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Dynapenic Abdominal Obesity – Falls, Fractures, Muscle Function, and miRNA Profile

By

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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September 2022

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Abstract

Older adults have the highest risk of falls. Dynapenia and abdominal obesity (AO) are associated with increased risk of falls and (site-specific) fractures; a cumulative risk is hypothesised in older adults with both dynapenia and AO (DAO).

Aims: To identify (i) if older women with DAO have higher risk of falls and fall-related injury compared with normal weight, dynapenic, and AO, (ii) potential mechanisms (muscle size, strength, and fatigue) for increased falls risk, and (iii) whether microRNAs are implicated in the pathogenesis of DAO.

In the UK Biobank, dynapenia (OR 0.86; 95%CI 0.83-0.89) and AO (1.14; 1.09-1.18) Z-scores independently associated with retrospective falls. In women with obesity, dynapenia exacerbated the risk of lower extremity fractures (2.78; 1.77, 4.37) and negated protection against other fractures (1.06; 0.82, 1.38). In the English Longitudinal Study of Ageing, older women with DAO had increased risk of prospective falls (1.5; 1.1, 2.1) related to dynapenia (1.4; 1.1, 1.7) but not AO (1.1; 0.9, 1.4). Women with AO had greater knee extensor force per unit muscle (median ± IQR; 0.347 ± 0.048 N/cm^3) than normal weight women (0.270 ± 0.085) and DAO women (0.278 ± 0.170). DAO women had reduced knee flexor force per unit muscle (0.321 ± 0.131) compared to women with obesity (0.405 ± 0.107). Muscle volumes were similar between obese and DAO groups. Systematic review identified 24 miRNAs potentially associated with sarcopenic obesity. However, a panel of differentially expressed miRNAs could not be identified in women with DAO.

Women with DAO have a higher risk of falls and fractures and impaired knee extensor and flexor strength which may relate to differences in muscle quality. A distinct panel of microRNAs could not be identified in women with DAO. The utility of categorising older women as DAO at an individual level requires further consideration.

(Maximum 300 words)

Acknowledgements

I would like to thank Dr Jennie Walsh for her encouragement, support, and supervision. Your ability to make decisions and resolve problems has been inspiring. I would like to thank my secondary supervisors Prof Dan Cuthbertson, Dr Kasia Goljanek-Whysall and Prof Claudia Mazza for their enthusiasm, encouragement, and continuous support. Thank you all for making my PhD experience truly enjoyable, even during a pandemic.

Thank you to CIMA (Medical Research Council and Versus Arthritis) for the funding, without which this would not have been possible.

I would like to thank everyone at the Academic Unit of Bone Metabolism, Biomedical Research Centre and Insigneo who supported me with these projects. Prof Eugene McCloskey, thank you for your input and guidance. Margaret, thank you for your general guidance with all things ethics, admin and DXA-related. Gill, thank you for being such a source of information. Ian, thank you for your support in the lab and being a great source of advice. Fatma, thank you for helping me to ship the samples (and the numerous failed attempts). A sincere thanks to everyone at the BRC, especially Sarah and the on-call nurses, for helping to ensure that all of the study visits ran so smoothly. Will, thank you for sharing your research with me to help make the MRI analysis achievable despite the delays. Tatiane and Ankita, thank you for your support with the systematic review.

Thank you to everyone at Sheffield Teaching Hospitals who helped with the initial study set-up (Aimee, Luke and Sarah), MRI scanning (Jonny and Sheree) and blood analysis (Ifti, Si). I would also like to extend my gratitude to all of the researchers and participants involved in the Muscle in Obesity, Fat and Bone, UK Biobank and English Longitudinal Study of Ageing studies.

Thank you to all of my friends and family who have been with me throughout this process. Esme, I am so grateful for our coffees.

To Mark – for your constant love, support and encouragement.

Contributions

Epidemiology Studies – ELSA, UK Biobank

I wrote the original protocols for the epidemiological analysis studies. I wrote and submitted the applications for data usage from the UK Biobank and ELSA (UK Data Service). For the English Longitudinal Study of Ageing (ELSA) project, input was provided by Dr Walsh, Prof Cuthbertson and Prof McCloskey. For the UK Biobank study, input was provided by Dr Walsh and Prof Cuthbertson. I wrote the initial manuscripts which were reviewed by all co-authors.

Muscle in Obesity Study

I wrote the original protocol for the Muscle in Obesity study. Scientific and practical input was provided by Dr Jennifer Walsh, Prof Claudia Mazza, Dr Kasia Goljanek-Whysall, and Prof Dan Cuthbertson.

I acquired ethical and research and development approvals for the study and I was responsible for site file maintenance, submission of amendments, and general study management. I obtained Medical Imaging and Medical Physics approval for the study. Dr Walsh was responsible for all clinical aspects of the study.

I wrote the participant information sheets, consent forms, and other supporting documentation. I was responsible for study recruitment; I arranged for Dr Walsh to send emails across the hospital trust and University of Sheffield, I put up poster advertisements, sent invite letters and arranged public engagement events. Where necessary, I telephone screened all volunteers prior to recruitment, wrote all study invitation and appointment letters and sent the participant documentation packs.

I booked and carried out all participant visits; I took informed consent from participants and performed all screening, anthropometry, muscle function tests, blood sampling and subsequent serum preparation. I was responsible for co-ordinating: participant travel, expenses, DXA scans and study visits. Dr Walsh requested MRI scans through the Radiology Department, STH. I completed all paper based data collection forms and was responsible for managing the participant source notes. I completed all data entry and spot checks.

All DXA scans were performed by Dr Margaret Paggiosi. Dr Paggiosi established the scanning protocols. DXA scan analysis was performed by Dr Paggiosi. All MRI scans were performed by STH radiographers using a protocol designed by Mr Andrew Fry (STH) for the PORTRAIT study (Prof Mazza and Prof Richard Eastell). William Henson and I performed MRI scan segmentation using a protocol

designed by William Henson, Insigneo, University of Sheffield. William Henson processed the MRI segmentations to obtain quantitative results. I processed the isokinetic dynamometry data using a MATLAB script written by Linda van Gelder, Insigneo, University of Sheffield. Biochemical analyses (C-Reactive Protein, lipid profile and glucose) were performed by Sheffield Teaching Hospitals Clinical Chemistry.

Systematic Review

I wrote the original protocol and registered the review on PROSPERO. I performed the first sift (titles and abstracts) which was verified by Ankita Duseja. Ankita Duseja and I independently performed the second sift (full manuscript) and risk of bias assessments. I completed data extraction which was verified by Ankita Duseja. I performed the synthesis of results and additional literature searches. I wrote the initial manuscript which was reviewed by all co-authors.

MicroRNA Analysis

I chose the microRNA measurement techniques. I developed the systematic approach to identify the panel of miRNAs for validation; Dr Goljanek-Whysall reviewed the systematic approach and we both chose the final miRNAs for validation. All microRNA analyses (RNA extraction, RT-qPCR, RNA-seq, bioinformatics and other statistical analyses) were performed by TAmiRNA GmBH (Vienna, Austria) with protocols designed by their team. The original FAB study and samples were collected by Dr Amy Evans (chief investigator) and Dr Walsh (primary investigator). The Muscle in Obesity samples were obtained from the Muscle in Obesity study previously described.

Published Manuscripts

Certified contribution details can be found at the start of each manuscript.

Statistical Analysis

I performed the statistical analysis for the Muscle in Obesity and epidemiological studies. For the study using ELSA, statistical support was provided by Prof McCloskey. For the Muscle in Obesity study, determination of the original power calculation was provided by Kathleen Baster, Sheffield Teaching Hospitals.

Dr Walsh, Prof Mazza, Dr Goljanek-Whysall and Prof Cuthbertson provided guidance and direction throughout this work.

Presentations and Publications

Oral Communications:

Dowling L, Goljanek-Whysall K, Walsh J. MicroRNAs in women with dynapenia and dynapenic abdominal obesity. [ACCEPTED] ICFSR 2022; Boston, USA.
Dowling L, Goljanek-Whysall K, Walsh J. MicroRNAs in women with dynapenia and dynapenic abdominal obesity. [ACCEPTED] The Mellanby Centre Annual Research Day 2022; Sheffield, UK.
Dowling L, Mazza C, Cuthbertston DJ, Goljanek-Whysall K, Walsh J. MicroRNAs in Sarcopenic Obesity. [ACCEPTED] CIMA Annual Meeting 2021; York, UK.
Dowling L, Cuthbertson DJ, Walsh J. Dynapenia and obesity as risk factors for falls and fall-related injuries [ACCEPTED] CIMA Spring Meeting 2021; Virtual.
Dowling L, Mazza C, Goljanek-Whysall K, Cuthbertston DJ, Walsh J. Muscle quantity, quality, and microRNA profile in dynapenic obese older women. [ACCEPTED] CIMA Epigenetics Meeting 2020. York, UK.
Dowling L, Mazza C, Goljanek-Whysall K, Cuthbertston DJ, Walsh J. 3 minute thesis [ACCEPTED] CIMA Online Meeting 2020; Virtual.
People's Choice Award

Oral Poster Presentations:

Dowling L, Cuthbertson DJ, Walsh J. Dynapenic Abdominal Obesity as a Risk Factor for Falls and Fall-related Injuries. [ACCEPTED] International Sarcopenia Translational Research Conference 2021; Virtual.
Dowling L, Cuthbertson DJ, Walsh J. Dynapenic Abdominal Obesity as a Risk Factor for Falls and Fall-related Injuries. University of Sheffield Medical School Research Day 2020; Sheffield, UK.
Duseja A, **Dowling L**, Vilaca T, Zarecki P, Eastell R, Hackl M. 2021 MicroRNAs in diabetic bone disease: A systematic review. Bone Reports, 14, p.100876.

Published Manuscripts:

Dowling L, McCloskey E, Cuthbertson DJ, Walsh JS, 2022. Dynapenic Abdominal Obesity as a Risk Factor for Falls. The Journal of Frailty & Aging, pp.1-6.
Dowling L, Duseja A, Vilaca T, Walsh JS, Goljanek-Whysall K., 2022. MicroRNAs in obesity, sarcopenia, and commonalities for sarcopenic obesity: a systematic review. Journal of Cachexia, Sarcopenia and Muscle, 13(1), pp.68-85.
Dowling L, Cuthbertson DJ, Walsh JS, 2022. Reduced muscle strength (dynapenia) in women with obesity confers a greater risk of falls and fractures in the UK Biobank. Obesity (Silver Spring), pp 1-10, doi:10.1002/oby.23609.

Declaration

This thesis is submitted in the form of the Alternative Format Thesis which includes academic manuscripts (published or unpublished), alongside traditional thesis sections.

I hereby certify that this thesis includes work submitted in scientific journals of which I am a co-author. I have included a written statement from co-authors, and verified by my supervisor Dr Jennifer Walsh. The contribution statements are included before each relevant manuscript.

Abbreviations

6MWD = 6 Minute Walking Distance

6MWT = 6 Minute Walking Test

aBMD = Areal Bone Mineral Density

ADL = Activities Of Daily Living

AGO = Argonuate

AKT = Protein Kinase B

ALM = Appendicular Lean Mass

AMPK = AMP-Activated Protein Kinase

ANOVA = Analysis Of Variance

AO = Abdominal Obesity

ASM = Appendicular Skeletal Mass

ATP = Adenosine Triphosphate

AUC = Area Under The Receiver Operating Characteristic Curve

BF = Body Fat

BF% = Body Fat Percentage

BFL = Biceps Femoris Long Head

BHS = Biceps Femoris Short Head

BMI = Body Mass Index

BPM = Beats Per Minute

BRC = Biomedical Research Centre

BW = Body Weight

CHD = Coronary Heart Disease

CI = Confidence Interval

CoV = Coefficient of Variation

CR = Caloric Restriction

CRF = Clinical Research Facility

CRP = C-reactive protein

D3Cr = D3 Creatine Dilution

DAO = Dynapenic Abdominal Obesity

DXA = Dual-Energy X-Ray Absorptiometry

EASO = European Association For The Study Of Obesity

eFI = Electronic Frailty Index

ELSA = English Longitudinal Study Of Ageing

ESPEN = European Society For Clinical Nutrition And Metabolism

EWGSOP1 = European Working Group On Sarcopenia In Older People (Cruz-Jentoft et al., 2010)

EWGSOP2 = European Working Group On Sarcopenia In Older People (Cruz-Jentoft et al., 2019)

Exp = Expression

FAB = Fat And Bone

FC = Fold Change

FDR = False Discovery Rate

FFQ = Food Frequency Questionnaire

FNIH = Foundation For The National Institutes Of Health

FOXO = Forkhead Box Protein

Fr = Frail

GS = gait speed

HDL = High Density Lipoprotein

HGS = Hand Grip Strength

HIIT = High Intensity Interval Training

HR = Hazard Ratio

HRB = Health Research Board

hsa = Homo Sapiens

ht^2 = height squared

ICD = International Classification Of Disease

IFG = Impaired Fasting Glucose

IGF-1 = Insulin-Like Growth Factor 1

IGF-1R = Insulin-Like Growth Factor 1 Receptor

IL-6 = Interleukin 6

IMAT = Intermuscular Adipose Tissue

IQR = Inter-Quartile Range

IRC = Irish Research Council

Kg = Kilogram

KLF6 = Krüppel-like factor 6

LDL = Low Density Lipoprotein

LLM = Leg Lean Mass

LLM = Leg Lean Mass

LM = Lean Mass

Log2FC = Log2(Fold Change)

MDC = Minimal Detectable Change

MHC = Myosin Heavy Chain

MiR = miRNA, microRNA

mmu = Mus Musculus

MR = Magnetic Resonance

MRC = Medical Research Council

MRI = Magnetic Resonance Imaging

MSC = Mesenchymal Stem Cell

mTOR = Mammalian Target Of Rapamycin

N-F = Non-Frail

N-S = Non-Sarcopenic

ND = Not Documented

NGS = Next Generation Sequencing

NOS = Newcastle Ottawa Scale

NW = Normal Weight

Ob = Obese

OR = Odds Ratio

PGC-1α = Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha

PI3K = Phosphoinositide 3-Kinase

Pri-miRNA = Primary miRNA

PRISMA = Preferred Reporting Items For Systematic Review And Meta-Analysis

PROSPERO = Prospective Register For Systematic Reviews

PTEN = Phosphatase And Tensin Homolog

QC = Quality Control

Ref = Reference

RF = Rectus Femoris

RFN = Right Femoral Neck

RISC = RNA-Induced Silencing Complex

RLOA = Ratio Limit Of Agreement

RNA-Seq = RNA Sequencing

RNI = Recommended Nutrient Intake

RPE = Rating Scale Of Perceived Exertion

Rpm = Revolutions Per Minute (In Relation To Centrifuge)

RT-qPCR = Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction

S = Sarcopenic

SABE = Brazilian Health, Well-Being And Aging Study

SASP = Senescence-Associated Secretory Phenotype

SAT = Subcutaneous Adipose Tissue

SD = Standard Deviation

SDOC = Sarcopenia Definitions And Outcomes Consortium

SFI = Science Foundation Ireland

SM = Semimembranosus

SPPB = Short Physical Performance Battery

ST = Semitendinosus

STH = Sheffield Teaching Hospitals

STL = Standard Triangle Language

STS = Sit-To-Stand Time

T = Tesla

T2DM = Type 2 Diabetes Mellitus

TE1 = Echo Time 1

TE2 = Echo Time 2

TEAD1 = TEA Domain Family Member 1

TGF-β = Transforming Growth Factor Beta

TGF-BR1 = A Receptor For TGF-B

TNF-A = Tumour Necrosis Factor Alpha (A)

TRBP = TAR RNA binding protein

TUG = Timed Up And Go

UTR = Untranslated Region

VAT = Visceral Adipose Tissue

VI = Vastus Intermedius

VIBE = Volume Interpolated Breath-Hold Examination

VL = Vastus Lateralis

VM = Vastus Medialis

VO_2 Max = Maximal Oxygen Consumption

WB = Whole Body

WC = Waist Circumference

WHO = World Health Organisation

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Chapter 1
Introduction

Introduction

1.1 Ageing population

Older adults (age ≥65 years) are the fastest growing age group worldwide [1, 2]. Recent population estimates indicate that one in five people in the UK are aged ≥65 years [2]. By 2050, one in six people worldwide could be aged ≥65 years, further increasing to one in four in Europe and North America [1].

Ageing is associated with an increased level of fat mass and reduced muscle strength [3-6]. These changes are individually associated with deleterious health consequences. However, as both changes occur with ageing, it is important to understand how they interact with each other. Clinically, this is important as both increased adiposity and reduced muscle strength can individually result in falls and (site-specific) injuries [7-10]; therefore, a cumulative effect is likely to exist.

1.2 Falls and Injuries

Older adults have the highest risk of falls with up to one in three people aged ≥65 years and half of people aged ≥80 years falling yearly [11]. Of those falls, it is estimated that 5% result in fracture whereas 30-50% result in injury [12]. In the US, $50 billion is spent yearly on non-fatal falls [13] and it is estimated that £2.3 billion is spent yearly in the UK [11]. In addition to the associated economic and social costs, up to half of older adults who do not recover from a fall may face a loss of independence, functional decline and require residential care [14]. Therefore, with the focus of healthy ageing being to optimise functional ability and intrinsic capacity, the prevention of falls and their associated consequences is relevant to this aim [15].

To assess a person's risk of falling, due to numerous risk factors, a multifactorial assessment is recommended [11]. For fractures, validated tools such as FRAX [16] or QFracture [17] are recommended by NICE guidelines to assess risk [18]. However, not all falls result in fracture. With an ageing population, there is a growing need to be able to identify people predisposed to falls (fallers) so as to target fall prevention strategies. A greater understanding of the risk factors for falls and fall-related injuries may lead to more targeted screening, prevention and treatment strategies.

1.3 Effects of Ageing on Muscle Strength

Muscle strength is typically defined as maximal voluntary force, torque or power [19]. Muscle strength reduces at a rate three times greater (~3%) than muscle mass (~1%) per year [20] therefore indicating

that muscle size is not the only determinant of muscle strength. The numerous biological factors determining muscle strength can broadly be categorised as muscular or neural [21] (Figure 1).

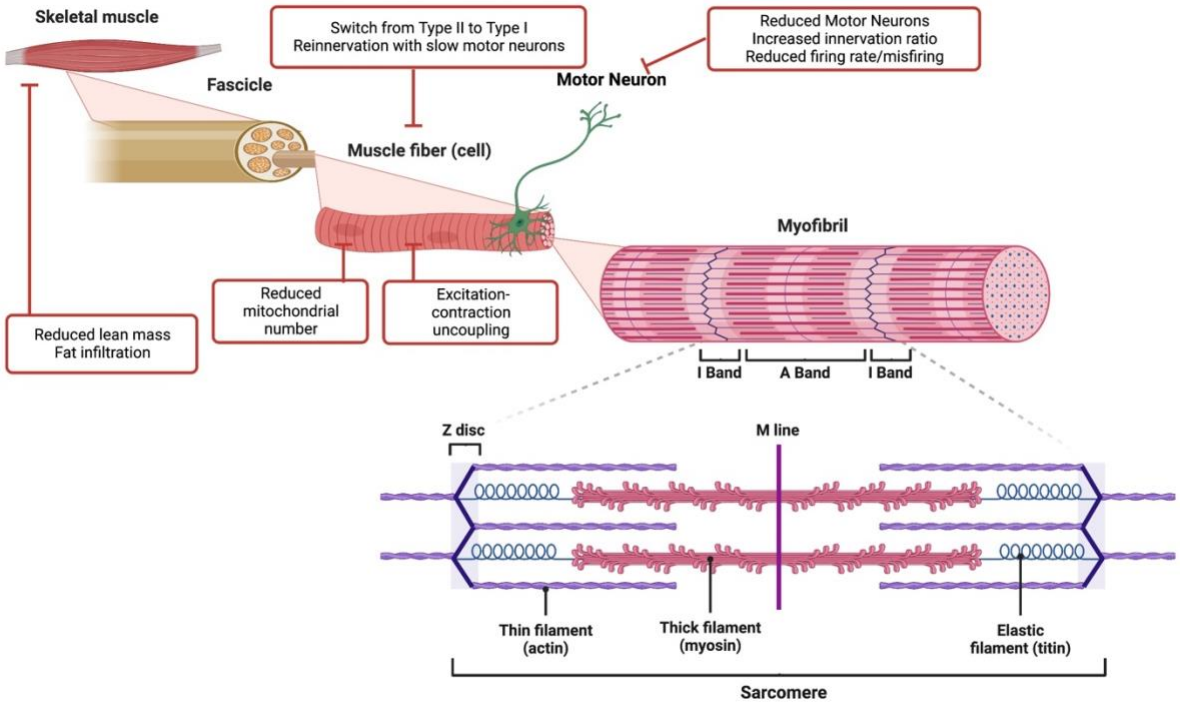

Figure 1 Muscular and neurological factors affecting muscle strength
Created with BioRender (https://biorender.com/).

1.3.1 Muscular factors

Changes In Muscle Fibre Type And Length

Ageing muscles are characterised by a reduced number and size of myofibers with a greater proportion of slow twitch Type I fibres compared with fast twitch Type II fibres [22]. Although there remains some controversy as others have shown either similar [23] or no change in muscle fibre type quantity [24], the general consensus is that ageing is associated with reduced Type II but not Type I muscle fibre cross sectional area in older men [24-26]. The specific atrophy of Type II muscle fibres in older men can result in the cross sectional area of Type II fibres becoming smaller than that of Type I muscle fibres [24] thereby disturbing the normal relationship between these muscle fibres [26]. The exact mechanism of these changes is unclear but age-related motor unit remodelling may play a role. This remodelling results in denervation of Type II muscle fibres with reinnervation by Type I motor neurons [27]. Moreover, there is a greater proportion of intra- and intermuscular fat infiltration [24, 28] combined with a reduction in Type II muscle fibre satellite cells [29]. This general shift towards a

slow muscle fibre type can affect a person's ability to produce rapid powerful contractions, such as to prevent a fall.

Impairment of excitation-contraction

Excitation-contraction coupling refers to the translation of a neural signal into muscle contraction and the subsequent force development [30]. The muscle fibre action potential activates the voltage-sensitive dihydropyridine receptors on the cell membrane (sarcolemma) which open the ryanodine receptors on the sarcoplasmic reticulum [30]. Calcium is released from the sarcoplasmic reticulum and binds to troponin C thereby leading to muscle contraction and force production. Following the contraction phase, the calcium is returned back to the sarcoplasmic reticulum allowing the muscle to relax [30]. Disruption to any of these events could lead to sub-optimal muscle activation and thus decreased muscle quality (force per unit tissue area) or strength [21]. Dysregulation of calcium release from the sarcoplasmic reticulum is the subject of intense investigation and has been proposed to explain deficits in muscle quality in aged muscle [21, 31]. This dysregulation may be related to the loss or reduced activity of dihydropyridine receptors with ageing [31]. Loss of dihydropyridine receptors results in an uncoupling from ryanodine receptors and less calcium is then released in response to muscle excitation [21, 31]. When less calcium is supplied to contractile proteins, a reduced contractile force is produced [21, 31]. Excitation-contraction uncoupling may contribute to the reduction in muscle strength observed in ageing.

Muscular Fat Infiltration

Ageing is associated with greater fat infiltration or adipocyte content between muscle groups (intermuscular adipose tissue), within muscle groups (intramuscular adipose tissue) and within muscle fibres (intramyocellular lipid) [20, 32, 33].

Intermuscular fat increases with age regardless of whether an individual loses, gains or maintains body weight [28]. However, five year longitudinal data in older adults (n=1678) have not observed a relationship between this greater intermuscular fat infiltration and strength loss but rather that strength losses are better related to muscle cross sectional area [28]. The authors noted, however, that the lack of association observed may have related to the small size of the intermuscular fat depot or the variability in both the fat depot and muscle torque changes [28]. At present though, it is unclear whether intermuscular fat is a marker of metabolic dysfunction or whether it is directly involved in sarcopenia or muscle contractility [28].

Intramuscular adipose tissue, referring to fat located between muscle fibres, is associated with reduced strength, falls and fractures [33-35]. However, the mechanism of this association is unknown. It has been proposed that ectopic fat accumulation may induce a localised pro-inflammatory state with the secretion of cytokines thus leading to a pathway for reduced muscle strength [21, 36].

Intramyocellular lipid refers to the accumulation of lipid droplets (0.20–0.50 μm^2) within myofibers [37, 38]. In endurance trained athletes, intramyocellular lipid content is high, particularly in Type I muscle fibres, which is thought to relate to its role as a source of energy during exercise [37, 39]. However, intramyocellular lipid content also increases in response to dietary fat content and circulating fatty acid content [39]. Under such conditions, intramyocellular lipid content is associated with insulin resistance, inflammation and impaired muscle function [38, 39]. In older adults with obesity, intramyocellular lipid content is higher compared to normal weight counterparts and is negatively correlated with muscle contraction velocity and power [40].

Mitochondrial Dynamics

Mitochondrial function (i.e. the ability to produce adenosine triphosphate (ATP)) as well as mitochondrial quantity is important for physical function [30]. Muscular mitochondrial content (measured as mitochondrial DNA and mRNA transcription) is inversely related to age [41]. Mitochondrial ATP production and VO_2 max (maximal oxygen consumption) also declines with age, with a positive association between both [41]. Indeed, transcriptional analysis comparing healthy older adults and sarcopenic older adults found that mitochondrial bioenergetic dysfunction was the major mechanism of sarcopenia [42].

1.3.2 Neurological factors

Motor Units and Ageing

A motor unit refers to a motor neuron and the skeletal muscle fibres which are innervated by the motor neuron's axon terminal [27]. Motor units work together to contract a single muscle. Age-related alterations in the motor unit have been linked with reductions in maximal voluntary torque [43]. Ageing is associated with estimates of 40-60% fewer functioning motor units [27, 44]. However, there are limitations with the quantification of motor units. Surface electromyography, which is the most commonly used technique, is subject to cross-contamination of electrical activity from other muscles and to the insulating effects of connective and adipose tissue [27]. Therefore, these estimates should be interpreted with caution.

In addition to reduced functional motor units, there are also more muscle fibres per motor unit (increased innervation ratio) with ageing [43]. This increased innervation ratio relates to the denervation of Type II muscle fibres with reinnervation from slow motor neurons [27]. As muscle fibre type is largely determined by the innervating neuron, this leads to an overall motor unit remodelling to a slow muscle fibre phenotype [45].

Discharge Rate, Central Activation and Ageing

In older adults, motor units exhibit a ~20-35% reduced maximal discharge (firing rate) in smaller hand and larger leg muscle groups during maximal isometric contractions [46, 47]. This reduced maximal firing rate may relate to the potential inability of ageing motor units to fire fast enough to produce a complementary response [48]. Recent work in younger men has found that immobilisation of one leg results in reduced and greater variability in the motor unit firing rate along with smaller motor unit areas [49]. This may have implications for older adults who experience hospital admissions or are more sedentary. However, similar immobilisation and bed-rest studies in older adults have focused on the muscular determinants of strength and more work is required [50].

Central activation refers to the ability to fully activate the available muscle [51]. Although ageing affects the determinants of central activation, that is the number and properties of motor units and their discharge rate, its impact on central activation capacity appears variable. Ageing has muscle-group specific effects which are likely related to differences in the function and physiological profiles (e.g. motor unit innervation) of these different muscle groups [51]. A recent meta-analysis of 54 studies found that older adults had reduced central activation of knee, elbow and plantar flexors but not ankle and dorsiflexors compared with younger adults [51]. However, 70% of the studies included showed non-significant results [51]. Methodological differences and large age-related variability within and between older adults may explain non-significant findings in small studies [51]. Variability itself in central activation may be concerning for older adults. As most tasks require only one attempt, an inadequate or variable activation could lead to a failed attempt with adverse effects [51].

Ageing has a medium overall effect size (Cohen's D -0.63) on knee extensor central activation [51]. This reduction is modest relative to the loss of muscle mass associated with ageing and raises questions around its significance [51]. It is thought that a small loss in central activation may represent a greater reduction in muscle strength [51]. Indeed, a recent cross-sectional study comparing younger, older and weak older adults found that weak older adults have a reduced ability to fully activate their leg extensor muscles [48]. The authors used incrementally increasing electrical stimuli to identify the

level of voluntary inactivation and estimated that ~33% of the between-subject variability in leg extensor strength was explained by neural excitability, similar to the contribution of thigh lean mass [48]. This requires further confirmation but potentially offers another route for interventions targeted at the nervous system which may be stand-alone or complementary to strength training.

Changes in the motor cortex

Ageing is associated with changes in the motor cortex which are likely to affect age-related reductions in motor performance [21]. Ageing is associated with cortical hypoexcitability and a reduced ability to modulate activity of inappropriate motor networks [52]. Others have shown the relationship between numerous cognition tests and hand-grip strength; this relationship has even been extended to white brain matter [52]. Whether, or how, these changes affect strength loss remains to be determined.

1.3.3 Other factors

The aetiology of all of these biological changes and the outcome of poor physical function is multi-factorial. Psychological (e.g. depression, motivation), pathological (e.g. back pain, arthritis), environmental (e.g. dietary intake, physical activity) and sociological factors can also impact physical function [21, 53].

1.4 Sarcopenia

Sarcopenia is a disease of reduced muscle function and mass recognised by the International Classification of Diseases (M62.84) [4, 22]. Originally defined as a condition of reduced muscle mass [54], it is increasingly acknowledged that muscle function is integral to the definition [4, 55]. This is perhaps an unsurprising development as longitudinal studies in ageing have shown that muscle strength reduces at a greater rate compared with lean mass [20]. Also, low lean mass is not a specific hallmark of sarcopenia and could be suggestive of malnutrition or cachexia [56, 57]. Numerous definitions have been proposed to identify sarcopenia [4, 55, 58-61]. The latest definition proposed by the European Working Group on Sarcopenia in Older People (EWGSOP) is the only definition endorsed by a variety of international societies including the European Geriatric Medicine Society, The European Society for Clinical Nutrition and Metabolism and the International Association for Gerontology and Geriatrics European Region [4, 22]. Using this definition, sarcopenia is thought to affect 3.2-26.3% of older adults – with highest rates found in studies of very old adults or where bioelectrical impedance was used [4, 62, 63].

More recently, the Sarcopenia Definition and Outcomes Consortium (SDOC) have proposed a definition and cut-offs based on associations with functional outcomes, specifically gait speed [58, 64, 65]. This definition did not identify lean mass as a potent predictor for slowness as predictive performance was comparable to age [65]. The EWGSOP2 and SDOC definitions for sarcopenia are the most widely accepted definitions; however, the former is thought not to identify enough individuals and associations with adverse outcomes are weakened [66-68] whereas the latter is thought to identify too many individuals for targeted treatment [64]. A new international working group, the Global Leadership Initiative in Sarcopenia (GLIS), has recently been formed to bring together the numerous sarcopenia working groups and develop a common definition which can be used worldwide [69].

Table 1 Definitions of sarcopenia proposed by international working groups

Working Group	**Criteria**	**Cut-off**
Sarcopenia Definition and Outcomes Consortium [58]	1. Low muscle strength (hand-grip) 2. Low physical performance (gait speed)	Specific cut-off values for proposed measures.
European Working Group on Sarcopenia in Older People (EWGSOP1) [60]	1. Low muscle mass 2. Low muscle strength 3. Low physical performance	Several cut-offs defined for proposed measures.
European Working Group on Sarcopenia in Older People (EWGSOP2) [4]	1. Low muscle strength 2. Low muscle quantity or quality 3. Low physical performance	Specific cut-off values for proposed measures.
Foundation for the National Institute of Health (FNIH) [55]	1. Low muscle strength 2. Low muscle mass Outcome: Low physical performance	Specific cut-off values for proposed measures. Note: recommended cut-off for low muscle mass as appendicular lean mass adjusted for body mass index.
International Working Group on Sarcopenia and Society of Sarcopenia, Cachexia and Wasting Disorders in Older People [70]	1. Low muscle mass 2. Low muscle performance	Specific cut-off values for proposed measures.

Asian Working Group on Sarcopenia [59]	1. Low muscle mass 2. Low muscle strength 3. Low physical performance	Specific cut-off values for proposed measures in Asian populations.

1.5 Obesity

Obesity is a disease of excess adiposity or body fat which affects one in four UK adults [5]. Obesity is associated with not only a greater risk of clinical complications (type 2 diabetes mellitus, hypertension, cancer [6]) but also biomechanical alterations and a greater risk of falls [8, 71-74]. Obesity is most commonly defined using clinical surrogate measures of body fat [5]. Whole-body obesity is defined using body mass index (BMI) (≥ 30kg/m^2) and abdominal obesity using high waist circumference (female >88cm or male >102cm) [5]. The prevalence of abdominal obesity increases with age and affects 47-51% of men and 56-62% of women aged over 55 years [5].

Using objective measures of body composition, obesity is defined by a high body fat percentage; >25-30% in males and >35-40% in females are amongst the most commonly used definitions [75, 76]. Abdominal adiposity is thought to be more detrimental to health [77-79]. Abdominal adiposity contains visceral adipose tissue (VAT) accounting for 5-8% of total fat in women [78]. VAT includes fat beneath the abdominal muscle wall, around the viscera and inside the intra-abdominal organs [77]. VAT contains proinflammatory cells (e.g. tumour necrosis factor-α (TNF-α), C-reactive protein (CRP) and interleukin-6 (IL-6)) and has increased vascularisation and innervation [78]. VAT is associated with a greater risk of obesity-related complications such as insulin resistance, hypertriglyceridemia, and coronary heart disease [77, 78]. Abdominal obesity also includes subcutaneous adipose tissue (SAT) which is less pro-inflammatory compared with VAT [78]. Moreover, greater abdominal obesity (waist circumference) has been linked to a greater loss of skeletal muscle mass in older adults and there may be an inter-relationship between low muscle strength and abdominal obesity [79].

Assessment of body fat percentage is not always feasible and clinical surrogates such as BMI and waist circumference are most often used in clinical practice [6, 80]. However, BMI alone fails to discriminate fat and lean mass, is not an adequate biomarker of abdominal obesity, and can lead to misclassification [75, 81]. Waist circumference is reflective of abdominal obesity and is able to identify high body fat percentage with high sensitivity and specificity [75]. Abdominal obesity as waist-to-hip ratio, in addition to BMI, has been recommended in the latest National Institute of Clinical Excellence Guidelines on Obesity [6]; previous guidance only recommended consideration of abdominal obesity [80].

1.6 Sarcopenic Obesity

Sarcopenic obesity comprises a phenotype of both obesity and sarcopenia (typically low lean mass) [82]. It is thought that sarcopenic obesity has the cumulative risks associated with both phenotypes [83]. Rates of sarcopenic obesity in older adults vary between 2.75% to over 20% and this discrepancy is due to differences in diagnostic criteria [84]. Although the first consensus definition for sarcopenia was proposed over 10 years ago [60], a consensus definition of sarcopenic obesity has only been proposed this year [76]. The delay in reaching a consensus on a definition of sarcopenic obesity has likely been exacerbated by heterogeneity in the literature. This is best highlighted in a recent review by Donini *et al.* (2020) who identified 19 measures of sarcopenia and 10 measures of adiposity used in 75 studies to define sarcopenic obesity [84].

Table 2 Top five most commonly used variables of sarcopenia and obesity which are combined to define sarcopenic obesity

Sarcopenia	**Obesity**
ASM/wt	Body Mass Index
ASM/ht^2	Fat Mass
ASM/ht^2 plus GS or HGS	Waist Circumference
Fat free mass index	BMI or Fat Mass
Mid-upper arm circumference plus GS or HGS	BMI or Waist Circumference

ASM = Appendicular Skeletal Mass; GS = gait speed; HGS = Hand-grip strength; wt = weight; ht = height. Adapted from Donini et al. (2020) [84].

Although this new consensus definition is momentous for the advancement of sarcopenic obesity research and clinical application, specific cut-offs and preferred tests (e.g. sit-to-stand time or hand-grip strength) have not been specified by the consensus group [76]. The consensus group proposed several measures and cut-offs which should be chosen based on the setting. Moreover, the definition proposed is based on expert opinion and whether it is predictive of clinical outcomes remains to be determined.

1.7 Dynapenic Abdominal Obesity (DAO)

1.7.1 Definition and Prevalence

Dynapenic Abdominal Obesity (DAO) refers to a phenotype of both abdominal obesity and low muscle strength [85, 86]. The prevalence of DAO is thought to range from 3.6-21.7% [87, 88]. Highest

prevalence rates (~20%) are reported in small studies of females whereas larger epidemiological studies report a prevalence in the range of 4-10% [88-90].

The concept of dynapenia was originally proposed by Manini and Clark in 2008 [19, 21]. The authors defined dynapenia as the age-related loss of strength, where strength is defined as the maximal force or power produced voluntarily [19, 21]. Dynapenia was proposed in response to a growing awareness of a disconnect between changes in muscle mass and strength [20]. In addition to overall muscle size, the various other nervous and muscular system mechanisms which contribute to muscle weakness in older adults have been outlined earlier (Section 1.3.2 Neurological factors). In 2012, the authors proposed an algorithm to identify dynapenia which includes screening using hand-grip strength followed by advanced testing using knee extensor strength [21]. Since then numerous studies have considered the role of dynapenia in the context of abdominal obesity, DAO, in relation to clinical outcomes. More recently, studies of DAO have adopted FNIH cut-off criteria for low hand-grip strength (HGS; 16kg female, 26kg male) [55] which are similar to EWGSOP2 criteria (16kg female, 27kg male) [4]. Abdominal obesity is typically defined as >102cm in men and >88cm in women (Table 3).

Table 3: Common Criteria used to Identify Dynapenic Abdominal Obesity

	Men	Women	Reference
Low muscle strength	-	<20.67kg	[87, 91]
Hand grip strength			
	<26kg	<16kg	[89, 90, 92-94]
	<33kg	<19kg	[95]
Leg muscle strength	<20.58kg	<11.66kg	[96]
	<15.33kg	<8.33kg	[86]
Abdominal obesity	-	>88cm	[87, 91]
Waist Circumference			
	>102cm	>88cm	[89, 90, 92-94]
	>99cm	>95cm	[95]
	>100cm	>87cm	[86, 96]

1.7.2 DAO Compared with Sarcopenic Obesity

DAO as a phenotype may be more relevant than sarcopenic obesity for several reasons. Firstly, DAO avoids the limitations and artefacts of identifying low muscle mass in obesity. Appendicular lean mass is most often used to identify sarcopenia in obesity – and included in the recent consensus definition [76]. However, it is becoming increasingly apparent with novel techniques (D3 creatine dilution; D3Cr)

that dual-energy X-ray absorptiometry (DXA) measured lean mass may not be a suitable surrogate for muscle mass with inconsistent associations with outcomes [97, 98]. However, this novel D3Cr method has predominantly been assessed in very old men and data on women is lacking with only small samples of very old, normal weight women available [99, 100]. Further research is also required on the sources of variability with D3Cr for optimal muscle mass calculation [101].

Compounding this lean mass artefact, there are particular challenges in identifying low muscle mass in people with obesity. Absolute lean or muscle mass is fundamentally correlated with body size. Several attempts have been made to adjust lean or muscle mass for body size with adjustments including division by height^2, weight, and BMI; however, these adjustments either maintain, strengthen or invert the relationship with BMI [53]. Furthermore, intermuscular thigh adipose tissue increases with obesity [102] and muscle strength per unit of lean mass declines with obesity [103] – indicating that although muscle quantity increases with obesity, the quality of this muscle may not. Therefore, the use of absolute measures of lean/muscle mass to identify sarcopenia in obesity may not be appropriate.

There is also growing general consensus regarding the definition of DAO in the literature whereas sarcopenic obesity has been defined by heterogenous definitions and the latest consensus definition has yet to be related to clinical outcomes. DAO also avoids the use of BMI, which fails to distinguish lean and fat mass whereas greater waist circumference has been linked to a greater loss of skeletal muscle mass in older adults [79]. This link may indicate an inter-relationship between dynapenia and abdominal obesity [79]. DAO may be a more clinically relevant condition given the already well-established adverse effects of central adiposity, which is closely related to fat mass, on health [104] compared with the known limitations of BMI and its failure to differentiate lean and fat mass. DAO has the potential for wide clinical applicability due to its ease of assessment

DAO may also be a more relevant phenotype due to the growing debate for greater focus being placed on muscle function, or dynapenia, rather than lean mass [19, 21]. This is also evident in the latest sarcopenia definitions whereby consensus groups have proposed that dynapenia is sufficient to trigger clinical intervention [4] or that the definition of sarcopenia be based on muscle function alone [58].

1.7.3 Clinical Outcomes – Falls and Fractures

It has been proposed that people with DAO have the cumulative risks associated with both dynapenia [7, 105, 106] and abdominal obesity [8, 86, 95, 107]. DAO is associated with a greater risk of falls in older adults compared to those with dynapenia, abdominal obesity, or neither phenotype [87, 89, 91, 108]. Poor physical function is a risk factor for falls and fracture in older adults with obesity [109-112]. In a large prospective study of English adults, participants with DAO had slower gait speed than both obese or dynapenic-only adults; however, the rate of decline was similar over 14 years [108].

Individuals with DAO report both greater fall risk factors [87] and prevalence of falls [89-91] than individuals with dynapenia or obesity alone. The study by Zhang et al. (2021) [108] requires consideration as although a greater risk of falls, recurrent falls, and fall-related injury was reported over 14 years – the authors did not allow for movement or re-classification of participants between groups over this time. Furthermore, changes in clinical factors which may confound the relationship over time were not considered. Thus, the true effect of DAO on falls is still unclear.

Table 4 A summary table of studies on the effects of dynapenic abdominal obesity on risk of falls

n (% F) Study*	Study Design	Definition	Findings	Reference
217 (100%)	Cross-sectional	Dynapenia = HGS ≤ 20.67 kg. AO = WC >88cm.	Women with DAO - greatest falls risk (assessed by decreased timed up and go, sit-to-stand time, postural balance) and fear of falls.	[87]
1046 (60.9%)	5-year follow-up	Dynapenia = HGS <26kg men, <16kg women. AO = WC >102cm men, >88cm women.	Older adults with DAO had highest risk for single fall, but lowest risk for recurrent falls.	[89]
3723 (54%) SABE	8-10 year follow-up	Dynapenia = HGS <26kg men, <16kg women. AO = WC >102cm men, >88cm women.	DAO - highest reported falls at baseline compared with normal weight. Greatest difference in the rate of ADL change, compared to those with dynapenia, abdominal obesity or neither.	[90]
201 (100%)	18 month follow-up	Dynapenia = HGS ≤ 20.67 kg. AO = WC >88cm.	In the overall group of older women: 27.5% fell. DAO highest risk of falls (HR 3.595), Dynapenic (HR 2.283), AO (HR 1.348)	[91]

4987 (54.7%) ELSA	14-year follow-up	Dynapenia = HGS <26kg men, <16kg women. AO = WC >102cm men, >88cm women.	Classification at baseline. DAO and Dynapenic participants had a higher risk of falls compared with normal weight/strength controls. DAO had a higher risk of recurrent (2+) falls and fall-related injury.	[108]
551 (42%)	Cross-sectional	Dynapenia = HGS <28 kg men, <18 kg women AO = WC ≥90cm men, ≥85cm women (Asian cut-offs)	Only DAO was associated with a worse performance on the Tinetti Performance Oriented Mobility Assessment.	[113]

** if applicable. Abbreviations: %F = Percentage Female; HGS = hand grip strength; AO = abdominal obesity; WC = waist circumference; DAO = dynapenic abdominal obesity; ADL = activities of daily living; HR = hazard ratio; ELSA = English Longitudinal Study of Ageing; SABE = Brazilian Health, Well-being and Aging Study.*

Moreover, whether the increased falls risk associated with DAO translates into greater fracture risk remains to be confirmed in longitudinal studies. A recent systematic review aimed to determine whether sarcopenic obesity was associated with an increased risk of vertebral and non-vertebral fractures [114]. Notably, the definition of sarcopenic obesity in this review was heterogeneous and included definitions of dynapenic obesity. The authors found that risk of non-vertebral fracture was similar between people with obesity and sarcopenic obesity, suggesting no cumulative effect. However, obesity is associated with a greater risk of some fractures - ankle, lower leg and proximal humerus [10, 115, 116]; yet a reduced risk at the wrist, hip, and spine [9, 10, 117]. Thus, these fractures should be considered accordingly in people with obesity. Nonetheless, based on the current evidence of greater fracture risk observed in dynapenia [7] and abdominal adiposity [107, 118, 119] separately, a greater cumulative risk seems likes a reasonable hypothesis [108]. It is also noteworthy that, given the body compositional and biomechanical differences [120, 121], the effects of DAO on falls and fall-related injuries have not been analysed and compared between sexes.

1.7.4 Other Clinical Outcomes

Older adults with DAO have the highest risk of hospitalisation [95, 96] and disability [88, 90, 95, 96] compared with non-dynapenic or obese counterparts. Other longitudinal studies have shown that participants with DAO have the highest mortality risk compared to those with dynapenia or obesity alone [86, 94]. A study of Brazilian older adults found that participants with DAO were more likely to have low high density lipoprotein (HDL) cholesterol plasma concentration, hypertriglyceridemia, hyperglycaemia, high glycated-haemoglobin and metabolic syndrome than those with dynapenia or

obesity alone [122]. However, these effects appear to be mediated by the abdominal obese phenotype. In support of this hypothesis, a study of even older adults (70+y) found no extra effect of dynapenia on components of metabolic syndrome (low density lipoprotein (LDL) or HDL cholesterol, triglycerides, total cholesterol, glucose) and a beneficial effect (i.e. reduction) on waist circumference and diastolic blood pressure in dynapenic obese participants [123]. However, these participants were classified according to higher cut-points for HGS (20kg female, 32kg for males) and BMI (30kg/m^2). In a younger cohort (50y +) using an alternative definition of dynapenia (lowest tertile of leg extension strength) in the context of DAO, the authors found that participants with DAO and obesity had a similar risk of having low HDL-cholesterol, hypertriglyceridemia, hyperglycaemia, and metabolic syndrome but a lower risk of high diastolic pressure [124]. Thus, dynapenia does not appear to exacerbate the effects of abdominal obesity on components of metabolic syndrome/health.

1.7.5 Mechanistic Factors

DAO has primarily been examined from an epidemiological perspective with limited, detailed phenotypic information. Therefore, the mechanism of the hypothesised increased falls risk is unclear. The available evidence suggests that women with DAO have slower timed up and go (TUG) times, higher prevalence of reduced reaction times (61.6% vs. 39.2%) [87] and slower gait speed [108] compared to women with obesity only. Women with DAO and obesity have similar fat-free mass [87, 91], postural balance, and impaired sit-to-stand time [87].

In people with obesity, multiple adaptations of gait have been observed which result in functional impairments, poor muscle coordination, and increased muscle fatigue [72]. Excess adiposity interferes with multi-joint coordination and muscles critical for balance control [73]. The location of adiposity is also relevant from a biomechanical perspective as central adiposity may result in an anterior shift of the body's centre of mass [125]. This is particularly significant as an increased magnitude of ankle torque is then required for stabilisation in the upright position [125]. If a corrective ankle torque cannot be produced, the person with obesity is more susceptible to reduced stability and falls [126]. There is some evidence to suggest that individuals with obesity are more vulnerable to falling than their normal weight counter-parts when using an ankle strategy to recover dynamic balance [125, 127]. Dynapenia (low hand-grip strength) is also associated with slow gait speed [65]. As both dynapenia and obesity are associated with functional and biomechanical impairments, people with DAO may experience similar or cumulative effects of both phenotypes. Overall, there is a paucity of information on the detailed phenotype of people with DAO using gold-standard methods for muscle

function, quantity and quality. Understanding the phenotype of older adults with DAO has the potential to identify strategies for intervention and reduction of falls risk.

1.7.6 Pathogenesis

Overview

The pathogenesis of DAO is multi-factorial (Figure 2) and likely involves a bidirectional relationship between dynapenia and obesity. Ageing is associated with a gradual loss of lean mass [20] which results in reduced total energy expenditure [128, 129]. Further reductions in total energy expenditure result from reduced physical activity and oxidative capacity – changes which promote the development of obesity [128]. In women, the menopause is associated with increased body mass, fat mass and reduced fat free mass [120]. This redistribution of fat results in a greater waist circumference [120]. Abdominal obesity is associated with reduced muscle strength in older adults [130] and greater decline in muscle strength in older men [79]. Other factors implicated in the development of sarcopenia include a reduced appetite with ageing [22], anabolic resistance [131], and insulin resistance [132]. Some other factors implicated in the development of DAO are discussed in more detail below.

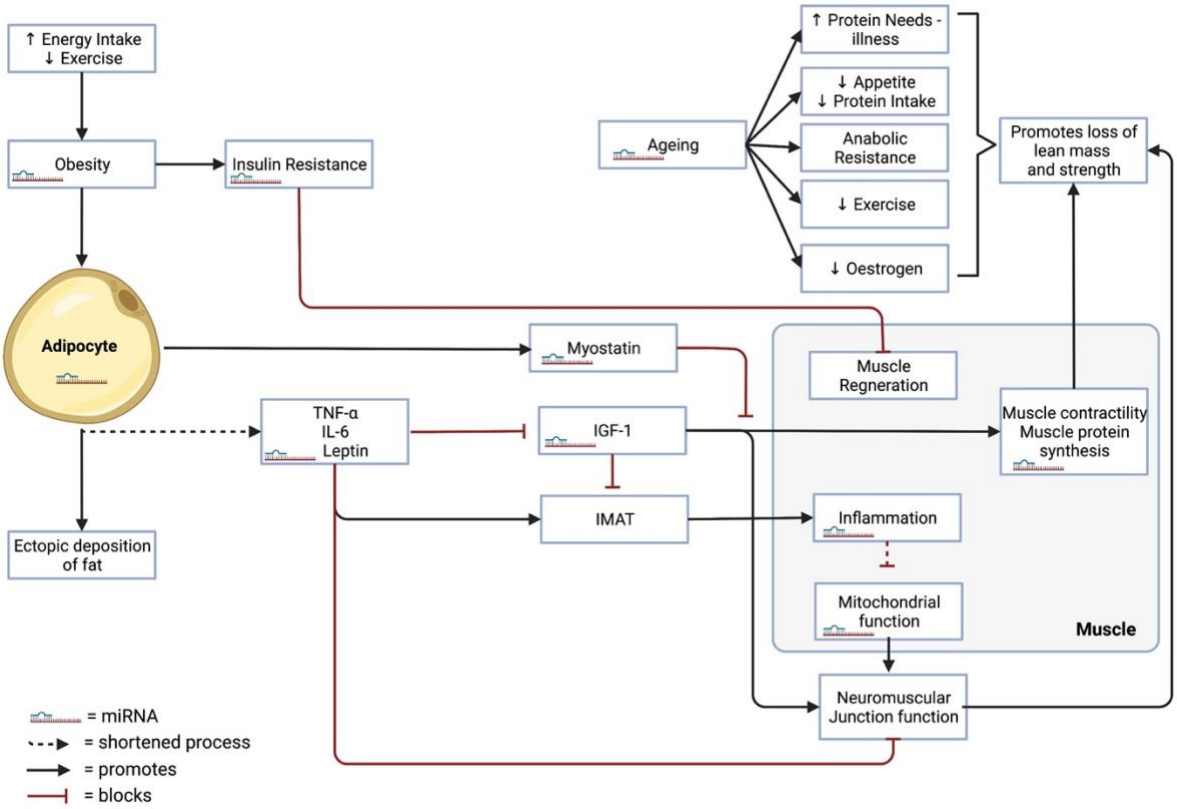

Figure 2 Schematic showing potential pathways to the development of dynapenic abdominal obesity

Adapted from [129, 133]. MiRNA symbol indicates that miRNAs have been implicated in the process, tissue or phenotype. Abbreviations: IGF-1 = insulin-like growth factor-1; IMAT = intermuscular adipose tissue; TNF-α = tumour necrosis factor-α; IL-6 = interleukin-6. Created with BioRender (https://biorender.com/).

IGF-1

Insulin-like growth factor-1 (IGF-1) is one of the most important regulators of muscle growth and repair [134]. The binding of IGF-1 to its receptor (IGF-1R) activates several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K) [135]. Downstream targets of the PI3K/AKT (protein kinase B) pathway include the forkhead box protein (FOXO) family which regulates processes such as autophagy and protein degradation [135] and the mammalian target of rapamycin (mTOR) which is implicated in protein synthesis [135]. IGF-1 is affected by diet and declines with age [136]. The synthesis of systemic IGF-1 in the liver is growth hormone dependent whereas IGF-1 produced outside of the liver acts locally [134].

Transgenic mice which over-express IGF-1 have increased muscle mass, cross-sectional area, and muscle force which is maintained into older age [137]. In older men and women, those in the lowest tertile of IGF-1 have a 4-fold increased risk of being sarcopenic (adjusted for factors such as age, sex, BMI) [138]. In another study, IGF-1 levels were lower in older women with sarcopenia (low muscle strength and lean mass), but not men, compared to those without sarcopenia; however, the association in women was lost when adjusted for age and risk of malnutrition [139]. Reduced IGF-1 also increases the risk of frailty in men [140].

Myostatin

Myostatin is a member of the transforming growth factor beta (TGF-β) superfamily of cytokines. Myostatin is predominantly expressed in skeletal muscle and is a key negative regulator of skeletal muscle growth [141]. Obesity is associated with higher levels of myostatin which also has implications for insulin sensitivity e.g. inhibited GLUT4 expression [142, 143]. Increased myostatin is associated with low lean mass and frailty in men but this has not been observed in women [144]. Myostatin expression may therefore play a key role in the development of sarcopenic obesity.

Phase two trials for myostatin inhibitors have shown promising results as a treatment for aberrant body composition but this does not appear to translate into improved muscle strength [145-148]. However, improved six minute walking distance (6MWD) was observed in those who could only walk a short distance at baseline suggesting that greatest benefit may be seen in the weakest [146]. Similar body compositional improvements following other bimagrumab (monoclonal antibody for myostatin receptor) interventions have also been found in people with Type 2 Diabetes [147] and patients following hip fracture [148]. Myostatin inhibitors may offer a promising treatment alternative for low muscle mass and obesity but perhaps not dynapenia.

Oestrogen

It is thought that menopause and the subsequent reduction in oestrogen are associated with loss of lean mass [120]. However, the relationship between the decline in oestrogen levels and muscle mass is not well understood. One hypothesis is that subsequent increases in pro-inflammatory cytokines (e.g. tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6)) are implicated [149]. Alternatively, oestrogen may play a more direct effect on protein synthesis as skeletal muscle has oestrogen receptors [149]. However, the effect of hormone replacement therapy on sarcopenic parameters has been conflicting with some studies showing a positive effect on muscle power and lean mass [150] but not strength [151], while others also found no effect on lean mass [152, 153].

Inflammation

A high waist circumference is associated with a greater loss of muscle mass [79]. Both a high BMI and greater waist circumference are associated with elevated levels of IL-6, C-reactive protein (CRP), TNF-α and leptin [129, 154]. A recent meta-analysis found that sarcopenia was associated with elevated levels of CRP but not IL-6 or TNF-α [155]. There were less studies available on IL-6 and TNF-α in this review and thus greater heterogeneity. However, others have shown that increased levels of IL-6 are associated with reduced IGF-1 [156]. Low levels of IGF-1 and high levels of IL-6 are associated with a greater risk of walking limitation, mobility disability, and disability in activities of daily living in older women [156]. Moreover, inflammation (IL-6) has been linked with in vitro senescence of Schwann cells [133]. Neurological factors are well-acknowledged determinants of muscle strength (See Section: 1.3.2 Neurological factors) and a growing area in sarcopenia and dynapenia research [48, 49, 157, 158].

The presence of senescent cells, which are permanently in cell-cycle arrest, in multiple tissues also increases with ageing. Senescent cells can exhibit a proinflammatory phenotype secreting proinflammatory cytokines, chemokines and proteases (senescence-associated secretory phenotype; SASP) thus contributing to chronic inflammation [159, 160]. There is a potential role for SASPs in the pathogenesis of dynapenia [159].

Both ageing [28] and obesity [36, 129] result in ectopic fat deposition. Ectopic fat deposition, or muscular fat infiltration, is associated with lower muscle strength and power [33]. It is unclear how this occurs but may relate to a more localised inflammatory environment created by cytokines acting in an autocrine or paracrine effect [36]. This inflammatory environment may lead to insulin resistance which in turn can promote fat mass gain and the loss of muscle mass [82, 154].

1.7.7 MicroRNAs

Biogenesis

MicroRNAs (miRs, miRNAs) are short, non-coding RNAs which regulate gene expression at the post-transcriptional level [161]. To date, 2,654 miRNAs have been identified in humans [162]. MiRNAs regulate gene expression through the repression of translation and acceleration of target mRNA degradation [163]. It is thought that they regulate around two thirds of protein-coding genes in the human genome – meaning that they are likely to regulate many physiological processes (e.g. Figure 2) [164]. MiRNAs and mRNAs have a 'many-to-many' relationship meaning that a single miRNA can regulate many targets and a single gene can be targeted by many miRNAs [26].

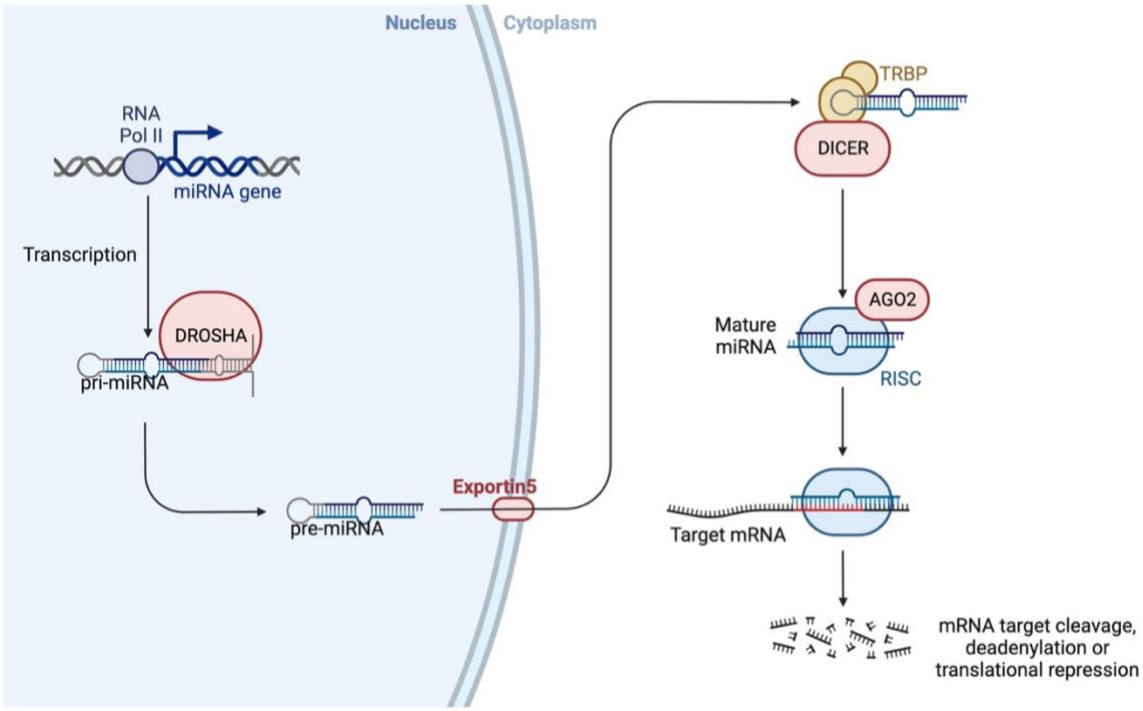

Figure 3 Biogenesis of microRNAs

Adapted from Krol et al. (2010) [165] and created with BioRender (https://biorender.com/).
Abbreviations: miRNA = microRNA; TRBP = TAR RNA binding protein; AGO2 = argonuate2; RISC = RNA-induced silencing complex.

The enzyme Drosha cleaves primary miRNA (pri-miRNA) precursors in the nucleus to form a pre-miRNA transcript [165]. The pre-miRNA transcript is transported from the nucleus to the cytoplasm where it is further cleaved by the enzyme Dicer to create a 19- to 24-bp miRNA duplex [166]. The miRNA duplex is unwound and one mature miRNA strand is integrated into a protein complex called the RNA-induced silencing complex (RISC) creating miRISC [165, 166]. The other strand of the miRNA duplex, which has not been incorporated, is often degraded [26]. The argonuate (AGO) protein family

bind to small noncoding RNAs such as miRNAs and lead them to RISC [165]. AGO and GW182 are also core components of miRISC [165]. Once incorporated, miRNAs guide RISC to targeted mRNA transcripts and bind to partially complementary sequences usually contained within the 3' – untranslated region (UTR) [165].

MiRNAs regulate gene expression through the repression of translation and acceleration of target mRNA degradation [163]. The miRNAs within RISC pair to complementary sites on mRNAs thereby inhibiting the translation of these mRNAs into their respective proteins. This occurs by deadenylation of the target mRNA(s) and in some cases de-capping and mRNA degradation from the 5' end [163]. In addition, miRNAs can target many genes and genes can be targeted by many miRNAs [166]. Numerous algorithms exist to predict these interactions (e.g. TargetScan, miRanda and miRWalk); however, there can be a lack of overlap between predictions [167].

Nomenclature

MiRNAs are assigned progressive numerical identifiers [168]. Three or four letter prefixes are used to denote the species e.g. hsa (homo sapiens) or mmu (mus musculus) [168]. Where miRNAs differ at only one or two positions, a lettered suffix is used e.g. miR-27a and miR-27b [168]. The use of -3p or -5p denotes the two different mature miRNA sequences which arise from the opposite arms of the same hairpin precursors [168]. With growing interest in the field of miRNAs, miRBase, a database of miRNAs, has undergone various updates; there has been a 5-fold increase in reads mapping to microRNA loci in the most recent release [162]. In addition, some microRNAs have been reviewed and removed from the database as their pattern does not support that of a miRNA (e.g. miR-6087, miR-4532, miR-4461, miR-1273a) [162].

Tissue specific expression and myomiRs

Different cells may exclusively express certain miRNAs; however, the expression of one miRNA may be misinterpreted as ubiquitous. This is due to the presence of numerous cell types (e.g. red blood cells, fibroblasts, adipocytes) within tissues and the ability for cellular cross-talk [169].

Some miRNAs are muscle specific – miR-1, miR-133a, miR-133b, miR-206 (skeletal muscle specific), miR-208a (cardiac-specific), miR-208b, miR-486, miR-499a, miR-499b – and have roles in regulating phenotypic changes that occur in muscle fibre type and mass [170, 171]. Mice with double knockout of the genes for miR-208b and miR-499, show a significant loss of slow Type I myofibers in combination

with reduced expression of β myosin heavy chain (β-MHC - a slow ATPase) mRNA and proteins [172]. Active adults show greater levels of fatigue resistant Type I (*Myh7 and Myh7b*) muscle fibre gene expression and miR-499 than sedentary controls. Moreover, greater miR-499 expression is associated with Type I muscle fibre type percentage, ATP_{max} and VO_{2max}, measures of muscle endurance [173]. MiR-1 and miR-133a are involved in myogenesis [174]. In a rat model of local muscle injury, local injection of double-stranded miR-1, miR-133a and miR-206 was shown to accelerate muscle regeneration and prevent fibrosis [175]. MiR-133a can also promote slow-to-fast muscle fibre type switching through its actions on TEA domain family member 1 (TEAD1), a transcription enhancer factor, in mice and C2C12 cells [176].

Cellular cross-talk

MicroRNAs are capable of exerting paracrine-like functions. Evidence from rodent studies has shown that adipose-derived miRNAs can be transported to a variety of cells including hepatocytes, myocytes, and macrophages [177, 178]. Likewise, skeletal muscle-derived miRNAs can be taken up by adipose tissue [179]. Functionally, this inter-organ cross-talk has been implicated in lipid metabolism, insulin resistance, and adipogenesis [177-179], thus suggesting a role for miRNAs in the pathogenesis of DAO.

MicroRNAs are an exciting area of research due to the potential of antagomiRs which have already been explored as pharmacological options in conditions such as cardiovascular disease and cancer [180]. To date, however, microRNAs have not been explored in relation to the pathogenesis of either DAO or sarcopenic obesity. There is evidence that ageing is associated with altered miRNA levels in the muscle and that this may have a detrimental impact on muscle quality and quantity [181-183]. Independently, sarcopenia and obesity are associated with altered miRNA levels [184, 185]. However, the influence of obesity or adiposity on the microRNA profile of older adults and whether this translates into functional impairment has not yet been established.

1.7.8 Management and Treatment of DAO

There are a limited number of interventions specifically designed for older adults with DAO. One study in older adults with dynapenic obesity (low hand-grip strength and high body fat percentage) found that high intensity interval training (HIIT) improved function (6-minute walking distance, 4m walking test, timed up and go, sit-to-stand test), body composition (lean mass, leg lean mass, waist circumference; total, android and gynoid fat mass) and muscle function (lower limb power) [186]. HIIT and L-citrulline further improved hand-grip and quadriceps strength [186]. Due to limited evidence in people with this phenotype and due to heterogeneity in the definition of sarcopenia (low lean mass

and/or low muscle strength), findings from interventions in sarcopenia and obesity may provide insight to treatments for DAO.

The proposed treatments for sarcopenia broadly centre around dietary and exercise interventions. A recent umbrella review which focused on nutritional interventions proposed that leucine, its metabolite β-Hydroxy β-methylbutyric acid, creatine, protein or protein with progressive resistance exercise could be used as treatments for sarcopenia [187]. Another umbrella review focusing on exercise interventions proposed that resistance training, multi-modal training (e.g. resistance and balance) and supervised blood flow restriction training could be used to treat sarcopenia [188]. This is echoed in a systematic review of interventions used to treat fallers whereby exercise and multifactorial interventions were recommended [189]. A final umbrella review of pharmacological treatments for sarcopenia proposed vitamin D and testosterone supplementation only where clinical deficiency is present [190]. Notably, high dose vitamin D is associated with a greater falls risk [189]. In addition to these reviews, there is growing interest in denosumab, an osteoporosis treatment, which may be associated with a lower falls risk although the exact mechanism remains unclear [191]. In summary, dietary and exercise interventions are shown to be effective in the treatment of sarcopenia or for the reduction of falls risk; however, there are a lack of pharmacological options. A 600 kcal reduction in dietary intake is recommended in National Health Service guidance for the treatment of obesity [6]. Increasing moderate exercise is recommended and pharmacological options are recommended if diet and exercise interventions fail [6]. Dietary interventions without concomitant resistance exercise can exacerbate dynapenia and lead to loss of areal bone mineral density (aBMD) [192-194].

Adherence to exercise interventions is poor amongst older adults [195, 196]. Barriers to exercise include baseline depression or stress, poor family support, obesity, fatigue, and poor health [196]. Dietary interventions can be affected by increased satiety, changes in the appetite hormone ghrelin, convenience, altered smell/taste/sight, mood, general health and dentition in older adults [197, 198]. These barriers thereby limit the generalisability and effectiveness of diet and exercise as therapeutic options. With a growing population of older adults, more treatment options are required to treat or combat the development of dynapenia in the presence of obesity in older adults.

1.8 Thesis overview

The number of older adults is increasing worldwide. Older adults have the highest risk of falls. Falls can result in fracture or other injury and are associated with both high economic and social costs. With an ageing population it is important to understand risk factors for falls in order to identify suitable screening and prevention strategies. Ageing is associated with increased abdominal obesity and reduced muscle strength. These changes are individually associated with falls and obesity is associated with site-specific fracture risk. A greater understanding of dynapenia, abdominal obesity, and how these age-related changes interact with each other is required to target effective screening and intervention strategies for people at risk of falling.

Firstly, this thesis used cohort studies to determine whether there was a cumulative effect of dynapenia and abdominal obesity (DAO) on falls risk and whether dynapenia modulated the site-specific fracture risk and greater aBMD observed in older women with obesity. To date, the evidence in this area suggests an increased incidence of falls whereas the relationship with fractures is unclear as obese-prone and obese-protective fractures are often grouped together. This information will provide a greater understanding of fracture risk in a population with a growing prevalence of obesity.

Subsequently, a detailed phenotyping study was undertaken using gold-standard techniques in order to identify patterns of physical performance which may relate to falls risk. DAO has primarily been examined from an epidemiological context or in smaller studies which have not used gold-standard techniques. Isokinetic dynamometry was used to measure strength and magnetic resonance imaging (MRI) measured muscle volumes. Linkage of these measurements allowed for a better understanding of the determinants of muscle strength in older women with obesity. A greater understanding of muscle function and quantity in older women with DAO may offer insight to alternative intervention strategies.

To date, the role of miRNAs in DAO (or sarcopenic obesity) has not been examined. A systematic review was undertaken to examine whether there was a plausible role for miRNAs in the development of DAO and to identify a panel of miRNAs which may be implicated. The microRNA profile of women with DAO was measured in serum using RNA-sequencing (RNA-seq) and Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) to determine whether there were any differences compared to normal weight, dynapenic or obese women. Using different techniques and

two cohorts of women enabled greater confidence in our findings. A greater understanding of the pathogenesis underlying DAO has the potential to identify novel therapeutic agents.

1.9 Long Term Research Goals

The ultimate goal of this area of research is to prevent falls and fractures. Understanding how dynapenia and abdominal obesity, phenotypes associated with ageing, interact and relate to falls and fracture risk has the potential to lead to effective and appropriate screening strategies in this growing proportion of the population. Preventing falls also has the potential to maintain intrinsic capacity and physical function. A greater understanding of the mechanism of falls in older adults with dynapenia and obesity as well as the pathogenesis underlying this phenotype has the potential to lead to alternative intervention strategies and novel therapeutic targets.

1.10 Thesis Aims and objectives

To determine the relationship between dynapenia, abdominal obesity and DAO, and falls and fracture risk in older women (Chapter 2).

a) To identify whether obesity and dynapenia have additive effects on falls risk, greater than either component alone.
b) To identify whether dynapenia modulates the positive effects of obesity on aBMD.
c) To determine whether the fracture pattern observed in women living with obesity is modulated by dynapenia by examining the relationship with:
    i. Obese-prone fractures (lower-extremity).
    ii. Obese-protective fractures (other fractures).

To detail the phenotype of women with DAO to determine where deficiencies in physical performance related to falls lie such that targeted intervention strategies may be proposed (Chapter 3).

To detail the phenotype of women with DAO; to compare measures of:

a) muscle function – quadriceps muscle strength (absolute and expressed relative to total body weight, lean mass and muscle volume) and fatigue from isokinetic dynamometry and dynamic muscle fatigue from the 6-minute walking test
b) muscle structure – absolute volume of quadriceps muscles

between normal weight, obese and DAO older women.

To provide an overview of the literature on miRNAs in the context of sarcopenic obesity / dynapenic abdominal obesity (Chapter 4).

a) To conduct a systematic review to identify differentially expressed miRNAs in obesity and sarcopenia and whether there are any commonly expressed miRNAs between these conditions.

To identify a panel of miRNAs which may be implicated in the pathogenesis of DAO in older women (Chapter 5).

a) To conduct RNA-seq to identify the range of miRNAs expressed in women with normal weight, dynapenia, obesity and DAO, in order to identify a panel of miRNAs which may be implicated in the pathogenesis of DAO.

b) To combine findings from the systematic review, RNA-seq analysis and evidence in the literature to identify a panel of miRNAs which are differentially expressed in DAO for validation using RT-qPCR.

c) To validate a panel of miRNAs which may be implicated in the pathogenesis DAO in both the same cohort of women and an external group of women using RT-qPCR.

Chapter 2

Falls and Fractures in DAO - UK Biobank and English Longitudinal Study of Ageing Analyses

2.1 Introduction

The purpose of this chapter was to determine whether dynapenic abdominal obesity (DAO) was associated with falls and fractures in older adults. As outlined in the Introduction (Chapter 1), a cumulative effect of dynapenia and obesity on falls risk is suspected in older adults with DAO [87, 89, 91, 108]; however, whether this translates into fall-related injury or fracture has not been determined. A greater understanding of falls and fracture risk in a population with a growing prevalence of obesity has the potential to reduce the associated economic burden through targeted prevention strategies. Such interventions also have the potential to maintain intrinsic capacity and physical function in older adults.

To determine whether the combination of dynapenia and abdominal obesity (DAO) results in a greater risk of falls and fall-related injuries, data from the UK Biobank and English Longitudinal Study of Ageing (ELSA) were used. Briefly, the UK Biobank and ELSA were chosen as they are both UK based studies with data available on the outcomes of interest (falls and fall-related injuries) in large numbers of well-characterised older adults. The UK Biobank is a large (n = ~500,000) study of adults in the UK with detailed phenotyping data (e.g. DXA, MRI, and health questionnaires) which started in 2006 and is ongoing. The UK Biobank has a low response rate (5.5% response rate), limited ethnic diversity, lower overall prevalence of overweight and obesity than the UK population and potential healthy volunteer bias (e.g. lower rates of diabetes, narrow socioeconomic backgrounds) [199]. ELSA is a broadly representative, longitudinal study of older adults in England which began in 2002/3, has two-yearly waves, and inclusion of refreshment samples to maintain representativeness [200]. ELSA collects large swathes of information on health, disability, economic status, social support, and household structure [200]; however, ELSA has less information on more objective measurements of health and phenotype (e.g. no body composition measurements) and the sample size is much smaller than the UK Biobank. Both the UK Biobank and ELSA have self-reported measurements of falls; ELSA in the previous two years and the UK Biobank in the previous 12 months. Self-reported fall-related injuries in the previous two years are reported in ELSA, whereas self-reported fall-related fractures in the previous five years are reported in the UK Biobank. Therefore, both studies were used to increase the applicability and robustness of any findings.

To address our aims we used data from the UK Biobank at Instance 2. An application was submitted to the UK Biobank (Application 65223) for the variables of interest (outlined in the manuscript). Following execution of the Material Transfer Agreement, the dataset was downloaded in February

2021. The UK Biobank has ethical approval from the North West Multi-centre Research Ethics Committee as a Research Tissue Bank approval – separate ethical approval was not required for the study presented in this chapter. Instance 2 (2014 – ongoing) was used as it contained information on body composition and areal bone mineral density (aBMD). Due to low numbers who completed both Instance 2 and either Instance 1 or Instance 3, a cross-sectional, retrospective approach was used. Participants who reported one or more falls in the past 12 months were classified as fallers. Fractures relating to falls (previous five years) were based on self-report. Validation of fractures was not possible using International Classification of Diseases (ICD) numbers which were only available for those admitted to hospital. Fractures were considered as obese-prone (leg and ankle) or obese-protective (arm, wrist, spine, hip and other bones) based on previous evidence of the site-specific nature of fractures in people with obesity. We hypothesised that dynapenia increased the risk of obese-prone, lower extremity fractures and counteracted the protective effects of obesity on all other, obese-protective fractures and speculated that this may relate to differences in either falls risk or aBMD.

Next, we sought to confirm our findings using a prospective approach in a representative cohort of older adults. The English Longitudinal Study of Ageing (ELSA) was chosen which is a representative study of free-living older adults in England. Baseline data was obtained from wave 6 (2012/13), self-reported falls and fall-related injury data (outcome measures) were obtained from wave 7 (2014/15) i.e. after a 2 year follow-up period to allow for a longitudinal study design. These waves had the most complete data for the relationships we wanted to study. The dataset was downloaded from the UK Data Service. Waves 6 and 7 obtained approval from the National Research Ethics Committee Service Committee South Central – Berkshire; separate ethical approval was not required for the study presented here. Participants with one or more falls were classified as fallers. Descriptive information was not available for fall-related injuries so the types of injuries sustained is unknown.

Findings from the UK Biobank are presented followed by findings from ELSA as published or accepted manuscripts. Tables and figures are presented at the end of each manuscript.

2.2 Aims and Hypotheses

Aims

1. To determine whether abdominal obesity and dynapenia have independent effects on falls risk.
2. To determine whether the fracture pattern observed in women living with abdominal obesity is modulated by dynapenia.
3. To determine whether aBMD differs in women with both abdominal obesity and dynapenia compared to women who are obese only.

Hypotheses

1. Obesity and dynapenia have independent effects on falls risk.
2. Dynapenia increases the risk of lower extremity fractures and counteracts the protective effects of obesity on all other fractures.
3. Dynapenia in older women with obesity is associated with lower aBMD compared to women with obesity only.

References:

UK Biobank Study:

Dowling, L.., Cuthbertson, D.J. and Walsh, J.S., 2022. Reduced muscle strength (dynapenia) in women with obesity confers a greater risk of falls and fractures in the UK Biobank. Obesity (Silver Spring), pp 1-10, doi:10.1002/oby.23609.

Study using ELSA:

Dowling, L., McCloskey, E., Cuthbertson, D.J. and Walsh, J.S., 2022. Dynapenic Abdominal Obesity as a Risk Factor for Falls. The Journal of Frailty & Aging, pp.1-6.

Contribution details

Title	Reduced muscle strength (dynapenia) in women with obesity confers a greater risk of falls and fractures in the UK Biobank
Publication Status	Obesity – accepted, in press.
Authors	Lisa Dowling[1], Daniel Cuthbertson[2], Jennifer Walsh[1] [1]The University of Sheffield, Sheffield, UK [2]The University of Liverpool, Liverpool, UK
Authors roles	Conceptualisation: LD, JW; Methodology: LD, JW; Formal Analysis: LD: Supervision: DC, JW; Writing – original draft: LD; Writing – review and editing: all authors; Approval of final manuscript: all authors.
Student author	Lisa Dowling

Student author statement:
In publication I, the candidate, planned and wrote the manuscript, co-author reviewed and provided comments on the manuscript.

Name Lisa Dowling	Signature  Date 15/09/2022

Co-author statement:
I hereby declare that I am aware that the work in the manuscript entitled: "Reduced muscle strength (dynapenia) in women with obesity confers a greater risk of falls and fractures in the UK Biobank" of which I am co-author, will form part of the PhD thesis by the PhD student Lisa Dowling who made a major contribution to the work stated above.

Co-author name
Dan Cuthbertson
Jennifer Walsh

Supervisor confirmation
I have seen email or other correspondence from all co-authors confirming their certifying authorship.

Name Dr Jennifer Walsh	Signature  Date 15/09/2022

Title page

1. **Title of the manuscript**

   2.3 “Reduced muscle strength (dynapenia) in women with obesity confers a greater risk of falls and fractures in the UK Biobank”

2. **All authors’ full name(s), highest academic degree(s) and affiliations;**

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4. **Main text word count**

   3574

5. **Number of data elements**

   5

6. **Conflicts of Interest**

   Disclosure: The authors declare no conflict of interest

2.3.1 Study Importance Questions

What is already known about this subject?

- People with obesity have a greater risk of fractures at the ankle, lower leg and proximal humerus but lower risk of other fractures although the mechanisms are unclear.
- Low muscle strength (dynapenia) is associated with a greater risk of fracture.
- A recent systematic review found no cumulative effect of obesity and dynapenia on non-vertebral fractures; however this group of fractures has heterogeneous risk factors and mechanisms, especially in obesity.

What are the new findings in your manuscript?

- Dynapenia, irrespective of bone mineral density, further increases the risk of lower extremity (ankle and leg) fractures in women with obesity.
- Dynapenia counteracts the lower risk of all other fractures in women with obesity.

How might your results change the direction of research or the focus of clinical practice?

- These findings highlight the independent risks of these phenotypes (obesity and dynapenia) and contribute to the understanding of the site-specific fracture risk in this group.
- Clinically, these results highlight the importance of increasing physical activity and exercise in weight management programs.

2.3.2 Abstract

Objective: This study aimed to determine the independent effects of obesity and dynapenia on falls risk, areal bone mineral density, and fracture risk (lower extremity or all other fractures).

Methods: A total of 16,147 women (aged 60-82 years) from the UK Biobank were categorized by handgrip strength (HGS; dynapenia status: HGS ≤21kg) and body weight (BMI: normal weight, overweight, or obesity). Multiple logistic regression models examined the association between dynapenia or dynapenic obesity and self-reported falls (previous 12 months), lower extremity fractures, and all other fractures (previous five-years).

Results: A total of 3,793/16,147 women fell, and 1,413/15,570 (9.1%) eligible women experienced fall-related fractures. Obesity (odds ratio [OR] 1.25, 95% CI: 1.12-1.38) and dynapenia (OR 0.87; 95% CI: 0.77-0.98) were both independently associated with greater lower extremity fracture risk, independently of areal bone mineral density. However, considering all other fracture sites, obesity conferred protection (OR 0.77; 95% CI: 0.61-0.96), except in those with low HGS, who had an equivalent fracture risk to those of normal weight (OR 1.06; 95% CI: 0.82-1.38).

Conclusion: Dynapenia further increases the increased risk of leg and ankle fractures in obesity, and counteracts the protective effects of obesity on fracture risk at all other sites (wrist, arm, hip, spine, other bones).

2.3.3 Introduction

Obesity is associated with a greater risk of fracture at the ankle, lower leg and proximal humerus [10, 115, 116] yet a lower fracture risk at the wrist, hip, and spine [9, 10, 117]. A more injurious fall type may explain this site-specific fracture risk in the knowledge that obesity is associated with greater areal bone mineral density (BMD) and stronger bone structure, such as denser cortices and increased trabecular number and thickness [9, 201, 202]. A greater understanding of fracture risk in a population with a growing prevalence of obesity could reduce the economic burden of fracture through targeted prevention strategies; the medical and social cost of fragility fractures alone is estimated at £4.4bn per year in the UK [203].

The measurement of lean mass in obesity is problematic owing to scaling with body size [53], and it is also becoming increasingly apparent that lean mass may not be the best surrogate of muscle mass [97]. Dynapenia, or low muscle strength, is recommended in recent reports and definitions of sarcopenia [4, 58]. As muscle strength reduces at a greater rate than muscle mass with age, and as this reduction is only partially explained by muscle mass, the concept of dynapenia has been identified as a distinct condition [21]. Dynapenic abdominal obesity refers to the combination of low muscle strength and obesity which is estimated to affect 3.6 to 23.4% of older adults [87, 88, 95, 111, 204]. Prevalence is higher in smaller studies [87, 204] approaching ~10% in large and population based-studies [88, 95, 111]. There is a bidirectional relationship between obesity and dynapenia: obesity may exacerbate dynapenia (e.g., through secretion of pro-inflammatory cytokines), whereas dynapenia may exacerbate weight gain with an impaired ability to undertake physical activity [129].

Independently, dynapenia and obesity are associated with a greater risk of falls [8, 105, 106] with reports suggesting a synergistic effect on falls risk [87, 89, 204]. Dynapenia is associated with a greater risk of all fractures [7], whereas obesity is associated with site-specific fracture risk [9, 10, 115-117] and greater BMD [9, 201]. Limited studies have explored the independent effects of dynapenia and obesity on BMD or fracture risk. A recent systematic review [114] reported no difference in lumbar spine BMD (eight studies included; total n = 9014) between groups with obesity and sarcopenic obesity; the clinical significance of a statistically significant reduction in femoral neck BMD was unclear (six studies included; total n = 5608). It should be noted that heterogenous definitions of sarcopenic obesity (low lean mass, low muscle strength, a combination of both) were included in this review. In addition, the risk of non-vertebral fracture was similar in people with sarcopenic or dynapenic obesity compared with people with or without obesity, suggesting no cumulative effect. However, these two

studies and respective sub-groups of people with sarcopenic obesity (n = 100-128) were small [111, 112], and nonvertebral fractures have heterogeneous risk factors and mechanisms, especially in obesity (e.g., obesity increases ankle fracture risk but decreases hip fracture risk).

The aim of this study was to determine whether the fracture pattern observed in women living with obesity is modulated by dynapenia. In order to address this aim, we examined the independent effects of obesity and dynapenia on falls, BMD, and fracture risk. Fractures were divided into lower extremity fractures (obesity-prone) and all other fractures (largely obesity-protective). We hypothesized that dynapenia increased the risk of lower extremity fractures and counteracted the protective effects of obesity on all other fractures and speculated that this may relate to differences in either falls risk or BMD.

2.3.4 Materials and methods

Participants

A description of the UK Biobank has been published elsewhere [199]. Briefly, between 2006 and 2010 the UK Biobank recruited ~500,000 participants (5.5% response rate) aged 40-69 years from the general UK population [199]. Participants attended 1 of 22 assessment centres across the UK where they completed a touch-screen questionnaire and an interview, underwent assessment of physical measures, and provided biological samples [199]. The results presented here are from the imaging visit, which began in 2014 and is ongoing. Recent comparison with population-based studies suggests that risk factor associations in the UK Biobank are generalisable [205]. Participants who were male (due to the low incidence of fractures; <3%), aged <60 years, had a body mass index (BMI) <18.5kg/m^2 (due to the association between low BMI and fracture risk and the low number of participants within this group), or had missing data for BMI, waist circumference, hand-grip strength (HGS), or the question on prior falls were excluded. The UK Biobank was approved by the North West Multicenter Research Ethics Committee, UK. Written informed consent was obtained prior to study entry.A Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Checklist is included [206] (Table 5).

Measures

Falls and fractures

Participants were asked whether they had any falls in the last year. Participants could respond that they had no falls, only one fall, or more than one fall. Participants with one or more falls were classified as “fallers”, and those with no falls or those who could not recall were classified as “non-fallers”. Participants were asked whether they had fractured or broken any bones in the last 5 years. Participants were subsequently asked whether the fracture had resulted from a fall (i.e., from standing height). Participants who responded that the fracture had resulted from a fall were classified as “injurers”. Participants who responded that the fracture had not resulted from a fall or that they did not know were excluded from the fracture analysis. Participants were asked to report the fractured bone site or sites (i.e. spine, hip, wrist, leg, ankle, arm, or other bones). Based on previous literature [9, 10, 115-117], fracture types were classified as (i) lower extremity (ankle, leg) fractures, sites that are ‘obesity-prone’ and (ii) other fractures (all other sites) or ‘obesity-protective’. Participants with ankle or leg fractures were preferentially categorized into the lower extremity fracture group irrespective of whether other sites were fractured.

Covariates

Sociodemographic factors included age, self-reported diabetes, alcohol status and smoking status (prefer not to answer, never, previous, current). Individuals who preferred not to answer or did not know were assumed to never have smoked (n = 75), to never had alcohol (n = 4) or not to have diabetes (n = 49). Weight was measured using a Tanita BC-418 MA body composition analyser (Tanita Europe, Amsterdam, the Netherlands) without shoes and heavy outer clothing. Height was measured using a Seca 202 height measure (Seca, Hamburg, Germany). BMI was calculated as weight (kilograms) divided by height (meters squared). Waist circumference was measured at the level of the umbilicus using a Wessex nonstretchable sprung tape. Maximal HGS was measured once on both right and left hands using a Jamar J00105 hydraulic hand dynamometer (Lafayette Instrument Co., Lafayette, Indiana); the maximal measure from either hand was used in this analysis. The UK Biobank protocol for data entry included computer-generated warnings for implausible measurements. Dual-energy X-ray absorptiometry was performed using a GE-Lunar iDXA (GE Healthcare, Madison, Wisconsin). Ethnicity was identified from the available baseline data of the UK Biobank as only 22% had this information at the imaging visit. Owing to large numbers, ethnicity was grouped as White (British, Irish, White, any other white background) or other ethnic group (prefer not to answer, Asian or Asian British, Chinese, other ethnic group, White and Black Caribbean, White and Black African, White and Asian, any other mixed background, Indian, Pakistani, any other Asian background, Caribbean, African, and those with missing data).

Dynapenia and obesity

Dynapenia was defined as the lowest tertile of hand-grip strength (≤21kg). A tertile approach was chosen, similar to others [95, 111, 207], owing to a lack of consensus and to allow for an exploratory approach. Normal weight, overweight, and obesity were classified according to consensus definitions based on BMI [80].

Statistical Analysis

A $\chi 2$ test was used for comparison among categorical variables. Comparison among more than two groups was conducted using either one-way ANOVA with Tukey post hoc test or Kruskal-Wallis test with Dunn post hoc test. Multiple logistic regression was used to examine the association between measures of obesity, dynapenia, or dynapenic obesity and self-reported falls in the past 12 months, lower extremity fractures, and all other fractures in the past 5 years. Regression models were adjusted

for age, measurement site, smoking status, self-reported diabetes status, and alcohol status, with results expressed as odds ratios (OR). Additional adjustment included right femoral neck BMD, HGS or BMI. Physical activity (moderate activity minutes per day) was initially considered as a confounder for both falls and fractures but adjustment did not alter results or conclusions and it was deemed that the analysis was more impaired by missing or incomplete data for physical activity (n = 2,791). Significance was accepted at $p < 0.05$. Analysis was undertaken using Stata V16.1 (StataCorp LLC, College Station, Texas).

Individual variable models Measures of obesity and dynapenia were explored separately as continuous variables. For continuous measures of dynapenia or obesity, z scores were calculated as an individual's result minus the population mean, divided by the population standard deviation.

Dynapenia by BMI category models We included BMI categories divided by dynapenia status. There were six subgroups: 1) individuals with normal weight and no dynapenia; 2) individuals with dynapenia only; 3) individuals with overweight and without dynapenia; 4) individuals with overweight and dynapenia; 5) individuals with obesity only; and 6) individuals with dynapenic obesity. The group with normal weight, without dynapenia, was used as the reference group.

2.3.5 Results

Baseline characteristics Of the 17,175 women at the imaging visit aged 60 years or older, participants who did not answer the question about prior falls (n = 152), had a BMI <18.5kg/m^2 (n = 206; 2 of whom experienced fractures from falls), or had incomplete measures for HGS (n = 617), BMI (n = 51), or waist circumference (n = 2) were excluded (Figure 6*, Supplementary*). A total of 16,147 women (aged 60-82 years) were included and categorized according to dynapenia status (lowest tertile of HGS*;* <21kg) and BMI categories (normal weight, overweight, or obesity; Table 6). BMI ranged from 18.5-69.6kg/m^2. Participants with dynapenia were older than counterparts in the same BMI category ($p < 0.001$). BMI, waist circumference and physical activity (minutes per day) were similar between participants with or without dynapenia in the same BMI category. Participants with dynapenic obesity ($p < 0.05$) and dynapenic overweight ($p < 0.01$) had lower BMD and T scores than counterparts in the same BMI category. Participants with normal weight dynapenia had similar L1-L4 BMD ($p = 0.052$) and T-scores ($p = 0.053$) but lower femoral neck BMD and T-scores ($p < 0.001$). However, differences were not clinically relevant (<5% or <0.5 T-score) [208]. The proportion of fallers increased with both dynapenia and obesity status ($p < 0.001$).

Falls and Fractures. A total of 3793 (23.5%) women fell, of whom 1062 reported more than one fall. Across all BMI categories, dynapenia appeared to increase the risk of falling (Figure 4). In the past 5 years, 577 women reported a fracture from causes other than a fall and were excluded from the fracture analysis. Thus, 1413 of 15,570 (9.1%) eligible women reported a fracture due to a fall. The proportion of injurers according to BMI and HGS tertile is presented in Table 7. Injurers were classified as lower extremity (n = 295; 1.9%) or other fractures (n = 1,118; 7.2%). Of the 295 lower extremity injurers, 58 (19.6%) women also reported other fractures (spine, arm, wrist, other bones), and 9 (3%) reported both ankle and leg fractures. In total, 1572 fractures were recorded. The most frequently reported fractures were “other bones” (577; 37%), followed by wrist (461; 29%) and ankle (230; 15%).

Association between falls and measures of muscle strength and obesity The association between measures of obesity and dynapenia as continuous variables and falls, lower extremity fractures and all other fracture risk are shown in Table 8. In a multivariable model, obesity, as determined either by greater BMI (OR 1.12; 95% CI 1.08, 1.16) or waist circumference (OR 1.14; 95% CI 1.09, 1.18), and lower HGS (OR 0.86; 95% CI 0.83, 0.90) had independent effects on falls risk.

Obesity or dynapenia as continuous variables, irrespective of BMD, were associated with a greater risk of lower extremity fractures with evidence of an independent effect of both obesity and dynapenia

(Table 8). In contrast, obesity was protective of all other fractures, whereas low HGS appeared to be associated with a greater risk. These findings remained when analyzed according to BMI category, with an increased risk of lower extremity fracture and lower risk of other fractures in people with overweight or obesity (Table 9 and Table 10). Mediation analyses showed that BMI and HGS as z scores partially mediate each other (direct and indirect effects $p < 0.05$) in their associations with both lower extremity or other fracture risk.

Association between fractures and dynapenia according to BMI category. Compared with the normal weight group, overweight and obesity were associated with a greater risk of lower extremity fractures (Figure 5a), with the greatest risk estimate seen in people with obesity (OR 2.08; 95% CI: 1.39-3.11) or with dynapenic obesity (OR 2.78; 95% CI: 1.77-4.37). The risk of lower extremity fracture among individuals with normal weight and dynapenia was also greater than those with normal weight alone (OR 1.69; 95% CI: 1.12-2.54). These findings support our continuous analysis whereby obesity and dynapenia appear to have individual and independent negative associations with lower extremity fracture.

Findings from the categorical analysis of BMI and dynapenia (Figure 5b) confirm our continuous analysis (Table 8) that BMI within the obesity category was protective of all other fractures (OR 0.77, 95% CI: 0.61-0.96) but this effect was negated by the presence of low HGS in which fracture risk was similar to that of the normal weight group (OR 1.06, 95% CI 0.82, 1.38). Participants who were overweight with (OR 0.90, 95% CI: 0.73-1.12) or without dynapenia (OR 0.88, 95% CI: 0.74-1.04) had a similar risk of all other fractures to women with normal weight. However, individuals with normal BMI and dynapenia had a greater risk of all other fractures (OR 1.29; 95% CI: 1.07-1.55) than those who were normal weight with normal strength. Owing to the large BMI range within this study, additional categorical analysis excluding participants with a BMI ≥ 40kg/m^2 (n = 188) found similar findings for both lower extremity and other fractures (Figure 7).

2.3.6 Discussion

In this large, cross-sectional, retrospective study of women (aged 60-82y), we have demonstrated the independent effects of obesity and dynapenia on risk of falls. We have shown that dynapenia confers an increased risk of all types of fracture. In contrast, the effects of obesity on fracture risk are site-specific. Lower extremity fracture risk is increased with both obesity (whether measured using BMI or waist circumference) and dynapenia irrespective of BMD. For all other fractures (wrist, arm, spine, hip, other bones), obesity is associated with a reduced risk of fracture except when accompanied by dynapenia.

A recent systematic review has shown that individuals with 'sarcopenic obesity' (obesity with reduced muscle mass or strength) have a similar risk of nonvertebral fractures to individuals with obesity alone [114] thus suggesting that there is no cumulative effect of sarcopenia on fracture risk. However, the available studies were relatively small, containing small subgroups [111, 112] and failing to consider the site-specific nature of fractures in people with obesity. Our findings are particularly novel as we have used a large cohort and recognized the site-specific fracture risk in people living with obesity. Our results reinforce the evidence regarding the site-specific nature of fractures in people with obesity, with their greater risk of sustaining ankle or leg fractures [10, 115, 116] but a reduced risk of other fractures [9, 10, 117]. However, our findings of the interaction between obesity and dynapenia on fracture risk are novel, namely that obesity increases the risk of lower extremity fractures (ankle, leg) and that dynapenia negates the positive effect of obesity on other fracture risk (wrist, arm, hip, spine, other bones). Consideration of both body weight and muscle strength are clearly relevant for determining and stratifying fall and fracture risk.

It is unclear the mechanism by which dynapenia may negate the protective effect of obesity on risk of other fractures. BMD is a well-acknowledged risk factor for fracture [208]; however, measurement of BMD is problematic in people with obesity due to confounding by body mass and bone size [209]. However, other quantitative computed tomography methods also support higher BMD, denser cortices, and increased trabecular thickness and number in people with obesity [202]. Comparison was made between groups with obesity and dynapenic obesity who would both be subject to the same measurement artifact, and, similar to previous studies [210, 211], BMD was reduced in the group with dynapenic obesity although the difference was small and unlikely to translate into a clinically significant difference in fracture risk [208]. The pathophysiology of dynapenia is multifactorial and can result from malnutrition, reduced physical activity, or co-morbid disease [129]; therefore, it is possible that these factors may also contribute to the negating effect of dynapenia although we were unable

to consider these in our analysis. Our findings tentatively suggest that the greater risk of fracture may relate to the cumulative falls risk observed, bearing in mind the differing recall period of both outcomes in the UK Biobank.

The majority of fractures result from falls from a standing height [212]. Separately, obesity and dynapenia are associated with a greater risk of falls [8, 105, 106] with growing evidence of a cumulative effect on falls risk, greater than from either phenotype alone [87, 89, 204]. Our findings support the notion of a greater impact or more injurious fall type in people with obesity [213]. Biomechanically, abdominal obesity is associated with an anterior shift in the centre of mass requiring greater torque for stabilisation [125]. Moreover, reduced soft tissue padding at the lower extremities, in combination with a high impact fall, may render these sites more liable to fracture in obesity [115]. Our findings are similar to those of Nielson et al. [214] who demonstrated that obesity was associated with greater risk of lower extremity fracture in 5995 older men (mean follow-up 7-years), but, in contrast with our results, the effect of obesity was attenuated by reduced physical performance and prior fracture history. Objective measures of lower limb performance were not available in the UK Biobank, and, as such, it requires further consideration as to whether other physical performance measures, especially of the lower extremities, may better relate to lower extremity fractures in women.

Our findings have important clinical implications suggesting that lifestyle interventions that target low muscle strength (either preventing or improving dynapenia), either alone or with measures to reduce excess body weight, could reduce both the risk of falls and fractures in women. Although evidence in younger adults suggests that modest weight reduction achieves greater benefits in balance [215, 216], findings in older adults with obesity have shown that weight loss alone exacerbates dynapenia [194]. Furthermore, weight loss without concomitant resistance or weight-bearing exercise can result in loss of BMD, particularly around the hip [192, 193], thereby potentially counteracting the reduction in fracture risk derived from a lesser falls risk. Notwithstanding, achieving a healthy weight is a public health priority considering the association of obesity with multiple chronic conditions/comorbidities (e.g., type 2 diabetes, cardiovascular disease, cancer [80]) in addition to falls and fractures risk. Indeed, the greatest gains in physical performance were derived from combined dietary and physical activity interventions [217] further highlighting the relevance of increasing physical activity and functional measurements, particularly of muscle strength, in weight management programs.

The limitations of this study must be acknowledged. Firstly, we were unable to validate the occurrence of fractures as International Classification of Diseases codes were only available for those who were admitted to hospital. Therefore, it is possible that participants may have misclassified or under-reported (e.g., spine) their (self-reported) fracture sites. Moreover, individuals with lower extremity fractures also had other fractures and therefore this may have affected associations. Secondly, this was a retrospective study and the inferences drawn should be viewed with caution as dynapenia or obesity may have occurred secondary to a fall or a fracture rather than being a driving pathophysiological factor. Longitudinal analysis was not feasible due to either an inadequate sample size at both visits or variability in baseline measurement collection time (5-14 years) prior to the outcome (previous 5 years). Thirdly, HGS is a surrogate measure of lower limb strength [21] but can be affected by other factors such as nutrition. Next, we could not replicate this present analysis in males due to the low prevalence of fractures in this group; therefore further work is required to confirm these findings in male individuals. Finally, the limitations of the UK Biobank in relation to generalisability must be considered which include its low-response rate, limited ethnic diversity, lower overall prevalence of overweight and obesity than the UK population and potential healthy volunteer bias (e.g., lower rates of diabetes, narrow socioeconomic backgrounds). However, strengths of the UK Biobank include its significant sample size and the detailed phenotyping available with evidence supporting that risk factor associations may be generalisable [205].

We have demonstrated that obesity and dynapenia have independent effects on falls risk but the relationship between obesity, dynapenia and fracture risk is anatomically site-specific. Dynapenia negates the protective effects of obesity on fractures of the wrist, arm, hip, spine, and other bones, suggesting that greater falls risk or other risk factors, rather than differences in BMD, may explain the negative effect of dynapenia. In contrast, both obesity and dynapenia are associated with a greater risk of lower extremity fractures, independently of BMD, suggesting fall type or fall force may be a mediating factor. These findings require validation in prospective analysis and in men but may have important clinical implications relating to risk identification and prevention of falls and fractures in a growing population living with overweight and obesity.

Acknowledgements

This research has been conducted using the UK Biobank Resource under Application Number 65223.

Funding

This work was supported by a studentship from the Medical Research Council (MRC) and Versus Arthritis as part of the Medical Research Council Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing (CIMA) [MR/R502182/1]. The MRC Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing is a collaboration between the Universities of Liverpool, Sheffield and Newcastle.

Table 5 (Supplementary) STROBE Statement—Checklist of items that should be included in reports of cohort studies

	Item No	Recommendation	✓ / Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	✓
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	✓
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓
		(*b*) Describe any methods used to examine subgroups and interactions	✓
		(c) Explain how missing data were addressed	✓
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	Fig S2
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Fig S1
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Tab1
		(b) Indicate number of participants with missing data for each variable of interest	Fig S1
		(c) Summarise follow-up time (eg, average and total amount)	N/A

Outcome data		15* Report numbers of outcome events or summary measures over time	✓ & Tab 1
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Supp Tab 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	✓, Fig1,3
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓
Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

**Give information separately for exposed and unexposed groups.*

Note: *An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PloS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.*

Table 6 Characteristics of participants according to BMI category and dynapenia status

	Normal Weight (BMI = 18.5-24.9kg/m^2)				**Obese** (BMI ≥ 30kg/m^2)				**Overweight** (BMI = 25-29.9kg/m^2)			
	Normal Strength		**Dynapenic**		**Normal Strength**		**Dynapenic**		**Normal Strength**		**Dynapenic**	
	N = 5279 (32.7%)		N = 2252 (14%)		N = 1812 (11.2%)		N = 985 (6.1%)		N = 3941 (24.4%)		N = 1878 (11.6%)	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age (years)	67	(7)	69	(8)	66	(7)	68	(8)	67	(8)	69	(8)
BMI (kg/m^2)	22.7	(2.5)	22.8	(2.5)	32.9	(4.2)	32.6	(4.1)	27.0	(2.3)	27.1	(2.5)
Waist Circumference (cm)	75	(10)	75	(9)	99	(12)	99	(12)	86	(10)	86	(10)
Hand-grip Strength (kg)	26	(6)	18	(4)	26	(5)	18	(4)	26	(5)	18	(4)
Ethnicity, n (%)												
White	5191	(98)	2182	(97)	1751	(97)	961	(97)	3857	(98)	1823	(97)
Other Ethnic Group	88	(2)	70	(2)	61	(3)	24	(3)	84	(2)	56	(3)
Bone Mineral Density*												
L1-L4 BMD (g/cm^2)	1.04	(0.21)	1.02	(0.21)	1.19	(0.25)	1.16	(0.25)	1.11	(0.23)	1.09	(0.23)
L1-L4 T-Score	-1.19	(1.76)	-1.31	(1.78)	0.04	(2.05)	-0.15	(2.10)	-0.56	(1.89)	-0.72	(1.93)
RFN BMD (g/cm^2)	0.83	(0.14)	0.81	(0.14)	0.93	(0.17)	0.90	(0.16)	0.88	(0.16)	0.86	(0.15)
RFN T-score	-1.27	(1.19)	-1.42	(1.21)	-0.44	(1.43)	-0.71	(1.37)	-0.88	(1.31)	-1.03	(1.28)
Physical activity level												
Mod Activity (mins/day)**	60	(60)	60	(60)	40	(40)	45	(40)	60	(50)	60	(60)
Mod Activity (days/wk)#	5	(4)	5	(4)	4	(4)	3	(3)	4	(3)	4	(5)
Falls, n (%)	1051	(19.91)	576	(25.58)	465	(25.66)	294	(29.85)	887	(22.51)	520	(27.69)
More than one fall n (%)	253	(4.79)	167	(7.41)	141	(7.78)	86	(8.73)	238	(6.04)	177	(9.42)
All Fractures, n	470	-	291	-	152	-	114	-	361	-	184	-
Lower extremity n	58	-	47	-	42	-	31	-	85	-	41	-
Other fractures n	412	-	244	-	110	-	83	-	276	-	143	-

Diabetes, n (%)	77	(1.46)	48	(2.13)	185	(10.21)	119	(12.08)	150	(3.81)	111	(5.91)
Smokers, n (%)												
Never	3462	(65.58)	1523	(67.63)	1091	(60.21)	619	(62.84)	2484	(63.03)	1218	(64.86)
Previous	1707	(32.34)	669	(29.71)	683	(36.69)	344	(34.92)	1361	(34.53)	626	(33.33)
Current	110	(2.08)	60	(2.66)	38	(2.10)	22	(2.23)	96	(2.44)	34	(1.81)
Alcohol, n (%)												
Never	181	(3.43)	125	(5.55)	103	(5.68)	67	(6.80)	153	(3.88)	121	(6.44)
Previous	159	(3.01)	80	(3.55)	73	(4.03)	63	(6.40)	132	(3.35)	84	(4.47)
Current	4939	(93.56)	2047	(90.90)	1636	(90.29)	855	(86.80)	3656	(92.77)	1673	(89.08)

**Missing measurements = L1-L4 BMD: n = 3439; L1-L4 T-score: n = 3445; RFN BMD n = 3271; RFN T-score n = 3278. **1334 participants did not know/answer this question, 2357 participants had missing measurements. #578 participants did not know/answer this question. Abbreviations: BMI = Body Mass Index; BMD = Bone Mineral Density; RFN = Right Femoral Neck; Mod = Moderate; wk = week.*

Table 7 Proportion of participants classified by both tertiles of handgrip strength and body mass index who were identified as being in the all other fractures or lower extremity fractures group.

	All Other Fractures		
	Normal weight	**Obese**	**Overweight**
HGS Tertiles	**N (%)**	**N (%)**	**N (%)**
High (≥26kg)	191 (6.95)	45 (4.74)	142 (6.70)
Medium (21-25.9kg)	178 (7.68)	55 (6.77)	106 (6.22)
Low (0-20.9kg)	202 (9.41)	75 (7.81)	124 (6.84)
	Lower Extremity Fractures		
	Normal weight	**Obese**	**Overweight**
HGS Tertiles	**N (%)**	**N (%)**	**N (%)**
High (≥26kg)	27 (0.98)	25 (2.63)	39 (1.84)
Medium (21-25.9kg)	31 (1.13)	17 (2.09)	44 (2.58)
Low (0-20.9kg)	41 (1.49)	30 (3.13)	41 (2.26)

Abbreviations: HGS = hand-grip strength

Table 8 Associations, expressed as odds ratios, between Z-scores of BMI, waist circumference, hand-grip strength and falls, lower extremity fractures and all other fractures

	Fall				Lower extremity fractures				All other fractures			
	OR	**[95%.**	**Interval]**	**P**	**OR**	**[95%.**	**Interval]**	**P**	**OR**	**[95%.**	**Interval]**	**P**
Model 1												
BMI	1.12	1.08	1.16	**0.000**	1.25	1.13	1.39	**0.000**	0.91	0.85	0.97	**0.005**
Waist Circumference	1.14	1.10	1.18	**0.000**	1.27	1.14	1.42	**0.000**	0.96	0.90	1.02	0.190
HGS	0.86	0.83	0.89	**0.000**	0.86	0.76	0.97	**0.015**	0.90	0.84	0.96	**0.002**
Model 1 + HGS Z-Scores												
BMI	1.12	1.08	1.16	**0.000**	1.25	1.12	1.38	**0.000**	0.91	0.85	0.97	**0.004**
Waist Circumference	1.14	1.09	1.18	**0.000**	1.27	1.13	1.41	**0.000**	0.96	0.90	1.02	0.168
HGS *	0.86	0.83	0.90	**0.000**	0.87	0.77	0.98	**0.023**	0.90	0.84	0.96	**0.001**
Model 1 + BMD												
BMI	-	-	-	-	1.30	1.15	1.46	**0.000**	0.98	0.91	1.06	0.584
Waist Circumference	-	-	-	-	1.33	1.17	1.52	**0.000**	1.02	0.95	1.10	0.601
HGS	-	-	-	-	0.84	0.74	0.97	**0.014**	0.92	0.86	0.99	**0.033**

*Z-score for HGS = 5.71kg, BMI = 4.56kg/m^2, Waist Circumference = 11.57cm. Model 1: adjusted for age, measurement centre, diabetes status, smoking status and alcohol status. * HGS = Model 1 + BMI Z-scores. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength; BMD = right femoral neck bone mineral density.*

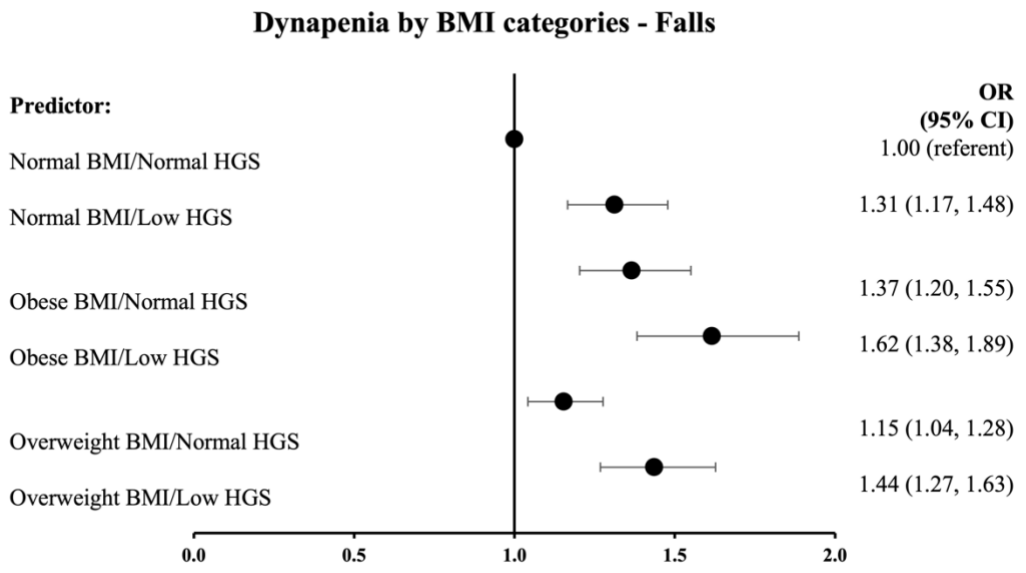
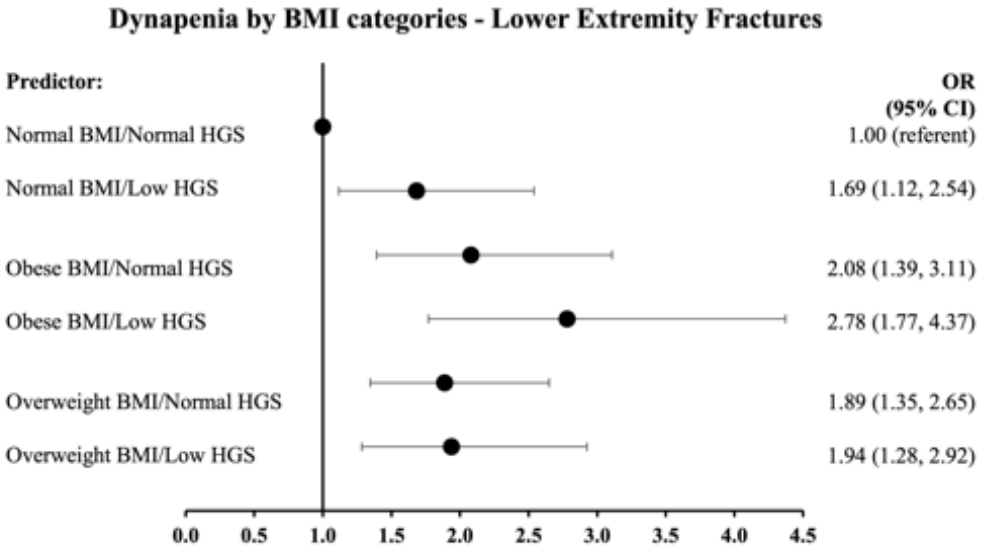


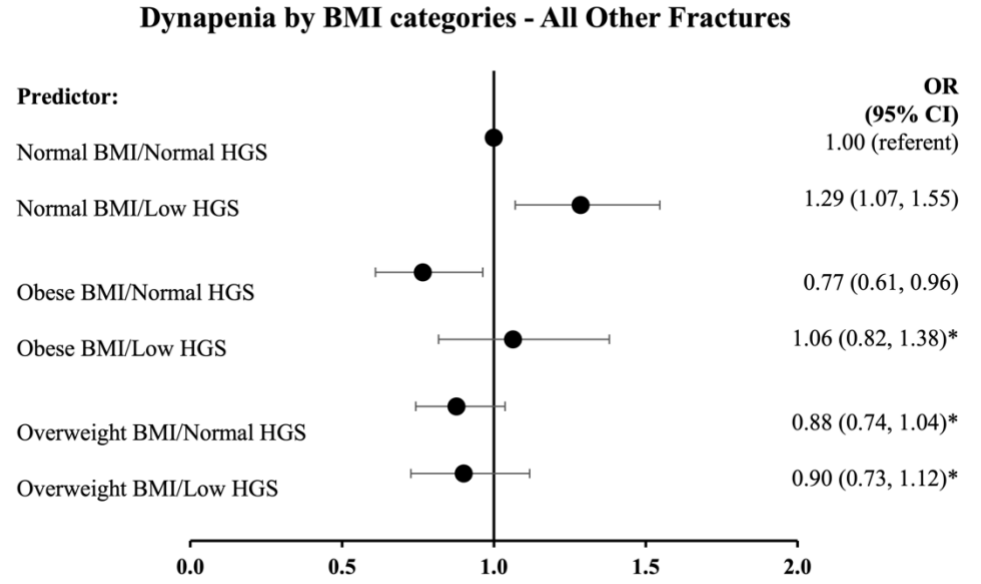
Figure 4 Association between dynapenia by BMI categories and falls risk

Adjusted for age, measurement centre, smoking status, self-reported 'diabetes' status, alcohol status.

** p > 0.05. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength.*



(a) Lower extremity fractures (ankle, leg)



(b) All other fractures (hip, spine, wrist, arm, other bones)

Figure 5 Association between (a) lower extremity or (b) all other fractures by dynapenia and obesity status

Adjusted for age, measurement centre, smoking status, self-reported 'diabetes' status, alcohol status.

**p > 0.05. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength.*

Supplementary Tables

Table 9 (Supplementary) Association between lower extremity fractures and obesity

	Normal Weight	**Overweight**				**Obese**			
BMI Categories	**Ref**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**
Model 1	1	1.57	1.20	2.06	**0.001**	1.90	1.39	2.60	**0.000**
Model 1 + RFN BMD	1	1.55	1.14	2.10	**0.005**	2.03	1.43	2.91	**0.000**
Model 1 + dynapenia (HGS)	1	1.57	1.20	2.06	**0.001**	1.88	1.38	2.57	**0.000**
Waist Categories		**Medium Risk**				**High Risk**			
Model 1	1	1.38	1.03	1.85	**0.033**	1.69	1.27	2.25	**0.000**
Model 1 + RFN BMD	1	1.39	1.00	1.93	0.053	1.83	1.32	2.54	**0.000**
Model 1 + dynapenia (HGS)	1	1.37	1.02	1.84	**0.037**	1.67	1.25	2.22	**0.000**

Model 1 – Adjusted for age, measurement centre, smoking status, self-reported 'diabetes' status, alcohol status. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength; RFN BMD = right femoral neck bone mineral density. Waist categories refer to normal (<80cm), medium risk (80-88cm), high risk (>88cm) [80].

Table 10 (Supplementary)Association between all other fractures and obesity

	Normal	Overweight				Obese			
BMI Categories	**Ref**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**
Model 1	1	0.81	0.71	0.93	**0.003**	0.79	0.66	0.95	**0.011**
Model 1 + RFN BMD	1	0.86	0.73	1.00	0.058	0.96	0.78	1.18	0.685
Model 1 + dynapenia (HGS)	1	0.81	0.71	0.93	**0.002**	0.78	0.66	0.94	**0.008**
Waist Categories		**Medium Risk**				**High Risk**			
Model 1	1	0.91	0.78	1.05	0.201	0.92	0.79	1.07	0.275
Model 1 + RFN BMD	1	0.99	0.83	1.17	0.867	1.05	0.88	1.25	0.609
Model 1 + dynapenia (HGS)	1	0.90	0.78	1.05	0.180	0.91	0.78	1.06	0.232

Model 1 – Adjusted for age, measurement centre, smoking status, self-reported 'diabetes' status, alcohol status. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength. Waist categories refer to normal (<80cm), medium risk (80-88cm), high risk (>88cm) [80].

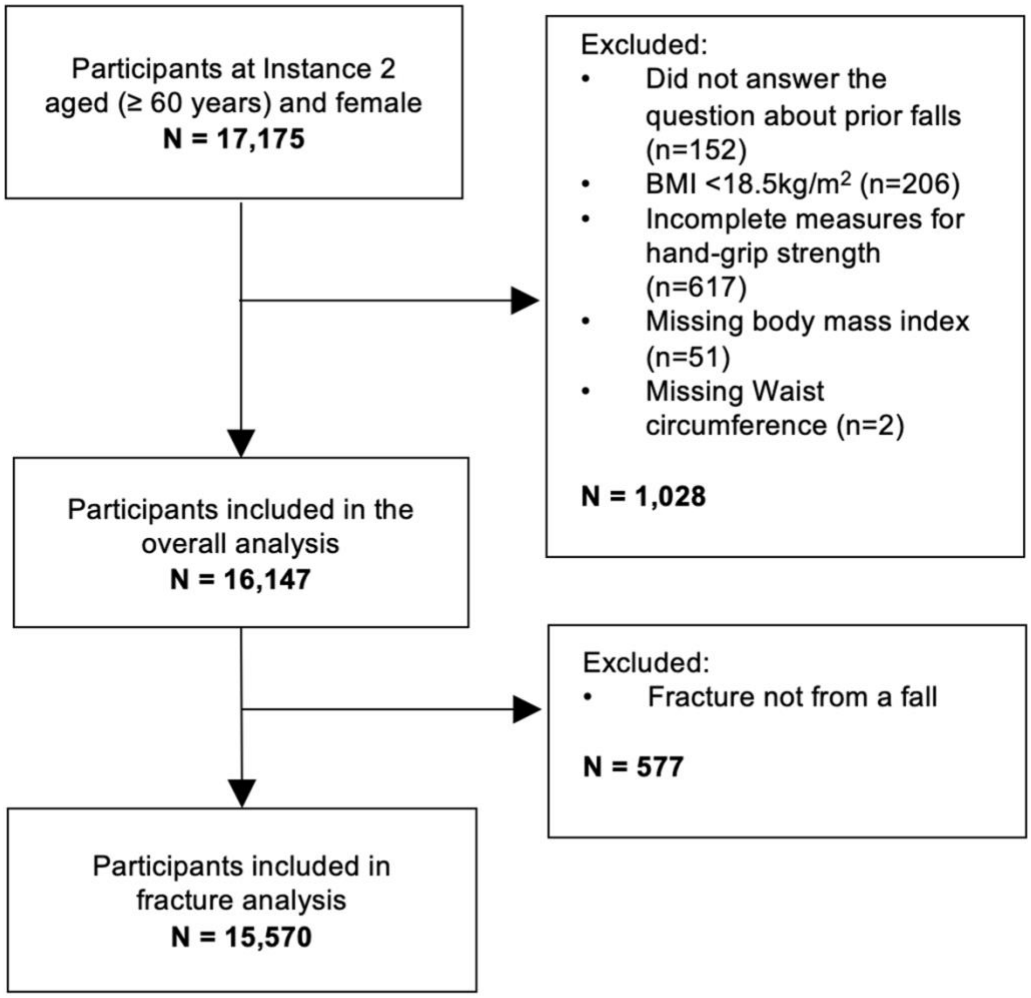
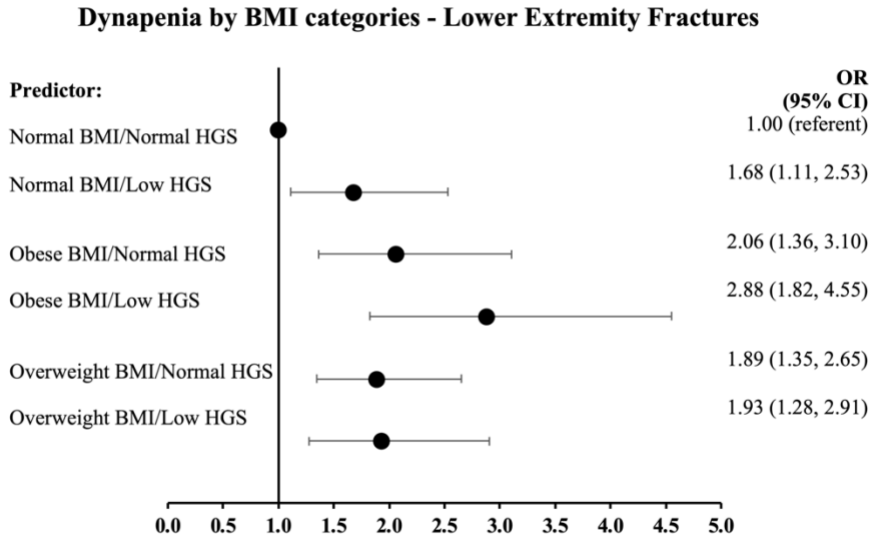
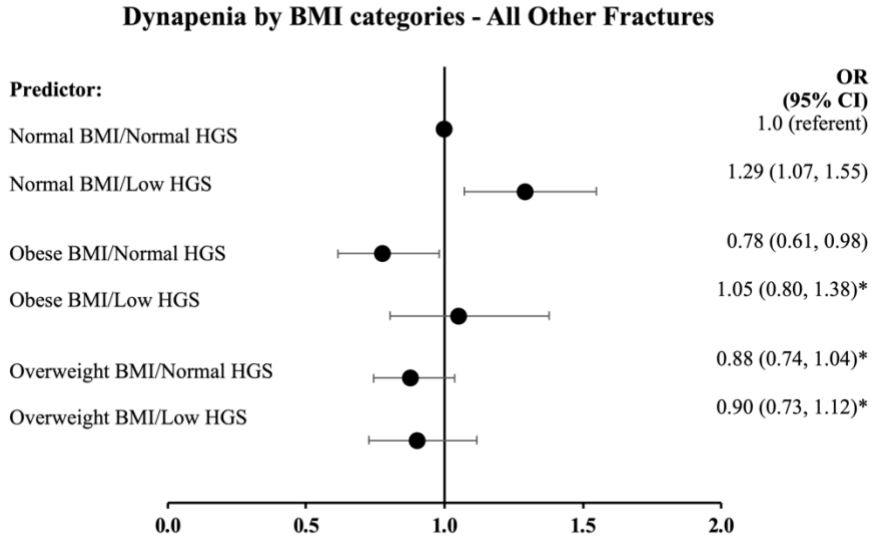


Figure 6 (Supplementary) Flow diagram of number of individuals at each stage examined for eligibility



(a) Lower extremity fractures (ankle, leg)



(b) All other fractures (hip, spine, wrist, arm, other bones)

Figure 7 (Supplementary) Association between (a) lower extremity or (b) all other fractures by dynapenia and obesity status excluding those with BMI ≥ 40kg/m^2 (n = 188)

*Adjusted for age, measurement centre, smoking status, self-reported 'diabetes' status, alcohol status. * p > 0.05. Abbreviations: BMI = Body Mass Index; HGS = Hand Grip Strength.*

Contribution details

Title	Dynapenic Abdominal Obesity as a Risk Factor for Falls
Publication Status	Published in Journal of Frailty and Aging https://doi.org/10.14283/jfa.2022.18
Authors	Lisa Dowling[1], Eugene McCloskey[1], Daniel Cuthbertson[2], Jennifer Walsh[1] [1]The University of Sheffield, Sheffield, UK [2]The University of Liverpool, Liverpool, UK
Authors roles	Conceptualisation: LD, JW; Methodology: LD, EM, JW; Formal Analysis: LD: Supervision: EM, DC, JW; Writing – original draft: LD; Writing – review and editing: all authors; Approval of final manuscript: all authors.
Student author	Lisa Dowling

Student author statement:
In publication I, the candidate, planned and wrote the manuscript, co-author reviewed and provided comments on the manuscript.

Name Lisa Dowling	Signature  Date 12/05/22

Co-author statement:
I hereby declare that I am aware that the work in the manuscript entitled: "Dynapenic Abdominal Obesity as a Risk Factor for Falls" of which I am co-author, will form part of the PhD thesis by the PhD student Lisa Dowling who made a major contribution to the work stated above.

Co-author name
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Supervisor confirmation
I have seen email or other correspondence from all co-authors confirming their certifying authorship.

Name Dr Jennifer Walsh	Signature  Date 12/05/22

Title page.

1. **Title of the manuscript**

   2.4 “Dynapenic Abdominal Obesity as a risk factor for falls”

2. **All authors’ full name(s), highest academic degree(s) and affiliations;**

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4. **Main text word count**

   3183 (including abstract, excluding title page and data elements)

5. **Number of data elements**
   4 (3 supplementary)

2.4.1 Abstract

Background: Obesity and low muscle strength (dynapenia) are independently associated with greater falls risk. It remains unclear whether dynapenia and obesity have an additive effect on falls risk, greater than either phenotype alone. **Objectives:** To determine whether a combination of abdominal obesity with dynapenia, dynapenic abdominal obesity (DAO), confers a greater risk of falls than either obesity or dynapenia alone in both men and women.

Design: An observational cohort study was conducted. **Setting and Participants:** Data from English adults (n=4239, 60-87 years) who took part in the English Longitudinal Study of Ageing were included. **Measurements**: Dynapenia, was defined as hand-grip strength <20kg (female), <30kg (male). Abdominal obesity was defined as waist circumference >88cm (female), >102cm (male). Data on falls and fall-related injuries over a 2-year follow-up were collected. Multiple logistic regression analyses were performed adjusting for age and sex, with results expressed as odds ratios (OR) and areas under the receiver operating characteristic curve (AUC).

Results: Falls occurred in 1049 participants, with 284 reporting a related injury during follow-up. DAO was associated with greater OR of falls in men (OR 2.1, 95% Confidence Intervals (CI) 1.3–3.2). Dynapenia rather than obesity was associated with falls in women, with greatest OR observed in those with low hand-grip strength (OR 1.36, 95% CI 1.09–1.71). Individual discrimination was low for measures of obesity or dynapenia either alone or in combination (AUC 0.51–0.58). There was no relationship between fall-related injuries and obesity or dynapenia.

Conclusion: Our findings suggest a synergistic effect of obesity with dynapenia on falls risk in men but not women.

2.4.2 Introduction

Falls are a major public health concern for older adults. Approximately 30% of older adults (> 65 years) fall yearly [11]. Worldwide data suggest that five percent of falls result in fracture and 30-50% of falls may result in minor injury [12]. Falls and their consequences represent a huge economic burden to the UK National Health Service, estimated at £2.3bn yearly [11]. With an ageing population, there is a growing need to be able to identify people predisposed to falls (fallers) in order to implement fall prevention strategies.

Individually, abdominal obesity [218, 219] and dynapenia (poor muscle strength) [105, 106] are associated with a greater risk of falling. Dynapenic abdominal obesity (DAO) is a phenotype of both low muscle strength and abdominal adiposity [92, 94, 96] thought to have the cumulative risk of both dynapenia and obesity. DAO is associated with worsening disability [92], hospitalisation [96], and mortality risk [94] and some studies suggest that individuals with DAO have a greater risk of falling than those with either obesity or dynapenia alone [87, 89, 204]. However, these studies on falls were small and did not consider if there were differences between men and women.

DAO may be a more clinically relevant phenotype given the already well-established adverse effects of central adiposity [220]. Moreover, excess body fat, particularly abdominally, can exacerbate both dynapenia [154, 221] and frailty [222]. This may relate to the low grade inflammation associated with obesity which can exacerbate loss of muscle mass and promote fat mass gain [129, 154].

Whether there is a cumulative risk of both dynapenia and obesity on falls risk requires further consideration. In this study, our primary aim was to determine whether DAO conferred a greater risk of falls than either obesity or dynapenia alone in both men and women. We hypothesised that men and women with both dynapenic and obese phenotypes have a greater risk of falling.

2.4.3 Methods

Participants

A description of the English Longitudinal Study of Ageing (ELSA) has previously been published [200]. In response to an ageing population, ELSA was designed to provide high quality longitudinal data in research areas focused on social status, physical and mental health, cognitive function and biology, in order to inform policy [200]. Briefly, participants living in England were drawn by postcode and stratified by health authority and socioeconomic status. The survey began in 2002/3 with subsequent waves at two-yearly intervals. Refreshment samples were added to maintain representativeness of people aged 50-75 years. ELSA is the first longitudinal study of older adults in England which is broadly representative of the English population [200].

Data collection

Data collected by ELSA includes information on health, disability, economic status, social support and household structure [200]. The main survey encompassed a face-to-face interview and paper self-completion questionnaire. Subsequent follow up occurred at different time points in 'waves'. At waves 2, 4, 6 and 8, eligible participants (those who remained living in private households in England) were offered a follow-up visit by a qualified nurse. All participants gave written informed consent. For this present analysis we used the anthropometric measures, functional tests, sociodemographic and clinical characteristics at wave 6 (2012/13) as our baseline and self-reported falls at wave 7 (2014/15) as our outcome measure i.e. after a 2 year follow up period. These waves had the most complete data for the relationships we wanted to study. Exclusion criteria were applied to the potential participants as described in Figure 8.

Measures

Falls and injuries At wave 7 participants aged 60 years and above were asked if, for any reason, they had fallen in the past two years or since the date of their last interview. This question was used to derive an outcome variable for incident falls since baseline (wave 6). If participants responded that they had fallen in the past two years, they were subsequently asked whether that fall resulted in an injury that required medical attention. Participants who could not recall whether they had a fall or injury were assumed to not have had a fall or injury in the analysis.

Independent variables

Sociodemographic factors included age and sex. Available self-reported data on co-morbidities included "diabetes or high blood sugar", coronary heart disease (CHD), arthritis (including osteoarthritis and rheumatism) and stroke (cerebrovascular disease). BMI was calculated as weight (kg) divided by height (m^2). Two measurements of waist circumference (WC) were recorded; a third was taken if the initial two measurements differed by 3cm or more. The average of these measurements was used in the analyses. Three measurements of grip strength were taken on both dominant and non-dominant hands using an isometric hand-grip strength device (Smedley) and a standing position was used for the majority of participants [3]. The maximum of these measurements was recorded as maximum hand-grip strength (HGS). A minimum of three hand-grip strength measurements was required for inclusion in this present study. The time taken to stand up and sit down five times from a firm chair without using arms was recorded as sit-to-stand (STS) time.

Dynapenia, obesity and dynapenic obesity

Dynapenia was defined as a hand-grip strength <20kg for women and <30kg for men [60], abdominal obesity as waist circumference >88cm for women and >102cm for men [80] and dynapenic abdominal obesity as the presence of both dynapenia and abdominal obesity. All participants were classed into one of four sub-groups (normal weight/non-dynapenic, dynapenic only, abdominal obese only and dynapenic abdominal obese).

EWGSOP2 criteria for dynapenia

Exploratory analysis was undertaken to determine whether alternative EWGSOP2 consensus measures of dynapenia (STS <15s; hand-grip strength <16kg for women and <27kg for men) [4] were also predictive of falls.

Statistical Analysis

Comparison between two groups was undertaken using either an independent t-test or Mann-Whitney U test; a chi-square test was used for categorical variables. Comparison between more than two groups was conducted using either one-way ANOVA with Tukey's post hoc test or Kruskal-Wallis test with Dunn's post hoc test. Multiple logistic regression was used to examine the association between measures of obesity, dynapenia or dynapenic abdominal obesity and incident falls in the next two years. Regression models were adjusted for age and sex, with results expressed as odds-ratios (OR) and discriminatory ability expressed as the area under the receiver operating characteristic curve

(AUC). Significance was accepted at $p<0.05$. Analysis was undertaken using Stata V16.1 (StataCorp 2019).

To address our aims, the following models were used

Individual variable models

Measures of obesity and dynapenia were explored separately as continuous or categorical variables. For continuous measures of dynapenia or obesity, Z-scores were calculated as an individual's result minus the population mean, divided by the population standard deviation. The use of Z-scores aimed to allow greater comparability between measures with different units e.g. centimetres, kilograms. Categorical variables of obesity and dynapenia were dichotomous for presence or absence of the phenotype according to consensus definitions. The non-obese or non-dynapenic groups were used as the reference group for the relevant analyses.

DAO models

The four sub-groups previously described, namely non-obese/non-dynapenic, dynapenic only, abdominal obese only, and DAO were included. The non-obese/non-dynapenic groups were used as the reference groups.

2.4.4 Results

Baseline characteristics A total of 8054 participants took part in the interviews and nurse visits at wave 6. Of the 5911 adults aged >60 years, 4239 adults aged 60–87 years had the complete data necessary for the analysis at waves 6 and 7 (Figure 8). Of these, 46% were male and 54% female (Table 11). Body mass index was similar between male and female participants, however men had greater waist circumference, hand-grip strength and quicker sit-to-stand times. More male participants reported that they had "diabetes or high blood sugar" or coronary heart disease (12.9% and 11.4%, respectively) than women (8.4% and 7.3%, respectively). More women reported that they had arthritis (including osteoarthritis and rheumatism; 47.4%) than men (31.4%). A similar proportion of men and women reported previously having a stroke (cerebral vascular disease).

Prevalence of abdominal obesity and dynapenia According to waist circumference, 53% (n=2241) were obese (59% of women, 46% of men, $p<0.001$). The proportion of adults with dynapenia was 16.9% (n=720; 21% of women, 13% of men, $p<0.001$).

Incident falls Twenty five percent of participants (n=1049) reported one or more falls in the two years between waves 6 and 7. Of those who fell, 60.5% fell once and 21.4% fell twice. More women fell (n=636, 28%) than men (n=413, 21%; $p<0.001$) but men reported a higher average number of falls (3.1 ± 11.7) than women (2.1 ± 8.2; $p=0.047$). Of those who fell, 27% (n=284) reported an injury that required medical attention; more women who fell reported injury (n=197, 31%) than men (n=87, 21%, $p<0.001$).

Association between falls, dynapenia and obesity The associations between abdominal obesity and dynapenia as continuous variables, and falls incidence, are shown in Table 12. In the whole cohort, abdominal obesity and dynapenia were individually associated with falls incidence. A clear difference in the associations emerged when the analyses were explored by sex. Higher waist circumference was significantly predictive of falls in men but not women. Lower hand-grip strength showed similar relationships with falls incidence in men and women but, in contrast to the overall analysis, did not reach statistical significance in either sex.

Next, we categorised participants according to the consensus definitions of abdominal obesity and dynapenia and examined the association with falls (Table 12). Similar patterns emerged whereby abdominal obesity was associated with falls in men but not women. Low HGS predicted falls in women but not in men.

Association between falls and dynapenic abdominal obesity According to the definition of DAO, participants were normal weight (neither obese nor dynapenic) (n=1671, 45.2% female), dynapenic only (n=327, 57.8% female), abdominal obese only (n=1848, 56.9% female), and DAO (n=393, 72.3% female). Waist circumference and BMI were similar between normal weight or obese groups with and without dynapenia. However, men who were classified as having DAO had a lower BMI (29.8 ± 3.1 kg/m^2) than the obese only group (31.4 ± 3.9 kg/m^2; p=0.0098). Dynapenic and DAO sub-groups were significantly older than normal weight and obese sub-groups, respectively.

In the total study population, both high WC and low HGS were significantly and independently associated with falls (Figure 9a). Thus, in a multivariate model containing both classifications, abdominal obesity (OR 1.3; 95% CI 1.1, 1.5) and dynapenia (OR 1.4; 95% 1.1, 1.6) were independently associated with falls incidence. All sub-groups of DAO had a higher OR of falling compared with the normal weight/non-dynapenic reference groups, independently of age and sex (Figure 9a). The combination of both dynapenia and obesity (DAO) had the strongest association (OR 1.7; 95% CI 1.3, 2.2) with falls incidence (Figure 9a.) compared to those classified as normal weight (neither obese nor dynapenic).

Analysis by sex identified that the combination of abdominal obesity and dynapenia was associated with falls incidence in men (Figure 9b). In contrast, both the joint-model analysis and four sub-group model confirmed that dynapenia rather than abdominal obesity was better associated falls in women (Figure 9c). The OR for dynapenia and DAO in women was similar, however the dynapenic sub-group was not significant.

Discriminative ability of dynapenia, obesity and dynapenic obesity AUC analysis was used to determine how well continuous measures of dynapenia, obesity or definitions of dynapenic abdominal obesity predicted falls at an individual level. The receiver operating characteristic curves (ROC) demonstrated that the discriminative ability of dynapenia (AUC 0.51; 95% CI 0.49–0.53) or abdominal obesity was low (AUC 0.57; 95% CI 0.55–0.59) For the definition of DAO, AUC was also low (0.56; 95% CI 0.54–0.58) indicating poor discriminative ability. The AUC was similarly low when the group was divided by sex (data not shown).

EWGSOP2 As part of an exploratory analysis, we examined the relationship between the more recent EWGSOP2 consensus criteria of dynapenia (STS, HGS) (22) and falls. Using EWGSOP2 criteria

(HGS <27kg (M), <16kg (F)), 7% (n=316) had low HGS (8% of women, 7% of men, p=0.076) and 14% had slow STS (<15s; 16% of women, 11% of men, p<0.001). Low HGS was not associated with falls in men or women. Sit-to-stand time predicted falls in women but this was not significant in men in either individual variable or joint model analysis (Supplementary Table 13).

2.4.5 Discussion

In this observational cohort study of English adults aged 60–87 years, we found that individuals with DAO had a greater odds of falling compared with normal weight adults. However, a key finding of this study was the sex-specific relationship between abdominal obesity, dynapenia and falls: the combination of abdominal obesity and dynapenia, or DAO, was only predictive of falls in men. In contrast, dynapenia alone rather than abdominal obesity was a stronger predictor of falls in women.

In line with others [92, 96, 111], prevalence of dynapenic abdominal obesity was 9% in this cohort. This coexistence of dynapenia and abdominal obesity is concerning considering the aggravating [154, 221, 222] and potential synergistic effects of both phenotypes [84]. In agreement with previous studies [87, 89, 204], we have demonstrated that DAO and its individual components are associated with falls incidence. Our novel findings that DAO is only predictive of falls in men with a lack of association observed between abdominal adiposity and falls in women, differ from the prospective study by Gadelha et al. [204] which found that the combination of dynapenia and abdominal obesity was associated with a greater risk of falling in older women. However, this study was small (n=201) and the individual phenotypes of dynapenia and abdominal obesity were not associated with falls. Other studies either did not consider sex separately [89] or looked at associations with falls risk rather than falls incidence [87].

There is reasonable correlation between upper and lower limb strength [21], thus potentially explaining the association between hand-grip strength and falls in both men and women. Additionally, hand-grip strength is associated with other factors (e.g. poor nutritional status [56]) which also associate with falls [12]. However, this does not explain the discordant effects of abdominal obesity in men and women.

One possible explanation for our observed sexual dimorphism may relate to differences in fat distribution (and thus body shape) in men and women influencing an individual's biomechanical movement. Menegoni et al. [121] found that greater BMI was associated with greater anterior-posterior instability in men and women whereas greater centre of pressure displacement and medio-lateral instability was only observed in men. The authors hypothesised that male (android) and female (gynoid) patterns of body fat distribution may explain this observation with an android shape characterised by greater mass/load over the hips and thus medio-lateral instability [12]. Moreover, increased body mass, particularly abdominally, requires greater ankle torque to maintain stability [12, 121]. Therefore, with regards to clinical intervention, although strength and balance training is

currently recommended for falls prevention [80], carefully monitored weight management may provide another important consideration [125] given that abdominal obesity is an additional independent predictor of falls in men.

Finally, we explored and compared the definition of DAO using the latest consensus definitions of low muscle strength [4]. The latest EWGSOP2 consensus suggests that dynapenia is sufficient to initiate clinical assessment and intervention [4]. It is therefore interesting that low hand-grip strength using EWGSOP2 criteria did not predict falls in our study. In agreement with others [66, 68], our findings suggest that further research is required to understand how these cut-offs associate with functional outcomes. In addition, we observed sex-specific differences for sit-to-stand time suggesting that functional measures relate differently to falls incidence in men and women. These results suggest that, for the outcome of falls, hand-grip strength and sit-to-stand time may not be comparable surrogates as proposed [4].

The limitations of this study must be acknowledged. First, falls and fall-related injuries were self-reported and are subject to recall bias and thus may be inaccurate. Second, participants with incomplete measures were excluded and reasons for non-completion were not always available. Third, an age-range of 60–87 years may be considered a heterogenous group in relation to falls prevalence [28, 223] and body composition [224]. However, a linear relationship was found between age and falls incidence, thus age was included as a covariate in our models. Fourth, analyses were exploratory and have not been corrected for multiple comparisons. Lastly, the effect of co-morbidities and other confounding factors (e.g. cognition, frailty, nutrition) requires further consideration. However, we did not include frailty as an independent variable as the Fried Frailty phenotype includes a measure of muscle strength and thus both are correlated.

Overall, dynapenia was associated with falls in men and women, whereas a link between abdominal obesity and falls was only evident in men. Consequently, a synergistic effect of abdominal obesity and dynapenia, DAO, and falls was only found in men. The use of these anthropometric and functional measures to identify patients at risk of falls is appealing due to their ease of clinical application and associations with adverse outcomes. A single cut-off approach has been utilised by numerous consensus committees [4, 58, 60] and further improves clinical acceptability and awareness. However, individual discrimination was low in this study suggesting that, in their current form, these measures may better serve as an adjunctive tool to clinical decision making [7]. Of clinical relevance, our findings suggest that aside from targeting regular physical activity and strength training in later life to prevent

dynapenia, weight maintenance and obesity prevention provide another potentially important public health intervention that may reduce the risk of falls in older people. This seems particularly relevant in older men considering our findings. The current challenge remains to find a way to operationalise a functionally-relevant definition of dynapenic abdominal obesity at an individual level and identify optimal treatment strategies.

Acknowledgements

The English Longitudinal Study of Ageing was developed by a team of researchers based at University College London, NatCen Social Research, the Institute for Fiscal Studies, the University of Manchester and the University of East Anglia. The data were collected by NatCen Social Research. The funding is currently provided by the National Institute on Aging in the US, and a consortium of UK government departments coordinated by the National Institute for Health Research. Funding has also been received by the Economic and Social Research Council.

Funding:

This work was supported by a studentship from the Medical Research Council (MRC) and Versus Arthritis as part of the Medical Research Council Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing (CIMA) [MR/R502182/1]. The MRC Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing is a collaboration between the Universities of Liverpool, Sheffield and Newcastle.

Conflict of interest:

None to declare.

Ethical standard:

ELSA has received ethical approval from the South Central — Berkshire Research Ethics Committee (21/SC/0030, 22nd March 2021).

Table 11 Descriptive characteristics of participants

	All n = 4239		**Male** n = 1960		**Female** n = 2279		**P**
	Mean	SD	Mean	SD	Mean	SD	
Clinical measures							
Age (y)	69.4	(6.6)	69.3	(6.6)	69.4	(6.6)	0.715
BMI (kg/m^2)	28.2	(4.8)	28.1	(4.2)	28.2	(5.2)	0.917
Waist circumference (cm)	96.3	(13.1)	101.9	(11.5)	91.5	(12.4)	**<0.001**
Functional measures							
Sit-to stand test (s)	11.4	(4.1)	10.9	(3.7)	11.8	(4.4)	**<0.001**
Max hand-grip strength (kg)	30.9	(10.5)	39.3	(8.5)	23.8	(5.7)	**<0.001**
Gait Speed (m/s)	1.0	(0.2)	1.0	(0.2)	0.9	(0.2)	**<0.001**
Medical co-morbidities							
Diabetes/high blood glucose (%)	444	(10.5)	252	(12.9)	192	(8.4)	**<0.001**
Stroke n (%)	152	(3.6)	74	(3.8)	78	(3.4)	0.536
CHD n (%)	391	(9.2)	224	(11.4)	167	(7.3)	**<0.001**
Arthritis n (%)	1696	(40.0)	615	(31.4)	1081	(47.4)	**<0.001**

Characteristics are presented as mean and standard deviation or n (%). P-value refers to comparisons between men and women. Abbreviations: BMI = Body Mass Index; WC = Waist Circumference; STS = Sit-to-Stand; HGS = Hand-grip strength; CHD = Coronary Heart Disease (angina or myocardial infarction). Note: 4035 participants had gait speed measurements.

Table 12 Associations (expressed as odds ratios) between continuous measures (Z-scores) and consensus definitions of abdominal obesity or dynapenia and falls, by sex

	All (n=4239)			**Male (n=1960)**			**Female (n=2279)**		
Adjusted	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P
Obesity									
WC (cm)	1.13	[1.10-1.22]	**0.001**	1.27	[1.13-1.44]	**<0.001**	1.06	[0.96-1.17]	0.225
Abdominal Obesity	1.27	[1.10-1.47]	**0.001**	1.47	[1.18-1.84]	**0.001**	1.14	[0.94-1.37]	0.176
Dynapenia									
HGS (kg)	0.87	[0.78-0.98]	**0.022**	0.89	[0.76-1.03]	0.129	0.87	[0.72-1.04]	0.128
Dynapenia	1.36	[1.13-1.63]	**0.001**	1.36	[1.00-1.87]	0.053	1.36	[1.09-1.71]	**0.008**

Logistic regression models adjusted for age and sex. Abdominal obesity is defined as WC >88cm for women, >102 for men; comparison is made to the reference non-abdominal obese group. Dynapenia is defined as HGS <20kg for women, <30kg for men; comparison is made to the reference non-dynapenic group. Abbreviations: OR = odds ratio; CI = confidence interval; BMI = Body Mass Index; WC = Waist Circumference; HGS = Hand-grip strength.

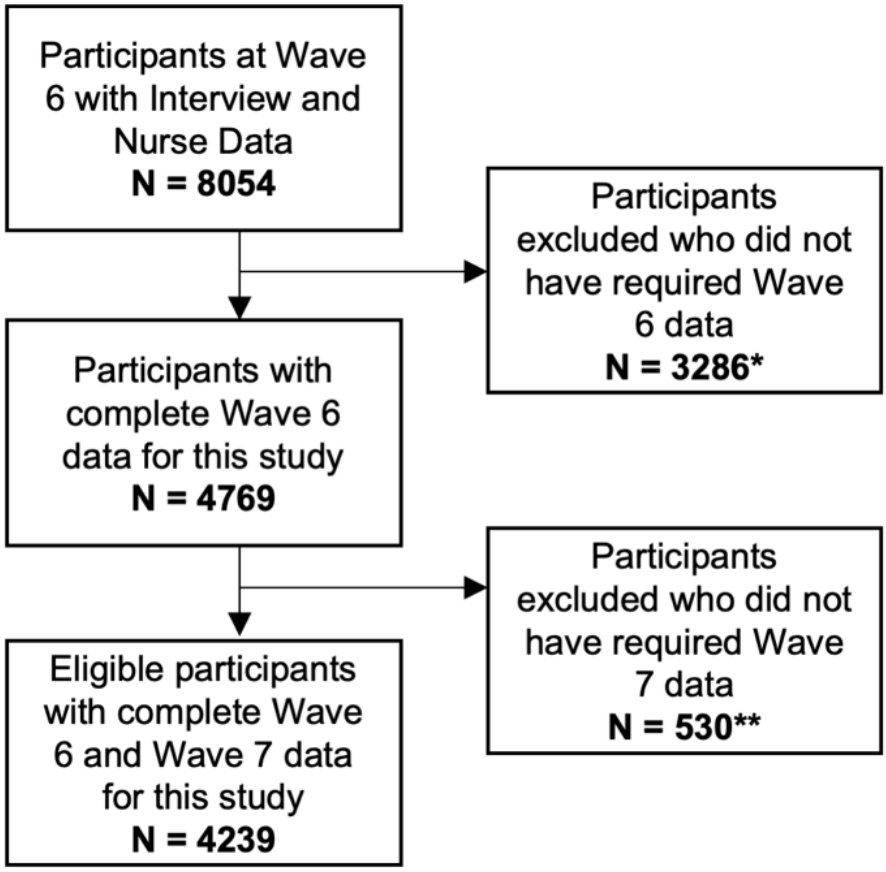
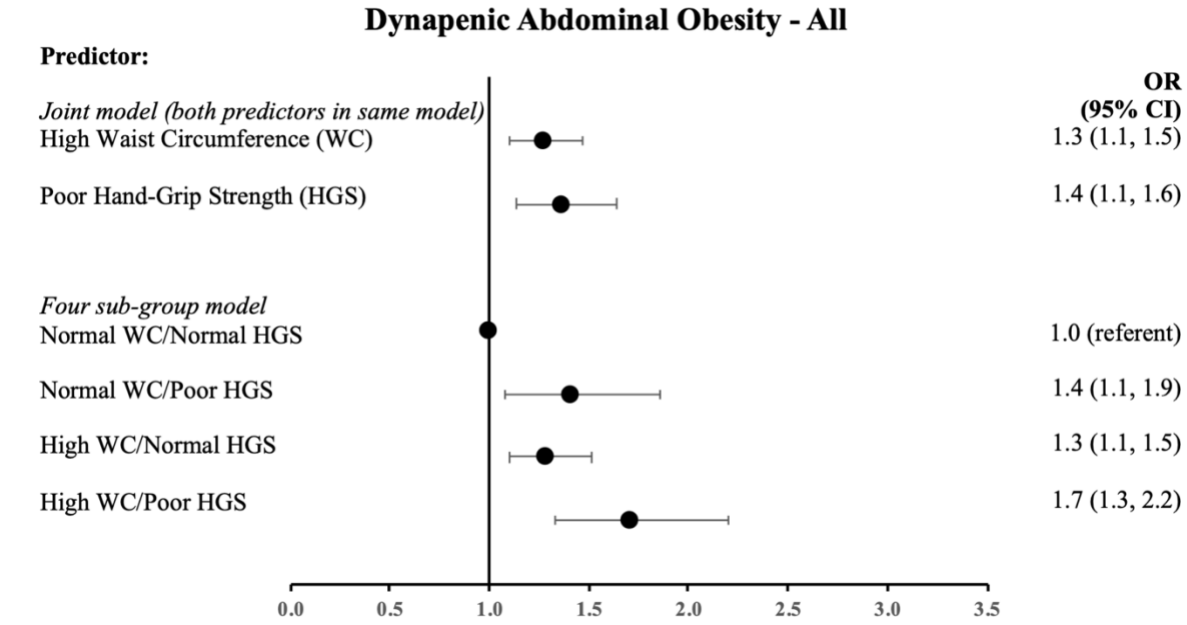
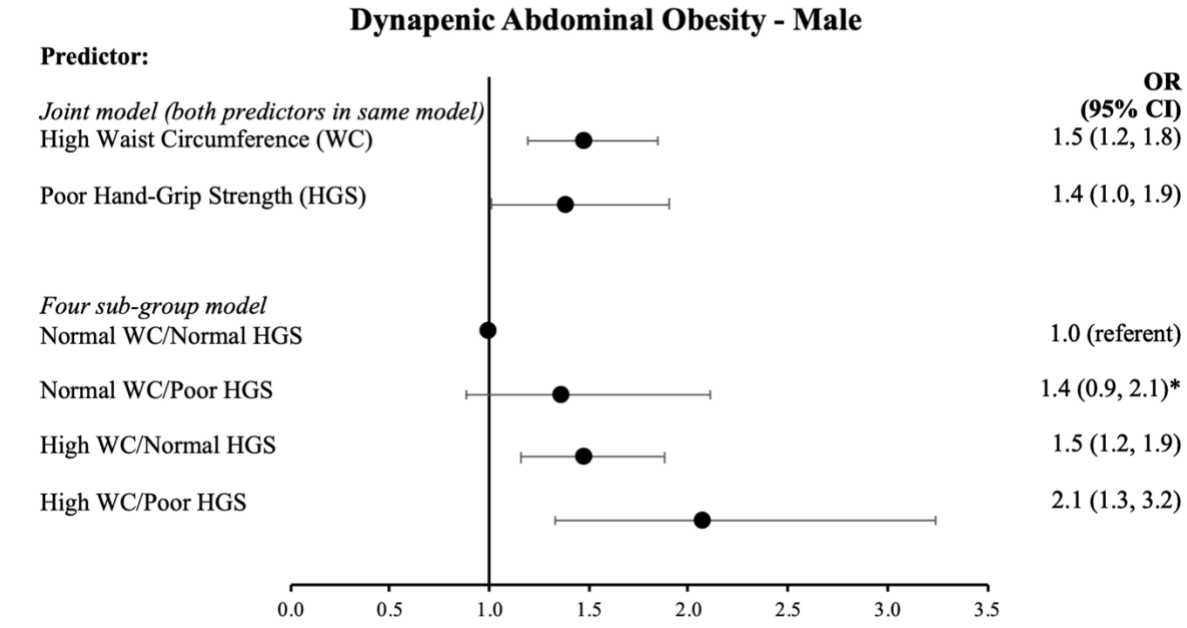


Figure 8 Flow diagram of number of individuals at each stage examined for eligibility.

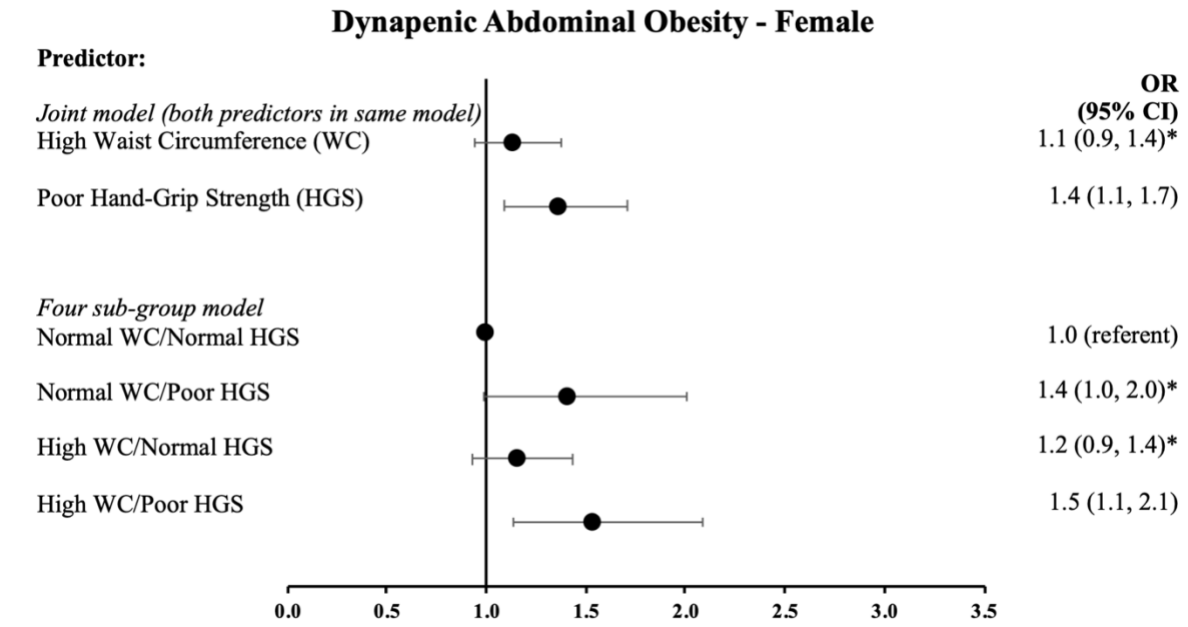
** Participants were excluded if they were < 60 years old (n=2143), had either no measurement, an incomplete or a zero measurement for waist circumference (n=0), body mass index or body mass index < 18.5kg/m^2 (n = 241), hand-grip strength (n=112), sit-to-stand time (n=789) or if they had refused to answer the question about falls in the previous 2 years (n=0). ** 530 participants were excluded if they did not complete, or refused to answer, the question about falls in the previous 2 years.*


Dynapenic Abdominal Obesity - All
Predictor:
OR
(95% CI)
Joint model (both predictors in same model)
High Waist Circumference (WC)
1.3 (1.1, 1.5)
Poor Hand-Grip Strength (HGS)
1.4 (1.1, 1.6)
Four sub-group model
Normal WC/Normal HGS
1.0 (referent)
Normal WC/Poor HGS
1.4 (1.1, 1.9)
High WC/Normal HGS
1.3 (1.1, 1.5)
High WC/Poor HGS
1.7 (1.3, 2.2)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

(a)


Dynapenic Abdominal Obesity - Male
Predictor:
OR
(95% CI)
Joint model (both predictors in same model)
High Waist Circumference (WC)
1.5 (1.2, 1.8)
Poor Hand-Grip Strength (HGS)
1.4 (1.0, 1.9)
Four sub-group model
Normal WC/Normal HGS
1.0 (referent)
Normal WC/Poor HGS
1.4 (0.9, 2.1)*
High WC/Normal HGS
1.5 (1.2, 1.9)
High WC/Poor HGS
2.1 (1.3, 3.2)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

(b)



(c)

Figure 9 Association between Dynapenic Abdominal Obesity and falls.

*Multiple logistic regression adjusted for age (b, c) and also sex (a). Dependent variable: Self-reported fall (yes/no). Joint-model independent variables: abdominal obesity expressed as high waist circumference (WC; >102cm M, >88cm F) and dynapenia expressed as poor hand-grip strength (HGS; <30kg M, <20kg F); the non-obese or non-dynapenic group was set as the reference. Four sub-group model: a categorical variable of dynapenic abdominal obesity (DAO); the normal WC/normal HGS group was set as the reference. Dynapenic Abdominal Obesity = WC (>102cm M, >88cm F) and HGS (<30kg M, <20kg F). Abbreviations: OR = odds ratio; CI = confidence interval. * Not significantly different from reference group.*

Supplementary Material:

Table 13 (Supplementary) Associations between consensus definitions of abdominal obesity or dynapenia (sit-to-stand time) and falls, by sex

		Obesity measure			Dynapenia measure		
Model	**Adjusted**	OR	[95% CI]	P	OR	[95% CI]	P
	Male						
1	Sit-to-stand time (STS)	-	-	**-**	1.36	[0.98-1.87]	0.064
2	WC / STS	1.45	[1.16-1.81]	**0.001**	1.28	[0.92-1.77]	0.139
	Female						
1	Sit-to-stand time (STS)	-	-	-	1.84	[1.44-2.35]	**<0.001**
2	WC / STS	1.09	[0.90-1.32]	0.364	1.82	[1.43-2.33]	**<0.001**

Consensus measures of abdominal obesity and dynapenia (STS < 15 seconds) as dichotomous variables were added to (1) individual (dynapenia only) or (2) joint logistic regression models (dynapenia and abdominal obesity), adjusted for age. The respective non-obese or non-dynapenic group was used as the reference. Abbreviations: OR = odds ratio; CI = confidence interval; WC = Waist Circumference.

Table 14 (Supplementary) Unadjusted associations (expressed as odds ratio) between characteristics, variables of obesity or dynapenia and risk of falls, by sex

	All (n=4239)			Male (n=1960)			Female (n=2279)		
Unadjusted	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P
Age (y)	1.29	[1.21-1.38]	**<0.001**	1.37	[1.23-1.53]	**<0.001**	1.23	[1.13-1.35]	**<0.001**
Sex (female)	1.45	[1.26-1.67]	**<0.001**	-			-		
Fall history	2.75	[2.37-3.20]	**<0.001**	3.42	[2.70-4.34]	**<0.001**	2.30	[1.88-2.80]	**<0.001**
Obesity									
Body Mass Index (kg/m^2)	1.09	[1.02-1.17]	**0.012**	1.21	[1.07-1.36]	**0.002**	1.04	[0.95-1.13]	0.387
Waist circumference (cm)	1.04	[0.97-1.11]	0.318	1.27	[1.12-1.43]	**<0.001**	1.06	[0.96-1.17]	0.227
Dynapenia									
Hand-grip strength (kg)	0.77	[0.71-0.83]	**<0.001**	0.76	[0.67-0.88]	**<0.001**	0.76	[0.64-0.90]	**0.001**
Sit-to-stand time (s)	1.32	[1.23-1.41]	**<0.001**	1.25	[1.11-1.40]	**<0.001**	1.33	[1.22-1.44]	**<0.001**

Measures of obesity, dynapenia and age are presented as Z-scores. Abbreviations: OR = odds ratio; CI = confidence interval.

2.5 Summary

In the UK Biobank, older women with obesity (BMI or waist circumference) had a greater risk of falls and lower extremity fractures; these risks were exacerbated by the presence of dynapenia. Abdominal obesity was not associated with other fractures. A greater BMI had a protective effect on all other fractures but this was negated by the presence of dynapenia. In women with obesity, dynapenia was not associated with clinically significant differences in bone mineral density compared to women with obesity-only. We were unable to replicate the findings from the UK Biobank in the English Longitudinal Study of Ageing. Obesity (BMI or waist circumference) was not associated with falls in older women whereas low hand-grip strength and slow sit-to-stand time were associated with an increased risk.

There are several reasons for our discordant findings. Firstly, an exploratory approach was used in the UK Biobank (tertiles of hand-grip strength) whereas in ELSA, consensus criteria were used (EWGSOP1 hand-grip strength [225]). An exploratory approach was used as, unlike sarcopenia, there is no formal consensus definition for dynapenia. Dynapenia is most commonly defined using hand-grip strength [89, 90, 92-94] (although other methods are sometimes used e.g. knee extension strength [96]). Several cut-offs for low hand-grip strength have been proposed by different working groups using different methodology – those proposed by EWGSOP1 [60], EWGSOP2 [4], and SDOC [58]. As these cut-offs have not been evaluated specifically in obesity and fracture prediction – tertiles were used so as not to make assumptions about thresholds. This is an approach which has been adopted by others [95, 111, 207]. ELSA was used to confirm the findings from the UK Biobank and to test consensus cut-offs. As EWGSOP2 criteria, most commonly used in DAO, was not associated with falls and fractures we used EWGSOP1 criteria. The EWGSOP1 criteria for low hand-grip strength approximate to the UK Biobank tertiles. The retrospective UK Biobank and prospective ELSA study designs also limit our comparisons in addition to differences in recall periods, sample sizes, and overall population representativeness.

Compared with the literature, a large systematic review and meta-analysis (n=1,758,694) found that obesity (BMI) was associated with an increased risk of falls [226]. This meta-analysis grouped men and women and, as shown in the ELSA study presented whereby obesity was associated with falls in men but not women, different associations may exist. A large retrospective study by Handrigan et al. (2017) found that BMI was not associated with falls in older women (n=9,461) in either unadjusted or adjusted models [74]. Indeed, controversy remains regarding the association between obesity and falls risk and this may relate to study design, for example, the original study's aims or phrasing of the

question regarding falls. The controversy may also relate to the location of body fat. Cho et al. (2018) (n=3,383) found that abdominal obesity was associated with a 1.37-fold increased risk (odds ratio) of retrospective falls whereas global obesity (BMI) was not associated with falls when included in the model [218]. The falls risk associated with abdominal obesity may relate to biomechanical differences [125]. Indeed, there has been a recent call to consider the presence of both global obesity (BMI) and abdominal obesity in individuals due to their independent associations with other factors such as mortality [6, 81].

In summary, our findings together with the literature suggest that abdominal obesity is associated with an increased risk of falls [125, 218, 227, 228] and lower extremity fractures; the risk of both clinical outcomes is exacerbated by the presence of dynapenia. Overall obesity (BMI) has a protective effect on other fractures but this is negated by the presence of dynapenia. Clinically, as dynapenia modulates the relationship of obesity with falls and fractures, consideration of both phenotypes is required to determine and stratify risk. Furthermore, lifestyle interventions targeting low muscle strength, either alone or with measures to reduce excess body weight, could reduce the risk of falls and fractures in women. However, more work is now required to elucidate the specific reasons for the increased risk of falls in older women with obesity. Identifying what and where deficits in muscle function exist, as well as their causes, can help identify the most effective screening and treatment strategies. This information can be used by clinicians and healthcare professionals to treat older adults with DAO. For example, understanding the phenotype of DAO using gold-standard techniques can help elucidate whether strengthening exercises focusing on specific muscle groups (e.g. hamstrings, quadriceps), minimal footwear which can improve ankle strength and balance [229], or interventions targeted at the nervous system (e.g. whole body vibration therapy [230]) or fat infiltration, may be effective. To date, however, the information available on older adults with DAO is from epidemiological studies or smaller studies which have not used gold-standard techniques. Therefore, detailed phenotyping studies using gold-standard techniques are required to identify potential interventions for older adults with DAO in order to reduce their risk of falls and fractures.

Chapter 3

Muscle strength, fatigue, and volume in DAO

3.1 Introduction

Older adults with dynapenic abdominal obesity (DAO) have a greater risk of falls, disability, hospitalisation, and mortality than older adults with either dynapenia or obesity alone [86-91, 94-96]. Dynapenia negates the protective effects of obesity on other fractures in women and increases the risk of lower extremity fractures in those with obesity (Chapter 2). We identified that this fracture risk may relate to an increased risk of falls (Chapter 2). However, the concept of DAO has primarily been examined from an epidemiological context using surrogate measures of adiposity, muscle strength, and function. A greater understanding of the phenotype of those with DAO in the context of physical and functional risk factors for falls may help elucidate the mechanism of greater falls risk in people with DAO. This information has the potential to inform interventions to reduce this risk.

Beyond epidemiological evidence, there is limited detailed characterisation of women with DAO. The available literature suggests that women with DAO and obesity have similar fat-free mass measured using DXA [87, 91]. Women with DAO have slower timed up and go (TUG) times and a higher prevalence of reduced reaction times (61.6% vs. 39.2%), but similar postural balance and prevalence of impaired sit-to-stand time as women with obesity [87]. Metabolic health parameters such as high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, glucose, and prevalence of metabolic syndrome are similar between older adults with dynapenic obesity (high BMI and low hand-grip strength) and obesity [123]. Similar findings were reported by Da Silva Alexandre et al. [122] in older adults with DAO. Overall, however, there is a paucity of information on the detailed phenotype of people with DAO using gold standard methods for muscle function and quantity. Muscle strength and fatigue can be objectively measured using gold-standard isokinetic dynamometry. A 6-minute walking test can be used to measure how muscles tire or fatigue in a setting more closely related to 'real-life'. Gold-standard MRI can be used to quantify the volume of specific leg muscles.

Lower extremity strength is critical for basic tasks of functional mobility and particularly relevant to outcomes such as falls and fall-related injury as the lower limbs are primarily responsible for postural control [231]. The inverted pendulum model is a valid postural control strategy which is based on the theory that the body is rigid and rotates on the ankle joint [231]. The ankle muscles maintain posture by generating torque to correct any disturbance which may displace the body's centre of mass. If balance cannot be corrected with the ankle strategy alone, the knees and hips are progressively engaged [232]. Isokinetic dynamometry is the gold standard for strength assessment as it can isolate the torque produced by a specific joint (e.g. hip, knee, ankle). Isokinetic dynamometry offers more

specific information than sit-to-stand which involves knee extension, dorsiflexion, trunk flexion, and hip rotation [233]. Thus, characterisation of strength and endurance deficits in this model may contribute to understanding the greater risk of falls in people with DAO.

It is generally accepted that absolute strength measured using isokinetic dynamometry is greater in people with obesity than in lean counterparts [234, 235]. However, absolute strength fails to account for the effect of body size. Moreover, muscle strength is influenced by factors such as muscle volume, geometry, fat infiltration, neural factors and overall muscle quality [21, 22]. Muscle quality is a multi-faceted concept which includes factors such as glucose and protein metabolism, fat infiltration, muscle contractility, fatigability and structural composition [236]. To date, there is no specific definition of muscle quality but the concept of specific strength (peak torque divided by body weight, lean mass or muscle cross sectional area) is most often used in the field of ageing and sarcopenia [236]. Thus, specific strength is used to interpret absolute strength in the context of overall body size and can provide an insight into muscle quality.

Based on the metric of specific strength, people with both sarcopenia [237] and obesity [234, 235] tend to have lower muscle quality. However, caution is required when extrapolating these findings to women with DAO due to differing definitions and age ranges in the available studies. Furthermore, the most common definitions of specific strength used are knee extensor/flexor torque adjusted for whole body mass, DXA acquired appendicular lean mass (ALM) [234, 235] or muscle cross-sectional area from a single magnetic resonance (MR) slice [238]. DXA derived ALM can include arm or calf mass and it is increasingly acknowledged that lean mass is an imprecise surrogate measure of muscle mass [239]. Depending on the slice used, a single midthigh MR slice has been shown to have an error of 10% when estimating muscle volume [240].

A better understanding of the relationship between strength and muscle volume can offer insight into both the pathophysiology of dynapenia in obesity and the increased risk of falls observed. MR imaging can be used to calculate muscle volume [241]. Others have shown that total thigh muscle volume is highly correlated with power (sit-to-stand time, leg push power) and knee extensor muscle strength (hand-held dynamometer) in healthy older women [242]. However, whilst this study included individuals with obesity, it did not specifically consider whether the relationship is altered in those with obesity [242]. Moreover, the thigh muscle volume calculated was not specific to the muscles being utilised i.e. knee flexors were included when the knee extensors were the most relevant muscle

group to the functions measured [242]. Thus, muscle quality (as specific strength) is still not fully understood in obesity and has not been explored in those with DAO.

Muscle fatigue refers to the decline in muscular capacity to generate strength [243]. Age, BMI, gender and pain status influence fatigue-related decline as well as protocol-related factors such as the nature of the task (static/dynamic) and the muscle groups measured [243]. Many daily and physical activities require repetitive contractions of the lower limb muscles (e.g. walking, stair climbing) which could fatigue a person with abdominal obesity and increase their susceptibility to falls. There is evidence that functional (dynamic e.g. from walking) and localised (static e.g. from weights) fatigue impairs balance in older adults [244, 245]. In a study of obese younger adults undertaking voluntary isometric quadriceps contractions, a lower threshold to fatigue was also shown [234]. However, others have shown contradictory findings [234, 243]. No difference was found between obese and non-obese younger adults when fatigue was electrically stimulated [234]. Similarly, there was no difference in a voluntary isometric force-control fatigue test of the knee extensors between older women with and without obesity [243]. Overall, there is a lack of information on muscle fatigue in abdominally obese older adults with and without low muscle strength. This information is of importance as a lower threshold to fatigue may impair physical performance, specifically balance and gait, and also provide an insight into muscle quality. A 6-minute walking test can look at how muscles tire or fatigue in a 'dynamic' setting or one that is more closely related to 'real-life'. Localised or static fatigue using isokinetic dynamometry can provide a more objective measurement of how specific leg muscles fatigue.

Summary

DAO is a phenotype associated with a greater risk of fractures compared to those with obesity-only which may result from a higher risk of falls. However, DAO has primarily been examined from an epidemiological perspective with little characterisation using gold-standard techniques. A detailed phenotyping analysis of older women with DAO may help elucidate potential mechanisms (strength, fatigue, and body composition) underlying the increased risk of falls and subsequent fractures in this phenotype. Understanding mechanisms which contribute to an increased risk of falls has the potential to identify suitable interventions.

3.2 Aims and Hypotheses

Aims

Identify specific muscle features of DAO which may relate to the increased risk of falls in older women with DAO, compared with normal weight, obese and DAO older women

1. **Muscle strength** – knee extensor and flexor muscle strength (torque and force; absolute and expressed relative to total body weight, lean mass and muscle volume).
2. **Muscle fatigue** – specific muscle fatigue from isokinetic dynamometry (number of repetitions) and dynamic fatigue from a 6-minute walking test (distance walked).
3. **Muscle volume** – volume of knee extensors and flexors (absolute, adjusted to thigh volume).

Hypotheses

1. Dynapenia AND obesity in older women is associated with greater impairments in muscle function (i.e. knee extensor and flexor muscle strength and fatigue) compared with older women who have EITHER normal weight OR obesity.
2. Dynapenia AND obesity in older women is associated with reduced muscle volume (reduced absolute volume of knee extensor and flexor muscles) compared with older women who have EITHER normal weight OR obesity.

3.3 Methodology

3.3.1 Muscle in Obesity Study Design

The Muscle in Obesity Study was a single centre, observational, cross-sectional study. The study was conducted at the Royal Hallamshire Hospital (Biomedical Research Centre) and Northern General Hospital (Clinical Research Facility, Radiology Department) in Sheffield, UK. This study was registered on ClinicalTrials.gov (NCT04300504). The serum samples of participants from this study were used for microRNA analysis in Chapter 5.

The total study sample consisted of 27 older women recruited from the general population. Participants were normal weight (waist circumference ≤88cm, BMI 18.5-25kg/m^2) and obese (waist circumference >88cm and BMI 30-40kg/m^2) older women (age 60-80 years) further stratified by presence or absence of dynapenia (dynapenia identified by sit-to-stand time >15s). Of these participants, 10 were classified as normal weight without dynapenia, 10 abdominal obese without dynapenia and 7 dynapenic abdominal obese (DAO). Poor sit-to-stand time, rather than hand-grip strength, was chosen as this measurement of dynapenia could be assessed remotely during the Covid-19 pandemic. Interchangeability of these tools to measure low muscle strength (dynapenia) is supported by international sarcopenia guidelines [4].

Participants were assigned to one of the following study groups:

1. Normal weight, non-dynapenic = waist circumference ≤88cm, BMI 18.5-25kg/m^2, sit-to-stand time <15s
2. Non-dynapenic obese = waist circumference >88cm, BMI 30-40kg/m^2, sit-to-stand time <15s
3. Dynapenic abdominally obese = waist circumference >88cm, BMI 30-40kg/m^2, sit-to-stand time >15s

3.3.2 Ethical Approval

Ethical approval was granted by the Leeds West Research Ethics Committee (REC Reference 20/YH/0274). Virtual primary screening (telephone) did not require written consent due to self-report. All participants provided written informed consent prior to either secondary screening or enrolment to the study and in accordance with Good Clinical Practice guidelines.

3.3.3 Inclusion and Exclusion Criteria

Inclusion Criteria:

Ages 60-80 years

This group includes post-menopausal women who are particularly susceptible to fractures [10, 117, 246].

Women

Women have both a higher risk of falling [223, 247] and a greater risk of fracture [246] than men. We therefore chose to study women.

White/Caucasian

Ethnicity is known to affect measurements such as body composition, strength [224] and microRNA profile [248]. Therefore, we chose to recruit White/Caucasians only to avoid confounding by ethnicity.

BMI 18.5-25 AND waist circumference <88cm i.e. normal weight AND abdominally obese; BMI 30-40kg/m^2 AND waist circumference >88cm i.e. obese AND not abdominally obese

The World Health Organisation (WHO) BMI and waist circumference obesity classifications were used to group normal weight and obese individuals. Whilst it is generally accepted that the obesity component of dynapenic abdominal obesity is based on WHO classifications for waist circumference only, groups were stratified according to both waist circumference and BMI. Evidence from previous studies conducted in the research group suggested that recruitment based on either BMI or waist circumference would result in the groups overlapping [202]. By selecting on both criteria, the groups were obese and abdominally obese and normal weight and not abdominally obese to avoid potential confounding i.e. inclusion of obese participants in the non-obese group.

Sufficiently mobile to undergo scanning and biomechanical testing

Able to remain motionless during scans

Able and willing to participate in the study and provide written informed consent

Exclusion Criteria:

History of any long-term immobilisation (duration greater than 2 weeks in the past 12 months)

Periods of prolonged (> 10 days) muscle disuse lead to a 0.5-0.6% daily loss of lean mass [50]. This is especially pertinent as this may exceed the yearly (~1-2%) estimated reduction in muscle mass [224].

History of hospital admission in the past 3 months

Hospital admission may result in muscle disuse or inflammation, which could affect body composition or strength. Evidence suggests that even at 3 months, reductions in muscle strength and muscle mass were still observed in some [249].

History of recent significant weight loss (5% in 3 months or 10% in 6 months)

This is considered as clinically significant weight loss and indicates malnutrition [250]. Weight loss has been shown to alter microRNA expression [251] and malnutrition results in decreased muscle mass and adverse functional outcomes [56].

Diabetes mellitus

Individuals with diabetes mellitus exhibit a different microRNA profile to those without [252].

History of current conditions which may affect muscle metabolism

- **Malabsorption syndromes e.g. inflammatory bowel disease, pancreatic insufficiency etc.**
- **Chronic renal disease**
- **Diagnosed eating disorder**

Conditions which prevent the undertaking or analysis of the MRI and DXA scans or the interpretation of their results e.g. hip prosthesis, metal implants etc.

Conditions which prevent the undertaking of the fatigue protocols e.g. hypertension etc.

Use of medications or treatment known to affect muscle metabolism

- **Anabolic steroids, glucocorticoids, antiretrovirals etc.**

Excessive alcohol intake defined as greater than 21 units per week

Competitive athlete, defined as participating in competitive sport at amateur or professional level

Conditions which prevent the undertaking of a 6-minute walking test e.g. unstable angina or myocardial infarction during the previous month.

3.3.4 Study Procedures

Participants attended the Biomedical Research Centre, Royal Hallamshire Hospital, Sheffield for Visit 1, and the Clinical Research Facility and Radiology Department, Northern General Hospital, Sheffield for Visit 2 and MRI Visit, respectively.

Table 15 Overview of study visits and procedures

Visit	Timeframe	Procedures
Pre	Minimum 24hr before Visit 1	Primary Screening
Pre	Prior to Visit 1	Secondary Screening and informed consent including height, weight, hip and waist circumference, BMI, hand grip strength
1	Baseline	Standard Health questionnaire, Muscular Strength and Fatigue Tests (isokinetic dynamometer and fatigue protocol), Questionnaires (physical activity, food frequency, falls), Fasting blood sample
2	7 days to 6 months after Visit 1	DXA, muscle function tests (SPPB, timed up and go), 6-minute walk test with wearable gait sensors
MRI	Prior to, or within 6 months of Visit 1	Magnetic Resonance Imaging

Abbreviations: hr = hour; BMI = Body Mass Index; DXA = dual-energy X-ray absorptiometry; SPPB = short physical performance battery.

Anthropometry

Height was measured in metres to the nearest 0.1cm using a wall-mounted stadiometer (Seca 242, Seca, Birmingham, UK). Weight was measured in kilograms (kg) to the nearest 0.1kg using a mechanical scale (Salter, Kent, UK). Hip circumference was measured standing to the nearest 0.5cm at the widest point of the hips – an average of up to three measures was taken.

Anthropometric Measures of Adiposity

Body Mass Index

BMI = weight (kg) / height^2 (m^2).

Waist Circumference

Waist circumference was measured with a non-stretchable tape (Seca 303; Seca, Hamburg, Germany). Measurements were made with the participant standing, to the nearest 0.5cm, midway between the

uppermost border of the iliac crest and the lower border of the costal margin at the end of exhalation – an average of up to three was taken.

Sit-to-stand Time

Poor sit-to-stand time was chosen to identify dynapenia as recommended by international sarcopenia guidelines [4] and this measurement could be assessed remotely during the Covid-19 pandemic. Participants were asked to stand up and sit down on a slightly padded chair (47cm height from floor to seat, 47cm depth, 19cm height of back, 46cm width) five times as quickly as possible without stopping and with arms folded across their chest. They had a maximum of one minute and the sit-to-stand test was ended after this time. Time to complete five sit-to-stands was recorded.

Hand-grip Strength

Hand-grip was measured using a Takei Physical Fitness Test (GRIP-D item# 5101, Takei Scientific Instruments Co. Ltd., Tokyo, Japan) in a seated position according to a standardised protocol [253]. The forearm was supported by the arm of the chair and wrist just over the edge in a neutral position. The base of the dynamometer was supported by the researcher. Participants were asked to squeeze the dynamometer as hard as they could. Participants started with the right hand and alternated hands until three were completed on each side. The maximum value was reported [3].

Questionnaires

Health questionnaire

A standard health and lifestyle questionnaire (Metabolic Bone Centre medical questionnaire) was completed to collect data on demographics, medication usage, smoking status and presence of co-morbidities.

Physical activity questionnaire

Participants completed a physical activity questionnaire to establish habitual levels. The EPAQ2 is a self-completed questionnaire which is representative of the past year. The questionnaire consists of three sections detailing activity at home, work and recreation. Validation studies have been completed in a similar age group (60-64 y) and have found similar activity ranking compared with heart rate and movement sensors [254].

Comparisons of moderate activity (mins/day) were compared with the Chief Medical Officer's recommendations (150 mins/day) [255].

Falls history

A history of falls was taken from all participants. Participants were advised on the definition of a fall based on NICE guidelines [256]. Participants were then asked a standardised question to determine whether they fell in the last 3 or 12 months [247, 257].

"Have you fallen in the last [three months / year]?"

Food frequency questionnaire

Participants completed a validated food frequency questionnaire which was used to estimate their average energy, macronutrient and micronutrient intakes. EPIC-Norfolk is a food frequency questionnaire designed to measure a participant's usual food intake during the previous year and has been validated in this age group (45-75 years) [258]. Both a strength and a limitation of this questionnaire is that the food list and portion sizes represent an adult population with an established eating pattern who follow a traditional UK diet. FETA GPL v2.53 was used to calculate nutrient intakes [259].

Estimated total dietary protein intake was adjusted to body weight (g/kg) and compared with the UK Recommended Nutrient Intake (RNI) (0.75 g/kg) [260] and the European Society for Clinical Nutrition and Metabolism (ESPEN) recommendation for older adults (1.0 – 1.2 g/kg) [261].

3.3.5 Assessment of Muscle Mass with Imaging

DXA

Principles of DXA

DXA involves a radiation source aimed at a radiation detector immediately beneath. The X-rays contain both high and low photon energies. The participant is placed in the path of the radiation beam and the photons are either absorbed or scattered, termed Compton scattering. This attenuation of the radiation beam is then related to body composition (BMD, fat mass, fat-free mass) [262, 263].

DXA is advocated by the World Health Organisation as the gold-standard for assessment of osteoporosis with validated reference ranges available [264]. It is also recommended in the

measurement of lean mass for the assessment of sarcopenia [4]. It has proven good precision, stable calibration, short scan times and low radiation dose [262].

DXA Protocol

A Hologic Horizon (Hologic Inc., Bedford, MA, USA) DXA scanner was used for all scans. Prior to the scan, participants were asked to wear clothes without any metal items (e.g. clips, zips, buttons) or offered a gown. For the total body scan, the participant lay supine, in a straight and central position on the scan table, with the head placed near the top of the table. Arms were placed alongside but not touching the body or hips with hands placed palm down. For the total hip scan, participants lay supine on the bed with the proximal femur internally rotated and leg flat on the scanner bed.

DXA Outcomes

Bone area (cm^2), bone mineral content (g) and mean areal bone mineral density (g/cm^2) were determined for the whole body (minus head) and total hip. Total, trunk and appendicular lean and fat mass (kg) were determined for the whole body and limbs.

DXA Calibration

The DXA scanner was calibrated according to standard operating procedures. Briefly, the scanner was calibrated daily or prior to each scan using the Hologic spine phantom. A range of 1.5% was deemed acceptable.

Limitations of DXA

Limitations exist with the use of DXA. Firstly, due to there being only two X-ray attenuation processes, DXA can only distinguish two types of tissue – bone and fat [262]. Therefore, DXA is able to distinguish bone from soft tissue but, if the composition of the tissue over-laying bone is not known, this introduces an artefact to the BMD measurement [262]. In this case, an assumption is made that soft tissue over-laying bone has the same fat mass : lean mass ratio as that for non-bone pixels of the same region [265]. Secondly, DXA is based on the assumption that hydration of lean mass is constant at 0.73 [266] and is therefore influenced by hydration status [263]. Lastly, DXA measures 'lean mass' rather than muscle or protein mass which can vary independently of the true change in protein mass [265] and lean mass is increasingly acknowledged as not being the best surrogate of muscle mass in men [97].

Limitations of DXA in obesity

Practical limitations relate to the size of the scanning area for common scanners – 0.66m (26”) width and 1.95m (77”) height with weight limits of 450lb (204kg) for the Hologic Horizon used in this study. Participants who exceeded the weight limit were therefore not recruited. Positioning within bed width limits can be difficult in participants with obesity; a half body scan can be used in such instances. However, error can be introduced with this method if the midlines are misaligned or where there are anatomical differences between the two halves.

Areal BMD measurements are confounded by anterior-posterior body thickness which absorbs the X-ray beam resulting in increased attenuation. Studies which have examined the effect of fat layering on phantoms have found increasing aBMD with increased fat layers [267]. Lastly, DXA computes an ‘areal BMD’ (aBMD) measurement and is therefore unable to provide information on trabecular or cortical mass.

MRI

Principles of MRI

Most MRI scanners use cryogenic superconducting magnets; the strength of the magnetic field produced (B_0) is measured in Tesla (T) and scanners are typically 1.5T or 3T [268, 269]. The source of the magnetic resonance (MR) signal which generates the clinical images mainly comes from hydrogen molecules [268]. Hydrogen nuclei contain one proton which emits a positive charge and is constantly spinning. Each proton has a nuclear spin which is normally randomly orientated and which can align with the scanner’s magnetic field [268]. Pulsed application of a second radiofrequency magnetic field (B_1) perpendicular to the static magnetic field emitted by the MR scanner (B_0) excites the nuclei [268]. The radiofrequency energy is absorbed by the nucleus and results in a transition from higher to lower energy levels. The energy absorbed or emitted by the nuclei emits a voltage which can be detected [269]. The protons move *in phase* which means in the same direction and at the same time as each other rather than random directions [268]. Once the radiofrequency pulse is switched off, the protons fall *out of phase* and return to a lower energy state or, relax [268].

Relaxation describes the process in which the nuclear ‘spin’ returns to thermal equilibrium after absorption of the radiofrequency energy [269]. The two types of relaxation are longitudinal and transverse relaxations described by the time constants, T1 and T2, respectively [269].

The Dixon method used in this study is a fat-suppression technique designed to achieve uniform fat suppression [270]. Both in phase and opposed phase images are simultaneously acquired. Thus, four images are created:

In-phase = water + fat

Out-of-phase = water – fat

Fat only = in-phase –out-of-phase

Water only = in phase + out-of-phase

The water only image can be used as a fat-suppressed image. The fat only image can be combined with other weightings (e.g. T1, T2) to give fat suppression and quantification.

MRI Scanning Protocol

Participants were scanned on a 1.5T Siemens Magnetom Avanto (Siemens AG, Erlangen, Germany) or 1.5T Siemens Magnetom Aera (Siemens AG, Erlangen, Germany) MRI scanner using 3D T1-weighted volume interpolated breath-hold examination (VIBE) sequences with in-phase and out-of-phase echoes. Acquisition was in five stations to cover from iliac crests to base of feet. Sequence parameters were: in-plane resolution 1.1 x 1.1 mm; matrix 416 x 416; flip angle 10°; repetition time of 7.7 ms; echo time 1 (TE1) 2.6 ms; echo time 2 (TE2) 4.8 ms; 1 average; acceleration factor 2; bandwidth 1 300 Hz/px; bandwidth 2 320 Hz/px. To reduce scanning time, the joints were acquired with a higher resolution (pixel size 1.05 mm^2, slice spacing 3.00 mm) than the long bone sections (pixel size 1.15 mm^2, slice spacing 5.00 mm) (Appendix 1).

MRI Segmentation Protocol

The MRI sequences were stacked in MATLAB (MathWorks, Natick, MA) forming one continuous 3D image from hip to foot, firstly by homogenising the resolution of each of the imaging sequences taken from the different sections to be 1.00×1.00×1.00 mm^3 through tri-linear interpolation (interp3, MATLAB 2006a). The fields of view of the images across the five sequences were equated by wrapping the images in blank data (greyscale value of 0), referencing the spatial metadata of the images to retain the relative subject position across the imaging sequences for each subject. The homogenised sequences were concatenated in the longitudinal direction, removing half of any overlapping volume from each section where the fields of view overlapped to reduce the effect of MR imaging bias.

Similar to others [271], muscle volume (cm^3) was determined for the following muscle groups:

Knee extensors:

Vastus medialis (VM), Vastus lateralis (VL), Vastus intermedius (VI), and Rectus femoris (RF).

Knee flexors:

Hamstrings (biceps femoris long head (BFL), biceps femoris short head (BFS), Semitendinosus (ST), and Semimembranosus (SM).

The knee flexors sartorius, gracilis, popliteus, and gastrocnemius muscles were not segmented due to their size or reliability [241]. Both extensor and flexor groups consisted of the four muscles that contribute most to the joint motion they permit [272]. Leg muscles are shown in Figure 10.

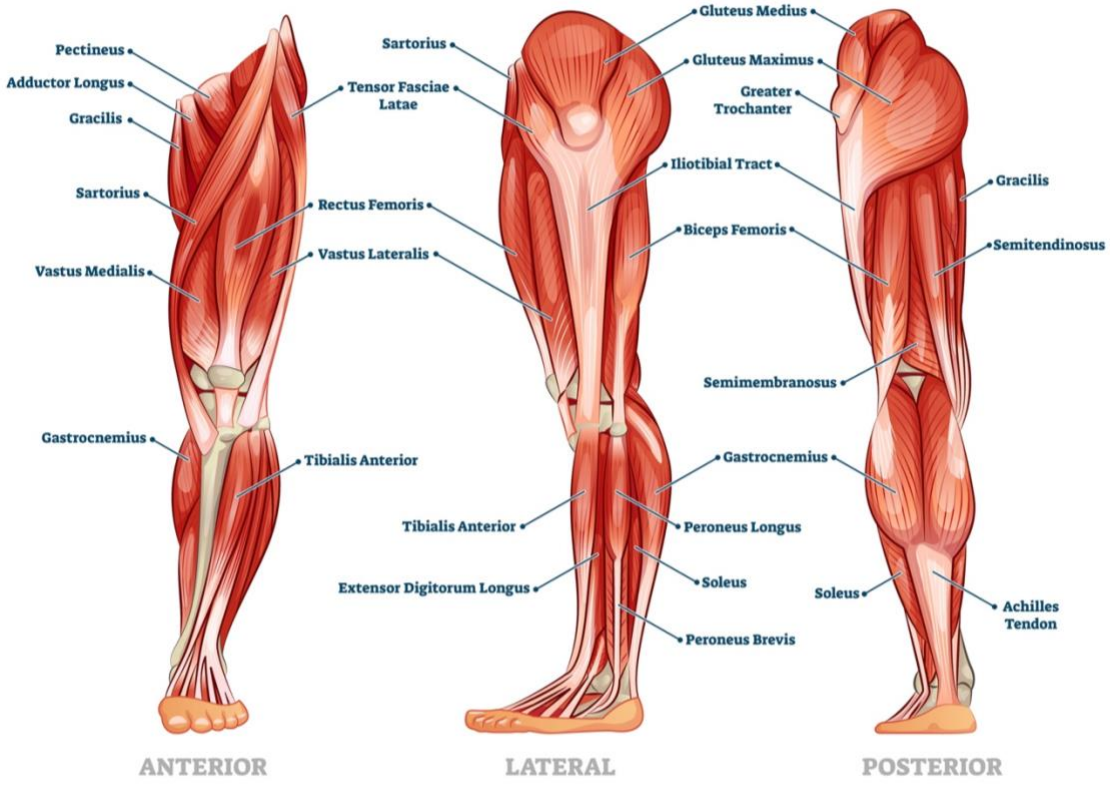


Figure 10 Leg muscles of left leg

The leg used for isokinetic dynamometry testing was segmented from the MRI sequences - in most cases this was the dominant leg. The two muscle groups were segmented from the pre-processed MRI sequences manually using 3DSlicer, an open-source manual segmentation software (3D Slicer v5.0.2, 3D Slicer, Boston, Massachusetts, USA). The muscles were individually highlighted using the paint tool within the segment editor module (Figure 11). The muscles were exported as Standard Triangle Language (STL) elements, and the volume of both muscle groups were subsequently calculated using MATLAB v11.0 R2021b (MathWorks, Natick, MA), for all subjects.

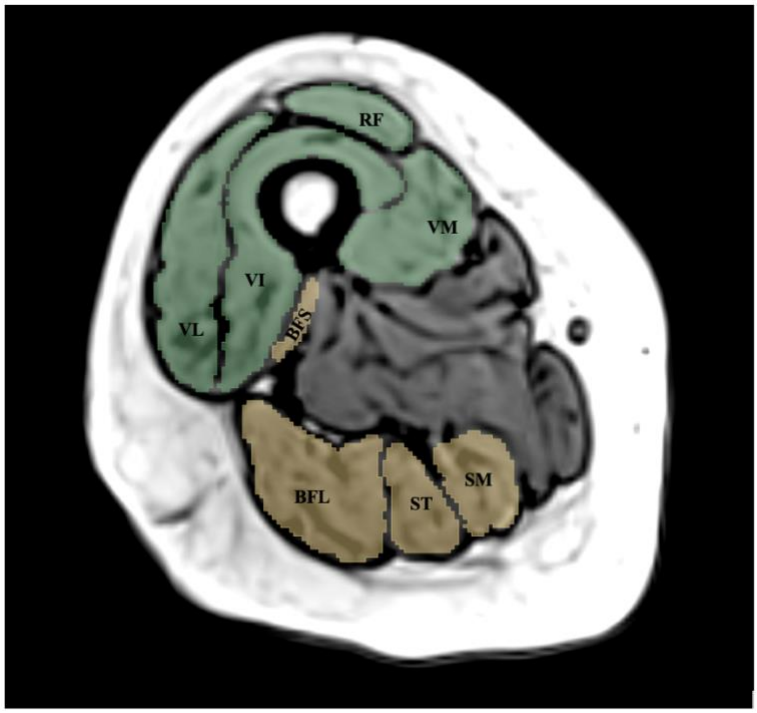


Figure 11 Example of MRI segmentation with muscles annotated, cross-sectional
Knee extensors (green): Vastus medialis (VM), Vastus lateralis (VL), Vastus intermedius (VI), Rectus femoris (RF); Knee flexors (yellow): biceps femoris long head (BFL), biceps femoris short head (BFS), Semitendinosus (ST), Semimembranosus (SM). MRI = Magnetic Resonance Imaging.

Figure 12 Example of MRI segmentation, sagittal plane
Knee extensors (green) and knee flexors (yellow). MRI = Magnetic Resonance Imaging.

The muscles were segmented from the MR images of the 27 participants by two expert operators. There are noted repeatability issues when performing muscle segmentation, primarily resulting from inter-operator variability [241]. Therefore, both the inter-operator and intra-operator repeatability were found, through calculation of the Coefficient of Variation (CoV) of the muscle volume of repeated segmentations. Firstly, the intra-operator repeatability of the segmentation procedure was assessed whereby one operator segmented both the knee flexors and extensors from one subject three times. The inter-operator repeatability was assessed by both operators segmenting the flexors and extensors from three subjects. The muscle volumes were calculated from the repeated segmentations, normalised (against the mean muscle volume found for both flexors and extensors within each of the subjects), and the CoV was calculated as the ratio of standard deviation and mean for the normalised muscle volumes. The CoV for all tests are presented in Table 16.

Table 16 Inter-operator and intra-operator repeatability, as coefficient of variation (CoV), muscle group segmentation from magnetic resonance imaging

Muscle group	**Intra-operator CoV (%)**	**Inter-operator CoV (%)**
Flexors	1.24	4.83
Extensors	3.39	4.77

Both intra- and inter-operator CoV were less than 5%, for both muscle groups, characterising the repeatability of the muscle segmentation procedure.

To calculate thigh volume, the top of the femoral head and the top of the patella of the leg used for isokinetic testing were located from the MRI sequences, for each participant. A single-level threshold was applied to the thigh section, masking only the tissue of the subject. The mask was pre-processed, filling holes within the mask which resulted from tissue with a greyscale value below the threshold. The pre-processed mask was used to calculate the thigh volume by summing the number of pixels within the mask. Using this measure of thigh volume, the percentage of flexor and extensor volume within the thigh volume was calculated.

Limitations of MRI

MRI protocols can be used to quantify and contrast differing body composition compartments [273]. MRI measurements of adipose tissue [273, 274], interstitial adipose tissue, and fat-free muscle mass [273] have been validated in post-mortem cadaver studies with good agreement – average difference for adipose tissue [0.076 ± 0.071 (SD) kg] [274]; and no significant difference for interstitial adipose tissue and fat-free muscle mass [273]. However, MRI is costly, requires specialist technicians and is

unsuitable for participants with MRI-incompatible implanted devices (e.g. pacemakers, metal implants). MRI is uncomfortable (enclosed space and noise) and other practical limitations include size and weight limits. The Magnetom Aera 1.5T (Siemens AG, Erlangen, Germany) scanner has a table length of 205cm and bore size (diameter of the hole) of 70cm. The Magnetom Avanto^{fit} 1.5T (Siemens AG, Erlangen, Germany) scanner has a table length of 204 cm and bore size of 60cm. Both scanners have weight limits of 250kg. Therefore participants outside these ranges were not recruited.

Figure 13 Magnetom Aera 1.5T magnetic resonance imaging scanner

Image courtesy of Siemens Healthineers.

3.3.6 Strength and Physical Performance Tests

Dynamometry

Principles of Dynamometry

Dynamometers operate through opposable electromagnetic plates which are capable of restricting movement velocity regardless of the magnitude of torque exerted [275]. It is possible to pre-define the range of motion, contractile mode (isometric, concentric, eccentric) and repetition numbers.

Figure 14 Biodex System 4 Pro Dynamometer used for testing procedures in this study

Definitions

Force, muscle: a straight-line push or pull exerted by muscle, typically expressed in pounds (lb) or Newtons (N) [276].

Torque, muscle: The rotating effect of force. A force applied to an object creates a torque that rotates the object [276].

Fatigue, muscle: Reduced force-generating capacity of muscle [245].

Isokinetic: This test measures torque through a range of motion at a constant angular velocity [277]. The machine applies a torque response which adapts to the subject's effort ensuring that the limb velocity increases or decreases consistently. This is the standard for muscle assessment.

Isotonic: This test allows limb velocity to vary while a constant force is applied. This better relates to functional task simulation whereby normal activities involve moving a constant weight at differing velocities.

Isometric: This test measures the maximal voluntary contraction against an immovable force [277].

Concentric: A type of contraction whereby muscles shorten [276].

Eccentric: A type of contraction whereby muscles lengthen. Concentric contractions tend to be weaker than eccentric contractions [276].

Set: A group of consecutive repetitions (reps).

Repetition (rep): One complete exercise movement e.g. one kick.

Rationale for Dynamometry Protocol

Many daily and physical activities require repetitive contractions of the lower limb muscles (e.g. walking, stair climbing) which could fatigue a person with abdominal obesity and increase their susceptibility to falls. Localised fatigue using an isokinetic dynamometer can provide a more objective measurement of how specific leg muscles tire. There are a limited number of studies exploring voluntary localised (knee) fatigue in older adults with heterogeneity amongst the protocols available.

The knee joint was chosen due to its involvement in postural control [231] and normal locomotion [278]. The protocol chosen was based on that of Bellew and Fenter (2006) [245]. Bellew and Fenter (2006) [245] tested knee extensor and flexor fatigue of older normal weight adults (average 77y) using a previously described protocol for the ankle [279, 280]. Bellew and Fenter (2006) used a 180 ^{o}/s velocity in contrast with 60^{o}/s described in the original protocols [279, 280] which may relate to the older adults tested [245]. Others have pre-specified the number of repetitions. For example, in the study by Mafiuletti et al. (2007) [234], obese younger adults completed 50 consecutive quadriceps contractions at 180 ^{o}/s. Caution is needed when prescribing a number of contractions to fatigue as participants may experiencing different levels of fatigue. Therefore, an individualised approach was used for this present study.

Dynamometry Protocol

Measurements of voluntary maximal isometric knee extensor and knee flexor torque and isokinetic fatigue of the quadriceps were taken at the Royal Hallamshire Hospital, Sheffield using the Biodex System 4 Pro (Biodex Medical Systems, Inc, Shirley, New York). For all tests the participants had straps around the pelvis, chest, and mid-thigh to avoid contribution from other muscles and extraneous body movements. The resistance pad was placed 1-2 cm immediately superior to the medial malleolus. The dominant leg was chosen for fatigue testing unless otherwise specified or requested by the

participant. To allow the software to account for the effects of gravity the participants leg was placed at 40° and participants were asked to relax.

Figure 15 Example of positioning for isokinetic dynamometry procedures
Product Image courtesy of Biodex Medical Systems, Inc.

Voluntary isometric strength testing:

Voluntary isometric strength of dominant knee extensors and flexors were measured. Participants were asked to undertake one set of five concentric and eccentric repetitions at 60°/s [235, 280] with five seconds of activation and a 10 second break between each repetition. Peak torque was calculated from the maximum torque produced during the voluntary isometric strength test and this measurement was used to determine fatigue. Participants had a 10-15 minute rest period between isometric and isokinetic testing.

To calculate the maximum muscle force (F_{max}) generated by the flexors and extensors, the distance (r) between the pivot and lever arm (the calf length) was measured. The calf length was calculated from the MR imaging sequences, measuring the distance from the knee to the ankle. Maximum voluntary knee extensor and flexor torque (τ_{max}) were determined from isokinetic testing. The maximum force was calculated for both the flexors and extensors using the mechanical force-torque relationship, $F_{max} = \tau_{max}/r$.

Voluntary Isokinetic fatigue testing:

Voluntary muscle fatigue of the knee extensors was induced by repetitive submaximal contractions at 180°/s until fatigue i.e. the torque output dropped below 50% of the calculated peak torque from the

voluntary isometric strength test [245, 279, 280]. Visual feedback and standardised verbal encouragement were provided throughout the procedure.

The number of repetitions performed and strength produced at each repetition during the isokinetic test was calculated using MATLAB v11.0 R2021b (MathWorks, Natick, MA) (Appendix 2). Counting of repetitions started from the first repetition above 50% and ended at the repetition before the three repetitions <50% (i.e. the signal for the end of the test) – therefore the start and endpoint was > 50% of the maximum contraction.

Limitations of Dynamometry

The main sources of error which can impair reproducibility of results include:

1. Machine-related inconsistencies
    i. Intra-model reproducibility – this refers to the tester's ability to test a subject reliably [281]. Others have shown good reproducibility across all speeds but differences can exist between testers [281]. The intra-model reproducibility was not measured during this study so bias may exist.
    ii. Inter-model reproducibility – this refers to the difference between different types of machines [282]. A single machine was used for this study so therefore this limitation is only relevant for comparison with other findings.
2. Subject Variation
    i. Pain e.g. muscle soreness, pain related to joints (e.g. arthritis).
    ii. Familiarisation. Some suggest that reliability is enhanced when several practice tests are provided prior to data collection [283]. In one study, knee extension/flexion was measured on five occasions using the Biodex 3 and, although no significant effect of learning was found for knee flexion, knee extension was highest at the first measurement [284].
    iii. Motivation of the participant and level of feedback provided is known to affect the results obtained [277, 283].
    iv. In older women, repeated measurement at the knee has shown a ratio limit of agreement (RLOA; the amount an individual's score varies with repeated measurement), of 20.5%, 23.0% and 22.8% for peak concentric (90°/s), eccentric (-90°/s) and isometric torque (0°/s), respectively [285]. Hartmann et al [286] also showed RLOAs of 35.4% (-12.7, 16.6) and 22.1% (-18.9, 22.1) for knee flexion and

extension (60°/s) in older men and women. They further attributed 2.0% and 0.8% to systematic bias and 14.6% and 19.7% to random error. These findings are similar to those of Lund et al. (2005) (-13.6, 14.7) and (-16.3, 17) for flexion and extension at (60°/s) after two learning phases and 20 minutes apart [284].

3. Testing procedure errors
    i. Poor/inconsistent stabilisation – stabilisation of the participant with appropriate straps is important to avoid the contribution of other muscles to the test and to secure the bones where the activated muscles originate [283].
4. Protocol variations
    i. Differences in rest periods can affect results. For isometric tests, a 10 second rest was allowed between each repetition whereas a 10-15 minute rest was allowed between isometric and isokinetic tests. In older men, maximal voluntary isometric torque returns to baseline after a 10 minute rest [287].
5. Inter-tester reliability
    i. Difference between examiners is associated with variability [281]. A single researcher completed all measurements for this study so therefore this is limitation is only relevant for comparison with other findings.

Calibration of Isokinetic Dynamometer

The Biodex System 4 Pro was calibrated monthly according to manufacturer specified procedures.

Isokinetic Dynamometer Outcomes

The following results were obtained:

Isokinetic (fatigue test):

- Number of repetitions
- Peak torque at each repetition – extension (away) and flexion (towards)

Isometric (strength test):

- Peak torque at each repetition – extension (away) and flexion (towards)
- Peak torque (maximum of five repetitions) – extension (away) and flexion (towards)

Short Physical Performance Battery

Participants completed the Short Physical Performance Battery (SPPB) which consists of three tests – balance, sit-to-stand test, and gait speed. The SPPB is an objective, valid and reliable assessment tool to evaluate lower extremity ability in older adults [288]. Safety and suitability of participants to

perform tests was assessed, those considered too unsteady or weak did not participate. The scores range from 0 (worst performance) to 12 (best performance). An SPPB score <10 is associated with all-cause mortality [289].

Sit-to-stand component: Participants were asked to stand up and sit down on a chair up to five times without stopping with arms folded across their chest. Participants were positioned towards the front of the chair and advised to complete the test as quickly as possible. They had a maximum of one minute and the chair stand test was ended after this time. Time to complete chair stands was recorded. The same chair was used for all participants with 47cm height, 46cm width and 47cm seat depth.

Balance component: The balance component comprised three stages. Participants began with feet together. They were asked to maintain this position for ten seconds without moving their feet while maintaining balance. If the participant was able to complete the feet together stage they were asked to repeat the procedure in semi tandem for ten seconds. If the participant was able to complete semi tandem they were asked to repeat the procedure in full tandem. Demonstration and assistance was provided by the researcher at each stage for the participant to initially achieve balance as required. If a participant was unable to complete a stage they did not progress to the next stage and the balance test was ended.

Gait speed component: A 10m course was measured out using a tape measure; two markers were placed at each end with a chair at one end if needed. The participant was asked to walk at their usual pace between the markers two times with a break in between. The participant could use usual walking aids. A stopwatch recorded how long it took the participant to reach the far marker.

A standardised script was used to describe all components of the SPPB [290].

Timed up and go

Participants sat on a chair. A point 3m away was marked on the floor. The participants were asked to stand up, walk 3m and return to their sitting position as quickly as they could [291]. The length of time to complete this task was recorded. The average of up to three attempts was recorded. Regular footwear and walking aids were allowed. The researcher walked behind the participant during the test to provide assistance if required. A cut-off to identify those at high risk of falls remains an area of uncertainty. Community dwelling older women should be able to complete the test within 12 seconds

[292] although EWGSOP2 propose a threshold of ≥ 20s based on data from the same study to determine low performance [4].

6-minute walk test (6MWT):

Rationale

There is a lack of information on muscle fatigue in abdominally obese older adults with and without low muscle strength. This information is of importance as a lower threshold to fatigue may impair physical performance, specifically balance and gait. A 6-minute walking test (6MWT) can look at how muscles tire or fatigue in a 'dynamic' setting or one that is more closely related to 'real-life'.

The 6MWT is used to measure the distance that a person can quickly walk on a flat, hard surface in six minutes [293]. The test is self-paced and participants do not achieve maximal exercise capacity. The 6MWT is easy to administer and reflective of activities of daily living. The 6MWT assesses the global and integrated responses of systems utilised during exercise – pulmonary, cardiovascular, neuromuscular units and muscle metabolism [293]. However, the test is unable to provide specific information on the individual function of different organs or systems [293].

The 6MWT is an outcome measure used by several regulatory agencies to assess drugs aimed at peripheral vascular disease and pulmonary hypertension [61]. The 6MWT is associated with limited activities of daily living (ADL), cognition and all-cause mortality in older adults [294, 295]. A clinically significant change is 50m in persons who can walk at least 100m [61].

Contraindications to the 6MWT included unstable angina or myocardial infarction during the previous month or a resting heart rate > 120 beats per minute (BPM) or systolic blood pressure >180 mmHG and a diastolic blood pressure of more than 100 mmHG [293].

6MWT Protocol

A 30m enclosed corridor is recommended as shorter distances require the patient to turn around more often leading to a reduced distance walked [293]. Due to reduced capacity at the clinical testing facility related to Covid-19, a 25m enclosed corridor was used. Standardised phrasing from the American Thoracic Society was used for reproducibility as the level of encouragement given affects the distance walked [293].

The participants were asked to walk as fast as possible while maintaining a comfortable walking pace [293]. Heart rate was measured at the beginning and end of the test (if possible, within 15s after the end of the test). Borg Rating Scale of Perceived Exertion (RPE) was measured at the beginning and end of the 6MWT [296]. Reasons for immediately stopping a 6-minute walk test included the following: (1) chest pain, (2) intolerable dyspnoea, (3) leg cramps, (4) staggering, (5) diaphoresis, (6) pale or ashen appearance and, (7) patient request.

6MWT Outcomes

The outcome of the 6-minute walk test was distance walked (m). Gait sensors (DynaPort MoveMonitor, McRoberts; The Hague, The Netherlands) were also worn to determine more subtle differences in gait; however, the files were contaminated for the majority of participants and could not be used.

Limitations of the 6MWT

Reproducibility can be affected by variations in instructions and the level of encouragement given; a standardised script was therefore used to improve reliability [293]. A 30m course is recommended; however, a 25m course was used meaning that participants had to turn more frequently. This may have affected the total number of metres walked. All participants used the same 25m course for comparability; care needs to be taken when comparing the results of this study with those using 30m courses. Lastly, as this was a 'voluntary' fatigue protocol, participants may not have fully exerted themselves and therefore not have become fatigued.

3.3.7 Biochemistry

Blood Sampling Procedure

Blood samples (4 x 5ml) were taken (BD Vacutainer® SST II Tubes) between 08:00 and 10:00 using a 23g butterfly needle after an overnight fast. Samples were allowed to clot at room temperature for 30 minutes and were then centrifuged at 1800g for 10 minutes. Where the blood could not be centrifuged immediately after clotting, the tubes were refrigerated at 4^{o}C for up to four hours [297]. Serum samples were aliquoted and stored at -80^{o}C until analysis.

Blood Testing

Blood testing was conducted by Sheffield Teaching Hospital Clinical Chemistry Laboratories. Tests for glucose, C-reactive protein (CRP), fasting lipids (HDL cholesterol, LDL cholesterol) were performed on

a Roche/Hitachi Cobas 8000 system with the Roche C702 Chemistry Module (Roche Diagnostics GmBH, Mannheim, Germany). The Cobas 8000 is a fully automated system for clinical chemistry analysis and the C702 Chemistry Module can perform photometric assays for a wide range of analytes at high-throughput. The assays used for blood testing are listed in Appendix 3.

3.3.8 Statistics

Power Calculations

Published data [298] on microRNA levels in ageing was used to calculate a sample size of 8 per group to identify an effect size of 2 with 80% power and Alpha level set at 0.01 two-sided. This was based on a 1.5 fold-change difference and a common SD of 0.75. We estimated a 20% attrition rate and therefore aimed to recruit n=10 per group to account for this. The findings in this chapter refer to secondary and exploratory outcome measures.

Analysis

Variables are reported as median ± inter-quartile range (IQR). Differences between variables across groups were determined using either consensus cut-offs (e.g. Table 17) or Kruskal-Wallis one-way analysis of variance test without adjustment [299]. Statistical analysis was undertaken using GraphPad Prism 9 (Version 9.4.1) for Mac, GraphPad Software, San Diego, California USA, www.graphpad.com. Statistical significance was accepted as $p < 0.05$.

Table 17 Summary of measures and cut-offs used to identify low muscle strength and physical function

Metric	Cut-off	Rationale
Strength		
Isometric extensor torque	< 1.01 (maximum extensor torque / body mass)	Discriminator of severe mobility limitation i.e. two consecutive reports of difficulty or inability to walk ¼ of a mile or climb 10 steps [300].
Hand grip strength	< 16kg	EWGSOP2 criteria for low muscle strength (dynapenia) based on -2.5 SD below peak adult strength [4].
	< 20kg	SDOC criteria for low muscle strength (dynapenia) based on associations with gait speed [58].
Performance		
6 minute walking distance	< 300m	A commonly used cut-off in clinical studies [146] based on the fifth percentile for older adults [294].
	Individual equations	There is a lack of standardised cut-offs and consensus on normative values. Two commonly accepted equations were used. Both equations were based on cohorts with a BMI range of 18.5-35kg/m^2; One [301] included those aged 40-80 years whereas the other [294] included those aged 68+ years. - 6MWD = 1,017 m − (6.24 × BMI) − (5.83 × age) [- 113m for lower limit of normal] [301] - 6MWD = 493m + (220 x Height) - (0.93 x Weight) - (5.3 x Age) [- 100m for lower limit of normal] [294]
SPPB	≤8 point score	EWGSOP2 criteria for low physical performance. Based on work by [289, 302].
	<10 point score	A cut-off of <10 is associated with mortality [289] and disability risk [302].
TUG	≥20 s	EWGSOP2 criteria for low physical performance. Community dwelling older women had a TUG time < 20 seconds [292].

***Abbreviations**: EWGSOP2 = European Working Group on Sarcopenia in Older People; SDOC = Sarcopenia Definitions and Outcomes Consortium; BMI = Body Mass Index; 6MWD = 6 Minute Walking Distance; SPPB = Short Physical Performance Battery; TUG = Timed up and Go; SD = standard deviation.*

3.4 Results

3.4.1 Characteristics

In total, 132 women were screened for participation in this study; 102 were ineligible (Figure 16). At the end of recruitment, a further three participants (dynapenic only) were excluded due to insufficient recruitment in this group. One participant (DAO) did not complete Visit 2 due to a change in her health circumstances.

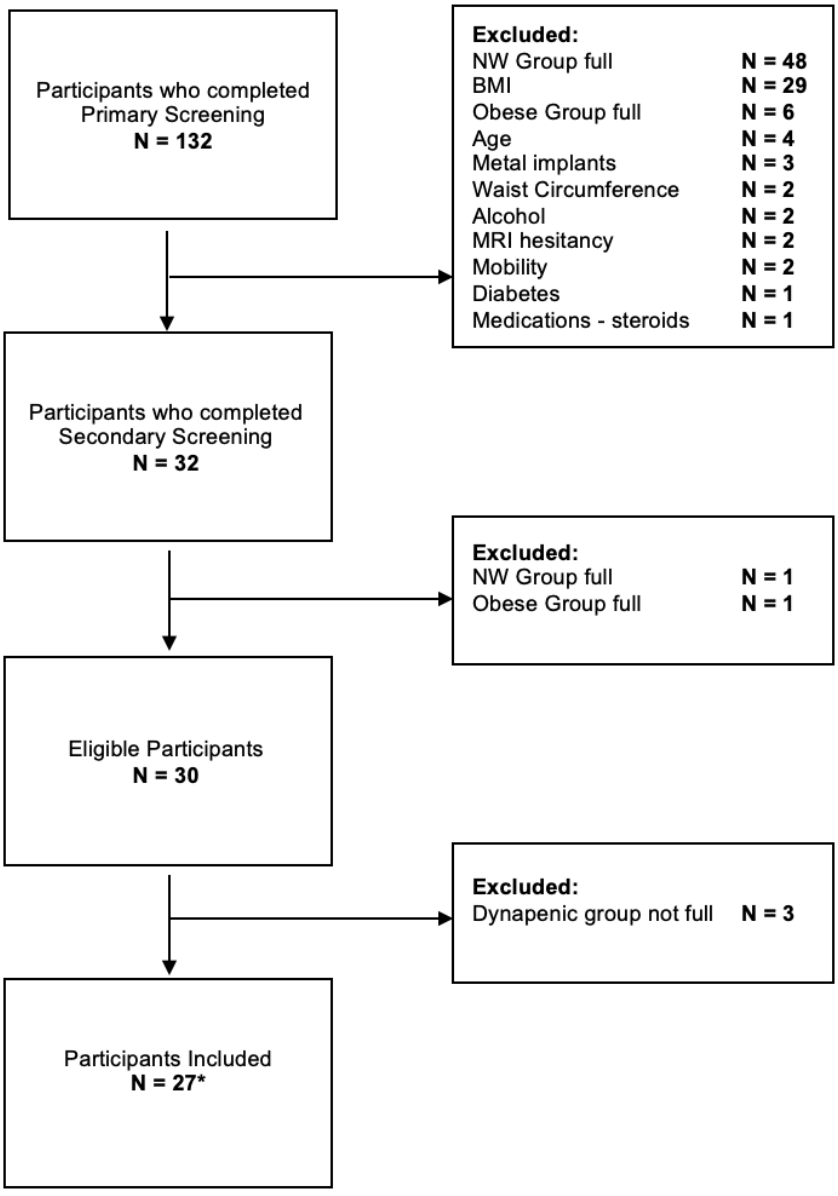


Figure 16 CONSORT diagram

** One participant did not attend visit 2 due to health reasons. Abbreviations: NW = Normal Weight; BMI = Body Mass Index; MRI = Magnetic Resonance Imaging.*

Table 18 Characteristics of participants

	Normal weight (n=10)		**Obese** (n=10)		**DAO** (n=7)	
	Median	IQR	Median	IQR	Median	IQR
Age (y)	64.5	7	64.5	8	68	16
Age 60-69y (n)	8		8		4	
Age 70-79y (n)	2		2		3	
Anthropometry						
Weight (kg)	58.5	10.4	84.5	22	92.0	17.5
Height (m)	1.61	0.05	1.62	0.11	1.62	0.08
BMI (kg/m^2)	21.43	3.11	32.19	4.59	36.92	7.37
Obese Class I (n)	-		8		3	
Obese Class II (n)	-		2		4	
Waist circumference (cm)	76	4	100.5	9	110	20
Body Composition*						
Lean Mass (kg)	40.70	4.90	45.05	11.86	47.76	5.23
ALM (kg)	23.67	5.01	33.41	11.57	34.95	3.00
ALM/ht^2 (kg/m^2)	9.00	1.32	13.08	2.98	13.21	0.99
ALM/weight (%)	41.59	3.12	39.30	2.72	37.65	5.94
Fat Mass (kg)	19.40	8.10	40.34	11.40	46.31	9.83
Fat Mass/Weight (%)	34.12	7.17	47.22	1.75	50.11	3.92
Trunk Fat Mass (kg)	8.86	3.37	19.29	4.38	24.36	2.56
Android Fat Mass (kg)	1.28	0.56	3.27	1.01	4.35	0.42
Gynoid Fat Mass (kg)	3.88	1.04	6.44	2.35	7.26	0.41
WB aBMD (cm^2)	1.03	0.17	1.04	0.17	0.91	0.22
Total Hip aBMD (cm^2)	0.84	0.15	0.88	0.08	0.84	0.24
Function						
5 STS Time(s)	9.32	2.50	11.15	3.70	17.50	3.06
Gait speed (m/s)*	1.38	0.26	1.20	0.14	1.09	0.23
TUG (s)*	7.03	1.13	7.61	1.35	9.61	1.91
SPPB*	11	0	11.00	1.00	8.5	1.0
Max HGS (kg)	21.25	2.8	20.95	11.30	19.1	3.8
6MWT distance (m)*	583	54	493	100	434	33
Lifestyle*						
Moderate activity (mins/day)	122.61	89.68	89.76	103.69	28.93	48.80
Protein Intake (g/day)	1.24	0.25	1.02	0.65	0.74	0.44
Clinical						
Osteoarthritis* (n)	1		2		2	
Fall in past year (n)	1		0		1	
Systolic (mmHg)**	122.6	21.5	150.5	24	137.0	20.0
Diastolic (mmHg)**	78.5	10.5	91.5	6	83	4
Pulse (BPM)**	67.5	7.5	77	14	79	21
Glucose (mmol/l)	4.8	0.9	5.3	0.4	5.6	0.6
CRP (mg/l)	0.5	0.2	3.2	1.8	3.0	1.5
LDL Cholesterol (mmol/l)	3.7	0.7	3.2	1.1	3.5	1.7
HDL Cholesterol (mmol/l)	2.0	0.3	1.7	0.5	1.62	0.71

** Missing measurements for n = 1 (DAO). ** Missing measurements for n=2 with DAO and n = 1 with obesity for systolic blood pressure and pulse; missing measurements for n = 2 with obesity for diastolic blood pressure. Osteoarthritis was self-reported. Abbreviations: DAO = Dynapenic Abdominal Obese; IQR = Inter-Quartile Range; ALM = Appendicular Lean Mass; aBMD = areal Bone Mineral Density; STS = Sit-to-stand; TUG = Timed up and Go; SPPB = Short Physical Performance Battery; HGS = Hand-grip Strength; 6MWT = 6 Minute Walking Test; BPM = Beats per minute; LDL = Low Density Lipoprotein; HDL = High Density Lipoprotein.*

Twenty seven women aged between 60-79y were included in this study (Table 18). The majority of all groups were aged between 60-69y but, compared with the other groups, a greater proportion of those with DAO (n = 3/7) were aged between 70-79y. The majority of women with obesity were obese class I (BMI 30-34.9kg/m^2; n = 8/10) whereas the majority of those with DAO were obese class II (BMI 35-39.9kg/m^2; n = 4/7). One person fell in the normal weight and DAO groups in the past 12 months.

The majority of the group with DAO (n = 5/7) and two participants with obesity had metabolic syndrome according to International Diabetes Federation criteria [303]. Blood glucose was normal (≤ 6.1mmol/l) for all normal weight participants.

Forty six days (median) passed between Visit 1 and the MRI visit (range: -11 – 104 days; n = 27) and 37.5 days between Visit 1 and Visit 2 (range: 12 – 98 days; n = 26). Participants completed the study in 49 days (range: 24 – 104 days; n = 27). Weight change between Visit 1 and Visit 2 was < 5% (median 1.2%; range: - 3.9% – 4.6%). Weights were not recorded for n = 6/27 MRI visits. Weight change between Visit 1 and the MRI visit was -0.49% (range -4.62 - 5.56%). The body weight difference between Visit 1 and the MRI visit for one participant was 5.56% (3kg); however, reliability is uncertain as this was over five days.

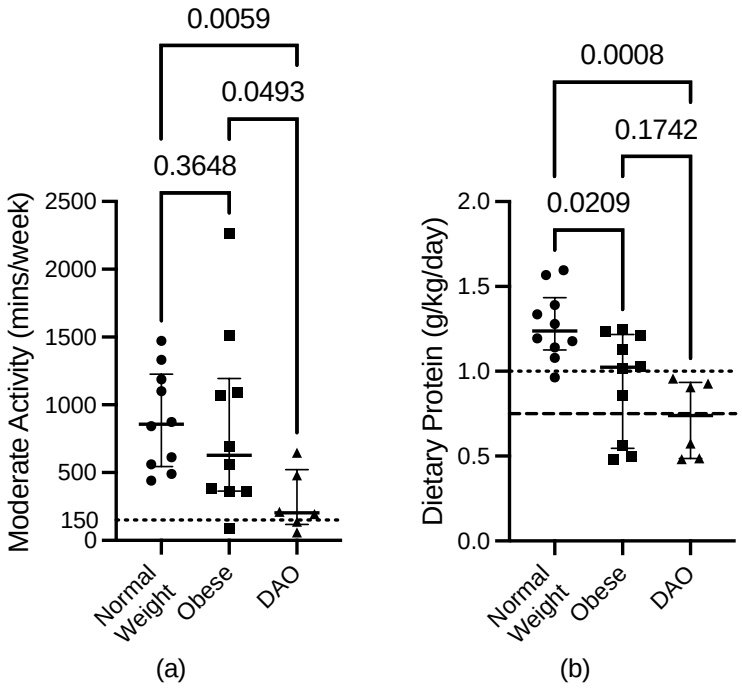


Figure 17 Number of participants meeting (a) moderate physical activity and (b) protein requirements, by group

(a) Dotted line refers to the Chief Medical Officer's recommendations for moderate physical activity (150 mins/week) [255] (b) dashed line refers to UK reference nutrient intake for protein (0.75 g/kg body weight/day) [260]; dotted line refers to European recommendations for older adults (1.0-1.2g/kg body weight/day) [261]. N = 1 missing from DAO Group. Abbreviations: DAO = Dynapenic Abdominal Obese.

Based on the self-reported physical activity questionnaire, all normal weight participants achieved public health recommendations for moderate physical activity (150 mins/week [255]) (Figure 16). One person with obesity and two with DAO failed to achieve this recommendation. Women with DAO reported less moderate physical activity (202.53 ± 341.60 mins/week) than either the group with normal weight (858.27 ± 627.72 mins/week; p = 0.006) or obesity (628.31 ± 725.82 mins/week; p = 0.049).

Based on the self-reported food frequency questionnaire, all participants with normal weight achieved the RNI for dietary protein (0.75 g/kg/day) [260] and the majority (n=9/10) exceeded the recommendations for older adults (1.0-1.2 g/kg/day) [261]. The majority of older women with obesity exceeded the RNI (n=7/10) and older adult specific recommendation (n=6/10). In contrast, only half of those with DAO (n = 3/6) exceeded the RNI but none met the older adult recommendation.

3.4.2 Body Composition

Total Lean and Fat Mass

All participants with obesity and DAO had a high body fat percentage (BF%; > 40%). One participant with normal weight had high BF% (40.49%; Figure 18a). Whole body lean mass as a percentage of total body weight of women with normal weight (67.0 ± 7.11%), obesity (53.47 ± 1.79%) and DAO (50.68 ± 2.97%) are shown in Figure 18b.

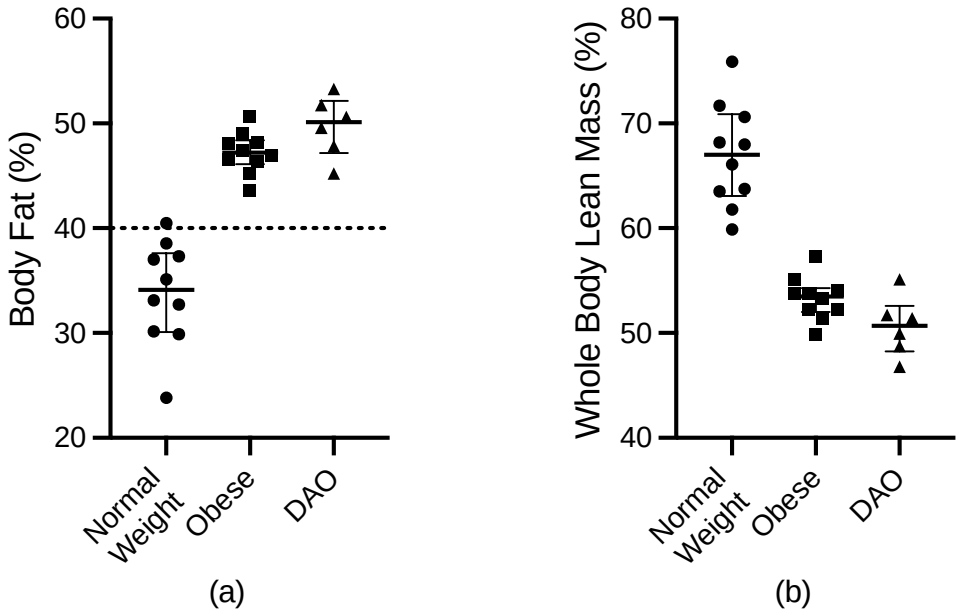


Figure 18 Overall body composition (a) Body Fat Percentage (b) Lean Mass Percentage (kg/m^2). Median ± inter-quartile range. Dashed line refers to the ESPEN/EASO threshold for obesity (> 40%). Abbreviations: DAO = Dynapenic Abdominal Obese; ESPEN = European Society for Clinical Nutrition and Metabolism; EASO = European Association for the Study of Obesity.

Appendicular Lean Mass

All participants had normal lean mass according to EWGSOP2 (ALM, ALM/ht^2) and ESPEN/EASO (ALM/weight) criteria (Figure 19).

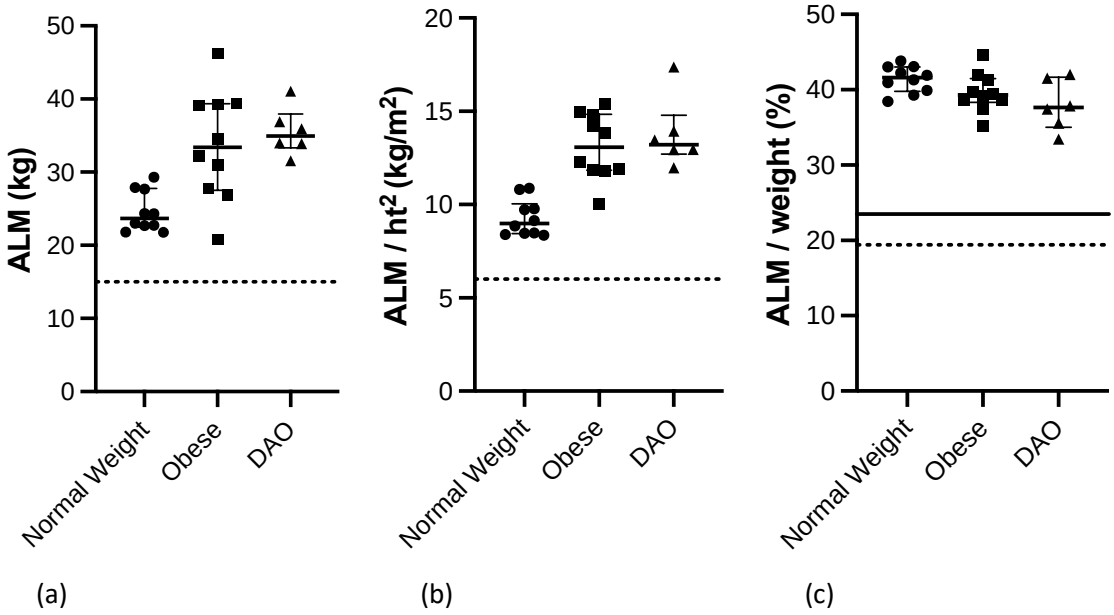
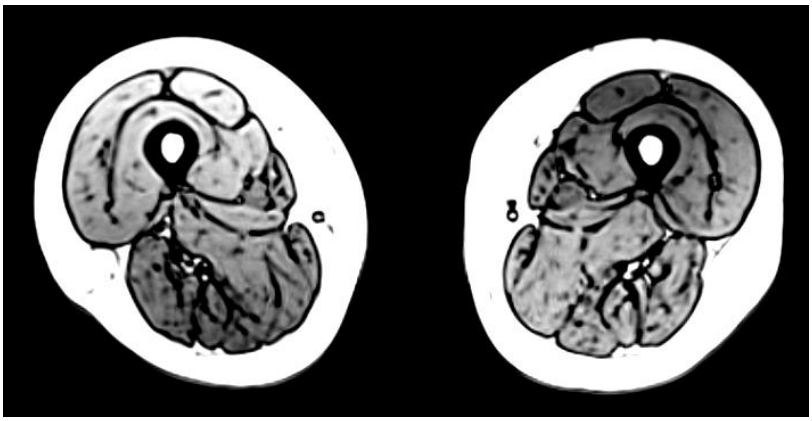


Figure 19 Appendicular lean mass expressed as (a) absolute mass and adjusted for (b) height and (c) weight

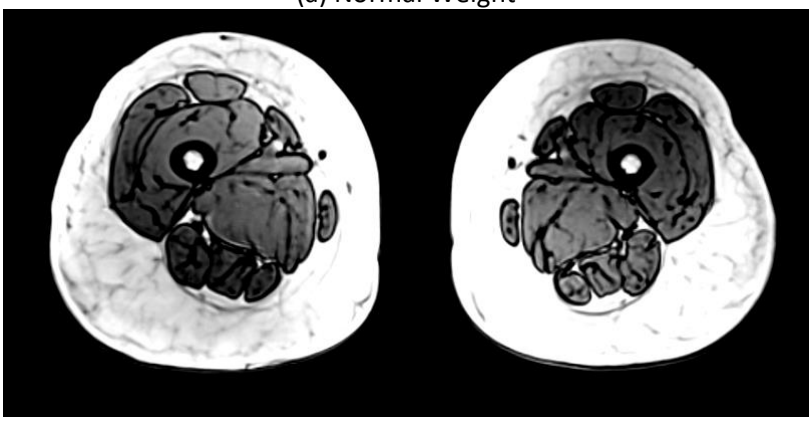
Median ± inter-quartile range. (a) ALM; dashed line refers to EWGSOP2 cut-off for low lean mass (< 15kg) [4]; (b) ALM/height^2 (ht^2); dashed line refers to EWGSOP2 cut-off for low ALM/ht^2 (< 6kg/m^2) [4]; (c) ALM/wt; dashed line refers to ESPEN/EASO recommended cut-off for low ALM/wt (< 19.4%) and solid line refers to a Caucasian-specific cut-off (< 23.47%) [76]. Abbreviations: DAO = Dynapenic Abdominal Obese; ALM = Appendicular lean mass; EWGSOP2 = European Working Group on Sarcopenia in Older People 2; ESPEN = European Society for Clinical Nutrition and Metabolism; EASO = European Association for the Study of Obesity.

MRI Measured Muscle Volume

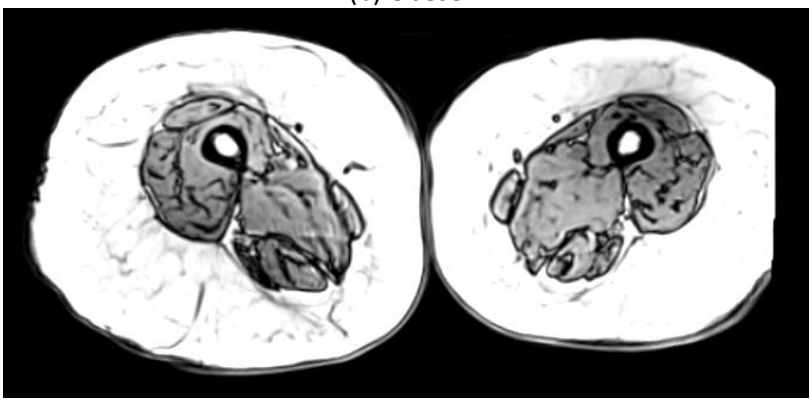
Examples of cross-sectional MR slices of the thigh of participants with normal weight, obesity and DAO are shown in Figure 20. Examples of segmentation can be seen in the MRI Scanning Protocol section. Scans were performed on the 1.5T Siemens Magnetom Avanto (Siemens AG, Erlangen, Germany; n = 9) or 1.5T Siemens Magnetom Aera (Siemens AG, Erlangen, Germany; n = 18).



(a) Normal Weight



(b) Obese



(c) DAO

Figure 20 Example MR slices from participants with (a) normal weight (b) obesity (c) DAO
Abbreviations: MR = magnetic resonance; DAO = Dynapenic Abdominal Obesity.

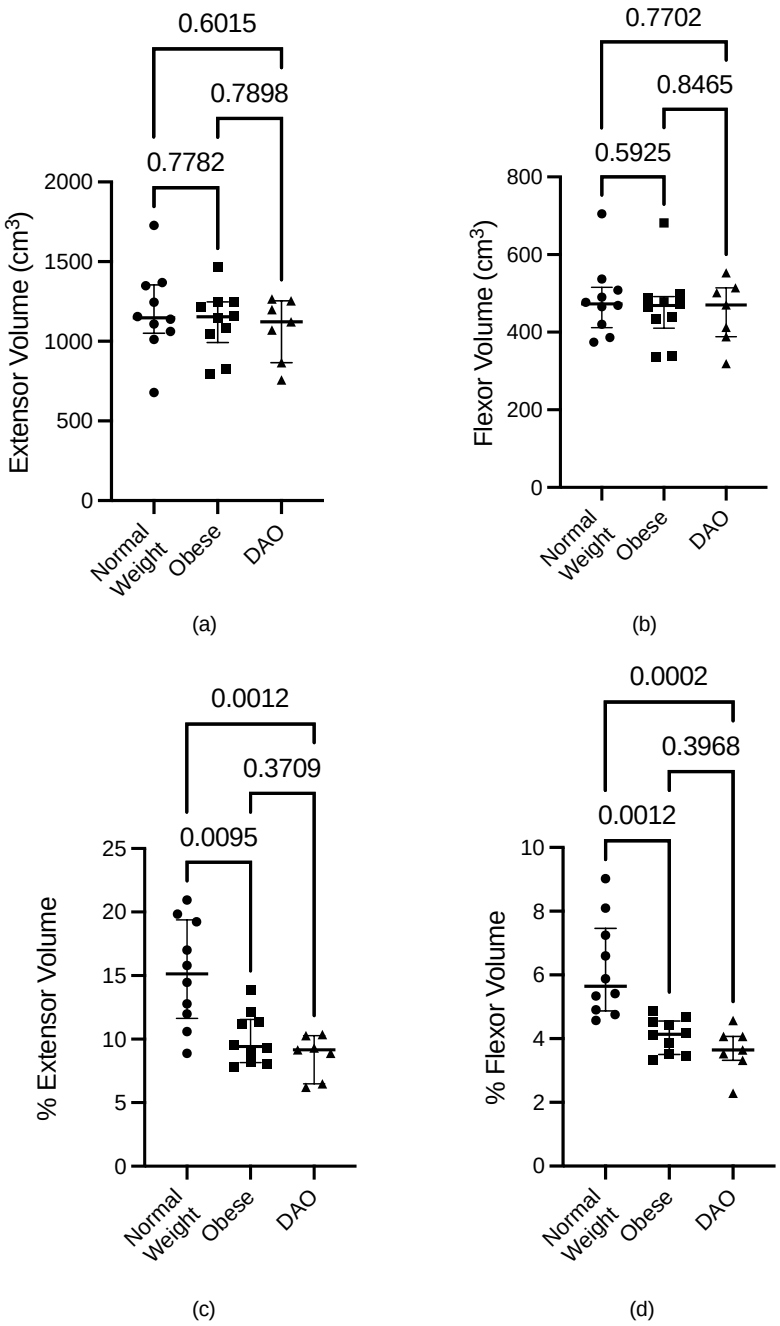


Figure 21 Muscle volume from magnetic resonance imaging of (a) knee extensors, (b) knee flexors, (c) knee extensors relative to total thigh volume, and (d) knee flexors relative to total thigh volume.

Median ± inter-quartile range. Abbreviations: DAO = Dynapenic Abdominal Obesity.

The volume of the knee extensors and flexors in the dominant leg is reported in all cases except for one woman with DAO (non-dominant leg) who had lymphoedema (Figure 21). Both knee extensors and flexors as a proportion of the total thigh volume were lower in those with obesity or DAO compared to those with normal weight (Figure 21c,d). Women with obesity and DAO had similar

percentage extensor (9.42 ± 3.17% vs. 9.16 ± 3.80% p = 0.37) and flexor (4.14 ± 0.99% vs. 3.65 ± 0.76% p = 0.4) volumes.

3.4.3 Physical Function

All participants had normal gait speed but there was a trend for reduced gait speed in those with obesity (1.20 ± 0.14 m/s; p = 0.038) and DAO (1.09 ± 0.23 m/s; p < 0.01) compared to those with normal weight (1.38 ± 0.26 m/s; Figure 22).

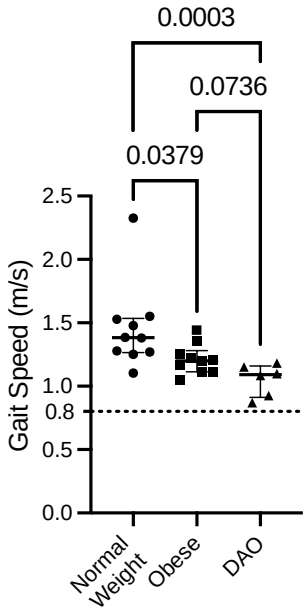


Figure 22 Gait speed (m/s) of participants, according to groups

Median ± inter-quartile range. The dashed line refers to the EWGSOP2 cut-off for slow gait speed (< 0.8 m/s) [4]. N=1 with DAO and n=1 with obesity did not complete this test due to health reasons. Abbreviations: DAO = Dynapenic Abdominal Obesity; EWGSOP2 = European Working Group on Sarcopenia in Older People.

Two thresholds were applied to identify a low SPPB score (< 10 points or < 8 points); the normal weight and obese groups were in the normal group for both SPPB thresholds. All of those with DAO scored < 10 points and this related to sit-to-stand time as all participants scored full points for gait speed whereas only one point was lost by one participant for the balance component. Using the cut-off of < 8 points advocated by EWGSOP2, three participants with DAO were classified as having a low SPPB score. Participants in all groups had a normal TUG time (< 20s).

Approximately half of the normal weight and obese groups (n = 4/10) had low hand-grip strength using SDOC criteria (< 20kg); conversely just under half of participants in the DAO group (n = 3/7) would have normal strength using this metric (Figure 23). Using EWGSOP2 criteria (< 16kg), one person with normal weight or DAO and two with obesity had low hand-grip strength; conversely the majority of participants with DAO had normal hand-grip strength (n = 6/7).

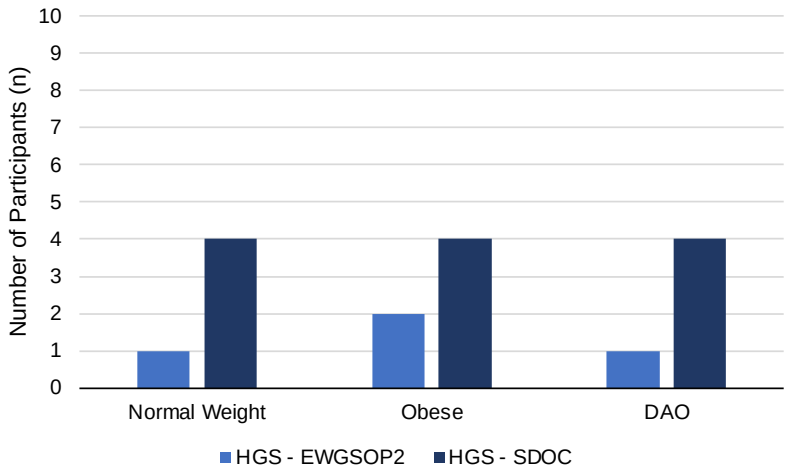


Figure 23 Number of participants within each group with low hand-grip strength

Abbreviations: DAO = Dynapenic Abdominal Obesity; HGS = Hand-grip strength; EWGSOP2 = European Working Group on Sarcopenia in Older People 2 (<16 kg) [4]; SDOC = Sarcopenia Definition and Outcomes Consortium (<20 kg) [58]

Strength

Isometric Torque

The dominant leg was used in all cases except for one woman with DAO who had lymphoedema. Maximum concentric (away) torque for normal weight (103.8 ± 36.0 Nm), obese (120.6 ± 43.9 Nm) and DAO (99.4 ± 37.5 Nm) groups are shown in Figure 24a.

The majority of women with DAO (n = 5/7) had low knee extensor (quadricep) strength (torque/body weight < 1.01 Nm/kg) (Figure 24b). All participants with normal weight or obesity had normal strength; however, unlike those with normal weight, some women with obesity approached the threshold (n = 4/10) for low muscle strength.

Following adjustment for lean mass of the leg (Figure 24c), older women with DAO had lower extensor strength (6.19 ± 1.49 Nm/kg) compared to those with normal weight (7.96 ± 2.31 Nm/kg; $p < 0.01$) or

obesity (8.35 ± 2.63 Nm/kg; $p < 0.01$). Women with obesity and normal weight had similar strength when adjusted for body weight and lower limb lean mass (Figure 24b,c). However, women with obesity produced more torque per unit muscle when adjusted for extensor volume than normal weight women (0.106 ± 0.020 Nm/cm^3 vs 0.085 ± 0.027; $p = 0.028$; Figure 24d). Older women with DAO produced less torque per unit muscle (0.092 ± 0.034 Nm/cm^3) than women with obesity ($p = 0.04$) but this was similar to those with normal weight ($p = 0.9$).

Maximum eccentric (towards) torque for normal weight (62.5 ± 22.5 Nm), obese (56.95 ± 17.2 Nm) and DAO (48.5 ± 24.6 Nm) groups are shown in Figure 25a. Flexor (hamstring) torque relative to body weight was lower in women with DAO (0.48 ± 0.21 Nm/kg; $p < 0.001$) and obesity (0.60 ± 0.20 Nm/kg; $p = 0.01$) compared to normal weight women (0.94 ± 0.31 Nm/kg; Figure 25b). Flexor torque relative to lower limb lean mass was significantly reduced in older adults with DAO (3.05 ± 1.41 Nm/kg) compared to those with normal weight (4.72 ± 1.38 Nm/kg; $p < 0.01$) or obesity (3.85 ± 0.71 Nm/kg, $p < 0.01$; Figure 25c). Older women with DAO produced less flexor torque output per unit muscle (0.101 ± 0.039 Nm/cm^3) than obese (0.121 ± 0.016 Nm/cm^3; $p = 0.03$) or normal weight (0.130 ± 0.039 Nm/cm^3; $p = 0.02$) women (Figure 25d). Flexor torque per unit muscle was similar between obese and normal weight groups (Figure 25d).

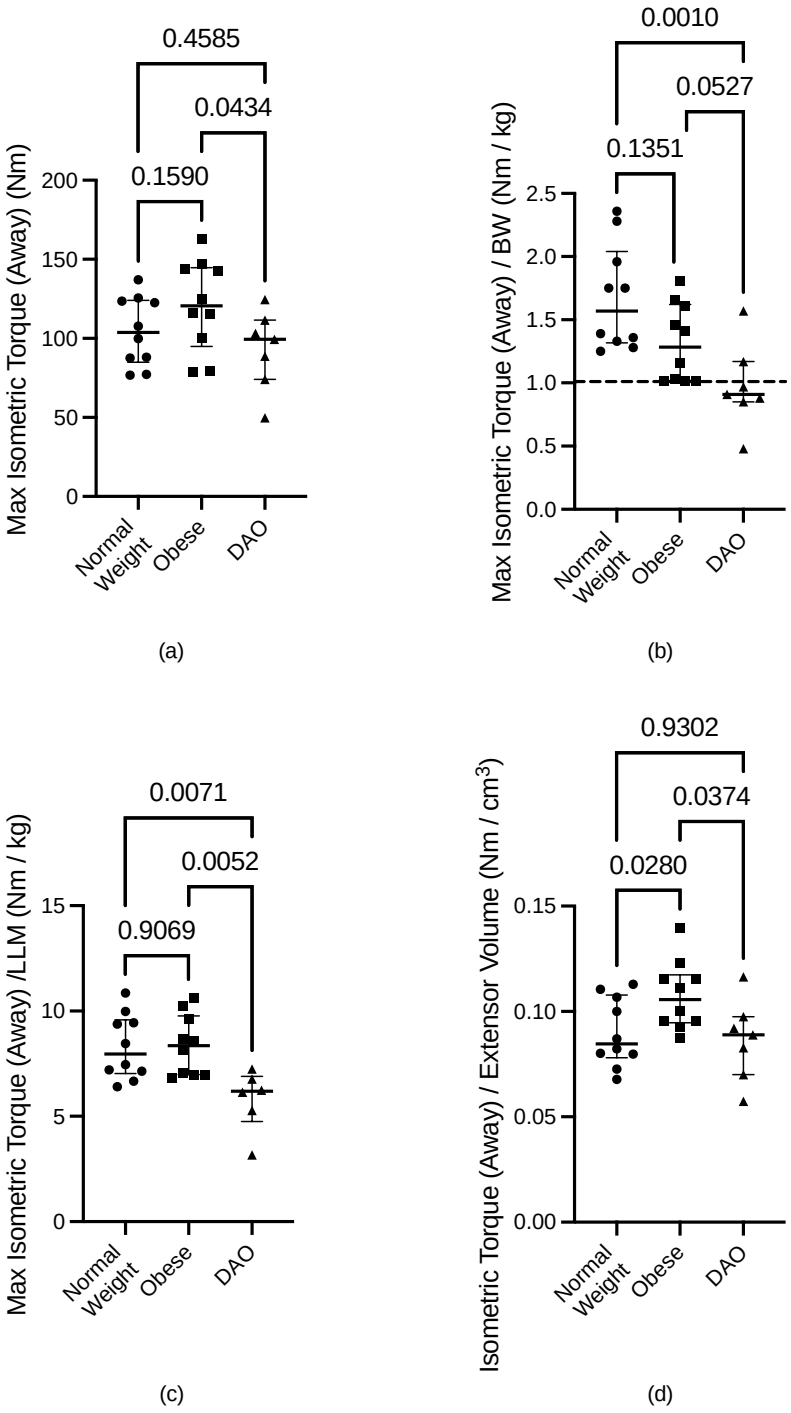


Figure 24 Isometric extensor torque (a) absolute and adjusted for (b) body weight (c) lower limb lean mass and (d) MRI extensor muscle volume

Median ± inter-quartile range. One participant with DAO is missing in (c) due to health reasons. (b) The horizontal line refers to a threshold of < 1.01 Nm/kg indicating high risk of severe mobility limitation (defined as difficulty walking ¼ mile) and gait speed < 1.22m/s [300]. Abbreviations: DAO = Dynapenic Abdominal Obesity; BW = body weight; LLM = leg lean mass.

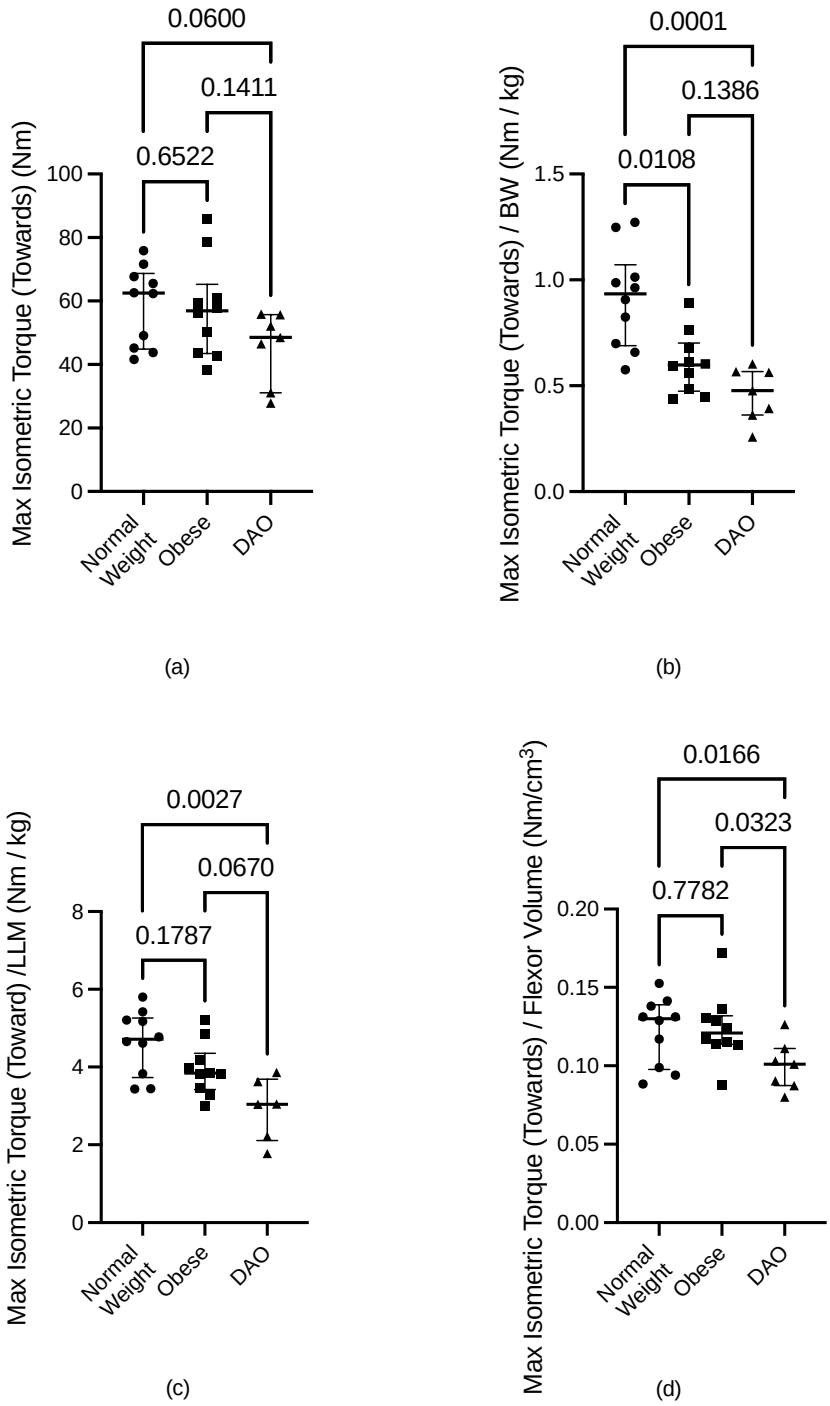


Figure 25 Isometric flexor torque (a) absolute and adjusted for (b) body weight (c) lower limb lean mass and (d) MRI flexor muscle volume

Median ± inter-quartile range. One participant with DAO is missing in (c) due to health reasons. Abbreviations: DAO = Dynapenic Abdominal Obesity; BW = body weight; LLM = leg lean mass.

Isometric Force

Torque is a product of force and the lever arm (calf) (force x lever arm) and an individual with a longer lever arm (calf) can produce greater torque than those with shorter levers. Thus, to determine the relationship between muscle volume and strength, without confounding by variations in calf length, force per unit muscle volume was determined (Figure 26).

Women with obesity produced greater force per unit extensor muscle (0.347 ± 0.048 N/cm^3) than those with normal weight (0.270 ± 0.085 N/cm^3; p = 0.02) (Figure 26a) but flexor force was similar (Figure 26b). Older women with DAO (0.278 ± 0.130 N/cm^3) had reduced extensor force output per unit compared to those with obesity (p = 0.026) and their output was more similar to the normal weight group (p= 0.96) (Figure 26a).

Older women with DAO (0.321 ± 0.131 N/cm^3) had reduced flexor force output per unit muscle compared to those with obesity (0.407 ± 0.103 N/cm^3; p = 0.033) but similar to those with normal weight (0.405 ± 0.107 N/cm^3; p = 0.057) (Figure 26b).

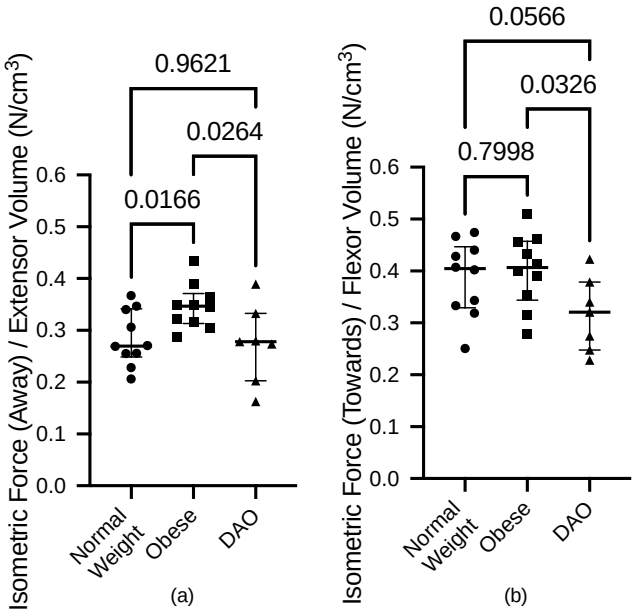


Figure 26 Isometric force by study group (a) extensor force adjusted for extensor muscle volume (b) flexor force adjusted for flexor muscle volume

Median ± inter-quartile range. Abbreviations: DAO = Dynapenic Abdominal Obesity.

Compared to older women with normal weight, women with obesity have lower knee extensor volume as a proportion of their thigh volume (Figure 21c; p = 0.011) but greater extensor force per unit muscle (Figure 26a; p = 0.001).

Compared to older women with obesity, women with DAO had a similar proportion of knee extensor and flexor volume relative to thigh volume (Figure 21c,d) but less extensor and flexor force output per unit muscle (Figure 26a,b).

Endurance

Isokinetic endurance

Compared with alternative fatigue protocols, participants in all groups failed to achieve 50 repetitions [304] whereas only one participant in both obese and DAO groups completed (more than) 30 repetitions away [305]. All groups completed a similar number of repetitions away and towards (Figure 27a, b).

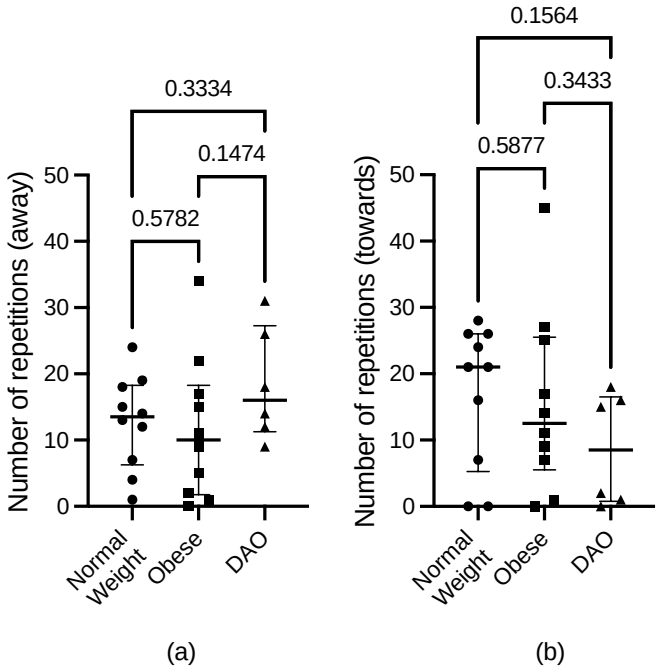


Figure 27 Number of repetitions (a) away and (b) towards completed during the isokinetic fatigue test

Median ± inter-quartile range. One participant with DAO – data was lost. Abbreviations: DAO = Dynapenic Abdominal Obesity.

6 Minute Walking Test

The normal weight group walked further (583 ± 54 m) than both the obese (493 ± 100 m; $p < 0.05$) and DAO (434 ± 33 m; $p < 0.01$) groups (Figure 28). All participants exceeded 300m, a commonly used cut-off in interventional studies, and the predicted fifth percentile for their age, gender and weight [294, 301].

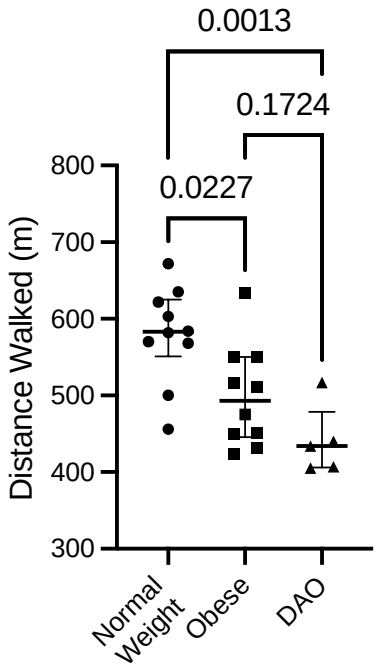


Figure 28 Distance walked during the 6-minute walk test, by group
Median ± inter-quartile range. Two people were excluded from the DAO groups (n = 1 contraindicated due to very high blood pressure [293]; n = 1 did not attend Visit 2 due to change in health circumstances). Abbreviations: DAO = Dynapenic Abdominal Obesity.

When compared to the predicted median distance expected for their age, gender and weight, the majority of participants in all groups exceeded this (Figure 29a,b). The median difference in actual 6MWD compared with the predicted distance for normal weight, obese and DAO groups was 84.4m, 51.8 and 43.7m, respectively, for the Enright & Sherrill (1998) equations. The median difference for normal weight, obese and DAO groups was 133.0m, 61.0m and 40.0m, respectively, for the Enright et al. (2003) equations. Only one participant with DAO failed to achieve the median using both equations.

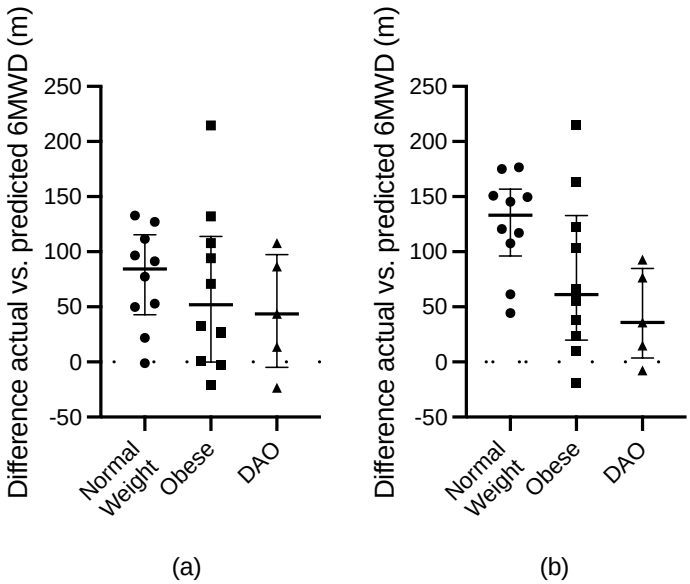


Figure 29 Difference between actual and predicted distance walked during 6 minute walking test, by group

Median ± inter-quartile range. (a) Absolute distance walked (b) Enright & Sherrill 1998 (c) Enright et al. 2003. Two people were excluded from the DAO groups (n = 1 contraindicated due to very high blood pressure [293]; n = 1 did not attend Visit 2 due to change in health circumstances). Abbreviations: DAO = Dynapenic Abdominal Obesity; 6MWD = 6 minute walking distance.

3.5 Discussion

This is the first well-characterised study of older women with DAO using gold-standard body composition and strength measurement techniques. The overall aim of this study was to identify physical and functional differences which may relate to the increased risk of falls observed in women with DAO. Overall, we failed to find a distinctive pattern of physical function which distinguishes women with DAO from those with obesity or normal weight – gait speed, balance, timed up and go test, hand-grip strength, and muscle fatigue were similar between groups. However, women with DAO had lower relative strength (torque/body weight) than women with obesity. Furthermore, using gold-standard techniques, our findings showed that obesity conferred benefits to knee extensor torque/force output per unit muscle but this benefit was lost in those with DAO. This benefit of obesity was not seen for the knee flexors; women with DAO had lower knee flexor force output per unit muscle than women with obesity. We hypothesise that these differences in women with DAO may relate to altered muscle quality as muscle volumes were similar compared to women with obesity. Therefore, deficits in knee extensor and flexor strength may impair postural control through the inverted pendulum model. We speculate that these deficits may contribute to the increased risk of falls observed in this cohort.

Using gold standard isokinetic dynamometry, we found that women with DAO had impaired knee extensor muscle torque (relative to body weight [300] and leg lean mass). Knee extensor torque is functionally important for climbing stairs and rising from a chair [306, 307]. Therefore, given that women with DAO were identified by their low sit-to-stand time, this reduction in torque is perhaps expected. However, the knee flexors and extensors are also important for postural stability (i.e. inverted pendulum model [231]) and normal locomotion [278]. Older adults who fell during a laboratory-induced trip were found to have lower knee extensor and flexor strength [308, 309] as well as lower plantar- and dorsiflexor strength [308]. Reduced knee flexor torque has also been reported in both older self-reported fallers [310] and those with a fear of falling [311]. In contrast, others have found that the rate of torque development for flexors [312], power, and sit-to-stand [313], rather than absolute strength, differed between fallers and non-fallers. Differences in study design and characteristics may explain the differences between the association of strength and falls. The study by Bohrer et al. (2022) [308] included those with an obese BMI whereas Simpkins et al. (2022) [313] measured those who were overweight. Furthermore, the studies which found no association between knee strength looked at retrospective falls which can be affected by recall bias and reverse causality [310, 313] whereas others used prospective designs [308, 309]. Thus, the increased falls risk observed

in those with DAO may relate to reduced knee extensor and flexor strength which may impair the inverted pendulum model of postural control.

We next sought to determine whether there were differences in the force production capacity of muscle groups between participants. Women with obesity produced more extensor force per unit muscle compared to those with normal weight. However, our findings suggested that this benefit was lost or reduced in women with DAO who were more similar to the normal weight group. Furthermore, there was no beneficial effect of obesity for knee flexors but women with DAO exhibited reduced flexor strength per unit muscle. There are several possible reasons why these reductions may be seen in older women with DAO. The knee extensors and flexors are used in normal locomotion [278] and our findings may relate to the reductions in physical activity reported by women with DAO. Others have shown that older women with obesity who are sedentary have less absolute knee extensor torque than those who are active, supporting the hypothesis of greater fat mass acting as a form of resistance training [314, 315]. As muscle volume was similar between obese and DAO groups, altered muscle quality (e.g. altered motor unit size, central activation, and muscle fibre type) may also explain the reduced knee extensor and flexor force output [28, 158]. For example, neural deficits are thought to explain one third of the variance in knee extensor strength, similar to thigh lean mass (DXA) in older adults [48]. It is also possible that despite similar muscle volumes, women with DAO have less active muscle due to fat infiltration. Muscular fat infiltration (computed tomography derived muscle attenuation) is associated with peak torque, rate of torque development [316], strength, and mobility impairment independently of muscle size [33, 102]. Muscular fat infiltration may also create an altered hormonal milieu and thus affect force output [36, 82, 317]. Therefore, differences in knee flexor/extensor function in women with DAO may not fully relate to differences in muscle size but also differences in muscle quality and neural deficits [48]. Further consideration of muscle quality parameters in women with DAO is required as this may offer alternative treatment options.

The finding of increased extensor force per unit muscle in obesity is perhaps counterintuitive given the large body of evidence showing greater intramuscular fat in people with obesity with subsequent negative effects on strength [33-35, 102, 316, 318]. One hypothesis for the increased extensor force (per unit muscle) observed in those with obesity is that the extra body weight acts as a form of constant training stimulating favourable muscle adaptations [315]. Our findings tentatively support this hypothesis using gold standard techniques. From a morphological perspective, changes in muscle fibre type may explain this increased force as obesity is associated with a higher proportion of Type IIa myofibers [319-321]. The power output from Type IIa myofibers is approximately three times

greater than Type I fibres or 1.5 times greater relative to cross sectional area [322]. Together, it may be hypothesised that muscles in obesity may be stronger due to differences in muscle fibre composition. However, studies on muscle fibre composition in obesity tend to be in younger and middle-aged adults [319-321] with very limited information in older adults with obesity [24, 40]. Gueugneau et al. (2014) [24] reported a similar proportion of Type I and IIa fibres (vastus lateralis) between men with and without metabolic syndrome; however, Type IIa muscle fibre cross-sectional area was larger in men with metabolic syndrome. In contrast, another study of older adults with obesity by Choi et al. (2016) [40] found a lower proportion of Type IIa muscle fibres compared to those with normal weight. Furthermore, the force production ability of individual muscle fibres (vastus lateralis) was 28% and 25% lower in Type I and IIa fibres, respectively, in older adults with obesity compared to those with normal weight and this related to intramyocellular lipid concentration [40]. These findings are in contrast with our results which may relate to study differences. Choi et al. (2016) [40] grouped both men and women, the obese group ranged from 27-35kg/m^2, and isokinetic torque rather than isometric force was measured. Moreover, whilst the vastus lateralis is the largest of the quadriceps muscles, compositional differences in other quadriceps muscles may exist in those with obesity and this requires consideration. More work is required to understand the relationship between obesity, strength and the mechanisms underlying force and power output. Understanding the determinants of muscle strength in older adults with obesity can provide greater insight into interventions to improve muscle strength.

We next sought to determine if there were differences in localised fatigue between groups. With repetitive movements, such as in a localised fatigue protocol, older adults show reduced contractile velocity [323] and slower recovery [324]. In our study, however, we found that all groups performed equally in the isokinetic fatigue test suggesting no effect of obesity on localised fatigue. There is limited information available on localised fatigue in older adults with obesity and the studies available have used heterogenous protocols. Paolillo et al. (2012) [235] found that postmenopausal women with obesity are more fatigue resistant than their lean counterparts; however, all participants completed a standardised rather than individualised fatigue protocol. Bellew and Fenter (2006) [245] found that an individualised fatigue protocol resulted in reduced dynamic balance measured using a lower extremity reach test.

The 6MWT was used to evaluate the global response of the body's systems during a dynamic fatiguing exercise relevant to postural balance [231, 293]. Whilst the obese and DAO groups walked a shorter distance than the normal weight group, there was no difference in distance walked between obese

and DAO groups. This finding reflects the reduced gait speed also observed in the obese and DAO groups compared to the normal weight group. As we were unable to measure specific parameters of gait related to the 6MWT, it is possible that we missed more subtle gait alterations relevant to falls risk which were not observed by quantification of distance walked. Others have shown reduced minimum foot clearance, greater step variability, and asymmetry in leg function (i.e. nondominant leg used for stability) after six minutes of fast walking [325]. In addition to reduced gait speed, people with obesity also exhibit multiple gait adaptations including a shorter stride length, shorter swing time, slower stride rate, and increased stride width [126, 326, 327]. Therefore, there may be further obesity-related alterations which could explain the higher risk of falls associated with DAO. However, further confirmation of the association between DAO, gait speed as well as measurement of gait-related spatio-temporal parameters is required.

Due to its accessibility, safety, and accuracy, DXA is the preferred technique to measure lean mass with consensus definitions of low lean mass proposed [4, 76, 186]. However, it is increasingly argued that lean mass is a poor surrogate measure of muscle mass due to inconsistent associations with clinical outcomes [97, 98]. There are further challenges when considering lean mass in those with obesity or dynapenia. In obesity, lean mass is highly correlated with body weight and adjustments either invert or strengthen this relationship [53, 239]. Lean mass is also not pre-requisite to the definition of dynapenia [19] relating to the fact that reductions in lean mass do not fully account for reductions in muscle strength [224]. Therefore, it is unsurprising that participants with DAO did not have low lean mass based on either the EWGSOP2 or ESPEN/EASO definitions [4, 76]. These findings do, however, provide further support for the concept of dynapenia. The use of lean mass cut-offs as currently formulated in those with obesity would fail to identify those with low strength and function.

In order to overcome one of the primary limitations of DXA-acquired lean mass (i.e. that it measures both muscle and other non-fat soft tissue), we used gold-standard MR imaging to determine whether there were differences between thigh muscle volume between groups. No difference was found between any group for absolute knee extensor and flexor volume. It is difficult to compare our findings with the literature due to heterogeneity in measurement and reporting of MRI measured thigh muscle (as total muscle volume, individual muscle volume or cross-sectional area) [241, 242, 271]. Similar to others [53], knee extensor and flexor volumes as a percentage of the total thigh were significantly reduced in those with obesity or DAO compared to women with normal weight. However, there was no difference between older women with DAO and those with obesity. Together, these findings

support our previous argument that muscle quality is an important determinant of function in older adults with obesity.

3.5.1 Limitations

There are several limitations to this research. The low number of participants in this study limits our ability to make definitive comparisons. The sample size was calculated to identify a difference in microRNA levels and the findings presented should be considered as exploratory. For this reason, we also did not adjust our statistical tests for multiple comparisons [299]. Next, we were unable to include a group with normal weight/dynapenia due to (Covid-19) recruitment delays which limits our ability to determine a 'dynapenic' signature. Further, due to Covid-19, we used sit-to-stand instead of hand-grip strength to screen participants to reduce unnecessary travel/exposure, potential transmission and due to reduced healthcare capacity. Whilst hand-grip strength and sit-to-stand are included as measures of strength in consensus definitions of sarcopenia or sarcopenic obesity [4, 76], sit-to-stand time was not included in the original definition of dynapenia [19]. The gait accelerometery data was contaminated for a large number of participants and could not be used; therefore, we were unable to look in more detail at the effects of DAO and fatigue on gait-specific parameters during the 6MWT. We focused on specific aspects of muscle strength and function; other risk factors for falls such as other measures of muscle function (e.g. rate of torque development), proprioception, or vision were not considered and warrant consideration in future research. Lastly, we measured strength and muscle volume in the dominant leg; there is evidence that differences in muscle volume [241] and muscle function [325] exist between an individual's legs and this should be considered in future research. However, there are strengths to this study. This is the first well-characterised study of women with DAO using gold standard techniques in an attempt to understand the increased risk of falls in this phenotype. Furthermore, this study is novel as muscle strength measured using isokinetic dynamometry and muscle volume measured using MRI have not been reported or connected in the context of DAO or sarcopenic obesity.

3.6 Conclusion

In this study of older women with normal weight, obesity and DAO we attempted to identify patterns of physical function which may elucidate the mechanism for the increased falls risk in women with DAO. Women with DAO appear to have deficits in knee extensors and flexors compared to women with obesity. Specifically, women with DAO have lower relative strength (torque/body weight) than women with obesity. Women with DAO lose the obesity-related benefits on knee extensor force

output and furthermore have reduced knee flexor force compared to women with obesity. Therefore, we hypothesise that deficits in knee extensor and flexor strength may impair postural control. Further work in a larger cohort is required to confirm these findings. Consideration of ankle and hip strength (other key components of the inverted pendulum model) and detailed gait parameters are required. Overall, however, we failed to find a distinctive pattern of physical function which distinguishes women with DAO from those with obesity or normal weight. Therefore, it is unclear whether DAO represents a truly distinct phenotype and whether this heterogeneity may partially explain the poor performance of DAO to predict outcomes at an individual level.

Chapter 4

MicroRNAs in sarcopenia and obesity, commonalities for sarcopenic obesity

4.1 Introduction

The purpose of this chapter was to determine whether there is a potential role for microRNAs in the pathogenesis of dynapenic abdominal obesity (DAO). In Chapter 2 we identified a greater risk of fracture in women with DAO compared to those with obesity only. This increased risk of fracture may be related to the higher risk of falls observed rather than altered aBMD. In Chapter 3, we completed a detailed phenotyping study of older women with DAO using gold-standard techniques in order to identify patterns of physical function which may relate to the previously observed falls risk. Reduced knee extensor and flexor strength were identified and it was speculated that this may result from reduced muscle quality. To date, diet and exercise are the main treatment options available for obesity or dynapenia, with very limited pharmacological therapies. Due to varying adherence and abilities to comply with these interventions, alternative treatment strategies are required for an ageing population with growing obesity levels. A greater understanding of the pathogenesis underlying DAO has the potential to identify alternative targets for intervention.

This chapter systematically reviewed the available published evidence in both obesity and sarcopenia to determine if there were any commonly expressed microRNAs which may be relevant to DAO.

The systematic review is presented as a published manuscript. Tables and figures are located at the end of the manuscript.

Contribution details

Title	MicroRNAs in obesity, sarcopenia, and commonalities for sarcopenic obesity: a systematic review
Publication Status	Published in Journal of Cachexia, Sarcopenia and Muscle, Volume 13, Issue 1, February 2022 https://doi.org/10.1002/jcsm.12878
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Student author statement:
In publication I, the candidate, planned and wrote the manuscript, co-author reviewed and provided comments on the manuscript.

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Co-author statement:
I hereby declare that I am aware that the work in the manuscript entitled: "MicroRNAs in obesity, sarcopenia, and commonalities for sarcopenic obesity: a systematic review" of which I am co-author, will form part of the PhD thesis by the PhD student Lisa Dowling who made a major contribution to the work stated above.

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Title

4.2 “MicroRNAs in obesity, sarcopenia and commonalities for sarcopenic obesity – a systematic review”

Journal name

Journal of Cachexia, Sarcopenia and Muscle

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4.2.1 Abstract

Background: Sarcopenic obesity is a distinct condition of sarcopenia in the context of obesity, with the cumulative health risks of both phenotypes. Differential expression of microRNAs (miRNAs) has been reported separately in people with obesity and sarcopenia and may play a role in the pathogenesis of sarcopenic obesity. However, this has not been explored to date. This study aimed to identify differentially expressed miRNAs reported in serum, plasma, and skeletal muscle of people with obesity and sarcopenia and whether there are any commonalities between these conditions.

Methods: We performed a systematic review on EMBASE and MEDLINE (PROSPERO, CRD42020224486) for differentially expressed microRNAs (fold change >1.5 or p-value < 0.05) in (i) sarcopenia or frailty and (ii) obesity or metabolic syndrome. The functions and targets of miRNAs commonly changed in both conditions, in the same direction, were searched using PubMed.

Results: Following de-duplication, 247 obesity and 42 sarcopenia studies were identified for full-text screening. Screening identified 36 obesity and 6 sarcopenia studies for final inclusion. A total of 351 miRNAs were identified in obesity and 157 in sarcopenia. Fifty-five miRNAs were identified in both obesity and sarcopenia – by sample-type, 48 were found in plasma and one each in serum and skeletal muscle. Twenty-four miRNAs were identified from 10 of the included studies as commonly changed in the same direction (22 in plasma and one each in serum and skeletal muscle) in obesity and sarcopenia. The majority of miRNA validated targets identified in the literature search were members of the phosphoinositide 3-kinase/protein kinase B and transforming growth factor beta signalling pathways. The most common targets identified were insulin-like growth factor (miR-424-5p, miR-483-3p, miR-18b-5p) and members of the SMAD family (miR-483-3p, miR-92a-3p, miR-424-5p). The majority of commonly changed miRNAs were involved in protein homeostasis, mitochondrial dynamics, determination of muscle fibre type, insulin resistance and adipogenesis.

Conclusion: Twenty-four miRNAs were identified as commonly dysregulated in obesity and sarcopenia with functions and targets implicated in the pathogenesis of sarcopenic obesity. Given the adverse health outcomes associated with sarcopenic obesity, understanding the pathogenesis underlying this phenotype has the potential to lead to effective screening, monitoring, or treatment strategies. Further research is now required to confirm whether these miRNAs are differentially expressed in older adults with sarcopenic obesity.

Key Words: MicroRNA; Sarcopenia; Obesity; Frailty; Metabolic syndrome

4.2.2 Introduction

Sarcopenic obesity is a condition of excess fat mass and sarcopenia [4, 129]. Differing definitions of sarcopenia have been proposed with growing consensus on the importance of muscle function [4, 58]. Sarcopenic obesity is more commonly found amongst older adults; however, it can also be found in younger adults during both acute and chronic disease, or intermittent weight cycling [84]. Dependent on the definition used, sarcopenic obesity is thought to range in prevalence from 2.75% to over 20% [84]. Of clinical importance, sarcopenic obesity may have the cumulative risk of both sarcopenia and obesity [328]. Growing evidence supports this with a greater risk of falls, hospitalisation, worsening disability and all-cause mortality reported [95, 114, 329].

The aetiology of sarcopenic obesity is complex and not fully understood (see Batsis and Villareal and Zamboni et al. [83, 129] for detailed reviews). Ageing is associated with changes in body composition including a loss of lean mass, increased body fat and muscular fat infiltration, with a subsequent reduction in resting metabolic rate [28, 129]. Reduced physical activity and malnutrition (including over- or under-nutrition and malabsorption) associated with ageing contribute to a gradual increase in body fat [129] and the development of sarcopenia [4]. Moreover, excess body fat or obesity can exacerbate sarcopenia [4, 221]. Obesity is associated with low-grade inflammation with the secretion of tumour necrosis factor, leptin, and C-reactive protein [129]. Leptin elevates the levels of pro-inflammatory cytokines which cause a reduction in the anabolic effects of insulin-like growth factor 1 (IGF-1) [129]. This inflammation leads to insulin resistance, further exacerbated by muscle catabolism, which promotes fat mass and loss of muscle mass [129, 154]. As such, changes associated with ageing, obesity, and sarcopenia as well as interrelationships between these phenotypes can contribute to the pathogenesis of sarcopenic obesity.

MicroRNAs (miRNAs, miRs) are short, noncoding RNAs which can regulate gene expression at a post-transcriptional level [161]. To date, 2654 miRNAs have been discovered which are predicted to regulate two thirds of the human genome [162, 164]. Therefore, many miRNAs may modulate many physiological processes. There is evidence that ageing changes miRNA levels in the muscle and that these changes may have a detrimental impact on muscle quality and quantity [159, 298]. However, the influence of obesity or adiposity on the microRNA profile of older adults and whether this translates into functional impairment has not yet been established. Evidence from rodent studies has demonstrated that adipose-derived miRNAs can be transported via exosomes to a variety of host cells including myocytes, hepatocytes, and macrophages [18-20]. Likewise, skeletal muscle-derived

miRNAs can be taken up by adipose tissue [21]. Functionally, this inter-organ cross-talk has been implicated in insulin resistance, adipogenesis and lipid metabolism [177-179, 330] thus suggesting a role for miRNAs in the pathogenesis of sarcopenic obesity. MicroRNAs are an exciting area of research due to the potential of antagomiRs which are already been explored as pharmacological options in conditions such as cardiovascular disease and cancer [180, 331].

The primary aim of this systematic review was to identify differentially expressed miRNAs reported in plasma, serum, or skeletal muscle of adults with obesity or sarcopenia to determine common microRNA changes between these phenotypes. As this is an emerging area with limited research, studies reporting (i) sarcopenia or frailty and (ii) obesity or metabolic syndrome were included due to similarities between definitions. A secondary aim of this review was to identify the targets and functions of these differentially expressed miRNAs.

4.2.3 Methods

Protocol registration:

The Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) statement was followed as a reference protocol standard [332]. A PRISMA flow chart is included. Our protocol was registered at the International Prospective Register for Systematic Reviews PROSPERO, with registration number CRD42020224486; available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020224486.

Bibliographic search and eligibility criteria:

This systematic review consisted of two searches performed on MEDLINE and EMBASE (last searched 6 January 2021). Part 1 searched for studies of sarcopenia or frailty using the following terms: MicroRNA/miR/miRNA AND “sarcopenia”, “muscle strength”, “frail”, “ageing”/“older adult”/“aged” AND “muscle”, “muscle weakness”, OR “dynapenia”. Part 2 searched for studies of obesity or metabolic syndrome using the following terms: MicroRNA/miR/miRNA combined with “obesity” OR “metabolic syndrome”. The multipurpose function was used for keywords and MeSH terms were used where available. Articles were limited to the English language and human studies. Eligible studies enrolled adult participants (>18y) with sarcopenia, frailty, metabolic syndrome, or obesity and comparable non-sarcopenic/frail or non-obese/metabolic syndrome controls as outlined in (Table 21, Supplementary). Studies were excluded if the primary condition of interest was not sarcopenia, frailty, metabolic syndrome, or obesity but instead an unrelated disease or condition, for example,. type 1 diabetes, cancer, or pregnancy, which may have confounded findings. Study groups containing some, but not all, type 2 diabetes participants were included. The considered biological fluids and tissues were serum, plasma, and skeletal muscle. Results on tissue samples or cell lines were excluded. Observational (cohort and case control) studies or intervention studies with relevant baseline results were included.

Study Selection:

Following deduplication, all selected titles and abstracts were screened to identify articles for full-text screening. A second reviewer verified a random sample of articles included in the first sift. The second sift, which consisted of full text screening, was independently conducted by two reviewers to confirm that the criteria for the condition of interest, samples, age-group, and outcome measure in the study met eligibility criteria. In cases where the same or similar results were reported in more than one

study, the publication with the most information was included and the other rejected for duplication. Authors of papers with insufficient information relating to eligibility criteria or outcome measures for this review were contacted. If a reply was not received within one month, only the information reported in the paper was included (e.g. incomplete list of miRNAs) or else the paper was rejected if eligibility criteria remained unclear (e.g. age group). A more comprehensive list of miRNA results was obtained from one study following email correspondence [251]. Disagreements between reviewers which could not be solved with discussion were resolved with a third reviewer by consensus.

Data extraction:

A standardised form was used to extract trial features (authors, published year, and country), patient characteristics (age and sex), RNA extraction and detection methods, the subset of differentially expressed miRNAs between the two conditions. Extracted information was verified by a second reviewer.

Risk of bias in individual studies:

Two reviewers assessed the quality and risk of bias of individual studies using the Newcastle Ottawa Scale (NOS) with an additional star for validation of results within the study using either two measurement methods or two study groups (Table 22, Supplementary). Due to the type of studies included, all studies were given a star for the question on non-response rate. Disagreements between reviewers which could not be solved through discussion were resolved with a third reviewer by consensus.

Summary Measures:

The outcome measure was differentially expressed miRNAs in human skeletal muscle, plasma, or serum with at least a 1.5-fold change or $P < 0.05$ measured using RT-qPCR, next generation sequencing (NGS) or microarray. Circulating (serum and plasma) miRNAs may be useful as non-invasive biomarkers whereas miRNAs found in muscle may provide a mechanistic insight into sarcopenic obesity.

Because of differences in nomenclature between studies, we used the information for previous miRNA IDs on miRbase (Release 22.1; October 2018) to clarify and update the nomenclature of included miRNAs which did not specify whether they were -3p or -5p [162]. The BioVenn online interface was

then used to identify potentially overlapping miRNAs [333]. Overlapping miRNAs that were differentially expressed in both obesity and sarcopenia were further classified by tissue/fluid type and as either differentially expressed in (i) the same direction (e.g. upregulated), (ii) different directions (e.g. up-regulated in obesity and down-regulated in sarcopenia), (iii) conflicting directions (e.g. both up- and down-regulated in one condition but not the other), or (iv) unclear (e.g. differences in nomenclature limited interpretation).

Synthesis of results and additional analyses:

MiRNAs that were differentially expressed in the same direction in obesity and sarcopenia were identified for further investigation. A literature search was conducted on PubMed to identify validated target genes or functions of these miRNAs with regard to muscle, sarcopenia or frailty and obesity, metabolic syndrome, or insulin resistance. A narrative synthesis of the findings from the studies and in the context of sarcopenic obesity, their target genes and metabolic pathways implicated is provided.

4.2.4 Results

Bibliographic Search

The bibliographic search in MEDLINE and EMBASE retrieved 4097 obesity-related papers and 2357 sarcopenia-related papers published before 6 January 2021. Following de-duplication, 2971 papers were screened for obesity and 2019 for sarcopenia. Following screening, 247 obesity studies and 42 sarcopenia studies were included for full text review. In total, 36 studies were identified for obesity and six for sarcopenia (Figure 30). MiRNAs dysregulated in both sarcopenia and obesity were identified from 10 studies [251, 334-342].

Studies were conducted in Korea [251], China [339], Singapore [336], New Zealand [335, 337], the USA [338, 341], Spain [334], and the UK [340]. The majority of obesity studies used World Health Organisation criteria for obesity [251, 334, 336, 337] although the criteria for two studies was unclear [335, 342]. The sarcopenia studies used Fried Frailty Score [338], Asian Working Group for Sarcopenia [339] and European Working Group on Sarcopenia in Older People 2010 [340, 341] criteria. Four studies were conducted in women only [334, 335, 337, 341] and three studies in men only [336, 340, 342]. Two studies recruited both men and women [251, 338] and one study did not report the sex of participants [339]. Based on the reported average age, obese participants would not be defined as older adults, age ≥ 65 years, [251, 334-337]. Sarcopenia studies recruited older adults [338-340] although one used a younger cut-off of 60 to 85 years [341]. Only three studies validated its findings [334, 337, 339].

MiRNAs reported as dysregulated in the context of sarcopenia and obesity

A total of 351 miRNAs were identified in obesity and 157 in sarcopenia (Figure 31). Fifty-five potential miRNAs were identified in both obesity and sarcopenia. When examined by sample type, 48 overlapping miRNAs were identified in plasma and one each in serum and skeletal muscle (vastus lateralis). Sixteen plasma miRNAs were expressed in differing directions in obesity and sarcopenia. Eight plasma miRNAs in obesity, which were also present in sarcopenia, were expressed in conflicting directions. Two plasma miRNAs could not be determined with confidence due to the nomenclature in the studies (miR-328, miR-215). Therefore, across six obesity [251, 334-337, 342] and four sarcopenia [338-341] studies, we manually identified 24 miRNAs differentially expressed in the same direction. Twenty-two of these miRNAs were found in plasma and one each in serum and skeletal muscle (Table 19). Of these overlapping miRNAs, only miR-23a-3p was reported in more than one tissue (serum and plasma) of both sarcopenia and obesity; however, in plasma, conflicting directions were reported in obesity. The majority of overlapping miRNAs were identified in two studies, one of which used RT-

qPCR [336] and the other used RNA-seq [338]. Exosomal miRNAs were reported by three studies [251, 334, 338]. Twenty-two of the 24 overlapping miRNAs were found in one study of frailty using plasma exosomes [338].

Two miRNAs may also be commonly expressed in obesity and sarcopenia but differences in nomenclature limited our understanding. Plasma miR-328-3p [336] and miR-328 [335] were down- and up-regulated in obesity, respectively and miR-328 was downregulated in sarcopenia [339]. Plasma miR-215 was upregulated in both obesity [335] and sarcopenia [338], miR-215-5p was also upregulated in obesity [336]. However, as we could not determine whether miR-215 was -3p or -5p using the previous ID section on miRbase we classified this miRNA as an unclear match. A list of the top externally validated circulating (plasma or serum) miRNAs in obesity or sarcopenia is available in Table 23, Supplementary.

Assessment of risk of bias

The majority of studies scored ≤ 6 on the NOS (2 [251], 3 [335, 342], 4 [338], 5 [336], 6 [340], 6 [341]), two received a star for validation (4* [334], 6* [337]) and one study scored > 6 (8* [339]). All studies lost a mark for failing to comment on the representativeness of cases [251, 334-342], five of six obesity studies lost a mark for failing to adequately define how controls were selected [251, 334-336, 342] and only four studies received marks for adequately describing how exposure was ascertained [336, 339-341]. All studies, except three [334, 336, 342], received a mark for adequately describing the case definition.

Validated target genes, metabolic pathways and functions of miRNAs

Validated target genes of the miRNAs of interest, in relation to sarcopenia, obesity and related conditions (e.g. insulin resistance, inflammation, and cachexia) where possible, were identified by conducting a literature search using PubMed (Table 20). The majority of validated targets identified in the literature search were members of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) and transforming growth factor beta (TGF-β) signalling pathways. The most common targets identified were IGF-1 (miR-424-5p, miR-483-3p, and miR-18b-5p) and members of the SMAD family (miR-483-3p, miR-92a-3p, and miR-424-5p). MiRNAs also targeted phosphatase and tensin homolog (PTEN) (miR-296-3p and miR-499) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) both directly (miR-23a-3p) and indirectly (miR-499 via Fnip). The forkhead box protein (FOXO) family was targeted by miR193b-5p. AMP-activated protein kinase (AMPK) was also targeted directly (miR-1224-5p) and indirectly (miR-499 via Fnip). The majority of commonly expressed miRNAs

were involved in protein homeostasis, mitochondrial dynamics, determination of muscle fibre type, insulin resistance, and adipogenesis.

4.2.5 Discussion

In this systematic review, we identified 24 miRNAs which are differentially expressed in both sarcopenia and obesity. These findings are particularly novel as miRNAs have not yet been explored in the context of sarcopenic obesity. The common dysregulation of the miRNAs identified in this review may therefore provide clues to understand the pathogenesis of sarcopenic obesity. To address this aim, a search was subsequently undertaken to understand the functions of these 24 miRNAs in relation to muscle/sarcopenia and adiposity/obesity. For some miRNAs, there were limited or no studies in the context of obesity or sarcopenia and therefore their relevance in relation to sarcopenic obesity is still unclear at present (miR-29b-2-5p, miR-378c, miR-4732-5p, miR-487a-3p, miR-550a-3p, miR-576-5p, and miR-589-5p). Other miRNAs have been shown to be differentially regulated in related diseases or metabolic responses, for example, in chronic obstructive pulmonary disease or amyotrophic lateral sclerosis (miR-1246) [343, 344], in response to a high fat meal (miR-145-5p) [345] or exercise (miR-766-3p) [346, 347]. However, we found that the majority of these commonly expressed miRNAs were involved in protein homeostasis, mitochondrial dynamics, determination of muscle fibre type, insulin resistance, and adipogenesis – processes implicated in the development of sarcopenic obesity [83, 129]. The targets identified were predominantly found in the PI3K/AKT and TGF-β pathways.

Protein homeostasis

IGF-1 is one of the most important mediators of muscle growth and repair [134]; however, IGF-1 declines with age [136]. MiRNAs identified to be upregulated in both obesity and sarcopenia target IGF-1, leading to its inhibition. The miRNAs miR-18b-5p, miR-483-3p, and miR-424-5p target IGF-1 *in vitro* [348-351]. Functionally, *in vitro* studies have shown that miR-483-3p inhibits bovine myoblast cell proliferation through the IGF1/PI3K/AKT pathway [349] and promotes apoptosis in hyperglycaemic cardiomyocytes [348]. Up-regulation of miR-483-3p causes a reduction in muscle diameter in mice and is also upregulated in muscle wasting conditions in humans [340]. These studies therefore suggest that upregulation of these miRNAs could be detrimental to muscle metabolism.

We found that miRNAs implicated in both obesity and sarcopenia regulate several targets of the PI3K/AKT pathway, which is involved in protein homeostasis [135]. Downstream of AKT, FoxO3 is targeted by miR-193b-5p [352]. The FOXO family is implicated in many processes included cell cycle, apoptosis, autophagy and muscle atrophy [135, 352]. In muscle, FOXO proteins are important mediators of two major proteolytic cellular pathways – the autophagy-lysosome and ubiquitin-proteasome systems [135]. These pathways are critical for quality control of sarcomeric proteins

[135]. Interestingly, miR-23a-3p targets the muscle atrophy genes atrogin-1/MAFbx1 and MURF-1 which are downstream targets of FOXO [353]. Ectopic overexpression of miR-23a-3p counteracts muscle atrophy in dexamethasone treated myotubes and glucocorticoid treated mice [353]. In addition to direct targeting, miRNAs have been reported to target regulators of the FOXO family. MiR-199a-5p, which is in sarcopenia and obesity, targets and suppresses Sirt1 [354, 355] which is responsible for the deacetylation of FOXO; suppression of Sirt1 results in cellular senescence *in vitro* [354]. Upregulation of miR-199a-5p promotes apoptosis and ROS formation *in vitro* [355]. Dysregulation in the PI3K/AKT/FOXO pathway may have implications on muscle atrophy and muscle quality control with implications for sarcopenia.

In mature adult muscle, TGF-β is a potent regulator of muscle atrophy which impairs skeletal muscle regeneration through inhibition of satellite cell proliferation, myofiber fusion, and expression of some muscle-specific genes [356]. Multiple miRNAs commonly expressed in sarcopenia and obesity target this pathway. MiR-499 targets TGF-βR1, a receptor for TGF-β [357]. Knockdown of this receptor inhibits myogenic differentiation in C2C12 cells [357]. Downstream of TGF-βR1, Smad4 is downregulated by miR-483-3p to induce apoptosis *in vitro* [358]. Two miRNAs upregulated in obesity and sarcopenia, miR-424-5p [340] and miR-92a-3p [359], target Smad7, a strong inhibitor of the TGF-β pathway which in turn inhibits SMAD2/3. Therefore, in sarcopenia and obesity, the TGF-β pathway may be inhibited by miR-483-3p but promoted by miR-424-5p, miR-92a-3p and the downregulation of miR-499. It is unclear what effect this may have in relation to the pathogenesis of sarcopenic obesity but known targets of TGF-β pathway include the muscle atrophy genes, atrogin-1 and MuRF-1.

Mitochondrial dynamics

MiRNAs dysregulated in both obesity and sarcopenia regulate mitochondrial biogenesis. MiR-196a-5p is highly expressed in myoblasts and suppresses PGC1β, a regulator of mitochondrial biogenesis [360]. MiR-499a-5p, which is down-regulated in sarcopenia/obesity, targets Fnip1 which in turn inhibits AMPK with subsequent reduced activation of PGC-1α [361]. Functionally, inhibition of Fnip1 by miR-499a-5p results in improved mitochondrial function in myocytes and improved mitochondrial capacity in mice with muscular dystrophy [361]. In adults with amyotrophic lateral sclerosis, of which mitochondrial dysfunction is considered an important factor in its pathogenesis, miR-23a-3p is elevated [362] similar to adults with obesity and sarcopenia [251, 341]. MiR-23a-3p targets PGC-1α with inhibition of its downstream signalling of mitochondrial biogenesis [362]. Likewise, miR-92a-3p [359] and miR-424-5p [340] target SMAD7, an antagonist of SMAD2/3. Functionally, miR-92a-3p inhibits mitochondrial content and oxygen consumption of brown adipocytes [359]. MiR-499a-5p also

inhibits mitochondrial fission and apoptosis in cardiomyocytes exposed to an anti-tumour drug by targeting p21, thus preventing cardiotoxicity [363]. Taken together, the dysregulation observed in these miRNAs in obesity and sarcopenia may lead to impaired mitochondrial function.

Fibre type switching

Ageing is associated with a switch from a fast muscle-fibre phenotype to one of a slow muscle-fibre type [22] whereas obesity is associated with a greater proportion of fast, Type II, muscle fibres [319]. Overexpression of miR-499 in vitro is associated with conversion of fast myofibers to slow through targeting of Sox6 and Thrap1 [172, 364]. In mice, knockout of miR-499 and miR-208b results in a loss of slow Type I myofibers and an increase in fast Type IIx/d and IIb myofibers [172]. In humans, miR-499 is associated with a slow muscle fibre phenotype [365] and is elevated in patients with Duchenne's muscular dystrophy [366] and chronic obstructive pulmonary disease [367]. It is interesting that miR-499 is under-expressed in both sarcopenia and obesity in light of the different muscle fibre properties of obesity and sarcopenia. However, it must be noted that this miRNA was reported in plasma rather than skeletal muscle where levels may be different.

Insulin resistance

Obesity is associated with insulin resistance which can also impair muscle regeneration [129, 154]. Several miRNAs identified as commonly up-regulated in sarcopenia and obesity affect glucose metabolism and are associated with insulin resistance [368-370]. MiR-197-3p can regulate glucose metabolism by suppressing PCSK1/3 to inhibit GIP and GLP-1 production, incretin hormones implicated in the pathogenesis of diabetes [368]. Overexpression of miR-424-5p is associated with decreased insulin-induced glycogen synthesis in hepatocytes [369]. In young adults, miR-193b-5p is also negatively correlated with BMI, plasma glucose levels and insulin response [370]. In contrast, downregulation of two miRNAs in obesity and sarcopenia may be beneficial for glucose metabolism – miR-199a-5p and miR-499 [371, 372]. MiR-199a-5p is upregulated in diabetes and in vitro studies have shown that miR-199a-5p targets and represses GLUT4, a glucose transporter isoform which increases glucose transport in response to insulin [371]. MiR-499 targets both PTEN and PTENP1; PTENP1 can act as a 'sink' for miR-499 to allow glucose metabolism [372]. It is unclear what effect these miRNAs may have in relation to the pathogenesis sarcopenic obesity.

Adiposity and adipogenesis

Gains in body fat and intramyocellular lipid deposition is characteristic of ageing, obesity and sarcopenia [22, 28, 129]. MiRNAs differentially expressed in both obesity and sarcopenia were associated with parameters of adiposity. Body mass index is correlated with miR-1246 [373] and miR-193b-5p [370] in adults. MiR-92a is negatively correlated with brown adipose tissue activity in young adults [374] and inhibition of miR-92a upregulates brown adipocyte differentiation *in vitro* [359]. MiR-499a-5p promotes myogenic rather than adipogenic differentiation in skeletal muscle stem cells [375]. MiR-1224-5p contributes to hepatic lipid accumulation in mice by targeting AMPKα1 [376].

Limitations

The limitations of this study must be considered. Firstly, the heterogeneity and low quality of the studies identified in this review must be acknowledged. In some cases, matches were found between younger or female obese studies and older or predominantly male sarcopenia studies. As such, it is unclear how the interaction of age or sex have impacted our findings. It is known that age affects miRNA profiles so perhaps older obese adults have differing miRNA profiles than younger obese adults, likewise males and females may exhibit differing profiles within the same condition. Secondly, we only included studies that had significantly different microRNAs, and therefore, we may have missed studies with non-significant miRNAs, which may dispute our findings. However, this approach is commonly accepted [184, 185]. Thirdly, due to the large number of overlapping miRNAs identified, we chose to discuss miRNAs, which were commonly dysregulated in the same direction in both conditions. The overlapping miRNAs identified were not externally validated, and therefore, our results should be viewed with caution and in need of further validation. MiRNAs which were reported as being expressed in conflicting directions in obesity may be due to differences between study methodology. Therefore, these miRNAs also warrant consideration in future research. Because of the large number of overlapping miRNAs, we chose to search for functions and targets in the context of obesity and sarcopenia and therefore may have omitted findings from other conditions, which may be relevant to sarcopenic obesity. However, a strength of our approach is that we focused on sarcopenia, obesity, and related conditions or diseases to focus our narrative review. It is possible that frailty and sarcopenia have differing miRNA profiles; however, due to limited studies and a similar clinical manifestation it was deemed that information available on frailty may be useful in this context. In addition, the majority of miRNAs identified in sarcopenia/frailty were found in exosomes. There is evidence to suggest that some miRNAs appear to be preferentially recruited to exosomes whereas others are retained within the original cell [377]. However, due to a limited number of studies

conducted in sarcopenia we therefore opted to use a more open definition and a less specific outcome measure to avoid missing potentially relevant findings.

Conclusions

The pathogenesis underlying sarcopenic obesity in not fully understood. This is the first study to examine the potential role of miRNAs in the context of sarcopenic obesity and thus offers a novel perspective on this topic. We have provided an overview of the field and identified a panel of miRNAs which may be implicated in sarcopenic obesity. Given the synergistic effect of sarcopenia and obesity on the risk of adverse health outcomes (falls, hospitalisation, worsening disability and all-cause mortality), understanding the pathogenesis of sarcopenic obesity has the potential to lead to effective screening, monitoring or treatment strategies. However, this systematic review was exploratory, and further work is now required to validate the findings presented here in older adults with sarcopenic obesity.

Acknowledgements

The authors of this manuscript certify that they comply with the ethical guidelines or authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle [378].

Funding

K.G-W is funded by Science Foundation Ireland (SFI) FFFP: 19/FFP/6709, Irish Research Council (IRC): IRCLA/2017/101, Health Research Board (HRB): COV19-2020-060 and Dunhill Medical Trust: R545/0217. A.D. is funded by FIDELIO, an MSCA Innovative Training Network, receiving funding from the European Union's Horizon 2020 programme under Grant Agreement no. 860898. This work was supported by a studentship from the Medical Research Council (MRC) and Versus Arthritis as part of the Medical Research Council Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing (CIMA) [MR/R502182/1]. The MRC Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing is a collaboration between the Universities of Liverpool, Sheffield and Newcastle.

Conflict of Interest

None declared.

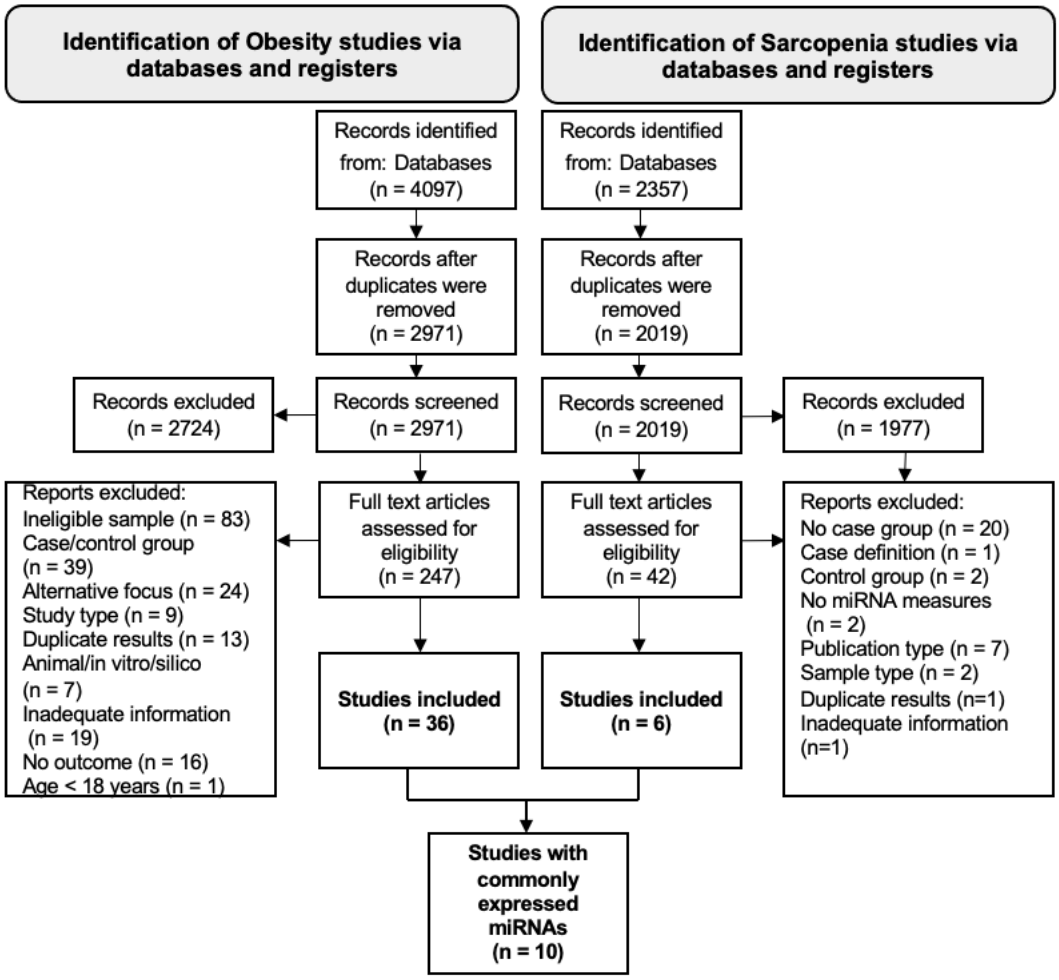
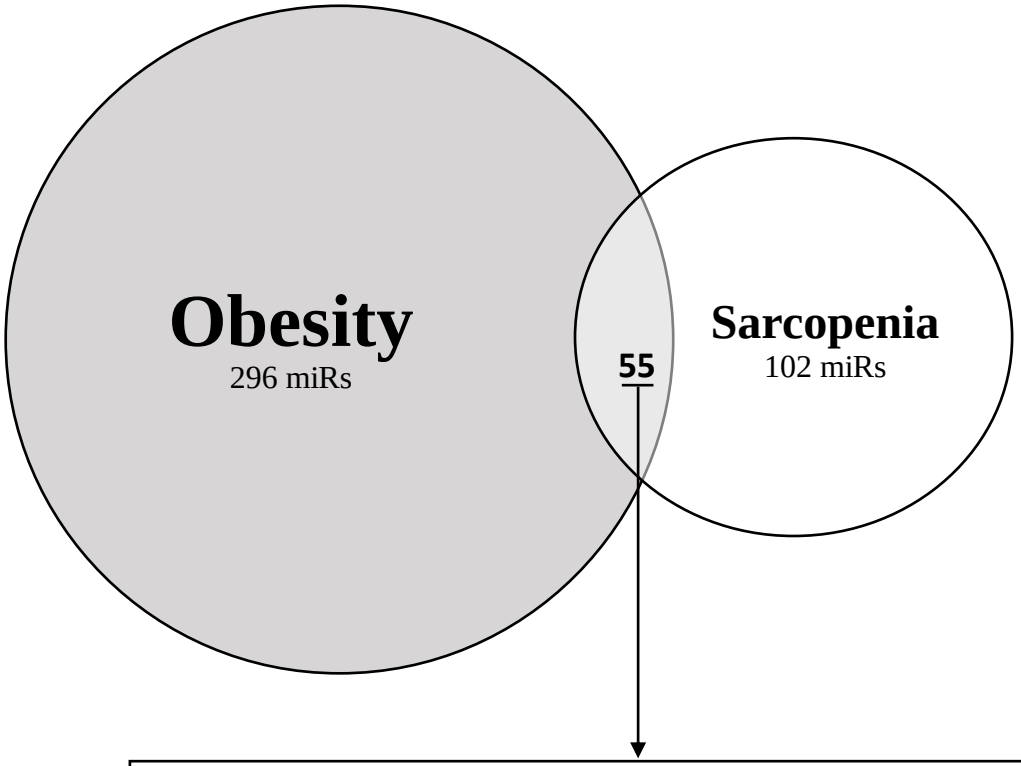


Figure 30 PRISMA flow chart for obesity/metabolic syndrome and sarcopenia/frailty parts of the systematic review

(a)



(b)

Same Direction		**Different Direction**		**Conflicting Direction**	**Unclear**
Plasma:		**Plasma**:		**Plasma**:	**Plasma**:
miR-106a-5p ↑	miR-301b-3p ↓	miR-133b	miR-411-3p	miR-154-5p	miR-328
miR-1224-5p ↑	miR-378c ↑	miR-136-3p	miR-485-5p	miR-155-5p	miR-215
miR-1246 ↑	miR-4732 ↑	miR-181c-5p	miR-495-3p	miR-194-5p	
miR-145-5p ↑	miR-483-3p ↑	miR-205-5p	miR-503-5p	miR-222-3p	
miR-18b-5p ↑	miR-487a-3p ↓	miR-21-3p	miR-664a-3p	miR-23a-3p	
miR-193b-5p ↑	miR-499a-5p ↓	miR-299-3p		miR-374b-5p	
miR-196a-5p ↑	miR-550a-3p ↑	miR-331-3p		miR-532-5p	
miR-197-3p ↑	miR-576-5p ↑	miR-361-5p		miR-625-5p	
miR-199a-5p ↓	miR-589-5p ↑	miR-362-5p			
miR-296-3p ↑	miR-766-3p ↑	miR-369-5p			
miR-29b-2-5p ↑	miR-92a-3p ↑	miR-379-3p			
Serum: miR-23a-3p ↑					
Vastus Lateralis: miR-424-5p ↑					

Figure 31 (a) Venn diagram of miRNAs commonly expressed in all tissues in both obesity and sarcopenia; (b) miRNAs by sample type (plasma, serum or vastus lateralis) found in both obesity and sarcopenia

Abbreviations: ↑refers to over-expressed ↓ refers to under-expressed. Note: Since the publication of several studies included in this review, some reported miRs have been removed from the latest version of miRbase (e.g. miR-4461, miR-4532, miR-6087), this does not affect overlapping miRs.

Table 19 Summary characteristics of studies with overlapping miRNAs in the same direction

		Obese					Sarcopenia				
	miRNA	**Country**	**Obesity Definition**	**N (%female)**	**Age (years)**	**Log2FC**	**Country**	**Sarcopenia Definition**	**N (%female)**	**Age (years)**	**Log2FC**
	Plasma										
1	**miR-106a-5p**	Spain [334]	BMI ≥ 30 kg/m^2	Ob 12 (100%) Lean 19 (100%)	Range 30-70 49.9 ± 11.3 45.1 ± 15.1	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.81
2	**miR-18b-5p**	New Zealand [335]	ND	Ob 11 (100%) Lean 12 (100%)	41 ± 5 44 ± 9	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	4.00
3	**miR-193b-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	1.89	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.96
4	**miR-197-3p**	New Zealand [335]	ND	Ob 11 (100%) Lean 12 (100%)	41 ± 5 44 ± 9	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.85
5	**miR-199a-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	-1.324	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	-0.74
6	**miR-483-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	1.413	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.92
7	**miR-499**	New Zealand [335]	BMI >30 kg/m^2	Ob 80 (100%) Lean 80 (100%)	52.5±10.5# 53.0± 13.5#	ND (Down)	China [339]	AWGS	S 93 (ND) N-S 93 (ND)	≥65 76.15 ± 0.58* 76.19 ± 0.58*	Down
8	**miR-550a-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.782	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.08
9	**miR-576-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.501	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.24

10	**miR-589-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.274	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.81
11	**miR-92a-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.571	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	0.78
12	**miR-1224-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.987	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	0.49
13	**miR-1246**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	1.254	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.23
14	**miR-145-5p**	New Zealand [335]	ND	Ob 11 (100%) Lean 12 (100%)	41 ± 5 44 ± 9	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.23
15	**miR-196a-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.885	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.48
16	**miR-296-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	1.049	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	0.20
17	**miR-29b-2-5p**	New Zealand [335]	ND	Ob 11 (100%) Lean 12 (100%)	41 ± 5 44 ± 9	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	3.68
18	**miR-301b-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	-0.973	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	-1.00
19	**miR-378c**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.676	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	2.41
20	**miR-4732-5p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	0.88	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	2.97

21	**miR-487a-3p**	Singapore [336]	BMI ≥ 27.5 kg/m^2	Ob 9 (0%) Lean 9 (0%)	28.4 ± 1.6* 23.2 ± 0.2*	-1.3	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	-1.03
22	**miR-766-3p**	New Zealand [335]	ND	Ob 11 (100%) Lean 12 (100%)	41 ± 5 44 ± 9	ND (Up)	USA [338]	Fried Frailty Phenotype	Fr 7 (0%) N-F 7 (29%)	Range 71-89 85.6 ± 3.8 76 ± 6.5	1.14
	Serum										
23	**miR-23a-3p**	Korea [251]	BMI ≥ 35 kg/m^2	Ob 16 (56%) Lean 18 (72%)	Range 30-59 31.3 ± 8.76 38.6 ± 7.9	2.81	USA [341]	EWGSOP 2010	S 12 (100%) N-S 51 (100%)	Range 60-85 ND ND	1.66 (NS)
	Vastus Lateralis										
24	**miR-424-5p**	ND [342]	ND	Ob 5 (0%) Lean 5 (0%)	ND	ND (Up)	UK [340]	EWGSOP 2010	S 5 (0%) N-S 59 (0%)	Range 68-76	Up

*Age is presented as mean ± SD unless specified. *SEM, # median ± IQR. Abbreviations: BMI = body mass index; Exp = expression; Ref = reference; Ob = obese; Fr = Frail; N-F = non-frail; S = Sarcopenic; N-S = non-sarcopenic; ND = not documented. EWGSOP = European Working Group on Sarcopenia in Older People; AWGS = Asian Working Group for Sarcopenia*

Table 20 Functions and predicted targets of miRNAs which are differentially expressed in the same direction in obesity and sarcopenia

miRNA (Family)	Cluster	↑↓	Function in relation to obesity/adiposity/insulin resistance or sarcopenia/muscle/exercise	Sample	Target
Plasma					
miR-106a-5p (miR-17)	miR-106a, 18b, 20b, 19b-2, 92a-2, 363	↑	Downregulated in polycystic ovary syndrome (PCOS) [379] Elevated in aged muscles (mice) and dexamethasone-treated myotubes; agomir results in downregulation of both myogenic regulatory factors (MyoD, MyoG and MyHC) and phosphorylation of AKT and decreased myotube size [380]	Plasma Exosomes [379] C2C12 cells [380] mice [380]	PIK3R1 [380]
miR-1224-5p (miR-1224)	N/A	↑	Upregulated in the liver of obese and high-fat diet fed mice, contributes to hepatic lipid accumulation by targeting AMPKα1 [376]	Mice [376]	AMPKα1 [376]
miR-1246 (miR-1246)	N/A	↑	Downregulated in patients with chronic obstructive pulmonary disease (COPD) and emphysema (n=20) [343] and amyotrophic lateral sclerosis (ALS) patients (n=14) [344] Upregulated in diabetic nephropathy patients (n=23); positively correlated with BMI [373]	Serum [343, 373] Plasma EVs [344]	
miR-145-5p (miR-145)	miR-145, -143	↑	Limited studies on obesity/sarcopenia Upregulated in normal weight women (n=11) following a high energy/fat breakfast [345]	Plasma [345]	
miR-18b-5p (miR-17)	miR-106a, 18b, 20b, 19b-2, 92a-2, 363	↑	Limited studies on obesity/sarcopenia Upregulated in PCOS [381] and relapsing multiple sclerosis (MS), may be involved in inflammatory pathways [382] SORBS2 identified as a target in diabetic nephropathy model cells [383] Targets and inhibits IGF-1, suppressing the activation of p-Akt, p-MEK and p-ERK1/2 in vitro [351]	Serum [381, 382] HGMCs/HRGECs [383] HRECs [351]	SORBS2 [383] IGF-1 [351]
miR-193b-5p (miR-193)	miRs-193b, 365a	↑	Limited studies on obesity/sarcopenia Weak negative correlations with BMI, plasma glucose levels and insulin response to OGTT in younger adults [370]. Targets and decreases expression of FoxO3 in cells, regulating cell cycle and cell proliferation [352]	Subcutaneous adipose tissue [370] BRL-3A [352]	FoxO3 [352]
miR-196a-5p (miR-196)	N/A	↑	High level of expression in myoblasts, suppresses mitochondrial biogenesis and its master regulator, PGC1β, and ND4. Suppresses osteoclast formation induced by RANKL in Raw264.7 cells [360]	C2C12 cells [360] Raw264.7 cells [360]	
miR-197-3p (miR-197)	N/A	↑	Increased after high intensity resistance exercise in young adults [384] Upregulation inhibits GIP and GLP-1 production through suppression of PCSK1/3 [368]	Serum [384] STC-1 cells [368]	
miR-199a-5p (miR-199)	miR-214	↓	Overexpression of AKT down-regulates miR-199a-5p with a subsequent increase in targets Sirt1 and HiF-1α in cardiomyocytes [385] Downregulated in mild and terminal stage ALS [386] and patients with Parkinson's disease [387] Upregulated in middle-aged adults with T2DM; in vitro studies showed miR-199a regulates cellular glucose uptake by targeting and suppressing GLUT4 [371] Upregulated in rat pancreatic β-cells exposed to high glucose, promotes apoptosis and ROS formation, suppresses SIRT1 [355] Inhibition results in decreased myogenic differentiation and increased MyoD1 and Pax7 in human myoblasts. High levels	Cardiomyocytes [385] Serum [386] Plasma [371] Induced pluripotent stem cells [387] Rat pancreatic β-cells [355] Myoblasts, HEK293T cells, Zebrafish [388]	Sirt1 [354, 355, 385] HiF-1α [385] GLUT4 [371]

			inhibit WNT signalling in HEK293T cells. Overexpression in zebrafish results in disorganisation and detachment of myofibers [388]		
miR-296-3p (miR-296)	miR-296, -298	↑	Upregulated in PCOS; reduction in miR-296-3p promotes cell proliferation [389]	Human granulosa cells [389] Human granulosa-like tumour cells [389]	PTEN [389]
miR-29b-2-5p (miR-29)	miR-29b-2 miR-29c	↑	Limited studies in the context of muscle/obesity Targets STAT3 in a fibroblast cell line [390]	L929 cells [390]	STAT3 [390]
miR-301b-3p (miR-130)	miR-301b miR-130	↓	Decreased during myogenic differentiation; may be involved in muscle differentiation by regulating Rb1cc1 [391]	Chicken myoblasts [391]	Rb1cc1 [391]
miR-378c	N/A	↑	Studies not identified in the context of muscle/obesity.		
miR-4732-5p (miR-4732)	miR-4732, -144, -451a, -451b	↑	Studies not identified in the context of muscle/obesity.		
miR-483-3p (miR-483)	N/A	↑	Upregulated in hyperglycaemic mice and cardiomyocytes. Overexpression downregulates IGF-1, thus promoting apoptosis in hyperglycaemic cardiomyocytes [348] Overexpression inhibits bovine myoblast cell proliferation through the *IGF1/PI3K/AKT* pathway; knockdown of miR-483 enhances the expression of myogenic maker genes *MyoD1, MyoG, MyHC* [349] Elevated in Duchenne's Muscular Dystrophy [392]	Mice, H9c2 cell line [348] Bovine myoblasts [349] Serum [392]	IGF-1 [348, 349]
miR-487a-3p (miR-154)	miR-1185-1, -1185-2, -381, -487a, -487b, -539, -889, -544a, -655, -382, -154, -496, -377	↓	Studies not identified in the context of muscle/obesity.		
miR-499a (miR-499) ***MyomiR***	miR-499a, -499b Encoded in slow myosin heavy chain genes (*Myh7b*)– restricted to T1 fibres (expressed in T1 fibres only)	↓	Elevated in patients and carriers (mothers) with Duchenne's muscular dystrophy [366], COPD (n=103) and significantly correlated with NFKB p50 [367] Affected by aerobic exercise - no changes after acute bout in young men [393]; decreased following acute bout with weight vest with/without nutritional supplementation [171]; increased in male marathon runners (n=21) after competitive marathon competition [394] Increased after essential amino acid ingestion in young adults (n=7) [181] Associated with a slow muscle fibre phenotype in human muscle [365] Double knockout miR-499/miR-208b mice lost slow type I myofibers with a concomitant increase in fast type IIx/d and IIb myosin isoforms; forced expression of miR-499 converted fast myofibers to slow. Sox6 helps mediate the actions of miR-499 on slow myofiber gene programming [172] Targets Thrap1 to promote slow muscle fibre type [364]	Plasma [366, 367, 394] Serum [393] Vastus lateralis [171, 181, 365] Mice [172, 361, 363, 365, 372, 395] C2C12 cells [172, 357, 364] SMSCs [375] H9c2 cells [363] Murine liver cells NCTC1469 [372, 395]	Sox6 [172] Thrap1 [172, 364] p21[363] TGFβR1 [357] PRDM16 [375] Fnip1 [361] PTEN [395] PTENP1 [372]

			Targets TGFβR1, a known regulator of skeletal myoblast development. Knockdown of TGFβR1 inhibits myogenic differentiation in C2C12 cells [357] Targets PRDM16 which subsequently promotes myogenic, rather than brown adipogenic, differentiation in mouse skeletal muscle stem cells (SMSCs) [375] Promotes mitochondrial function. Targets Fnip1, a negative regulator of mitochondrial function in myocytes, which leads to activation of PGC-1α. Fnip1 inhibition stimulates oxygen consumption rates, a sign of mitochondrial function, in myocytes. Mice with muscular dystrophy bred with miR-499 mice exhibit improved mitochondrial capacity, restored slow-oxidative muscle fibre programming and greater muscle functionality assessed with treadmill distance [361] Knockdown of p21, a target of miR-499, decreases mitochondrial fission and cell death in cardiomyocytes exposed to Doxorubicin, anti-tumour drug [363] PTENP1, a target gene of miR-499, expression is enhanced in diabetic and obese mouse models resulting in impaired Akt/GSK activation and glycogen synthesis contributing to insulin resistance [372] Downregulation was observed in diabetic mouse models. Downregulation *in vitro* was shown to impair the insulin signalling, Akt/GSK pathway and glycogen synthesis. PTEN was identified as a target [395]		
miR-550a-3p (miR-550)	miR-550a-1, -550b-1	↑	Limited studies in muscle/obesity Downregulated in patients with sporadic ALS [396] Associated with parameters of bone formation and microstructure parameters (mineral apposition ratio, bone surface, trabecular bone volume) [397] Downregulated in postmenopausal women with fractures older than 6 months; excellent discrimination of patients with low traumatic fractures [398]	Peripheral blood [396] Serum [397, 398]	
miR-576-5p (miR-576)	N/A	↑	Studies not identified in the context of muscle/obesity.		
miR-589-5p (miR-589)	N/A	↑	Limited studies in muscle/obesity Decreased upon TGF-β stimulation in control fibroblasts, with no effect seen in COPD fibroblasts [399]	Fibroblasts [399]	
miR-766-3p (miR-766)	N/A	↑	Decreased in older (60-73 years; n=51) compared with younger (19-42 years; n=55) or long-lived (90-102 years; n=51) adults [400]. Overexpressed in older adult human dermal fibroblasts (HDFs) [401] Decreased after 12 weeks of endurance training in young men (n=32) [346] Increased in sedentary T2DM adults (40-70years; n=24) who undertook either 4 month resistance or aerobic training [347]	PBMCs [400] HDFs [401] HeLa cells [401] Plasma [346, 347]	SIRT6 [401]
miR-92a-3p (miR-92a)	miR-17-92	↑	Anti-miR, MRG-110, was tested in adult men and found to counteract the repression of known miR-92a-3p targets, ITGA5 and CD93. Elevated levels of DDIT4, an inhibitor of mTOR were found in cells treated with MRG-110 [402]	Whole blood [402] $CD4^+$ T Cells [402] Plasma [346, 403, 406]	ITGA5 [402] CD93 [402] SMAD7 [359]

			In a systematic review, downregulated following bariatric surgery [403] Decreased following 20-week aerobic exercise training (n=20) [404], 12 weeks of endurance training in young men (n=32) [346] and a 6-week cycling training in young men (n=24) [405] No change following 5-month aerobic training in obese older adults (n=33); however changes in miR-92a positively correlated with changes in gait speed following intervention [406] MiR-92a targets SMAD7, inhibition of miR-92a led to increased mitochondrial content and oxygen consumption of brown adipocytes; inhibition of miR-92a led to promotion of SMAD7 and subsequent suppression of p-SMAD3/SMAD3. Inhibition of miR-92a promoted differentiation of brown adipocytes [359]. Negatively correlated with BAT activity in young adults (n=41); downregulated in the serum exosomes of mice with active BAT [374] Gradually upregulated with age (22y, 40y, 59y and 70y) in males and females [407]	Serum [374, 404, 407] C2C12 cells [359] Vastus lateralis [405] Mice [374]	
Serum					
miR-23a-3p (miR-23)	miR-23 ~ 27 ~ 24	↑	Significantly downregulated in SAT and VAT of obese participants and significantly correlated with measures of adiposity (BMI, waist circumference, insulin measures). Involved in the regulation of PTEN although the molecular mechanism is unclear [408] In young men (n=7), increased following resistance or endurance exercise and protein ingestion [409] Increased following EAA ingestion alone [181] Decreased after an acute bout of endurance exercise in young adults (n=9) [410] Upregulated in ALS. Targets PGC-1α with subsequent effects on mitochondrial biogenesis and activity [362] Protects muscles from atrophy by targeting atrogin-1/MAFbx1 and MURF-1. Overexpression counteracts muscle atrophy induced by dexamethasone in myotubes and glucocorticoids in mice [353]	VAT, SAT [408] Vastus lateralis [181, 362, 410] Mice [353, 362] Adipocytes [408] C2C12 cells [353]	Atrogin-1/MAFbx1 [353], MURF-1 [353] PGC-1α [362, 411]
Vastus Lateralis					
miR-424-5p (miR-322)	miR-424, -503, 542, 450a-2, 450a-1, 450b	↑	Downregulated in young women with PCOS (n=24) [381]. No difference between obese (n=21) and NW (n=19) women but correlated with waist circumference [412] Increased in cachectic cancer patients [413] Upregulated in muscle wasting conditions – ICU acquired weakness and COPD. Over-expression causes a reduction in muscle diameter of mice [340] Saturated fat/high fat diet impairs insulin signalling (INSR and IRS-1) and upregulated miR-424-5p in hepatocytes and mice. Overexpression causes a significant decrease in insulin-induced glycogen synthesis in hepatocytes. INSR is a direct target [369] Targets IGF-1 in mice and human myocytes [350]	Serum [381] Mice [340, 369] Vastus lateralis [340, 413] SAT [412], Plasma [412], Hepatocytes [369] C2C12 cells [350] Human myoblasts [350]	SMAD7 [340] INSR [369] IGF-1 [350]

Abbreviations: ↑ = up-regulated in sarcopenia/obesity, ↓ = down-regulated in sarcopenia/obesity; HGMCs = human glomerular mesangial cells; HRGECs = human renal glomerular endothelial cells; HRECs = human retinal endothelial cells; PBMC = peripheral blood mononuclear cells.

Table 21 (Supplementary) Eligible definitions/criteria for conditions studied

Condition	**Definition**
Sarcopenia	Due to heterogeneous definitions, studies which define sarcopenia as low lean muscle mass, low muscle strength, or both will be included.
Frailty	- The use of gait speed (taking more than 5 seconds to walk 4 m using usual walking aids if appropriate) or Gait speed <0.8m/s - Timed up and go test - The PRISMA 7 questionnaire (with a cut-off score of >3) - Clinical frailty scale - Edmonton frail scale or Reported Edmonton frail scale - Electronic Frailty Index (eFI) - Fried criteria for frailty
Obesity	- High BMI - High fat mass or % body fat mass - High waist circumference - High visceral fat area
Metabolic Syndrome	International Diabetes Federation Consensus Worldwide Definition of the Metabolic Syndrome (2017)

Table 22 (Supplementary) Interpretation of Newcastle-Ottawa Quality Assessment Scale for Case Control Studies in the context of this study

Selection	**Interpretation**
1) Is the case definition adequate? a) yes, with independent validation * b) yes, e.g. record linkage or based on self reports c) no description	a) Refers to a consensus/validated/endorsed definition or uses the IDF definition for MetS, or clearly uses the WHO or Asia-Pacific cut-offs (Asian populations only) for BMI.
2) Representativeness of the cases a) consecutive or obviously representative series of cases * b) potential for selection biases or not stated	a) States that the participants have been recruited consecutively or are representative.
3) Selection of Controls a) community controls * b) hospital controls c) no description	a) States that the participants have been recruited from the community or are free living or it can be reasonable assumed that the participants are from the community b) Participants who are inpatients or attending clinics c) No description of how participants were recruited
4) Definition of Controls a) no history of disease (endpoint) * b) no description of source	a) Study either defines that participants did not have the disease (e.g. non-obese, lean), or indicates with the definition that the participants did not have the disease b) No description of the definition for controls e.g. 'healthy controls'
Comparability	
1) Comparability of cases and controls on the basis of the design or analysis a) study controls for **age*** b) study controls for **gender** *	Study either states that the participants were matched based on age/gender or the study is e.g. female only.
Exposure	
1) Ascertainment of exposure a) secure record (eg surgical records) * b) structured interview where blind to case/control status * c) interview not blinded to case/control status d) written self report or medical record only e) no description	a) Independently measured in the study b-e) as described
2) Same method of ascertainment for cases and controls a) yes * b) no	a) Same method of ascertainment can be assumed for both groups even if not explicitly said. b) No description for ascertainment of exposure
3) Non-Response rate a) same rate for both groups * b) non respondents described c) rate different and no designation	Due to the study design, all studies were given a mark for this question.
Additional - Validation	
1) Validated a) two methods (e.g. qPCR and RNA-seq) * b) two separate study groups * c) not validated	If a star is given, this is not included in the Newcastle-Ottawa Scale score rather as a " * " at the end of the score (e.g. 6*)

Abbreviations: WHO = World Health Organisation, IDF = International Diabetes Federation, BMI = Body Mass Index

Table 23 (Supplementary) Top externally validated circulating (plasma or serum) miRNAs in obesity and sarcopenia

	miRNA	Count	Up	Down
Obesity				
1	miR-21-5p	9	[1-3]	[4-9]
2	miR-320a-3p	6	[10, 11]	[3, 12-14]
3	miR-122-5p	6	[1, 7, 15, 16]	[2, 8]
4	miR-221-3p	6	[2, 17]	[4, 6, 8, 13]
5	miR-126-3p	5	[2, 3, 8]	[6, 18]
6	miR-146a-5p	5	[2, 19]	[5, 6, 11]
7	miR-155-5p	5	[2, 20, 21]	[22, 23]
8	miR-192-5p	5	[1, 6, 7]	[2, 12]
9	miR-27a-3p	5	[1, 2, 14]	[6, 13]
10	miR-92a-3p	5	[1, 6, 10, 24]	[12]
Sarcopenia				
1	miR-23a-3p	2	[25, 26]	

The top 10 validated miRNAs are shown for obesity/metabolic syndrome. In sarcopenia, only one miRNA was reported by more than one study. Count refers to the number of separate studies reporting differential expression of the miRNA.

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Chapter 5

MicroRNAs in Dynapenic Abdominal Obesity - Testing

5.1 Introduction

In older adults, dynapenia (low muscle strength) and abdominal obesity are independently associated with a greater risk of falls, hospitalisation and mortality; the combination of both phenotypes, dynapenic abdominal obesity (DAO), is associated with a cumulative risk [86, 89-91, 94-96]. However, the pathogenesis of DAO is not fully understood. Factors such as age, diet, physical activity, insulin resistance, altered IGF-1 and myostatin levels have all been implicated in the development of high fat mass and reduced muscle mass and strength with age [129]. A greater understanding of the pathogenesis of DAO has the potential to lead to the identification of novel treatment strategies.

MicroRNAs are short, non-coding RNAs (ribonucleic acids) which regulate gene expression at the post-transcriptional level [161]. In humans, 2,654 miRNAs have been identified which are thought to regulate around two thirds of protein-coding genes in the human genome [162]. This means that miRNAs are likely to regulate many physiological processes [164] and are potentially implicated in the development of dynapenia and DAO. To date, however, the role of miRNAs in people with both dynapenia and obesity has not been examined.

Muscle specific microRNAs (myomiRs) are involved in regulating phenotypic changes that occur with muscle fibre type and mass [170, 171] (see Chapter 1, Section 1.7.7 MicroRNAs). As such, differential expression of miRNAs may be implicated in the loss of muscle mass (sarcopenia) [182, 185, 298]. Moreover, compared to younger adults, the miRNA profile of skeletal muscle in older adults is altered both at baseline and in response to protein intake and exercise [182, 298]. A recent systematic review identified 13 differentially expressed miRNAs in the plasma, serum, and skeletal muscle of people with sarcopenia/frailty which were associated with grip strength, self-reported exhaustion, and gait speed [185].

Another systematic review identified 33 differentially expressed miRNAs in people with obesity [184]. Bioinformatic analyses showed that these differentially expressed microRNAs may play a role in fatty acid metabolism and PI3K-Akt signalling [184]. Akt is an insulin-activated kinase and dysregulation is associated with insulin resistance and tumorigenesis and thus the authors proposed a potential pathway to Type 2 Diabetes and other obesity-related comorbidities [184]. Bariatric surgery results in a significant reduction in exosomal miRNAs which are associated with insulin receptor signalling [251, 414, 415] indicating that weight loss can lead to improvements in miRNA profiles.

Based on the evidence in ageing [182, 298], sarcopenia [185], and obesity [184] there appears to be a potential role for miRNAs in the pathogenesis of dynapenia and DAO. This is further supported by the results from Chapter 4 which identified 24 miRNAs commonly changed in the same direction in obesity and sarcopenia. The majority of these miRNAs were involved in protein homeostasis, mitochondrial dynamics, muscle fibre type determination, insulin resistance, and adipogenesis – processes implicated in the pathogenesis of DAO (see Chapter 1, 1.7.6 Pathogenesis). It remains unclear if these miRNAs are differentially expressed in people with dynapenia and DAO.

In addition to actual changes within skeletal muscle [182, 298] and adipose tissue [169], miRNA transport and intercellular crosstalk may contribute to the pathogenesis of dynapenia and DAO. MiRNAs can be released into circulation complexed with Argonuate2 (Ago) proteins, high-density lipoproteins, or packaged within an exosome [169, 416, 417]. Exosomes released into the extracellular space can diffuse in the blood stream or bodily fluids to be taken up into different organs such as the liver, lungs and heart. Numerous metabolic organs release exosomes including skeletal muscle [418] and adipose tissue [169, 419].

Adipose tissue is a major source of miRNAs which are released into circulation (exosomal miRNAs) and in mice it has been shown that differing depots exhibit differing miRNA signatures [169]. In rodent studies, adipose-derived miRNAs can be transported in exosomes to various other cell types including myocytes, macrophages, and hepatocytes [177, 178, 330]. Similarly, skeletal muscle-derived miRNAs can be taken up by adipose tissue [179]. Wang et al. (2013) [420] incubated C2C12 cells with adipocyte-derived culture containing miR-130b and subsequently found elevated levels of miR-130b in the C2C12 cells. Functionally, this crosstalk has been associated with insulin resistance, adipogenesis, and lipid metabolism [177, 179]. These findings are particularly meaningful as they further support the role of miRNAs in the pathogenesis of DAO.

In summary, miRNAs can regulate many physiological processes with differential expression independently reported in ageing, obesity and sarcopenia. MiRNAs can travel in blood circulation, be taken up by other cells, and regulate physiological processes involved in DAO (e.g. insulin resistance, adipogenesis and lipid metabolism). A greater understanding of the miRNA expression of people with dynapenia and DAO may provide insights into its pathogenesis and lead to novel treatment strategies. Additional treatment strategies have the potential to reduce the burden of increased falls, hospitalisation and mortality risk in people with these phenotypes. To date, however, the miRNA profile of women with dynapenia or dynapenic abdominal obesity (or sarcopenic obesity) has not been determined.

5.2 Aims and Hypotheses

Aims:

1. To compare the miRNA profile, measured by RNA-seq, in a cohort of older women with normal weight, dynapenia, abdominal obesity, and DAO.
2. To identify a panel of miRNAs for further validation with RT-qPCR by combining the RNA-seq results, a literature search, and a systematic review (Chapter 4).
3. To validate the expression of a panel of miRNAs in both the same cohort of women and an external cohort of older women (Chapter 3) using RT-qPCR.

Hypotheses:

1. A panel of differentially expressed miRNAs can be identified and validated in women with DAO compared to those who are obese only and these miRNAs relate to processes involved in the pathogenesis of DAO.
2. A panel of differentially expressed miRNAs can be identified and validated in women with dynapenia compared to those who are normal weight only and these miRNAs relate to processes involved in the pathogenesis of dynapenia.
3. There are similarities between the panels of differentially expressed miRNAs in DAO and dynapenia.

5.3 Methods

5.3.1 Overview

To address the aims previously outlined, an exploratory study, internal validation study (Fat and Bone study, described below), and external validation study (Muscle in Obesity study, Chapter 3) were undertaken to measure the microRNA profile of serum samples from normal weight, dynapenic, abdominally obese and DAO older women. The methods section which follows provides a description of these studies, rationale for the methodological choices, and describes the specific methods used.

5.3.2 FAB Study

The Fat and Bone (FAB) Study was a single-centre, observational, cross-sectional, case-control study conducted between 2011-2013 in South Yorkshire [202]. Cases were obese women (BMI 30-40kg/m^2) and controls were those with a normal BMI (18.5-24.9kg/m^2) aged 55-70 years and post-menopausal. Body composition was measured using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA, USA). All participants provided written informed consent prior to participation. Ethical approval was granted by Sheffield Research Ethics Committee. Ethical approval for this study was granted by the South Yorkshire North Derbyshire Musculoskeletal Biobank.

Participant selection

Non-obese women were consecutively chosen based on recruitment date, if the DAO definition criteria of this present study were fulfilled and absence of either impaired fasting glucose (≥6.1mmol/l) if normal weight [421] or metabolic syndrome if obese [303]. Further, participants were preferentially selected if detailed phenotyping was available i.e. quantitative computed tomography and DXA results.

Definitions

Participants were chosen based on the criteria of DAO with the most consensus in the literature – high waist circumference (>88cm) and low hand-grip strength using EWGSOP2 criteria (<16kg) [4].

In addition to being abdominally obese, participants were also required to be obese according to BMI (30-40kg/m^2). Thus, participants were both obese and abdominally obese, or non-obese (BMI 18.5-24.9kg/m^2) and non-abdominally obese. Due to small numbers, this approach was chosen to avoid potential confounding of results.

Four groups were created:

1. Normal weight, non-dynapenic = waist circumference ≤88cm, BMI 18.5-25kg/m^2, hand grip strength ≥16kg
2. Normal weight, dynapenic = waist circumference ≤88cm, BMI 18.5-25kg/m^2, hand grip strength <16kg
3. Non-dynapenic obese = waist circumference >88cm, BMI 30-40kg/m^2, hand grip strength ≥16kg
4. Dynapenic abdominally obese = waist circumference >88cm, BMI 30-40kg/m^2, hand grip strength <16kg

5.3.3 Muscle in Obesity Study

The Muscle in Obesity study is described in detail in Chapter 3. Briefly, 27 older women (age 60 – 79 years) were recruited from the South Yorkshire area. Participants were excluded from recruitment if they had a history of long-term immobilisation, recent hospital admission (3 months), recent significant weight loss, diabetes mellitus, medications affecting muscle metabolism, conditions limiting test procedures (e.g. hypertension), or were currently partaking in competitive sport. Ethical approval was granted by the Leeds West Research Ethics Committee (REC Reference 20/YH/0274).

Definitions

Due to Covid-19, sit-to-stand time (> 15 seconds) was used to determine dynapenia instead of hand-grip strength due to the ability to conduct this measurement remotely. Hand-grip strength and sit-to-stand time are both advocated measures of dynapenia [4]. Abdominal obesity was defined as in the FAB study. Due to Covid-19 related delays, we were unable to successfully recruit sufficient participants with dynapenia.

Three groups were created:

1. Normal weight, non-dynapenic = waist circumference ≤88cm, BMI 18.5-25kg/m^2, sit-to-stand time < 15 seconds
2. Non-dynapenic obese = waist circumference >88cm, BMI 30-40kg/m^2, sit-to-stand time < 15 seconds
3. Dynapenic abdominally obese = waist circumference >88cm, BMI 30-40kg/m^2, sit-to-stand time > 15 seconds

Power Calculations

Power calculations were used to calculate the sample size for independent sample t-tests using nQuery (v5) software. Alpha level was set at 0.01%, two-sided, to adjust for multiple comparisons.

We used published data [298] to calculate a sample size of 8 per group to identify an effect size of 2 with 80% power and Alpha level set at 0.01% two-sided. This was based on a 1.5 fold-change difference and a common SD of 0.75.

The proportion of participants identified in some groups was less than eight. The findings of this study are therefore exploratory.

5.3.4 Choice of Sample Type

Serum vs. plasma

There is strong debate over the preferred biofluid (whole blood, serum or plasma) to measure circulating miRNAs.

For serum samples, miRNAs may be released from platelets or red blood cells into serum during the coagulation process [422]. For plasma samples, it is possible to accidentally aspirate cells. Platelets are also a major source of miRNAs in plasma [423]. If plasma is not cleared of platelets prior to freezing, attempts to remove the platelets can be futile as even one freeze-thaw cycle is enough to release miRNAs from the platelets [423]. For certain peptidomic studies, platelet-poor plasma is desirable due to less degradation *ex vivo* [424] and this has also been shown for miRNAs [423]. In addition to the issue of platelets, numerous methodologies exist to prepare plasma; however, these are not always adequately described in the literature leading to differences in reported expression levels [423]. In patients receiving heparin where plasma was prepared with EDTA, reductions were found in both endogenous miRNAs and exogenous spike-in controls (cel-miR-39) [425]. Heparin can inhibit the reverse transcriptase and polymerase enzymes used in PCR. Removal of heparin is therefore required but this can reduce RNA yield and residual heparin may remain [426].

Common to both serum and plasma samples, miRNAs found in vesicles can be released during incubation of between 1-3 hours (n=3), such conditions are typical of clinical settings [427].

Some have shown that the quality, content and profile of miRNAs in human plasma and serum appear similar with similar expression of the top most highly expressed miRNAs reported [428]. Others have

shown that plasma exhibits a more diverse miRNA profile but is also more affected by white blood cell contamination [429]. Comparison between studies using serum and those using plasma should be interpreted with caution in view of the previous methodological considerations.

For this study serum was chosen because, unlike plasma, serum extraction protocols are largely standard and comparison can be made to other serum studies. Serum appears less prone to platelet and white blood cell contamination than plasma.

The FAB study was chosen as it is a well characterised cohort of women who fit the criteria for dynapenic abdominal obesity. Only serum was collected and available from the FAB study.

Circulatory vs. extracellular vesicle miRNAs

The majority of miRNAs found in circulation are bound to Ago proteins or within RISC [430]. In plasma, approximately 3% of miRNAs are located in exosomes [430]. Others have shown that exosomal miRNAs from a variety of human sources (plasma, seminal fluid, dendritic cells, mast cells, and ovarian cancer cells) account for less than one molecule per exosome [431]. Therefore, we chose to measure circulating miRNAs as limiting the measurement of miRNAs to only those in extracellular vesicles ignores the vast majority found outside of these vesicles.

5.3.5 Blood Sampling

Fat and Bone Study

Blood samples were collected at the first visit, following an overnight fast. Blood samples were collected between 08:00-10:00 and allowed to clot at room temperature for 30 minutes before being centrifuged at 3000 rpm for 10 minutes. Following adoption by the South Yorkshire North Derbyshire Musculoskeletal (SYNDM) Biobank, samples were stored at -80^{o} C.

Muscle in Obesity Study

Blood samples were collected at the first visit, following an overnight fast. Blood samples were collected between 08:00-10:00 and allowed to clot at room temperature for 30 minutes. Samples were centrifuged at 1800 g for 10 minutes. Where the blood could not be centrifuged immediately after clotting, the tubes were refrigerated at 4^{o} C for up to four hours [297]. Serum samples were aliquoted and stored at -80^{o} C until analysis.

MicroRNAs have shown diurnal and weekly variability [432] and thus standardised timings help reduce this effect. MicroRNAs have also been shown to be stable at -25°C for at least 40 years [433, 434].

5.3.6 Overview of miRNA measurement methods

There are three common approaches for quantifying miRNAs – microarray, RT-qPCR and RNA-seq.

Microarray

Overview

Microarray was one of the first methods identified for parallel analysis of large numbers of miRNAs. The 3' end of miRNAs are tagged and detection of individual miRNAs is by hybridisation of the tags to capture probes, complementary DNA oligonucleotides, arrayed on a slide or beads [435].

Strengths and limitations

Microarrays are generally less expensive than RNA-seq but cannot be used to determine absolute quantification or to identify novel miRNAs. Due to limited specificity, initial observations should be validated by additional methods e.g. real-time quantitative polymerase chain reaction (RT-qPCR) or northern blot [435].

RT-qPCR

Overview

RNA is reverse transcribed into cDNA, followed by a quantitative polymerase chain reaction (qPCR) and real-time (RT) monitoring of the reaction product accumulation [435].

Strengths and limitations

Unlike RNA-seq, the miRNA of interest needs to be specified in advance; however, reactions can be carried out in a high-throughput form using commercially available plates or cards. RT-qPCR has good reproducibility although variation occurs across different platforms [436]. It is also considered the gold-standard validation technique for validation of microarray and RNA-seq findings. However, there is some debate over whether RT-qPCR really is the gold standard or is even needed to validate RNA-seq if steps are performed to a high standard [437].

A large study comparing RNA-seq with RT-qPCR for 18,080 protein coding genes found that 15-20% of genes were non-concordant [438]. Non-concordance was defined as differential expression in

opposite directions or differential expression in only one method. Of the 15-20% non-concordant genes, ~80% had a fold change lower than 2. Of those with a fold change >2, the majority were lowly expressed. The authors concluded that a small fraction (1.8%) of non-concordant genes had lower expression and were shorter. Generally, there is good concordance (80-95%) between RNA-seq and RT-qPCR methods [436].

Figure 32 Roche LightCycler 96 instrument for RT-qPCR
Photograph courtesy of Roche.

RNA-seq

Overview

RNA-seq involves preparation of a small RNA cDNA library from the sample of interest which is subsequently parallel sequenced (see Figure 34). Bioinformatic analysis is used to determine both known and novel miRNA and provide relative quantification.

Strengths and limitations

RNA-seq is costly and requires significant computational infrastructure but has a high level of accuracy and can identify novel miRNAs [435]. RNA-seq shows good reproducibility [436] even across laboratories using a standardised protocol [439]

Figure 33 Illumina NextSeq 550 for RNA-seq
Image courtesy of Illumina, Inc.

5.3.7 Sources of error – microRNA measurement

Potential sources of error in miRNA expression studies include:

Biological variation

MiRNA expression responds to exercise and meal consumption [182] and varies with disease status [440] and age [183].

Rhythmic variation

MiRNAs can exhibit rhythmic variation throughout the day [432]. Heegaard *et al.* (2016) measured 92 plasma miRNAs in 24 young men, 3-hourly over 24 hours. Of the 79 miRNAs which could be measured reliably, one third (n=26) displayed a rhythmic pattern throughout the day [432]

Intra-individual variation

Yoon *et al.* (2007) [441] demonstrated that miRNAs also vary within an individual at the same time of day. The authors measured 95 microRNAs in the cerebrospinal fluid of nine healthy volunteers at baseline and 48 hours later, demonstrating that 12/95 miRNAs were significantly different intra-individually [441].

Cellular expression is not ubiquitous

Different cells may exclusively express certain miRNAs and their expression may be misinterpreted as ubiquitous due to the presence of numerous cell types (e.g. red blood cells, fibroblasts, adipocytes) within tissues [442] and the ability for cellular cross-talk [169].

Technical variation

RNA extraction

Sample contamination or RNA degradation may occur due to RNAses. Quality control can be assessed using spike-in controls or gel electrophoresis to analyse the ratio of 28S to 18S ribosomal bands [443].

Reverse transcription bias

Bias in reverse transcription can result from input quantity, reverse transcriptase enzyme or kit and priming methods [444].

Amplification and GC bias

PCR amplification introduces bias whereby not all fragments are amplified with equal efficiency leading to potential over- and under-representation of certain fragments [445]. Furthermore, fragments with a high GC or AT content may be under-represented or lost in library preparation due to greater efficiency with the amplification of GC-neutral fragments [445]. Next generation sequencing (NGS) approaches which do not use PCR have been suggested. This approach avoids both amplification and GC bias; however, increases the workload, cost, and input material required [445, 446]. Optimisation of the PCR step is sufficient to reduce bias without the need to increase input quantity [446].

Adapter ligation bias or 3' end bias

RNA fragments are first ligated with the 3' adapter followed by the 5' adapter; however, there is a preference towards the 3' adapter by single stranded ligation sites [445]. This is termed 3' adapter bias. One solution is to randomise the 3' end of the 5' adapter and likewise, the 5' end of the 3' adapter [445]. Alternatively, a circularisation approach may be used.

In this present study, RealSeq Biofluids (SomaGenics, California, USA) was used which utilises a single adapter and circularisation approach [447]. A combo adapter, which includes the primer, is ligated onto the 3' end of the miRNA and this new miRNA-adapter product is circularised via intra-molecular ligation. RT-qPCR amplification of these circles generates a library for analysis in standard NGS platforms. This process removes the need for a 5'-adapter ligation step – of which the ligation steps are the main contributors to library preparation bias [447]. Standard two-adapter protocols can result in a high rate of dimer (adapter-adapter) formation thus requiring a purification step. The reproducibility of small RNA-seq experiments is diminished by this gel purification step.

Compared with other methods, RealSeq®-AC, for cellular RNA, both identified and accurately quantified the most miRNAs (71.8%) – the next best method for quantification was SMARTer (Takara Bio) (48%); however, this performed worst for miRNA detection [447].

Sample storage duration

Unlike larger RNAs, miRNAs are stable in serum stored at -25^{o}C for up to 40 years [433, 448]. Sample variability tends to be more affected by pre-processing factors such as differing clotting times, haemolysis or contamination with serum preservatives e.g. iodoacetate [433]. The study by Keller et al. 2017 [448] used a longitudinal approach to dissect the influence of age and storage length on

miRNA stability. Although the majority of miRNAs were more affected by the age of the participant, a small proportion had variation related to storage time. However, this analysis was conducted on a subset of the whole group (n=30/90) and may have been affected by selection bias.

Endogenous and exogenous controls

MicroRNAs represent only a small fraction of RNA in a sample which can vary between samples. The use of a housekeeping gene (endogenous control) for normalisation can remove differences due to sample collection, variation in the amount of starting material, and quality of RNA thus allowing the identification of true changes in gene expression [449]. However, there is a lack of a standardised microRNA endogenous control.

Small RNAs such as U6, SNORD43, SNORD44, and SNORD48 have previously been used as endogenous controls [450]. However, these are structurally different from miRNAs and have different properties in terms of transcription, processing, and tissue-specific patterns [449]. The use of U6 as a normaliser has been questioned due to variability in its expression and as it is more affected by freeze-thaw cycles than miRNAs [451]. Identification of reference miRNAs is complex and findings may not be generalisable – a reference gene in one cohort may not be appropriate in others and multiple genes may be required to obtain more reliable data [450]. For example, miR-16-5p and let-7a-5p have been proposed as endogenous references in T-Cell acute lymphoblastic leukaemia [452] whereas let-7f has been proposed as a marker of ageing in bone [453]. Another study normalised the plasma of sarcopenic and dynapenic older adults to miR-133a and miR-21 although it is unclear how these were chosen besides being a myomiR and inflammation-related miR, respectively [454]. The authors found that normalisation to these endogenous controls or an exogenous spike in (cel-miR-39-3p) did not affect quantification.

Exogenous controls (spike-in controls) can be used to measure the quality of the testing protocols and to normalise findings. For examples spike-in controls can be added prior to RNA extraction and RT-qPCR stages. Most commonly used controls are UniSp4 and cel-miR-39-3p (from *C. Elegans*) which are used for normalisation as well as other proprietary controls [450, 454].

5.3.8 RNA-seq and Bioinformatics

RNA-seq and bioinformatics analysis was conducted by TamiRNA GmBH (Vienna, Austria). An overview of the protocol is shown in Figure 34 and is described in detail below.

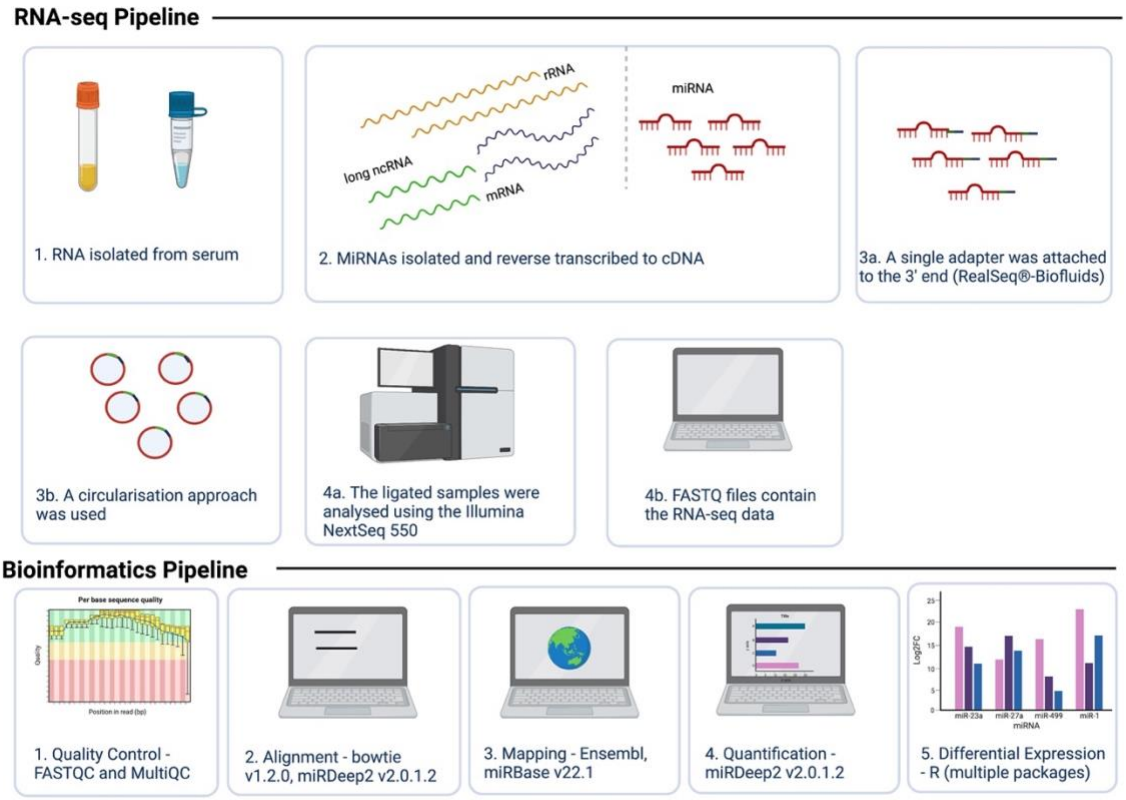

Figure 34 RNA-seq and bioinformatics pipelines

Created with BioRender (https://biorender.com/).

RNA-seq Pipeline

Step 1: Isolation of RNA from the biological sample.

Total RNA was extracted from serum samples using the miRNeasy Mini Kit (Qiagen, Germany). Briefly, 200 µL serum was homogenized with 1000 µL Qiazol. Synthetic RNA oligonucleotide mix obtained from the miRCURY Spike-In kit (Qiagen, Germany) was added to each sample at equimolar amounts prior to RNA extraction. These spike-ins were used to monitor RNA extraction efficiency. After incubation at room temperature for 15 minutes, 200 µL chloroform was added to the lysates followed by cooled centrifugation at 12,000g for 15 minutes at 4°C. Next, 650 µL of the upper aqueous phase was mixed with 7 µL glycogen (5 mg/mL) to enhance precipitation. Samples were transferred to a miRNeasy mini column, and RNA was precipitated with ethanol followed by automated washing with proprietary RPE and RWT buffers in a QIAcube liquid handling robot (Qiagen, Germany). Finally, total RNA was eluted in 30 µL nuclease free water and stored at -80°C until further analysis.

Step 2 & 3: Library Preparation and Adapter Ligation (circularisation approach)

This step involves isolation of the desired RNA molecules, reverse-transcription of the RNA to cDNA, fragmenting or amplifying randomly primed cDNA molecules, and ligating sequencing adaptors. Total RNA (8.5 µL) was used as an input for small RNA-sequencing library preparation. To each RNA sample 1 µl of miND® spike-in standards (TAmiRNA, Austria) was added prior to small RNA library preparation. The RNA-seq library was prepared using the RealSeq Biofluids kit (RealSeq Biosciences, United States) according to manufacturers' instructions.

Step 4: RNA-seq

Adapter-ligated libraries were amplified with 17 PCR cycles using barcoded Illumina reverse primers in combination with the Illumina forward primer. Library quality control was performed using DNA1000 Chip (Agilent, United States). An equimolar pool consisting of all sequencing libraries was prepared and sequenced on an Illumina NextSeq 550 (Illumina, United States) with 75 bp single-end reads.

The Illumina NextSeq 550 platform has four lane flow cells. Each lane is coated with oligonucleotides which are complementary to the library adapters. The Illumina NextSeq 550 was programmed for 190 million read outputs per run with a depth of approximately 7 million reads per sample.

FASTQ-format files containing the RNA-seq data were generated. The bioinformatics pipeline followed.

Bioinformatics Pipeline

Step 1: Quality Control

Variation in RNA-seq data can arise from a variety of sources from the quality of the RNA inputted through to the NGS platform used. To control for technical sources of variability it is becoming increasingly standard practice to use a positive control or 'spike-in' for the sequencing libraries [443]. The following positive controls were used – UniSp2, UniSp4, UniSp5, and six TamiRNA proprietary spike-ins (miND® spike-in standards; TAmiRNA, Austria).

After demultiplexing, FASTQC v0.11.1 (Babraham Institute Bioinformatics Group) was used for quality assessment of the sequencing data. FASTQC uses a traffic light system to highlight potential sources of error or contamination. If the ends of reads are found to be of poor quality, there is considerable adapter contamination and various tools can be used to trim the data. MultiQC v1.10 then combined

the results generated from FASTQC into a single report [455]. The Illumina adapters were trimmed using Cutadapt v3.3 [456] and filtered for a minimum length of 17 nt.

Steps 2 & 3: Alignment, mapping and quality assessment

Mapping steps were performed with bowtie v1.3.0 [457] and miRDeep2 v2.0.1.2 [458]. Reads were mapped first against the genomic reference GRCh38.p12 provided by Ensembl [459] allowing for two mismatches and subsequently miRBase v22.1 [168] filtered for miRNAs of human origin only (hsa), allowing for one mismatch. For a general RNA composition overview, non-miRNA mapped reads were mapped against RNAcentral [460] and then assigned to various RNA species of interest.

Step 4: Quantification

For quantification miRDeep2 v2.0.1.2 was used [458]. MiRDeep2 was designed for read mapping but also contains a standalone Quantifier module which sums up read counts for known miRNAs in RNA-seq data.

Step 5: Differential expression (statistical analysis)

Statistical analysis of pre-processed RNA-seq data was conducted with R v4.0 and the packages pheatmap v1.0.12, pcaMethods v1.82, and genefilter v1.72. Differential expression analysis was completed with edgeR v3.32 [461] using quasi-likelihood negative binomial generalized log-linear model functions. DESeq2, an R package, was used for independent filtering adapted for use with edgeR to remove low abundance miRNAs and subsequently improve the false discovery rate (FDR) [462]. Counts were normalised to one million mapped reads (reads per million; RPM) before differential expression testing to account for gene size (longer genes will have more reads) and library size (greater depth will have more reads). The FDR was set at < 0.1 due to the exploratory nature of this study.

5.3.9 Identification of an endogenous miRNA control

The Normfinder add-in for Microsoft Excel was used to identify an endogenous control from the RNA-seq data [463]. Normfinder uses analysis of variance (ANOVA) on log-transformed expression values to calculate the stability value. Normfinder ranks normalisation genes according to their expression stability in a given sample with a given experimental design. The authors did not recommend a specific cut-off; however, an acceptable stability value of < 0.15 has been adopted [464].

5.3.10 Identification of miRNAs for RT-qPCR analysis

To identify a panel of miRNAs which may be relevant in the pathogenesis of dynapenic abdominal obesity, three search strategies were utilised:

1. All microRNAs which had a log2FC > 0.58 or < -0.58 (equivalent to a fold change of 1.5) in the DAO/obese comparison were listed. These microRNAs were compared with the dynapenic/normal weight comparison results. If these microRNAs were (i) differentially expressed (FDR < 0.1) in the dynapenic/normal weight comparison and (ii) expressed in the same direction in both comparisons, they were included in the literature search. These miRs were thought to represent those which may reflect a 'dynapenic' phenotype, but failed to reach statistical significance in the DAO group.

2. The top ten (both over- and under-expressed) most highly differentially expressed microRNAs in the dynapenic/normal weight comparison.

3. Significantly different microRNAs in the dynapenic/normal weight comparison which were common to the systematic review.

Searches were conducted with RStudio (v2022.02) using RISmed (version 2.3.0) and EUtilsSummary to locate articles including the miRNA of interest published between 2000-2021 on PubMed. The title and abstracts were firstly screened, followed by full-text review of relevant articles to identify validated target genes or functions of these miRNAs with regards to muscle, sarcopenia, or frailty, and obesity, metabolic syndrome, or insulin resistance. Based on the evidence found, the list was reduced to nine microRNAs for further analysis by RT-qPCR.

5.3.11 RT-qPCR

An overview of the RT-qPCR method is shown in Figure 35. For reverse transcription, cDNA was synthesized using the miRCURY RT Kit (Qiagen, Germany). Reaction conditions were set according manufacturer instructions – 2 µL of total RNA were used as input in a 10 µL reaction. Quantitative PCR reactions were set up using miRCURY SYBR® green master mix and commercial LNA-enhanced primer assays (Qiagen, Germany). Two spike ins were added: UniSp4 was added during RNA extraction and cel-miR-39-3p was added prior to RT and qPCR. RT-qPCR reactions were performed in a 96-well plate format in a Roche LC96 instrument (Roche, Germany) with the following temperature settings: 95°C for 2 min, 45 cycles of 95°C for 10 s and 56°C for 60 s, followed by melting curve analysis. Cq-values

were normalized to RNA spike-in control level, by subtracting the individual miRNA Cq-value from RNA spike-in Cq, thus obtaining delta-Cq values that were used for data analysis. See Appendix 3 for details of assay and reagents used.

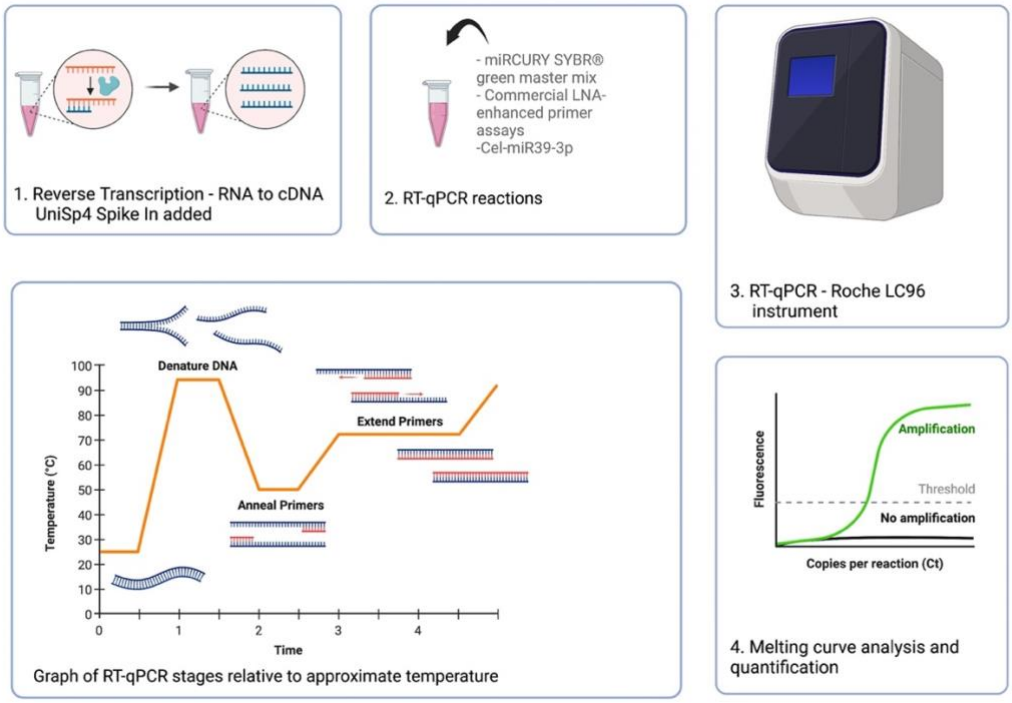

Figure 35 Schematic of RT-qPCR process

Abbreviations: RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction. Created with BioRender (https://biorender.com/).

5.3.12 Statistics

Differential expression of RNA-seq results are described (Section - Step 5: Differential expression (statistical analysis)) above. Comparisons between four groups was conducted using a Kruskal Wallis one-way ANOVA with corrected Dunn's post-hoc test using GraphPad Prism 9 (Version 9.4.1) for Mac, GraphPad Software, San Diego, California USA, www.graphpad.com. Correlations between miRNAs and measures of adiposity and strength were determined using Spearman's correlation with Bonferroni post-hoc test (Stata v16.1, StataCorp, Texas, USA). Statistical significance for differential expression of RNA-seq results was accepted at a FDR < 0.1 and for RT-qPCR at $p < 0.05$.

5.4 Results

This study includes results from two separate cohorts – the Fat and Bone (FAB) Study and Muscle in Obesity Study. Serum samples from older women in the FAB study (n = 23) were used for exploratory miRNA analysis (RNA-seq), identification of an endogenous miRNA control and internal validation of selected miRNAs (RT-qPCR). Serum samples from older women in the Muscle in Obesity study (n = 27) were used for external validation of the selected miRNAs using RT-qPCR. The results from the FAB study are presented followed by the results from the Muscle in Obesity Study.

5.5 FAB Study

5.5.1 Characteristics

A sub-sample of 23 women aged 59-75 years were included from the FAB Study (Table 24). The majority of all groups were aged less than 70y. All participants with obesity (n=5) were obese class I (BMI = 30 – 34.9kg/m^2) whereas the majority (n = 3/5) of women with DAO were obese class II (BMI = 35 – 39.9kg/m^2). Participants with normal weight had normal body fat percentage (BF% < 40%); one participant with dynapenia had high BF% (44.2%). One person with obesity had normal BF% (39.5%) whereas all participants with DAO had high BF% (range 46.5 – 51.0%). Participants with normal weight had a normal sit-to-stand (STS) time whereas two participants in all other groups had a slow STS time (> 15s).

Table 24 Characteristics of participants in the Fat and Bone exploratory study

	Normal weight N=7		**Dynapenic** N=6		**Obese** N=5		**DAO** N=5	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age (y)	66.6	5.7	63.5	9.5	64.5	1.3	63.5	9.0
Age < 70y (n)	6		4		4		3	
Age ≥ 70y (n)	1		2		1		2	
Anthropometry								
Weight (kg)	59.3	5.6	56.8	6.8	78.6	5.2	87.0	4.8
Height (m)	161.0	6.9	158.6	6.9	161.3	4.9	156.3	3.5
BMI (kg/m^2)	22.6	1.2	22.9	2.9	30.8	0.7	36.3	3.3
Obese Class I (n)	-		-		5		2	
Obese Class II (n)	-		-		0		3	
Waist Circumference (cm)	77.0	8.0	82.8	11.9	93.2	1.9	104.0	4.5
Body Composition								
WB Total Fat (kg)	20.3	5.7	21.7	0.6	34.0	0.6	42.3	2.8
Trunk Fat (kg)	8.0	3.1	10.1	2.3	16.2	1.7	20.9	1.3
Body Fat (%)	33.1	8.7	37.5	2.9	43.3	2.5	47.7	3.3
BF% > 40 % (n)	0		1		4		5	
Android Fat (kg)	1.2	0.6	1.5	0.5	2.8	0.7	3.6	0.3
Gynoid Fat (kg)	3.5	1.2	3.7	0.7	5.5	0.8	6.6	0.3
Function								
Max HGS (kg)	18.3	6.9	13.0	2.8	16.6	6.3	14.7	4.5
STS time (s)	11.5	1.4	11.8	5.0	13.2	3.3	13.3	5.3
STS time > 15s (n)	0		2		2		2	
Clinical								
IGF-1 (ng/l)	118.5	87.4	136.0	39.8	158.3	33.0	88.1	26.5
Leptin (pgm/ml)	585.8	547.2	1348.0	1261.2	2505.7	1.636.1	4988.5	3373.7
Glucose (mmol/l)	5.2	0.8	5.3	0.2	5.3	0.1	5.4	0.2

Abbreviations: DAO = dynapenic abdominal obese; BMI = body mass index; HGS = hand grip strength; STS = sit-to-stand time; WB = whole body; BF% = body fat percentage; IGF-1 = insulin-like growth factor 1.

5.5.2 Exploratory: RNA-seq

Quality Control

Quality control of the RNA-seq analysis was acceptable (Appendix 4). The per base sequence content (Appendix 4c) relates to bias in the first ~22 reads due to the spike-in control. All reads have an acceptable mean quality score (>30) until 30-35bp (Appendix 4d) and acceptable per sequence quality score (Appendix 4e).

Differential Expression

In the RNA-seq analysis, 25 miRNAs were upregulated and 13 downregulated in the dynapenic group compared with the normal weight group (FDR < 0.1). The full list is located in Table 25**.**

There were no differentially expressed miRNAs (normalised to one million mapped reads; RPM) when the DAO group was compared with the obese or normal weight group (Figure 36). Likewise, there were no differentially expressed miRNAs when the obese group was compared with the normal weight group. Two further comparisons were made which compared (i) all women with obesity to all women who were normal weight and (ii) all women with dynapenia to all women with normal strength – no differences were found in either comparison.

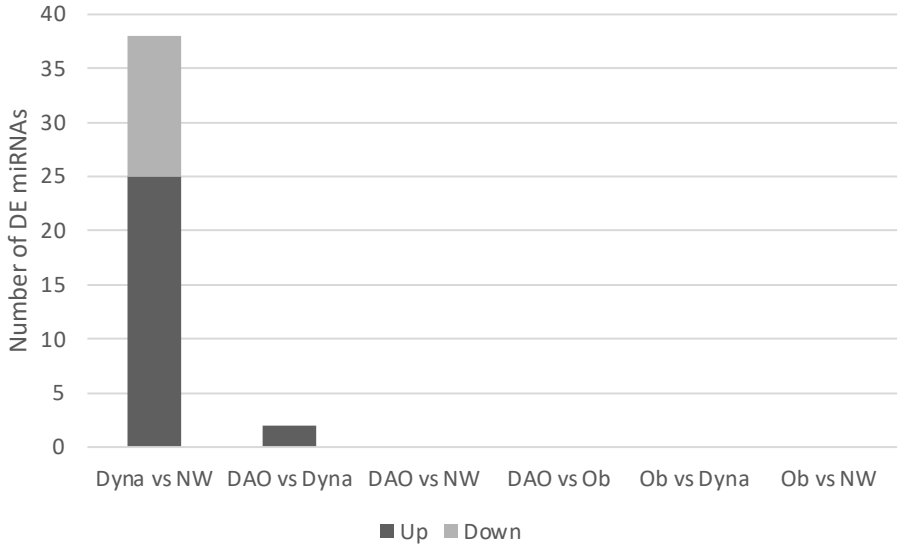

Figure 36 Summary of differential expression analysis

Abbreviations: DE = differentially expressed; Dyna = dynapenic; NW = normal weight; DAO = dynapenic abdominal obese; Ob = obese.

Table 25 List of differentially expressed miRNAs (dynapenia and normal weight)

miRNA	logFC	P	FDR
hsa-miR-369-3p	-1.9	0.00077	0.071
hsa-miR-92a-3p	0.85	0.001	0.071
hsa-miR-142-3p	-0.87	0.0016	0.071
hsa-miR-199a-5p	-1.2	0.0018	0.071
hsa-miR-25-3p	0.64	0.0019	0.071
hsa-miR-543	-1.7	0.0025	0.071
hsa-miR-16-2-3p	0.87	0.003	0.071
hsa-miR-143-3p	-0.88	0.0037	0.071
hsa-miR-15a-5p	0.77	0.0038	0.071
hsa-miR-107	0.75	0.0046	0.071
hsa-miR-376a-3p	-1.5	0.0051	0.071
hsa-miR-199b-5p	-0.96	0.0052	0.071
hsa-miR-126-3p	-0.54	0.0052	0.071
hsa-miR-486-5p	0.92	0.0054	0.071
hsa-miR-101-3p	0.56	0.0056	0.071
hsa-miR-654-3p	-1.5	0.0057	0.071
hsa-miR-425-5p	0.47	0.0062	0.071
hsa-miR-410-3p	-1.5	0.0064	0.071
hsa-miR-376c-3p	-1.5	0.0072	0.072
hsa-miR-339-5p	-0.82	0.0072	0.072
hsa-miR-136-3p	-1.1	0.0079	0.075
hsa-miR-30e-3p	-0.77	0.01	0.092
hsa-miR-487b-3p	-1.6	0.011	0.092
hsa-miR-16-5p	0.72	0.011	0.092
hsa-miR-660-5p	0.48	0.012	0.095
hsa-miR-411-5p	-1.2	0.013	0.098
hsa-miR-409-3p	-1.5	0.014	0.098
hsa-let-7i-5p	0.46	0.014	0.098
hsa-miR-15b-3p	0.71	0.015	0.098
hsa-miR-382-5p	-1.4	0.015	0.098
hsa-miR-26a-5p	-0.69	0.016	0.098
hsa-miR-191-5p	-0.60	0.016	0.098
hsa-miR-744-5p	-0.73	0.016	0.098
hsa-miR-30d-5p	0.46	0.017	0.099
hsa-miR-30b-5p	-0.69	0.018	0.099
hsa-miR-127-3p	-1.4	0.018	0.099
hsa-miR-199a-3p	-0.76	0.019	0.099
hsa-miR-199b-3p	-0.75	0.019	0.099

Abbreviations: hsa = homo sapiens; FC = fold change; FDR = false discovery rate.

Endogenous control

To determine an endogenous control, the stability of the miRNAs measured using RNA-seq was assessed. The top ten most stable miRNAs, based on the lowest stability values, are listed in Table 26. Using data from the systematic review (Chapter 4), nine of these miRNAs were found to be differentially expressed in people with obesity in external studies (Table 26). The miRNAs were not found in the sarcopenia arm of the systematic review (Chapter 4); however, one miRNA (miR-186-5p) not identified in the systematic review was shown to be involved in myogenesis *in vitro* and *in vivo* [465]. Based on these findings, an endogenous control could not be identified with sufficient confidence for use in the RT-qPCR validation study.

Table 26 Most stably expressed miRNAs from RNA-seq data

	MiRNA	Stability value	Systematic review study
1	miR-19a-3p	0.079	[335, 336]
2	miR-186-5p	0.080	-
3	miR-19b-3p	0.087	[335, 466]
4	miR-421	0.102	[251, 335]
5	miR-148b-3p	0.105	[335, 336]
6	miR-500a-3p	0.107	[335, 467]
7	miR-140-3p	0.107	[335, 336, 468]
8	miR-652-3p	0.108	[336, 469]
9	miR-502-3p	0.110	[335, 336]
10	miR-128-3p	0.110	[336]

The top ten most stable miRNAs identified by NormFinder. Systematic review study refers to external studies of obesity or sarcopenia that have identified these miRNAs as differentially expressed.

Identification of miRNAs for validation

A panel of miRNAs was identified for validation using RT-qPCR. To identify this panel a systematic approach was undertaken to filter the results of the RNA-seq analysis as outlined below (Figure 37). The targets and functions of the resulting microRNAs were then determined through a literature search of PubMed.

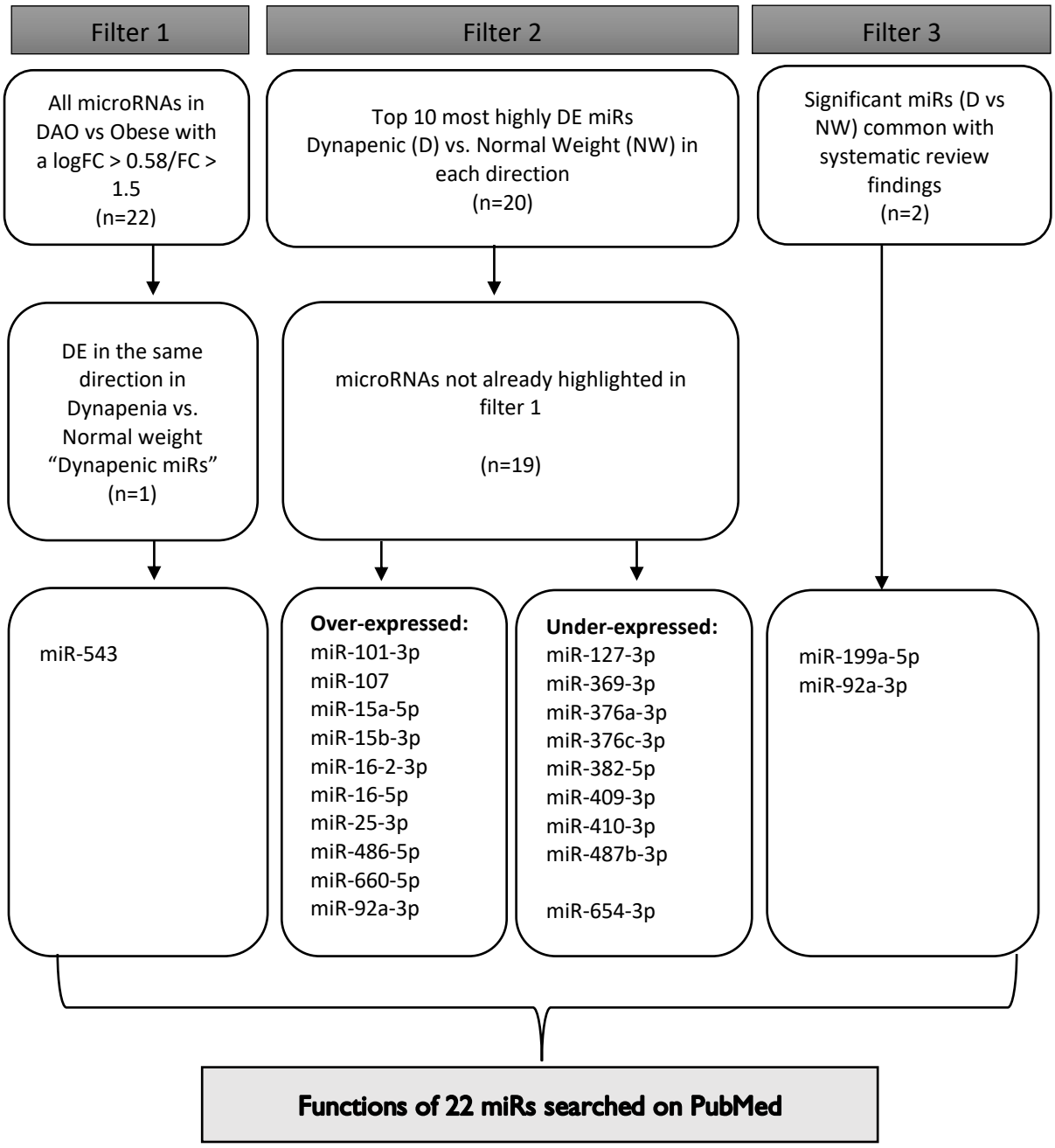

Figure 37 Search strategy for microRNAs for further RT-qPCR validation.

Abbreviations: RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction; DE = differentially expressed; D = dynapenic; NW = normal weight; DAO = dynapenic abdominal obese.

Nine miRNAs were identified for further RT-qPCR measurement. The log2FC and FDR of these miRNAs are presented in Table 27.

Table 27 Fold-change of miRs selected for RT-qPCR validation

	Dynapenic vs. Normal Weight		DAO vs Obese		Obese vs. Normal Weight	
miRNA	**Log2FC**	**(FDR)**	**Log2FC**	**(FDR)**	**Log2FC**	**(FDR)**
miR-543	-1.732	(0.071)	-0.6065	(0.995)	-0.2623	(0.929)
miR-107	0.7548	(0.071)	-0.04539	(0.995)	0.3947	(0.929)
miR-16-5p	0.7235	(0.092)	-0.1793	(0.995)	0.3895	(0.929)
miR-486-5p	0.9196	(0.071)	0.1069	(0.995)	0.1976	(0.929)
miR-127-3p	-1.435	(0.099)	0.2056	(0.995)	-0.645	(0.929)
miR-376c-3p	-1.462	(0.072)	-0.4089	(0.995)	-0.4332	(0.929)
miR-382-5p	-1.373	(0.098)	-0.2376	(0.995)	-0.596	(0.929)
miR-92a-3p	0.984	(0.071)	-0.02306	(0.995)	0.2113	(0.929)
miR-199a-5p	-1.058	(0.071)	0.1343	(0.995)	-0.688	(0.929)

Abbreviations: DAO = Dynapenic Abdominal Obese; RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction; FC = fold change; FDR = false discovery rate.

Relevant functions and targets of the miRNAs selected for validation are presented in Table 28. All miRNAs had roles in muscle (e.g. myogenesis, exercise, muscle atrophy), obesity, or other roles relevant to DAO (e.g. glucose metabolism, lipemia, mitochondrial function). Two miRNAs were also identified as potentially involved in sarcopenic obesity from the systematic review (miR-92a-3p, miR-199a-5p).

Table 28 Summary of functions and targets of microRNAs selected for RT-qPCR validation

miRNA	RPM	Function/Target
miR-543	94.9	Promotes C2C12 cell differentiation by targeting Krüppel-like factor 6 (KLF6) [470]. Associated with post-prandial lipemia in mice [471]. Promotes bovine myoblast differentiation [472].
miR-107	8030.3	Systematic review (Chapter 4) – upregulated in two and downregulated in one study of obesity [335, 336, 468]. In the soleus muscle, it is strongly associated with exercise performance-related physiological and metabolic functions. Higher levels found in high energy capacity mice [473]. Overexpression inhibits bovine myoblast differentiation and protects cells from apoptosis [474]. Endurance exercise increased expression of miR-107 in mice (quadriceps femoris) [475].
miR-16-5p	129975.7	Upregulated in mice who underwent caloric restriction (CR), may modulate anti-inflammatory effects of CR [476]. Decreased after aerobic exercise in obese older adults [406]. In a meta-analysis, it is downregulated after bariatric surgery [403]. Intervention of resistance training and whey protein, skeletal muscle miR-16-5p increased with exercise in older men [477]. In chickens, miR-16-5p directly targets SESN1 to regulate the p53 signalling pathway, affecting myoblast proliferation and apoptosis [478].
miR-486-5p	42229.6	Attenuates muscle atrophy and improves strength (C2C12 myotubes, mice) [479]. Targets PTEN and FOXO1, downregulated in sarcopenic mice [480]. Involved in myoblast differentiation via targeting MRTF-A [481]. Highly expressed in skeletal muscle (vastus lateralis) of middle aged men but not associated with strength [482]. Increased expression in plasma exosomes in older men who regularly exercise [483]. Increased in skeletal muscle following carbohydrate and protein feeding in younger men; targets PTEN [484]. Increased *in vitro* expression with high glucose; inhibits SIRT1 which promotes adipose-derived-MSC senescence [485].
miR-127-3p	50.7	Upregulated in VAT of women with T2DM/IFG [486]. Inhibits myoblast proliferation – targets Sept7 *in vitro* [487]. Regulates myoblast proliferation and differentiation by targeting Vamp2 [488]. Regulates myocyte proliferation via KMT5a [489].
miR-376c-3p	228.5	Attenuates muscle atrophy by targeting Atrogin-1. Promotes myotube thickening, improves muscle function, force and fatigue resistance in older mice [490]
miR-382-5p	153.1	Upregulated in patients with muscular dystrophy, predicted to be involved in mitochondrial damage using bioinformatics [491]. Inhibition leads to mitonuclear protein imbalance and activation of the mitochondrial unfolded protein response *in vitro* [492].
miR-92a-3p	58395.5	Identified in the systematic review (upregulated) as a potential miRNA involved in sarcopenic obesity (Chapter 4).
miR-199a-5p	624.4	Identified in the systematic review (downregulated) as a potential miRNA involved in sarcopenic obesity (Chapter 4).

Reads per million (RPM) is expressed as median of the total group (n=27). Abbreviations: MSC = mesenchymal stem cell; VAT = visceral adipose tissue; T2DM = type two diabetes mellitus; IFG = impaired fasting glucose; FOXO1 = forkhead box protein 1; PTEN = phosphatase and tensin homolog.

5.5.3 Internal Validation: RT-qPCR

Quality Control

Quality control of experimental processes was determined from exogenous spike-in controls – UniSp4 and cel-miR-39-3p. Both spike-in controls were homogenous across all samples (Figure 38). RNA spike-in control (UniSp4) was used for normalization to adjust for analytical noise using the equation: $Cq = Cq^{(UniSp4)} - Cq^{(miRNA)}$.

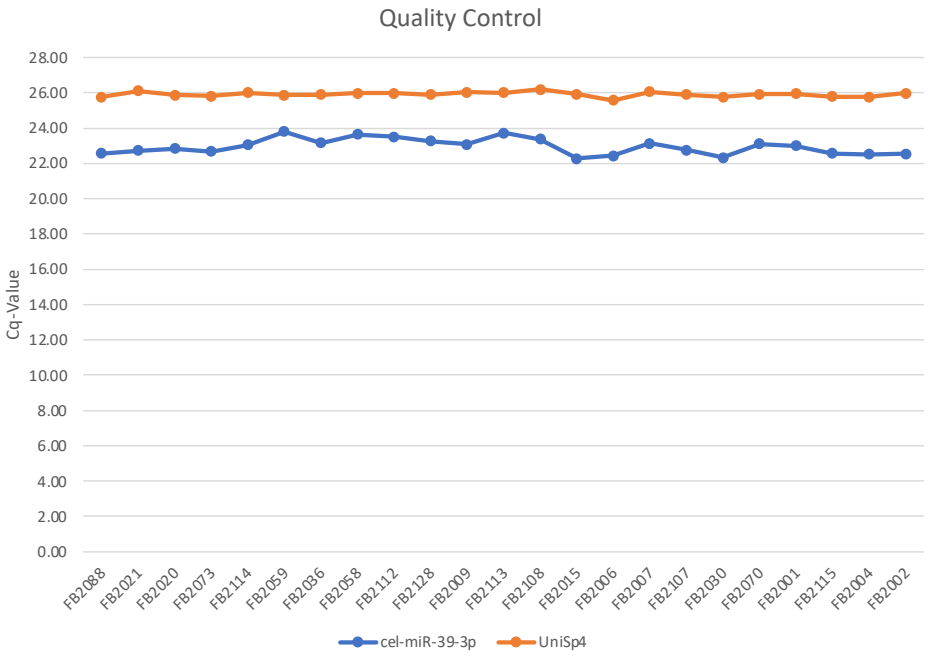

Figure 38 Quality control according to exogenous spike-in controls (UniSp4 and cel-miR-39-3p) for RT-qPCR - FAB Study

The horizontal axis refers to individual participants. Abbreviations: FAB = Fat and Bone Study; RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction.

Differential expression

The median and inter-quartile range for each miRNA in each of the four groups (normal weight, dynapenic, obese, DAO) are listed in Table 29. Of the nine microRNAs selected, there were no significantly different miRNAs between any group (Figure 39). Melting curves for individual microRNAs are located in Appendix 5.

Table 29 Normalised Cq-values of microRNAs from RT-qPCR

	Normal weight		**Dynapenic**		**Obese**		**DAO**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
miR-543	-8.18	1.11	-10.00	0.77	-9.15	5.32	-9.03	2.13
miR-107	-1.56	1.42	-1.79	0.81	-2.32	0.54	-2.11	0.38
miR-16-5p	4.88	0.90	4.42	0.53	4.71	1.49	3.90	0.40
miR-486-5p	1.21	1.13	0.85	1.13	1.40	1.73	0.72	0.38
miR-127-3p	-7.91	2.23	-8.32	1.42	-10.27	1.58	-8.21	0.65
miR-376c-3p	-4.31	2.40	-4.83	1.28	-5.25	1.51	-3.52	0.86
miR-382-5p	-7.13	1.18	-8.02	1.86	-7.88	3.78	-6.72	2.01
miR-92a-3p	1.97	0.80	1.72	0.58	1.88	1.24	1.52	0.08
miR-199a-5p	-4.27	0.69	-4.93	1.19	-4.70	1.39	-4.23	0.45

Abbreviations: RT-qPCR = real time quantitative polymerase chain reaction; IQR = inter-quartile range; DAO = Dynapenic Abdominal Obese.

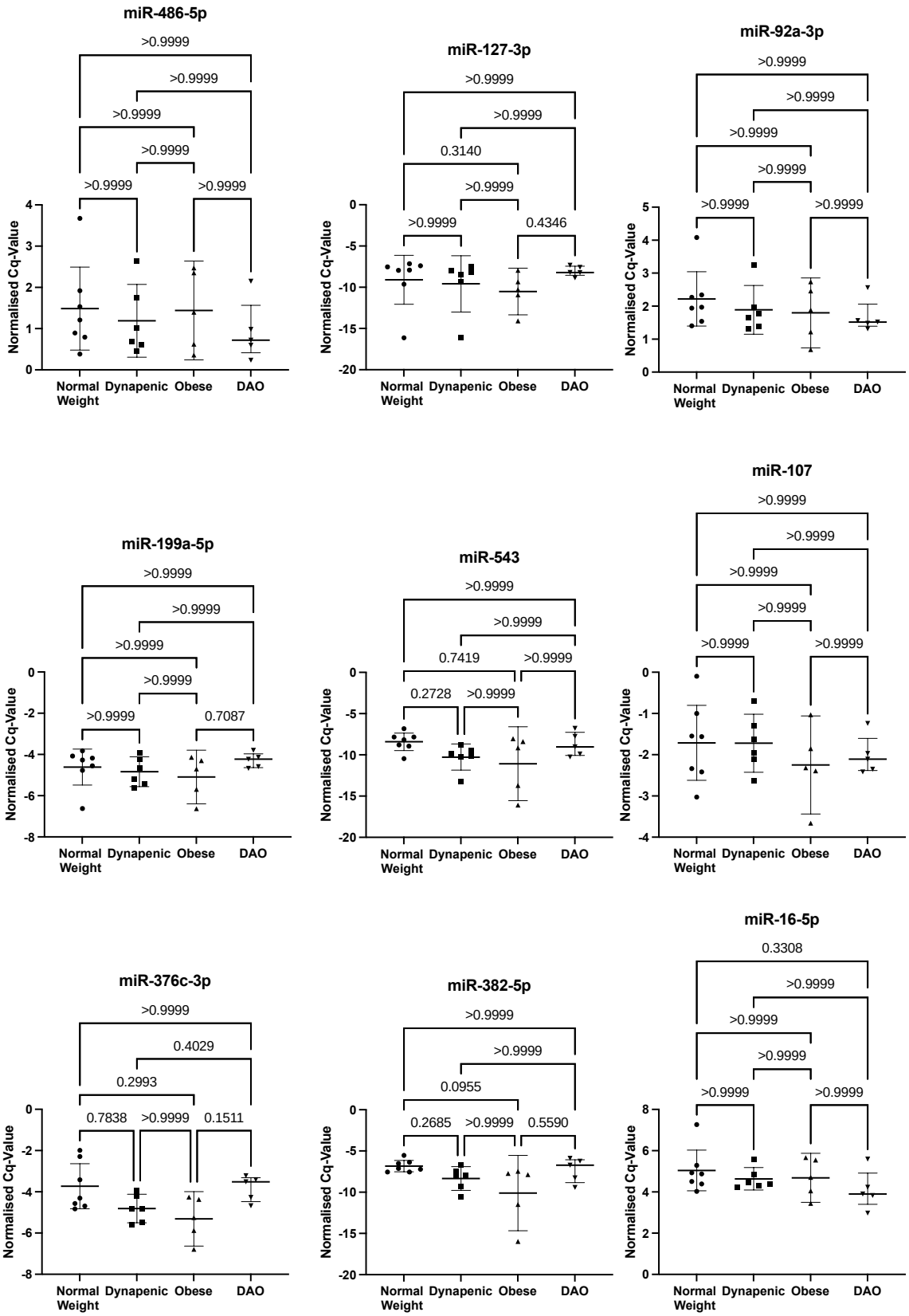

Figure 39 MicroRNAs Validated with RT-qPCR – Fat and Bone Study

Median ± Inter-quartile Range. Abbreviations: DAO = Dynapenic Abdominal Obese.

Relationship between miRNAs, strength and body fat

There were monotonic correlations between miR-92a-3p and hand-grip strength (r=1.00, p<0.001) in those with DAO, and miR-376c-3p with body fat percentage in those with obesity (r=1.00, p<0.001). Although there were suggestions of strong - very strong relationships between microRNAs and sit-to-stand time, all failed to meet statistical significance.

Table 30 Correlation between miRNAs from the Fat and Bone (exploratory) study and measures of strength and adiposity

	miR-486-5p	**miR-127-3p**	**miR-92a-3p**	**miR-199a-5p**	**miR-543**	**miR-107**	**miR-376c-3p**	**miR-382-5p**	**miR-16-5p**
Hand grip strength (kg)	r	r	r	r	r	r	r	r	r
Normal Weight	0.00	-0.57	-0.04	-0.14	0.04	-0.25	0.18	0.31	-0.04
Dynapenic	0.03	0.43	0.26	0.09	-0.60	0.26	0.03	-0.26	0.26
Obese	-0.70	-0.90	-0.70	-0.20	-0.60	-0.20	-0.30	-0.20	-0.60
DAO	0.40	0.30	**1.00***	-0.50	0.40	0.10	0.40	0.00	0.90
Sit-to-stand time (s)									
Normal Weight	0.04	0.23	0.00	0.18	0.27	0.32	0.59	0.22	-0.13
Dynapenic	-0.43	0.31	-0.20	0.83	0.20	0.31	0.26	0.77	-0.20
Obese	-0.10	0.30	-0.10	0.10	0.70	0.10	0.90	0.60	-0.20
DAO	-0.30	0.10	-0.60	0.70	-0.30	0.20	-0.30	-0.10	-0.20
Body Fat (%)									
Normal Weight	-0.07	0.32	0.11	-0.36	-0.07	-0.18	-0.36	-0.41	0.29
Dynapenic	0.43	-0.31	0.20	-0.83	-0.20	-0.31	-0.26	-0.77	0.20
Obese	0.30	0.40	0.30	0.50	0.90	0.50	**1.00***	0.50	0.10
DAO	0.10	-0.30	-0.20	-0.10	0.10	-0.60	0.10	0.30	-0.60

*Abbreviations: DAO = dynapenic abdominal obese. * p < 0.001*

5.5.4 Comparison of experimental methods – RNA-seq and RT-qPCR

There were very weak/weak correlations between RNA-seq and RT-qPCR results which did not reach statistical significance. The association between RNA-seq (expressed as log2 transformed RPM) and RT-qPCR (Ct values normalised to UniSp4) results was very weak for miR-92a-3p (r=-0.01), miR-127-

3p (r=0.04), miR-199a-5p (r=0.04), miR-486-5p (r=0.06), miR-107 (r=-0.13), and miR-16-5p (r=-0.12), and weak for miR-382-5p (r=0.22), miR-376c-3p (r=0.29), and miR-543 (r=0.34). Comparisons were also made between the log-transformed copy number from RNA-seq and normalised delta-Cq values, all associations remained very weak/weak and not statistically significant.

A comparison of the log2FC and delta-Cq values for the validation panel of miRNAs is shown in Appendix 6.

5.5.5 Minimal detectable change

Using a measure for the minimal detectable change for HGS (5kg), participants with DAO (n=3/5), dynapenia (n=4/6), obesity (n=3/5) and normal weight (n=4/7) may have been mis-classified (Figure 40). Three women with normal weight and two with dynapenia may be considered to be abdominally obese, or medium risk, using the threshold of 80cm.

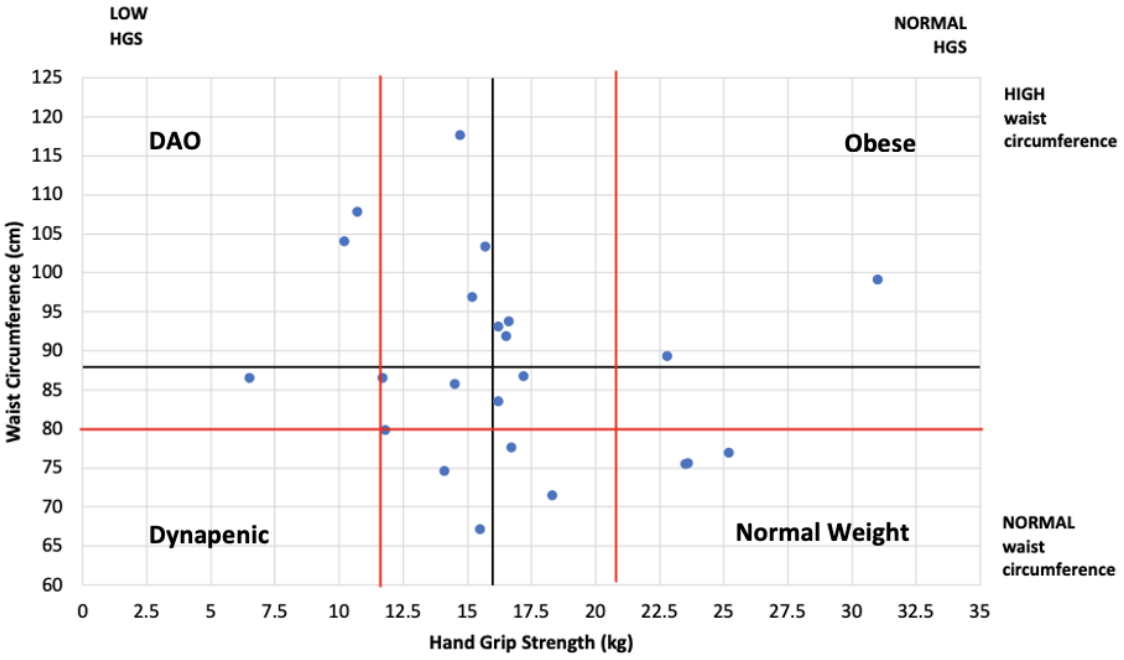

Figure 40 Hand-grip strength (kg) versus waist circumference (cm) for all participants
The black horizontal line shows the cut-off used for high and low waist circumference (>88cm) and the red horizontal line shows the alternative clinical cut-off for medium risk waist circumference (>80cm) [80, 303]. The black vertical line shows the cut-off for dynapenia (<16kg) and the red vertical lines show the minimal detectable change (± 5kg) in hand grip strength [493].

5.6 Muscle in Obesity Study

5.6.1 Characteristics

For external validation, the miRNA panel was measured in the serum of the older women from the Muscle in Obesity study. The characteristics of participants in the Muscle in Obesity study are described in Chapter 3. Twenty seven women aged 60-79y were included, 10 with normal weight, 10 with obesity, and 7 with DAO (high waist circumference and poor sit-to-stand time). The majority of the group with DAO (n = 5/7) and two participants with obesity had metabolic syndrome according to International Diabetes Federation criteria [303]. Participants with normal weight did not have impaired fasting glucose (> 6.1mmol/l).

5.6.2 External Validation: RT-qPCR

Quality Control

Quality control of experimental processes was determined from exogenous spike-in controls – UniSp4 and cel-miR-39-3p. Both spike-in controls were homogenous across all samples (Figure 41). RNA spike-in control (UniSp4) was used for normalization to adjust for analytical noise using the equation: Cq = $Cq^{(UniSp4)} - Cq^{(miRNA)}$.

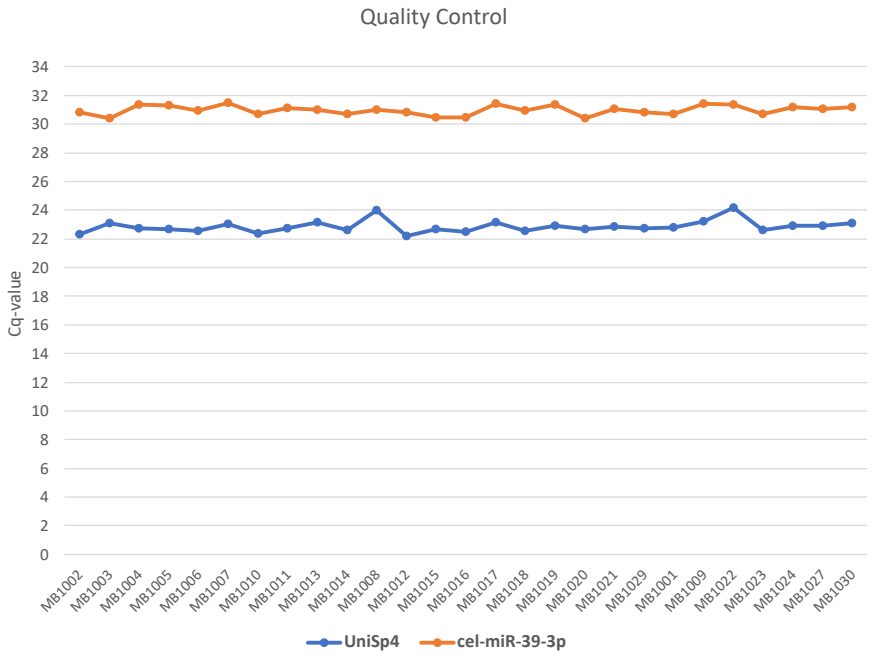

Figure 41 Quality control according to exogenous spike-in controls (UniSp4 and cel-miR-39-3p) for RT-qPCR – Muscle in Obesity Study

Horizontal axis refers to individual participants. Abbreviations: RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction.

Differential Expression

There were no significant differences in miRNA expression between any group for the selected miRNA panel (Figure 42). Melting curves for individual microRNAs are located in Appendix 7.

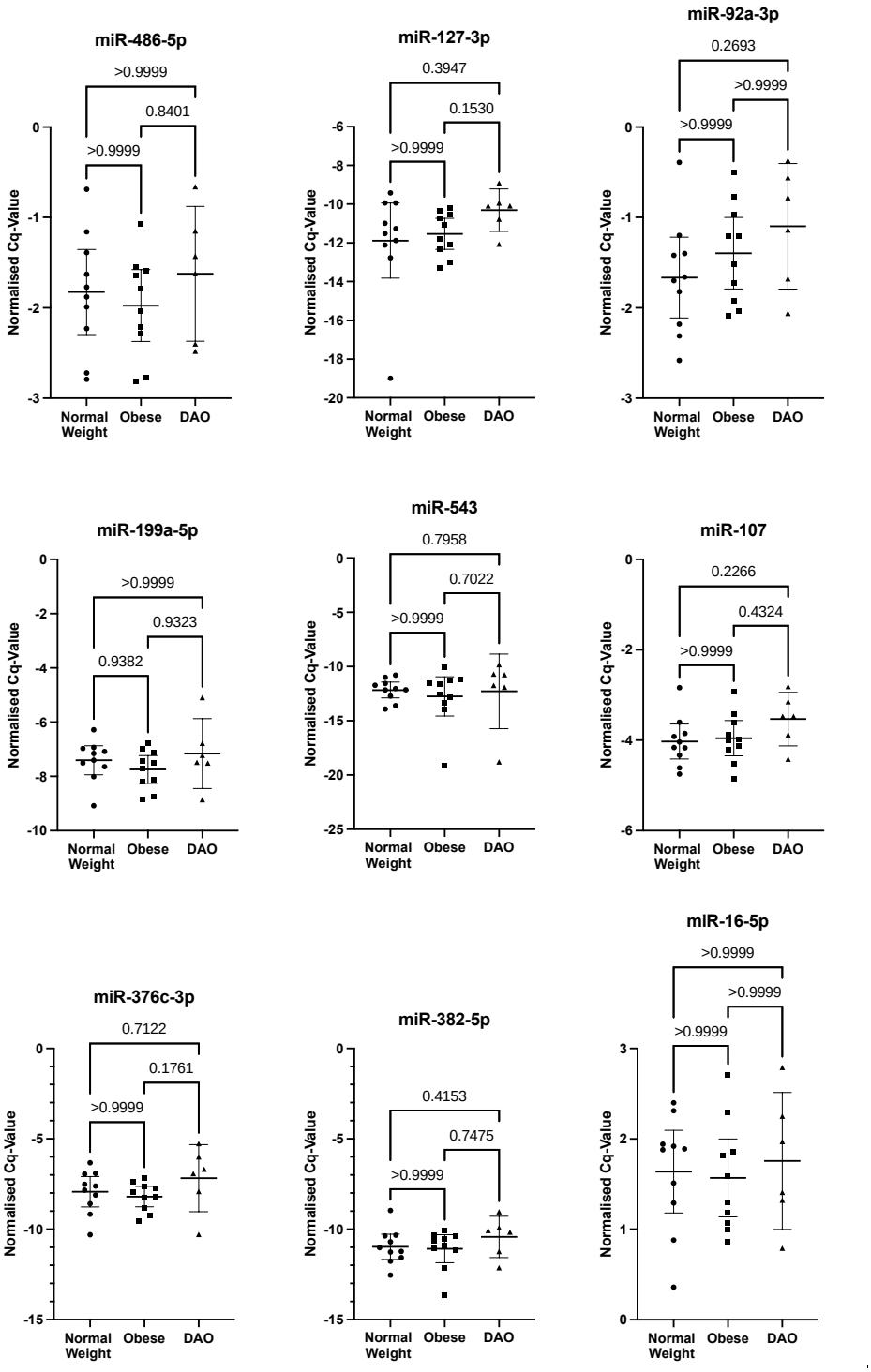

Figure 42 MicroRNAs Validated with RT-qPCR – Muscle in Obesity Study

Median ± Inter-quartile Range. Abbreviations: DAO = Dynapenic Abdominal Obese.

Due to differences in the definition of dynapenia between the exploratory and external cohort study, the relationship between HGS and miRNA expression levels were examined (Table 31). The relationship between the panel of miRNAs and sit-to-stand time and body fat percentage was also explored to confirm findings from the exploratory study.

There were monotonic correlations between miR-376c-3p with body fat percentage in those with obesity (r=1.00, p<0.001). Although there were suggestions of strong to very strong relationships between miRNAs and both HGS (miR-127-3p, miR-92a-3p, miR-543) and sit-to-stand time (miR-199a-5p, miR-382-5p), all failed to meet statistical significance.

Table 31 Correlation between miRNAs from the Muscle in Obesity (validation) study and measures of strength and adiposity

	miR-486-5p	**miR-127-3p**	**miR-92a-3p**	**miR-199a-5p**	**miR-543**	**miR-107**	**miR-376c-3p**	**miR-382-5p**	**miR-16-5p**
Hand grip strength (kg)	r	r	r	r	r	r	r	r	r
Normal Weight	0.00	-0.57	-0.04	-0.14	0.04	-0.25	0.18	0.31	-0.04
Obese	0.03	0.43	0.26	0.09	-0.60	0.26	0.03	-0.26	0.26
DAO	-0.70	-0.90	-0.70	-0.20	-0.60	-0.20	-0.30	-0.20	-0.60
Sit to stand time (s)									
Normal Weight	0.04	0.23	0.00	0.18	0.27	0.32	0.59	0.22	-0.13
Obese	-0.43	0.31	-0.20	0.83	0.20	0.31	0.26	0.77	-0.20
DAO	-0.10	0.30	-0.10	0.10	0.70	0.10	0.90	0.60	-0.20
Body Fat (%)									
Normal Weight	-0.07	0.32	0.11	-0.36	-0.07	-0.18	-0.36	-0.41	0.29
Obese	0.30	0.40	0.30	0.50	0.90	0.50	**1.00***	0.50	0.10
DAO	0.10	-0.30	-0.20	-0.10	0.10	-0.60	0.10	0.30	-0.60

*Abbreviations: DAO = dynapenic abdominal obesity. * p < 0.001*

5.7 Discussion

To the best of our knowledge, this is the first observational, cross-sectional study of older women which aimed to examine the miRNA profile of those with DAO, or within the context of sarcopenic obesity. We found that women with DAO do not have a significantly different miRNA profile compared to women with obesity, normal weight, or dynapenia. We confirmed these findings in an external cohort of women. However, we found that women with dynapenia had a panel of miRNAs which were significantly differently expressed (RNA-seq) compared to those with normal weight but this could not be validated with RT-qPCR.

The aim of this study was to identify whether there was a panel of miRNAs which were differentially expressed in women with DAO. There are several possible explanations why we did not find a difference in this group. Firstly, in both cohorts, the group with DAO was small and the findings of this study should be considered as exploratory. DAO also represents a phenotype associated with impaired metabolic health (hypertriglyceridemia, hyperglycaemia, and metabolic syndrome [122]) which can affect the miRNA profile [494]. Another key consideration is within the definition of dynapenic abdominal obesity itself – whilst there was a 5kg/m^2 gap of BMI between the normal weight and obese groups, there was no such gap between the definitions of dynapenia (HGS <16kg or STS > 15s) or abdominal obesity (>88cm). Although there is no consensus on an acceptable minimal detectable change (MDC) in hand-grip strength, findings from the Canadian Study of Ageing propose a 5kg difference in HGS [493]. The implication to this current study is that a gap, similar to that of BMI, may have avoided potential confounding by mis-classification of participants as the majority of all groups were close to the cut-off for dynapenia. Lastly, it could also be hypothesised that DAO represents a heterogenous phenotype with no distinct miRNA pattern.

In an attempt to overcome any potential effects of incorrect categorisation, the relationship between the selected miRNAs, strength, and body fat percentage was examined. Positive associations were found between miR-376c-3p and body fat percentage in those with obesity in both the exploratory and validation studies. This miRNA does not seem to have been identified as differentially expressed in other studies of obesity. MiR-376c-3p has been shown to improve muscle function and force in older mice [490]; however, whether it has functional relevance in older adults with dynapenia or obesity is unclear. MiR-92a-3p, which was also identified in the systematic review (Chapter 4), was positively associated with hand-grip strength in those with DAO but this could not be validated in the external cohort. MiR-92a-3p gradually increases with age [407], decreases with exercise in young men

[346, 404, 405] but not in obese older men [406]. It is also down-regulated following bariatric surgery [403]. In the external cohort, there were no significant correlations between the miRNAs measured and hand-grip strength. These findings tentatively suggest that the differential expression results were not confounded by different levels of hand-grip strength; however, further studies using larger cohorts are required.

Dynapenia and loss of muscle strength is multi-factorial relating to ageing, nutrition, physical activity, neurological changes and muscular changes (e.g. muscle fibre type and fat infiltration) [21, 129]. Functionally, dynapenia is associated with a greater risk of falls, fractures, hospitalisation and mortality [7, 86, 89-91, 94-96]. Therefore, understanding the pathogenesis of dynapenia in normal weight individuals is also of clinical importance in order to identify novel treatment strategies. We identified 38 miRNAs which were differentially expressed between normal weight and dynapenic women using RNA-seq. Six of the top 20 most highly differentially expressed miRNAs were selected for validation due to their roles in muscle (e.g. myogenesis, exercise, muscle atrophy), obesity, or other related roles (e.g. glucose metabolism, lipemia, and mitochondrial function). However, differential expression of these miRNAs could not be confirmed either internally or externally therefore it remains unclear whether these miRNAs are truly involved in the pathogenesis of dynapenia.

It is unclear why we found such disparity between the RT-qPCR and RNA-seq results as others have shown that both techniques have strong correlations [495]. Our disparity was further supported by observing that some miRNAs identified as expressed in one direction with RNA-seq were expressed in another direction using RT-qPCR. This phenomenon, termed non-concordant genes/miRNAs, was also reported by Everaert et al. (2017) [438]. In that study, 15% of protein coding genes validated using qPCR were non-concordant – these genes had low expression, had a fold-change < 2, and were shorter. One potential explanation in our study may relate to differences in normalisation techniques between methods – reads per million (RNA-seq) or to an exogenous spike-in control (RT-qPCR). An endogenous control may have resulted in better correlation between the two methods. However, there are no universally validated endogenous controls and different miRNAs are suggested in different conditions [452, 453]. We failed to identify an endogenous control from the RNA-seq analysis as the most stable miRNAs were identified by others in studies of obesity or muscle metabolism. Further research should focus on the identification of an endogenous miRNA in this phenotype.

Despite the significant evidence that people with obesity exhibit differences in miRNAs when compared to people who are normal weight [184], we found no difference between normal weight and obese groups. However, the results from our systematic review (Chapter 4) highlighted that all of the included miRNA studies in those with obesity were conducted in younger and middle-aged adults. It could also be speculated that the women with obesity in the FAB study were more 'healthy' given the inclusion and exclusion criteria applied as well as a more general healthy volunteer bias – or conversely the normal weight group was less healthy thus leading to homogeneity between groups. Furthermore, a waist circumference between 80-88cm in women is still considered high [80, 303] and some women with normal weight could be classified as abdominally obese. Alternatively, we may not have used the most appropriate definition of obesity as both BMI and waist circumference are surrogate measures of adiposity and body fat percentage is preferred [75]. Based on body fat percentage, some normal weight/dynapenic and obese/normal strength women could be re-categorised as obese and normal weight respectively.

5.7.1 Limitations

The small sample sizes and exploratory nature of this study must be acknowledged as limitations. To better understand the pathogenesis of DAO, a muscle biopsy could have provided a more direct insight as the origin of these serum miRNAs is unknown. To determine a panel of miRNAs for validation, functions of selected miRNAs were identified in the literature; however, this was based on different settings and samples (e.g. rodents, muscle cells, older adults) and may not be generalisable to older women. Haemolysis status of the samples was uncertain. The Muscle in Obesity study contained women with metabolic syndrome which may have confounded our findings. Some miRNAs in the validation study had low expression levels (<100 RPM: miR-127-3p, miR-543; <1000 RPM: miR-199a-5p, miR-376c-3p, miR-382-5p) meaning the fold-change difference may be inflated and less biologically relevant. However, eight of these nine miRNAs measured in this study were also identified as differentially expressed in other studies of obesity [251, 335, 336, 468, 469]. Lastly, due to differences in the dynapenia definition between the FAB and Muscle in Obesity studies, the external validation study is not a true validation study. However, there are strengths to this study. This is the first study to explore and attempt to validate the role of miRNAs in women with dynapenia or DAO using a systematic approach. Secondly, participants with impaired fasting glucose (normal weight) or metabolic syndrome (obese) were excluded from the FAB study to avoid confounding as these diseases exhibit their own individual miRNA profiles [494, 496]. MiRNAs which had low expression levels in all groups, and no biologically or statistically significant relevance, were filtered out of the

RNA-seq analysis to improve power [462]. Lastly, the relationships between validated miRNAs and components of DAO or other relevant surrogate measures were explored as continuous variables.

5.8 Conclusion

In conclusion, this is the first study to explore and attempt to validate the role of miRNAs in women with dynapenia or DAO. There were no significantly different miRNAs between women with DAO and obesity and although miRNAs were different between women with dynapenia and normal weight using RNA-seq, these could not be validated. This study raises several possible conclusions. Despite the increased risk of adverse outcomes, women with DAO or dynapenia may not have a distinct miRNA pattern. Alternatively, the effect of this phenotype (DAO or dynapenia) on miRNA expression is smaller than predicted and a much larger group is required to find an effect. Lastly, the relationship between miRNAs and strength, and perhaps obesity, should be considered on a scale rather than by dichotomous characterisation (i.e. presence or absence of the phenotype). Further work is required in external groups and men to confirm our findings and also using muscle samples to determine whether differences exist at the site of interest.

Chapter 6

Discussion

6.1 Overview

This thesis sought to identify whether obesity and dynapenia (dynapenic abdominal obesity) interact and have cumulative effects on falls and fall-related injury/fractures. Subsequently, this thesis sought to identify opportunities for intervention by attempting to elucidate both potential mechanisms for the increased falls risk observed (muscle function and size) and the pathogenesis (microRNAs) underlying DAO and thus offer insight into novel treatment strategies.

Together, the studies included in this thesis are unique in their multi-disciplinary approach to study the phenotype of women with DAO. The UK Biobank and ELSA studies were the first large cohort studies to examine falls, aBMD, and fractures in older adults with DAO (Chapter 2). The Muscle in DAO study was the first detailed phenotyping study of DAO using gold-standard techniques (e.g. MRI, isokinetic dynamometry; Chapter 3). The systematic review was the first study to examine and propose a role for miRNAs in the pathogenesis of sarcopenic obesity/DAO (Chapter 4). The studies presented in Chapter 5 were the first to measure, compare, and validate the miRNA profiles of older women with DAO using two techniques and in two cohorts.

6.1.1 Falls and Fractures in DAO: UK Biobank and English Longitudinal Study of Ageing (ELSA)

The UK Biobank and ELSA studies (Chapter 2) were the first studies to examine fall-related injuries in older adults with DAO by sex, acknowledging the site-specific fracture risk in obesity. By studying the association between DAO and fractures grouped by anatomical site (obese-protective or prone), it was possible to identify whether dynapenia modified these relationships. This thesis further sought to understand the mechanism of this increased fracture risk. In doing so this was also the first study to report aBMD of older women with DAO and to examine falls risk in people with DAO by sex – previously only an increased prevalence had been reported [87, 89, 91]. Since commencement, one other study has studied the 14-year prospective risk of DAO on falls – but not according to sex [108]. Studying the effects of DAO in males and females separately allowed for the consideration of biological differences (e.g. fat distribution, strength differences).

We found that older women with DAO have a higher risk of retrospective falls but aBMD was similar when compared to those with obesity (UK Biobank). Older women with DAO have a higher risk of lower extremity fractures compared to women with obesity and normal strength. Using BMI as a measure of overall obesity instead of waist circumference in the definition of DAO, we were also able to extend this finding to all other fracture types. By acknowledging the specific fracture pattern of

people with obesity, we identified that dynapenia exacerbated the risk of lower extremity fractures but counteracted the protective effects of other fractures in those with abdominal obesity and overall obesity. We hypothesised that the increased fracture risk observed may relate to the increased risk of falls as aBMD was similar.

We attempted to confirm our findings in a smaller, representative cohort of older adults in England (ELSA). However, our findings in ELSA showed that obesity as either increased waist circumference or BMI was not prospectively associated with falls in women. Furthermore, DAO performed poorly at an individual level to identify fallers indicating limited clinical utility. We also found no association between DAO and fall-related injury in ELSA, which may relate to the heterogenous nature of this category.

The limitations of these studies need to be acknowledged. The outcomes measured in the UK Biobank and ELSA were self-reported (history of falls and fall-related injury or fractures) and thus affected by recall bias. Comparison between these studies requires consideration of the differences in recall periods for the outcomes of interest (falls, injuries) and study design (retrospective and prospective). Whilst ELSA is representative of the English population, the UK Biobank is prone to healthy volunteer bias, limited ethnic diversity, low response rate, and lower prevalence of overweight/obesity, and therefore generalisability may be a concern. The UK Biobank study was a retrospective study and inferences drawn require validation in prospective studies. Furthermore, simple regression models were used for clinical applicability and the effects of co-morbidities and other confounding factors (e.g. cognition, frailty, nutrition, and physical activity) require further consideration. We were unable to validate the occurrence of fractures as International Classification of Diseases (ICD) codes were only available for those who were admitted to hospital; moreover, the context and timeframe of those fractures (ICD codes) could not be determined limiting their utility. Therefore, it is possible that participants may have misclassified or incorrectly reported their (self-reported) fracture sites. The UK Biobank analysis could also not be replicated in men due to the low prevalence of fractures reported. However, the UK Biobank study has strengths including its large sample size, detailed phenotyping and there is evidence suggesting that risk factor associations may be generalisable [205].

6.1.2 Muscle strength, fatigue and volume in DAO

DAO has primarily been studied from either an epidemiological perspective or small scale studies which have not used gold-standard techniques. The Muscle in DAO study was the first study to examine women with DAO using gold standard techniques (MRI, DXA, and isokinetic dynamometry)

which measure muscle function, size, and quality. Linkage of muscle volume from MRI and strength from isokinetic dynamometry is a novel approach, not used in people with this phenotype before, which provided insight to the determinants of muscle strength and the pathogenesis of DAO. This approach allowed us to speculate potential causes for the increased risk of falls and fractures observed as well as to determine whether the definition of DAO identifies a truly distinct phenotype.

Older women with DAO had lower knee extensor strength relative to body weight than women with obesity or normal weight. Considering strength output relative to the size of the quadriceps, women with obesity produced more force per unit muscle than those with normal weight. However, similar to the findings from the UK Biobank, this beneficial effect of obesity appeared to be lost in those with DAO. Furthermore, knee flexor force per unit muscle was also worse in women with DAO compared to those with obesity. As there was no difference in muscle volumes between women with obesity and DAO, these findings suggest that differences in muscle quality explain strength deficits in those with DAO. However, there were no other distinctive differences when older women with DAO were compared to those with obesity or normal weight. All groups had normal gait speed and exceeded the predicted lower limits of the 6-minute walking test distance.

There are limitations to the Muscle in DAO study. The study was designed to identify differences in the microRNA profile of women with DAO. The muscle function and composition measurements are secondary and exploratory outcomes and as such any findings and differences require confirmation in larger cohort studies. This work is also limited by study design changes related to COVID-19 (See Section 6.2 Impact of COVID-19). Further, we were unable to fill the dynapenic group due to recruitment delays (COVID-19) and thus cannot give a complete picture of the potential contributions of the individual phenotypes of dynapenia and obesity to muscle impairments in DAO. The study was designed for all participants to complete visits within six months – all participants completed the study in less time than this (approximately two-three months). Nevertheless, weight fluctuations were observed between visits and whilst differing weighing scales, clothes, time of day and fasting status may explain this variation, true weight change cannot be ruled out. Thus, the relationship between strength and body composition is limited by the potential impact of these weight changes. It is also unclear if the force/muscle volume reduction observed in those with DAO is clinically significant (e.g. relevant to falls), it is possible that other joints may compensate for the reduced strength observed. Lastly, the accelerometery (gait) data was unusable for the majority of participants and was subsequently omitted. Therefore, it is unclear if reduced gait speed in those with obesity and those with DAO results from similar impairments of gait.

6.1.3 MicroRNAs – Systematic Review

The systematic review was the first study to look at miRNAs in the context of sarcopenic obesity and DAO in order to elucidate the mechanisms underlying the pathogenesis of DAO. To date, miRNAs have been considered in either sarcopenia or obesity. Due to a lack of studies in sarcopenic obesity (or DAO), the approach taken in this review allowed us to systematically identify whether there was a plausible role for miRNAs in sarcopenic obesity based on evidence and to identify specific miRNAs which may be implicated. Since publication, only one review has mentioned that there may be a potential role for miRNAs in the pathogenesis sarcopenic obesity [497].

Using a systematic approach, 24 miRNAs were identified as commonly changed in the same direction in both obesity and sarcopenia. The miRNAs identified were found to have validated functions in protein homeostasis, mitochondrial dynamics, determination of muscle fibre type, insulin resistance, and adipogenesis, with potential implications in the pathogenesis of sarcopenic obesity/DAO. As the miRNA profiles of women with both phenotypes had not been reported in the literature, these findings were considered to be exploratory.

The heterogeneity and low quality of the studies identified in the systematic review must be considered. In some cases, matches were found between younger or female obese studies and older or predominantly male sarcopenia studies. As such, it is unclear how the interaction of age or sex has impacted our findings. Furthermore, due to limited available evidence, different sample types (exosomes, serum, plasma) and study characteristics (e.g. age, gender) were included and subsequently compared. Next, only miRNAs with a fold change > 1.5 or $p < 0.05$ were included; therefore, we may have missed studies which could have introduced bias. The commonly changed miRNAs identified are in need of external validation, and as such the results should be viewed as in need of further validation. Lastly, due to the large number of overlapping miRNAs, we chose to search for functions and targets in the context of obesity and sarcopenia and may have omitted other relevant findings.

6.1.4 MicroRNAs - Testing

Following on from the results of the systematic review, the FAB and Muscle in Obesity studies were the first studies to measure the miRNA panel of those with DAO and compare levels to those to women with normal weight, dynapenia, or obesity. Using RNA-seq and literature searches, a panel of microRNAs was identified for validation which may have been implicated in the pathogenesis of DAO.

We further attempted to validate these findings using a second measurement technique (RT-qPCR) and an external cohort.

There was no difference in the microRNA profile (RNA-seq) of women with DAO compared to those with normal weight, dynapenia or obesity. This was confirmed in two validation studies; within the same cohort using an alternative technique (RT-qPCR) and in a second cohort of women. We were unable to identify a suitable endogenous control for RT-qPCR. It may be that DAO does not have a sufficiently distinct miRNA signature. Alternatively, the difference between groups is smaller than expected and a larger sample size is required. A panel of 38 miRNAs were found which were differentially expressed between dynapenic and normal weight older women; however, these could not be confirmed in the validation studies.

The FAB miRNA study is limited by the small number of participants included and findings should only be considered as exploratory. We were unable to identify an endogenous control and therefore the difference in normalisation methods between the RNA-seq and RT-qPCR analyses should be considered a limitation. We used a systematic approach to identify a panel of miRNAs which may be implicated in dynapenia and DAO; however, expression levels were low for some selected miRNAs. Therefore, despite published evidence of effects which may relate to DAO, these miRNAs may not be biologically relevant. As there is a many-to-many relationship between miRNAs and mRNAs, we chose to identify validated functions of potential miRNAs from published literature rather than bioinformatic analysis. Whilst this approach has strengths, some functions were identified in cell and rodent-based research and it is possible that these roles may not be replicated in humans. We attempted to validate these miRNA findings in an external cohort; however, due to Covid-19, changes were required to the study design whereby we used a different definition of dynapenia thus this external study cannot be considered a true validation. In an attempt to overcome this, we examined the relationship between these miRNAs and hand-grip strength (the original definition of dynapenia) and found no relationship. Furthermore, due to Covid-19 related delays we failed to recruit the full sample size for the DAO group and thus these findings should also be considered exploratory.

6.2 Impact of COVID-19

The COVID-19 pandemic primarily affected the clinical study (Chapter 3). Recruitment was delayed by 12-18 months which meant that there was not sufficient time to achieve full recruitment. Recruitment was also affected by the limited opportunity for public engagement events due to the effect of Covid-19 (e.g. social groups meeting online rather than in person). Additionally, changes to the study design

were required. Firstly, we chose to use sit-to-stand to identify dynapenia rather than hand-grip strength – this was due to the ability to complete this measurement over the phone and therefore reduce the transmission risk of COVID-19. Both methods are deemed to be interchangeable based on the EWGSOP2 consensus for low muscle strength [4]. Secondly, we originally aimed to determine the miRNA profile in the muscle (*vastus lateralis*) for a more direct understanding of how miRNAs relate to muscle in obesity. However, due to the increased demand on the local Clinical Research Facility (CRF) for COVID-19 related research, we opted to measure the miRNA profile in serum as blood samples could be obtained safely in the available facilities. Thirdly, the corridor length for the 6MWT is recommended to be 30m in length; however, we could only use a 25m corridor due to CRF capacity related to COVID-19 research. Lastly, MRI scanning capacity for research was reduced at Sheffield Teaching Hospitals. Therefore, two different scanners were used for this study and the scan also could not be performed on the same day as the strength assessments potentially impacting the specific strength calculations.

6.3 Future Work

Epidemiological evidence has shown that consideration of both body weight and muscle strength is relevant for determining and stratifying fall and fracture risk. Clinically, interventions targeting low muscle strength, either alone or with measures to reduce excess body weight, could reduce the risk of both falls and fractures in women. However, definitions of DAO do not perform well at an individual level to either identify risk or to characterise a specific phenotype using gold-standard techniques. Future work is required to determine whether a definition of DAO can be determined which can offer utility at an individual level in relation to fall and fracture risk identification. Currently, DAO is identified with clinically acceptable surrogate measurements of muscle strength and abdominal obesity. The diagnostic cut-offs for these surrogates have not been obtained from data on fallers, but rather from associations with other factors (e.g. waist circumference and metabolic health [303]) or arbitrary cut-offs (e.g. 2.5 standard deviations below peak adult hand-grip strength [3, 4]). Furthermore, a single cut-off of hand-grip strength or sit-to-stand time is recommended in consensus definitions of sarcopenia and sarcopenic obesity regardless of body weight [4, 76]. Such an approach may lead to mis-identification of dynapenia in people with higher body weight where requirements are different [125, 498]. Therefore, future work should aim to better characterise known fallers and people with fall-related fractures, with different body weights. The aim of this work would be to use gold-standard techniques to identify muscle function (dynapenia-related) and body composition (adiposity-related) characteristics, according to body weight and sex, which better relate to fallers and could be used in risk-identification tools.

Areas of future work which should be considered in studies characterising people who have fallen or had fractures are listed below. These areas have the potential to provide insight for both screening and intervention strategies.

- Linkage of objective, gold-standard measures of muscle strength and size (i.e. isokinetic dynamometry and MRI or computed tomography), relevant to postural control may offer potential for risk identification. For screening purposes, these measurements could be adapted to clinically acceptable surrogate techniques, for example, ultrasonography and knee strength measured using a hand-grip dynamometer.
- Multiple components of the inverted pendulum model (ankle, knee, and hip joint) and rate of torque development require consideration. There is evidence of joint torque and power redistribution with ageing [499] and whilst those with DAO appeared to show reduced specific knee extensor and flexor force in this thesis, other joints may compensate and thus be stronger.
- Whilst obesity and DAO appeared to have similar trends for slow gait speed, there may be more subtle differences which could be captured with accelerometery or more detailed gait analysis (e.g. force plate, pressure insoles). For example, a greater understanding of how gait measures known to associate with falls risk such as step length, step duration [500], and gait smoothness (index of harmonicity) [501] alter with muscle fatigue is required. These measurements could identify characteristics for screening purposes and also alternative interventional targets (e.g. minimal footwear [229]). In clinical practice, wearable tri-axial accelerometers could be used to measure these characteristics.
- Work should also aim to determine if, and to what extent, factors such as voluntary and actual central activation (e.g. electromyography), fat infiltration (e.g. computed tomography, MRI) and differences in muscle fibres (e.g. muscle biopsy) are implicated in strength output. A greater understanding of the determinants of strength in older adults with particular focus on neuromuscular function is required. Understanding the pathophysiology of reduced specific strength may offer insight into the utility of alternative treatment strategies for example targeting the nervous system (e.g. whole body vibration therapy [230]) or fat infiltration.
- The microRNA profile of muscle (*vastus lateralis*) in fallers should be measured to better determine if there is tissue specific effect. A greater understanding of the pathogenesis underlying dynapenia in people with obesity who have fallen may provide insight to novel therapeutic options.

More robust methods to capture fall incidence and their circumstances (e.g. monthly recall postcards, phone calls) are required and should be considered in future epidemiological studies. For example, certain fall-related circumstances may not raise clinical concern; however, such information is missing from epidemiological studies and the person would be classified as a faller. As such, this may contribute to the poor performance of DAO measurements at an individual level.

Due to the multi-factorial nature of falls, future work should consider whether aspects of dynapenia and abdominal obesity in previously validated falls and fracture screening tools (e.g. FRAX [16], Tinetti Performance Oriented Mobility Assessment [502]) improves risk identification.

Lastly, future work is required to confirm the findings presented in this thesis in men and people from different ethnic backgrounds. Specifically, work should aim to determine whether DAO is associated with an increased risk of fractures in men. Due to the low prevalence of fractures in men in the UK Biobank, it was not possible for us to determine whether the relationship between DAO and fractures was also present.

Chapter 7

Conclusion

Conclusion

Epidemiological evidence has shown that DAO is associated with an increased risk of falls in men and women. Compared to women with obesity, risk is higher in those with DAO for lower extremity fractures but similar for all other fractures. An obese BMI has a protective effect on all other fractures but this benefit is negated by the presence of dynapenia. Therefore, consideration of both body weight and muscle strength are relevant for determining and stratifying fall and fracture risk. However, current definitions of DAO do not combine with a distinct pathophysiology at an individual level. As currently defined, DAO describes a heterogeneous syndrome as there was overlap in gait speed, six minute walking distance, and hand-grip strength compared to obese and normal weight phenotypes. Furthermore, when considering the miRNA profile of women with DAO, despite published evidence of microRNAs being implicated in both sarcopenic and obese phenotypes, we did not identify a DAO-specific profile. Some specific features of muscle function were found which may be a useful focus to understand people at risk of falling. Women with obesity had greater knee extensor force per unit muscle than those with normal weight but this beneficial effect was lost in women with DAO. Knee flexor force per unit muscle was also lower in women with DAO compared to obese or normal weight women. The reduced muscle force observed may relate to differences in muscle quality as muscle volumes were similar between women with obesity and DAO. The finding of similar muscle volumes between groups despite different functional capacity lends further support to the need to consider and prioritise muscle function measurements in people with obesity. Thus, DAO may be a meaningful concept in the identification of people at risk of falls and fractures. However, more work is needed to understand the pathophysiology of fallers of different body weights (specifically relating to muscle function and body composition characteristics) to enable definitions of DAO that work at an individual level for case finding.

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Appendices

Appendix 1 MRI Protocol Parameters provided by Sheffield Teaching Hospitals

The Magnetic Resonance Imaging (MRI) protocol described in Chapter 3 was designed by a radiologist at Sheffield Teaching Hospitals.

Hips:
3mm Slice
Repetition Time :7.64
Echo Time 1: (2.59) - Echo Time 2: (4.79)
1 average
Matrix 416 x 416
Flip:10
Acceleration factor: 2
Bandwidth 1: (300) 2: (315)

Long Bones:
5mm Slice
Repetition Time :7.76
Echo Time 1: (2.59) Echo Time 2: (4.82)
2 averages
Matrix 416 x 416
Flip:10
Acceleration factor: 2
Bandwidth 1: (300) 2: (310)

Appendix 2 MATLAB Script used to analyse isokinetic dynamometry tests

```
%% pksmax and pksmin are the values you are looking for after running the full code

%% Reading of data
% This block will read in the data, so change the name between ' ' to the file you want to read in
clear all
close all

data=readtable('NAME.txt');

%% Cutting of the data
%This will plot the data, a cross will come up the screen and you need to
%click twice making sure the data you want to keep falls between the two
%points you just clicked. If you want to keep the beginning, just click as
%close to zero as you can

figure
%2 is column number on excel
torque= table2array(data(:,2));
plot(torque);
[loc, num]=ginput(2);
torque(loc(2):end)=[];
torque(1:loc(1))=[];

%% Finding the peaks
%You will need to set the distance you expect between the peaks, you can
%see this in the figure that just appeared at the previous step. You
%further need to set the minimum peak height (seems to differ for the
%participants). With the minimum peak height you need to be careful in
%whether the data is positive of negative to start with (when positive you
%will need to add a '-' minus sign to the data. When the data is negative
%to start with the number should be positive.
PeakDistance=90;
%what the minimum peak height should be - may need to change depending on
```

```
%person
PeakHeigthMax=20;
%same as height max
PeakHeigthMin=-15;

figure
plot(torque)
[pksmax,locmax]=findpeaks(torque,'MinPeakDistance',PeakDistance,'MinPeakHeight',PeakHeigthMa
x);
hold on
plot(locmax,pksmax,'r*')
[pksmin,locmin]=findpeaks(-
torque,'MinPeakDistance',PeakDistance,'MinPeakHeight',PeakHeigthMin);
pksmin=-pksmin;
hold on
plot(locmin,pksmin,'g*')
%%

meanpeak=mean(pksmax)
maxpeak=(max(pksmax))
stdpeak=std(pksmax)

meanpeakmin=mean(pksmin)
maxpeakmin=(min(pksmin))
stdpeakmin=std(pksmin)
```

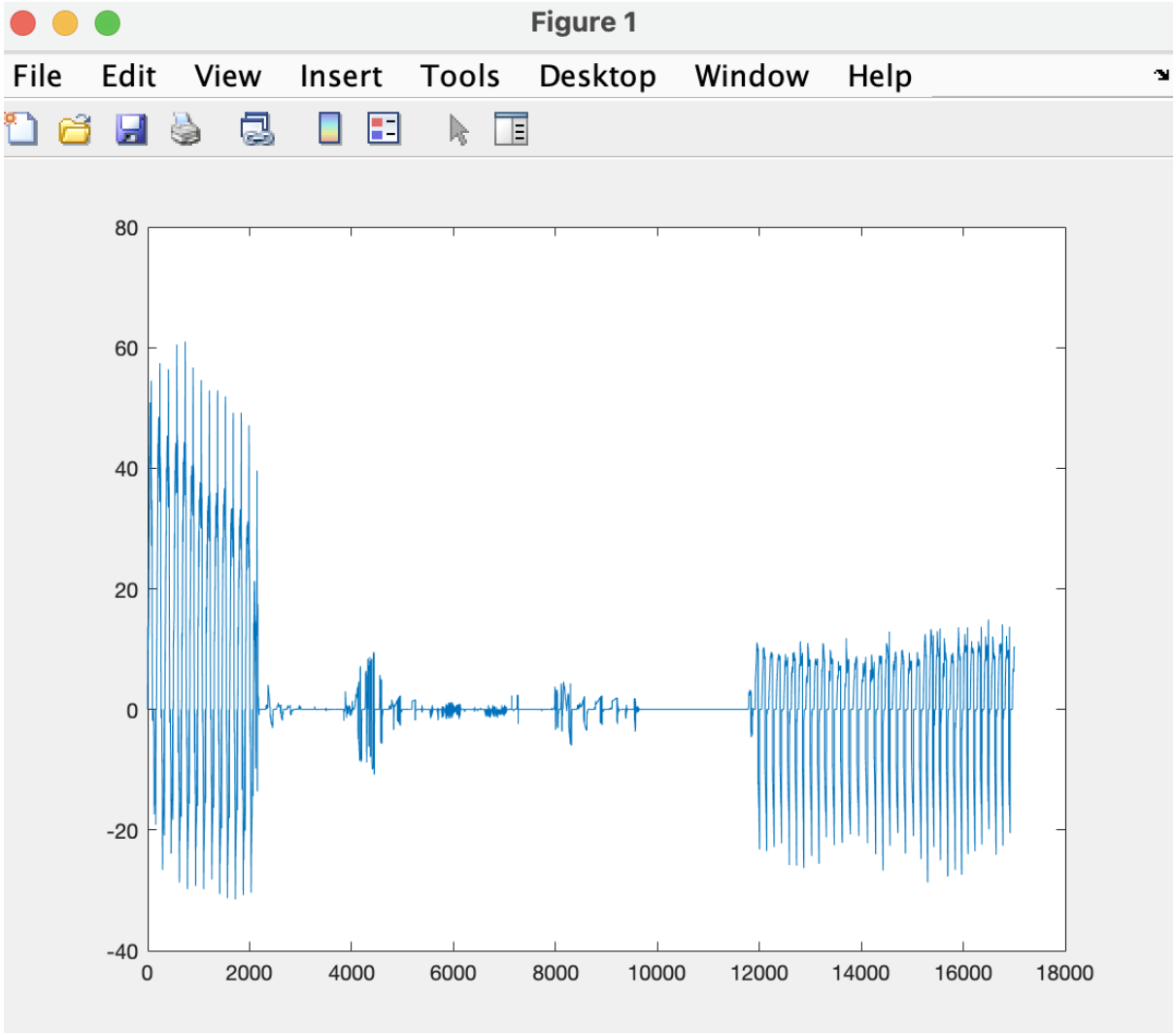


Figure A: Screenshot example of peaks produced during isokinetic fatigue test in Matlab

The horizontal axis refers to time in milliseconds (ms); the vertical axis refers to torque (Nm). The isokinetic dynamometer was programmed so that 50 repetitions needed to be completed before the test could end. The peaks between 0-2000 ms were produced by the participant; the peaks at the end were completed by the investigator and not used in the analysis. The participant's peaks were cut for further analysis as described in the script.

Appendix 3 Assays and reagents used for Muscle in Obesity clinical chemistry and miRNA analyses

RT-qPCR
RNA Spike-In Kit: Cat: 339390
https://www.qiagen.com/de-at/search/products/?query=339390

miRCURY LNA RT Kit: Cat: 339340
https://www.qiagen.com/de-at/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/mirna-qpcr-assay-and-panels/mircury-lna-rt-kit/?catno=339340

miRCURY LNA SYBR® Green PCR Kit (Master Mix) Cat: 339347
https://www.qiagen.com/de-at/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/mirna-qpcr-assay-and-panels/mircury-lna-sybr-green-pcr-kits/?catno=339347

miRNA Primer: miRCURY LNA miRNA PCR Assay
https://geneglobe.qiagen.com/at/product-groups/mircury-lna-mirna-pcr-assays

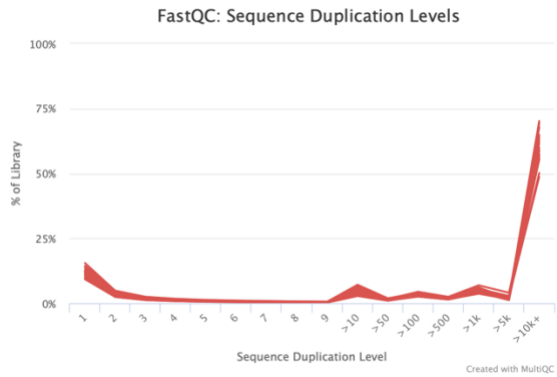
MiRNA	MiRBase accession and mature miRNA sequence
hsa-miR-107	MIMAT0000104: 5'AGCAGCAUUGUACAGGGCUAUCA
hsa-miR-127-3p	MIMAT0000446: 5'UCGGAUCCGUCUGAGCUUGGCU
hsa-miR-16-5p	MIMAT0000069: 5'UAGCAGCACGUAAAUAUUGGCG
hsa-miR-199a-5p	MIMAT0000231: 5'CCCAGUGUUCAGACUACCUGUUC
hsa-miR-376c-3p	MIMAT0000720: 5'AACAUAGAGGAAAUUCCACGU
hsa-miR-382-5p	MIMAT0000737: 5'GAAGUUGUUCGUGGUGGAUUCG
hsa-miR-486-5p	MIMAT0002177: UCCUGUACUGAGCUGCCCCGAG
hsa-miR-543	MIMAT0004954: 5'AAACAUUCGCGGUGCACUUCUU
hsa-miR-92a-3p	MIMAT0000092: 5'UAUUGCACUUGUCCCGGCCUGU

Muscle in Obesity Study
C-reactive Protein – CRP4 Tina-quant C-Reactive Protein IV (Roche)

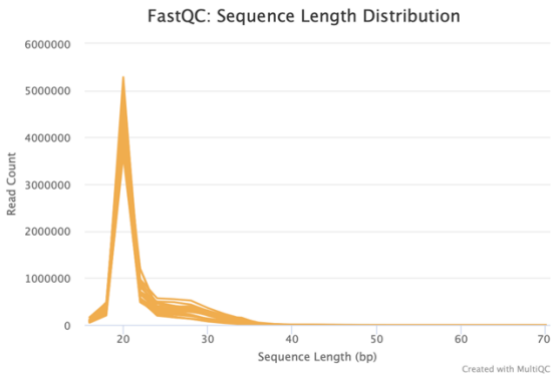
Glucose - Glucose HK Gen. 3 (Roche)

Lipid Profile – Trigl Triglycerides (Roche), HDLC4 HDL-Cholesterol Gen.4 (Roche), CHOL2 Cholesterol Gen.2 (Roche)

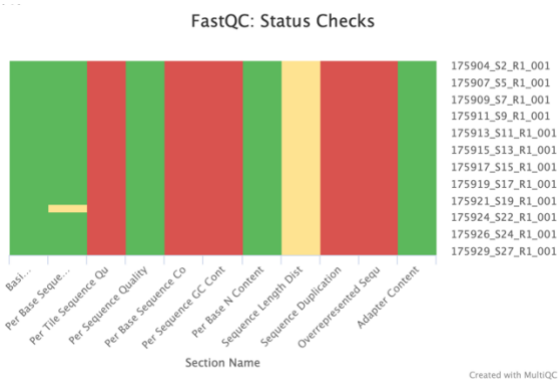
Appendix 4 Summary of RNA-seq quality checks



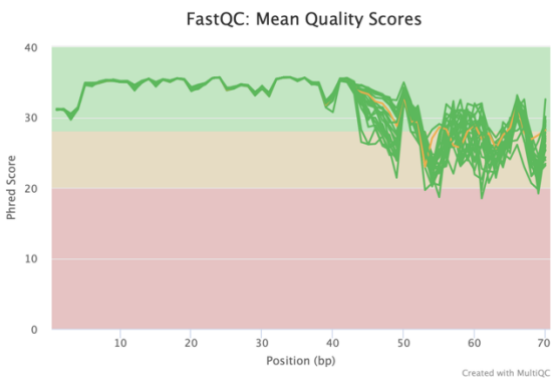
(a) Fast QC Sequence Duplication levels



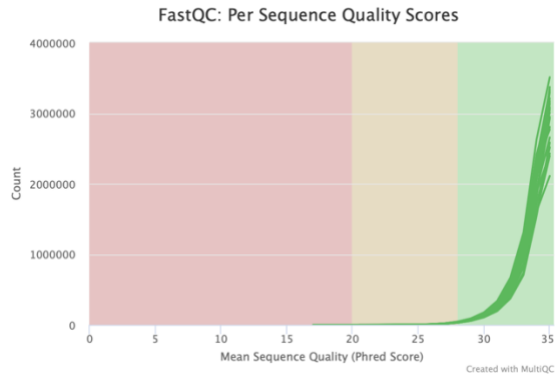
(b) Fast QC Sequence Length Distribution



(c) FastQC: Status Check



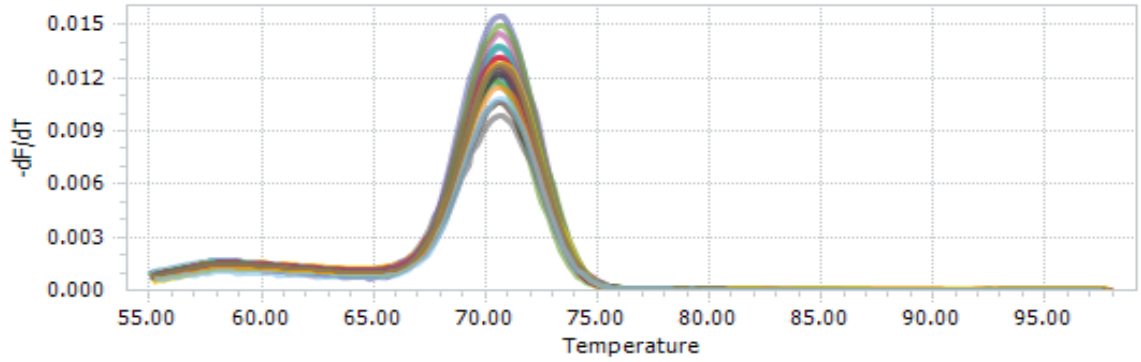
(d) FastQC: Mean Quality Scores



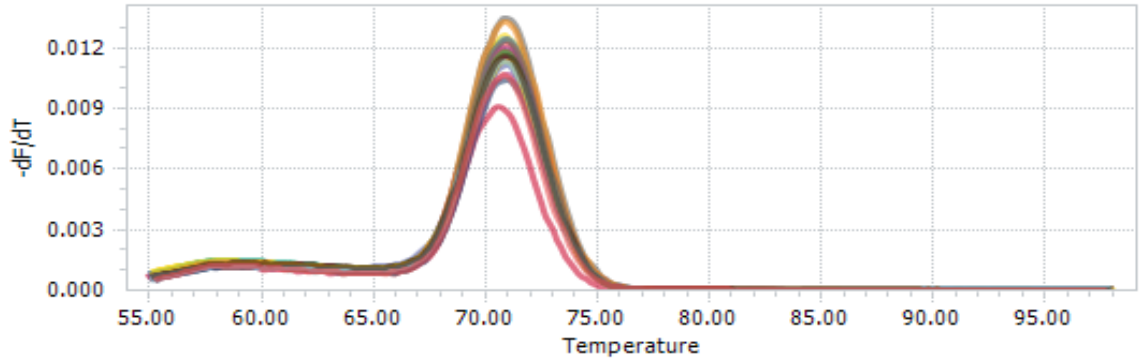
(e) FastQC: Per Sequence Quality Scores

Most reads have a high mean quality score (>30) until 30-35bp (d) and per sequence quality score (e).

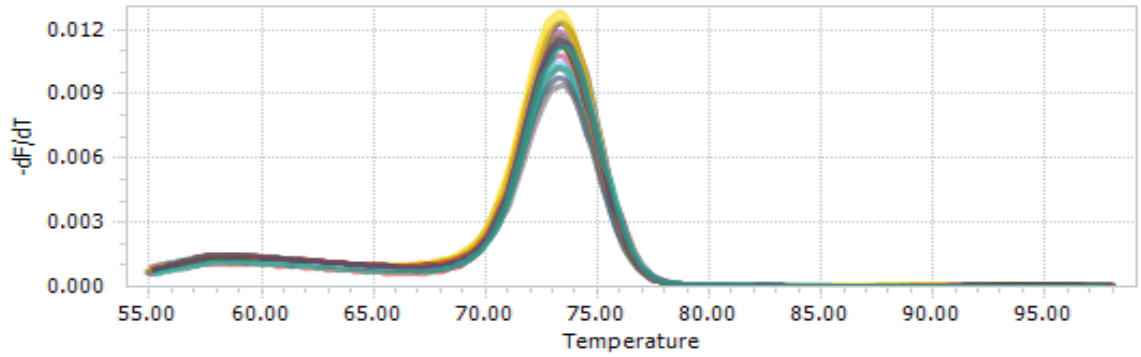
Appendix 5 Melt curves for RT-qPCR for Fat and Bone cohort



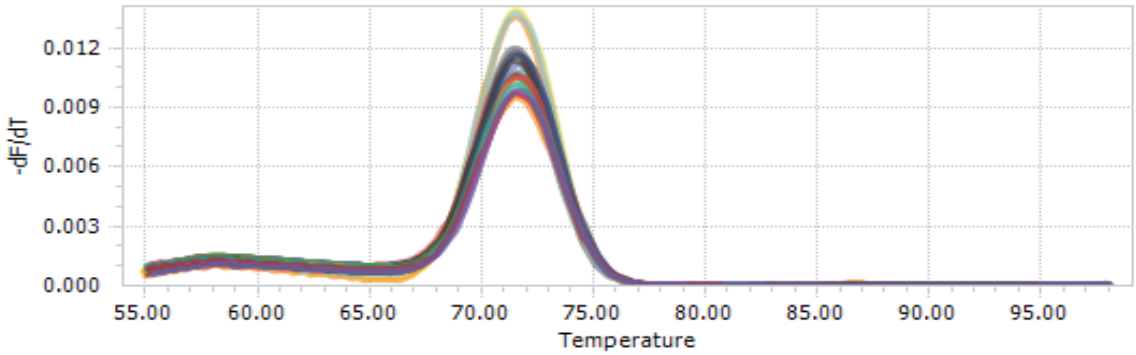
(a) Cel-miR-39-3p



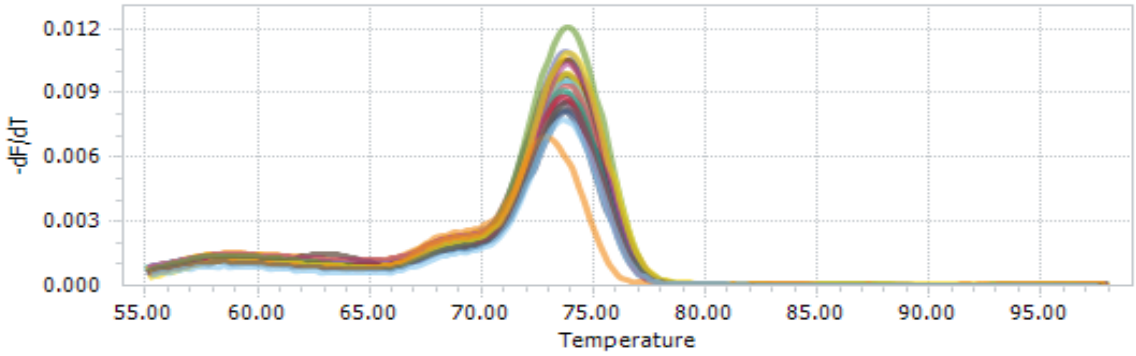
(b) UniSp4



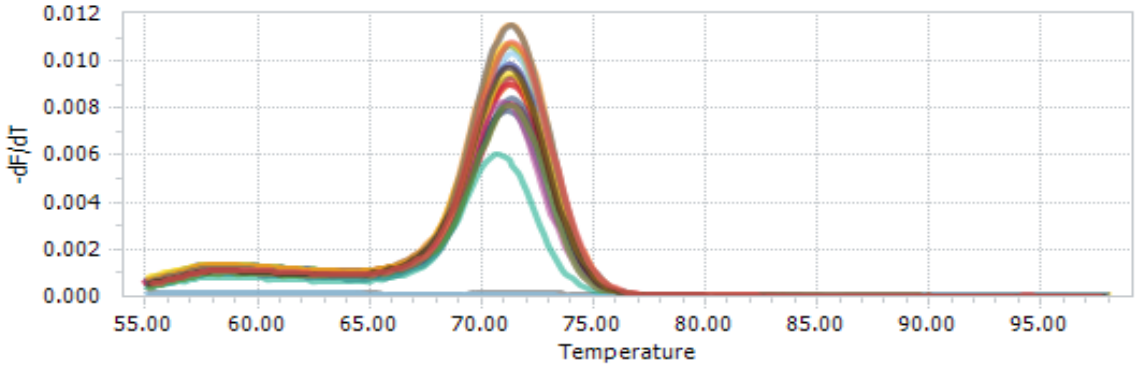
(c) MiR-92a-3p



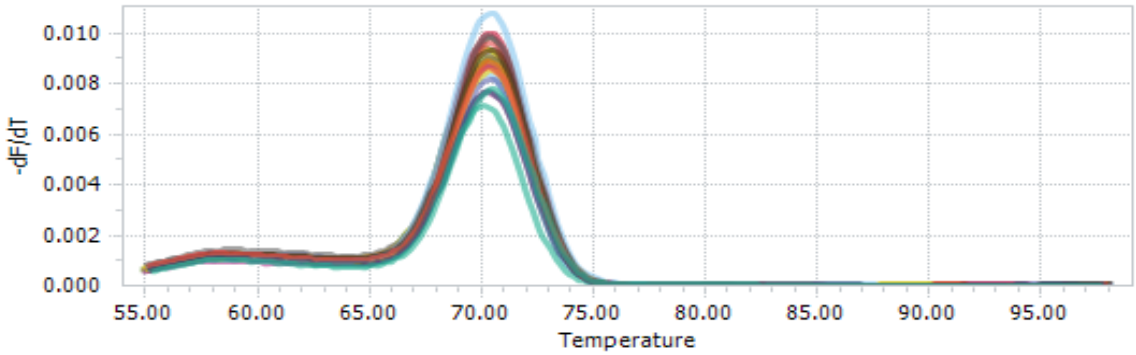
(d) miR-16-5p



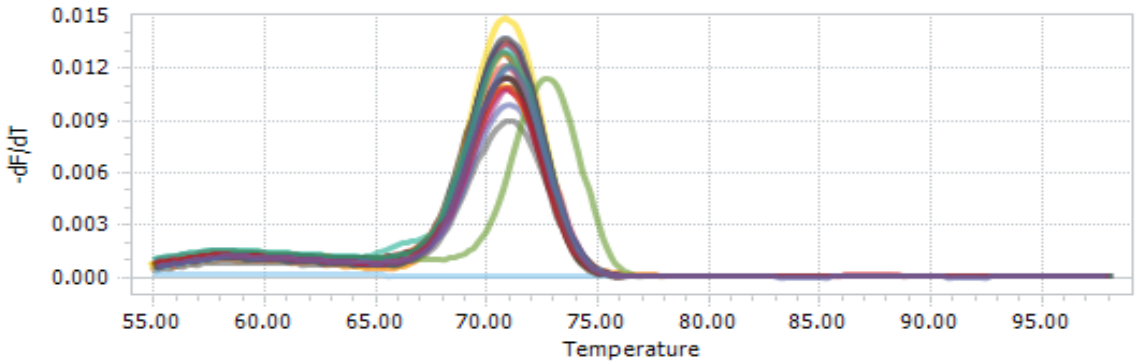
(e) miR-486-5p



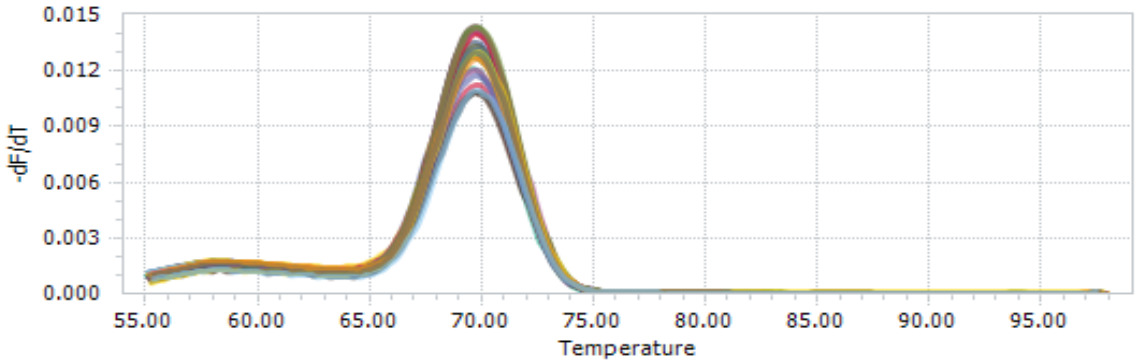
(f) miR-127-3p



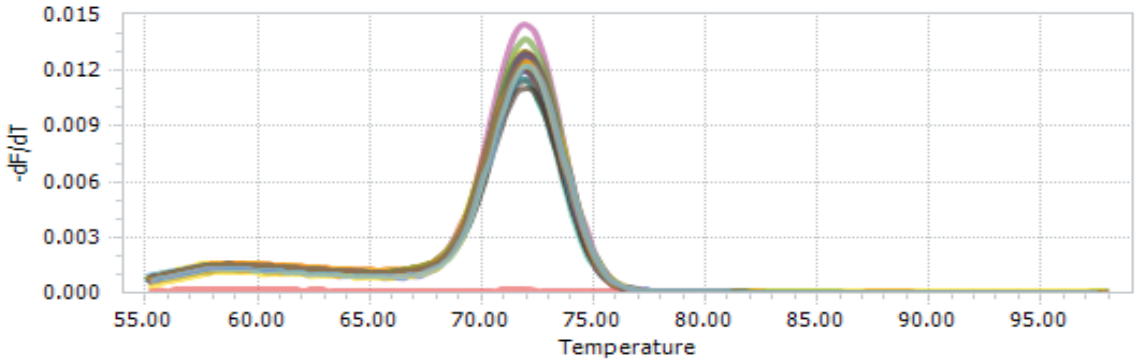
(g) miR-107



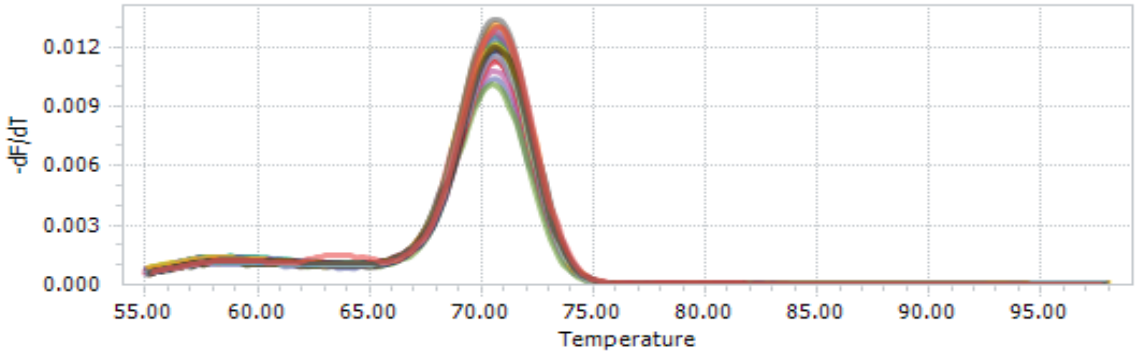
(h) miR-543



(i) miR-199a-5p



(j) miR-382-5p



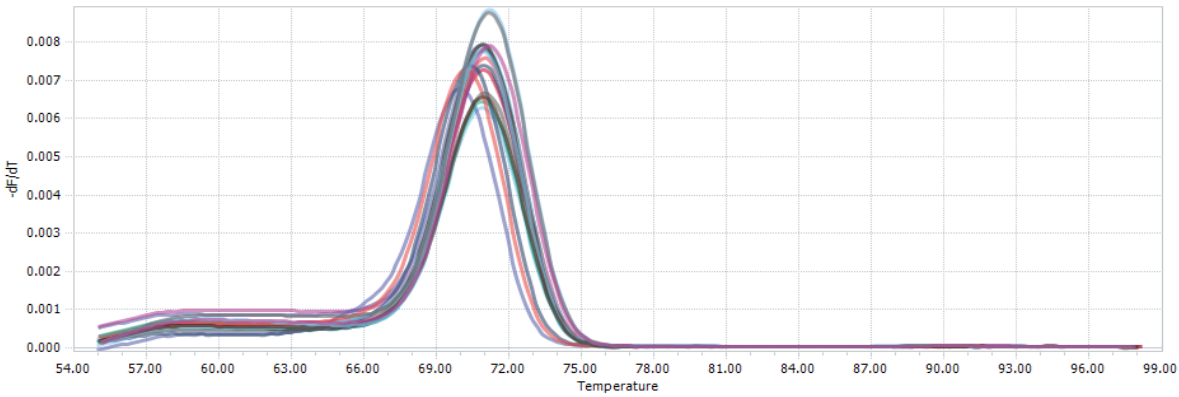
(k) miR-376c

Appendix 6 Comparison of Log2FC results from RT-qPCR and RNA-seq – Fat and Bone Study

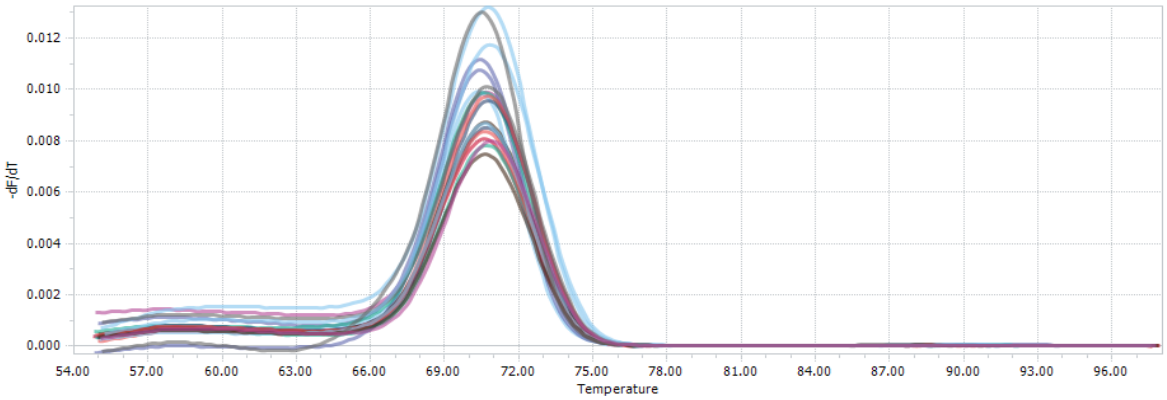
	miR-486-5p	**miR-127-3p**	**miR-92a-3p**	**miR-199a-5p**	**miR-543**	**miR-107**	**miR-376c-3p**	**miR-382-5p**	**miR-16-5p**
RT-qPCR									
D vs NW	-0.321	0.075	-0.233	0.069	0.288	0.006	0.367	0.287	-0.120
DAO vs Ob	-0.621	-0.386	-0.091	-0.248	-0.341	-0.157	-0.475	-0.468	-0.190
Ob vs NW	-0.044	0.208	-0.306	0.143	0.397	0.392	0.510	0.563	-0.106
RNA-seq									
D vs NW	0.920	-1.435	0.984	-1.058	-1.732	0.755	-1.462	-1.373	0.724
DAO vs Ob	0.107	0.206	-0.023	0.134	-0.607	-0.045	-0.409	-0.238	-0.179
Ob vs NW	0.198	-0.645	0.211	-0.688	-0.262	0.395	-0.433	-0.596	0.390

Abbreviations: RT-qPCR = reverse transcription quantitative real-time polymerase chain reaction; D = dynapenic; NW = normal weight; DAO = dynapenic abdominal obese; Ob = obese.

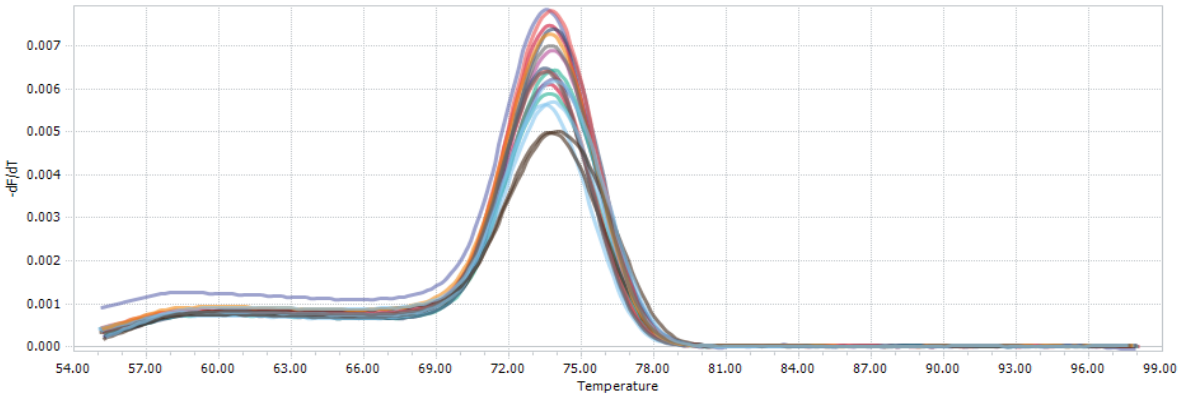
Appendix 7 Melt curves for RT-qPCR for Muscle in Obesity



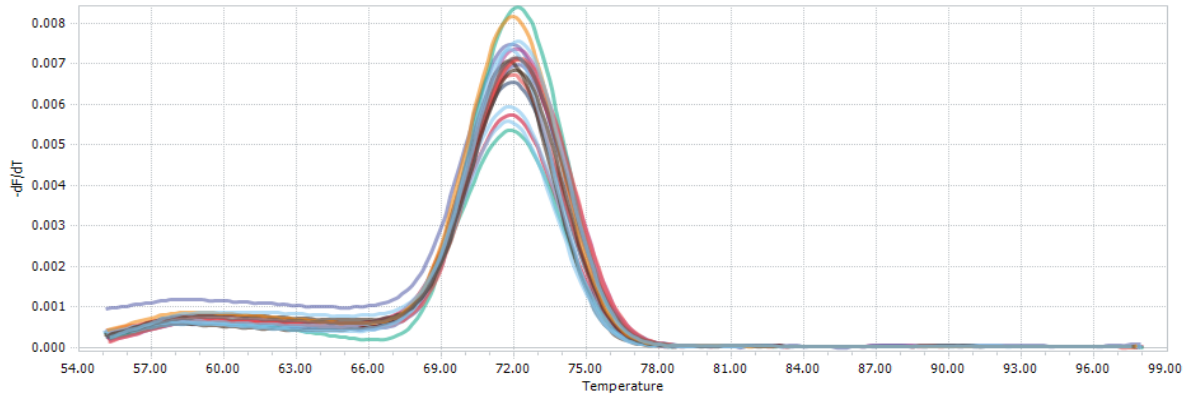
(a) Cel-miR-39-3p



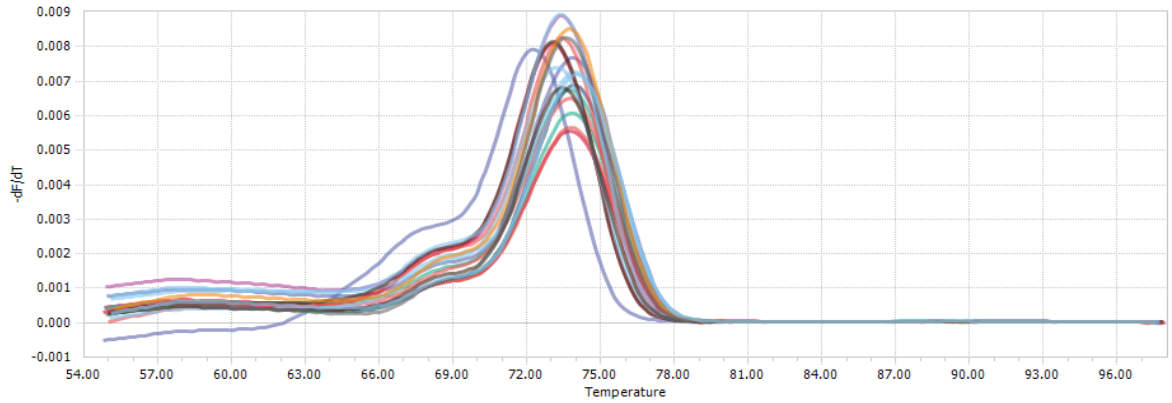
(b) UniSp4



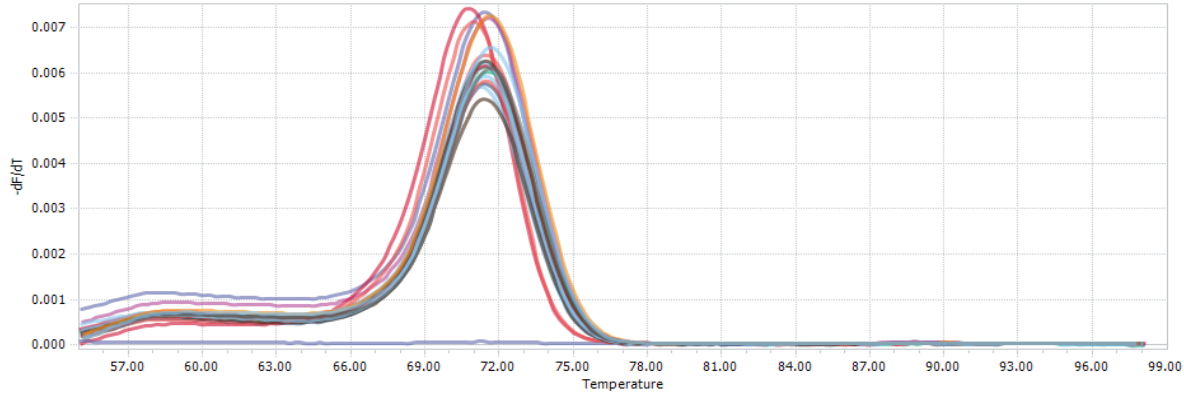
(c) MiR-92a-3p



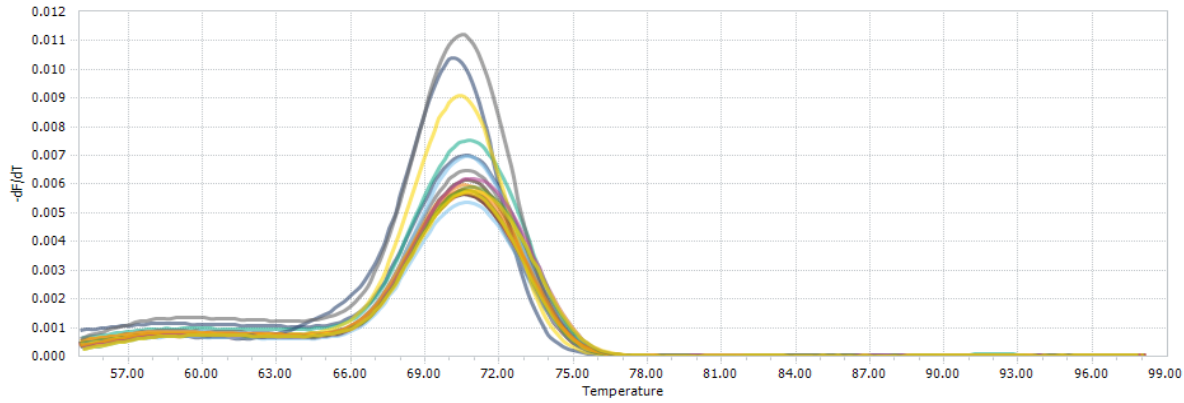
(d) miR-16-5p



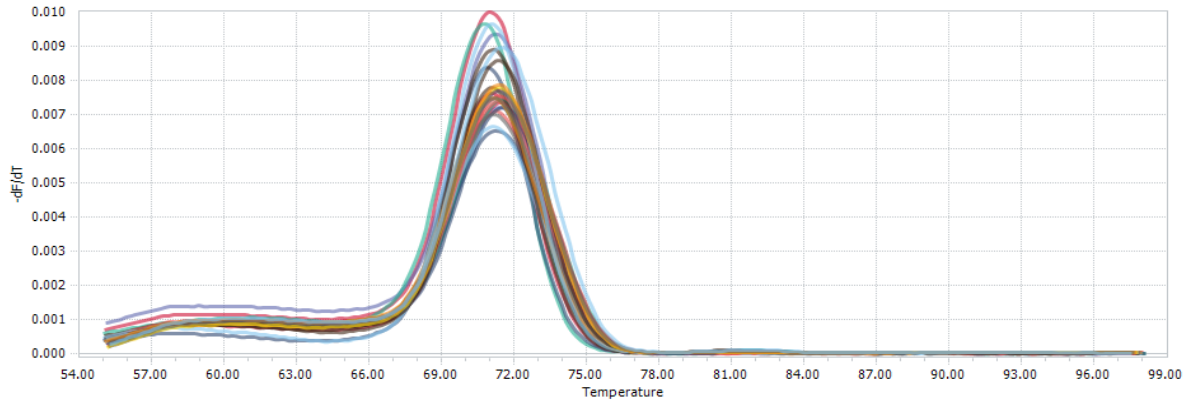
(e) miR-486-5p



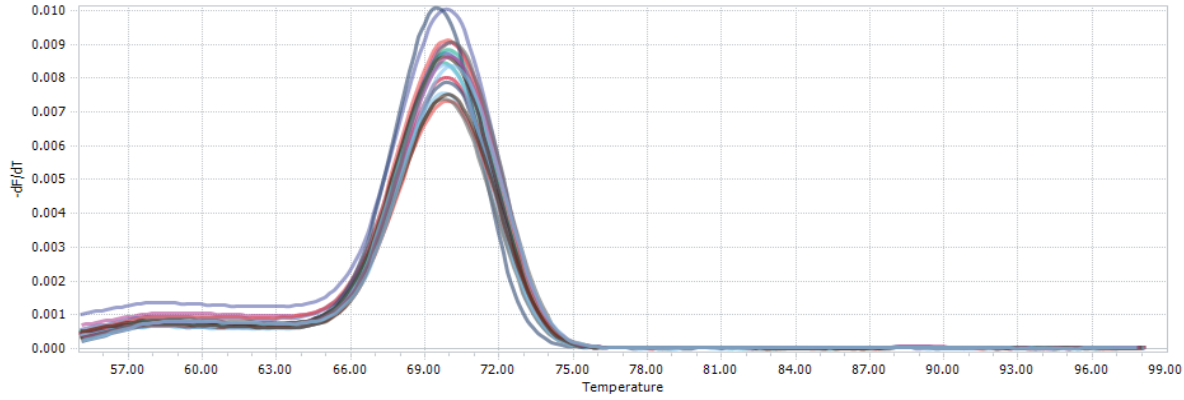
(f) miR-127-3p



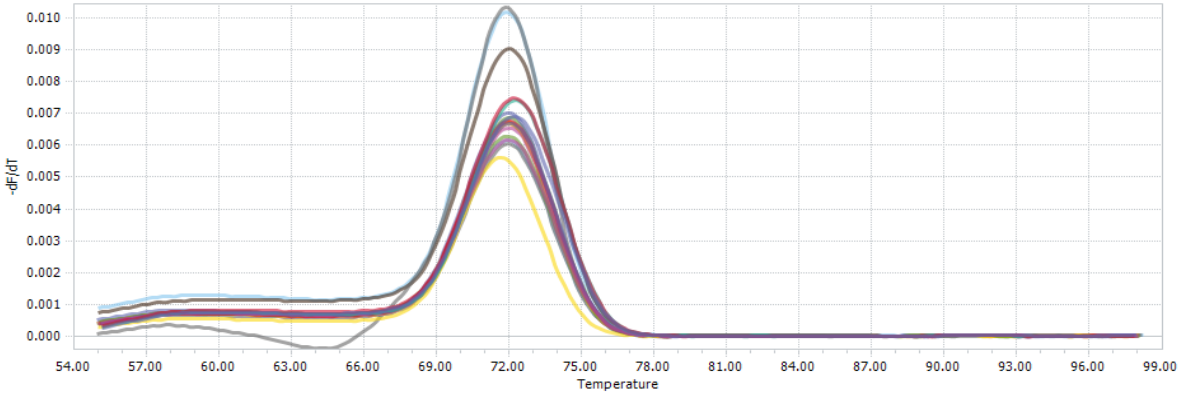
(g) miR-107



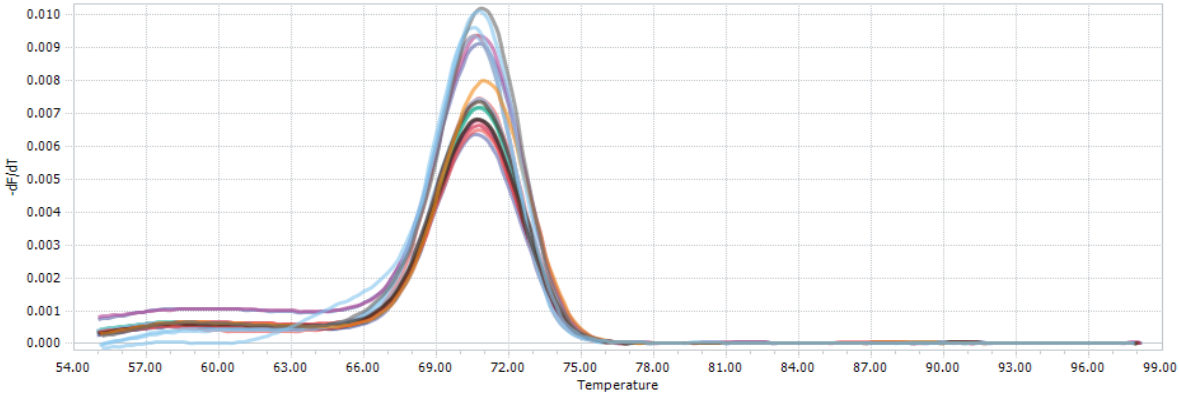
(h) miR-543



(i) miR-199a-5p



(j) miR-382-5p



(k) miR-376c-3p