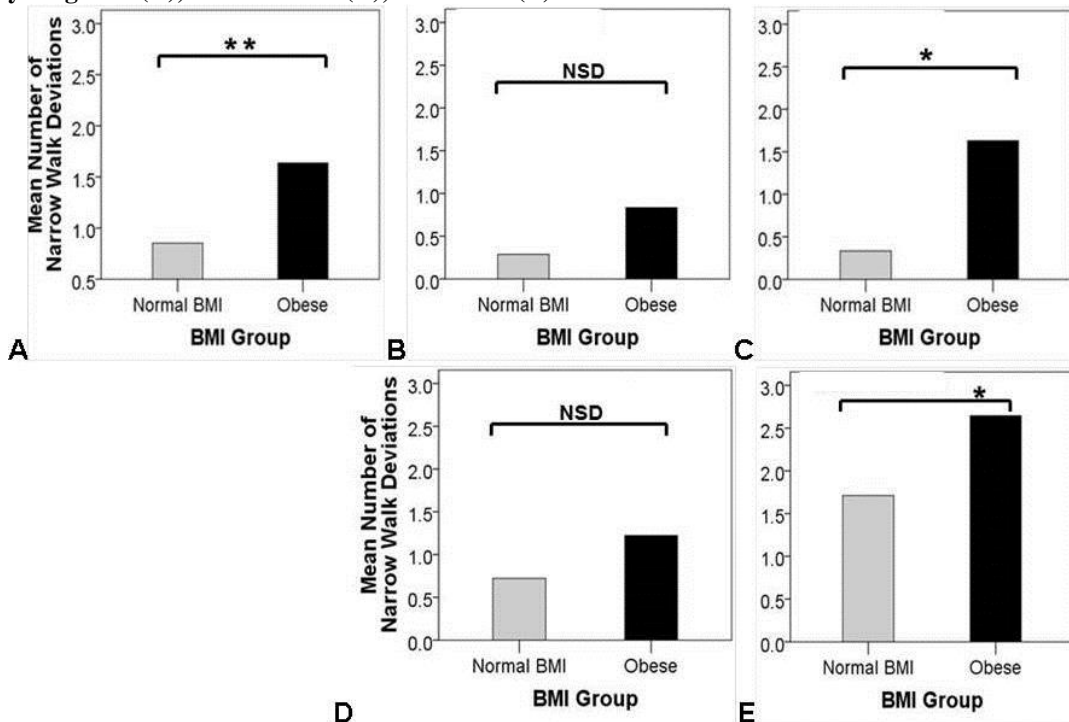
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## Balance

Obese individuals had poorer balance assessed by the narrow walk test than normal BMI individuals ( $p < 0.01$  all and older, younger did not differ) (Figure 68). Older adults had poorer balance than young adults ( $p < 0.001$ ). Men had poorer balance than women ( $p < 0.001$ ). There were no interactions between age or gender and the effect of BMI on balance. Multiple linear regression showed the effect of BMI on balance remained significant after adjusting for age and gender ( $p < 0.001$ ).

**Figure 68: Balance in normal BMI and obese individuals determined by number of deviations on a narrow walk. Groups combined (A), young women (B), young men (C), older women (D), older men (E)<sup>1,2</sup>**



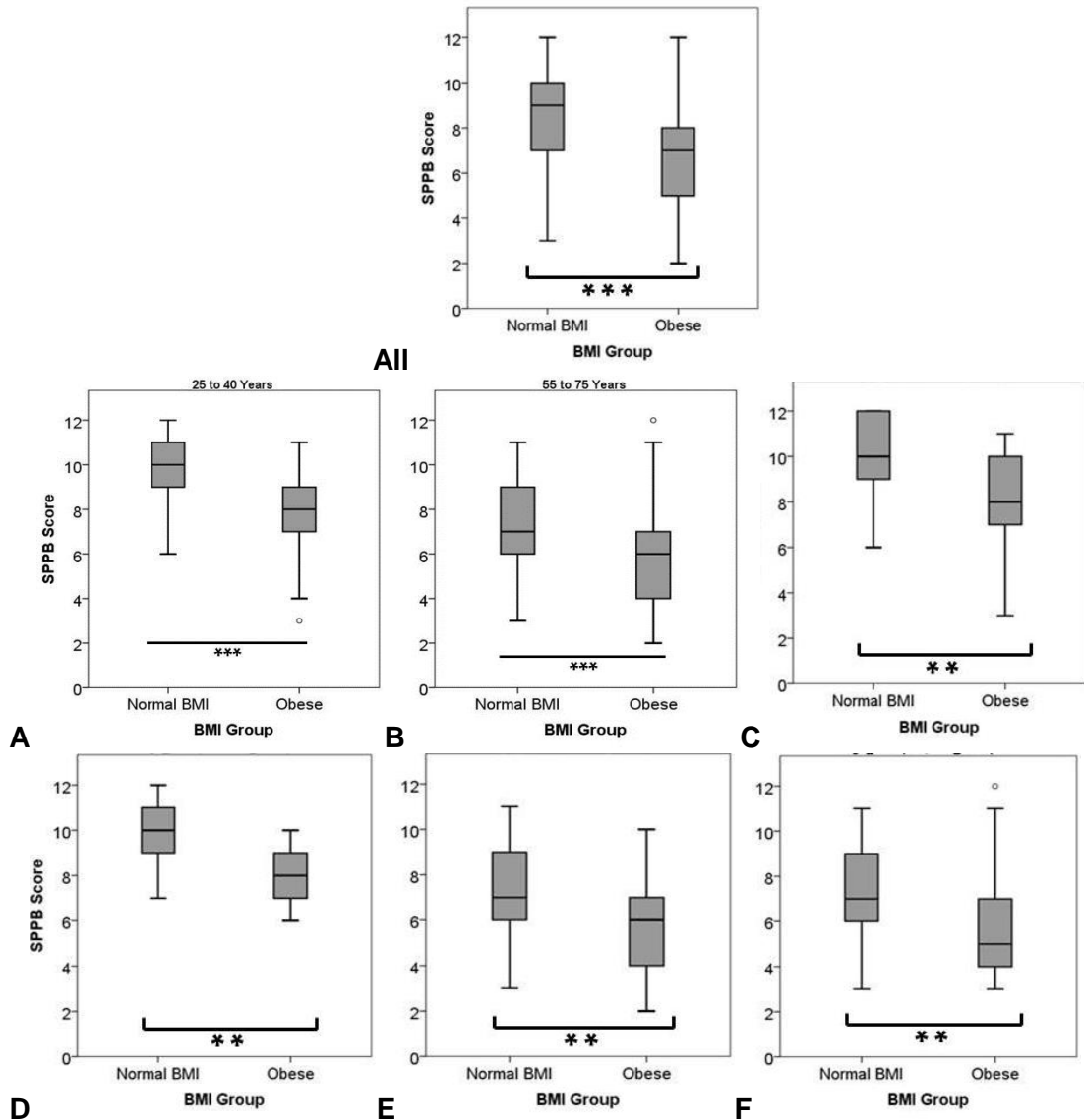
## SPPB Score

Obese individuals had lower SPPB scores than normal BMI individuals (all  $p < 0.001$ ) (Figure 69). Older adults had lower SPPB scores than young adults ( $p < 0.001$ ). There was no effect of gender on SPPB score or interactions between age or gender and BMI on SPPB score. The relationship between BMI and SPPB score remained significant after adjusting for age and gender ( $p < 0.001$ ). A model consisting of age, BMI and PTH best predicted SPPB score (adjusted R square 0.473, overall model  $p < 0.001$ , BMI beta -0.478,  $p < 0.001$ , age: beta -0.511,  $p < 0.001$ , PTH: beta 0.125,  $p < 0.05$ ).

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

**Figure 69: SPPB score of normal BMI and obese individuals**  
**Groups combined (All), young (A), older (B), young women (C), young men (D), older women (E), older men (F)<sup>1,2</sup>**



### Associations Between LM and Physical Performance in Obesity

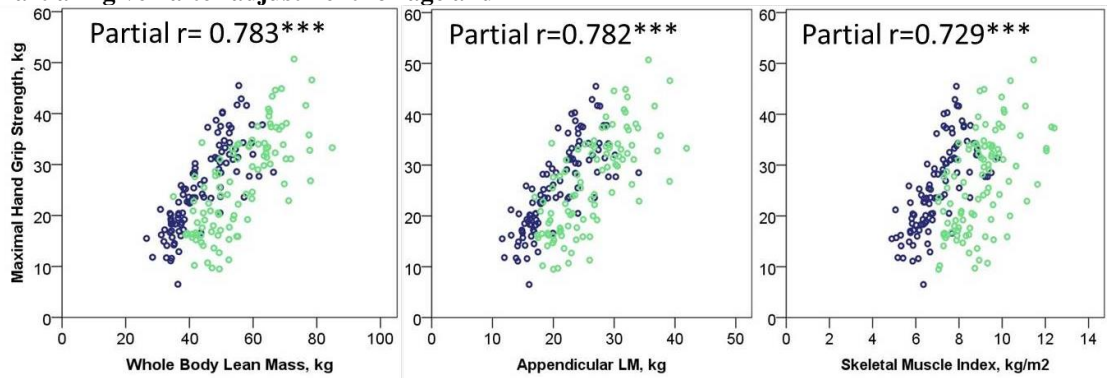
Grip strength was associated with SPPB score ( $r=0.157$ ,  $p<0.05$ ), gait speed ( $r=0.351$ ,  $p<0.001$ ), balance ( $r=0.160$ ,  $p<0.05$ ) and chair stand time ( $r=-0.198$ ,  $p<0.01$ ).

WBLM, ALM and SMI were positively associated with grip strength (WBLM  $r=0.963$ , ALM  $r=0.704$ , SMI  $r=0.498$ , all  $p<0.001$ ). After adjustment for age and BMI, associations between LM and grip strength remained positive (all  $p<0.001$ ) (Figure 70).

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , NSD no significant difference

**Figure 70: Association between LM and grip strength**  
**WBLM (Left), ALM (Centre), SMI (Right), normal BMI=blue, obese =green**  
**Partial r given after adjustment for age and BMI**

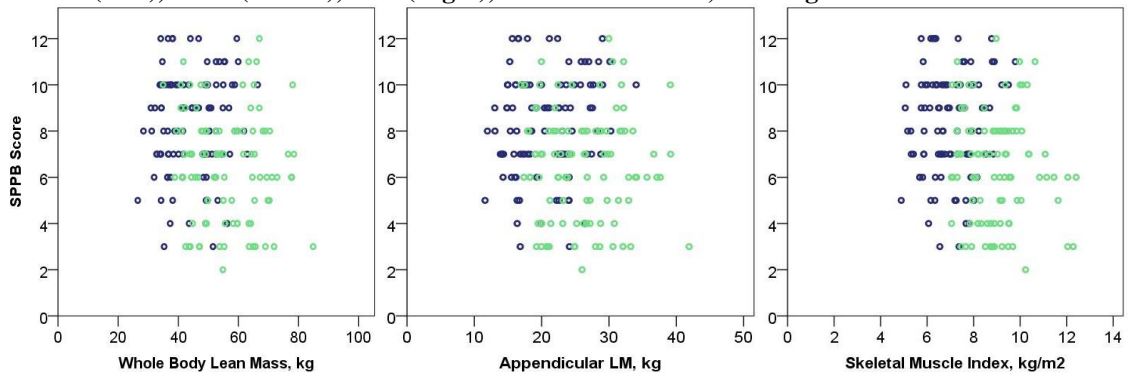


Chair stand time was associated with gait speed ( $r=-0.596$ ,  $p<0.001$ ) and poor balance ( $r=1.71$ ,  $p<0.05$ ). Slower gait was associated with poorer balance ( $r=-0.184$ ,  $p<0.05$ ).

In unadjusted analyses, WBLM, ALM and SMI were positively associated with number of walk deviations (WBLM  $r=0.348$ , ALM  $r=0.317$ , SMI  $r=0.342$ , all  $p<0.001$ ), but not with chair stand time or gait speed. After adjustment for age and BMI, WBLM, ALM and SMI were positively associated with number of walk deviations (partial correlations: WBLM  $r=0.266$ , ALM  $r=0.258$ , both  $p<0.001$ , SMI  $r=0.230$ ,  $p<0.01$ ) and gait speed (partial correlations: WBLM  $r=0.166$ , ALM  $r=0.182$ , both  $p<0.05$ , SMI  $r=0.190$ ,  $p<0.01$ ), but there remained no association with chair stand time.

WBLM and SMI were negatively correlated with SPPB score (WBLM  $r=-0.179$ ,  $p<0.05$ , SMI  $r=-0.222$ ,  $p<0.01$ ), but there was no association between ALM and SPPB score (Figure 71). After adjustment for age and BMI, WBLM, ALM and SMI were no longer associated with SPPB score.

**Figure 71: Association between LM and SPPB score**  
**WBLM (Left), ALM (Centre), SMI (Right), normal BMI=blue, obese =green**



### Sarcopenia in Obesity

Based on the EWGOSP definition, 2% of the total study population were pre-sarcopenic, 4% sarcopenic and 7.1% severely sarcopenic. There was a significant association between BMI group and sarcopenia status (Chi square,  $p < 0.01$ ). 5.1% of young normal BMI individuals were sarcopenic. 3.3% of older normal BMI individuals were pre-sarcopenic, 3.3% sarcopenic and 11.7% severely sarcopenic. None of the obese individuals were sarcopenic, due to their greater LM.

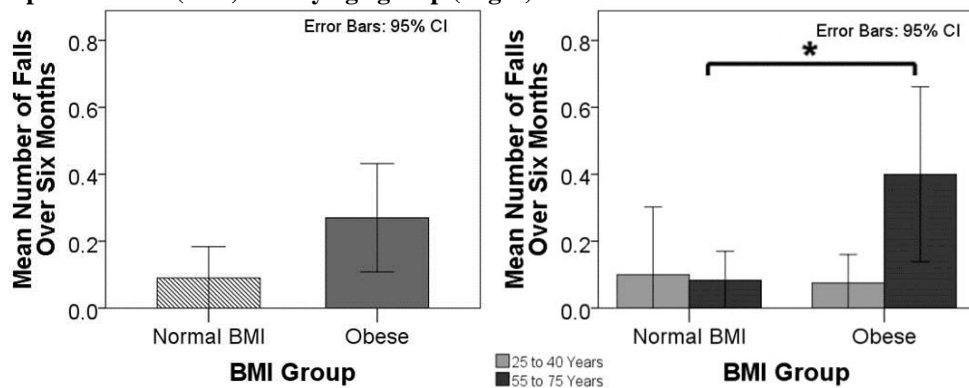
Based on SMI alone (SMI criteria: women  $< 5.67 \text{ kg/m}^2$ , men  $< 7.23 \text{ kg/m}^2$ ), 13% of all, 5% of young and 18.3% of older adults with a normal BMI were sarcopenic. No obese individuals were classed as sarcopenic, due to their greater LM. There was a significant association between BMI group and sarcopenia status (Chi square  $p < 0.001$ ).

In contrast, based on SPPB score alone, as a proxy for dynapenia, 49.5% of all, 20.5% of young and 68.3% of older normal BMI adults were dynapenic. 82.8% of all, 64.1% of young and 95% of older obese individuals were dynapenic. There was a significant association between BMI group and dynapenia status (Chi square,  $p < 0.01$ ).

### Falls in Obesity

Older obese adults reported more falls than older adults with a normal BMI (ages combined  $p = 0.066$ , younger NSD, older  $p < 0.05$ ) (Figure 72). 15% of all obese adults, 5% of young obese adults and 10% of older obese adults had fallen in the 6 months prior to recruitment, compared to 5%, 1.3% and 3.3% in the respective normal BMI groups. There was no effect of gender on reported number of falls.

**Figure 72: Mean number of falls in normal BMI and obese individuals**  
Age groups combined (Left) and by age group (Right)



### **Risk Factors for Falls in Obesity**

Number of falls was not associated with grip strength, balance or LM (WBLM, ALM and SMI). Number of falls was associated with chair stand time ( $r=0.204$ ,  $p<0.001$ ), gait speed ( $r= -0.214$ ,  $p<0.01$ ) and SPPB score ( $r= -0.233$ ,  $p<0.01$ ).

There was no difference in alcohol intake between obese and normal BMI groups, with a mean (SD) 6.46 (6.1) unit intake per week in obese and 7.18 (6.4) units per week in normal BMI individuals. There was no association between alcohol intake and falls.

There was a similar prevalence of neurological conditions ( $n=6$  obese,  $n=7$  normal BMI), respiratory conditions including asthma and COPD ( $n=14$  obese,  $n=13$  normal BMI), psychological conditions including depression ( $n=2$  obese,  $n=3$  normal BMI) between groups. Musculoskeletal conditions including osteoarthritis away from study sites were more common in obesity ( $n=23$  obese,  $n=13$  normal BMI).

There was no difference in the number of hours of physical activity completed by obese and normal BMI groups (obese mean (SD) 8.5 (15.2) hours, normal BMI mean (SD) 10.5 (14.7) hours). There was no association between number of hours of activity and falls. There was no difference in estimated METs (1 MET being the amount of oxygen consumed while sitting at rest) expended per week between obese (mean (SD) 35.05 (69.8) METs) and normal BMI individuals (mean (SD) 47.01 (62.7) METs). There was no association between METs expended per week and falls.

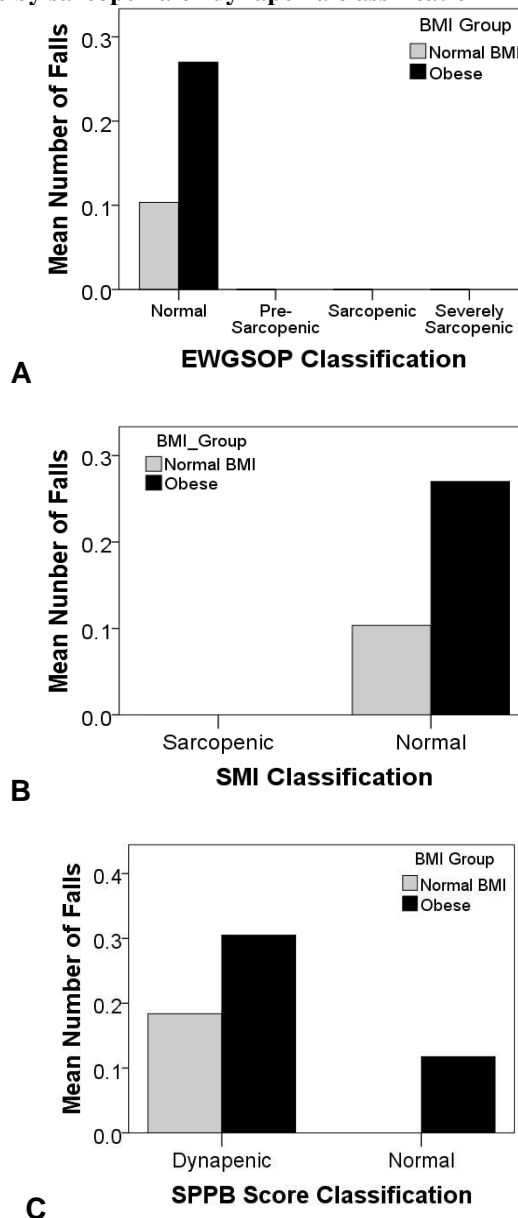
Obese individuals had lower habitual mobility scores (mean (SD) 22.57 (2.9)) than normal BMI individuals (mean (SD) 23.55 (2.1)) indicating greater difficulty completing habitual activities such as walking, climbing stairs and personal care (all  $p<0.01$ ). Older adults had lower mobility scores than younger adults (Univariate GLM,  $p<0.01$ ). There was no effect of gender on habitual mobility score. After adjusting for age, the effect of BMI on habitual mobility score remained significant ( $p<0.001$ ). Habitual mobility score was negatively correlated with number of falls ( $r= -0.393$ ,  $p<0.001$ ).



## Sarcopenia and Falls in Obesity

Figure 73 shows the number of falls reported by obese and normal BMI individuals, by the EWGSOP and alternative definitions. Figure A illustrates that obese individuals reported more falls than those with a normal BMI, but were not classified as sarcopenic by the EWGSOP criteria (due to their higher LM). Figure B further demonstrates that obese individuals fell more, despite greater LM. Figure C shows that obese individuals with poor physical performance, irrespective of LM, reported more falls.

**Figure 73: Number of falls by sarcopenia or dynapenia classification**

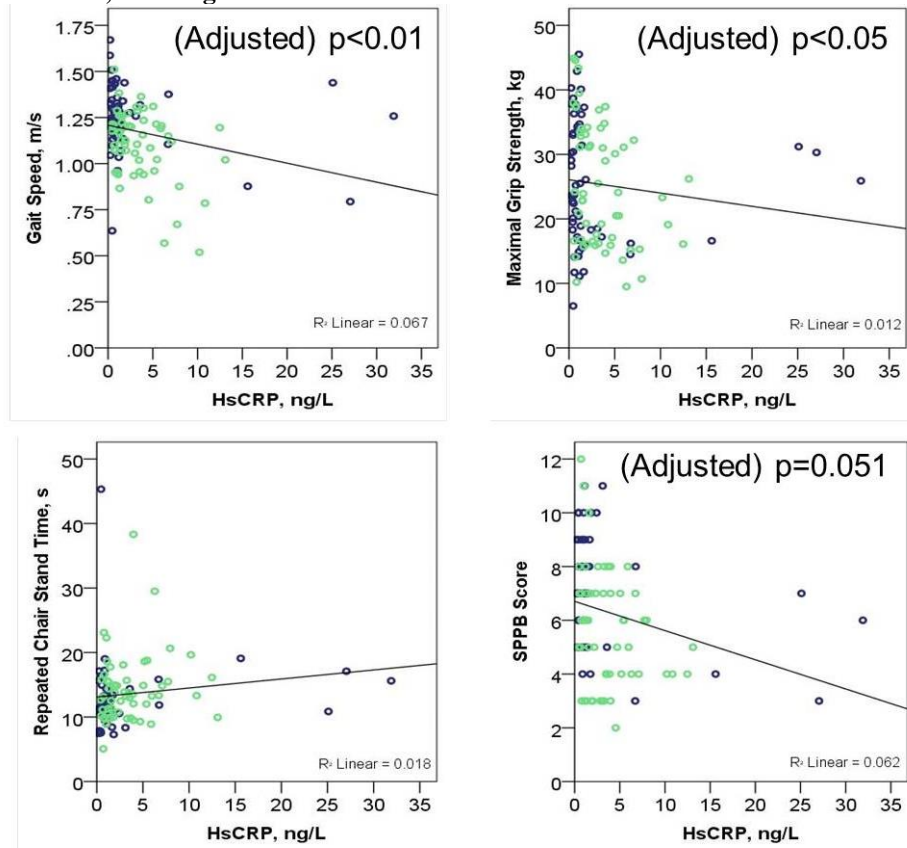


## Potential Biochemical Mediators of Poor Physical Function in Obesity Inflammation, LM and Physical Performance

HsCRP was not correlated with WBLM or ALM but was positively associated with SMI ( $r=0.249$ ,  $p<0.01$ ). After adjustment for age and BMI, HsCRP was negatively correlated with WBLM ( $r=-0.210$ ), ALM ( $r=-0.223$ ) and SMI ( $r=-0.224$ ) (all  $p<0.05$ ).

HsCRP was negatively associated with gait speed ( $r=-0.361$ ,  $p<0.001$ ) and grip strength ( $r=-0.252$ ,  $p<0.01$ ), and positively associated with chair stand time ( $r=0.227$ ,  $p<0.05$ ). HsCRP was inversely associated with SPPB score ( $r=-0.354$ ,  $p<0.001$ ). After adjustment for age and BMI, HsCRP remained negatively associated with gait speed and grip strength, but was no longer associated with chair stand time or SPPB score (Figure 74).

**Figure 74: Associations between HsCRP and physical function**  
Normal BMI=blue, obese =green

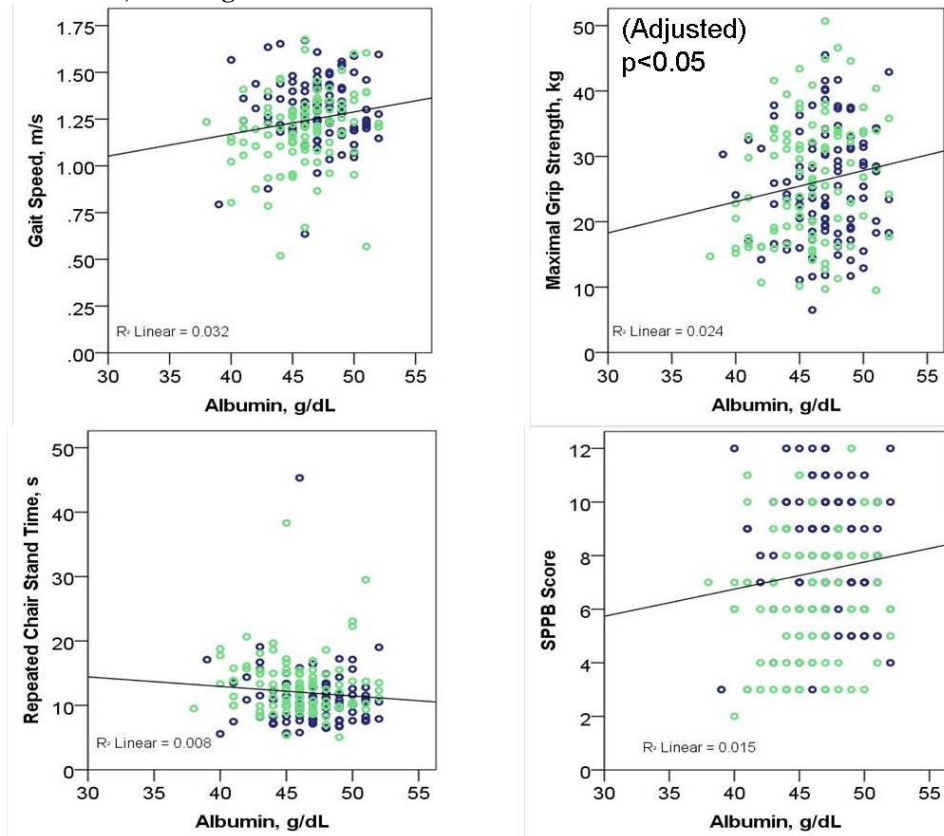


### Albumin, LM and Physical Performance

Albumin was inversely correlated with HsCRP ( $r=-0.448$ ,  $p<0.001$ ) but not associated with WBLM, ALM or SMI. After adjustment for age and BMI, albumin was positively associated with WBLM ( $r=0.263$ ), ALM ( $r=0.264$ ) and SMI ( $r=0.276$ ) (all  $p<0.001$ ).

Albumin was positively correlated with gait speed ( $r=0.171$ ), grip strength ( $r=0.151$ ) and SPPB score ( $r=0.159$ ) (all  $p<0.05$ ) but not associated with chair stand time. After adjustment for age and BMI, albumin was positively associated with grip strength ( $r=0.127$ ,  $p<0.05$ ) but not associated with gait speed, chair stand time or SPPB score (Figure 75).

**Figure 75: Associations between albumin and physical function**  
Normal BMI=blue, obese =green

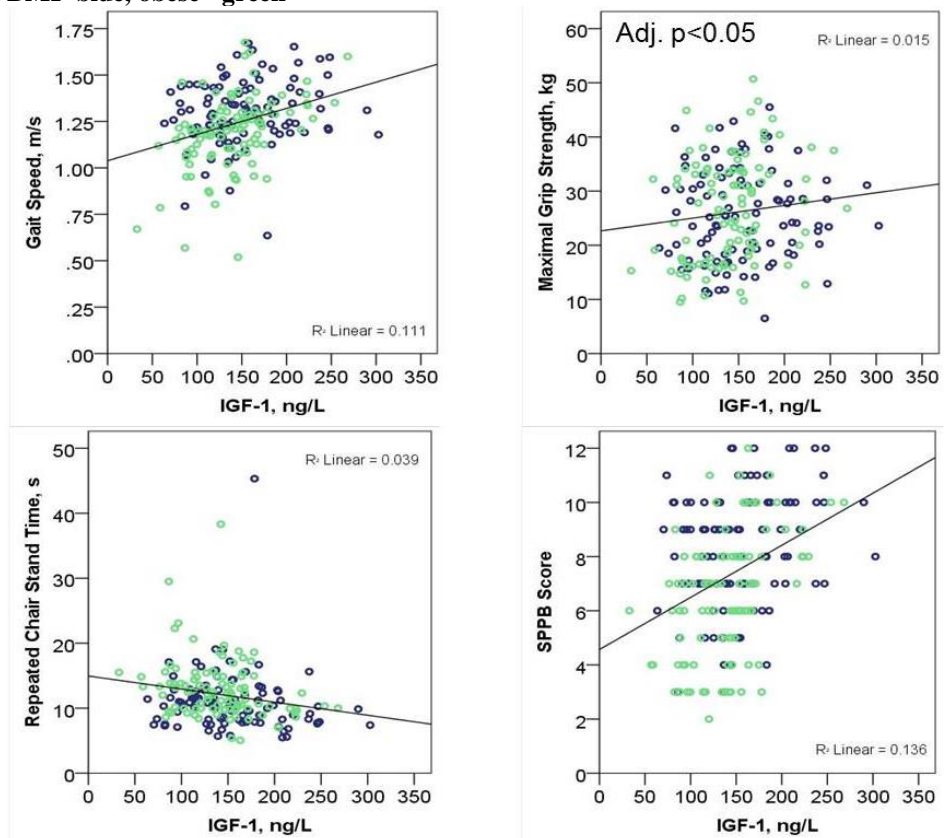


### IGF-I, LM and Physical Performance

IGF-I was not associated with WBLM, ALM or SMI. After adjustment for age and BMI, IGF-I was positively correlated with WBLM ( $r=0.195$ ,  $p<0.01$ ), ALM ( $r=0.215$ ,  $p<0.001$ ) and SMI ( $r=0.233$ ,  $p<0.001$ ).

IGF-I was associated with gait speed ( $r=0.329$ ,  $p<0.001$ ), grip strength ( $r=0.145$ ,  $p<0.05$ ), chair stand time ( $r=-0.267$ ,  $p<0.001$ ) and SPPB score ( $r=0.327$ ,  $p<0.001$ ) (Figure 76). After adjustment for age and BMI, IGF-I was positively associated with grip strength ( $r=0.128$ ,  $p<0.05$ ) but not associated with gait speed, chair stand time or SPPB score.

**Figure 76: Associations between IGF-I and physical function**  
Normal BMI=blue, obese =green

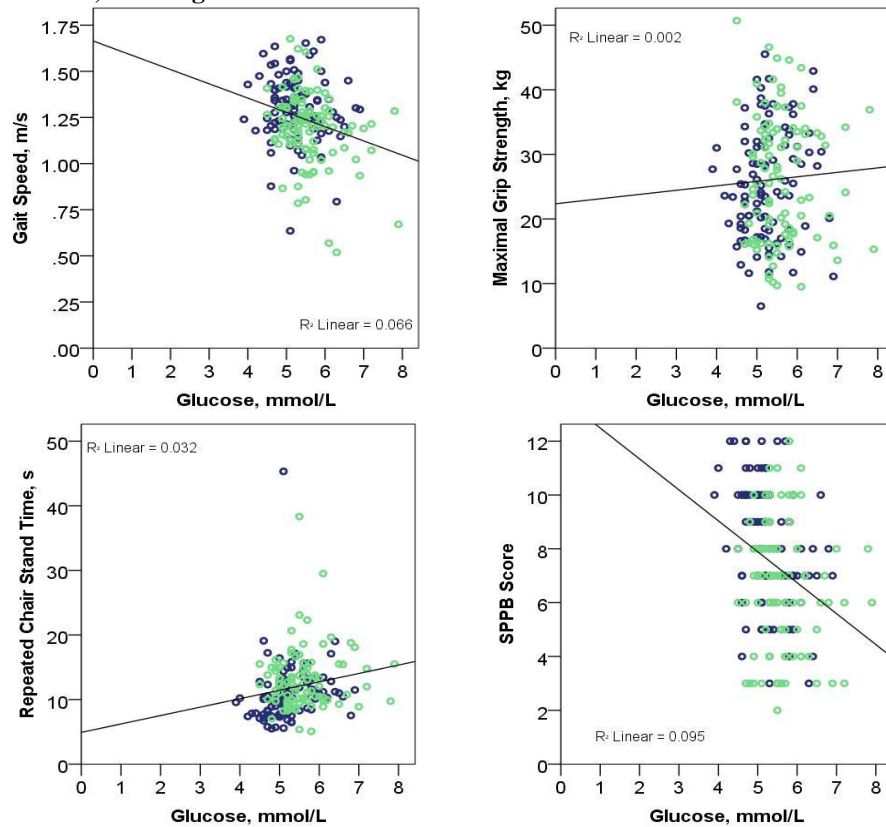


### Glucose, LM and Physical Performance

Glucose was positively associated with WBLM ( $r=0.199$ ,  $p<0.01$ ), ALM ( $r=0.154$ ,  $p<0.05$ ) and SMI ( $r=0.217$ ,  $p<0.01$ ). After adjustment for age and BMI, glucose remained positively associated with WBLM ( $r=0.167$ ,  $p<0.01$ ), ALM ( $r=0.154$ ,  $p<0.05$ ) and SMI ( $r=0.138$ ,  $p<0.05$ ).

Glucose was associated with gait speed ( $r=-0.255$ ,  $p<0.01$ ), chair stand time ( $r=0.246$ ,  $p<0.01$ ) and SPPB score ( $r=-0.288$ ,  $p<0.001$ ) (Figure 77). Glucose was not associated with grip strength. After adjustment for age and BMI, glucose was not associated with gait speed, grip strength, chair stand time or SPPB score.

**Figure 77: Associations between glucose and physical function**  
Normal BMI=blue, obese =green

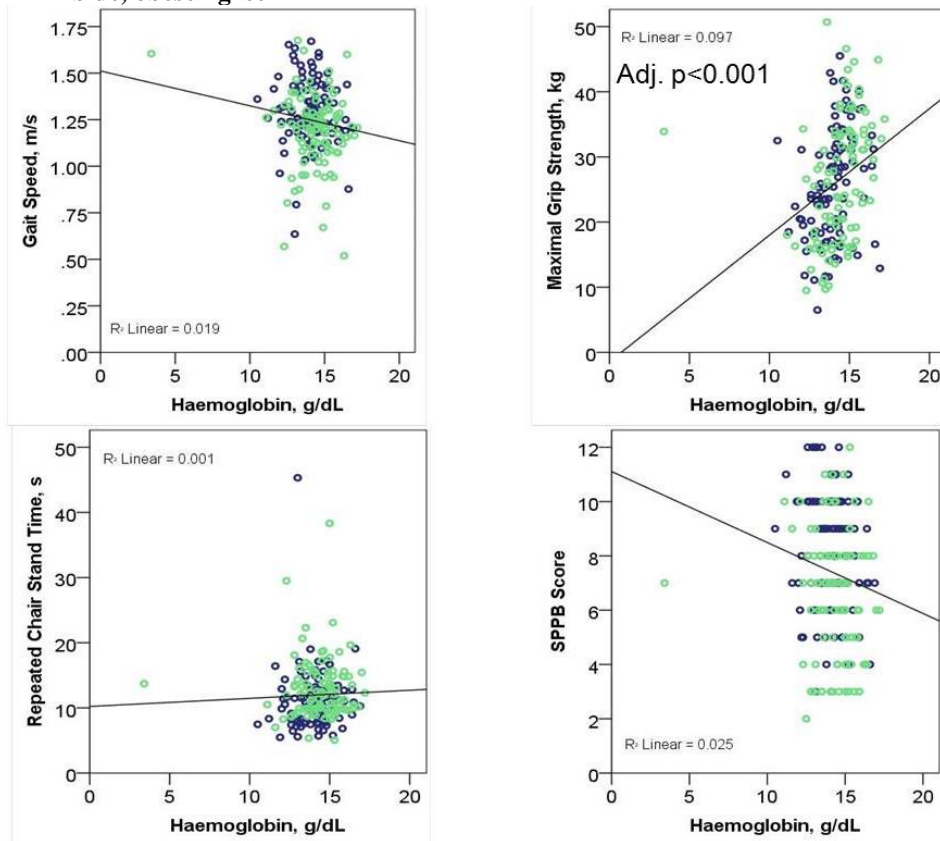


## Haemoglobin, LM and Physical Performance

Obese individuals had higher haemoglobin than normal BMI individuals ( $p < 0.01$  all, NSD younger,  $p < 0.01$  older). Haemoglobin was positively associated with WBLM ( $r = 0.289$ ), ALM ( $r = 0.283$ ) and SMI ( $r = 0.260$ ) (all  $p < 0.001$ ). After adjustment for age and BMI, haemoglobin remained positively associated with WBLM ( $r = 0.373$ ), ALM ( $r = 0.374$ ) and SMI ( $r = 0.379$ ) (all  $p < .001$ ).

Haemoglobin was not correlated with gait speed or chair stand time. Haemoglobin was positively correlated with grip strength ( $r = 0.437$ ,  $p < 0.001$ ) and inversely with SPPB score ( $r = -0.183$ ,  $p < 0.05$ ) (Figure 78). After adjustment for age and BMI, haemoglobin remained positively associated with grip strength only ( $r = 0.262$ ,  $p < 0.001$ ).

**Figure 78: Associations between haemoglobin and physical function**  
Normal BMI=blue, obese =green

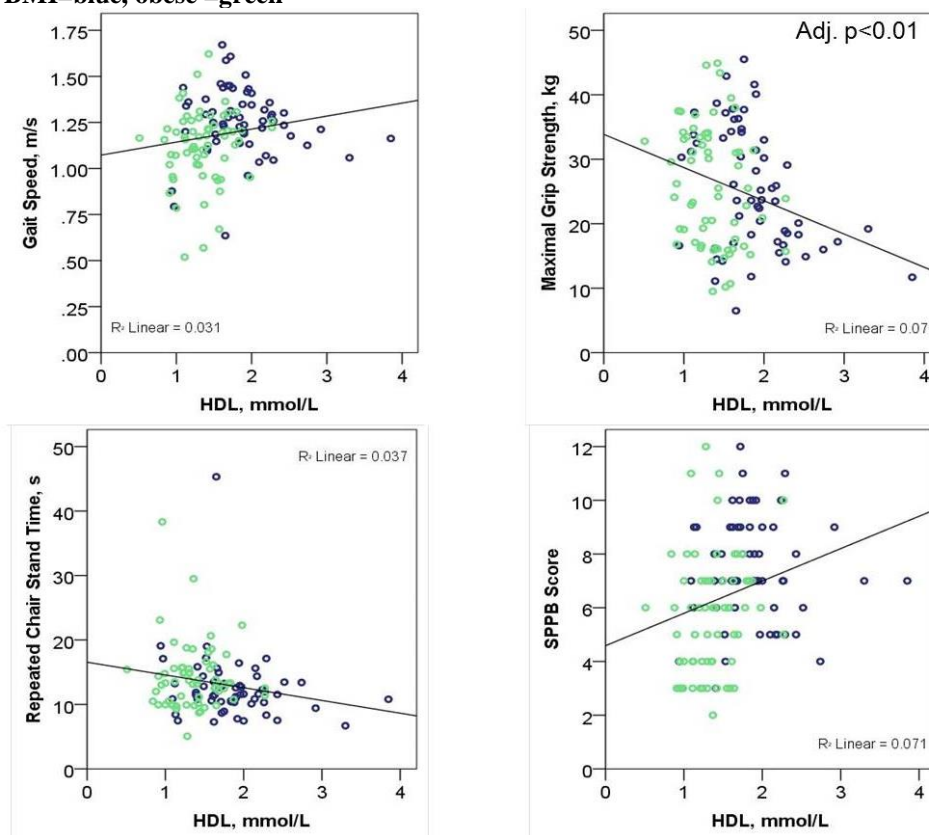


### HDL, LM and Physical Performance

HDL was inversely associated with WBLM ( $r=-0.581$ ), ALM ( $r=-0.557$ ) and SMI ( $r=-0.597$ ) (all  $p<0.001$ ). After adjustment for age and BMI, HDL remained inversely associated with WBLM ( $r=-0.392$ ), ALM ( $r=-0.364$ ) and SMI ( $r=-0.351$ ) (all  $p<0.001$ ).

HDL was inversely associated with grip strength ( $r=-0.227$ ) and chair stand time ( $r=-0.186$ ) (both  $p<0.05$ ) and positively associated with gait speed ( $r=0.257$ ,  $p<0.01$ ) and SPPB score ( $r=0.343$ ,  $p<0.001$ ) (Figure 79). After adjustment for age and BMI, HDL was inversely associated with grip strength only ( $r=-0.277$ ,  $p<0.01$ ).

**Figure 79: Associations between HDL and physical function**  
Normal BMI=blue, obese =green



### CK, LM and Physical Performance

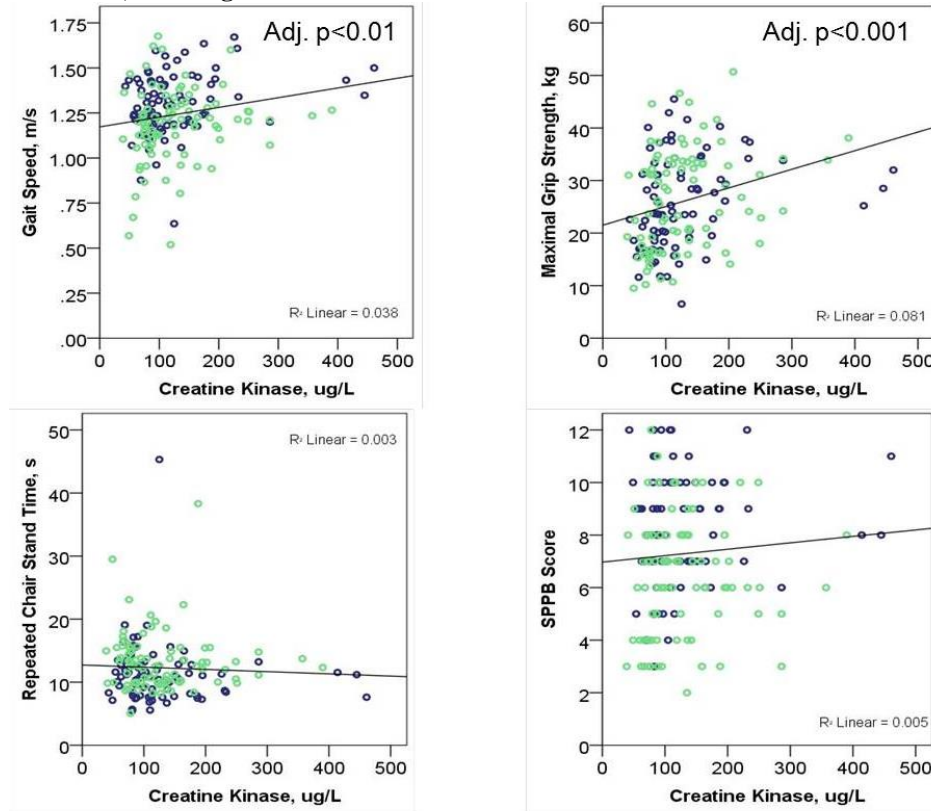
CK did not differ between obese and normal BMI individuals. CK was positively associated with WBLM ( $r=0.328$ ), ALM ( $r=0.349$ ) and SMI ( $r=0.284$ ) (all  $p<0.001$ ). After adjustment for age and BMI, CK remained positively associated with WBLM ( $r=0.407$ ), ALM ( $r=0.415$ ) and SMI ( $r=0.430$ ) (all  $p<0.001$ ).

CK was positively correlated with gait speed ( $r=0.204$ ,  $p<0.01$ ) and grip strength ( $r=0.374$ ,  $p<0.001$ ) but was not associated with chair stand time or SPPB score (Figure 80). After adjustment for age and BMI, CK remained positively associated with gait speed



( $r=0.176$ ,  $p<0.01$ ) and grip strength ( $r=0.326$ ,  $p<0.001$ ) but not associated with chair stand time or SPPB score.

**Figure 80: Associations between CK and physical function**  
Normal BMI=blue, obese =green



## E2, LM and Physical Performance

WBLM was positively associated with totE2 ( $r=0.177$ ,  $p<0.01$ ), fE2 ( $r=0.229$ ,  $p<0.001$ ), and bioE2 ( $r=0.241$ ,  $p<0.001$ ). ALM was positively associated with totE2 ( $r=0.199$ ,  $p<0.01$ ), fE2 ( $r=0.248$ ,  $p<0.001$ ), and bioE2 ( $r=0.263$ ,  $p<0.001$ ). SMI was positively associated with totE2 ( $r=0.144$ ,  $p<0.05$ ), fE2 ( $r=0.222$ ,  $p<0.001$ ), and bioE2 ( $r=0.228$ ,  $p<0.001$ ). After adjustment for age and BMI, (or age, BMI and gender), totE2, fE2 and bioE2 were no longer associated with WBLM, ALM or SMI.

E2 was associated with chair stand time (totE2:  $r=-0.194$ , fE2:  $r=-0.163$ , bioE2:  $r=-0.177$ , all  $p<0.01$ ), grip strength (totE2:  $r=0.136$ , fE2:  $r=0.140$ , bioE2:  $r=0.150$ , all  $p<0.05$ ), gait speed (bioE2:  $r=0.125$ ,  $p<0.05$ ) and SPPB score (totE2:  $r=0.161$ , fE2:  $r=0.131$ , bioE2:  $r=0.153$ , all  $p<0.05$ ). After adjustment for age and BMI, (or age, BMI and gender), E2 was no longer associated with grip strength, gait speed, chair stand time or SPPB score.



### **25OHD, LM and Physical Performance**

25OHD was inversely associated with WBLM ( $r=-0.305$ ), ALM ( $r=-0.300$ ) and SMI ( $r=-0.332$ ) (all  $p<0.001$ ). After adjustment for age and BMI, 25OHD was no longer associated with WBLM, ALM or SMI.

25OHD was not correlated with gait speed, grip strength, chair stand time or SPPB score before or after adjustment for age and BMI.

### **Adiposity, Physical Performance and Falls**

To determine associations between adiposity and physical performance, WBFM, HsCRP, albumin, IGF-I, totE2, haemoglobin, HDL and glucose were entered into a stepwise multiple linear regression model with SPPB score as the dependent variable. WBFM was a significant determinant of SPPB (overall model  $p<0.001$ , adjusted R square 0.199, effect of WBFM: beta  $-0.454$ ,  $p<0.001$ ). Adding totE2 to the model improved the prediction of SPPB score (overall model  $p<0.001$ , adjusted R square 0.245, effect of WBFM: beta  $-0.439$ ,  $p<0.001$ , effect of totE2: beta  $-0.230$ ,  $p<0.01$ ).

To determine the association between adiposity and falls, WBFM, totE2 and PTH were entered into a stepwise multiple linear regression model with number of falls as the dependent variable. The model showed WBFM was a significant determinant of reported number of falls (overall model  $p<0.05$ , adjusted R square 0.024, effect of WBFM: beta  $0.170$ ,  $p<0.05$ ).

After adjusting for age, mobility score and SPPB score, BMI was no longer a predictor of number of falls. The only significant predictor of number of falls was mobility score (overall model  $p<0.001$ , adjusted R square 0.164, effect of mobility score: beta  $-0.410$ ,  $p<0.001$ ), suggesting that the effect of BMI on falls is mediated through poor physical performance.

## Summary of Results

A summary of the results of Chapter 5 is provided in Table 17

**Table 17: Summary of results Chapter 5**

The effects of BMI, age and gender and interactions between age or gender and BMI on muscle mass, physical performance and number of falls.

Variable	Effect of BMI (%difference)	Effect of Age	Effect of Gender	Interactions
WBLM	↑ (27.3%)	↑ in younger	↑ in men	-
ALM	↑ (27.6%)	↑ in younger	↑ in men	-
SMI	↑ (28.2%)	↑ in younger	↑ in men	Gender-BMI <sup>1</sup>
Prevalence of sarcopenia	↓			
Gait Speed	↓ (-8.3%)	↑ in younger	-	Age-BMI <sup>2</sup>
Chair Stand Time	↑ (17.4%)	↑ in older	-	Age-BMI <sup>3</sup>
Grip Strength	- (3.3%)	↑ in younger	↑ in men	-
Narrow walk deviations	↑ (91.8%)	↑ in older	↑ in men	-
SPPB Score	↓ (-21.6%)	↑ in younger	-	-
Habitual Mobility	↓ (-4.2%)	↑ in younger	-	-
Number of falls	↑(older)	↑ in older		
HsCRP	↑(older)	-	-	-
Albumin	↓	↑ in younger	↑ in men	-
IGF-I	↓	↑ in younger	-	-
Total E2	-	↑ in younger	↑ in women	Age-BMI <sup>2</sup>
Haemoglobin	↑			
HDL	↓(older)	-	↑ in women	-
CK	-			
25OHD	↓	↑ in older	-	-
Glucose	↑	↑ in older	-	-

Data shown as a percentage of the mean of the normal BMI group

<sup>1</sup> Greater effect of BMI in women than men

<sup>2</sup> Greater effect of BMI in older adults

<sup>3</sup> Greater effect of BMI on chair stand time in younger adults

## **Discussion**

The results of this Chapter show that physical performance and habitual mobility are worse in obese adults than adults with a normal BMI. Poor physical performance and mobility might be associated with a greater fall frequency in older adulthood and could affect fall kinetics. Poor physical performance in obese adults is not due to low LM; with obese adults having greater LM of the total body and appendicular regions. Poor physical performance may contribute to the greater risk of ankle and proximal humerus fracture in obese adults compared to those with a normal BMI, through associations with the number of falls and possibly by affecting fall direction and fall kinetics.

Several risk factors for falls were assessed including alcohol intake, medical conditions and medication use associated with greater risk of falls and physical activity participation. Greater number of falls in obesity was not due to greater alcohol intake. Having an alcohol intake of no more than 21 units per week was one of the inclusion criteria for the study; hence alcohol intakes were low to moderate. Although positive associations between adiposity and alcohol consumption are reported in high intake or binge drinkers, moderate consumption is typically negatively or not associated with adiposity (503). Greater number of falls in obesity was not due to greater prevalence of medical conditions known to increase the risk of falls, which was similar between normal BMI and obese groups. Individuals with medical conditions known to affect bone metabolism were excluded from the study and this may have included those with conditions influencing falls risk. Other conditions which are more common in obesity and associated with greater risk of falls, such as depression, may have led to individuals invited to participate in this study having declined to do so as a result of their condition (504, 505). Therefore the obese individuals in this sample may be of better general health than the general obese population. As obese individuals had poorer physical function and fell more often, the difference between obese and normal BMI groups was perhaps even underestimated. Greater physical activity has been shown to have a strong influence on balance and is generally associated with fall prevention (506). However, physical activity may be associated with greater fall risk, through exposure to environments predisposing to a fall. In the general population, it would have been expected that physical activity levels would be lower in obesity than those with a normal BMI. When recruiting to the study, individuals participating in competitive sport or moderate intensity physical activity for greater than seven hours per week were excluded. Therefore, no between-groups differences in physical activity were expected and differences in physical performance resulting from physical activity, rather than adiposity were minimised. As there was no difference in weekly physical activity duration or METs expended per week between

groups, greater number of falls in obesity cannot be attributed to physical activity. It is therefore most likely that the greater number of falls in obesity was due to poor physical performance.

The finding that obese older adults fall often than older adults with a normal BMI is consistent with previous findings (23, 49, 261, 278). Poor physical performance in obesity has also been shown by others. In the EIPDOS and NHANES III studies, obese women were more likely to report difficulties in performing habitual activities than women with a normal BMI (507, 508) and this was also reported in men with moderate obesity in the NHANES III cohort (508). Obese men and women have previously been shown to have lower SPPB scores than non-obese individuals (509).

Poor physical performance may be partly attributed to higher HsCRP in obese adults which was inversely associated with gait speed. Poor physical performance was not attributed to low 25OHD in obesity. There are several other potential causes of poor physical performance in obesity, with different causes associated with poor performance in different components of the performance battery:

Effective completion of the chair stand test requires the individual to retain control of their centre of mass whilst generating forward and upward motion. This might require greater effort in obesity, where there may be an anteriorly displaced centre of mass, especially in those with abdominal obesity. As there appears not to be greater muscle strength in obesity, this may further increase the effort required to complete the chair stand test effectively. Using kinematic and kinetic data, Sibella et al. showed that although normal weight individuals completed a repeated stand test with forward trunk flexion and a hip joint torque approximately twice that of the knee, in obesity, participants positioned their feet posteriorly (closer to the level of the back of the chair), resulting in greater knee torque with reduced trunk flexion (510). Participants in the FAB study were instructed to begin the test with their feet flat and knees bent at a 90° angle. Participants were instructed not to lift their feet when returning to the seated position, so as not to 'rock' and gather momentum for the forthcoming stand. Therefore any differences in foot positioning in the present study are perhaps unlikely or extremely discrete.

Slower gait speed in obesity is consistent with the findings of others and is associated with an altered step frequency and a shorter stride length (506, 511, 512). As the energy expenditure to facilitate movement of greater body mass is higher in obesity compared to normal BMI individuals, obese individuals exhibit "a longer stance phase, shorter

swing phase and a greater period of double support” when walking (506, 511, 513). Obesity has also been associated with greater step width, reflecting the greater leg girth and possibly in an attempt to improving dynamic balance (506, 513). Differences in gait speed and gait kinematics in obesity are plausible adaptations to compensate for impaired dynamic balance as a result of body size, composition and distribution or joint pain (514).

Poor balance in obesity may be attributed to differences in body fat distribution affecting the body’s centre of mass. Corbeil et al. used mathematical modelling to show that when the body’s centre of mass is displaced in an anterior direction, greater ankle torque was required to stabilise the body, increasing the risk of falls (477). As greater BMI does not necessarily result in anterior displacement of the centre of mass, illustrated by Gravante et al., the distribution of adiposity is likely to be a key factor in determining falls risk attributed to altered centre of mass (515).

Additionally, common alterations in foot structure in obesity, such as pes planus, greater rear-foot eversion and foot abduction, can affect postural stability and gait (478, 506, 512, 516, 517). Pes planus results from weakened ligaments in the foot being placed under greater loading conditions in obesity. Greater peak plantar pressure with greater body mass has been reported during both stance and dynamic states (515, 517-519) with greater plantar pressures reported under the mid-foot in particular. Greater rear-foot eversion may cause additional strain in musculo-tendinous structures of the lower limb. High BMI exerts greater loading forces which have been linked to greater muscle-tendon unit stiffness (520), although others report no association between BMI and muscle-tendon unit stiffness (516). Greater muscle-tendon unit stiffness can affect plantar landing kinematics and plantar pressure, foot posture, motion accuracy and overall joint stability (518, 520). Greater tendon and/or ligament stiffness may contribute to greater risk of falls through a lack of joint stability due to decreased visco-elasticity. The role of ligament and tendon stiffness in obesity, particularly surrounding ankle fracture, remains to be fully characterised.

There was no difference in grip strength between obese and normal BMI individuals in the present study, indicating that in obesity, grip strength might be poor indicator of overall physical function. This finding is consistent with that of another study (521), although others have shown associations between grip strength and BMI (483, 522). The relationship between obesity and muscle strength may be site-specific as there is evidence to support greater trunk and lower extremity muscle strength in obesity (464,

506). Relatively low muscle strength for LM in obesity may be attributed to reduced oxidative capacity and decreased capillary density of muscle fibres (506). There may also be a reduced neuromuscular activation of motor units, although it has been shown that there is no difference in the contraction of muscles fibres in obese and normal BMI individuals following percutaneous stimulation, suggesting that the muscle fibres are capable of contraction but lack stimulation in obesity (523).

Overall, there is a strong suggestion that physical function may be impaired in obesity. With the potential for poor physical performance to increase the risk of falls and ultimately fractures, identification of individuals at greatest risk is important. Although sarcopenia has been associated with greater risk of falls in the general population (524), the results of this Chapter show that greater fall frequency in obesity was not due to sarcopenia. Obese individuals had higher LM than individuals with a normal BMI and, as low LM must be observed for functional decline to be recognised by the EWGSOP consensus definition, consequently no obese individuals were sarcopenic (249). This finding was consistent with that of Newman et al. who found none of the obese participants in a 70-79 years age group subsample of the Health ABC study to be sarcopenic due to their greater LM (525). Zoico et al. found that half of elderly women with high FM but normal LM had impaired functional performance compared to healthy premenopausal women (526). Clearly, having 'normal' or above 'normal' LM does not necessarily confer protection against functional limitation and poor physical performance in obese adults.

In obesity, high FM appears more influential than low LM with respect to mobility and physical performance. Similarly, in the Cardiovascular Health Study, high FM predicted disability in older men and women, whereas low fat-free mass did not (527). Muscle strength was shown to be a stronger predictor of functional limitation and poor health than LM (528). In the Health ABC Study, although grip strength and physical performance were associated with the risk of hospitalisation, LM and thigh muscle cross sectional area were not risk factors for hospitalisation (529). Cawthon et al. reported that clustered factors of adiposity and body size were strongly associated with disability risk, whilst greater strength and larger lean body size were not associated with disability risk (530). Support for studying muscle function over LM to determine physical performance and falls risk has also been provided by exercise intervention studies which have shown that it is possible to maintain muscle strength and density despite a concurrent loss of LM with age (530).

Whether definitions of sarcopenia should be amended to account for FM is a topic of considerable debate. Both LM and FM are altered with weight change, typically with a

1:4 increase of LM to FM (531) and greater LM is required to enable movement of greater body mass. Adipose status has been shown to heavily influence LM whilst negatively affecting physical performance (506). Therefore, it may be argued that to determine whether an individual has insufficient LM to function adequately, as is the aim of current sarcopenia guidelines, adiposity should be taken into account. Although the EWGSOP consensus acknowledges that LM alone is inadequate to identify functional decline, the current definition of sarcopenia does not consider adipose status (249).

By not adjusting for FM, others have reported a failure to identify a large proportion of the population at risk of falls and fracture (276). Newman et. al found that adjusting ALM for height and FM resulted in the classification of a greater proportion of obese individuals as sarcopenic than the use of SMI where ALM is adjusted for height squared irrespective of adiposity (525). Recognising an individual's adiposity and applying adiposity adjusted cut-offs for LM and/or a combination of risk factors for falling rather than a dependence on low LM, may improve identification of obese individuals at risk of falls and fractures (276).

However, at present there is no agreed method of correcting LM for adiposity, when assessing sarcopenia. Other authors have suggested identifying sarcopenic individuals as those with a LM which is at least two standard deviations from the mean of a gender specific, young population. This could have been developed from the 25 to 40 year old obese group applied to the older obese adults in the present study, such that muscle mass thresholds are relative to BMI. Further research is needed to identify the most appropriate LM and FM compartments to be considered in any adjustment, as the distribution of adiposity will likely affect the outcome and the resultant relationship with physical function.

Sarcopenic obesity describes an increase in FM combined with a net loss in body LM, with approximately 8.4% of women and 13.5% of men affected by sarcopenic obesity beyond 80 years of age (276, 509). Due to the small sample size, it was not possible to study the effect of sarcopenic obesity on physical function or fall frequency from the present data. Sarcopenic obesity is associated with impaired physical performance and increased disability risk (276, 532, 533). From the results of this Chapter, where the obese population have a high LM with impaired physical function, it is plausible that physical function may be considerably worse in sarcopenic obesity with a high ratio of FM to LM resulting in increased demand "on an inadequate locomotor system" (276).

However, as the results of this Chapter suggest that function is driven by factors other than LM, the relationship between LM and function in sarcopenia may not be linear.

In a state of low LM with high FM, it might be hypothesised that functional outcomes in sarcopenic obesity are worse than in non-sarcopenic obesity (534). Sarcopenic obesity has been associated with poorer physical function (521). Sarcopenic obese women were more likely to report difficulty in performing habitual activities than obese non-sarcopenic women in the EIPDOS cohort, though generally not significantly so, and there was a small number of sarcopenic obese-women in this sample (507). In the same cohort, sarcopenic obese women were more likely to report difficulty performing habitual activities than sarcopenic non-obese women (507). Poor physical performance may be partly attributed to sarcopenic obese individuals having a longer forefoot contact phase and a shorter initial contact phase, greater oscillation in the metatarsals compared to non-obese and obese non-sarcopenic individuals (532). However, sarcopenic obesity was not associated with functional performance in older women (526) or with self-reported difficulty in completing habitual activities (508). There was no difference in physical function or SPPB score between sarcopenic obese women and men and non-sarcopenic obese individuals but obese sarcopenic individuals had a poorer SPPB score than non-sarcopenic, non-obese individuals and obese sarcopenic women had a poorer SPPB score than non-obese sarcopenic women (509).

The only biochemical factor to predict physical performance in obesity was totE2. While others have suggested that adipokines and inflammatory cytokines may mediate a link between adiposity and muscle strength (528), assuming that potential mediators of a relationship between adiposity, LM and function in non-obese individuals extrapolate to obese individuals may not be a suitable approach when the relationship between adiposity, LM and function is dissociated in obesity compared to normal BMI individuals.

The strengths of this work include the use of DXA based estimates of LM and the use of validated methods to determine muscle strength and function to provide an accurate assessment of LM and muscle function. While other studies have investigated LM, sarcopenia and physical performance in obesity, and some with a non-obese control group, the individually-matched study design and control for physical activity in this study provides a more clear understanding of the differences between obese and normal BMI groups attributed to adiposity rather than confounding factors.



There are some limitations to the work in this Chapter. While there were statistically significant correlations between biochemical factors and physical function variables, the correlations only accounted for a very small proportion of the variance in the relationship. Alternative factors may better explain associations between muscle mass and physical performance in obesity.

Study participants had to be mobile and willing to travel to attend the research facility to participate. It must be acknowledged that this may result in a degree of sample bias and that the results may not be generalisable to the whole population.

Data on fall history was only collected for the six months prior to recruitment. Collecting falls data over a longer period of time might enable a more accurate assessment of fall prevalence in the study population. Due to the small number of reported falls, it was not possible to calculate meaningful hazard ratios for falls in the obese and non-obese groups. No data was collected on fall direction. Whilst relying on participants to recall such information may prove problematic, simply determining whether participants fell forwards, backwards or sideways would enable us to better understand the way obese individuals fall, which could then be related to fracture prevalence.

One of the study inclusion criteria was that participants must not have fractured in the twelve months preceding recruitment (Chapter 2). Although any fall could cause fracture, only five to ten percent do result in a fracture (535). As any individuals who reported fracture in the twelve months prior when screened, were excluded, the true number of falls experienced by both BMI groups in the population is likely to have been underestimated by at least five to ten percent. As none of the falls recorded in the study resulted in fracture, it was necessary to assume no difference between a fall resulting in fracture and a fall with no fracture implication. However, this is unlikely to be the case as whether or not a fall results in fracture is not due to chance, but due to differences in fall arrest, fall kinematics and skeletal adequacy.

The degree of difficulty experienced when completing habitual activities was self-reported and self-perceived, which may affect the validity of these results.

Physical activity was assessed as both hours per week and METs per week to give an overall indication of the energy expended by physical activity taking into account intensity and duration. A pre-determined MET value was identified from the literature, based on an individual weighing 70kg, for each of the activities reported in the lifestyle

questionnaire and the duration of reported activity. METs should be calculated as the amount of oxygen consumed per kg weight times the duration (minutes) and so the lack of adjustment for the individual's body weight may have affected the accuracy of the MET results, such that METs of the obese group were underestimated.

The risk of proximal humerus fracture may be related to slower reaction time in obesity. Slower reaction time may predispose an individual to proximal humerus fracture if there is inability to outstretch the forearm arm in time to break their fall (535). Berrigan et al. showed that rapid arm movement time was slower in obese versus normal BMI individuals and that rapid arm movement in obesity was accompanied anterior movements of the trunk (536). These findings support the hypothesis that obese individuals fracture their proximal humerus more frequently due to poor balance and slower arm movement, resulting in fall impact on the shoulder, rather than the wrist. Although obese individuals may have greater soft tissue thickness at the upper body which may infer some protection against proximal humerus fracture, this protection may be outweighed by greater body weight and increased fall forces. The effect of soft tissue thickness on proximal humerus fracture has not been studied to date. Incorporating a reaction time task into the study would have enhanced our understanding of the present findings in relation to fracture risk.

While the assessment of gait speed by six metre walk is a validated method, when participants begin the walk at the zero metre mark, reaction time and time to initiate motion may influence the overall six metre walk time. Whilst this may be considered a reflection of physical performance, completing the six metre walk with a preceding two metre lead up may give more accurate reflection of true gait speed, as has been suggested by others (537). Using deviations from a narrow walk course to assess balance allows a relatively simple assessment of mediolateral balance. Although mediolateral balance is important for dynamic activities such as gait (506), and would be the balance axis involved in a sideways fall resulting in proximal humerus fracture, the differences in centre of mass discussed earlier in this discussion suggest that balance in alternative axes may also be affected and of interest in obesity. No motion analysis was performed in this cohort to assess gait or rear-foot eversion in more detail. Motion capture can be troublesome in obese populations due to the incurrance of error from movement of the cutaneous markers with increasing subcutaneous adiposity.

Fat infiltration of the muscle was not determined in the present study. Intramuscular adiposity increases with increasing BMI and has been associated with poorer muscle

strength, power and physical function (453-456, 538). Muscle adiposity could be determined from the abdominal CT scan described in Chapter 4 using the psoas, erector spinae, rectus abdominus, internal and external oblique and transversus abdominus muscle groups. Determining peripheral muscle fat could also be done using the HR-pQCT imaging of the tibia described in Chapter 2.

In conclusion, by standard definitions of sarcopenia, obesity appears protective of sarcopenia due to greater LM, but physical function scores and habitual mobility are impaired, such that obese adults may be at greater risk of dynapenia. Adjusting SMI for FM and/ or determining muscle quality rather than mass, may better identify obese adults at greatest risk of poor physical performance. Older obese adults fall more frequently than non-obese adults. There are physiological differences between obese and normal BMI adults, which may contribute to poor physical function. Poor physical performance might be associated with greater fall frequency and differences in fall kinetics, and explain the greater risk of proximal humerus and ankle fracture in obese adults.

## **CHAPTER 6:**

# **VITAMIN D METABOLISM IN OBESITY**

## **CHAPTER 6: Vitamin D Metabolism in Obesity**

### **Background**

Vitamin D status is a determinant of calcium absorption and may also have a role in immunity and inflammation. In the UK, a small proportion of our vitamin D comes from dietary sources such as oily fish, eggs and fortified foods (vitamin D<sub>3</sub>) and from dietary supplements. However, the majority of our vitamin D comes from exposing the skin to UVB rays (wavelength 290 to 310 nm). UVB exposure stimulates the conversion of 7-dehydrocholesterol to pre-D<sub>3</sub> when the B-ring of 7-dehydrocholesterol is broken. Pre-D<sub>3</sub> isomerises to become vitamin D<sub>3</sub> and is released into the circulation. In the liver, vitamin D<sub>3</sub> is converted to 25OHD by one of four cytochrome P450 (CYP) enzymes; CYP2R1, CYP3A4, CYP27A1, or CYP2J2 before being released to circulate bound to vitamin D binding protein (DBP) or albumin (539) (Figure 81).

### **25OHD Insufficiency**

Low 25OHD results in a decrease in intestinal calcium absorption, which lowers serum calcium. This leads to increased PTH, which results in increased bone turnover and a loss of bone mineral, as calcium is released into the circulation to increase and subsequently maintain serum calcium while intestinal calcium absorption is low. This process of demineralisation is associated with bone weakness as seen in rickets and osteomalacia and is a risk factor for osteoporosis. Low 25OHD is associated with several chronic diseases, some cancers, and higher all-cause and cardiovascular mortality, however there is a lack of *in-vivo* evidence to imply a causal role of low 25OHD in such conditions (540, 541).

### **Deficient, Insufficient or Replete**

Several definitions of vitamin D adequacy have been published, most of which are based on PTH and skeletal responses to 25OHD. Debate concerning such thresholds is ongoing and it is unknown what 25OHD concentration should be considered sufficient for the alternative functions of vitamin D where there is less evidence for such effects *in-vivo*. 25OHD levels greater than 50 nmol/l have been proposed as adequate by the US Institute of Medicine, with levels of 30 to 50 nmol/l considered insufficient and below 30 nmol/l as deficient (542, 543), although the US Endocrine Society recommends a level greater than 75 nmol/l be deemed sufficient, 50 to 75 nmol/l insufficient and below 50 nmol/l deficient (544). For the purposes of this Chapter, the Institute of Medicine classifications will be used.

Variations in measurements of 25OHD by different immunoassays have been reported (545) and consequently there has been a move to standardise the method for measuring serum 25OHD and a move toward using international standards to improve precision.

### **Groups at High Risk of Low 25OHD**

Vitamin D insufficiency is common in the UK, particularly during the winter months due to low UVB exposure (546). Specific groups have been identified as being at particularly high risk of vitamin D insufficiency, including children under 3 years of age, adults over 65 years of age, pregnant and lactating women, individuals who avoid sun exposure, or live at high latitudes, have darkly-pigmented skin or with chronic kidney disease (547). Recently, those with genetic polymorphisms in the vitamin D receptor (VDR), vitamin D binding protein (DBP) or calcium-sensing receptor and obese individuals have been identified as being at greater risk of low 25OHD (548).

### **25OHD in Obesity**

Obese individuals have low circulating total 25OHD and this has been found across different ethnicities and worldwide (407, 549-558). However, low total 25OHD in obesity does not appear to be associated with the typical effects of vitamin D deficiency on the skeleton. In this thesis it has been shown that obese individuals have higher PTH, but despite this, bone turnover is lower; with greater BMD at all sites measured (Chapters 3 and 4). This raises questions over whether the traditional vitamin-D endocrine axis is altered in obese individuals and whether low total 25OHD is the most appropriate marker of true vitamin D status in obesity. Others have reported similar findings. In obese premenopausal women, while there were inverse associations between total 25OHD and BMI, total abdominal adiposity and SAT, there was no association between total 25OHD and L4 Tb.vBMD or PINP (269). There was no apparent effect of lower 25OHD on bone microarchitecture or bone mechanical properties in obese men (268). If the vitamin D axis is indeed altered in obesity, such that bone turnover, and consequently BMD, are unaffected by lower 25OHD, there may no requirement to supplement obese individuals with vitamin D for skeletal benefit.

Although much of the literature has focused on associations between total circulating 25OHD and health outcomes, most 25OHD circulates bound to binding proteins; DBP and albumin. Only 0.02 to 0.05% of total 25OHD is free, or unbound, with approximately 10% bioavailable (bound to albumin) (559). Free and bioavailable fractions may be important as these are the fractions which can be considered biologically available as only free lipophilic ligands are able to cross cell membranes and exert physiological

effects through intercellular interactions (560). It is currently unknown how, or if, the free and bioavailable fractions of 25OHD in obesity differ compared to those of non-obese groups and whether differences in DBP concentration or DBP genotype contribute to such differences.

### **Potential Causes of Low 25OHD in Obesity**

#### **Low UVB Exposure**

Although it has been established that following UVB exposure, synthesis of vitamin D in obesity is normal and so low total 25OHD is not a consequence of impaired cutaneous synthesis of D<sub>3</sub> (407), obese people may receive less exposure to UVB radiation than individuals with a normal BMI (561). Immobility and sedentary lifestyles may limit the exposure of obese individuals to the outdoors. Obese individuals who do go outdoors may be less likely to expose large areas of skin due to the social stigma of obesity.

#### **Low Dietary and/or Supplementary Intake**

Low total 25OHD could result from a low intake of dietary vitamin D in obesity (562). Obese individuals may be more likely to consume high calorie foods of low nutritional content. Obese individuals may also be less likely to take vitamin D containing supplements which might also contribute to lower 25OHD.

#### **Low Vitamin D Binding Protein Concentration**

Circulating DBP levels and binding of 25OHD to DBP may be inhibited by inflammatory cytokines and triglycerides, which are higher in obesity (563-565). Although it is not yet clear whether obesity affects DBP levels or free 25OHD (566, 567), lower concentrations of binding proteins for 25OHD in obese individuals may positively affect free 25OHD, resulting in normal free 25OHD despite low total 25OHD.

#### **Greater Volumetric Dilution**

As vitamin D is fat soluble and stored in adipose tissue (568), greater adiposity could be providing a greater pool for the volumetric dilution of 25OHD (569). Passive distribution into a larger pool may result in lower circulating 25OHD in obesity, as illustrated by the blunted response of obese individuals to vitamin D supplementation (557, 570-573) and particularly by the blunted difference in summer and winter 25OHD levels in obesity (396). Diminished summer rises in circulating 25OHD may be suggestive of volumetric dilution as despite a larger body surface area and no difference in cutaneous synthesis of D<sub>3</sub> from UVB exposure compared to non-obese individuals, circulating 25OHD is lower in obesity (407).

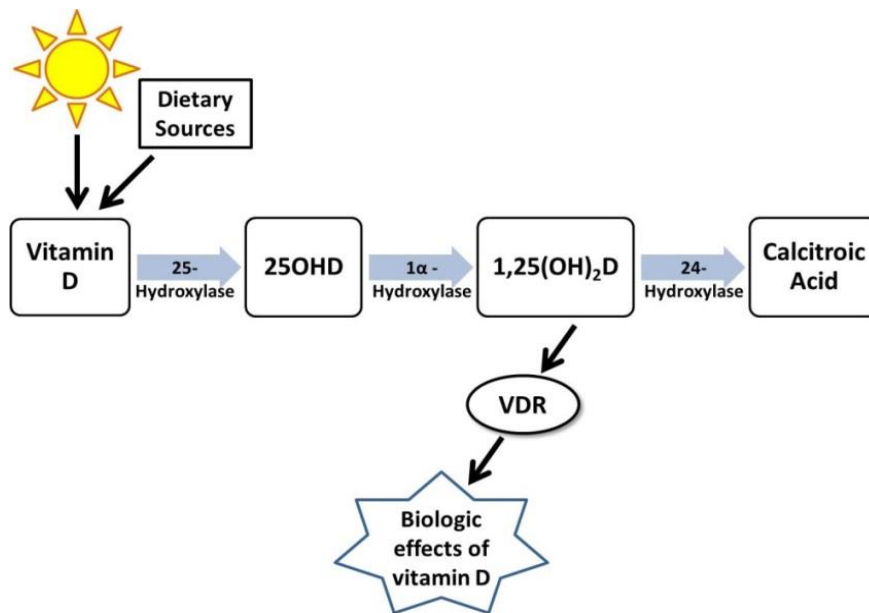
### Potential Consequences of Low 25OHD in Obesity

While the results of Chapters 3 to 5 suggest that lower 25OHD does not have a detrimental effect on skeletal parameters or muscle function in obesity, this will be investigated in more detail in this Chapter.

### Total 1,25-dihydroxyvitamin D in Obesity

25OHD is hydroxylated to the active hormone 1,25-dihydroxyvitamin D ( $1,25(\text{OH})_2\text{D}$ ) by  $1\alpha$ -hydroxylase, the enzymatic product of the CYP27B1 gene. This  $1\alpha$ -hydroxylation takes place mainly in the kidney, but also at extra-renal sites. Although 25OHD is able to bind to the VDR, the binding affinity of  $1,25(\text{OH})_2\text{D}$  for the VDR is approximately one thousand times greater than that of 25OHD. When  $1,25(\text{OH})_2\text{D}$  binds to the VDR, the biologic effects of vitamin D are initiated with the ultimate normalisation of serum calcium concentrations (Figure 81).

**Figure 81: Simplified illustration of vitamin D metabolism**  
Adapted from (574)



24-hydroxylase, a product of the CYP24A1 gene, catabolises 25OHD to  $24,25(\text{OH})_2\text{D}$  and degrades  $1,25(\text{OH})_2\text{D}$  to calcitroic acid. The production of  $1\alpha$ -hydroxylase as a result of increased CYP27B1 expression is increased by PTH to raise circulating  $1,25(\text{OH})_2\text{D}$  levels and PTH supresses production of 24-hydroxylase through decreased expression of CYP24A1 to decrease catabolism of  $1,25(\text{OH})_2\text{D}$  to calcitroic acid. The effects of PTH are mediated by those of FGF-23, which supresses production of  $1\alpha$ -hydroxylase and



induces production of 24-hydroxylase through increased expression of CYP24A1 to catabolise 1,25(OH)<sub>2</sub>D to calcitric acid (574).

The majority of studies have shown inverse associations between adiposity and total 1,25(OH)<sub>2</sub>D (441, 549, 556, 558, 575-577), although only two studies show lower total 1,25(OH)<sub>2</sub>D in an obese group compared to a non-obese group (441, 578). Whether 1,25(OH)<sub>2</sub>D levels are more strongly associated with certain fat compartments remains unclear (110, 268, 269).

Only 0.2 to 0.6% of total 1,25(OH)<sub>2</sub>D is free, or unbound (560). It is currently unknown how, or if, the free and bioavailable fractions of 1,25(OH)<sub>2</sub>D in obesity differ compared to those of non-obese groups and whether differences in DBP concentration or DBP genotype contribute to such differences.

### **Low 25OHD, Muscle Mass and Physical Performance**

Low vitamin D status in obesity may be associated with the poor muscle function and physical performance observed in Chapter 5. Although total 25OHD was not associated with WBLM, ALM or SMI, or with physical function (Chapter 5), only total 25OHD, unadjusted for seasonality was investigated. There might be effects of free 25OHD and total and free 1,25(OH)<sub>2</sub>D on both LM and muscle function in obesity which could contribute to the greater number of falls reported in older obese adults.

Vitamin D appears to be indirectly associated with muscle function through associations with calcium and phosphate (579). Vitamin D may also have direct effects on muscle function through activation of VDRs which are found in muscle tissue. Activation of skeletal muscle VDRs by 1,25(OH)<sub>2</sub>D has been shown to stimulate the activation of pathways involved in calcium metabolism and myogenesis (579). Furthermore, skeletal muscle has been shown to express CYP27B1, indicating that skeletal muscle may be an important site for the conversion of 25OHD to 1,25(OH)<sub>2</sub>D (579). Vitamin D may also increase myocyte mitochondrial oxidative function (580).

Vitamin D may play a role in the maintenance of LM and/or muscle function over time. Scott et al. reported that higher baseline 25OHD was associated with better maintenance of lower-limb strength but was not associated with maintenance of appendicular lean mass (ALM) (581, 582). This finding may be relevant in obesity where there is low 25OHD and poor muscle performance, despite greater appendicular LM (ALM) (Chapter 5). Whether 25OHD is associated with LM in obesity is unclear, with a recent study of

obese premenopausal women suggesting there is no association between 25OHD and thigh muscle cross sectional area (269).

Vitamin D insufficiency is also associated with greater muscle fat infiltration (484, 581). However, the relationship between obesity, low 25OHD and physical function may not be explained by muscle fat infiltration as in obese premenopausal women there was no association between 25OHD and thigh muscle density as an indicator of muscle adiposity (269).

25OHD levels have generally been positively associated with muscle strength (583-585), although some report null (586) or inverse associations (587). Total 1,25(OH)<sub>2</sub>D may also be positively associated with measures of physical function (587). Vitamin D supplementation in those who are vitamin D deficient is positively associated with muscle strength (588) and balance (588, 589). Supplementation of those who are vitamin D replete does not appear to yield the same benefits; with the supplementation of vitamin D only improving muscle strength in those with a low 25OHD at baseline (<25 nmol/L) (589, 590).

### **Low 25OHD and Falls**

Low 25OHD is associated with greater falls risk in older adults and the elderly (394, 401, 581, 591, 592), as is lower 1,25(OH)<sub>2</sub>D (587). The current evidence indicates that vitamin D supplementation in people who are vitamin D deficient protects against falls in older people (589, 591, 593) and supplementation with both vitamin D and calcium may further reduce fall risk (593). Bischoff-Ferrari et al. concluded that whether supplemental vitamin D was cholecalciferol or ergocalciferol or an active form of vitamin D (1 $\alpha$ -hydroxycalciferol or 1,25-dihydroxycholecalciferol) may affect reductions in falls risk, although overall, a significant risk reduction of 19 to 22% was reported (594). Evidence for benefit in terms of falls risk in young adults is less clear (591, 595). Whether low 25OHD is associated with greater risk of falls in younger adults and in obesity is unknown. It is unclear whether supplementing obese adults with vitamin D to prevent falls due to impaired physical function would be beneficial.

## **Research Question, Aims and Hypotheses**

Research Question:

**Is obesity associated with both low total and free vitamin D and if so, what are the potential causes and musculoskeletal consequences of low vitamin D in obesity?**

Aims

1. To establish whether obese people have low total 25OHD and 1,25(OH)<sub>2</sub>D.
2. To investigate whether obese people have low free 25OHD and 1,25(OH)<sub>2</sub>D.
3. To determine the potential cause(s) of low 25OHD and/or 1,25(OH)<sub>2</sub>D in obesity.
4. To investigate associations between 25OHD and BMD, bone structure and bone strength to establish whether there are any skeletal consequences of low 25OHD in obesity.
5. To investigate associations between total and free 25OHD and 1,25(OH)<sub>2</sub>D and physical performance.
6. To determine whether body fat distribution is associated with 25OHD.

Hypotheses:

1. Total 25OHD and 1,25(OH)<sub>2</sub>D are lower in obesity.
2. Lower dietary vitamin D intake and greater pool size are associated with low 25OHD levels in obesity.
3. As result of lower dietary vitamin D intake, greater pool size and lower DBP and albumin, free 25OHD and 1,25(OH)<sub>2</sub>D are lower in obesity.
4. There are no skeletal implications of low circulating total 25OHD in obesity.
5. Low total and free 25OHD and 1,25(OH)<sub>2</sub>D are associated with lower SPPB score.

## **Methods**

All participants from the original FAB Study described in Chapter 2 were invited to participate in the vitamin D sub-study. Participants who no longer met the original study inclusion criteria were excluded. Participants taking prescribed vitamin D were ineligible. Eligible participants attended for a single visit to the Clinical Research Facility, Northern General Hospital, Sheffield, in the autumn (September to October 2012) or spring (April to May 2013). Weekly average sunlight hours for the study periods can be found in Appendix 3.

Height (cm) and weight (kg) were measured and BMI calculated, as described in Chapter 2. Triceps skinfold thickness (mm) was measured and used as an indicator of peripheral subcutaneous adiposity. Waist and hip circumference were measured and WHR used as a marker of visceral adiposity, as described in Chapter 2. BMD, bone structure, bone strength, body composition and physical function were assessed in the original FAB study, as described in Chapter 2.

UVB exposure, SPF habits, supplementary vitamin D and dietary vitamin D intake were determined from questionnaires (Appendix 1, described fully in Chapter 2). Dietary vitamin D data was analysed using the DIETQ software program (Tinuviel Software, Warrington, UK).

### **Total 25OHD and 1,25(OH)<sub>2</sub>D**

The serum concentration of total 25OHD is most commonly measured to determine vitamin D status. High circulating total 25OHD is associated with high cellular 1,25(OH)<sub>2</sub>D, which reflects the physiologically relevant vitamin D status. Although 25OHD has an effect on calcium absorption, this effect appears to be mediated by indirect actions, whereas 1,25(OH)<sub>2</sub>D has a direct effect on calcium absorption (596). Whereas 25OHD acts a static marker of vitamin D status, reflecting supply and expenditure, 1,25(OH)<sub>2</sub>D is affected by vitamin D intake, circulating 25OHD, 25-hydroxylase and 1 $\alpha$ -hydroxylase activity. Therefore, although 1,25(OH)<sub>2</sub>D may therefore provide a better indicator of the biological status of vitamin D, the lesser physiologic variability of total 25OHD relative to 1,25(OH)<sub>2</sub>D explains why total 25OHD is the most commonly measured metabolite (574). Total 25OHD was measured by automated ECLIA (Cobas e411, Roche Diagnostics, Mannheim, Germany). Total 1,25(OH)<sub>2</sub>D was measured by manual immunoassay (Immunodiagnostic Systems, Boldon, UK).

### **25OHD<sub>2</sub> and 25OHD<sub>3</sub>**

Although the measurement of total 25OHD reflects overall 25OHD status, both 25OHD<sub>3</sub> and 25OHD<sub>2</sub> contribute to this measurement and immunoassays may not distinguish the two forms. 25OHD<sub>3</sub> and 25OHD<sub>2</sub> were therefore measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the laboratory of the Institute of Human Development, University of Manchester, UK.

### **DBP and DBP Genotyping**

DBP was measured by manual immunoassay (Human Vitamin DBP Quantikine ELISA, R&D Systems, UK). Genetic variation in the DBP gene has given rise to several DBP genotypes. The ancestral form of DBP, or group specific component (Gc), Gc1f has undergone two amino acid changes; a D432E (previously D416E) and a T436K (previously T420K) change to form Gc1s and Gc2, respectively (559). Circulating DBP concentrations vary by haplotype, with Gc2 associated with the least abundant DBP and Gc1f the most DBP (109, 559, 567). Genetic variations in the DBP gene are also associated with circulating total 25OHD levels (109, 567). Differences in circulating levels of 25OHD by DBP genotype are most likely the result of different affinities of DBP for 25OHD by DBP haplotype (559, 597). Gc1f has the highest affinity for 25OHD with a dissociation constant (Kd) of 0.9 nM, compared to Gc1s (Kd 1.7 nM) and Gc2 with the lowest affinity for 25OHD (Kd 2.8 nM) (559). Gc1f also has the highest affinity for 1,25(OH)<sub>2</sub>D (Kd 56 nM) compared to Gc1s (Kd 160 nM) and again, Gc2 the lowest affinity (Kd 240 nM) (559, 597-599). If the conversion of 25OHD to 1,25(OH)<sub>2</sub>D is substrate dependent, dependence of physiological availability of 25OHD on DBP genotype may make DBP an important determinant of 1,25(OH)<sub>2</sub>D (255). DBP genotyping was performed in the laboratories of the Sheffield Diagnostic Genetics Service, Sheffield Children's Hospital, as described in Chapter 2.

### **Free 25OHD**

Free 25OHD was measured by immunoassay (Future Diagnostics BV, Wijchen, Netherlands) in the laboratory of Future Diagnostics (Wijchen, Netherlands) (254). (Chapter 2)

### **Free 25OHD and 1,25(OH)<sub>2</sub>D**

Free 25OHD and 1,25(OH)<sub>2</sub>D were measured by immunoassay as described in Chapter 2. Free and bioavailable fractions may be important as these are the fractions which can be considered biologically available and able to exert physiological effects through intercellular interactions (560). Free 25OHD and free 1,25(OH)<sub>2</sub>D were also calculated using the following equations (255, 256):

$$\text{Free 25OHD} = \text{Total 25OHD} / (1 + (6 \cdot 10^5 \times \text{albumin}) + (7 \cdot 10^8 \times \text{DBP}))$$

$$\text{Free 1,25(OH)}_2\text{D} = \text{Total 1,25(OH)}_2\text{D} / (1 + (5.4 \cdot 10^4 \text{M}^{-1} \times \text{albumin}) + (3.7 \cdot 10^7 \text{M}^{-1} \times \text{DBP}))$$

CTX, PINP, OC (Cobas e411, Roche Diagnostics, Mannheim, Germany) and BAP (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK) were measured to assess bone

turnover. Albumin, creatinine, calcium, phosphate, PTH, CK, triglycerides, lipid profile, IGF-I, HsCRP were measured as described in Chapter 2.

### **Statistical Analysis**

Mean (SD) age, height and BMI of the study sample were calculated by age, gender and BMI group. All variables were assessed for normality and log transformed where necessary.

Measured and calculated free 25OHD were compared using correlation, Passing-Bablok regression and Bland-Altman plots. Independent samples t-tests, or Mann-Whitney U-tests, were used to determine differences between normal BMI and obese individuals. Paired t-tests were not used in this Chapter as not all participants from the FAB study took part in the sub-study and restricting analysis to complete pairs of normal BMI and obese individuals reduced the sample size.

Analysis of variance or Kruskal Wallis tests were used to determine differences by DBP diplotype or genotype. Univariate general linear models were used to identify significant effects of age and gender on vitamin D outcomes and interactions between age, gender and BMI on vitamin D outcomes. Vitamin D was adjusted for time of visit (i.e. September to October or April to May).

Multiple linear regression was used to see if the relationship between BMI and the outcome variable changed after adjusting for age (as a continuous variable) and gender. In multiple regression analysis, covariates which had a variance inflation factor  $\geq 10$  or tolerance statistic  $\leq 0.1$  were removed from the model.

Percentage difference between the mean of the normal BMI group and that of the obese group for principal outcomes was calculated to facilitate comparisons of the magnitude of the difference between variables.

Analysis was performed using IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.). Passing-Bablok regression was performed in MedCalc (Version 13.0.2, MedCalc, Belgium). Significance was accepted when  $p < 0.05$ .

The sub-study had 90% power at 5% two-sided significance to detect a minimum statistically significant correlation coefficient of 0.22.

## Results

The overall sample consisted of 167 individuals of which 82 had a normal BMI and 85 were obese. Characteristics of the study population are shown in Table 18. Obese and normal BMI individuals were of a similar age and height (Table 18). Compared to the full population described in Chapter 3, mean ages were similar although the sub-study sample was slightly younger (approximately 1 to 2 years difference between the group means). In the vitamin D sample there was a larger difference in height between normal BMI and obese young women (approximately 2 cm difference between means) and older men were slightly taller (approximately 1 cm difference between the means). The FAB and sub-study samples were similar in terms of BMI.

**Table 18: Characteristics of the vitamin D sub-study sample by age, gender and BMI group. Data shown as mean (SD)**

	Women				Men			
	25 to 40 Years		55 to 75 Years		25 to 40 Years		55 to 75 Years	
	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese
n	16	16	27	28	12	13	27	28
Age, years	36.16 (3.8)	36.96 (5.1)	67.12 (5.7)	66.83 (5.7)	34.70 (4.8)	33.95 (4.4)	66.63 (5.1)	66.53 (5.8)
Height, cm	166.18 (6.5)	165.48 (6.8)	161.10 (4.5)	159.81 (4.1)	177.66 (7.8)	180.26 (7.4)	174.43 (6.5)	174.58 (7.7)
BMI, kg/m <sup>2</sup>	22.47 (1.5)	34.94 (5.1)	22.49 (1.6)	36.02 (5.0)	23.16 (1.4)	33.39 (3.4)	23.51 (1.3)	35.16 (3.8)

### Total 25OHD in Obesity

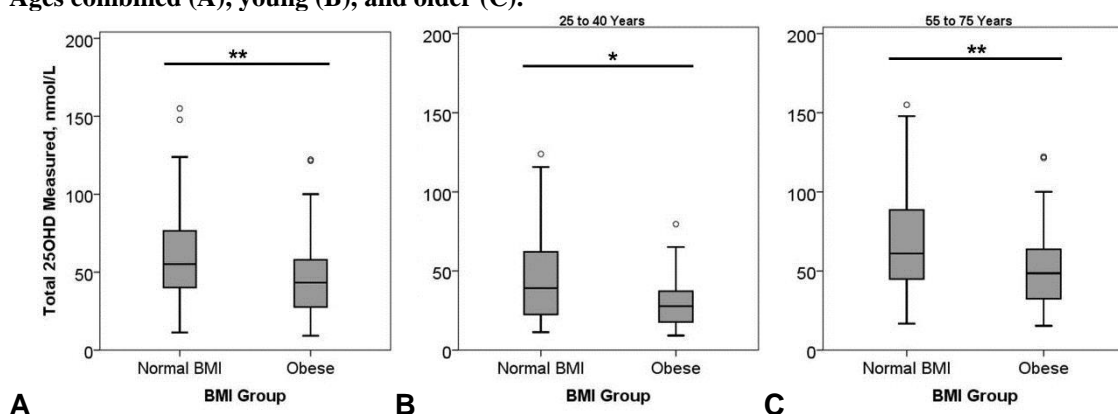
Table 19 shows the distribution of 25OHD status within the overall sample and by age and BMI group.

**Table 19: Vitamin D status of the sub-study sample by age and BMI group. Data shown as percentage**

		Deficient (<30 nmol/l)	Insufficient (30-50 nmol/l)	Replete (>50 nmol/l)
Overall		22.7	30.1	47.2
25 to 40 Years	Normal BMI	33.3	33.3	33.3
	Obese	57.7	30.8	11.5
55 to 75 Years	Normal BMI	3.7	25.9	70.4
	Obese	19.6	32.1	48.2

Obese individuals had lower total 25OHD than individuals with a normal BMI ( $p < 0.01$  overall,  $p < 0.05$  younger,  $p < 0.01$  older) (Figure 82). Older adults had a higher total 25OHD than younger adults ( $p < 0.001$ ). Women had a higher total 25OHD than men ( $p < 0.01$ ). There was no interaction between age or gender and the effect of BMI on total 25OHD. Multiple linear regression showed the relationship between BMI and total 25OHD was significant after adjusting for age (as a continuous variable) and gender (overall model:  $p < 0.001$ , adjusted R square 0.298, effect of BMI: beta -0.307,  $p < 0.001$ ).

**Figure 82: Total 25OHD in normal BMI and obese individuals**  
Ages combined (A), young (B), and older (C).<sup>1,2</sup>



### Total 25OHD<sub>2</sub> and 25OHD<sub>3</sub> in Obesity

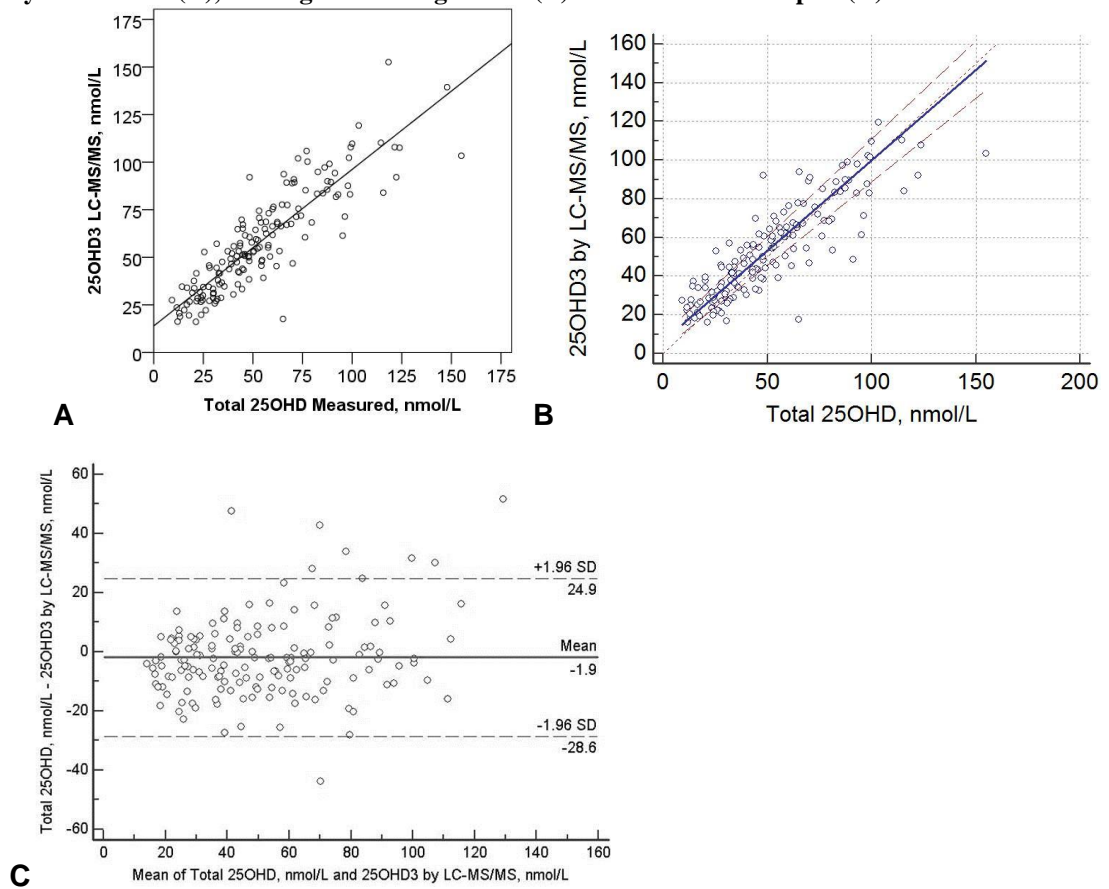
Total 25OHD<sub>2</sub> was undetectable in the majority of samples (detectable in  $n=2$  (1.2%)) and so was not included in the statistical analysis. Total 25OHD<sub>3</sub> by LC-MS/MS was strongly correlated and showed good agreement with total 25OHD by immunoassay ( $p < 0.001$ , R square 0.771) (Figure 83).

Obese individuals had a lower 25OHD<sub>3</sub> than those with a normal BMI ( $p < 0.01$  overall,  $p < 0.05$  younger and older) (Figure 84). Older adults had a higher 25OHD<sub>3</sub> than younger adults ( $p < 0.001$ ). There was no effect of gender on 25OHD<sub>3</sub> or interactions between age, gender and BMI on 25OHD<sub>3</sub>. Multiple linear regression showed the relationship between BMI and 25OHD<sub>3</sub> was significant after adjusting for age (as a continuous variable) (overall model:  $p < 0.001$ , adjusted R square 0.310, effect of BMI: beta -0.266,  $p < 0.001$ ).

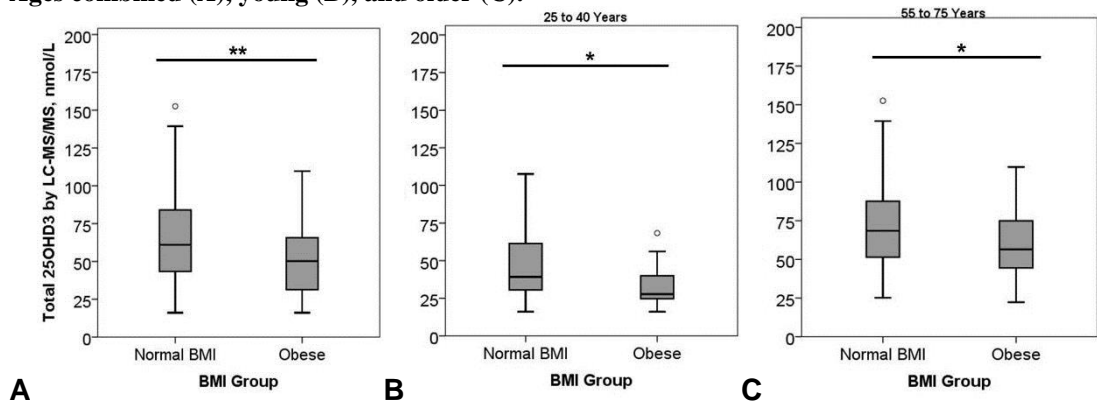
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)  
<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference



**Figure 83: Association between total 25OHD and 25OHD<sub>3</sub> by correlation (A), Passing-Bablok regression (B) and Bland-Altman plot (C)**



**Figure 84: Total 25OHD<sub>3</sub> by LC-MS/MS in normal BMI and obese individuals Ages combined (A), young (B), and older (C).<sup>1,2</sup>**



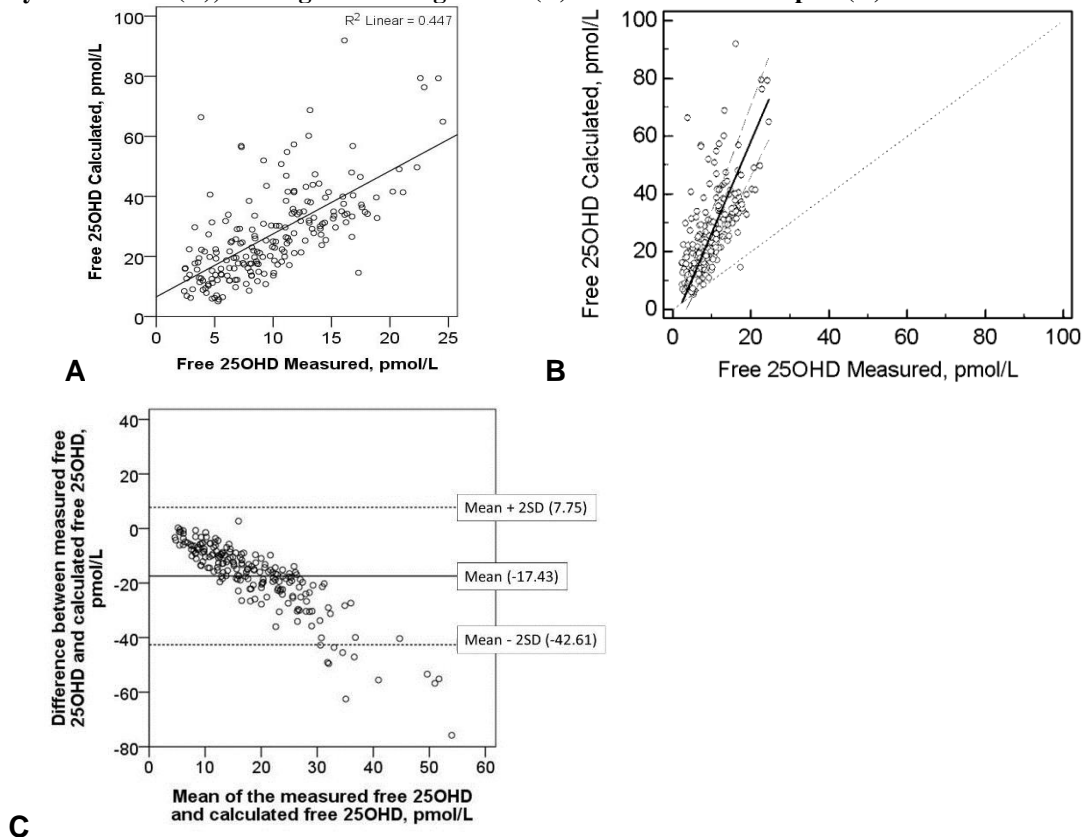
<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \*p<0.05, \*\*p<0.01. \*\*\*p<0.001, NSD no significant difference

## Free 25OHD in Obesity

Measured free 25OHD results were compared to those for calculated free 25OHD. Although correlation between the methods was strong ( $R^2 = 0.447$ ,  $p < 0.001$ ), there was a significant difference between free 25OHD by measured and calculated methods ( $p < 0.001$ ), with the measured method giving lower free 25OHD results, particularly at higher average free 25OHD levels (Figure 85).

**Figure 85: Association between measured and calculated free 25OHD by correlation (A), Passing-Bablok regression (B) and Bland-Altman plot (C)**

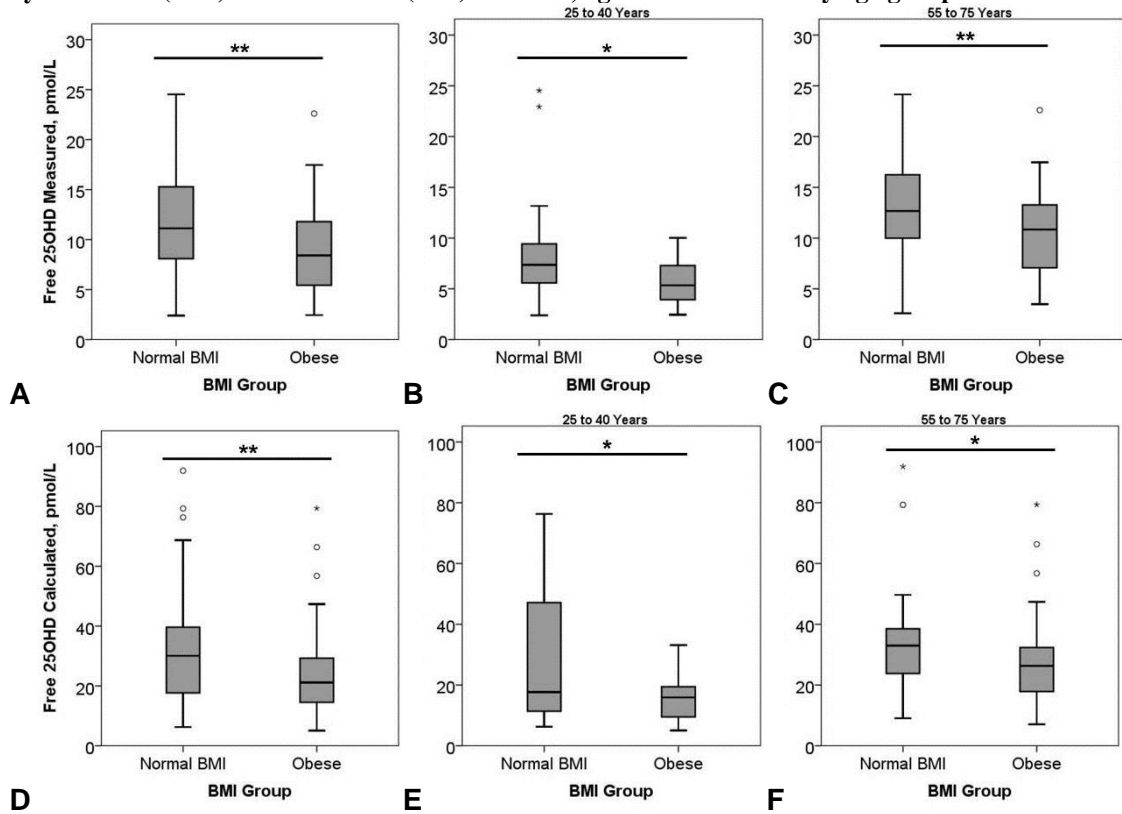


Obese individuals had lower free 25OHD than individuals with a normal BMI ( $p < 0.01$  overall (measured and calculated),  $p < 0.05$  younger (measured and calculated), older  $p < 0.01$  measured,  $p < 0.05$  calculated) (Figure 86). Older adults had a higher free 25OHD than younger adults ( $p < 0.001$  measured,  $p < 0.01$  calculated). There was no effect of gender on measured free 25OHD, but women had a higher calculated free 25OHD than men ( $p < 0.01$ ). There were no interactions between age or gender and the effect of BMI on measured free 25OHD.

Multiple linear regression showed the relationship between BMI and measured free 25OHD was significant after adjusting for age (overall model  $p < 0.001$ , adjusted  $R^2 = 0.269$ , effect of BMI  $\beta = -0.286$ ,  $p < 0.001$ ). Multiple linear regression showed the

relationship between BMI and calculated free 25OHD was significant after adjusting for age and gender (overall model  $p < 0.001$ , adjusted R square 0.234, effect of BMI beta - 0.294,  $p < 0.001$ ).

**Figure 86: Free 25OHD in normal BMI and obese individuals by measured (A-C) and calculated (D-F) methods, ages combined and by age group.<sup>1,2</sup>**



## Possible Causes of Low Vitamin D in Obesity

### Dietary Vitamin D Intake in Obesity

There was no difference in dietary vitamin D intake between normal BMI and obese individuals (Figure 87). Dietary calcium intake was not different in obese individuals compared to normal BMI individuals. There was no effect of age or gender on dietary calcium intake.

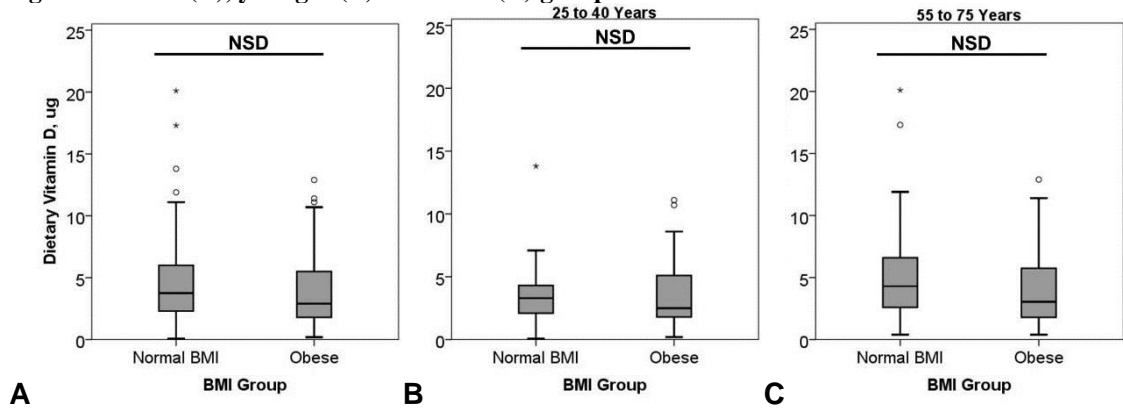
There was no difference in self-reported energy intake and therefore no difference in the amount of dietary vitamin D consumed per kcal of total energy intake, between normal BMI and obese individuals. Older adults had a greater dietary vitamin D intake ( $p < 0.05$ ) and a greater intake of vitamin D per kcal of total intake ( $p < 0.05$ ) than younger adults.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ . \*\*\* $p < 0.001$ , NSD no significant difference

Women had a greater intake of vitamin D ( $p < 0.05$ ) and a greater intake of vitamin D per kcal of total intake ( $p < 0.01$ ) than men. There were no interactions between age, gender and BMI on vitamin D intake.

**Figure 87: Dietary vitamin D intake in normal BMI and obese individuals**  
Ages combined (A), younger (B) and older (C) groups.<sup>1,2</sup>

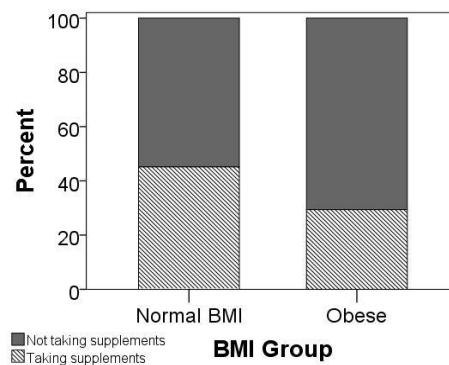


After adjusting for age and gender, BMI was not a significant predictor of vitamin D intake or intake of vitamin D per kcal of total intake, with age the only significant predictor of vitamin D intake (overall model:  $p < 0.05$ , adjusted R square 0.025, effect of age: beta 0.177,  $p < 0.05$ ) and age and gender predicting vitamin D intake per kcal of total intake (overall model:  $p < 0.01$ , adjusted R square 0.074, effect of age: beta 0.217,  $p < 0.01$ , effect of gender: beta -0.2,  $p < 0.01$ ).

### Supplementation in Obesity

Obese individuals were less likely to take supplements containing vitamin D than normal BMI individuals ( $p < 0.05$ ) (Figure 88). Older adults took more supplements containing vitamin D than young adults ( $p < 0.05$ ). There was no effect of gender on supplement habits. Multiple linear regression adjusting for age and gender showed an effect of BMI on whether people were taking vitamin D containing supplements (overall model  $p < 0.05$ , adjusted R square 0.031, effect of BMI beta -0.189,  $p < 0.05$ ).

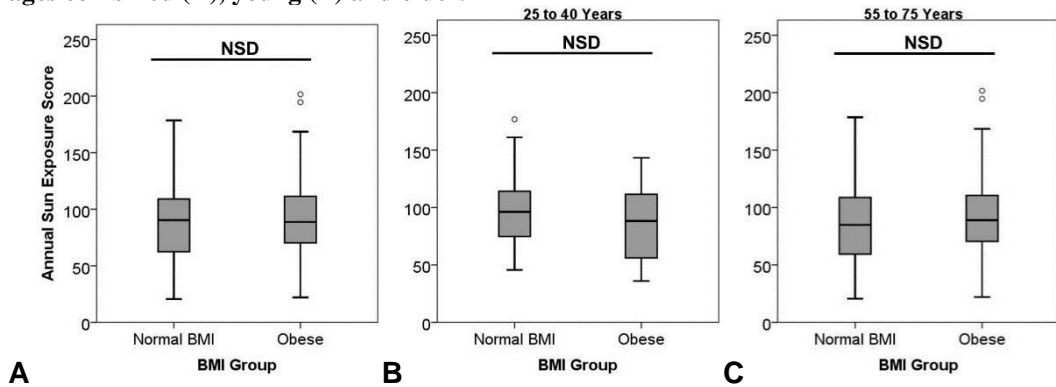
**Figure 88: Percentage of normal BMI and obese individuals taking supplements containing vitamin D**



## UVB Exposure in Obesity

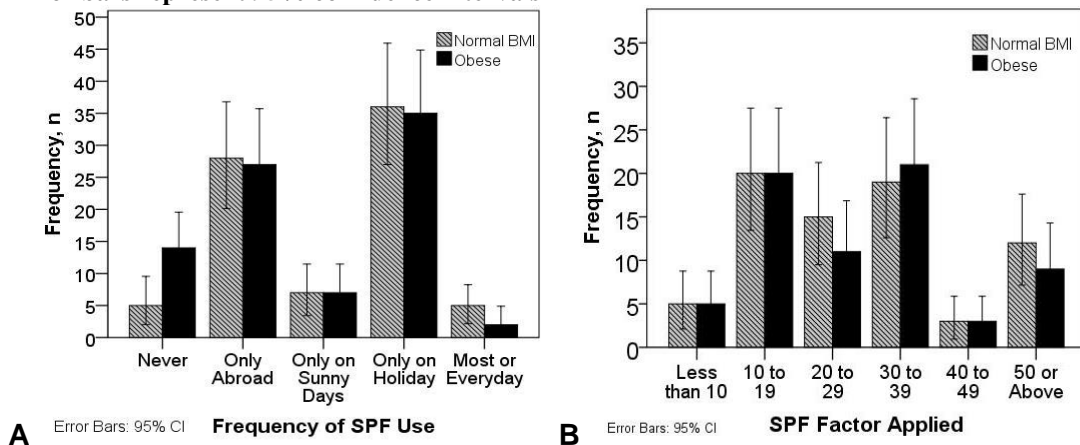
There was no difference in sun exposure between normal BMI and obese individuals (Figure 89). There was no effect of age or gender on annual sun exposure score.

**Figure 89: Annual sun exposure of normal BMI and obese individuals ages combined (A), young (B) and older.<sup>1,2</sup>**



98.8% of the sample never used sunbeds. The remaining 1.2% used sunbeds less than once per month. There was no difference in the frequency of sun protection factor (SPF) use between normal BMI and obese groups (Figure 90A). Women wore SPF more often than men ( $p < 0.01$ ). There was no difference in the SPF factor applied between normal BMI and obese, by gender, or by age (Figure 90B).

**Figure 90: Sun protection factor habits of normal BMI and obese individuals**  
Error bars represent 95% confidence intervals

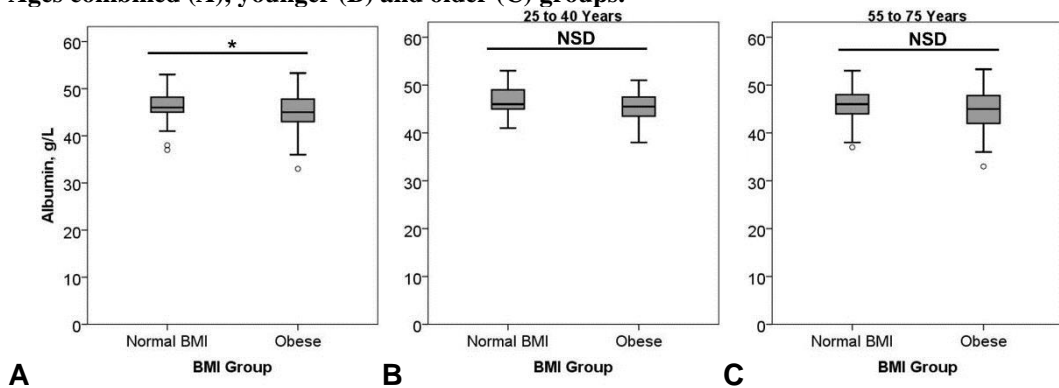


<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)  
<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference

## Vitamin D Binding Proteins in Obesity

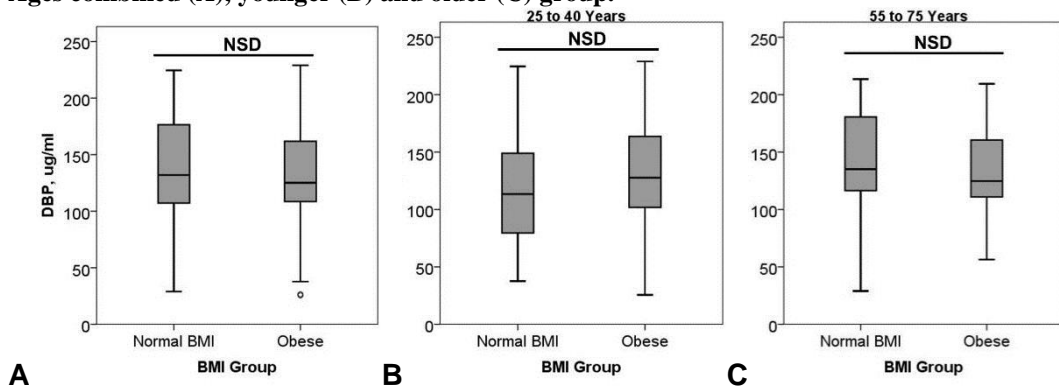
Obese individuals had lower albumin than normal BMI individuals ( $p < 0.05$  overall, NSD younger, NSD older) (Figure 91). There was no effect of age on albumin. Men had higher albumin than women ( $p < 0.001$ ). There were no interactions between age, gender and BMI with albumin. Multiple linear regression showed the relationship between BMI and albumin was significant after adjusting for age and gender (overall model:  $p < 0.001$ , adjusted R square 0.073, effect of BMI: beta -0.169,  $p < 0.05$ ).

**Figure 91: Albumin in normal BMI and obese individuals**  
Ages combined (A), younger (B) and older (C) groups.<sup>1,2</sup>



There was no difference in DBP concentration between normal BMI and obese individuals (Figure 92). There was no effect of age or gender on DBP. Adjusting for age and gender did not affect the relationship between BMI and DBP.

**Figure 92: DBP in normal BMI and obese individuals**  
Ages combined (A), younger (B) and older (C) group.<sup>1,2</sup>



Albumin was not correlated with total or free 25OHD, but was positively correlated with total 1,25(OH)<sub>2</sub>D ( $r = 0.169$ ,  $p < 0.05$ ). DBP was positively correlated with total 25OHD

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference.

( $r=0.223$ ,  $p<0.01$ ),  $25\text{OHD}_3$  ( $r=0.231$ ,  $p<0.01$ ) and total  $1,25(\text{OH})_2\text{D}$  ( $r=0.163$ ,  $p<0.05$ ) and negatively correlated with free  $25\text{OHD}$  (calculated,  $r=-0.237$ ,  $p<0.01$ ) and free  $1,25(\text{OH})_2\text{D}$  ( $r=-0.438$ ,  $p<0.001$ ).

### DBP Genotype

Distribution of DBP diplotypes and genotypes are presented in Table 20. The most common diplotype was GC2-1s and the least common was GC1f-1f. The most common genotype was GC1-1 and the least common was GC2-2 (Table 21).

**Table 20: Percentage distribution of the DBP diplotypes for the rs4588 and rs7041 SNPs by age, gender and BMI group. Most common diplotype for each group shown in bold.**

	Women					Men			
	Total	25 to 40 Years		55 to 75 Years		25 to 40 Years		55 to 75 Years	
		Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese
1s-1s	30.5	18.8	<b>37.5</b>	<b>37.0</b>	<b>32.1</b>	<b>33.3</b>	15.4	29.6	32.1
1f-1f	1.2	0.0	6.3	0.0	0.0	0.0	7.7	0.0	0.0
1s-1f	16.2	18.8	12.5	11.1	25.0	16.7	15.4	22.2	7.1
2-2	9.6	18.8	6.3	7.4	7.1	16.7	0.0	11.1	10.7
2-1s	<b>35.9</b>	<b>25.0</b>	31.3	<b>37.0</b>	<b>32.1</b>	25.0	<b>53.8</b>	<b>37.0</b>	<b>42.9</b>
2-1f	6.6	18.8	6.3	7.4	3.6	8.3	7.7	0.0	7.1

There was no difference in age, height, BMI, WHR, triceps skinfold, sun exposure, dietary intake of vitamin D, calcium or D per kcal of total energy intake, supplement usage, albumin, eGFR, CK, creatinine, PTH, calcium, phosphate, IGF-I, HsCRP, lipid profile or BTMs between the DBP diplotypes or genotypes (all  $p>0.05$ ).

**Table 21: Percentage distribution of the DBP genotypes for the rs4588 and rs7041 SNPs, by age, gender and BMI group. Most common genotype for each group shown in bold.**

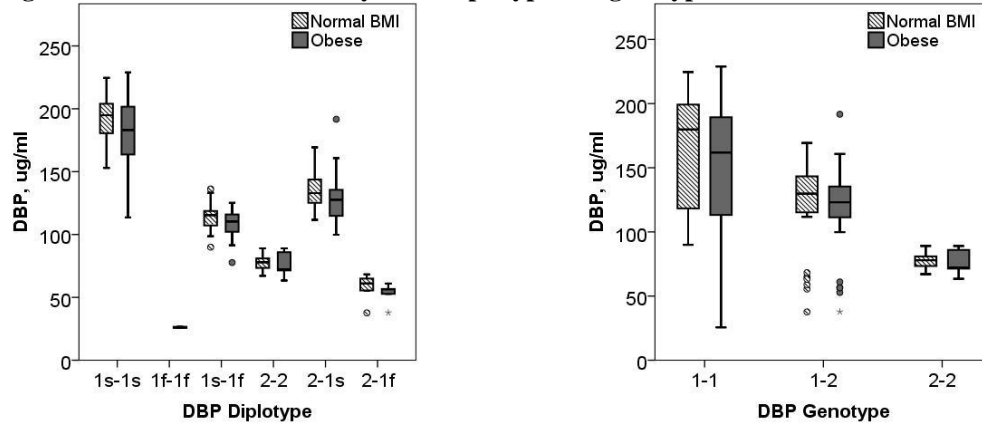
Genotype	Women					Men			
	Total	25 - 40 Years		55 - 75 Years		25 - 40 Years		55 - 75 Years	
		Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese	Norm. BMI	Obese
1-1	<b>47.9</b>	37.5	<b>56.3</b>	<b>48.1</b>	<b>57.1</b>	<b>50.0</b>	38.5	<b>51.9</b>	39.3
1-2	42.5	<b>43.8</b>	37.5	44.4	35.7	33.3	<b>61.5</b>	37.0	<b>50.0</b>
2-2	9.6	18.8	6.3	7.4	7.1	16.7	0.0	11.1	10.7

### DBP concentration by Genotype

DBP concentration differed between the diplotypes ( $p<0.001$ ) and genotypes ( $p<0.001$ ). Post-hoc testing of pairwise comparisons showed a significant difference in DBP

between the six diplotypes (all  $p < 0.001$  except between Gc1s-1f and Gc2-1s where  $p < 0.05$ ) and between the three genotypes (Gc1-1 and Gc2-2  $p < 0.001$ , Gc1-1 and Gc1-2  $p < 0.001$ , Gc1-2 and Gc2-2  $p < 0.01$ ) (Figure 93).

**Figure 93: DBP concentration by DBP Diplotype and genotype**

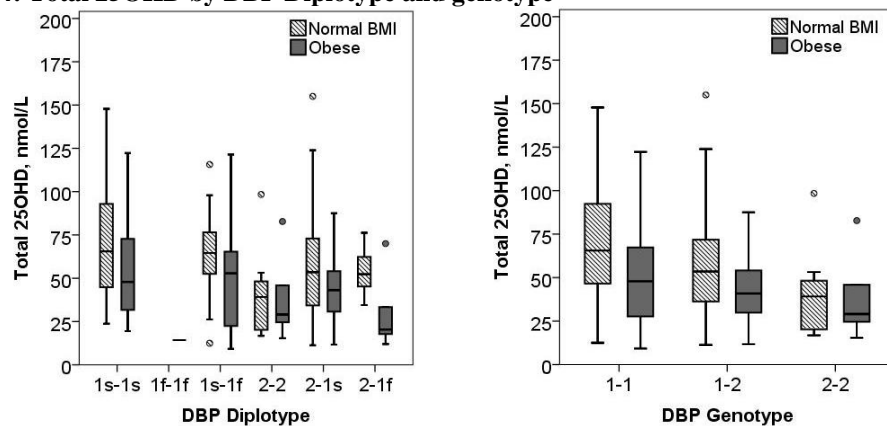


Age and diplotype, but not gender or BMI, were determinants of DBP (overall model  $p < 0.001$ , adjusted R square 0.309, effect of age: beta 0.130,  $p < 0.05$ , effect of diplotype: beta -0.538,  $p < 0.001$ ). Age and genotype, but not gender or BMI, were determinants of DBP (overall model  $p < 0.001$ , adjusted R square 0.247, effect of age: beta 0.145,  $p < 0.05$ , effect of genotype: beta -0.477,  $p < 0.001$ ).

### Total 25OHD by DBP Genotype

Total 25OHD differed between the diplotypes ( $p < 0.05$ ) and genotypes ( $p < 0.01$ ). Post-hoc testing of pairwise comparisons showed a difference between all six diplotype groups ( $p < 0.05$ ) and between the Gc1-1 and Gc1-2 ( $p < 0.05$ ), and Gc1-1 and Gc2-2 ( $p < 0.05$ ) but not between Gc1-2 and Gc2-2 (Figure 94).

**Figure 94: Total 25OHD by DBP Diplotype and genotype**



Multiple linear regression showed age, gender, BMI and DBP diplotype had significant effects on total 25OHD (overall model  $p < 0.001$ , adjusted R square 0.328, effect of age:



beta 0.437,  $p < 0.001$ , effect of gender: beta -0.128,  $p < 0.05$ , effect of BMI: beta -0.309,  $p < 0.001$ , effect of diplotype: beta -0.184,  $p < 0.01$ ). Age, BMI and DBP genotype, but not gender, had significant independent effects on total 25OHD (overall model  $p < 0.001$ , adjusted R square 0.331, effect of age: beta 0.439,  $p < 0.001$ , effect of BMI: beta -0.320,  $p < 0.001$ , effect of genotype: beta -0.225,  $p < 0.001$ ).

### Free 25OHD by DBP Genotype

Free 25OHD (measured and calculated) did not differ by diplotype or genotype, before and after adjusting for age, gender and BMI. BMI and age, but not gender, diplotype or genotype, were significant predictors of measured free 25OHD (overall model  $p < 0.001$ , adjusted R square 0.269, effect of BMI: beta -0.286,  $p < 0.001$ , effect of age: beta 0.452,  $p < 0.001$ ) and calculated free 25OHD (overall model  $p < 0.001$ , adjusted R square 0.234, effect of BMI: beta -0.294,  $p < 0.001$ , effect of age: beta 0.381,  $p < 0.001$ , effect of gender: beta -0.149,  $p < 0.05$ ).

### Total 1,25(OH)<sub>2</sub>D by DBP Genotype

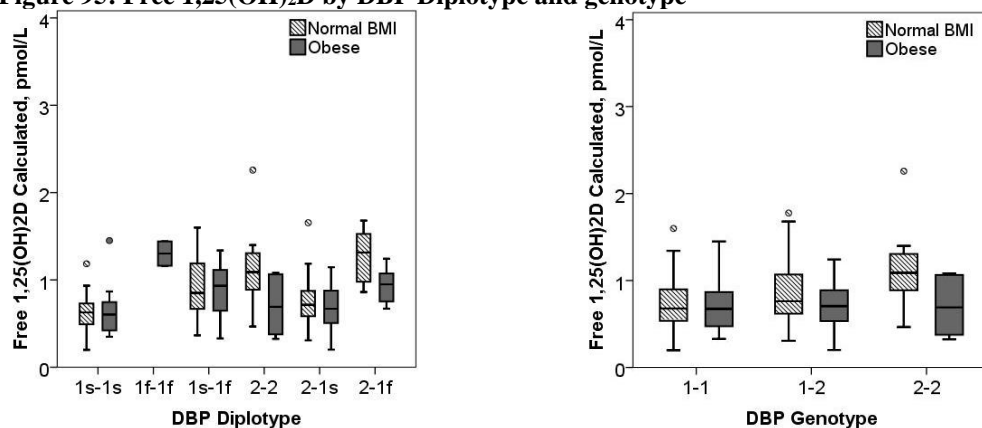
Total 1,25(OH)<sub>2</sub>D did not differ by diplotype or genotype (both  $p > 0.05$ ).

BMI and age were significant predictors of total 1,25(OH)<sub>2</sub>D after adjustment for age and gender (overall model  $p < 0.01$ , adjusted R square 0.063, effect of BMI: beta -0.210,  $p < 0.01$ , effect of age: 0.179  $p < 0.05$ ). BMI, age, and genotype were significant predictors of total 1,25(OH)<sub>2</sub>D after adjustment for gender (overall model  $p < 0.001$ , adjusted R square 0.082, effect of BMI: beta -0.220,  $p < 0.01$ , effect of age: 0.170  $p < 0.05$ , effect of genotype -0.156,  $p < 0.05$ ).

### Free 1,25(OH)<sub>2</sub>D by DBP Genotype

Free 1,25(OH)<sub>2</sub>D differed by diplotype ( $p < 0.001$ ) but not by genotype (Figure 95).

Figure 95: Free 1,25(OH)<sub>2</sub>D by DBP Diplotype and genotype



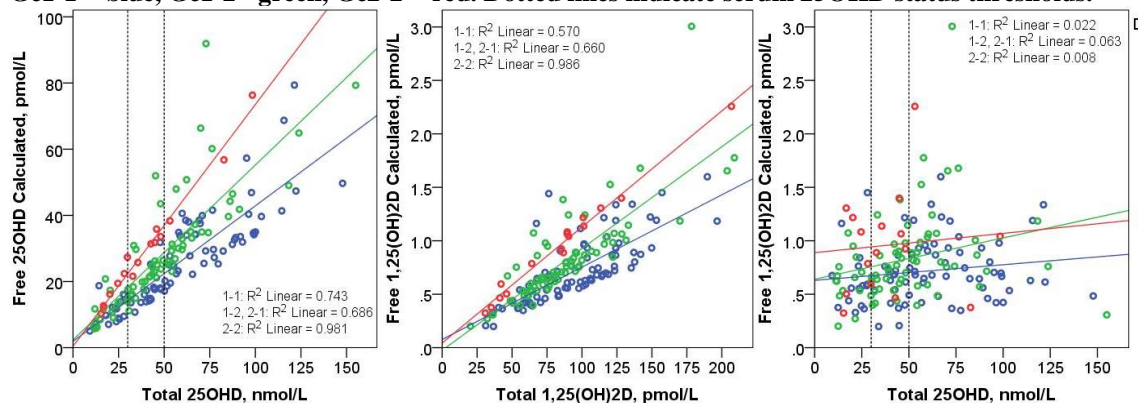
Post-hoc testing of pairwise comparisons showed a difference between all six diplotypes (all  $p < 0.05$  except between Gc1s-1s and Gc2-1f where  $p < 0.001$ ). BMI and diplotype or genotype were predictors of free 1,25(OH)<sub>2</sub>D after adjustment for age and gender (diplotype model  $p < 0.01$ , adjusted R square 0.069, effect of BMI: beta -0.156,  $p < 0.05$ , effect of diplotype: 0.231,  $p < 0.01$ ) (genotype model  $p < 0.01$ , adjusted R square 0.069, effect of BMI: beta -0.153,  $p < 0.05$ , effect of diplotype: 0.160,  $p < 0.01$ ).

### Associations Between Total and Free 25OHD and 1,25(OH)<sub>2</sub>D by DBP Diplotype and Genotype

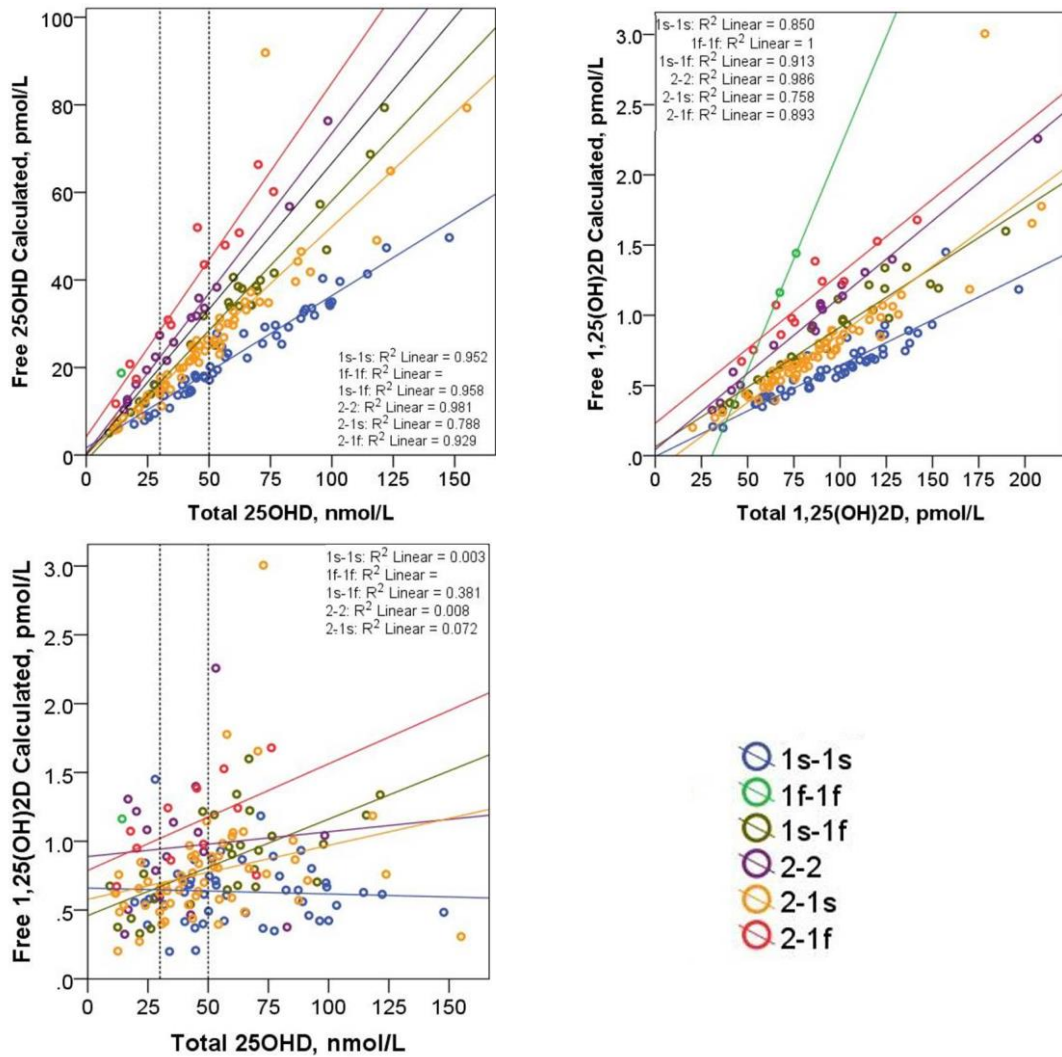
Figure 96 and Figure 97 illustrate the variation in free 25OHD for a given total 25OHD by DBP diplotype and genotype, and the variation in free 1,25(OH)<sub>2</sub>D for a given total 1,25(OH)<sub>2</sub>D by diplotype and genotype.

Gc1s-1s and Gc1-1 were consistently found to have lower free 25OHD or 1,25(OH)<sub>2</sub>D for a given total 25OHD or 1,25(OH)<sub>2</sub>D, while Gc2-1f and Gc2-2 showed the highest free concentration for a given total concentration.

**Figure 96: Total and free 25OHD and 1,25(OH)<sub>2</sub>D by DBP genotype**  
**Gc1-1 = blue, Gc1-2 = green, Gc2-2 = red. Dotted lines indicate serum 25OHD status thresholds.**



**Figure 97: Total and free 25OHD and 1,25(OH)<sub>2</sub>D by DBP Diplotype**  
 Dotted lines indicate serum 25OHD status thresholds.

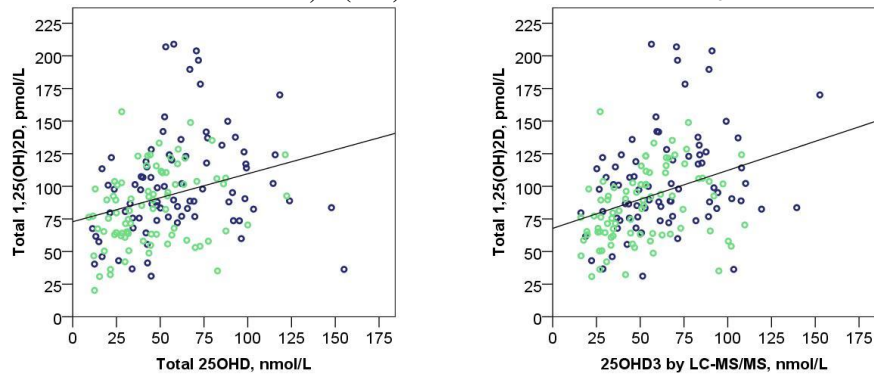


### Consequences of Low 25OHD in Obesity

#### Total 1,25(OH)<sub>2</sub>D in Obesity

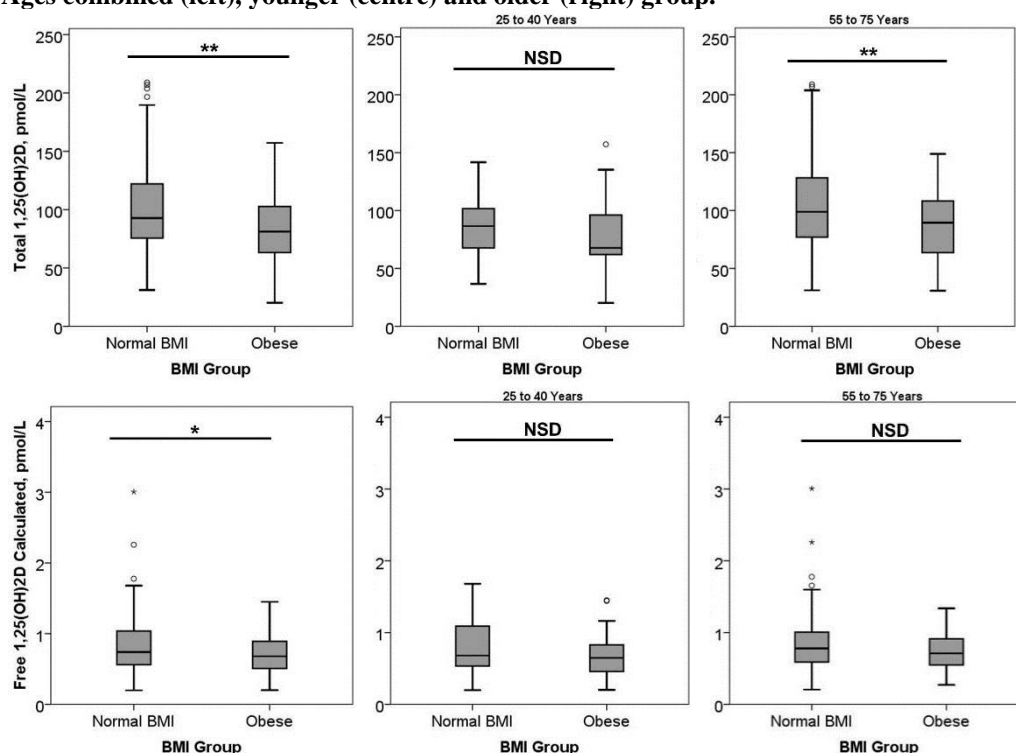
Total 1,25(OH)<sub>2</sub>D was positively correlated with total 25OHD ( $p < 0.001$ ,  $r = 0.384$ ) and 25OHD<sub>3</sub> ( $p < 0.001$ ,  $r = 0.869$ ) (Figure 98).

**Figure 98: Association between total 1,25(OH)<sub>2</sub>D and 25OHD and 25OHD<sub>3</sub>**



Obese individuals had lower total 1,25(OH)<sub>2</sub>D than normal BMI individuals (p<0.01 overall, NSD younger, p<0.01 older) (Figure 99). Older adults had higher total 1,25(OH)<sub>2</sub>D than younger adults (p<0.01). There was no effect of gender or interactions between age, gender and BMI on total 1,25(OH)<sub>2</sub>D.

**Figure 99: Total and free 1,25(OH)<sub>2</sub>D in normal BMI and obese individuals**  
**Ages combined (left), younger (centre) and older (right) group.<sup>1,2</sup>**



Multiple linear regression models showed the relationship between BMI and total 1,25(OH)<sub>2</sub>D was significant after adjusting for age and gender (overall model p<0.01, adjusted R square 0.058, effect of BMI: beta -0.210, p<0.01, effect of age: beta 0.179, p<0.05, effect of gender: beta -0.014, p=0.850).

BMI remained significant when albumin, PTH, eGFR and phosphate, the principal determinants of total 1,25(OH)<sub>2</sub>D, were added to the model (overall model p<0.01, adjusted R square 0.082, effect of BMI: beta -0.213, p<0.01, effect of age: beta 0.257, p<0.01, effect of albumin: beta 0.181, p<0.05, no significant effect of gender, PTH, eGFR or phosphate).

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \*p<0.05, \*\*p<0.01. \*\*\*p<0.001, NSD no significant difference.

### Free 1,25(OH)<sub>2</sub>D in Obesity

Obese individuals had lower calculated free 1,25(OH)<sub>2</sub>D than individuals with normal BMI ( $p < 0.05$  overall, NSD younger, NSD older). There was no effect of gender or age on free 1,25(OH)<sub>2</sub>D.

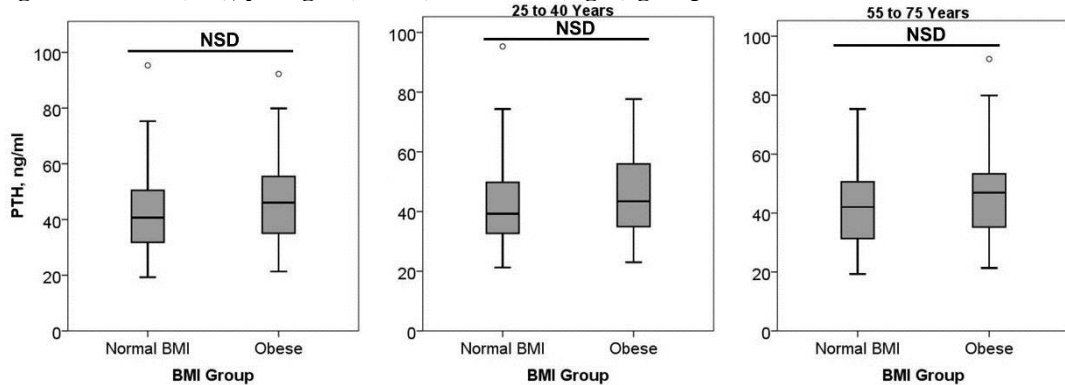
Multiple linear regression showed BMI was the only significant predictor of free 1,25(OH)<sub>2</sub>D after adjusting for age and gender (overall model  $p < 0.05$ , adjusted R square 0.021, effect of BMI: beta -0.165,  $p < 0.05$ ).

With further adjustment for age, gender, PTH, albumin, eGFR and phosphate the model was no longer significant and a stepwise linear regression model showed that BMI was the only significant predictor of free 1,25(OH)<sub>2</sub>D (overall model  $p < 0.05$ , adjusted R square 0.027, beta -181).

### Vitamin D and PTH in Obesity

There was no difference in PTH between normal BMI and obese individuals (Figure 100). There was no effect of age or gender on PTH. There was an interaction between gender and BMI on PTH, such that PTH was higher in obese women than normal BMI women but lower in obese men than normal BMI men ( $p < 0.05$ ).

**Figure 100: PTH in normal BMI and obese individuals**  
Ages combined (left), younger (centre) and older (right) group.<sup>1,2</sup>



After adjusting for age and gender there remained no significant association between BMI and PTH (adjusted R square -0.11). PTH was not correlated with total or free 25OHD or 1,25(OH)<sub>2</sub>D. Multiple linear regression models showed that PTH was not determined by age, gender, BMI, total or free 25OHD or 1,25(OH)<sub>2</sub>D.

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference.

### **Vitamin D and Bone Turnover in Obesity**

Obese individuals had lower CTX than normal BMI individuals ( $p < 0.01$  overall,  $p = 0.053$  younger,  $p < 0.05$  older). Young adults had higher CTX than older adults ( $p < 0.05$ ), but there was no effect of gender on CTX (Figure 101).

Obese individuals had lower OC than normal BMI individuals ( $p < 0.001$  overall,  $p < 0.01$  younger,  $p < 0.05$  older). There was no effect of age or gender on OC. (Figure 101)

There was no difference in PINP or BAP between obese and normal BMI individuals, or by age or gender (Figure 101).

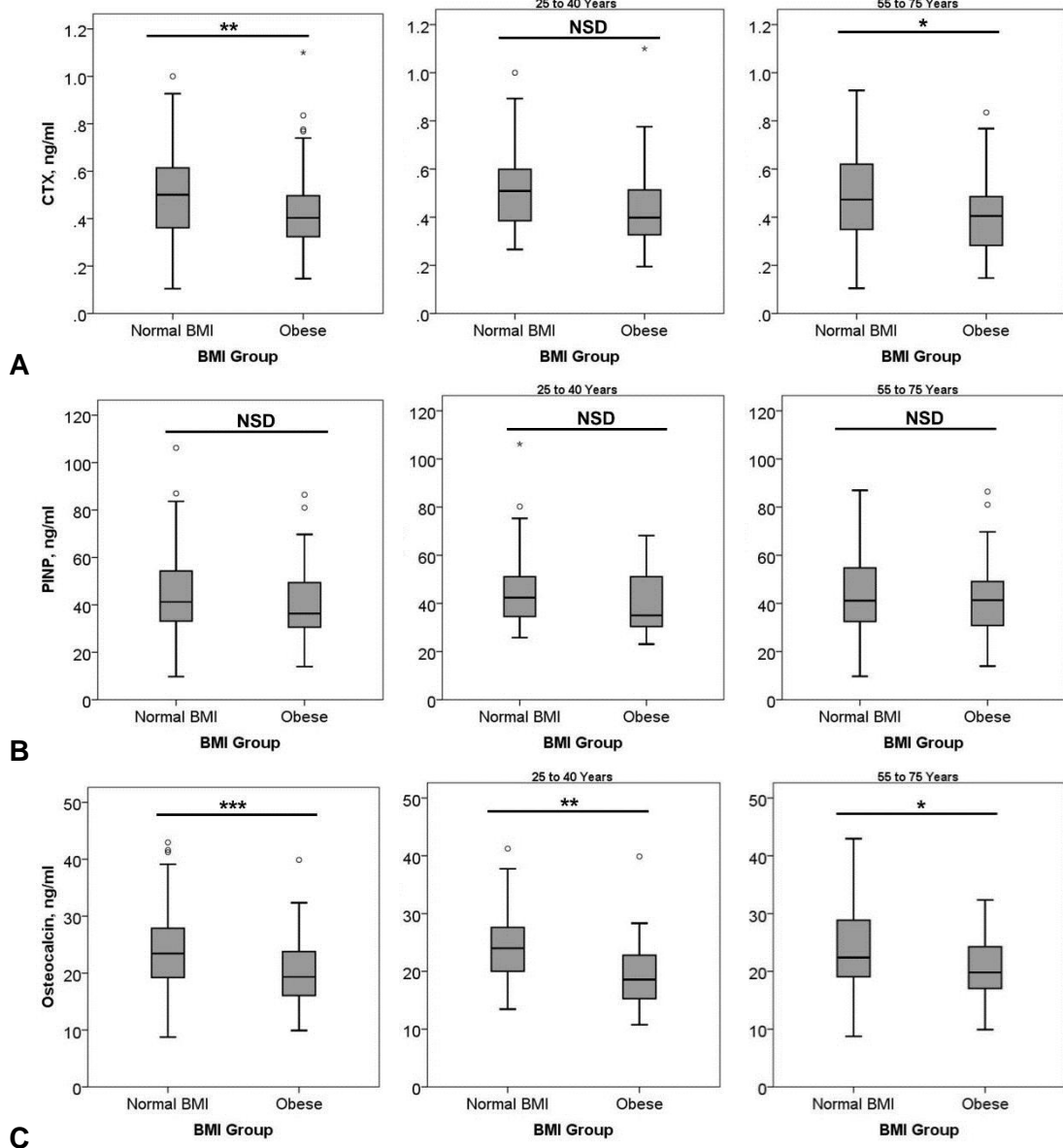
In the overall sample, total or free 25OHD and  $1,25(\text{OH})_2\text{D}$  were not correlated with CTX, PINP, OC or BAP in unadjusted analyses. In obese individuals alone, free 25OHD was negatively correlated with BAP in unadjusted analyses ( $r = -0.235$ ,  $p < 0.05$  measured,  $r = -0.225$ ,  $p < 0.05$  calculated). Adjusting for age, gender and BMI, total 25OHD was inversely related to PINP ( $r = -0.180$ ,  $p < 0.05$ ). There remained no association between total 25OHD and CTX, OC or BAP, or between free 25OHD, total and free  $1,25(\text{OH})_2\text{D}$  and any of the BTMs after adjustment.

A multiple linear regression model accounting for the effects of age, gender, BMI, total 25OHD and PTH significantly predicted CTX (overall model  $p < 0.01$ , adjusted R square 0.067) and BMI was a significant predictor within the model (beta -0.196,  $p < 0.05$ ).

A multiple linear regression model accounting for the effects of age, gender, BMI, total 25OHD and PTH significantly predicted OC (overall model  $p < 0.05$ , adjusted R square 0.057) and BMI was a significant predictor within the model (beta -0.279,  $p < 0.001$ ).

Adjustment for age and gender did not affect the relationship between PINP and BMI or BAP and BMI. PINP or BAP were not predicted by models of age, gender, BMI, total 25OHD and PTH.

**Figure 101: BTMs in normal BMI and obese sub-study individuals CTX (A), P1NP (B) and OC (C) by ages combined (left), young (centre) and older (right).<sup>1,2</sup>**



### Bone Density, Microstructure and Strength by DBP Genotype

There was no difference in aBMD, vBMD, bone microarchitecture or bone strength by DBP diplotype or genotype (ANOVA  $p > 0.05$ ). After adjusting for age, gender and BMI, there was a significant association between DBP diplotype and WB aBMD (overall model  $p < 0.001$ , adjusted R square=0.397, effect of diplotype: beta -0.126,  $p < 0.05$ ), and between DBP diplotype and tibial estimated failure load (overall model  $p < 0.001$ , adjusted R square=0.608, effect of diplotype: beta -0.135,  $p < 0.01$ ), but all other associations

<sup>1</sup> Box and whisker plots showing the median, interquartile range and outliers (defined as being more than one and a half interquartile ranges above or below the interquartile range)

<sup>2</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , NSD no significant difference.

between DBP diplotype or genotype and aBMD, vBMD, bone microarchitecture or bone strength remained non-significant.

aBMD (Figure 102), and vBMD (Figure 103) were negatively correlated with measures of total and free 25OHD and 1,25(OH)<sub>2</sub>D (supported by Table 22). The negative association between 25OHD and BMD was unexpected, but possibly explained by obesity causing both low 25OHD and high BMD. Multiple linear regression showed that after adjustment for age, gender and BMI, total 25OHD was not a significant predictor of WB (beta 0.075), TH (beta -0.001) or LS aBMD (beta 0.038) (tolerance statistic= 0.751, 0.751, 0.766 respectively) or of tibia vBMD (beta -0.066), radius vBMD (-0.155) or LS vBMD (beta -0.056).

Multiple linear regression showed that after adjustment for age, gender and BMI, total 25OHD was not a significant predictor of estimated failure load at the radius (beta 0.038) or tibia TH (beta 0.055).

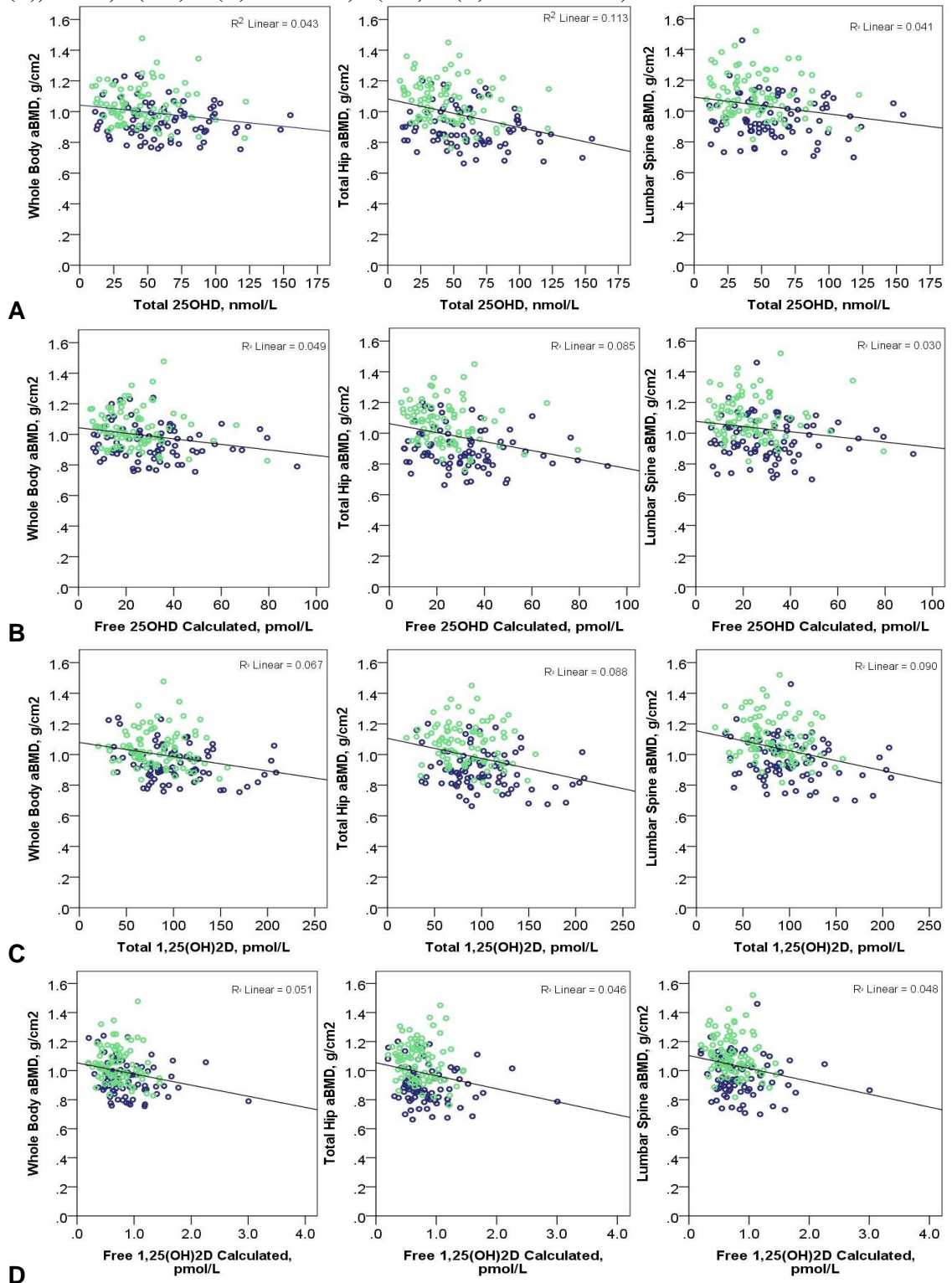
**Table 22: Correlation coefficients between BMD and vitamin D metabolites**

	Total 25OHD	Free 25OHD Measured	Free 25OHD Calculated	25OHD <sub>3</sub>	Total 1,25(OH) <sub>2</sub> D	Free 1,25(OH) <sub>2</sub> D
WB aBMD	-0.168*	NS	-0.170*	-0.141*	-0.200**	-0.181**
TH aBMD	-0.292***	-0.214*	-0.270***	-0.285***	-0.236***	-0.179**
LS aBMD	-0.181**	-0.161*	-0.166*	-0.202**	-0.238***	-0.185**
Radius vBMD	-0.266***	-0.237**	-0.234**	-0.227**	0.189**	-0.140*
Tibia vBMD	-0.288***	0.273**	-0.273***	-0.309***	-0.194**	-0.181**
LS vBMD	NS	NS	NS	NS	NS	NS

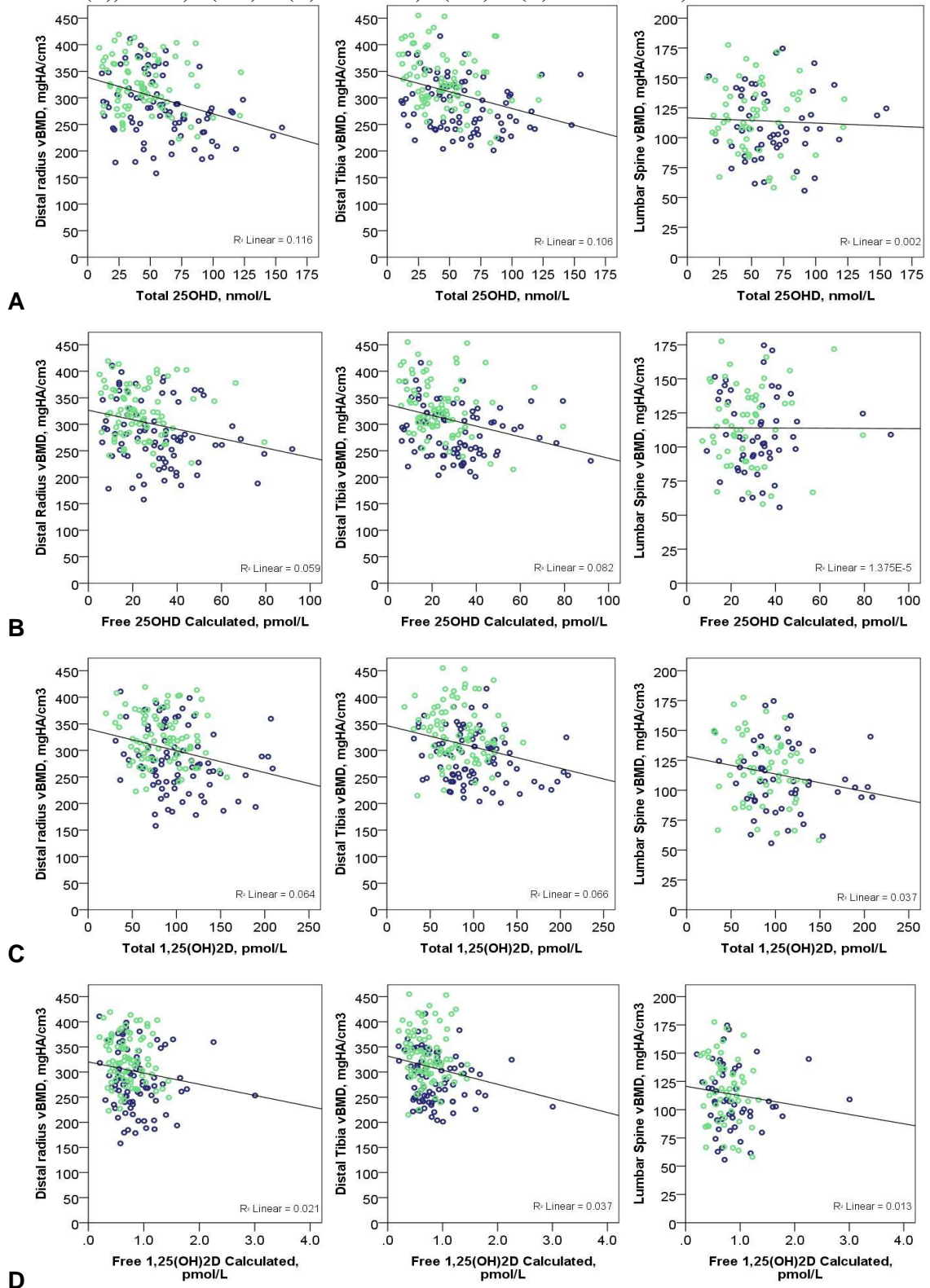
\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, NS not significant



**Figure 102: Associations between vitamin D and aBMD**  
 At the whole body (Left), total hip (centre) and lumbar spine (right), by total 25OHD (A), free 25OHD (B), total 1,25(OH)<sub>2</sub>D (C) and free 1,25(OH)<sub>2</sub>D (D). Green=obese, blue = normal BMI



**Figure 103: Associations between vitamin D and vBMD**  
 At the distal radius (Left), distal tibia (centre) and lumbar spine (right), by total 25OHD (A), free 25OHD (B), total 1,25(OH)<sub>2</sub>D (C) and free 1,25(OH)<sub>2</sub>D (D). Green=obese, blue = normal BMI



### Vitamin D and Biochemical Factors in Obesity

Total 25OHD ( $r=0.254$ ,  $p<0.01$ ), free 25OHD (measured  $r=0.245$ ,  $p<0.01$ , calculated  $r=0.188$ ,  $p<0.05$ ) and 25OHD<sub>3</sub> ( $r=0.221$ ,  $p<0.05$ ) were positively correlated with adjusted calcium. Adjusted calcium was not associated with total or free 1,25(OH)<sub>2</sub>D. Total and free 25OHD and 1,25(OH)<sub>2</sub>D were not correlated with IGF-I, phosphate, creatinine or CK.

### Is Vitamin D Affected by Serum Lipids or Inflammatory Markers?

TC was not correlated with total or free 25OHD or 1,25(OH)<sub>2</sub>D. HDL was positively correlated with total ( $r=0.260$ ,  $p<0.01$ ) and free 25OHD ( $r=0.206$ ,  $p<0.01$ ), and total ( $r=0.273$ ,  $p<0.01$ ) and free 1,25(OH)<sub>2</sub>D ( $r=0.180$ ,  $p<0.05$ ). TC:HDL ratio and triglycerides were negatively correlated with total ( $r=-0.241$ ,  $p<0.01$ ) and free 25OHD ( $-0.243$ ,  $p<0.01$ ) and total ( $r=-0.265$ ,  $p<0.05$ ) and free 1,25(OH)<sub>2</sub>D ( $r=-0.215$ ,  $p<0.05$ ). Triglycerides remained associated with total 25OHD after adjustment for age, gender, BMI, TC, HDL and LDL (overall model  $p<0.001$ , adjusted R square 0.303, effect of triglycerides: beta -0.164,  $p<0.05$ ). TC, HDL and LDL were not significant predictors of total 25OHD after adjustment for age, gender, BMI and triglycerides.

HsCRP was positively correlated with VAT and SAT (both  $p<0.001$ ) and inversely associated with albumin ( $r=-0.193$ ,  $p<0.05$ ). HsCRP was not correlated with total or free 25OHD but was negatively correlated with total 1,25(OH)<sub>2</sub>D ( $r=-0.227$ ,  $p<0.01$ ) and free 1,25(OH)<sub>2</sub>D ( $r=-0.226$ ,  $p<0.05$ ). After adjusting for age, gender, BMI and albumin, HsCRP remained a predictor of 1,25(OH)<sub>2</sub>D ( $p<0.01$ , adjusted R square 0.046) and the only significant predictor of free 1,25(OH)<sub>2</sub>D ( $p<0.01$ , adjusted R square 0.045).

### Vitamin D and Body Fat Distribution in Obesity

Significant correlations between adipose compartments and vitamin D metabolites are shown in (Table 23). No significant correlations were observed for WHR, VAT or VAT:SAT ratio.

**Table 23: Correlations between fat compartments and vitamin D metabolites**  
Data shown as r values

	Total 25OHD	Free 25OHD	25OHD <sub>3</sub>	Total 1,25(OH) <sub>2</sub> D	Free 1,25(OH) <sub>2</sub> D
WB FM	-0.253**	-0.270**	-0.272**	-0.210**	NS
Trunk FM	-0.231**	-0.243**	-0.222**	-0.181*	NS
SAT	-0.270**	-0.271**	-0.221**	-0.207**	-0.178*
Triceps skinfold	-0.222**	-0.210**	-0.249**	NS	NS

\* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , NS not significant

### **Vitamin D and Physical Performance in Obesity**

SPPB score was not correlated with total or free 25OHD (measured or calculated), 25OHD<sub>3</sub> or total 1,25(OH)<sub>2</sub>D, but was positively correlated with free 1,25(OH)<sub>2</sub>D ( $r=0.173$ ,  $p<0.05$ ).

After adjustment for age, gender and BMI, SPPB score was positively correlated with total 1,25(OH)<sub>2</sub>D ( $r=0.234$ ,  $p<0.01$ ) and free 1,25(OH)<sub>2</sub>D ( $r=0.217$ ,  $p<0.01$ ). However, the addition of total or free 1,25(OH)<sub>2</sub>D did not improve the model described in Chapter 5 for the prediction of SPPB score.

Gait speed was negatively correlated with 25OHD<sub>3</sub> by LC-MS/MS ( $r=-0.176$ ,  $p<0.05$ ) and grip strength was negatively correlated with free 25OHD ( $r=-0.156$ ,  $p<0.05$ ), but after adjustment for age, gender and BMI no measures of physical function were correlated with total or free 25OHD, 25OHD<sub>3</sub>, or 1,25(OH)<sub>2</sub>D.

Adding 25OHD<sub>3</sub> did not improve the model for predicting gait speed (multiple linear regression with gait as the dependent variable, age and BMI as independent variables; overall model  $p<0.001$ , adjusted R square 0.272, with age, BMI and 1,25(OH)<sub>2</sub>D as independent variables; overall model  $p<0.001$ , adjusted R square 0.277; beta 1,25(OH)<sub>2</sub>D -0.028,  $p=0.729$ ).

Adding free 25OHD slightly improved the model for predicting grip strength (multiple linear regression with grip strength as the dependent variable, age, gender and BMI as independent variables; overall model  $p<0.001$ , adjusted R square 0.571, with age, gender, BMI and 1,25(OH)<sub>2</sub>D as independent variables; overall model  $p<0.001$ , adjusted R square 0.580; beta free 25OHD -0.031,  $p=0.614$ ).

### **Summary of Results**

A summary of the general findings of this Chapter is given in Table 24

**Table 24: Summary of results of Chapter 6**  
**The effects of BMI, age and gender and interactions between age or gender and BMI on 25OHD levels and potential causal factors and consequences of low 25OHD in obesity.**

Variable	Effect of BMI (%difference)	Effect of Age	Effect of Gender	Interactions
Total 25OHD	↓(-25.5)	↑in older	↑women	-
25OHD <sub>3</sub>	↓(-20.8)	↑in older	-	-
Free 25OHD (M)	↓(-23.1)	↑in older	-	-
Free 25OHD (C)	↓(-24.9)	↑in older	↑women	-
Dietary intake	-	↑in older	↑women	-
Supplement use	↓	↑in older	-	-
Sun exposure	-	-	-	-
Albumin	↓(-2.9)	-	↑men	-
DBP concentration	- (-4.1)	-	-	-
DBP genotype	-	-	-	-
Total 1,25(OH) <sub>2</sub> D	↓(-17.4)	↑in older	-	-
Free 1,25(OH) <sub>2</sub> D	↓(-16.3)	-	-	-
PTH	- (+6.4)	-	-	Gender-BMI <sup>1</sup>
CTX	↓(-14.3)	↑in younger	-	-
PINP	- (-8.6)	-	-	-
OC	↓(-17.5)	-	-	-
BAP	- (-1.1)	-	-	-

Data shown as a percentage of the mean of the normal BMI group

M=measured free 25OHD, C= calculated free 25OHD

<sup>1</sup> PTH higher in obese than normal BMI women, lower in obese than normal BMI men

## **Discussion**

Total 25OHD levels are lower in obesity. Associations between 25OHD and adiposity have been widely reported (395, 402, 405, 407, 549-551, 558, 575, 600-602) and were recently supported by the results of a meta-analysis of the association between 25OHD and BMI in healthy adults (603). Several smaller studies of pre-bariatric surgery patients have shown low 25OHD in obese individuals compared to non-obese controls (405, 565).

Free 25OHD levels are also lower in obesity. This is the first study to investigate the difference in free 25OHD between obese individuals and a non-obese control group and the first study to report associations between adiposity and measured free 25OHD.

Younger people have lower total and free 25OHD than older people. Whilst some studies have reported greater total 25OHD with increasing age (601, 604), there is a greater body of evidence to support inverse associations between 25OHD and age (395, 605, 606). Need et al. reported an increase in 25OHD between the ages ~45 to ~65 years but this was followed by a significant decline in 25OHD to ~75 years (601). In older adults, vitamin D status was inversely associated with BMI, WC, FM, FM corrected for height

and percentage FM to fat free mass, but none of these associations were observed in younger adults age 20 to 40 years (557). Lower total 25OHD in younger people may be due to lower dietary intakes in this age group. Lower dietary vitamin D intakes were reported in Danish teenage girls compared to elderly women (607). Vitamin D intake increased with age in adults aged 25 to 69 years (575, 608). The finding of lower 25OHD in younger adults is consistent with that of a large study of British adults which found those aged 50 to 64 years consumed more oily fish than any other age group and that although low 25OHD was widespread in all age groups, "it was most noticeable in older children, young adults (including women of childbearing age) and older people living in institutions", with the young adults in the SACN report being of a similar age to those in the present study (609). There may be more rapid clearance of 25OHD in young people, which could lower circulating 25OHD.

Women had higher 25OHD than men, as previously shown (610, 611), although others have reported higher total 25OHD in men (109). There were no differences in 25OHD<sub>3</sub> or measured free 25OHD by gender, although calculated free 25OHD was lower in men. Previous suggestions that differences in 25OHD status by gender are a result of greater adiposity for a given BMI in women appear counterintuitive, as this would result in men having a greater serum 25OHD, opposing the results of this study (603). As women had a greater dietary intake of vitamin D than men, this may explain such findings although additional evidence to support gender differences in vitamin D intake is conflicting (608, 612, 613).

The most likely cause of low 25OHD in obesity is greater volume of distribution resulting from greater SAT (WBFM, SAT and triceps skinfold) (but not visceral fat), acting as a sump. 25OHD is inversely associated with waist circumference (404), WHR (402) total abdominal FM (269), SAT (269, 404, 549) and VAT (404, 549, 614). During a 2.6 year longitudinal study of 25OHD, adiposity, adipokines and lipids in older adults, those with incident vitamin D deficiency had a greater BMI and trunk FM than individuals who remained vitamin D replete, while subjects with recovery of vitamin D deficiency had lower BMI and WBFM and trunk FM (600). Measuring 25OHD<sub>3</sub> in the fat tissue of pre-bariatric surgery patients by LC-MS/MS, Blum et al. found that serum and fat tissue concentrations of 25OHD<sub>3</sub> were positively correlated (615). This demonstrates that fat tissue does indeed function as a depot for vitamin D storage. Thus although obese individuals have lower circulating 25OHD, the positive association between serum and fat tissue concentrations of 25OHD<sub>3</sub> is likely explained by the greater number of adipocytes each with a relatively lower concentration of 25OHD in obesity. The concept

of volumetric dilution, rather than sequestration of 25OHD into adipose tissue has also been proposed (569). The circulating 25OHD response to vitamin D supplementation has been shown to be blunted in obesity (557, 570, 572, 573, 575) and there is less seasonal variation in circulating 25OHD into the winter months (601). Greater adiposity is also associated with lower 25OHD after irradiation (407). The larger pool size in obesity may require a greater loading dose to achieve adequate circulating levels.

Lower 25OHD in obesity was not due to lower dietary intake of vitamin D. High BMI has been linked to lower vitamin D intakes in younger and older adults (395, 608, 616). In older American adults, the major modifiable predictors of low vitamin D status were being obese, having a low dietary and supplementary vitamin D intake, and physical inactivity (617). However, there was no difference in dietary or supplementary vitamin D intake between a group of young women with 25OHD <90nmol/L and an average BMI of 28.6 kg/m<sup>2</sup> and a group with 25OHD >90nmol/L and an average BMI 26.0 kg/m<sup>2</sup> (618). No difference in vitamin D intake was observed between obese and non-obese pre and postmenopausal women (596). The lack of difference in vitamin D intake was not due to obese adults consuming more vitamin D through consumption of more kcal, as obese people reported consuming the same amount of vitamin D per kcal of their total dietary intake as the normal weight group. As reported total kcal intake was not different between the groups, this may raise doubt over the validity of the reported kcal intakes and obese individuals may be more likely to under-report kcal intake. Overall vitamin D intakes were low; with the mean dietary vitamin D considerably below recommended intakes by the US Institute of Medicine (15µg per day) and Endocrine Society (37.5-50.0µg per day).

The lower prevalence of supplementation in obesity may contribute to the lower circulating total and free 25OHD. 25OHD<sub>2</sub> was detectable by LC-MS/MS in very few participants. Low rates of detection were also reported by Schwartz et al., who found a 6% detection rate (560). Obese people were shown to take fewer dietary supplements in the NHANES study (2003-2006 data), with 48% of obese compared to 56% of those with a normal BMI taking supplements (619). Although this analysis excluded supplementary calcium and vitamin D, general supplementary habits may be generalizable to vitamin D supplementation. Others have also reported lower supplementation in those with high BMI, particularly in women (402, 608), although the present results indicate no gender difference in supplement intake. Older people take more supplements containing vitamin D than younger people, which was also found in the NHANES study (613, 619).

There was no difference in UVB exposure between obese and normal BMI groups. Skin synthesis of vitamin D in response to UVB exposure was found not to differ between normal BMI and obese adults (407) and therefore a lack of difference in exposure would be expected to result in a lack of difference in circulating vitamin D, which was not the case. Kull et al. reported that individuals with the greatest percentage body fat and those with a BMI greater than 30 kg/m<sup>2</sup> were more likely to avoid sunbathing altogether, and there was a significant difference in the body fat percentage and BMI between those who exposed their arms and face and those who exposed their whole body when sunbathing (561). Hours of sunlight exposure were negatively correlated with BMI in postmenopausal women, but not significantly so (601). However, in a UK study, sunlight exposure was also found not to vary with BMI (620) and others report no difference in sun exposure between normal BMI and obese groups (602). Harris et al. found that while men were exposed to UVB for more hours per week than women, percentage FM and age were not significant predictors of hours of sun exposure and the percentage of skin exposed did not differ across quartiles of percentage FM (616). SPF use was no different between normal BMI and obese groups, similar to the findings of Harris and Dawson-Hughes (616). UVB exposure may play more of a role in the relationship between obesity and vitamin D status in settings where UVB is more intense such as in Saudi Arabia (395).

Obese people had lower albumin, and albumin was inversely associated with HsCRP. Therefore it is likely that the lower albumin was due to the greater inflammation observed in obesity as a result of greater SAT and VAT. Previous studies have identified links between low 25OHD and inflammation, with DBP being inversely associated with CRP and inflammation (564). Typically, lower albumin would be expected to result in a greater proportion of free 25OHD, and in a state where 25OHD is low, such as obesity, may be expected to normalise or increase free 25OHD. However, this was not the case and lower free fractions were observed in obesity. Lower free fractions may be better explained by DBP levels, than albumin. DBP bound fractions are stronger determinants of the biological effects of vitamin D metabolites than those which are albumin bound, due to the higher affinity of DBP to 25OHD and 1,25(OH)<sub>2</sub>D placing greater restriction on the ability of vitamin D metabolites to cross cell membranes, compared to the affinities for albumin.

There was no difference in DBP between normal BMI and obese groups. Similarly there was no association between DBP and BMI (109, 550), FM (109) or percentage FM (109) in previous studies. However, positive associations between DBP, BMI and FM (567)



and negative associations between DBP and BMI (255) have also been reported. DBP was positively associated with total 25OHD in the present study. Positive association between DBP and total 25OHD was also reported by Bolland et al. in women, but not in men (109), while others have found no association between total 25OHD and DBP (550, 567) and have argued that the lack of association between DBP and total 25OHD is somewhat similar to the lack of association between DBP and 25OHD observed in other conditions where vitamin D is low and yet DBP is not abnormal (621). DBP was positively associated with total 1,25(OH)<sub>2</sub>D in both the present study and previous work (567). As the major transporter of vitamin D metabolites in the circulation, DBP being inversely correlated with free 25OHD and 1,25(OH)<sub>2</sub>D was expected. Although DBP levels do not explain low total 25OHD in obesity, DBP may be a significant contributor to the low free 25OHD and free 1,25(OH)<sub>2</sub>D in obesity as despite the positive correlations between total 25OHD and DBP, DBP levels are not lower despite lower total 25OHD, resulting in a greater proportion of DBP to 25OHD and so a lower proportion of free 25OHD in obesity. Powe et al. proposed that 25OHD may regulate the production of DBP to regulate the free or bioavailable fraction of 25OHD when levels are low (255). The results of the present study do not appear to support this, as despite low 25OHD, DBP are 'unchanged' relative to those of normal BMI.

The results suggest that the interaction between 25OHD and DBP in obesity is not affected by CK; with no difference in CK between normal BMI and obese groups and there was no association between total or free 25OHD or 1,25(OH)<sub>2</sub>D and CK. However, the interaction between 25OHD and DBP may be affected by lipids. While there was no difference in TC in obesity, HDL was lower and inversely associated with total and free 25OHD and 1,25(OH)<sub>2</sub>D. Triglycerides were elevated in obesity and negatively associated with total and free 25OHD and 1,25(OH)<sub>2</sub>D, although after adjustment only the association between total 25OHD and triglycerides remained. A longitudinal study found incident vitamin D deficiency was predicted positively by triglycerides, although after adjustment for seasonality, gender, age, BMI, smoking and physical activity, this association was no longer significant (600). There was no association between TC, HDL, LDL or TC:HDL ratio and incident vitamin D deficiency (600). Recovery of vitamin D deficiency was negatively predicted by triglycerides and TC:HDL ratio (600). Annual change in 25OHD was associated with TC:HDL ratio in univariate analyses and positively associated with HDL and negatively with IL-6 and TC:HDL ratio after adjustment for baseline 25OHD, seasonality, gender, age, BMI, smoking, sun exposure and activity (600). Overall, it appears that a more inflammatory environment could reduce the availability of active vitamin D in obesity.

There was no effect of age on vitamin D binding proteins, as found by Bolland et al. (109). However whilst Bolland et al. reported higher DBP in women than men, this was not seen in the present study (109).

The DBP genotype distribution in this study sample was similar to that previously described (622). Gc1s and Gc2 have been shown to be the most common alleles (622), and in this study the most common diplotype was Gc2-1s followed by Gc1s-1s, the same as reported by Lauridsen et al. (598). The most common genotype was Gc1-1 and the least common, Gc2-2 as previously reported (598, 622). There was no difference in genotype or diplotype between normal and obese groups. Lauridsen et al. found no difference in BMI by DBP genotype and no difference in skinfold thickness at various sites by genotype (598). These results suggest that DBP genotype is unlikely to explain low free 25OHD levels in obesity.

DBP levels differed by DBP diplotype and genotype, with the highest concentration of DBP in the Gc1s-1s diplotype and the Gc1-1 genotype, as previously shown (598). Although Gc1f-1f was previously associated with the highest DBP concentration, there were very few participants with this diplotype in the study. The lowest DBP was seen in those with Gc2-1f diplotype and Gc2-2 genotype, as expected (598).

While total 25OHD differed by diplotype and genotype, with higher 25OHD in those with the Gc1-1 genotype, free 25OHD did not differ by diplotype or genotype. This can be explained by Gc1-1 being associated with the highest total 25OHD but also with the highest DBP concentration and so there was no difference in free 25OHD by genotype. Despite this logical explanation, Lauridsen et al. found Gc1-1 to be associated with the highest total 25OHD but also with the highest free 25OHD, possibly attributed to differences in the proportion of 25OHD to DBP compared to the present work (598). In Belgian men no differences were reported in 25OHD by DBP genotype (567).

It was hypothesised that higher affinity forms of DBP would result in lower circulating 1,25(OH)<sub>2</sub>D. However, there was no difference in 1,25(OH)<sub>2</sub>D by DBP diplotype or genotype, although the low prevalence of Gc1f-1f, meant that the group with the highest affinity was underrepresented. Others have reported higher levels of total 1,25(OH)<sub>2</sub>D in those with the Gc1-1 genotype (567, 598) and Chun et al. have shown that “Gc1f-1f is better able to maintain serum levels of vitamin D metabolites as a consequence of more efficient retention of 25OHD<sub>3</sub> and 1,25(OH)<sub>2</sub>D after megalin-mediated uptake of DBP by

kidney cells” (623). Although others found no difference in free 1,25(OH)<sub>2</sub>D by genotype (598), after adjustment, free 1,25(OH)<sub>2</sub>D differs by diplotype and genotype, explained by Gc1-1 having the highest total 1,25(OH)<sub>2</sub>D but concurrently the highest DBP and highest affinity constant, and so the lowest free 1,25(OH)<sub>2</sub>D, while the opposite was true for Gc2-2.

Differences in the association between free and total 25OHD and free and total 1,25(OH)<sub>2</sub>D by diplotype and genotype illustrate the potential for more accurate assessment of true vitamin D sufficiency by acknowledging of the effect of DBP genotype on the free fraction. Although reference ranges for free 25OHD sufficiency have not been proposed, more detailed assessment of 25OHD status would improve on current practice of determining sufficiency solely by measuring total 25OHD (622). Chun et al. have shown by mathematical modeling, that “an individual with 100 nM total 25D and Gc1f-1f DBP genotype would have less free 25D than an individual with 50 nM total 25D carrying either Gc1s-2 or Gc2-2 genotype” (624). Chun et al. showed that at a 25OHD of 50 nM, free 25OHD as a percentage of total 25OHD varied by DBP genotype from 0.026 to 0.074%, which is somewhat considerable given the that free 25OHD typically accounts for 0.02 to 0.05% of total 25OHD (624). The same trend was observed for 1,25(OH)<sub>2</sub>D, where at 100 pM, free 1,25(OH)<sub>2</sub>D constituted 0.4 to 1.3% of total 1,25(OH)<sub>2</sub>D (624). As the association between total and free values differed increasingly by diplotype and genotype with increasing concentration of total 25OHD in the present study, characterising DBP traits may be particularly important for individuals with sufficient to replete total 25OHD but a Gc1s-1s or 1-1 genotype.

DBP diplotype was associated with WB aBMD and estimated failure load at the tibia but not with other skeletal parameters. This is consistent with the findings of Taes et al. who showed no difference in BMD or BTMs by DBP diplotype or genotype in Belgian men (567), and with the findings of Lauridsen et al. who reported no difference in aBMD by DBP genotype at all sites measured (598).

Overall, the results of this Chapter suggest that low 25OHD associated with high BMI is not due to associations with vitamin D binding proteins. As there was no difference in genotype or diplotype between normal and obese groups, DBP genotype is unlikely to explain low 25OHD in obesity.

There was no difference in PTH between normal BMI and obese groups and there was no association between PTH and total or free 25OHD or 1,25(OH)<sub>2</sub>D. PTH is generally

positively associated with adiposity and inversely associated with 25OHD (395, 402, 441, 555, 612, 625-627). Pitroda et al. and Cohen et al. reported no difference in PTH across tertiles of percentage body fat and trunk FM respectively, although despite trends towards low 25OHD with adiposity, these were not significant and so perhaps no difference in PTH would be expected as a consequence (273, 625). Other studies involving obese participants have reported lower 25OHD accompanied by higher PTH levels in those with the highest BMI (396, 402). Bell et al. and Bolland et al. have shown that PTH levels are associated with adiposity independently of 25OHD levels (626, 627) and the lack of association between PTH and total and free 1,25(OH)<sub>2</sub>D was also reported by Lauridsen et al. (598). Phosphate, a down-regulator of PTH independent of calcium, was lower in the obese group, and might contribute to there being no difference in PTH between the groups (625).

Pitroda et al. suggested that the duration of adiposity may affect the relationship between 25OHD and PTH, with chronic 25OHD deficiency contributing to enlargement of the parathyroid gland and greater PTH (625). Duration of adiposity has not been studied in the FAB cohort, but whether participants had remained at a constant weight, lost weight, gained weight or lost and gained weight in the year before recruitment was not associated with PTH. More detailed studies of weight history may provide an insight into possible causes of 'normal' PTH despite low 25OHD in obesity.

The most notable consequence of low total and free 25OHD was low total 1,25(OH)<sub>2</sub>D, suggesting that the conversion of 25OHD to 1,25(OH)<sub>2</sub>D in obesity is substrate dependent, as suggested by Lagunova et al. who found total 25OHD was the strongest predictor of total 1,25(OH)<sub>2</sub>D levels in overweight and obese individuals (578). Inverse associations between 1,25(OH)<sub>2</sub>D and BMI (556, 558, 575-577, 602), WBFM (556) and SAT (549) have previously been reported, with lower 1,25(OH)<sub>2</sub>D reported in obese men and women than in non-obese participants (441, 611). In mice, leptin has been shown to reduce 1 $\alpha$ -hydroxylase expression in the kidneys; lowering serum 1,25(OH)<sub>2</sub>D (600). Whether leptin plays a role in renal 1 $\alpha$ -hydroxylase expression in obese humans is unclear, and warrants further research.

In young adults, Bell et al. showed that while 25OHD was lower, urinary calcium was also lower and serum PTH and 1,25(OH)<sub>2</sub>D higher in obese than non-obese participants (626). Interestingly, Shapses et al. found that compared to women with a normal BMI, obese women had lower 25OHD and higher PTH, but there was no difference in 1,25(OH)<sub>2</sub>D between the groups (596). By a radioisotope method they showed that the

obese women had a higher true fractional calcium absorption than non-obese women, positively associated with PTH but not with 25OHD or 1,25(OH)<sub>2</sub>D (596). Further conflicting results have been shown by Grethen et al. who found that compared to controls, obese people awaiting bariatric surgery had a 25OHD and serum calcium which was not different to that of the controls, but despite this, their PTH was higher and their 1,25(OH)<sub>2</sub>D lower (441). However, the controls in this study consisted of six obese, four overweight and ten normal BMI individuals, not matched for height, and therefore it is perhaps unexpected that there was a difference in findings compared to the results of the present study.

Although younger adults had lower total 25OHD than older adults, there was no difference in PTH by age group and 1,25(OH)<sub>2</sub>D was lower in younger people. This may reflect a lower rate of 1 $\alpha$ -hydroxylation of 25OHD in younger people or simply the substrate dependent nature of the conversion of 25OHD to 1,25(OH)<sub>2</sub>D.

It is clear that as previously indicated (626, 628), the vitamin D endocrine system and the vitamin D-PTH association in particular, is altered in obesity, however a lack of consistency in findings makes it difficult to confirm the way in which it is altered. Studies sampling serum across seasons, involving multiple ethnicities and not controlling for stature with regard to volumetric dilution, may contribute to this lack of consistency in reported findings.

Participants with hyperparathyroidism were excluded from the FAB study due to the skeletal effects of high PTH potentially confounding the effect of adiposity on bone. Whether excluding these individuals explains why others report differences between normal BMI and obese groups which were not observed in this work is unclear, but this would not explain why lower 25OHD fails to drive higher PTH in the obese group in this study. Although 1,25(OH)<sub>2</sub>D acts in a negative feedback mechanism to limit production of 25OHD from vitamin D, low 1,25(OH)<sub>2</sub>D in obesity suggests that low 25OHD is not due to lower hepatic 25-hydroxylation of vitamin D inhibited by elevated levels of 1,25(OH)<sub>2</sub>D and PTH as previously described (626, 629).

Lower free 1,25(OH)<sub>2</sub>D in obesity may be a consequence of lower 1,25(OH)<sub>2</sub>D combined with no difference in DBP in obese people.

The results of this Chapter showed no difference in CTX (after adjustment) or PINP in obesity, although osteocalcin was lower in the obese group compared to the normal

weight group. Typically, high PTH as a result of vitamin D deficiency leads to greater bone turnover and bone loss and this pathway has been observed in obesity previously (395, 396). The results of this Chapter conflict with the findings presented in Chapter 4, and those of another study (358), both of which showed lower bone turnover in obesity. It is most likely that no differences in BTMs were observed in the obese group compared to the normal BMI group because in this smaller, unpaired sample, PTH was not different between the groups and therefore failed to drive higher bone turnover. The finding that younger adults had higher CTX than older adults might be explained by bone turnover being higher in early adulthood due to the period of consolidation, despite bone turnover typically increasing with age. As there was no difference in CTX by age group in the main study (Chapter 4), this difference may be an effect of the smaller sample size and lack of pairing in the vitamin D sub-study analysis.

When the results of this Chapter and those of Chapter 3 are considered together, there appear to be no adverse skeletal consequences of low 25OHD in obesity, with greater BMD, favourable bone microstructure and greater bone strength in obese individuals compared to those with a normal BMI.

Although 1,25(OH)<sub>2</sub>D has a direct positive effect on calcium absorption, low 1,25(OH)<sub>2</sub>D in obesity does not seem to be restricting calcium absorption to a detrimental effect on the skeleton (596). Others have reported no association between 1,25(OH)<sub>2</sub>D and BMD (255).

After adjustment for potential confounders, total 25OHD was not a significant predictor of BMD. These results are supported by other studies which have shown associations between adiposity, particularly SAT, and 25OHD but no effect of 25OHD on BMD at peak bone mass (110), in obese premenopausal women (269) or in postmenopausal women (630). Similarly, Lenders et al. showed that obese adolescents maintained a normal BMD despite having low 25OHD, although in this study, PTH decreased with increasing VAT (631). Irrespective of adiposity, Arabi et al. found that after adjustment for age, height, LM, and PTH, there was no effect of total 25OHD on BMD, with the exception of the trochanter in men and suggested that the negative effects of low vitamin D on BMD in the general elderly population are mediated through PTH levels and LM and not through independent effects of 25OHD (632). In a longitudinal study, Arabi et al. reported no correlation between mean total 25OHD and change in BMD except at the trochanter ( $r = 0.19$ ,  $p < 0.01$ ) from baseline to follow up at four years, while mean PTH was negatively correlated with change in BMD after adjustment, indicating that PTH, and not

25OHD, predicted bone loss (633). The lack of association between low 25OHD and skeletal parameters in obesity suggests that supplementation of vitamin D for skeletal benefit may not be necessary in those with a high BMI.

SPPB score was not associated with measures of 25OHD and although SPPB score was positively associated with free 1,25(OH)<sub>2</sub>D, inclusion of 1,25(OH)<sub>2</sub>D did not improve the model for predicting SPPB. Therefore low 25OHD does not appear to explain poor physical function in obesity. While some have reported associations between 25OHD and physical performance (583, 584, 589, 634-636), others have reported similar findings to the current work, with no association between low 25OHD and physical function (586, 587, 637). Kwon et al. reported that concomitant lower albumin and 25OHD were associated with lower muscle strength and poorer balance (638), supporting the findings of the present study. Low vitamin D status in obesity may not be associated with falls. Greater risk of some fractures in obesity, possibly associated with poor physical function, greater prevalence of falls and/or differences in fall kinetics may be better explained by factors other than low 25OHD, such as altered foot structure and centre of mass, as proposed in Chapter 5.

Despite evidence to support vitamin D supplementation in fall prevention; this may not be beneficial in obesity. Bischoff-Ferrari et al. found that if supplementation did not raise 25OHD to at least 60nmol/L, there may be no reduction in falls risk (594), and given the evidence that supplementation response is blunted in obesity, this may further demonstrate that there is little benefit of supplementation with vitamin D in obesity for the prevention of falls.

The strengths of this work include the two short (one month) recruitment windows which enabled measurement of vitamin D to be controlled for seasonal variation. All blood sampling was performed between 08:00 and 10:00, reducing the confounding effects of diurnal variation, particularly on 1,25(OH)<sub>2</sub>D and DBP which has been ignored by others (550). Although matched pairing was not applied to the analysis for this Chapter to increase sample size, all participants were sampled from those originally recruited to the FAB study which prospectively recruited individually-matched pairs. Therefore, even though it cannot be said that participants were matched in this analysis, age and height were similar between groups. Having also controlled for socioeconomic status through postcode matching within the original sample, differences in diet, supplement use and sun exposure habits were less likely to be attributed to differences in socioeconomic status and more likely reflections of adiposity related traits. Individuals who used of

hormonal contraceptives in the year prior to recruitment to the FAB study or prior to the vitamin D visit were excluded. As DBP is higher in those using hormonal contraceptives, this strengthens the analysis of the role of DBP in the relationship between adiposity and vitamin D in young women (639).

Having two methods to determine free 25OHD increased confidence in the free 25OHD observations. Although the measured free 25OHD was consistently lower than that of the calculated method, this is most likely due to calculated free 25OHD being subject to assay variation in total 25OHD, DBP and albumin due to the calculation by which it is determined. The relationship between measured and calculated free 25OHD was very recently described by Schwartz et al. in a group of healthy individuals and others with conditions known to affect albumin and DBP concentrations (560). The authors reported that calculated free 25OHD was overestimated compared to measured free 25OHD, as found in the present study, and that this could be due to error from assuming a single affinity constant for DBP, based on knowledge that different DBP diplotypes have different affinities for 25OHD (560). Schwartz et al. concluded that the use of measured free 25OHD was favourable over calculated methods but as the majority of the current literature presents calculated free 25OHD, performing both analyses provided data for meaningful comparison with previous findings (560). Using LC-MS/MS to determine 25OHD<sub>2</sub> and 25OHD<sub>3</sub> provides a more comprehensive assessment of total 25OHD than traditional assay methods where the fractional components of total 25OHD may not be distinguished.

A strength, but also a limitation, of this work is that only Caucasian individuals were studied. There are differences in body fat distribution and there may be differences in the strength of the relationship between 25OHD and obesity in different ethnicities (549, 550, 556, 577, 640). As a result, the findings of this Chapter may not be generalisable to the entire population or to alternate ethnic groups.

Polysulfone badges may have provided a more accurate quantification of UVB exposure than the questionnaire method used in this study, however the study timeframe did not allow for this.

There are several alternative hypotheses which have been proposed to explain low 25OHD in obesity which have not been addressed by the work in this Chapter and which warrant further investigation. Firstly, hepatic synthesis of 25OHD may occur at a lower rate in obesity, possibly contributed to by a higher prevalence of non-alcoholic fatty liver



disease in obesity, which is associated with lower 25OHD (628). No indicator of liver function was measured and so this could not be investigated as a potential mechanism.

HOMA-IR was greater in the obese group, and insulin resistance may down-regulate the expression of 25-hydroxylase in the liver, reducing 25OHD formation (600). However, without measures of 25-hydroxylase (or 1 $\alpha$ -hydroxylase) expression it was not possible to investigate this further.

In obese women, fat tissue itself has been shown to have altered expression of enzymes for both the formation and catabolism of 25OHD and 1,25(OH)<sub>2</sub>D compared to non-obese women (539). Lower expression of the 25-hydroxylase CYP2J2 in the SAT of obese women may indicate less 25-hydroxylation, while decreased expression of the 1 $\alpha$ -hydroxylase CYP27B1 in SAT these women might suggest lower conversion of 25OHD to 1,25(OH)<sub>2</sub>D, potentially contributing to lower circulating 25OHD and 1,25(OH)<sub>2</sub>D levels in obesity (539). Although there were no differences in CYP24A1 in adipose tissue between the normal BMI and obese women, if greater FM was taken into account, it may be that obese people do degrade more vitamin D within fat tissue, further supporting a role for altered enzymatic expression in adipose tissue in contributing to lower 25OHD and 1,25(OH)<sub>2</sub>D levels in obesity (539).

The type of fat consumed in the diet may affect the serum 25OHD response to 25OHD<sub>3</sub> supplementation; with monounsaturated fatty acids raising and polyunsaturated fats lowering the effect of supplementation on serum levels (641). Fatty acid consumption was not investigated in this study but may be an important factor in understanding the blunted 25OHD response to supplementation in obesity.

Production of 1,25(OH)<sub>2</sub>D is inhibited by calcium, phosphate, and FGF-23 (441). FGF-23 was not measured in the present study. As FGF-23 is positively associated with FM (642) it may contribute to lower 1,25(OH)<sub>2</sub>D in obesity.

Although DBP diplotypes vary in their affinities for 25OHD and 1,25(OH)<sub>2</sub>D, such variations in affinity were not taken into account when calculating an individual's free 25OHD or free 1,25(OH)<sub>2</sub>D. Assuming a constant affinity of DBP for 25OHD or 1,25(OH)<sub>2</sub>D may result in a degree of inaccuracy in the analysis of the relationship between free metabolites and DBP diplotype.

Analysing the effect of variation in the DBP gene by grouping DBP diplotypes into genotypes was advantageous for simplifying the analysis. However, merging Gc1f-1f, Gc1f-1s and Gc1s-1s to Gc1-1 effectively results in the analysis of the effect of the rs4588 SNP alone, as Gc1-1 always has threonine at the 436 position and Gc2-2 always has lysine at the 436 position (643). For example, comparing Gc1-1, with Gc1-2 and Gc2-2 is actually comparing ttcc, gtcc and ggcc (GC1-1) with ttca and gtca (Gc1-2) with ttaa (Gc2-2); the T436K change is that governing any effect. There may be greater value in studying DBP diplotype, over genotype.

Aside from the skeletal effects of vitamin D, vitamin D has also been shown to have effects on glucose metabolism, immune function, the risk of some cancers, cognitive function, CVD and mortality (644). Whether low 25OHD in obesity has consequences for any of these non-skeletal effects is unknown and warrants further research.

In conclusion, this Chapter provides novel evidence that both total and free 25OHD are lower in obese adults compared to adults with a normal BMI. This is most likely due to volumetric dilution or greater pool size and is not due to differences in dietary vitamin D intake or UVB exposure, although supplementary D intakes are lower in obesity. Concentrations of the vitamin D binding proteins DBP and albumin, may contribute to the lower free 25OHD and  $1,25(\text{OH})_2\text{D}$  seen in obesity. PTH does not appear to mediate the effect of 25OHD on BMD. DBP genotype may play an important role in the association between total and free vitamin D fractions, but is not a determinant of low 25OHD in obesity. There do not appear to be any skeletal consequences of low 25OHD in obesity and low 25OHD does not seem to be a mediator of the negative effect of obesity on physical performance. Obese people will need higher loading doses of vitamin D to achieve the same circulating levels as individuals with a normal BMI, but it is not clear that there would be musculoskeletal benefits to achieving the same circulating 25OHD level as individuals with a normal BMI.

## **CHAPTER 7:**

## **DISCUSSION**

## **CHAPTER 7: Discussion**

### **Main Findings**

I sought to better understand in this thesis why obese people are protected against some fractures but at greater risk of fracture at other sites and identify potential drivers of the site-specific differences in fracture risk observed in obesity. Obese adults have greater bone density than normal BMI individuals at all sites measured. Greater bone density is due to greater trabecular density; due to greater trabecular number and lower trabecular spacing, with no difference in trabecular thickness. Older obese adults also have greater cortical density, due to greater tissue mineral density, and lower cortical porosity at the tibia. Younger and older obese adults have greater Ct.Th at the tibia, and Ct.Th is also greater at the radius in older obese adults. There is no difference in bone size between obese and normal BMI individuals.

There was a greater effect of obesity on bone density and bone microstructural parameters in older adults than younger adults. Whilst patterns of bone microarchitecture were consistent between the distal radius and distal tibia in the older adults, younger adults appeared to exhibit less significant effects of obesity at the radius compared to the tibia. Despite this, bone strength is greater at both the radius and tibia in both younger and older obese adults. In older adults, the magnitude of the difference between normal BMI and obese individuals was similar at the distal radius and distal tibia suggesting the effect of obesity is not mediated by greater mechanical loading. Despite greater BMD and favourable bone microarchitecture, the effect of obesity on BMD and bone microarchitectural parameters and bone strength is not commensurate to greater body weight in obesity. However, assuming a linear relationship between body weight and bone microstructural response may be inappropriate. The greater effect of obesity on bone density and structural parameters in older adults suggests that the predominant effect of obesity is to reduce bone loss.

Reduced bone loss may be a consequence of lower bone turnover, shown by lower bone resorption and lower bone formation. Coupling index is not affected by obesity in young adults, but the positive coupling index in older obese adults may contribute to the greater effect of obesity on BMD and microstructure in this age group. Lower bone resorption in obesity was associated with greater adiposity and not with greater lean mass. While all adipose depots were positively associated with bone density and favourably with bone microstructural outcomes, subcutaneous abdominal adiposity appeared to exert the greatest protective effect against bone resorption.

The association between subcutaneous abdominal adipose tissue and bone resorption was accounted for by circulating leptin levels. It might be that leptin lowers bone resorption by increasing OPG, preventing the binding of RANKL with RANK and thus inhibiting osteoclastogenesis, although there was no difference in OPG between obese and normal BMI adults. There may also be positive effects of leptin on osteoblastogenesis. Higher circulating leptin and the greater effect of adiposity on leptin in older adults than young adults may contribute to the greater effect of obesity on bone density and microarchitectural parameters in older adults. E2 also appears to exert a protective effect against bone resorption, independent of the effect of leptin. There was no effect of insulin, IGF-I, glucose or adiponectin on bone resorption.

Older obese adults fell more frequently in the six months prior to recruitment than the older adults with a normal BMI. It has been shown that despite having greater lean mass, there is no difference in muscle strength between normal BMI and obese adults and physical performance is impaired in obese individuals. Thus, obese individuals may be better classified as dynapenic than sarcopenic. Poor physical performance in obesity may be partly mediated by higher HsCRP which is associated with slower gait.

Obese adults have low total 25OHD. There is no difference in DBP between obese and normal BMI adults, but obese adults have lower albumin, a likely result of greater inflammation. Despite this, free 25OHD is lower in obesity. There is no difference in dietary vitamin D intake or sun exposure habits between obese and normal BMI groups, suggesting that the primary cause of low 25OHD in obesity is greater volumetric dilution. Obese adults take fewer vitamin D containing supplements than those with a normal BMI which may also contribute to low 25OHD levels. The consequences of low circulating 25OHD in obesity were primarily low total and free 1,25(OH)<sub>2</sub>D, associated with higher PTH than normal BMI individuals.

Despite low 25OHD associated with high PTH, there do not appear to be adverse skeletal consequences of low 25OHD in obesity; with low 25OHD associated with lower bone turnover, greater BMD, favourable microarchitecture and greater bone strength. Low total and free 25OHD are not associated with physical performance. Although low total and free 1,25(OH)<sub>2</sub>D were associated with poorer SPPB score, inclusion of total and free 1,25(OH)<sub>2</sub>D did not improve the prediction of SPPB score.

Poor physical performance despite greater muscle mass (dynapenia) in obesity may predispose older obese adults to falls. Lower risk of hip and vertebral fracture in obesity

might be partly attributable to greater bone density, favourable cortical and trabecular microarchitecture and greater bone strength. Greater soft tissue thickness may also afford protection at central sites. The greater risk of ankle and proximal humerus fracture in obesity, despite greater bone density, favourable microarchitecture and greater bone strength at all sites measured, may be attributable to the effects of poor physical function on fall frequency and differences in fall kinetics in obese individuals. There may be less protection afforded by greater soft tissue thickness at these sites in obesity. Low vitamin D does not appear to adversely affect skeletal metabolism or physical function in obesity.

These findings suggest that the most appropriate direction for fracture prevention to take in obesity might be to develop improved fall prevention strategies which recognise impaired physical performance in obese individuals. Although bone strength in obesity is not commensurate to greater body weight when investigated in a linear fashion, it is likely that bone is sufficiently adapted to habitual loading forces in those with high BMI. To further reduce the risk of fracture attributable to skeletal determinants, obese individuals may need to achieve a phenotype which is sufficiently adapted to fall forces rather than habitual loading forces, which is likely to be unattainable and would not be maintainable physiologically. Vitamin D supplementation may be unlikely to benefit the musculoskeletal system in obesity.

### **How the Study was Unique**

This is the first study to investigate associations between obesity and vBMD and bone microarchitecture in both younger and older, men and women against a matched control group of non-obese individuals. Since this study began, others have studied the effects of obesity on vBMD and microarchitecture in specific groups of younger obese women (269), younger obese men (268), younger and older obese and non-obese women matched on age (261) and younger and older men and women who were not specifically obese or non-obese (267). By studying younger and older adults it has been possible to identify potential differences in the relationship between obesity and bone metabolism in younger and older adults, and allude to the possible mechanisms by which obesity may exert protective effects on the skeleton.

This study has investigated a range of possible mechanisms by which obesity may affect bone density and microstructure, including the role of various body fat compartments determined by imaging and anthropometry, associations with bone turnover and biochemical factors.

This is the first study to investigate differences in SPPB score in an obese group against a group of individually matched non-obese controls. Others have investigated the effect of adiposity by BMI on physical performance (472, 645). However, the design of the FAB study ensured a good representation of obese individuals, a group which can be underrepresented in population based studies. Having matched normal BMI and obese adults by height and age was a further strength. Controlling for such factors is important to be able to draw conclusions regarding the effect of adiposity on physical performance outcomes.

25OHD<sub>3</sub> by LC-MS/MS has not previously been determined in an obese population. This study is the first to investigate the free fraction of 25OHD in obese individuals compared to matched normal BMI controls. Determining the free fraction of 25OHD provides a better understanding of the biological availability of 25OHD and may be considered more physiologically relevant than total 25OHD. This was also the first study to measure free 25OHD, rather than use calculated free 25OHD in relation to BMI. Measuring free 25OHD is advantageous, as calculating free 25OHD may incur measurement error from the total 25OHD, albumin and DBP assays and makes assumptions about the binding affinities of these proteins for 25OHD which may be inaccurate. This combined error most likely explains the difference observed in free 25OHD determined by measured and calculated free 25OHD methods. This is the first study to investigate free 1,25(OH)<sub>2</sub>D levels in obesity.

### **Future Work**

Further research is required to confirm the mechanisms by which obesity is protective against hip and vertebral fracture. Following the identification of leptin as a potential mechanism by which subcutaneous abdominal fat might affect bone resorption and ultimately bone density and microstructure, an interventional study of weight change would be valuable to investigate how changes in the subcutaneous fat compartment affect leptin expression and concentration, bone resorption and bone microarchitecture. Others have shown that bariatric surgery induced weight loss leads to cortical bone loss (47), decreases in adipocyte size (646) and changes leptin expression which are associated with bone resorption (434), but which may be disproportionate to the loss of fat mass, possibly linked to a nonlinear relationship between adipocyte volume and leptin expression (647, 648). A single study linking weight loss, body composition analysis, bone microarchitecture, bone turnover, leptin and E2 levels may help to confirm whether the mechanisms identified in this work are indeed causal mechanisms by which obesity affects bone metabolism. Future research may involve the study of leptin administration

to prevent weight loss induced bone resorption which may drive cortical bone loss. Although an initial study has shown this may not be successful, further research is warranted (649).

Further studies are needed to understand how locomotor characteristics, plantar pressures, static postural stability and dynamic balance in obesity influence gait and fall kinematics in obese adults. Better understanding of fall kinematics and kinetics in obesity are needed to improve current FE models by developing more realistic models based on true fall patterns in obesity. This could help to better explain the site-specific fracture risk in obesity. The building of more sophisticated FE models from which bone strength can be estimated and fracture risk predicted is an area which warrants further research. Such models would be particularly useful for predicting ankle fractures, where fractures most commonly occur as a result of torsion rather than a vertical compression which is the action simulated by current FE models. Generating FE models which do not assume uniformity of bone is likely to be more complex but would also be beneficial for accurate fracture prediction. Others have begun to developing FE models which take into account soft tissue padding at sites of impact (274). Standing hip width at the level of the greater trochanter has been measured in all participants in the FAB study to determine soft tissue thickness at the hip and will be incorporated into in-house FE models to provide a more realistic estimate of fracture risk than is achievable using present models.

More research is required to characterise the role of ligament ossification and muscle-tendon unit stiffness in ankle fracture in obesity. Understanding the role of ligaments and tendons in physical performance and associations with falls may facilitate such properties to be incorporated into finite element models. Again, this would be particularly beneficial for FE models of the ankle.

Muscle fat infiltration will be studied in this study population to try to better understand why physical performance and muscle function are lower in obesity despite greater muscle mass. Further research is needed to clarify the role of brown fat and bone marrow fat compartments in obese adults compared to normal BMI populations.

Whether weight history, or duration of obesity, affects associations between obesity and bone density, structure and strength is unclear and warrants further research.

Further research is required to quantify 24,25(OH)<sub>2</sub>D in obese adults compared to those with a normal BMI to determine associations between obesity and the catabolism of



1,25(OH)<sub>2</sub>D. Further research to investigate whether leptin affects renal 1 $\alpha$ -hydroxylase expression in humans, as has been shown in mice, is warranted to better understand the causes of low 1,25(OH)<sub>2</sub>D in obesity.

### **Limitations**

The main limitation of this work is the cross-sectional, observational study design. Differences observed between obese and normal BMI individuals are associated with obesity rather than caused by obesity.

The smaller sample of young adults compared to that of older adults was a result of difficulty in recruiting eligible adults from this age group. Employment, use of hormonal contraceptives, childcare commitments and lower levels of health engagement were the key factors for lower recruitment rates in this group. Most recruitment of older adults was done through GP practices, with small catchment areas. As a result, highly selective matching based on postcode could be employed for older adult pairs. However, young adults were typically university and hospital staff and visitors, often living within a large radius. This made postcode matching of younger adults difficult. Alternative methods of adjusting for socioeconomic status may have been better suited to the young adult group.

Although visits 1 and 2 were completed within 28 days, it is acknowledged that serum samples were not collected at the same time point as bone imaging. However, most participants attended for visit 2 within 1 to 2 weeks of their first visit and it is unlikely that a 28 day interval would lead to discrepancies in measurements. Biochemical data from the vitamin D sub-study were related to imaging data taken up to 2 years prior. It was not possible to repeat imaging due to radiation constraints.

I was unable to analyse the hip QCT scans due to difficulties in trimming soft tissue from the image prior to quantification of the bone. This meant reliance on hip aBMD which may be affected by greater soft tissue thickness, rather than vBMD, and the inability to determine bone microstructural properties at the hip.

Assessments of physical performance in this study were chosen as they are established components of a previously determined physical performance battery. More sophisticated motion analysis techniques might have enabled a more detailed understanding of movement patterns in obese individuals and may help to better understand fall patterns in obesity. Motion capture can be troublesome in obese individuals due to cutaneous marker error from soft tissue movement. Some simple

exercises might have given further insights into physical performance in obesity in relation to falls; testing reaction times and postural sway for example. Incorporating a measure of lower body strength may have been valuable in the context of falls during habitual activity.

Fall history was only collected for the six months prior to the first study visit. One year fall history might have given more generalisable data, although there are limitations associated with participant recall over longer periods. None of the participants had fractured in the year before recruitment and so none of the falls reported in the six months prior to recruitment resulted in fracture. Excluding individuals with fracture may have limited the findings to an extent, as obese individuals who experience more falls but are seemingly protected against fracture at most sites were eligible, while normal BMI individuals at greater risk of fracture at most sites may have been more frequently excluded. To have determined fracture site, fracture cause and perceived fall direction of those who were ineligible due to prior fracture might have been interesting.

Due to the large number of participants to recruit to the vitamin D sub-study and requirement for serum sampling to be done in the fasted state with control for circadian variation, the sub-study had to be run over two roughly one-month periods either side of the winter. As recruitment to the main study was more rapid in the older group, the majority of the participants entering the first vitamin D sub-study period were in the older age group. Weekly sunlight hours were shown to be similar during the two study periods (Appendix 3). The assessment of sun exposure did not take into account sun exposure received from holidays abroad due to difficulties quantifying the UVB exposure accurately especially with respect to SPF habits.

The free 25OHD assay used has not been widely validated to date. However the results were comparable to those attained using the calculated method and the discrepancy between measured and calculated free 25OHD was similar to that reported by others (560).

There may be alternative causes of low 25OHD in obesity which were not investigated, such as faster metabolic clearance of 25OHD, altered enzymatic activity, or decreased hepatic 25-hydroxylation (626). Shorter 25OHD half-life in obesity could explain lower circulating 25OHD. However, lower 1,25(OH)<sub>2</sub>D in obesity, despite higher PTH, might suggest that the metabolic clearance of 25OHD is not increased.

The findings of this work may not be applicable to children, peri-menopausal or menopausal women, or to elderly adults.

### **Conclusion**

Obese adults have a lower risk of hip and vertebral fracture which might be explained by greater aBMD, vBMD, favourable bone microarchitecture and greater bone strength at these sites. Greater BMD and bone microarchitecture appear to be a consequence of lower bone resorption, mediated by the effects of leptin and oestrogen on bone resorption. The greater risk of ankle and proximal humerus fracture in obesity might be explained by poor physical function in obesity despite greater LM compared to individuals with a normal BMI. Poor physical function may contribute to greater fall frequency and affect fall kinematics in obese individuals which may predispose to a greater risk of fracture, despite greater BMD, favourable bone microarchitecture and greater bone strength at all sites measured. Low 25OHD due to greater volumetric dilution in obesity and lower supplementary vitamin D intake does not appear to exert negative consequences on bone density, structure and strength, nor does it affect physical function.

## **CHAPTER 8:**

## **REFERENCES**

## **References**

1. Silverman SL. Quality-of-life issues in osteoporosis. *Curr Rheumatol Rep.* 2005;7(1):39-45.
2. Geusens P. Osteoporosis: clinical features. *Minerva Med.* 2008;99(2):167-75.
3. Borgstrom F, Lekander I, Ivergard M, Strom O, Svedbom A, Alekna V, et al. The International Costs and Utilities Related to Osteoporotic Fractures Study (ICUROS)--quality of life during the first 4 months after fracture. *Osteoporos Int.* 2013;24(3):811-23.
4. Compston JE, Flahive J, Hooven FH, Anderson FA, Jr., Adachi JD, Boonen S, et al. Obesity, Health-Care Utilization, and Health-Related Quality of Life After Fracture in Postmenopausal Women: Global Longitudinal Study of Osteoporosis in Women (GLOW). *Calcif Tissue Int.* 2013.
5. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, et al. A meta-analysis of previous fracture and subsequent fracture risk. *Bone.* 2004;35(2):375-82.
6. Boskey AL, Robey PG. The Composition of Bone. In: Rosen CJ, Bouillon R, Compston J, Rosen V, editors. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Eighth ed: John Wiley & Sons; 2013. p. 49-56.
7. Jones HH, Priest JD, Hayes WC, Tichenor CC, Nagel DA. Humeral hypertrophy in response to exercise. *J Bone Joint Surg Am.* 1977;59(2):204-8.
8. Parfitt AM. The two faces of growth: benefits and risks to bone integrity. *Osteoporos Int.* 1994;4(6):382-98.
9. Walsh JS, Henry YM, Fatayerji D, Eastell R. Lumbar spine peak bone mass and bone turnover in men and women: a longitudinal study. *Osteoporos Int.* 2009;20(3):355-62.
10. Ferrari S, Rizzoli R, Slosman D, Bonjour JP. Familial resemblance for bone mineral mass is expressed before puberty. *J Clin Endocrinol Metab.* 1998;83(2):358-61.
11. Eriksen EF. Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease. *Endocr Rev.* 1986;7(4):379-408.
12. Cooper C. Osteoporosis: disease severity and consequent fracture management. *Osteoporos Int.* 2010;21 Suppl 2:S425-9.
13. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltsev N. The diagnosis of osteoporosis. *J Bone Miner Res.* 1994;9(8):1137-41.
14. Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, et al. Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. *J Bone Miner Res.* 2008;23(1):17-29.
15. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. *J Clin Endocrinol Metab.* 2007;92(5):1640-6.
16. Colon-Emeric CS, Saag KG. Osteoporotic fractures in older adults. *Best Pract Res Clin Rheumatol.* 2006;20(4):695-706.
17. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos Int.* 2005;16(11):1330-8.
18. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. *The Lancet.* 2002;359(9319):1761-7.
19. Patsch JM, Kiefer FW, Varga P, Pail P, Rauner M, Stupphann D, et al. Increased bone resorption and impaired bone microarchitecture in short-term and extended high-fat diet-induced obesity. *Metabolism.* 2010.
20. Miller P, Papapoulos S. Osteoporosis. In: Miller P, Papapoulos S, editors. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Eighth Edition ed. Oxford, UK: John Wiley & Sons, Inc.; 2013. p. 343-533.

21. Korpelainen R, Korpelainen J, Heikkinen J, Vaananen K, Keinanen-Kiukaanniemi S. Lifelong risk factors for osteoporosis and fractures in elderly women with low body mass index--a population-based study. *Bone*. 2006;39(2):385-91.
22. Adami S, Giannini S, Giorgino R, Isaia GC, Maggi S, Sinigaglia L, et al. Effect of age, weight and lifestyle factors on calcaneal quantitative ultrasound in premenopausal women: the ESOPPO study. *Calcif Tissue Int*. 2004;74(4):317-21.
23. Beck TJ, Petit MA, Wu G, LeBoff MS, Cauley JA, Chen Z. Does obesity really make the femur stronger? BMD, geometry, and fracture incidence in the women's health initiative-observational study. *J Bone Miner Res*. 2009;24(8):1369-79.
24. Burger H, de Laet CE, van Daele PL, Weel AE, Witteman JC, Hofman A, et al. Risk factors for increased bone loss in an elderly population: the Rotterdam Study. *Am J Epidemiol*. 1998;147(9):871-9.
25. Malabanan AO, Rosen HN, Vokes TJ, Deal CL, Alele JD, Olinginski TP, et al. Indications of DXA in women younger than 65 yr and men younger than 70 yr: the 2013 Official Positions. *J Clin Densitom*. 2013;16(4):467-71.
26. Schousboe JT, Gourlay M, Fink HA, Taylor BC, Orwoll ES, Barrett-Connor E, et al. Cost-effectiveness of bone densitometry among Caucasian women and men without a prior fracture according to age and body weight. *Osteoporos Int*. 2013;24(1):163-77.
27. Waugh EJ, Lam MA, Hawker GA, McGowan J, Papaioannou A, Cheung AM, et al. Risk factors for low bone mass in healthy 40-60 year old women: a systematic review of the literature. *Osteoporos Int*. 2009;20(1):1-21.
28. Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, et al. A meta-analysis of the association of fracture risk and body mass index in women. *J Bone Miner Res*. 2014;29(1):223-33.
29. Prieto-Alhambra D, Premaor MO, Fina Aviles F, Hermosilla E, Martinez-Laguna D, Carbonell-Abella C, et al. The association between fracture and obesity is site-dependent: a population-based study in postmenopausal women. *J Bone Miner Res*. 2012;27(2):294-300.
30. Nielson CM, Marshall LM, Adams AL, Leblanc ES, Cawthon PM, Ensrud K, et al. BMI and fracture risk in older men: the osteoporotic fractures in men (MrOS) study. *Journal of Bone and Mineral Research*. 2010(1523-4681 (Electronic)).
31. Misra M, Katzman DK, Cord J, Manning SJ, Mendes N, Herzog DB, et al. Bone metabolism in adolescent boys with anorexia nervosa. *J Clin Endocrinol Metab*. 2008;93(10):3029-36.
32. Misra M, Soyka LA, Miller KK, Herzog DB, Grinspoon S, De Chen D, et al. Serum osteoprotegerin in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*. 2003;88(8):3816-22.
33. Howgate DJ, Graham SM, Leonidou A, Korres N, Tsiridis E, Tsapakis E. Bone metabolism in anorexia nervosa: molecular pathways and current treatment modalities. *Osteoporos Int*. 2013;24(2):407-21.
34. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. *Int J Obes (Lond)*. 2008;32(2):211-22.
35. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet*. 2005;366(9491):1059-62.
36. Eaton SB, Eaton SB, 3rd. Paleolithic vs. modern diets--selected pathophysiological implications. *Eur J Nutr*. 2000;39(2):67-70.
37. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet*. 2011;377(9765):557-67.
38. Kopelman P, Jebb SA, Butland B. Executive summary: Foresight 'Tackling Obesity: Future Choices' project. *Obes Rev*. 2007;8 Suppl 1:vi-ix.

39. Butland B, Jebb, S., Kopelman, P., McPherson, K., Thomas, S., Mardell, J., Parry, V. Tackling Obesities: Future Choices - Project Report. Report. London: Government Office for Science, 2007 Oct 2007. Report No.
40. The NHS Information Centre. Health Survey for England 2009: Health and Lifestyles. Summary of Key Findings. 2010.
41. Rithirangsiroj K, Panyakhamlerd K, Chaikittisilpa S, Chaiwatanarat T, Taechakraichana N. Osteoporosis in different age-groups and various body mass index (BMI) ranges in women undergoing bone mass measurement at King Chulalongkorn Memorial Hospital. *J Med Assoc Thai.* 2012;95(5):644-9.
42. Premaor MO, Ensrud K, Lui L, Parker RA, Cauley J, Hillier TA, et al. Risk factors for nonvertebral fracture in obese older women. *J Clin Endocrinol Metab.* 2011;96(8):2414-21.
43. Morin S, Leslie WD, Manitoba Bone Density P. High bone mineral density is associated with high body mass index. *Osteoporos Int.* 2009;20(7):1267-71.
44. Ong T, Sahota O, Tan W, Marshall L. A United Kingdom perspective on the relationship between body mass index (BMI) and bone health: A cross sectional analysis of data from the Nottingham Fracture Liaison Service. *Bone.* 2014;59:207-10.
45. Meyer HE, Sogaard AJ, Falch JA, Jorgensen L, Emaus N. Weight change over three decades and the risk of osteoporosis in men: the Norwegian Epidemiological Osteoporosis Studies (NOREPOS). *Am J Epidemiol.* 2008;168(4):454-60.
46. Wu F, Ames R, Clearwater J, Evans MC, Gamble G, Reid IR. Prospective 10-year study of the determinants of bone density and bone loss in normal postmenopausal women, including the effect of hormone replacement therapy. *Clin Endocrinol (Oxf).* 2002;56(6):703-11.
47. Stein EM, Carrelli A, Young P, Bucovsky M, Zhang C, Schrope B, et al. Bariatric surgery results in cortical bone loss. *J Clin Endocrinol Metab.* 2013;98(2):541-9.
48. Morin S, Tsang JF, Leslie WD. Weight and body mass index predict bone mineral density and fractures in women aged 40 to 59 years. *Osteoporos Int.* 2009;20(3):363-70.
49. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, et al. Obesity is not protective against fracture in postmenopausal women: GLOW. *Am J Med.* 2011;124(11):1043-50.
50. Premaor MO, Pilbrow L, Tonkin C, Parker RA, Compston J. Obesity and fractures in postmenopausal women. *J Bone Miner Res.* 2010;25(2):292-7.
51. Compston JE, Flahive J, Hosmer DW, Watts NB, Siris ES, Silverman S, et al. Relationship of weight, height, and body mass index with fracture risk at different sites in postmenopausal women: The global longitudinal study of osteoporosis in women (GLOW). *J Bone Miner Res.* 2013.
52. Armstrong ME, Cairns BJ, Banks E, Green J, Reeves GK, Beral V. Different effects of age, adiposity and physical activity on the risk of ankle, wrist and hip fractures in postmenopausal women. *Bone.* 2012;50(6):1394-400.
53. Armstrong ME, Spencer EA, Cairns BJ, Banks E, Pirie K, Green J, et al. Body mass index and physical activity in relation to the incidence of hip fracture in postmenopausal women. *J Bone Miner Res.* 2011;26(6):1330-8.
54. Premaor MO, Compston JE, Fina Aviles F, Pages-Castella A, Nogues X, Diez-Perez A, et al. The association between fracture site and obesity in men: a population-based cohort study. *J Bone Miner Res.* 2013;28(8):1771-7.
55. Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, et al. A meta-analysis of the association of fracture risk and body mass index in women. *J Bone Miner Res.* 2013.
56. Tang X, Liu G, Kang J, Hou Y, Jiang F, Yuan W, et al. Obesity and risk of hip fracture in adults: a meta-analysis of prospective cohort studies. *PLoS One.* 2013;8(4):e55077.

57. Szulc P, Varennes A, Delmas PD, Goudable J, Chapurlat R. Men with metabolic syndrome have lower bone mineral density but lower fracture risk--the MINOS study. *J Bone Miner Res.* 2010;25(6):1446-54.
58. Gnudi S, Sitta E, Lisi L. Relationship of body mass index with main limb fragility fractures in postmenopausal women. *J Bone Miner Metab.* 2009;27(4):479-84.
59. King CM, Hamilton GA, Cobb M, Carpenter D, Ford LA. Association between ankle fractures and obesity. *J Foot Ankle Surg.* 2012;51(5):543-7.
60. Strauss EJ, Frank JB, Walsh M, Koval KJ, Egol KA. Does obesity influence the outcome after the operative treatment of ankle fractures? *J Bone Joint Surg Br.* 2007;89(6):794-8.
61. Reid IR. Relationships among body mass, its components, and bone. *Bone.* 2002;31(5):547-55.
62. Williams AR, Weiss NS, Ure CL, Ballard J, Daling JR. Effect of weight, smoking, and estrogen use on the risk of hip and forearm fractures in postmenopausal women. *Obstet Gynecol.* 1982;60(6):695-9.
63. Kato I, Toniolo P, Zeleniuch-Jacquotte A, Shore RE, Koenig KL, Akhmedkhanov A, et al. Diet, smoking and anthropometric indices and postmenopausal bone fractures: a prospective study. *Int J Epidemiol.* 2000;29(1):85-92.
64. Nielson CM, Srikanth P, Orwoll ES. Obesity and fracture in men and women: an epidemiologic perspective. *J Bone Miner Res.* 2012;27(1):1-10.
65. Premaor M, Parker RA, Cummings S, Ensrud K, Cauley JA, Lui LY, et al. Predictive value of FRAX for fracture in obese older women. *J Bone Miner Res.* 2013;28(1):188-95.
66. Maheshwari R, Mack CD, Kaufman RP, Francis DO, Bulger EM, Nork SE, et al. Severity of injury and outcomes among obese trauma patients with fractures of the femur and tibia: a crash injury research and engineering network study. *J Orthop Trauma.* 2009;23(9):634-9.
67. Batsis JA, Huddleston JM, Melton LJ, 3rd, Huddleston PM, Larson DR, Gullerud RE, et al. Body mass index (BMI) and risk of noncardiac postoperative medical complications in elderly hip fracture patients: a population-based study. *J Hosp Med.* 2009;4(8):E1-9.
68. Jamal Sepah Y, Umer M, Khan A, Ullah Khan Niazi A. Functional outcome, mortality and in-hospital complications of operative treatment in elderly patients with hip fractures in the developing world. *Int Orthop.* 2010;34(3):431-5.
69. Compston J. Obesity and bone. *Curr Osteoporos Rep.* 2013;11(1):30-5.
70. Prieto-Alhambra D, Premaor MO, Aviles FF, Castro AS, Javaid MK, Nogues X, et al. Relationship between mortality and BMI after fracture: a population-based study of men and women aged  $\geq 40$  years. *J Bone Miner Res.* 2014;29(8):1737-44.
71. Meyer HE, Tverdal A, Falch JA. Body height, body mass index, and fatal hip fractures: 16 years' follow-up of 674,000 Norwegian women and men. *Epidemiology.* 1995;6(3):299-305.
72. Bliuc D, Nguyen ND, Milch VE, Nguyen TV, Eisman JA, Center JR. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. *Jama.* 2009;301(5):513-21.
73. Bouxsein ML, Szulc P, Munoz F, Thrall E, Sornay-Rendu E, Delmas PD. Contribution of trochanteric soft tissues to fall force estimates, the factor of risk, and prediction of hip fracture risk. *J Bone Miner Res.* 2007;22(6):825-31.
74. Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int.* 2002;13(10):835-40.
75. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr.* 2007;86(5):1530-8.
76. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *Int J Obes Relat Metab Disord.* 2000;24(5):627-32.



77. Dimitri P, Wales J, Bishop N. Fat and Bone in Children - Differential Effects of Obesity on Bone Size and Mass According to Fracture History. *J Bone Miner Res.* 2009.
78. Pirro M, Fabbriani G, Leli C, Callarelli L, Manfredelli MR, Fioroni C, et al. High weight or body mass index increase the risk of vertebral fractures in postmenopausal osteoporotic women. *J Bone Miner Metab.* 2010;28(1):88-93.
79. Nunez NP, Carpenter CL, Perkins SN, Berrigan D, Jaque SV, Ingles SA, et al. Extreme obesity reduces bone mineral density: complementary evidence from mice and women. *Obesity (Silver Spring).* 2007;15(8):1980-7.
80. Woo DG, Lee BY, Lim D, Kim HS. Relationship between nutrition factors and osteopenia: Effects of experimental diets on immature bone quality. *J Biomech.* 2009;42(8):1102-7.
81. Lorincz C, Reimer RA, Boyd SK, Zernicke RF. High-fat, sucrose diet impairs geometrical and mechanical properties of cortical bone in mice. *Br J Nutr.* 2010;103(9):1302-8.
82. Ionova-Martin SS, Do SH, Barth HD, Szadkowska M, Porter AE, Ager JW, 3rd, et al. Reduced size-independent mechanical properties of cortical bone in high-fat diet-induced obesity. *Bone.* 2010;46(1):217-25.
83. Li KC, Zernicke RF, Barnard RJ, Li AF. Effects of a high fat-sucrose diet on cortical bone morphology and biomechanics. *Calcif Tissue Int.* 1990;47(5):308-13.
84. Demigne C, Bloch-Faure M, Picard N, Sabboh H, Besson C, Remesy C, et al. Mice chronically fed a westernized experimental diet as a model of obesity, metabolic syndrome and osteoporosis. *Eur J Nutr.* 2006;45(5):298-306.
85. Zernicke RF, Salem GJ, Barnard RJ, Schramm E. Long-term, high-fat-sucrose diet alters rat femoral neck and vertebral morphology, bone mineral content, and mechanical properties. *Bone.* 1995;16(1):25-31.
86. Frost HM. Mechanical determinants of bone modeling. *Metab Bone Dis Relat Res.* 1982;4(4):217-29.
87. Skerry TM. One mechanostat or many? Modifications of the site-specific response of bone to mechanical loading by nature and nurture. *J Musculoskelet Neuronal Interact.* 2006;6(2):122-7.
88. Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. *Calcif Tissue Int.* 1985;37(4):411-7.
89. Lee KD. Applications of mesenchymal stem cells: an updated review. *Chang Gung Med J.* 2008;31(3):228-36.
90. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *Am J Med.* 2009;122(5):409-14.
91. Griffith JF, Yeung DK, Tsang PH, Choi KC, Kwok TC, Ahuja AT, et al. Compromised bone marrow perfusion in osteoporosis. *J Bone Miner Res.* 2008;23(7):1068-75.
92. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. *Exp Biol Med (Maywood).* 2001;226(6):507-20.
93. Kassi E, Papavassiliou AG. A possible role of osteocalcin in the regulation of insulin secretion: human in vivo evidence? *J Endocrinol.* 2008;199(2):151-3.
94. Fukumoto S, Martin TJ. Bone as an endocrine organ. *Trends Endocrinol Metab.* 2009;20(5):230-6.
95. Walsh JS, Henriksen DB. Feeding and Bone. *Arch Biochem Biophys.* 2010.
96. Reid IR. Relationships between fat and bone. *Osteoporos Int.* 2008;19(5):595-606.
97. Gomez R, Lago F, Gomez-Reino J, Dieguez C, Gualillo O. Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases. *J Mol Endocrinol.* 2009;43(1):11-8.
98. Thomas T, Burguera B. Is leptin the link between fat and bone mass? *J Bone Miner Res.* 2002;17(9):1563-9.
99. Kawai M, Devlin MJ, Rosen CJ. Fat targets for skeletal health. *Nat Rev Rheumatol.* 2009;5(7):365-72.

100. Reid IR. Fat and Bone. *Arch Biochem Biophys*. 2010.
101. Frost M, Abrahamsen B, Nielsen TL, Frystyk J, Flyvbjerg A, Hagen C, et al. Adiponectin and peak bone mass in men: a cross-sectional, population-based study. *Calcif Tissue Int*. 2010;87(1):36-43.
102. Sayers A, Timpson NJ, Sattar N, Deanfield J, Hingorani AD, Smith GD, et al. Adiponectin and its association with bone mass accrual in childhood. *J Bone Miner Res*. 2010.
103. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Yano S, Sugimoto T. Relationships between serum adiponectin levels versus bone mineral density, bone metabolic markers, and vertebral fractures in type 2 diabetes mellitus. *Eur J Endocrinol*. 2009;160(2):265-73.
104. Richards JB, Valdes AM, Burling K, Perks UC, Spector TD. Serum adiponectin and bone mineral density in women. *J Clin Endocrinol Metab*. 2007;92(4):1517-23.
105. Sodi R, Hazell MJ, Durham BH, Rees C, Ranganath LR, Fraser WD. The circulating concentration and ratio of total and high molecular weight adiponectin in post-menopausal women with and without osteoporosis and its association with body mass index and biochemical markers of bone metabolism. *Clin Biochem*. 2009;42(13-14):1375-80.
106. Luo XH, Guo LJ, Xie H, Yuan LQ, Wu XP, Zhou HD, et al. Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. *J Bone Miner Res*. 2006;21(10):1648-56.
107. Gomez-Ambrosi J, Rodriguez A, Catalan V, Fruhbeck G. The bone-adipose axis in obesity and weight loss. *Obes Surg*. 2008;18(9):1134-43.
108. Schett G. Effects of inflammatory and anti-inflammatory cytokines on the bone. *Eur J Clin Invest*. 2011;41(12):1361-6.
109. Bolland MJ, Grey AB, Ames RW, Horne AM, Mason BH, Wattie DJ, et al. Age-, gender-, and weight-related effects on levels of 25-hydroxyvitamin D are not mediated by vitamin D binding protein. *Clin Endocrinol (Oxf)*. 2007;67(2):259-64.
110. Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab*. 2009;94(1):67-73.
111. Seibel MJ, Robins SP. Serum Undercarboxylated Osteocalcin and the Risk of Hip Fracture. *J Clin Endocrinol Metab*. 1997;82(3):717-8.
112. Yeap B, Chubb SA, Flicker L, McCaul K, Ebeling P, Beilby J, et al. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia and triglyceride levels. *Eur J Endocrinol*. 2010.
113. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Ozaki R, Tanaka K, et al. Low plasma phylloquinone concentration is associated with high incidence of vertebral fracture in Japanese women. *J Bone Miner Metab*. 2008;26(1):79-85.
114. Yamauchi M, Yamaguchi T, Nawata K, Takaoka S, Sugimoto T. Relationships between undercarboxylated osteocalcin and vitamin K intakes, bone turnover, and bone mineral density in healthy women. *Clin Nutr*. 2010.
115. An JJ, Han DH, Kim DM, Kim SH, Rhee Y, Lee EJ, et al. Expression and regulation of osteoprotegerin in adipose tissue. *Yonsei Med J*. 2007;48(5):765-72.
116. Ashley DT, O'Sullivan EP, Davenport C, Devlin N, Crowley RK, McCaffrey N, et al. Similar to adiponectin, serum levels of osteoprotegerin are associated with obesity in healthy subjects. *Metabolism*. 2011;60(7):994-1000.
117. Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr Diabetes Rev*. 2006;2(4):367-73.
118. Russell M, Mendes N, Miller KK, Rosen CJ, Lee H, Klibanski A, et al. Visceral fat is a negative predictor of bone density measures in obese adolescent girls. *J Clin Endocrinol Metab*. 2010;95(3):1247-55.
119. Gilsanz V, Chalfant J, Mo AO, Lee DC, Dorey FJ, Mittelman SD. Reciprocal relations of subcutaneous and visceral fat to bone structure and strength. *J Clin Endocrinol Metab*. 2009;94(9):3387-93.

120. Araujo AB, Travison TG, Harris SS, Holick MF, Turner AK, McKinlay JB. Race/ethnic differences in bone mineral density in men. *Osteoporos Int.* 2007;18(7):943-53.
121. Travison TG, Beck TJ, Esche GR, Araujo AB, McKinlay JB. Age trends in proximal femur geometry in men: variation by race and ethnicity. *Osteoporos Int.* 2008;19(3):277-87.
122. Wulan SN, Westerterp KR, Plasqui G. Ethnic differences in body composition and the associated metabolic profile: a comparative study between Asians and Caucasians. *Maturitas.* 2010;65(4):315-9.
123. Demeter S, Leslie WD, Lix L, MacWilliam L, Finlayson GS, Reed M. The effect of socioeconomic status on bone density testing in a public health-care system. *Osteoporos Int.* 2007;18(2):153-8.
124. Brennan SL, Henry MJ, Wluka AE, Nicholson GC, Kotowicz MA, Williams JW, et al. BMD in population-based adult women is associated with socioeconomic status. *J Bone Miner Res.* 2009;24(5):809-15.
125. Brennan SL, Henry MJ, Wluka AE, Nicholson GC, Kotowicz MA, Pasco JA. Socioeconomic status and bone mineral density in a population-based sample of men. *Bone.* 2010;46(4):993-9.
126. Brennan SL, Pasco JA, Urquhart DM, Oldenburg B, Wang Y, Wluka AE. Association between socioeconomic status and bone mineral density in adults: a systematic review. *Osteoporos Int.* 2011;22(2):517-27.
127. Alver K, Sogaard AJ, Falch JA, Meyer HE. The Oslo Health Study: Is bone mineral density higher in affluent areas? *Int J Equity Health.* 2007;6:19.
128. Pearson D, Taylor R, Masud T. The relationship between social deprivation, osteoporosis, and falls. *Osteoporos Int.* 2004;15(2):132-8.
129. Wang MC, Dixon LB. Socioeconomic influences on bone health in postmenopausal women: findings from NHANES III, 1988-1994. *Osteoporos Int.* 2006;17(1):91-8.
130. Brennan SL, Henry MJ, Kotowicz MA, Nicholson GC, Zhang Y, Pasco JA. Incident hip fracture and social disadvantage in an Australian population aged 50 years or greater. *Bone.* 2011;48(3):607-10.
131. Farahmand BY, Persson PG, Michaelsson K, Baron JA, Parker MG, Ljunghall S, et al. Socioeconomic status, marital status and hip fracture risk: a population-based case-control study. *Osteoporos Int.* 2000;11(9):803-8.
132. Brennan SL, Henry MJ, Nicholson GC, Kotowicz MA, Pasco JA. Socioeconomic status and risk factors for obesity and metabolic disorders in a population-based sample of adult females. *Prev Med.* 2009;49(2-3):165-71.
133. Tamaki J, Iki M, Fujita Y, Kouda K, Yura A, Kadowaki E, et al. Impact of smoking on bone mineral density and bone metabolism in elderly men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. *Osteoporos Int.* 2011;22(1):133-41.
134. Kiel DP, Zhang Y, Hannan MT, Anderson JJ, Baron JA, Felson DT. The effect of smoking at different life stages on bone mineral density in elderly men and women. *Osteoporos Int.* 1996;6(3):240-8.
135. Lorentzon M, Mellstrom D, Haug E, Ohlsson C. Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. *J Clin Endocrinol Metab.* 2007;92(2):497-503.
136. Wust RC, Winwood K, Wilks DC, Morse CI, Degens H, Rittweger J. Effects of smoking on tibial and radial bone mass and strength may diminish with age. *J Clin Endocrinol Metab.* 2010;95(6):2763-71.
137. Szulc P, Debiecse E, Boutroy S, Vilauphiou N, Chapurlat R. Poor trabecular microarchitecture in male current smokers: the cross-sectional STRAMBO study. *Calcif Tissue Int.* 2011;89(4):303-11.
138. Hoidrup S, Prescott E, Sorensen TI, Gottschau A, Lauritzen JB, Schroll M, et al. Tobacco smoking and risk of hip fracture in men and women. *Int J Epidemiol.* 2000;29(2):253-9.

139. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. *Osteoporos Int.* 2005;16(2):155-62.
140. Vestergaard P, Mosekilde L. Fracture risk associated with smoking: a meta-analysis. *J Intern Med.* 2003;254(6):572-83.
141. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcif Tissue Int.* 2001;68(5):259-70.
142. Marshall LM, Zmuda JM, Chan BK, Barrett-Connor E, Cauley JA, Ensrud KE, et al. Race and ethnic variation in proximal femur structure and BMD among older men. *J Bone Miner Res.* 2008;23(1):121-30.
143. Barrett-Connor E, Siris ES, Wehren LE, Miller PD, Abbott TA, Berger ML, et al. Osteoporosis and fracture risk in women of different ethnic groups. *J Bone Miner Res.* 2005;20(2):185-94.
144. Looker AC, Melton LJ, 3rd, Harris T, Borrud L, Shepherd J, McGowan J. Age, gender, and race/ethnic differences in total body and subregional bone density. *Osteoporos Int.* 2009;20(7):1141-9.
145. Gilsanz V, Skaggs DL, Kovanlikaya A, Sayre J, Loro ML, Kaufman F, et al. Differential effect of race on the axial and appendicular skeletons of children. *J Clin Endocrinol Metab.* 1998;83(5):1420-7.
146. Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab.* 1999;84(12):4702-12.
147. Rush EC, Freitas I, Plank LD. Body size, body composition and fat distribution: comparative analysis of European, Maori, Pacific Island and Asian Indian adults. *Br J Nutr.* 2009;102(4):632-41.
148. Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, Lambert EV, et al. BMI, fat and muscle differences in urban women of five ethnicities from two countries. *Int J Obes (Lond).* 2007;31(8):1232-9.
149. Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, et al. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. *Obesity (Silver Spring).* 2011;19(2):402-8.
150. Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL, Jr., Ravussin E, et al. Racial differences in abdominal depot-specific adiposity in white and African American adults. *Am J Clin Nutr.* 2010;91(1):7-15.
151. Sumner AE, Micklesfield LK, Ricks M, Tambay AV, Avila NA, Thomas F, et al. Waist circumference, BMI, and visceral adipose tissue in white women and women of African descent. *Obesity (Silver Spring).* 2011;19(3):671-4.
152. Carroll JF, Chiapa AL, Rodriguez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. *Obesity (Silver Spring).* 2008;16(3):600-7.
153. Lu H, Fu X, Ma X, Wu Z, He W, Wang Z, et al. Relationships of percent body fat and percent trunk fat with bone mineral density among Chinese, black, and white subjects. *Osteoporos Int.* 2011;22(12):3029-35.
154. Armbrecht G, Belavy DL, Backstrom M, Beller G, Alexandre C, Rizzoli R, et al. Trabecular and cortical bone density and architecture in women after 60 days of bed rest using high-resolution pQCT: WISE 2005. *J Bone Miner Res.* 2011;26(10):2399-410.
155. Belavy DL, Beller G, Ritter Z, Felsenberg D. Bone structure and density via HR-pQCT in 60d bed-rest, 2-years recovery with and without countermeasures. *J Musculoskelet Neuronal Interact.* 2011;11(3):215-26.
156. Beller G, Belavy DL, Sun L, Armbrecht G, Alexandre C, Felsenberg D. WISE-2005: bed-rest induced changes in bone mineral density in women during 60 days simulated microgravity. *Bone.* 49. United States: 2011 Elsevier Inc; 2011. p. 858-66.
157. Nagaraja MP, Risin D. A review: The current state of bone loss research: Data from spaceflight and microgravity simulators. *J Cell Biochem.* 2012.

158. Sievanen H. Immobilization and bone structure in humans. *Arch Biochem Biophys.* 503. United States: 2010 Elsevier Inc; 2010. p. 146-52.
159. Rittweger J, Simunic B, Bilancio G, De Santo NG, Cirillo M, Biolo G, et al. Bone loss in the lower leg during 35 days of bed rest is predominantly from the cortical compartment. *Bone.* 44. United States 2009. p. 612-8.
160. Rittweger J, Felsenberg D. Recovery of muscle atrophy and bone loss from 90 days bed rest: results from a one-year follow-up. *Bone.* 44. United States 2009. p. 214-24.
161. Rittweger J, Beller G, Armbrecht G, Mulder E, Buehring B, Gast U, et al. Prevention of bone loss during 56 days of strict bed rest by side-alternating resistive vibration exercise. *Bone.* 46. United States: 2009 Elsevier Inc; 2010. p. 137-47.
162. Spatz JM, Fields EE, Yu EW, Divieti Pajevic P, Bouxsein ML, Sibonga JD, et al. Serum sclerostin increases in healthy adult men during bed rest. *J Clin Endocrinol Metab.* 97. United States 2012. p. E1736-40.
163. Ivaska KK, Gerdhem P, Akesson K, Garnero P, Obrant KJ. Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women. *J Bone Miner Res.* 2007;22(8):1155-64.
164. Obrant KJ, Ivaska KK, Gerdhem P, Alatalo SL, Pettersson K, Vaananen HK. Biochemical markers of bone turnover are influenced by recently sustained fracture. *Bone.* 36. United States 2005. p. 786-92.
165. Ingle BM, Hay SM, Bottjer HM, Eastell R. Changes in bone mass and bone turnover following ankle fracture. *Osteoporos Int.* 10. England 1999. p. 408-15.
166. Ingle BM, Hay SM, Bottjer HM, Eastell R. Changes in bone mass and bone turnover following distal forearm fracture. *Osteoporos Int.* 10. England 1999. p. 399-407.
167. Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM. Changes in bone mass and bone turnover following tibial shaft fracture. *Osteoporos Int.* 2006;17(3):364-72.
168. Yu-Yahiro JA, Michael RH, Dubin NH, Fox KM, Sachs M, Hawkes WG, et al. Serum and urine markers of bone metabolism during the year after hip fracture. *J Am Geriatr Soc.* 49. United States 2001. p. 877-83.
169. Prentice A. Maternal calcium metabolism and bone mineral status. *Am J Clin Nutr.* 2000;71(5 Suppl):1312S-6S.
170. Moller UK, Vieth Streym S, Mosekilde L, Rejnmark L. Changes in bone mineral density and body composition during pregnancy and postpartum. A controlled cohort study. *Osteoporos Int.* 2012;23(4):1213-23.
171. Black AJ, Topping J, Durham B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. *J Bone Miner Res.* 2000;15(3):557-63.
172. Oliveri B, Parisi MS, Zeni S, Mautalen C. Mineral and bone mass changes during pregnancy and lactation. *Nutrition.* 20. United States 2004. p. 235-40.
173. Cross NA, Hillman LS, Allen SH, Krause GF, Vieira NE. Calcium homeostasis and bone metabolism during pregnancy, lactation, and postweaning: a longitudinal study. *Am J Clin Nutr.* 1995;61(3):514-23.
174. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. *J Bone Miner Res.* 2000;15(1):129-37.
175. Mendez RO, Gallegos AC, Cabrera RM, Quihui L, Zozaya R, Morales GG, et al. Bone Mineral Density Changes in Lactating Adolescent Mothers During the First Postpartum Year. *Am J Hum Biol.* 2013.
176. Affinito P, Tommaselli GA, di Carlo C, Guida F, Nappi C. Changes in bone mineral density and calcium metabolism in breastfeeding women: a one year follow-up study. *J Clin Endocrinol Metab.* 1996;81(6):2314-8.
177. Sowers M, Eyre D, Hollis BW, Randolph JF, Shapiro B, Jannausch ML, et al. Biochemical markers of bone turnover in lactating and nonlactating postpartum women. *J Clin Endocrinol Metab.* 1995;80(7):2210-6.

178. Laskey MA, Price RI, Khoo BC, Prentice A. Proximal femur structural geometry changes during and following lactation. *Bone*. 48. United States: 2010 Elsevier Inc; 2011. p. 755-9.
179. Sowers M, Corton G, Shapiro B, Jannausch ML, Crutchfield M, Smith ML, et al. Changes in bone density with lactation. *JAMA*. 1993;269(24):3130-5.
180. Laskey MA, Prentice A. Bone mineral changes during and after lactation. *Obstet Gynecol*. 94. United States 1999. p. 608-15.
181. Prentice A, Jarjou LM, Stirling DM, Buffenstein R, Fairweather-Tait S. Biochemical markers of calcium and bone metabolism during 18 months of lactation in Gambian women accustomed to a low calcium intake and in those consuming a calcium supplement. *J Clin Endocrinol Metab*. 1998;83(4):1059-66.
182. Hamann C, Kirschner S, Gunther KP, Hofbauer LC. Bone, sweet bone--osteoporotic fractures in diabetes mellitus. *Nat Rev Endocrinol*. 8. England 2012. p. 297-305.
183. Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res*. 2007;22(9):1317-28.
184. Vestergaard P, Rejnmark L, Mosekilde L. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. *Calcif Tissue Int*. 2009;84(1):45-55.
185. Ahmed LA, Joakimsen RM, Berntsen GK, Fonnebo V, Schirmer H. Diabetes mellitus and the risk of non-vertebral fractures: the Tromso study. *Osteoporos Int*. 2006;17(4):495-500.
186. de L, II, van der Klift M, de Laet CE, van Daele PL, Hofman A, Pols HA. Bone mineral density and fracture risk in type-2 diabetes mellitus: the Rotterdam Study. *Osteoporos Int*. 2005;16(12):1713-20.
187. Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Bauer DC, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. *Arch Intern Med*. 165. United States 2005. p. 1612-7.
188. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab*. 91. United States 2006. p. 3404-10.
189. Schwartz AV, Hillier TA, Sellmeyer DE, Resnick HE, Gregg E, Ensrud KE, et al. Older women with diabetes have a higher risk of falls: a prospective study. *Diabetes Care*. 2002;25(10):1749-54.
190. Yamagishi S. Role of advanced glycation end products (AGEs) in osteoporosis in diabetes. *Curr Drug Targets*. 12. Netherlands 2011. p. 2096-102.
191. Bredella MA, Fazeli PK, Miller KK, Misra M, Torriani M, Thomas BJ, et al. Increased bone marrow fat in anorexia nervosa. *J Clin Endocrinol Metab*. 94. United States 2009. p. 2129-36.
192. Ecklund K, Vajapeyam S, Feldman HA, Buzney CD, Mulkern RV, Kleinman PK, et al. Bone marrow changes in adolescent girls with anorexia nervosa. *J Bone Miner Res*. 2010;25(2):298-304.
193. Fazeli PK, Bredella MA, Misra M, Meenaghan E, Rosen CJ, Clemmons DR, et al. Preadipocyte factor-1 is associated with marrow adiposity and bone mineral density in women with anorexia nervosa. *J Clin Endocrinol Metab*. 95. United States 2010. p. 407-13.
194. Misra M, Klibanski A. Bone metabolism in adolescents with anorexia nervosa. *J Endocrinol Invest*. 34. Italy 2011. p. 324-32.
195. Misra M, Prabhakaran R, Miller KK, Goldstein MA, Mickley D, Clauss L, et al. Weight gain and restoration of menses as predictors of bone mineral density change in adolescent girls with anorexia nervosa-1. *J Clin Endocrinol Metab*. 93. United States 2008. p. 1231-7.
196. Halvorsen I, Platou D, Hoiseth A. Bone mass eight years after treatment for adolescent-onset anorexia nervosa. *Eur Eat Disord Rev*. 2012;20(5):386-92.

197. Hartman D, Crisp A, Rooney B, Rackow C, Atkinson R, Patel S. Bone density of women who have recovered from anorexia nervosa. *Int J Eat Disord.* 28. United States: 2000 John Wiley & Sons, Inc.; 2000. p. 107-12.
198. Olmos JM, Valero C, del Barrio AG, Amado JA, Hernandez JL, Menendez-Arango J, et al. Time course of bone loss in patients with anorexia nervosa. *Int J Eat Disord.* 2010;43(6):537-42.
199. Maurel DB, Boisseau N, Benhamou CL, Jaffre C. Alcohol and bone: review of dose effects and mechanisms. *Osteoporos Int.* 2012;23(1):1-16.
200. Turner RT. Skeletal response to alcohol. *Alcohol Clin Exp Res.* 2000;24(11):1693-701.
201. Ganry O, Baudoin C, Fardellone P. Effect of alcohol intake on bone mineral density in elderly women: The EPIDOS Study. *Epidemiologie de l'Osteoporose. Am J Epidemiol.* 2000;151(8):773-80.
202. Manson JE, Martin KA. Postmenopausal Hormone-Replacement Therapy. *New England Journal of Medicine.* 2001;345(1):34-40.
203. Bolton-Smith C, McMurdo ME, Paterson CR, Mole PA, Harvey JM, Fenton ST, et al. Two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women. *J Bone Miner Res.* 2007;22(4):509-19.
204. Ludescher B, Machann J, Eschweiler GW, Vanhofen S, Maenz C, Thamer C, et al. Correlation of fat distribution in whole body MRI with generally used anthropometric data. *Invest Radiol.* 2009;44(11):712-9.
205. Berker D, Koparal S, Isik S, Pasaoglu L, Aydin Y, Erol K, et al. Compatibility of different methods for the measurement of visceral fat in different body mass index strata. *Diagn Interv Radiol.* 2010;16(2):99-105.
206. Roopakala MS, Suresh A, Ashtalakshmi, Srinath, Ashok, Giridhar, et al. Anthropometric measurements as predictors of intraabdominal fat thickness. *Indian J Physiol Pharmacol.* 2009;53(3):259-64.
207. Clarys JP, Provyn S, Marfell-Jones MJ. Cadaver studies and their impact on the understanding of human adiposity. *Ergonomics.* 2005;48(11-14):1445-61.
208. Martin AD, Janssens V, Caboor D, Clarys JP, Marfell-Jones MJ. Relationships between visceral, trunk and whole-body adipose tissue weights by cadaver dissection. *Ann Hum Biol.* 2003;30(6):668-77.
209. Wang J, Thornton JC, Kolesnik S, Pierson RN, Jr. Anthropometry in body composition. An overview. *Ann N Y Acad Sci.* 2000;904:317-26.
210. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. *Jama.* 2002;288(15):1889-97.
211. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *Bmj.* 1996;312(7041):1254-9.
212. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. *Lancet.* 1993;341(8837):72-5.
213. Crabtree NJ, Leonard MB, Zemel BS. Dual-energy X-ray absorptiometry. In: Sawyer AJ, Bachrach LK, Fung EB, editors. *Current Clinical Practice: Bone Densitometry in Growing Patients: Guidelines for Clinical Practice.* Totowa, New Jersey, USA: Humana; 2006.
214. Ferrar L, Jiang G, Adams J, Eastell R. Identification of vertebral fractures: an update. *Osteoporos Int.* 2005;16(7):717-28.
215. Knapp KM, Welsman JR, Hopkins SJ, Fogelman I, Blake GM. Obesity increases precision errors in dual-energy X-ray absorptiometry measurements. *J Clin Densitom.* 2012;15(3):315-9.
216. Knapp KM, Welsman JR, Hopkins SJ, Shallcross A, Fogelman I, Blake GM. Obesity Increases Precision Errors in Total Body Dual-Energy X-Ray Absorptiometry Measurements. *J Clin Densitom.* 2014.

217. Yu EW, Thomas BJ, Brown JK, Finkelstein JS. Simulated increases in body fat and errors in bone mineral density measurements by DXA and QCT. *J Bone Miner Res.* 2011.
218. Brownbill RA, Ilich JZ. Measuring body composition in overweight individuals by dual energy x-ray absorptiometry. *BMC Med Imaging.* 2005;5(1):1.
219. Bolotin HH, Sievanen H, Grashuis JL. Patient-specific DXA bone mineral density inaccuracies: quantitative effects of nonuniform extraosseous fat distributions. *J Bone Miner Res.* 2003;18(6):1020-7.
220. Blake GM, Griffith JF, Yeung DK, Leung PC, Fogelman I. Effect of increasing vertebral marrow fat content on BMD measurement, T-Score status and fracture risk prediction by DXA. *Bone.* 2009;44(3):495-501.
221. Bredella MA. Perspective: the bone-fat connection. *Skeletal Radiol.* 2010.
222. Beck T. Measuring the structural strength of bones with dual-energy X-ray absorptiometry: principles, technical limitations, and future possibilities. *Osteoporos Int.* 2003;14 Suppl 5:S81-8.
223. Laskey MA, Lyttle KD, Flaxman ME, Barber RW. The influence of tissue depth and composition on the performance of the Lunar dual-energy X-ray absorptiometer whole-body scanning mode. *Eur J Clin Nutr.* 1992;46(1):39-45.
224. Javed F, Yu W, Thornton J, Colt E. Effect of fat on measurement of bone mineral density. *Int J Body Compos Res.* 2009;7(1):37-40.
225. Pocock NA, Noakes KA, Majerovic Y, Griffiths MR. Magnification error of femoral geometry using fan beam densitometers. *Calcif Tissue Int.* 1997;60(1):8-10.
226. Griffiths MR, Noakes KA, Pocock NA. Correcting the magnification error of fan beam densitometers. *J Bone Miner Res.* 1997;12(1):119-23.
227. A KW. *Computed Tomography.* 2nd ed. Germany: Publicis Corporate Publishing; 2005.
228. Husband JE, Fry IK. *Computed Tomography of The Body, A Radiographical and Clinical Approach.* First ed. London, UK: Macmillan Publishers Ltd; 1981.
229. Armstrong P, Wastie M, Rockall A. *Diagnostic Imaging.* 6th ed. Chichester, UK: Wiley-Blackwell; 2009.
230. GEHealthcare. *A Guide to CT Radiation Dose Management.* 2012.
231. Tothill P, Pye DW. Errors due to non-uniform distribution of fat in dual X-ray absorptiometry of the lumbar spine. *Br J Radiol.* 1992;65(777):807-13.
232. Yang L, Burton AC, Bradburn M, Nielson CM, Orwoll ES, Eastell R. Distribution of bone density in the proximal femur and its association with hip fracture risk in older men: the osteoporotic fractures in men (MrOS) study. *J Bone Miner Res.* 2012;27(11):2314-24.
233. Amin S, Kopperdhal DL, Melton LJ, 3rd, Achenbach SJ, Therneau TM, Riggs BL, et al. Association of hip strength estimates by finite-element analysis with fractures in women and men. *J Bone Miner Res.* 2011;26(7):1593-600.
234. Lang TF, Guglielmi G, van Kuijk C, De Serio A, Cammisa M, Genant HK. Measurement of bone mineral density at the spine and proximal femur by volumetric quantitative computed tomography and dual-energy X-ray absorptiometry in elderly women with and without vertebral fractures. *Bone.* 2002;30(1):247-50.
235. Orwoll ES, Marshall LM, Nielson CM, Cummings SR, Lapidus J, Cauley JA, et al. Finite element analysis of the proximal femur and hip fracture risk in older men. *J Bone Miner Res.* 2009;24(3):475-83.
236. Black DM, Bouxsein ML, Marshall LM, Cummings SR, Lang TF, Cauley JA, et al. Proximal femoral structure and the prediction of hip fracture in men: a large prospective study using QCT. *J Bone Miner Res.* 2008;23(8):1326-33.
237. Cheung AM, Adachi JD, Hanley DA, Kendler DL, Davison KS, Josse R, et al. High-resolution peripheral quantitative computed tomography for the assessment of bone strength and structure: a review by the Canadian Bone Strength Working Group. *Curr Osteoporos Rep.* 2013;11(2):136-46.



238. Liu XS, Cohen A, Shane E, Yin PT, Stein EM, Rogers H, et al. Bone density, geometry, microstructure and stiffness: Relationships between peripheral and central skeletal sites assessed by DXA, HR-pQCT, and cQCT in premenopausal women. *J Bone Miner Res.* 2010.
239. Cohen A, Dempster DW, Muller R, Guo XE, Nickolas TL, Liu XS, et al. Assessment of trabecular and cortical architecture and mechanical competence of bone by high-resolution peripheral computed tomography: comparison with transiliac bone biopsy. *Osteoporos Int.* 2010;21(2):263-73.
240. Nishiyama KK, Shane E. Clinical imaging of bone microarchitecture with HR-pQCT. *Curr Osteoporos Rep.* 2013;11(2):147-55.
241. Tjong W, Kazakia GJ, Burghardt AJ, Majumdar S. The effect of voxel size on high-resolution peripheral computed tomography measurements of trabecular and cortical bone microstructure. *Med Phys.* 2012;39(4):1893-903.
242. Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab.* 2005;90(12):6508-15.
243. Macneil JA, Boyd SK. Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. *Bone.* 2008;42(6):1203-13.
244. Nishiyama KK, Macdonald HM, Hanley DA, Boyd SK. Women with previous fragility fractures can be classified based on bone microarchitecture and finite element analysis measured with HR-pQCT. *Osteoporos Int.* 2013;24(5):1733-40.
245. Boutroy S, Van Rietbergen B, Sornay-Rendu E, Munoz F, Bouxsein ML, Delmas PD. Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with wrist fracture in postmenopausal women. *J Bone Miner Res.* 2008;23(3):392-9.
246. Pistoia W, van Rietbergen B, Lochmuller EM, Lill CA, Eckstein F, Ruegsegger P. Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images. *Bone.* 2002;30(6):842-8.
247. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol.* 1994;49(2):M85-94.
248. Guralnik JM, Ferrucci L, Pieper CF, Leveille SG, Markides KS, Ostir GV, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *J Gerontol A Biol Sci Med Sci.* 2000;55(4):M221-31.
249. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing.* 2010;39(4):412-23.
250. Hannon R, Eastell R. Preanalytical variability of biochemical markers of bone turnover. *Osteoporos Int.* 2000;11 Suppl 6:S30-44.
251. Szulc P, Bauer DC, Eastell R. Biochemical Markers of Bone Turnover in Osteoporosis. In: Rosen CJ, Bouillon R, Compston J, Rosen V, editors. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Eighth ed: John Wiley & Sons; 2013. p. 297-302.
252. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, et al. International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. *Clin Chem Lab Med.* 2011;49(8):1271-4.
253. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. Validity of free testosterone and free estradiol determinations in serum

- samples from postmenopausal women by theoretical calculations. *Cancer Epidemiol Biomarkers Prev.* 2002;11(10 Pt 1):1065-71.
254. Swinkels L MA, Martens M, Parsons G, Rosmalen F. An immunoassay for free 25-hydroxy vitamin D. 43rd Oak Ridge Conference Emerging Technologies for 21st Century Diagnostics: April 14–15 2011 American Association for Clinical Chemistry 2011.
  255. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, et al. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* 2011;26(7):1609-16.
  256. Bhan I, Powe CE, Berg AH, Ankers E, Wenger JB, Karumanchi SA, et al. Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients. *Kidney international.* 2012;82(1):84-9.
  257. Bakker I, Twisk JW, Van Mechelen W, Kemper HC. Fat-free body mass is the most important body composition determinant of 10-yr longitudinal development of lumbar bone in adult men and women. *J Clin Endocrinol Metab.* 2003;88(6):2607-13.
  258. Alcala C, Yanez M, Devoto E, Sostin C, Zeballos L, Santos JL. Obesity as a protective factor for postmenopausal osteoporosis. *Int J Obes Relat Metab Disord.* 1996;20(11):1027-32.
  259. Dogan A, Nakipoglu-Yuzer GF, Yildizgoren MT, Ozgirgin N. Is age or the body mass index (BMI) more determinant of the bone mineral density (BMD) in geriatric women and men? *Arch Gerontol Geriatr.* 2010.
  260. Yu Z, Zhu Z, Tang T, Dai K, Qiu S. Effect of body fat stores on total and regional bone mineral density in perimenopausal Chinese women. *J Bone Miner Metab.* 2009;27(3):341-6.
  261. Sornay-Rendu E, Boutroy S, Vilayphiou N, Claustrat B, Chapurlat RD. In obese postmenopausal women, bone microarchitecture and strength are not commensurate to greater body weight: the Os des Femmes de Lyon (OFELY) study. *J Bone Miner Res.* 2013;28(7):1679-87.
  262. Barrera G, Bunout D, Gattas V, de la Maza MP, Leiva L, Hirsch S. A high body mass index protects against femoral neck osteoporosis in healthy elderly subjects. *Nutrition.* 2004;20(9):769-71.
  263. Kirchengast S, Peterson B, Hauser G, Knogler W. Body composition characteristics are associated with the bone density of the proximal femur end in middle- and old-aged women and men. *Maturitas.* 2001;39(2):133-45.
  264. Greco EA, Fornari R, Rossi F, Santiemma V, Prossomariti G, Annoscia C, et al. Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index. *Int J Clin Pract.* 2010;64(6):817-20.
  265. Sukumar D, Schluskel Y, Riedt CS, Gordon C, Stahl T, Shapses SA. Obesity alters cortical and trabecular bone density and geometry in women. *Osteoporos Int.* 2011;22(2):635-45.
  266. Bacchetta J, Boutroy S, Guebre-Egziabher F, Juillard L, Drai J, Pelletier S, et al. The relationship between adipokines, osteocalcin and bone quality in chronic kidney disease. *Nephrol Dial Transplant.* 2009;24(10):3120-5.
  267. Ng AC, Melton LJ, 3rd, Atkinson EJ, Achenbach SJ, Holets MF, Peterson JM, et al. Relationship of adiposity to bone volumetric density and microstructure in men and women across the adult lifespan. *Bone.* 2013;55(1):119-25.
  268. Bredella MA, Lin E, Gerweck AV, Landa MG, Thomas BJ, Torriani M, et al. Determinants of bone microarchitecture and mechanical properties in obese men. *J Clin Endocrinol Metab.* 2012;97(11):4115-22.
  269. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, et al. Determinants of bone mineral density in obese premenopausal women. *Bone.* 2011;48(4):748-54.

270. Madeira E, Mafort TT, Madeira M, Guedes EP, de Oliveira Moreira R, de Mendonca LM, et al. Lean mass as a predictor of bone density and microarchitecture in adult obese individuals with metabolic syndrome. *Bone*. 2013.
271. Jamsa T, Jalovaara P, Peng Z, Vaananen HK, Tuukkanen J. Comparison of three-point bending test and peripheral quantitative computed tomography analysis in the evaluation of the strength of mouse femur and tibia. *Bone*. 1998;23(2):155-61.
272. Burghardt AJ, Kazakia GJ, Ramachandran S, Link TM, Majumdar S. Age- and gender-related differences in the geometric properties and biomechanical significance of intracortical porosity in the distal radius and tibia. *J Bone Miner Res*. 2010;25(5):983-93.
273. Cohen A, Dempster DW, Recker RR, Lappe JM, Zhou H, Zwahlen A, et al. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. *J Clin Endocrinol Metab*. 2013;98(6):2562-72.
274. Majumder S, Roychowdhury A, Pal S. Hip fracture and anthropometric variations: dominance among trochanteric soft tissue thickness, body height and body weight during sideways fall. *Clin Biomech (Bristol, Avon)*. 2013;28(9-10):1034-40.
275. Majumder S, Roychowdhury A, Pal S. Effects of trochanteric soft tissue thickness and hip impact velocity on hip fracture in sideways fall through 3D finite element simulations. *J Biomech*. 2008;41(13):2834-42.
276. Binkley N, Krueger D, Buehring B. What's in a name revisited: should osteoporosis and sarcopenia be considered components of "dysmobility syndrome?". *Osteoporos Int*. 2013;24(12):2955-9.
277. Marcus RL, Addison O, Dibble LE, Foreman KB, Morrell G, Lastayo P. Intramuscular adipose tissue, sarcopenia, and mobility function in older individuals. *J Aging Res*. 2012;2012:629637.
278. Himes CL, Reynolds SL. Effect of obesity on falls, injury, and disability. *J Am Geriatr Soc*. 2012;60(1):124-9.
279. Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat Rec*. 1987;219(1):1-9.
280. Wang MC, Bachrach LK, Van Loan M, Hudes M, Flegal KM, Crawford PB. The relative contributions of lean tissue mass and fat mass to bone density in young women. *Bone*. 2005;37(4):474-81.
281. Nakaoka D, Sugimoto T, Kaji H, Kanzawa M, Yano S, Yamauchi M, et al. Determinants of bone mineral density and spinal fracture risk in postmenopausal Japanese women. *Osteoporos Int*. 2001;12(7):548-54.
282. Khosla S, Atkinson EJ, Riggs BL, Melton LJ, 3rd. Relationship between body composition and bone mass in women. *J Bone Miner Res*. 1996;11(6):857-63.
283. Fu X, Ma X, Lu H, He W, Wang Z, Zhu S. Associations of fat mass and fat distribution with bone mineral density in pre- and postmenopausal Chinese women. *Osteoporos Int*. 2010.
284. Reid IR, Ames R, Evans MC, Sharpe S, Gamble G, France JT, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women - a key role for fat mass. *J Clin Endocrinol Metab*. 1992;75(1):45-51.
285. Pluijm SM, Visser M, Smit JH, Popp-Snijders C, Roos JC, Lips P. Determinants of bone mineral density in older men and women: body composition as mediator. *J Bone Miner Res*. 2001;16(11):2142-51.
286. Taaffe D. C., J. A., Danielson, M., Nevitt, M. C., Lang, T. F., Bauer, D. C., Harris, T. B. Race and sex effects on the association between muscle strength, soft tissue, and bone mineral density in healthy elders: the Health, Aging, and Body Composition Study. *Journal of Bone and Mineral Research*. 2001;16(0884-0431 (Print)):1343-52.
287. Taaffe D, Cauley, J. A., Danielson, M., Nevitt, M. C., Lang, T. F., Bauer, D. C., Harris, T. B. Race and sex effects on the association between muscle strength, soft tissue, and bone mineral density in healthy elders: the Health, Aging, and Body

- Composition Study. *Journal of Bone and Mineral Research*. 2001;16(0884-0431 (Print)):1343-52.
288. Xu L, Nicholson P, Wang QJ, Wang Q, Alen M, Cheng S. Fat mass accumulation compromises bone adaptation to load in Finnish women - a cross-sectional study spanning three generations. *J Bone Miner Res*. 2010.
  289. Douchi T, Yamamoto S, Oki T, Maruta K, Kuwahata R, Yamasaki H, et al. Difference in the effect of adiposity on bone density between pre- and postmenopausal women. *Maturitas*. 2000;34(3):261-6.
  290. Van Pelt RE, Evans EM, Schechtman KB, Ehsani AA, Kohrt WM. Contributions of total and regional fat mass to risk for cardiovascular disease in older women. *Am J Physiol Endocrinol Metab*. 2002;282(5):E1023-8.
  291. Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *Br J Nutr*. 2008;99(5):931-40.
  292. Warming L, Ravn P, Christiansen C. Visceral fat is more important than peripheral fat for endometrial thickness and bone mass in healthy postmenopausal women. *Am J Obstet Gynecol*. 2003;188(2):349-53.
  293. Marques EA, Moreira P, Wanderley F, Pizarro AN, Leao-Rosas JP, Mota J, et al. Appendicular fat mass is positively associated with femoral neck bone mineral density in older women. *Menopause*. 2012;19(3):311-8.
  294. Marwaha RK, Garg MK, Tandon N, Mehan N, Sastry A, Bhadra K. Relationship of body fat and its distribution with bone mineral density in Indian population. *J Clin Densitom*. 2013;16(3):353-9.
  295. Kuwahata A, Kawamura Y, Yonehara Y, Matsuo T, Iwamoto I, Douchi T. Non-weight-bearing effect of trunk and peripheral fat mass on bone mineral density in pre- and post-menopausal women. *Maturitas*. 2008;60(3-4):244-7.
  296. Yanazume Y, Kawamura Y, Kuwahata A, Yonehara Y, Matsuo T, Iwamoto I, et al. Difference in non-weight-bearing effects on bone mineral density between trunk and peripheral fat mass in women with polycystic ovary syndrome. *J Obstet Gynaecol Res*. 2010;36(2):352-6.
  297. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev*. 2010;11(1):11-8.
  298. Kim HY, Choe JW, Kim HK, Bae SJ, Kim BJ, Lee SH, et al. Negative association between metabolic syndrome and bone mineral density in Koreans, especially in men. *Calcif Tissue Int*. 2010;86(5):350-8.
  299. von Muhlen D, Safii S, Jassal SK, Svartberg J, Barrett-Connor E. Associations between the metabolic syndrome and bone health in older men and women: the Rancho Bernardo Study. *Osteoporos Int*. 2007;18(10):1337-44.
  300. Zillikens MC, Uitterlinden AG, van Leeuwen JP, Berends AL, Henneman P, van Dijk KW, et al. The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density. *Calcif Tissue Int*. 2010;86(2):116-25.
  301. Kim CJ, Oh KW, Rhee EJ, Kim KH, Jo SK, Jung CH, et al. Relationship between body composition and bone mineral density (BMD) in perimenopausal Korean women. *Clin Endocrinol (Oxf)*. 2009;71(1):18-26.
  302. Chung W, Lee J, Ryu OH. Is the negative relationship between obesity and bone mineral content greater for older women? *J Bone Miner Metab*. 2013.
  303. Yoo HJ, Park MS, Yang SJ, Kim TN, Lim KI, Kang HJ, et al. The differential relationship between fat mass and bone mineral density by gender and menopausal status. *J Bone Miner Metab*. 2011.
  304. Filip R, Raszewski G. Bone mineral density and bone turnover in relation to serum leptin, alpha-ketoglutarate and sex steroids in overweight and obese postmenopausal women. *Clin Endocrinol (Oxf)*. 2009;70(2):214-20.
  305. Chang CS, Chang YF, Wang MW, Chen CY, Chao YJ, Chang HJ, et al. Inverse relationship between central obesity and osteoporosis in osteoporotic drug naive

- elderly females: The Tianliao Old People (TOP) Study. *J Clin Densitom.* 2013;16(2):204-11.
306. Tarquini B, Navari N, Perfetto F, Piluso A, Romano S, Tarquini R. Evidence for bone mass and body fat distribution relationship in postmenopausal obese women. *Arch Gerontol Geriatr.* 1997;24(1):15-21.
  307. Goodpaster BH, Krishnaswami S, Harris TB, Katsiaras A, Kritchevsky SB, Simonsick EM, et al. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. *Arch Intern Med.* 2005;165(7):777-83.
  308. Karelis AD, St-Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET. Metabolic and body composition factors in subgroups of obesity: what do we know? *J Clin Endocrinol Metab.* 2004;89(6):2569-75.
  309. Goldenstein J, Kazakia G, Majumdar S. In vivo evaluation of the presence of bone marrow in cortical porosity in postmenopausal osteopenic women. *Ann Biomed Eng.* 2010;38(2):235-46.
  310. Compston JE. Bone marrow and bone: a functional unit. *J Endocrinol.* 2002;173(3):387-94.
  311. Griffith JF, Yeung DK, Antonio GE, Lee FK, Hong AW, Wong SY, et al. Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. *Radiology.* 2005;236(3):945-51.
  312. Wang YX, Griffith JF, Kwok AW, Leung JC, Yeung DK, Ahuja AT, et al. Reduced bone perfusion in proximal femur of subjects with decreased bone mineral density preferentially affects the femoral neck. *Bone.* 2009;45(4):711-5.
  313. Wren TA, Chung SA, Dorey FJ, Bluml S, Adams GB, Gilsanz V. Bone marrow fat is inversely related to cortical bone in young and old subjects. *J Clin Endocrinol Metab.* 2011;96(3):782-6.
  314. Lecka-Czernik B. Marrow fat metabolism is linked to the systemic energy metabolism. *Bone.* 2012;50(2):534-9.
  315. Hudak CS, Sul HS. Pref-1, a gatekeeper of adipogenesis. *Front Endocrinol (Lausanne).* 2013;4:79.
  316. Sul HS. Minireview: Pref-1: role in adipogenesis and mesenchymal cell fate. *Mol Endocrinol.* 2009;23(11):1717-25.
  317. Abdallah BM, Jensen CH, Gutierrez G, Leslie RG, Jensen TG, Kassem M. Regulation of human skeletal stem cells differentiation by Dlk1/Pref-1. *J Bone Miner Res.* 2004;19(5):841-52.
  318. O'Connell J, Lynch L, Hogan A, Cawood TJ, O'Shea D. Preadipocyte factor-1 is associated with metabolic profile in severe obesity. *J Clin Endocrinol Metab.* 2011;96(4):E680-4.
  319. Faje AT, Fazeli PK, Katzman D, Miller KK, Breggia A, Rosen CJ, et al. Inhibition of Pref-1 (preadipocyte factor 1) by oestradiol in adolescent girls with anorexia nervosa is associated with improvement in lumbar bone mineral density. *Clin Endocrinol (Oxf).* 2013;79(3):326-32.
  320. Fazeli PK, Bredella MA, Freedman L, Thomas BJ, Breggia A, Meenaghan E, et al. Marrow fat and preadipocyte factor-1 levels decrease with recovery in women with anorexia nervosa. *J Bone Miner Res.* 2012;27(9):1864-71.
  321. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, et al. Vertebral Bone Marrow Fat Is Positively Associated With Visceral Fat and Inversely Associated With IGF-1 in Obese Women. *Obesity (Silver Spring).* 2010.
  322. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med.* 2009;360(15):1509-17.
  323. Bredella MA, Gill CM, Rosen CJ, Klibanski A, Torriani M. Positive effects of brown adipose tissue on femoral bone structure. *Bone.* 2014;58:55-8.

324. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med.* 2009;360(15):1500-8.
325. Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. *Int J Obes (Lond).* 2013.
326. Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, et al. Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest.* 2013;123(8):3404-8.
327. Yoneshiro T, Aita S, Matsushita M, Okamatsu-Ogura Y, Kameya T, Kawai Y, et al. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring).* 2011;19(9):1755-60.
328. Villarroya J, Cereijo R, Villarroya F. An endocrine role for brown adipose tissue? *Am J Physiol Endocrinol Metab.* 2013;305(5):E567-72.
329. Bredella MA, Fazeli PK, Freedman LM, Calder G, Lee H, Rosen CJ, et al. Young women with cold-activated brown adipose tissue have higher bone mineral density and lower Pref-1 than women without brown adipose tissue: a study in women with anorexia nervosa, women recovered from anorexia nervosa, and normal-weight women. *J Clin Endocrinol Metab.* 2012;97(4):E584-90.
330. Lee P, Brychta RJ, Collins MT, Linderman J, Smith S, Herscovitch P, et al. Cold-activated brown adipose tissue is an independent predictor of higher bone mineral density in women. *Osteoporos Int.* 2013;24(4):1513-8.
331. Motyl KJ, Bishop KA, DeMambro VE, Bornstein SA, Le P, Kawai M, et al. Altered thermogenesis and impaired bone remodeling in Misty mice. *J Bone Miner Res.* 2013;28(9):1885-97.
332. Khosla S, Oursler MJ, Monroe DG. Estrogen and the skeleton. *Trends Endocrinol Metab.* 2012;23(11):576-81.
333. Reid I. Adipose Tissue and Bone. *Clinical Review in Bone and Mineral Metabolism.* 2009;7:207-9.
334. Thomas T, Burguera B, Melton LJ, 3rd, Atkinson EJ, O'Fallon WM, Riggs BL, et al. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone.* 2001;29(2):114-20.
335. Mohiti-Ardekani J, Soleymani-Salehabadi H, Owlia MB, Mohiti A. Relationships between serum adipocyte hormones (adiponectin, leptin, resistin), bone mineral density and bone metabolic markers in osteoporosis patients. *J Bone Miner Metab.* 2013.
336. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* 2004;34(3):376-83.
337. Hamrick MW, Della-Fera MA, Choi YH, Pennington C, Hartzell D, Baile CA. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. *J Bone Miner Res.* 2005;20(6):994-1001.
338. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med.* 1999;341(12):879-84.
339. Agbaht K, Gurlek A, Karakaya J, Bayraktar M. Circulating adiponectin represents a biomarker of the association between adiposity and bone mineral density. *Endocrine.* 2009;35(3):371-9.
340. Jurimae J, Jurimae T, Leppik A, Kums T. The influence of ghrelin, adiponectin, and leptin on bone mineral density in healthy postmenopausal women. *J Bone Miner Metab.* 2008;26(6):618-23.
341. Weiss LA, Barrett-Connor E, von Muhlen D, Clark P. Leptin predicts BMD and bone resorption in older women but not older men: the Rancho Bernardo study. *J Bone Miner Res.* 2006;21(5):758-64.

342. Biver E, Salliot C, Combescure C, Gossec L, Hardouin P, Legroux-Gerot I, et al. Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2011;96(9):2703-13.
343. Thomas T, Burguera B, Melton LJ, 3rd, Atkinson EJ, O'Fallon WM, Riggs BL, et al. Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. *Metabolism.* 2000;49(10):1278-84.
344. Baumgartner RN, Ross RR, Waters DL, Brooks WM, Morley JE, Montoya GD, et al. Serum leptin in elderly people: associations with sex hormones, insulin, and adipose tissue volumes. *Obes Res.* 1999;7(2):141-9.
345. Lorentzon M LK, Mellström D, Ohlsson C. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. *Journal of Bone and Mineral Research.* 2006;21(12):1871-8.
346. Blum M, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, et al. Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int.* 2003;73(1):27-32.
347. Kontogianni MD, Dafni UG, Routsias JG, Skopouli FN. Blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in perimenopausal women. *J Bone Miner Res.* 2004;19(4):546-51.
348. Jurimae J, Rembel K, Jurimae T, Rehand M. Adiponectin is associated with bone mineral density in perimenopausal women. *Horm Metab Res.* 2005;37(5):297-302.
349. Wu N, Wang QP, Li H, Wu XP, Sun ZQ, Luo XH. Relationships between serum adiponectin, leptin concentrations and bone mineral density, and bone biochemical markers in Chinese women. *Clin Chim Acta.* 2010;411(9-10):771-5.
350. King GA, Deemer SE, Thompson DL. Relationship between leptin, adiponectin, bone mineral density, and measures of adiposity among pre-menopausal Hispanic and Caucasian women. *Endocr Res.* 2010;35(3):106-17.
351. Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin--a key adipokine in the metabolic syndrome. *Diabetes Obes Metab.* 2006;8(3):264-80.
352. Napoli N, Pedone C, Pozzilli P, Lauretani F, Ferrucci L, Incalzi RA. Adiponectin and bone mass density: The InCHIANTI study. *Bone.* 2010.
353. Register TC, Divers J, Bowden DW, Carr JJ, Lenchik L, Wagenknecht LE, et al. Relationships between serum adiponectin and bone density, adiposity and calcified atherosclerotic plaque in the African American-Diabetes Heart Study. *J Clin Endocrinol Metab.* 2013;98(5):1916-22.
354. Huang KC, Cheng WC, Yen RF, Tsai KS, Tai TY, Yang WS. Lack of independent relationship between plasma adiponectin, leptin levels and bone density in nondiabetic female adolescents. *Clin Endocrinol (Oxf).* 2004;61(2):204-8.
355. Hassager C, Christiansen C. Influence of soft tissue body composition on bone mass and metabolism. *Bone.* 1989;10(6):415-9.
356. Ricci TA, Heymsfield SB, Pierson RN, Jr., Stahl T, Chowdhury HA, Shapses SA. Moderate energy restriction increases bone resorption in obese postmenopausal women. *Am J Clin Nutr.* 2001;73(2):347-52.
357. Black A, Allison DB, Shapses SA, Tilmont EM, Handy AM, Ingram DK, et al. Calorie restriction and skeletal mass in rhesus monkeys (*Macaca mulatta*): evidence for an effect mediated through changes in body size. *J Gerontol A Biol Sci Med Sci.* 2001;56(3):B98-107.
358. Papakitsou EF, Margioris AN, Dretakis KE, Trovas G, Zoras U, Lyritis G, et al. Body mass index (BMI) and parameters of bone formation and resorption in postmenopausal women. *Maturitas.* 2004;47(3):185-93.
359. Ardawi MS, Al-Kadi HA, Rouzi AA, Qari MH. Determinants of serum sclerostin in healthy pre- and postmenopausal women. *J Bone Miner Res.* 2011;26(12):2812-22.

360. Ribot C, Tremollieres F, Pouilles JM, Bonneau M, Germain F, Louvet JP. Obesity and postmenopausal bone loss: The influence of obesity on vertebral density and bone turnover in postmenopausal women. *Bone*. 1987;8(6):327-31.
361. Ribot C, Tremollieres F, Pouilles JM. The effect of obesity on postmenopausal bone loss and the risk of osteoporosis. *Adv Nutr Res*. 1994;9:257-71.
362. Tremollieres FA, Pouilles JM, Ribot C. Vertebral postmenopausal bone loss is reduced in overweight women: a longitudinal study in 155 early postmenopausal women. *J Clin Endocrinol Metab*. 1993;77(3):683-6.
363. Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol*. 2013;228(6):1149-53.
364. Zhou M, Ma X, Li H, Pan X, Tang J, Gao Y, et al. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. *Eur J Endocrinol*. 2009;161(5):723-9.
365. Movahed A, Larijani B, Nabipour I, Kalantarhormozi M, Asadipooya K, Vahdat K, et al. Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: the crosstalk between bone and energy metabolism. *J Bone Miner Metab*. 2012;30(6):683-91.
366. Hwang YC, Jeong IK, Ahn KJ, Chung HY. Circulating osteocalcin level is associated with improved glucose tolerance, insulin secretion and sensitivity independent of the plasma adiponectin level. *Osteoporos Int*. 2012;23(4):1337-42.
367. Bao Y, Ma X, Yang R, Wang F, Hao Y, Dou J, et al. Inverse relationship between serum osteocalcin levels and visceral fat area in Chinese men. *J Clin Endocrinol Metab*. 2013;98(1):345-51.
368. Kim SH, Lee JW, Im JA, Hwang HJ. Serum osteocalcin is related to abdominal obesity in Korean obese and overweight men. *Clin Chim Acta*. 2010;411(23-24):2054-7.
369. Amrein K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J Clin Endocrinol Metab*. 2012;97(1):148-54.
370. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner Res*. 2008;23(6):860-9.
371. Kramer I, Loots GG, Studer A, Keller H, Kneissel M. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *J Bone Miner Res*. 2010;25(2):178-89.
372. Gardner JC, van Bezooijen RL, Mervis B, Hamdy NA, Lowik CW, Hamersma H, et al. Bone mineral density in sclerosteosis; affected individuals and gene carriers. *J Clin Endocrinol Metab*. 2005;90(12):6392-5.
373. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. *J Clin Endocrinol Metab*. 2010;95(4):1991-7.
374. Sheng Z, Tong D, Ou Y, Zhang H, Zhang Z, Li S, et al. Serum sclerostin levels were positively correlated with fat mass and bone mineral density in central south Chinese postmenopausal women. *Clin Endocrinol (Oxf)*. 2012;76(6):797-801.
375. Durosier C, van Lierop A, Ferrari S, Chevalley T, Papapoulos S, Rizzoli R. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. *J Clin Endocrinol Metab*. 2013;98(9):3873-83.
376. Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int*. 2013;24(2):489-94.
377. Register TC, Hruska KA, Divers J, Bowden DW, Palmer ND, Carr JJ, et al. Sclerostin is positively associated with bone mineral density in men and women



- and negatively associated with carotid calcified atherosclerotic plaque in men from the african american-diabetes heart study. *J Clin Endocrinol Metab.* 2014;99(1):315-21.
378. Szulc P, Boutroy S, Vilayphiou N, Schoppet M, Rauner M, Chapurlat R, et al. Correlates of bone microarchitectural parameters and serum sclerostin levels in men: the STRAMBO study. *J Bone Miner Res.* 2013;28(8):1760-70.
  379. Kawai M, Rosen CJ. The IGF-I regulatory system and its impact on skeletal and energy homeostasis. *J Cell Biochem.* 2010;111:14-9.
  380. Morcov C, Vulpoi C, Branisteanu D. Correlation between adiponectin, leptin, insulin growth factor-1 and bone mineral density in pre and postmenopausal women. *Rev Med Chir Soc Med Nat Iasi.* 2012;116(3):785-9.
  381. Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nasser P, et al. Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. *J Bone Miner Res.* 2009;24(8):1481-92.
  382. Walsh JS, Paggiosi MA, Eastell R. Cortical consolidation of the radius and tibia in young men and women. *J Clin Endocrinol Metab.* 2012;97(9):3342-8.
  383. Kudo O, Fujikawa Y, Itonaga I, Sabokbar A, Torisu T, Athanasou NA. Proinflammatory cytokine (TNFalpha/IL-1alpha) induction of human osteoclast formation. *J Pathol.* 2002;198(2):220-7.
  384. Kudo O, Sabokbar A, Pocock A, Itonaga I, Fujikawa Y, Athanasou NA. Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. *Bone.* 2003;32(1):1-7.
  385. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. *J Clin Endocrinol Metab.* 2008;93(5):1952-8.
  386. Pino AM, Rios S, Astudillo P, Fernandez M, Figueroa P, Seitz G, et al. Concentration of adipogenic and proinflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. *J Bone Miner Res.* 2010;25(3):492-8.
  387. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev.* 2002;23(1):90-119.
  388. de Pablo P, Cooper MS, Buckley CD. Association between bone mineral density and C-reactive protein in a large population-based sample. *Arthritis Rheum.* 2012;64(8):2624-31.
  389. Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int.* 2005;16(10):1263-71.
  390. Rolland T, Boutroy S, Vilayphiou N, Blaizot S, Chapurlat R, Szulc P. Poor trabecular microarchitecture at the distal radius in older men with increased concentration of high-sensitivity C-reactive protein--the STRAMBO study. *Calcif Tissue Int.* 2012;90(6):496-506.
  391. Ishii S, Cauley JA, Greendale GA, Crandall CJ, Danielson ME, Ouchi Y, et al. C-reactive protein, bone strength, and nine-year fracture risk: data from the Study of Women's Health Across the Nation (SWAN). *J Bone Miner Res.* 2013;28(7):1688-98.
  392. Barbour KE, Boudreau R, Danielson ME, Youk AO, Wactawski-Wende J, Greep NC, et al. Inflammatory markers and the risk of hip fracture: the Women's Health Initiative. *J Bone Miner Res.* 2012;27(5):1167-76.
  393. Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, Pahor M, et al. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. *J Bone Miner Res.* 2007;22(7):1088-95.
  394. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev.* 2001;22(4):477-501.

395. Ardawi MS, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. *Osteoporos Int.* 2012;23(2):675-86.
396. Macdonald HM, Mavroei A, Barr RJ, Black AJ, Fraser WD, Reid DM. Vitamin D status in postmenopausal women living at higher latitudes in the UK in relation to bone health, overweight, sunlight exposure and dietary vitamin D. *Bone.* 2008;42(5):996-1003.
397. Larrosa M, Gomez A, Casado E, Moreno M, Vazquez I, Orellana C, et al. Hypovitaminosis D as a risk factor of hip fracture severity. *Osteoporos Int.* 2012;23(2):607-14.
398. van Schoor NM, Visser M, Pluijm SM, Kuchuk N, Smit JH, Lips P. Vitamin D deficiency as a risk factor for osteoporotic fractures. *Bone.* 2008;42(2):260-6.
399. Boonen S, Vanderschueren D, Cheng XG, Verbeke G, Dequeker J, Geusens P, et al. Age-related (type II) femoral neck osteoporosis in men: biochemical evidence for both hypovitaminosis D- and androgen deficiency-induced bone resorption. *J Bone Miner Res.* 1997;12(12):2119-26.
400. Sakuma M, Endo N, Oinuma T. Serum 25-OHD insufficiency as a risk factor for hip fracture. *J Bone Miner Metab.* 2007;25(3):147-50.
401. Snijder MB, van Schoor NM, Pluijm SM, van Dam RM, Visser M, Lips P. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. *J Clin Endocrinol Metab.* 2006;91(8):2980-5.
402. Ardawi MS, Qari MH, Rouzi AA, Maimani AA, Raddadi RM. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre- and postmenopausal women. *Osteoporos Int.* 2011;22(2):463-75.
403. Beydoun MA, Boueiz A, Shroff MR, Beydoun HA, Wang Y, Zonderman AB. Associations among 25-hydroxyvitamin D, diet quality, and metabolic disturbance differ by adiposity in adults in the United States. *J Clin Endocrinol Metab.* 2010;95(8):3814-27.
404. Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, McCabe EL, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes.* 2010;59(1):242-8.
405. Goldner WS, Stoner JA, Thompson J, Taylor K, Larson L, Erickson J, et al. Prevalence of vitamin D insufficiency and deficiency in morbidly obese patients: a comparison with non-obese controls. *Obes Surg.* 2008;18(2):145-50.
406. Orwoll E, Nielson CM, Marshall LM, Lambert L, Holton KF, Hoffman AR, et al. Vitamin D deficiency in older men. *J Clin Endocrinol Metab.* 2009;94(4):1214-22.
407. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *AmJClinNutr.* 2000;72(3):690-3.
408. Caron-Jobin M, Morisset AS, Tremblay A, Huot C, Légaré D, Tchernof A. Elevated serum 25(OH)D concentrations, vitamin D, and calcium intakes are associated with reduced adipocyte size in women. *Obesity (Silver Spring).* 2011;19(7):1335-41.
409. Eaton CB, Young A, Allison MA, Robinson J, Martin LW, Kuller LH, et al. Prospective association of vitamin D concentrations with mortality in postmenopausal women: results from the Women's Health Initiative (WHI). *Am J Clin Nutr.* 2011;94(6):1471-8.
410. Rico H, Arribas I, Casanova FJ, Duce AM, Hernandez ER, Cortes-Prieto J. Bone mass, bone metabolism, gonadal status and body mass index. *Osteoporos Int.* 2002;13(5):379-87.
411. Sowers MR, Zheng H, Greendale GA, Neer RM, Cauley JA, Ellis J, et al. Changes in bone resorption across the menopause transition: effects of reproductive hormones, body size, and ethnicity. *J Clin Endocrinol Metab.* 2013;98(7):2854-63.
412. Cifuentes M, Johnson MA, Lewis RD, Heymsfield SB, Chowdhury HA, Modlesky CM, et al. Bone turnover and body weight relationships differ in normal-weight

- compared with heavier postmenopausal women. *Osteoporos Int.* 2003;14(2):116-22.
413. Revilla M, Villa LF, Sanchez-Atrio A, Hernandez ER, Rico H. Influence of body mass index on the age-related slope of total and regional bone mineral content. *Calcif Tissue Int.* 1997;61(2):134-8.
  414. Viljakainen H, Ivaska KK, Paldanius P, Lipsanen-Nyman M, Saukkonen T, Pietilainen KH, et al. Suppressed bone turnover in obesity - a link to energy metabolism? A case-control study. *J Clin Endocrinol Metab.* 2014;jc20133097.
  415. Goulding A, Taylor RW. Plasma leptin values in relation to bone mass and density and to dynamic biochemical markers of bone resorption and formation in postmenopausal women. *Calcif Tissue Int.* 1998;63(6):456-8.
  416. Burguera B, Hofbauer LC, Thomas T, Gori F, Evans GL, Khosla S, et al. Leptin reduces ovariectomy-induced bone loss in rats. *Endocrinology.* 2001;142(8):3546-53.
  417. Martin A, de Vittoris R, David V, Moraes R, Begeot M, Lafage-Proust MH, et al. Leptin modulates both resorption and formation while preventing disuse-induced bone loss in tail-suspended female rats. *Endocrinology.* 2005;146(8):3652-9.
  418. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology.* 1999;140(4):1630-8.
  419. Gannage-Yared MH, Yaghi C, Habre B, Khalife S, Noun R, Germanos-Haddad M, et al. Osteoprotegerin in relation to body weight, lipid parameters insulin sensitivity, adipocytokines, and C-reactive protein in obese and non-obese young individuals: results from both cross-sectional and interventional study. *Eur J Endocrinol.* 2008;158(3):353-9.
  420. Dimitri P, Wales JK, Bishop N. Adipokines, bone-derived factors and bone turnover in obese children; evidence for altered fat-bone signalling resulting in reduced bone mass. *Bone.* 2010(1873-2763 (Electronic)).
  421. Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. *J Clin Endocrinol Metab.* 2001;86(7):3162-5.
  422. Khosla S, Arrighi HM, Melton LJ, 3rd, Atkinson EJ, O'Fallon WM, Dunstan C, et al. Correlates of osteoprotegerin levels in women and men. *Osteoporos Int.* 2002;13(5):394-9.
  423. Li H, Matheny M, Nicolson M, Tumer N, Scarpace PJ. Leptin gene expression increases with age independent of increasing adiposity in rats. *Diabetes.* 1997;46(12):2035-9.
  424. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab.* 1996;81(9):3424-7.
  425. Moller N, O'Brien P, Nair KS. Disruption of the relationship between fat content and leptin levels with aging in humans. *J Clin Endocrinol Metab.* 1998;83(3):931-4.
  426. Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, et al. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab.* 1997;82(2):579-84.
  427. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996;334(5):292-5.
  428. Hamilton BS, Paglia D, Kwan AY, Deitel M. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat Med.* 1995;1(9):953-6.
  429. Van Harmelen V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonnqvist F, et al. Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes.* 1998;47(6):913-7.

430. Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S. Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes*. 1997;46(3):342-7.
431. Baumgartner RN, Waters DL, Morley JE, Patrick P, Montoya GD, Garry PJ. Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. *Metabolism*. 1999;48(3):378-84.
432. Taes YE, Lapauw B, Vanbillemont G, Bogaert V, De Bacquer D, Zmierzczak H, et al. Fat mass is negatively associated with cortical bone size in young healthy male siblings. *J Clin Endocrinol Metab*. 2009;94(7):2325-31.
433. Wong IP, Nguyen AD, Khor EC, Enriquez RF, Eisman JA, Sainsbury A, et al. Neuropeptide Y is a critical modulator of leptin's regulation of cortical bone. *J Bone Miner Res*. 2013;28(4):886-98.
434. Bruno C, Fulford AD, Potts JR, McClintock R, Jones R, Cacucci BM, et al. Serum markers of bone turnover are increased at six and 18 months after Roux-en-Y bariatric surgery: correlation with the reduction in leptin. *J Clin Endocrinol Metab*. 2010;95(1):159-66.
435. Heshmati HM, Khosla S, Robins SP, O'Fallon WM, Melton LJ, 3rd, Riggs BL. Role of low levels of endogenous estrogen in regulation of bone resorption in late postmenopausal women. *J Bone Miner Res*. 2002;17(1):172-8.
436. Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. *J Bone Miner Res*. 2005;20(8):1334-41.
437. Argoud T, Boutroy S, Claustrat B, Chapurlat R, Szulc P. Association between sex steroid levels and bone microarchitecture in men - the STRAMBO study. *J Clin Endocrinol Metab*. 2014;jc20133233.
438. Szulc P, Uusi-Rasi K, Claustrat B, Marchand F, Beck TJ, Delmas PD. Role of sex steroids in the regulation of bone morphology in men. The MINOS study. *Osteoporos Int*. 2004;15(11):909-17.
439. Vandendput L, Lorentzon M, Sundh D, Nilsson ME, Karlsson MK, Mellstrom D, et al. Serum Estradiol Levels are Inversely Associated with Cortical Porosity in Older Men. *J Clin Endocrinol Metab*. 2014;jc20141319.
440. Vandewalle S, Taes Y, Van Helvoirt M, Debode P, Herregods N, Ernst C, et al. Bone size and bone strength are increased in obese male adolescents. *J Clin Endocrinol Metab*. 2013;98(7):3019-28.
441. Grethen E, Hill KM, Jones R, Cacucci BM, Gupta CE, Acton A, et al. Serum leptin, parathyroid hormone, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, bone alkaline phosphatase, and sclerostin relationships in obesity. *J Clin Endocrinol Metab*. 2012;97(5):1655-62.
442. Modder UI, Hoey KA, Amin S, McCreedy LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. *J Bone Miner Res*. 2011;26(2):373-9.
443. Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab*. 2003;88(4):1730-6.
444. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science*. 2005;307(5708):426-30.
445. Thommesen L, Stunes AK, Monjo M, Grosvik K, Tambursten MV, Kjobli E, et al. Expression and regulation of resistin in osteoblasts and osteoclasts indicate a role in bone metabolism. *J Cell Biochem*. 2006;99(3):824-34.
446. Zhang H, Xie H, Zhao Q, Liao EY, Xie GQ, Wu XP, et al. Relationships between serum adiponectin, apelin, leptin, resistin, visfatin levels and bone mineral density,

- and bone biochemical markers in postmenopausal Chinese women. *J Endocrinol Invest*.
447. Peng XD, Xie H, Zhao Q, Wu XP, Sun ZQ, Liao EY. Relationships between serum adiponectin, leptin, resistin, visfatin levels and bone mineral density, and bone biochemical markers in Chinese men. *Clin Chim Acta*. 2008;387(1-2):31-5.
  448. Oh KW, Lee WY, Rhee EJ, Baek KH, Yoon KH, Kang MI, et al. The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men. *Clin Endocrinol (Oxf)*. 2005;63(2):131-8.
  449. Delbono O, O'Rourke KS, Ettinger WH. Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. *J Membr Biol*. 1995;148(3):211-22.
  450. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol* (1985). 2001;90(6):2157-65.
  451. Gallagher D, Kuznia P, Heshka S, Albu J, Heymsfield SB, Goodpaster B, et al. Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. *Am J Clin Nutr*. 2005;81(4):903-10.
  452. Lang T, Cauley JA, Tylavsky F, Bauer D, Cummings S, Harris TB. Computed tomographic measurements of thigh muscle cross-sectional area and attenuation coefficient predict hip fracture: the health, aging, and body composition study. *J Bone Miner Res*. 2010;25(3):513-9.
  453. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *J Gerontol A Biol Sci Med Sci*. 2005;60(3):324-33.
  454. Visser M, Kritchevsky SB, Goodpaster BH, Newman AB, Nevitt M, Stamm E, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *J Am Geriatr Soc*. 2002;50(5):897-904.
  455. Tuttle LJ, Sinacore DR, Mueller MJ. Intermuscular adipose tissue is muscle specific and associated with poor functional performance. *J Aging Res*. 2012;2012:172957.
  456. Hilton TN, Tuttle LJ, Bohnert KL, Mueller MJ, Sinacore DR. Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus, and peripheral neuropathy: association with performance and function. *Phys Ther*. 2008;88(11):1336-44.
  457. Addison O, Marcus RL, Lastayo PC, Ryan AS. Intermuscular Fat: A Review of the Consequences and Causes. *Int J Endocrinol*. 2014;2014:309570.
  458. Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr*. 2009;90(6):1579-85.
  459. von Haehling S, Morley JE, Anker SD. From muscle wasting to sarcopenia and myopenia: update 2012. *J Cachexia Sarcopenia Muscle*. 2012;3(4):213-7.
  460. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-56.
  461. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr*. 1997;127(5 Suppl):990s-1s.
  462. Clark BC, Manini TM. What is dynapenia? *Nutrition*. 2012;28(5):495-503.
  463. Rolland YM, Perry HM, 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. *J Gerontol A Biol Sci Med Sci*. 2007;62(3):330-5.
  464. Chen L, Nelson DR, Zhao Y, Cui Z, Johnston JA. Relationship between muscle mass and muscle strength, and the impact of comorbidities: a population-based, cross-sectional study of older adults in the United States. *BMC Geriatr*. 2013;13(1):74.

465. Sjoblom S, Suuronen J, Rikkinen T, Honkanen R, Kroger H, Sirola J. Relationship between postmenopausal osteoporosis and the components of clinical sarcopenia. *Maturitas*. 2013;75(2):175-80.
466. Miyakoshi N, Hongo M, Mizutani Y, Shimada Y. Prevalence of sarcopenia in Japanese women with osteopenia and osteoporosis. *J Bone Miner Metab*. 2013;31(5):556-61.
467. Walsh MC, Hunter GR, Livingstone MB. Sarcopenia in premenopausal and postmenopausal women with osteopenia, osteoporosis and normal bone mineral density. *Osteoporos Int*. 2006;17(1):61-7.
468. Di Monaco M, Vallerio F, Di Monaco R, Tappero R. Prevalence of sarcopenia and its association with osteoporosis in 313 older women following a hip fracture. *Arch Gerontol Geriatr*. 2011;52(1):71-4.
469. Szulc P, Beck TJ, Marchand F, Delmas PD. Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men--the MINOS study. *J Bone Miner Res*. 2005;20(5):721-9.
470. Landi F, Cruz-Jentoft AJ, Liperoti R, Russo A, Giovannini S, Tosato M, et al. Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from the SIRENTE study. *Age Ageing*. 2013;42(2):203-9.
471. Rosengren BE, Ribom EL, Nilsson JA, Mallmin H, Ljunggren O, Ohlsson C, et al. Inferior physical performance test results of 10,998 men in the MrOS Study is associated with high fracture risk. *Age Ageing*. 2012;41(3):339-44.
472. Karlsson MK, Ribom E, Nilsson JA, Ljunggren O, Ohlsson C, Mellstrom D, et al. Inferior physical performance tests in 10,998 men in the MrOS study is associated with recurrent falls. *Age Ageing*. 2012;41(6):740-6.
473. de Rekeneire N, Visser M, Peila R, Nevitt MC, Cauley JA, Tylavsky FA, et al. Is a fall just a fall: correlates of falling in healthy older persons. The Health, Aging and Body Composition Study. *J Am Geriatr Soc*. 2003;51(6):841-6.
474. Cawthon PM, Fullman RL, Marshall L, Mackey DC, Fink HA, Cauley JA, et al. Physical performance and risk of hip fractures in older men. *J Bone Miner Res*. 2008;23(7):1037-44.
475. Schafer AL, Vittinghoff E, Lang TF, Sellmeyer DE, Harris TB, Kanaya AM, et al. Fat infiltration of muscle, diabetes, and clinical fracture risk in older adults. *J Clin Endocrinol Metab*. 2010;95(11):E368-72.
476. Sheu Y, Marshall LM, Holton KF, Caserotti P, Boudreau RM, Strotmeyer ES, et al. Abdominal body composition measured by quantitative computed tomography and risk of non-spine fractures: the Osteoporotic Fractures in Men (MrOS) study. *Osteoporos Int*. 2013;24(8):2231-41.
477. Corbeil P, Simoneau M, Rancourt D, Tremblay A, Teasdale N. Increased risk for falling associated with obesity: mathematical modeling of postural control. *IEEE Trans Neural Syst Rehabil Eng*. 2001;9(2):126-36.
478. Butterworth PA, Landorf KB, Gilleard W, Urquhart DM, Menz HB. The association between body composition and foot structure and function: a systematic review. *Obes Rev*. 2013.
479. Cesari M, Incalzi RA, Zamboni V, Pahor M. Vitamin D hormone: a multitude of actions potentially influencing the physical function decline in older persons. *Geriatr Gerontol Int*. 2011;11(2):133-42.
480. Visser M, Kritchevsky SB, Newman AB, Goodpaster BH, Tylavsky FA, Nevitt MC, et al. Lower serum albumin concentration and change in muscle mass: the Health, Aging and Body Composition Study. *Am J Clin Nutr*. 2005;82(3):531-7.
481. Hubbard RE, O'Mahony MS, Savva GM, Calver BL, Woodhouse KW. Inflammation and frailty measures in older people. *J Cell Mol Med*. 2009;13(9b):3103-9.
482. Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A, Giorgino F. The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. *J Endocrinol*. 2010;205(3):201-10.

483. Onder G, Liperoti R, Russo A, Soldato M, Capoluongo E, Volpato S, et al. Body mass index, free insulin-like growth factor I, and physical function among older adults: results from the iLSIRENTE study. *Am J Physiol Endocrinol Metab.* 2006;291(4):E829-34.
484. Gilsanz V, Kremer A, Mo AO, Wren TA, Kremer R. Vitamin D status and its relation to muscle mass and muscle fat in young women. *J Clin Endocrinol Metab.* 2010;95(4):1595-601.
485. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JF, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes.* 2004;53(2):294-305.
486. Gaens KH, Stehouwer CD, Schalkwijk CG. Advanced glycation endproducts and its receptor for advanced glycation endproducts in obesity. *Curr Opin Lipidol.* 2013;24(1):4-11.
487. Haus JM, Carrithers JA, Trappe SW, Trappe TA. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *J Appl Physiol* (1985). 2007;103(6):2068-76.
488. Dalal M, Ferrucci L, Sun K, Beck J, Fried LP, Semba RD. Elevated serum advanced glycation end products and poor grip strength in older community-dwelling women. *J Gerontol A Biol Sci Med Sci.* 2009;64(1):132-7.
489. Cawthon PM, Ensrud KE, Laughlin GA, Cauley JA, Dam TT, Barrett-Connor E, et al. Sex hormones and frailty in older men: the osteoporotic fractures in men (MrOS) study. *J Clin Endocrinol Metab.* 2009;94(10):3806-15.
490. Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys Williams G, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci.* 2004;59(3):242-8.
491. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci.* 2002;57(5):M326-32.
492. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med.* 2006;119(6):526.e9-17.
493. Landi F, Russo A, Cesari M, Pahor M, Bernabei R, Onder G. HDL-cholesterol and physical performance: results from the ageing and longevity study in the sirente geographic area (iLSIRENTE Study). *Age Ageing.* 2007;36(5):514-20.
494. Volpato S, Ble A, Metter EJ, Lauretani F, Bandinelli S, Zuliani G, et al. High-density lipoprotein cholesterol and objective measures of lower extremity performance in older nondisabled persons: the InChianti study. *J Am Geriatr Soc.* 2008;56(4):621-9.
495. Senechal M, Dionne IJ, Brochu M. Dynapenic abdominal obesity and metabolic risk factors in adults 50 years of age and older. *J Aging Health.* 2012;24(5):812-26.
496. Hittel DS, Hathout Y, Hoffman EP, Houmard JA. Proteome analysis of skeletal muscle from obese and morbidly obese women. *Diabetes.* 2005;54(5):1283-8.
497. Kim JY, Shin S, Han K, Lee KC, Kim JH, Choi YS, et al. Relationship between socioeconomic status and anemia prevalence in adolescent girls based on the fourth and fifth Korea National Health and Nutrition Examination Surveys. *Eur J Clin Nutr.* 2013.
498. Cheng HL, Bryant CE, Rooney KB, Steinbeck KS, Griffin HJ, Petocz P, et al. Iron, hepcidin and inflammatory status of young healthy overweight and obese women in Australia. *PLoS One.* 2013;8(7):e68675.
499. Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S, et al. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. *J Am Geriatr Soc.* 2004;52(5):719-24.

500. Haslam A, Hausman DB, Davey A, Elaine Cress M, Johnson MA, Poon LW. Associations between anemia and physical function in Georgia centenarians. *J Am Geriatr Soc.* 2012;60(12):2362-3.
501. Bowling CB, Muntner P, Bradbury BD, Kilpatrick RD, Isitt JJ, Warriner AH, et al. Low hemoglobin levels and recurrent falls in U.S. men and women: prospective findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. *Am J Med Sci.* 2013;345(6):446-54.
502. Scharf G, Heineke J. Finding good biomarkers for sarcopenia. *J Cachexia Sarcopenia Muscle.* 2012;3(3):145-8.
503. Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. *Nutr Rev.* 2011;69(8):419-31.
504. Russo A, Cesari M, Onder G, Zamboni V, Barillaro C, Pahor M, et al. Depression and physical function: results from the aging and longevity study in the Sirente geographic area (ilSIRENTE Study). *J Geriatr Psychiatry Neurol.* 2007;20(3):131-7.
505. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry.* 2010;67(3):220-9.
506. Wearing SC, Hennig EM, Byrne NM, Steele JR, Hills AP. The biomechanics of restricted movement in adult obesity. *Obes Rev.* 2006;7(1):13-24.
507. Rolland Y, Lauwers-Cances V, Cristini C, Abellan van Kan G, Janssen I, Morley JE, et al. Difficulties with physical function associated with obesity, sarcopenia, and sarcopenic-obesity in community-dwelling elderly women: the EPIDOS (EPIDemiologie de l'OSteoporose) Study. *Am J Clin Nutr.* 2009;89(6):1895-900.
508. Davison KK, Ford ES, Cogswell ME, Dietz WH. Percentage of body fat and body mass index are associated with mobility limitations in people aged 70 and older from NHANES III. *J Am Geriatr Soc.* 2002;50(11):1802-9.
509. Bouchard DR, Dionne IJ, Brochu M. Sarcopenic/obesity and physical capacity in older men and women: data from the Nutrition as a Determinant of Successful Aging (NuAge)-the Quebec longitudinal Study. *Obesity (Silver Spring).* 2009;17(11):2082-8.
510. Sibella F, Galli M, Romei M, Montesano A, Crivellini M. Biomechanical analysis of sit-to-stand movement in normal and obese subjects. *Clin Biomech (Bristol, Avon).* 2003;18(8):745-50.
511. DeVita P, Hortobagyi T. Obesity is not associated with increased knee joint torque and power during level walking. *J Biomech.* 2003;36(9):1355-62.
512. Lai PP, Leung AK, Li AN, Zhang M. Three-dimensional gait analysis of obese adults. *Clin Biomech (Bristol, Avon).* 2008;23 Suppl 1:S2-6.
513. Spyropoulos P, Pisciotto JC, Pavlou KN, Cairns MA, Simon SR. Biomechanical gait analysis in obese men. *Arch Phys Med Rehabil.* 1991;72(13):1065-70.
514. Jadelis K, Miller ME, Ettinger WH, Jr., Messier SP. Strength, balance, and the modifying effects of obesity and knee pain: results from the Observational Arthritis Study in Seniors (oasis). *J Am Geriatr Soc.* 2001;49(7):884-91.
515. Gravante G, Russo G, Pomara F, Ridola C. Comparison of ground reaction forces between obese and control young adults during quiet standing on a baropodometric platform. *Clin Biomech (Bristol, Avon).* 2003;18(8):780-2.
516. Faria A, Gabriel R, Abrantes J, Bras R, Moreira H. The relationship of body mass index, age and triceps-surae musculotendinous stiffness with the foot arch structure of postmenopausal women. *Clin Biomech (Bristol, Avon).* 2010;25(6):588-93.
517. Hills AP, Hennig EM, Byrne NM, Steele JR. The biomechanics of adiposity--structural and functional limitations of obesity and implications for movement. *Obes Rev.* 2002;3(1):35-43.



518. Hills AP, Hennig EM, McDonald M, Bar-Or O. Plantar pressure differences between obese and non-obese adults: a biomechanical analysis. *Int J Obes Relat Metab Disord.* 2001;25(11):1674-9.
519. Arnold JB, Causby R, Pod GD, Jones S. The impact of increasing body mass on peak and mean plantar pressure in asymptomatic adult subjects during walking. *Diabet Foot Ankle.* 2010;1.
520. Faria A, Gabriel R, Abrantes J, Bras R, Moreira H. Triceps-surae musculotendinous stiffness: relative differences between obese and non-obese postmenopausal women. *Clin Biomech (Bristol, Avon).* 2009;24(10):866-71.
521. Sternfeld B, Ngo L, Satariano WA, Tager IB. Associations of body composition with physical performance and self-reported functional limitation in elderly men and women. *Am J Epidemiol.* 2002;156(2):110-21.
522. Hulens M, Vansant G, Lysens R, Claessens AL, Muls E, Brumagne S. Study of differences in peripheral muscle strength of lean versus obese women: an allometric approach. *Int J Obes Relat Metab Disord.* 2001;25(5):676-81.
523. Blimkie CJ, Sale DG, Bar-Or O. Voluntary strength, evoked twitch contractile properties and motor unit activation of knee extensors in obese and non-obese adolescent males. *Eur J Appl Physiol Occup Physiol.* 1990;61(3-4):313-8.
524. Landi F, Liperoti R, Russo A, Giovannini S, Tosato M, Capoluongo E, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the iSIRENTE study. *Clin Nutr.* 2012;31(5):652-8.
525. Newman AB, Kupelian V, Visser M, Simonsick E, Goodpaster B, Nevitt M, et al. Sarcopenia: alternative definitions and associations with lower extremity function. *J Am Geriatr Soc.* 2003;51(11):1602-9.
526. Zoico E, Di Francesco V, Guralnik JM, Mazzali G, Bortolani A, Guariento S, et al. Physical disability and muscular strength in relation to obesity and different body composition indexes in a sample of healthy elderly women. *Int J Obes Relat Metab Disord.* 2004;28(2):234-41.
527. Visser M, Langlois J, Guralnik JM, Cauley JA, Kronmal RA, Robbins J, et al. High body fatness, but not low fat-free mass, predicts disability in older men and women: the Cardiovascular Health Study. *Am J Clin Nutr.* 1998;68(3):584-90.
528. Koster A, Ding J, Stenholm S, Caserotti P, Houston DK, Nicklas BJ, et al. Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? *J Gerontol A Biol Sci Med Sci.* 2011;66(8):888-95.
529. Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS, et al. Do muscle mass, muscle density, strength, and physical function similarly influence risk of hospitalization in older adults? *J Am Geriatr Soc.* 2009;57(8):1411-9.
530. Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS, et al. Clustering of strength, physical function, muscle, and adiposity characteristics and risk of disability in older adults. *J Am Geriatr Soc.* 2011;59(5):781-7.
531. Heymsfield SB, Gonzalez MC, Shen W, Redman L, Thomas D. Weight loss composition is one-fourth fat-free mass: a critical review and critique of this widely cited rule. *Obes Rev.* 2014.
532. Monteiro MA, Gabriel RC, Sousa MF, Castro MN, Moreira MH. Temporal parameters of the foot roll-over during walking: influence of obesity and sarcopenic obesity on postmenopausal women. *Maturitas.* 2010;67(2):178-85.
533. Monteiro M, Gabriel R, Aranha J, Neves e Castro M, Sousa M, Moreira M. Influence of obesity and sarcopenic obesity on plantar pressure of postmenopausal women. *Clin Biomech (Bristol, Avon).* 2010;25(5):461-7.
534. Domiciano DS, Figueiredo CP, Lopes JB, Caparbo VF, Takayama L, Menezes PR, et al. Discriminating sarcopenia in community-dwelling older women with high frequency of overweight/obesity: the Sao Paulo Ageing & Health Study (SPAH). *Osteoporos Int.* 2013;24(2):595-603.

535. Kim KJ, Ashton-Miller JA. Biomechanics of fall arrest using the upper extremity: age differences. *Clin Biomech (Bristol, Avon)*. 2003;18(4):311-8.
536. Berrigan F, Simoneau M, Tremblay A, Hue O, Teasdale N. Influence of obesity on accurate and rapid arm movement performed from a standing posture. *Int J Obes (Lond)*. 2006;30(12):1750-7.
537. Bohannon RW, Williams Andrews A. Normal walking speed: a descriptive meta-analysis. *Physiotherapy*. 2011;97(3):182-9.
538. Kidde J, Marcus R, Dibble L, Smith S, Lastayo P. Regional muscle and whole-body composition factors related to mobility in older individuals: a review. *Physiother Can*. 2009;61(4):197-209.
539. Wamberg L, Christiansen T, Paulsen SK, Fisker S, Rask P, Rejnmark L, et al. Expression of vitamin D-metabolizing enzymes in human adipose tissue -- the effect of obesity and diet-induced weight loss. *Int J Obes (Lond)*. 2013;37(5):651-7.
540. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Archives of internal medicine*. 2008;168(12):1340-9.
541. Ginde AA, Scragg R, Schwartz RS, Camargo CA, Jr. Prospective study of serum 25-hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older U.S. adults. *Journal of the American Geriatrics Society*. 2009;57(9):1595-603.
542. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. The National Academies Press; Washington DC. 2011.
543. National Osteoporosis Society. Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management. 2013.
544. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*. 2011;96(7):1911-30.
545. Roth HJ, Schmidt-Gayk H, Weber H, Niederau C. Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography-tandem mass spectrometry as a reference. *Ann Clin Biochem*. 2008;45(Pt 2):153-9.
546. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *AmJClinNutr*. 2007;85(3):860-8.
547. Prentice A. Vitamin D deficiency: a global perspective. *Nutr Rev*. 2008;66(10 Suppl 2):S153-64.
548. Update on Vitamin D. Position statement by the Scientific Advisory Committee on Nutrition. 2007. The Stationery Office.
549. Young KA, Engelman CD, Langefeld CD, Hairston KG, Haffner SM, Bryer-Ash M, et al. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. *J Clin Endocrinol Metab*. 2009;94(9):3306-13.
550. Winters SJ, Chennubhatla R, Wang C, Miller JJ. Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women. *Metabolism*. 2009;58(4):438-42.
551. Vilarrasa N, Vendrell J, Maravall J, Elió I, Solano E, San José P, et al. Is plasma 25(OH) D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population? *Endocrine*. 2010;38(2):235-42.
552. Vilarrasa N, Maravall J, Estepa A, Sánchez R, Masdevall C, Navarro MA, et al. Low 25-hydroxyvitamin D concentrations in obese women: their clinical significance and relationship with anthropometric and body composition variables. *J Endocrinol Invest*. 2007;30(8):653-8.

553. van Dam RM, Snijder MB, Dekker JM, Stehouwer CD, Bouter LM, Heine RJ, et al. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: the Hoorn Study. *Am J Clin Nutr.* 2007;85(3):755-61.
554. Valiña-Tóth AL, Lai Z, Yoo W, Abou-Samra A, Gadegbeku CA, Flack JM. Relationship of vitamin D and parathyroid hormone with obesity and body composition in African Americans. *Clin Endocrinol (Oxf).* 2010;72(5):595-603.
555. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab.* 2005;90(7):4119-23.
556. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab.* 2004;89(3):1196-9.
557. Forsythe LK, Livingstone MB, Barnes MS, Horigan G, McSorley EM, Bonham MP, et al. Effect of adiposity on vitamin D status and the 25-hydroxycholecalciferol response to supplementation in healthy young and older Irish adults. *Br J Nutr.* 2012;107(1):126-34.
558. Konradsen S, Ag H, Lindberg F, Hexeberg S, Jorde R. Serum 1,25-dihydroxy vitamin D is inversely associated with body mass index. *Eur J Nutr.* 2008;47(2):87-91.
559. Chun RF, Peercy BE, Orwoll ES, Nielson CM, Adams JS, Hewison M. Vitamin D and DBP: The free hormone hypothesis revisited. *J Steroid Biochem Mol Biol.* 2013.
560. Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, et al. A comparison of direct and calculated free 25(OH) Vitamin D levels in clinical populations. *J Clin Endocrinol Metab.* 2014;jc20133874.
561. Kull M, Kallikorm R, Lember M. Body mass index determines sunbathing habits: implications on vitamin D levels. *InternMedJ.* 2009;39(4):256-8.
562. Goldner WS, Stoner JA, Thompson J, Taylor K, Larson L, Erickson J, et al. Prevalence of vitamin D insufficiency and deficiency in morbidly obese patients: a comparison with non-obese controls. *ObesSurg.* 2008;18(2):145-50.
563. Speeckaert MM, Taes YE, De Buyzere ML, Christophe AB, Kaufman JM, Delanghe JR. Investigation of the potential association of vitamin D binding protein with lipoproteins. *AnnClinBiochem.* 2010;47(Pt 2):143-50.
564. Waldron JL, Ashby HL, Cornes MP, Bechervaise J, Razavi C, Thomas OL, et al. Vitamin D: a negative acute phase reactant. *Journal of clinical pathology.* 2013;66(7):620-2.
565. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. *InternEmergMed.* 2011.
566. Winters SJ, Chennubhatla R, Wang C, Miller JJ. Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women. *Metabolism.* 2009;58(4):438-42.
567. Taes YE, Goemaere S, Huang G, Van P, I, De BD, Verhasselt B, et al. Vitamin D binding protein, bone status and body composition in community-dwelling elderly men. *Bone.* 2006;38(5):701-7.
568. Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, et al. Vitamin D(3) in fat tissue. *Endocrine.* 2008;33(1):90-4.
569. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring).* 2012;20(7):1444-8.
570. Gallagher JC, Yalamanchili V, Smith LM. The effect of vitamin D supplementation on serum 25(OH)D in thin and obese women. *J Steroid Biochem Mol Biol.* 2013;136:195-200.

571. Maki KC, Rubin MR, Wong LG, McManus JF, Jensen CD, Lawless A. Effects of vitamin D supplementation on 25-hydroxyvitamin D, high-density lipoprotein cholesterol, and other cardiovascular disease risk markers in subjects with elevated waist circumference. *Int J Food Sci Nutr.* 2011;62(4):318-27.
572. Didriksen A, Grimnes G, Hutchinson MS, Kjaergaard M, Svartberg J, Joakimsen RM, et al. The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, BMI, and baseline levels. *Eur J Endocrinol.* 2013;169(5):559-67.
573. Harel Z, Flanagan P, Forcier M, Harel D. Low vitamin D status among obese adolescents: prevalence and response to treatment. *J Adolesc Health.* 2011;48(5):448-52.
574. Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys.* 2012;523(1):9-18.
575. Jorde R, Sneve M, Emaus N, Figenschau Y, Grimnes G. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromsø study. *Eur J Nutr.* 2010;49(7):401-7.
576. Lee P, Greenfield JR, Seibel MJ, Eisman JA, Center JR. Adequacy of vitamin D replacement in severe deficiency is dependent on body mass index. *Am J Med.* 2009;122(11):1056-60.
577. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, et al. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab.* 2008;93(9):3381-8.
578. Lagunova Z, Porojnicu AC, Vieth R, Lindberg FA, Hexeberg S, Moan J. Serum 25-hydroxyvitamin D is a predictor of serum 1,25-dihydroxyvitamin D in overweight and obese patients. *J Nutr.* 2011;141(1):112-7.
579. Ceglia L, Harris SS. Vitamin D and its role in skeletal muscle. *Calcif Tissue Int.* 2013;92(2):151-62.
580. Sinha A, Hollingsworth KG, Ball S, Cheetham T. Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. *The Journal of clinical endocrinology and metabolism.* 2013;98(3):E509-13.
581. Scott D, Sanders KM, Ebeling PR. Vitamin D, muscle function, and falls in older adults: does reduced deposition of intramuscular adipose tissue influence the relationship? *J Clin Endocrinol Metab.* 2013;98(10):3968-70.
582. Scott D, Blizzard L, Fell J, Ding C, Winzenberg T, Jones G. A prospective study of the associations between 25-hydroxy-vitamin D, sarcopenia progression and physical activity in older adults. *Clin Endocrinol (Oxf).* 2010;73(5):581-7.
583. von Hurst PR, Conlon C, Foskett A. Vitamin D status predicts hand-grip strength in young adult women living in Auckland, New Zealand. *J Steroid Biochem Mol Biol.* 2013;136:330-2.
584. Mithal A, Bonjour JP, Boonen S, Burckhardt P, Degens H, El Hajj Fuleihan G, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. *Osteoporos Int.* 2013;24(5):1555-66.
585. Redzic M, Lewis RM, Thomas DT. Relationship between 25-hydroxyvitamin D, muscle strength, and incidence of injury in healthy adults: a systematic review. *Nutr Res.* 2013;33(4):251-8.
586. Annweiler C, Beauchet O, Berrut G, Fantino B, Bonnefoy M, Herrmann FR, et al. Is there an association between serum 25-hydroxyvitamin D concentration and muscle strength among older women? Results from baseline assessment of the EPIDOS study. *J Nutr Health Aging.* 2009;13(2):90-5.
587. Faulkner KA, Cauley JA, Zmuda JM, Landsittel DP, Newman AB, Studenski SA, et al. Higher 1,25-dihydroxyvitamin D<sub>3</sub> concentrations associated with lower fall rates in older community-dwelling women. *Osteoporos Int.* 2006;17(9):1318-28.

588. Muir SW, Montero-Odasso M. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. *J Am Geriatr Soc.* 2011;59(12):2291-300.
589. Dawson-Hughes B. Serum 25-hydroxyvitamin D and muscle atrophy in the elderly. *Proc Nutr Soc.* 2012;71(1):46-9.
590. Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int.* 2011;22(3):859-71.
591. Girgis CM, Clifton-Bligh RJ, Turner N, Lau SL, Gunton JE. Effects of vitamin D in skeletal muscle: falls, strength, athletic performance and insulin sensitivity. *Clinical endocrinology.* 2014;80(2):169-81.
592. Suzuki T, Kwon J, Kim H, Shimada H, Yoshida Y, Iwasa H, et al. Low serum 25-hydroxyvitamin D levels associated with falls among Japanese community-dwelling elderly. *J Bone Miner Res.* 2008;23(8):1309-17.
593. Murad MH, Elamin KB, Abu Elnour NO, Elamin MB, Alkatib AA, Fatourechi MM, et al. Clinical review: The effect of vitamin D on falls: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2011;96(10):2997-3006.
594. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *Bmj.* 2009;339:b3692.
595. Gallagher JC. Vitamin D deficiency and muscle strength: are they related? *The Journal of clinical endocrinology and metabolism.* 2012;97(12):4366-9.
596. Shapses SA, Sukumar D, Schneider SH, Schluskel Y, Brodin RE, Taich L. Hormonal and dietary influences on true fractional calcium absorption in women: role of obesity. *Osteoporos Int.* 2012;23(11):2607-14.
597. Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). *Hum Genet.* 1993;92(2):183-8.
598. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcified tissue international.* 2005;77(1):15-22.
599. Carpenter TO, Zhang JH, Parra E, Ellis BK, Simpson C, Lee WM, et al. Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* 2013;28(1):213-21.
600. Ding C, Parameswaran V, Blizzard L, Burgess J, Jones G. Not a simple fat-soluble vitamin: Changes in serum 25-(OH)D levels are predicted by adiposity and adipocytokines in older adults. *J Intern Med.* 2010;268(5):501-10.
601. Need AG, Morris HA, Horowitz M, Nordin C. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr.* 1993;58(6):882-5.
602. Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH. Low circulating vitamin D in obesity. *Calcif Tissue Int.* 1988;43(4):199-201.
603. Saneei P, Salehi-Abargouei A, Esmailzadeh A. Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. *Obes Rev.* 2013;14(5):393-404.
604. González-Gross M, Valtueña J, Breidenassel C, Moreno LA, Ferrari M, Kersting M, et al. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr.* 2012;107(5):755-64.
605. Looker AC. Body fat and vitamin D status in black versus white women. *J Clin Endocrinol Metab.* 2005;90(2):635-40.
606. Maggio D, Cherubini A, Lauretani F, Russo RC, Bartali B, Pierandrei M, et al. 25(OH)D Serum levels decline with age earlier in women than in men and less

- efficiently prevent compensatory hyperparathyroidism in older adults. *J Gerontol A Biol Sci Med Sci.* 2005;60(11):1414-9.
607. Andersen R, Brot C, Jakobsen J, Mejbom H, Molgaard C, Skovgaard LT, et al. Seasonal changes in vitamin D status among Danish adolescent girls and elderly women: the influence of sun exposure and vitamin D intake. *Eur J Clin Nutr.* 2013;67(3):270-4.
  608. Kamycheva E, Joakimsen RM, Jorde R. Intakes of calcium and vitamin d predict body mass index in the population of Northern Norway. *J Nutr.* 2003;133(1):102-6.
  609. (SACN) SACoN. *The Nutritional Wellbeing of the British Population.* United Kingdom: 2008.
  610. Johnson LK, Hofsø D, Aasheim ET, Tanbo T, Holven KB, Andersen LF, et al. Impact of gender on vitamin D deficiency in morbidly obese patients: a cross-sectional study. *Eur J Clin Nutr.* 2012;66(1):83-90.
  611. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res.* 2009;29(9):3713-20.
  612. Kamycheva E, Sundsfjord J, Jorde R. Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *Eur J Endocrinol.* 2004;151(2):167-72.
  613. Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, et al. Estimation of total usual calcium and vitamin D intakes in the United States. *J Nutr.* 2010;140(4):817-22.
  614. Freedman BI, Wagenknecht LE, Hairston KG, Bowden DW, Carr JJ, Hightower RC, et al. Vitamin d, adiposity, and calcified atherosclerotic plaque in african-americans. *J Clin Endocrinol Metab.* 2010;95(3):1076-83.
  615. Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, et al. Vitamin D(3) in fat tissue. *Endocrine.* 2008;33(1):90-4.
  616. Harris SS, Dawson-Hughes B. Reduced sun exposure does not explain the inverse association of 25-hydroxyvitamin D with percent body fat in older adults. *J Clin Endocrinol Metab.* 2007;92(8):3155-7.
  617. Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, et al. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *J Steroid Biochem Mol Biol.* 2010;121(1-2):462-6.
  618. Rodríguez-Rodríguez E, Navia B, López-Sobaler AM, Ortega RM. Vitamin D in overweight/obese women and its relationship with dietetic and anthropometric variables. *Obesity (Silver Spring).* 2009;17(4):778-82.
  619. Bailey RL, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR, et al. Dietary supplement use in the United States, 2003-2006. *J Nutr.* 2011;141(2):261-6.
  620. Macdonald HM, Mavroei A, Aucott LA, Diffey BL, Fraser WD, Ormerod AD, et al. Skin color change in Caucasian postmenopausal women predicts summer-winter change in 25-hydroxyvitamin D: findings from the ANSAViD cohort study. *J Clin Endocrinol Metab.* 2011;96(6):1677-86.
  621. Haddad JG, Jr., Walgate J. Radioimmunoassay of the binding protein for vitamin D and its metabolites in human serum: concentrations in normal subjects and patients with disorders of mineral homeostasis. *J Clin Invest.* 1976;58(5):1217-22.
  622. Chun RF. New perspectives on the vitamin D binding protein. *Cell Biochem Funct.* 2012;30(6):445-56.
  623. Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, et al. Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. *J Clin Endocrinol Metab.* 2010;95(7):3368-76.
  624. Chun RF, Peercy BE, Adams JS, Hewison M. Vitamin D Binding Protein and Monocyte Response to 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D: Analysis by Mathematical Modeling. *PLoS ONE.* 2012;7(1):e30773.

625. Pitroda AP, Harris SS, Dawson-Hughes B. The association of adiposity with parathyroid hormone in healthy older adults. *Endocrine*. 2009;36(2):218-23.
626. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest*. 1985;76(1):370-3.
627. Bolland MJ, Grey AB, Ames RW, Horne AM, Gamble GD, Reid IR. Fat mass is an important predictor of parathyroid hormone levels in postmenopausal women. *Bone*. 2006;38(3):317-21.
628. Earthman CP, Beckman LM, Masodkar K, Sibley SD. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int J Obes (Lond)*. 2012;36(3):387-96.
629. Bell NH, Shaw S, Turner RT. Evidence that 1,25-dihydroxyvitamin D<sub>3</sub> inhibits the hepatic production of 25-hydroxyvitamin D in man. *J Clin Invest*. 1984;74(4):1540-4.
630. Hosseinpanah F, Rambod M, Hossein-nejad A, Larijani B, Azizi F. Association between vitamin D and bone mineral density in Iranian postmenopausal women. *J Bone Miner Metab*. 2008;26(1):86-92.
631. Lenders CM, Feldman HA, Von Scheven E, Merewood A, Sweeney C, Wilson DM, et al. Relation of body fat indexes to vitamin D status and deficiency among obese adolescents. *Am J Clin Nutr*. 2009;90(3):459-67.
632. Arabi A, Baddoura R, Awada H, Salamoun M, Ayoub G, El-Hajj Fuleihan G. Hypovitaminosis D osteopathy: is it mediated through PTH, lean mass, or is it a direct effect? *Bone*. 2006;39(2):268-75.
633. Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G. PTH level but not 25 (OH) vitamin D level predicts bone loss rates in the elderly. *Osteoporos Int*. 2012;23(3):971-80.
634. Houston DK, Cesari M, Ferrucci L, Cherubini A, Maggio D, Bartali B, et al. Association between vitamin D status and physical performance: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2007;62(4):440-6.
635. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. *Am J Clin Nutr*. 2004;80(3):752-8.
636. Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, et al. Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab*. 2007;92(6):2058-65.
637. Mathei C, Van Pottelbergh G, Vaes B, Adriaensen W, Gruson D, Degryse JM. No relation between vitamin D status and physical performance in the oldest old: results from the Belfrail study. *Age Ageing*. 2013;42(2):186-90.
638. Kwon J, Suzuki T, Yoshida H, Kim H, Yoshida Y, Iwasa H. Concomitant lower serum albumin and vitamin D levels are associated with decreased objective physical performance among Japanese community-dwelling elderly. *Gerontology*. 2007;53(5):322-8.
639. Moller UK, Streyms S, Jensen LT, Mosekilde L, Schoenmakers I, Nigdikar S, et al. Increased plasma concentrations of vitamin D metabolites and vitamin D binding protein in women using hormonal contraceptives: a cross-sectional study. *Nutrients*. 2013;5(9):3470-80.
640. Renzaho AM, Halliday JA, Nowson C. Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: a systematic review. *Nutrition*. 2011;27(9):868-79.
641. Niramitmahapanya S, Harris SS, Dawson-Hughes B. Type of dietary fat is associated with the 25-hydroxyvitamin D<sub>3</sub> increment in response to vitamin D supplementation. *J Clin Endocrinol Metab*. 2011;96(10):3170-4.
642. Mirza MA, Alsio J, Hammarstedt A, Erben RG, Michaelsson K, Tivesten A, et al. Circulating fibroblast growth factor-23 is associated with fat mass and dyslipidemia

- in two independent cohorts of elderly individuals. *Arterioscler Thromb Vasc Biol.* 2011;31(1):219-27.
643. Gozdzik A, Zhu J, Wong BY, Fu L, Cole DE, Parra EJ. Association of vitamin D binding protein (VDBP) polymorphisms and serum 25(OH)D concentrations in a sample of young Canadian adults of different ancestry. *J Steroid Biochem Mol Biol.* 2011;127(3-5):405-12.
644. Brouwer-Brolsma EM, Bischoff-Ferrari HA, Bouillon R, Feskens EJ, Gallagher CJ, Hypponen E, et al. Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonisation of dietary reference intakes for vitamin D across Europe. *Osteoporos Int.* 2013;24(5):1567-77.
645. Chen KY, Acra SA, Donahue CL, Sun M, Buchowski MS. Efficiency of walking and stepping: relationship to body fatness. *Obes Res.* 2004;12(6):982-9.
646. Verhoef SP, Camps SG, Bouwman FG, Mariman EC, Westerterp KR. Physiological response of adipocytes to weight loss and maintenance. *PLoS One.* 2013;8(3):e58011.
647. Edwards C, Hindle AK, Fu S, Brody F. Downregulation of leptin and resistin expression in blood following bariatric surgery. *Surg Endosc.* 2011;25(6):1962-8.
648. Klempel MC, Varady KA. Reliability of leptin, but not adiponectin, as a biomarker for diet-induced weight loss in humans. *Nutr Rev.* 2011;69(3):145-54.
649. Conroy R, Girotra M, Shane E, McMahon DJ, Pavlovich KH, Leibel RL, et al. Leptin administration does not prevent the bone mineral metabolism changes induced by weight loss. *Metabolism.* 2011;60(9):1222-6.



**Appendix**

**1) Sunlight Questionnaire**

**Sunlight Exposure Record**

Initials .....	Date .....	Subject Number .....
----------------	------------	----------------------

How often are you usually out-doors in sunny weather? Which parts of your body do you usually expose? Please tick the boxes which best describe your behaviour in the current and preceding months.

		Often (1)	Occasionally (2)	Seldom / Never (3)	Skin Exposure				
					Head	Hands	Arms	Legs	Torso
Spring	March	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	April	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	May	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Summer	June	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	July	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	August	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Autumn	September	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	October	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	November	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Winter	December	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	January	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	February	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Prompts for completion of sunlight exposure record  
 If it is sunny, are you generally in or out? Walks, shopping, gardening, sitting in the sun etc.  
 If sunny but cool/windy, do you muffle up (hat/gloves/tights) ?  
 If it is sunny and warm do you usually go outside with short-sleeves ? With bare legs ? With a hat ?  
 Do you sun-bathe, exposing your torso (stomach / back) to the sun ?

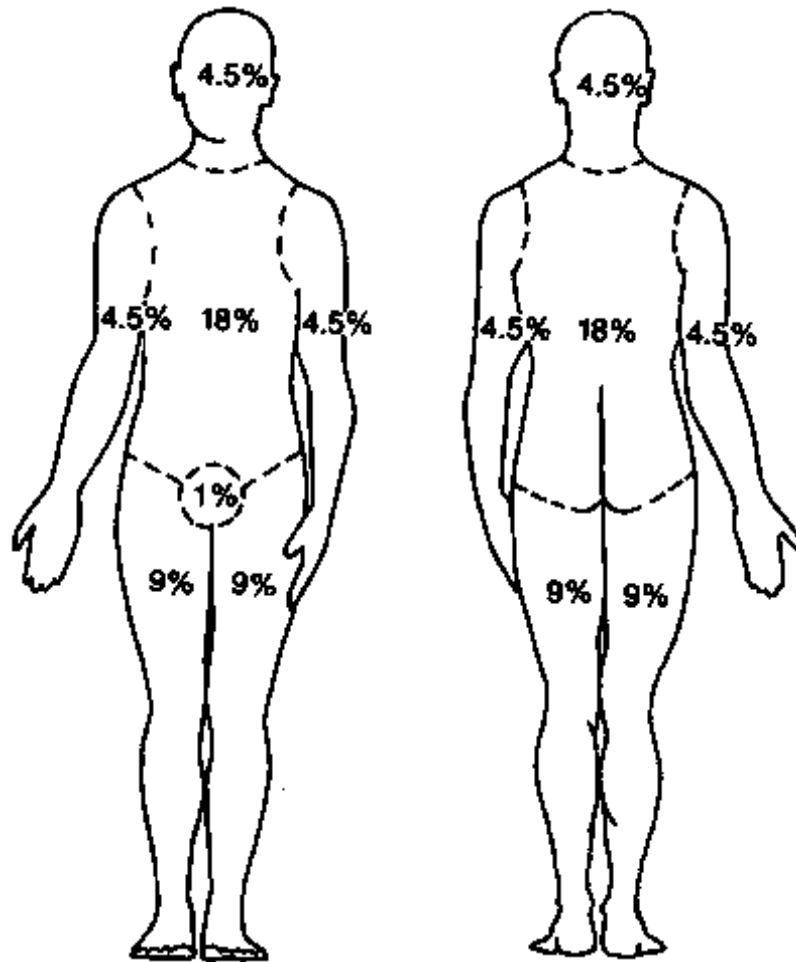
## Holiday Details (Follows on from Sunlight Exposure)

Initials .....      Date .....      Subject Number .....

Please list any holiday visits abroad which you have taken in the last 6 months

Dates of Visit Month / Year	Town and Country Visited	No. of hours in sun / day	Skin Exposure				
			(1) Head	(2) Hands	(3) Arms	(4) Legs	(5) Torso
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

## 2) Quantification of UVB Exposure: the Rule of Nines



### 3) Sunlight Hours

Average number of hours of intense sunlight (irradiance measurement >120 w/m<sup>2</sup>) in Sheffield over the duration of the vitamin D sub-study visits.

Data kindly provided by Weston Park Weather Station, Sheffield.

<b>Week beginning</b>	<b>Mean daily hours of sunlight</b>
17/09/12	3.9
24/09/12	2.7
01/10/12	5.3
08/10/12	3.7
15/10/12	5.4
22/10/12	1.1
01/04/13	5.7
08/04/13	1.9
15/04/13	6.6
22/04/13	4.9
29/04/13	7.2
06/05/13	6.1
13/05/13	5.7
<b>Total</b>	<b>4.6</b>