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Fracture risk assessment in people living with HIV infection

By:

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Abstract

Background

People living with HIV (PLWH) are at higher risk of reduced bone mineral density (BMD) and fragility fracture compared to the general population. Possible causes include: an increased prevalence of general fracture risk factors (GFRFs) in PLWH; a direct effect of HIV; and a contributory role of antiretroviral therapy.

HIV guidelines recommend FRAX® (www.sheffield.ac.uk/FRAX) for fracture risk assessment in PLWH. FRAX®, however, incorporates GFRFs but not HIV disease-specific factors.

Hypotheses

1. Both HIV disease-specific factors and GFRFs contribute to reduced BMD and fracture risk in PLWH.
2. FRAX® correlates poorly with BMD in PLWH.

Methods

Phase One: The prevalence of GFRFs and fractures were recorded in PLWH. FRAX® 10-year osteoporotic fracture probabilities (FRAX® scores) were calculated.

Phase Two: A subset of the Phase One cohort were recruited proportionately by race, gender and FRAX® scores (low, intermediate and high) for dual-energy X-ray absorptiometry BMD measurements, vertebral fracture risk assessment and blood and urine sampling for biochemical and immunological markers. T-cell and monocyte subsets were assessed using flow cytometry.

Results

Phase One (n = 625): GFRFs were prevalent, but FRAX® scores and fragility fracture prevalence were low.

Phase Two (n = 114): FRAX®-incorporated GFRFs and increased cumulative

protease inhibitor exposure (but no other HIV disease-specific factor) were significant independent determinants of reduced BMD. Non-classical monocytes were also associated with reduced BMD. There was a significant negative correlation between FRAX® scores and BMD in black patients (p=0.003 for lumbar spine and total hip) and between FRAX® hip scores and total hip BMD in white patients (p=0.030). Total hip BMD differed significantly between patients with low FRAX® hip scores (0.999 ± .113 g cm^{-2}) and high FRAX® hip scores (0.882 ± .136g cm^{-2}) (p<0.001).

Conclusions

FRAX®-incorporated GFRFs were the predominant determinants of reduced BMD. FRAX® correlated well with BMD and may be of value for fracture risk assessment in specific HIV-positive patient subgroups.

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25-OH-D	25-hydroxyvitamin D
AIDS	acquired immune deficiency syndrome
ALP	alkaline phosphatase
ART	antiretroviral therapy
ARV	antiretroviral
AZT	zidovudine
β-hCG	β-human chorionic gonadotrophin
BHIVA	British HIV Association
BMD	bone mineral density
BMI	body mass index
BSA	bovine serum albumin
CD4 cell count	$CD3^+/CD4^+$ cell count
CD4 %	$CD3^+/CD4^+$ cell percentage of all $CD3^+$ cells
CD4:CD8	$CD3^+/CD4^+$ cell to $CD3^+/CD8^+$ cell ratio
CD8 cell count	$CD3^+/CD8^+$ cell count
CD8 %	$CD3^+/CD8^+$ cell percentage of all $CD3^+$ cells
COPD	chronic obstructive pulmonary disease
CTx	C-terminal telopeptide of collagen type 1
DF	disoproxil fumarate
DXA	dual-energy X-ray absorptiometry
EACS	European AIDS Clinical Society
eGFR	estimated glomerular filtration rate
ERK	extracellular signal-regulated kinase
Gnas	guanine nucleotide-binding protein alpha stimulating
Got	glutamate oxaloacetate transaminase
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
hs-CRP	highly sensitive CRP
IBD	inflammatory bowel disease
IKK	I kappa B kinase
IFN-γ	interferon-γ

IGF-1	insulin-like growth factor
IL-4	interleukin-4
IL-6	interleukin-6
IL-8	interleukin-8
INI	integrase inhibitor
IRIS	immune reconstitution inflammatory syndrome
JNK-1	c-Jun NH_2-terminal kinase-1
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
M-CSF	macrophage colony stimulating factor
MIP-1	macrophage inflammation protein-1
MSM	men who have sex with men
NICM	non-infectious co-morbidity
NFATc1	nuclear factor of activated T-cells, cytoplasmic 1
NF-κB	nuclear factor kappa B
NNRTI	non-nucleoside reverse transcriptase inhibitor
NOGG	National Orthopaedic Guideline Group
NRTI	nucleoside/nucleotide reverse transcriptase inhibitor
OPG	osteoprotegerin
P1NP	procollagen type 1 N-terminal propeptide
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCP	pneumocystis jiroveci pneumonia
PCR	polymerase chain reaction
PI	protease inhibitor
PI-3K	phosphoinositide-3-kinase
PLWH	people living with HIV
PPI	proton pump inhibitor
PRTD	proximal renal tubular dysfunction
PTH	parathyroid hormone
RANK	receptor of activated NF-κB
RANKL	receptor of activated NF-κB ligand
ROS	reactive oxygen species
RUNX-2	runt-related transcription factor-2

s.d.	standard deviation
Snord 32A	small nucleolar RNA C/D box 32A
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STH	Sheffield Teaching Hospitals NHS Foundation Trust
TAF	tenofovir alafenamide
TIMP-3	tissue inhibitor of metalloproteinase-3
TNF-α	tumour necrosis factor-α
TRAIL	TNF-related apoptosis-inducing ligand
TRAF	TNF receptor associated factor
TRAP	tartrate-resistant acid phosphatase
TRP	tubular reabsorption of phosphate
uRBP	urinary retinal binding protein
VFA	vertebral fracture risk assessment
VpR	viral protein R

List of publications and presentations

Publications

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1. Introduction

There have been dramatic advances in the management of HIV infection over the past few decades, such that the long term outlook for a person diagnosed with HIV today differs significantly to that of a person diagnosed with HIV in the 1980s or early 1990s.

In the 1980s, in the absence of any effective treatment, a new diagnosis of HIV infection was, for the majority of patients, a terminal diagnosis. Patients almost inevitably developed severe immunodeficiency – Acquired Immune Deficiency Syndrome (AIDS) – and ultimately died from opportunistic infections, such as pneumocystic jirvocei pneumonia (PCP), or from AIDS-associated malignancies, such as Kaposi's sarcoma.

Over the past few decades, mortality in people living with HIV (PLWH) has, however, declined dramatically (UNAIDS/WHO 2017). This was initially as a result of improved prophylaxis and treatment of opportunistic infections, but was considerably accelerated with the introduction of effective combination antiretroviral therapy (ART) in the mid-1990s (Palella *et al.* 1998).

ART has improved significantly in terms of its efficacy and toxicity since its introduction in the mid-1990s. A patient established on ART in the mid-1990s could expect to have to take multiple antiretroviral (ARV) tablets multiple times a day, with associated significant gastrointestinal and other side effects and a high risk of developing longer term ART-related toxicity, including lipodystrophy. ART was also then only commenced in patients with a low CD4 cell count, initially below 200 cells μL^{-1}, leaving patients not on ART with an unsuppressed HIV viraemia. On account of ARV-related toxicity and pill-burden, it was less easy for PLWH to adhere to taking their ART fully and missed doses resulted in a higher likelihood of HIV resistance development and HIV virological failure. PLWH were commonly switched to alternative ART regimens on account of toxicity and/or resistance. In contrast, a person diagnosed with HIV in the UK today is established on ART immediately, irrespective of CD4 cell count, with a much shorter period of unsuppressed

HIV viraemia. Furthermore, ART regimens currently available are more efficacious, with a higher barrier to the development of HIV resistance, have significantly less day to day side effects and are, more often than not, once daily regimens compromising as little as one or two tablets taken once daily, significantly improving adherence and the likelihood of achieving good HIV control.

In the era of effective and more widely available ART and a markedly reduced incidence of opportunistic infections, PLWH now have significantly improved life expectancies (Nakagawa *et al.* 2012). In the UK, PLWH should now expect to have a normal life expectancy, similar to HIV-negative people, providing that they are diagnosed in good time, established on ART and have and undetectable HIV viral load and a good CD4 cell count (May *et al.* 2014).

It has become evident that a spectrum of non-infectious co-morbidities (NICMs) – complications less related to extremes of immunosuppression but instead associated with inflammatory changes and accelerated ageing and more prevalent in PLWH than in the general population – can also cause significant morbidity and mortality in PLWH (Guaraldi *et al.* 2011). Reduced bone mineral density (BMD) has emerged as one of these NICMs (Amorosa and Tebas 2006) and there is concern that, as PLWH age, they will experience a higher incidence of fragility fractures and related morbidity and mortality. The surveillance and treatment of NICMs, including reduced BMD and fragility fractures, will increasingly be a focus of HIV care as the HIV-positive population ages.

This introduction discusses the evidence linking reduced BMD and fragility fractures to HIV infection and ART, with an emphasis on the extent of the problem, the risk factors, the pathogenic mechanisms and risk assessment.

1.1 The extent of the problem

1.1.1 Prevalence

Cross-sectional studies have consistently demonstrated a significantly higher prevalence of reduced BMD in PLWH when compared to age-, race- and sex-matched HIV-negative controls (Table 1.1) (Tebas *et al.* 2000; Carr *et al.* 2001; Knobel *et al.* 2001; Moore *et al.* 2001; Nolan *et al.* 2001; Loiseau-Peres *et al.* 2002; Bruera *et al.* 2003; Fernandez-Rivera *et al.* 2003; Mondy *et al.* 2003; Teichmann *et al.* 2003; Vescini *et al.* 2003; Amiel *et al.* 2004; Brown *et al.* 2004; Dolan *et al.* 2004; Madeddu *et al.* 2004; Konishi *et al.* 2005; Yin *et al.* 2005; Arnsten et al. 2006; Bolland et al. 2006; Fausto *et al.* 2006; Garcia Aparicio *et al.* 2006; Arnsten et al. 2007; Cazanave *et al.* 2008; Jones *et al.* 2008; Calmy *et al.* 2009; Teichman *et al.* 2009; Libois *et al.* 2010; Grijsen *et al.* 2013; Negredo *et al.* 2014). The prevalence of reduced BMD in PLWH – defined as a T-score < -1.0 when measured by Dual-Energy X-ray Absorptiometry (DXA) measurements at the lumbar spine, total hip or both – has varied widely between published cohorts, ranging from 21.2% (Grijsen *et al.* 2013) to 87.5% (Knobel *et al.* 2001). There is large heterogeneity between these cohorts, however, with respect to patient gender, race, age and body mass index (BMI), and the nature of the HIV-negative control groups, amongst other factors (Goh *et al.* 2018).

One meta-analysis of pooled prevalence data from eleven cross-sectional studies published prior to 2006 demonstrated an overall prevalence of 67% reduced BMD and 15% osteoporosis (T-score ≤ -2.5) in 884 HIV-positive patients (67% male, predominantly white race, mean age 39.6 years, mean BMI 24.1 kg m^{-2}) when compared to 654 HIV-negative age and sex-matched controls, with odds ratios of 6.4 (95% CI 3.7, 11.3) and 3.7 (95% CI 2.3, 5.9) for reduced BMD and osteoporosis respectively (Brown and Qaqish 2006). A more recent meta-analysis of 29 studies (Goh *et al.* 2018), which included four studies that included predominantly black HIV-positive patients (Arnsten *et al.* 2006, Arnsten *et al.* 2007, Jones *et al.* 2008, Libois *et al.* 2010), also demonstrated a significantly higher prevalence of reduced BMD in PLWH

compared to matched HIV-negative controls, albeit with slightly lower odds ratios of 2.4 (95% CI 2.0, 3.8) for lumbar spine BMD and 3.4 (95% CI 2.2, 3.8) for total hip BMD.

1.1.2 Fracture prevalence and incidence

There is growing evidence in support of a higher incidence of all fractures and fragility fractures (defined as a fracture occurring in adult life either spontaneously, or arising from trauma which, in a healthy individual, would not have resulted in a fracture, i.e. from a fall of standing height or less) in PLWH compared with the general population. Whilst early cross-sectional studies reported a very low or even zero incidence of any fracture after HIV diagnosis, based on retrospective self-reporting (Carr *et al.* 2001; Loiseau-Peres *et al.* 2002; Teichmann *et al.* 2003), subsequent larger studies have reported an increased fracture rate in PLWH compared to HIV-negative controls (Triant *et al.* 2008, Young *et al.* 2011 and Hansen *et al.* 2012). A meta-analysis of thirteen studies has reported a modest increase in fracture incidence overall in PLWH compared with HIV-negative controls, with pooled incidence rate ratios of 1.58 (95% CI 1.25, 2.00) and 1.35 (95% CI 1.10, 1.65) for all fractures and for fragility fractures respectively (Shiau *et al.* 2013).

One population-based study, conducted in a large US-based healthcare system, collected data on all vertebral, hip and wrist fractures, identified from a patient database using ICM-9-CM codes, occurring in patients attending both inpatient and outpatient healthcare settings over 54 months. 8,525 HIV-positive patients were compared to 2,208,792 HIV-negative individuals in groups matched by age (by decade) and ethnicity (Triant *et al.* 2008). In nearly all age and ethnicity groups, there was a significantly greater fracture prevalence (approximately 1.5 to 2-fold) in HIV-positive individuals. Of interest, the extent of the difference in all fracture prevalence between the HIV-positive and -negative groups progressively increased with each successive decade of age, in men after the age of 40 years and in women between 50 and 70 years, suggesting that fracture susceptibility may increase

as the HIV population ages. Similarly, a more recent cohort study, comparing HIV-positive male patients to age- and sex-matched HIV-negative controls, identified no significant difference in fracture incidence by HIV status between men aged 40 to 49 years old, but a significantly higher incidence of all fractures and fragility fractures in HIV-positive men aged 50 to 59 years old compared to HIV-negative men of the same age – adjusted incidence rate ratios of 2.06 (95% CI 1.49, 2.84) and 2.06 (95% CI 1.21, 3.50) for all fractures and for fragility fractures respectively (Gonciulea *et al.* 2017).

Study reference		Location	HIV-positive patients							HIV-negative controls			
First author	Year		n	% male	Mean age years	% on ART	% reduced BMD	% osteo-paenia	% osteo-porosis	n	% reduced BMD	% osteo-paenia	% osteo-porosis
Tebas *et al.*	2000	USA	95	100	-	-	40.0	-	-	17	29.0	-	-
Carr *et al.*	2001	Australia	222	100	43.0	85.6	51.0	22.0	3.0	n/a	-	-	-
Knobel *et al.*	2001	Spain	80	73	41.0	67.5	87.5	67.5	21.2	100	30.0	25.0	5.0
Moore *et al.*	2001	UK	105	71	40.0[a]	86.0	71.0	58.0	13.0	n/a	-	-	-
Nolan *et al.*	2001	Australia	183	100	-	84.7	54.1	38.8	15.3	n/a	-	-	-
Loiseau-Peres *et al.*	2002	France	47	66	41.5	-	68.1	59.6	8.5	47	34.0	31.9	2.1
Bruera *et al.*	2003	Argentina	111	89	34.3	70.2	73.0[b]	57.7[b]	15.3[b]	31	15.4[b]	15.4[b]	0.0[b]
Fernandez-Rivera *et al.*	2003	Spain	89	71	37.0[a]	87.6	43.0	42.0	1.0	n/a	-	-	-
Mondy *et al*.	2003	USA	125	86	41.5	-	45.6	-	-	n/a	-	-	-
Teichmann *et al.*	2003	Germany	50	0	37.4	0.0	76.0[d]	62.0[d]	14.0[d]	50	4.0[d]	4.0[d]	0.0[d]
Vescini *et al.*	2003	Italy	70	49	41.0	94.3	48.6	40.0	8.6	n/a	-	-	-
Amiel *et al.*	2004	France	148	100	41.5	67.6	82.0	66.0	16.0	81	36.0	32.0	4.0
Brown *et al.*	2004	USA	51	85	40.1	100.0	62.7	54.9	7.8	22	31.8	31.8	0.0
Dolan *et al.*	2004	USA	84	0	41.0	81.0	64.0	54.0	10.0	63	35.0	30.0	5.0

[a] Median values [b] Femoral neck [c] Total hip [d] Lumbar spine

Table 1.1. Summary of cross-sectional studies reporting prevalence of reduced BMD in HIV-positive patients compared with HIV-negative controls (continued on next page)

Study reference		Location	HIV-positive patients							HIV-negative controls			
First author	Year		n	% male	Mean age years	% on ART	% reduced BMD	% osteo-paenia	% osteo-porosis	n	% reduced BMD	% osteo-paenia	% osteo-porosis
Madeddu *et al.*	2004	Italy	172	65	38.5	88.4	59.3	44.8	14.5	64	7.8	7.8	0.0
Konishi *et al.*	2005	Japan	39	100	41.4	79.5	25.7	23.1	2.6	n/a	-	-	-
Yin *et al.*	2005	USA	31	0	56.0	-	77.4	67.4	10.0[c]	186	56.0	55.0	1.0[c]
Arnsten *et al.*	2006	USA	263	0	44.0	-	27.0	-	-	232	19.0	-	-
Bolland *et al.*	2006	New Zealand	59	100	50.1	-	32.2	28.8	3.4	118	22.0	21.2	0.8
Fausto *et al.*	2006	Italy	161	64	38.6	70.2	49.7	-	-	n/a	-	-	-
Garcia Aparicio *et al.*	2006	Spain	30	100	41.0	56.7	57.0	-	-	n/a	-	-	-
Arnsten *et al.*	2007	USA	328	100	54.7	-	55.0	-	-	231	51.0	-	-
Cazanave *et al.*	2008	France	492	73	43.0[a]	93.1	80.5	53.7	26.8	n/a	-	-	-
Jones *et al.*	2008	USA	57	60	61.0	-	67.0[d]	39.0[d]	28.0[d]	47	39.0[d]	26.0[d]	13.0[d]
Calmy *et al.*	2009	Australia	153	99	48.0[a]	100.0	42.0	36.0	4.0	n/a	-	-	-
Teichmann *et al.*	2009	Germany	80	100	-	-	35.0	35.0	0.0	20	0.0	0.0	0.0
Grijsen *et al.*	2013	Netherlands	147	100	-	-	21.2	-	-	30	13.0	-	-
Negredo *et al.*	2014	Spain	232	79	28.0[a]	-	67.3	56.5	10.7	75	54.7	50.7	4.0

[a] Median values [b] Femoral neck [c] Total hip [d] Lumbar spine

Table 1.1. (continued from previous page)

1.2 Risk factors for reduced BMD in HIV infection

1.2.1 General risk factors

Validated risk factors for reduced BMD and fragility fracture are well established for the general population (Kanis *et al.* 2005). In addition to previous fragility fracture and low BMD at the femoral neck, these include: increasing age; low BMI; parental history of hip fracture; glucocorticoid exposure; rheumatoid arthritis; current smoking; alcohol consumption ≥ 3 units per day; hypogonadism, including post-menopausal status in women; prolonged immobility; malabsorption and liver cirrhosis (Kanis *et al.* 2005). In addition, other well reported associations exist, including vitamin D deficiency, opiate (Pedrazzoni *et al.* 1993) and other substance dependence (Whyte *et al.* 2009) and the use of selective serotonin uptake inhibitor (SSRI) antidepressants (Haney *et al.* 2010). Multiple studies show that many of these risk factors are increased in HIV-positive populations, such as rates of smoking (Fuster *et al.* 2009; Levine *et al.* 2010), opiate use (Cooper *et al.* 2003) and depression requiring SSRI treatment (Pence *et al.* 2006). Alcohol and other substance misuse are associated with high-risk sexual intercourse and consequently increased HIV incidence, particularly amongst men who have sex with men (MSM) (Mimiaga *et al.* 2008; Shuper *et al.* 2010). Patients presenting late in their illness with AIDS usually have a low BMI (Maas *et al.* 1998). Immune reconstitution inflammatory syndrome (IRIS) may require prolonged courses of glucocorticoid therapy (Meintjes *et al.* 2008), as may treatment of HIV-associated malignancies (Weiss *et al.* 2006). Chronic diarrhoea resulting in malabsorption and nutritional deficiency can occur secondary to opportunistic infections or HIV directly. Androgen deficiency was common in the pre-ART era in men presenting with AIDS-associated wasting (Dobs *et al.* 1996) and is still seen in HIV-infected men on ART presenting with weight loss (Rietschel *et al.* 2000).

Vitamin D deficiency (25-hydroxyvitamin D (25-OH-D) <50 nmol l^{-1}) is highly prevalent in PLWH, with reported prevalence ranging from 38% (Manion *et al.* 2017) to 89% (Cervero *et al.* 2018). There are conflicting reports as to

whether vitamin D deficiency is more prevalent in PLWH than in the general population, however (Sherwood *et al.* 2012, Hidron *et al.* 2015). Whilst the key determinants of vitamin D deficiency in PLWH are the same as for the general population, including black race (Welz *et al.* 2010, Sherwood *et al.* 2012) and reduced sunlight exposure (Paul *et al.* 2010, Welz *et al.* 2010), HIV disease-specific determinants of vitamin D have also been identified: these include nadir CD4 cell count less than 200 cells μl^{-1} and cumulative exposure to the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (Welz *et al.* 2010, Wohl *et al.* 2014). Whether or not vitamin D deficiency contributes to the increased prevalence of reduced BMD in PLWH remains unclear: one South African study has reported a significant correlation between lower 25-OH-D levels and total hip BMD (Dave *et al.* 2015), whereas other studies have not observed any significant association (Sherwood *et al.* 2012, Cotter *et al.* 2014).

Considering the high prevalence of the above general risk factors in PLWH, it could be argued that the increased prevalence of reduced BMD in PLWH is unsurprising. Certainly low BMI (Nolan *et al.* 2001; Mondy *et al.* 2003; Dolan *et al.* 2004; Fausto *et al.* 2006; Cazanave *et al.* 2008; Brown *et al.* 2009; Yin *et al.* 2012) and low weight before starting ART (Carr *et al.* 2001), increasing age (Fausto *et al.* 2006; Cazanave *et al.* 2008), non-black race (Brown *et al.* 2009), steroid use (Mondy *et al.* 2003), smoking (Mondy *et al.* 2003; Dolan *et al.* 2006), low testosterone in men (Calmy *et al.* 2009), post-menopausal state (Yin *et al.* 2012), hepatitis B or C co-infection (Lo Re *et al.* 2009; Sharma *et al.* 2010), heroin (Sharma *et al.* 2010) and methadone use (Sharma *et al.* 2011) have all been reported, using multivariate logistic regression models, to be independent risk factors for decreased BMD or increased fragility fractures in PLWH.

The majority of comparative cross-sectional studies, however, have compared HIV-positive individuals to age-, sex-, ethnicity-, BMI- and, for females, menopausal state-matched HIV-negative controls and have still identified a significant decrease in BMD in PLWH, suggesting the existence of independent HIV disease-specific and potentially also ART-associated factors

in the pathogenesis of decreased BMD in PLWH. It is very challenging, however, to separate HIV disease-specific factors from the confounding effect of general risk factors, which are over-represented in the HIV-positive population.

1.2.2 HIV disease-specific risk factors

1.2.2.1 Evidence supporting an independent role of HIV infection

As well as establishing the contribution of general osteoporosis risk factors within the HIV-positive population, cross-sectional and longitudinal studies have also related changes in BMD to HIV disease-specific factors, for example duration of HIV infection, HIV viral load and CD4 cell count, to determine whether these represent independent risk factors for reduced BMD in PLWH and consequently whether HIV infection is a risk factor for reduced BMD in its own right.

The extent of the rise in CD4 cell count was directly proportional to the BMD increase at the lumbar spine in one longitudinal study of patients on ART, with no significant relationship between BMD and weight or fat change, suggesting a negative effect of HIV on BMD and a potentially beneficial role for ART (Dolan *et al.* 2006). The BMD increase in this study was also independently associated with having an undetectable HIV viral load. In support of this finding, a high HIV viral load at the time of DXA correlated positively with reduced BMD in one cross-sectional study (Fausto *et al.* 2006), although neither high HIV viral load nor low CD4 cell count were associated with reduced BMD in other studies (Knobel *et al.* 2001; Moore *et al.* 2001; Teichmann *et al.* 2003; Yin *et al.* 2010). The relationship with ART is complex, however, with a low or undetectable HIV viral load – perhaps a surrogate marker for patients taking ART – being identified as an independent risk factor for reduced BMD in two other studies (Mondy *et al.* 2003; Cazanave *et al.* 2008).

A low nadir CD4 cell count has been shown to be an independent risk factor for both reduced BMD and increased fracture incidence after adjustment for BMI (Moore *et al.* 2001; Cazanave *et al.* 2008; Brown *et al.* 2009; Dao *et al.* 2010; Young *et al.* 2011). Nadir CD4 cell count could act as a surrogate marker for the duration of HIV infection, which is usually difficult to accurately determine as it often has little relationship with the date of HIV diagnosis. Despite this, time from date of HIV diagnosis and, by implication, prolonged exposure to unsuppressed HIV was also found to be an independent risk factor for loss of BMD in three studies (Bruera *et al.* 2003; Mondy *et al.* 2003; Dolan *et al.* 2006). Nevertheless, duration from diagnosis could also act as a surrogate for the length of ART exposure. It could equally be argued that low nadir CD4 count acts merely as a marker for other established risk factors for decreased BMD, such as low BMI, immobility or chronic diarrhoea.

1.2.2.2 How HIV-1 infection might directly influence bone turnover

Osteoblast and osteoclast function is influenced by a number of factors modulated during HIV-1 infection**,** including pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), expression of RANKL (receptor of activated NF-κB ligand) and osteoprotegerin (OPG), vitamin D and calcium metabolism and hormone levels (Amorosa and Tebas 2006; Pan *et al.* 2006a). Although there is not convincing evidence that osteoblasts or osteoclasts are directly infected, their function may be modulated by a variety of HIV proteins (see below). The HIV envelope glycoprotein, gp 120, can induce TNF-α dependent apoptosis of osteoblast cell lines or primary cells (Gibellini *et al.* 2008). Conversely, a different *in vitro* study showed no evidence that gp120 induced osteoblast apoptosis, but instead increased osteoblast proliferation, which was inhibited by inhibition of the gp120 receptor, CXCR4 (Cummins *et al.* 2011). The difference in the findings of these two studies may be explained by relative differences in osteoblast cell surface expression of CXCR4 – relatively low surface expression in the study by Gibellini *et al.* (2008) and relatively high in the study by Cummins *et al.* (2011). gp120 may also increase osteoblast alkaline phosphatase (ALP)

activity and cell proliferation and decrease cellular apoptosis via Wnt/β-catenin signalling (Butler *et al.* 2013).

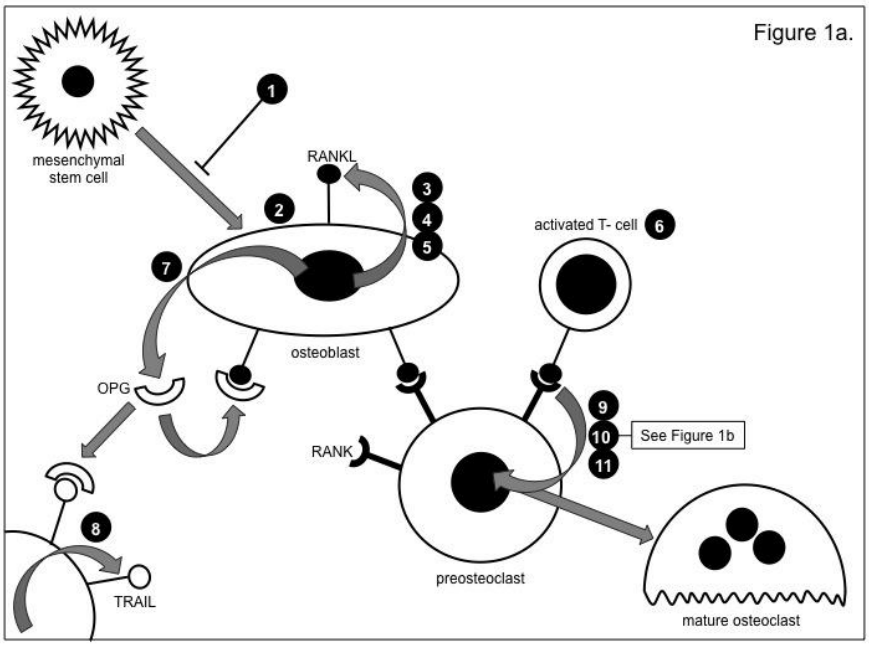
Inflammatory conditions modify bone metabolism, including a variety of factors released by T cells and macrophages such as TNF-α, interferon-γ (IFN-γ), interleukin-4 (IL-4), macrophage inflammation protein-1 (MIP-1) and RANKL (Pan *et al.* 2006b). Markers of bone resorption in advanced HIV infection correlate with TNF-α levels (Aukrust *et al.* 1999). During HIV infection, the cytokine profile favours TNF-α expression and increased viral replication, whilst there is a shift towards a T_H2 cytokine balance, with decreased production of IFN-γ (Clerici and Shearer 1993). This leads to less IFN-γ-induced downregulation of RANKL (Takayanagi *et al.* 2000) and, in turn, increased binding of RANKL to RANK, which is expressed on the surface of preosteoclasts; increased RANKL-RANK binding subsequently results in increased osteoclast activity and bone resorption (Wei *et al.* 2005).

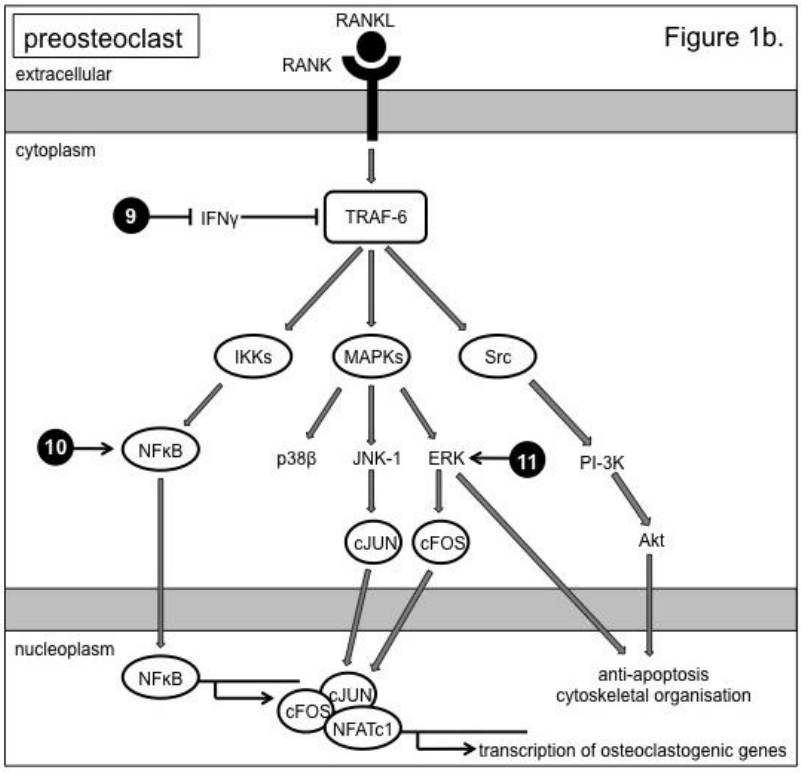
OPG acts as decoy receptor, binding to RANKL and preventing RANKL-RANK binding and osteoclast activation (Theoleyre *et al*. 2004). A number of studies suggest HIV proteins can shift the OPG/RANKL ratio in favour of RANKL-mediated osteoclastic activation (Gibellini *et al.* 2007). HIV Vpr (viral protein R), a factor needed for viral replication, being required for the nuclear import of the HIV-1 pre-integration complex, upregulates RANKL, potentiating glucocorticoid-induced stimulation of RANKL (Fakruddin and Laurence 2005). gp120 also stimulates RANKL (Fakruddin and Laurence 2003). ARV-naïve HIV-positive patients have increased serum RANKL levels and reduced OPG/RANKL ratios, which correlate with the HIV viral load and the Z-score obtained by densitometry (Gibellini *et al.* 2007). RANKL is not only produced by osteoblasts but also activated T cells, which represent a likely source of enhanced RANKL expression in light of their increased numbers during HIV infection (Kong *et al.* 2000).

Although the natural inhibitor of RANKL, OPG, is also enhanced in the serum of ART-naïve patients (Gibellini *et al.* 2007), increased binding of OPG to another factor upregulated by HIV, TNF-related apoptosis-inducing ligand

(TRAIL), in preference to RANKL, limits its availability to inhibit osteoclast activation by RANKL (Yang *et al.* 2003; Herbeuval *et al.* 2005). RANK (receptor of activated NF-κB) signalling via tumour necrosis factor receptor-associated factor 6 (TRAF-6) facilitates nuclear factor kappa B (NF-κB) activation and phosphorylates (activates) c-Jun NH_2-terminal kinase (JNK) 1 and Akt facilitating osteoclastogenesis (Wong *et al.* 1999; Srivastava *et al.* 2001). gp120 may also stimulate RANKL via activation of extracellular signal-regulated kinase (ERK) signalling (Fakruddin and Laurence 2003). The HIV-1 Tat protein has also been shown to enhance RANKL/macrophage colony stimulating factor (M-CSF)-induced osteoclast differentiation by increasing mRNA expression of specific osteoclast differentiation markers (Gibellini *et al.* 2010). Nevertheless, the specific RANK signalling events activated by HIV-1 are still being delineated.

RANKL appears to limit the susceptibility of mitochondria to oxidative stress induced dysfunction in response to nucleos(t)ide reverse transcriptase inhibitors (NRTIs) by mechanisms that do not involve alterations in levels of mitochondrial superoxide dismutase (SOD) (Pan *et al.* 2006). Whilst these observations have been made in macrophage cell lines, if replicated in osteoclasts, they suggest that a potential mechanism of enhanced osteoclastogenesis in response to RANKL could therefore be maintenance of mitochondrial metabolism, despite increasing cell stress, and therefore maintenance of osteoclast viability and prevention of apoptotic death, in contrast to the TNF-α dependent apoptosis triggered by HIV in osteoblasts (Gibellini *et al.* 2008). HIV proteins gp120 and the gag structural protein p55 suppress osteoblast activity in cell lines with upregulation of runt-related transcription factor-2 (RUNX-2) and decreased release of RANKL (Cotter *et al.* 2007). p55 also suppresses osteoblast differentiation from mesenchymal stem cells (Cotter *et al.* 2007). The mechanisms by which HIV-1 infection might directly or indirectly influence bone turnover are summarised in Figures 1.1a and 1.1b.





Figures 1.1a and 1.1b. Mechanisms by which HIV-1 infection might directly or indirectly influence bone turnover. *(1) HIV protein p55 suppresses osteoblast differentiation; (2) HIV envelope protein gp120 induces TNF-α dependent osteoblast apoptosis; (3) TNF-α upregulates RANKL; (4) decreased production of IFN-γ and decreased IFN-γ-mediated downregulation of RANKL; (5) HIV proteins gp120 and VpR upregulate RANKL; (6) increased numbers of activated T-cells in HIV infection;(7) pro-inflammatory cytokines upregulate osteoblast OPG secretion;(8) upregulation of TRAIL with increased OPG-TRAIL binding limiting RANKL-OPG binding; (9) decreased IFN-γ-mediated proteosomal degradation of TRAF-6; (10) TNF-α activates NF-κB signalling; and (11) gp120 activates ERK signalling downstream of RANK-RANKL binding, enhancing osteoclast survival and differentiation.*

1.2.3 Role of antiretroviral therapy

It would seem logical that ART should stabilise BMD loss through suppression of HIV viraemia, immune recovery and a reduction in the immune activation and inflammation associated with increased bone turnover and BMD loss in PLWH (described in Section 1.2.2.2). Mixed conclusions from cross-sectional and longitudinal studies, however, suggest that the relationship between ART and BMD in PLWH is more complex.

1.2.3.1 Cross-sectional data for and against a role for antiretroviral therapy

An early meta-analysis of pooled data from ten cross-sectional studies conducted between 2000 and 2005 (Table 1.2), compared 824 ART-treated (experienced) patients with 202 ART-naïve patients and found a significant reduction in BMD in the ART-treated patients, odds ratio 2.5 (95% CI 1.8, 3,7) (Brown and Qaqish 2006). BMI was similar between ART-experienced and ART-naïve groups**,** with the exception of one study (Garcia Aparicio *et al.* 2006), although there was no adjustment for other potentially important confounding factors such as patient age or duration of HIV infection.

Further analyses have examined individual classes of ARVs (individual ARVs are listed by drug class in Table 1.3). Pooled meta-analysis of 14 studies demonstrated significantly lower BMD in protease inhibitor (PI)-treated patients (n = 791), odds ratio 1.5 (95% CI 1.1, 2.0), compared with non-PI-treated patients (n = 410) (Brown and Qaqish 2006). An Australian cross-sectional study of 153 predominantly male HIV-positive patients also identified boosted PI use (median duration 51 months) as an independent risk factor for reduced BMD (Calmy *et al.* 2009). The same study observed an association between the use of tenofovir disoproxil fumarate (DF) and increased bone turnover, but with no significant association between tenofovir DF use (median duration 28 months) and BMD. A more recent, albeit smaller, Spanish cross-sectional study identified the combined use of a boosted PI with tenofovir DF as an independent risk factor for reduced BMD in HIV-

positive patients (Cervero *et al*. 2018); the same study also identified a history of tenofovir DF use, but not PI use, as a separate risk factor.

PI-naïve patients on NNRTI-containing ART did not have an increased risk of reduced BMD when compared to ART-naïve patients (Bongiovanni *et al.* 2006).

Three large retrospective analyses have explored the relationship between ART exposure and fracture risk. Only one identified an increased risk of fragility fracture with cumulative exposure to ARVs on multivariate analysis and specifically only with cumulative exposure to tenofovir DF or to the ritonavir-boosted PI lopinavir (Bedimo *et al.* 2012); another, however, found no association between tenofovir DF exposure and fracture incidence, (Gedmintas *et al.* 2017). Furthermore, one study actually identified a reduced fracture risk in ART-treated HIV-positive patients compared to those not on treatment (Mundy *et al.* 2012).

NRTIs	**NNRTIs**	**PIs**	**Newer drug classes**
Abacavir Didanosine Emtricitabine Lamivudine Stavudine Tenofovir disoproxil fumarate (TDF) Tenofovir alafenamide (TAF) Zidovudine	Efavirenz Etravirine Nevirapine Rilpivirine	Amprenavir Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir/ritonavir Nelfinavir Ritonavir Saquinavir Tipranavir	***Chemokine receptor antagonists*** Maraviroc ***Entry inhibitors*** Enfuvirtide (T20) ***Integrase inhibitors*** Dolutegravir Elvitegravir/cobicistat Raltegravir

Table 1.3. Antiretroviral drugs listed by drug class (NRTIs = nucleos(t)ide reverse transcriptase inhibitors; NNRTIs = non-nucleoside reverse transcriptase inhibitors; PIs = protease inhibitors)

Study reference		Location	ART-experienced		ART-naïve		Comparative analysis	
First author	Year		n	% reduced BMD	n	% reduced BMD	Odds ratio (95% CI)	p-value
Carr *et al.*	2001	Australia	189	25	32	6	-	NS
Knobel *et al.*	2001	Spain	54	98	26	69	-	NS
Bruera *et al.*	2003	Argentina	78	69	33	55	-	NS
Fernandez-Rivera *et al.*	2003	Spain	78	44	11	27	-	NS
Vescini *et al.*	2003	Italy	66	74	4	50	-	-
Amiel *et al.*	2004	France	100	77	48	50	-	NS
Madeddu *et al.*	2004	Italy	152	63	20	35	-	NS
Fausto *et al.*	2006	Italy	113	51	48	46	2.61 (0.66,10.27)	0.17
Garcia Aparicio *et al.*	2006	Spain	17	53	13	62	-	NS
Cazanave *et al.*	2008	France	458	-	34	-	0.28 (0.06, 1.31)	0.11
Zuccotti *et al.*	2010	Italy	71	-	15	-	-	NS

NS = not significant

Table 1.2. Summary of cross-sectional studies comparing prevalence of reduced BMD in ART-naïve and in ART-treated HIV-positive patients (NS = not significant)

1.2.3.2 Longitudinal data for and against a role for antiretroviral therapy

Longitudinal studies examining the changes in BMD over time have been conducted in both HIV-positive patients well established on ART (ART-stable) and in patients who are ART-naïve.

Studies conducted in ART-stable patients have confirmed lower baseline BMD in PLWH when compared to HIV-negative controls (Dolan *et al.* 2006; Yin *et al.* 2010). Over 2 to 2.5 years follow up, however, neither of these studies identified any significant difference in BMD decline between ART-stable HIV-positive patients and HIV-negative controls. Moreover, a recent meta-analysis, pooling the results of six longitudinal studies, concluded that BMD was, in general, stable in ART-stable patients, when followed up for a minimum of 48 weeks (Bolland *et al.* 2011).

Longitudinal studies in ART-naïve HIV-positive patients have shown a different picture, however. The Gilead 903 Study compared tenofovir DF with stavudine in combination with lamivudine and efavirenz in ART-naïve individuals (Gallant *et al.* 2004). This study identified an initial significant loss in BMD at 24 weeks at the lumbar spine and at 48 weeks at the total hip in both the tenofovir DF and the stavudine-treated groups, with significantly greater reduction at the lumbar spine in the tenofovir DF-treated group. Subsequently stabilisation of bone loss was observed in both groups, followed by partial recovery of BMD at the lumbar spine in both treatment groups, although BMD did not return to baseline over the 144 weeks of follow up. In the same study, BMD at the total hip remained static over time. The more recent ASSERT and ACTG A5224s studies also identified a similar pattern of BMD loss with subsequent stabilisation at both the total hip and lumbar spine in ART-naïve patients randomised to either tenofovir DF/emtricitabine-based ART or abacavir/lamivudine-based ART, with again greater initial BMD loss at both the total hip and lumbar spine in the tenofovir DF-treated group (Stellbrink *et al.* 2010; McComsey *et al.* 2011).

Greater initial BMD decline has also been observed following initiation of boosted PI-containing ART regimens when compared with PI-sparing NNRTI-containing ART regimens (Duvivier *et al.* 2009, McComsey *et al.* 2011).

A meta-analysis of longitudinal BMD measurements pooled from 37 studies of treatment-naïve patients starting ART has demonstrated this initial accelerated but short-term loss of BMD in the first one to two years following ART initiation as a generalised pattern (Bolland *et al.* 2011). Moreover, two studies comparing ART-naïve HIV-positive patients who immediately start ART with those who defer starting ART – the large multinational START trial (Hoy *et al.* 2017) and a smaller study in patients with primary HIV infection (Vlot *et al.* 2018) – both identified a significantly steeper decline in BMD loss in the immediate treatment groups compared to the deferred treatment groups.

The same pattern of BMD loss seen in treatment-naïve patients initiating ART has also been observed in switch studies, in which patients already established on ART undergo an ART regimen change, again with more profound effects seen with tenofovir DF. One Australian study randomised 357 HIV-positive individuals already on ART to change to either tenofovir DF/emtricitabine-based ART or abacavir/lamivudine-based ART (Martin *et al.* 2009). At 96 weeks, there was a significant difference between the two treatment arms, with a significant net reduction in BMD at the total hip with tenofovir DF-emtricitabine at 48 and 96 weeks compared to a non-significant net increase in the abacavir-lamivudine group. These results were not adjusted for change in BMI, however, and a modest reduction in weight and lean mass was observed in the tenofovir DF-emtricitabine group.

1.2.3.3 Trends in bone turnover markers before and after initiation of antiretroviral therapy

The levels of bone turnover markers, including osteocalcin and procollagen type 1 N-terminal propeptide (P1NP) – both markers of bone formation – and

C-terminal telopeptide of collagen type 1 (CTx) – a sensitive marker of bone resorption – have also been measured in PLWH both before and after initiation of ART.

In one US study of 52 white male patients, in whom HIV acquisition and seroconversion occurred under observation, ostoecalcin levels measured both before and after the time of HIV seroconversion were found to be significantly lower after seroconversion compared to before seroconversion, with no observed significant change in P1NP or CTx, suggesting an overall reduction in bone formation following HIV seroconversion (Slama *et al .*2017). This was consistent with another study that demonstrated that ART-naïve PLWH have lower level bone turnover markers, including P1NP and CTx, compared to HIV-negative age-, gender- and BMI-matched healthy controls (Zhang *et al.* 2013). Furthermore, a Norwegian study of 73 ART-naïve HIV-positive patients observed markedly low osteocalcin levels, as well as increased CTx levels, in patients with more advanced HIV infection with high unsuppressed plasma HIV RNA and low CD4+ T cell counts (Aukrust *et al.*), supporting the notion that bone formation is reduced relative to bone resorption in the context of unsuppressed HIV infection.

Following initiation of ART, however, the levels of bone turnover markers – including both markers of bone formation and bone resorption – appear to increase. Increases in osteocalcin, P1NP, CTx and bone-specific ALP were observed in 26 male patients initiating ART during primary HIV infection, with significantly higher levels of bone turnover markers observed 24 weeks after ART initiation compared with 9 patients in whom ART was not initiated (Vlot *et al.* 2018). A similar trend of significant increases in osteocalcin, P1NP, CTx and bone-specific ALP after initiation of ART was observed in a large study of 833 HIV-positive patients followed up for 144 weeks, with bone turnover marker levels peaking at 48 or 96 weeks following ART initiation and then stabilising (Tebas *et al.* 2015). Furthermore, the magnitude of bone turnover marker increase was greater in patients commencing tenofovir DF-containing ART than abacavir containing ART. Bone turnover markers have also been observed to be higher in patients established on PI-containing ART compared

with non-PI containing ART (Kinai *et al.* 2017). Switching patients from tenofovir DF-containing ART to non-tenofovir DF-containing ART has resulted in a reduction in levels of bone turnover markers in several large studies, with associated improvements in BMD measuerments (Bloch *et al.* 2014, Negredo *et al.* 2015, McComsey *et al.* 2018).

1.2.3.4 How antiretroviral therapy might influence bone turnover

The mechanisms by which ART might influence bone turnover remain unclear. Proposed mechanisms are summarised in Figures 1.2a, 1.2b and 1.2c.

Most information exists for the use of PIs, but results have been conflicting and the relevance of culture models incubated with PIs *in vitro* debated. The PIs nelfinavir and indinavir have been shown to decrease osteoblast activity *in vitro* (Malizia *et al.* 2007). Gene array experiments of primary osteoblasts suggest tissue inhibitor of metalloproteinase-3 (TIMP-3) is upregulated whilst siRNA inhibition of TIMP-3 reverses these effects, enhancing calcium deposition and production of ALP (Malizia *et al.* 2007). A further study from the same group implicated upregulation of the pro-inflammatory cytokine genes monocyte chemoattractant protein (MCP)-1 and interleukin-8 (IL-8) in osteoblasts as a contributory factor (Malizia *et al.* 2007).

Results with PIs are clouded by differences in responses to different agents; in one study nelfinavir and lopinavir reduced osteoblast activity and OPG secretion, whilst ritonavir, indinavir, saquinavir and nelfinavir, but not lopinavir or amprenavir induced osteoclast activity (Jain and Lenhard 2002). Despite these observations there is not clear cut evidence from clinical trials that PIs trigger bone demineralization (Amorosa and Tebas 2006). One potential explanation may be that ritonavir also appears to block RANK signalling, blocking recruitment of TRAF-6 recruitment to RANK-containing lipid rafts and inhibiting Akt signalling pathways that lead to NF-κB activation and oseoclastogenesis (Wang *et al.* 2004). Interestingly, ritonavir did not alter

signalling via other pathways downstream of RANK such as JNK-1 or ERK in this study and indinavir had no effect on osteoclastogenesis. The exact effects of PIs on RANK signalling is, however, complex since, at a lower dose than used in the prior study**,** ritonavir and, in addition, saquinavir reversed the physiological inhibition to RANK signalling provided by IFN-γ mediated proteasomal degradation of TRAF-6 and restored JNK1 activation (Fakruddin and Laurence 2003). Potentiation of signalling at these doses of PI might be relevant in the context of relatively preserved IFN-γ levels, as might appear early in disease or after some reversal of HIV-induced perturbation of cytokine bias by ART has reduced the HIV viral load.

Microarray analysis suggested inhibition of osteoblast and enhancement of osteoclast function could involve changes in canononical Wnt signalling, with inhibition of β-catenin nuclear translocation, a proposed mechanism by which Wnt suppresses osteoclastic differentiation, contributing to the increased osteoclast activity (Modarresi *et al.* 2009) (Figures 1.2b and 1.2c). These array studies suggested ritonavir upregulates non-canonical Wnt proteins 5B and 7B, which antagonise canonical Wnt signalling and enhance ubiquitination (and hence degradation) of β-catenin with the overall effect of preventing the inhibitory effect of β-catenin on osteoclast differentiation. Ritonavir-mediated upregulation of Wnt proteins 5A and 5B in human and murine osteoclast precursors has since been demonstrated elsewhere (Santiago *et al.* 2012). Since canonical Wnt also facilitates osteoblast release of the soluble RANKL decoy OPG, the authors speculated that alterations in Wnt signalling could also effect osteoclast development by indirect effects on osteoblasts, but this remains to be tested directly. Ritonavir also upregulated the inhibitor of apoptosis Bcl-X_L in this study.

Although PIs may affect molecular pathways involved in homeostasis *in vitro*, the physiological consequences are not yet clear. One group in whom these changes may translate into alterations of measured BMD, however, may be children. HIV-positive children or adolescents receiving PI-containing ART had decreased OPG/RANKL ratios, as compared to HIV-negative controls,

which normalised after PI-therapy was switched to the NNRTI efavirenz (Mora *et al.* 2007).

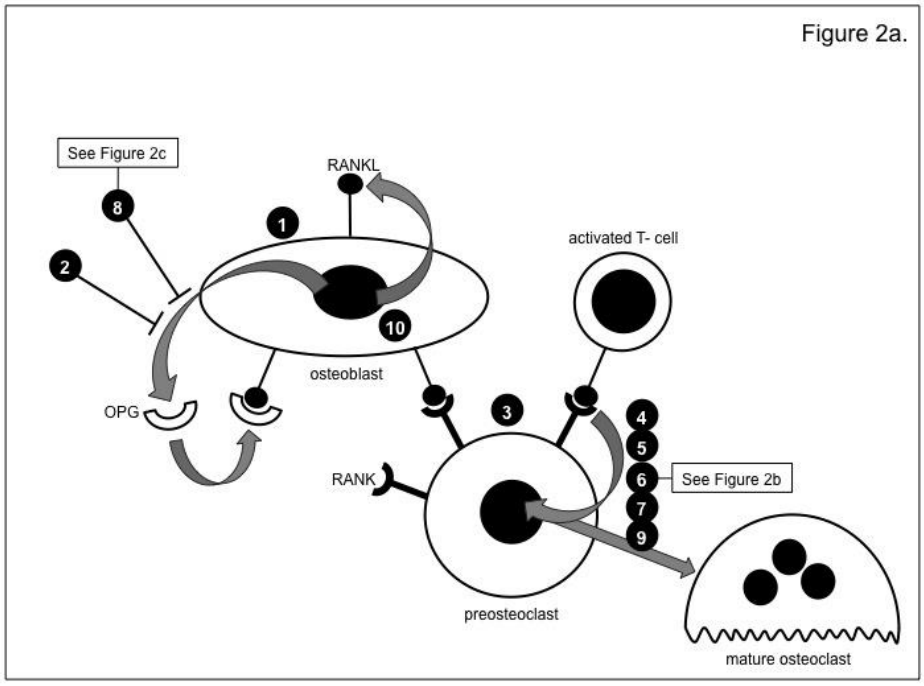
Zidovudine enhances RANKL-dependent osteoclastogenesis in cell lines and primary cells, as evidenced by increased expression of the tartrate-resistant acid phosphatase (TRAP) promoter and also osteoclastic activity and loss of BMD (Pan *et al.* 2004; Pan *et al.* 2006). As discussed above, RANKL could prevent reactive oxygen species (ROS) induced mitochondrial damage in the presence of NRTIs such as zidovudine, thus preventing osteoclast apoptosis (Pan *et al.* 2006). Some, but not other studies, have identified a link between nucleoside-induced impairment of osteoblast function and mitochondrial toxicity (Carr *et al.* 2001; Cossarizza and Moyle 2004). RANKL may prevent nucleoside analogue induced mitochondrial toxicity, explaining the lack of mitochondrial toxicity observed (Pan *et al.* 2006).

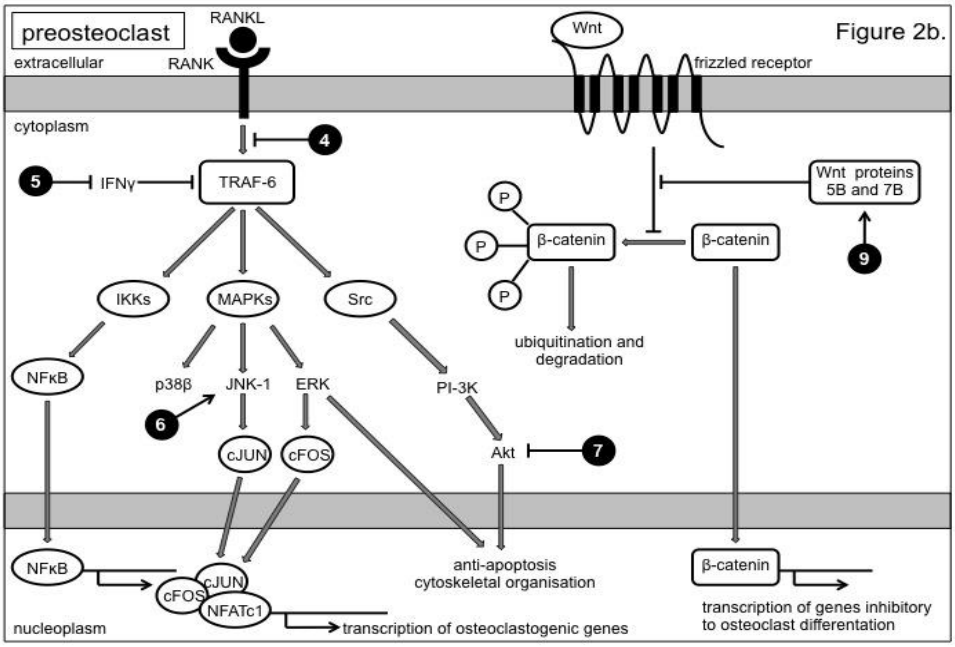
There is currently relatively less information on the molecular mechanisms of tenofovir's potential effects on bone homeostasis. Tenofovir alters the transcriptional profile of osteoblasts, altering genes involved in cell signalling, cell cycle and amino acid metabolism (Grigsby *et al.* 2010a). A recent gene array analysis of primary osteoclasts identified genes downregulated by tenofovir, including guanine nucleotide-binding protein alpha stimulating (Gnas), a G-protein receptor involved in mitogen-activated protein kinase (MAPK)/ERK signalling, whose loss of function has been associated with impaired osteoblast activity**,** and glutamate oxaloacetate transaminase (Got) 2, a mitochondrial enzyme whose loss of function has also been linked to impaired bone homeostasis (Grigsby *et al.* 2010b). Although not further verified**,** a further gene downregulated in this study was small nucleolar RNA C/D box 32A (Snord 32A), a transcript implicated in gene expression, RNA processing and protein trafficking.

The ability of tenofovir to induce either isolated renal phosphate wasting or a Fanconi-like syndrome with proximal renal tubular dysfunction (PRTD) has been reported in case reports in association with osteomalacia or reduced BMD (summarised by Fux *et al.* (Fux 2007)) and has led to speculation that

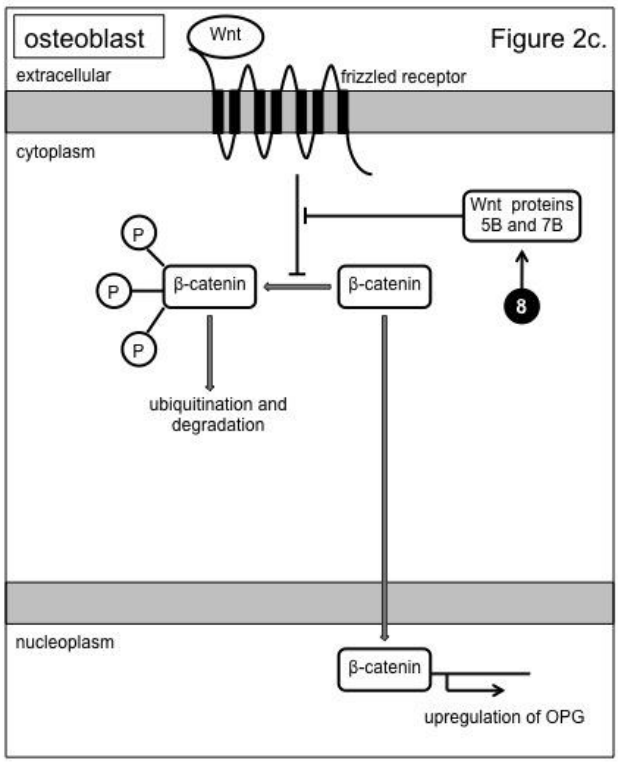
the BMD loss seen in association with tenofovir DF might be caused by renal phosphate wasting. The prevalence of PRTD in HIV-positive patients on tenofovir DF is reportedly rare, however (Woodward *et al.* 2009). Furthermore, bone abnormalities were reported in only 0.1% of 10,343 HIV-positive patients taking tenofovir DF in a four-year prospective study (Nelson *et al.* 2007), although subclinical bone abnormalities were not reported.

There is little information on the mechanism by which other factors may influence bone turnover. Both PI- and non-PI -containing ART have been associated with impairment of vitamin D metabolism (Cozzolino *et al.* 2003; Gyllensten *et al.* 2006; Conesa-Botella *et al.* 2010). PIs may reduce 25α-hydroxylase activity in hepatocyte and 1α-hydroxylase in monocyte cell lines (Cozzolino *et al.* 2003). Efavirenz may accelerate catabolism of 1,25-hydroxyvitamin D_3 through the upregulation of 25-hydroxyvitamin D_3-24-hydroxylase (CYP24) (Barrett *et al.* 2002; Gyllensten *et al.* 2006).





Figures 1.2a and 1.2b (above) and 1.2c (next page). Mechanisms by which antiretrovirals might influence bone turnover. *(1) Nelfinavir and indinavir decrease osteoblast activity in vitro; (2) nelfinavir and lopinavir downregulate OPG secretion; (3) ritonavir, indinavir, saquinavir and nelfinavir induce osteoclast activity; (4) ritonavir prevents downstream RANK intracellular signalling by blocking TRAF-6 recruitment to RANK lipid rafts; (5) low dose ritonavir and saquinavir inhibit IFN-γ-mediated proteasomal degradation of TRAF-6 and (6) restore JNK-1 activation; (7) ritonavir inhibits Akt signalling; (8) ritonavir upregulates non-canonical Wnt proteins 5B and 7B, antagonising canonical Wnt signalling and inhibition of β-catenin ubiquitination and degradation, with decreased β-catenin-mediated OPG release in osteoblasts and (9) increased osteoclast differentiation; (10) tenofovir downregulates Gnas which alters MAPK/ERK signalling, impairing osteoblast activity.*



1.2.3.4 Newer antiretrovirals and bone mineral density

Patients on ART have historically received two NRTI drugs in combination with either a boosted PI or an NNRTI. In recent years, however, integrase inhibitors (INIs) – a newer class of ARVs – are being increasingly used alongside NRTIs in place of PIs or NNRTIs in patients newly starting or switching ART. Furthermore, the use of dual therapy and NRTI-sparing ART regimens is also increasing, as evidence in support of their efficacy and more favourable safety profile is emerging.

Cobicistat-boosted elvitegravir – an INI – co-formulated with tenofovir DF/emtricitabine resulted in significantly less BMD loss at both 48 and 96 weeks following ART initiation in ART-naïve patients compared with tenofovir DF/emtricitabine co-administered with ritonavir-boosted atazanavir (Rockstroh *et al.* 2013). Furthermore, raltegravir – another INI – resulted in significantly less BMD loss at 48 weeks following ART initiation in ART-naïve patients

compared with tenofovir DF/emtricitabine when each were combined with ritonavir-boosted darunavir (Bernadino *et al.* 2015).

Even more recently, tenofovir alafenamide (TAF) – a new tenofovir pro-drug and alternative NRTI to tenofovir DF – has become available for clinical use. Phase 3 studies demonstrated significantly less BMD loss at 48 weeks and 96 weeks following initiation of TAF-containing ART in ART-naïve HIV-positive patients compared with tenofovir DF-containing ART (Sax *et al.* 2015, Wohl *et al.* 2016). Whether or not use of TAF in place of tenofovir DF will result in reduced fragility fracture incidence over time, however, remains to be seen.

1.3 Fracture risk assessment in PLWH

PLWH have a higher prevalence of reduced BMD and a higher incidence of fragility fractures than the general population. To date, however, there have been very few published studies on the use of osteoporosis risk or fracture risk assessment tools in this population.

The WHO Collaborating Centre for Metabolic Bone Diseases in Sheffield has undertaken a comprehensive review and a number of meta-analyses of published risk factors within the general population. The outcome of this work, the FRAX® tool (www.shef.ac.uk/FRAX) has been validated as an effective tool for predicting a patient's fracture risk based upon their age, gender, BMI, menopausal status and other validated general osteoporosis risk factors, with or without BMD results at the femoral neck if available (Kanis *et al.* 2008a; Johansson *et al.* 2009; Kanis *et al.* 2009). The tool is valid for men and women aged 40 to 90 years and provides an estimate of an individual's ten-year probability of major osteoporotic fracture (hip, clinical vertebral, wrist or proximal humerus) or hip fracture alone. The output of the tool has been coupled to a guideline from the National Osteoporosis Guideline Group in the UK (NOGG) to determine who should be considered for BMD assessment and/or therapy to reduce fracture risk (Kanis *et al.* 2008b; Compston *et al.* 2009). Of note, the FRAX® tool is one of several well validated fracture risk

assessment calculators that are available, which also include the Garvan Institute Fracture Risk Calculator (Nguyen *et al.* 1993) and, for UK use only, the QFracture®-2016 risk calculator (Hippisley-Cox and Coupland 2012).

British HIV Association (BHIVA) Guidelines recommend the use of FRAX® for fracture risk assessment in PLWH (BHIVA 2016). Guidelines published by the European AIDS Clinical Society (EACS) in 2017 also recommend FRAX® score calculation in HIV-positive patients aged 40 years or more, with the added recommendation that HIV infection is incorporated into FRAX® as a "secondary osteoporosis" risk factor (EACS 2017). As FRAX® does not incorporate HIV-disease specific risk factors, however, the appropriateness of its use in PLWH remains a subject of research.

Only two studies have compared FRAX®-calculated 10-year probabilities of fragility fracture (estimated incidence) to observed 10-year fragility fracture incidence in PLWH (Yin *et al.* 2016, Yang *et al.* 2018). Each of these two US studies – one exclusively in older male patients and the other in female patients – concluded that FRAX® was less accurate at predicting observed 10-year fragility fracture incidence in PLWH than in HIV-negative controls, particularly in older and higher risk patients. Furthermore, each study also observed that the sensitivity of FRAX® to predict observed fragility fracture incidence was improved in PLWH by incorporating HIV infection into FRAX® as a "secondary osteoporosis" risk factor. Of note, one of these was a retrospective study in which data on parental hip fracture and the presence of other "secondary osteoporosis" risk factors was not available and therefore not included in FRAX® calculations (Yin *et al.* 2016). In addition, there was a significantly higher prevalence of HCV co-infection – an additional fracture risk factor (Lo Re *et al.* 2012) also not incorporated into FRAX® – in the HIV-positive men. Moreover, observed fragility fracture incidence was actually quite low.

Where FRAX® is not used, screening with DXA is currently recommended for the following HIV-positive patient groups: post-menopausal women; men more than or equal to 50 years of age; individuals with a history of fragility

fracture; individuals with a high risk for falls; individuals with clinical hypogonadism; and those who have been on oral glucocorticoid treatment (at least 5 mg prednisolone or equivalent) for more than three months (EACS 2017). DXA screening could also be considered in younger men with other risk factors, namely low BMI and/or nadir CD4 count <200 cells μL^{-1} (Mary-Krause *et al.* 2012).

1.4 Summary of current evidence

The prevalence of reduced BMD and incidence of fragility fractures are increased in PLWH compared with HIV-negative controls. There are many potential explanations for this (Figure 1.3) – an increased prevalence of general osteoporosis risk factors in the HIV-positive population, a likely direct effect of HIV infection itself and, in addition, a possible contributory role of ART – with accelerated bone loss within the first one to two years following ART initiation or switch – with potential mechanisms identified for both HIV infection and ART. The interplay between these multiple factors and their relative contribution to reduced BMD and increased fracture risk in PLWH, however, remains unclear.

At present there is no adequate risk assessment tool validated in the HIV-positive population to help predict who is at risk of reduced BMD or fragility fracture and who may benefit from BMD assessment and preventative treatments.

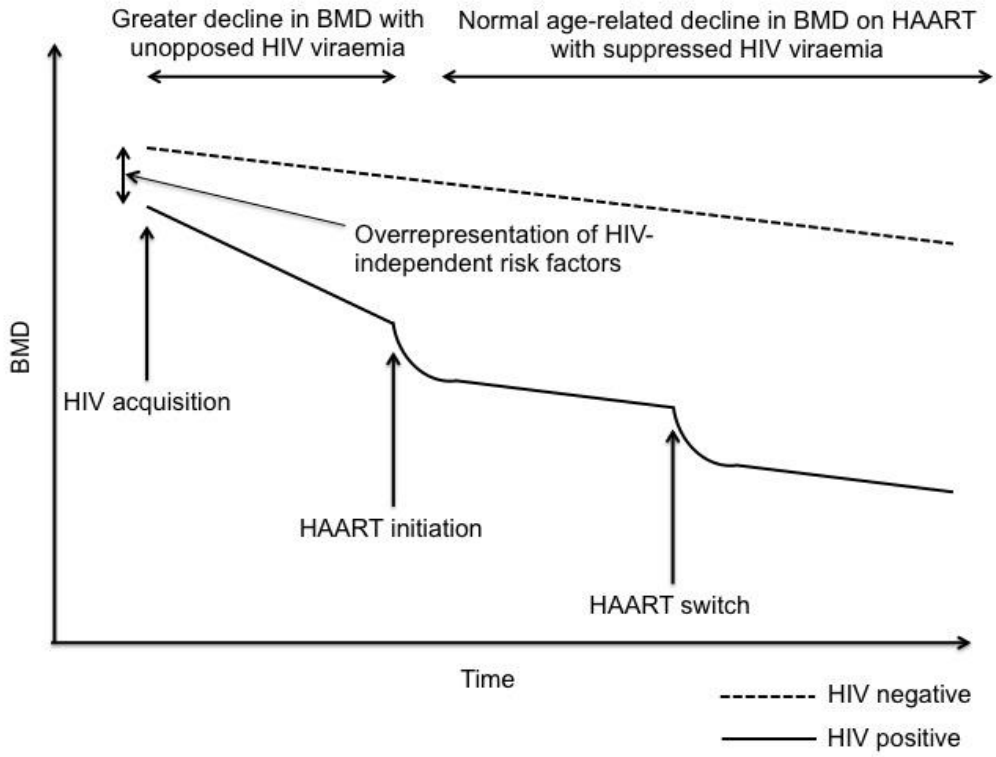


Figure 1.3. Factors contributing to reduced BMD in people living with HIV over time compared with HIV-negative individuals (adapted from Mallon et al. 2010). (HAART = highly-active antiretroviral therapy)

1.5 Study hypotheses and aims

Primary hypotheses:

1. BMD in PLWH is determined by HIV disease-related risk factors – related to HIV infection or its treatment – as well as established general risk factors.
2. 10-year probability of major osteoporotic fractures calculated using FRAX® (derived from general fracture risk factors alone) correlates poorly with BMD in PLWH.

Secondary hypotheses:

1. The prevalence or distribution of both general risk factors and HIV-disease specific risk factors are similar in both black and white, black male and white male and black female and white female PLWH.
2. The effects of both general risk factors and HIV-disease specific risk factors on BMD and on fracture prevalence are similar in both black and white, black male and white male and black female and white female PLWH.
3. The correlation between 10-year probability of major osteoporotic fractures calculated using FRAX® with BMD is similar in both black and white PLWH.

Aims

1. To determine the prevalence of both general risk factors, HIV disease-specific factors and fractures in PLWH in the Sheffield HIV Cohort (Chapter 3).
2. To assess the relationship between BMD and both general and HIV-disease specific clinical risk factors in order to establish the key clinical determinants of BMD in PLWH in the Sheffield HIV Cohort (Chapter 4).

3. To assess the relationship between BMD and both general and HIV-disease specific biochemical markers, including 25-OH-D, in order to establish the key biochemical determinants of BMD in PLWH in the Sheffield HIV Cohort (Chapter 5).
4. To assess the relationship between BMD and markers of inflammation and immune activation in order to establish the key inflammatory or immunological determinants of BMD in PLWH in the Sheffield HIV Cohort (Chapter 6).
5. To asses the correlation between 10-year probability of major osteoporotic and hip fractures calculated using FRAX® with and without BMD in PLWH in the Sheffield HIV Cohort (Chapter 7).

More specific aims are detailed within the introductions to results chapters 3, 4, 5, 6 and 7 respectively.

2. Materials and Methods

2.1 Scientific Peer Review, Ethics and Research and Development approvals

The protocol for this study underwent successful local scientific peer review and was approved by the regional Research Ethics Committee (reference 12/YH/0131) and the Sheffield Teaching Hospitals NHS Foundation Trust (STH) Research Department.

2.2 Phase One: Assessment of patient demographics, fracture prevalence and prevalence of risk factors for reduced BMD

HIV-positive patients within the Sheffield HIV Cohort, attending STH outpatient clinics in the Department of Infection and Tropical Medicine and the Department of Genitourinary Medicine, were recruited prospectively over a period of twelve months, from 1 October 2009 to 30 September 2010. Clinical data was collected by patient interview within routine clinic appointments and by subsequent medical record review. Data was collected and recorded on a pre-designed questionnaire.

Baseline patient demographics were recorded, including gender, age, race and country of origin. Patients' height and weight were measured and their BMI calculated.

HIV-specific data were recorded, including the date of HIV diagnosis, the likely route of HIV transmission, the current HIV viral load, the current CD4 cell count, the nadir CD4 cell count, the presence of hepatitis B or C co-infection, any history of lipodystrophy and a full ART history. Current CD4 cell count was analysed by the STH Cell Markers Laboratory; the most recent CD4 cell count within six months before or after the clinic appointment was recorded. HIV viral load (plasma HIV RNA) was quantified using polymerase chain reaction (PCR) by the STH Virology Laboratory using the *COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 (TaqMan 96)* (Roche Molecular Systems, Inc.) with a lower limit of detection of 20 copies of HIV

RNA ml^{-1}; the most recent plasma HIV RNA within six months before or after the clinic appointment was recorded.

The presence of general osteoporotic risk factors (Table 2.1) was established, including the history and nature of any previous fractures and specifically any fragility fractures (defined in Chapter 1, Section 1.1.2) sustained in adulthood. The number of falls within the preceding 12 months was also recorded.

- Prior fragility fracture
- Parental history of hip fracture
- Rheumatoid arthritis
- Current tobacco smoking
- Alcohol consumption ≥3 units per day
- Significant ever use of glucocorticoids (>3 months at a dose equivalent to prednisolone 5mg daily or more)
- One or more other disorder strongly associated with osteoporosis (grouped in FRAX® under "secondary osteoporosis" risk factors):
  - hypogonadism
  - chronic diarrhoea (≥ 1 month)
  - malabsorption
  - inflammatory bowel disease
  - organ transplant recipient
  - untreated longstanding hyperthyroidism
  - type 1 diabetes mellitus
  - chronic obstructive pulmonary disease (COPD)
  - prolonged immobility (≥ 1 month)
  - liver cirrhosis

Table 2.1. General risk factors for fragility fracture, as incorporated into FRAX®

Venous blood was obtained and tested for total 25-OH D (25-OH-D_2 and 25-OH-D_3). Testosterone was tested in male patients (to assess for hypogonadism – see below). Blood samples were analysed by the STH

Clinical Chemistry Laboratory. 25-OH D was analysed using the *COBAS*® *Elecsys Vitamin D Total Assay*, an electrochemiluminescence binding assay, with a lower limit of quantification of 7.5 nmol l^{-1} and an upper limit of quantification of 175.0 nmol l^{-1}. Patients with a history of current or previous vitamin D and/or calcium replacement or supplementation were excluded from subsequent 25-OH D and related biochemical data analyses.

The 10-year probabilities of major osteoporotic fractures and hip fractures were calculated (without femoral neck BMD and without including HIV as a "secondary osteoporosis" risk factor) using FRAX® (www.sheffield.ac.uk/FRAX), by inputting demographic and clinical data (Figure 2.1). Country specific FRAX® calculators were used, where available, according to a patient country of origin. The US Black FRAX® calculator was used for all patients of black race, in the absence of any other country-specific calculator specific to patients of black race.

Definite male hypogonadism was defined as a patient with a previous confirmed diagnosis of male hypogonadism, or with morning (pre-10am) testosterone of <12nmol l^{-1} in combination with hypogonadal symptoms, i.e. erectile dysfunction, loss of morning erections or loss of sexual libido. Possible male hypogonadism was defined as a patient with a random (later than 10am) testosterone of <12nmol l^{-1} but without subsequent confirmatory pre-10am testosterone, in combination with hypogonadal symptoms. Female hypogonadism was defined as premature menopause, i.e. onset < 45 years of age.

The presence of other general risk factors for reduced BMD not incorporated into FRAX® was also recorded, including current or previous: Depo-Provera® use (female patients only); anticonvulsant therapy; hyperparathyroidism; growth hormone deficiency; rosiglitazone or pioglitazone use; SSRI antidepressant use; use of a proton pump inhibitor (PPI) for more than five years; anastrozole, letrozole or exemestane use; opiate dependence; cannabis use or other recreational drug use.

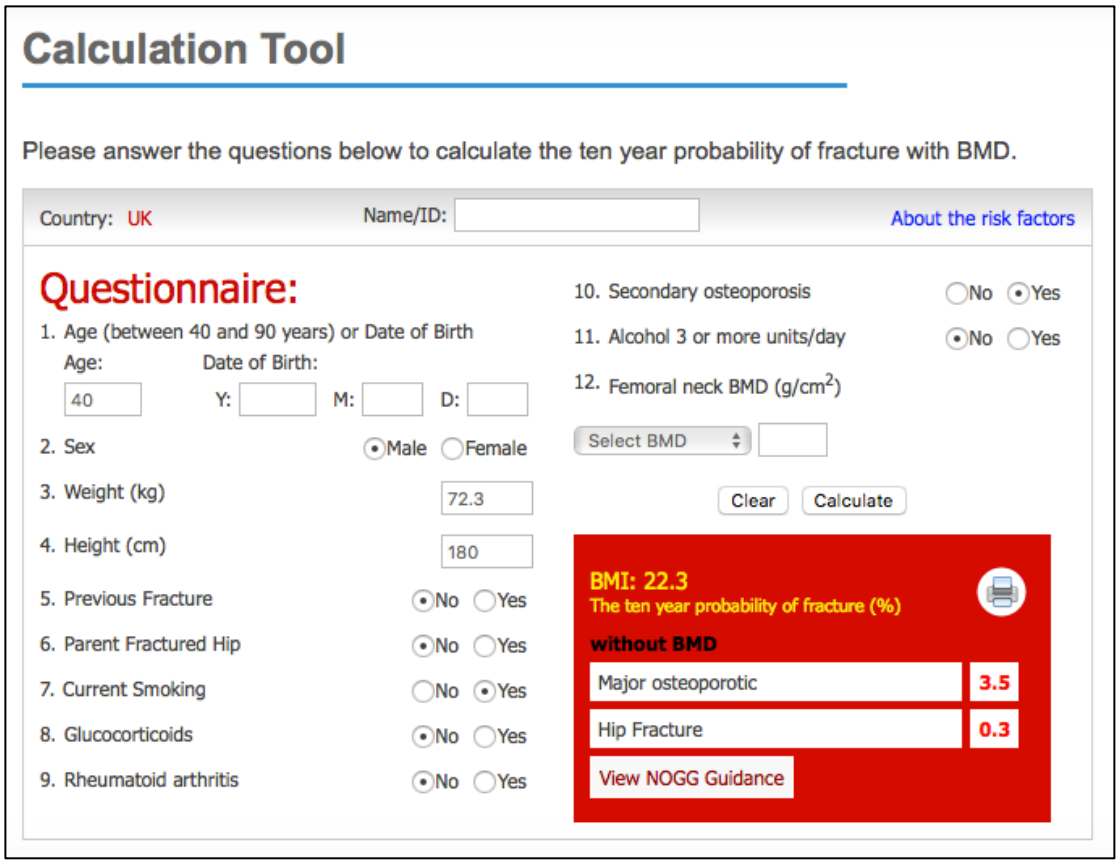


Figure 2.1. Example of data entry into the FRAX® calculator, without femoral neck BMD, to generate 10-year probability of major osteoporotic fracture and hip fracture (www.sheffield.ac.uk/FRAX)

2.3 Selection of patients for further analysis within study Phase Two

2.3.1 Phase Two patient sample size calculation and selection strategy

Patient selection for the Phase Two study was informed by a sample size calculation, to ensure that analysis would be possible to assess the difference in BMD between three equal groups of patients (tertiles) with either low, intermediate or high FRAX®-calculated 10-year probability of major osteoporotic fracture.

The expected difference in BMD between the lowest and highest FRAX® probability tertiles is at least one population standard deviation (s.d.) (approximately 0.1g cm^{-2}) which approximates to a doubling of fracture risk,

but even smaller differences are of clinical relevance. A total of 39 patients per tertile would allow detection of a 0.65 s.d. difference with 80% power at a significance of $p < 0.05$. The sample size was therefore set at 40 patients per tertile.

Patients were recruited for Phase Two study analysis from the Phase One study cohort based upon Phase One FRAX®-calculated 10-year probabilities of major osteoporotic fracture (without femoral neck BMD data), with the aim of recruiting patients equally (1:1:1) from low, intermediate and high fracture risk groups, to create equal-sized low, intermediate and high fracture risk tertiles of 40 patients each (120 patients in total) for Phase Two study analysis.

In order to be able to compare patients of black race *versus* white race, patient recruitment from the Phase One study population for Phase Two study analysis was also stratified simultaneously to recruit equal numbers of black and white patients, i.e. 60 within each racial subgroup.

2.3.2 Phase Two inclusion criteria

All HIV-1 seropositive patients aged 18 or above, receiving their HIV care within the Sheffield HIV Cohort, were potentially eligible for recruitment into Phase Two of the study.

2.3.3 Phase Two exclusion criteria

The following patients were excluded from the Phase Two study:

- HIV-2 seropositive individuals;
- patients of non-black non-white race;
- patients currently or previously on bone replacement therapy (e.g. a bisphosphonate);

- patients currently or previously on hormone replacement therapy (including testosterone replacement therapy in men);
- patients for whom clinical data was incomplete, e.g. an incomplete ART history;
- patients with concurrent illness or recent vaccination within the preceding 14 days of their study visit;
- patients on current oral or inhaled glucocorticoid therapy;
- pregnant females;
- patients with a history of any known condition that would interfere with the acquisition and/or assessment of DXA scans at either the hip, spine or whole body site, including more than one prosthesis (a single prosthesis being acceptable);
- bilateral fractures or replacement at the hip.

Patients with a history of current or previous vitamin D and/or calcium replacement or supplementation were not excluded from the Phase Two study, but were excluded from 25-OH D and related biochemical data analyses.

2.4 Methods for Phase Two

2.4.1 Phase Two recruitment, patient information and informed consent

Phase Two study patients were recruited over a 25-month period, from 30 April 2013 to 31 May 2015. Patients were approached during their routine HIV outpatient clinic attendances. Patient study eligibility was assessed by questionnaire. Eligible patients were provided with a Patient Information Sheet. Interested and eligible patients were offered a separate study visit appointment. Patients were instructed to attend between 8am and 10am, following an overnight fast. At the study visit, patient eligibility was re-confirmed. Pregnancy was excluded in pre-menopausal female patients by

same day urine β-human chorionic gonadotrophin (β-hCG) testing. Patient informed consent was obtained from all study participants before proceeding with further study assessments.

2.4.2 Re-assessment of patient demographics, fracture prevalence and prevalence of risk factors for reduced BMD

The same clinical data, as collected for Phase One of the study (Section 2.2), was re-collected for all Phase Two patients by patient interview and by medical record review. Data was collected and recorded using the same pre-designed questionnaire.

2.4.3 Venous blood and urine sampling

Venous blood and midstream urine was collected from fasted study participants between 8am and 10am.

Venous blood samples were tested for: CD4 cell count and percentage, CD8 cell count and percentage and CD4:CD8 ratio (performed by STH Cell Markers Laboratory); HIV viral load (performed by STH Virology Laboratory – see Section 2.2 for details); total 25-OH-D (see Section 2.2 for details), PTH, ALP, corrected calcium, phosphate, liver function, renal function (including creatinine), glucose, bicarbonate, insulin-like growth factor-1 (IGF-1), testosterone (in male volunteers only), sex hormone binding globulin, luteinising hormone, follicule stimulating hormone and oestradiol (performed by STH Biochemistry Laboratory); highly sensitive CRP (hs-CRP) (performed by STH Immunology Laboratory) and D-dimer (performed by STH Coagulation Laboratory). An additional 40ml of blood was collected in a pre-heparinised Falcon tube for further analysis (see Section 2.4.4).

In addition to dipstick testing for the presence of β-hCG in premenopausal women, to exclude pregnancy prior to undergoing imaging studies, urine was

also tested by dipstick for the presence of glucose and protein. Urine was subsequently analysed (in parallel with serum creatinine and phosphate) for protein, albumin, microalbumin, phosphate and creatinine, to enable calculations of protein and albumin creatinine ratios and tubular resoprtion of phosphate (analysis and calculations performed by STH Clinical Chemistry Laboratory). Urine was also analysed for retinal binding protein (performed by STH Immunology Laboratory).

2.4.4 Peripheral blood mononuclear cell isolation

Whole blood (obtained as described in Section 2.4.3) was subjected to density centrifugation (RAD 500 at 4°C for 23 minutes) using Ficoll-Paque to separate plasma and peripheral blood mononuclear cells (PBMCs). Plasma was separated and transferred to cryovials as 1ml aliquots and stored at -80°C, for subsequent interleukin-6 (IL-6) measurement (see Section 2.4.6). Freshly isolated PBMCs were washed twice in phosphate buffered saline (PBS) and resuspended in PBS + 0.1% bovine serum albumin (BSA) (FACS buffer) at a concentration of $1x10^6$ cells ml^{-1}.

2.4.5 Flow cytometry

The percentage of activated $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells was assessed by expression of CD25 and HLA-DR – an MHC class II cell surface receptor (the gating strategy for $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cell analysis is displayed in Figure 2.2). The percentage of monocytes within classical, non-classical and intermediate populations was determined using relative CD14 and CD16 expression (the monocyte gating strategy is displayed in Figure 2.3).

PBMCs resuspended in FACS buffer at a concentration of $1x10^6$ cells ml^{-1} (obtained in Section 2.4.3) underwent further centrifugation (RAD 500 at 4°C for 10 minutes). Cell pellets were resuspended in 100µl FACS buffer and

incubated for 45 minutes with 2 µl of mouse anti-human antibodies (1µg ml^{-1}) against cell surface markers, summarised in Table 2.2, and with 1 µl UV/DMSO (LIVE/DEAD Fixable Blue Cell Stain Kit, UV excitable, Life technologies). Unstained cells, single antibody stained cells and "fluorescence minus one" stained cells were processed simultaneously as controls. 2 µl of mouse anti-human antibodies (1µg ml^{-1}) was added to compensation beads (ArC Amine Reactive and AbC anti-mouse compensation beads, Life Technologies) in 100µl FACS buffer and incubated simultaneously. Following incubation, cells and beads were washed twice in FACS buffer and fixed in 1% paraformaldehyde.

Flow cytometric measurements were performed using a four-colour LSRII flow cytometer (BD Biosciences). Forward and side scatter light was used to identify cell populations by size and granularity. Flow cytometric measurements were used to determine the percentage of CD25 and HLA-DR expression in both $CD3^+/CD4^+$ and $CD3^+/CD8^+$ viable lymphocytes, and to determine the relative percentages of classical, intermediate and non-classical viable monocytes using their relative CD14 and CD16 expression. In all flow cytometry experiments, 50,000 events were captured and analysed with *FlowJo*™ software version 10.0.6 (Tree Star, Inc.).

Human cell marker	Fluorochrome	Laser/voltage	Manufacturer
CD3	fluorescin isothiocyanate (FITC)	Blue 530	eBioscience
CD4	peridinin chlorophyll protein (PerCP)-eFluor® 710	Blue 695	eBioscience
CD8	Brilliant Violet 421	Violet 450	BioLegend
CD14	allophycocyanin (APC)-eFluor® 780	Red 780	eBioscience
CD16	PE (phycoerythrin)-Cy7	Blue 780	eBioscience
CD25	PE	Blue 575	eBioscience
HLA-DR	Alexa Fluor®780	Red 730	eBioscience

Table 2.2. Mouse anti-human antibodies used to determine cell surface marker expression

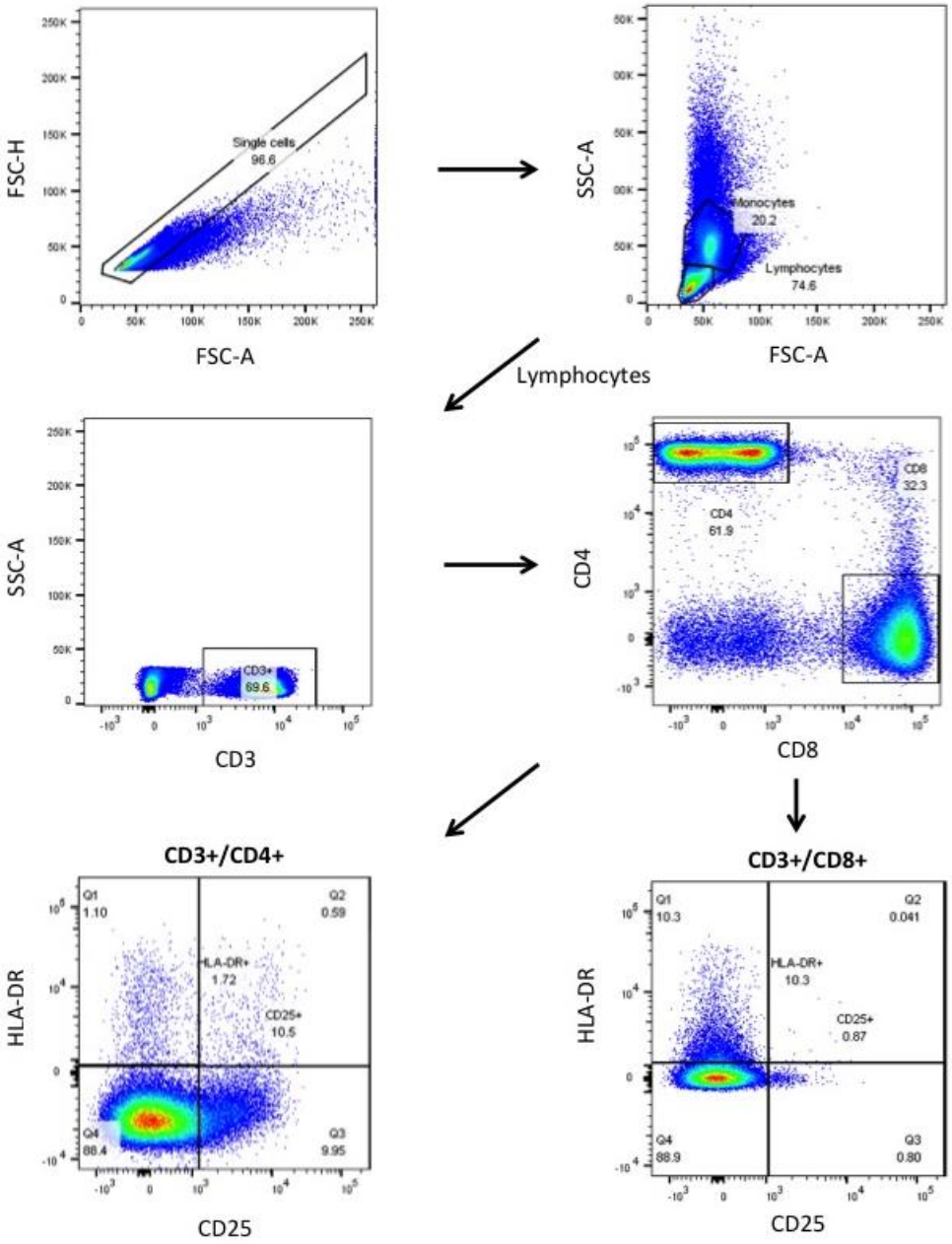


Figure 2.2. Flow cytometry gating strategy for $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cell analysis of CD25 and HLA-DR expression

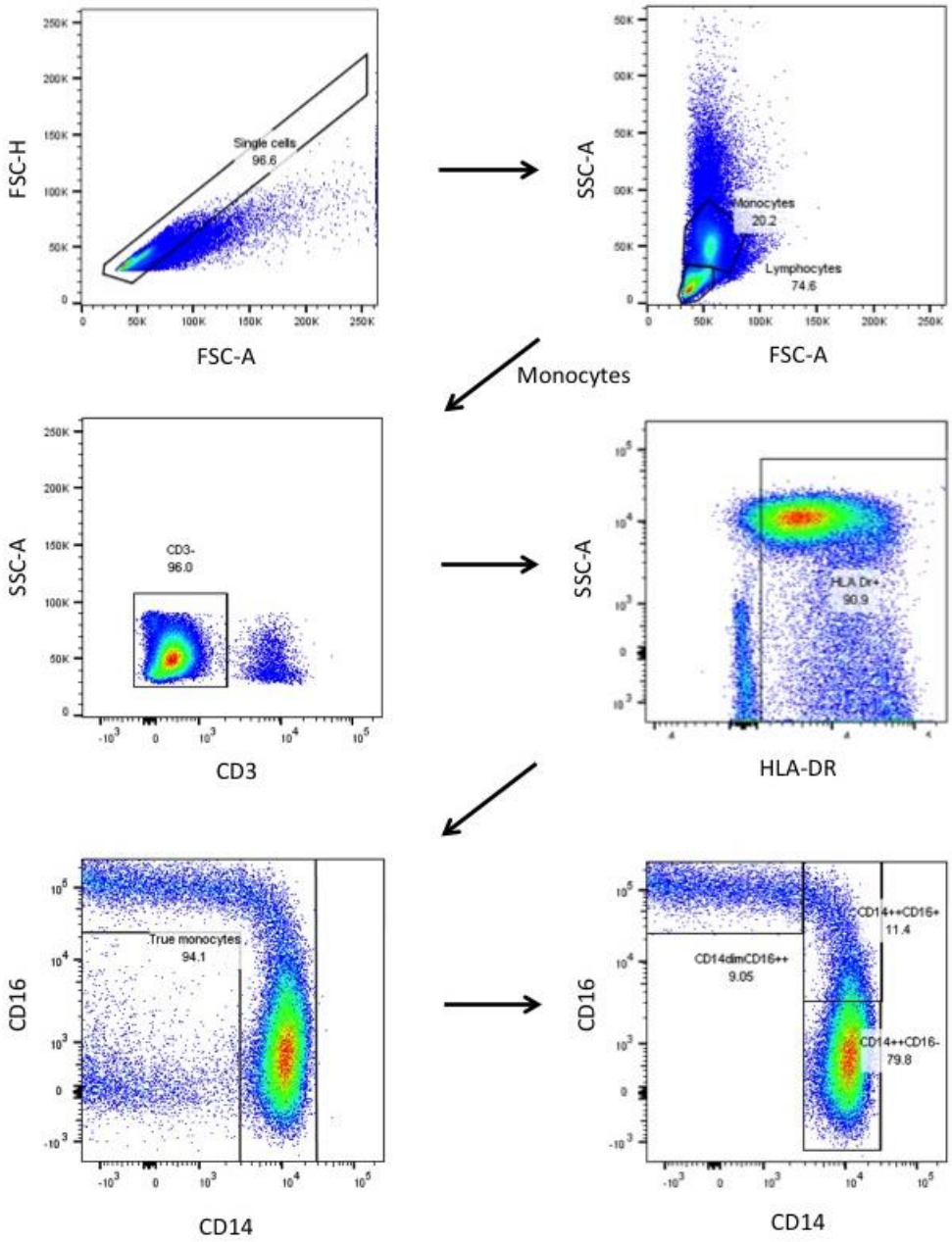


Figure 2.3. Flow cytometry gating strategy for analysis of monocyte subpopulations

2.4.6 Measurement of interleukin-6

IL-6 was quantified on plasma samples (obtained as described in Section 2.4.4) from a subset of Phase Two study patients using the Quantiglow® IL-6 specific enzyme-linked immunosorbant assay (QS6000B, R&D Systems). Methods were carried out in accordance with the standard protocol provided by the manufacturer.

Standards were generated by serial dilution of a reconstituted IL-6 top standard (30,000 pg ml^{-1}). The standard curve produced included values of 1500, 300, 60, 12, 2.4 and 0.48 pg ml^{-1}.

100 µl of assay diluent was added to each well of the 96-well IL-6 microplate. 100 µl of either thawed Phase Two study patient plasma or a standard solution was then added to each well. Wells were then sealed and incubated at room temperature on a horizontal orbital plate shaker (Helidorph Rotamax, 150 rpm) for 2 hours. Following incubation, the microplate was washed four times with wash buffer. 200 µl of IL-6 conjugate was then added to each well. Wells were re-sealed and incubated at room temperature on the plate shaker for a further 3 hours. Following the second incubation, the washing step was repeated. 100 µl of working glow reagent (4 ml glow reagent A and 8ml glow reagent B) was then added to each well. The wells were then re-sealed and the microplate immediately wrapped in foil and incubated at room temperature for a further 15 minutes.

Following incubation, the microplate was transferred to the Varioskan® *Flash* (Thermoskan Scientific) plate reader. Relative light units were measured using the following settings: 1 minute lag time; 0.5 second per well read time; summation mode; auto gain. Results were collected using *Scan it RE for Varioskan®* version 2.4.3 software. Data was analysed using *Graph Pad Prism* version 6.0, within which a standard curve was generated and Phase Two study patient plasma IL-6 concentration was extrapolated.

2.4.7 Imaging

DXA was used to assess BMD at the lumbar spine, total hip, femoral neck and total body and to assess body composition. The prevalence of sub-clinical vertebral fractures was investigated by vertebral fracture assessment (VFA). All examinations were performed using a Discovery A bone densitometer (Hologic Inc., Bedford, MA, USA).

2.4.8 Calculation of 10-year probabilities of major osteoporotic fractures and hip fractures using FRAX®

10-year probabilities of major osteoporotic fractures and hip fractures were re-calculated using FRAX® (as described for Phase One study patients in Section 2.2), initially without femoral neck BMD and without including HIV as a "secondary osteoporosis" risk factor. FRAX® probabilities were then recalculated with incorporation of HIV as a "secondary osteoporosis" risk factor (Figure 2.4) (for patients with one or other significant disorder significantly associated with reduced BMD (not including HIV), "secondary osteoporosis" was already included within the original FRAX® calculation and therefore FRAX® probabilities were not altered by inclusion of HIV). Finally, FRAX® probabilities were recalculated with inclusion of femoral neck BMD data, but without including HIV as a "secondary osteoporosis" risk factor (Figure 2.5).

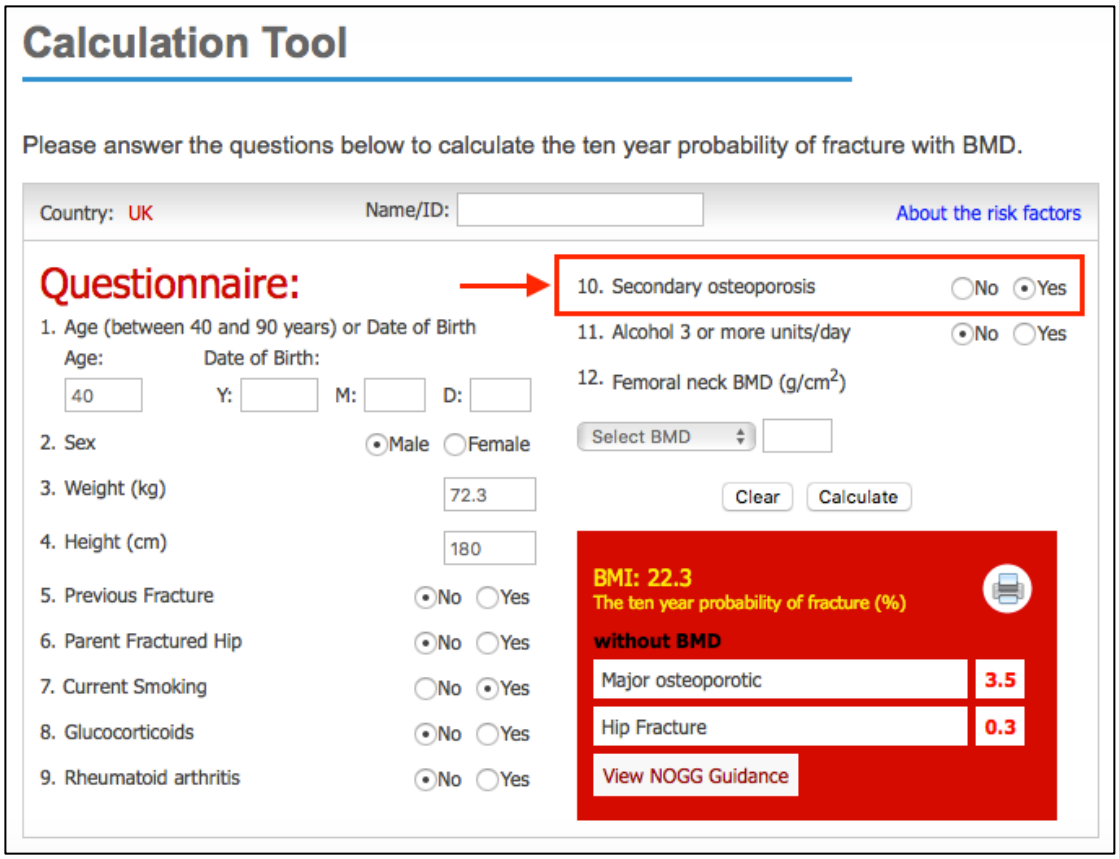


Figure 2.4. Example of data entry into the FRAX® calculator, without femoral neck BMD, but with inclusion of HIV as a "secondary osteoporosis" risk factor, to generate 10-year probability of major osteoporotic fracture and hip fracture (www.sheffield.ac.uk/FRAX)

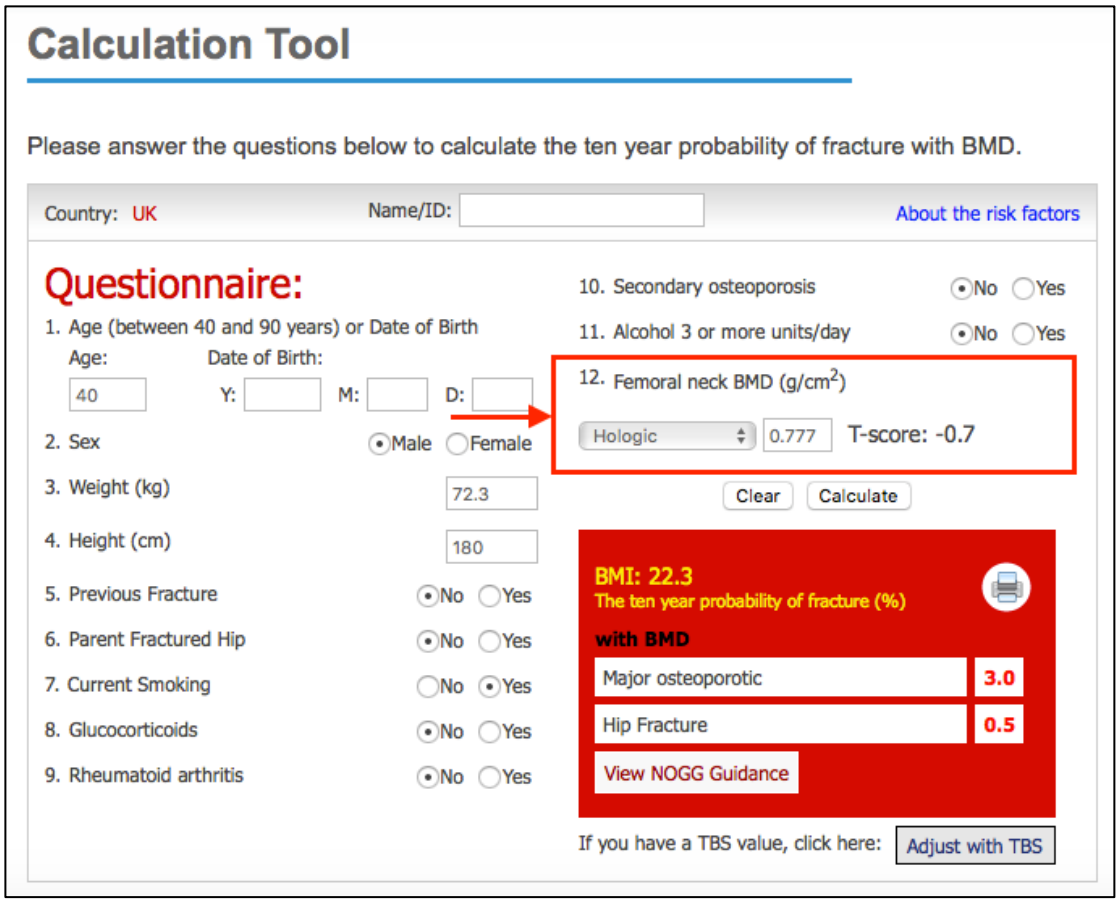


Figure 2.5. Example of data entry into the FRAX® calculator, with femoral neck BMD, to generate 10-year probability of major osteoporotic fracture and hip fracture (www.sheffield.ac.uk/FRAX)

2.5 Statistical Methods

Statistical analyses were performed using *IBM® SPSS® Statistics* software (version 25). Statistical significance was defined as a two-tailed *p*-value < 0.05.

Continuous variables were assessed for normality using histograms and normality plots. Pearson correlation was used to assess the relationship between two parametric continuous variables; Spearman correlation was used to assess the relationship between two continuous variables including at least one non-parametric variable. The difference in the distribution of a parametric continuous variable between two populations was analysed using the independent sample (unpaired) T Test. The difference in the distribution of a non-parametric continuous variable between two populations was analysed using the Mann-Whitney U Test.

Respective analyses were performed for all patients within each of the Phase One and Phase Two study populations, as well as within black and white racial subgroups and within black male, black female, white male and white female race / gender subgroups.

The primary outcome measure within the Phase Two study was BMD, measured at the lumbar spine, hip and femoral neck and for total body. Independent predictors of BMD were assessed using a generalised linear regression model (further details provided in Chapter 4, Section 4.9). Generalised linear regression models were also used to determine independent predictors of 25-OH-D (using Phase One data) and serum phosphate and tubular resoprtion of phosphate (using Phase Two data) (further details provided in Chapter 5).

The correlations between FRAX® probabilities (with and without inclusion of HIV as a “secondary osteoporosis” risk factor) and BMD were calculated in all Phase Two patients and within black and white patient subgroups. Differences in BMD between Phase Two patients within either low, intermediate or high

FRAX® probability tertiles were also analysed. The percentage change in FRAX® probabilities calculated with femoral neck BMD compared to without femoral neck BMD was described within black and white patient subgroups.

3. Prevalence of general and HIV disease-specific fracture risk factors and fractures in people living with HIV in Sheffield

3.1 Introduction

In the era of widely available and effective combination ART, the incidence of opportunistic infections in PLWH has significantly decreased (Palella *et al.* 1998) and, with access to ART, life expectancy for PLWH is now expected to be similar to people who are HIV-negative (Nakagawa *et al.* 2012). Over the past two decades, however, it has become evident that a spectrum of NICMs, including reduced BMD and increased fragility fractures, can also cause significant morbidity and mortality in PLWH and that these NICMs are more prevalent in PLWH than in age-, gender- and ethnicity-matched controls (Guaraldi *et al.* 2011).

On account of a higher prevalence of reduced BMD (Brown and Qaqish 2006) there is concern that, as PLWH age, they will experience a higher incidence of fragility fracture and increased associated morbidity and mortality compared to the general population. Several large retrospective population studies have documented an increased incidence of all fractures and specifically fragility fractures (defined by site of fracture) in PLWH *vs.* age- and sex-matched HIV-negative controls (Triant *et al.* 2008, Young *et al.* 2011, Hansen *et al.* 2012) and, in addition, have observed an increase in fracture incidence with increased age in PLWH (Triant *et al.* 2008).

Reduced BMD and increased fracture incidence in PLWH are most likely attributable to a combination of factors, including an over-representation of general fracture risk factors – both those incorporated into the FRAX® assessment tool (Table 2.1) and those not incorporated into FRAX® – as well as HIV disease-specific fracture risk factors, including ART-related risk factors and non-ART-related risk factors (Goh *et al.* 2016, Hoy and Young 2016).

With respect to general fracture risk factors, multiple studies have shown that many of these are more prevalent in PLWH such as rates of smoking (Fuster

et al. 2009; Levine *et al.* 2010), alcohol and other substance misuse, particularly amongst MSMs (Mimiaga *et al.* 2008; Baliunas *et al.* 2009; Browne and Wechsberg 2010; Reisner *et al.* 2010; Shuper *et al.* 2010).

The relative contribution of these general risk factors to reduced BMD and increased fracture risk in PLWH relative to the contribution of HIV disease-specific risk factors – including nadir CD4 count and past history of AIDS-defining illness (Cazanave *et al.* 2008, Gedmintas *et al.* 2017), HCV co-infection (Lo Re V *et al.* 2009, Gedmintas *et al.* 2017) and exposure to specific ARVs, including PIs (Brown and Qaqish 2006, McComsey *et al.* 2011, Bedimo *et al.* 2012) and tenofovir DF (Gallant *et al.* 2004, McComsey *et al.* 2011, Cervero *et al.* 2018) – is, however, not known. It is therefore not yet established whether the use of FRAX® as a tool for assessment of fragility fracture risk – incorporating general fracture risk factors only – is appropriate in PLWH, in spite of FRAX® being recommended for use in PLWH in both national and international HIV guidelines (BHIVA 2016, EACS 2017).

The Sheffield HIV Cohort is more than 50% Black and more than 40% female, in contrast to the predominantly white male HIV cohorts in which reduced BMD and/or increased fracture risk have mostly been reported. BMD, fracture risk and fracture incidence may therefore differ significantly within the Sheffield HIV Cohort compared to other published HIV cohorts, particularly if fracture risk factor prevalence differs significantly between patients of different race and gender.

This chapter aims to answer the following questions:

1. How prevalent are fracture risk factors and fractures in PLWH within the Sheffield HIV Cohort?
2. Is there a difference in the prevalence of different fracture risk factors in PLWH according to race and gender?
3. Can an overexpression of general fracture risk factors account for fragility fracture incidence alone, and therefore could FRAX® be a valid tool for the assessment of fragility fracture risk in PLWH, or do additional non- FRAX®

incorporated general risk factors and/or HIV disease-specific risk factors contribute also?

3.2 Phase One study population demographics: race, ethnicity, gender, age, height, weight and BMI

625 patients were recruited to Phase One of the study from the Sheffield HIV Cohort. 53.0% were of black race (n = 331: Black African = 323; Black British = 6; Black Caribbean = 2), 43.8% of white race (n = 274: White British or White Irish = 248; White European = 21; White Other = 5) and 3.2% of non-black non-white race (n = 20: mixed ethnicity = 9; South East Asian = 6; Afghan = 2; Arabic = 1; Indian = 1; Sri Lankan = 1). Patients originated from 48 different countries: 51.8% of patients originated from African countries (n = 324); 41.8% from the UK or Republic of Ireland (n = 261); and 6.4% from other countries (n = 40) (as listed in Table 3.1).

Black patients were predominantly female (65.0%), compared with white and non-black non-white patients who were predominantly male (82.5% and 65.0% respectively).

Patient age, height, weight and BMI are summarised by patient race and gender in Table 3.2. The distribution of patient age, height, weight and BMI in black male, black female, white male and white female patient subgroups are compared in Figures 3.1 to 3.4. On account of the relatively small number of patients of non-black non-white race recruited, statistical analyses were not performed within this racial subgroup.

The mean patient age was 40.7 ± 9.6 years. White patients were significantly older than black patients overall ($p = 0.002$), although there was no significant difference in patient age between black male and black female ($p = 0.076$), white male and white female ($p = 0.185$), black male and white male ($p = 0.109$) and black female and white female ($p = 0.642$) patient subgroups.

Continent / country	n	**Continent** / country	n	**Continent** / country	n
Africa	**324**	**Europe**	**281**	**Central America**	**2**
Zimbabwe	188	United Kingdom	259	Jamaica	2
Zambia	25	Portugal	9		
Eritrea	14	Italy	2	**South America**	**4**
Ethiopia	11	Poland	2	Argentina	1
Somalia	11	Republic of Ireland	2	Brazil	1
South Africa	9	Slovakia	2	Chile	1
Cameroon	8	Spain	2	Guyana	1
Congo	8	Denmark	1		
Nigeria	8	Germany	1		
Kenya	6	Greece	1		
Malawi	6				
Uganda	5	**Asia & Middle East**	**11**		
Burundi	4	Thailand	3		
Ivory Coast	4	Afghanistan	2		
Liberia	4	Malaysia	2		
Tanzania	3	Burma	1		
Angola	2	India	1		
Sierra Leone	2	Iraq	1		
Botswana	1	South Korea	1		
D.R.C.	1				
The Gambia	1	**Australasia**	**1**		
Ghana	1	Australia	1		
Mauritius	1				
Mozambique	1				
Senegal	1				

Table 3.1. Country of birth of patients within the Phase One study population (D.R.C. = Democratic Republic of Congo)

The mean patient BMI was 26.6 ± 5.1 kg m^{-2}. Black patients had a significantly higher BMI than white patients ($p < 0.001$), with black females having a significantly higher BMI than both black males and white females ($p < 0.001$ and $p = 0.025$ respectively), although black male BMI was not significantly higher than white male BMI ($p = 0.185$), nor was white female BMI significantly higher than white male BMI ($p = 0.068$).

	All (n = 625)	Black (n = 331)		White (n = 274)		Non-Black Non-White (n =20)	
		Male (n = 116)	Female (n = 215)	Male (n = 226)	Female (n = 48)	Male (n = 13)	Female (n = 7)
Mean age ± s.d. *years*	40.7 ± 9.6	40.7 ± 9.0	38.9 ± 7.9	42.8 ± 10.5	40.5 ± 12.4	37.3 ± 9.5	35.1 ± 9.3
Mean height ± s.d. *m*	171 ± 9.8[a]	1.76 ± 0.07[b]	1.63 ± 0.06[c]	1.78 ± 0.07[d]	1.63 ± 0.08	1.76 ± 0.05	1.63 ± 0.11
Mean weight ± s.d. *kg*	78.0 ± 15.6[e]	80.0 ± 15.7[f]	76.7 ±15.4[g]	80.03 ± 14.8	71.4 ± 18.0[h]	75.1 ± 7.9	66.4 ± 23.3
Mean BMI ± s.d. *kg m^{-2}*	26.6 ± 5.1[i]	25.7 ± 4.7[j]	28.7 ± 5.5[k]	25.3 ± 4.4[l]	26.6 ± 5.4[m]	24.3 ± 2.3	24.7 ± 6.9

[a]5 missing values [b]1 missing value [c]3 missing values [d]1 missing value
[e]3 missing values [f]1 missing value [g]1 missing value [h]1 missing value
[i]6 missing values [j]1 missing value [k]3 missing values [l]1 missing value [m]1 missing value

Table 3.2. Age, height, weight and BMI for all (n=625), black (n=331), white (n=274) and non-black non-white (n=20) patient subgroups within the Phase One study population

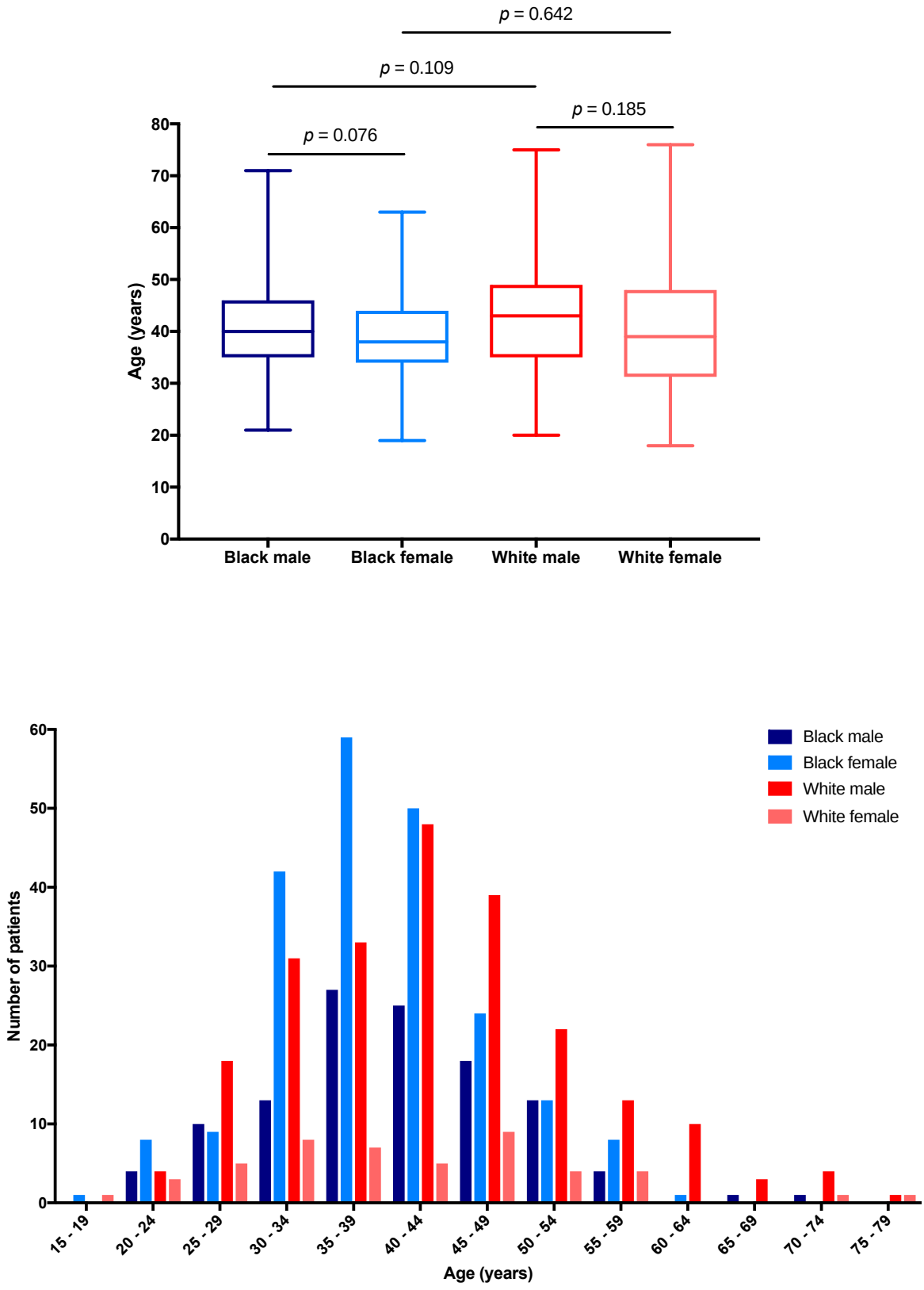


Figure 3.1. Distribution of age in black male (n = 116), black female (n = 215), white male (n = 226) and white female (n = 48) patients within the Phase One study population

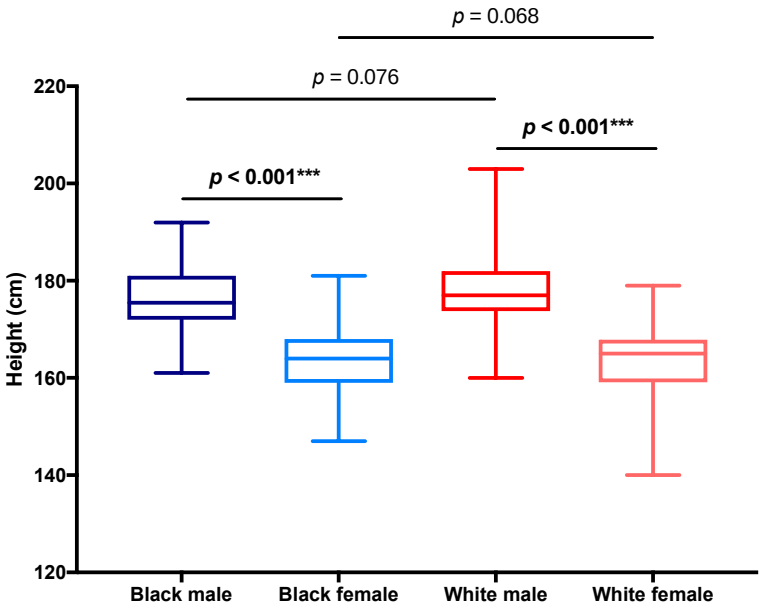


Figure 3.2. Distribution of height in black male (n = 115), black female (n = 212), white male (n = 225) and white female (n = 48) patients within the Phase One study population

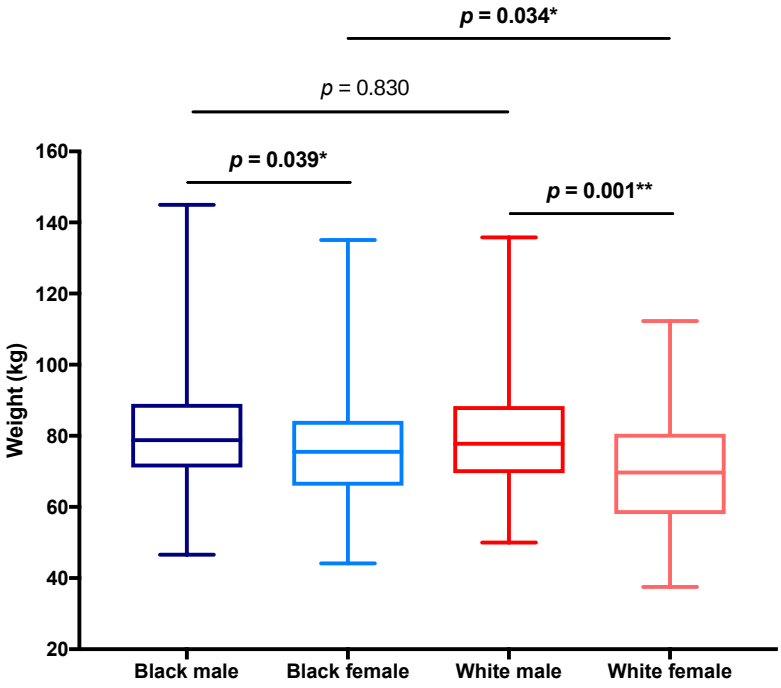


Figure 3.3. Distribution of weight in black male (n = 115), black female (n = 214), white male (n = 226) and white female (n = 47) patients within the Phase One study population

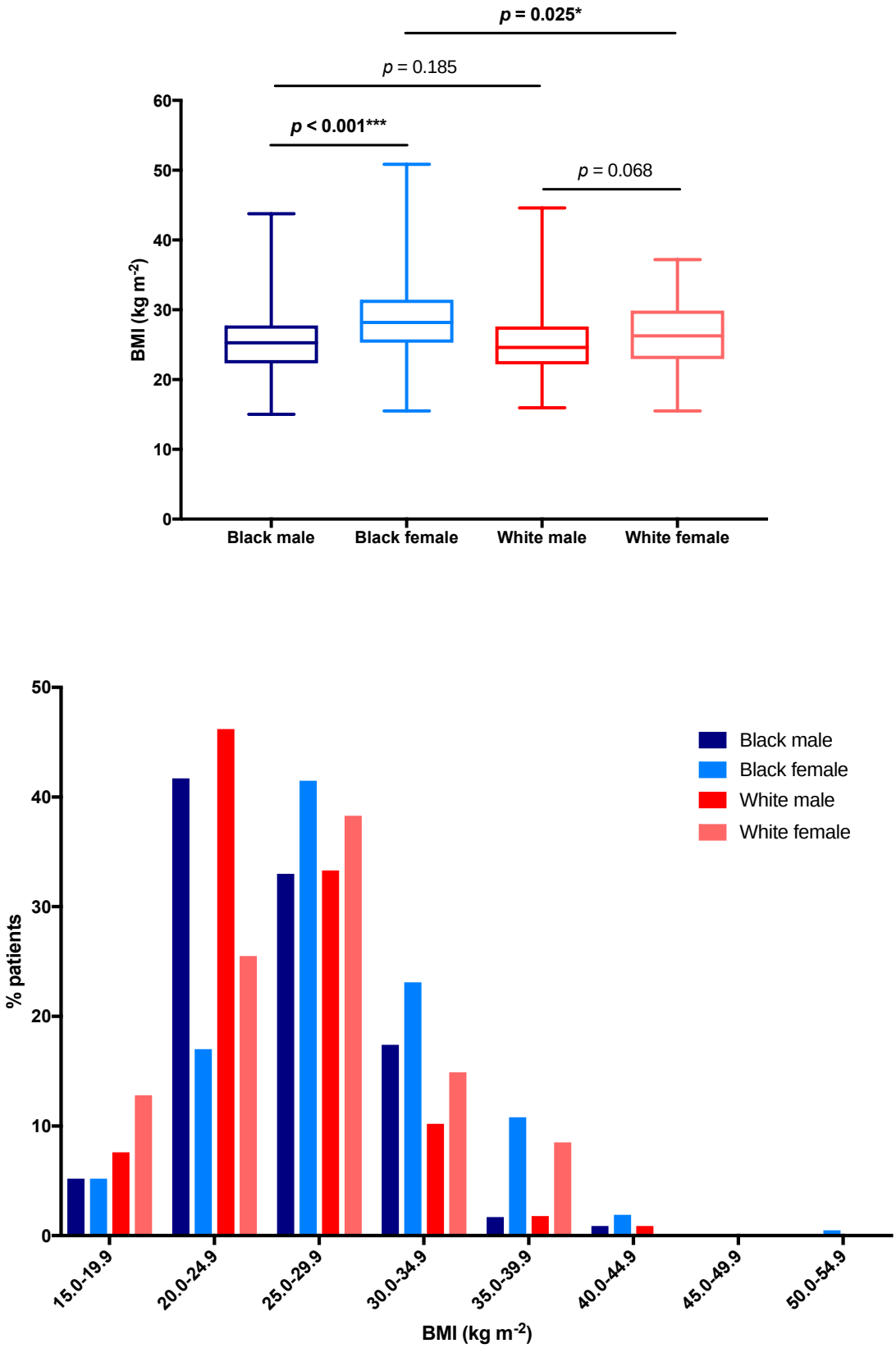


Figure 3.4. Distribution of BMI in black male (n = 115), black female (n = 214), white male (n = 226) and white female (n = 47) patients within the Phase One study population

3.3 *Prevalence of HIV disease-specific risk factors*

HIV disease-specific risk factors for reduced BMD and/or fragility fracture for the Phase One study population are summarised by race and gender in Table 3.3. The distributions of nadir CD4 cell count and current CD4 cell count in black male, black female, white male and white female patient subgroups are compared in in Figure 3.5 and Figure 3.6 respectively.

The median nadir CD4 cell count for all recruited patients was 217 cells μL^{-1} (range 1 to 1050). The nadir CD4 cell count was significantly lower in black patients than in white patients ($p < 0.001$) and in black males than in white males ($p < 0.001$), but was not significantly lower in black females compared with white females ($p = 0.098$), black males compared with black females ($p = 0.095$) or white males compared with white females ($p = 0.538$).

The median current cell CD4 count for all recruited patients was 457 cells μL^{-1} (range 2 to 1842). Whilst there was no significant difference in current CD4 count between black and white patients overall ($p = 0.054$), current CD4 cell count was significantly lower in black males compared with both white males ($p = 0.002$) and black females ($p = 0.001$) respectively, with no significant difference between white and black females ($p = 0.154$) or between white males and white females ($p = 0.199$).

78.2% of recruited patients had a history of having ever been on ART, 73.8% were currently taking ART and 63% of patients had a suppressed HIV viral load, i.e. plasma HIV RNA < 40 copies ml^{-1} on their most recent sample. A slightly greater proportion of black patients had a history of prior ART, were on current ART and had a suppressed HIV viral load compared with white patients, both overall and when comparing black males with white males and black females with white females. The proportions of patients with a history of prior ART, on current ART and with a suppressed HIV viral load were slightly higher in both black males and white males compared with black females and white females respectively.

	All (n = 625)	Black (n = 331)		White (n = 274)		Non-Black Non-White (n = 20)	
		Male (n = 116)	Female (n = 215)	Male (n = 226)	Female (n = 48)	Male (n = 13)	Female (n = 7)
Median nadir CD4 cell count	217[a]	187[b]	198[c]	248[d]	265[e]	304	144
(range) *cells μL^{-1}*	(1 – 1050)	(2 – 578)	(2 294)	(1 – 1050)	(1 – 1020)	(8 – 856)	(33 – 423)
Median current CD4 cell count	457[f]	405	488[g]	468	550	382	344
(range) *cells μL^{-1}*	(2 – 1842)	(2 – 1019)	(16 – 1189)	(13 – 1842)	(4 – 1144)	(91 – 921)	(106 – 632)
Ever ART *n (%)*	489 (78.2)	98 (84.5)	178 (82.8)	169 (74.8)	34 (70.8)	7 (53.8)	4 (57.1)
Current ART *n (%)*	461 (73.8)	96 (82.8)	160 (74.4)	164 (72.6)	29 (60.4)	7 (53.8)	4 (57.1)
Plasma HIV RNA *copies ml^{-1}*	393 (63.0)[h]	78 (67.2)	149 (69.6)[i]	133 (58.8)	24 (50.0)	5 (38.5)	4 (57.1)

[a]19 missing values
[b]2 missing values
[c]9 missing values
[d]6 missing values
[e]2 missing values
[f]1 missing value
[g]1 missing value
[h]1 missing value
[i]1 missing value

Table 3.3. Nadir and current CD4 cell count, ART exposure and current plasma HIV RNA for all (n = 625), black (n = 331), white (n = 274) and non-black non-white (n = 20) patients within the Phase One study population

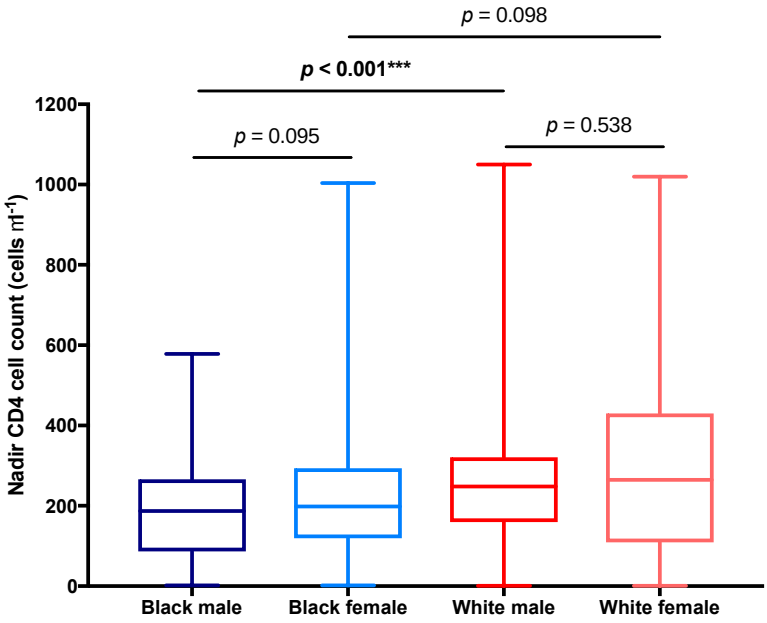


Figure 3.5. Distribution of nadir CD4 cell count in black male (n = 114), black female (n = 206), white male (n = 220) and white female (n = 44) patients within the Phase One study population

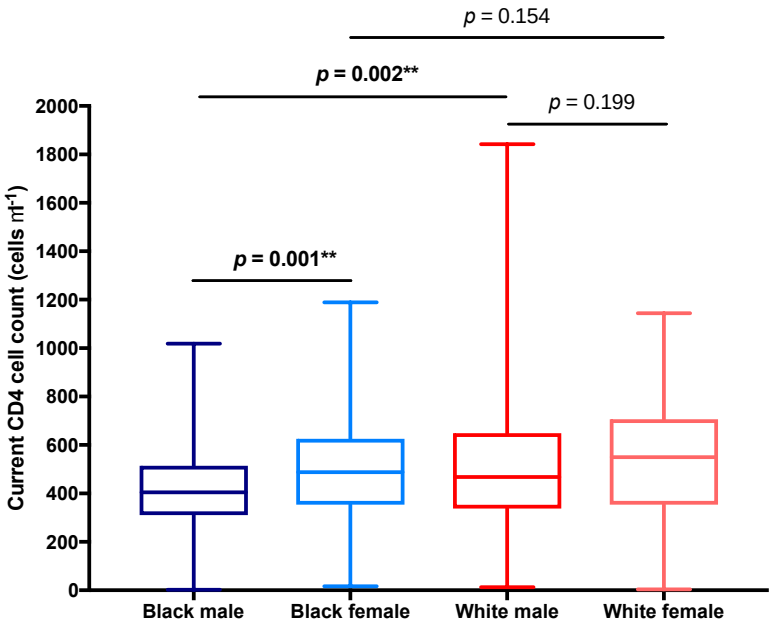


Figure 3.6. Distribution of current CD4 cell count in black male (n = 116), black female (n= 214), white male (n = 226) and white female (n = 48) patients within the Phase One study population

Current and ever ARV exposure is summarised for all patients by ARV drug class in Table 3.4 and by main NRTI in Table 3.5.

Approximately twice as many patients were either currently on or had ever taken the NRTI tenofovir DF compared with the NRTI abacavir. Approximately twice as many patients were either currently on or had ever taken an NNRTI compared with a PI. Very small proportions of patients had a history of exposure, either currently or previously, to ARVs from the INI, CCR5 receptor antagonist or fusion inhibitor drug classes.

3.4 Falls prevalence

The number of self-reported falls, classified into no falls, one single fall or multiple falls, within the preceding 12 months is detailed in Table 3.6 for all patients and by patient race and gender subgroups. Overall, 541 patients (86.7%) reported no falls, 47 (7.5%) one single fall and 36 (5.8%) multiple falls (median 5, range 2 to 365). There was no difference in the relative proportions of patients reporting no falls *vs.* one single fall *vs.* multiple falls when comparing black male, black female, white male and white female patient subgroups (Figure 3.7).

In general, neither age nor BMI had a significant bearing as to whether patients self-reported no falls, one single fall or multiple falls in the preceding 12 months, neither in all Phase One patients, nor within black male, black female, white male and white female subgroups overall, except within black female patients in whom patients reporting multiple falls were significantly older than patients reporting one single fall ($p = 0.037$) and within white female patients in whom patients reporting multiple falls had a significantly higher BMI than patients reporting either no falls ($p = 0.014$) or one single fall ($p = 0.033$) (Figures 3.8 and 3.9).

ARV class	Exposure	All (n = 625)	Black (n = 331)		White (n = 274)		Non-Black Non-White (n = 20)	
			Male (n = 116)	Female (n = 215)	Male (n = 226)	Female (n = 48)	Male (n = 13)	Female (n = 7)
NRTI	**Current** *n* (%)	455 (72.8)	95 (81.9)	161 (74.9)	161 (71.2)	28 (58.3)	7 (53.8)	4 (57.1)
	Ever *n* (%)	488 (78.1)	98 (84.5)	178 (82.8)	167 (73.9)	34 (70.8)	7 (53.8)	4 (57.1)
NNRTI	**Current** *n* (%)	302 (48.3)	75 (64.7)	101 (47.0)	102 (45.1)	15 (31.3)	6 (46.2)	3 (42.9)
	Ever *n* (%)	394 (63.0)	91 (78.4)	137 (63.7)	134 (59.3)	22 (45.8)	7 (53.8)	3 (42.9)
PI	**Current** *n* (%)	168 (26.9)	24 (20.7)	61 (28.3)	66 (29.2)	16 (33.3)	0 (0.0)	1 (14.3)
	Ever *n* (%)	240 (38.4)	31 (26.7)	95 (44.2)	91 (40.3)	21 (43.8)	1 (7.7)	1 (14.3)
INI	**Current** *n* (%)	11 (1.8)	0 (0.0)	0 (0.0)	10 (4.4)	1 (2.1)	0 (0.0)	0 (0.0)
	Ever *n* (%)	13 (2.1)	1 (0.9)	0 (0.0)	11 (4.9)	1 (2.1)	0 (0.0)	0 (0.0)
CCR5 receptor antagonist	**Current** *n* (%)	3 (0.5)	0 (0.0)	2 (0.9)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
	Ever *n* (%)	4 (0.6)	0 (0.0)	3 (1.4)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Fusion inhibitor	**Current** *n* (%)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
	Ever *n* (%)	7 (1.1)	0 (0.0)	0 (0.0)	5 (2.2)	2 (4.2)	0 (0.0)	0 (0.0)

Table 3.4. Antiretroviral class for all, black, white and non-black non-white patients within the Phase One study population (NRTI = nucleos(t)ide reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, INI = integrase inhibitor)

NRTI	Exposure	All (n = 625)	Black (n = 331)		White (n = 274)		Non-Black Non-White (n = 20)	
			Male (n = 116)	Female (n = 215)	Male (n = 226)	Female (n = 48)	Male (n = 13)	Female (n = 7)
Tenofovir DF	**Current** *n* (%)	294 (47.0)	58 (50.0)	88 (40.9)	119 (52.7)	21 (43.8)	4 (30.8)	4 (57.1)
	Ever *n* (%)	319 (51.0)	62 (53.4)	97 (45.1)	126 (55.8)	26 (54.2)	4 (30.8)	4 (57.1)
Abacavir	**Current** *n* (%)	142 (22.7)	35 (30.2)	58 (27.0)	38 (16.8)	7 (14.5)	3 (23.1)	0 (0.0)
	Ever *n* (%)	219 (35.0)	48 (41.3)	85 (39.5)	70 (31.0)	12 (25.0)	3 (23.1)	1 (14.3)
Zidovudine	**Current** *n* (%)	33 (5.3)	10 (8.6)	23 (10.7)	15 (6.7)	4 (8.3)	1 (7.7)	1 (14.3)
	Ever *n* (%)	142 (22.7)	35 (30.2)	107 (49.8)	74 (32.7)	20 (41.7)	3 (23.1)	1 (14.3)

Table 3.5. Nucleos(t)ide reverse transcriptase inhibitor (NRTI) exposure for all, black, white and non-black non-white patients within the Phase One study population (tenofovoir DF = tenofovir disoproxil fumarate)

	All (n = 624)[a]	Black (n = 330)[a]		White (n = 274)		Non-Black Non-White (n = 20)	
		Male (n = 116)	Female (n = 214)[a]	Male (n = 226)	Female (n = 48)	Male (n = 13)	Female (n = 7)
No falls *n (%)*	541 (86.7)	105 (90.5)	187 (87.4)	192 (84.9)	38 (79.2)	12 (92.3)	7 (100.0)
Single fall *n (%)*	47 (7.5)	8 (6.9)	15 (7.0)	16 (7.1)	7 (14.6)	1 (7.7)	0 (0.0)
Multiple falls *n (%)*	36 (5.8)	3 (2.6)	12 (5.6)	18 (8.0)	3 (6.2)	0 (0.0)	0 (0.0)

[a] 1 missing value [a] 1 missing value

Table 3.6. Number of self-reported falls within the preceding 12 months by patient race and gender within the Phase One study population

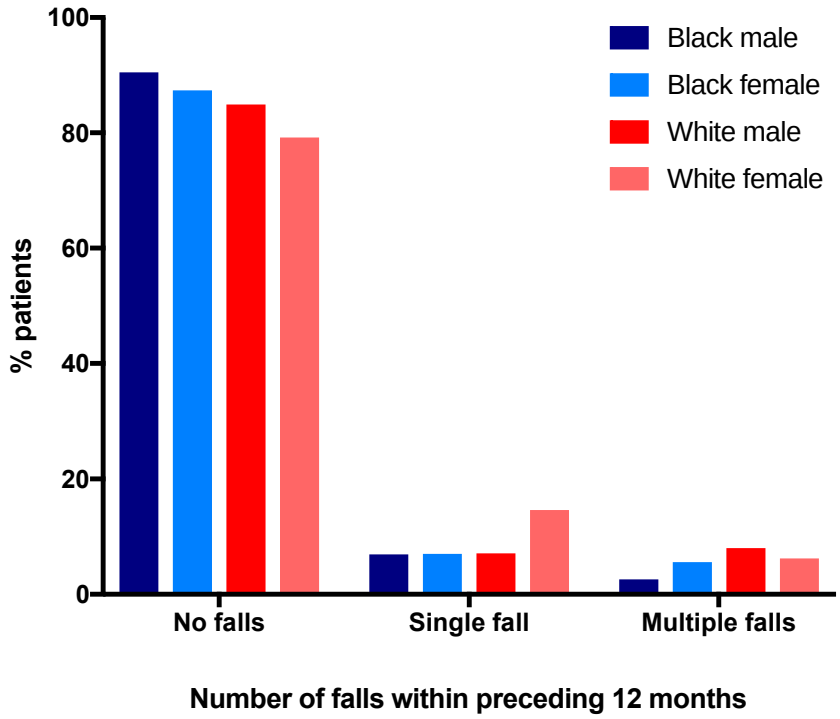


Figure 3.7. Percentage of black male (n = 116), black female (n = 214), white male (n = 226) and white female (n = 48) patients within the Phase One study population reporting no falls, one single fall or multiple falls within the preceding 12 months

3.5 Fracture prevalence

The median number of self-reported fractures in adulthood was 0 (range 0 – 6) in 566 patients for whom fracture history was provided. The number and proportion of patients having sustained either no, one or multiple fractures are detailed by patient race and gender subgroups in Table 3.7 and Figure 3.10. A higher proportion of white patients had sustained a single fracture in adulthood than either black or non-black non-white patients and within each racial subgroup a higher proportion of male patients had sustained a single fracture in adulthood than female patients. Only a small proportion of patients reported multiple fractures in adulthood overall, with a higher proportion of multiple fractures reported in white patients compared with black patients (there were no cases of multiple fractures reported in non-black non-white patients).

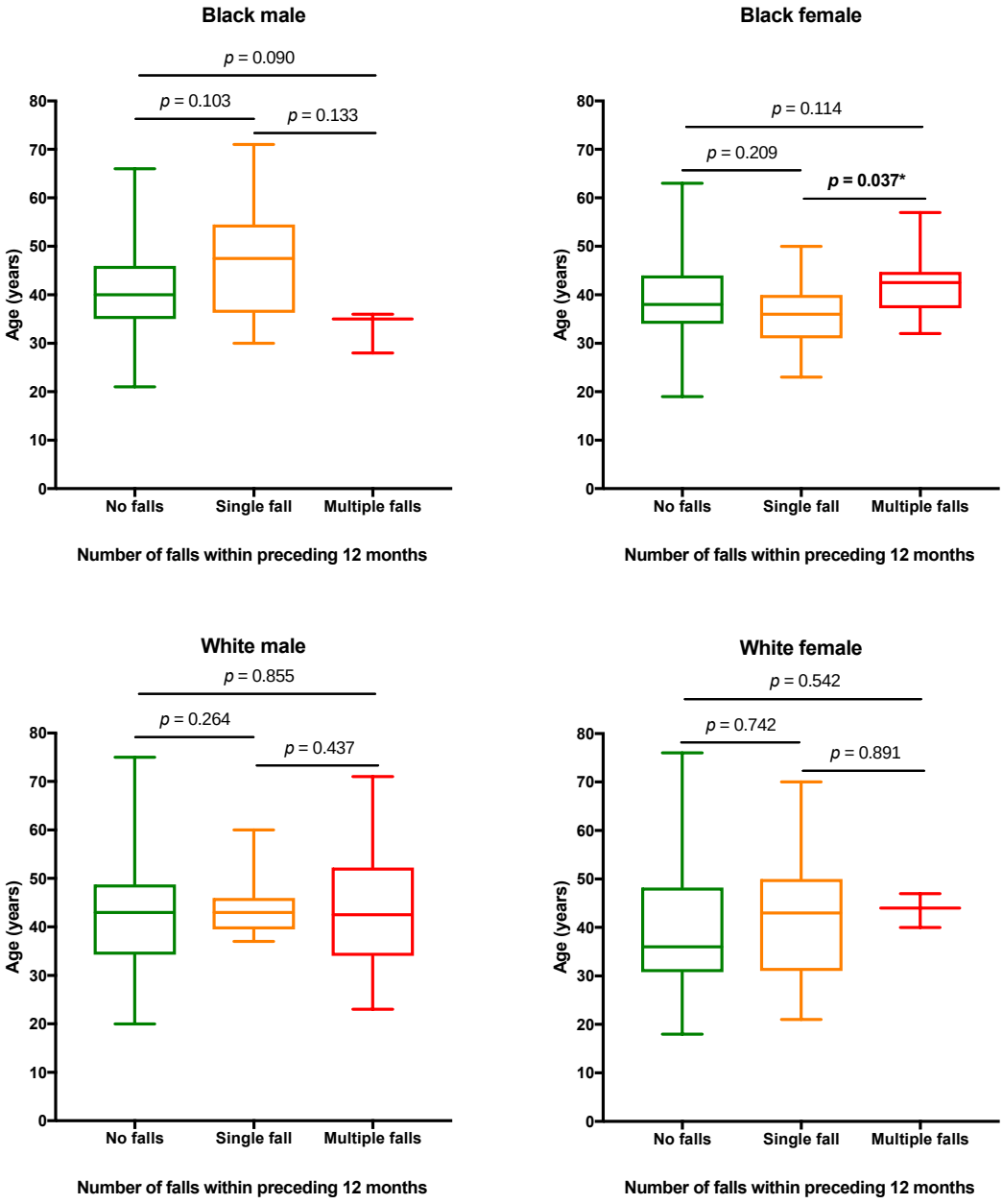


Figure 3.8. Age distribution of patients reporting no falls, one single fall or multiple falls within the preceding 12 months in black male (n = 116), black female (n = 214), white male (n = 226) and white female (n = 48) patients within the Phase One study population

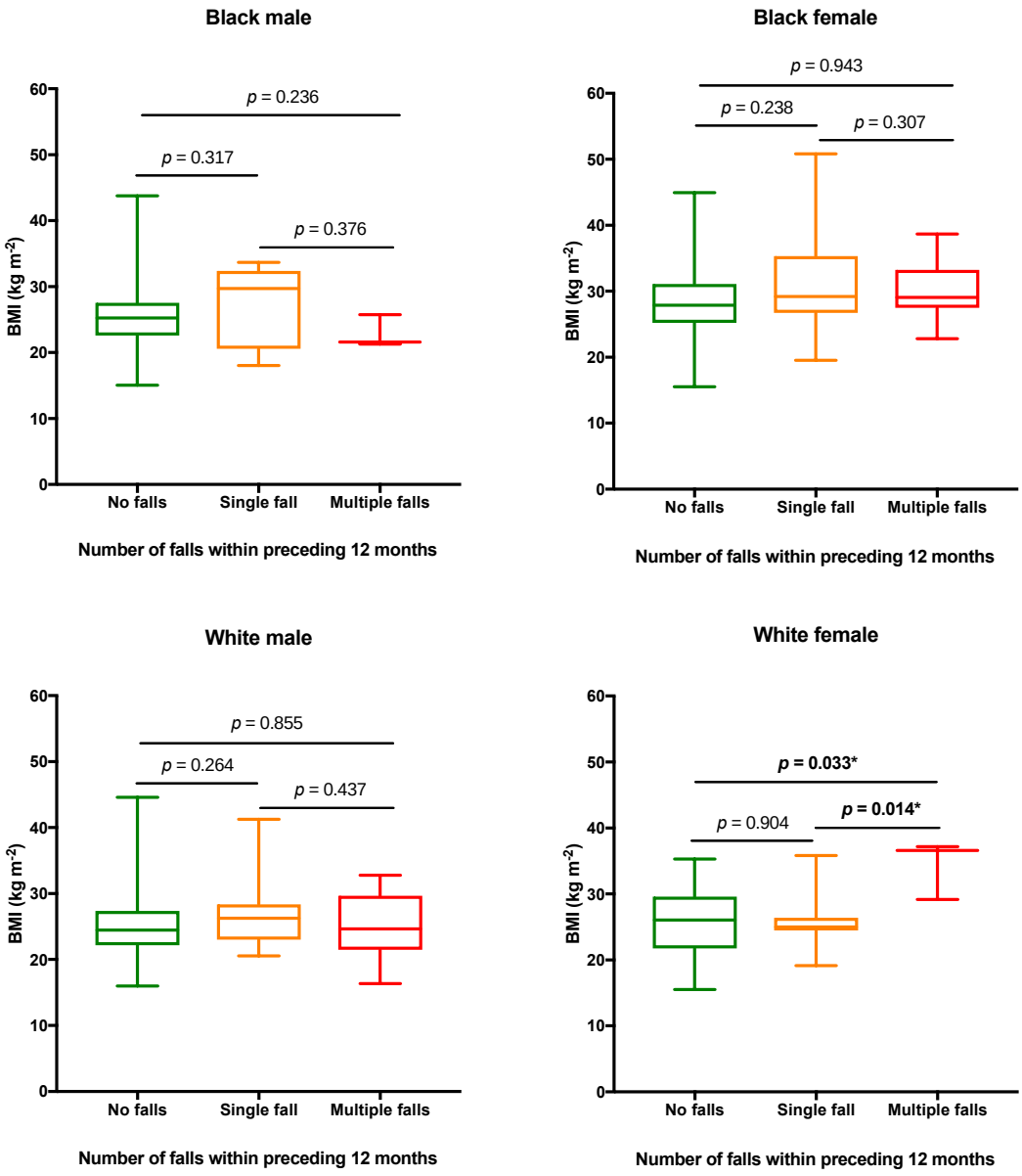


Figure 3.9. BMI distribution of patients reporting no falls, one single fall or multiple falls within the preceding 12 months in black male (n = 115), black female (n = 211), white male (n = 225) and white female (n = 47) patients within the Phase One study population

	All (n = 566)	Black (n = 292)		White (n = 254)		Non-Black Non-White (n = 20)	
		Male (n = 101)	Female (n = 191)	Male (n = 209)	Female (n = 45)	Male (n = 13)	Female (n = 7)
No fracture *n (%)*	456 (80.6)	90 (89.1)	180 (94.2)	132 (63.2)	35 (77.8)	12 (92.3)	7 (100.0)
Single fracture *n (%)*	83 (14.7)	10 (9.9)	6 (3.1)	59 (28.2)	7 (15.5)	1 (7.7)	0 (0.0)
Multiple fractures *n (%)*	27 (4.8)	1 (1.0)	5 (2.6)	18 (8.6)	3 (6.7)	0 (0.0)	0 (0.0)

Table 3.7. Number of self-reported fractures in adulthood by patient race and gender within the Phase One study population

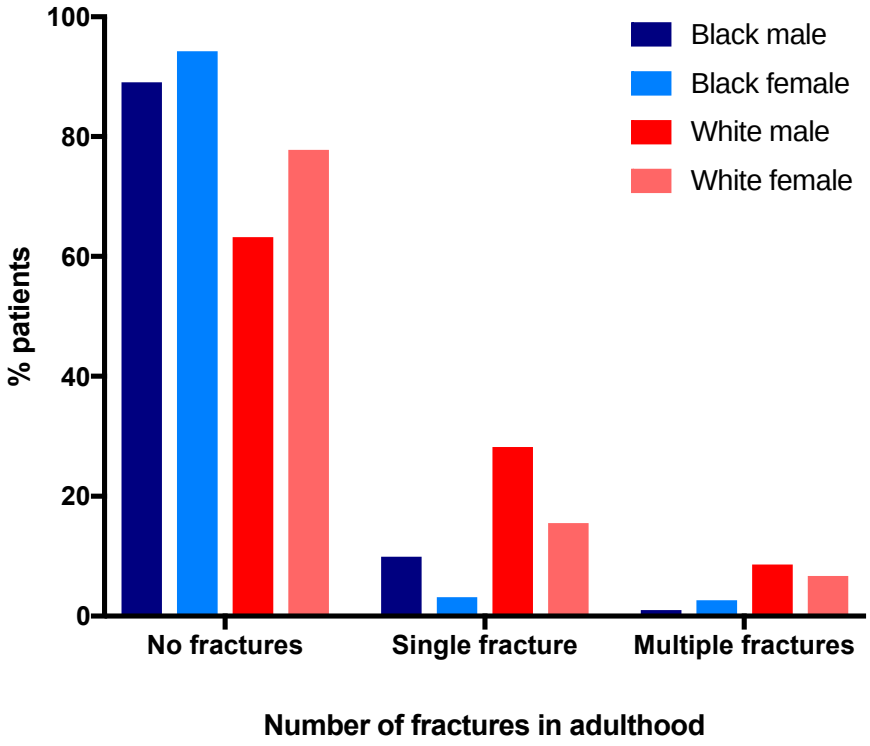


Figure 3.10. Percentage of black male (n = 101), black female (n = 191), white male (n = 209) and white female (n = 45) patients within the Phase One study population reporting no fractures, one single fracture or multiple fractures in adulthood

151 fractures were reported in 110 (19.4%) of patients. The median age of first fracture and most recent fracture was 28 years (range 18 to 56) and 32 years (range 18 to 58) respectively. Patients reporting one single fracture in adulthood (n = 83) and patients reporting multiple fractures in adulthood (n = 27) were significantly older than patients reporting no fractures in adulthood (n = 456) (p = 0.040 and p = 0.001 for single fracture and multiple fractures respectively). Patients reporting multiple fractures in adulthood were significantly older than those reporting no fractures in adulthood in both black female and white male patient subgroups (p = 0.036 and p = 0.046 respectively), but with no other significant association of patient age and number of fractures within subgroups otherwise (Figure 3.11).

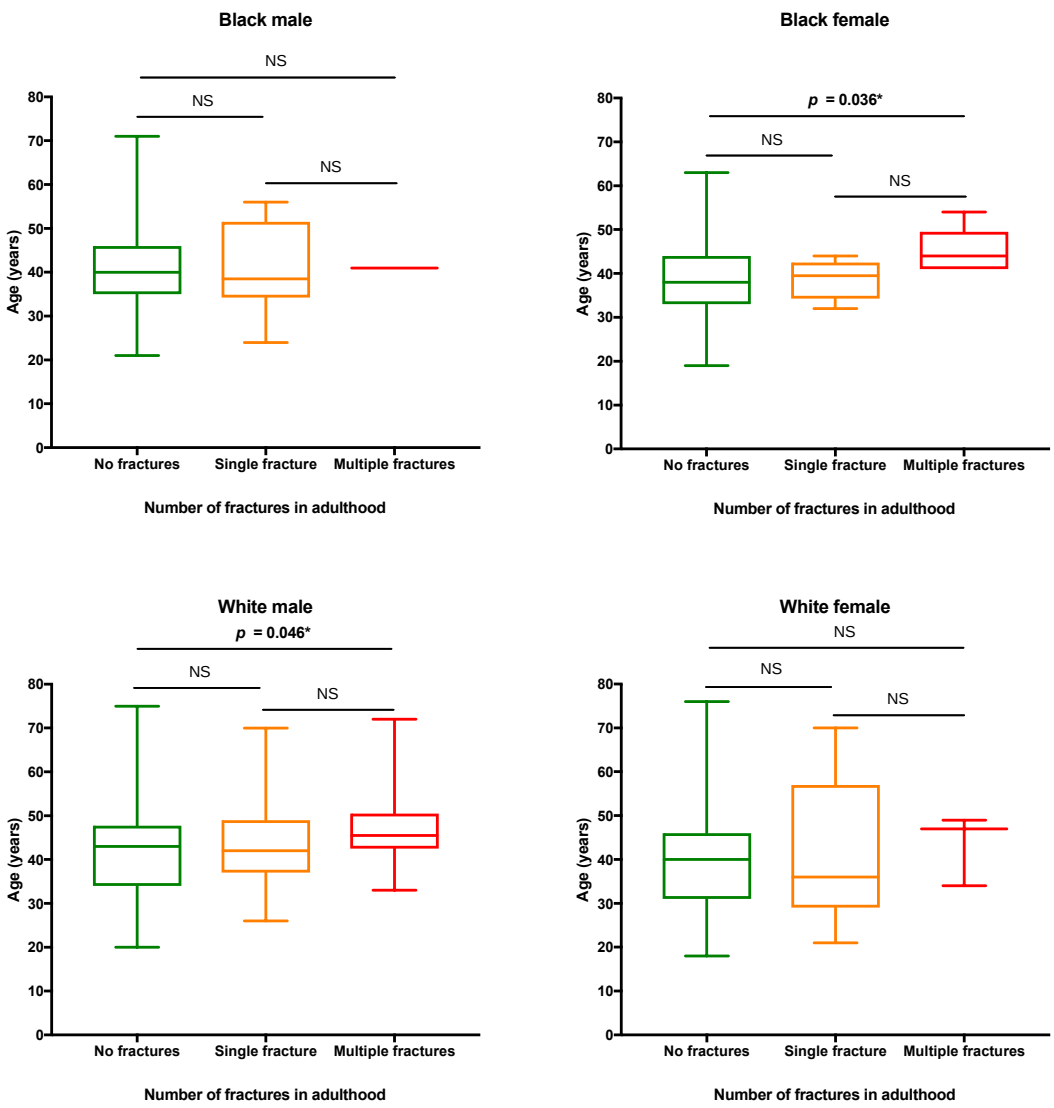


Figure 3.11. Age distribution of patients reporting no fractures, one single fracture or multiple fractures in adulthood in black male (n = 101), black female (n = 191), white male (n = 209) and white female (n = 45) patients within the Phase One study population (NS = not significant)

Whilst there was a trend to white female patients reporting multiple fractures to be older also, this was not statistically significant.

BMI was significantly higher in patients reporting multiple fractures in adulthood compared to those reporting one single fracture in adulthood only ($p = 0.014$), but with no significant difference in BMI between patients reporting no fractures versus one single fracture ($p = 0.079$), or between patients reporting no fractures versus multiple fractures ($p = 0.114$). Furthermore, BMI was not significantly different in patients reporting no fractures *vs.* patients reporting one single fracture within any patient race-gender subgroup either, although BMI was significantly higher in black female patients reporting multiple fractures versus those reporting no fractures ($p = 0.009$, Figure 3.12).

The body region and specific site of each reported fracture is detailed in Figure 3.13 and Table 3.8 respectively. The vast majority of fractures were traumatic in nature. Only five patients (0.8 %) reported fragility fractures in adulthood, two black patients (both female) and three white patients (two female and one male), median age 50 years (range 41 to 57). Further details regarding these five individuals, the site of their fragility fractures, their associated risk factors and FRAX®-derived fracture risk are summarised in Table 3.9.

Four of the patients with past fragility fracture had at least one FRAX®-incorporated general fracture risk factor; the fifth (black female) patient had two non-FRAX®-incorporated general fracture risk factors – SSRI and Depo-Provera® use – as well as three HIV disease-specific risk factors, namely low current and nadir CD4 cell count and an unsuppressed HIV viral load. FRAX®-calculated probabilities of both major osteoporotic and hip fractures were high in the three white patients and relatively high (compared with other black patients in the Phase One study population) in the two black patients.

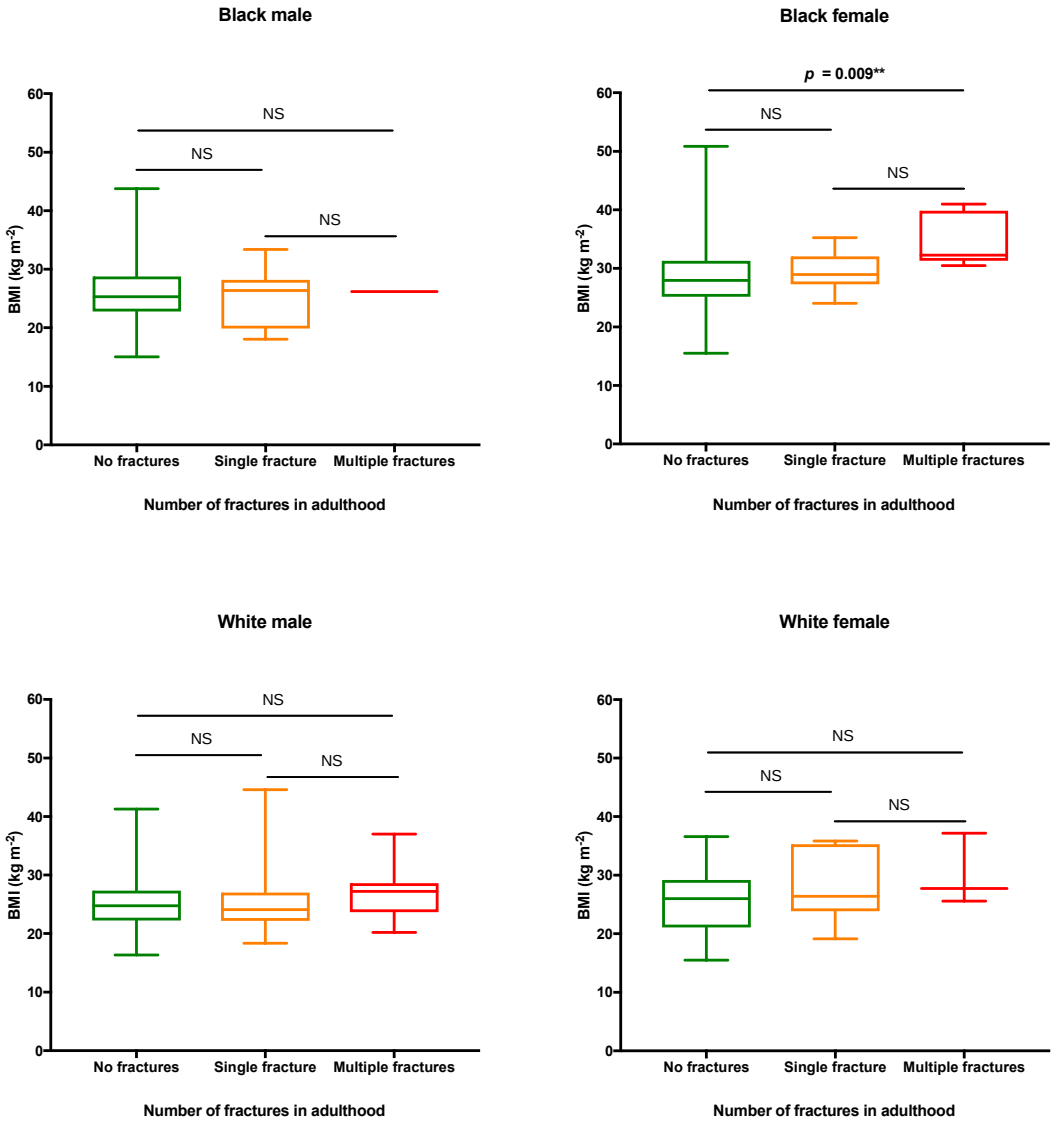


Figure 3.12. BMI distribution of patients reporting no fractures, one single fracture or multiple fractures in adulthood in black male (n = 100), black female (n = 188), white male (n = 208) and white female (n = 44) patients within the Phase One study population (NS = not significant)

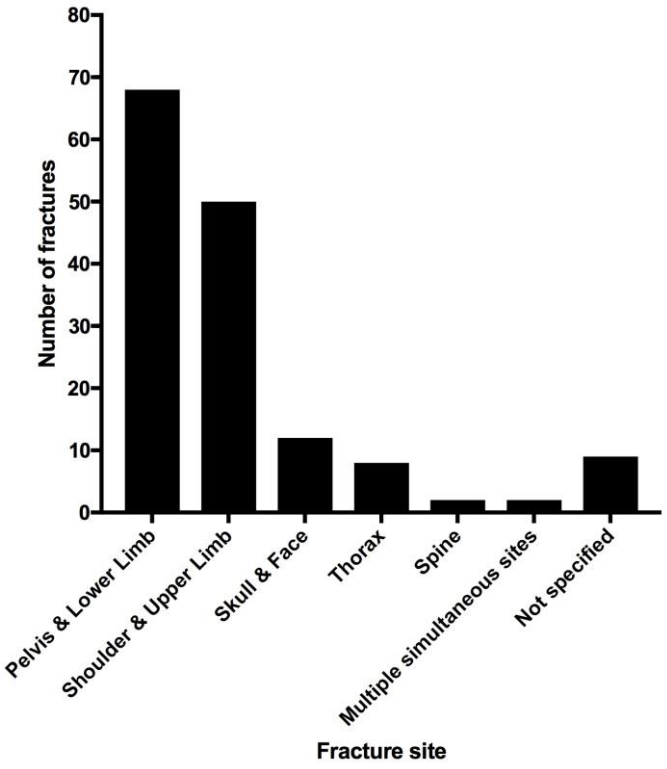


Figure 3.13. Body region sites of self-reported fractures in adulthood in patients within the Phase One study population (151 fractures self-reported in 110 of 566 patients)

Body region / site	n	**Body region** / site	n	**Body region** / site	n
Pelvis and lower limb	**68**	**Shoulder and upper limb**	**50**	**Skull / facial**	**12**
Tibia and/or fibula / ankle	27	Wrist / scaphoid	19	Jaw	5
Foot / toe(s)	25	Hand / finger(s) / thumb	19	Nose	3
Femur	6	Shoulder / scapula	3	Maxilla	2
Not specified lower limb	5	Humerus	3	Skull	1
Patella	2	Forearm	3	Eye socket	1
Hip	1	Not specified upper limb	3		
Pelvis	1				
Thorax	**8**	**Vertebral**	**2**	**Site not specified**	**9**
Rib(s)	5				
Clavicle	2	**Multiple simultaneous sites**	**2**		
Sternum	1				

Table 3.8. Specific sites of self-reported fractures in adulthood in patients within the Phase One study population (151 fractures self-reported in 110 of 566 patients)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Number of fragility fractures	1	1	1	1	2
Site of fracture(s)	Femur	Hip	Vertebral	Arm	Humerus; vertebral
Age at time of fracture(s) *years*	49	32	56	28	40
Current age *years*	50	57	58	41	42
Gender	Female	Female	Male	Female	Female
Ethnicity	White British	White British	White British	Black African	Black British
Country of origin	UK	UK	UK	Zimbabwe	UK
BMI *kg m^{-2}*	19.1	26.3	33.2	41.0	32.1
FRAX® 10-year probability of major osteoporotic fracture (not including BMD)	13.0	18.0	13.0	1.0	1.5
FRAX® 10-year probability of hip fracture (not including BMD)	4.4	4.8	2.6	0.2	0.2
FRAX® incorporated risk factor(s)	Low BMI; current smoker; chronic diarrhoea; malabsorption	Prednisolone use; current smoker	Prednisolone use; IBD	Type 1 DM	-
Other (non-FRAX® incorporated) risk factors	SSRI use	-	ITU stay	-	SSRI use; depo-provera use (12 years) + secondary amenorrhoea
Current CD4 *cells μL^{-1}*	896	569	851	552	182
Nadir CD4 *cells μL^{-1}*	257	569	105	441	138
Ever ART	Yes	No	Yes	Yes	No
Current ART	Yes	No	Yes	Yes	No
Months of ART	53	0	31	3	0
HIV RNA <40 copies ml^{-1}	Yes	No	Yes	No	No

Table 3.9. Characteristics of the five individuals with a history of fragility fracture within the Phase One study population

3.6 Prevalence of general fracture risk factors

The percentage prevalence of FRAX®-incorporated validated general (i.e. not HIV disease-specific) fracture risk factors (excluding age, BMI, race and gender), as listed in Table 2.1, is shown for Phase One patients by patient race and gender in Figure 3.14. Further detail as to the percentage prevalence of individual "other risk factors", i.e. other disorders strongly associated with osteoporosis (included in FRAX® under the label of "Secondary Osteoporosis") is shown by patient race and gender in Figure 3.15.

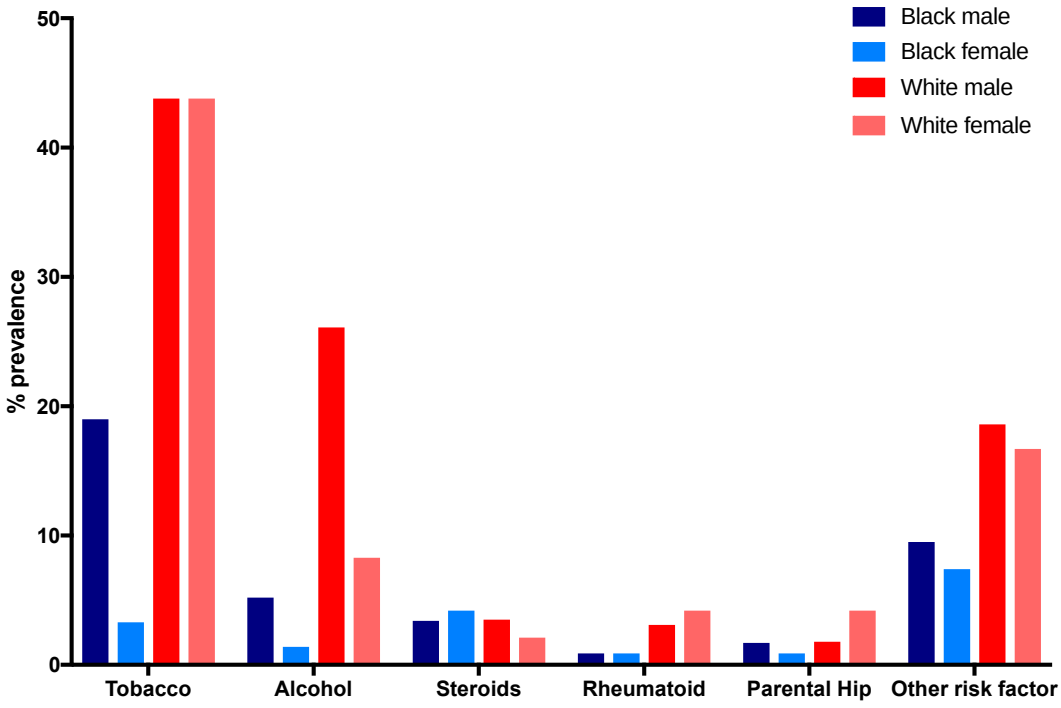


Figure 3.14. Percentage prevalence of FRAX®-incorporated HIV-independent validated osteoporotic risk factors for black male (n = 116), black female (n = 215), white male (n = 226) and white female (n = 48) patients within the Phase One study population. "Tobacco" = current tobacco smoking; "Alcohol" = alcohol consumption ≥3 units per day; "Steroids" = significant oral glucocorticoid steroid use; "Rheumatoid" = rheumatoid disease; "Parental Hip" = parental family history of hip fracture; "Other risk factor" = at least one of: hypogonadism, chronic diarrhoea, prolonged immobility, malabsorption, inflammatory bowel disease, chronic obstructive pulmonary disease, cirrhosis, type 1 diabetes mellitus, untreated hyperthyroidism, organ transplant recipient or osteogenesis imperfecta.

The percentage prevalence of definite and possible male hypogonadism (defined in Section 2.2) is shown by patient race in Figure 3.16. The percentage prevalence of female hypogonadism (premature menopause onset at age less than 45 years), as well of menopause (any age of onset) and other non-FRAX® incorporated female-specific risk factors for reduced BMD, is shown by patient race in Figure 3.17.

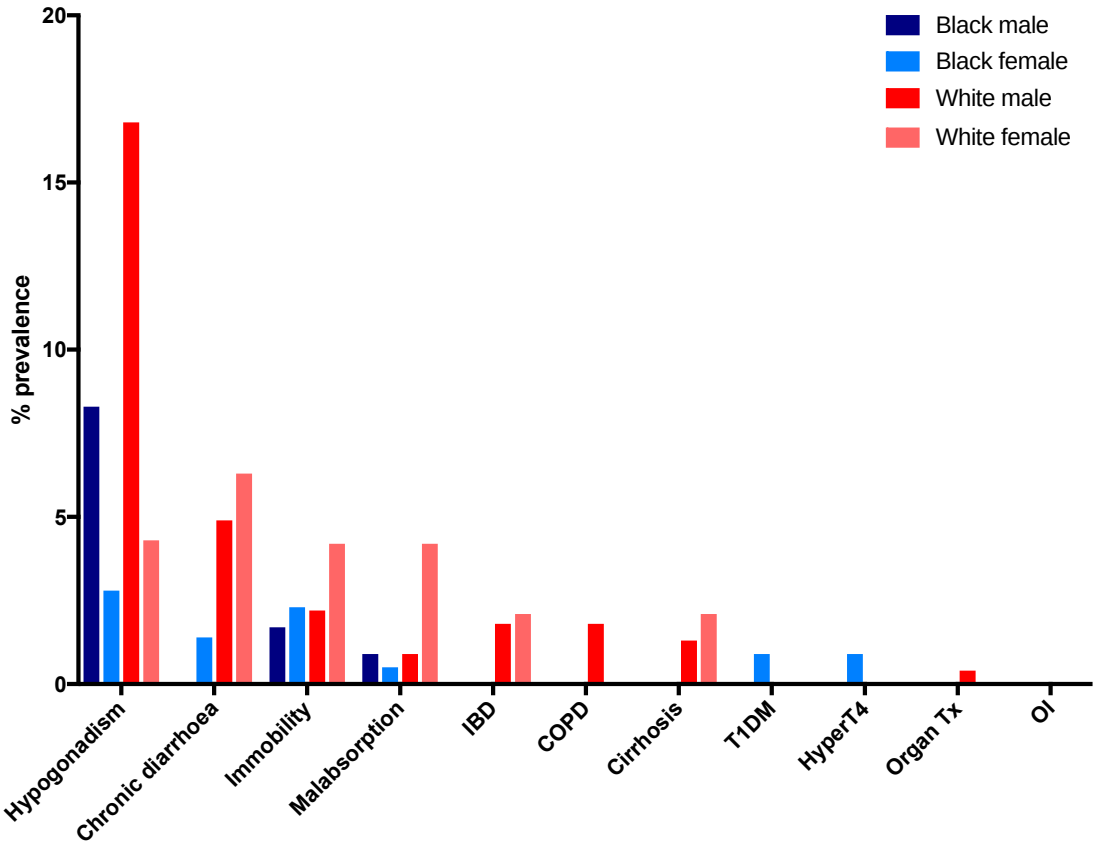


Figure 3.15. Percentage prevalence of FRAX®-incorporated "other" HIV-independent validated osteoporotic risk factors for black male (n = 116), black female (n = 215), white male (n = 226) and white female (n = 48) patients within the Phase One study population. IBD = inflammatory bowel disease; COPD = chronic obstructive pulmonary disease; T1DM = type 1 diabetes mellitus; HyperT4 = untreated hyperthyroidism; Organ Tx = organ transplant recipient; OI = osteogenesis imperfecta in adulthood.

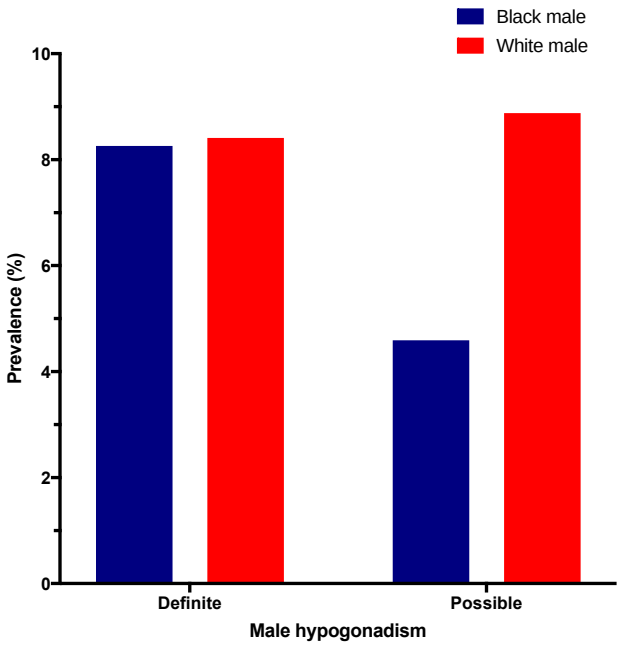


Figure 3.16. Percentage prevalence of definite and possible male hypogonadism in black (n = 109) and white (n = 214) male patients within the Phase One study population

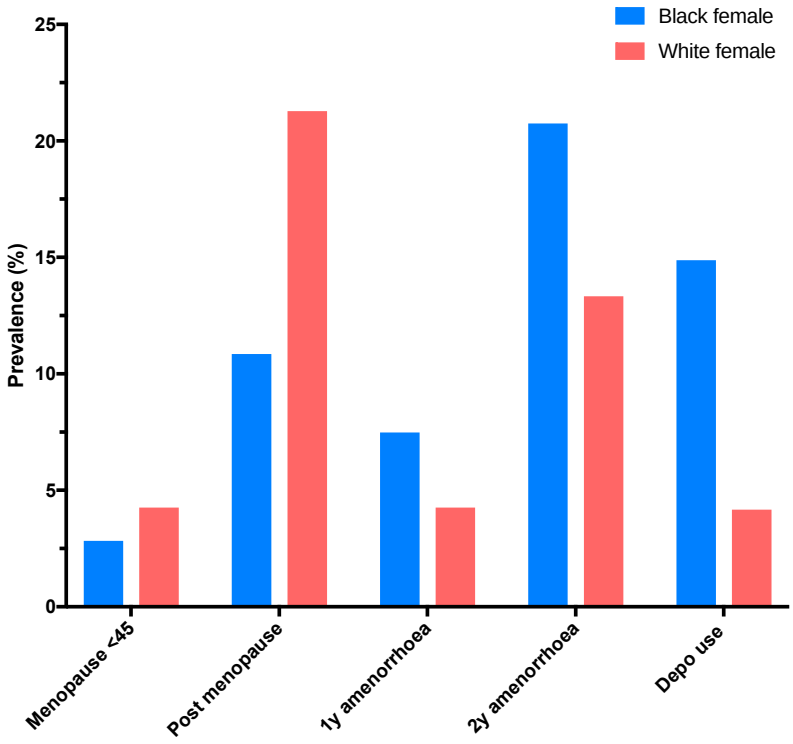


Figure 3.17. Percentage prevalence of female hypogonadism (menopause onset <45 years of age) and of other female-specific non-FRAX® incorporated fracture risk factors in black (n=215) and white (n=48) female patients within the Phase One study population. "Depo use" = ever use of Depo-Provera®.

Current tobacco smoking, “other risk factors” (as one grouping) and alcohol consumption more than or equal to three units per day were the most prevalent general fracture risk factors within Phase One patients (excluding age, BMI and ethnicity) – 24.2%, 13.0% and 11.7% prevalence respectively – although tobacco smoking and alcohol consumption were much more prevalent in white patients (43.8% and 23.0% prevalence respectively) than in black patients (8.8% and 2.7% prevalence respectively) and in male patients (34.7% and 18.6% prevalence respectively) than in female patients (10.4% and 2.6% prevalence respectively). Significant glucocorticoid use was more prevalent in female patients (10.4%) than in male patients (3.4%).

Amongst the “other risk factors”, current or previous hypogonadism was the most prevalent risk factor for patients of both black and white race. Male hypogonadism was confirmed in 8.6% of male patients, with a possible but unconfirmed diagnosis in a further 7.4%, with little difference in prevalence observed between black and white male patients. Premature menopause was reported in 3.0% of female patients overall, with a slightly higher prevalence in white female patients (4.3%) versus black female patients (2.8%). 12.4% of all female patients were post-menopausal, again with a higher prevalence in white females (21.3%) versus black females patients (10.7%). History of primary amenorrhoea, secondary amenorrhoea and Depo-Provera® use were all more common in black female patients (7.5%, 20.8% and 14.9% respectively) than in white female patients, however (4.3%, 13.3% and 4.2% respectively).

Of other recorded general fracture risk factors not incorporated into FRAX®, current or previous SSRI antidepressant use, cannabis use, “other” (non-cannabis non-opiate) recreational drug use and opiate dependence were the most frequently reported overall (Figure 3.18), although more so in male patients compared with female patients and in patients of white race compared to patients of black race with respect to SSRI and cannabis use, with little or no self-reported “other” recreational drug or opiate use reported in black patients.

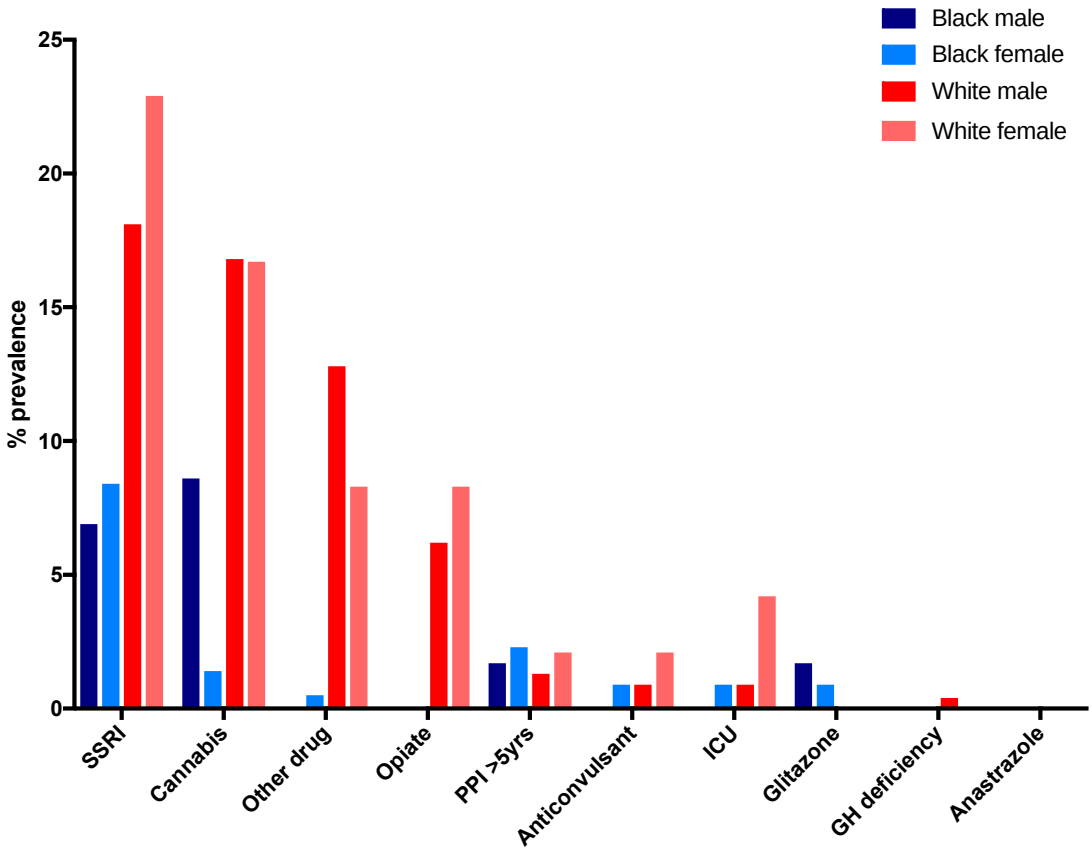


Figure 3.18. Percentage prevalence of non-FRAX®-incorporated general fracture risk factors for black male (n = 116), black female (n = 215), white male (n = 226) and white female (n = 48) patients within the Phase One study population. "SSRI" = ever use of a selective serotonin reuptake inhibitor; "cannabis" = ever use of cannabis; "other drug" = ever use of non-cannabis non-opiate recreational drug; "opiate" = history of opiate dependency; "PPI>5yrs" = use of proton pump inhibitor continuously for >5 years; "anticonvulsant" = ever use of anticonvulsant drug; "ICU" = past intensive care unit inpatient admission; "glitazone" = ever use of a glitazone drug; "GH deficiency" = history of confirmed growth hormone deficiency diagnosis; "anastrazole' = ever use of anastrazole, letrozole or exemestane.

3.7 FRAX®-calculated probability of major osteoporotic fractures and hip fracture

The distribution of FRAX®-calculated 10-year probabilities (not incorporating BMD measurement) of major osteoporotic fracture and of hip fracture are shown for the Phase One study population in Figures 3.19 and 3.20 respectively, grouped by patient race and gender. (Further detail with respect to which FRAX® calculator was used for each patient, dependent on patient race and country of origin, is included in Section 2.2.)

The median FRAX® -calculated 10-year risk of major osteoporotic fracture was 1.6% (range 0.2 – 19.0) for all patients (n = 619), 0.6% (range 0.2 – 7.0) for black patients (n = 327), 2.6% (range 0.6 – 19.0) for white patients (n = 272) and 2.2% (range 0.3 – 3.7) for non-black non-white patients (n = 20). Black patients had significantly lower 10-year risk of major osteoporotic fracture compared to white patients ($p < 0.001$). There was no significant difference in FRAX® -calculated 10-year risk of major osteoporotic fracture by gender between black male and black female patients or between white male and white female patients, however (Figure 3.19).

The median FRAX® -calculated 10-year risk of hip fracture was 0.1% (range 0.0 – 8.4) for all patients, 0.0% (range 0.0 – 1.5) for black patients, 0.3% (range 0.0 – 8.4) for white patients and 0.2% (range 0.0 – 0.3) for non-black non-white patients. Black patients had significantly lower risk of hip fracture compared with white patients ($p < 0.000$), but there was no significant difference in risk of hip fracture between black male and black female patients or between white male and white female patients (Figure 3.20).

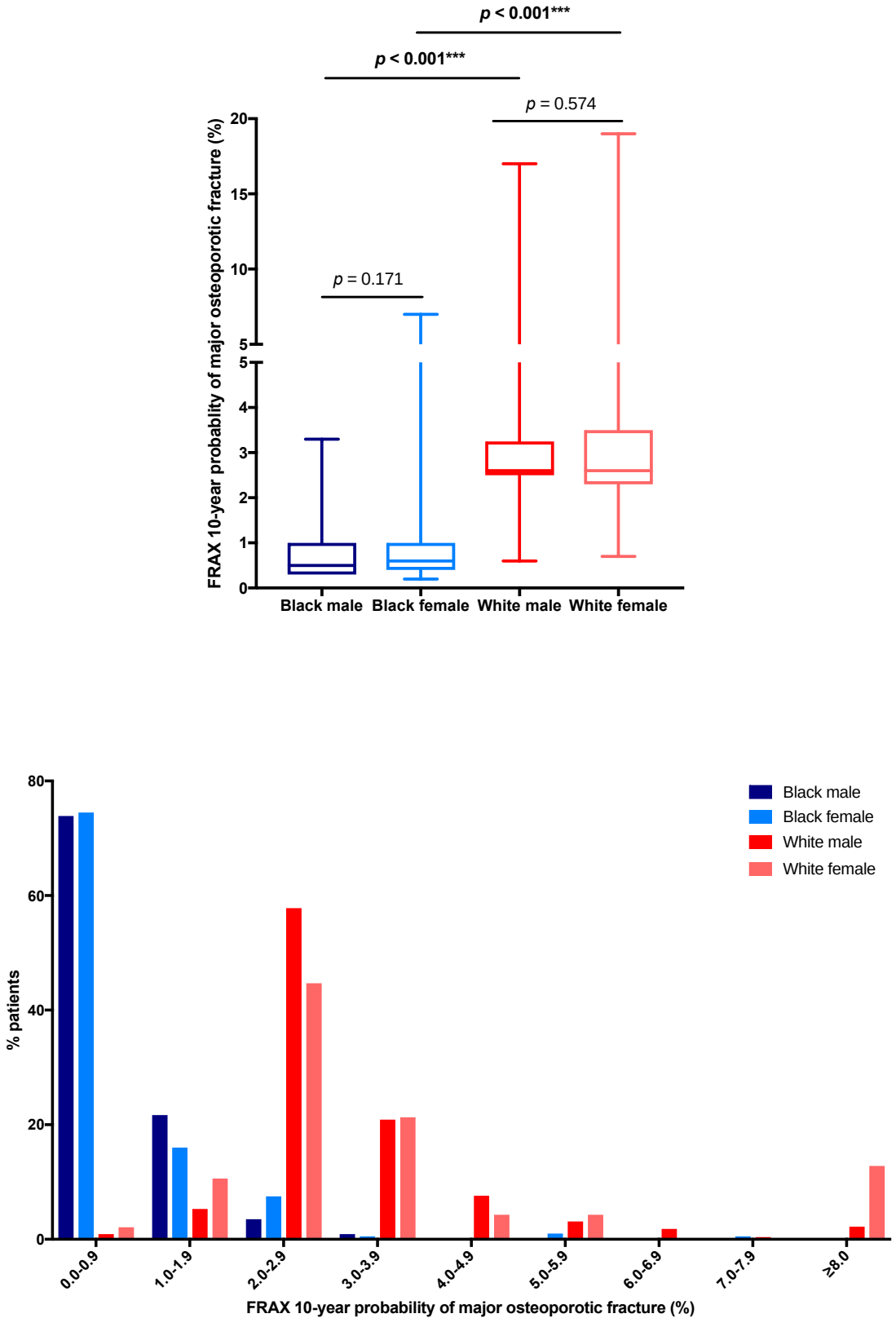


Figure 3.19. Distribution of FRAX® calculated 10-year probabilities of major osteoporotic fracture (not incorporating BMD) in black male (n = 115), black female (n = 212), white male (n = 225) and black female (n = 47) patients within the Phase One study population

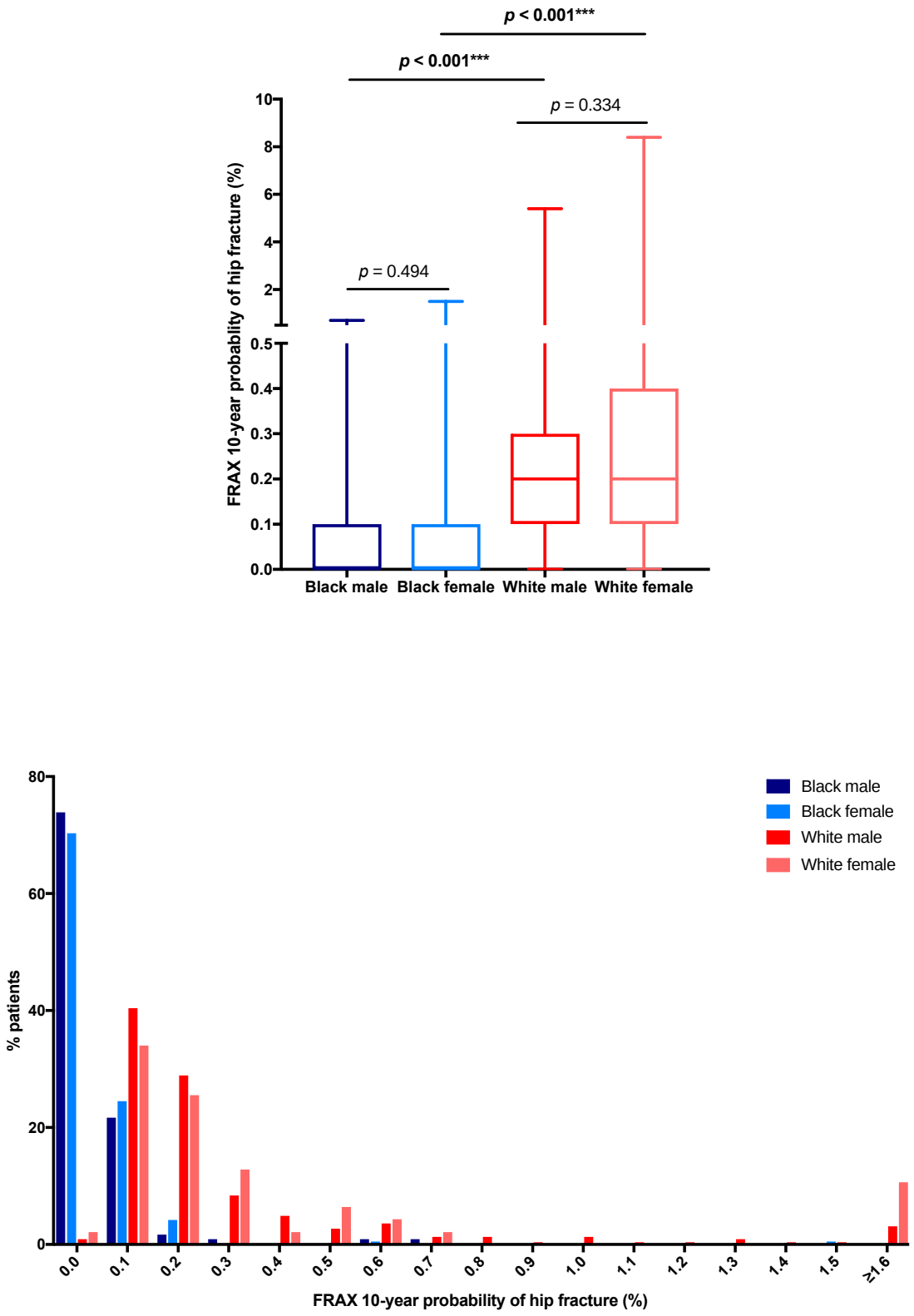


Figure 3.20. Distribution of FRAX® calculated 10-year probabilities of hip fracture (not incorporating BMD) in black male (n = 115), black female (n = 212), white male (n = 225) and black female (n = 47) patients within the Phase One study population

3.8 Discussion

The population recruited to Phase One of this study (n = 625), representing approximately 80% of PLWH within the Sheffield HIV Cohort (n = circa 800 at time of Phase One study recruitment), was heterogenous with respect to race, country of origin, gender and age, with a reasonably balanced representation of both black and white patients (53.0% and 43.8% respectively) and male and female patients (56.8% male). Black patients were predominantly female and generally younger than white patients, who were predominantly male and generally older. The majority of patients were overweight, i.e. BMI >25 kg m^{-2}, with BMI significantly higher in black female patients compared with both black male and white female patients.

In terms of potential HIV disease-specific fracture risk factors, black patients, and specifically black male patients, had significantly lower nadir CD4 cell count compared with white patients, perhaps on account of increased time from HIV acquisition to diagnosis and initiation of ART in immigrant black African patients (Page *et al*. 2009), in addition to $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cell counts in general being lower in patients of black race compared to patients of white race and in males compared with females (Bosire *et al.* 2013). Overall, the proportions of patients on current ART (73.8%) and with a suppressed HIV viral load (63.0%) within the study population were lower than expected. In light of changes to national ARV prescribing policy since 2017, with all patients now qualifying for ART initiation at the time of HIV diagnosis, the proportion of patients taking ART and with a suppressed HIV viral load would now be expected to be much higher.

Regarding specific ARV use, approximately twice as many patients had been exposed to an NNRTI compared to a PI and to the NRTI tenofovir DF compared to the NRTI abacavir, with very low numbers of patients having been exposed to other newer ARV drug classes, including to INIs. Again, more recent changes to ARV availability, ARV drug cost and national ARV prescribing policy would most likely now result in far greater INI use with less NNRTI use. With some ARVs now available in generic form and with

pressures to save costs within UK healthcare systems, the use of abacavir – available as a generic drug ahead of tenofovir DF – is also likely to have now increased within this cohort relative to tenofovir DF use. Of further note, this study was conducted before the availability of the new tenofovir pro-drug TAF, the availability of which would also be expected to result in reduced tenofovir DF use.

1 in 6 patients reported having fallen in the preceding 12 months and the majority of these patients reported falling only once in the past year, with very few patients recording more frequent falls. In contrast to other published data (Sharma *et al.* 2018), older age did not result in significantly increased fall frequency in the Phase One study population overall, although older age was significantly associated with increased fall frequency in black females. As the Phase One cohort was relatively young (mean age 40.7 ± 9.6 years), however, the effects of age on falls frequency may not have been as apparent, on account of the relatively low proportion of older patients included in the Phase One analysis in comparison with the study by Sharma *et al.* (2018) (median age 49 years). More specific assessments of frailty were not assessed within the Phase One population and therefore no comparison of frailty with falls frequency was possible.

There was a relatively high proportion of patients with FRAX®-incorporated general fracture risk factors in the Phase One study population, most notably current tobacco smoking, significant alcohol consumption and hypogonadism. Current tobacco smoking and significant alcohol consumption were more prevalent in white patients compared with black patients, tobacco smoking was more prevalent in black males than in black females and significant alcohol consumption was more prevalent in both black males and white males than in black females and white females respectively. Hypogonadism was more prevalent in males than females, although in males this also included “possible” as well as “definite” hypogonadism (defined in Chapter 2, Section 2.2). History of parental hip fracture was uncommon overall, perhaps reflecting the young age of patients within the cohort and therefore also the relatively young age of their parents who would not yet have reached an age

to be significantly more likely to sustain a hip fracture. Furthermore, the parents of black African patients may have died prematurely before reaching old age and being at risk of hip fracture. Black African patients may also have lost contact with their parents following migration to the UK and therefore parental fracture history might be less accurate for these patients.

There was also a high prevalence of non-FRAX®-incorporated general osteoporotic risk factors in this study population, including SSRI use, cannabis use, opiate dependence and non-cannabis non-opiate recreational drug use, again more so in male and white patients than in female and black patients, as well as secondary amenorrhoea and Depo-Provera® use in predominantly black female patients.

In spite of a high prevalence of FRAX®-incorporated general osteoporotic risk factors, FRAX®-derived 10-year probabilities of both major osteoporotic fracture and hip fracture (FRAX® scores) were low overall, although with some higher risk outlier patients, in this relatively young population in whom the majority were overweight. Black patients had significantly lower FRAX® scores than white patients, in part reflecting younger age, higher BMI and a lower prevalence of FRAX®-incorporated general osteoporotic risk factors than white patients, but also due to the use of a black-race specific FRAX®-calculator (US Black) in black patients, assuming higher BMD and therefore lower fragility fracture risk in black populations versus non-black populations. Whether or not it is appropriate to use the US Black FRAX®-calculator in black patients who have immigrated to the UK from sub-Saharan Africa is unknown. One study compared lumbar spine and hip BMD in healthy black females living in Zimbabwe to black females living in the US and found BMD to be lower in black females living in Zimbabwe, although the black females living in Zimbabwe also had significantly lower BMI (Mukwasi *et al.* 2015). One would expect weight and BMI to increase in black Africans following migration to the US or to other developed countries such as the UK; the findings of this study are therefore not necessarily applicable to black African immigrants to the UK from Zimbabwe or other sub-Saharan African countries, for whom use of the

US Black FRAX®-calculator following immigration and weight gain may well be appropriate.

Almost 1 in 5 patients reported a history of fracture in adulthood, with fracture frequency significantly greater with older age; the vast majority of these were traumatic non-fragility fractures, however, with only a very small proportion of patients (0.8%, n = 5) with a history of fragility fracture. Fragility fractures occurred in young as well as in older patients (median age of occurrence 40 years old, range 28 to 56 years). Of note, all fiver patients with a history of fragility fracture had at least one general fracture risk factor, although HIV disease-specific fracture risk factors were also present in these patients.

The generally low FRAX® scores observed within the Phase One study population and the low prevalence of fragility fractures, as well as the observation that all five patients with a history of fragility fracture had at least one general fracture risk factor and three had high FRAX® scores, could support the hypothesis that general fracture risk factors play a more significant role in reduced BMD and fragility fracture risk than HIV disease-specific risk factors in PLWH. This could suggest that the widely reported increased prevalence of reduced BMD and increased fracture incidence in HIV-positive patient cohorts may be more due to an over representation of general fracture risk factors within HIV-positive populations than HIV disease-specific risk factors. FRAX® could, therefore, be a valid tool for the assessment of fragility fracture risk in PLWH.

Drawing these conclusions from the Phase One study population data is limited, however, by the generally low prevalence of fragility fractures, the absence of an HIV-negative control group to compare the prevalence of general fracture risk factors, the lack of BMD data and the lack of longitudinal data, with FRAX® predicting future fragility fracture risk and not historic fragility fracture prevalence. It remains also possible, therefore, that although FRAX® scores were low, the high prevalence of general risk factors, when combined with superimposed HIV disease-specific risk factors, could put many more

patients at risk of sustaining a fragility fracture in the future as this cohort ages.

3.9 Conclusions

1. General fracture risk factors were highly prevalent within the Sheffield HIV Cohort, although FRAX® scores and fragility fracture prevalence was very low (0.8%), reflecting the young age of the study population; it is likely, however, that fracture risk and incidence will increase as the Sheffield HIV Cohort ages.
2. There is a difference in the prevalence of specific fracture risk factors between patients of different race and gender, e.g. higher BMI in black females (protective) and increased smoking and alcohol consumption in white and male patients, therefore specific sub-populations of PLWH are likely to be at higher risk of fragility fracture than PLWH in general.
3. Whilst general fracture risk factors could account for fragility fracture incidence alone and FRAX® could potentially be a valid tool for the assessment of fragility fracture risk in this population, general fracture risk factors would need to be over represented in PLWH in general to explain the increased prevalence of fragility fractures observed compared to the general population. Without an HIV-negative control group to compare general fracture risk factor prevalence, however, this cannot be demonstrated.

4. Clinical determinants of bone mineral density in people living with HIV in Sheffield

4.1 Introduction

BMD is consistently lower in PLWH than in age- and sex-matched HIV-negative controls (Brown and Qaqish 2006, Goh *et al.* 2018). The extent of reduced BMD in PLWH varies widely between published cohorts, however, with reduced BMD prevalence (T-score < -1.0) ranging from 21.2% (Grijsen *et al.* 2013) up to 87.5% (Knobel *et al.* 2001). There is large heterogeneity between different HIV cohorts from which BMD data have been reported with respect to patient gender, race, age and BMI, amongst other factors, however (Goh *et al.* 2018). The prevalence of osteopaenia (T-score < -1.0 and > -2.5) and osteoporosis (T-score ≤ -2.5) in PLWH was 52% and 15% respectively (67% reduced BMD overall) in one meta-analysis of pooled data from eleven cross-sectional studies published prior to 2006 (pooled cohort: n = 884, 67% male, mean age 39.6 years, mean BMI 24.1 kg m^{-2}) (Brown and Qaqish 2006), but with only one of the eleven studies including a significant number of black patients (Dolan *et al.* 2004). In four more recently published studies that included a majority of black patients (range 54% to 75%) (Arnsten *et al.* 2006, Arnsten *et al.* 2007, Jones *et al.* 2008, Libois *et al.* 2010), prevalence of reduced BMD ranged from 27% in one study (n = 263, 100% female, mean age 44 ± 5 years) (Arnsten *et al.* 2006) to 67% in another (n = 57, 60% male, mean age 61 ± 5 years) (Jones *et al.* 2008).

FRAX®-incorporated general fracture risk factors – well established predictors of BMD and fracture risk in the general population (Kanis *et al.* 2005) – have also been shown to be significantly associated with reduced BMD and/or increased fracture risk in PLWH, including older age, non-Black race, female gender, low BMI, smoking, steroid exposure and hypogonadism (Arnsten *et al.* 2007; Calmy *et al.* 2009, Carr *et al.* 2015, Cazanave *et al.* 2008, Dolan *et al.* 2006, Mondy *et al.* 2003). It is postulated that an overexpression of these general risk factors in PLWH may contribute to the increased prevalence of reduced BMD in PLWH compared to the general population (Mallon *et al.*

2010). Other factors that have been associated with reduced BMD and/or increased fracture risk in the general population, for example SSRI use (Haney *et al.* 2010), opiate use (Pedrazzoni *et al.* 1993) and other non-opiate recreational drug use (Reece *et al*. 2009), may also be over-represented in PLWH (Cooper *et al.* 2003, Pence *et al.* 2006) and therefore also contribute. In addition, HBV and HCV co-infection (Lo Re *et al.* 2009; Sharma *et al.* 2010) and sexual activity between men (Grijsen *et al.* 2013), each over-represented in PLWH overall, have all been reported to be independent risk factors for decreased BMD or increased fractures in PLWH.

HIV disease-specific risk factors are also likely to contribute to the increased prevalence of reduced BMD in PLWH, however. Low nadir CD4 cell count (Cazanave *et al*. 2008), an unsuppressed HIV viral load (Fausto *et al.* 2006) increased time since HIV diagnosis (Mondy *et al.* 2003, Dolan *et al.* 2006) and the presence of lipodystrophy (Huang *et al.* 2001) have each been significantly associated with reduced BMD in PLWH. Whilst ART can reverse or stabilise bone mineral loss by suppression of HIV viraemia and reduction of HIV-associated inflammation and bone resorption (Gibellini *et al.* 2007, Gibellini *et al.* 2008), the relationship between ART and BMD is more complex.

There is now consensus of opinion that, irrespective of the combination of ARVs used to treat HIV, BMD declines during the first one to two years following ART initiation or switch, before subsequent stabilisation (Bolland *et al.* 2011). The rate of initial BMD decline, however, is greater with certain ARVs compared to others, e.g. with tenofovir DF compared to other NRTIs (Gallant *et al*. 2004, McComsey *et al.* 2011, Stellbrink *et al.* 2010) or with PIs compared to NNRTIs (Duvivier *et al.* 2009, McComsey *et al.* 2011). Furthermore, the effects of PIs on reduced BMD appear to be independent of the co-administered booster (ritonavir or cobicistat) effect of increasing tenofovir plasma levels when tenofovir DF and boosted PIs are co-administered (Rockstroh *et al.* 2013).

The prevalence of reduced BMD has never previously been assessed in the Sheffield HIV Cohort. It is therefore not known how the burden of reduced BMD in this heterogenous population (54% black, 55% male) of relatively young age (mean 40.7 years old) and high BMI (mean 26.6 kg m^{-2}) compares to other published HIV cohorts. Furthermore, the relative contribution of general versus HIV disease-specific risk factors to reduced BMD has not been determined in this cohort and the best approach to identify PLWH within Sheffield at highest risk of reduced BMD and future fragility fractures is yet to be established.

This chapter aims to answer the following questions:

1. How representative is the Phase Two study population of the larger Phase One study population, i.e. can Phase Two study conclusions be extrapolated to the wider Sheffield HIV Cohort?
2. What is the distribution of BMD in the Phase Two study population, including the prevalence of osteopaenia and osteoporosis, are there differences between race / gender subgroups and how do BMD measurements compare to other published HIV cohorts and to the general population?
3. What is the effect of general fracture risk factors – both those incorporated into FRAX® and those not incorporated into FRAX® – and HIV disease-specific factors, including ARV exposure, on BMD in the Phase Two study population and are there differences between race / gender subgroups?

4.2 Phase Two study population demographics and comparison to Phase One study population

114 patients were recruited to Phase Two of the study, 52 (45.6%) of black race and 62 (54.4%) of white race. Black patients were 92.3% black African and 7.7% black British (97.6% and 1.8% respectively in Phase One study population), with 50% from Zimbabwe (58% in Phase One). White patients were 91.9% white British or white Irish (92.7% in Phase One), 5.7% white European (7.7% in Phase One), with one patient from each of Malta, Portugal and Slovakia, and 3.2% white other (1.8% in Phase One) with one patient from Australia and one from Chile.

The distribution of patient age, height, weight and BMI is detailed within black male, black female, white male and white female patient subgroups and compared with the larger Phase One study population in Table 4.1. The proportions of black male, black female, white male and white female patients recruited to Phase Two of the study were representative of the proportions within the larger Phase One study population. Both overall and between each race / gender-matched subgroup, Phase Two patients were, on average, older than Phase One patients (mean age 47.9 ± 10.8 years *versus* 40.7 ± 9.6 years for all patients) and black female patients in the Phase Two population were overall heavier and had a higher BMI than black female patients in the Phase One population.

Within the Phase Two study population, there was no significant difference in age between each of the four race / gender subgroups (Table 4.2). Black males and white males were significantly taller than black females and white females respectively ($p < 0.001$ for both); black males and white females were, on average, shorter than white males and black females respectively, although not significantly ($p = 0.061$ and $p = 0.273$ respectively). Black females were, on average, heavier compared to both black males and white females, but this difference was only significant between black females and white females ($p = 0.017$). Black females also had a higher BMI than white females, although not significantly ($p = 0.052$). Black female BMI was

	Phase One study population[1] (n=605)				Phase Two study population (n=114)			
	Black (n=331)		White (n=274)		Black (n=52)		White (n=62)	
	Male (n=116)	Female (n=215)	Male (n=226)	Female (n=48)	Male (n=15)	Female (n=37)	Male (n=52)	Female (n=10)
Race/gender subgroup % of study population	19.2	35.5	37.4	7.9	13.2	32.5	45.6	8.8
Mean age ± sd	40.7 ± 9.0	38.9 ± 7.9	42.8 ± 10.5	40.5 ± 12.4	49.3 ± 9.9	44.8 ± 8.5	49.9 ± 12.1	46.6 ± 11.6
(range) *years*	(21 – 71)	(19 – 63)	(20 – 75)	(18 – 76)	(31 – 72)	(32 – 63)	(32 – 76)	(22 – 62)
Mean height ± sd	1.76 ± 0.07	1.63 ± 0.06	1.78 ± 0.07	1.63 ± 0.08	1.74 ± 0.06	1.62 ± 0.04	1.77 ± 0.06	1.60 ± 0.06
(range) *m*	(161 – 192)	(147 – 181)	(160 – 203)	(140 – 179)	(1.63 – 1.82)	(1.49 – 1.70)	(1.61 – 1.90)	(1.50 – 1.71)
Mean weight ± sd	80.0 ± 15.7	76.7 ±15.4	80.03 ± 14.8	71.4 ± 18.0	79.7 ± 15.4	83.5 ± 16.3	78.9 ± 12.0	69.6 ± 13.3
(range) *kg*	(46.6 – 145.0)	(44.4 – 135.1)	(50.0 – 135.8)	(37.5 – 112.3)	(56.8 – 117.6)	(48.3 – 115.1)	(51.1 – 110.0)	(46.7 – 86.7)
Mean BMI ± sd	25.7 ± 4.7	28.7 ± 5.5	25.3 ± 4.4	26.6 ± 5.4	26.3 ± 4.53	31.7 ± 5.65	25.1 ± 3.3	27.3 ± 6.2
(range) *kg* m^{-2}	(15.0 – 43.8)	(15.5 – 50.9)	(16.0 – 44.6)	(15.5 – 37.2)	(20.6 – 37.1)	(20.4 – 42.7)	(16.9 – 34.0)	(18.5 – 38.5)

1. Excluding 20 non-black non-white patients

Table 4.1. Distributions of race, gender, age, height, weight and BMI within the Phase One population versus the Phase Two study population

Patient race / gender subgroup	**n**	**Age** *years*		**Height** *m*		**Weight** *kg*		**BMI** *kg m^{-2}*	
		Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
All patients	114	47.9 ± 10.8	-	1.70 ± 0.09	-	79.7 ± 14.3	-	27.6 ± 5.42	-
Black male	15	49.3 ± 9.9	.148	1.74 ± 0.06	**<.001**	79.7 ± 15.4	.419	26.3 ± 4.53	**.002**
Black female	37	44.8 ± 8.5		1.62 ± 0.04		83.5 ± 16.3		31.7 ± 5.65	
Black male	15	49.3 ± 9.9	.994	1.74 ± 0.06	.061	79.7 ± 15.4	.872	26.3 ± 4.53	.489
White male	52	49.9 ± 12.1		1.77 ± 0.06		78.9 ± 12.0		25.1 ± 3.3	
Black female	37	44.8 ± 8.5	.404	1.62 ± 0.04	.273	83.5 ± 16.3	**.017**	31.7 ± 5.65	.052
White female	10	46.6 ± 11.6		1.60 ± 0.06		69.6 ± 13.3		27.3 ± 6.2	
White male	52	49.9 ± 12.1	.688	1.77 ± 0.06	**<.001**	78.9 ± 12.0	**.028**	25.1 ± 3.3	.358
White female	10	46.6 ± 11.6		1.60 ± 0.06		69.6 ± 13.3		27.3 ± 6.2	

Table 4.2. Differences in distribution of age, height, weight and BMI between black male, black female, white male and white female patients within the Phase Two study population

significantly higher than black males, however (p = 0.002). White males were significantly heavier than white females (p = 0.028), but with no significant difference in BMI between the two subgroups.

BMI increased with age in all patients, although not significantly (r = 0.144, p = 0.128); this was also observed in all patient race / gender subgroups and was significant in black female patients (p = 0.041); the rate of BMI increase with age was steeper in female patients (r = 0.337 and r = 0.333 in black and white females respectively) than in male patients (r = 0.166 and r = 0.257 in black and white males respectively) (Figure 4.1).

4.3 BMD measurements, T-scores and Z-scores by race and gender

The distributions of lumbar spine, total hip, femoral neck and total body BMD measurements in the four race / gender patient subgroups are shown in Table 4.3 and Figure 4.2.

Whilst mean total body BMD was lower in female patients compared with male patients of the same race and in white patients compared with black patients of the same gender, there were no statistically significant differences between any patient subgroup. There were no significant differences in lumbar spine, total hip or femoral neck BMD between patients of the same race but different gender, or of the same gender but different race.

The percentage prevalence of normal BMD (T-score ≥ -1.0), osteopaenia (T-score < -1.0 and > -2.5) and osteoporosis (T-score ≤ -2.5) for lumbar spine and total hip are shown for each patient subgroup in Figure 4.3. Reference range data used to calculate black male and black female T-scores were sourced from Kelly *et al.* (1990) and Looker *et al.* (1998) for lumbar spine and total hip respectively. The percentage prevalence of Z-score ≥ -1.0, Z-score < -1.0 and > -2.5 and Z-score ≤ -2.5 for each BMD site are shown for each patient subgroup in Figure 4.4. The differences in distribution of T- and Z-

scores between patient subgroups are shown in Tables 4.4 and 4.5 respectively and in Figure 4.5.

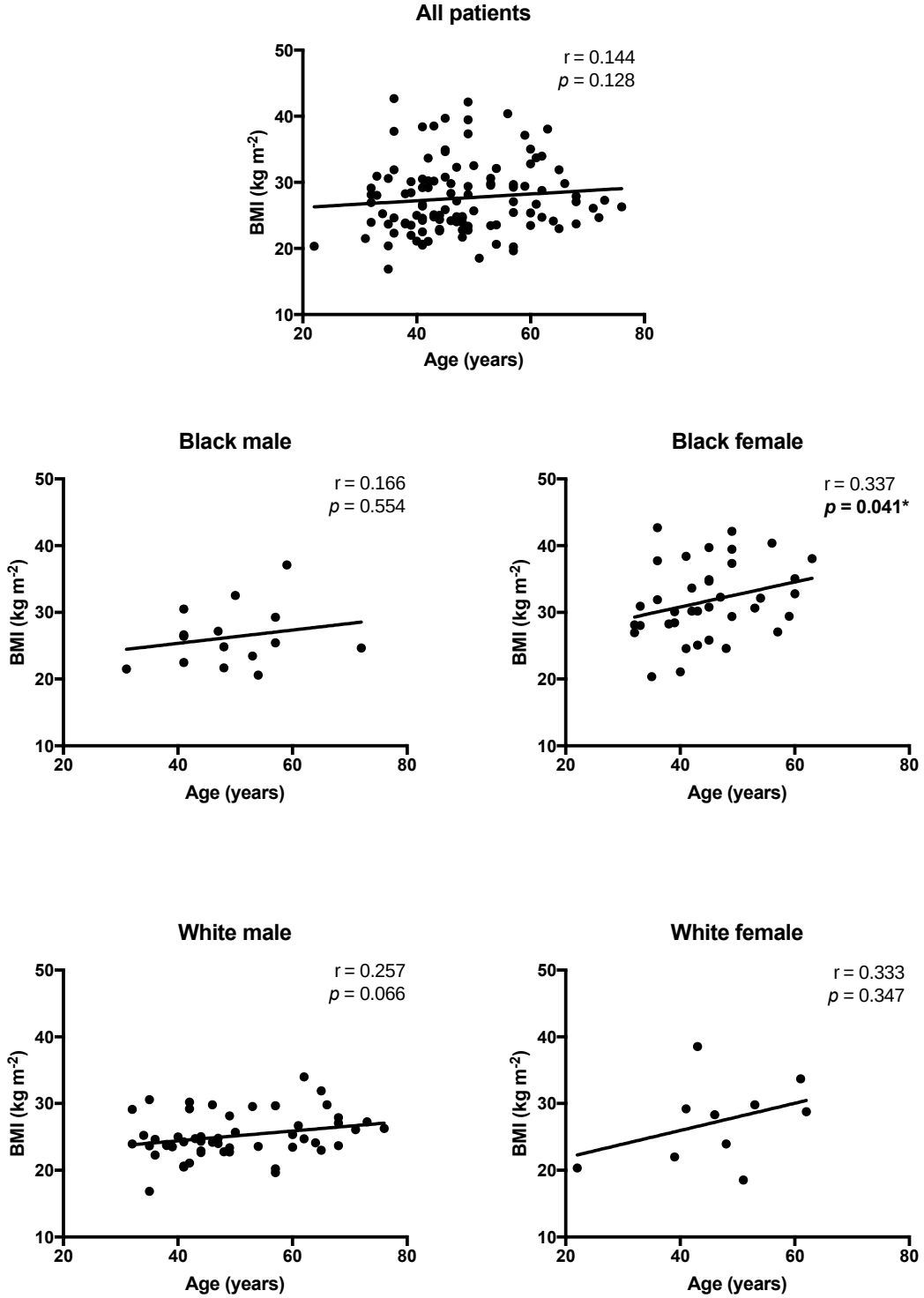


Figure 4.1. Relationship between age and BMI in all patients (n = 114), black males (n = 15), black females (n = 37), white males (n = 52) and white females (n = 10) within the Phase Two study population

Patient race / gender subgroup	**n**	**Lumbar spine BMD** *g cm^{-2}*		**Total hip BMD** *g cm^{-2}*		**Femoral neck BMD** *g cm^{-2}*		**Total body BMD** *g cm^{-2}*	
		Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
All patients	114	1.004 ± .130	-	0.959 ± .133	-	0.813 ± .135	-	1.102 ± .102	-
Black male	15	0.970 ± .142	.123	0.991 ± .152	.926	0.835 ± .169	.579	1.123 ± .087	.548
Black female	37	1.035 ± .131		0.987 ± .115		0.860 ± .136		1.106 ± .981	
Black male	15	0.970 ± .142	.515	0.991 ± .152	.180	0.835 ± .169	.109	1.123 ± .087	.461
White male	52	0.994 ± .120		0.937 ± .131		0.777 ± .106		1.102 ± .100	
Black female	37	1.035 ± .131	.919	0.987 ± .115	.840	0.860 ± .136	.785	1.106 ± .981	.214
White female	10	0.990 ± .159		0.927 ± .162		0.788 ± .166		1.055 ± .143	
White male	52	0.994 ± .120	.362	0.937 ± .131	.190	0.777 ± .106	.163	1.102 ± .100	.197
White female	10	0.990 ± .159		0.927 ± .162		0.788 ± .166		1.055 ± .143	

Table 4.3. Differences in distribution of lumbar spine, total hip, femoral neck and total body BMD measurements between black male, black female, white male and white female patients within the Phase Two study population

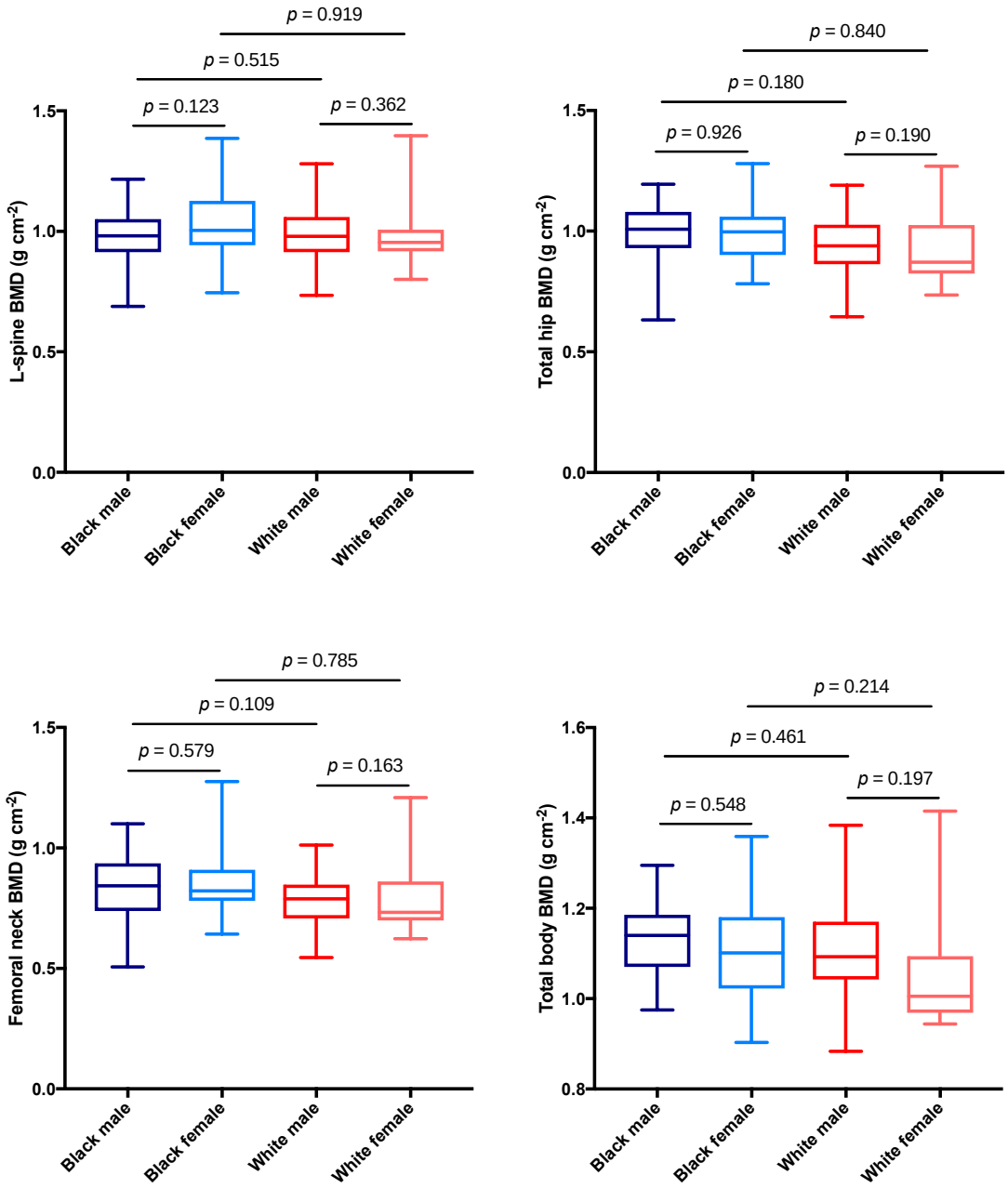


Figure 4.2. Difference in distribution of lumbar (L-) spine, total hip, femoral neck and total body BMD in black male (n=15), black female (n=37), white male (n=52) and white female (n=10) patients within the Phase Two study population

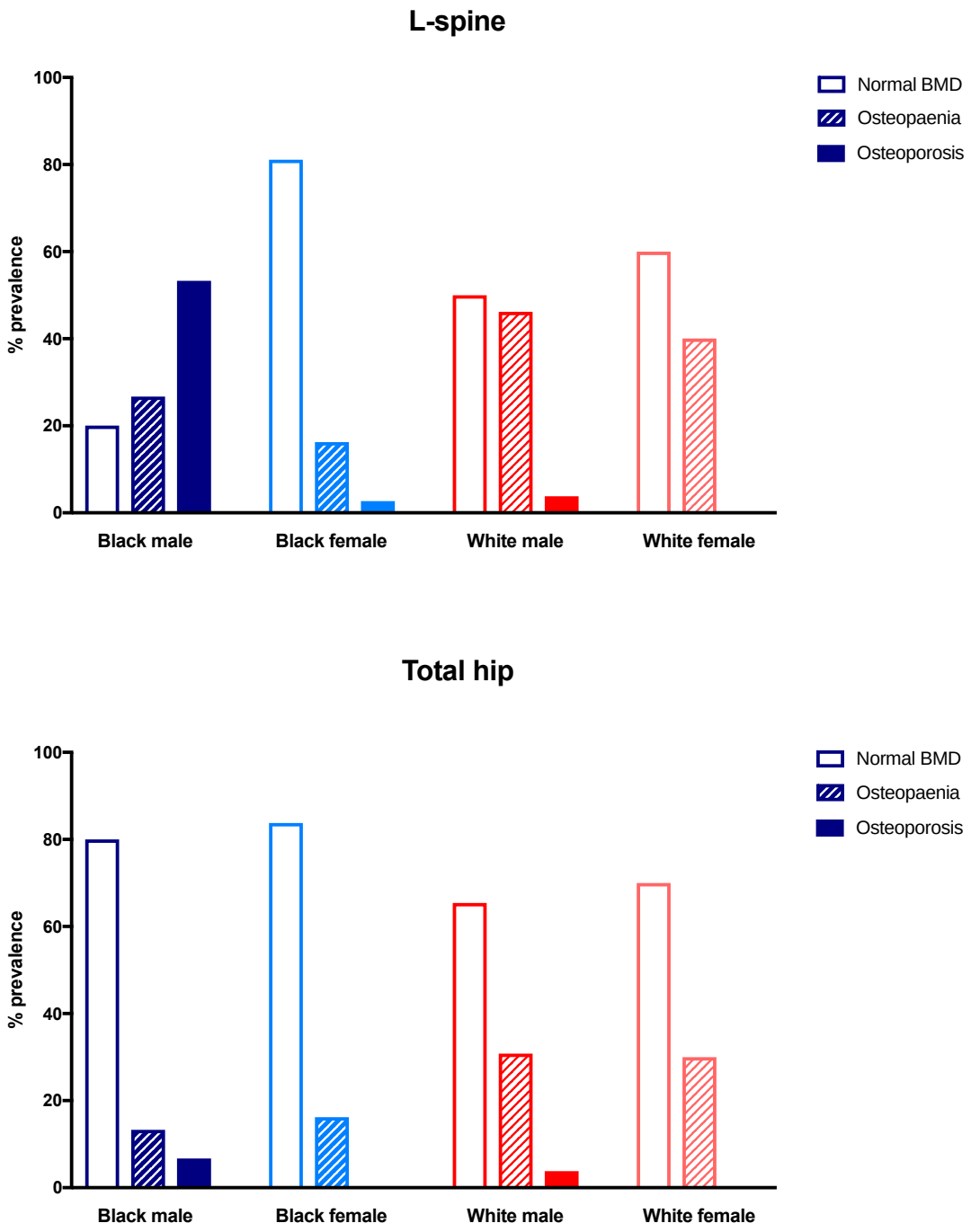


Figure 4.3. Percentage prevalence of normal BMD (T-score ≥ -1.0), osteopaenia (T-score < -1.0 and > -2.5) and osteoporosis (T-score ≤ -2.5) for lumbar (L-) spine and total hip in black male (n=15), black female (n=37), white male (n=52) and white female (n=10) patients within the Phase Two study population

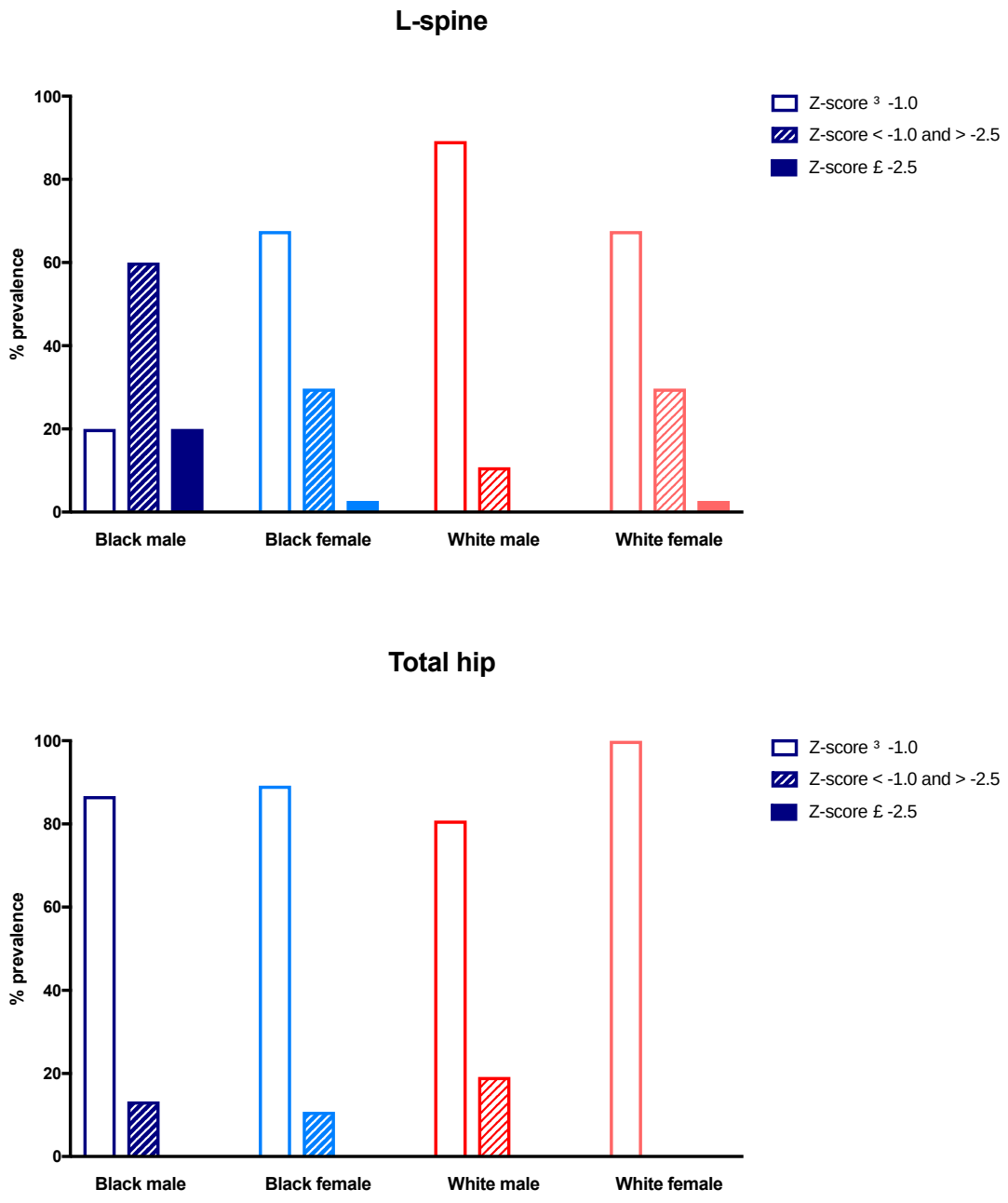


Figure 4.4. Percentage prevalence of Z-scores ≥ -1.0, between < -1.0 and > -2.5 and ≤ -2.5 for lumbar (L-) spine and total hip in black male (n=15), black female (n=37), white male (n=52) and white female (n=10) patients within the Phase Two study population

Patient race / gender subgroup	n	Lumbar spine T-score		Total hip T-score	
		Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
All patients	114	-0.627 ± 1.225	-	-0.344 ± 1.143	-
Black male	15	-1.107 ± 1.305	.**010**	-0.380 ± 1.111	.069
Black female	37	-0.111 ± 1.187		0.249 ± 1.104	
Black male	15	-1.107 ± 1.305	.473	-0.380 ± 1.111	.162
White male	52	-0.867 ± 1.079		-0.788 ± 0.948	
Black female	37	-0.111 ± .1.187	.307	0.249 ± .1.104	.318
White female	10	-0.570 ± 1.460		-0.170 ± 1.378	
White male	52	-0.867 ± 1.079	.455	-0.788 ± 0.948	.085
White female	10	-0.570 ± 1.460		-0.170 ± 1.378	

Table 4.4. Differences in distribution of lumbar spine and total hip T-scores between black male, black female, white male and white female patients within the Phase Two study population

Patient race / gender subgroup	n	Lumbar spine Z-score		Total hip Z-score	
		Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
All patients	114	-0.557 ± 1.299	-	-0.096 ± 0.973	-
Black male	15	-1.680 ± 1.201	**.002**	-0.313 ± 0.971	.205
Black female	37	-0.386 ± 1.307		0.049 ± 0.901	
Black male	15	-1.680 ± 1.201	**.001**	-0.313 ± 0.971	.818
White male	52	-0.488 ± 1.115		-0.250 ± 0.927	
Black female	37	-0.386 ± 1.307	.280	0.049 ± 0.901	.216
White female	10	0.140 ± 1.512		0.490 ± 1.273	
White male	52	-0.488 ± 1.115	.129	-0.250 ± 0.927	**.034**
White female	10	0.140 ± 1.512		0.490 ± 1.273	

Table 4.5. Differences in distribution of lumbar spine, total hip and total body Z-scores between black male, black female, white male and white female patients within the Phase Two study population

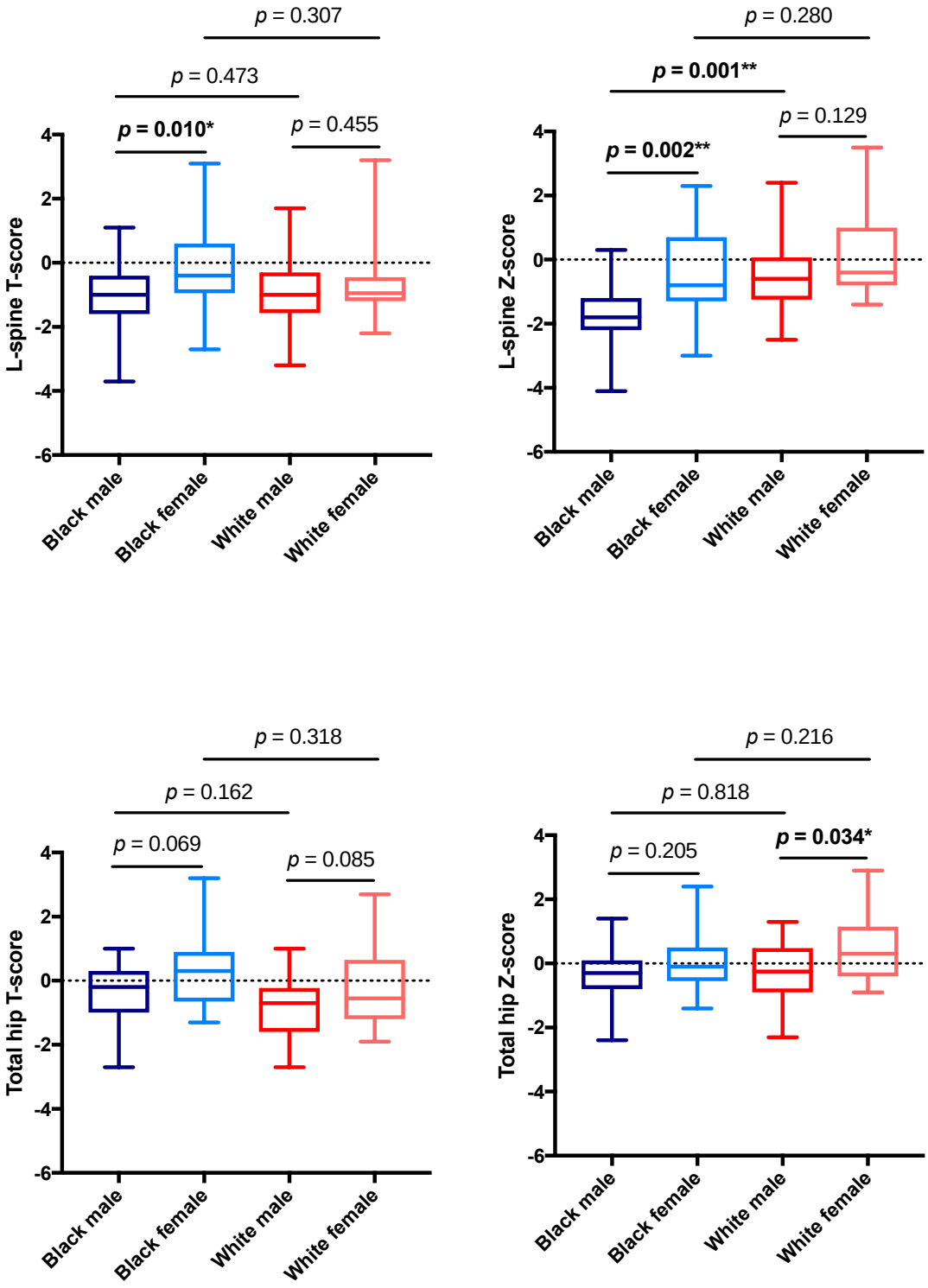


Figure 4.5. Difference in distribution of lumbar (L-) spine and total hip T- and Z-scores in black male (n=15), black female (n=37), white male (n=52) and white female (n=10) patients within the Phase Two study population

In the Phase Two study population overall, the prevalence of osteopaenia (T-score <-1 and >-2.5) and osteoporosis (T-score ≤-2.5) respectively were 33.3% and 9.6% for lumbar spine and 23.7% and 2.7% for total hip.

Eight (53.3%) black male patients had osteoporosis at the lumbar spine and a further four (26.7%) had lumbar spine osteopaenia, leaving only three (20.0%) patients with normal lumbar spine BMD as defined by T-score. Lumbar spine T-scores were lower in black males than in white males and significantly lower than in black females (p = 0.010), with contrastingly low proportions of patients with lumbar spine osteoporosis in black females, white males and white females (2.7%, 3.8% and 0.0% respectively). A greater proportion of black female patients had normal lumbar spine BMD than white female patients (81.1% versus 60.0%). Lumbar spine Z-scores were also significantly lower in black males compared with both black females (p = 0.002) and white males (p = 0.001).

In contrast, the distribution of hip T- and Z-scores were more similar between the four patient subgroups, with very few patients with hip osteoporosis across all subgroups; the proportion of patients with normal total hip BMD was higher in both black male and female patients compared with white male and female patients (80.0% and 83.8% versus 65.4% and 70.0% respectively).

4.4 Age, height, weight and BMI as determinants of BMD in PLWH

Correlations between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD are shown in Table 4.6 for all Phase Two patients and for each patient race / gender subgroup in Appendix 1, Tables A1.1 to A1.4.

All patients (n = 114)	**Lumbar spine BMD** *g cm^{-2}*		**Total hip BMD** *g cm^{-2}*		**Femoral neck BMD** *g cm^{-2}*		**Total body BMD** *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age	-0.158	.094	-0.107	.258	-0.187	**.046**	-0.149	.115
Height	0.039	.684	0.045	.638	-0.060	.524	0.174	.064
Weight	0.472	**<.001**	0.523	**<.001**	0.453	**<.001**	0.312	**.001**
BMI	0.391	**<.001**	0.469	**<.001**	0.471	**<.001**	0.212	**.023**

Table 4.6. Relationship between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114)

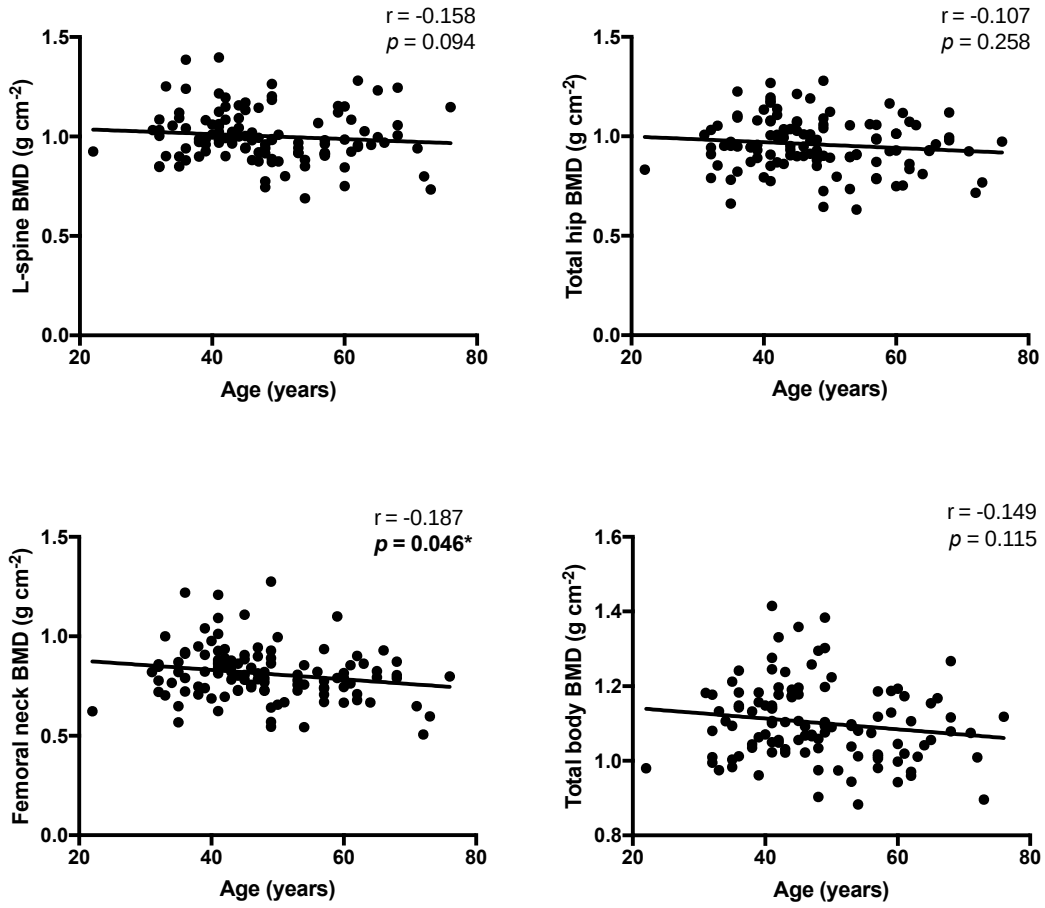


Figure 4.6. Relationship between age and lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114)

Whilst BMD decreased at each site with increasing patient age, this was only significant in all patients at the femoral neck ($p = 0.046$) (Figure 4.6), with a steeper decline in BMD with increasing age seen in black male patients (comparative lumbar spine BMD subgroup data shown in Figure 4.7) but with no significant correlation between age and BMD within any race / gender subgroup (Appendix 1, Tables A1.1 to A1.4).

Whereas BMD did not change significantly with height, neither in all patient analysis nor within race / gender subgroup analyses, BMD significantly increased with both increases in weight and BMI at each site in all patients ($p < 0.001$, $p < 0.001$, $p < 0.001$ and $p = 0.001$ for weight and lumbar spine, total

hip, femoral neck and total body BMD respectively; $p < 0.001$, $p < 0.001$, $p < 0.001$ and $p = 0.023$ for BMI and lumbar spine, total hip, femoral neck and total body BMD respectively), with a similar relationship between BMD and weight and BMD and BMI (relationship between BMD and BMI shown for all patients in Figure 4.8). A similar relationship was observed between weight and BMD and BMI and BMD within each race / gender subgroup, although weight and BMI correlations with BMD were not statistically significant at every BMD site within every subgroup (Appendix 1, Tables A1.1 to A1.4).

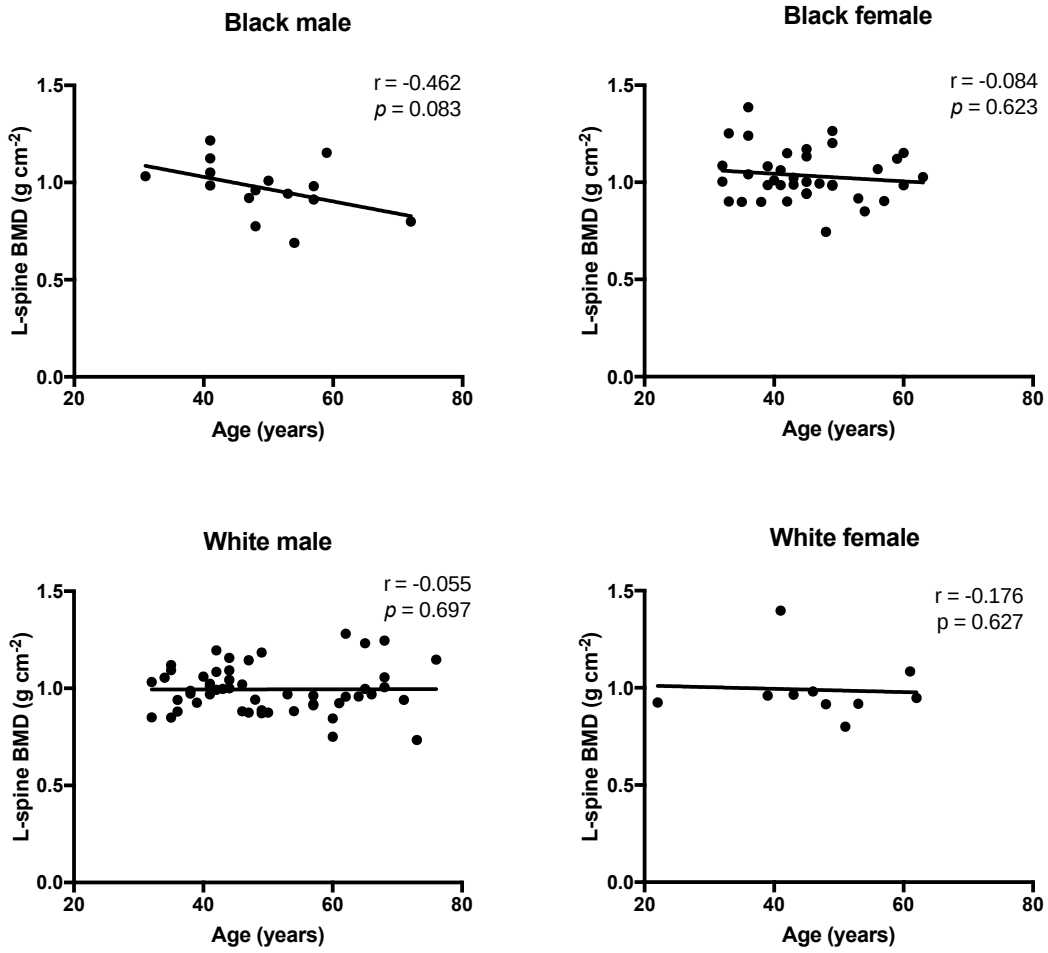


Figure 4.7. Relationship between age and lumbar (L-) spine BMD in black male (n = 15), black female (n = 37), white male (n = 52) and white female (n = 10) patients within the Phase Two study population

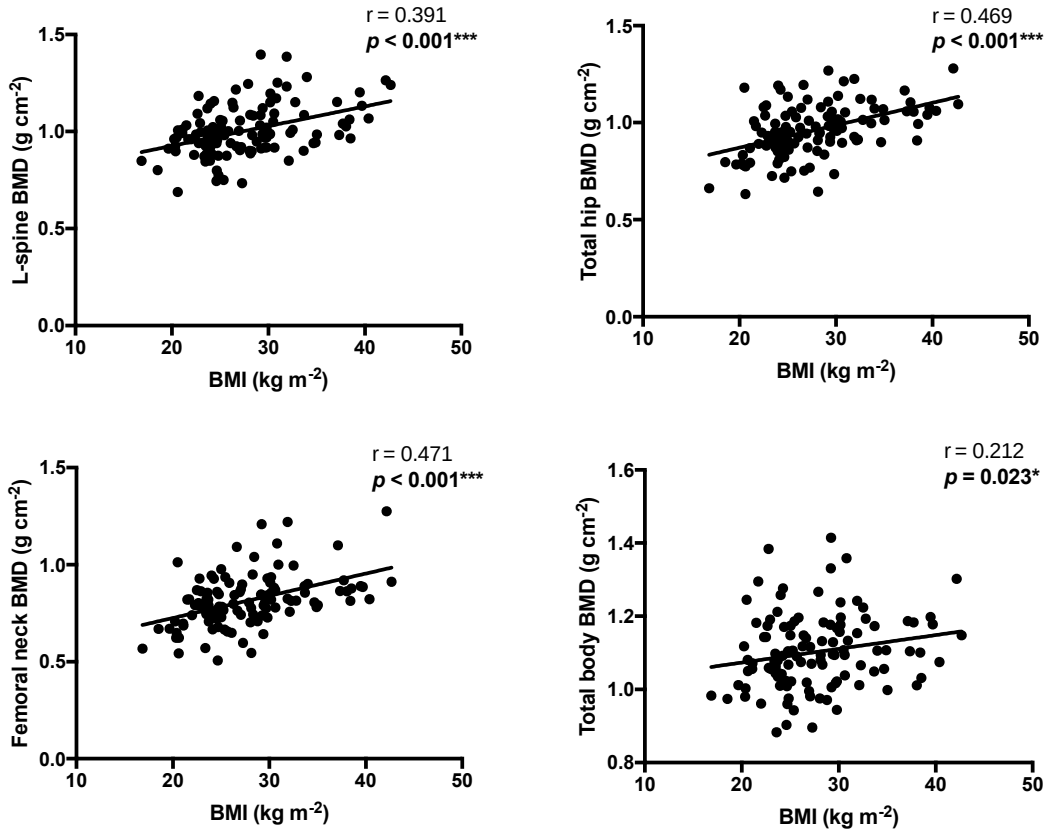


Figure 4.8. Relationship between BMI and lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114)

4.5 FRAX®-incorporated general fracture risk factors as determinants of BMD in PLWH

The differences in lumbar spine, total hip, femoral neck and total body BMD according to either the presence or absence of FRAX®-incorporated general fracture risk factors are shown for all Phase Two recruited patients in Tables 4.7 and 4.8. (The effects of race, gender, age and BMI on BMD – also FRAX®-incorporated general fracture risk factors – have already been described in Sections 4.3 and 4.4.) Differences in BMD by risk factor presence or absence within each of the four race / gender patient subgroups are detailed in Appendix 1, Tables A1.5 to A1.12.

Whilst there was a trend to lower BMD at the lumbar spine, total hip and femoral neck in current smokers compared with non-smokers (Figure 4.9), the differences in BMD were not significant at any site ($p = 0.249$, $p = 0.139$ and $p = 0.091$ for lumbar spine, total hip and femoral neck respectively), nor were these differences significant within any patient subgroup. Average current alcohol consumption ≥3 units per day was associated with lower BMD at all sites in all patients, however, and significantly so for total hip and femoral neck BMD in all patients ($p = 0.017$ and $p = 0.002$ respectively) (Figure 4.10); reduced BMD with increased alcohol consumption was more marked in black male patients ($p = 0.010$, $p = 0.004$ and $p = 0.005$ for lumbar spine, total hip and femoral neck respectively), although with no significant difference observed at any site in white male patients ($p = 0.884$, $p = 0.449$ and $p = 0.248$ for lumbar spine, total hip and femoral neck respectively) (Figure 4.11). (No black female patients and only one white female patient reported consumption of ≥3 units per day.)

Significant steroid exposure (defined in Table 2.1), contrary to expectation, was associated with higher BMD at all sites in all patients and this was statistically significant for total body BMD ($p = 0.045$) (Figure 4.12). Six of the seven patients with significant steroid exposure were white males, in whom the increase in BMD compared with the 46 white males without significant steroid exposure was significant for total body ($p = 0.007$). The six white male patients with significant steroid exposure were, on average, younger (mean age 46.2 ± 10.0 years) compared with the 46 male patients without significant steroid exposure (mean age 50.4 ± 12.4 years) and with a higher BMI (mean 26.9 ± 4.4 kg m^{-2} *vs.* 24.9 ± 3.6 kg m^{-2}), although not significantly. Of the six patients with significant steroid exposure, none had a diagnosis of rheumatoid arthritis, one had a diagnosis of inflammatory bowel disease (IBD) and one had COPD respectively, compared with one patient with rheumatoid arthritis, no patients with IBD and one patient with COPD in those without significant steroid exposure.

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current smoking	No	92 (80.7)	1.010 ± .127	.249	0.968 ± .129	.139	0.823 ± .134	.091	1.100 ± .101	.704
	Yes	22 (19.3)	0.975 ± .143		0.922 ± .144		0.768 ± .135		1.109 ± .109	
Current alcohol ≥3 units d^{-1}	No	95 (83.3)	1.013 ± .129	.105	0.973 ± .132	**.017**	0.830 ± .135	**.002**	1.108 ± .106	.146
	Yes	19 (16.7)	0.959 ± .133		0.893 ± .117		0.727 ± .103		1.071 ± .078	
Fragility fracture history	No	104 (91.2)	1.102 ± .122	**.029**	0.966 ± .125	.081	0.817 ± .131	.250	1.107 ± .102	.061
	Yes	10 (8.8)	0.918 ± .185		0.889 ± .190		0.765 ± .168		1.044 ± 0.90	
Significant steroid exposure	No	107 (93.9)	0.999 ± .130	.179	0.957 ± .130	.407	0.811 ± .136	.595	1.097 ± .100	**.045**
	Yes	7 (6.1)	1.068 ± .127		0.999 ± .172		0.839 ± .134		1.177 ± .124	
Parental hip fracture	No	111 (97.4)	1.003 ± .131	.745	0.959 ± .130	.666	0.813 ± .134	.661	1.101 ± .103	.558
	Yes	3 (2.6)	1.028 ± .140		0.992 ± .240		0.779 ± .191		1.136 ± .105	
Rheumatoid arthritis	No	113 (97.4)	1.005 ± .130	.235	0.962 ± .130	**.024**	0.815 ± .133	.067	1.103 ± .102	.245
	Yes	1 (2.6)	0.849		.662		0.567		0.983	
Other disorders*	No	75 (65.8)	1.016 ± .133	.150	0.981 ± .134	**.016**	0.836 ± .142	**.010**	1.119 ± .102	**.012**
	Yes	39 (34.2)	0.979 ± .123		0.918 ± .122		0.768 ± .108		1.069 ± .096	

*other disorders strongly associated with osteoporosis, including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant receipt and osteogenesis imperfecta (no patients recruited to Phase Two study with any of the latter four disorders)

Table 4.7. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114) according to presence or absence of FRAX®-incorporated general fracture risk factors

Fracture risk factor*	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Chronic diarrhoea	No	99 (86.8)	1.003 ± .135	.909	0.967 ± .134	.110	0.820 ± .137	.144	1.107 ± .104	.219
	Yes	15 (13.2)	1.007 ± .098		0.908 ± .114		0.765 ± .112		1.072 ± .091	
Prolonged immobility	No	101 (88.6)	1.009 ± .125	.169	0.967 ± .013	.072	0.820 ± .133	.111	1.103 ± .101	.727
	Yes	13 (11.4)	0.957 ± .046		0.897 ± .151		0.756 ± .142		1.093 ± .115	
Hypogonadism	No	103 (90.4)	1.008 ± .133	.272	0.962 ± .134	.503	0.817 ± .138	.304	1.108 ± .103	.051
	Yes	11 (9.6)	0.962 ± .093		0.933 ± .126		0.773 ± .094		1.044 ± .743	
Male hypogonadism *(n=67)*	No	60 (89.6)	0.991 ± .130	.687	0.951 ± .137	.664	0.794 ± .126	.438	1.114 ± .099	.095
	Yes	7 (10.4)	0.971 ± .058		0.927 ± .139		0.755 ± .114		1.048 ± .055	
Female hypogonadism *(n=47)*	No	43 (91.5)	1.032 ± .136	.246	0.977 ± .129	.632	0.849 ± .150	.553	1.100 ± .109	.286
	Yes	4 (8.5)	0.948 ± .148		0.945 ± .117		0.803 ± .038		1.039 ± .111	
Chronic obstructive pulmonary disease	No	112 (98.3)	1.004 ± .132	.814	0.960 ± .133	.544	0.814 ± .135	.376	1.102 ± .103	.566
	Yes	2 (1.7)	0.982 ± .034		0.903 ± .131		0.728 ± .087		1.061 ± .026	
Malabsorption	No	113 (99.1)	1.004 ± .131	.626	0.960 ± .133	.796	0.814 ± .135	.225	1.102 ± .103	.793
	Yes	1 (0.9)	0.940		0.925		0.649		1.075	
Inflammatory bowel disease	No	113 (99.1)	1.001 ± .128	**.032**	0.958 ± .133	.391	0.812 ± .135	.515	1.102 ± .103	.969
	Yes	1 (0.9)	1.1281		1.073		0.901		1.106	
Untreated hyperthyroidism	No	113 (99.1)	1.005 ± .131	.445	0.960 ± .133	.511	0.813 ± .135	.601	1.103	.237
	Yes	1 (0.9)	0.904		0.872		0.742		.981	

Table 4.8. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114) according to presence or absence of FRAX®-incorporated "other disorders strongly associated with osteoporosis"

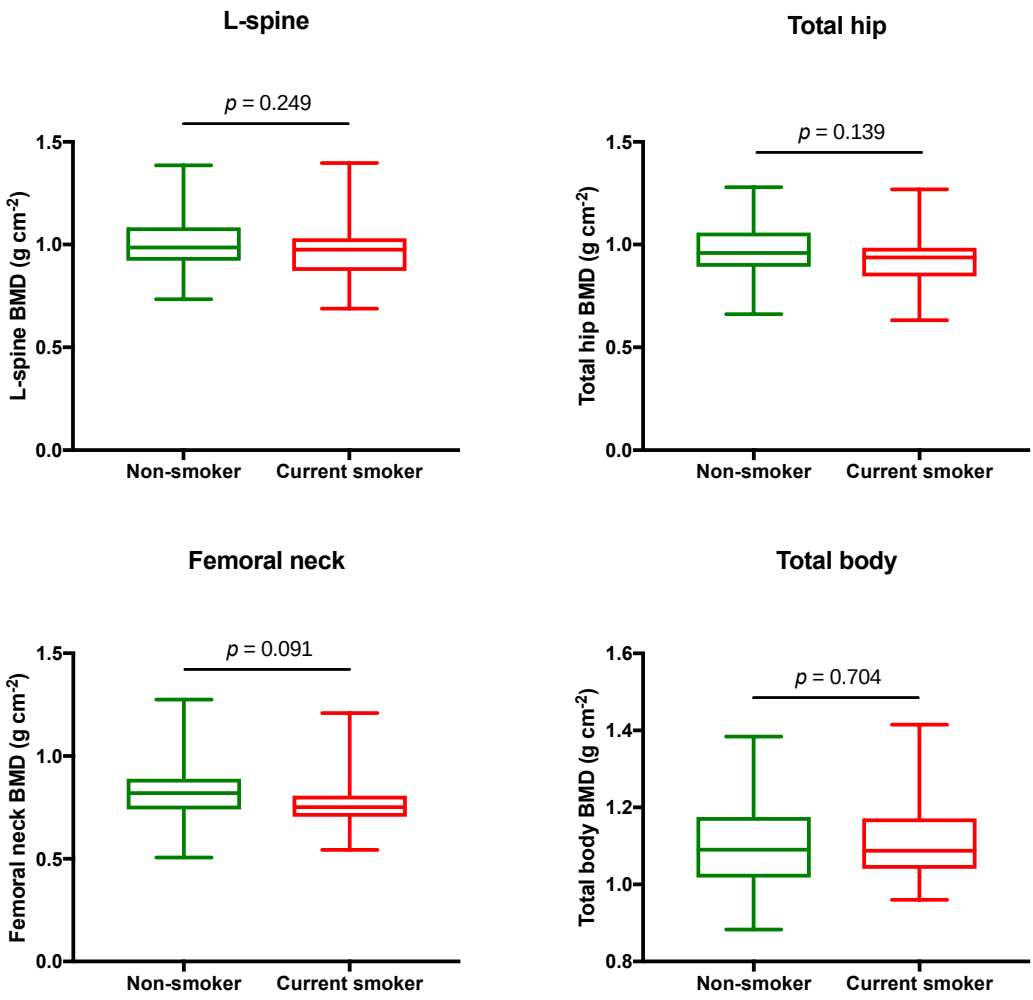


Figure 4.9. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in non-smokers (n=92) compared to current smokers (n=22) within the Phase Two study population

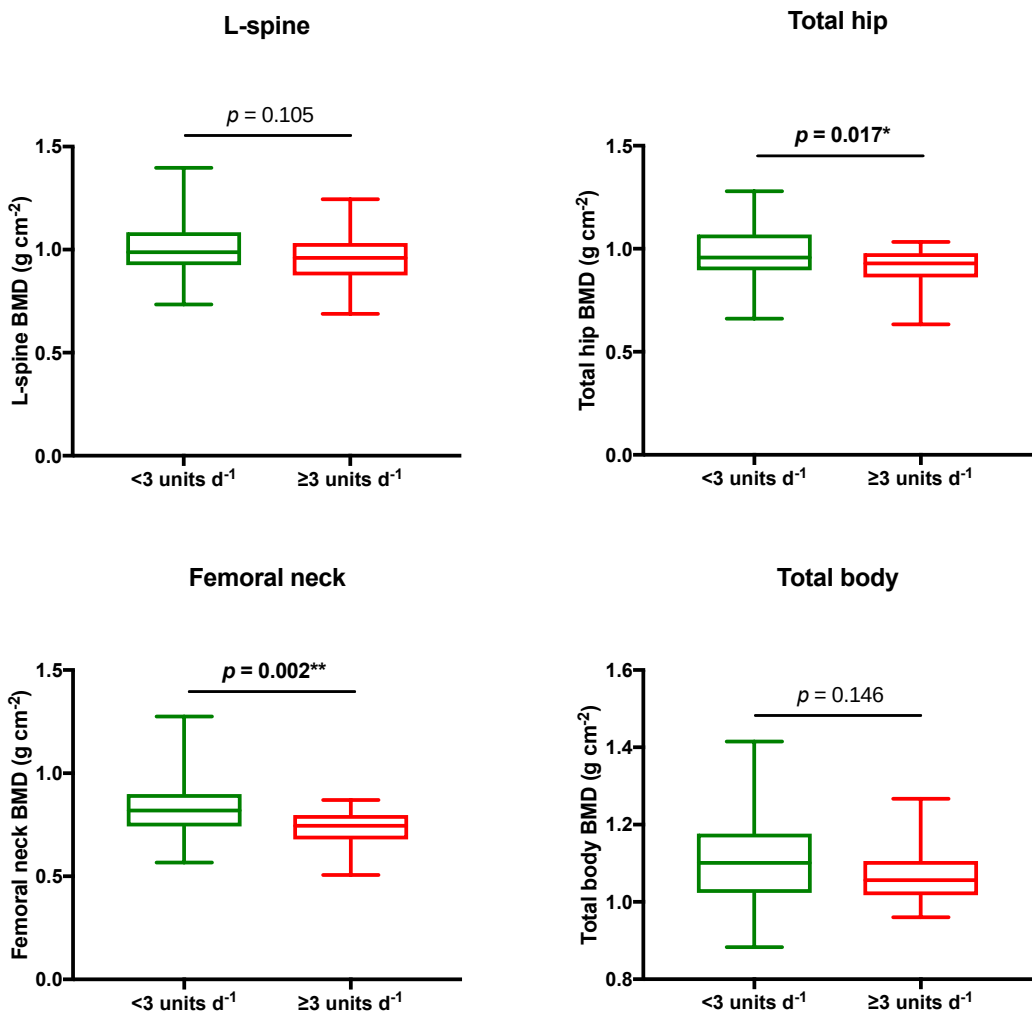


Figure 4.10. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients consuming on average <3 units alcohol per day (n=95) compared to patients consuming on average ≥3 units alcohol per day (n=19)

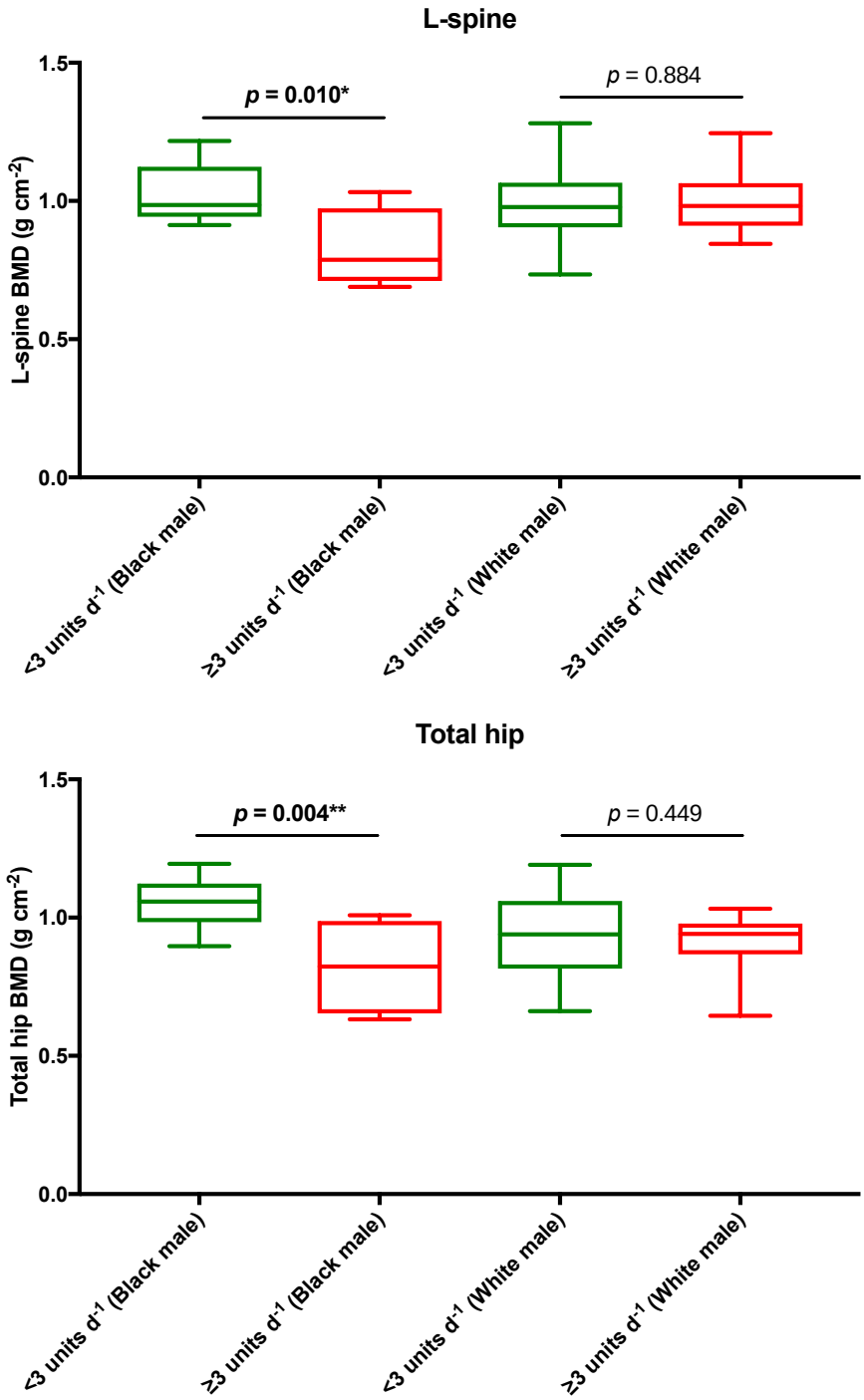


Figure 4.11. Differences in lumbar (L-) spine and total hip BMD in black males consuming on average <3 units alcohol per day (n=11) compared to black males consuming on average ≥3 units alcohol per day (n=4) and in white males consuming on average <3 units alcohol per day (n=38) compared to white males consuming on average ≥3 units alcohol per day (n=14) within the Phase Two study population

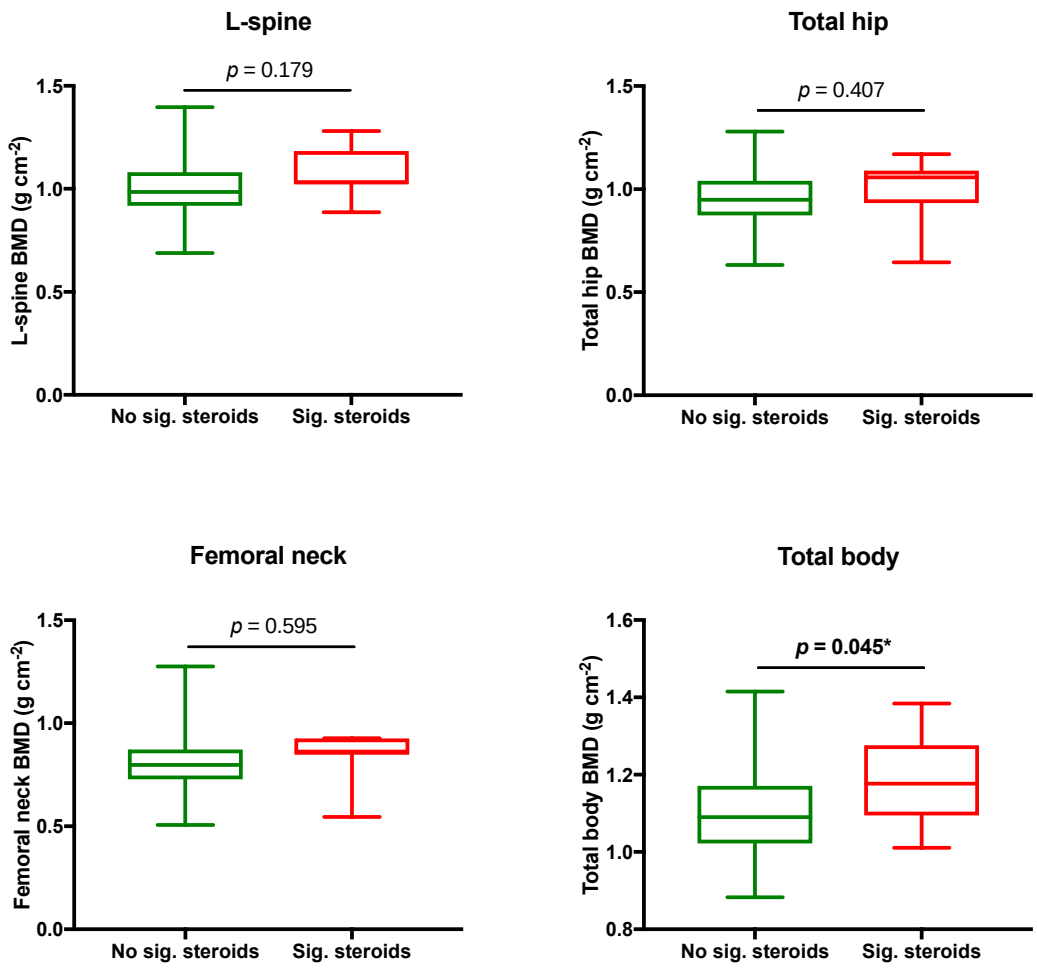


Figure 4.12. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients without significant steroid exposure (n=107) compared to patients with significant steroid exposure (n=7)

Rheumatoid arthritis was associated with reduced BMD at all sites and with significantly reduced BMD at both the total hip and femoral neck (p = 0.024 and p = 0.067 respectively), although in one patient only compared with 113 without rheumatoid arthritis. Of note, the 35-year old white male patient with rheumatoid arthritis had never been treated with steroids (treated with the anti-TNF-α monoclonal antibody infliximab), but had a very low BMI (16.9 kg m^{-2}).

The prevalence of Phase Two recruited patients reporting a history of parental hip fracture was only 2.6% (n = 3) – one black female and two white male patients – and there was no significant difference in BMD at any site comparing these three patients with the 111 patients without parental hip fracture history (Table 4.7).

39 patients with a history of current or past "other disorders strongly associated with osteoporosis" (as defined by and incorporated into FRAX®) had lower lumbar spine BMD (p = 0.150) and significantly lower total hip, femoral neck and total body BMD (p = 0.016, p = 0.010 and p = 0.012 respectively) compared with 75 patients without a history of "other disorder" (Figure 4.13). Within patient race / gender subgroups, however, a significant reduction in BMD in patients with a history of "other disorder" was only observed in black female patients (eight patients (21.6%) with a history of "other disorder") and only for total body BMD (p = 0.007).

With respect to each specific "other disorder", when analysing all Phase Two patients collectively (Table 4.8), the only significant difference identified was increased BMD at the lumbar spine in the one white male patient with a diagnosis of IBD (p = 0.032) when compared to the 113 patients without IBD (the patient with IBD also had a history of significant steroid exposure). Although not significant, there was also a trend to reduced lumbar spine, total hip and femoral neck BMD in patients with a history of prolonged immobility compared to those without (p = 0.169, p = 0.072 and p = 0.111 respectively) (Figure 4.14) and reduced total body BMD in all patients with hypogonadism (p = 0.051) compared to patients without hypogonadism.

Within patient race / gender subgroups, prolonged immobility was only significantly associated with reduced BMD in black male patients (p = 0.035, p = 0.009 and p = 0.160 for lumbar spine, total hip and femoral neck BMD respectively), although with only one black male with a history of prolonged immobility (patient 54 years old, with below average BMI 20.6 kg m^{-2} and a current smoker) compared with 14 black males without.

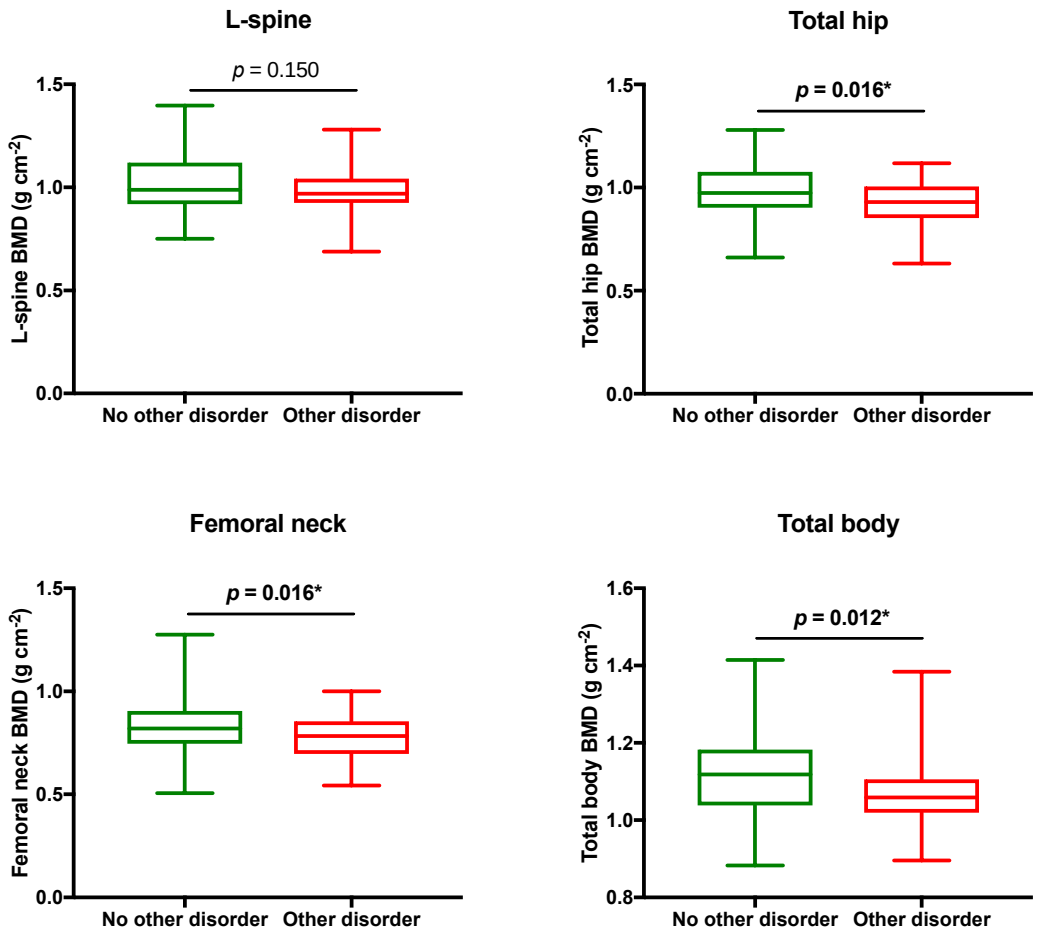


Figure 4.13. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients without (n=75) compared to patients with (n=39) a significant history of FRAX®-defined "other disorders strongly associated with osteoporosis", including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant receipt and osteogenesis imperfecta (no patients recruited to Phase Two study with any of latter four disorders)

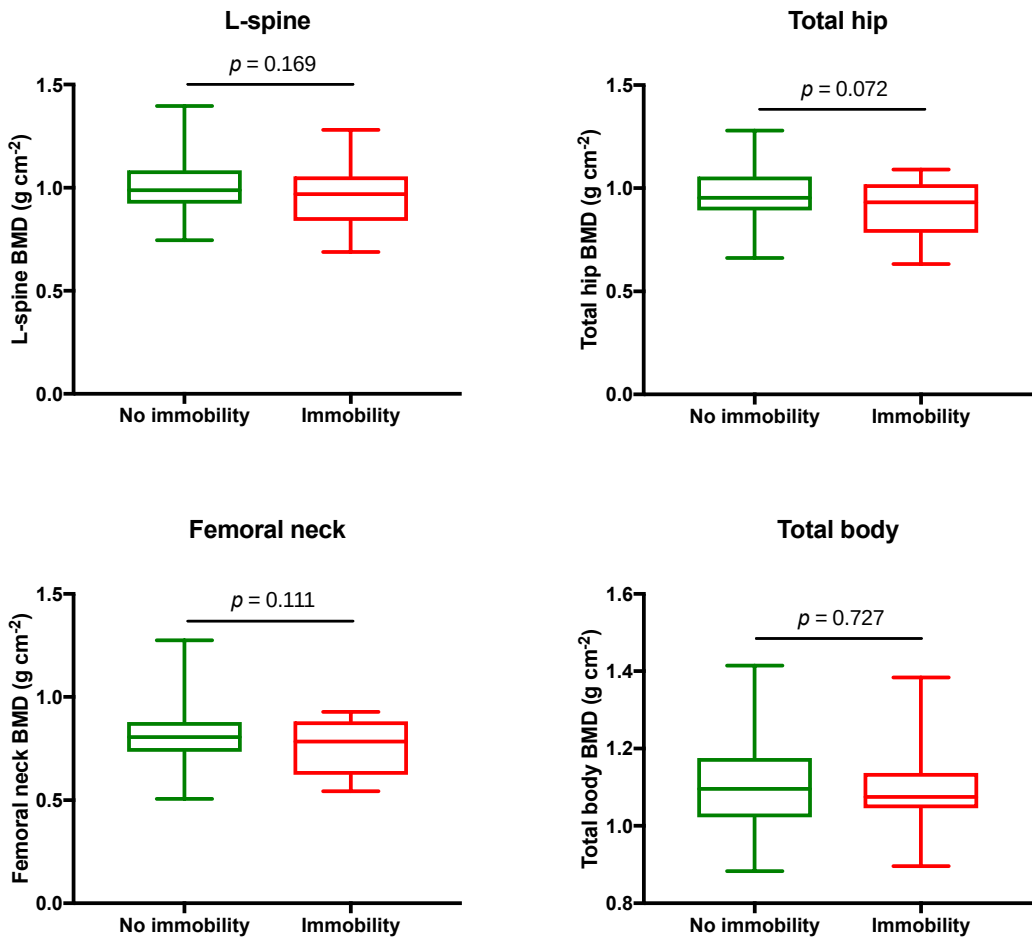


Figure 4.14. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients without (n=101) compared to those with (n=13) a history of prolonged immobility

Female hypogonadism (premature menopause, onset before 45 years of age), reported in three (8.1%) of black female patients, was associated with significantly reduced total body BMD (p = 0.037), compared with black females without hypogonadism. No such difference was seen in white female patients (p = 0.418), albeit with only one white female patient reporting premature menopause, nor when analysing black and white female patients together (p = 0.286).

Current or past male hypogonadism was not associated with any significant difference in BMD at any site in black male patients, white male patients or in male patients analysed together.

Ten (8.8%) patients had sustained at least one fragility fracture in the Phase Two study population overall: two (13.3%), two (5.4%), four (7.7%) and two (20.0%) patients within black male, black female, white male and white female subgroups respectively. Three old fragility fractures were diagnosed on vertebral fracture assessment only without patient self-reported history; one, four and five fragility fractures were reported at the hip, wrist and lower thoracic or lumbar spine respectively. Patients with fragility fracture history had, overall, lower BMD at each site compared with patients without a fragility fracture history (Figure 4.15), although this was only significant at the lumbar spine (p = 0.029). Within each patient race / gender subgroup, BMD was also lower at each BMD site in patients with a history of fragility fracture, but only significantly lower in black female patients for lumbar spine BMD only (p = 0.033).

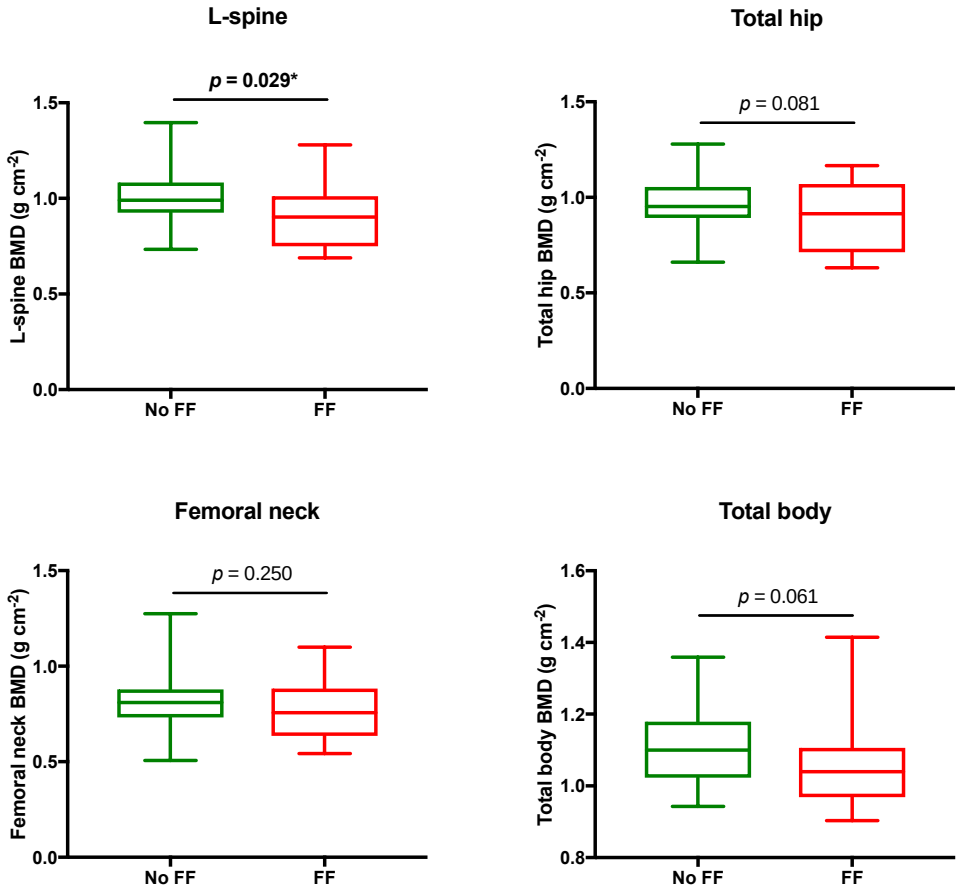


Figure 4.15. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients without a history of fragility fracture (n=104) compared to those with a history of fragility fracture (n=10) (FF = fragility fracture)

4.6 Non-FRAX®-incorporated general fracture risk factors as determinants of BMD in PLWH

The effects of ever use of SSRIs, use of a PPI for five years or longer, ever use of cannabis, ever significant use of opiates, ever "other" recreational drug use (excluding cannabis and opiates) and (for female patients) a history of primary or secondary amenorrhoea or of Depo-Provera® use – other general fracture risk factors not incorporated into FRAX® – on BMD are detailed for all patients in Table 4.9 and for each patient race gender subgroup in Appendix 1, Tables A1.13 to A1.16.

In all Phase Two patients, neither SSRI exposure, PPI exposure for five years or more, ever use of cannabis, nor significant opiate use had any observable effect on BMD at any site. In black male patients, ever cannabis use (n = 2) was associated with a significantly greater total body BMD in black male patients (p = 0.040) and PPI exposure for five years or more (n = 1) was associated with a significantly lower femoral neck BMD (p = 0.039); conversely in black female patients, PPI exposure for five years or more (n = 2) was associated with significantly greater femoral neck BMD (p = 0.013). Ever use of other (non-cannabis, non-opiate) recreational drugs, almost exclusively reported in white male patients only (n = 13, 25.0%) excepting one white female patient, was associated with lower BMD at all sites, but not significantly.

In female patients, neither a history of primary amenorrhoea, secondary amenorrhoea, nor self-reported ever use of Depo-Provera® had any significant effect on BMD at any site or within either racial subgroup.

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
SSRI exposure	No	79 (69.3)	1.005 ± .116	.914	0.961 ± .115	.815	0.814 ± .118	.824	1.103 ± .090	.879
	Yes	35 (30.7)	1.002 ± .160		0.955 ± .168		0.808 ± .169		1.100 ± .127	
PPI exposure more than 5 years	No	106 (93.0)	1.004 ± .126	.923	0.965 ± .129	.546	0.813 ± .128	.867	1.103 ± .102	.772
	Yes	8 (7.0)	0.999 ± .188		0.932 ± .183		0.805 ± .220		1.092 ± .112	
Cannabis use	No	92 (80.7)	1.005 ± .123	.842	0.964 ± .132	.433	0.816 ± .137	.570	1.096 ± .095	.183
	Yes	22 (19.3)	0.999 ± .162		0.939 ± .135		0.798 ± .130		1.128 ± .130	
Other recreational drug use	No	100 (87.7)	1.011 ± .130	.094	0.967 ± .134	.084	0.820 ± .139	.139	1.107 ± .106	.153
	Yes	14 (12.3)	0.949 ± .127		0.902 ± .110		0.763 ± .091		1.065 ± .063	
Significant opiate use	No	112 (98.2)	1.004 ± .130	.735	0.959 ± .131	.713	0.813 ± .135	.953	1.102 ± .102	.846
	Yes	2 (1.8)	0.973 ± .243		0.994 ± .278		0.807 ± .194		1.116 ± .201	
Primary amenorrhoea *(n=47)*	No	40 (85.1)	1.019 ± .140	.482	0.968 ± .126	.433	0.836 ± .139	.339	1.090 ± .110	.502
	Yes	7 (14.9)	1.059 ± .126		1.010 ± .149		0.893 ± .175		1.121 ± .111	
Secondary amenorrhoea *(n=47)*	No	26 (55.3)	1.024 ± .157	.971	0.976 ± .139	.907	0.845 ± .157	.991	1.089 ±.128	.683
	Yes	21 (44.7)	1.026 ± .112		0.972 ± .114		0.854 ± .131		1.102 ± .083	
Depo-provera® use *(n=47)*	No	31 (66.0)	1.018 ± .136	.627	0.972 ± .131	.841	0.849 ± .150	.795	1.091 ± .110	.717
	Yes	16 (34.0)	1.039 ± .142		0.980 ± .122		0.837 ± .138		1.103 ± .028	

Table 4.9. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n=114) according to presence or absence of non-FRAX®-incorporated other general fracture risk factors

4.7 HIV disease-specific factors unrelated to antiretroviral therapy as determinants of BMD in PLWH

The relationship between time since HIV diagnosis and nadir CD4 cell count with lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients is shown in Table 4.10 (race / gender subgroup analyses are included in Appendix 1, Tables A1.17, A1.19, A1.21 and A1.23). The differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients according to the presence or absence of other HIV disease-specific factors unrelated to ART are detailed in Table 4.11 (subgroup analyses are included in Appendix 1, Tables A1.18, A1.20, A1.22 and A1.24).

Whilst BMD at each site decreased with increased time since HIV diagnosis, this relationship was not significant in all Phase Two patients; within subgroup analyses, the rate of BMD decline with increasing time since HIV diagnosis was steeper in black male patients, with a significant correlation for lumbar spine ($p = 0.027$), than in the other three subgroups within which there was no significant correlation. This mirrored the relationship seen between BMD and age (correlation of lumbar spine BMD with age and time since HIV diagnosis respectively compared in black males and black females in Figure 4.16). There was a significant correlation between time since HIV diagnosis and age in all patients ($r = 0.331$, $p < 0.001$) (Figure 4.17).

There was no observed significant relationship between nadir CD4 cell count and BMD at any site in all patients, and this was also observed in all subgroups, with the exception of black males in whom, contrary to expectation, a significant decline in BMD at both the total hip and femoral neck ($p = 0.029$ and = 0.004 respectively) was seen with an increase in nadir CD4 cell count. The relationship between nadir CD4 cell count and femoral neck BMD is demonstrated within each patient subgroup in Figure 4.18.

HIV disease-specific fracture risk factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Time since HIV diagnosis *years*	-0.133	.158	-0.061	.520	-0.079	.406	-0.077	.413
Nadir CD4 cell count[a] *cells μL^{-1}*	-0.094	.323	-0.023	.811	-0.120	.206	-0.032	.742

[a] 2 missing values (n=112)

Table 4.10. Relationship between time since HIV diagnosis and nadir CD4 cell count and lumbar spine, hip, femoral neck and total body BMD in all Phase Two patients (n = 114)

HIV disease-specific fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Mode of HIV acquisition	Hetero	74 (64.9)	1.021 ± .139	.087	0.973 ± .129	.208	0.835 ± .143	**.030**	1.105 ± .105	**.019**
	MSM	38 (33.3)	0.977 ± .107		0.939 ± .139		0.777 ± .108		1.099 ± .099	
Serious illness at time of HIV diagnosis	No	82 (71.9)	0.999 ± .133	.507	0.958 ± .124	.880	0.807 ± .121	.490	1.098 ± .106	.538
	Yes	32 (28.1)	1.017 ± .125		0.962 ± .155		0.827 ± .167		1.111 ± .094	
Previous intensive care admission	No	106 (93.0)	1.000 ± .129	.360	0.958 ± .134	.649	0.813 ± .136	.843	1.101 ± .106	.643
	Yes	8 (7.0)	1.045 ± .147		0.980 ± .124		0.803 ± .131		1.118 ± .045	
Chronic HBV infection	No	110 (96.5)	1.005 ± .127	.662	0.961 ± .131	.441	0.814 ± .135	.520	1.102 ± .104	.930
	Yes	4 (3.5)	0.976 ± .224		0.909 ± .187		0.770 ± .159		1.098 ± .075	
Current or past chronic HCV infection	No	112 (98.2)	1.006 ± .130	.179	0.961 ± .133	.455	0.814 ± .136	.478	1.101 ± .101	.653
	Yes	2 (1.8)	0.881 ± .112		0.889 ± .131		0.745 ± .107		1.135 ± .227	
Lipodystrophy	No	102 (89.5)	1.002 ± .128	.728	0.961 ± .133	.772	0.813 ± .139	.943	1.102 ± .105	.992
	Yes	12 (10.5)	1.016 ± .154		0.949 ± .135		0.810 ± .094		1.102 ± .082	

Table 4.11. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114) according to presence or absence of HIV disease-specific fracture risk factors ("Hetero" = HIV acquisition through sexual intercourse between a man and woman, "MSM" = HIV acquisition through sexual intercourse between men)

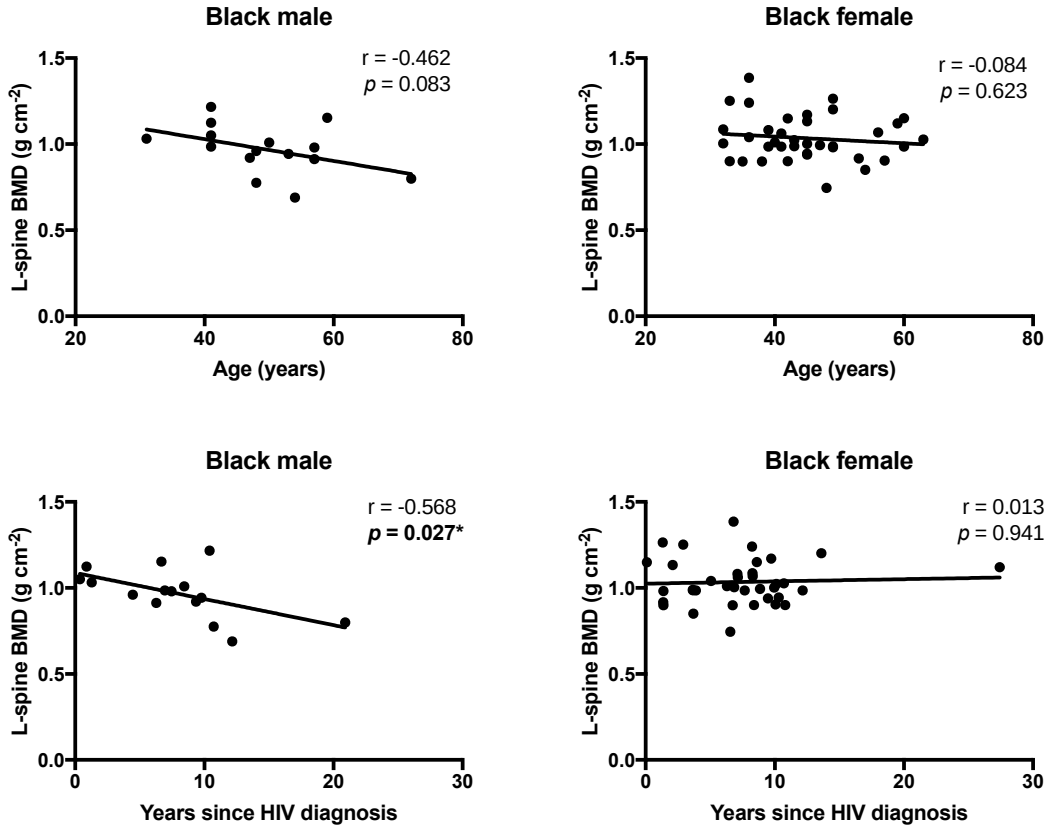


Figure 4.16. Correlation of lumbar (L-) spine BMD with age and time since HIV diagnosis respectively in black males (n = 15) and black females (n = 37) within the Phase Two study population

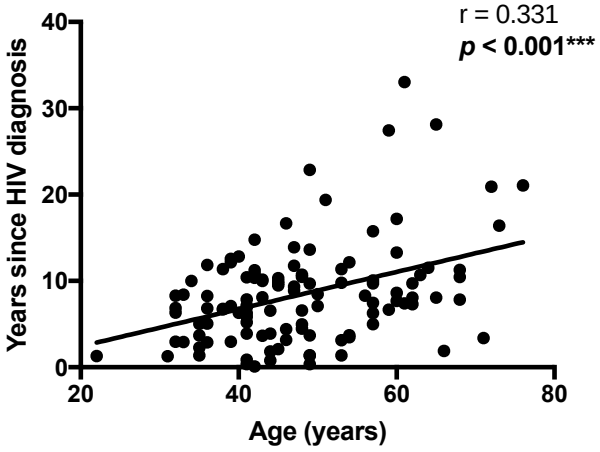


Figure 4.17. Relationship between age and time since HIV diagnosis in all patients (n = 114) within the Phase Two study population

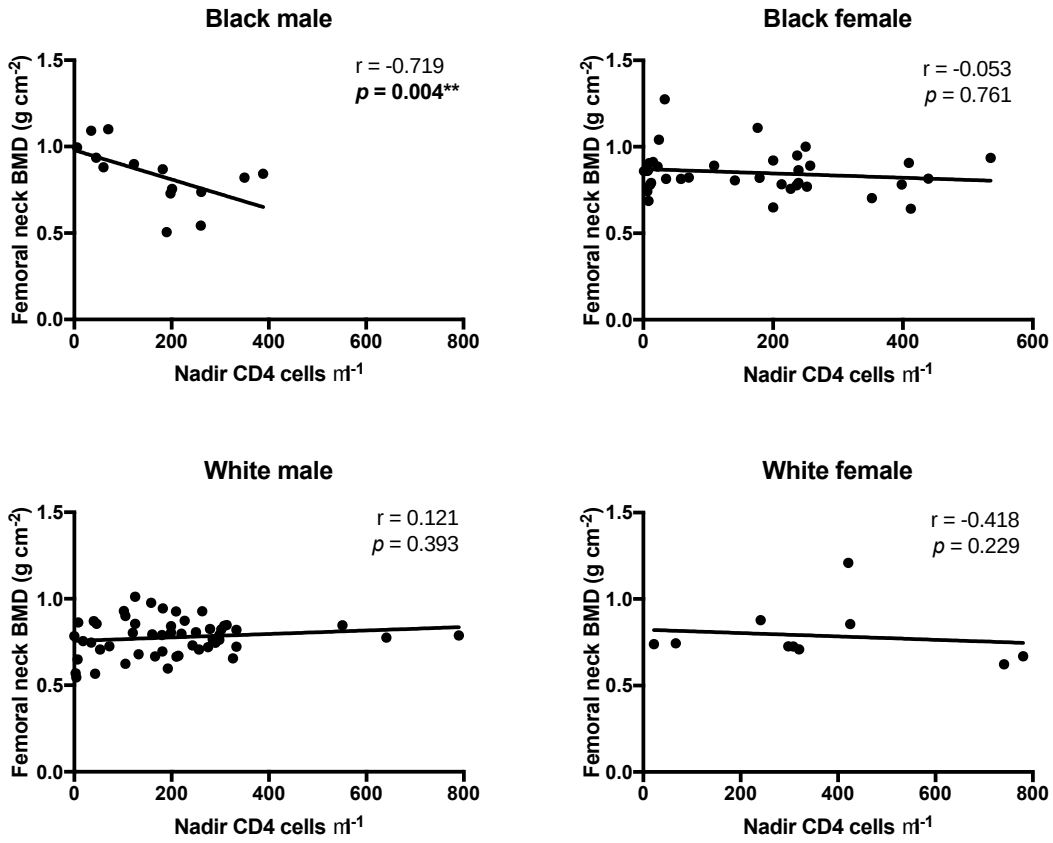


Figure 4.18. Relationship between nadir CD4 cell count and femoral neck BMD in black male (n = 14), black female (n = 36), white male (n = 52) and white female patients (n = 10) within the Phase Two study population

Of other HIV disease-specific factors, only mode of HIV acquisition had a significant effect on BMD (Table 4.11), with significantly lower femoral neck and total body BMD in patients who acquired HIV infection via sexual intercourse between men *versus* patients who acquired HIV infection via sexual intercourse between a man and a woman ($p = 0.030$ and $p = 0.019$ for femoral neck and total body respectively). 38 patients who acquired HIV infection via sexual intercourse between men where almost universally white male (97.4%), however, with the exception of one black male, in contrast to the 74 patients who acquired HIV infection via sexual intercourse between a man and a woman who were predominantly black and female. Within the white male subgroup there was no significant difference in BMD at any site

between 37 white male patients who acquired HIV infection via sexual intercourse between men and 14 white male patients who acquired HIV infection via sexual intercourse between a man and a woman. (Only one Phase Two recruited patient acquired HIV infection via injecting drug use and one via an unknown route, with no other modes of transmission reported.)

Neither serious illness at time of HIV diagnosis, previous intensive care admission, chronic HBV infection, current or past chronic HCV infection or lipodystrophy (lipoatrophy and/or lipohypertrophy) had any significant relationship with BMD at any site in all patients. Of note, the prevalence of some risk factors, notably chronic HBV infection (3.5%, n = 4) and current or past chronic HCV infection (1.8%, n = 2), were low in Phase Two recruited patients. Whilst chronic HBV infection was associated with significantly lower BMD at the lumbar spine and total hip in black male patients, only one patient with chronic HBV infection was included within this subgroup; this 54-year old patient had other risk factors, including low BMI (20.6kg m^{-2}), being a current smoker and with an average alcohol consumption ≥3 units per day.

4.8 Antiretroviral therapy and related HIV disease-specific factors as determinants of BMD in PLWH

The effects of ART on BMD are summarised for all Phase Two patients in Tables 4.12 to 4.19. Further race / gender subgroup analyses are detailed in Appendix 1, Tables A1.25 to A1.56.

There was no significant difference between BMD at any site in Phase Two patients currently on or ever exposed to ART versus those not currently on or never exposed to ART respectively, nor was there a significant difference in BMD at any site between patients with suppressed plasma HIV RNA versus patients with unsuppressed HIV RNA (Table 4.12). Of note, numbers of patients not currently on ART, never exposed to ART or with detectable plasma HIV RNA either above 40 copies ml^{-1} or above 200 copies ml^{-1} were

relatively small within the Phase Two study population (n = 4, n = 3, n = 12 and n = 8 respectively).

Overall, there was no significant change in BMD at each site with either increased continuous time on ART or increased cumulative time ever on ART at the time of BMD measurement (Table 4.13), with a similar relationship observed within each race / gender subgroup except for black males, in whom BMD decreased at each site with increased ART exposure (significant between total body BMD and cumulative ART exposure, $p = 0.046$) (Tables A1.26, A1.42, A1.33 and A1.50). The relationship between quantitative ART exposure and BMD in all patients and within each race / gender subgroup (Figure 4.19) was similar to that of age and BMD described in Section 4.4 (Figure 4.6 and 4.7).

All but one of the 110 Phase Two patients currently on ART were also on at least one NRTI and all of the 111 patients ever on ART had been on at least one NRTI at one point. There were no significant differences in BMD between patients currently on or ever exposed to an NRTI compared with those not currently on or never exposed to an NRTI, with only very few patients not currently on or never exposed an NRTI for comparison (Table 4.14). The relationship between BMD at each site with both continuous and cumulative NRTI exposure was also similar to that seen with any ART (Table 4.15). No significant variation was observed within any of the four race / gender subgroups (Tables A1.27, A1.28, A1.35, A1.36, A1.43, A1.44, A1.51 and A1.52).

With respect to specific NRTI exposure, there was no significant difference in BMD observed between patients either currently on or ever exposed to either tenofovir DF, abacavir or zidovudine (AZT) compared to patients either not currently on or never exposed to tenofovir DF, abacavir or AZT, except for current AZT and femoral neck BMD, with the four patients currently on AZT (all male, one black and three white) having significantly lower femoral neck BMD than the 110 patients not on AZT ($p = 0.043$) (Table 4.14, Figure 4.20). This difference was attributed to the one black male currently on AZT, who

had lower BMD at all sites compared with the 14 black males not on AZT (lumbar spine and hip BMD significantly lower, $p = 0.035$ and $p = 0.009$ respectively, Table A1.27). No difference in BMD was observed within the white male patient subgroup between patients currently on or not currently on AZT (Table A1.43). The black male patient currently on AZT was the same 54-year old current smoker with significant average alcohol consumption, current HBV infection and low BMI, described in Section 4.7.

There was no significant difference observed in the relationship between either the duration of continuous or cumulative tenofovir DF, abacavir or AZT exposure and BMD at any site (Table 4.15) (comparative relationships with cumulative tenofovir DF, abacavir and AZT duration and lumbar spine BMD are illustrated in Figure 4.21).

51.8% Phase Two patients (n = 59) were currently on an NNRTI and 78.1% (n = 89) had ever been exposed to an NNRTI. The mean BMD in patients currently on or ever exposed to an NNRTI, currently on efavirenz or currently on or ever exposed to nevirapine was higher for each BMD site than in patients not currently on or never exposed to NNRTIs, efavirenz and nevirapine respectively (Table 4.16). Although differences in BMD were not significant between respective groups, the differences in BMD between patients currently on an NNRTI versus not currently on an NNRTI approached significance at the lumbar spine, total hip and femoral neck ($p = 0.095$, $p = 0.061$, $p = 0.083$) (Figure 4.22). Total hip, femoral neck and total body BMD were significantly higher in black male patients currently on an NNRTI (n = 8) than in black male patients not currently on an NNRTI (n = 7) ($p = 0.032$, $p = 0.031$, $p = 0.035$ for total hip, femoral neck and total body respectively) (Table A1.29) and lumbar spine BMD was significantly higher in black female patients currently on nevirapine (n = 3) than black females not currently on nevirapine (n = 34) ($p = 0.044$) (Table A1.37).

HIV antiretroviral therapy-related factor	Exposure or presence	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current ART	No	4 (3.5)	1.028 ± .089	.704	1.003 ± .072	.508	0.802 ± .124	.873	1.139 ± .044	.461
	Yes	110 (96.5)	1.003 ± .132		0.958 ± .134		0.813 ± .136		1.100 ± .104	
Ever ART	No	3 (2.6)	1.056 ± .084	.481	0.980 ± .069	.782	0.809 ± .151	.959	1.150 ± .047	.413
	Yes	111 (97.4)	1.003 ± .131		0.959 ± .134		0.813 ± .135		1.100 ± .103	
Plasma HIV RNA <40 copies ml^{-1}	No	12 (10.5)	0.998 ± .122	.872	0.998 ± .157	.294	0.828 ± .193	.683	1.131 ± .081	.296
	Yes	102 (89.5)	1.004 ± .132		0.955 ± .130		0.811 ± .128		1.099 ± .105	
Plasma HIV RNA <200 copies ml^{-1}	No	8 (7.0)	1.105 ± .149	.803	1.006 ± .160	.308	0.824 ± .225	.800	1.127 ± .090	.487
	Yes	106 (93.0)	1.003 ± .130		0.956 ± .131		0.812 ± .127		1.100 ± .103	

Table 4.12. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114) according to exposure to or presence of general HIV antiretroviral therapy (ART)-related factors

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on ART[a]	-0.091	.339	-0.115	.229	-0.077	.421	-0.116	.224
Cumulative number of months ever on ART[a]	-0.061	.524	-0.035	.713	-0.029	.765	-0.063	.507

[a] 2 missing values (n=112)

Table 4.13. Relationship between continuous number of months on antiretroviral therapy (ART) at time of BMD measurement or cumulative number of months ever on ART with lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114)

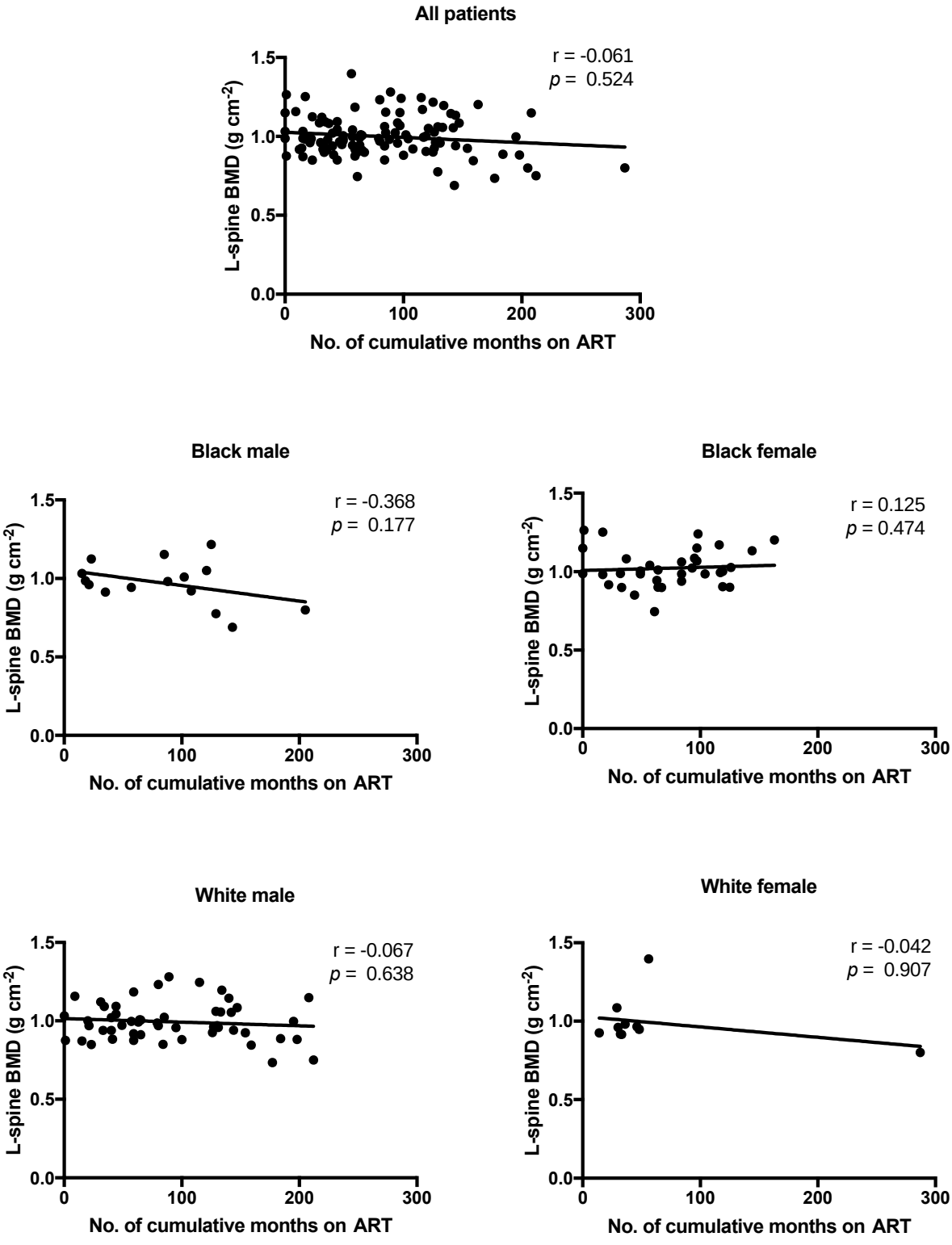


Figure 4.19. Relationship between cumulative antiretroviral therapy (ART) exposure and lumbar (L-) spine BMD in all (n = 112), black male (n = 15), black female (n = 35), white male (n = 52) and white female (n = 10) patients within the Phase Two study population

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NRTI	No	5 (4.4)	1.043 ± .075	.498	0.962 ± .117	.963	0.835 ± .163	.712	1.150 ± .040	.284
	Yes	109 (95.6)	1.001 ± .132		0.959 ± .134		0.812 ± .134		1.100 ± .104	
Ever NRTI	No	3 (2.6)	1.056 ± .084	.481	0.980 ± .069	.782	0.809 ± .151	.959	1.150 ± .047	.967
	Yes	111 (97.4)	1.003 ± .131		0.959 ± .134		0.813 ± .135		1.100 ± .103	
Current TDF	No	35 (30.7)	1.006 ± .141	.915	0.963 ± .125	.864	0.818 ± .131	.787	1.107 ± .090	.750
	Yes	79 (69.3)	1.003 ± .126		0.958 ± .137		0.810 ± .138		1.100 ± .108	
Ever TDF	No	28 (24.6)	1.031 ± .133	.210	0.987 ± .113	.212	0.841 ± .127	.200	1.122 ± .089	.243
	Yes	86 (75.4)	0.995 ± .129		0.951 ± .138		0.803 ± .136		1.096 ± .106	
Current ABC	No	82 (71.9)	1.006 ± .125	.769	0.962 ± .134	.746	0.815 ± .138	.761	1.105 ± .106	.663
	Yes	32 (28.1)	0.998 ± .146		0.953 ± .131		0.806 ± .128		1.095 ± .094	
Ever ABC	No	62 (54.4)	1.010 ± .128	.571	0.969 ± .134	.384	0.820 ± .146	.530	1.103 ± .105	.928
	Yes	52 (45.6)	0.996 ± .134		0.948 ± .132		0.804 ± .121		1.101 ± .100	
Current AZT	No	110 (96.5)	1.008 ± .128	**.043**	0.961 ± .130	.392	0.815 ± .134	.352	1.101 ± .103	.793
	Yes	4 (3.5)	0.875 ± .143		0.903 ± .221		0.751 ± .159		1.115 ± .108	
Ever AZT	No	72 (63.2)	1.009 ± .123	.553	0.958 ± .129	.905	0.814 ± .139	.869	1.097 ± .096	.511
	Yes	42 (36.8)	0.994 ± .143		0.961 ± .141		0.810 ± .129		1.110 ± .114	

Table 4.14. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114) according to current or ever exposure to at least one nucleos(t)ide reverse transcriptase inhibitor (NRTI), tenofovir DF (TDF), abacavir (ABC) and zidovudine (AZT)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NRTI[a]	-0.065	.497	-0.084	.376	-0.052	.588	-0.113	.237
Cumulative number of months ever on NRTI[a]	-0.076	.427	-0.054	.569	-0.028	.772	-0.101	.289
Continuous number of months on TDF	-0.067	.480	-0.135	.154	-0.101	.284	-0.160	.089
Cumulative number of months ever on TDF	-0.094	.317	-0.136	.151	-0.107	.257	-0.155	.100
Continuous number of months on ABC[b]	0.007	.945	-0.013	.893	-0.025	.795	-0.010	.918
Cumulative number of months ever on ABC[b]	-0.030	.750	-0.048	.613	-0.054	.569	-0.013	.888
Continuous number of months on AZT	-0.163	.083	-0.069	.464	-0.071	.453	0.012	.900
Cumulative number of months ever on AZT[a]	-0.051	.595	0.028	.771	0.025	.790	0.039	.685

[a] 2 missing values (n=112)
[b] 1 missing value (n=113)

Table 4.15. Relationship between continuous number of months on or cumulative number of months ever on either at least one NRTI (nucleos(t)ide reverse transcriptase inhibitors), tenofovir DF (TDF), abacavir (ABC) or zidovudine (AZT) with lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114)

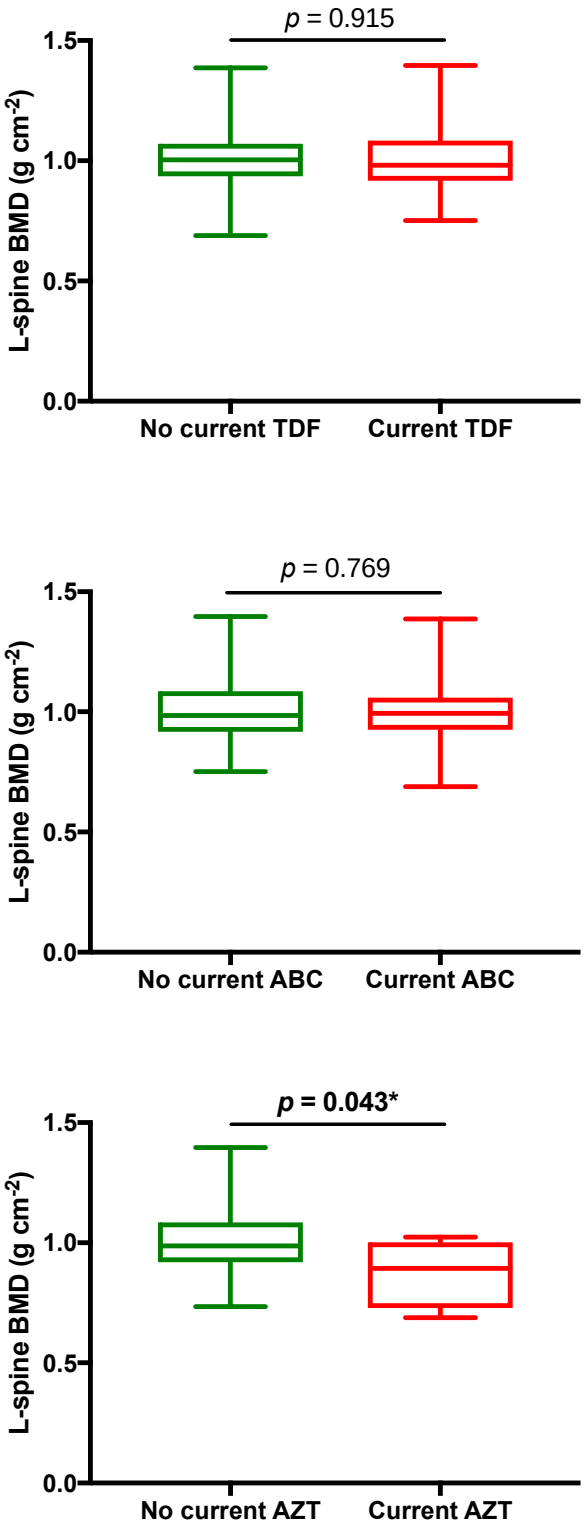


Figure 4.20. Differences in lumbar (L-) spine BMD in all Phase Two patients (n = 114) either not currently on or currently on tenofovir DF (TDF) (no current TDF: n = 28; current TDF: n = 86), abacavir (ABC) (no current ABC: n = 82; current ABC: n = 32) or zidovudine (AZT) (no current AZT: n = 110; current AZT: n = 4)

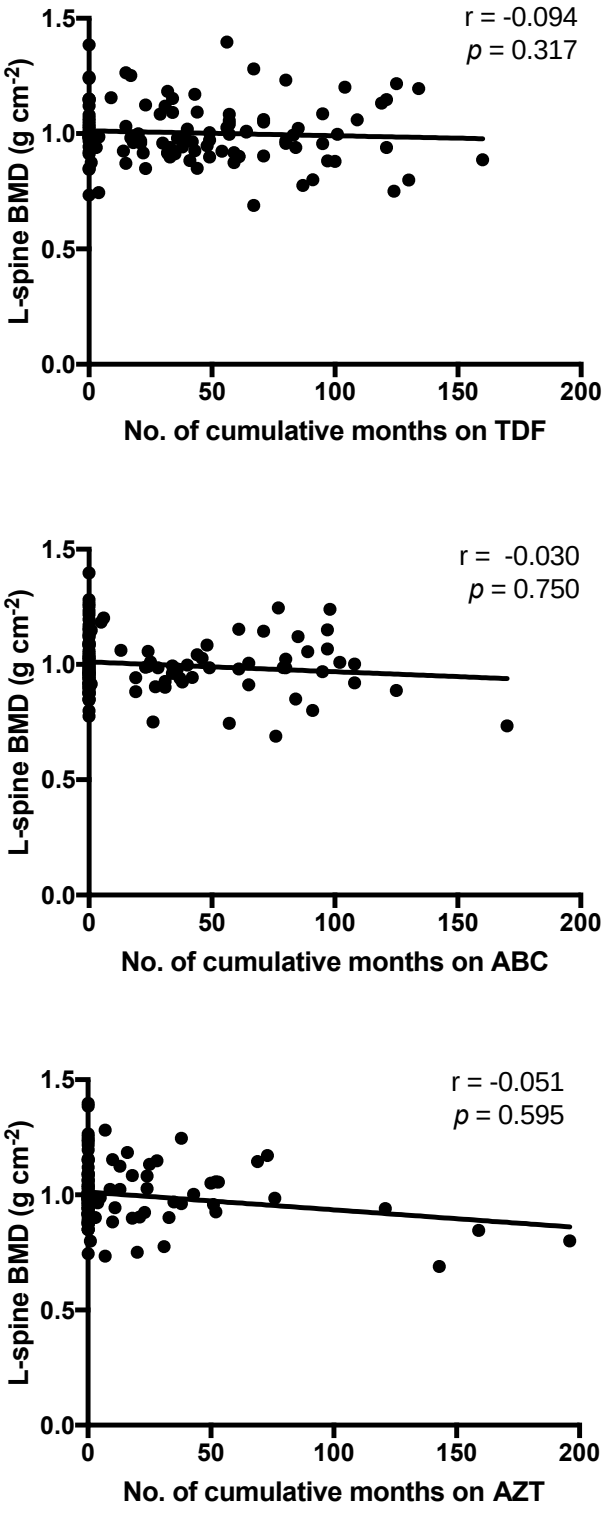


Figure 4.21. Relationship between lumbar (L-) spine BMD and cumulative exposure to tenofovir DF (TDF) (n = 114), abacavir (ABC) (n = 113) and zidovudine (AZT) (n = 112) within the Phase Two study population

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NNRTI	No	55 (48.2)	0.983 ± .118	.095	0.935 ± .132	.061	0.790 ± .132	.083	1.099 ± .089	.790
	Yes	59 (51.8)	1.023 ± .139		0.982 ± .131		0.834 ± .135		1.104 ± .115	
Ever NNRTI	No	25 (21.9)	0.970 ± .125	.148	0.929 ± .146	.190	0.789 ± .151	.319	1.098 ± .084	.816
	Yes	89 (78.1)	1.013 ± .131		0.968 ± .128		0.819 ± .130		1.103 ± .107	
Current EFV	No	76 (66.7)	0.996 ± .129	.403	0.952 ± .137	.406	0.807 ± .140	.557	1.105 ± .098	.638
	Yes	38 (33.3)	1.018 ± .134		0.974 ± .125		0.823 ± .125		1.096 ± .112	
Ever EFV	No	43 (37.7)	1.006 ± .144	.886	0.946 ± .144	.394	0.804 ± .156	.591	1.115 ± .101	.289
	Yes	71 (62.3)	1.002 ± .122		0.968 ± .126		0.818 ± .122		1.094 ± .103	
Current NVP	No	105 (92.1)	1.001 ± .127	.378	0.958 ± .130	.663	0.809 ± .132	.733	1.100 ± .102	.493
	Yes	9 (7.9)	1.041 ± .175		0.978 ± .168		0.850 ± .175		1.125 ± .117	
Ever NVP	No	92 (80.7)	0.998 ± .125	.341	0.958 ± .130	.783	0.807 ± .130	.354	1.093 ± .095	.068
	Yes	22 (19.3)	1.028 ± .154		0.966 ± .149		0.837 ± .155		1.138 ± .124	

Table 4.16. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114) according to current or ever exposure to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NNRTI[a]	0.161	.089	0.147	.120	0.133	.159	-0.014	.883
Cumulative number of months ever on NNRTI[b]	0.174	.066	0.178	.061	0.168	.076	-0.011	.911
Continuous number of months on EFV	0.098	.300	0.075	.430	0.048	.611	-0.070	.462
Cumulative number of months ever on EFV[a]	0.088	.351	0.128	.177	0.120	.205	-0.090	.343
Continuous number of months on NVP[a]	0.041	.667	-0.023	.808	0.012	.902	-0.052	.583
Cumulative number of months ever on NVP[a]	0.065	.491	0.006	.949	0.050	.600	0.134	.158

[a]1 missing value (n=113)
[b]2 missing values (n=112)

Table 4.17. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) with lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114)

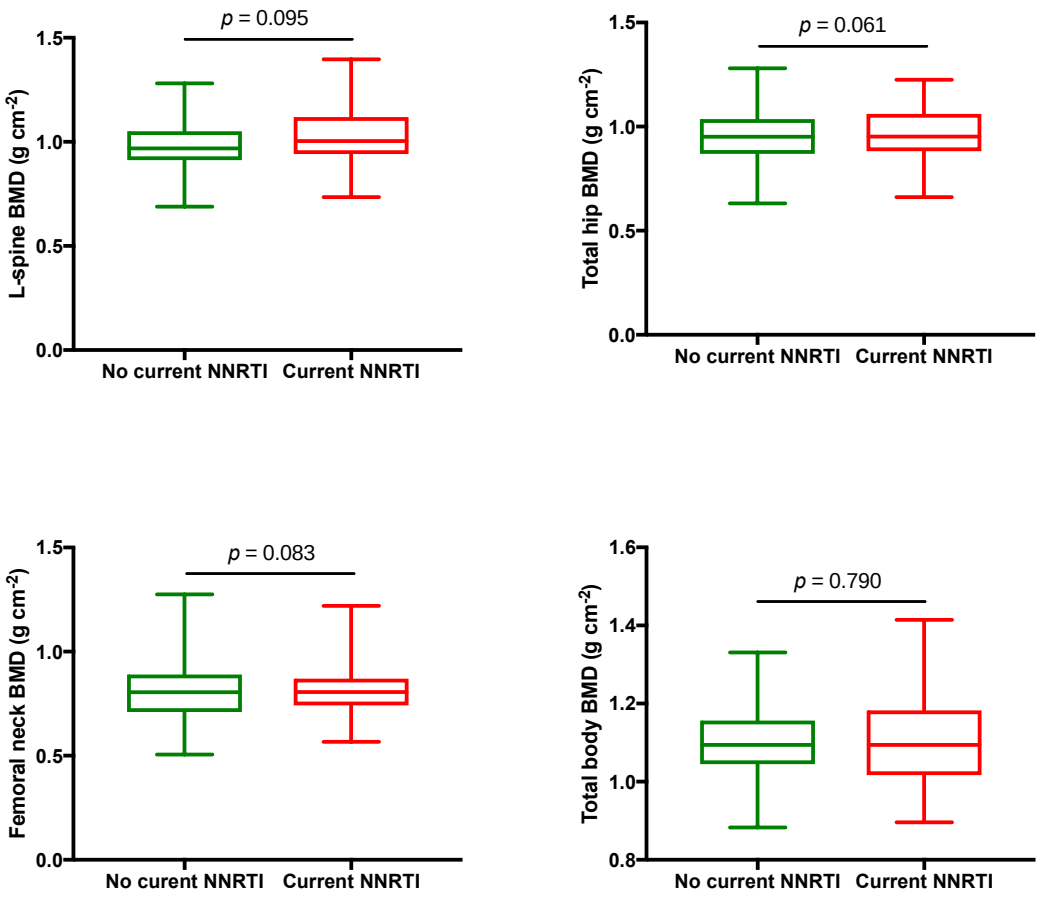


Figure 4.22. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD between Phase Two patients currently on a nucleoside reverse transcriptase inhibitor (NNRTI) (n = 59) versus those not currently on an NNRTI (n = 55)

The relationship between continuous or cumulative NNRTI exposure and BMD was more complex and differed for different BMD sites and within different patient subgroups. In all Phase Two patients, neither the duration of continuous or cumulative NNRTI, nevirapine or efavirenz exposure had any significant relationship with BMD at any site, although with a general trend of slight increase in lumbar spine, total hip and femoral neck BMD (but no change in total body BMD) with increased duration of exposure to an NNRTI, nevirapine or efavirenz (Table 4.17). In black male patients, increased continuous number of months on an NNRTI was associated with a significant increase in total hip, femoral neck and total body BMD (r = 0.575 p = 0.025, r = 0.590 p = 0.021 and r = 0.570 p = 0.027 respectively) (Table A1.30). In black

female patients, increased continuous or cumulative exposure to efavirenz was associated with a decrease in BMD at all sites, which was significant for total body BMD (r = -0.344 *p* = 0.037 and r = -0.458 *p* = 0.005 for continuous and cumulative efavirenz exposure respectively) (Table A1.30).

In contrast to NNRTI exposure, the mean BMD in patients with current exposure to a PI (n = 49) or who had ever been exposed to a PI (n = 67) was, overall, less than in patients without current PI exposure (n = 65) or ever PI exposure (n = 47), although differences in BMD were not significant at any site (Table 4.18). Increased continuous exposure to a PI was associated with a significant reduction in lumbar spine and total hip BMD (r = -0.240 *p* = 0.011 and r = -0.220 *p* = 0.019 respectively), however, and increased cumulative exposure to a PI was associated with a significant reduction in lumbar spine, total hip and femoral neck BMD (r = -0.247 *p* = 0.009, r = -0.271 *p* = 0.004 and r = -0.229 *p* = 0.015 respectively) (Table 4.19, Figure 4.23). Of note, 23 Phase Two patients currently on a PI were not concurrently on an NNRTI and 32 patients currently on an NNRTI were not concurrently on a PI (27 patients were on both concurrently and 32 patients were on neither).

Only a small proportion of Phase Two patients were either currently on or had ever taken an INI (8.8% (n = 10) and 13.2% (n = 15) respectively). Current INI use was associated with lower BMD overall and significantly lower femoral neck BMD (*p* = 0.022) (Table 4.19, Figure 4.24). Furthermore, the duration of continuous exposure to an INI was associated with reduced BMD overall and this relationship was significant for femoral neck BMD (r = -0.210, *p* = 0.025). Patients had only been on an INI for a relatively short duration (median 13 months, interquartile range 4 to 54 months). Nine patients were currently on raltegravir and only one patient currently on elvitegavir/cobicistat (the latter co-formulated and therefore by default co-administered with tenofovir DF and emtricitabine). Three patients were concurrently on an INI and a PI (no patients were concurrently on an INI and an NNRTI).

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current PI	No	65 (57.0)	1.018 ± .129	.163	0.977 ± .127	.114	0.821 ± .136	.439	1.100 ± .103	.836
	Yes	49 (43.0)	0.984 ± .131		0.937 ± .138		0.801 ± .134		1.104 ± .103	
Ever PI	No	47 (41.2)	1.022 ± .126	.209	0.986 ± .120	.069	0.830 ± .127	.263	1.108 ± .099	.622
	Yes	67 (58.8)	0.991 ± .133		0.940 ± .139		0.801 ± .140		1.098 ± .106	
Current INI	No	104 (91.2)	1.007 ± .133	.435	0.966 ± .133	.074	0.821 ± .134	**.022**	1.104 ± .106	.526
	Yes	10 (8.8)	0.973 ± .101		0.888 ± .118		0.720 ± .108		1.082 ± .049	
Ever INI	No	99 (86.8)	1.004 ± .133	.979	0.961 ± .130	.784	0.814 ± .127	.832	1.100 ± .104	.645
	Yes	15 (13.2)	1.003 ± .113		0.951 ± .156		0.806 ± .186		1.113 ± .094	

Table 4.18. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114) according to current or ever exposure to at least one protease inhibitor (PI) or integrase inhibitor (INI)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on PI[a]	-0.240	**.011**	-0.220	**.019**	-0.164	.083	-0.052	.588
Cumulative number of months ever on PI[b]	-0.247	**.009**	-0.271	**.004**	-0.229	**.015**	-0.111	.245
Continuous number of months on INI	-0.096	.308	-0.154	.101	-0.210	**.025**	-0.048	.614
Cumulative number of months ever on INI	-0.016	.864	-0.036	.704	-0.055	.559	0.048	.612

[a]1 missing value (n=113)
[b]2 missing values (n=112)

Table 4.19. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one protease inhibitor (PI) or integrase inhibitor (INI) with lumbar spine, total hip, femoral neck and total body BMD in all Phase Two patients (n = 114)

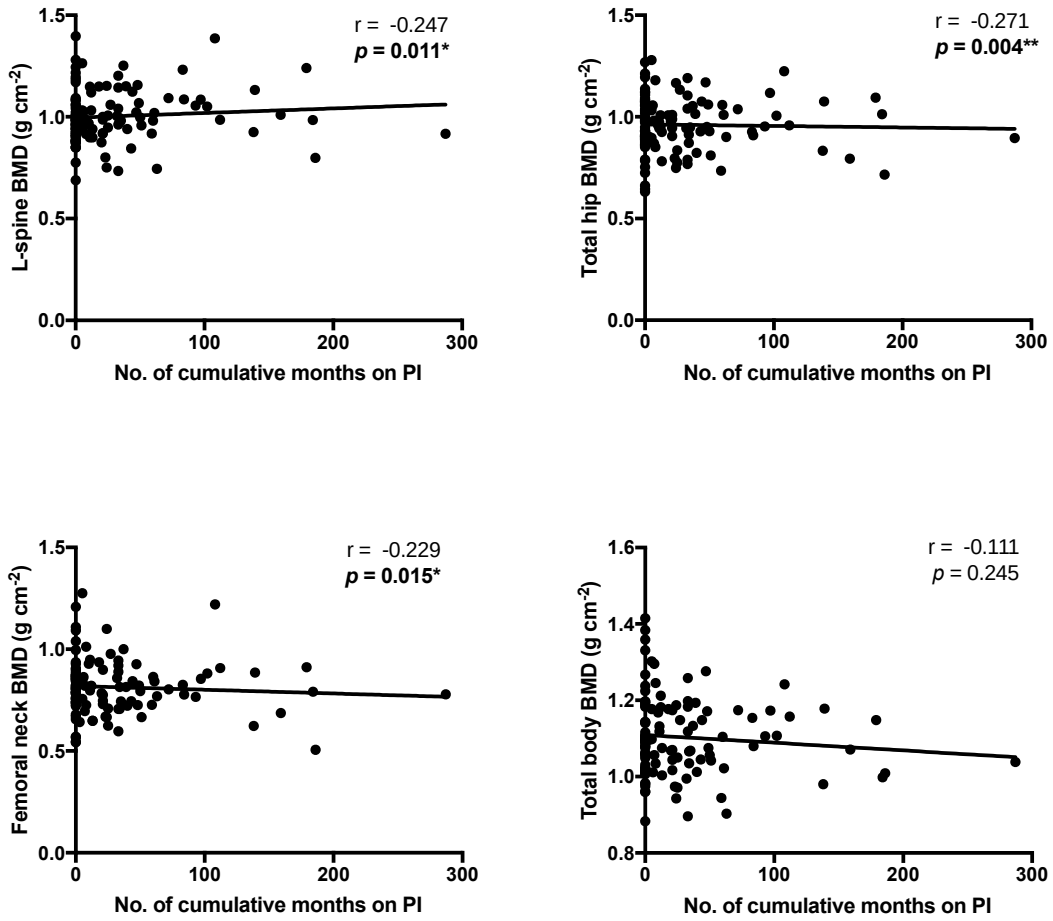


Figure 4.23. Relationship between lumbar (L-) spine, total hip, femoral neck and total body BMD and cumulative exposure to a protease inhibitor (PI) in Phase Two patients (n = 112)

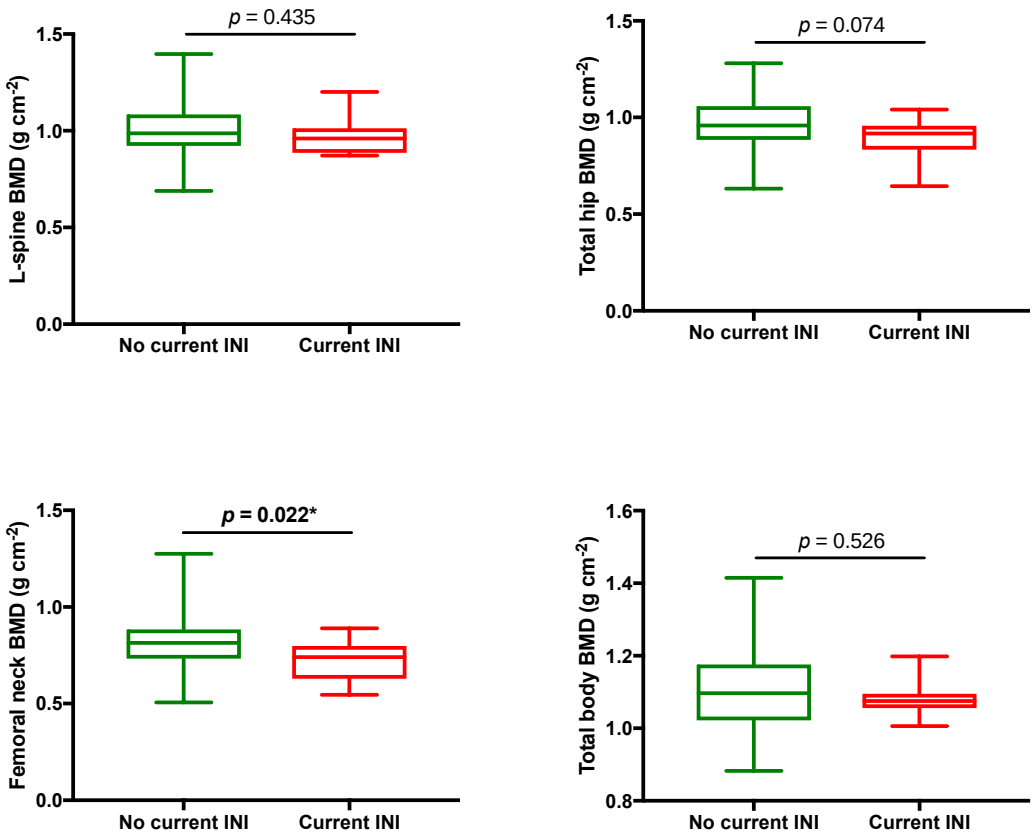


Figure 4.24. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD between Phase Two patients currently on an integrase inhibitor (INI) (n = 10) versus those not currently on an INI (n = 104)

4.9 Summary of clinical determinants of BMD by multivariate analysis

General and HIV disease-specific clinical factors found to have either significant ($p < 0.05$) or borderline significant ($p \geq 0.05$ and < 0.10) association with BMD at one or more site the Phase Two study population are listed in Table 4.20.

Risk factor	BMD site			
	Lumbar spine	Total hip	Femoral neck	Total body
Age	.094	.258	**.046**	.115
Height	.684	.638	.524	.064
Weight	**<.001**	**<.001**	**<.001**	**.001**
Body mass index	**<.001**	**<.001**	**<.001**	**.023**
Alcohol ≥ 3 units d^{-1}	.105	**.017**	**.002**	.146
Prior fragility fracture	**.029**	.081	.250	.061
Steroid exposure	.179	.407	.595	**.045**
Rheumatoid arthritis	.235	**.024**	.067	.245
"Other disorders"	.150	**.016**	**.010**	**.012**
MSM HIV acquisition	.087	.208	**.030**	**.019**
Current AZT	**.043**	.392	.352	.793
Cumulative AZT	.595	**.028**	**.025**	.685
Continuous PI	**.011**	**.019**	.083	.588
Cumulative PI	**.009**	**.004**	**.015**	.245
Current INI	.435	.074	**.022**	.526
Continuous INI	.308	.101	**.025**	.614
Current smoking	.249	.139	.091	.704
Other drug use	.094	.084	.139	.153
Continuous TDF	.480	.154	.284	.089
Continuous AZT	.083	.464	.453	.900
Current NNRTI	.095	.061	.083	.790
Continuous NNRTI	.089	.120	.159	.883
Cumulative NNRTI	.066	.061	.076	.911
Ever PI	.209	.069	.263	.622

Table 4.20. General and HIV disease-specific factors with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) associations with BMD at one or more site from univariate analysis

Risk factors with significant or borderline associations with BMD at each site were taken forward for multivariate analysis within separate generalised linear models for each of lumbar spine, total hip, femoral neck and total body BMD. BMI was put forward for multivariate analysis, a product of both height and weight, which were not put forward separately. For other risk factors that were not independent, e.g. continuous, cumulative and ever PI exposure, only the risk factor with the most significant association on univariate analysis was taken forward for multivariate analysis.

Although not significantly associated with BMD at every site in univariate analysis, age was included in multivariate analysis models for BMD at each site, as a potential confounder for time-dependent risk factors, e.g. continuous or cumulative ARV exposures.

Backward elimination was used to determine risk factors with significant association with BMD within multivariate analysis models (detailed in Appendix A1.2), with stepwise elimination continuing until all residual risk factors achieved significance of $p < 0.10$. Age was maintained in each model, irrespective of significance, for reasons explained above. Risk factors with significant ($p < 0.05$) or borderline significant ($p \geq 0.05$ and < 0.10) association with BMD following multivariate analysis are shown for lumbar spine, total hip, femoral neck and total body in Tables 4.21, 4.22, 4.23 and 4.24 respectively.

For both lumbar spine and total body BMD, the only significant covariates or factors associated with BMD following multivariate analysis were general fracture risk factors (i.e. not HIV disease-specific) and, furthermore, these were all FRAX®-incorporated general fracture risk factors. Both reduced BMI and a history of fragility fracture were both significantly associated with reduced BMD for both lumbar spine ($p < 0.001$ for BMI and $p = 0.005$ for fragility fracture history) and total body ($p = 0.030$ for BMI and $p = 0.018$ for fragility fracture history); in addition, history of one or more other disorder associated with osteoporosis was significantly associated with reduced total body BMD ($p = 0.012$) and significant steroid exposure was significantly associated with increased total body BMD ($p = 0.002$).

Covariate / factor	Factor present	Estimated marginal mean *g/cm²*	Standard Error	Wald Chi-Square	*p*-value
BMI	-	-	-	26.347	**<.001**
Fragility fracture history	No	1.007	0.011	9.317	.**005**
	Yes	0.896	0.035		

Table 4.21. Covariates and factors with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) association with lumbar spine BMD following multivariate analysis

Covariate / factor	Factor present	Estimated marginal mean *g/cm²*	Standard Error	Wald Chi-Square	*p*-value
BMI	-	-	-	26.669	**<.001**
Fragility fracture history	No	0.843	0.055	4.286	**.038**
	Yes	0.766	0.063		
Rheumatoid arthritis	No	0.921	0.018	4.687	**.030**
	Yes	0.688	0.108		
Other disorder	No	0.832	0.056	6.086	**.014**
	Yes	0.777	0.058		
Cumulative no. of months ever on AZT	-	-	-	3.404	.065
Cumulative no. of months ever on PI	-	-	-	8.484	**.004**

Table 4.22. Covariates and factors with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) association with total hip BMD following multivariate analysis (AZT = zidovudine; PI = boosted protease inhibitor)

Covariate / factor	Factor present	Estimated marginal mean g/cm^2	Standard Error	Wald Chi-Square	*p*-value
BMI	-	-	-	24.874	**<.001**
Age	-	-	-	3.026	.082
Rheumatoid arthritis	No	0.805	0.011	2.991	.084
	Yes	0.614	0.113		
Other disorder	No	0.738	0.057	5.994	**.014**
	Yes	0.681	0.059		
Cumulative no. of months ever on AZT	-	-	-	3.154	.076
Cumulative no. of months ever on PI	-	-	-	7.728	**.007**

Table 4.23. Covariates and factors with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) association with femoral neck BMD following multivariate analysis (AZT = zidovudine; PI = boosted protease inhibitor)

Covariate / factor	Factor present	Estimated marginal mean *g/cm²*	Standard Error	Wald Chi-Square	*p*-value
BMI	-	-	-	4.727	**.030**
Fragility fracture history	Yes	1.149	0.019	5.549	**.018**
	No	1.074	0.032		
Significant steroid exposure	Yes	1.054	0.016	9.277	**.002**
	No	1.168	0.036		
Other disorders	Yes	1.136	0.023	6.242	**.012**
	No	1.087	0.023		

Table 4.24. Covariates and factors with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) association with total BMD following multivariate analysis

Covariates and factors with significant or borderline significant association with either total hip or femoral neck BMD were similar for each and overlapped with covariates and factors significantly associated with lumbar spine and total body BMD. As for lumbar spine and total body, reduced BMI was significantly associated with reduced BMD at both total hip ($p < 0.001$) and femoral neck ($p < 0.001$). History of one or more other disorders associated with osteoporosis was also significantly associated with reduced BMD for both total hip ($p = 0.014$) and femoral neck ($p = 0.014$). Fragility fracture history was also significantly associated with reduced total hip BMD ($p = 0.038$) but not femoral neck BMD. Rheumatoid arthritis was significantly associated with reduced BMD, independent of BMI, for total hip ($p = 0.030$), with borderline significant association for femoral neck ($p = 0.084$). Age, whilst included in multivariate analysis models for each BMD site, was not significantly associated with reduced BMD for any site, although borderline significant association between increased age and reduced BMD was observed for femoral neck *(*$p = 0.082$).

In addition to FRAX®-incorporated general fracture risk factors, greater cumulative duration of months ever on a PI was also significantly associated with reduced BMD for both total hip ($p = 0.004$) and femoral neck ($p = 0.007$). Borderline significant association between greater cumulative duration of months ever on AZT and reduced BMD was also observed for both total hip ($p = 0.065$) and femoral neck ($p = 0.076$). No other HIV disease-specific covariate or factor was significantly associated with BMD at any site following multivariate analysis.

Phase Two patient subgroups were too small to allow race / gender-specific multivariate analysis.

4.10 Discussion

The Phase Two study population was representative of the wider Phase One study population and therefore also of the Sheffield HIV Cohort with respect to

relative proportions of patients by race and gender (excluding the small numbers of non-black non-white patients in the Phase One cohort who were excluded from Phase Two analysis). The Phase Two study population was, however, on average older and, with respect to black female patients, heavier and with a higher BMI than the Phase One study population.

The older age of the Phase Two study population relative to Phase One in part reflects targeted recruitment into Phase Two of the study, which aimed to ensure equal proportions of high, intermediate and low fracture risk patients respectively. This recruitment strategy therefore resulted in an over-representation of higher fracture risk patients – and therefore also older patients – in Phase Two relative to Phase One. It was also more challenging to recruit younger patients to attend for additional study visits for BMD measurement outside routine clinic visits. Furthermore, Phase Two study visits were conducted between three to six years after demographic data was collected within the preceding Phase One study.

As weight and BMI increase with age, it follows that the older Phase Two population would also be heavier and have a higher BMI than the Phase One population, especially with respect to female patients. Phase Two data could therefore exaggerate the current burden of reduced BMD in the wider (and on average younger) Sheffield HIV Cohort. Higher BMI, however, specifically in black female patients, may offset the effects of older age on BMD in the Phase Two study population.

Fragility fracture prevalence was much higher in the Phase Two study population than the Phase One study population (8.8%, n = 10 *vs.* 0.8%, n = 5). The time lag (plus three to six years) between Phase One and Phase Two, the older mean age of the Phase Two cohort, the proportionally higher number of patients with either high or intermediate fracture risk recruited to Phase Two versus Phase One, as well as the inclusion of sub-clinical vertebral fractures detected only by vertebral fracture risk assessment in Phase Two (three of the ten fragility fractures reported in Phase Two), could all account for this difference.

The prevalence of reduced BMD within the Phase Two study population was 42.6% for lumbar spine (33.3% osteopaenia, 9.6% osteoporosis) and 26.4% for hip (23.7% osteopaenia, 2.7% osteoporosis). This places the Phase Two cohort in the lower half of the range of reduced BMD prevalence reported across other HIV cohorts (Goh *et al.* 2018) and less than the 67% prevalence reported from another meta-analysis of pooled data from predominantly Caucasian HIV patients (Brown and Qaqish 2006). When compared to other HIV cohorts with a higher proportion of black patients, the Phase Two observed reduced BMD prevalence was also lower, however, although one of these studies with reduced BMD prevalence of 67% had a comparatively much older HIV cohort (mean age 61.5 ± 5 years) (Jones *et al.* 2008).

Whilst there was a trend to lower BMD in white patients compared with black patients and white female patients compared with white male patients within the Phase Two study population, there was no significant difference in BMD at any site between any race / gender subgroup, perhaps due to relatively small patient numbers within comparative subgroups.

The prevalence of reduced BMD as determined by T-score did vary between race / gender subgroups at the lumbar spine – 80% in black males, 50% in white males, 40% in white females and 19% in black females – and at the hip – 35% in white males, 30% in white females, 20% in black males and 16% in black females. The higher prevalence of reduced lumbar spine BMD in black male Phase Two patients compared to other race / gender subgroups is likely to be exaggerated by the recruitment of proportionally more high or intermediate fracture risk (older) black males into the Phase Two study than low risk (younger) black males and therefore may not be reflective of the prevalence of reduced BMD in black males within the wider Sheffield HIV Cohort.

No comparative HIV-negative control group was included in this study, limiting comparisons of BMD to the general population. Meta-analyses of BMD in PLWH have consistently demonstrated a higher prevalence of and greater odds ratios for reduced BMD in PLWH when compared to age- and sex-

matched HIV-negative controls (Brown and Qaqish 2006, Goh *et al.* 2018). The mean lumbar spine and total hip Z-scores for Phase Two patients (Table 4.5) suggest BMD measurements below age-, sex- and ethnicity-matched population means for all patient race / gender subgroups at each BMD site (except for the mean total hip Z-score in black female patients which was normal). The range of Z-scores for each BMD site in Phase Two was wide, however, and the number of patients within each subgroup relatively small, especially with respect to black males and white females. Furthermore, using UK black population data as a comparator for black patients of African origin may be suboptimal: BMD measurements in healthy Zimbabwean females were lower than in US black female patients in another study, although BMI was also lower in Zimbabwean black females than in US black females (Mukwasi *et al.* 2015).

There was some commonality in significant determinants of BMD at each site within the Phase Two study population. Following multivariate analysis, BMI was a significant predictor of BMD at every site and the strongest predictor of BMD for lumbar spine, total hip and femoral neck BMD ($p < 0.001$ for each); fragility fracture history was also a significant predictor of BMD at the lumbar spine, total hip and for total body (although not femoral neck). The presence of one or more other disorders associated with osteoporosis (collectively, but not individually) was also a significant predictor of BMD for total hip, femoral neck and total body (although not lumbar spine). Rheumatoid arthritis was a significant independent predictor of total hip BMD (borderline significance for femoral neck). Significant steroid exposure, however, was only a significant predictor of total body BMD only ($p = 0.002$) and was, unusually, associated with significantly higher BMD than in patients without significant steroid exposure. The association of age with BMD was only borderline significant for femoral neck BMD only ($p = 0.082$). In terms of other general fracture risk factors, whilst alcohol consumption ≥ 3 units d^{-1} was significantly associated with reduced BMD at the hip and femoral neck on univariate analysis, this was not significant on multivariate analysis. Non-FRAX® general fracture risk factors, such as SSRI use, Depo-Provera® use in female patients and

recreational drug use, were not significantly associated with BMD in this cohort.

In addition to FRAX®-incorporated general fracture risk factors, the only other factor – and the only HIV disease-specific factor – significantly associated with reduced BMD was increased cumulative PI exposure (independent of age) with respect to total hip ($p = 0.004$) and femoral neck ($p = 0.007$) (not lumbar spine or total body). Increased cumulative AZT exposure was associated with reduced BMD at the total hip and femoral neck with borderline significance. Whilst increased continuous tenofovir DF exposure was significantly associated with reduced total body BMD in univariate analysis, this was seen on multivariate analysis.

These results again support the notion that general risk factors contribute more to BMD (and therefore also to fracture risk) than HIV disease-specific factors in PLWH. With all significant predictors of both lumbar spine and total body BMD included in FRAX®, "unmodified" FRAX® – i.e. without the addition of HIV as a "secondary osteoporosis" risk factor – could therefore be a valid tool to predict non-hip fragility fractures in this population. FRAX® could be a reasonable tool to predict hip and femoral neck fractures also – with the majority of significant determinants of total hip and femoral neck BMD also included in FRAX® – although perhaps with the need for some modification of FRAX® to incorporate cumulative PI exposure in order to improve hip fracture prediction accuracy. The relatively small size of the Phase Two study population, the relatively low prevalence of patients with some risk factors within this population (e.g. injecting drug use (0.9%); HCV coinfection (1.8%)) and the lack of equal and sufficiently sized groups of patients either with and without individual fracture risk factors for comparative analysis limit the robustness of these findings, however, and therefore the ability to apply these conclusions to the wider Sheffield HIV Cohort and to PLWH in general.

Individual race / gender subgroups were too small to adequately assess whether or not individual covariates or factors had a greater or lesser impact on BMD in any one race / gender subgroup compared to another.

4.11 Conclusions

1. The Phase Two study population was older than the Phase One study population and therefore Phase Two BMD measurements may be lower overall than they would be on average for the wider Sheffield HIV Cohort, although higher BMI in Phase Two patients versus Phase One patients may offset this.
2. The prevalence of reduced BMD in the Phase Two study population was lower than average when compared with data reported from other HIV cohorts, but, based on Z-scores, BMD was still lower than in the general population; there were no significant differences in BMD at any site between patients of different race and gender.
3. FRAX®-incorporated general fracture risk factors were more significant than both non-FRAX®-incorporated general fracture risk factors and HIV disease-specific factors in determining BMD (and therefore also fracture risk) in PLWH, although cumulative PI exposure was also a significant predictor of total hip and femoral neck BMD; FRAX® might therefore be a valid tool for predicting fragility fracture risk in PLWH, with or without modification for cumulative PI exposure to improve accuracy of hip fracture probability prediction.

5. Distribution, determinants and effects of 25-hydroxyvitamin D and other biochemical factors, including their effect on bone mineral density, in people living with HIV in Sheffield

5.1 Introduction

Vitamin D deficiency – defined as 25-OH-D <50 nmol l^{-1} – is highly prevalent within PLWH, with reported prevalence within HIV cohorts ranging from 38% (Manion *et al.* 2017) to 89% (Cervero *et al.* 2018). Whether or not vitamin D deficiency is more prevalent in PLWH than in the general population remains equivocal, with conflicting data reported (Sherwood *et al.* 2012, Hidron *et al.* 2015,). Indeed, many of the significant determinants of vitamin D deficiency in PLWH are not HIV disease-specific, including black race (Welz *et al.* 2010, Sherwood *et al.* 2012), reduced sunlight exposure (Paul *et al.* 2010, Welz *et al.* 2010), increased BMI (Dao *et al.* 2011) and renal insufficiency (Dao *et al.* 2011).

Emerging data, however, suggests that HIV disease-specific factors do contribute to 25-OH-D levels. 25-OH-D has been shown to be lower in HIV-positive patients on ART compared with age- and sex-matched ART-naïve HIV-infected patients (Garcia *et al.* 2006, Paul *et al.* 2010), implicating ART as a potential 25-OH-D determinant. NNRTI use (Mueller *et al.* 2010) and, moreover, exposure to efavirenz (but not nevirapine, nor the newer NNRTI rilpivirne) has been specifically implicated (Welz *et al.* 2010, Wohl *et al.* 2014), with potent induction of 25-hydroxylase (CYP3A4) and 24-hydroxylase (CYP24) enzymes by efavirenz causing accelerated catabolism of vitamin D metabolites (Gyllensten *et al.* 2006). Low nadir CD4 cell count is another HIV disease-specific factor found to be significantly associated with lower 25-OH-D levels (Welz *et al.* 2010). (The relationship between 25-OH-D and chronic inflammation and immune activation in HIV will be examined in Chapter 6.)

Data linking reduced levels of 25 OH-D to reduced BMD in PLWH remains less clear. A recent South African study demonstrated lower total hip (but not lumbar spine) BMD in patients with lower 25-OH-D (Dave *et al.* 2015), which

was in contrast to the findings of other studies in which no significant association was identified between BMD and 25-OH-D, after adjusting for general fracture risk factors (Sherwood *et al.* 2012, Cotter *et al.* 2014). A Southern Indian study linked 25-OH-D to hyperparathyroidism and hyperparathyroidism to reduced BMD in PLWH, although 25-OH-D was not associated with BMD directly (Paul *et al.* 2010). There is a paucity of published data regarding the effect of vitamin D deficiency on falls frequency and fracture prevalence in PLWH.

In addition to hyperparathyroidism in the context of vitamin D deficiency (Paul *et al.* 2010), hyperparathyroidism in PLWH has also been independently associated with the NRTI tenofovir DF, with higher PTH levels observed within the first 48 weeks following initiation of tenofovir DF/emtricitabine than following initiation of abacavir/lamivudine, but without any effect noted on serum and urine phosphate and calcium (Masia *et al.* 2012); this association may not necessarily persist in patients well established on tenofovir DF-based ART, however (Samarawickrama *et al.* 2018). Moreover, tenofovir DF has been associated with renal phosphate wasting (Fux *et al.* 2007), accelerated renal function decline (Calza *et al.* 2009) and, less commonly, PRTD (Fux *et al.* 2007, Calza *et al.* 2009) in PLWH and it has been postulated that renal phosphate wasting could be one mechanism by which tenofovir DF may cause greater bone loss than non-tenofovir DF containing ART in PLWH. The renal and bone effects of tenofovir DF are likely to be potentiated by co-administration with a boosted PI (Calmy *et al.* 2009, Calza *et al.* 2011, Mizushima *et al.* 2018). In one cross-sectional study, both lumbar spine and hip BMD were lower in patients with PRTD than in those without, but the differences in BMD were not significant (Calmy *et al.* 2009).

This chapter aims to answer the following questions:

1. What is the distribution of 25-OH-D within the Sheffield HIV Cohort and how does this compare to other HIV cohorts?

2. What are the general and HIV disease-specific determinants of 25-OH-D within the Sheffield HIV Cohort and do these differ to those identified in other HIV cohorts?

3. Are there significant associations between 25-OH-D and BMD, falls frequency and fracture prevalence within the Sheffield HIV Cohort?

4. What is the distribution of serum phosphate within the Sheffield HIV Cohort, what are its general and HIV disease-specific determinants – including assessment of association with tenofovir DF exposure – and what are its effects on BMD, falls frequency and fracture prevalence?

5. What is the distribution or prevalence of other “biochemical” factors – namely serum PTH, serum corrected calcium, renal phosphate wasting (measured as percentage of tubular reabsorption of phosphate (TRP)), PRTD and renal insufficiency (measured as race-adjusted estimated glomerular filtration rate (eGFR)) – within the Phase Two study population, what are their general and HIV disease-specific determinants and what are their effects on BMD?

5.2 Distribution, determinants and effects of 25-hydroxyvitamin D in PLWH

5.2.1 Distribution and determinants of 25-hydroxyvitamin D in PLWH

The distribution of 25-OH-D in the Phase One study cohort is shown in Table 5.1 (n = 575, excluding 33 patients for whom no 25-OH-D result was available and a further 17 patients either currently taking vitamin D supplementation or having taken vitamin D supplementation in the past). Table 5.1 and Figure 5.1 also detail the distribution of 25-OH-D by patient race / gender subgroups. The percentage prevalence of Phase One patients with 25-OH-D <25 nmol l^{-1} (severe deficiency), 25 – 49.9 nmol l^{-1} (non-severe deficiency), 50 – 74.9 nmol l^{-1} (insufficiency) and ≥75 nmol l^{-1} (normal) was 35.5%, 46.7%, 9.9% and 7.9% respectively. Greater proportions of black patients had 25-OH-D <25 nmol l^{-1} or <50 nmol l^{-1} compared to white patients (Figure 5.1), with 25-OH-D significantly lower in black males compared to white males ($p < 0.001$), in black females compared to white females ($p = 0.001$) and in black patients compared to white patients ($p < 0.001$). 25-OH-D was also significantly lower in female patients (of whom 78.2% were of black race) compared with male patients (of whom 64.3% were of white race) ($p < 0.001$), although with 25-OH-D also significantly lower in white females compared with white males ($p = 0.017$) (no significant difference observed in 25-OH-D between black males and black females).

There was a significant difference in 25-OH-D according to season of sampling (winter = December to February, spring = March to May, summer = June to August and autumn = September to November) with 25-OH-D significantly higher if sampled in summer as opposed to non-summer months ($p = 0.004$) (Table 5.2 and Figure 5.2). Atypically, some very high 25-OH-D values (greater than 150 nmol l^{-1}) were observed in patients sampled in autumn (in two patients) and in winter (one patient), with none in patients sampled in spring or summer. Whilst patients reporting current or previous vitamin D and/or calcium supplementation were excluded from analysis, no data was collected or available with respect to recent sunbed use or overseas travel.

	All (n = 575)	Black (n = 294)		White (n = 261)		Non-Black Non-White (n = 20)	
		Male (n = 107)	Female (n = 187)	Male (n = 216)	Female (n = 45)	Male (n = 13)	Female (n = 7)
Median 25-OH-D *nmol l^{-1}*	29.6	24.1	25.9	38.7	30.6	25.2	37.2
IQR	20.8 – 42.8	18.5 – 30.0	17.8 – 35.6	28.7 – 62.4	25.2 – 47.6	18.0 – 27.4	23.7 – 46.6
Range	9.9 – 191.1	9.9 – 75.6	9.9 – 85.3	11.5 – 191.1	13.1 – 116.7	9.9 – 41.5	19.4 – 104.3

Table 5.1. 25-hydroxyvitamin D (25-OH-D) for all (n = 575), black (n = 331), white (n = 274) and non-black non-white (n = 20) patient subgroups within the Phase One study population (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

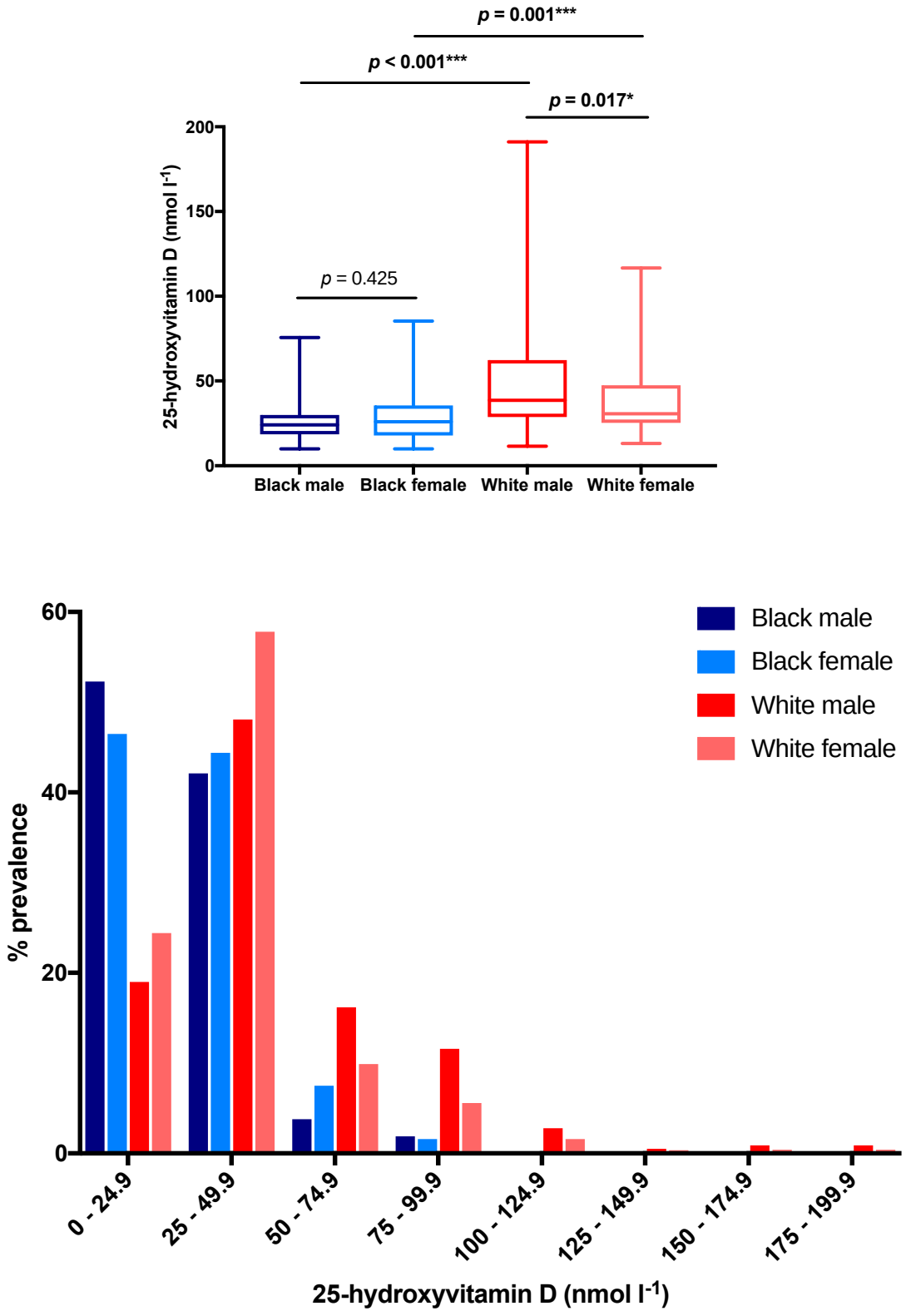


Figure 5.1. Distribution of and differences between 25-hydroxyvitamin D (25-OH-D) in black male (n = 107), black female (n=187), white male (n = 216) and white female (n= 45) patient subgroups within the Phase One study population (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

	Winter (n = 216)	Spring (n = 139)	Summer (n = 56)	Autumn (n = 164)
Median 25-OH-D *nmol l^{-1}*	29.0	28.7	33.8	30.2
IQR	20.0 – 41.8	20.7 – 39.6	27.0 – 57.9	20.1 – 41.5
Range	9.9 – 191.1	9.9 – 131.4	9.9 – 112.9	9.9 – 176.2

Table 5.2. Distribution of 25-hydroxyvitamin D (25-OH-D) within the Phase One study according to season of sampling (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1}) (winter = December to February, spring = March to May, summer = June to August and autumn = September to November)

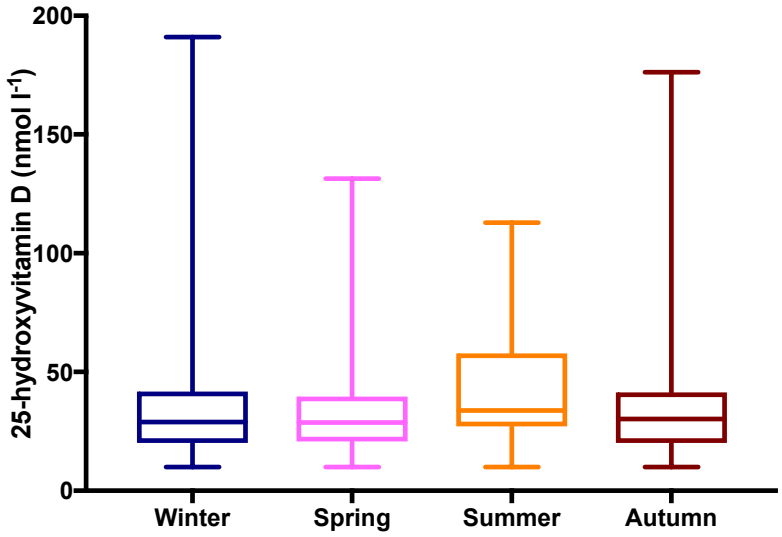


Figure 5.2. Distribution of 25-hydroxyvitamin D (25-OH-D) within the Phase One study population according to season of sampling (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1}) (winter = December to February (n = 216), spring = March to May (n = 139), summer = June to August (n = 56) and autumn = September to November (n = 164))

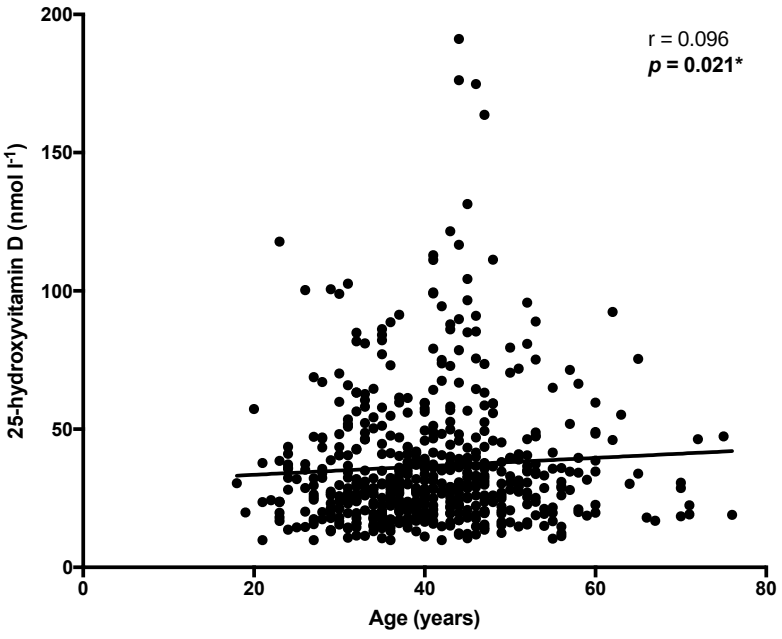


Figure 5.3. Relationship between 25-hydroxyvitamin D (25-OH-D) and patient age within the Phase One study population (n = 575) (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

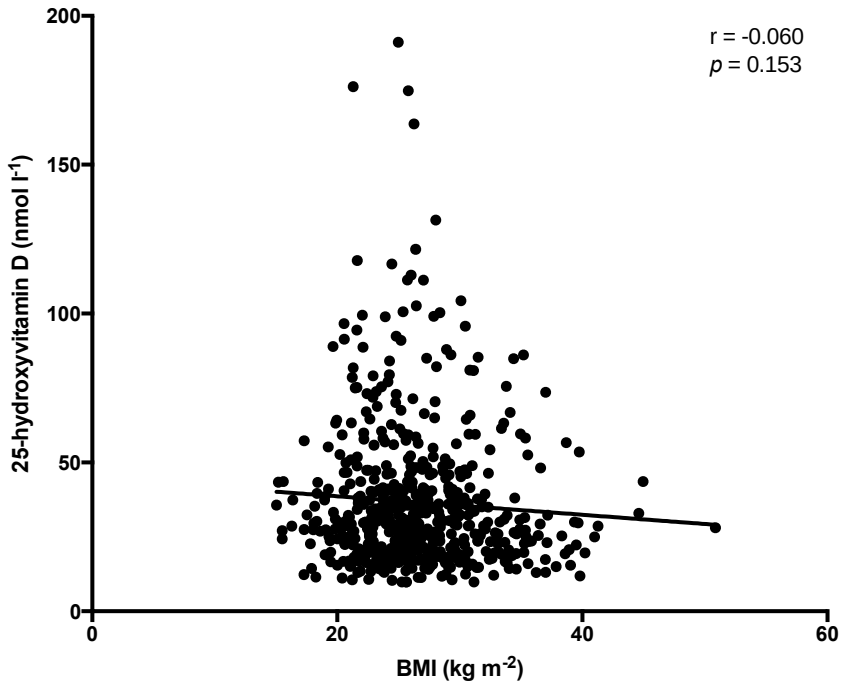


Figure 5.4. Relationship between 25-hydroxyvitamin D (25-OH-D) and patient body mass index (BMI) within the Phase One study population (n = 570) (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

There was a significant association between 25-OH-D and age, with an increase-in 25-OH-D with older age (r = 0.096, *p* = 0.021) (Figure 5.3), but there was no significant association between 25-OH-D and either patient weight (r = 0.057, *p* = 0.169) or BMI (r = -0.060, *p* = 0.153) (Figure 5.4).

With respect to HIV disease-specific factors, there was a significant positive correlation between 25-OH-D and nadir CD4 cell count (r = 0.097, *p* = 0.022) (Figure 5.5), but no significant association between 25-OH-D and current CD4 cell count (r = 0.069, *p* = 0.100), nor any significant difference in 25-OH-D between patients with or without HIV viral load <40 copies ml^{-1} (*p* = 0.228) or <200 copies ml^{-1} (*p* = 0.257), although median 25-OH-D and interquartile range of 25-OH-D were lower in patients with suppressed HIV viral load at either cut-off value than in patients with unsuppressed HIV viral load (Table 5.3).

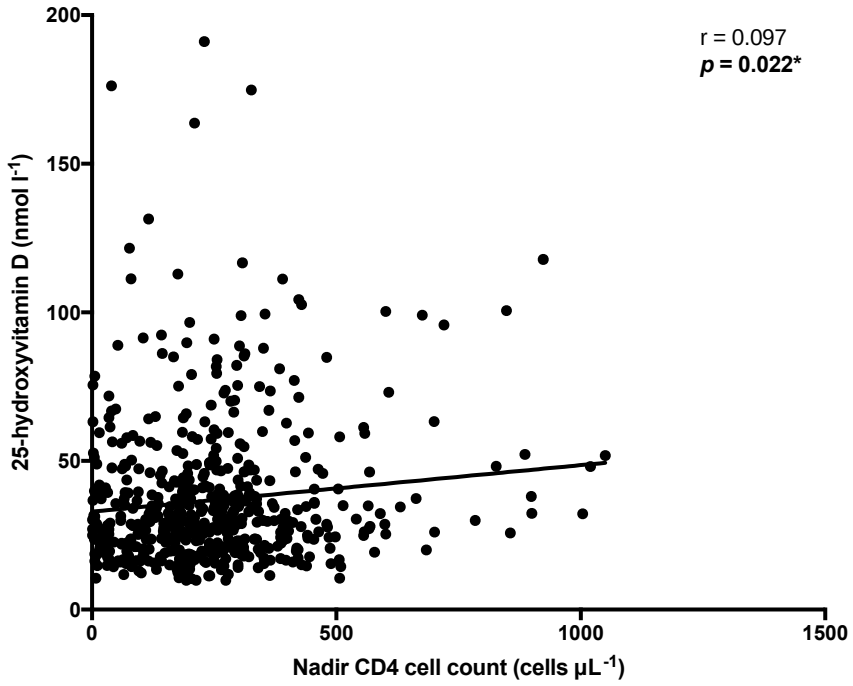


Figure 5.5. Relationship between 25-hydroxyvitamin D (25-OH-D) and patient nadir CD4 cell count (cells µL^{-1}) within the Phase One study population (n = 558) (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

25-OH-D was not significantly different in Phase One patients with either current or ever exposure to any ART or to any NRTI compared with Phase One patients without respective exposures (p = 0.067, p = 0.170, p = 0.088 and p = 0.0164 for current ART exposure, ever ART exposure, current NRTI exposure and ever NRTI exposures respectively), however median 25-OH-D and interquartile range of 25-OH-D were lower in patients with ART or NRTI exposures compared with patients without (Table 5.3). Neither the duration of continuous or cumulative ART had any significant association with 25-OH-D (r = -0.018, p = 0.672 and r = 1.000, p = 0.710 for continuous and cumulative ART exposure respectively) (continuous and cumulative duration of NRTI data not collected within the Phase One study population).

HIV disease-specific factor		*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
Plasma HIV RNA <40 copies ml^{-1}	No	218	31.3	21.4 – 31.3	9.9 – 176.2	.228
	Yes	357	29.1	20.7 – 40.8	9.9 – 191.1	
Plasma HIV RNA <200 copies ml^{-1}	No	176	30.3	22.3 – 46.9	9.9 – 117.8	.257
	Yes	399	29.3	20.5 – 41.1	9.9 – 191.1	
Current ART	No	152	32.0	22.8 – 47.2	9.9 – 176.2	.067
	Yes	423	29.1	20.4 – 40.9	9.9 – 191.1	
Ever ART	No	129	30.0	22.7 – 47.1	9.9 – 117.8	.170
	Yes	446	29.4	20.6 – 41.2	9.9 – 191.1	
Current NRTI	No	158	31.4	22.5 – 47.1	9.9 – 176.2	.088
	Yes	417	29.1	20.5 – 41.0	9.9 – 191.1	
Ever NRTI	No	130	30.3	22.9 – 47.1	9.9 – 117.8	.164
	Yes	445	29.4	20.6 – 41.2	9.9 – 191.1	
Current NNRTI	No	297	31.9	23.4 – 46.4	9.9 – 176.2	**<.001**
	Yes	278	27.3	19.2 – 39.5	9.9 – 191.1	

Table 5.3 (continued overleaf) Differences in 25-hydroxyvitamin D in patients with or without exposure to HIV disease-specific antiretroviral treatment (ART)-related factors within the Phase One study population (n = 575) (NRTI = nucleos(t)ide reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; EFV = efavirenz; NVP = nevirapine; 25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

HIV disease-specific factor		*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
Ever NNRTI	No	216	30.9	23.1 – 45.3	9.9 – 174.8	.051
	Yes	359	28.8	19.9 – 39.5	9.9 – 191.1	
Current EFV	No	376	31.3	23.1 – 46.2	9.9 – 191.1	**<.001**
	Yes	199	26.7	18.5 – 37.4	9.9 – 121.6	
Ever EFV	No	314	31.6	23.4 – 45.9	9.9 – 191.1	**<.001**
	Yes	261	27.4	19.2 – 39.7	9.9 – 131.4	
Current NVP	No	507	29.8	21.0 – 43.3	9.9 – 176.2	.488
	Yes	68	28.3	20.2 – 40.7	10.7 – 191.1	
Ever NVP	No	454	28.8	20.4 – 41.3	9.9 – 174.8	**.034**
	Yes	121	31.9	23.6 – 48.7	10.7 – 191.1	
Current PI	No	420	28.6	19.8 – 43.3	9.9 – 191.1	**.017**
	Yes	155	31.9	24.5 – 41.9	9.9 – 174.8	
Ever PI	No	357	28.6	20.1 – 42.2	9.9 – 191.1	.098
	Yes	218	31.4	22.5 – 43.3	9.9 – 176.2	

Table 5.3 (continued from overleaf) Differences in 25-hydroxyvitamin D_3 in patients with or without exposure to HIV disease-specific antiretroviral treatment (ART)-related factors within the Phase One study population (n = 575) (NRTI = nucleos(t)ide reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; EFV = efavirenz; NVP = nevirapine; 25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

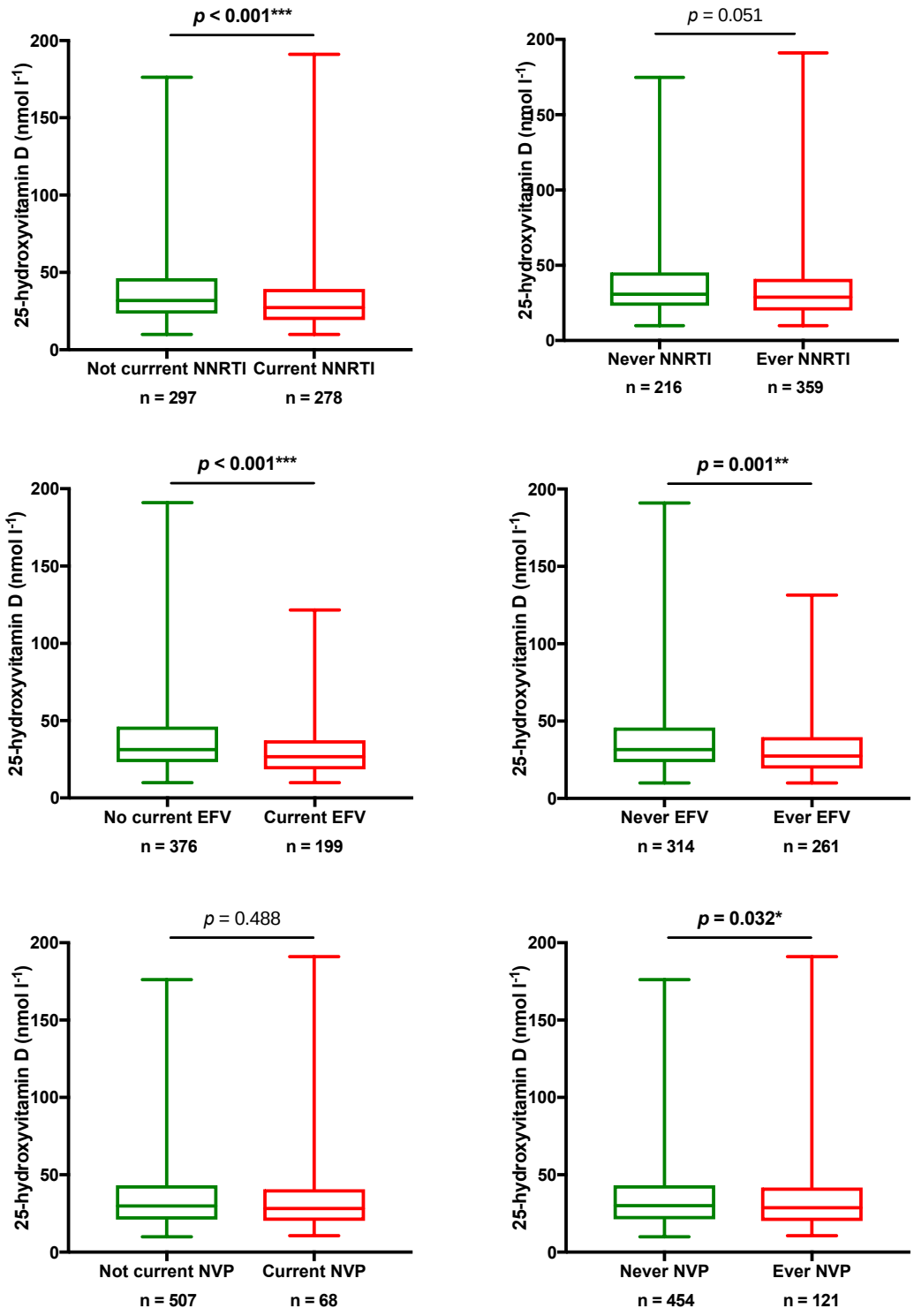


Figure 5.6. Distribution of 25-hydroxyvitamin D (25-OH-D) in Phase One patients not currently on versus currently on and never exposed to versus ever exposed to any non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

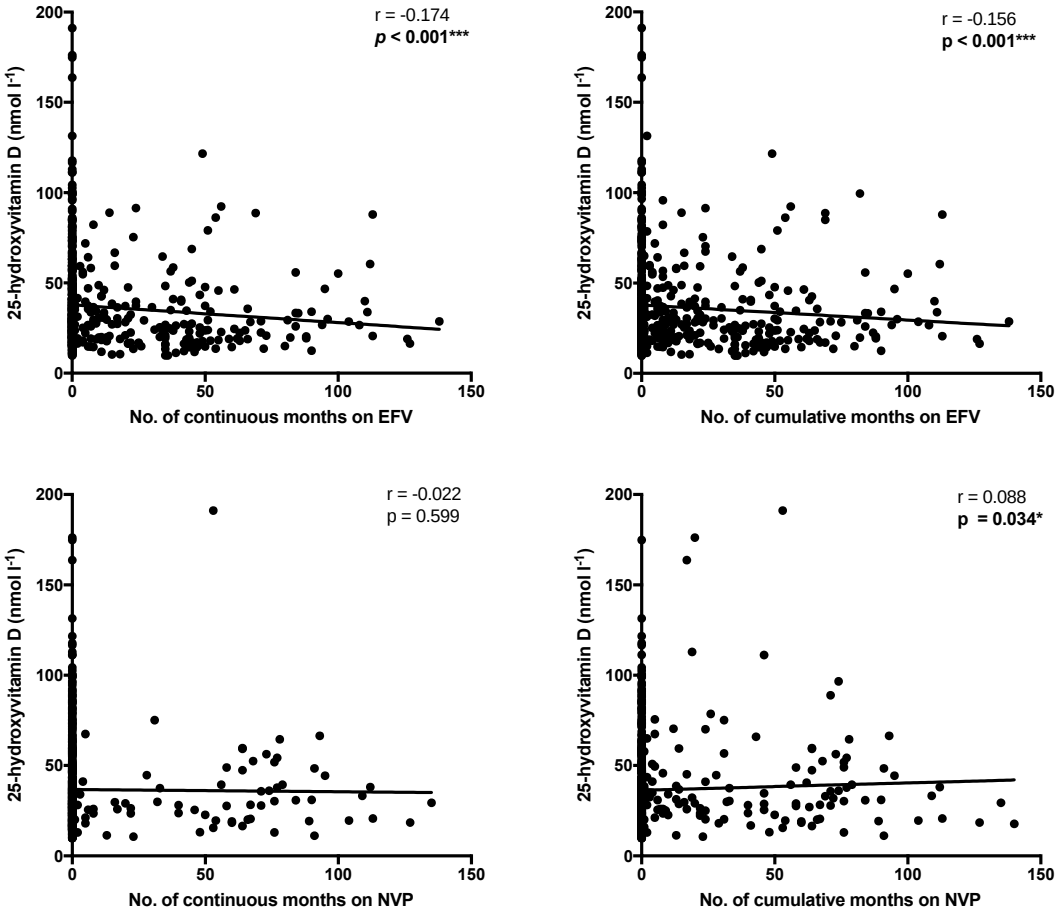


Figure 5.7. Relationship between 25-hydroxyvitamin D (25-OH-D) and both continuous and cumulative duration (months) of efavirenz (EFV) and nevirapine (NVP) in Phase One patients (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

25-OH-D was significantly lower in patients with current exposure to NNRTIs ($p < 0.001$), with current or ever efavirenz exposure ($p < 0.001$ and $p = 0.001$ respectively) and with ever nevirapine exposure ($p = 0.032$) compared with Phase One patients without respective exposures, but with no significant difference in 25-OH-D in patients with ever *versus* never NNRTI exposure ($p = 0.051$) or with current *versus* not current nevirapine exposure ($p = 0.488$) (Table 5.3 and Figure 5.6). Both longer continuous duration of efavirenz and longer cumulative duration of efavirenz exposures were associated with significantly lower 25-OH-D values (r = -0.174, $p < 0.001$ and r = -0.156, $p < 0.001$ respectively); the duration of continuous exposure to nevirapine had no significant relationship with 25-OH-D, however (r = -0.022, $p = 0.599$) and

longer cumulative duration of nevirapine exposure was associated with significantly higher 25-OH-D values (r = 0.038, *p* = 0.034) (Figure 5.7).

Phase One patients with current, but not ever, PI exposure had a significantly higher 25-OH-D than Phase One patients not currently on a PI (*p* = 0.017, Table 5.3 and Figure 5.8) (continuous and cumulative PI exposure not collected within the Phase One study population).

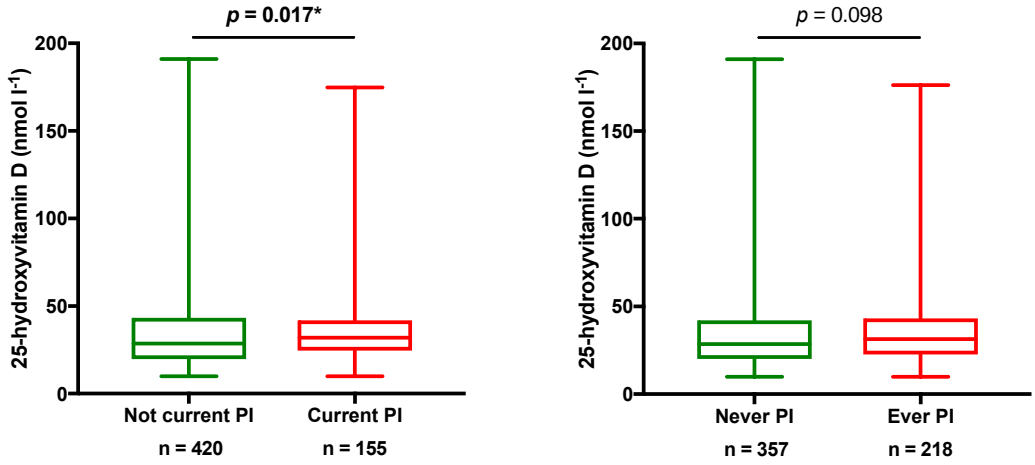


Figure 5.8. Distribution of 25-hydroxyvitamin D (25-OH-D) in Phase One patients not currently on versus currently on and never exposed to versus ever exposed to any protease inhibitor (PI) (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

The prevalence of patients with a history of either chronic diarrhoea, malabsorption or liver cirrhosis were low within the Phase One study population for whom 25-OH-D data was available for analysis: 2.6% (n = 15), 0.7% (n = 4) and 0.7% (n = 4) respectively. There was no significant difference in 25-OH-D between patients with or without a history of chronic diarrhoea, malabsorption or liver cirrhosis (*p* = 0.708, *p* = 0.257 and *p* = 0.065 respectively, Table 5.4).

Factor	Factor present	*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
Chronic diarrhoea	No	560	29.5	20.7 – 42.8	9.9 – 191.1	.708
	Yes	15	29.8	22.5 – 29.8	14.8 – 99.5	
Malabsorption	No	571	29.4	20.7 – 42.8	9.9 – 191.1	.257
	Yes	4	35.5	32.8 – 46.6	32.4 – 49.9	
Liver cirrhosis	No	571	29.4	20.7 – 42.6	20.7 – 42.6	.065
	Yes	4	43.4	39.0 – 56.2	38.6 – 59.3	

Table 5.4. Differences in 25-hydroxyvitamin D in patients with or without a history of chronic diarrhoea, malabsorption or liver cirrhosis within the Phase One study population (n = 575) (25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

Factor	Factor present	*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
Race-adjusted eGFR <90 ml min^{-1}	No	54	31.8	18.8 – 54.7	12.4 – 176.0	**<.001**
	Yes	27	46.6	35.8 – 83.5	14.8 – 151.3	
Race-adjusted eGFR <60 ml min^{-1}	No	78	36.8	25.1 – 69.9	12.4 – 176.0	.560
	Yes	3	35.6	-	35.1 – 82.6	

Table 5.5. Differences in 25-hydroxyvitamin D in patients with or without race-adjusted eGFR <90 ml min^{-1} or <60 ml min^{-1} within the Phase Two study population (n = 81) (25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

eGFR adjusted for race (x1.159 in patients of black race) was available for Phase Two study patients only (n = 114), of whom 81 patients had never received vitamin D supplementation and for whom analysis of 25-OH-D determinants was possible. Patients with race-adjusted eGFR <90 ml min^{-1} had significantly higher 25-OH-D than patients with race-adjusted eGFR ≥90 ml min^{-1} ($p < 0.001$) (Table 5.5). There was no significant difference in 25-OH-D between patients with race-adjusted eGFR <60 ml min^{-1} *versus* those with race-adjusted eGFR ≥60 ml min^{-1} ($p = 0.560$), although only three patients had race-adjusted eGFR <60 ml min^{-1} (Table 5.5).

Factors and co-variates found to have either significant ($p < 0.05$) or borderline significant ($p \geq 0.05$ and < 0.10) with 25-OH-D within the Phase One study population are summarised in Table 5.6.

Factor / covariate	***p*-value**	**Factor / co-variate**	***p*-value**
Black race	**<.001**	Current ART	.067
Female gender	**<.001**	Current NRTI	.088
Age	**.021**	Ever NNRTI	.051
Summer sampling	**.004**	Ever PI	.098
Nadir CD4 cell count	**.022**	Liver cirrhosis	.065
Current NNRTI	**<.001**		
Current EFV	**<.001**		
Ever EFV	**.001**		
No. mths continuous EFV	**<.001**		
No. mths cumulative EFV	**<.001**		
Ever NVP	**.032**		
No. mths cumulative NVP	**.034**		
Current PI	**.017**		

Table 5.6. Factors and co-variates with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) associations with 25-hydroxyvitamin D from univariate analysis within the Phase One study population

Factors and covariates with significant ($p < 0.05$) association with 25-OH-D were taken forward for multivariate analysis using a generalised linear model. Current NNRTI class exposure – non-independent of individual efavirenz and nevirapine exposure-related factors and covariates – was not taken forward for multivariate analysis. Furthermore, as no patients were on both efavirenz and nevirapine, nor on efavirenz and a PI or nevirapine and a PI simultaneously, efavirenz, nevirapine and PI exposure-related factors were also likely to be co-dependent; therefore, as efavirenz exposure had the strongest association with 25-OH-D on univariate analysis, only efavirenz exposure-related factors and not nevirapine or PI-exposure related factors were taken forward for multivariate analysis. Current efavirenz exposure was included over ever, continuous or cumulative efavirenz exposure. 20 non-black non-white patients were excluded from the multivariate analysis, which included only patients of black or white race.

Backward elimination was used to determine factors and covariates with significant association with 25-OH-D within the multivariate analysis model (detailed in Appendix A2.1), with stepwise elimination continuing until all residual risk factors achieved significance of $p < 0.05$. Factors and covariates with significant association with 25-OH-D following multivariate analysis are shown in Table 5.7. Only race (black *versus* white race), season of sampling (summer *versus* non-summer) and current efavirenz exposure were significantly associated with 25-OH-D on multivariate analysis, with summer sampling being associated with significantly higher 25-OH-D measurements and black race and current efavirenz exposure being associated with significantly lower 25-OH-D measurements.

Covariate / factor	Factor present	Estimated marginal mean *nmol l^{-1}*	Standard Error	Wald Chi-Square	*p*-value
White race	No	30.900	1.837	98.189	**<.001**
	Yes	49.964	2.001		
Summer season of sampling	No	35.847	1.0472	8.011	**.005**
	Yes	45.017	3.111		
Current efavirenz	No	43.247	1.741	7.825	**.005**
	Yes	37.617	2.126		

Table 5.7. Covariates and factors with significant (p < 0.05) association with 25-hydroxyvitamin D in the Phase One study population (n = 538) following multivariate analysis

5.2.2 Effects of 25-hydroxyvitamin D in PLWH

The relationships between 25-OH-D and lumbar spine, total hip, femoral neck and total body BMD within the Phase Two study population, excluding Phase Two patients ever treated with vitamin D supplementation, are shown in Figure 5.9. Whilst there was a negative correlation between 25-OH-D and BMD, this was not significant at any BMD site (Figure 5.9).

Furthermore, there was no significant correlation between 25-OH-D and BMD at any site in either black patients (r = 0.048, p = 0.798 for lumbar spine; r = -0.149, p = 0.425 for total hip; r = -0.066, p = 0.724 for femoral neck and r = 0.007, p = 0.968 for total body) or white patients (r = -0.230, p = 0.109 for lumbar spine; r = -0.162, p = 0.261 for total hip; r = -0.135, p = 0.349 for femoral neck and r = -0.086, p = 0.553 for total body) when analysed separately.

There was no significant difference in 25-OH-D in all, black and white Phase One patients self-reporting no falls, one single fall or multiple falls within the previous twelve months (Table 5.8).

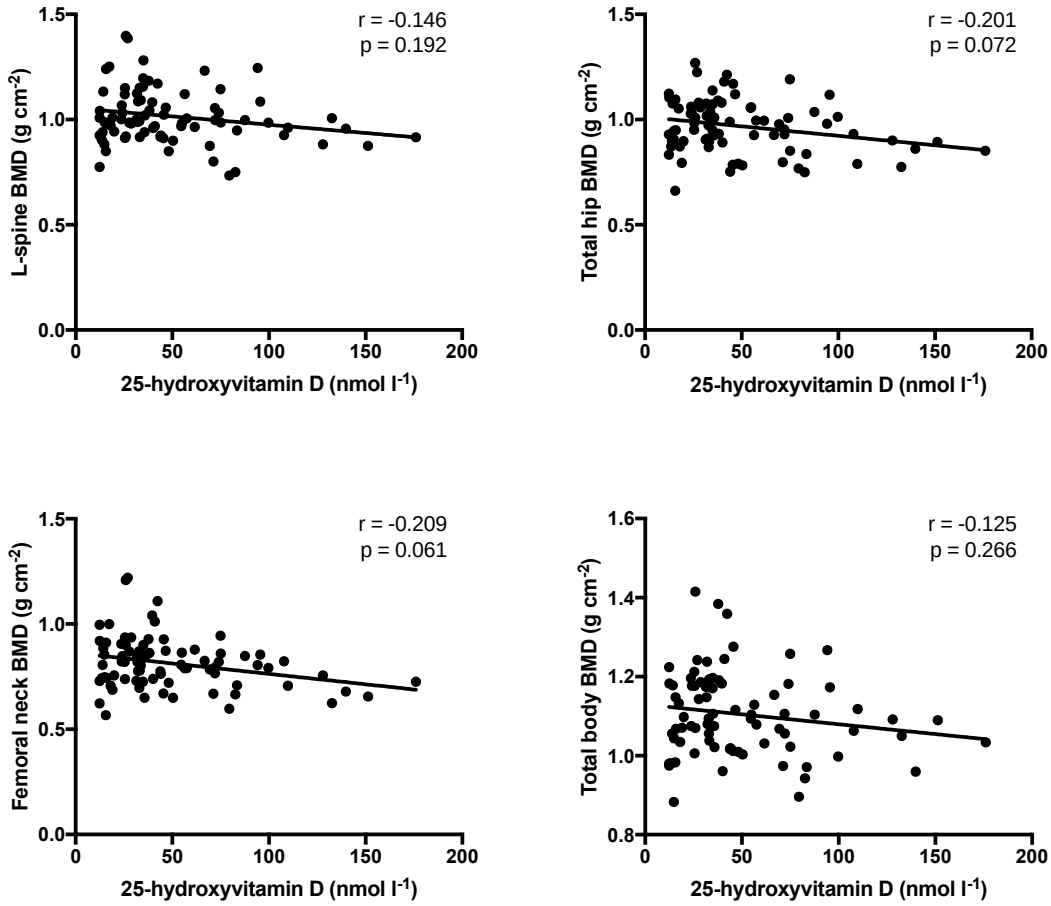


Figure 5.9. Relationship between 25-hydroxyvitamin D and lumbar (L-) spine, total hip, femoral neck and total body BMD in the Phase Two study population (n = 81)

Phase One patient group	Number of falls in last 12 months	*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
All patients	None	500	29.7	20.7 – 42.4	9.9 – 191.1	.199
	Single	41	26.8	20.8 – 39.7	11.8 – 91.4	
	Multiple	33	32.3	22.5 – 54.3	12.1 – 121.6	
Black patients	None	261	25.6	18.1 – 34.8	9.9 – 85.3	.492
	Single	20	24.2	16.7 – 27.4	14.6 – 50.3	
	Multiple	12	22.4	19.4 – 34.5	16.4 – 56.7	
White patients	None	220	37.4	27.6 – 57.7	11.5 – 191.1	.635
	Single	20	36.0	24.6 – 50.3	11.8 – 91.4	
	Multiple	21	38.1	28.7 – 85.6	12.1 – 121.6	

Table 5.8 Differences in 25-hydroxyvitamin D between all (n = 574), black (n = 293) and white (n = 261) Phase One patients self-reporting no falls, one single fall or multiple falls in last 12 months (25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

Phase One patient group	Number of fractures in adulthood	*n*	25-hydroxyvitamin D *nmol l^{-1}*			*p*-value
			Median	IQR	Range	
All patients	None	419	28.7	19.8 – 39.9	9.9 – 191.1	**<.001**
	Single	81	33.9	24.5 – 52.7	12.5 – 131.4	
	Multiple	25	40.5	25.9 – 60.4	17.1 – 99.1	
Black patients	None	241	24.5	17.8 – 32.1	9.9 – 81.0	.069
	Single	16	29.1	25.7 – 43.7	16.4 – 61.4	
	Multiple	4	23.0	18.1 – 48.8	17.1 – 56.7	
White patients	None	159	37.3	28.7 – 56.0	11.5 – 191.1	.409
	Single	64	35.9	24.4 – 62.5	12.5 – 131.4	
	Multiple	21	46.4	31.2 – 68.2	22.6 – 99.1	

Table 5.9 Differences in 25-hydroxyvitamin D between all (n = 525), black (n = 261) and white (n = 244) Phase One patients self-reporting no fractures, one single fracture or multiple fractures in adulthood (25-hydroxyvitamin D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

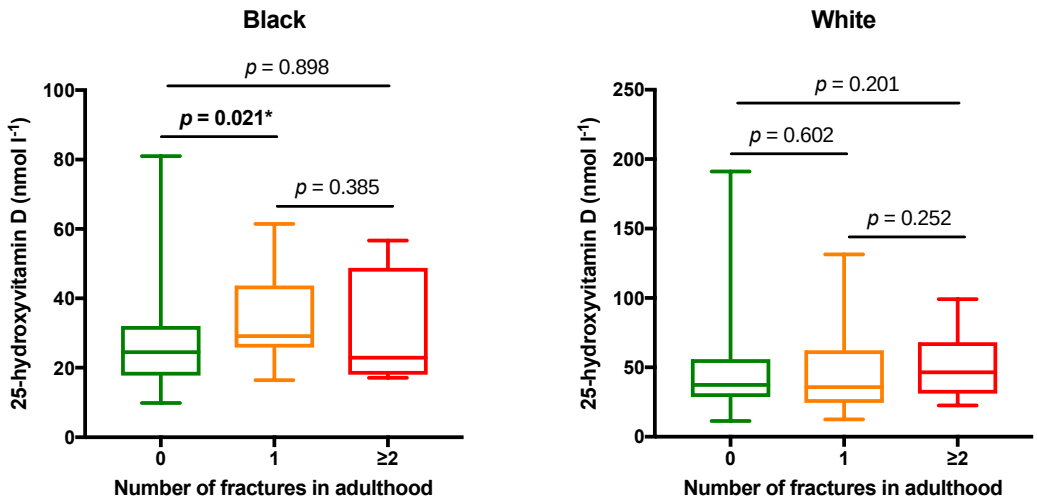


Figure 5.10. Distribution of 25-hydroxyvitamin D (25-OH-D) in black (n = 261) and white (n = 244) Phase One patients reporting no fractures, one single fracture or multiple fractures in adulthood (25-OH-D values <10.0 nmol l^{-1} (lowest limit of quantification) recorded as 9.9 nmol l^{-1})

Whilst there was a significant difference in 25-OH-D in all Phase One patients reporting either no fractures, one single fracture or multiple fractures in adulthood overall ($p < 0.001$) (Table 5.9), within black and white patient subgroups, this difference was only significant between black patients reporting no fractures (n = 241) and black patients reporting one single fracture (n = 16) ($p = 0.021$), with 25-OH-D higher in patients reporting one single fracture (Figure 5.10).

In terms of fragility fractures specifically, analysis of Phase Two patients (in whom vertebral fracture assessment was performed) revealed no significant difference in 25-OH-D between white patients without prior fragility fracture (n = 47, median 25-OH-D 45.4 nmol l^{-1}, IQR 32.9 – 79.5) and those with prior fragility fracture (n = 3, median 25-OH-D 61.6 nmol l^{-1}, range 35.1 – 82.6) ($p = 0.728$). (The other seven Phase Two patients with prior fragility fracture, including all four black patients with prior fragility fracture, were either previously on or currently on vitamin D supplementation and therefore excluded from analysis.)

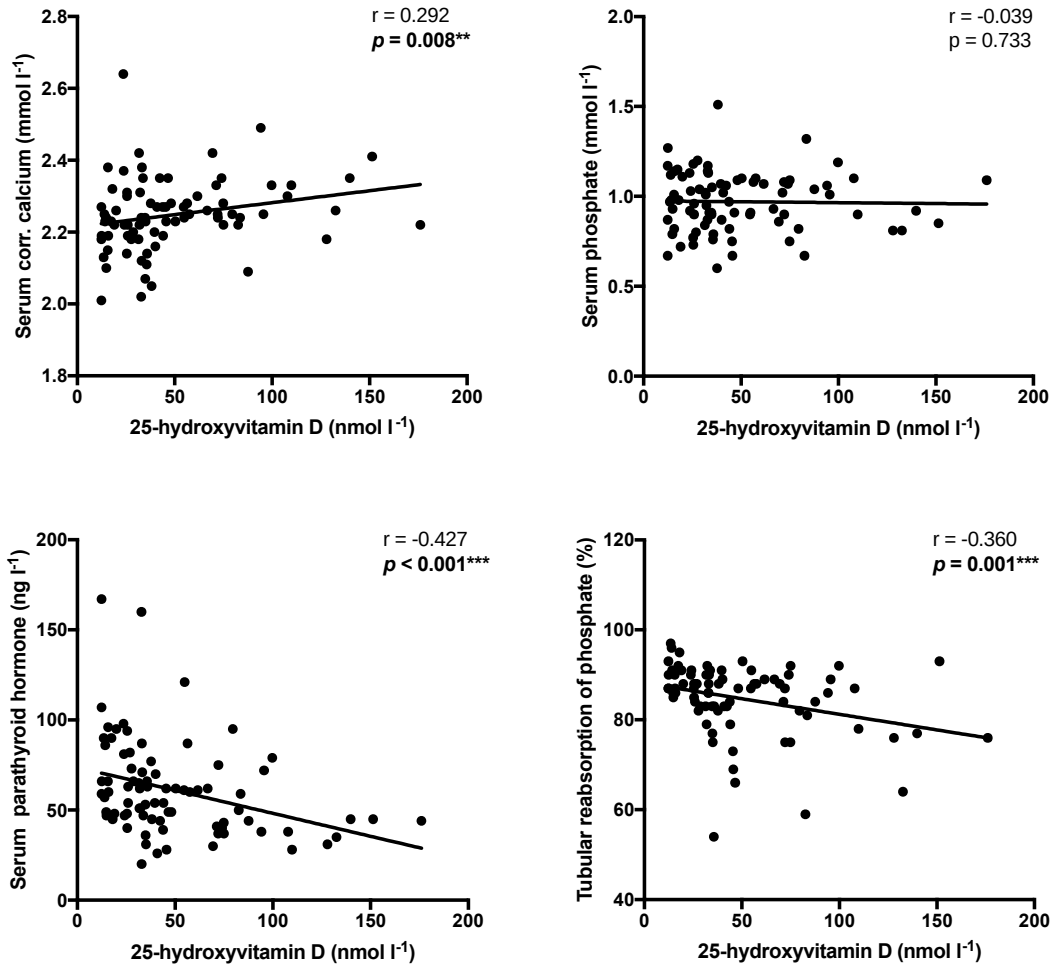


Figure 5.11. Relationship between 25-hydroxyvitamin D and serum correct calcium (corr. calcium), serum phosphate, serum parathyroid hormone and tubular reabsorption of phosphate (TRP) in the Phase Two study population (n = 81)

The relationships between 25-OH-D and serum corrected calcium, serum phosphate, TRP and serum PTH measurements are shown in Figure 5.11 for Phase Two patients (n = 81). Whilst there was no significant correlation between 25-OH-D and serum phosphate (r = -0.039, *p* = 0.733), there was a significant positive correlation between serum corrected calcium and 25-OH-D (r = 0.292, *p* = 0.008) and a significant negative correlation between serum PTH and 25-OH-D (r = -0.427, p < 0.001) and, unexpectedly, TRP and 25-OH-D (r = -0.360, *p* = 0.001). A significant negative correlation between TRP and 25-OH-D was also observed in white patients on subgroup analysis (r = -0.282, *p* = 0.047), but not in black patients (r = -0.051, *p* = 0.788).

5.3 Distribution, determinants and effects of serum phosphate in PLWH

5.3.1 Distribution and determinants of serum phosphate in PLWH

The distribution of fasted serum phosphate within the Phase Two study population (n = 81) is shown in Figure 5.12 (mean 0.97 ± 0.16 mmol l^{-1}, normal range 0.80 – 1.50 mmol l^{-1}). 12 (14.8%) Phase Two patients had mild hypophosphataemia (serum phosphate 0.60 to 0.79 mmol l^{-1}); no patients had serum phosphate measurements less than 0.60 mmol l^{-1}.

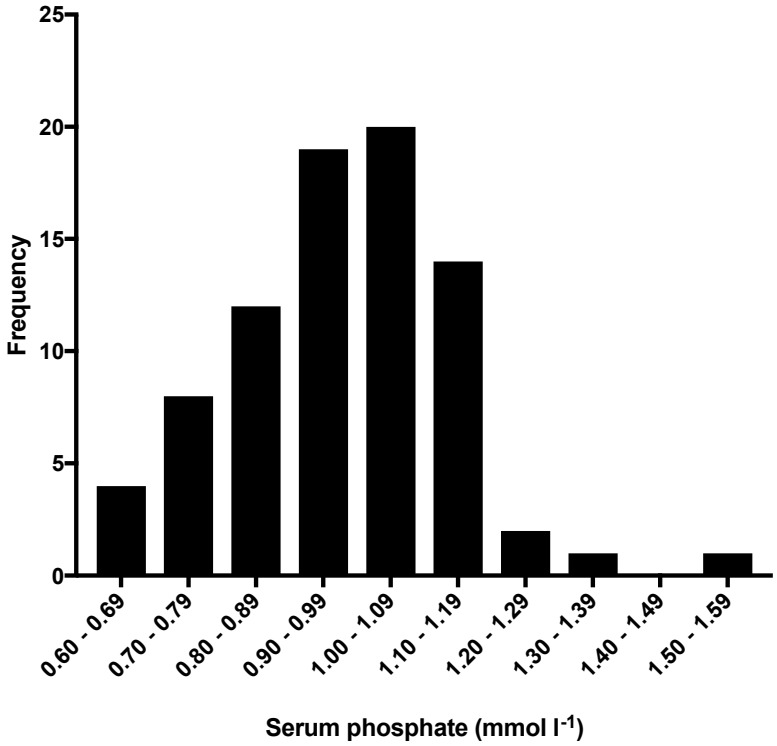


Figure 5.12. Distribution of serum phosphate in the Phase Two study population (n =81)

There was no significant association between serum phosphate and patient age (r = -0.154, *p* = 0.169), weight (r = -0.413, *p* = 0.202) or BMI (r = 0.090, *p* = 0.425). Serum phosphate was significantly higher in black patients (mean 1.02 ± 0.15 nmol l^{-1}, n = 31) than in white patients (mean 0.94 ± 0.17 nmol l^{-1}, n = 50) (*p* = 0.049) and in female patients (mean 1.05 ± 0.14 nmol l^{-1}, n = 31) than in male patients (mean 0.92 ± 0.16 nmol l^{-1}, n = 50) (*p* = 0.001). Serum

phosphate was not significantly different between black male (n = 10) and black female (n = 21), white male (n = 41) and white female (n = 9), black male and white male or black female and white female patients, however, although there was a trend to lower serum phosphate in white males (mean 0.92 ± 0.16 nmol l^{-1}) compared to white females (mean 1.07 ± 0.14 nmol l^{-1}) (Figure 5.13).

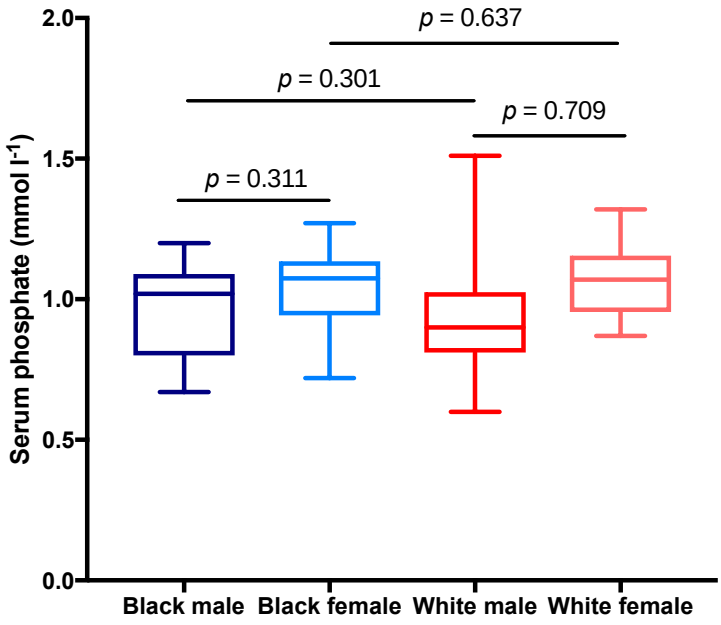


Figure 5.13. Distribution of serum phosphate in black male (n = 10), black female (n = 21), white male (n = 41) and white female (n = 9) Phase Two patients

Patients with race-adjusted eGFR <90 ml min^{-1} (n = 27) also had significantly lower serum phosphate (mean 0.90 ± 0.15 mmol l^{-1}) than patients with race-adjusted eGFR ≥90 ml min^{-1} (mean 1.01 ± 0.16 mmol l^{-1}, n = 54) (p = 0.006). Similarly, in patients with race-adjusted eGFR <60 ml min^{-1} (n = 3) serum phosphate was also lower (mean 0.83 ± 0.20 mmol l^{-1}) than in patients with race-adjusted eGFR ≥60 ml min^{-1} (mean 0.98 ± 0.16 mmol l^{-1}, n = 78), but not significantly (p = 0.123).

With respect to HIV disease-specific factors, nadir CD4 cell count had no significant effect on serum phosphate (r = 0.165, *p* = 0.147), although serum phosphate was significantly higher with higher current CD4 cell count (r = 0.355, *p* = 0.001), which was also observed in black and white patient subgroups separately (r = 0.409, *p* = 0.022 and r = 0.378, *p* = 0.007 respectively).

There was no significant difference in serum phosphate between patients with or without HIV viral load suppression with plasma HIV RNA <40 copies ml^{-1} (*p* = 0.707) or <200 copies ml^{-1} (*p* = 0.737). Furthermore, there was no identified significant difference in serum phosphate in patients with or without either current or ever ART, NRTI, tenofovir DF, abacavir, NNRTI, efavirenz, nevirapine or PI exposure, although serum phosphate ranges were lower in patients currently on or ever exposed to ART or an NRTI (only very few patients not on or never exposed to either) or to tenofovir DF, but not to abacavir (Table 5.10). There was also a trend to reduced serum phosphate with longer continuous or cumulative exposure to ART (r = -0.200, *p* = 0.077 and r = -0.216 and *p* = 0.056 respectively), NRTIs (r = -0.175, *p* = 0.123 and r = -0.213 and *p* = 0.060 respectively) and tenofovir DF (r = -0.213, *p* = 0.060 and r = -0.213 and *p* = 0.174 respectively), but not abacavir (r = 0.020, *p* = 0.857 and r = -0.031, *p* = 0.981 respectively) (Table 5.11 and Figure 5.14). Serum phosphate was also significantly lower with longer cumulative duration of NNRTI exposure (r = -0.233, p = 0.038).

HIV antiretroviral therapy-related factor	Exposure	n	Mean serum PO_4 ± s.d. *mmol* l^{-1}	*p*-value
Current ART	No	2	1.105 ± .106	.242
	Yes	79	0.967 ± .164	
Ever ART	No	2	1.105 ± .106	.242
	Yes	79	0.967 ± .164	
Current NRTI	No	4	1.045 ± .116	.354
	Yes	77	0.966 ± .166	
Ever NRTI	No	2	1.105 ± .106	.242
	Yes	79	0.967 ± .164	
Current TDF	No	26	0.988 ± .181	.498
	Yes	55	0.962 ± .156	
Ever TDF	No	23	0.998 ± .185	.346
	Yes	58	0.959 ± .155	
Current ABC	No	56	0.968 ± .153	.838
	Yes	25	0.976 ± .184	
Ever ABC	No	43	0.978 ± .145	.643
	Yes	38	0.961 ± .185	
Current NNRTI	No	35	0.970 ± .148	.975
	Yes	46	0.971 ± .177	
Ever NNRTI	No	14	1.034 ± .108	.109
	Yes	67	0.957 ± .171	
Current EFV	No	54	0.966 ± .171	.711
	Yes	27	0.980 ± .152	
Ever EFV	No	31	0.987 ± .143	.473
	Yes	50	0.960 ± .176	
Current NVP	No	72	0.974 ± .155	.545
	Yes	9	0.939 ± .232	
Ever NVP	No	62	0.976 ± .152	.593
	Yes	19	0.953 ± .203	
Current PI	No	45	0.969 ± .161	.928
	Yes	36	0.972 ± .169	
Ever PI	No	34	0.967 ± .173	.878
	Yes	47	0.973 ± .159	

Table 5.10. Differences in serum phosphate in Phase Two patients (n = 81) according to current or ever exposure to antiretroviral therapy (ART), individual ART classes and specific NRTIs and NNRTIs

HIV antiretroviral therapy-related factor	r	*p*-value
Continuous number of months on ART	-0.200	.077
Cumulative number of months ever on ART	-0.216	.056
Continuous number of months on NRTI	-0.175	.123
Cumulative number of months ever on NRTI	-0.213	.060
Continuous number of months on TDF	-0.212	.174
Cumulative number of months ever on TDF	-0.180	.109
Continuous number of months on ABC	0.020	.857
Cumulative number of months ever on ABC	-0.003	.981
Continuous number of months on NNRTI	-0.111	.329
Cumulative number of months ever on NNRTI	-0.233	**.038**
Continuous number of months on EFV	-0.013	.909
Cumulative number of months ever on EFV	-0.089	.430
Continuous number of months on NVP	-0.129	.254
Cumulative number of months ever on NVP	-0.079	.484
Continuous number of months on PI	-0.006	.955
Cumulative number of months ever on PI	-0.005	.967

Table 5.11. Relationship between continuous number of months on or cumulative number of months ever on antiretroviral therapy (ART), individual ART classes and specific nucleos(t)ide reverse transcriptase inhibitors (NRTIs), namely tenofovir disoproxil fumarate (TDF) and abacavir (ABC), and non-nucleoside reverse transcriptase inhibitors (NNRTIs), namely efavirenz (EFV) and nevirapine (NVP) with serum phosphate in Phase Two patients (n = 81)

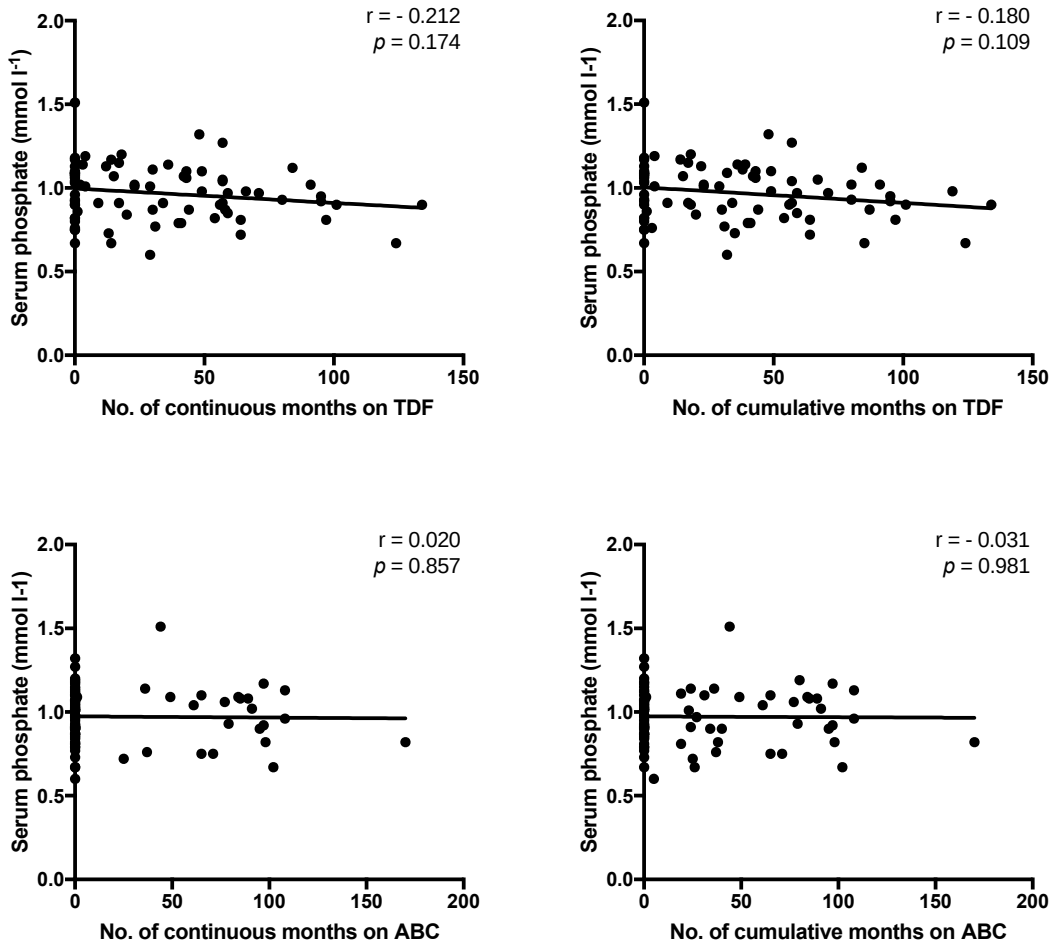


Figure 5.14. Relationship between serum phosphate and duration of continuous or cumulative exposure to tenofovir disoproxil fumarate (TDF) and abacavir (ABC) in the Phase Two study population (n = 81)

Factors and co-variates found to have either significant ($p < 0.05$) or borderline significant ($p \geq 0.05$ and < 0.10) association with serum phosphate within the Phase Two study population are summarised in Table 5.12. These factors and covariates were taken forward for multivariate analysis, as described for 25-OH-D in Section 5.2.1. ART exposure-related covariates were deselected in favour of more specific NRTI and NNRTI class exposure-related covariates. (Further detail is included in Appendix 2.2.)

Factors and covariates with significant association ($p < 0.05$) with serum phosphate following multivariate analysis were: female gender (associated with higher serum phosphate, $p = 0.020$), race-adjusted eGFR <90 ml min^{-1} (associated with lower serum phosphate, $p = 0.023$) and current CD4 cell count (increased CD4 cell count associated with higher serum phosphate, $p = 0.026$) (Table 5.13).

Factor / covariate	***p*-value**	**Factor / co-variate**	***p*-value**
White race	**.049**	No. of continuous mths on ART	.077
Female gender	**.001**	No. of cumulative mths on ART	.056
Race-adjusted eGFR <90 ml min^{-1}	**.006**	No. of cumulative mths on NRTI	.060
Current CD4 cell count	**.001**		
No. of cumulative mths on NNRTI	**.038**		

Table 5.12. Factors and co-variates with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) associations with serum phosphate from univariate analysis within the Phase Two study population

Covariate / factor	Factor present	Estimated marginal mean *mmol l^{-1}*	Standard Error	Wald Chi-Square	*p*-value
Female gender	No	0.926	0.029	5.436	**.020**
	Yes	1.010	0.030		
Race-adjusted eGFR <90 ml min^{-1}	No	1.009	0.020	5.201	**.023**
	Yes	0.928	0.304		
Current CD4 cell count	-	-	-	4.928	**.026**

Table 5.13. Covariates and factors with significant (p < 0.05) association with serum phosphate in the Phase Two study population (n = 81) following multivariate analysis

5.3.2 Effects of serum phosphate in PLWH

There was no significant relationship between serum phosphate and BMD at any site in the Phase Two study population (Figure 5.15). Furthermore, no relationship between serum phosphate and BMD at any site was observed within each of the four patient race / gender subgroups (data not shown).

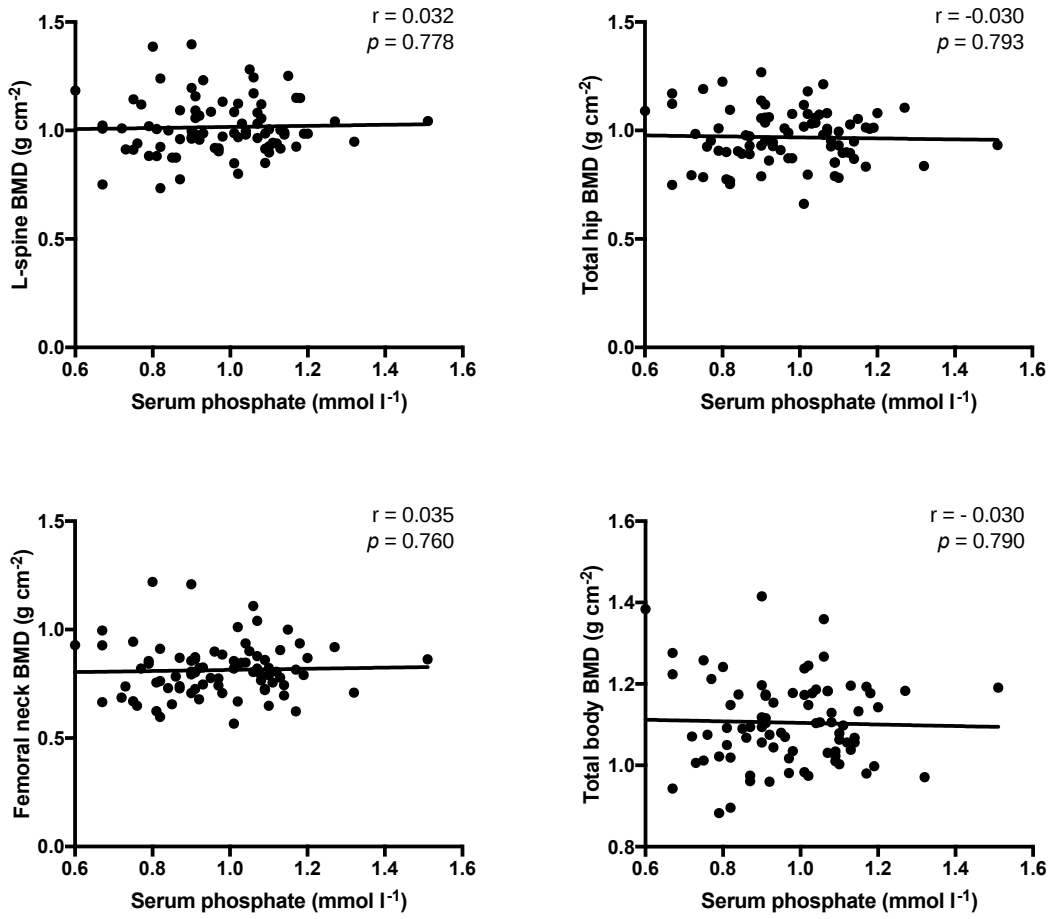


Figure 5.15. Relationship between serum phosphate and lumbar (L-) spine, hip, femoral neck and total BMD in the Phase Two study population (n = 81)

The relationships between serum phosphate and serum corrected calcium, PTH and TRP respectively are shown in Figure 5.16. There was no observed relationship between serum phosphate and serum corrected calcium. Serum parathyroid hormone levels decreased with increased serum phosphate, but

not significantly. There was a significant positive correlation between TRP and serum phosphate, however (r = 0.333, *p* = 0.003).

There was no significant difference in serum phosphate in Phase Two patients self-reporting no falls, one single fall or multiple falls within the previous 12 months (Table 5.14) or no fractures, one fracture or multiple fractures in adulthood (Table 5.15). The few included patients with a history of fragility fracture (n = 3) had lower mean serum phosphate (0.93 ± 0.23 mmol l^{-1}) compared to patients without (0.97 ± 0.16 mmol l^{-1}, n = 78) but this difference was not significant (*p* = 0.667).

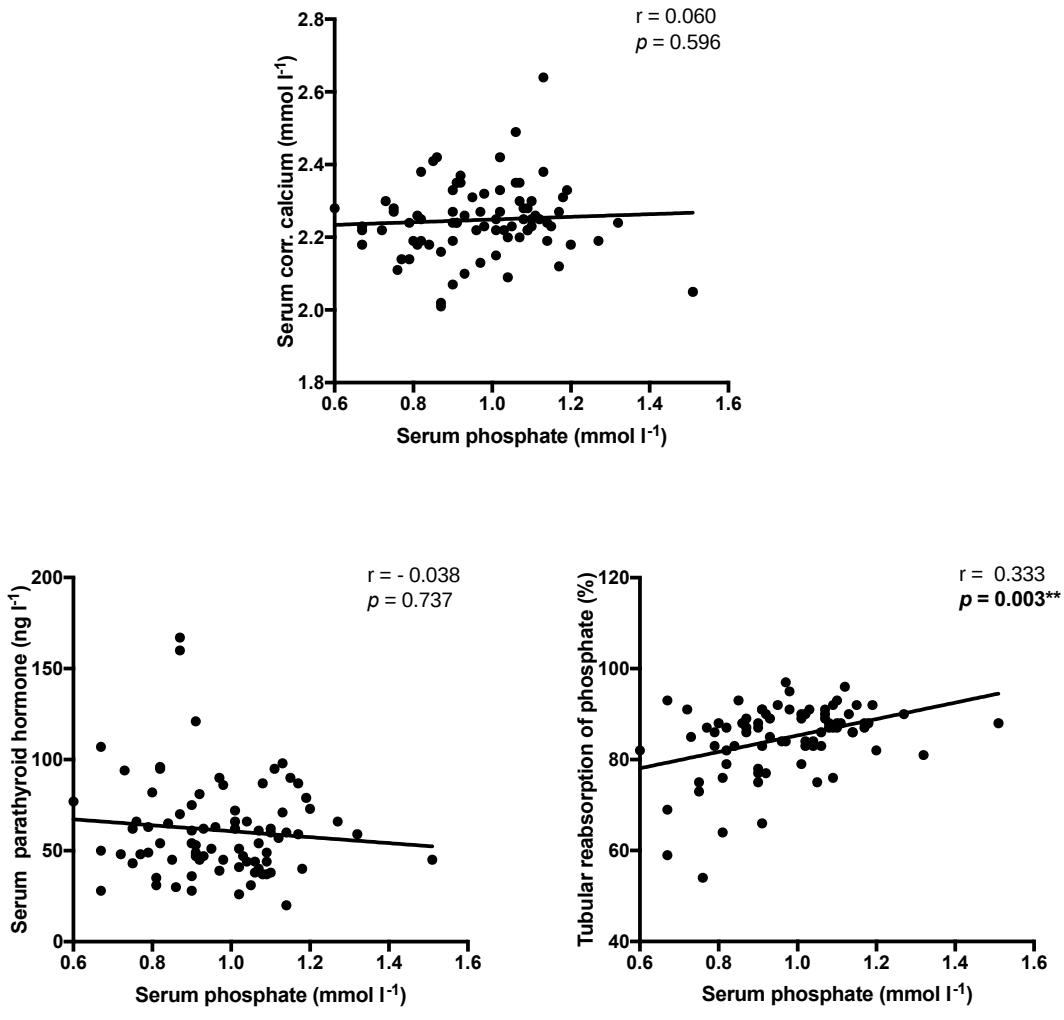


Figure 5.16. Relationship between serum phosphate and serum correct calcium (corr. calcium), serum parathyroid hormone and tubular reabsorption of phosphate (TRP) in the Phase Two study population (n = 81)

Phase Two patient group	Number of falls in last 12 months	*n*	Serum phosphate *mmol l*$^{-1}$		*p*-value
			Mean	s.d.	
All patients	None	68	0.974	.163	.878
	Single	5	0.944	.157	
	Multiple	8	.0953	.198	

Table 5.14. Differences in serum phosphate between all (n = 81) Phase Two patients self-reporting no falls, one single fall or multiple falls in last 12 months

Phase Two patient group	Number of fractures in adulthood	*n*	Serum phosphate *mmol l*$^{-1}$		*p*-value
			Mean	s.d.	
All patients	None	56	0.978	.155	.836
	Single	18	0.956	.197	
	Multiple	7	0.950	.165	

Table 5.15. Differences in serum phosphate between all (n = 81) Phase Two patients self-reporting no falls, one single fall or multiple fractures in adulthood

5.4 Distribution, determinants and effects of other biochemical markers in PLWH

5.4.1 Parathyroid hormone

Median PTH in all, black and white Phase Two patients was 57 ng l^{-1} (IQR 44.0 – 72.5), 73 ng l^{-1} (IQR 37.0 – 167.0) and 48.5 ng l^{-1} (IQR 38.8 – 61.3) respectively (normal range 15.0 – 65.0 ng l^{-1}), with PTH significantly higher in black patients than in white patients ($p < 0.001$). PTH was also significantly higher in black males (median 80.0 ng l^{-1}, IQR 60.0 – 98.0) than in white males (median 47.0 ng l^{-1} IQR 37.5 – 61.5) ($p = 0.001$), but with no significant difference between black females (median 71.0 ng l^{-1}, IQR 52.5 – 88.5) and white females (median 59.0 ng l^{-1}, IQR 49.0 – 65.5) ($p = 0.114$), between black males and black females ($p = 0.348$) or between white males and white females ($p = 0.113$).

The relationships between PTH and 25-OH-D and serum phosphate have been described in Sections 5.2.2 and 5.3.2 respectively. Predictably, there was a negative correlation between serum corrected calcium and PTH (r = -0.190, $p = 0.090$) and TRP increased significantly with increased PTH (r = 0.273, $p = 0.014$) (Figure 5.17).

There was no significant relationship identified between current or ever tenofovir DF exposure and serum PTH levels ($p = 0.567$ and $p = 0.297$ respectively). Whilst there was a negative correlation between both duration of continuous tenofovir DF exposure and duration of cumulative tenofovir DF exposure and serum PTH (r = -0.108 and r = -0.214 for continuous and cumulative tenofovir DF exposure respectively), which was in contrast to the positive correlation seen between both duration of continuous abacavir exposure and duration of cumulative abacavir exposure and PTH (r = 0.183 and r = 0.184 respectively), these correlations were not significant ($p = 0.336$ and $p = 0.055$ for continuous and cumulative tenofovir DF exposure respectively). Furthermore, after adjusting for 25-OH-D, the relationship

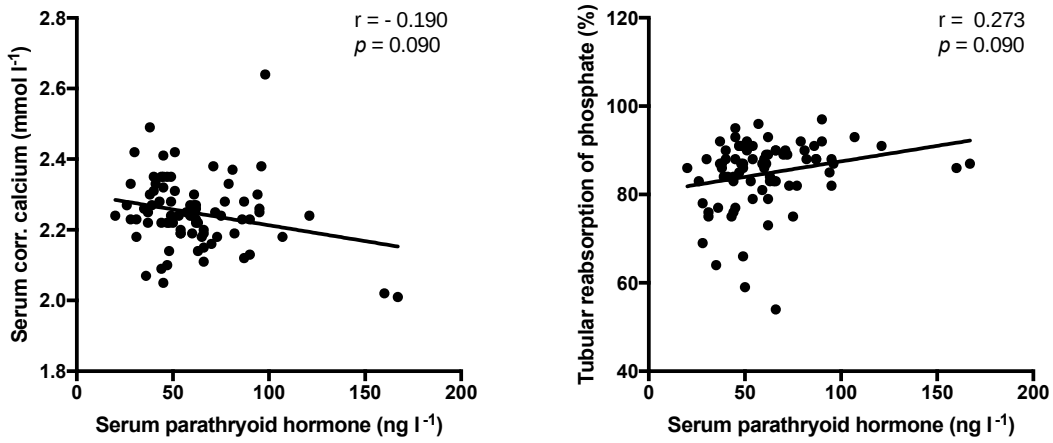


Figure 5.17. Relationship between serum parathyroid hormone and serum correct calcium (corr. calcium) and tubular reabsorption of phosphate (TRP) in the Phase Two study population (n = 81)

between the duration of cumulative tenofovir DF exposure and PTH became even less significant (p = 0.500).

There was no significant relationship observed between PTH and BMD at any site (r = 0.006, p = 0.956 for lumbar spine, r = 0.110, p = 0.330 for hip, r = 0.095, p = 0.400 for femoral neck and r = -0.068, p = 0.547 for total body).

5.4.2 Serum corrected calcium

Mean serum corrected calcium in Phase Two patients was 2.25 ± 0.10 mmol l^{-1} (normal range 2.20 – 2.60), with no significant difference observed between patient race / gender subgroups. The relationships between serum corrected calcium and 25-OH-D, serum phosphate and PTH have been described in Sections 5.2.2, 5.3.2 and 5.4.1 respectively. TRP increased with increased serum corrected calcium (r = 0.106), but not significantly (p = 0.348).

There was no significant relationship observed between serum corrected calcium and BMD at any site (r = 0.044, p = 0.699 for lumbar spine, r = 0.048,

p = 0.673 for total hip, r = -0.003, p = 0.979 for femoral neck and r = 0.080, p = 0.479 for total body).

5.4.3 Tubular reabsorption of phosphate

Median TRP in Phase Two patients was 87.0% (IQR 83 – 90, range 54 – 97) (normal range 82– 95). 21.3% patients (n = 17) had TRP <82%. TRP was significantly higher in black patients compared to white patients (p < 0.001), black females compared to white females (p = 0.004) and in black females compared to black males (p = 0.028) (Figure 5.18). TRP decreased significantly with age (r = -0.346, p = 0.002) and increased significantly with BMI (r = 0.247, p = 0.027) (Figure 5.19). TRP was significantly lower in patients with race-adjusted eGFR <90 ml min^{-1} compared to those with eGFR ≥ 90 ml min^{-1} (median 88.0%, IQR 76.0 – 97.0) (p < 0.001, Figure 5.20).

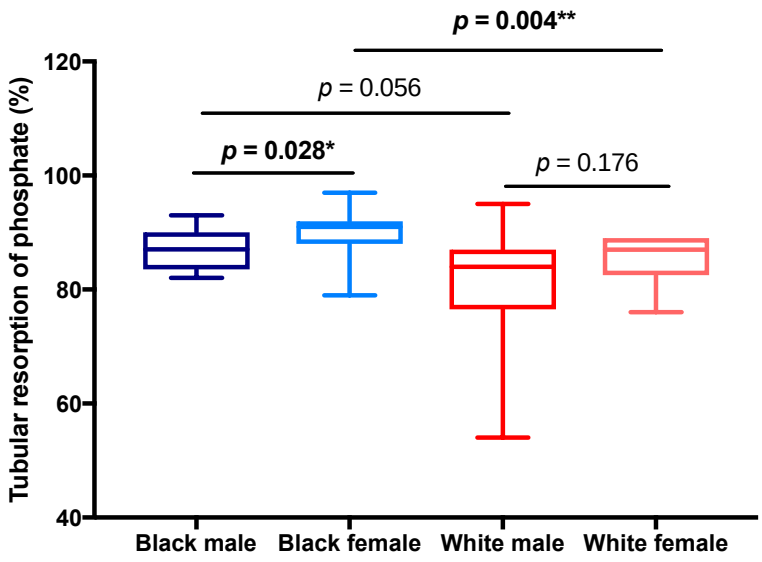


Figure 5.18. Distribution of tubular reabsorption of phosphate (TRP) in black males (n= 9), black females (n = 21), white males (n = 41) and white females (n = 9) within the Phase Two study population (n = 81)

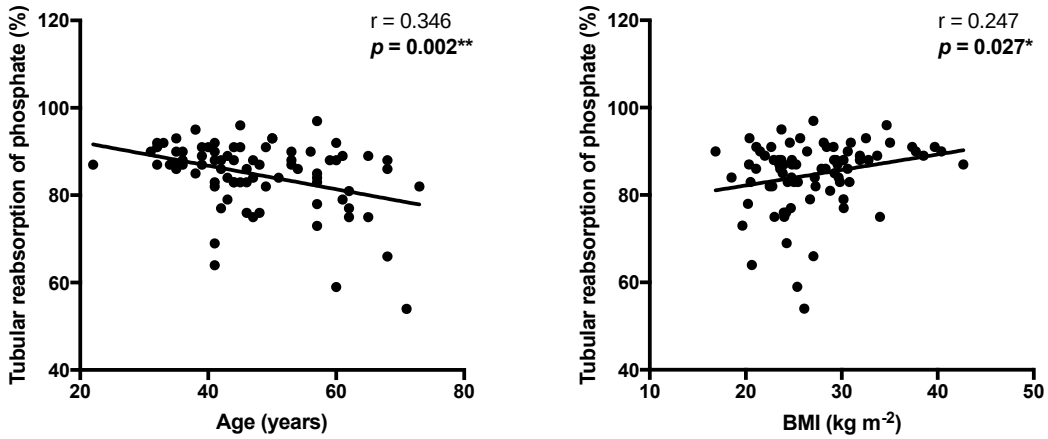


Figure 5.19. Relationship between tubular reabsorption of phosphate (TRP) with age and body mass index (BMI) in the Phase Two study population (n = 81)

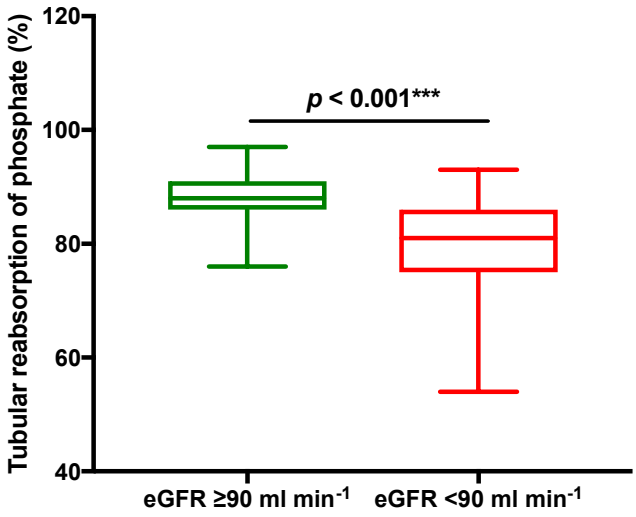


Figure 5.20. Distribution of tubular reabsorption of phosphate (TRP) in patients with race-adjusted eGFR ≥90 ml min^{-1} (n = 53) and <90 ml min^{-1} (n = 27) within the Phase Two study population (n = 81)

There was no difference in TRP between Phase Two patients currently on tenofovir DF compared to those not currently on tenofovir DF (p = 0.799), with a similar range of TRP observed in patients currently on abacavir to those currently on tenofovir DF (Table 5.16). Furthermore, there was no significant relationship between TRP and duration of continuous exposure to tenofovir DF (r = -0.024, p = 0.832) (Table 5.17).

Patients currently on efavirenz had a signifcantly higher percentage of TRP than those not currently on efavirenz (p = 0.040), but no significant difference was seen between patients currently on or not currently on nevirapine (p = 0.532) (Table 5.16). Furthermore, there was a trend to a lower percentage of TRP with longer continuous duration of efavirenz exposure (r = -0.219, p = 0.051), but again no relationship seen with nevirapine (Table 5.17).

In terms of ARV drug class exposure, whilst there was no significant relationship between duration of continuous exposure to NNRTIs combined and tubular phosphate resorption, a longer duration of continuous PI exposure was associated with a significantly higher percentage of tubular phosphate resorption (r = 0.254, p = 0.023) (Table 5.17).

The relationships between TRP and 25-OH-D, serum phosphate, PTH and corrected calcium have been described in Sections 5.2.2, 5.3.2, 5.4.1 and 5.4.2 respectively.

HIV antiretroviral therapy-related factor	Exposure	n	Tubular reabsorption of phosphate %		*p*-value
			Median	IQR	
Current TDF	No	25	87.0	82.5 – 88.0	.799
	Yes	55	87.0	82.0 – 90.0	
Current ABC	No	56	87.0	82.0 – 90.0	.584
	Yes	24	87.0	83.3 – 88.0	
Current NNRTI	No	34	86.0	77.5 – 89.3	.190
	Yes	46	87.5	82.8 – 90.0	
Current PI	No	44	86.0	79.0 – 88.0	.063
	Yes	36	88.0	83.3 – 90.8	
Current EFV	No	53	86.0	79.0 – 89.5	**.040**
	Yes	27	88.0	85.0 – 90.0	
Current NVP	No	71	87.0	83.0 – 90.0	.532
	Yes	9	83.0	80.5 – 89.0	

Table 5.16. Differences in tubular reabsorption of phosphate in Phase Two patients (n = 81) according to current exposure to tenofovir disoproxil fumarate (TDF), abacavir (ABC), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	r	*p*-value
Continuous number of months on TDF	-0.024	.832
Continuous number of months on ABC	-0.050	.660
Continuous number of months on NNRTI	0.079	.487
Continuous number of months on PI	0.254	**.023**
Continuous number of months on EFV	-0.219	.051
Continuous number of months on NVP	-0.097	.397

Table 5.17. Relationship between continuous number of months on tenofovir disoproxil fumarate (TDF), abacavir (ABC), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), efavirenz (EFV) and nevirapine (NVP) with tubular reabsorption of phosphate in Phase Two patients (n = 81)

Factors and co-variates found to have either significant ($p < 0.05$) or borderline significant ($p \geq 0.05$ and < 0.10) association with TRP within the Phase Two study population are summarised in Table 5.18 (serum phosphate ($p = 0.003$) excluded as a likely effect of TRP). These factors and covariates were taken forward for multivariate analysis, as described for 25-OH-D_3 in Section 5.2.1. (Further detail is included in Appendix 2.3.)

Factors and covariates with significant association ($p < 0.05$) with TRP following multivariate analysis were: female gender (associated with higher TRP, $p = 0.013$), race-adjusted eGFR <90 ml min^{-1} (associated with lower tubular resorption of phosphate, p <0.001) and current efavirenz (associated with higher TRP, $p = 0.031$) (Table 5.19).

Factor / covariate	***p*-value**	**Factor / co-variate**	***p*-value**
White race	**<.001**	Parathyroid hormone	.090
Female gender	**<.001**	No. of continuous months on EFV	.077
Age	**.002**	Current PI	.056
BMI	**.027**		
Race-adjusted eGFR <90 ml min^{-1}	**<.001**		
25-hydroxyvitamin D	**.001**		
Current EFV	**.040**		
No. of continuous months on PI	**.023**		

Table 5.18. Factors and co-variates with significant (p < 0.05) or borderline significant (p ≥ 0.05 and < 0.10) associations with tubular reabsorption of phosphate from univariate analysis within the Phase Two study population

Covariate / factor	Factor present	Estimated marginal mean %	Standard Error	Wald Chi-Square	*p*-value
Female gender	No	82.5	0.90	6.218	**.013**
	Yes	86.2	1.28		
Race-adjusted eGFR <90 ml min^{-1}	No	88.4	0.85	28.504	**<.001**
	Yes	80.2	1.35		
Current efavirenz	No	82.8	0.89	4.649	**.031**
	Yes	85.9	1.28		

Table 5.19. Covariates and factors with significant (p < 0.05) association with tubular reabsorption of phosphate in the Phase Two study population (n = 81) following multivariate analysis

There was no significant relationship observed between TRP and BMD at any site (r = 0.093, *p* = 0.411 for lumbar spine, r = 0.039, *p* = 0.731 for hip, r = 0.109, *p* = 0.338 for femoral neck and r = 0.013, *p* = 0.907 for total body).

5.4.4 Proximal renal tubular dysfunction

81 patients within the Phase Two study population without previous or current vitamin D supplementation were assessed for PRTD. Four biochemical markers were used to establish the presence or absence of PRTD, namely: isolated glycosuria in absence of plasma hyperglycaemia; serum bicarbonate <22 mmol l^{-1}; urinary retinal binding protein (uRBP) >15 mg l^{-1}; and TRP <82% in the absence of 25-OH-D < 50nmol l^{-1}. “Absence of PRTD” was defined as the absence of all four markers, “possible PRTD” was defined as the presence of one of the four markers and “probable PRTD” was defined as the presence of two or more of the four markers.

One patient was diagnosed with probable PRTD (isolated glycosuria, uRBP 65 mg l^{-1} and TRP 59% (25-OH D_3 82.6 nmol l^{-1}), 16 patients had possible PRTD (eight had low TRP with normal 25-OH-D, seven had serum bicarbonate <22 mmol l^{-1} and one had isolated glycosuria) and 56 patients did not have PRTD. A further eight patients had insufficient evidence to make a diagnosis of either absent or possible PRTD (no markers present, but one or more marker not tested). The one patient with probable PRTD was a 60-year old white male patient, BMI 25.4 kg m^{-2}, with current CD4 cell count 593 cells μl^{-1} and undetected plasma HIV RNA on ART (212 months continuous duration) comprising tenofovir DF (124 months continuous duration), etravirine (44 months continuous duration) and a boosted PI (16 months continuous duration). His fasting serum phosphate was also low (0.67 mmol l^{-1}). His eGFR was low (48 ml min^{-1}). He had sustained one fragility fracture (distal radius) aged 59 years old. He had no other FRAX®-incorporated general fracture risk factors, but had relatively elevated FRAX®-calculated 10-year probabilities of major osteoporotic fracture (7.7%) and hip fracture (1.2%), with BMD assessment advised.

BMD was significantly reduced at the lumbar spine in the one patient with probable PRTD compared with patients with absent or only possible PRTD (p = 0.046), but with no significant difference observed for total hip, femoral neck or total BMD (p = 0.086, p = 0.247 and p = 0.141 respectively) (Table 5.20). There was no significant difference in BMD between patients with absent PRTD compared with patients with possible PRTD at any site (p = 0.933, p = 0.220, p = 0.210 and p = 0.634 for lumbar spine, total hip, femoral neck and total body respectively).

5.4.5 Race-adjusted estimated glomerular filtration rate

The relationships between race-adjusted eGFR and different ARV exposures are summarised in Table 5.20. Phase Two patients with race-adjusted eGFR < 90 ml min^{-1} (n = 27) had longer median duration of both continuous and cumulative tenofovir DF exposure (43 months (IQR 0 – 59) and 48 months (IQR 0 – 67) respectively) than those with race-adjusted eGFR ≥ 90 ml min^{-1} (n = 54) (16 months (IQR 0 – 43) and 23 months (IQR 0 – 45) respectively), but this did not meet statistical significance (p = 0.068 and p = 0.069 for continuous and cumulative tenofovir DF exposure respectively). There was no noticeable difference in duration of continuous or cumulative exposure to abacavir, NNRTIs or PIs between patients with race-adjusted eGFR either < 90 ml min^{-1} or ≥ 90 ml min^{-1}, however.

There was no difference in BMD at any site between patients with race-adjusted eGFR < 90 ml min^{-1} or ≥ 90 ml min^{-1}, nor in patients with race-adjusted eGFR either < 60 ml min^{-1} or ≥ 60 ml min^{-1} (Tables 5.21 and 5.22).

Patient race / gender subgroup	n	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
		Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Absent PRTD	56	1.011 ± .135	.933	0.981 ± .126	.220	0.825 ± .132	.210	1.107 ± .133	.634
Possible PRTD	16	1.014 ± .100		0.936 ± .125		0.780 ± .103		1.092 ± .084	
Absent PRTD	56	1.011 ± .135	.062	0.981 ± .126	.075	0.825 ± .132	.239	1.107 ± .133	.157
Probable PRTD	1	0.751		0.749		0.666		0.943	
Possible PRTD	16	1.014 ± .100	**.022**	0.936 ± .125	.166	0.780 ± .103	.297	1.092 ± .084	.106
Probable PRTD	1	0.751		0.749		0.666		0.943	
Absent or possible PRTD	72	1.011 ± .128	**.046**	0.971 ± .126	.086	0.815 ± .127	.247	1.103 ± .107	.141
Probable PRTD	1	0.751		0.749		0.666		0.943	

Table 5.20. Distribution of lumbar spine, total hip, femoral neck and total body BMD measurements between patients without proximal renal tubular disorder (PRTD) (n = 56), patients with possible PRTD (n = 16) and patients with probable PRTD (n = 1) within the Phase Two study population

	Race-adjusted eGFR ≥90 ml min^{-1} n = 54	**Race-adjusted eGFR <90 ml min^{-1}** n = 27	***p*-value**
Median (IQR) duration of continuous TDF exposure *months*	16 (0 – 43)	43 (0 – 59)	.068
Median (IQR) duration of cumulative TDF exposure *months*	23 (0 – 45)	48 (0 – 67)	.069
Median (IQR) duration of continuous ABC exposure *months*	0 (0 – 45)	0 (0 – 65)	.816
Median (IQR) duration of cumulative ABC exposure *months*	0 (0 -52)	0 (0 – 65)	.952
Median (IQR) duration of continuous NNRTI exposure *months*	18 (0 – 53)	40 (0 – 97)	.156
Median (IQR) duration of cumulative NNRTI exposure *months*	38 (4 – 8)	47 (19 – 97)	.308
Median (IQR) duration of continuous PI exposure *months*	1 (0 – 39)	0 (0 – 25)	.560
Median (IQR) duration of cumulative PI exposure *months*	13 (0 – 48)	0 (0 – 33)	.107

Table 5.21. Differences in median duration of continuous or cumulative exposure to tenofovir disoproxil fumarate (TDF), abacavir (ABC), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) between Phase Two patients with either race-adjusted estimated glomerular filtration rate (eGFR) ≥90 ml min^{-1} (n = 54) or race-adjusted eGFR <90 ml min^{-1} (n = 27)

	Race-adjusted eGFR ≥90 ml min^{-1} n = 54	**Race-adjusted eGFR <90 ml min^{-1}** n = 27	***p*-value**
Mean lumbar spine BMD ± s.d. *g cm^{-2}*	1.020 ± .127	1.000 ± .136	.656
Mean total hip BMD ± s.d. *g cm^{-2}*	0.975 ± .119	0.957 ± .137	.542
Mean femoral neck BMD ± s.d. *g cm^{-2}*	0.828 ± .125	0.784 ± .114	.126
Mean total body BMD ± s.d. *g cm^{-2}*	1.117 ± .097	1.081 ± .118	.146

Table 5.22. Differences in lumbar spine, total hip, femoral neck and total body BMD between Phase Two patients with either race-adjusted estimated glomerular filtration rate (eGFR) ≥90 ml min^{-1} (n = 54) or race-adjusted eGFR <90 ml min^{-1} (n = 27)

	Race-adjusted eGFR ≥60 ml min^{-1} n = 78	Race-adjusted eGFR <60 ml min^{-1} n = 3	*p*-value
Mean lumbar spine BMD ± s.d. *g cm*$^{-2}$	1.017 ± .125	0.991 ± .269	.736
Mean total hip BMD ± s.d. *g cm*$^{-2}$	0.971 ± .124	0.916 ± .162	.459
Mean femoral neck BMD ± s.d. *g cm*$^{-2}$	0.816 ± .122	0.738 ± .141	.284
Mean total body BMD ± s.d. *g cm*$^{-2}$	1.107 ± .105	1.041 ± .087	.288

Table 5.23. Differences in lumbar spine, total hip, femoral neck and total body BMD between Phase Two patients with either race-adjusted estimated glomerular filtration rate (eGFR) ≥60 ml min^{-1} (n = 78) or race-adjusted eGFR <60 ml min^{-1} (n = 3)

5.5 Summary of biochemical determinants of BMD

Of the various biochemical parameters analysed, only the presence of probable PTRD was associated with a significant reduction in BMD and only at the lumbar spine. There was no significant reduction in BMD with reduced 25-OH-D, nor was there any significant relationship observed between BMD and serum phosphate, serum PTH, serum corrected calcium, TRP or race-adjusted eGFR.

5.6 Discussion

The prevalence of severe vitamin D deficiency, non-severe vitamin D deficiency and vitamin D insufficiency within the Phase One study population (35.5%, 46.7% and 9.9% respectively) was similar to the prevalence reported from another UK HIV cohort with a similar (albeit slightly higher) proportion of black *versus* white patients (reporting prevalence of 34.8%, 38.7% and 17.7% respectively) (Welz *et al.* 2010). In keeping with the findings of this and one other study (Welz *et al.* 2010, Sherwood *et al.* 2012), there were significantly

higher proportions of vitamin D deficiency and severe vitamin D deficiency in black patients (92.1% and 48.6% respectively) compared to white patients (69.7% and 19.9% respectively) within the Phase One study population. Other significant determinants of reduced 25-OH-D in the Phase One study population, namely non-summer sampling, efavirenz (but not nevirapine) exposure, were also consistent with published data from other HIV cohorts (Welz *et al.* 2010, Dao *et al.* 2011, Wohl *et al.* 2014);

As expected, there was a significant negative correlation between 25-OH-D and serum corrected calcium and a significant positive correlation with serum PTH. Unusually, however, there was also a significant negative correlation between 25-OH-D and TRP and not the expected positive correlation. The heterogeneity of the patient cohort with respect to its race and gender mix may have complicated the analysis of some of the biochemical data, masking or altering relationships between respective variables. Whilst race / gender subgroup analyses are possible, the smaller numbers within each subgroup increases the risk of analyses being skewed by outlier values, for example patients with very high 25-OH-D secondary to recent sunbed use or overseas travel, or from undisclosed supplement use.

In keeping with the majority of other published reports (Sherwood *et al.* 2012, Cotter, *et al.* 2014), there was also no identified significant association between 25-OH-D and BMD at any site. In addition, there was no significant association between 25-OH-D and falls frequency, although the numbers of patients reporting falls was low in this young cohort. Unexpectedly, 25-OH-D measurements were significantly higher in patients with increased fracture frequency overall, however fracture frequency was higher in white patients than in black patients in the Phase One study population (Section 3.5) and therefore differences in 25-OH-D most likely reflect differences in patient race between the no, single and multiple fracture patient groups. Whilst 25-OH-D was significantly lower in black patients reporting one single fracture in adulthood compared with no fractures, there was no significant difference between black patients reporting multiple fractures and no fractures. The very small numbers of black patients reporting single or multiple fractures in

adulthood increases the risk of results being skewed by outlier patients within the single or multiple fracture groups. Notably there was no significant difference in 25-OH-D measurements between patients with or without a prior fragility fracture, although the numbers of patients with prior fragility fracture included in the analysis were very low.

The majority of the Phase Two study population had normal serum phosphate, with only twelve patients (14.8%) having mild hypophosphataemia and no patients having moderate or severe hypophosphataemia. Whilst there was a trend to lower serum phosphate with increased tenofovir DF exposure, this was not significant. There was also a trend to lower serum phosphate with both increased NRTI exposure and ART exposure overall, reflecting the predominance of tenofovir DF use in Phase Two patient ART regimens over the main alternative NRTI abacavir, with which there was no observed relationship with serum phosphate. As hypophosphataemia is usually only transient, it is perhaps not surprising that no significant relationships were identified between serum phosphate and any ARV in otherwise stable and asymptomatic patients. Low race-adjusted eGFR was significantly associated with reduced serum phosphate, however, as was reduced tubular resorption of phosphate. As with 25-OH-D, serum phosphate had no significant effect on BMD or on falls frequency or fracture prevalence.

Current efavirenz use was significantly associated with increased TRP on multivariate analysis, although this is most likely to be an effect of increased TRP in the context of efavirenz-related lower 25-OH-D levels and secondary hyperparathyroidism. As with serum phosphate, TRP was also significantly lower in patients with race-adjusted eGFR <90ml min^{-1}. There was no significant effect of increased tenofovir DF exposure on TRP, however. It seems likely, therefore, that renal phosphate wasting reported in PLWH on tenofovir DF (Fux *et al.* 2007) could be idiosyncratic and not a general or dose-dependent effect of tenofovir DF. Of note, there was no observed effect of TRP on BMD.

A probable diagnosis of PRTD was made in only one patient out of 78 assessed within the Phase Two study population, in spite of two thirds of Phase Two patients being on tenofovir DF with or without a boosted PI. This is in accordance with other reports, which noted that PRTD in PLWH on tenofovir DF occurred very rarely (Nelson *et al.* 2007, Woodward *et al.* 2009). The absence of a universally accepted clinical definition of PRTD limits the ability to make too many comparisons with other studies, however. Whilst it is not possible to comment on significant associations of PRTD in PLWH from our own study, the one patient with probable PRTD did have several risk factors typically associated with PRTD in PLWH. These included increased age, prolonged exposure to tenofovir DF in combination with a PI and reduced eGFR (Calza *et al.* 2011), although reduced eGFR could have been an effect of PRTD and/or tenofovir DF also. A probable diagnosis of PRTD was the only “biochemical” factor significantly associated with reduced BMD in this cohort, although at the lumbar spine only. The one patient with probable PRTD had also sustained a recent fragility fracture, suggesting PRTD as both a risk factor for reduced BMD and fragility fractures in PLWH.

5.7 Conclusions

1. The prevalence of vitamin D deficiency within the Sheffield HIV cohort was high and akin to other HIV cohorts with similar patient demographics.
2. In concordance with other published data, black race, non-summer sampling and exposure to efavirenz were each significant independent predictors of reduced 25-OH-D; no novel determinants of reduced 25-OH-D in PLWH were identified within the Sheffield HIV Cohort, however.
3. 25-OH-D was not a significant determinant of BMD at any site; in addition, 25-OH-D was not significantly associated with falls frequency or fragility fracture prevalence.
4. Few patients had hypophosphataemia, with no moderate or severe hypophosphataemia identified.

5. Race-adjusted eGFR <90 ml min^{-1}, male gender and low current CD4 cell count were each significant independent predictors of serum phosphate; whilst there was a trend to lower serum phosphate in patients with increased tenofovir DF exposure, this was not significant.
6. Serum phosphate was not a significant determinant of BMD at any site, nor was serum phosphate significantly associated with falls frequency or fracture prevalence.
7. Increased exposure to tenofovir DF was associated with reduced race-adjusted eGFR (borderline significance only), but not reduced TRP.
8. Of other biochemical factors examined, including serum PTH, serum corrected calcium, TRP, PRTD and race-adjusted-eGFR, only probable PRTD, identified in one patient only, was significantly associated with BMD, with BMD significantly reduced at the lumbar spine, but at no other site. PRTD may also be a risk factor for fragility fracture.

6. Inflammatory and immunological determinants of bone mineral density and 25-hydroxyvitamin D_3 in people living with HIV in Sheffield

The IL-6 analysis work included in this chapter was performed by Dr Rebecca Marlor, with the assistance of Katie Cooke. Dr Marlor has kindly agreed for me to include her IL-6 results within my wider analysis.

6.1 Introduction

Immunological markers typically associated with either late HIV presentation or poor HIV disease control – namely low current CD4 cell count <200 cells μl^{-1}, low nadir CD4 cell count and low baseline CD4 cell count prior to initiation of ART – have been independently associated with either reduced BMD or higher fragility fracture incidence in PLWH (Cazanave *et al.* 2008, Yong *et al.* 2011, Grant *et al.* 2013, Borges *et al.* 2017, Gedmentas *et al.* 2017). This suggests that HIV infection, independent of any effect of ART, contributes to the higher prevalence of reduced BMD and fragility fracture incidence seen in PLWH compared with the general population, either directly or through HIV-mediated immune dysregulation and associated inflammation. Indeed, mechanisms have been identified detailing how HIV proteins, activated immune cells and pro-inflammatory cytokines can alter the balance between osteoclast and osteoblast activity, mediated through RANK, RANKL, OPG and TRAIL, increasing bone turnover and bone loss, summarised by Stone *et al.* (2010) and detailed in Chapter 1, Section 1.2.2.2.

In longitudinal studies of previously ART-naïve HIV-positive patients, higher baseline levels of the pro-inflammatory cytokine IL-6 and of hs-CRP, a marker of inflammation, at ART initiation have each been associated with increased bone loss after one and two years of ART treatment (Hileman *et al.* 2014, Brown *et al.* 2015). Whilst there is growing evidence to support persisting immune activation and a chronic pro-inflammatory state in HIV-positive patients well established on ART with immune recovery (Brenchley *et al.*

2006), evidence in support of an ongoing association between pro-inflammatory markers and reduced BMD in patients stable on ART is lacking. In contrast to IL-6, which decreases with increasing time on ART in PLWH, hs-CRP levels remain stable (Hattab *et al.* 2014) and therefore could, arguably, indicate ongoing inflammation that could contribute to longer-term bone loss beyond ART initiation. In one cross-sectional study of 142 HIV-positive patients stable on ART with a median ART duration of 56 months and with mean CD4 cell count 604 cells μl^{-1}, there was, however, no significant correlation between BMD and hs-CRP, nor IL-6, nor other pro-inflammatory markers, including D-dimer and TNF-α (Erlandson et *al.* 2014). Of note, whilst there is no existing evidence supporting an association between D-dimer and BMD in PLWH, D-dimer has been implicated in the pathogenesis of other NICMs in PLWH, notably cardiovascular disease (Ford *et al.* 2010).

A low $CD3^+/CD4^+$ cell to $CD3^+/CD8^+$ cell ratio (CD4:CD8) has also been independently associated with a higher risk of NICMs in PLWH established on ART, including cardiovascular disease, renal disease and non-AIDS-malignancies, (Serrano-Villar *et al.* 2014a, Serrano-Villar *et al.* 2014b, Castilho *et al.* 2016), but with no data published to date describing the relationship between CD4:CD8 and either BMD or fracture incidence in PLWH.

As already observed with IL-6 and hs-CRP, higher baseline expression of some T cell activation markers – specifically CD38 and HLA-DR in $CD3^+/CD4^+$ T-cell – also resulted in greater BMD loss at 96 weeks following ART initiation in previously ART-naïve HIV-positive patients, although at the lumbar spine only (Brown *et al.* 2015). There is conflicting data regarding the association of T cell activation and BMD in PLWH stable on ART. One cross-sectional study observed no association between BMD and the percentage expression of CD38 and HLA-DR in $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells (Erlandson *et al.* 2014), whereas another identified a significant and independent negative correlation between BMD and each of $CD3^+/CD4^+$ T cell expression of CD28, $CD3^+/CD4^+$ T cell expression of HLA-DR and $CD3^+/CD8^+$ T cell expression of HLA-DR (Gazzola *et al.* 2013).

As with D-dimer and CD4:CD8, a higher peripheral blood percentage of non-classical monocytes ($CD14^{dim}$ CD16++) relative to other monocyte population subsets has been associated with an increased risk of cardiovascular disease in PLWH stable on ART (Chow *et al.* 2016, Zungsontiporn *et al.* 2016). There is a paucity of published data describing the association between monocyte population subsets and BMD in PLWH, however, with just one cross-sectional study of HIV-positive patients stable on ART observing no significant relationship between monocyte population subsets and BMD (Erlandson *et al.* 2014).

The relationship between vitamin D, immune activation and inflammation in PLWH is complex, with reduced vitamin D levels likely to contribute to increased immunological hyperactivity and, in turn, chronic immune activation and inflammation likely to contribute to reduced vitamin D, a result of reduced synthesis and plasma concentrations of vitamin D binding protein – a negative acute phase protein – in response to inflammation (Hang *et al.* 1998). Elevated levels of proinflammatory cytokines and other markers of inflammation, including IL-6, TNF-α, hs-CRP and D-dimer, have been associated with severe vitamin D deficiency in PLWH, including in patients stable on ART (Ansemant *et al.* 2013, Manion *et al.* 2017). Furthermore, increased percentages of non-classical monocytes have also been associated with reduced 25-OH-D levels in PLWH (Manion *et al.* 2017).

This chapter aims to answer the following questions:

1. What are the distributions and general and HIV-disease specific associations of inflammatory markers, T cell CD4 and CD8 subsets, T cell activation markers and peripheral blood monocyte population subsets in PLWH within the Sheffield HIV Cohort?
2. What is the relationship between inflammatory markers, T cell CD4 and CD8 subsets, T cell activation markers and monocyte subsets with BMD and 25-OH-D in PLWH within the Sheffield HIV Cohort?

6.2 Distribution and associations of inflammatory markers and their relationships with BMD and 25-hydroxyvitamin D in PLWH

6.2.1 Distribution and general, immunological and HIV disease-specific associations of inflammatory markers in PLWH

The distributions of D-dimer (normal range: 0 – 500 μg l^{-1}), hs-CRP (normal range: 0 – 3 mg l^{-1}) and IL-6, measured within the Phase Two study population, are summarised in all patients and within each patient race / gender subgroups in Table 6.1. Differences in the distributions of D-dimer, hs-CRP and IL-6 between patient race / gender subgroups are highlighted in Figures 6.1 to 6.3.

D-dimer was significantly higher in females than in males overall ($p = 0.006$), but this difference was only significant between black males and black females on patient race / gender subgroup analysis ($p = 0.018$) and not significant between white males and white females ($p = 0.117$) (Figure 6.2). Hs-CRP was also significantly higher in females than in males ($p < 0.001$) and in black patients than in white patients ($p < 0.001$), with hs-CRP also significantly higher in black males compared to white males ($p = 0.044$) and in black females compared to white females ($p = 0.008$), but with no statistically significant difference between black males and black females or between white males and white females (Figure 6.3). IL-6 was also higher in females compared to males and in black patients compared to white patients, but these differences were not of statistical significance (Figure 6.4).

D-dimer, hs-CRP and IL-6 each had a significant positive correlation with each other ($r = 0.231$ $p = 0.032$ for D-dimer and hs-CRP, $r = 0.412$ $p = 0.010$ for D-dimer and IL-6 and $r = 0.649$, $p < 0.001$ for hs-CRP and IL-6).

The relationships between D-dimer, hs-CRP and IL-6 with general factors, immunological markers and HIV disease-specific factors are summarised in Tables 6.2 to 6.5.

		All	Black male	Black female	White male	White female
D-dimer *μg l^{-1}*	*n*	88	13	32	35	9
	Median	250	208	295	222	327
	IQR	78 – 8772	140 – 258	196 – 595	158 – 311	197 – 1433
hs-CRP *mg l^{-1}*	*n*	89	12	32	36	9
	Median	1.98	1.98	4.72	1.18	1.42
	IQR	0.98 – 4.69	1.09 – 7.81	2.54 – 8.22	0.82 – 2.32	0.51 – 3.36
IL-6 *pg ml^{-1}*	*n*	41	4	11	23	3
	Median	0.716	0.610	0.911	0.596	3.051
	IQR	0.506 – 1.503	0.288 – 0.880	0.667 – 1.473	0.452 – 1.522	-

Table 6.1. Distribution of D-dimer, highly sensitive CRP (hs-CRP) and interleukin-6 (IL-6) in all patients, black male, black female, white male and white female patients within the Phase Two study population

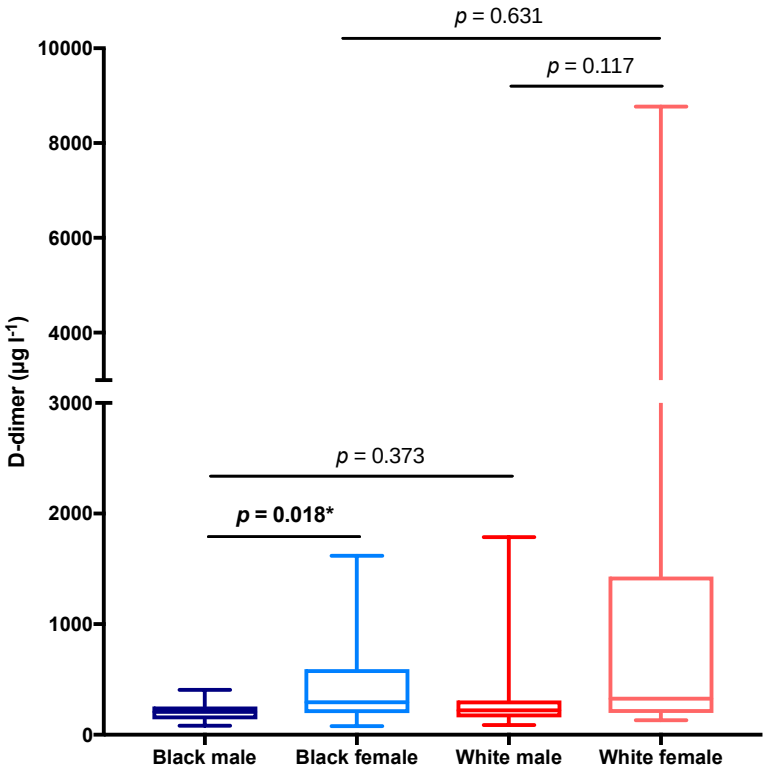


Figure 6.1. Distribution of D-dimer in black males (n = 13), black females (n = 32), white males (n = 35) and white females (n = 9) within the Phase Two study population

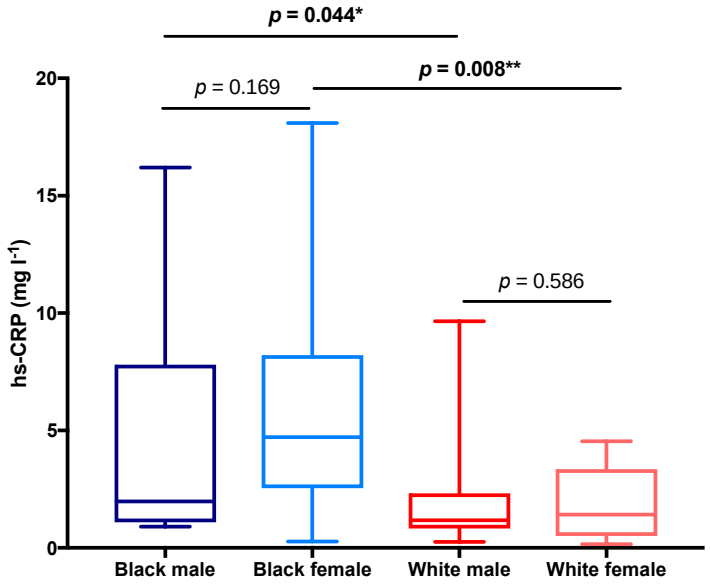


Figure 6.2. Distribution of highly sensitive CRP (hs-CRP) in black males (n = 12), black females (n = 32), white males (n = 56) and white females (n = 9) within the Phase Two study population

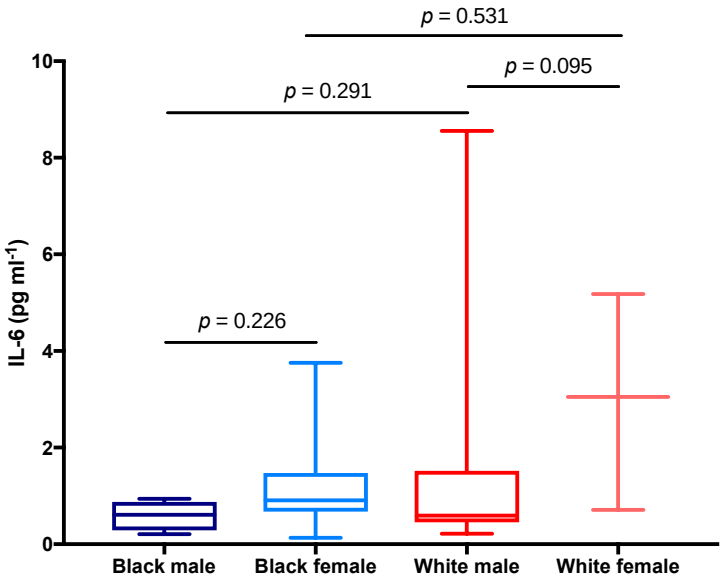


Figure 6.3. Distribution of interleukin-6 (IL-6) in black males (n = 4), black females (n = 11), white males (n = 23) and white females (n = 3) within the Phase Two study population

There was a significant positive correlation between both D-dimer and hs-CRP with both BMI and percentage subtotal body fat (p = 0.002 and p = 0.018 for D-dimer with BMI and percentage subtotal body fat respectively; p <0.001 for hs-CRP with BMI and percentage subtotal body fat respectively) (Figure 6.4).

Of note, the differences observed in D-dimer between black males and black females and in hs-CRP between black and white females were no longer significant after adjusting for BMI (p = 0.201 and p = 0.135 respectively); the difference in hs-CRP between black and white males, however, remained significant after adjusting for BMI (p = 0.007).

Neither D-dimer, hs-CRP nor IL-6 had any significant relationship with current $CD3^+/CD4^+$ cell count (CD4 cell count), current $CD3^+/CD4^+$ cell percentage of all $CD3^+$ cells (CD4 %), current $CD3^+/CD8^+$ cell count (CD8 cell count), current $CD3^+/CD8^+$ cell percentage of all $CD3^+$ cells (CD8 %), nor current CD4:CD8 (Table 6.2). There was, however, a significant negative correlation between hs-CRP and nadir CD4 cell count (r = -0.234, p = 0.027) (Figure 6.5).

	D-dimer (n = 88)		**hs-CRP** (n = 89)		**IL-6** (n = 41)	
	r	*p*-value	r	*p*-value	r	*p*-value
Age *years*	0.185	.084	0.120	.262	0.279	.077
Weight *kg*	0.193	.071	0.205	.054	0.106	.508
Body mass index *kg m^{-2}*	0.324	**.002**	0.446	**<.001**	0.257	.105
Sub-total body fat *%*	0.252	**.018**	0.541	**<.001**	0.307	.051
Nadir CD4 cell count *cells µl^{-1}*	0.010	.929	-0.234	**.027**	0.075	.639
Current CD3$^+$/CD4$^+$ cell count *cells µl^{-1}*	-0.054	.620	-0.083	.441	-0.100	.533
Current CD3$^+$/CD4$^+$ cell %	-0.041	.707	-0.108	.312	-0.109	.497
Current CD3$^+$/CD8$^+$ cell count *cells µl^{-1}*	-0.002	.983	0.131	.222	0.152	.344
Current CD3$^+$/CD8$^+$ cell %	0.041	.708	0.162	.129	0.175	.274
Current CD4:CD8 ratio	-0.071	.511	-0.143	.180	-0.142	.376
Number of years since HIV diagnosis	0.028	.795	0.106	.323	0.201	.207
Number of months continuous ART exposure	-0.040	.711	0.151	.160	0.118	.462
Number of months continuous TDF exposure	-0.128	.236	0.109	.308	0.116	.472
Number of months continuous ABC exposure	0.083	.444	-0.033	.757	0.014	.930
Number of months continuous NNRTI exposure	-0.092	.397	0.105	.328	0.157	.328
Number of months continuous PI exposure	0.113	.299	0.144	.181	0.134	.404

Table 6.2. Relationships between general, immunological and HIV disease-specific factors and D-dimer, highly sensitive CRP (hs-CRP) and interleukin-6 (IL-6) in the Phase Two study population (ART = antiretroviral therapy; TDF = tenofovir disoproxil fumarate; ABC = abacavir, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor)

Factor		n	D-dimer *μg l*$^{-1}$		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	8	349	180 – 1024	.289
	Yes	80	248	180 – 387	
Plasma HIV RNA <200 copies ml^{-1}	No	6	405	184 – 405	.197
	Yes	82	248	179 – 370	
Current ART	No	2	310	153 – 310	1.000
	Yes	86	250	180 – 398	
Current TDF	No	24	268	181 – 655	.440
	Yes	64	250	177 – 352	
Current ABC	No	65	250	176 – 349	.260
	Yes	23	317	184 – 674	
Current NNRTI	No	40	258	177 – 459	.666
	Yes	48	243	180 – 338	
Current PI	No	39	222	169 – 327	.191
	Yes	49	263	189 – 404	

Table 6.3. Differences in D-dimer according to the presence or absence of HIV disease-specific factors in the Phase Two study population (ART = antiretroviral therapy; TDF = tenofovir disoproxil fumarate; ABC = abacavir, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor)

Factor		n	hs-CRP *mg l^{-1}*		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	9	5.48	1.34 – 9.86	.079
	Yes	80	1.83	0.97 – 4.28	
Plasma HIV RNA <200 copies ml^{-1}	No	7	5.48	1.33 – 9.80	.256
	Yes	82	1.92	0.97 – 4.35	
Current ART	No	3	1.33	-	.219
	Yes	86	2.51	0.99 – 4.71	
Current TDF	No	25	1.35	0.87 – 5.82	.616
	Yes	64	2.55	1.04 – 4.65	
Current ABC	No	66	0.99	2.53 – 4.67	.623
	Yes	23	1.41	0.86 – 5.81	
Current NNRTI	No	40	1.57	0.89 – 4.60	.473
	Yes	49	2.53	1.00 – 4.71	
Current PI	No	40	1.49	0.99 – 4.58	.465
	Yes	49	2.57	0.93 – 4.75	

Table 6.4. Differences in highly sensitive CRP (hs-CRP) according to the presence or absence of HIV disease-specific factors in the Phase Two study population (ART = antiretroviral therapy; TDF = tenofovir disoproxil fumarate; ABC = abacavir, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor)

Factor		n	IL-6 *pg ml^{-1}*		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	6	0.938	0.688 – 2.687	.284
	Yes	35	0.701	0.493 – 1.484	
Plasma HIV RNA <200 copies ml^{-1}	No	5	0.936	0.556 – 3.043	.360
	Yes	36	0.707	0.500 – 1.481	
Current ART	No	2	0.614	-	.549
	Yes	39	0.716	0.519 – 1.522	
Current TDF	No	27	0.614	0.493 – 1.522	.602
	Yes	14	0.716	0.510 – 1.420	
Current ABC	No	8	0.716	0.493 – 1.522	.924
	Yes	23	0.766	0.510 – 1.420	
Current NNRTI	No	27	0.707	0.458 – 1.230	.318
	Yes	14	0.717	0.559 – 2.330	
Current PI	No	18	0.701	0.460 – 1.473	.478
	Yes	23	0.751	0.577 – 2.004	

Table 6.5. Differences in interleukin-6 (IL-6) according to the presence or absence of HIV disease-specific factors in the Phase Two study population (ART = antiretroviral therapy; TDF = tenofovir disoproxil fumarate; ABC = abacavir, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor)

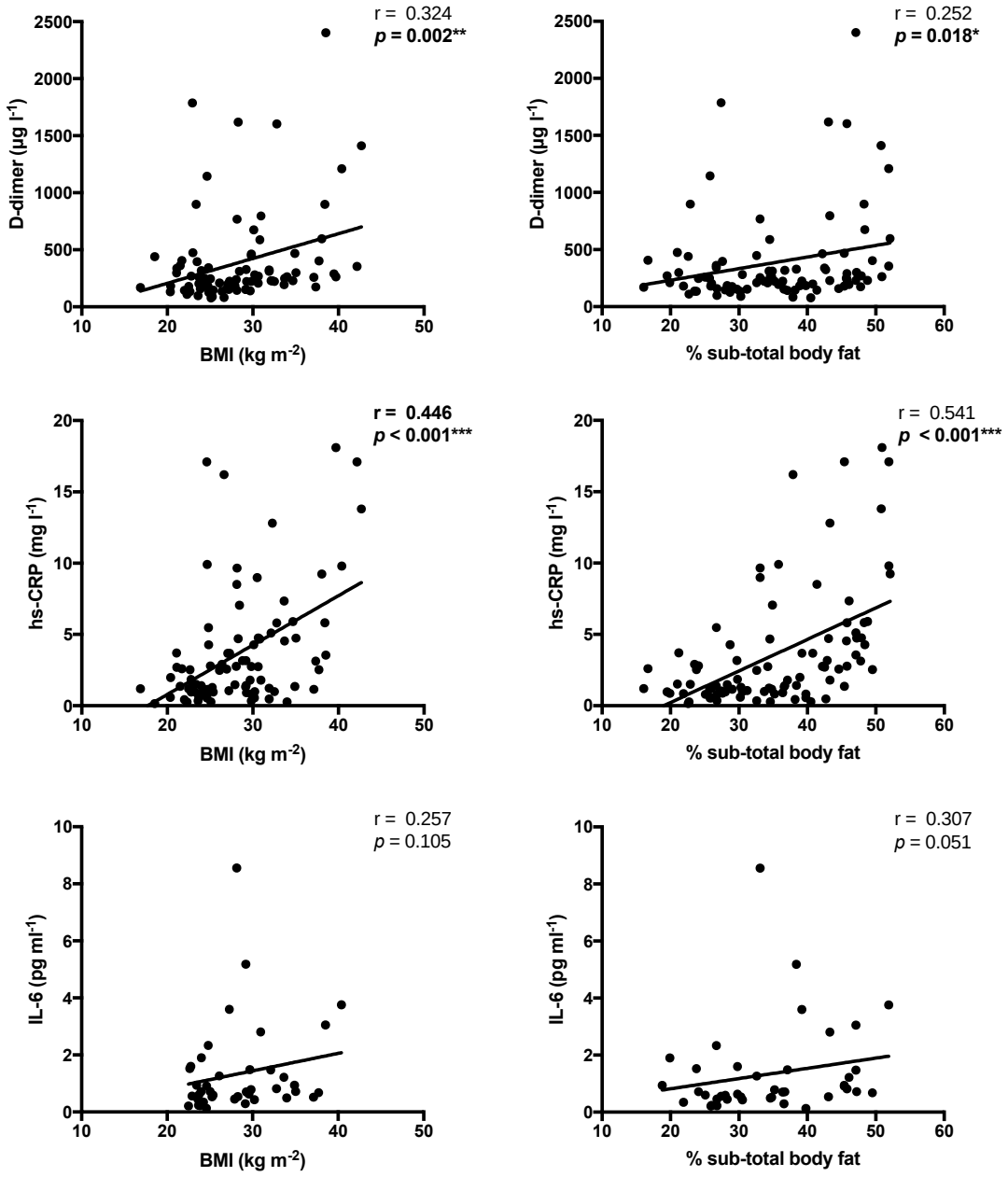


Figure 6.4. Relationships between D-dimer, highly sensitive CRP (hs-CRP) and interleukin-6 (IL-6) and body mass index (BMI) and percentage subtotal fat measurements in the Phase Two study population

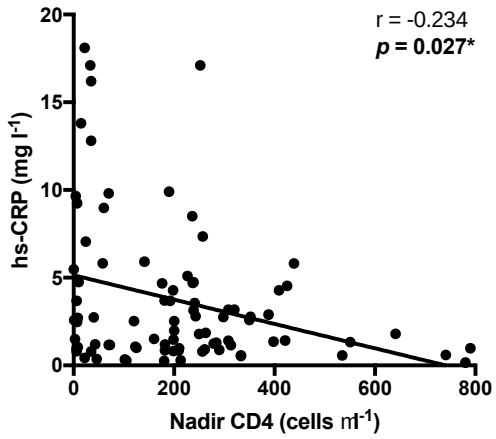


Figure 6.5. Relationship between nadir CD4 cell count and highly sensitive CRP (hs-CRP) within the Phase Two study population (n = 87)

Median D-dimer, hs-CRP and IL-6 were all higher in Phase Two patients with plasma HIV RNA greater than 40 copies ml^{-1} and 200 copies ml^{-1} compared with patients with plasma HIV RNA les than 40 copies ml^{-1} and 200 copies ml^{-1} respectively, but with only few patients with unsuppressed plasma HIV RNA within the Phase Two study population, these differences were not significant (Tables 6.3 to 6.5). The vast majority of Phase Two patients were currently on ART, including most of the patients with detectable plasma HIV RNA, with no significant difference in D-dimer, hs-CRP or IL-6 observed in patients either currently on or not currently on ART. Furthermore, there was no significant association with any inflammatory marker and the duration of continuous or cumulative ART exposure, nor were there any ARV class or drug-specific associations observed (Tables 6.2 to 6.5).

6.2.2 Relationships between inflammatory markers and BMD and 25-hydroxyvitamin D in PLWH

No significant association was observed between D-dimer, hs-CRP or IL-6 with BMD at any site or with 25-OH-D (Table 6.6) (the relationships between hs-CRP and lumbar, total hip, femoral neck and total body BMD are shown in Figure 6.6).

	D-dimer (n = 88)		**hs-CRP** (n = 89)		**IL-6** (n = 41)	
	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	0.125	.246	0.015	.888	-0.167	.298
Total hip BMD *g cm^{-2}*	0.041	.702	0.076	.478	0.011	.947
Femoral neck BMD *g cm^{-2}*	0.139	.197	0.046	.666	-0.121	.451
Total body BMD *g cm^{-2}*	1.196	.067	0.002	.982	-0.134	.403
25-hydroxyvitamin D *nmol l^{-1}*	0.137[a]	.297	0.003[b]	.980	0.080[c]	.653

[a] 28 missing values [b] 27 missing values [c] 7 missing values

Table 6.6. Relationships between lumbar spine, total hip, femoral neck and total body bone mineral density (BMD) and 25-hydroxyvitamin D and D-dimer, highly sensitive CRP (hs-CRP) and interleukin-6 (IL-6) in the Phase Two study population

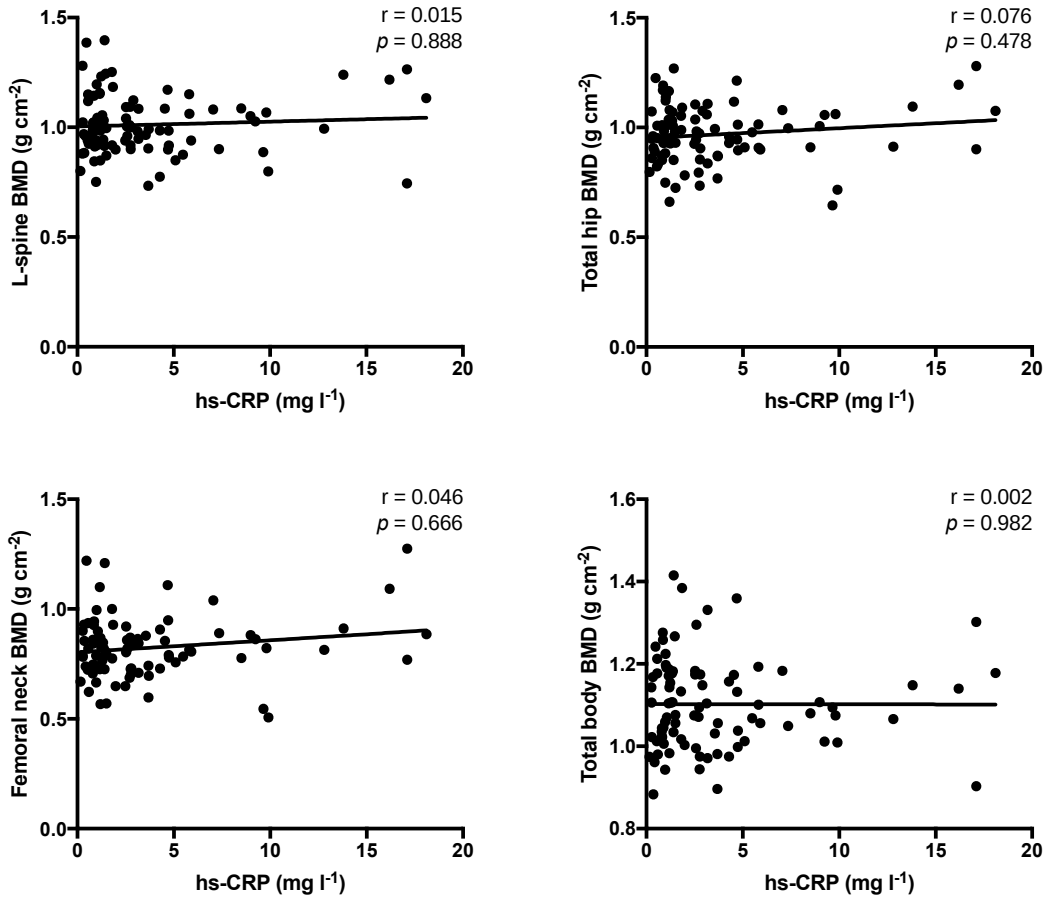


Figure 6.6. Relationship between highly sensitive CRP (hs-CRP) and lumbar (L-) spine, total hip, femoral neck and total body BMD within the Phase Two study population (n = 89)

6.3 Distribution and associations of T cell CD4 and CD8 subsets and their relationships with BMD and 25-hydroxyvitamin D in PLWH

6.3.1 Distribution and general and HIV disease-specific associations of T cell CD4 and CD8 subsets in PLWH

The distributions of current CD4 cell count, current CD4 %, current CD8 cell count, current CD8 % and CD4:CD8 for all patients, black males, black females, white males and white females within the Phase Two study population are shown in Table 6.7. There was no significant difference in the distribution of current CD4 cell count, CD4 %, CD8 cell count, CD8% or

CD4:CD8 between black and white patients (p = 0.760, p = 0.574, p = 0.488, p = 0.716 and p = 0.607 respectively). Within patient race / gender subgroups, the only significant differences identified were between black females and white females, with significantly higher CD8 % (p = 0.023) and lower CD4:CD8 (p = 0.033) in black females compared with white females, and between white males and white females, with significantly lower CD4 cell count (p = 0.021), higher CD8 % (p = 0.017) and lower CD4:CD8 (p = 0.012) in white males compared with white females. (The distribution of nadir CD4 cell count has already been described within the Phase One and Phase Two study populations, including within patient race / gender subgroups, in Chapters 3 and 4 respectively.)

Other general and HIV disease-specific associations of current CD4 cell count, CD4 %, CD8 cell count, CD8% and CD4:CD8 within the Phase Two study population are summarised in Tables 6.8 to 6.13. CD4 cell count, CD4 % and CD4:CD8 each had a significant positive correlation with nadir CD4 cell count. In addition, CD4 cell count, CD4 % and CD4:CD8 all increased with longer duration of continuous or cumulative ART and were higher in patients with suppressed plasma HIV RNA and in patients with either current or ever ART exposure than in patients with unsuppressed plasma HIV RNA or without ART exposure (significant correlations detailed in Tables 6.8 to 6.13). In contrast, CD8 % decreased with longer duration of ART and was lower in patients with suppressed plasma HIV RNA or with ART exposure than in patients with unsuppressed plasma HIV RNA or without ART exposure (significant correlations detailed in Tables 6.8 to 6.13). CD4 cell count, CD4 % and CD4:CD8 all decreased with age, but not significantly (Table 6.8). There was no significant association between BMI and T cell CD4 and CD8 subsets (Table 6.8).

		All (n = 114)	Black males (n = 15)	Black females (n = 37)	White males (n = 52)	White females (n = 10)
$CD3^+/CD4^+$ cell count *cells μl^{-1}*	*Mean ± s.d.*	622 ± 236	525 ± 161	651 ± 236	597 ± 237	792 ± 247
$CD3^+/CD4^+$ %	*Mean ± s.d.*	31 ± 10	31 ± 7	32 ± 9	30 ± 11	36 ± 7
$CD3^+/CD8^+$ cell count *cells μl^{-1}*	*Median (IQR)*	796 (555 – 1031)	583 (449 – 933)	802 (615 – 995)	815 (549 – 1180)	682 (473 – 973)
$CD3^+/CD8^+$ %	*Median (IQR)*	40 (33 – 50)	41 (31 – 50)	39 (34 – 49)	42 (36 – 53)	31 (27 – 40)
CD4:CD8	*Median (IQR)*	0.806 (0.514 – 1.090)	0.866 (0.582 – 1.044)	0.759 (0.555 – 1.164)	0.722 (0.437 – 1.036)	1.149 (0.851 – 1.499)

Table 6.7. Distribution of current $CD3^+/CD4^+$ cell count, current $CD3^+/CD4^+$ cell percentage, current $CD3^+/CD8^+$ cell count, current $CD3^+$/ $CD8^+$ percentage and current $CD3^+/CD4^+$ cell to $CD3^+/CD8^+$ cell count ratio (CD4:CD8) in all patients (n = 114), black males (n = 15), black females (n = 37), white males (n = 52) and white females (n = 10) within the Phase Two study population

	Current $CD3^+/CD4^+$ cell count		Current $CD3^+/CD4^+$ percentage		Current $CD3^+/CD8^+$ cell count		Current $CD3^+/CD8^+$ percentage		CD4:CD8	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age *years*	-0.179	.057	-0.018	.851	-0.172	.067	-0.010	.914	-0.001	.994
Body mass index *kg* m^{-2}	0.021	.828	0.019	.839	-0.078	.409	-0.144	.126	0.096	.312
Years since HIV diagnosis	0.153	.103	0.131	.166	0.007	.939	-0.062	.515	0.135	.153
Nadir CD4 cell count *cells* μl^{-1}	0.410	**<.001**	0.410	**<.001**	0.060	.523	-0.058	.543	0.282	**.002**
Duration of continuous ART *mo.*	0.175	.065	0.225	**.017**	-0.137	.149	-0.224	**.018**	0.270	**.004**
Duration of cumulative ART *mo.*	0.153	.107	0.170	.073	-0.080	.399	-0.162	.089	0.210	**.026**

Table 6.8. Relationships between general and HIV disease-specific factors and current $CD3^+/CD4^+$ cell count, current $CD3^+/CD4^+$ cell percentage, current $CD3^+/CD8^+$ cell count, current $CD3^+/CD8^+$ percentage and current $CD3^+/CD4^+$ cell to $CD3^+/CD8^+$ cell count ratio (CD4:CD8) in the Phase Two study population (n = 114) (ART = antiretroviral therapy, mo. = months)

		n	Current $CD3^+/CD4^+$ cell count *cells* μl^{-1}		*p*-value
			Mean	s.d.	
Plasma HIV RNA <40 copies ml^{-1}	No	13	503	229	.053
	Yes	101	637	233	
Plasma HIV RNA <200 copies ml^{-1}	No	7	576	267	.582
	Yes	106	627	235	
Current ART	No	4	549	217	.533
	Yes	110	623	237	
Ever ART	No	3	600	236	.869
	Yes	111	623	237	

Table 6.9. Differences in current $CD3^+/CD4^+$ cell count according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART) in the Phase Two study population

		n	Current $CD3^+/CD4^+$ cell percentage		*p*-value
			Mean	s.d.	
Plasma HIV RNA <40 copies ml^{-1}	No	13	26	10	**.041**
	Yes	101	32	10	
Plasma HIV RNA <200 copies ml^{-1}	No	7	27	10	.200
	Yes	106	32	10	
Current ART	No	4	26	6	.304
	Yes	110	32	10	
Ever ART	No	3	28	5	.604
	Yes	111	31	10	

Table 6.10. Differences in current $CD3^+/CD4^+$ cell percentage according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART) in the Phase Two study population

		n	Current $CD3^+/CD8^+$ cell count *cells* μl^{-1}		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	13	938	683 – 1288	.050
	Yes	101	785	532 – 1013	
Plasma HIV RNA <200 copies ml^{-1}	No	7	987	898 – 1306	**.030**
	Yes	106	789	539 – 1025	
Current ART	No	4	1129	708 – 1481	.114
	Yes	110	791	548 - 1025	
Ever ART	No	3	1270	-	.183
	Yes	111	796	551 – 1023	

Table 6.11. Differences in current $CD3^+/CD8^+$ cell count according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART) in the Phase Two study population

		n	Current $CD3^+/CD8^+$ cell percentage		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	13	53	45 – 57	**.002**
	Yes	101	39	33 – 46	
Plasma HIV RNA <200 copies ml^{-1}	No	7	53	50 – 54	**.015**
	Yes	106	39	33 – 47	
Current ART	No	4	52	50 – 53	**.032**
	Yes	110	39	33 – 48	
Ever ART	No	3	53	-	**.049**
	Yes	111	39	33 – 48	

Table 6.12. Differences in current $CD3^+/CD8^+$ percentage according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART) in the Phase Two study population

		n	CD4:CD8		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	13	0.447	0.308 – 0.699	**.007**
	Yes	101	0.866	0.571 – 1.116	
Plasma HIV RNA <200 copies ml^{-1}	No	7	0.447	0.403 – 0.552	**.044**
	Yes	106	0.835	0.555 – 1.104	
Current ART	No	4	0.493	0.411 – 0.617	.069
	Yes	110	0.827	0.519 – 1.104	
Ever ART	No	3	0.552	-	.183
	Yes	111	0.821	0.515 – 1.092	

Table 6.13. Differences in current CD3$^+$/CD8$^+$ cell count ratio (CD4:CD8) according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART) in the Phase Two study population

6.3.2 Relationships between T cell CD4 and CD8 subsets and BMD and 25-hydroxyvitamin D in PLWH

No significant association was observed between current CD4 cell count, current CD4 %, current CD8 count, current CD8 % or current CD4:CD8 with BMD at any site (n = 114) or with 25-OH-D (n = 81) (Table 6.14). (The relationship between nadir CD4 cell count with both BMD and 25-OH-D_3 has already been described in Chapters 4 and 5 respectively.)

	Current $CD3^+/CD4^+$ cell count		Current $CD3^+/CD4^+$ percentage		Current $CD3^+/CD8^+$ cell count		Current $CD3^+/CD8^+$ percentage		CD4:CD8	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	0.120	.202	0.080	.399	-0.090	.339	-0.180	.055	0.125	.186
Total hip BMD *g cm^{-2}*	0.061	.521	0.034	.722	-0.035	.709	-0.199	.206	0.055	.558
Femoral neck BMD *g cm^{-2}*	0.059	.534	0.029	.758	-0.030	.749	-0.161	.086	0.072	.444
Total body BMD *g cm^{-2}*	0.071	.452	-0.043	.652	-0.006	.953	-0.058	.542	-0.024	.801
25-hydroxyvitamin D *nmol l^{-1}*	0.028[a]	.802	-0.060[a]	.595	0.083[a]	.462	0.037[a]	.742	-0.050[a]	.656

[a] 33 missing values

Table 6.14. Relationships between lumbar spine, total hip, femoral neck and total body bone mineral density (BMD) and 25-hydroxyvitamin D and current $CD3^+/CD4^+$ cell count, current $CD3^+/CD4^+$ cell percentage, current $CD3^+/CD8^+$ cell count, current $CD3^+/CD8^+$ percentage and current $CD3^+/CD4^+$ cell to $CD3^+/CD8^+$ cell count ratio (CD4:CD8) in the Phase Two study population (n = 114)

6.4 Distribution and associations of T cell activation markers and their relationships with BMD and 25-hydroxyvitamin D in PLWH

6.4.1 Distribution and general and HIV disease-specific associations of T cell activation markers in PLWH

The distributions of percentage $CD25^+$ $CD3^+/CD4^+$ T cells (% $CD25^+$ $CD3^+/CD4^+$), percentage HLA-DR^+ $CD3^+/CD4^+$ T cells (% HLA-DR^+ $CD3^+/CD4^+$), percentage $CD25^+$ $CD3^+/CD8^+$ T cells (% $CD25^+$ $CD3^+/CD8^+$) and percentage HLA-DR^+ $CD3^+/CD8^+$ T cells (% HLA-DR^+ $CD3^+/CD8^+$) are shown for all Phase Two patients and by race / gender patient subgroups in Table 6.15. Examples of populations with both low % $CD25^+$ and low % HLA-DR^+, relatively high % $CD25^+$ but low % HLA-DR^+ and low % $CD25^+$ but relatively high % HLA-DR^+ are demonstrated for $CD3^+/CD4^+$ lymphocyte populations in Figures 6.7a, 6.7b and 6.7c respectively.

CD25 data was available in 101 of the 114 patients within the Phase Two study population. HLA-DR data was only available in 26 patients, however, and in more black patients (n = 20) than white patients (n = 6). There was no significant difference in the % $CD25^+$ in either $CD3^+/CD4^+$ or $CD3^+/CD8^+$ T cells between any patient race / gender subgroup, nor in the % HLA-DR^+ in either $CD3^+/CD4^+$ or $CD3^+/CD8^+$ T cells between black males and black females (numbers of white patients with HLA-DR data were too small to allow meaningful comparisons).

The relationships between the percentage positive cells with each T cell activation marker and age, BMI, percentage of subtotal body fat, T cell CD4 and CD8 subsets and HIV disease-specific factors are summarised in Table 6.16. In addition, differences in % $CD25^+$ $CD3^+/CD4^+$, % HLA-DR^+ $CD3^+/CD4^+$, % $CD25^+$ $CD3^+/CD8^+$ and % HLA-DR^+ $CD3^+/CD8^+$ according to patient plasma HIV RNA and ART exposure are summarised in Tables 6.17 to 6.20.

		All (n = 101)	Black males (n = 14)	Black females (n = 30)	White males (n = 47)	White females (n = 10)
% $CD25^+$ $CD3^+/CD4^+$	*Median (IQR)*	10.3 (6.9 – 14.6)	12.3 (4.8 – 14.2)	9.4 (7.4 – 12.5)	11.0 (6.4 – 21.2)	9.7 (7.3 – 15.8)
% HLA-DR^+ $CD3^+/CD4^+$	*Median (IQR)*	1.8[a] (1.2 – 2.6)	2.08[b] (0.9 – 2.7)	1.6[c] (1.1 – 2.0)	1.9[d] (–)	2.5[e] (0.8 – 4.4)
% $CD25^+$ $CD3^+/CD8^+$	*Median (IQR)*	0.9 (0.4 – 2.1)	0.5 (0.3 – 1.0)	0.6 (0.3 – 1.1)	1.3 (0.6 – 3.0)	0.9 (0.4 – 2.0)
% HLA-DR^+ $CD3^+/CD8^+$	*Median (IQR)*	4.9[a] (2.6 – 8.1)	5.1[b] (3.4 – 9.2)	4.7[c] (2.1 – 6.6)	3.1[d] (–)	5.1[e] (1.8 – 9.8)

[a] 75 missing values [b] 6 missing values [c] 18 missing values [d] 45 missing values [e] 6 missing values

Table 6.15. Distribution of percentage $CD25^+$ $CD3^+/CD4^+$, percentage HLA-DR^+ $CD3^+/CD4^+$, percentage $CD25^+$ $CD3^+/CD8^+$ and percentage HLA-DR^+ $CD3^+/CD8^+$ in all patients (n = 101), black males (n = 14), black females (n = 30), white males (n = 47) and white females (n = 10) within the Phase Two study population

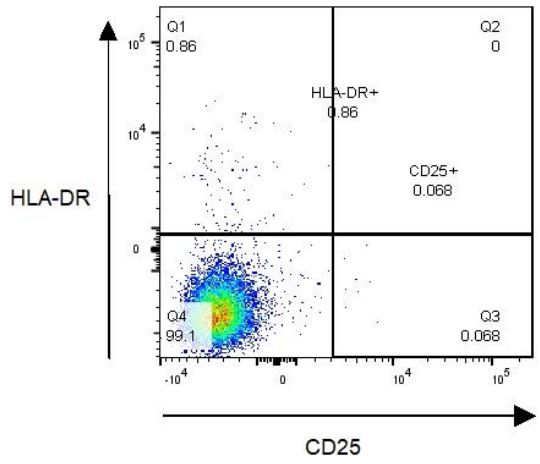


Figure 6.7a. Example of both low CD25⁺ and low HLA-DR⁺ percentage populations in CD3⁺/CD4⁺ T cells

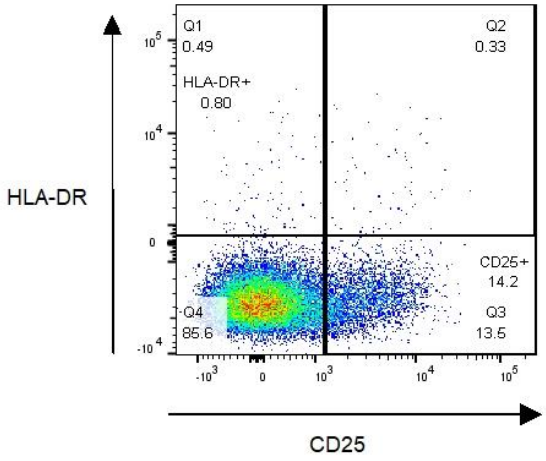


Figure 6.7b. Example of relatively high CD25⁺ but low HLA-DR⁺ percentage populations in CD3⁺/CD4⁺ T cells

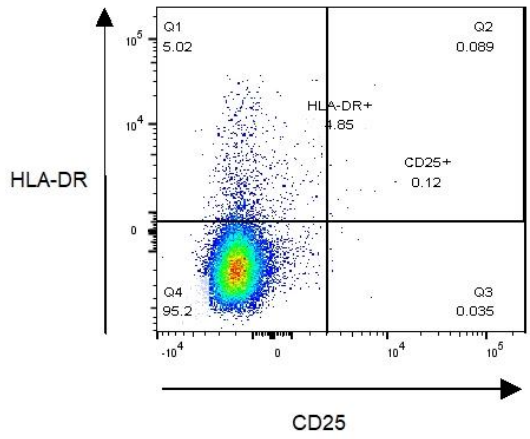


Figure 6.7c. Example of low CD25⁺ but relatively high HLA-DR⁺ percentage populations in CD3⁺/CD4⁺ T cells

	% $CD25^+$ $CD3^+/CD4^+$		% $HLA\text{-}DR^+$ $CD3^+/CD4^+$		% $CD25^+$ $CD3^+/CD8^+$		% $HLA\text{-}DR^+$ $CD3^+/CD8^+$	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age *years*	0.064	.525	0.436	**.026**	0.200	**.045**	0.182	.372
BMI *kg* m^{-2}	0.057	.573	0.067	.745	-0.065	.516	0.224	.271
Subtotal body fat *%*	-0.090	.369	0.032	.877	-0.230	**.021**	0.201	0.326
Nadir CD4 cell count *cells* μl^{-1}	-0.073	.471	0.030	.883	-0.143	.155	-0.038	.854
$CD3^+/CD4^+$ cell count *cells* μl^{-1}	-0.366	**<.001**	-0.111	.588	-0.187	.062	-0.147	.472
$CD3^+/CD4^+$ %	-0.410	**<.001**	-0.143	.485	-0.220	.027	-0.079	.701
$CD3^+/CD8^+$ cell count *cells* μl^{-1}	0.123	.220	0.006	.975	0.086	.395	0.037	.859
$CD3^+/CD8^+$ %	0.245	**.014**	-0.011	.958	0.122	.224	0.144	.481
CD4:CD8	-0.394	**<.001**	-0.080	.696	-0.204	**.041**	-0.171	.405
Years since HIV diagnosis	-0.013	.893	-0.032	.875	-0.148	.140	-0.136	.506
Duration of continuous ART *mo.*	-0.124	.221	-0.128	.541	0.082	.419	-0.118	.573
Duration of cumulative ART *mo.*	-0.059	.562	-0.098	.642	0.141	.161	-0.083	.693

Table 6.16. Relationships between general factors, immunological markers and HIV disease-specific factors and percentage $CD25^+$ $CD3^+/CD4^+$ (n = 101), percentage $HLA\text{-}DR^+$ $CD3^+/CD4^+$ (n = 26), % $CD25^+$ $CD3^+/CD8^+$ (n = 101) and percentage $HLA\text{-}DR^+$ $CD3^+/CD8^+$ (n = 26) in the Phase Two study population (ART = antiretroviral therapy, mo. = months)

		n	% $CD25^+$ $CD3^+/CD4^+$		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	12	13.1	7.5 – 21.6	.193
	Yes	89	10.3	6.6 – 14.4	
Plasma HIV RNA <200 copies ml^{-1}	No	7	10.3	5.85 – 21.9	.543
	Yes	93	10.3	5.69 – 14.5	
Current ART	No	4	12.4	7.0 – 20.0	.585
	Yes	97	10.3	6.9 – 14.6	
Current TDF	No	29	10.3	6.4 – 15.4	.705
	Yes	72	10.4	6.8 – 14.7	
Current ABC	No	75	10.7	7.1 – 14.7	.172
	Yes	26	8.4	5.3 – 14.4	
Current PI	Yes	55	9.9	6.1 – 16.0	.921
	No	46	10.4	10.4 – 14.4	
Current NNRTI	Yes	50	10.1	7.5 – 15.8	.374
	No	51	10.5	5.5 – 14.4	
Current EFV	Yes	69	10.3	7.3 – 15.9	.459
	No	32	10.6	4.5 – 14.0	
Current NVP	Yes	93	10.3	6.9 – 14.4	.403
	No	8	12.9	6.8 – 19.2	

Table 6.17. Differences in percentage $CD25^+$ $CD3^+/CD4^+$ T cells within the Phase Two study population according to plasma HIV RNA and current exposure to antiretroviral therapy (ART) and specific ART drugs and drug classes (TDF = tenofovir disoproxil; ABC = abacavir; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; EFV = efavirenz; NVP = nevirapine)

		n	% HLA-DR$^+$ CD3$^+$/CD4$^+$		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	3	2.9	-	.182
	Yes	23	1.7	1.1 – 2.2	
Plasma HIV RNA <200 copies ml^{-1}	No	2	2.5	-	.443
	Yes	24	1.8	1.1 – 2.5	
Current ART	No	1	1.7	-	.923
	Yes	25	1.8	1.2 – 2.0	
Current TDF	No	6	1.5	0.9 – 1.9	.123
	Yes	20	1.9	1.8 – 2.8	
Current ABC	No	20	1.9	1.5 – 2.7	.196
	Yes	6	1.1	0.9 – 2.3	
Current PI	No	5	2.0	1.1 – 2.5	.742
	Yes	21	1.7	1.2 – 2.6	
Current NNRTI	No	12	1.7	1.2 – 3.1	1.000
	Yes	14	1.9	1.1 – 2.2	
Current EFV	No	16	1.7	1.0 – 3.1	1.000
	Yes	10	1.9	1.3 – 2.2	
Current NVP	No	24	1.8	1.2 – 2.7	.554
	Yes	2	1.4	-	

Table 6.18. Differences in percentage HLA-DR$^+$ CD3$^+$/CD4$^+$ T cells within the Phase Two study population according to plasma HIV RNA and current exposure to antiretroviral therapy (ART) and specific ART drugs and drug classes (TDF = tenofovir disoproxil; ABC = abacavir; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; EFV = efavirenz; NVP = nevirapine)

		n	% $CD25^+$ $CD3^+/CD8^+$		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	12	1.1	0.6 – 1.3	.737
	Yes	89	0.9	0.4 – 2.1	
Plasma HIV RNA <200 copies ml^{-1}	No	7	1.0	0.6 – 1.3	.930
	Yes	93	0.9	0.4 – 2.1	
Current ART	No	4	0.8	0.2 – 2.7	.731
	Yes	97	0.9	0.4 – 2.1	
Current TDF	No	29	0.9	0.5 – 1.8	.982
	Yes	72	0.9	0.4 – 2.1	
Current ABC	No	75	0.9	0.4 – 2.4	.622
	Yes	26	0.8	0.5 – 1.6	
Current PI	No	55	1.0	0.5 – 2.2	.392
	Yes	46	1.7	0.3 – 2.0	
Current NNRTI	No	50	1.0	0.4 – 2.4	.105
	Yes	51	0.7	0.4 – 1.6	
Current EFV	No	69	1.0	0.4 – 2.1	.829
	Yes	32	0.7	0.4 – 2.0	
Current NVP	No	93	0.9	0.4 – 2.1	.466
	Yes	8	1.1	0.5 – 3.1	

Table 6.19. Differences in percentage $CD25^+$ $CD3^+/CD8^+$ T cells within the Phase Two study population according to plasma HIV RNA and current exposure to antiretroviral therapy (ART) and specific ART drugs and drug classes (TDF = tenofovir disoproxil; ABC = abacavir; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; EFV = efavirenz; NVP = nevirapine)

		n	% HLA-DR$^+$ CD3$^+$/CD8$^+$		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	3	6.0	-	.312
	Yes	23	4.9	2.2 – 8.1	
Plasma HIV RNA <200 copies ml^{-1}	No	2	7.1	-	.443
	Yes	24	4.9	2.3 – 7.7	
Current ART	No	1	3.8	-	.846
	Yes	25	5.0	2.4 – 8.2	
Current TDF	No	6	2.8	1.7 – 4.3	.072
	Yes	20	5.1	3.0 – 8.9	
Current ABC	No	20	5.1	3.4 – 8.9	**.006**
	Yes	6	2.0	1.4 – 3.5	
Current PI	No	5	5.0	2.5 – 5.7	1.000
	Yes	21	4.6	2.4 – 8.7	
Current NNRTI	No	12	4.2	2.7 – 8.5	.801
	Yes	14	5.0	2.7 – 8.1	
Current EFV	No	16	4.2	2.3 – 8.0	.816
	Yes	10	5.0	3.0 – 8.3	
Current NVP	No	24	4.9	2.7 – 8.3	.886
	Yes	2	4.5	-	

Table 6.20. Differences in percentage HLA-DR$^+$ CD3$^+$/CD8$^+$ T cells within the Phase Two study population according to plasma HIV RNA and current exposure to antiretroviral therapy (ART) and specific ART drugs and drug classes (TDF = tenofovir disoproxil; ABC = abacavir; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; EFV = efavirenz; NVP = nevirapine)

A significant positive correlation was observed between age and both % HLA-DR$^+$ CD3$^+$/CD4$^+$ T cells (r = 0.436, *p* = 0.026) and % CD25$^+$ CD3$^+$/CD8$^+$ T cells (r = 0.200, *p* = 0.045) (Table 6.16). Whilst there was no significant correlation between BMI and any individual T cell activation marker, there was a significant negative correlation between the percentage of subtotal body fat and % CD25$^+$ CD3$^+$/CD8$^+$ T cells (r = -0.230, *p* = 0.021) (Table 6.16).

There was a significant negative correlation between current CD4 cell count, CD4 % and CD4:CD8 and % CD25$^+$ CD3$^+$/CD4$^+$ T-cells (r = -0.366, *p* < 0.001 for current CD4 cell count, r = -0.410, *p* < 0.001 for CD4 % and r = -0.394, *p* < 0.001 for CD4:CD8) (Table 6.16).

There was no significant relationship between any T cell activation marker and the number of years since HIV diagnosis or continuous or cumulative duration of ART (Table 6.16). Furthermore, there was no significant difference in the percentage of positive cells with any T cell activation marker between patients either currently on or not currently on any ART or any specific ARV drug class or specific ARV drug (Tables 6.16 to 6.20), with the exception of the few patients currently on abacavir (n = 6), who had significantly higher % HLA-DR$^+$ CD3$^+$/CD8$^+$ T cells than patients not currently on abacavir (n = 20) (*p* = 0.006) (Table 6.20).

The relationships between the percentage of positive cells with any T cell activation marker and inflammatory markers – D-dimer, hs-CRP and IL-6 – are detailed in Table 6.21. (No IL-6 data was available in patients with HLA-DR data). There was a significant positive correlation between D-dimer and % CD25$^+$ CD3$^+$/CD8$^+$ T cells (r = 0.253, *p* = 0.022), hs-CRP and % HLA-DR$^+$ CD3$^+$/CD8$^+$ T cells (r = 0.572, *p* = 0.003) and IL-6 and CD25$^+$ CD3$^+$/CD4$^+$ T cells (r = 0.328, *p* = 0.039).

	% $CD25^+$ $CD3^+/CD4^+$			% HLA-DR^+ $CD3^+/CD4^+$			% $CD25^+$ $CD3^+/CD8^+$			% HLA-DR^+ $CD3^+/CD8^+$		
	n	r	*p*-value	n	r	*p*-value	n	r	*p*-value	n	r	*p*-value
D-dimer *µg* l^{-1}	82	0.181	.104	25	0.141	.502	80	0.253	**.022**	25	-0.048	.821
hs-CRP *mg* l^{-1}	83	0.128	.248	25	0.252	.225	83	-0.004	.974	25	0.572	**.003**
IL-6 *pg* ml^{-1}	40	0.328	**.039**	0	-	-	40	0.171	0.292	0	-	-

Table 6.21. Relationships between D-dimer, highly-sensitive CRP (hs-CRP) and interleukin-6 (IL-6) and percentage $CD25^+$ $CD3^+/CD4^+$, percentage HLA-DR^+ $CD3^+/CD4^+$, percentage $CD25^+$ $CD3^+/CD8^+$ and percentage HLA-DR^+ $CD3^+/CD8^+$ in the Phase Two study population

6.4.2 Relationships between T cell activation markers and BMD and 25-hydroxyvitamin D in PLWH

No significant association was observed between % $CD25^+$ $CD3^+/CD4^+$, % HLA-DR^+ $CD3^+/CD4^+$, % $CD25^+$ $CD3^+/CD8^+$ or % HLA-DR^+ $CD3^+/CD8^+$ T cells and BMD at any site (Table 6.22). There was, however, a trend to higher 25-OH-D with higher % $CD25^+$ $CD3^+/CD4^+$, % HLA-DR^+ $CD3^+/CD4^+$, % $CD25^+$ $CD3^+/CD8^+$ and % HLA-DR^+ $CD3^+/CD8^+$ T cell populations, with a significant positive correlation between 25-OH-D and both % HLA-DR^+ $CD3^+/CD4^+$ ($p < 0.001$) and % $CD25^+$ $CD3^+/CD8^+$ T cell populations ($p = 0.003$) (Table 6.22 and Figure 6.8). These associations remained significant after adjustment for patient race, season of 25-OH-D sampling, current efavirenz use and race-adjusted eGFR.

	% $CD25^+$ $CD3^+/CD4^+$		% $HLA\text{-}DR^+$ $CD3^+/CD4^+$		% $CD25^+$ $CD3^+/CD8^+$		% $HLA\text{-}DR^+$ $CD3^+/CD8^+$	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	-0.018	.855	-0.116[b]	.573	-0.124	.217	-0.065[e]	.751
Total hip BMD *g cm^{-2}*	0.013	.901	-0.117[b]	.568	-0.154	.124	-0.019[e]	.925
Femoral neck BMD *g cm^{-2}*	-0.049	.626	-0.202[b]	.322	-0.138	.170	-0.044[e]	.831
Total body BMD *g cm^{-2}*	0.048	.637	-0.050[b]	.807	-0.047	.637	0.130[e]	.526
25-hydroxyvitamin D *nmol l^{-1}*	0.205[a]	.082	0.800[c]	**<.001**	0.348[d]	**.003**	0.419[f]	.094

[a] 28 missing values
[b] 75 missing values
[c] 84 missing values
[d] 28 missing values
[e] 75 missing values
[f] 84 missing values

Table 6.22. Relationships between lumbar spine, total hip, femoral neck and total body bone mineral density (BMD) and 25-hydroxyvitamin D and percentage $CD25^+$ $CD3^+/CD4^+$, percentage $HLA\text{-}DR^+$ $CD3^+/CD4^+$, percentage $CD25^+$ $CD3^+/CD8^+$ and percentage $HLA\text{-}DR^+$ $CD3^+/CD8^+$ T cell populations in the Phase Two study population (n = 101).

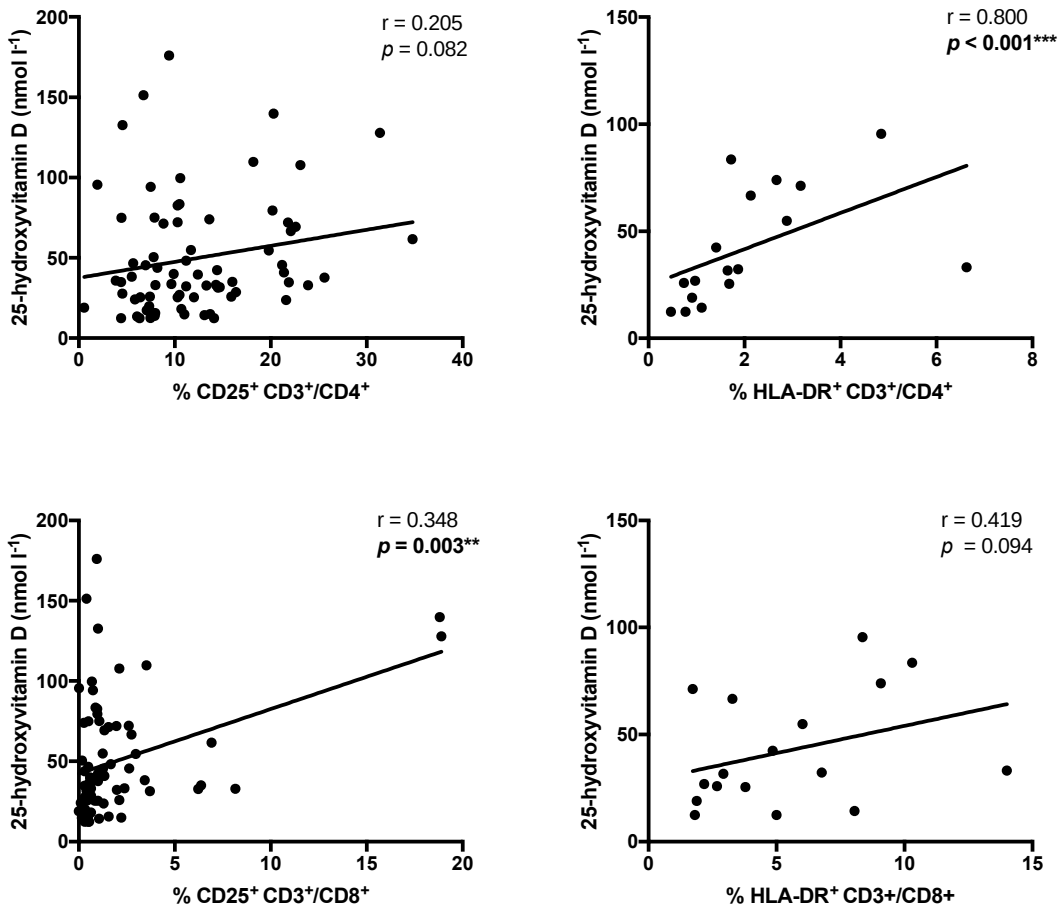


Figure 6.8. Relationship between 25-hydroxyvitamin D and percentage $CD25^+$ $CD3^+/CD4^+$ (n = 73), percentage HLA-DR^+ $CD3^+/CD4^+$ (n = 17), percentage $CD25^+$ $CD3^+/CD8^+$ (n = 73) and percentage HLA-DR^+ $CD3^+/CD8^+$ T cell populations (n = 17) in the Phase Two study population

6.5 Distribution and associations of peripheral blood monocyte population subsets and their relationships with BMD and 25-hydroxyvitamin D_3 in PLWH

6.5.1 Distribution and general and HIV disease-specific associations of peripheral blood monocyte population subsets in PLWH

The distribution of relative proportions (expressed as percentages) of peripheral blood monocyte population subsets – classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$) and non-classical ($CD14^{dim}$ $CD16^{++}$) – are detailed in Table 6.23 for all Phase Two patients in whom monocyte data was available (n = 26), as well as for black and white patient subgroups (n = 20 and n = 6 respectively). The percentage of classical monocytes was, overall, higher than the percentage of intermediate and non-classical monocytes and the percentage of non-classical monocytes was higher than the percentage of intermediate monocytes. There was no significant difference in the relative proportions of each monocyte population subset between black and white patients. Examples of patients with relatively high percentages of intermediate and non-classical monocyte population subsets are demonstrated in Figures 6.9a and 6.9b respectively.

The relationships between the percentage of each peripheral blood monocyte population subset with age, BMI, subtotal body fat percentage, T cell immunological markers and HIV disease-specific factors are summarised in Table 6.24. In addition, differences in the percentages of each peripheral blood monocyte population subset according to patient plasma HIV RNA and ART exposure are summarised in Tables 6.25 to 6.27. There was a significant positive correlation between the percentage of classical monocytes and current CD4 cell count (r = 0.490, p = 0.011), current CD4 % (r = 0.692, p < 0.001) and CD4:CD8 (r = 0.665, p < .001) and a significant negative correlation between the percentage of non-classical monocytes and current CD4 cell count (r = -0.458, p = 0.019), current CD4 % (r = -0.735, p < 0.001) and CD4:CD8 (r = -0.693, p < 0.001) (Table 6.24).

		All (n = 26)	Black (n = 20)	White (n = 6)
% Classical	*Median (IQR)*	78.6 (69.5 – 83.8)	77.2 (67.8 – 83.0)	81.4 (70.0 – 87.9)
% Intermediate	*Median (IQR)*	7.1 (5.5 – 10.3)	7.1 (5.8 – 11.0)	7.2 (3.1 – 8.9)
% Non-classical	*Median (IQR)*	12.7 (7.4– 19.4)	13.3 (7.7 – 20.8)	10.3 (3.6 – 22.9)

Table 6.23. Distribution of relative proportions (%) of classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$) and non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocyte population subsets in all patients (n = 26), black patients (n = 20) and white patients (n = 6) within the Phase Two study population for whom monocyte data was available

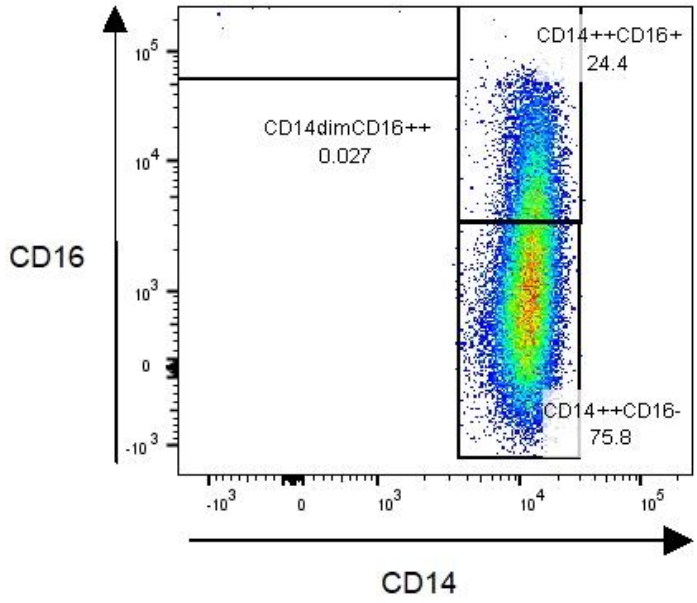


Figure 6.9a. Example of a relatively high percentage of intermediate ($CD14^{++}$ $CD16^{+}$) and low percentage of non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocytes

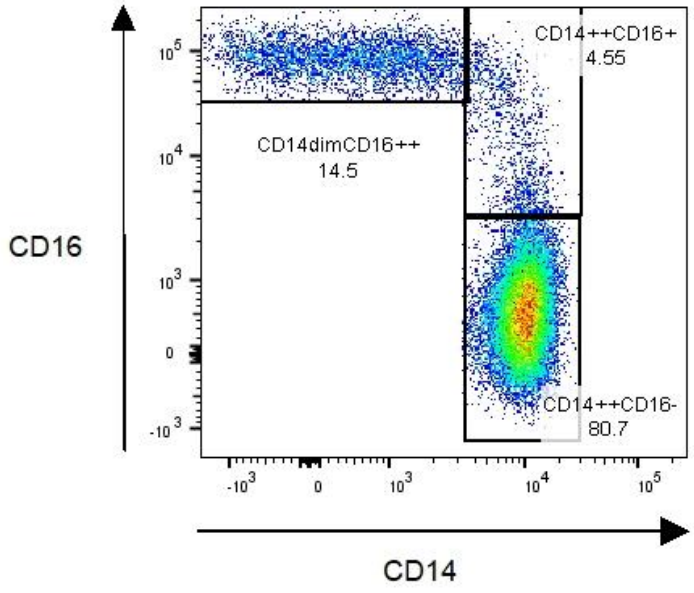


Figure 6.9b. Example of a relatively high percentage of non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocytes

	% Classical		% Intermediate		% Non-classical	
	r	*p*-value	r	*p*-value	r	*p*-value
Age *years*	-0.123	.549	-0.100	.628	0.179	.381
BMI *kg m^{-2}*	-0.004	.985	0.018	.931	0.003	.989
Subtotal body fat *%*	-0.167	.414	0.147	.472	0.061	.769
Nadir CD4 cell count *cells µl^{-1}*	0.076	.714	0.131	.525	-0.062	.765
CD3$^+$/CD4$^+$ cell count *cells µl^{-1}*	0.490	**.011**	-0.019	.927	-0.458	**.019**
CD3$^+$/CD4$^+$ %	0.692	**<.001**	-0.055	.789	-0.735	**<.001**
CD3$^+$/CD8$^+$ cell count *cells µl^{-1}*	-0.324	.107	0.130	.526	0.346	.083
CD3$^+$/CD8$^+$ %	-0.542	**.004**	0.149	.469	0.520	**.007**
CD4:CD8	0.665	**<.001**	-0.078	.705	-0.693	**<.001**
Years since HIV diagnosis	0.035	.866	-0.097	.637	-0.089	.665
Duration of continuous ART *mo.*	0.046	.827	-0.293	.156	-0.052	.807
Duration of cumulative ART *mo.*	-0.003	.990	-0.276	.182	-0.009	.965

Table 6.24. Relationships between general factors, immunological markers and HIV disease-specific factors and relative proportions (%) of classical (CD14^{++} CD16$^-$), intermediate (CD14^{++} CD16$^+$) and non-classical (CD14^{dim} CD16^{++}) peripheral blood monocyte population subsets in all patients within the Phase Two study population for whom monocyte data was available (n = 26) (mo. = months)

		n	% Classical		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	3	55.2	-	**.012**
	Yes	23	79.8	72.6 – 83.9	
Plasma HIV RNA <200 copies ml^{-1}	No	2	61.1	-	.074
	Yes	24	79.7	71.9 – 83.9	
Current ART	No	1	66.9	-	.462
	Yes	25	79.6	71.0 – 83.8	
Ever ART	No	1	66.9	-	.462
	Yes	25	79.6	71.0 – 83.8	

Table 6.25. Differences in relative proportion (%) of classical (CD14^{++} CD16^{-}) peripheral blood monocytes in all patients within the Phase Two study population for whom monocyte data was available (n = 26) according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART)

		n	% Intermediate		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	3	11.4	-	.352
	Yes	23	6.8	4.8 – 8.2	
Plasma HIV RNA <200 copies ml^{-1}	No	2	13.2	-	.074
	Yes	24	6.7	5.0 – 8.2	
Current ART	No	1	15.0	-	.231
	Yes	25	6.8	5.3 – 9.1	
Ever ART	No	1	15.0	-	.231
	Yes	25	6.8	5.3 – 9.1	

Table 6.26. Differences in relative proportion (%) of intermediate (CD14^{++} CD16^{+}) peripheral blood monocytes in all patients within the Phase Two study population for whom monocyte data was available (n = 26) according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART)

		n	% Non-classical		*p*-value
			Median	IQR	
Plasma HIV RNA <40 copies ml^{-1}	No	3	33.5	-	**.041**
	Yes	23	11.7	7.4 – 17.1	
Plasma HIV RNA <200 copies ml^{-1}	No	2	25.5	-	.222
	Yes	24	12.1	7.4 – 18.2	
Current ART	No	1	17.4	-	.615
	Yes	25	12.4	7.4 – 20.3	
Ever ART	No	1	17.4	-	.615
	Yes	25	12.4	7.4 – 20.3	

Table 6.27. Differences in relative proportion (%) of non-classical (CD14^{dim} CD16^{++}) peripheral blood monocytes in all patients within the Phase Two study population for whom monocyte data was available (n = 26) according to plasma HIV RNA and current or ever exposure to antiretroviral therapy (ART)

In contrast, there was a significant negative correlation between the percentage of classical monocytes and current CD8 % (r = -0.542, *p* = 0.004) and a significant positive correlation between the percentage of non-classical monocytes and current CD8 % (r = 0.520, *p* = 0.007) (Table 6.24).

The percentage of classical monocytes was significantly lower and the percentage of non-classical monocytes significantly higher in the few patients with unsuppressed plasma HIV RNA ≥ 40 copies ml^{-1} (n = 3) compared to those with suppressed plasma HIV RNA <40 copies ml^{-1} (n = 23) (*p* = 0.012 and *p* = 0.041 for percentage classical and percentage non-classical monocytes respectively) (Tables 6.25 and 6.27).

No significant relationship was observed between the relative proportion of any peripheral blood monocyte population subset and D-dimer or hs-CRP, as detailed in Table 6.28. (No IL-6 data was available in the 26 patients with monocyte data).

	% Classical		% Intermediate		% Non-classical	
	r	*p*-value	r	*p*-value	r	*p*-value
D-dimer *µg l*$^{-1}$	0.377	.099	-0.277	.180	-0.156	.456
hs-CRP *mg l*$^{-1}$	-0.115	.584	0.090	.670	-0.042	.842

Table 6.28. Relationships between D-dimer and highly-sensitive CRP (hs-CRP) and interleukin-6 (IL-6) and relative proportions (%) of classical (CD14^{++} CD16^{-}), intermediate (CD14^{++} CD16^{+}) and non-classical (CD14^{dim} CD16^{++}) peripheral blood monocyte population subsets for all patients within the Phase Two study population for whom monocyte data was available (n = 26)

6.5.2 Relationships between peripheral blood monocyte population subsets and BMD and 25-hydroxyvitamin D in PLWH

The relationship between the relative proportions (percentage) of classical, intermediate and non-classical peripheral blood monocyte population subsets with BMD and 25-OH D are detailed in Tables 6.29, 6.30 and 6.31 for all, black and white patients within the Phase Two study population for whom monocyte data was available.

In all patients, there was a trend to increased lumbar spine and total body BMD with a higher percentage of classical monocytes, coupled with a trend to reduced BMD at each site with a higher percentage of non-classical monocytes; the percentage of non-classical monocytes was significantly negatively correlated with lumbar spine BMD (r = -0.391, p = 0.048). A similar trend was observed in black patients, with a trend to higher BMD at all sites with a higher percentage of classical monocytes and lower BMD at all sites with a higher percentage of non-classical monocytes; significant correlations were observed between the percentage of classical monocytes and total BMD (r = 0.496, p = 0.026) and between non-classical monocytes and hip BMD (r = -0.477, p = 0.034). The relationship between lumbar spine BMD and the percentage of both classical and non-classical monocytes was similar in white patients; in contrast to black patients, however, total hip, femoral neck and total body BMD each decreased with a higher percentage of classical monocytes and increased with a higher percentage of non-classical monocytes.

There was no significant relationship observed between the relative proportions of classical, intermediate or non-classical peripheral blood monocyte population subsets and 25-OH-D in all patients or in black patients. In white patients, however, a significant negative correlation was seen between 25-OH-D_3 and the percentage of classical monocytes (r = -0.900, p = 0.037), with a trend to increased 25-OH-D with higher percentages of both intermediate and non-classical monocytes (r = 0.600, p = 0.285 and r = 0.800, p = 0.104 for intermediate and non-classical monocytes respectively).

	% Classical		% Intermediate		% Non-classical	
	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	0.271	.180	0.258	.203	-0.391	**.048**
Total hip BMD *g cm^{-2}*	0.095	.643	0.011	.959	-0.148	.470
Femoral neck BMD *g cm^{-2}*	0.093	.652	0.126	.539	-0.175	.391
Total body BMD *g cm^{-2}*	0.281	.164	0.012	.952	-0.179	.380
25-hydroxyvitamin D *nmol l^{-1}*	0.097	0.711	-0.065	.804	0.038	.885

Table 6.29. Relationships between lumbar spine, total hip, femoral neck and total bone mineral density (BMD) and 25-hydroxyvitamin D and relative proportions (%) of classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$) and non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocyte population subsets for all patients within the Phase Two study population for whom monocyte data was available (n = 26)

	% Classical		% Intermediate		% Non-classical	
	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	0.256	.276	0.083	.729	-0.435	.056
Total hip BMD *g cm^{-2}*	0.336	.148	-0.122	.609	-0.477	**.034**
Femoral neck BMD *g cm^{-2}*	0.247	.294	0.059	.806	-0.406	.076
Total body BMD *g cm^{-2}*	0.496	**.026**	-0.164	.490	-0.414	.070
25-hydroxyvitamin D_3 *nmol l^{-1}*	0.049	.880	-0.336	.286	0.063	.846

Table 6.30. Relationships between lumbar spine, total hip, femoral neck and total body bone mineral density (BMD) and 25-hydroxyvitamin D and between relative proportions (%) of classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$) and non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocyte population subsets for black patients within the Phase Two study population for whom monocyte data was available (n = 20)

	% Classical		% Intermediate		% Non-classical	
	r	*p*-value	r	*p*-value	r	*p*-value
Lumbar spine BMD *g cm^{-2}*	0.314	.544	0.714	.111	-0.486	.329
Total hip BMD *g cm^{-2}*	-0.543	.266	0.200	.704	0.429	.397
Femoral neck BMD *g cm^{-2}*	-0.314	.544	0.314	.544	0.257	.623
Total body BMD *g cm^{-2}*	-0.086	.872	-0.029	.957	0.143	.787
25-hydroxyvitamin D *nmol l^{-1}*	-0.900	**.037**	0.600	.285	0.800	.104

Table 6.31. Relationships between lumbar spine, total hip, femoral neck and total body bone mineral density (BMD) and 25-hydroxyvitamin D and between relative proportions (%) of classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$) and non-classical ($CD14^{dim}$ $CD16^{++}$) peripheral blood monocyte population subsets for white patients within the Phase Two study population for whom monocyte data was available (n = 6)

6.6 Discussion

The overwhelming majority of patients included in the Phase Two study population were well established on ART – median continuous ART duration 64 months (IQR 32 – 122) – with suppressed plasma HIV RNA – less than 40 copies ml^{-1} in 101 (88.6%) patients and less than 200 ml^{-1} in 107 (93.9%) patients – and with good immune reconstitution – median current CD4 cell count 616 cells μl^{-1} (IQR 437 – 783). Only four (3.5%) patients were not currently on ART and only three (2.6%) patients were ART-naïve. That no correlation was identified between BMD and either IL-6, hs-CRP or D-dimer within the ART-stable Phase Two study population is in keeping with the findings from one other cross-sectional study also performed in ART-stable patients (Erlandson *et al.* 2014). Interestingly, both hs-CRP and D-dimer increased significantly with increases in both BMI and percentage of subtotal body fat. As there is a strong positive correlation between BMI and BMD, larger BMD increases with increases in BMI may offset any BMD decreases secondary to higher levels of inflammation in ART-stable patients.

The findings of this study support the hypothesis that only pre-ART initiation levels of inflammatory markers are significantly associated with BMD in PLWH – with greater bone loss observed within the first two years of ART initiation with higher pre-ART levels of IL-6 and hs-CRP (Hileman *et al.* 2014, Brown *et al.* 2015) – and that levels of inflammatory markers in ART-stable patients are not significantly associated with BMD. Of note, IL-6 data was only analysed in a small subset of Phase Two patients (n = 41, 36.0%) and should ideally be assessed across the wider Phase Two cohort to further verify these findings. Longitudinal BMD data would also be valuable to confirm whether or not levels of inflammatory markers in ART-stable patients determine future bone loss.

No relationship was identified between either current CD4 cell count or CD4 cell percentage and BMD within the ART-stable Phase Two study population. Only four Phase Two patients had current CD4 cell counts <200 cells ul^{-1}, however, probably too few to assess a significant difference in BMD between

patients with low *versus* high current CD4 cell count, which has been observed elsewhere (Yong *et al.* 2011). No relationship was identified between nadir CD4 cell count and BMD either, however (Chapter 4), in spite of 58 (50.9%) Phase Two patients having a nadir CD4 cell count <200 cells μl^{-1}. As observed with markers of inflammation, it may be that whilst low nadir or ART baseline CD4 cell counts can influence the degree of bone loss within the first one to two years following ART initiation, this influence does not persist at a significant level beyond this time point and is therefore no longer seen in an ART-stable population. This could explain why, whilst an inverse CD4:CD8 (< 1) can persist following immune reconstitution in patients with low nadir CD4 counts, current CD4:CD8 (< 1 in 76 Phase Two patients) was also not significantly associated with BMD in this ART-stable population. CD4:CD8 does influence the risk of other NICMs in ART-stable HIV-positive populations, however (Serrano-Villar *et al.* 2014a, Serrano-Villar *et al.* 2014b, Castilho *et al.* 2016) and it may be justifiable to assess the relationship between CD4:CD8 ratio and BMD in PLWH a larger cohort.

In keeping with Erlandson *et al.* 2014, but in contrast to Gazzola *et al.* 2013, T cell activation markers were not found to correlate significantly with BMD in the Phase Two study population. Of note, whilst % HLA-DR^+ $CD3^+/CD4^+$ and % HLA-DR^+ $CD3^+/CD8^+$ T cell populations were assessed within each study, different "second" markers of T cell activation were used – % CD25 (in this Phase Two study), % CD28 (Gazzola *et al.* 2013) and % CD38 (Erlandson *et al.* 2014) – and therefore findings between these studies are not necessarily comparable. Moreover, HLA-DR data was only available in a small subset of the Phase Two study population (n = 26, 22.8%) and this small sample size is likely to negatively impact upon the potential to identify significant correlations. Furthermore, as activated T cells have a relatively short half-life before undergoing apoptosis (Carreño *et al.* 2006), real-time measurements in stable patients are unlikely to reflect past immune activation and therefore correlate poorly with BMD. The simultaneous measurement of bone turnover markers and BMD longitudinally could improve current understanding of the role of T cell activation in reduced BMD in PLWH.

A trend to an increased percentage of circulating non-classical ($CD14^{dim}$ $CD16^{++}$) monocytes with reduced BMD was observed within the Phase Two study population for all BMD sites, with a significant negative correlation observed between percentage of non-classical monocytes and lumbar spine BMD. Monocyte population subset data was available in only 26 Phase Two patients, however, of whom a majority (76.9%) were black African. This finding cannot necessarily be extrapolated to all white patients, therefore. A larger study (Erlandson *et al.* 2014), which included142 HIV-positive ART-stable patients, of whom 69% were of black race, demonstrated no correlation between percentage of non-classical monocytes and lumbar spine BMD

Of interest, having a higher percentage of non-classical monocytes was significantly associated with HIV disease-specific factors typically associated with either late HIV presentation or poorer HIV disease control, namely lower current CD4 cell count and CD4 %, lower CD4:CD8 and having an unsuppressed plasma HIV RNA. None of these factors in isolation have had independent significant associations with BMD identified within the Phase Two cross-sectional study of mostly ART-stable patients. Whilst these factors are recoverable with ART initiation, expanded non-classical monocyte population subsets can persist, with no change in non-classical monocyte population size at one year following ART initiation in one study (Han *et al.* 2009). Non-classical monocytes may therefore act as a lasting footprint of past immune dysregulation, increased bone turnover and BMD loss and therefore may correlate better with current BMD – a summation of past bone turnover activity – than current recovered CD4 cell count, CD4 %, CD4:CD8 or plasma HIV RNA.

No significant relationship was observed between 25-OH-D and inflammatory or immunological markers within this study. In contrast to expectation, 25-OH-D was higher (not lower) with higher levels of T cell immune activation, with a significant positive correlation observed between 25-OH-D and both % $HLA\text{-}DR^{+}$ $CD3^{+}/CD4^{+}$ and % $CD25^{+}$ $CD3^{+}/CD8^{+}$ T cell populations. Also contrary to expectation, 25-OH-D was higher (not lower) with an increased percentage of non-classical monocytes. These unexpected findings may be explained by the

small sample size of this study, particularly with respect to monocyte analysis, as well as a failure to adjust for unidentified confounding factors, in the context of multiple potential vitamin D predictors. These findings also demonstrate the complex, multidirectional and perhaps not always predictable relationship between vitamin D, the immune system and inflammation.

6.7 Conclusions

1. There was no significant association observed between inflammatory markers (D-dimer, hs-CRP or IL-6) with BMD or 25-OH-D.
2. There was no significant association observed between current CD4 cell count, current CD4 %, current CD8 cell count, current CD8 % or CD4:CD8 ratio with BMD or 25-OH-D.
3. There was no significant association between T cell activation markers (% $CD25^+$ $CD3^+/CD4^+$, % $HLA\text{-}DR^+$ $CD3^+/CD4^+$, % $CD25^+$ $CD3^+/CD8^+$ or % $HLA\text{-}DR^+$ $CD3^+/CD8^+$ T cell populations) with BMD; there was, however, a trend to increased 25-OH-D with increased percentage positivity of each T-cell activation marker which was significant for both % $HLA\text{-}DR^+$ $CD3^+/CD4^+$ and % $CD25^+$ $CD3^+/CD8^+$ T-cell populations independent of other predictors of 25-OH-D.
4. There was a trend to decreased BMD at each site with a higher percentage of peripheral blood non-classical monocytes, which was significant for lumbar spine BMD; a higher percentage of peripheral blood non-classical monocytes was significantly associated with factors typically associated with either late HIV presentation or poorer HIV disease control; overall, there was no significant relationship between the relative proportions of peripheral blood monocyte population subsets and 25-OH-D.

7. FRAX® as a tool for the assessment of fracture risk in people living with HIV

7.1 Introduction

The WHO FRAX® tool (www.shef.ac.uk/FRAX) has been validated as an effective tool for predicting fracture risk in men and women 40 to 90 years of age, incorporating clinical risk factors, with or without femoral neck BMD measurements if available (Kanis *et al.* 2008a; Johansson *et al.* 2009; Kanis *et al.* 2009). FRAX® provides an estimate of an individual's 10-year probability of major osteoporotic fracture (hip, clinical vertebral, wrist or proximal humerus) or hip fracture alone. In the UK, its output has been coupled to a guideline from the National Osteoporosis Guideline Group (NOGG) to determine who should be considered for BMD assessment and/or therapy to reduce fracture risk (Kanis *et al.* 2008b; Compston *et al.* 2009).

FRAX® is recommended for use in PLWH in both national and international HIV guidelines (BHIVA 2016; EACS 2017). EACS recommend FRAX® score calculation in PLWH aged 40 years or more, with the added recommendation to consider incorporating HIV infection into FRAX® as a "secondary osteoporosis" risk factor (EACS 2017). In addition to patients with high FRAX®-calculated 10-year probability of fracture, EACS also recommends BMD measurement directly, irrespective of FRAX®, in PLWH within any of the following groups: post-menopausal women; men more than or equal to 50 years of age; those with a history of fragility fracture; those with a high risk for falls; those with clinical hypogonadism; and those who have been on oral glucocorticoid treatment (at least 5 mg prednisolone daily or equivalent) for more than three months (EACS 2017).

FRAX® does not incorporate HIV-disease specific risk factors for fracture, however, and therefore the appropriateness of its use in PLWH remains a subject of research. Assessing FRAX® within HIV cohorts has been limited by a lack of longitudinal studies with sufficient follow up time to allow a comparison of baseline FRAX® probabilities with ten year actual fragility

fracture incidence. Furthermore, outside the context of large population studies, the incidence of fragility fractures is too small within studied HIV cohorts to support any meaningful analysis. FRAX® has therefore been compared to BMD – a sub-optimal yet pragmatic surrogate for actual ten year fracture incidence, with several FRAX®-incorporated risk factors mediating at least part of their effect by altering BMD.

An Australian cross-sectional study compared FRAX®-calculated 10-year probabilities of fragility fracture to BMD in 153 predominantly male HIV-positive patients (race not stated), median age 48 years and median BMI 24.5 kg m^{-2}, and found no significant difference in FRAX®-calculated probabilities between patients with normal BMD and those with reduced BMD (n = 65, 42.5%) (Calmy *et al.* 2009). Two smaller cross-sectional Italian studies also observed poor sensitivity of FRAX® for the prediction of HIV-positive patients with reduced BMD (Gazzola *et al.* 2010; Pepe *et al.* 2012). In one of these two studies, modification of FRAX®, with inclusion of HIV as a "secondary osteoporosis" risk factor, improved its sensitivity of reduced BMD prediction, but remained suboptimal (37.5% sensitivity with HIV as a "secondary osteoporosis" risk factor *versus* 22% without) (Gazzola *et al.* 2010).

Two studies in PLWH have compared FRAX®-calculated 10-year probabilities of fragility fracture (estimated incidence) to actual 10-year fragility fracture incidence (observed incidence). The first of these was a retrospective data review of exclusively male HIV-positive patients 50 to 70 years of age within the US Veterans Ageing Cohort Study Virtual Cohort (Yin *et al.* 2016). Osteoporotic fracture incidence was derived from ICD-9-CM diagnostic codes. Of note, data on parental hip fracture and the presence of other "secondary osteoporosis" risk factors were not available and therefore not included in FRAX® calculations, thereby lowering estimated fragility fracture incidence within the study population overall. Observed/estimated fragility fracture incidence ratios (O/E ratios) were compared in 24,451 HIV-positive males with age-matched HIV-negative male controls. FRAX® was found to be a poorer predictor of fragility fracture incidence in HIV-positive males *versus* HIV-negative males (O/E ratio 1.62, 95% CI 1.45, 1.81 *versus* O/E ratio 1.29, 95%

CI 1.19, 1.40). The observed fragility fracture incidence was actually quite low, however. Furthermore, there was a high prevalence of HCV co-infection within HIV-positive males – an additional potential fracture risk factor (Lo Re *et al.* 2012) not incorporated into FRAX®. The accuracy of FRAX® was worse in HIV-positive males with higher FRAX®-calculated 10-year probability of major osteoporotic fracture, including in older males. Of interest, the inclusion of HIV as a "secondary osteoporosis" risk factor improved the correlation between FRAX® and observed fragility fracture incidence in HIV-positive males (O/E ratio 1.20, 95% CI 1.08, 1.34).

The second of these studies is the only published prospective longitudinal study to date that compares FRAX®-calculated estimated fragility fracture risk and observed fracture incidence in PLWH (Yang *et al.* 2018). Similar to the retrospective male study, this exclusively female US study also demonstrated that FRAX® was a poorer predictor of fragility fracture incidence in 900 HIV-positive females than in 248 HIV-negative females (O/E ratio 5.05 compared with 3.26 for major osteoporotic fracture and 19.78 compared with 7.94 for hip fracture in HIV-positive and HIV-negative females respectively). Again, as seen in male patients, O/E ratios for HIV-positive females improved with the inclusion of HIV as a "secondary osteoporosis" risk factor, with further improvement observed for hip O/E ratios when femoral neck BMD data was also incorporated into FRAX® (O/E ratio 4.00).

There have been no publications to date comparing the performance of FRAX® – with or without inclusion of HIV as a "secondary osteoporosis" risk factor – in black *versus* white HIV-positive patients.

This chapter aims to identify whether there may be a subset of PLWH in whom FRAX® may be a useful tool for fracture risk assessment, by answering the following specific questions:

1. How well does FRAX® predict fragility fracture incidence – using BMD as a surrogate for actual fracture incidence – in the Sheffield HIV Cohort?

2. Does FRAX® perform better or worse in black *versus* white HIV-positive patients, in low fracture risk patients compared to high fracture risk patients, or for different BMD sites?

3. Does the inclusion of HIV into FRAX® as a “secondary osteoporosis” risk factor improve the performance of FRAX® in PLWH?

4. To what extent do FRAX® probabilities calculated with femoral neck BMD differ from FRAX® probabilities calculated without BMD and are differences between these greater or smaller in black HIV-positive patients compared to white HIV-positive patients or in low fracture risk patients compared to high fracture risk patients?

5. Could HIV disease-specific risk factors be used with FRAX® to improve its correlation with BMD and therefore to improve fracture risk assessment in PLWH?

7.2 Relationship between FRAX® 10-year probability of major osteoporotic and hip fracture (calculated without femoral neck BMD) and BMD in PLWH

The distributions of FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and FRAX® 10-year probability of hip fracture ("FRAX® hip") – both calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor – are shown in Table 7.1 for all (n = 114), black (n = 52) and white (n = 62) patients within the Phase Two study population. The US Black FRAX® tool was used in all black patients (in the absence of other black race country-specific tools being available). Country-specific FRAX® tools were used in white patients (UK tool for white British and Irish patients (n = 57); country-specific tools for individual patients from Australia, Chile, Malta, Portugal and Slovakia respectively). "FRAX® major" and "FRAX® hip" were significantly lower in black patients than in white patients ($p < 0.001$ for both).

The correlations between both "FRAX® major" and lumbar spine, total hip, femoral neck and total body BMD and between "FRAX® hip" and total hip and femoral neck BMD are shown in Table 7.2 for all, black and white patients within the Phase Two study population. In all patients and in black patients (Figures 7.1 and 7.2), there was a significant negative correlation between both "FRAX® major" with BMD at every site and "FRAX® hip" with BMD at the total hip and femoral neck. In white patients, however, there was no significant correlation between "FRAX® major" and BMD at any site (Figure 7.3). There was, however, a significant negative correlation between "FRAX® hip" and total hip BMD, although not between "FRAX® hip" femoral neck BMD (Figure 7.4).

		All patients (n = 114)	Black patients (n = 52)	White patients (n = 62)
"FRAX® major" %	Median	2.6	1.0	3.6
	IQR	1.1 – 4.1	0.7 – 1.9	2.6 – 6.0
	Range	0.5 – 15.0	0.5 – 6.2	1.5 – 15.0
"FRAX® hip" %	Median	0.2	0.0	0.4
	IQR	0.0 – 0.5	0.0 – 0.1	0.2 – 0.9
	Range	0.0 – 3.6	0.0 – 1.8	0.1 – 3.6

Table 7.1. Distribution of FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (both calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) in all (n = 114), black (n = 52) and white (n = 62) patients within the Phase Two study population

BMD site	All (n = 114)				Black (n = 52)				White (n = 62)			
	FRAX® major		FRAX® hip		FRAX® major		FRAX® hip		FRAX® major		FRAX® hip	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
L-spine	-0.243	**.009**	-	-	-0.405	**.003**	-	-	-0.120	.354	-	-
Total hip	-0.324	**<.001**	-0.384	**<.001**	-0.295	**.034**	-0.410	**.003**	-0.245	.055	-0.275	**.030**
Femoral neck	-0.408	**<.001**	-0.462	**<.001**	-0.434	**.001**	-0.545	**<.001**	-0.211	.100	-0.233	.068
Total body	-0.288	**.002**	-	-	-0.370	**.007**	-	-	-0.203	.114	-	-

Table 7.2. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (both calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in all (n = 114), black (n = 52) and white (n = 62) patients within the Phase Two study population

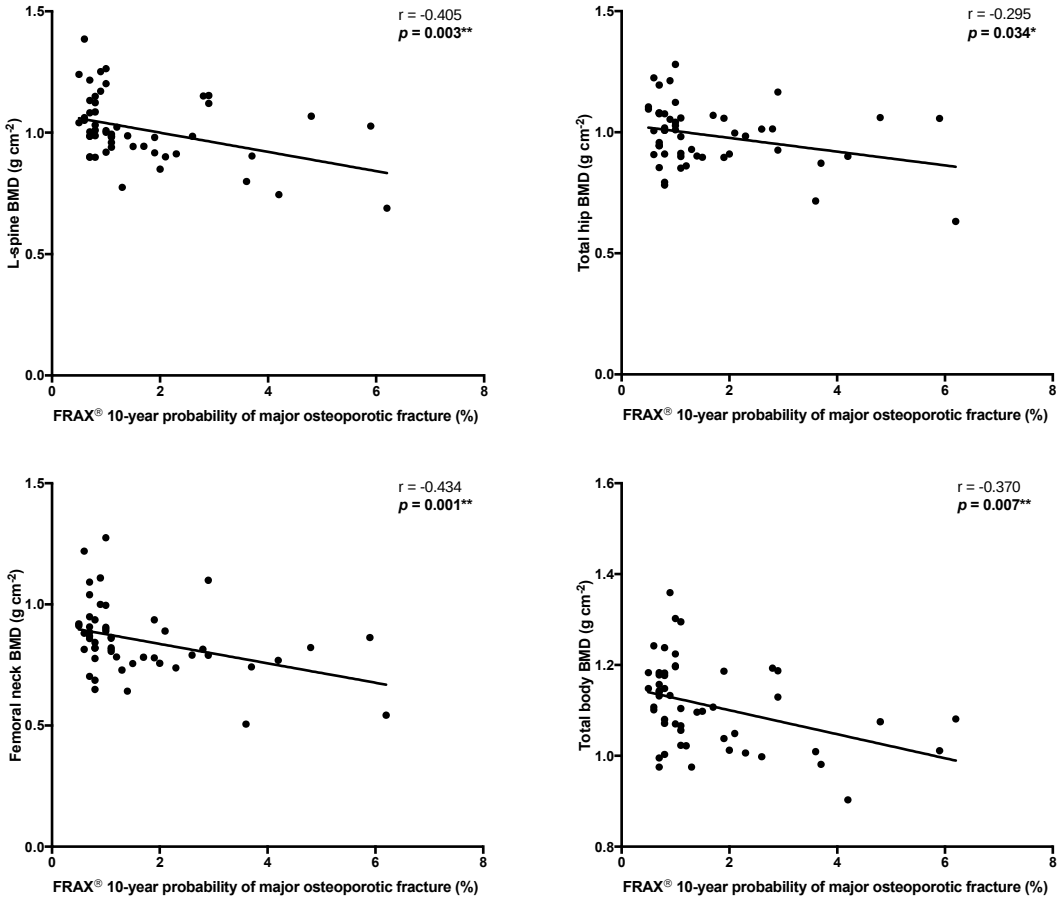


Figure 7.1. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in black (n = 52) patients within the Phase Two study population

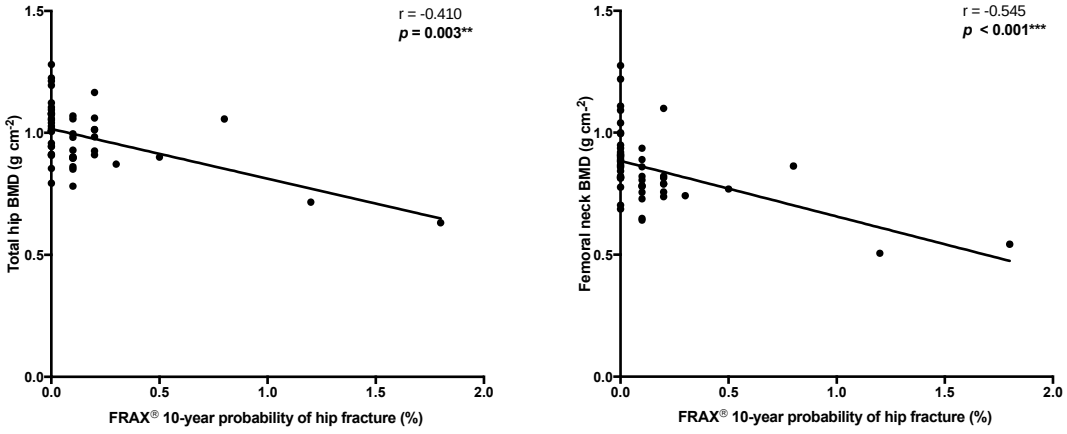


Figure 7.2. Relationship between FRAX® 10-year probability of hip fracture ("FRAX® hip") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and total hip and femoral neck BMD in black (n = 52) patients within the Phase Two study population

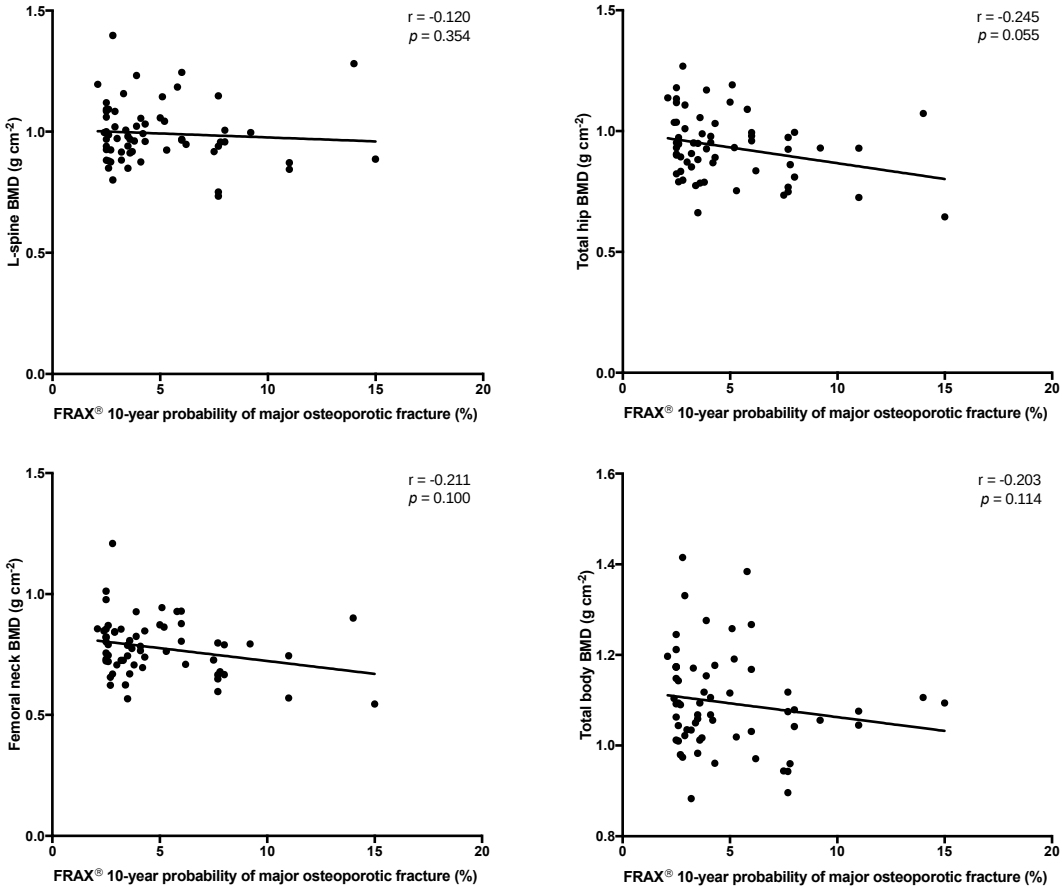


Figure 7.3. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in white (n = 62) patients within the Phase Two study population

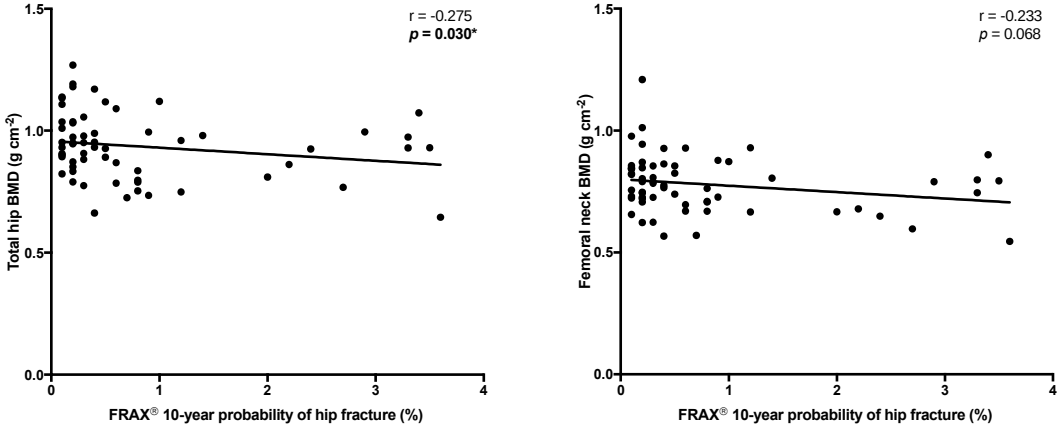


Figure 7.4. Relationship between FRAX® 10-year probability of hip fracture ("FRAX® hip") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and total hip and femoral neck BMD in white (n = 62) patients within the Phase Two study population

The relationship between FRAX® and BMD was also analysed by assessing the differences in BMD between Phase Two patients with either low, intermediate or high FRAX® probabilities.

In all patients (n = 114), low, intermediate and high "FRAX® major" probabilities were defined as ≤ 1.9% (n = 39), 2.0 – 3.6% (n = 39) and ≥ 3.7% (n = 36) and low, intermediate and high "FRAX® hip" probabilities were defined as ≤ 0.1% (n = 51), 0.2 – 0.4% (n = 32) and ≥ 0.5% (n = 31).

Whilst differences in both lumbar spine and total BMD between "FRAX® major" low, intermediate and high probability tertiles were not significant (F (2,111) = 2.315, p = 0.301 for lumbar spine; F (2,111) = 2.777, p = 0.067 for total BMD), differences between both total hip and femoral neck BMD and "FRAX® major" low, intermediate and high probability tertiles were significant (F (2,111) = 4.045, p = 0.020 for hip; and F (2,111) = 8.033, p = 0.001 for femoral neck) (Table 7.3 and Figure 7.5). There was good differentiation in both total hip and femoral neck BMD between both "FRAX® major" low and intermediate probability tertiles (p = 0.075 for total hip; p = 0.005 for femoral neck) and between "FRAX® major" low and high probability tertiles (p = 0.006 for total hip; p < 0.001 for femoral neck). There was no clear differentiation in total hip and femoral neck BMD between "FRAX® major" intermediate and high probability tertiles, however (p = 0.295 for total hip; p = 0.441 for femoral neck).

There were significant differences in both total hip and femoral neck BMD between "FRAX® hip" low, intermediate and high probability tertiles (F (2,111) = 8.785, p < 0.001 for total hip; F (2,111) = 9.658, p < 0.001 for femoral neck), with good differentiation in both total hip and femoral neck BMD between both "FRAX® hip" low and high probability tertiles (p < 0.001 for both total hip and femoral neck) and between "FRAX® hip" intermediate and high probability tertiles (p = 0.010 for total hip; p = 0.030 for femoral neck) (Table 7.4 and Figure 7.6). There was less clear differentiation in total hip and femoral neck BMD between "FRAX® hip" low and intermediate probability tertiles, however (p = 0.301 for total hip, p = 0.054 for femoral neck).

	n	L-spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	39	1.039 ± .124	.089	1.003 ± .117	.075	0.878 ± .138	**.005**	1.132 ± .090	.093
Intermediate	39	0.991 ± .124		0.952 ± .133		0.789 ± .132		1.094 ± .093	
Low	39	1.039 ± .124	.052	1.003 ± .117	**.006**	0.878 ± .138	**<.001**	1.132 ± .090	**.023**
High	36	0.979 ± .139		0.919 ± .138		0.767 ± .108		1.078 ± .108	
Intermediate	39	0.991 ± .124	.708	0.952 ± .133	.295	0.789 ± .132	.441	1.094 ± .093	.526
High	36	0.979 ± .139		0.919 ± .138		0.767 ± .108		1.078 ± .108	
One-way ANOVA	114	-	.103	-	**.020**	-	**.001**	-	.067

Table 7.3. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in all Phase Two study patients (n = 114) with either low, intermediate or high FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

	n	Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	51	0.999 ± .113	.301	0.863 ± .130	.054
Intermediate	32	0.971 ± .130		0.806 ± .129	
Low	51	0.999 ± .113	**<.001**	0.863 ± .130	**<.001**
High	31	0.882 ± .136		0.737 ± .116	
Intermediate	32	0.971 ± .130	**.010**	0.806 ± .129	**.030**
High	31	0.882 ± .136		0.737 ± .116	
One-way ANOVA	114	-	**<.001**	-	**<.001**

Table 7.4. Differences in total hip and femoral neck BMD in all Phase Two study patients (n = 114) with either low, intermediate or high FRAX® 10-year probability of hip fracture ("FRAX® hip") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

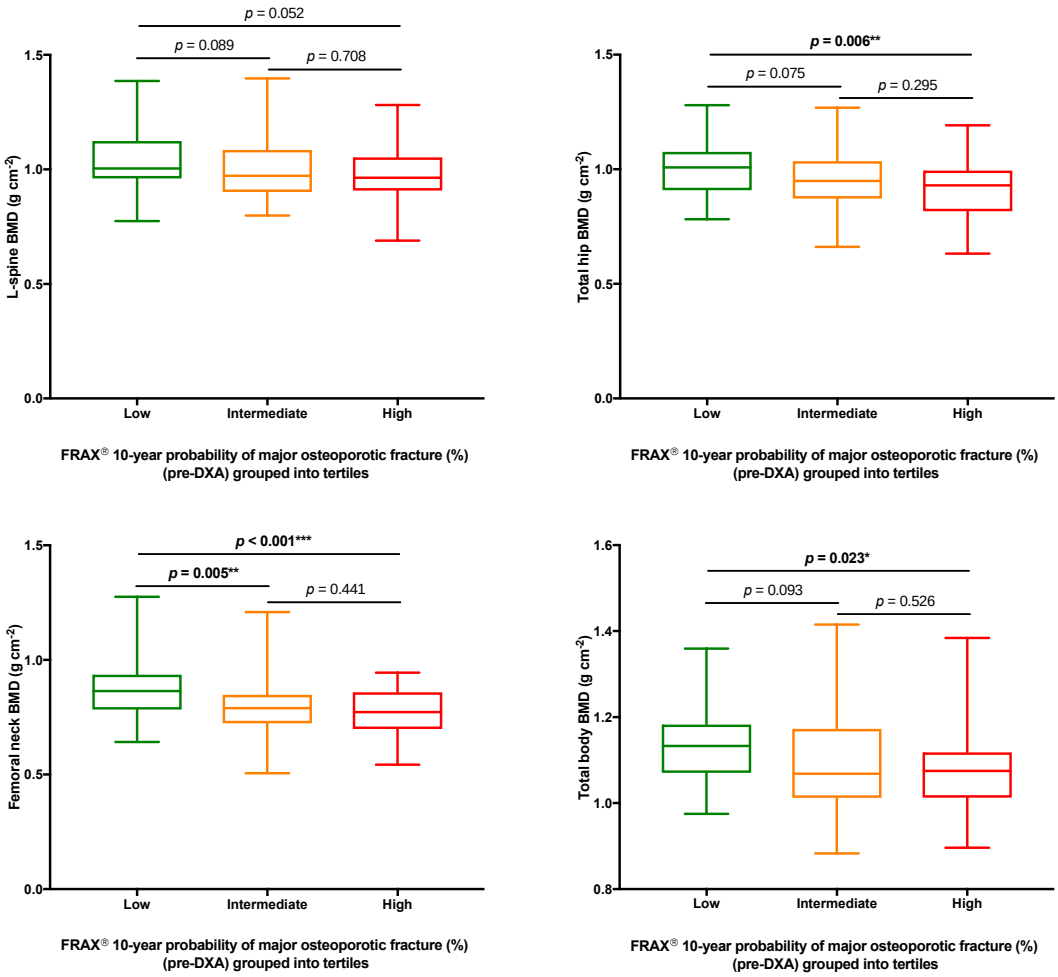


Figure 7.5. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in all Phase Two study patients (n = 114) with either low (n = 39), intermediate (n = 39) or high (n = 36) FRAX® 10-year probability of major osteoporotic fracture

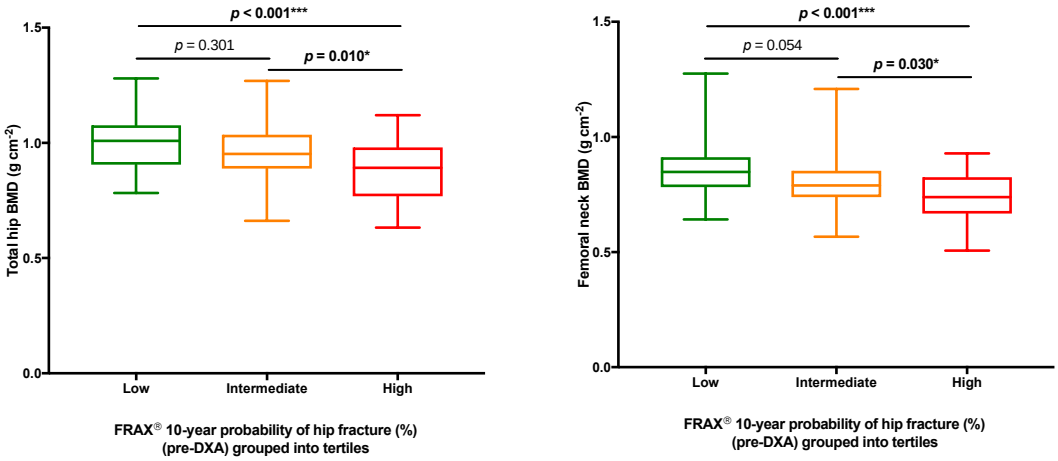


Figure 7.6. Differences in total hip and femoral neck BMD in all Phase Two study patients (n = 114) with either low (n = 51), intermediate (n = 32) or high (n = 31) FRAX® 10-year probability of hip fracture

Analysis by FRAX® probability tertiles was also performed within black and white patient subgroups. In black patients (n = 52), low, intermediate and high "FRAX® major" probabilities were defined as ≤ 0.8% (n = 20), 0.9 – 1.5% (n = 16) and ≥ 1.6% (n = 16) and low, intermediate and high "FRAX® hip" probabilities were defined as 0.0% (n = 28), 0.1% (n = 12) and ≥ 0.2% (n = 12). Differences in BMD between low, intermediate and high "FRAX® major" probabilities and between low, intermediate and high "FRAX® hip" probabilities are shown for black patients in Tables 7.5 and 7.6 respectively.

There was a significant difference in both lumbar spine and total body BMD between black patients with low, intermediate or high "FRAX® major" probabilities (F (2,49) = 3.683, p = 0.032 for lumbar spine; F (2,49) = 3.699, p = 0.032 for total body BMD) (Table 7.5). There was also a significant difference in both total hip and femoral neck BMD between patients with low, intermediate or high "FRAX® hip" probabilities (F (2,49) = 5.127, p = 0.010 for total hip; F (2,49) = 7.550, p = 0.001 for femoral neck) (Table 7.6).

Lumbar spine and total body BMD were significantly greater in black patients with low "FRAX® major" probabilities compared with patients with high "FRAX® major" probabilities (p = 0.011 for lumbar spine; p = 0.013 for total body BMD); total body BMD was also significantly greater in black patients with intermediate "FRAX® major" probabilities compared with high "FRAX® major" probabilities (p = 0.032) (Table 7.5 and Figure 7.7). Differences in both lumbar spine and total body BMD between black patients with either low or intermediate "FRAX® major" probabilities were not significant, however.

In comparison, total hip and femoral neck BMD were significantly greater in black patients with low "FRAX® hip" probabilities compared with those with either intermediate (p = 0.027 for total hip; p = 0.004 for femoral neck) or high (p = 0.005 for total hip; p = 0.004 for femoral neck) "FRAX® hip" probabilities, although with no significant difference in BMD between black patients with either intermediate or high "FRAX® hip" probabilities (Table 7.6 and Figure 7.8).

	n	L-spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	20	1.064 ± .122	.373	1.033 ± .119	.988	0.879 ± .134	.917	1.129 ± .074	.764
Intermediate	16	1.026 ± .132		1.003 ± .124		0.884 ± .154		1.139 ± .112	
Low	20	1.064 ± .122	**.011**	1.033 ± .119	.259	0.879 ± .134	.055	1.129 ± .074	**.013**
High	16	0.947 ± .137		0.955 ± .136		0.789 ± .137		1.060 ± .083	
Intermediate	16	1.026 ± .132	.107	1.003 ± .124	.304	0.884 ± .154	.074	1.139 ± .112	**.032**
High	16	0.947 ± .137		0.955 ± .136		0.789 ± .137		1.060 ± .083	
One-way ANOVA	52	-	**.032**	-	.446	-	.103	-	**.032**

Table 7.5. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two study population black patients with either low, intermediate or high FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

	n	**Total hip BMD** *g cm^{-2}*		**Femoral neck BMD** *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	28	1.036 ± .112	**.005**	0.917 ± .135	**.004**
Intermediate	12	0.927 ± .085		0.786 ± .087	
Low	28	1.036 ± .112	**.027**	0.917 ± .135	**.004**
High	12	0.938 ± .149		0.770 ± .150	
Intermediate	12	0.927 ± .085	.832	0.786 ± .087	.448
High	12	0.938 ± .149		0.770 ± .150	
One-way ANOVA	52	-	**.010**	-	**.001**

Table 7.6. Differences in total hip and femoral neck BMD in Phase Two study population black patients with either low, intermediate or high FRAX® 10-year probability of hip fracture ("FRAX® hip") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

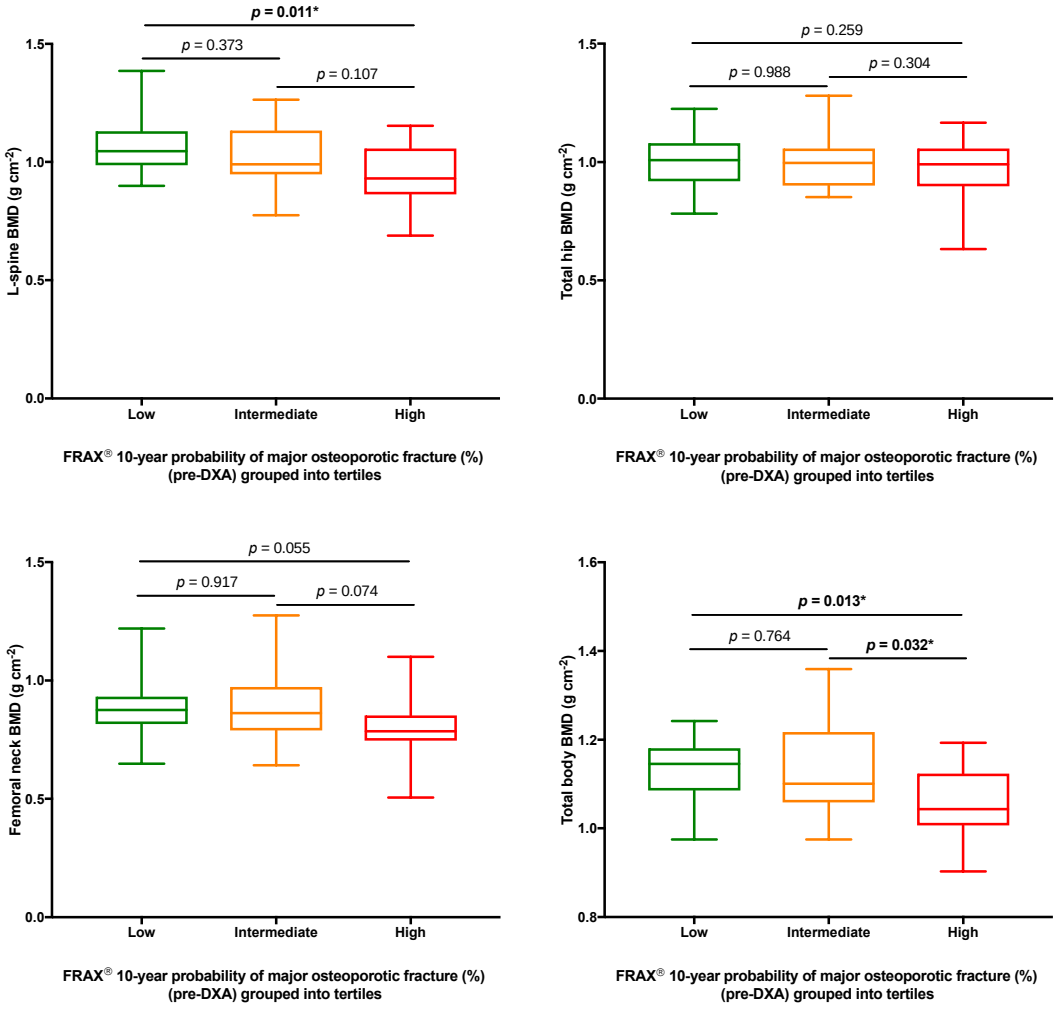


Figure 7.7. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two study population black patients with either low (n = 20), intermediate (n = 16) or high (n = 16) FRAX® 10-year probability of major osteoporotic fracture

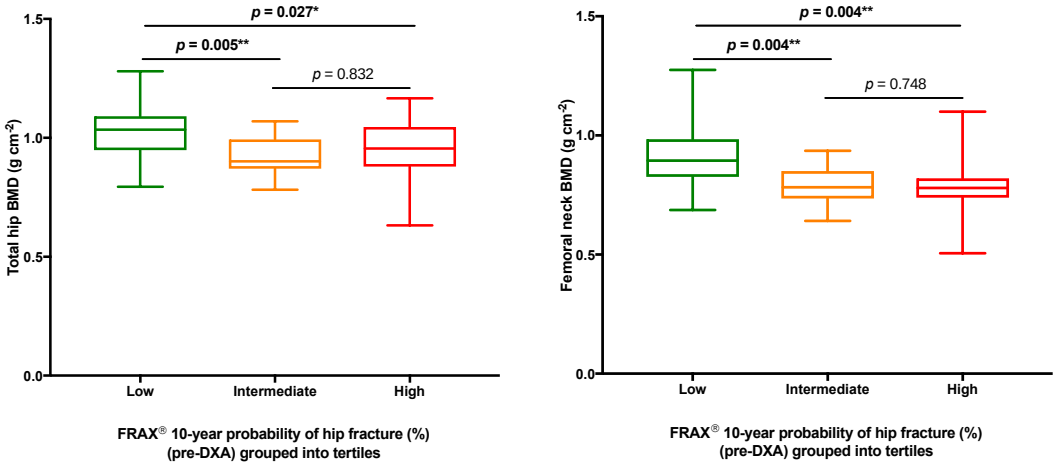


Figure 7.8. Differences in total hip and femoral neck BMD in Phase Two study population black patients with either low (n = 28), intermediate (n = 12) or high (n = 12) FRAX® 10-year probability of hip fracture

In white patients (n = 62), low, intermediate and high "FRAX® major" probabilities were defined as ≤ 2.8% (n = 19), 2.9 – 4.9% (n = 21) and ≥ 5.0% (n = 22) and low, intermediate and high "FRAX® hip" probabilities were defined as ≤ 0.2% (n = 24), 0.3 – 0.7% (n = 18) and ≥ 0.8% (n = 20). Differences in BMD between low, intermediate and high "FRAX® major" probabilities and between low, intermediate and high "FRAX® hip" probabilities are shown for white patients in Tables 7.7 and 7.8 respectively.

There was no significant difference in BMD at any site between white patients with low, intermediate or high "FRAX® major" probabilities (Table 7.7 and Figure 7.9). There was, however a significant difference in hip BMD between white patients with low, intermediate or high "FRAX® hip" probabilities (F (2,59) = 3.794, p = 0.028) (Table 7.8). Both total hip and femoral neck BMD were significantly greater in white patients with low "FRAX® hip" probabilities compared with high FRAX® hip" probabilities (p = 0.008 for total hip, p = 0.044 for femoral neck), but with no significant difference in BMD between white patients with either low or intermediate "FRAX® hip" probabilities, or with either intermediate or high "FRAX® hip" probabilities (Table 7.8 and Figure 7.10).

Correlations between BMD and "FRAX® major" or "FRAX® hip" were further analysed for Phase Two patients within low, intermediate and high "FRAX® major" or "FRAX® hip" probability tertiles (Table 7.9). In general, BMD correlated better with "FRAX® major" in patients within low and intermediate "FRAX® major" probability tertiles (Figures 7.11 and 7.12) and less well in patients within the high "FRAX® major" probability tertile. A not dissimilar pattern was observed for "FRAX® hip", with a significant correlation identified between both total hip and femoral neck BMD and "FRAX® hip" in patients within the low "FRAX® hip" probability tertile (r = -0.379, p = 0.006 for total hip; r = -0.498, p < 0.001 for femoral neck), but with no significant correlation between either total hip or femoral neck BMD with "FRAX® hip" in patients within either the intermediate or high "FRAX® hip" probability tertiles.

	n	L-spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	19	1.004 ± .141	.665	0.979 ± .137	.181	0.815 ± .138	.134	1.123 ± .106	.193
Intermediate	21	0.988 ± .092		0.924 ± .119		0.760 ± .085		1.080 ± .102	
Low	19	1.004 ± .141	.750	0.979 ± .137	.113	0.815 ± .138	.215	1.123 ± .106	.265
High	22	0.990 ± .143		0.908 ± .143		0.764 ± .120		1.084 ± .117	
Intermediate	21	0.988 ± .092	.956	0.924 ± .119	.688	0.760 ± .085	.898	1.080 ± .102	.910
High	22	0.990 ± .143		0.908 ± .143		0.764 ± .120		1.084 ± .117	
One-way ANOVA	62	-	.909	-	.218	-	.259	-	.385

Table 7.7. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two study population white patients with either low, intermediate or high FRAX® 10-year probability of major osteoporotic ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

	n	Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	24	0.988 ± .128	.136	0.817 ± .126	.162
Intermediate	18	0.926 ± .135		0.765 ± .108	
Low	24	0.988 ± .128	**.008**	0.817 ± .126	**.044**
High	20	0.881 ± .125		0.744 ± .103	
Intermediate	18	0.926 ± .135	.297	0.765 ± .108	.557
High	20	0.881 ± .125		0.744 ± .103	
One-way ANOVA	62	-	**.028**	-	.096

Table 7.8. Differences in total hip and femoral neck BMD in Phase Two study population white patients with either low, intermediate or high FRAX® 10-year probability of hip fracture ("FRAX® hip") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor)

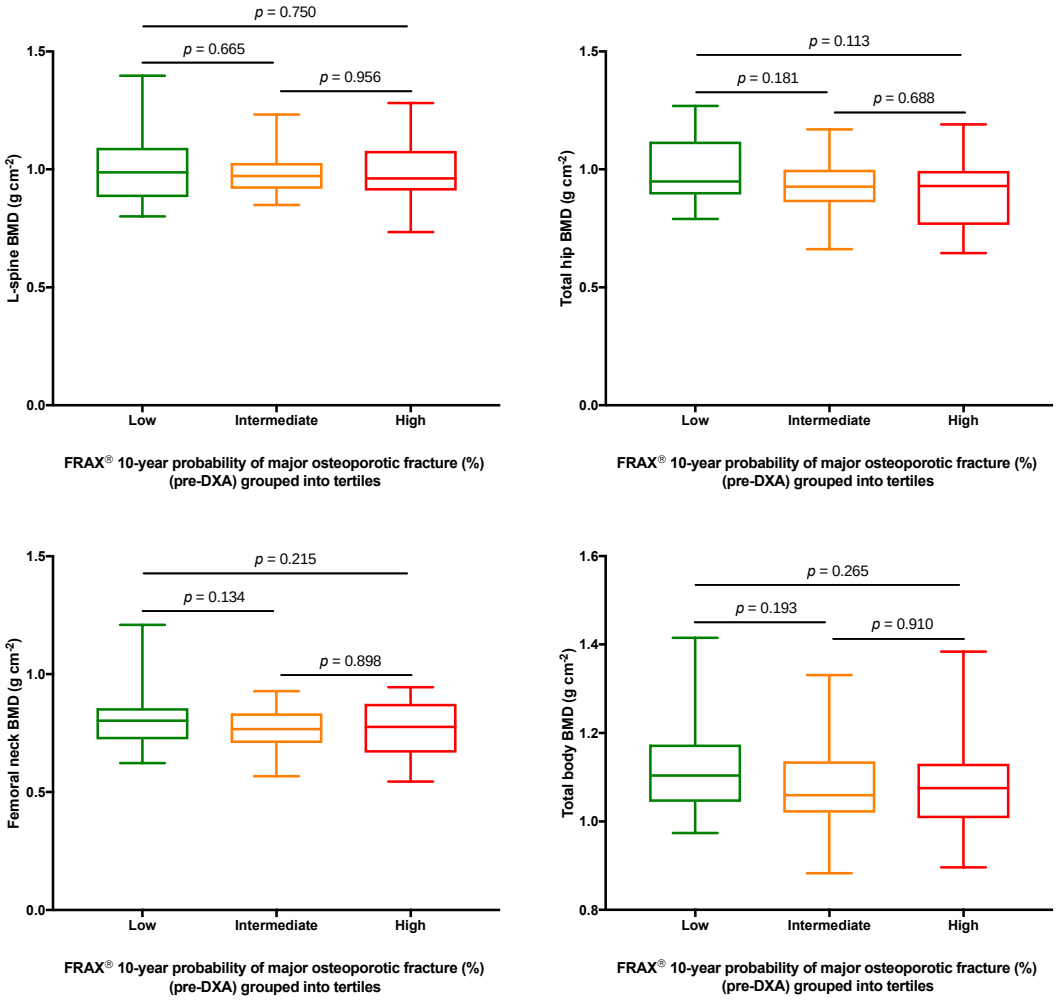


Figure 7.9. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two study population white patients with either low (n = 20), intermediate (n = 21) or high (n = 21) FRAX® 10-year probability of major osteoporotic fracture

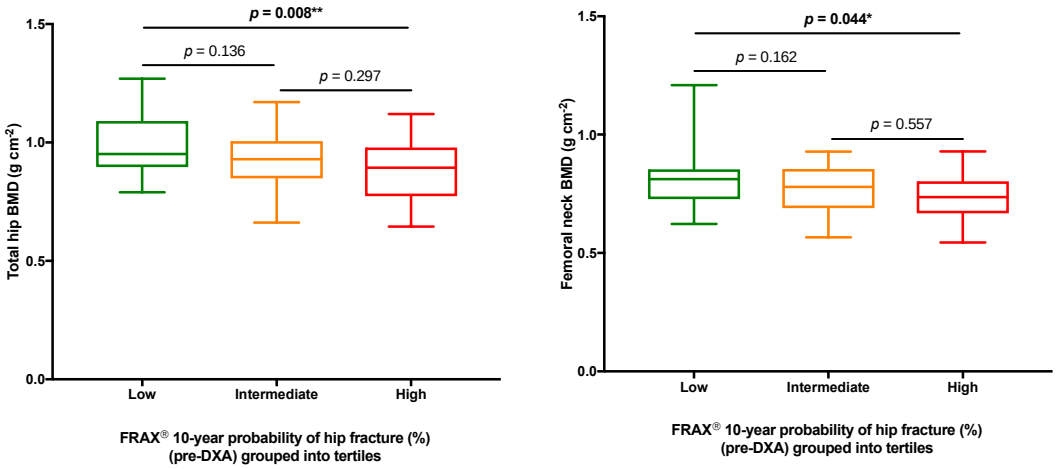


Figure 7.10. Differences in total hip and femoral neck BMD in Phase Two study population white patients with either low (n = 28), intermediate (n = 12) or high (n = 12) FRAX® 10-year probability of hip fracture

BMD site	Low FRAX® probability				Intermediate FRAX® probability				High FRAX® probability			
	FRAX® major (n = 39**)**		**FRAX® hip** (n = 51)		**FRAX® major** (n = 39)		**FRAX® hip** (n = 32)		**FRAX® major** (n = 36)		**FRAX® hip** (n = 31)	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
L-spine	-0.446	**.004**	-	-	-0.076	.664	-	-	-0.167	.330	-	-
Total hip	-0.294	.069	-0.379	**.006**	-0.382	**.017**	-0.187	.305	-0.281	.097	-0.205	.157
Femoral neck	-0.355	**.027**	-0.498	**<.001**	-0.393	**.013**	-0.119	.516	-0.301	.074	0.893	.399
Total body	-0.196	.231	-	-	-0.223	.172	-	-	-0.147	.394	-	-

Table 7.9. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (both calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients with either low, intermediate or high "FRAX® major" or "FRAX® hip" probabilities

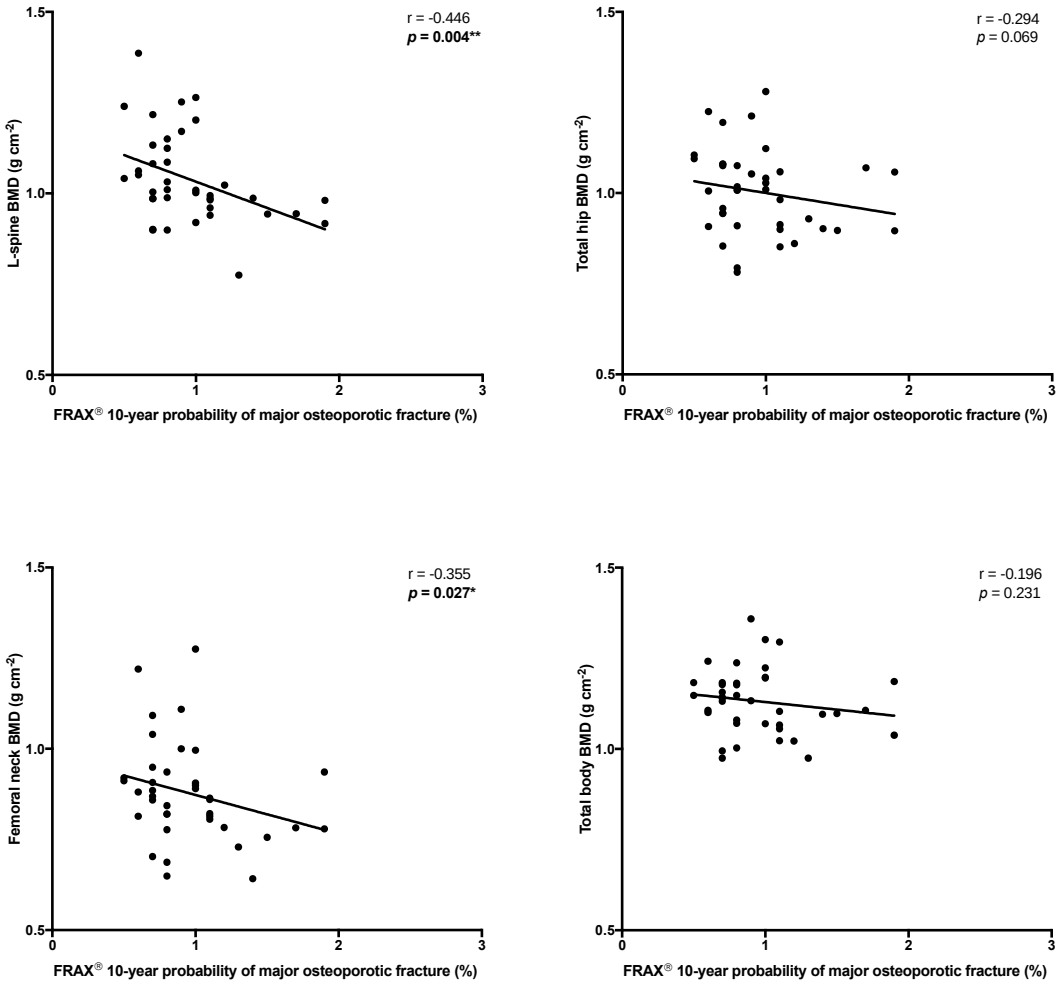


Figure 7.11. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients within the low "FRAX® major" probability tertile (n = 39)

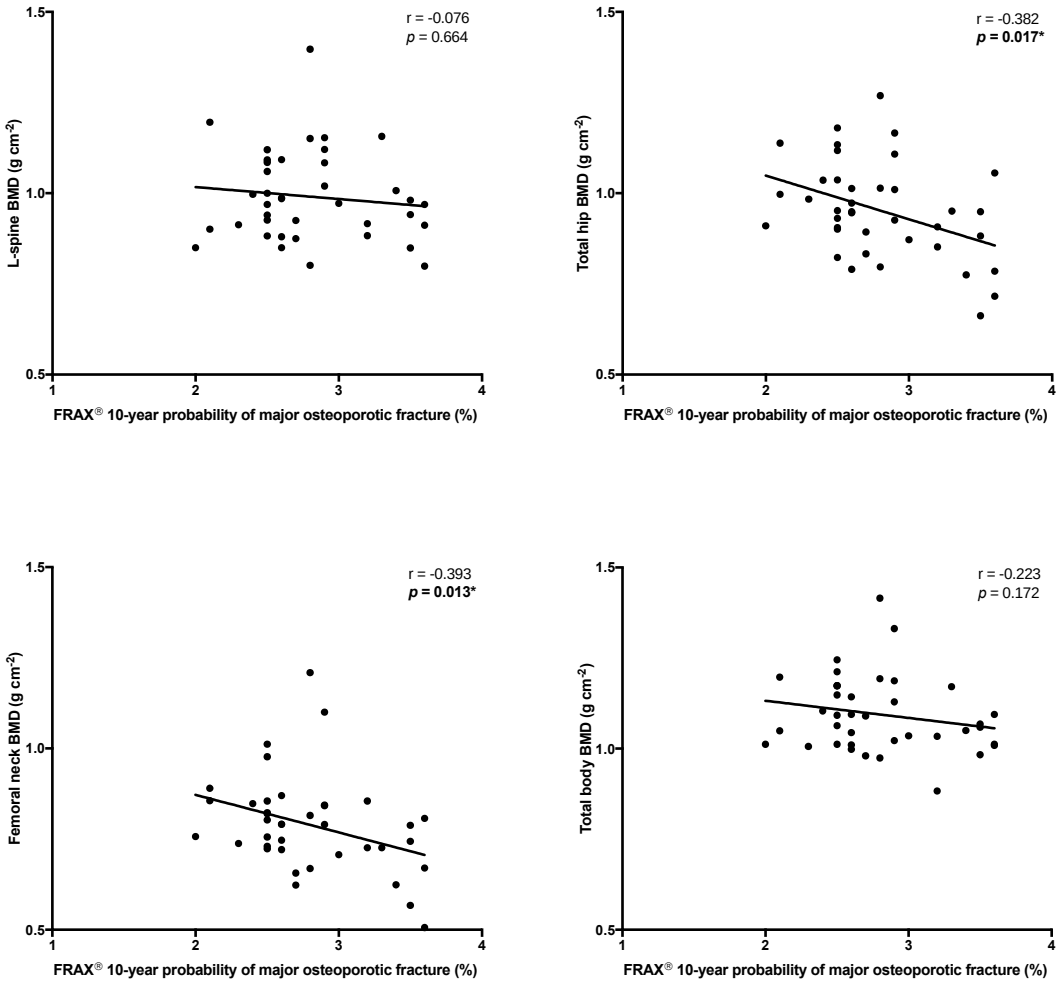


Figure 7.12. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") (calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in Phase Two patients within the intermediate "FRAX® major" probability tertile (n = 39)

7.3 Relationship between "modified" FRAX® 10-year probability of major osteoporotic and hip fracture (calculated with HIV as a "secondary osteoporosis" risk factor, but without femoral neck BMD) and BMD in PLWH

"Modified" FRAX® 10-year probability of major osteoporotic fracture ("modified FRAX® major") and FRAX® 10-year probability of fracture hip fracture ("modified FRAX® hip") were each calculated by incorporating HIV into FRAX® by inclusion of HIV as a "secondary osteoporosis" risk factor.

The distributions of "FRAX® major" and "FRAX® hip" are compared in Table 7.10 to the distributions of "modified FRAX® major" and "modified FRAX® hip" respectively for all, black and white Phase Two patients without one or more "other disorder strongly associated with osteoporosis" (defined in Chapter 4) (n = 75, n = 41 and n = 34 for all, white and black patients respectively). (Phase Two patients with one or more "other disorder strongly associated with osteoporosis" (n = 39, 34.2%) have already had FRAX® probabilities calculated with the presence of at least one "secondary osteoporosis" risk factor.) The changes in median "FRAX® major" and "FRAX® hip" by inclusion of HIV as a "secondary osteoporosis" risk factor were +0.2% and +0.1% respectively in black patients and +0.8% and +0.1% respectively in white patients.

The correlations between "modified FRAX® major" and "modified FRAX® hip" with lumbar spine, total hip, femoral neck and total body BMD are shown in Table 7.11 for all, black and white Phase Two patients without one or more "other disorder strongly associated with osteoporosis". In all patients and in both black and white patient subgroups, both "modified FRAX® major" and "modified FRAX® hip" correlated less well with BMD at each site than unmodified "FRAX® major" and "FRAX® hip" (compare with Table 7.2).

		All patients (n = 75)	Black patients (n = 41)	White patients (n = 34)
"FRAX® major" *%*	Median	2.4	1.0	2.9
	IQR	0.8 – 2.9	0.7 – 1.6	2.5 – 4.0
	Range	0.5 – 11.0	0.5 – 3.6	2.1 – 11.0
"Modified FRAX® major" *%*	Median	3.2	1.2	3.7
	IQR	1.1 – 3.9	1.0 – 2.0	3.3 – 5.5
	Range	0.6 – 15.0	0.6 – 5.2	2.8 – 15.0
"FRAX® hip" *%*	Median	0.1	0.0	0.2
	IQR	0.0 – 0.2	0.0 – 0.1	0.1 – 0.5
	Range	0.0 – 3.3	0.0 – 1.2	0.1 – 3.3
"Modified FRAX® hip" *%*	Median	0.2	0.1	0.3
	IQR	0.1 – 0.4	0.0 – 0.2	0.2 – 0.8
	Range	0.0 – 5.8	0.0 – 2.0	0.1 – 5.8

Table 7.10. Distributions of FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (each calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) compared to the distributions of "modified FRAX® major" and "modified FRAX® hip" (each calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor) in all (n = 75), black (n = 41) and white (n = 34) patients within the Phase Two study population without one or more "other disorder strongly associated with osteoporosis" prior to incorporation of HIV as a "secondary osteoporosis" risk factor

BMD site	All (n = 75)				Black (n = 41)				White (n = 34)			
	Modified FRAX® major		Modified FRAX® hip		Modified FRAX® major		Modified FRAX® hip		Modified FRAX® major		Modified FRAX® hip	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
L-spine	-0.199	.087	-	-	-0.266	.092	-	-	-0.114	.522	-	-
Total hip	-0.265	**.022**	-0.265	**.021**	-0.162	.311	-0.217	.172	-0.184	.299	-0.087	.626
Femoral neck	-0.363	**.001**	-0.360	**.002**	-0.321	**.040**	-0.358	**.022**	-0.166	.347	-0.070	.694
Total body	-0.188	.107	-	-	-0.076	.636	-	-	-0.173	.328	-	-

Table 7.11. Relationship between "modified" FRAX® 10-year probability of major osteoporotic ("modified FRAX® major") and hip fracture ("modified FRAX® hip") (each calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor) and lumbar (L-) spine, total hip, femoral neck and total body BMD in all (n = 75), black (n = 41) and white (n = 34) patients within the Phase Two study population without one or more "other disorder strongly associated with osteoporosis" prior to incorporation of HIV as a "secondary osteoporosis" risk factor

The distributions of “FRAX® major” and “FRAX® hip” are compared to the distributions of “modified FRAX® major” and “modified FRAX® hip” in Table 7.12 for all, black and white patients within the Phase Two study population, irrespective of the presence or absence of one or more “other disorder strongly associated with osteoporosis (n = 114, n = 52 and n = 62 for all, black and white patients respectively). This analysis includes 39 patients with one or more “other disorder strongly associated with osteoporosis” (in whom “modified FRAX® major” and “modified FRAX® hip” are unchanged compared “FRAX® major” and “FRAX® hip” respectively), alongside 75 patients without one or more “other disorder strongly associated with osteoporosis” (in whom “modified FRAX® major” and “modified FRAX® hip” are equal or greater than “FRAX® major” and “modified FRAX® hip” respectively). The changes in median “FRAX® major” and “FRAX® hip” by inclusion of HIV as a “secondary osteoporosis” risk factor were +0.7% and +0.1% respectively in all patients, +0.3% and +0.1% respectively in black patients and +0.6% and +0.0% respectively in white patients.

The correlations between “modified FRAX® major” and “modified FRAX® hip” with lumbar spine, total hip, femoral neck and total body BMD are shown for all, black and white Phase Two patients in Table 7.13 (compare with Table 7.2).

In all patients, there was no difference in correlation with BMD at any site between “FRAX® major” and “modified FRAX® major”, with each having a significant negative correlation with BMD at each site. “Modified FRAX® hip” correlated slightly less well with BMD at the total hip and femoral neck than “FRAX® hip”, albeit with a maintained significant negative correlation between “modified FRAX® hip” and total hip and femoral neck BMD

In black patients, “modified FRAX® major” and “modified FRAX® hip” correlated less well with BMD at each site than “FRAX® major” and “FRAX® hip”, with loss of significance of correlation between “modified FRAX® major” and lumbar spine, total hip and total body BMD, although with maintained significant negative correlation between “modified FRAX® major” and femoral

		All patients (n = 114)	Black patients (n = 52)	White patients (n = 62)
"FRAX® major" *%*	Median	2.6	1.0	3.6
	IQR	1.1 – 4.1	0.7 – 1.9	2.6 – 6.0
	Range	0.5 – 15.0	0.5 – 6.2	1.5 – 15.0
"Modified FRAX® major" *%*	Median	3.3	1.3	4.2
	IQR	1.4 – 5.1	1.0 – 2.5	3.3 – 6.4
	Range	0.6 – 15.0	0.6 – 6.2	1.4 – 15.0
"FRAX® hip" *%*	Median	0.2	0.0	0.4
	IQR	0.0 – 0.5	0.0 – 0.1	0.2 – 0.9
	Range	0.0 – 3.6	0.0 – 1.8	0.1 – 3.6
"Modified FRAX® hip" *%*	Median	0.3	0.1	0.4
	IQR	0.1 – 0.6	0 – 0.2	0.3 – 1.3
	Range	0.0 – 5.8	0 – 2.0	0.1 – 5.8

Table 7.12. Distributions of FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (each calculated without femoral neck BMD and without incorporating HIV as a "secondary osteoporosis" risk factor) compared to the distributions of "modified FRAX® major" and "modified FRAX® hip" (each calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor) in all (n = 114), black (n = 52) and white (n = 62) Phase Two patients, irrespective of the presence or absence of one or more "other disorder strongly associated with osteoporosis" prior to incorporation of HIV as a "secondary osteoporosis" risk factor

BMD site	All (n = 114)				Black (n = 52)				White (n = 62)			
	Modified FRAX® major		Modified FRAX® hip		Modified FRAX® major		Modified FRAX® hip		Modified FRAX® major		Modified FRAX® hip	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
L-spine	-0.271	**.004**	-	-	-0.348	**.012**	-	-	-0.237	.064	-	-
Total hip	-0.328	**<.001**	-0.334	**<.001**	-0.189	.181	-0.282	**.043**	-0.360	**.016**	-0.227	.077
Femoral neck	-0.413	**<.001**	-0.412	**<.001**	-0.404	**.003**	-0.460	**.001**	-0.241	.059	-0.174	.177
Total body	-0.256	**.006**	-	-	-0.240	.087	-	-	-0.213	.097	-	-

Table 7.13. Relationship between modified FRAX® 10-year probability of major osteoporotic fracture ("modified FRAX® major") and hip fracture ("modified FRAX® hip") (each calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor) with lumbar (L-) spine, total hip, femoral neck and total body BMD in all (n = 114), black (n = 52) and white (n = 62) Phase Two patients, irrespective of the presence or absence of one or more "other disorder strongly associated with osteoporosis" prior to incorporation of HIV as a "secondary osteoporosis" risk factor

neck BMD and between “modified FRAX® hip” and both total hip and femoral neck BMD.

In white patients, “modified FRAX® major” correlated better with BMD at all sites compared to “FRAX® major”, with significant negative correlation between “modified FRAX® major” and total hip BMD (p = 0.016). “Modified FRAX® hip” correlated less well with total hip and femoral neck BMD than “FRAX® hip” in white patients, however, with loss of significant correlation with total hip BMD.

Tables 7.14 and 7.15 and Figures 7.13 and 7.14 demonstrate the differences in BMD between white patients with either low, intermediate or high “modified FRAX® major” and “modified FRAX® hip” probabilities. Low, intermediate and high probabilities were defined as ≤ 2.8% (n = 22), 2.9 – 4.1% (n = 20) and ≥ 4.2% (n = 20) for “modified FRAX® major” and ≤ 0.1% (n = 18), 0.2 – 0.4% (n = 24) and ≥ 0.5% (n = 20) for “modified FRAX® hip”.

There was no significant difference in BMD at any site between white patients with low, intermediate or high “modified FRAX® major” probabilities on one-way analysis of variance (Table 7.14 and Figure 7.13), as previously observed in white patients for unmodified “FRAX® major” (Table 7.7 and Figure 7.9).

In contrast to unmodified “FRAX® hip” (Table 7.8 and Figure 7.10), there was no significant difference in total hip BMD between white patients with low, intermediate or high “modified FRAX® hip” probabilities on one-way analysis of variance (Table 7.15 and Figure 7.14). Total hip BMD was significantly greater in white patients with low “modified FRAX® hip” probabilities compared with those with high “modified FRAX® hip” probabilities, however (p = 0.045). Similarly to unmodified “FRAX® hip” (Table 7.8 and Figure 7.10), however, there was no significant difference in femoral neck BMD between white patients with low, intermediate or high “modified FRAX® hip” probabilities on one-way analysis of variance (Table 7.15 and Figure 7.14).

	n	L-spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	24	1.021 ± .141	.242	0.972 ± .146	.188	0.807 ± .132	.272	1.124 ± .108	.101
Intermediate	19	0.977 ± .089		0.919 ± .102		0.769 ± .082		1.072 ± .090	
Low	24	1.021 ± .141	.288	0.972 ± .146	.151	0.807 ± .132	.168	1.124 ± .108	.224
High	19	0.975 ± .136		0.906 ± .145		0.752 ± .123		1.080 ± .123	
Intermediate	19	0.977 ± .089	.960	0.919 ± .102	.758	0.769 ± .082	.627	1.072 ± .090	.814
High	19	0.975 ± .136		0.906 ± .145		0.752 ± .123		1.080 ± .123	
One-way ANOVA	62	-	.395	-	.236	-	.280	-	.239

Table 7.14. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in white Phase Two patients (n = 62) with either low, intermediate or high "modified" FRAX® 10-year probability of major osteoporotic fracture, calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor ("modified FRAX® major")

	n	Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*	
		Mean ± s.d.	*p*	Mean ± s.d.	*p*
Low	24	0.978 ± .129	.090	0.808 ± .122	.173
Intermediate	17	0.906 ± .141		0.758 ± .112	
Low	24	0.978 ± .129	**.045**	0.808 ± .122	.134
High	18	0.898 ± .126		0.755 ± .108	
Intermediate	17	0.906 ± .141	.852	0.758 ± .112	.926
High	18	0.898 ± .126		0.755 ± .108	
One-way ANOVA	62	-	.084	-	.214

Table 7.15. Differences in total hip and femoral neck BMD in white Phase Two patients (n = 62) with either low, intermediate or high "modified" FRAX® 10-year probability of hip fracture, calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor ("modified FRAX® hip")

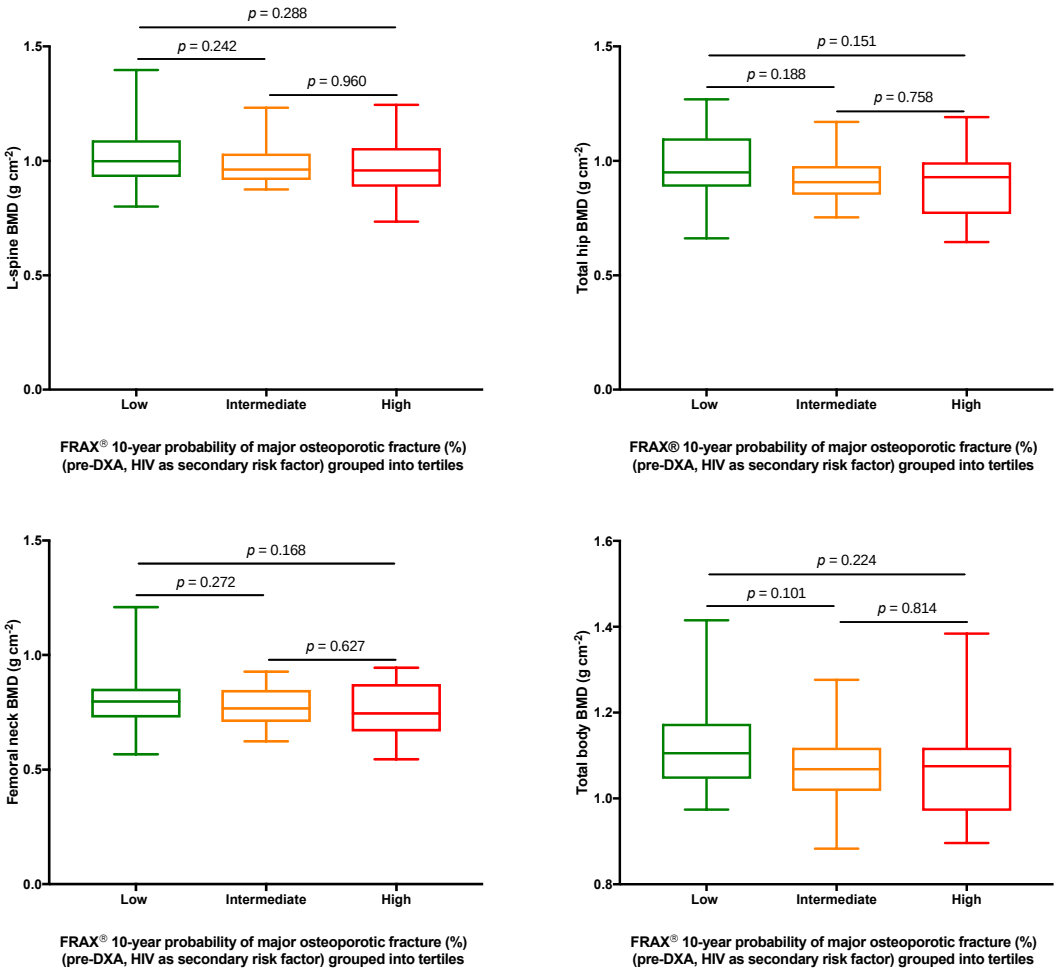


Figure 7.13. Differences in lumbar (L-) spine, total hip, femoral neck and total body BMD in white Phase Two patients with either low (n = 22), intermediate (n = 20) or high (n = 20) "modified" FRAX® 10-year probability of major osteoporotic fracture, calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor ("modified FRAX® major")

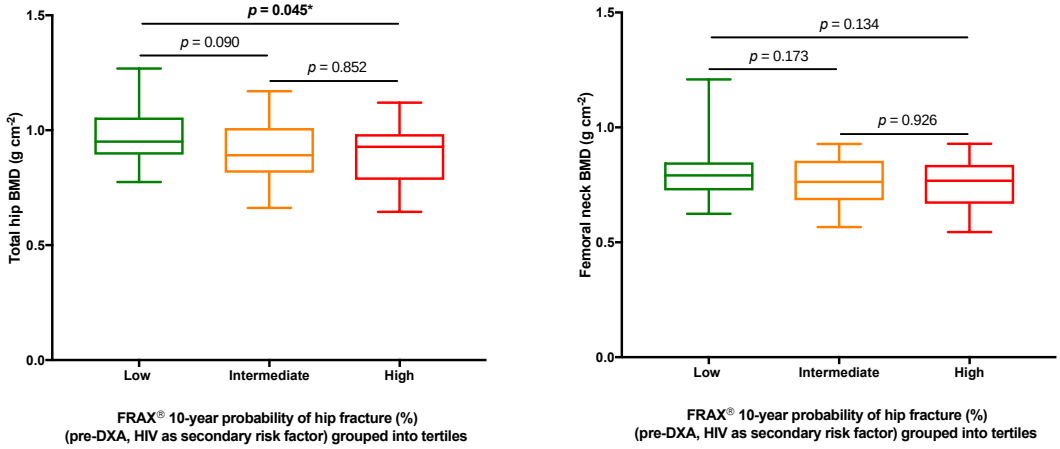


Figure 7.14. Differences in total hip and femoral neck BMD in white Phase Two patients with either low (n = 18), intermediate (n = 24) or high (n = 20) "modified" FRAX® 10-year probability of hip fracture, calculated without femoral neck BMD but with incorporation of HIV as a "secondary osteoporosis" risk factor ("modified FRAX® hip")

7.4 FRAX® 10-year probability of major osteoporotic and hip fracture calculated with femoral neck BMD compared to FRAX® 10-year probability of major osteoporotic and hip fracture calculated without femoral neck BMD in PLWH

The mean difference ± s.d. (positive or negative change) in "FRAX® major with BMD" compared to "FRAX® major" was 0.88 ± 2.02% in all patients, 0.36 ± 0.72% in black patients and 1.31 ± 2.59% in white patients. The mean difference in "FRAX® hip with BMD" compared to "FRAX® hip" was 0.59 ± 2.11% in all patients, 0.18 ± 0.61% in black patients and 0.93 ± 2.76% in white patients. The percentage change in "FRAX® major with BMD" and "FRAX® hip with BMD" compared to FRAX® 10-year probability of major osteoporotic and hip fracture calculated without femoral neck BMD ("FRAX® major" and "FRAX® hip") is shown in Figures 7.15 and 7.16 for black and white patients respectively.

In black patients, "FRAX® major with BMD" decreased by ≥ 1% in 2 (3.8%) patients, decreased by < 1 % in 13 (25%) patients, was unchanged in 10 (19.2%) patients, increased by < 1% in 25 (48.1%) patients and increased by ≥ 1% in 2 (3.8%) patients compared to "FRAX® major". "FRAX® hip with BMD" decreased by ≥ 0.5% in 1 (1.9%) patient, decreased by < 0.5 % in 13 (25.0%) patients, was unchanged in 31 (59.6%) patients, increased by < 0.5% in 5 (9.6%) patients and increased by ≥ 0.5% in 2 (3.8%) patients compared to "FRAX® hip".

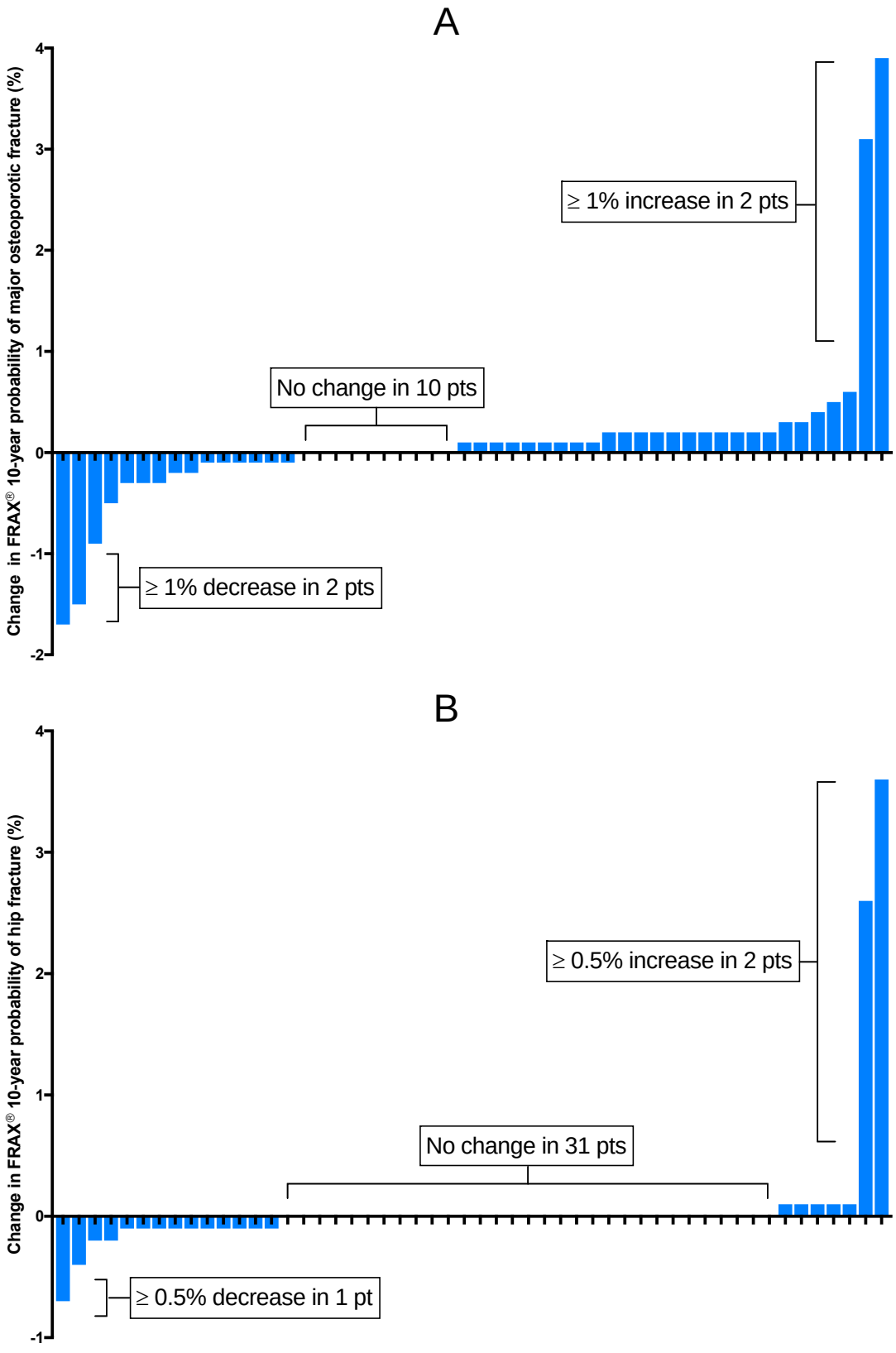


Figure 7.15. Percentage change in FRAX® 10-year probability of major osteoporotic (A) and hip fracture (B) when calculated with femoral neck BMD compared to without femoral neck BMD in black patients within the Phase Two study population (n = 52)

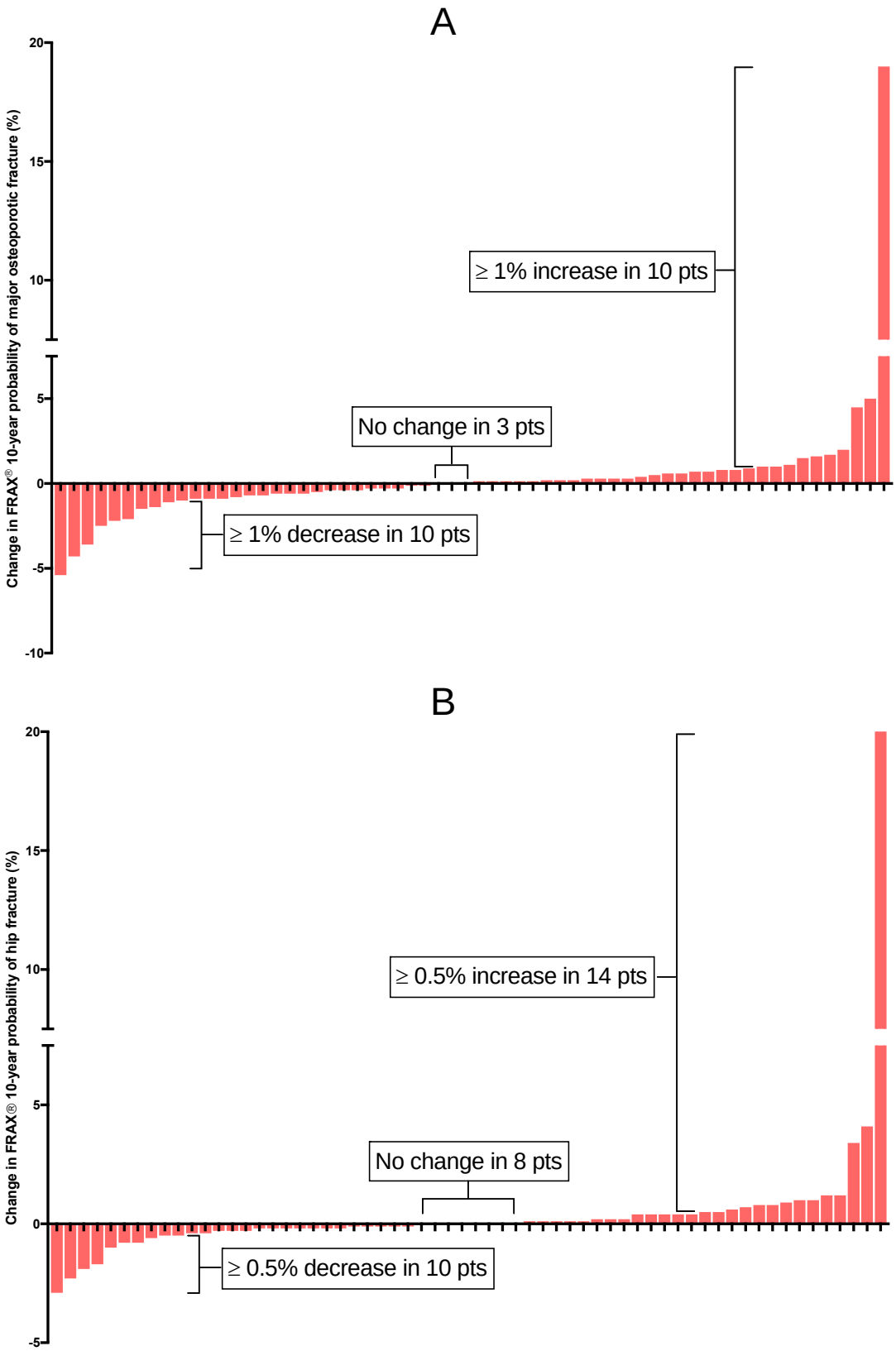


Figure 7.16. Percentage change in FRAX® 10-year probability of major osteoporotic (A) and hip fracture (B) when calculated with femoral neck BMD compared to without femoral neck BMD in white patients within the Phase Two study population (n = 62)

“FRAX® major with BMD” and “FRAX® hip with BMD” increased by ≥ 1% and ≥ 0.5% respectively in the same two black patients. Further characteristics of these two patients are detailed in Table 7.16. Both patients were in the high 10-year probability tertiles for both major osteoporotic and hip fracture in black patients, based on baseline “FRAX® major” and “FRAX® hip”, with each patient having two or more general fracture risk factors (age and alcohol excess in one and low BMI, prior fragility fracture, current smoking, alcohol excess and prolonged immobility in the other). One patient also had HIV disease-specific factors associated with reduced BMD within this cohort, namely long duration of cumulative PI exposure (186 months, with 130 months concurrent exposure to tenofovir DF) and a high percentage of non-classical monocytes (33.5%, with an associated low CD4:CD8 of 0.466).

In white patients, there was greater divergence between FRAX® probabilities calculated with femoral neck BMD compared to those calculated without femoral neck BMD than in black patients (Figure 7.16). “FRAX® major with BMD” decreased by ≥ 1% in 10 (16.1%) patients, decreased by < 1 % in 18 (29.0%) patients, was unchanged in 3 (4.8%) patients, increased by < 1% in 21 (33.9%) patients and increased by ≥ 1% in 10 (16.1%) patients compared to “FRAX® major”. “FRAX® hip with BMD” decreased by ≥ 0.5% in 10 (16.1%) patient, decreased by < 0.5 % in 17 (27.4%) patients, was unchanged in 8 (12.9%) patients, increased by < 0.5% in 13 (25.0%) patients and increased by ≥ 0.5% in 14 (26.9%) patients compared to “FRAX® hip”.

“FRAX® major with BMD” increased by ≥ 5% in two patients. “FRAX® hip with BMD” increased by ≥ 2.5% in the same two patients, as well as in one additional patient. Further characteristics of these three patients are detailed in Table 7.17. The patient with the largest increase in FRAX® scores following inclusion of BMD data had the highest baseline “FRAX® major” and “FRAX® hip” prior to BMD inclusion, with multiple general fracture risk factors. No data was available regarding non-classical monocytes in these three patients, although all three patients had low nadir CD4 cell counts and low CD4:CD8.

	Patient 1	Patient 2
FRAX® major *%*	3.6	6.2
FRAX® major with BMD *%*	7.5	9.3
Increase in FRAX® major with BMD *vs.* FRAX® major *%*	3.9	3.1
FRAX® major with BMD: FRAX® major ratio	2.1	1.5
FRAX® hip *%*	1.2	1.8
FRAX® hip with BMD *%*	3.8	5.4
Increase in FRAX® hip with BMD" *vs.* FRAX® hip *%*	2.6	3.6
FRAX® hip with BMD: FRAX® hip ratio	3.2	3.0
Gender	Male	Male
Age *years*	72	54
BMI *kg m^{-2}*	24.7	20.6
Other general fracture risk factors	Alcohol	Fragility fracture Smoking Alcohol "Other" (immobility)
Years diagnosed with HIV	20.9	12.2
Nadir CD4 cell count *cells µl^{-1}*	190	260
Current CD4 cell count *cells µl^{-1}*	437	657
CD4:CD8 ratio	0.466	0.740
% Non-classical monocytes	33.5	*No data available*
Hepatitis B or C co-infection	No	Yes (HBV)
Duration of cumulative PI exposure *months*	186	0
Duration of cumulative TDF exposure *months*	130	67

Table 7.16. Characteristics of black patients within the Phase Two study population with an increase in either "FRAX® major with BMD" by ≥ 1% compared to "FRAX® major" or "FRAX® hip with BMD" by ≥ 0.5% compared to "FRAX® hip"

	Patient 1	Patient 2	Patient 3
FRAX® major *%*	15.0	3.5	11.0
FRAX® major with BMD *%*	34.0	8.0	16.0
Increase in FRAX® major with BMD *vs.* FRAX® major *%*	19.0	4.5	5.0
FRAX® major with BMD: FRAX® major ratio	2.3	2.3	1.5
FRAX® hip *%*	3.6	0.4	0.7
FRAX® hip with BMD *%*	25.0	4.5	4.1
Increase in FRAX® hip with BMD" *vs.* FRAX® hip *%*	21.4	4.1	3.4
FRAX® hip with BMD: FRAX® hip ratio	6.9	11.3	5.9
NOGG guidance without BMD	Treat	Measure BMD	Measure BMD
NOGG guidance with BMD	Treat	Treat	Treat
Gender	Male	Male	Male
Age *years*	49	35	49
BMI *kg m^{-2}*	28.2	16.9	23.4
Other general fracture risk factors	Fragility fracture Smoking Steroids Alcohol "Other" (immobility & hypogonadim)	None	"Other" (chronic diarrhoea)
Years diagnosed with HIV	22.8	2.3	0.4
Nadir CD4 cell count *cells µl^{-1}*	4	43	3
Current CD4 cell count *cells µl^{-1}*	212	713	20
CD4:CD8 ratio	0.275	0.515	0.232
% Non-classical monocytes	*No data*	*No data*	*No data*
Hepatitis B or C co-infection	No	No	No
Duration of cumulative PI exposure *months*	0	0	0
Duration of cumulative TDF exposure *months*	160	23	15

Table 7.17. Characteristics of white patients within the Phase Two study population with an increase in either "FRAX® major with BMD" by ≥ 5% compared to "FRAX® major" or "FRAX® hip with BMD" by ≥ 2.5% compared to "FRAX® hip"

In these three white patients, as well as in the other 54 white British and Irish patients, for whom NOGG clinical guidance was available as part of the UK FRAX® tool calculation, inclusion of BMD data into FRAX® did not alter the ultimate clinical outcome in any patient with respect to treatment for reduced BMD, compared to clinical management based on guidance generated from FRAX® without BMD data (see Table 7.18).

The difference (positive or negative change) between "FRAX® major with BMD" and "FRAX® hip with BMD" and "FRAX® major" and "FRAX® hip" respectively was significantly greater in patients with higher baseline "FRAX® major" and "FRAX® hip" in all, black and white Phase Two patients (Figure 7.17).

NOGG clinical guidance		Number of patients (%)
Based on "FRAX® major / hip" (without BMD)	**Based on "FRAX® major / hip with BMD"**	
Lifestyle advice and reassure	Lifestyle advice and reassure	36 (63.2)
	Treat	0 (0.0)
Measure BMD	Lifestyle advice and reassure	15 (26.3)
	Treat	4 (7.0)
Treat	Lifestyle advice and reassure	0 (0.0)
	Treat	2 (3.5)

Table 7.18. National Osteoporosis Guideline Group (NOGG) clinical guidance based on FRAX® 10-year probability of major osteoporotic and hip fracture calculated without femoral neck BMD ("FRAX® major" and "FRAX® hip") compared with NOGG clinical guidance based on FRAX® 10-year probability of major osteoporotic and hip fracture calculated with femoral neck BMD ("FRAX® major with BMD" and "FRAX® hip with BMD") in white British and Irish patients within the Phase Two study population (n = 57)

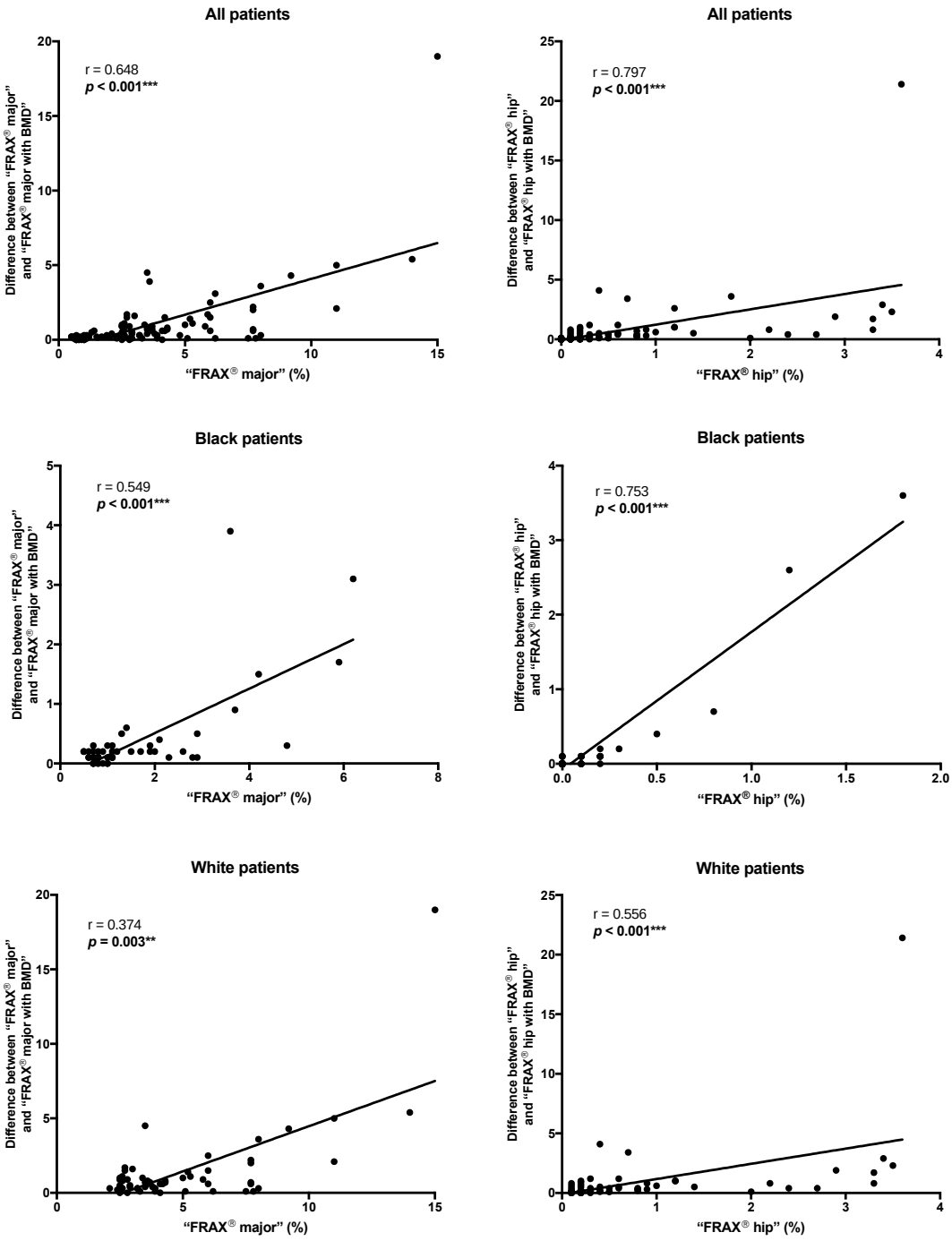


Figure 7.17. Relationship between FRAX® 10-year probability of major osteoporotic fracture ("FRAX® major") and hip fracture ("FRAX® hip") (each calculated without femoral neck BMD and without incorporating HIV as a "secondary risk factor") and the difference between "FRAX® major" and "FRAX® major with BMD" and between "FRAX® hip" and "FRAX® hip with BMD" respectively in all (n = 114), black (n = 52) and white (n = 62) patients within the Phase Two study population

7.5 Consideration of HIV disease-specific factors alongside FRAX® to improve fracture risk assessment in PLWH

Within the Phase Two study population, few HIV disease-specific factors were found to have a significant relationship with BMD: cumulative PI exposure (for total hip and femoral neck BMD only) (Chapter 4); probable PRTD (for lumbar spine BMD only and only identified in one patient) (Chapter 5); and percentage of peripheral blood non-classical monocytes (for lumbar spine BMD only).

Cumulative number of months of PI exposure was not a significant determinant of either total hip or femoral neck BMD when analysed alongside either "FRAX® major" and "FRAX® hip" in multivariate analysis ($p = 0.265$ and $p = 0.305$ with "FRAX® major" and "FRAX® hip" respectively for total hip BMD and $p = 0.258$ and $p = 0.304$ with "FRAX® major" and "FRAX® hip" respectively for femoral neck BMD), in which "FRAX® major" and "FRAX® hip" were both significant predictors of both total hip and femoral neck BMD ($p < 0.001$ and $p = 0.001$ with "FRAX® major" and "FRAX® hip" respectively for total hip BMD; $p < 0.001$ with both "FRAX® major" and "FRAX® hip" for femoral neck BMD).

The percentage of peripheral blood non-classical monocytes was a significant predictor of lumbar spine BMD ($p = 0.007$) when analysed alongside "FRAX® major" in a multivariate analysis, although analysis was only possible in the 26 patients in whom monocyte data was available and in only four white patients.

7.6 Discussion

FRAX® was designed to predict future fragility risk – not BMD. Given the paucity of fragility fractures within the Sheffield HIV Cohort, as well as the lack of prospective fragility fracture incidence data, the analysis within this chapter has focused on the relationship between FRAX® and BMD in PLWH, with BMD used as an acknowledged sub-optimal yet pragmatic alternative to ten

year fragility fracture incidence in order to allow the assessment of FRAX® within this HIV-positive population.

The significant negative correlations observed between “FRAX® major” and each BMD site and between “FRAX® hip” and both total hip and femoral neck BMD is perhaps unsurprising, considering that FRAX®-incorporated general fracture risk factors were the predominant determinants of BMD within this study population (Chapter 4). FRAX® correlated less well with BMD in white patients, however, particularly with respect to “FRAX® major” and both lumbar spine and total body BMD. FRAX® also correlated less well with BMD in high (although predominantly white) fracture risk patients than in low (although predominantly black) fracture risk patients. Furthermore, there was poorer differentiation in BMD between patients of low, intermediate or high “FRAX® major” or “FRAX® hip” probabilities in white patients compared to black patients, between “FRAX® major” and BMD at any site compared to “FRAX® hip” and total hip or femoral neck BMD and between intermediate and high risk patients than between either low and high risk patients or (in general, but not always) between low and intermediate risk patients.

The difference (positive or negative change) between FRAX® calculated with inclusion of femoral neck BMD compared to baseline FRAX® calculated without femoral neck BMD was also greater in white patients than in black patients and in higher fracture risk patients than in lower fracture risk patients, with the difference increasing almost exponentially with higher baseline FRAX®-probabilities, again suggesting that FRAX® correlates better with BMD in black and lower risk HIV-positive patients than in white and higher risk HIV-positive patients. Of note, however, the difference in FRAX® calculated with inclusion of femoral neck BMD compared to baseline FRAX® calculated without femoral neck BMD was not sufficient in any white patient, for whom NOGG clinical management guidance was available, to alter patient clinical outcome in terms of treatment for reduced BMD.

The observation that FRAX® correlates less well with BMD in white and higher risk patients is consistent with other published data. FRAX®-correlated poorly

with BMD in one cross-sectional study of almost entirely male and assumed predominantly white HIV-positive patients in Australia (Calmy *et al.* 2009). Moreover, in both longitudinal studies comparing FRAX® to 10-year fragility fracture incidence, FRAX® was less accurate in higher risk patients, including in older patients (Yin *et al.* 2016, Yang *et al.* 2018).

Why FRAX® correlates less well with BMD in both white and higher risk patients than in black and lower risk patients remains to be confirmed. It seems likely that, whilst perhaps not the main determinants of reduced BMD or of future fragility fracture risk, HIV disease-specific factors still do contribute to reduced BMD and fracture risk in PLWH. The effect of HIV disease-specific factors appears to be minimal when fracture risk attributed to general risk factors is low, but greater when fracture risk attributed to general risk factors is higher, with more exaggerated differences and greater discordance between FRAX® and BMD when fracture risk is high. As black patients generally have higher BMD and lower fracture risk compared to white patients – with FRAX® calculated using race-specific FRAX® tools – this could explain why, in general, FRAX® correlates better with BMD in black patients than in white patients.

Interestingly, modifying FRAX® by including HIV as a "secondary osteoporosis" risk factor, as recommended in some HIV clinical guidelines (EACS 2017), actually weakened the correlation between FRAX® and BMD in black patients and between "FRAX® hip" and hip and femoral neck BMD in white patients, with improvement in correlation observed only between "FRAX® major" and BMD at each site in white patients. One explanation for this observation could be that the addition of HIV as a "secondary osteoporosis" risk factor may overcompensate for any difference caused by HIV disease-specific factors between predicted fracture risk and BMD / actual fracture risk where the difference is small, e.g. in black patients or at the total hip or femoral neck, making "modified" FRAX® less accurate than unmodified FRAX®. Where that difference is larger, however, e.g. in white and higher risk patients or at the lumbar spine or for total body BMD, the addition of HIV as a "secondary osteoporosis" risk factor may partially, but incompletely, address

the difference caused by HIV disease-specific factors between predicted fracture risk and BMD / actual fracture risk, improving the correlation between FRAX® and BMD, although with correlations still not statistically significant.

Our observations are in contrast to the findings of both longitudinal studies, in which “modified” FRAX® was a better predictor of 10-year fragility fracture incidence than unmodified FRAX® (Yin *et al.* 2016, Yang *et al.* 2018). Neither of these two studies included data comparing the performance of FRAX® with or without modification in black *versus* white patient subgroups, however, presenting only pooled population data. In addition, patients in at least one of these studies were significantly older than patients within the Phase Two study population (Yin *et al.* 2016) and therefore with higher baseline fracture risk, in whom “modified” FRAX® may perform better than unmodified FRAX®. In that same study, the very high prevalence of HCV coinfection (33.2%) within the HIV-positive group – an established fracture risk factor (Lo Re *et al.* 2012) also not included within FRAX® – may also contributed to FRAX® performing less well in terms of prediction of actual fragility fracture incidence. The comparatively low prevalence of HCV coinfection within the Phase Two study population (no patients with current HCV infection and only two (1.8%) with past cleared HCV infection) may also explain why FRAX® seemed to perform better within this study, albeit in terms of BMD correlation as opposed to actual fragility fracture incidence.

The absence of an HIV-negative control group in this study precludes comparative analysis of the performance of FRAX® in PLWH *versus* the general population. Other published data suggest that FRAX® performs less well in PLWH compared to HIV-negative control groups, however (Yin *et al.* 2016, Yang *et al.* 2018). With respect to HIV disease-specific factors that may improve fracture risk assessment in PLWH, the duration of cumulative PI exposure did not significantly enhance the prediction of total hip or femoral neck BMD over and above FRAX® in the Phase Two study population in multivariate analysis, in spite of the duration of cumulative PI exposure being a significant independent determinant of total hip and femoral neck BMD in addition to FRAX®-incorporated general risk factors within the Phase Two

study population. The percentage of non-classical monocytes did enhance the prediction of lumbar spine BMD over and above FRAX®, however, albeit within a small subset of 26 Phase Two patients only for whom monocyte data was available and which included only four white patients. The interaction between the relative proportions of monocyte populations, BMD and FRAX® may therefore warrant further study in a larger population with a more equal balance of black and white patients.

Extrapolating these Phase Two study findings to inform recommendations for use in the wider clinical setting is limited by the lack of longitudinal 10-year fracture incidence data. The Phase Two study population is also relatively small and of a relatively young age with relatively low fracture risk overall, which could provide false reassurance as to the validity of FRAX® for use as a fracture risk assessment tool in PLWH.

Nevertheless, with a paucity of published data assessing the performance of FRAX® in PLWH and pending the availability of further prospective 10-year fragility fracture incidence data collected from larger HIV-positive populations, it would seem appropriate, in the interim, to recommend FRAX® as a tool to use for fracture risk assessment in PWLH, particularly in black patients and in lower risk patients, to recommend that modification of FRAX® by incorporating HIV as a “secondary osteoporosis” risk factor should only be considered in non-black patients to assess 10-year probability of non-hip major osteoporotic fracture and to recommend that FRAX® should be sufficient as a practical and inexpensive first-line screening tool to assess fracture risk in PLWH, to further identify patients who require BMD measurement and/or treatment for reduced BMD, without the need to “bypass” FRAX® and perform BMD measurements directly within specific HIV-positive patient groups.

7.7 Conclusions

1. "FRAX® major" and "FRAX® hip" correlate well with BMD at all sites in black patients and should be considered for use without modification for fracture risk assessment in PLWH of black race.
2. "FRAX® major" does not correlate well with BMD at any site in white patients and should not be used without modification for fracture risk assessment in PLWH of white race; "FRAX® hip" does correlate well with total hip BMD in white patients, however, and could be considered for use without modification for hip fracture risk assessment in PLWH of white race.
3. "Modified FRAX® major" and "modified FRAX® hip" – incorporating HIV as a "secondary osteoporosis" risk factor – correlate less well with BMD at all sites in black patients than unmodified "FRAX® major" and "FRAX® hip"; "modified FRAX® major" and "modified FRAX® hip" should therefore not be used for fracture risk assessment in PLWH of black race.
4. "Modified FRAX® major" has improved correlation with BMD at all respective sites in white patients than unmodified "FRAX® major" and could be used pragmatically for fracture risk assessment in PLWH of white race.
5. "Modified FRAX® hip" correlates less well with total hip and femoral neck BMD than unmodified "FRAX® hip" and should therefore not be used for hip fracture risk assessment in PLWH of white race.
6. The difference between "FRAX® major with BMD" and "FRAX® major" and between "FRAX® hip with BMD" and "FRAX® hip" was greater in white patients than in black patients and in patients with high baseline "FRAX® major" and "FRAX® hip" calculated fracture risk than in patients with low baseline fracture risk, but without change to patient clinical outcome; BMD measurement is therefore not necessary for initial fracture risk assessment in PLWH, in whom "FRAX® major" (incorporating HIV as a "secondary osteoporotic" risk factor in white patients) and "FRAX® hip" can be used instead.

7. There may a role for using the percentage of peripheral blood non-classical monocytes alongside FRAX® to improve the prediction of lumbar spine BMD and therefore major osteoporotic fracture risk assessment in PLWH, but evaluation in a larger number of patients and in more white patients is required.

8. Discussion

This thesis represents the first in depth analysis of bone health and fracture risk within the Sheffield HIV Cohort. One of the main attributes of the Sheffield HIV Cohort – and a key strength of this study – is its inclusion of large and relatively equal numbers of both black and white HIV-positive patients. This has enabled a more detailed exploration of the differences in fracture risk factor prevalence, BMD, FRAX® probabilities and the correlation of FRAX® probabilities to BMD within these two distinct subpopulations than in other studies performed in less heterogeneous HIV-positive cohorts.

This study has identified that the prevalence of general fracture risk factors differs between black and white HIV-positive patients living in the UK, with general risk factor prevalence higher in white patients and especially in white male patients. Moreover, BMI (inversely correlated to BMD and fracture risk) was significantly higher in black patients – more specifically in black female patients – compared with white patients. In keeping with these findings, fragility fracture risk attributed to general risk factors, calculated using FRAX®, was higher in white patients than in black patients (although with FRAX® probabilities calculated using race-specific tools already incorporating lower fracture risk in black patients independent of other risk factors). Whilst FRAX® probabilities were higher in white patients, they were still relatively low in the context of the relatively young age of our study population (mean 40.7 ± 9.6 years). Furthermore, within this low fracture risk population, there was a very low prevalence of fragility fractures, reported in five patients only (0.8%), all with at least one general risk factor and three with high FRAX® probabilities.

The Phase One study findings support the notion that general fracture risk factors are the key contributors to fracture risk in PLWH, over and above HIV disease-specific factors. One could therefore postulate that it is an overrepresentation of general risk factors in PLWH and less an effect of other HIV-disease specific factors that results in the higher prevalence of reduced BMD and increased fragility fracture incidence consistently reported in PLWH when compared to HIV-negative control populations (Brown and Qaqish 2006;

Triant *et al.* 2008; Goh *et al.* 2018). As our cohort ages and fracture risk increases, one would then expect fragility fracture incidence to increase, with a greater incidence in white patients with higher prevalence of general risk factors than in black patients. The absence of an HIV-negative control population within which to confirm lower comparative prevalence of general fracture risk factors precludes the ability to confirm this, however. Conversely, it would be reasonable to argue that HIV disease-specific factors do contribute, but that their effect on fracture risk is not yet apparent in our relatively young cohort; several studies have shown that divergence in fracture incidence between HIV-positive and HIV-negative populations may not occur until patients reach their fifth or sixth decades (Triant *et al.* 2008; Gonciulea *et al.* 2017).

The Phase Two study findings were also in agreement with the theory that general risk factors have a greater influence on fracture risk in PLWH than HIV-disease specific factors, however. In this cohort of relatively young age and high BMI, the prevalence of reduced BMD was lower in comparison to other HIV cohorts (Brown and Qaqish 2006; Goh *et al.* 2018), including cohorts with significant proportions of black patients (Arnsten *et al.* 2006, Arnsten *et al.* 2007, Jones *et al.* 2008, Libois *et al.* 2010). Moreover, we identified that the significant independent determinants of BMD in our cohort were all FRAX®-incorporated general risk factors, with BMI the strongest predictor of BMD at all sites. The only HIV disease-specific factor found to have a significant independent association with BMD within our cohort was cumulative PI exposure. It is possible that our study was not sensitive enough – through size or patient selection – to identify other HIV disease-specific factors that might have smaller effects on BMD. Our findings are in accordance with larger studies, however, which have also found general risk factors to be the main significant determinants of BMD and fracture incidence in PLWH (Womack *et al.* 2011; Carr *et al.* 2015) and PI exposure to be one of the most consistently identified HIV disease-specific factors also significantly associated with BMD and fracture incidence in PLWH (Brown and Qaqish 2006; Womack *et al*. 2011).

Regarding 25-OH-D, our findings with respect to its significant independent determinants in PLWH, namely race, season of sampling and efavirenz use, and its lack of relationship with BMD, are in line with other published data in PLWH (Welz *et al.* 2010; Dao *et al.* 2011; Sherwood *et al.* 2012; Cotter, *et al.* 2014; Wohl *et al.* 2014). Whilst vitamin D deficiency is undoubtedly highly prevalent in PLWH, which the results of this study support, it is less clear as to whether or not vitamin D deficiency is significantly more prevalent in PLWH compared to the general population and, moreover, whether or not vitamin D deficiency contributes to the higher prevalence of reduced BMD and increased fragility fracture incidence seen in PLWH. Vitamin D deficiency could still affect fracture risk independently to any effect on BMD and has been linked to an increase risk of hip fracture in older HIV-negative males (Cauley *et al.* 2010). More evidence is needed to determine whether or not vitamin D deficiency is associated with increased fragility fracture prevalence in PLWH, however, and whether or not blanket 25-OH-D testing still has a place in routine fracture risk assessment for all HIV-positive patients, as is currently recommended by some guidelines (EACS 2017).

The significant association between PRTD and reduced BMD, observed within in our study, has also been identified elsewhere (Calmy *et al.* 2009). Our diagnosis of possible PRTD in one Phase Two patient only, in spite of two thirds of patients being on tenofovir DF with or without a PI, also supports other publications reporting PRTD to be very rare in PLWH taking tenofovir DF (Nelson *et al.* 2007, Woodward *et al.* 2009).

In our ART-stable Phase Two cohort, we did not identify any correlation between BMD and markers of inflammation or T cell immune activation. This is in line with the observations of Erlandson *et al.* (2014) from another ART-stable cohort and consistent with the growing consensus that it is baseline levels of markers of inflammation and T cell immune activation prior to ART initiation – and not levels in patients well established on ART – that are the more important determinants of BMD (Hileman *et al.* 2014; Brown *et al.* 2015). We did not identify a significant relationship between CD4:CD8 and BMD either and to our knowledge this has not been reported previously. Moreover,

we did identify a significant negative correlation between the percentage of non-classical monocytes and lumbar spine BMD, another novel finding, albeit within a small subset of 26 Phase Two patients only. Our theory is that, unlike other immune activation markers, non-classical monocytes persist as a footprint of past inflammation and immune dysregulation – supported by data from Han *et al.* (2009) – and could therefore be used to identify patients who experienced greater bone loss following a longer period of uncontrolled HIV viraemia and associated heightened inflammation, immune activation and bone turnover prior to their HIV diagnosis and their initiation and stabilisation on ART, or during subsequent intermittent but unsustained viral replication and immune activation after ART initiation.

We acknowledge BMD as an imperfect means of assessing the validity of FRAX® in PLWH, but argue that other studies that have compared FRAX® to actual ten-year fragility fracture incidence in HIV-positive populations with low fracture incidence (Yin *et al.* 2016; Yang *et al.* 2018) are also imperfect. We maintain, therefore, that the use of BMD to assess the performance of FRAX® within this low fracture prevalence population, in whom ten-year fracture incidence data was also absent, was pragmatic and not unreasonable. Unlike the few other studies that have compared FRAX® with BMD in PLWH (Calmy *et al.* 2009; Gazzola *et al.* 2010; Pepe *et al.* 2012), our study was designed specifically to detect differences in BMD between patients within either low, intermediate and high risk FRAX® probability tertiles and to assess differences in the correlation between BMD and FRAX® probabilities between black and white patients. This is the first study to identify that FRAX® does correlate well with BMD in PLWH overall and specifically in black patients, patients with low FRAX® probabilities and for total hip and femoral neck assessment. Furthermore, there was significant differentiation in BMD between low and high fracture risk tertiles. Importantly, we also observed that modification of FRAX® by including HIV as a “secondary osteoporosis” risk factor – advised by some clinical guidelines (EACS 2017) – actually worsened the correlation between BMD and FRAX® for most patients. The main exception to this was the relationship between FRAX® and lumbar spine and total body BMD in white patients, where FRAX® did not correlate well and was improved by

including HIV as a “secondary osteoporosis” risk factor. The percentage change in FRAX® with femoral neck BMD compared to without was greater in white patients also, especially in higher fracture risk patients, but with no white patients transitioning from low to high fracture risk with inclusion of BMD data and therefore with no alteration to ultimate clinical outcome.

In conclusion, our findings support the notion that FRAX®-incorporated general fracture risk factors are the main determinants of bone health in PLWH and that FRAX® could be an appropriate fracture risk assessment tool for use in HIV clinical management, modifying with inclusion of HIV as a “secondary osteoporosis” risk factor only to assess major osteoporotic fracture risk in white patients. Validation of these findings with actual ten-year fracture incidence will be important. Whilst we will be in a position to collect ten-year follow up data from the Phase One study population from autumn 2019, ideally much larger longitudinal population studies with sufficiently high fracture incidence will be needed to validate the use of FRAX® in PLWH, requiring co-ordinated national or international prospective high quality data collection and, if possible, an HIV-negative control population.

HIV disease-specific factors probably do play a smaller role in bone health in PLWH, which is magnified in higher risk patients. The percentage of non-classical monocytes may be a useful marker to identify patients in whom past or intermittent low-level HIV inflammation and immune activation has had a significant impact on BMD and who might be at higher risk of fracture; our own future work should also focus on exploring this further in a larger subset of patients.

Ultimately, however, with global efforts now focusing on diagnosing and treating HIV earlier in its natural history and with increasing availability of effective PI- and tenofovir DF-free ART regimens, the difference in reduced BMD prevalence and fragility fracture incidence between HIV-positive and HIV-negative populations may diminish over time, giving confidence to HIV clinicians to adopt the same fracture risk assessment tools validated and used in the general population for use in PLWH.

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Appendices

Appendix 1. Chapter 4 supplementary results

Appendix 2. Chapter 5 supplementary results

Appendix 1. Chapter 4 supplementary results

A1.1 Phase Two race / gender patient subgroup analyses

Tables A1.1 to A1.4 show the relationship between age, weight, height and BMI and lumbar spine, total hip, femoral neck and total body BMD in black male, black female, white male and white female patient subgroups respectively.

Tables A1.5 to A1.16 detail the differences in lumbar spine, total hip, femoral neck and total body BMD in black male, black female, white male and white female patient subgroups according to the presence or absence of both FRAX®-incorporated and non-FRAX® general fracture risk factors.

Tables A1.17 to A1.24 relate to HIV disease-specific fracture risk factors unrelated to antiretroviral therapy. Tables A3.17, A3.19, A3.21 and A3.23 describe the relationship between time since HIV diagnosis and nadir CD4 count and lumbar spine, total hip, femoral neck and total body BMD in black male, black female, white male and white female patient subgroups respectively. Tables A1.18, A1.20, A1.22 and A1.24 detail the differences in lumbar spine, total hip, femoral neck and total body BMD in black male, black female, white male and white female patient subgroups according to the presence or absence of other fracture risk factors HIV disease-specific fracture risk factors unrelated to antiretroviral therapy.

Black male patients (n = 15)	**Lumbar spine BMD** *g cm^{-2}*		**Total hip BMD** *g cm^{-2}*		**Femoral neck BMD** *g cm^{-2}*		**Total body BMD** *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age	-0.462	.083	-0.321	.243	-0.200	.474	-0.155	.581
Height	0.338	.218	0.510	.052	0.364	.182	0.392	.149
Weight	0.531	**.042**	0.640	**.010**	0.649	**.009**	0.194	.488
BMI	0.436	**.104**	0.564	**.028**	0.632	**.011**	0.068	.810

Table A1.1. Relationship between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD in black male patients (n=15)

Black female patients (n = 37)	**Lumbar spine BMD** *g cm^{-2}*		**Total hip BMD** *g cm^{-2}*		**Femoral neck BMD** *g cm^{-2}*		**Total body BMD** *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age	-0.084	.623	0.058	.732	-0.213	.205	-0.079	.643
Height	0.248	.139	0.174	.302	0.178	.292	0.134	.428
Weight	0.457	**.004**	0.636	**.000**	0.390	**.017**	0.367	**.026**
BMI	0.405	**.013**	0.665	**.000**	0.398	**.015**	0.367	**.026**

Table A1.2. Relationship between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD in black male patients (n=37)

White male patients (n = 52)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age	-0.055	.697	-0.094	.507	-0.105	.461	-0.139	.327
Height	0.142	.314	0.148	.295	0.104	.464	0.168	.235
Weight	0.404	**.003**	0.348	**.011**	0.297	**.032**	0.191	.176
BMI	0.275	**.049**	0.292	**.036**	0.239	.089	0.128	.365

Table A1.3. Relationship between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD in white male patients (n=52)

White female patients (n = 10)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Age	-0.176	.627	-0.212	.556	-0.103	.777	-0.127	.726
Height	0.480	.160	0.271	.449	0.328	.355	0.480	.161
Weight	0.662	**.037**	0.671	**.034**	0.725	**.018**	0.596	.069
BMI	0.576	.082	0.503	.138	0.733	**.016**	0.273	**.446**

Table A1.4. Relationship between age, height, weight and BMI and lumbar spine, total hip, femoral neck and total body BMD in white female patients (n=10)

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current smoking	No	13 (86.7)	0.992 ± .128	.124	1.019 ± .125	.065	0.859 ± .160	.177	1.113 ± .079	.277
	Yes	2 (13.3)	0.824 ± .192		0.807 ± .247		0.682 ± .197		1.189 ± .151	
Current alcohol ≥3 units d^{-1}	No	11 (73.3)	1.023 ± .101	**.010**	1.052 ± .088	**.004**	0.903 ± .012	**.005**	1.146 ± .780	.100
	Yes	4 (26.7)	0.824 ± .147		0.821 ± .176		0.649 ± .150		1.062 ± .092	
Fragility fracture history	No	13 (86.7)	0.977 ± .119	.619	1.005 ± .117	.383	0.837 ± .143	.907	1.122 ± .092	.861
	Yes	2 (13.3)	0.921 ± .328		0.899 ± .378		0.821 ± .394		1.134 ± .075	
Significant steroid exposure	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Parental hip fracture	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Rheumatoid arthritis	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Other disorders*	No	12 (80.0)	0.992 ± .131	.258	1.020 ± .131	.145	0.864 ± .164	.200	1.138 ± .090	.203
	Yes	3 (20.0)	0.884 ± .183		0.874 ± .210		0.721 ± .170		1.065 ± .052	

*other disorders strongly associated with osteoporosis, including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant recipient and osteogenesis imperfecta (no patients recruited to Phase 2 with latter four disorders)

Table A1.5. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black male patients (n=15) according to presence or absence of FRAX®-incorporated general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Chronic diarrhoea	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Prolonged immobility	No	14 (93.3)	0.991 ± .124	**.035**	1.163 ± .121	**.009**	0.856 ± .154	.071	1.126 ± .898	.633
	Yes	1 (6.7)	0.689		0.632		0.543		1.081	
Male hypogonadism	No	13 (86.7)	0.968 ± .151	.904	0.990 ± .165	.967	0.839 ± .180	.828	1.133 ± .087	.259
	Yes	2 (13.3)	0.982 ± .098		0.995 ± .015		0.810 ± .101		1.057 ± .071	
Chronic obstructive pulmonary disease	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Malabsorption	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Inflammatory bowel disease	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Untreated hyperthyroidism	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.6. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black male patients (n=15) according to presence or absence of FRAX®-incorporated "other disorders strongly associated with osteoporosis"

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current smoking	No	36 (97.3)	1.040 ± .129	.157	0.989 ± .116	.502	0.863 ± .137	.449	1.108 ± .098	.339
	Yes	1 (2.7)	0.850		0.910		0.757		1.012	
Current alcohol ≥3 units d^{-1}	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Fragility fracture history	No	35 (94.6)	1.046 ± .124	**.033**	0.987 ± .117	.982	0.865 ± .139	.373	1.112 ± .095	.137
	Yes	2 (5.4)	0.845 ± .141		0.985 ± .120		0.776 ± .009		1.005 ± .144	
Significant steroid exposure	No	36 (97.3)	1.035 ± .133	.954	0.985 ± .116	.547	0.860 ± .138	.984	1.108 ± .098	.334
	Yes	1 (2.7)	1.027		1.057		0.863		1.011	
Parental hip fracture	No	36 (97.3)	1.034 ± .133	.801	0.985 ± .116	.524	0.861 ± .138	.778	1.107 ± .099	.755
	Yes	1 (2.7)	1.068		1.061		0.822		1.075	
Rheumatoid arthritis	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Other disorders*	No	29 (78.4)	1.046 ± .128	.319	0.100 ± .119	.244	0.868 ± .148	.494	1.128 ± .942	**.007**
	Yes	8 (21.6)	0.993 ± .145		0.945 ± .095		0.831 ± .080		1.026 ± .068	

*other disorders strongly associated with osteoporosis, including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant recipient and osteogenesis imperfecta (no patients recruited to Phase 2 with latter four disorders)

Table A1.7. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black female patients (n=37) according to presence or absence of FRAX®-incorporated general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Chronic diarrhoea	No	34 (91.9)	1.030 ± .131	.468	0.987 ± .117	.996	0.856 ± .140	.537	1.110 ± .100	.363
	Yes	3 (8.1)	1.088 ± .143		0.987 ± .117		0.908 ± .080		1.056 ± .067	
Prolonged immobility	No	35 (94.6)	1.035 ± .135	.934	0.989 ± .115	.705	0.861 ± .140	.840	1.109 ± .100	.407
	Yes	2 (5.4)	1.027 ± .058		0.956 ± .148		0.841 ± .027		1.049 ± .037	
Female hypogonadism	No	34 (91.9)	1.046 ± .126	.068	0.996 ± .116	.119	0.867 ± .140	.332	1.116 ± .094	**.037**
	Yes	3 (8.1)	0.903 ± .143		0.887 ± .023		0.786 ± .018		0.994 ± .080	
Chronic obstructive pulmonary disease	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Malabsorption	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Inflammatory bowel disease	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Untreated hyperthyroidism	No	36 (97.3)	1.038 ± .131	.320	0.990 ± .115	.318	0.863 ± .137	.387	1.109 ± .097	.201
	Yes	1 (2.7)	.904		.872		.742		.981	

Table A1.8. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black female patients (n=37) according to presence or absence of FRAX®-incorporated "other disorders strongly associated with osteoporosis"

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current smoking	No	36 (69.2)	0.997 ± .132	.763	0.945 ± .133	.503	0.788 ± .114	.272	1.103 ± .111	.923
	Yes	16 (30.8)	0.986 ± .089		0.918 ± .117		0.752 ± .085		1.100 ± .076	
Current alcohol ≥3 units d^{-1}	No	38 (73.1)	0.993 ± .125	.884	0.945 ± .141	.449	0.787 ± .113	.248	1.110 ± .109	.368
	Yes	14 (26.9)	0.998 ± .110		0.914 ± .099		0.749 ± .084		1.081 ± .074	
Fragility fracture history	No	48 (92.3)	0.999 ± .109	.362	0.944 ± .125	.165	0.782 ± .102	.222	1.107 ± .102	.260
	Yes	4 (7.7)	0.941 ± .234		0.849 ± .190		0.714 ± .149		1.047 ± .074	
Significant steroid exposure	No	46 (88.5)	0.985 ± .115	.079	0.930 ± .123	.290	0.769 ± .100	.156	1.089 ± .093	**.007**
	Yes	6 (11.5)	1.075 ± .138		0.990 ± .186		0.835 ± .146		1.205 ± .110	
Parental hip fracture	No	50 (96.2)	0.994 ± .119	.869	0.936 ± .125	.818	0.778 ± .102	.792	1.099 ± .100	.358
	Yes	2 (3.8)	1.008 ± .192		0.958 ± .329		0.757 ± .265		1.167 ± .129	
Rheumatoid arthritis	No	51 (98.1)	0.997 ± .119	.225	0.942 ± .126	**.032**	0.781 ± .103	**.045**	1.104 ± .100	.237
	Yes	1 (1.9)	0.849		0.662		0.567		0.983	
Other disorders*	No	30 (57.7)	0.995 ± .125	.967	0.956 ± .133	.232	0.795 ± .100	.156	1.107 ± .098	.696
	Yes	22 (42.3)	0.993 ± .116		0.911 ± .125		0.753 ± .112		1.096 ± .023	

*other disorders strongly associated with osteoporosis, including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant recipient and osteogenesis imperfecta (no patients recruited to Phase 2 with latter four disorders)

Table A1.9. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 white male patients (n=52) according to presence or absence of FRAX®-incorporated general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Chronic diarrhoea	No	43 (82.7)	0.993 ± .126	.939	0.948 ± .131	.172	0.790 ± .106	.050	1.101 ± .103	.930
	Yes	9 (17.3)	0.997 ± .089		0.882 ± .120		0.714 ± .087		1.105 ± .095	
Prolonged immobility	No	44 (84.6)	0.995 ± .109	.900	0.940 ± .129	.721	0.778 ± .097	.945	1.098 ± .094	.532
	Yes	8 (15.4)	0.989 ± .176		0.921 ± .149		0.774 ± .156		1.123 ± .138	
Male hypogonadism	No	47 (90.4)	0.997 ± .125	.587	0.941 ± .129	.517	0.781 ± .105	.341	1.108 ± .103	.188
	Yes	5 (9.6)	0.966 ± .051		0.900 ± .161		0.733 ± .122		1.045 ± .056	
Chronic obstructive pulmonary disease	No	50 (96.2)	0.995 ± .122	.886	0.938 ± .132	.710	0.779 ± .107	.516	1.104 ± .103	.558
	Yes	2 (3.8)	0.982 ± .034		0.903 ± .131		0.728 ± .087		1.061 ± .026	
Malabsorption	No	51 (98.1)	0.995 ± .121	.653	0.937 ± .132	.928	0.779 ± .106	.228	1.103 ± .102	.790
	Yes	1 (1.9)	0.940		0.925		0.649		1.075	
Inflammatory bowel disease	No	51 (98.1)	0.988 ± .114	**.014**	0.934 ± .131	.296	0.774 ± .106	.244	1.102 ± .102	.969
	Yes	1 (1.9)	1.281		1.073		0.901		1.106	
Untreated hyperthyroidism	No	52 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.10. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 white male patients (n=52) according to presence or absence of FRAX®-incorporated "other disorders strongly associated with osteoporosis"

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current smoking	No	7 (70.0)	0.962 ± .059	.433	0.888 ± .120	.259	0.732 ± .068	.105	1.019 ± .081	.241
	Yes	3 (30.0)	1.054 ± .308		1.020 ± .237		0.919 ± .272		1.140 ± .240	
Current alcohol ≥3 units d^{-1}	No	9 (90.0)	0.993 ± .169	.858	0.931 ± .171	.830	0.793 ± .175	.778	1.066 ± .148	.552
	Yes	1 (10.0)	0.960		0.891		0.739		0.961	
Fragility fracture history	No	8 (80.0)	1.000 ± .178	.661	0.943 ± .166	.569	0.784 ± .184	.901	1.072 ± .156	.489
	Yes	2 (20.0)	0.942 ± .033		0.864 ± .183		0.802 ± .107		0.988 ± .062	
Significant steroid exposure	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Parental hip fracture	No	10 (10.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Rheumatoid arthritis	No	10 (10.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Other disorders*	No	4 (40.0)	1.039 ± .239	.456	0.922 ± .237	.942	0.821 ± .263	.635	1.093 ± .218	.525
	Yes	6 (60.0)	.0957 ± .091		0.931 ± .116		0.766 ± .083		1.030 ± .082	

*other disorders strongly associated with osteoporosis, including: chronic diarrhoea, prolonged immobility, hyopgonadism, chronic obstructive pulmonary disease, malabsorption, inflammatory bowel disease, untreated hyperthyroidism, type 1 diabetes mellitus, liver cirrhosis, organ transplant recipient and osteogenesis imperfecta (no patients recruited to Phase 2 with latter four disorders)

Table A1.11. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 white female patients (n=10) according to presence or absence of FRAX®-incorporated general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Chronic diarrhoea	No	7 (70.0)	1.003 ± .193	.703	0.936 ± .192	.810	0.793 ± .197	.887	1.084 ± .165	.360
	Yes	3 (30.0)	0.958 ± .009		0.907 ± .080		0.775 ± .090		0.988 ± .038	
Prolonged immobility	No	8 (80.0)	1.014 ± .164	.358	0.941 ± .176	.625	0.808 ± .181	.472	1.063 ± .159	.730
	Yes	2 (20.0)	0.891 ± .128		0.873 ± .107		0.707 ± .053		1.021 ± .066	
Female hypogonadism	No	9 (90.0)	0.979 ± .165	.560	0.906 ± .156	.236	0.780 ± .175	.696	1.042 ± .146	.418
	Yes	1 (10.0)	1.085		1.118		0.855		1.173	
Chronic obstructive pulmonary disease	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Malabsorption	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Inflammatory bowel disease	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Untreated hyperthyroidism	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.12. Differences in lumbar spine, total hip, femoral neck and total body BMD in all Phase 2 white female patients (n=10) according to presence or absence of FRAX®-incorporated "other disorders strongly associated with osteoporosis"

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
SSRI exposure	No	13 (86.7)	0.985 ± .127	.302	1.017 ± .126	.087	0.854 ± .160	.284	1.128 ± .093	.627
	Yes	2 (13.3)	0.870 ± .256		0.819 ± .264		0.712 ± .239		1.094 ± .018	
PPI exposure more than 5 years	No	14 (93.3)	0.983 ± .139	.226	1.010 ± .138	.060	0.859 ± .148	**.039**	1.132 ± .084	.184
	Yes	1 (6.7)	0.799		0.716		0.506		1.009	
Cannabis use	No	13 (86.7)	0.966 ± .152	.793	0.990 ± .165	.970	0.838 ± .183	.898	1.106 ± .076	**.040**
	Yes	2 (13.3)	0.996 ± .051		0.995 ± .183		0.820 ± .001		1.239 ± .080	
Other recreational drug use	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Significant opiate use	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.13. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black male patients (n=15) according to presence or absence of non-FRAX®-incorporated other general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
SSRI exposure	No	29 (78.4)	1.033 ± .109	.939	0.972 ± .098	.125	0.848 ± .106	.306	1.109 ± .091	.692
	Yes	8 (21.6)	1.038 ± .204		1.042 ± .158		0.905 ± .219		1.093 ± .128	
PPI exposure more than 5 years	No	35 (94.6)	1.022 ± .119	**.013**	0.982 ± .111	.269	0.852 ± .125	.123	1.101 ± .098	.243
	Yes	2 (5.4)	1.254 ± .187		1.076 ± .211		1.000 ± .304		1.186 ± .080	
Cannabis use	No	36 (97.3)	1.035 ± .133	.962	0.984 ± .115	.305	0.859 ± .138	.664	1.104 ± .099	.432
	Yes	1 (2.7)	1.041		1.105		0.920		1.183	
Other recreational drug use	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Significant opiate use	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Primary amenorrhoea	No	30 (81.1)	1.029 ± .133	.591	0.982 ± .108	.574	0.852 ± .023	.481	1.102 ± .097	.655
	Yes	7 (18.9)	1.059 ± .126		1.010 ± .149		0.893 ± .174		1.121 ± .111	
Secondary amenorrhoea	No	17 (45.9)	1.041 ± .153	.787	1.000 ± .117	.532	0.872 ± .145	.641	1.101 ± .119	.809
	Yes	20 (54.1)	1.029 ± .113		0.976 ± .116		0.850 ± .132		1.109 ± .079	
Depo-provera® use	No	24 (64.9)	1.039 ± .142	.774	0.993 ± .125	.656	0.875 ± .157	.370	1.109 ± .113	.783
	Yes	13 (35.1)	1.026 ± .113		0.975 ± .097		0.832 ± .087		1.100 ± .067	

Table A1.14. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 black female patients (n=37) according to presence or absence of non-FRAX®-incorporated other general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
SSRI exposure	No	34 (65.4)	0.992 ± .121	.862	0.938 ± .121	.907	0.778 ± .102	.888	1.095 ± .090	.492
	Yes	18 (34.6)	0.998 ± .122		0.934 ± .152		0.774 ± .117		1.115 ± .121	
PPI exposure more than 5 years	No	49 (94.2)	0.995 ± .123	.791	0.935 ± .128	.690	0.773 ± .104	.312	1.100 ± .102	.511
	Yes	3 (5.8)	0.976 ± .019		0.966 ± .198		0.838 ± .157		1.140 ± .096	
Cannabis use	No	37 (71.2)	0.997 ± .104	.809	0.949 ± .135	.316	0.781 ± .111	.656	1.095 ± .097	.459
	Yes	15 (28.8)	0.988 ± .157		0.908 ± .120		0.766 ± .096		1.119 ± .112	
Other recreational drug use	No	39 (75.0)	1.009 ± .113	.111	0.948 ± .135	.281	0.781 ± .111	.628	1.111 ± .111	.238
	Yes	13 (25.0)	0.948 ± .132		0.903 ± .115		0.764 ± .094		1.073 ± .058	
Significant opiate use	No	51 (98.1)	0.991 ± .119	.210	0.932 ± .127	**.049**	0.774 ± .105	.112	1.099 ± .099	.120
	Yes	1 (1.9)	1.144		1.191		0.944		1.258	

Table A1.15. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 white male patients (n=52) according according to presence or absence of non-FRAX®-incorporated other general fracture risk factors

Fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
SSRI exposure	No	3 (30.0)	0.948 ± .033	.621	0.879 ± .061	.568	0.726 ± .017	.477	1.024 ± .049	.683
	Yes	7 (70.0)	1.007 ± .191		0.948 ± .191		0.814 ± .197		1.068 ± .171	
PPI exposure more than 5 years	No	8 (80.0)	1.017 ± .163	.306	0.948 ± .175	.450	0.809 ± .181	.460	1.077 ± .154	.364
	Yes	2 (20.0)	0.881 ± .112		0.844 ± .067		0.704 ± .049		0.968 ± .009	
Cannabis use	No	6 (60.0)	0.962 ± .065	.537	0.887 ± .132	.366	0.731 ± .074	.198	1.028 ± .084	.502
	Yes	4 (40.0)	1.031 ± .256		0.988 ± .204		0.874 ± .239		1.095 ± .215	
Other recreational drug use	No	9 (90.0)	0.993 ± .169	.858	0.931 ± .171	.830	0.793 ± .175	.778	1.066 ± .148	.522
	Yes	1 (10.0)	0.960		0.891		0.739		0.961	
Significant opiate use	No	9 (90.0)	1.011 ± .154	.232	0.942 ± .165	.429	0.801 ± .171	.485	1.064 ± .149	.582
	Yes	1 (10.0)	0.801		0.797		0.669		0.974	
Primary amenorrhoea	No	10 (10.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Secondary amenorrhoea	No	9 (90.0)	0.993 ± .169	.858	0.931 ± .171	.830	0.793 ± .175	.778	1.066 ± .148	.522
	Yes	1 (10.0)	0.960		0.891		0.739		0.961	
Depo-provera® use	No	7 (70.0)	0.945 ± .085	.190	0.897 ± .131	.399	0.758 ± .078	.422	1.028 ± .077	.390
	Yes	3 (30.0)	1.094 ± .263		0.998 ± .237		0.857 ± .310		1.118 ± .257	

Table A1.16. Differences in lumbar spine, total hip, femoral neck and total body BMD in Phase 2 white female patients (n=10) according to presence or absence of non-FRAX®-incorporated other general fracture risk factors

HIV disease-specific fracture risk factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Time since HIV diagnosis *years*	-0.568	**.027**	-0.322	.226	-0.311	.260	-0.489	.064
Nadir CD4 count[a] *cells/µL*	-0.455	.102	-0.582	**.029**	-0.719	**.004**	-0.248	.392

[a] 1 missing value (n=14)

Table A1.17. Relationship between time since HIV diagnosis and nadir CD4 count and lumbar spine, total hip, femoral neck and total body BMD in black male patients (n=15)

HIV disease-specific fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Mode of HIV acquisition	Hetero	14 (93.3)	0.971 ± .148	.945	0.991 ± .159	.954	0.836 ± .176	.933	1.111 ± .076	**.036**
	MSM	1 (6.7)	0.960		0.982		0.821		1.295	
Serious illness at time of HIV diagnosis	No	12 (80.0)	0.945 ± .118	.255	0.982 ±.127	.674	0.819 ± .118	.483	1.126 ± .090	.811
	Yes	3 (20.0)	1.056 ± .225		1.026 ± .268		0.899 ± .341		1.112 ± .092	
Previous intensive care admission	No	15 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Chronic HBV infection	No	14 (93.3)	0.990 ± .124	**.035**	1.016 ± .121	**.009**	0.856 ± .154	.071	1.126 ± .090	.633
	Yes	1 (6.7)	0.689		0.632		0.543		1.081	
Current or past chronic HCV infection	No	14 (93.3)	0.971 ± .148	.945	0.991 ± .159	.954	0.836 ± .176	.933	1.111 ± .076	**.036**
	Yes	1 (6.7)	0.960		0.982		0.821		1.295	
Lipodystrophy	No	13 (86.7)	0.971 ± .153	.935	0.993 ± .162	.901	0.834 ± .179	.930	1.121 ± .092	.759
	Yes	2 (13.3)	0.962 ± .027		0.977 ± .114		0.846 ± .127		1.142 ± .062	

Table A1.18. Differences in lumbar spine, total hip, femoral neck and total body BMD in black male patients (n=15) according to presence or absence of HIV disease-specific fracture risk factors ("Hetero" = HIV acquisition through sexual intercourse between a man and woman, "MSM" = HIV acquisition through sexual intercourse between men)

HIV disease-specific fracture risk factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Time since HIV diagnosis *years*	0.013	.941	0.007	.967	-0.044	.796	-0.060	.725
Nadir CD4 count[a] *cells/µL*	-0.177	.302	0.031	.859	-0.053	.761	0.083	.632

[a] 1 missing value (n=36)

Table A1.19. Relationship between time since HIV diagnosis and nadir CD4 count and lumbar spine, total hip, femoral neck and total body BMD in black female patients (n=37)

HIV disease-specific fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Mode of HIV acquisition	Hetero	37 (100.0)	-	-	-	-	-	-	-	-
	MSM	0 (0.0)	-		-		-		-	
Serious illness at time of HIV diagnosis	No	22 (59.5)	1.007 ± .138	.130	0.973 ± .103	.379	0.843 ± .133	.348	1.086 ± .092	.136
	Yes	15 (40.5)	1.074 ± .113		1.008 ± .132		0.886 ± .142		1.135 ± .102	
Previous intensive care admission	No	34 (91.9)	1.030 ± .131	.505	0.979 ± .117	.170	0.862 ± .141	.779	1.105 ± .102	.939
	Yes	3 (8.1)	1.084 ± .149		1.075 ± .018		0.839 ± .067		1.110 ± .037	
Chronic HBV infection	No	36 (97.3)	1.030 ± .130	.200	0.986 ± .116	.639	0.859 ± .138	.828	1.103 ± .098	.348
	Yes	1 (2.7)	1.202		1.041		0.890		1.198	
Current or past chronic HCV infection	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Lipodystrophy	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.20. Differences in lumbar spine, total hip, femoral neck and total body BMD black female patients (n=37) according to presence or absence of HIV disease-specific fracture risk factors ("Hetero" = HIV acquisition through sexual intercourse between a man and woman, "MSM" = HIV acquisition through sexual intercourse between men)

HIV disease-specific fracture risk factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Time since HIV diagnosis *years*	-0.136	.337	-0.043	.760	-0.047	.743	-0.102	.473
Nadir CD4 count *cells/µL*	0.071	.618	0.142	.314	0.121	.393	0.008	.958

Table A1.21. Relationship between time since HIV diagnosis and nadir CD4 count and lumbar spine, total hip, femoral neck and total body BMD in white male patients (n=52)

HIV disease-specific fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Mode of HIV acquisition	Hetero	14 (26.9)	1.043 ± .142	.085	0.935 ± .109	.947	0.789 ± .097	.697	1.125 ± .119	.340
	MSM	37 (71.2)	0.977 ± .108		0.938 ± .141		0.776 ± .110		1.094 ± .095	
Serious illness at time of HIV diagnosis	No	40 (76.9)	1.010 ± .125	.089	0.949 ± .123	.235	0.785 ± .098	.293	1.103 ± .106	.876
	Yes	12 (23.1)	0.942 ± .086		0.897 ± .152		0.748 ± .132		1.098 ± .084	
Previous intensive care admission	No	47 (90.4)	0.991 ± .117	.602	0.938 ± .132	.808	0.776 ± .101	.906	1.100 ± .105	.627
	Yes	5 (9.6)	1.021 ± .158		0.923 ± .126		0.782 ± .162		1.123 ± .054	
Chronic HBV infection	No	50 (96.2)	0.994 ± .121	.892	0.935 ± .133	.630	0.775 ± .108	.537	1.104 ± .102	.511
	Yes	2 (3.8)	1.006 ± .124		0.981 ± .011		0.823 ± .067		1.055 ± .054	
Current or past chronic HCV infection	No	52 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Lipodystrophy	No	42 (80.8)	0.986 ± .107	.339	0.935 ± .129	.868	0.771 ± .109	.397	1.104 ± .105	.771
	Yes	10 (19.2)	1.027 ± .168		0.943 ± .143		0.803 ± .093		1.094 ± .086	

Table A1.22. Differences in lumbar spine, total hip, femoral neck and total body BMD in white male patients (n=52) according to presence or absence of HIV disease-specific fracture risk factors ("Hetero" = HIV acquisition through sexual intecourse between a man and woman, "MSM" = HIV acquisition through sexual intercourse between men)

HIV disease-specific fracture risk factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Time since HIV diagnosis *years*	0.055	.881	0.248	.489	0.236	.511	0.285	.425
Nadir CD4 count *cells/µL*	-0.236	.511	-0.200	.580	-0.418	.229	0.212	.556

Table A1.23. Relationship between time since HIV diagnosis and nadir CD4 count and lumbar spine, total hip, femoral neck and total body BMD in white female patients (n=10)

HIV disease-specific fracture risk factor	Risk factor present	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Mode of HIV acquisition	Hetero	9 (90.0)	-	-	-	-	-	-	-	-
	MSM	0 (0.0)	-		-		-		-	
Serious illness at time of HIV diagnosis	No	8 (80.0)	0.994 ± .180	.862	0.929 ± .183	.948	0.780 ± .187	.686	1.065 ± .158	.681
	Yes	2 (20.0)	0.971 ± .015		0.920 ± .041		0.752 ± .003		1.015 ± .076	
Previous intensive care admission	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Chronic HBV infection	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Current or past chronic HCV infection	No	9 (90.0)	1.011 ± .154	.232	0.942 ± .165	.429	0.801 ± .171	.485	1.064 ± .149	.582
	Yes	1 (10.0)	0.801		0.797		0.669		0.974	
Lipodystrophy	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	

Table A1.24. Differences in lumbar spine, total hip, femoral neck and total body BMD in white female patients (n=10) according to presence or absence of HIV disease-specific fracture risk factors ("Hetero" = HIV acquisition through sexual intercourse between a man and woman, "MSM" = HIV acquisition through sexual intercourse between men)

HIV antiretroviral therapy-related factor	Exposure or presence	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current ART	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	15 (0.0)	-		-		-		-	
Ever ART	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	15 (0.0)	-		-		-		-	
Plasma HIV RNA <40 copies/ml	No	2 (13.3)	0.871 ± .102	.307	0.807 ± .128	.064	0.631 ± .176	.063	1.054 ± .063	.237
	Yes	13 (86.7)	0.985 ± .144		1.019 ± .139		0.867 ± .151		1.134 ± .087	
Plasma HIV RNA <200 copies/ml	No	1 (6.7)	0.799	.226	0.716	.060	0.506	**.039**	1.009	.184
	Yes	14 (93.3)	0.982 ± .139		1.010 ± .138		0.859 ± .148		1.132 ± .084	

Table A1.25. Differences in lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15) according to exposure to or presence of general HIV antiretroviral therapy (ART)-related factors

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on ART	-0.282	.308	-0.254	.362	-0.111	.694	-0.389	.152
Cumulative number of months ever on ART	-0.368	.177	-0.296	.283	-0.161	.567	-0.521	**.046**

Table A1.26. Relationship between continuous number of months on antiretroviral therapy (ART) at time of BMD measurement or cumulative number of months ever on ART with lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NRTI	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	15 (0.0)	-		-		-		-	
Ever NRTI	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	15 (0.0)	-		-		-		-	
Current TDF	No	4 (3.5)	0.900 ± .145	.263	0.956 ± .221	.611	0.843 ± .204	.915	1.140 ± .076	.669
	Yes	11 (96.5)	0.996 ± .139		1.003 ± .132		0.832 ± .166		1.117 ± .094	
Ever TDF	No	3 (20.0)	0.970 ± .046	.999	1.064 ± .057	.375	0.944 ± .049	.227	1.160 ± .080	.437
	Yes	12 (80.0)	0.970 ± .159		0.972 ± .165		0.808 ± .179		1.114 ± .090	
Current ABC	No	11 (96.5)	0.996 ± .139	.263	1.003 ± .132	.611	0.832 ± .166	.915	1.117 ± .094	.669
	Yes	4 (3.5)	0.900 ± .145		0.956 ± .221		0.843 ± .204		1.140 ± .076	
Ever ABC	No	9 (60.0)	0.984 ± .143	.659	0.997 ± .130	.850	0.811 ± .155	.517	1.112 ± .101	.544
	Yes	6 (40.0)	0.949 ± .151		0.981 ± .195		0.872 ± .197		1.141 ± .066	
Current AZT	No	14 (93.3)	0.990 ± .124	**.035**	1.016 ± .121	**.009**	0.856 ± .154	.071	1.126 ± .090	.633
	Yes	1 (6.7)	0.689		0.632		0.543		1.081	
Ever AZT	No	9 (60.0)	0.996 ± .092	.416	1.037 ± .088	.155	0.881 ± .114	.289	1.149 ± .086	.166
	Yes	6 (40.0)	0.932 ± .201		0.921 ± .208		0.767 ± .224		1.085 ± .081	

Table A1.27. Differences in lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15)) according to current or ever exposure to at least one nucleos(t)ide reverse transcriptase inhibitor (NRTI), tenofovir disoproxil fumarate (TDF), abacavir (ABC) and zidovudine (AZT)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NRTI	-0.104	.713	-0.100	.723	0.096	.732	-0.196	.483
Cumulative number of months ever on NRTI	-0.286	.302	-0.246	.376	-0.096	.732	-0.475	.074
Continuous number of months on TDF	0.227	.416	-0.088	.754	-0.130	.645	-0.243	.382
Cumulative number of months ever on TDF	-0.172	.540	-0.409	.130	-0.441	.100	-0.573	**.025**
Continuous number of months on ABC	-0.243	.383	0.096	.733	0.270	.330	0.101	.721
Cumulative number of months ever on ABC	-0.157	.576	0.105	.710	0.337	.220	0.155	.580
Continuous number of months on AZT	-0.433	.107	-0.433	.107	-0.371	.173	-0.186	.508
Cumulative number of months ever on AZT	-0.145	.606	-0.338	.217	-0.302	.274	-0.306	.267

Table A1.28. Relationship between continuous number of months on or cumulative number of months ever on either at least one NRTI (nucleos(t)ide reverse transcriptase inhibitors), tenofovir disoproxil fumarate (TDF), abacavir (ABC) or zidovudine (AZT) with lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NNRTI	No	7 (46.7)	0.920 ± .146	.212	0.903 ± .167	**.032**	0.738 ± .158	**.031**	1.074 ± .052	**.035**
	Yes	8 (53.3)	1.014 ± .132		1.067 ± .092		0.920 ± .135		1.167 ± .092	
Ever NNRTI	No	3 (20.0)	0.911 ± .218	.442	0.906 ± .239	.299	0.761 ± .192	.419	1.100 ± .042	.617
	Yes	12 (80.0)	0.985 ± .126		1.012 ± .130		0.854 ± .167		1.129 ± .096	
Current EFV	No	9 (60.0)	0.953 ± .148	.579	0.950 ± .173	.212	0.800 ± .189	.344	1.099 ± .067	.197
	Yes	6 (40.0)	0.996 ± .142		1.053 ± .098		0.888 ± .133		1.150 ± .107	
Ever EFV	No	4 (26.7)	0.929 ± .181	.515	0.944 ± .210	.495	0.805 ± .179	.693	1.121 ± .055	.957
	Yes	11 (73.3)	0.985 ± .132		1.010 ± .135		0.846 ± .173		1.124 ± .099	
Current NVP	No	14 (93.3)	0.969 ± .148	.940	0.986 ± .157	.666	0.828 ± .173	.559	1.119 ± .089	.478
	Yes	1 (6.7)	0.981		1.058		0.936		1.186	
Ever NVP	No	14 (93.3)	0.969 ± .148	.940	0.986 ± .157	.666	0.828 ± .173	.559	1.119 ± .089	.478
	Yes	1 (6.7)	0.981		1.058		0.936		1.186	

Table A1.29. Differences in lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15) according to current or ever exposure to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NNRTI	0.398	.141	0.575	**.025**	0.590	**.021**	0.570	**.027**
Cumulative number of months ever on NNRTI	0.186	.506	0.290	.294	0.319	.247	0.090	.751
Continuous number of months on EFV	0.197	.481	0.338	.217	0.214	.445	0.298	.280
Cumulative number of months ever on EFV	0.146	.604	0.150	.595	0.103	.716	-0.085	.764
Continuous number of months on NVP	0.000	1.000	0.124	.660	0.247	.374	0.247	.374
Cumulative number of months ever on NVP	0.000	1.000	0.124	.660	0.247	.374	0.247	.374

Table A1.30. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) with lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current PI	No	9 (60.0)	1.003 ± .128	.292	1.058 ± .091	**.030**	0.900 ± .140	.066	1.149 ± .101	.179
	Yes	6 (40.0)	0.921 ± .160		0.889 ± .178		0.738 ± .173		1.086 ± .046	
Ever PI	No	7 (46.7)	1.005 ± .075	.397	1.057 ± .071	.118	0.897 ± .123	.195	1.175 ± .088	**.027**
	Yes	8 (53.3)	0.940 ± .183		0.933 ± .185		0.781 ± .193		1.079 ± .060	
Current INI	No	14 (93.3)	0.974 ± .147	.694	0.991 ± .159	.967	0.842 ± .173	.572	1.132 ± .084	.172
	Yes	1 (6.7)	0.913		0.984		0.738		1.006	
Ever INI	No	14 (93.3)	0.974 ± .147	.694	0.991 ± .159	.967	0.842 ± .173	.572	1.132 ± .084	.172
	Yes	1 (6.7)	0.913		0.984		0.738		1.006	

Table A1.31. Differences in lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15) according to current or ever exposure to at least one protease inhibitor (PI) or integrase inhibitor (INI)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on PI	-0.395	.145	-0.536	**.039**	-0.403	.136	-0.483	.068
Cumulative number of months ever on PI	-0.350	.201	-0.471	.077	-0.358	.191	-0.614	**.015**
Continuous number of months on INI	-0.247	.374	-0.124	.660	-0.247	.374	-0.371	.173
Cumulative number of months ever on INI	-0.247	.374	-0.124	.660	-0.247	.374	-0.371	.173

Table A1.32. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one protease inhibitor (PI) or integrase inhibitor (INI) with lumbar spine, total hip, femoral neck and total body BMD in black male patients (n = 15)

HIV antiretroviral therapy-related factor	Exposure or presence	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current ART	No	3 (8.1)	1.027 ± .109	.918	0.993 ± .085	.925	0.787 ± .147	.337	1.127 ± .044	.706
	Yes	34 (91.9)	1.035 ± .134		0.987 ± .118		0.867 ± .136		1.104 ± .102	
Ever ART	No	2 (5.4)	1.069 ± .115	.713	0.955 ± .075	.690	0.789 ± .208	.456	1.137 ± .057	.655
	Yes	35 (94.6)	1.033 ± .133		0.989 ± .117		0.864 ± .135		1.104 ± .100	
Plasma HIV RNA <40 copies/ml	No	6 (16.2)	1.066 ± .122	.533	1.063 ± .124	.140	0.887 ± .214	.610	1.144 ± .085	.310
	Yes	31 (83.8)	1.029 ± .134		0.972 ± .109		0.855 ± .121		1.098 ± .100	
Plasma HIV RNA <200 copies/ml	No	5 (13.5)	1.083 ± .128	.388	1.064 ± .138	.109	0.891 ± .239	.591	1.151 ± .092	.269
	Yes	32 (86.5)	1.027 ± .132		0.975 ± .109		0.855 ± .119		1.099 ± .098	

Table A1.33. Differences in lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37) according to exposure to or presence of general HIV antiretroviral therapy (ART)-related factors

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on ART[a]	0.150	.391	0.092	.599	0.038	.829	0.017	.924
Cumulative number of months ever on ART[a]	0.125	.474	0.083	.635	0.001	.995	-0.025	.887

[a] 2 missing values (n=35)

Table A1.34. Relationship between continuous number of months on antiretroviral therapy (ART) at time of BMD measurement or cumulative number of months ever on ART with lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NRTI	No	3 (8.1)	1.073 ± .082	.605	0.997 ± .090	.883	0.873 ± .206	.870	1.152 ± .049	.402
	Yes	34 (91.9)	1.031 ± .135		0.986 ± .118		0.859 ± .133		1.102 ± .101	
Ever NRTI	No	2 (5.4)	1.069 ± .115	.713	0.955 ± .075	.690	0.789 ± .208	.456	1.137 ± .057	.655
	Yes	35 (94.6)	1.033 ± .133		0.989 ± .117		0.864 ± .135		1.104 ± .100	
Current TDF	No	16 (43.2)	1.060 ± .140	.312	0.999 ± .102	.665	0.866 ± .130	.814	1.110 ± .088	.815
	Yes	21 (56.8)	1.015 ± .124		0.980 ± .126		0.856 ± .144		1.102 ± .107	
Ever TDF	No	13 (35.1)	1.089 ± .124	.076	1.006 ± .105	.464	0.878 ± .141	.563	1.134 ± .067	.195
	Yes	24 (64.9)	1.007 ± .129		0.977 ± .121		0.850 ± .136		1.090 ± .109	
Current ABC	No	23 (62.2)	1.023 ± .122	.501	0.990 ± .117	.844	0.865 ± .146	.785	1.110 ± .105	.727
	Yes	14 (37.8)	1.054 ± .147		0.982 ± .117		0.852 ± .123		1.098 ± .090	
Ever ABC	No	17 (45.9)	1.032 ± .128	.938	0.995 ± .131	.703	0.879 ± .166	.439	1.118 ± .106	.493
	Yes	20 (54.1)	1.036 ± .137		0.980 ± .103		0.844 ± .107		1.095 ± .092	
Current AZT	No	37 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Ever AZT	No	22 (59.5)	1.045 ± .149	.554	0.986 ± .119	.936	0.867 ± .149	.729	1.097 ± .090	.504
	Yes	15 (40.5)	1.019 ± .103		0.989 ± .113		0.861 ± .120		1.119 ± .111	

Table A1.35. Differences in lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37) according to current or ever exposure to at least one nucleos(t)ide reverse transcriptase inhibitor (NRTI), tenofovir disoproxil fumarate (TDF), abacavir (ABC) and zidovudine (AZT)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NRTI[a]	0.131	.453	0.069	.693	0.013	.943	0.000	.998
Cumulative number of months ever on NRTI[a]	0.116	.507	0.076	.663	-0.004	.982	-0.026	.884
Continuous number of months on TDF	-0.176	.298	-0.194	.249	-0.164	.331	-0.164	.333
Cumulative number of months ever on TDF	-0.159	.348	-0.225	.180	-0.207	.219	-0.250	.136
Continuous number of months on ABC[b]	0.154	.370	-0.013	.939	-0.068	.694	0.027	.875
Cumulative number of months ever on ABC[b]	0.054	.754	0.004	.979	-0.098	.571	-0.085	.621
Continuous number of months on AZT	-	-	-	-	-	-	-	-
Cumulative number of months ever on AZT[a]	-0.096	.582	.114	.515	.115	.509	.109	.533

[a] 2 missing values (n=35)
[b] 1 missing value (n=36)

Table A1.36. Relationship between continuous number of months on or cumulative number of months ever on either at least one NRTI (nucleos(t)ide reverse transcriptase inhibitors), tenofovir disoproxil fumarate (TDF), abacavir (ABC) or zidovudine (AZT) with lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NNRTI	No	16 (43.2)	1.018 ± .118	.516	0.979 ± .112	.712	0.860 ± .141	.980	1.122 ± .078	.380
	Yes	21 (56.8)	1.047 ± .142		0.993 ± .120		0.861 ± .136		1.093 ± .111	
Ever NNRTI	No	7 (18.9)	1.054 ± .134	.665	1.025 ± .125	.336	0.901 ± .197	.388	1.166 ± .069	.069
	Yes	30 (81.1)	1.030 ± .132		0.978 ± .113		0.851 ± .121		1.092 ± .099	
Current EFV	No	23 (62.2)	1.050 ± .133	.384	1.001 ± .110	.353	0.879 ± .148	.282	1.126 ± .080	.104
	Yes	14 (37.8)	1.010 ± .130		0.964 ± .123		0.829 ± .112		1.072 ± .117	
Ever EFV	No	14 (37.8)	1.083 ± .144	.081	1.030 ± .114	.076	0.912 ± .176	.072	1.154 ± .074	**.018**
	Yes	23 (62.2)	1.005 ± .116		0.961 ± .110		0.829 ± .097		1.077 ± .101	
Current NVP	No	34 (91.9)	1.022 ± .122	**.044**	0.980 ± .111	.225	0.853 ± .127	.298	1.103 ± .099	.631
	Yes	3 (8.1)	1.180 ± .179		1.065 ± .158		0.940 ± .244		1.132 ± .095	
Ever NVP	No	29 (78.4)	1.024 ± .125	.335	0.970 ± .109	.080	0.840 ± .122	.124	1.091 ± .090	.184
	Yes	8 (21.6)	1.075 ± .155		1.060 ± .123		0.932 ± .169		1.158 ± .116	

Table A1.37. Differences in lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37) according to current or ever exposure to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NNRTI[a]	0.121	.481	0.029	.868	-0.096	.578	-0.228	.181
Cumulative number of months ever on NNRTI[b]	0.109	.535	0.008	.962	-0.101	.563	-0.311	.069
Continuous number of months on EFV	-0.074	.664	-0.150	.376	-0.199	.238	-0.344	**.037**
Cumulative number of months ever on EFV[a]	-0.161	.349	-0.224	.190	-0.249	.143	-0.458	**.005**
Continuous number of months on NVP[a]	0.174	.309	0.064	.713	-0.113	.511	-0.047	.785
Cumulative number of months ever on NVP[a]	0.094	.587	0.203	.234	0.125	.468	0.161	.348

[a] 1 missing value (n=36)
[b] 2 missing values (n=35)

Table A1.38. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) with lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current PI	No	23 (62.2)	1.048 ± .138	.432	0.982 ± .115	.760	0.845 ± .133	.381	1.093 ± .106	.333
	Yes	14 (37.8)	1.013 ± .121		0.995 ± .119		0.886 ± .143		1.126 ± .084	
Ever PI	No	15 (40.5)	1.074 ± .153	.134	1.010 ± .113	.323	0.879 ± .145	.486	1.107 ± .109	.933
	Yes	22 (59.5)	1.008 ± .110		0.971 ± .117		0.847 ± .132		1.105 ± .092	
Current INI	No	35 (94.6)	1.029 ± .132	.287	0.988 ± .117	.878	0.861 ± .140	.931	1.103 ± .100	.524
	Yes	2 (5.4)	1.132 ± .099		0.975 ± .094		0.852 ± .054		1.150 ± .069	
Ever INI	No	32 (86.5)	1.021 ± .131	.123	0.977 ± .109	.168	0.841 ± .120	**.025**	1.093 ± .096	**.042**
	Yes	5 (13.5)	1.119 ± .112		1.054 ± .144		0.985 ± .181		1.188 ± .074	

Table A1.39. Differences in lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37) according to current or ever exposure to at least one protease inhibitor (PI) or integrase inhibitor (INI)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on PI[a]	-0.272	.109	-0.001	.997	0.094	.586	0.144	.403
Cumulative number of months ever on PI[b]	-0.341	**.045**	-0.126	.470	-0.052	.768	0.065	.709
Continuous number of months on INI	0.215	.200	-0.018	.915	0.037	.828	0.161	.341
Cumulative number of months ever on INI	0.292	.080	0.204	.226	0.323	.051	0.359	**.029**

[a] 1 missing value (n=36)
[b] 2 missing values (n=35)

Table A1.40. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one protease inhibitor (PI) or integrase inhibitor (INI) with lumbar spine, total hip, femoral neck and total body BMD in black female patients (n = 37)

HIV antiretroviral therapy-related factor	Exposure or presence	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current ART	No	1 (1.9)	1.032	.753	1.031	.470	0.847	.509	1.177	.459
	Yes	51 (98.1)	0.993 ± .121		0.935 ± .131		0.775 ± .107		1.101 ± .101	
Ever ART	No	1 (1.9)	1.032	.753	1.031	.470	0.847	.509	1.177	.459
	Yes	51 (98.1)	0.993 ± .121		0.935 ± .131		0.775 ± .107		1.101 ± .101	
Plasma HIV RNA <40 copies/ml	No	4 (7.7)	0.960 ± .065	.553	0.995 ± .161	.363	0.838 ± .130	.237	1.152 ± .076	.308
	Yes	48 (92.3)	0.997 ± .123		0.932 ± .129		0.772 ± .104		1.098 ± .102	
Plasma HIV RNA <200 copies/ml	No	2 (3.8)	0.954 ± .111	.630	1.005 ± .038	.460	0.816 ± .044	.601	1.123 ± .077	.773
	Yes	50 (96.2)	0.999 ± .121		0.934 ± .133		0.775 ± .108		1.101 ± .102	

Table A1.41. Differences in lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52) according to exposure to or presence of general HIV antiretroviral therapy (ART)-related factors

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on ART	-0.152	.282	-0.195	.167	-0.158	.264	-0.218	.121
Cumulative number of months ever on ART	-0.067	.638	-0.040	.778	-0.019	.892	-0.065	.646

Table A1.42. Relationship between continuous number of months on antiretroviral therapy (ART) at time of BMD measurement or cumulative number of months ever on ART with lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NRTI	No	2 (3.8)	0.997 ± .049	.972	0.910 ± .171	.773	0.777 ± .099	.998	1.148 ± .042	.521
	Yes	50 (96.2)	0.994 ± .122		0.938 ± .131		0.777 ± .108		1.100 ± .102	
Ever NRTI	No	1 (1.9)	1.032	.753	1.031	.470	0.847	.509	1.177	.459
	Yes	51 (98.1)	0.993 ± .121		0.935 ± .131		0.775 ± .107		1.101 ± .101	
Current TDF	No	14 (26.9)	0.980 ± .128	.619	0.934 ± .120	.914	0.761 ± .092	.527	1.098 ± .101	.862
	Yes	38 (73.1)	0.999 ± .118		0.938 ± .136		0.783 ± .112		1.104 ± .102	
Ever TDF	No	12 (23.1)	0.985 ± .138	.772	0.946 ± .123	.777	0.775 ± .091	.952	1.098 ± .109	.883
	Yes	40 (76.9)	0.997 ± .116		0.934 ± .134		0.777 ± .111		1.103 ± .100	
Current ABC	No	41 (78.8)	0.995 ± .116	.889	0.936 ± .134	.969	0.781 ± .109	.558	1.104 ± .099	.764
	Yes	11 (21.2)	0.990 ± .138		0.938 ± .125		0.760 ± .099		1.094 ± .111	
Ever ABC	No	29 (55.8)	1.000 ± .116	.671	0.949 ± .125	.439	0.788 ± .112	.412	1.099 ± .090	.839
	Yes	23 (44.2)	0.986 ± .127		0.921 ± .138		0.763 ± .099		1.105 ± .116	
Current AZT	No	49 (94.2)	0.998 ± .121	.395	0.933 ± .130	.443	0.774 ± .107	.475	1.100 ± .100	.668
	Yes	3 (5.8)	0.936 ± .089		0.994 ± .155		0.820 ± .095		1.127 ± .130	
Ever AZT	No	33 (63.5)	0.987 ± .098	.593	0.924 ± .123	.352	0.766 ± .112	.357	1.090 ± .082	.265
	Yes	19 (36.5)	1.006 ± .152		0.959 ± .144		0.795 ± .095		1.123 ± .127	

Table A1.43. Differences in lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52) according to current or ever exposure to at least one nucleos(t)ide reverse transcriptase inhibitor (NRTI), tenofovir disoproxil fumarate (TDF), abacavir (ABC) and zidovudine (AZT)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NRTI	-0.127	.371	-0.142	.316	-0.126	.373	-0.238	.090
Cumulative number of months ever on NRTI	-0.102	.471	-0.085	.548	-0.042	.770	-0.475	.074
Continuous number of months on TDF	0.021	.884	-0.100	.479	-0.006	.967	-0.096	.499
Cumulative number of months ever on TDF	0.043	.765	0.001	.993	0.087	.540	0.005	.971
Continuous number of months on ABC	-0.020	.888	0.016	.910	-0.097	.492	-0.069	.629
Cumulative number of months ever on ABC	-0.066	.640	-0.118	.403	-0.160	.259	-0.051	.720
Continuous number of months on AZT	-0.130	.357	0.046	.744	0.063	.659	0.017	.903
Cumulative number of months ever on AZT	0.053	.708	0.103	.468	0.112	.428	0.112	.429

Table A1.44. Relationship between continuous number of months on or cumulative number of months ever on either at least one NRTI (nucleos(t)ide reverse transcriptase inhibitors), tenofovir disoproxil fumarate (TDF), abacavir (ABC) or zidovudine (AZT) with lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NNRTI	No	27 (51.9)	0.990 ± .112	.809	0.935 ± .135	.898	0.775 ± .118	.885	1.111 ± .095	.498
	Yes	25 (48.1)	0.998 ± .130		0.939 ± .128		0.779 ± .094		1.092 ± .108	
Ever NNRTI	No	9 (17.3)	0.942 ± .086	.157	0.886 ± .124	.206	0.741 ± .111	.265	1.092 ± .067	.740
	Yes	43 (82.7)	1.005 ± .124		0.947 ± .131		0.784 ± .105		1.104 ± .107	
Current EFV	No	37 (71.2)	0.985 ± .121	.376	0.935 ± .140	.907	0.780 ± .115	.774	1.110 ± .110	.391
	Yes	15 (28.8)	1.017 ± .117		0.940 ± .110		0.770 ± .083		1.083 ± .073	
Ever EFV	No	18 (34.6)	0.989 ± .138	.817	0.905 ± .138	.208	0.750 ± .116	.182	1.121 ± .114	.331
	Yes	34 (65.4)	0.999 ± .111		0.954 ± .125		0.791 ± .099		1.092 ± .093	
Current NVP	No	47 (90.4)	0.997 ± .117	.627	0.940 ± .127	.630	0.777 ± .105	.957	1.101 ± .097	.899
	Yes	5 (9.6)	0.969 ± .154		0.910 ± .176		0.779 ± .132		1.108 ± .147	
Ever NVP	No	40 (76.9)	0.991 ± .105	.719	0.948 ± .125	.281	0.782 ± .104	.569	1.094 ± .087	.293
	Yes	12 (23.1)	1.005 ± .164		0.901 ± .148		0.761 ± .117		1.129 ± .139	

Table A1.45. Differences in lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52) according to current or ever exposure to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NNRTI	0.073	.607	0.061	.667	0.066	.640	-0.103	.470
Cumulative number of months ever on NNRTI	0.155	.272	0.175	.214	0.201	.152	0.024	.866
Continuous number of months on EFV	0.137	.331	0.085	.547	0.002	.988	-0.073	.607
Cumulative number of months ever on EFV	0.182	.196	0.274	**.049**	0.258	.064	0.000	.999
Continuous number of months on NVP	-0.024	.867	-0.106	.453	0.016	.910	0.044	.757
Cumulative number of months ever on NVP	0.055	.700	-0.151	.286	-0.072	.611	0.152	.281

Table A1.46. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) with lumbar spine, hip, femoral neck and total body BMD in white male patients (n = 52)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current PI	No	27 (51.9)	0.992 ± .109	.875	0.939 ± .129	.902	0.771 ± .105	.701	1.088 ± .084	.318
	Yes	25 (48.1)	0.997 ± .133		0.934 ± .135		0.783 ± .110		1.117 ± .117	
Ever PI	No	22 (42.3)	0.995 ± .119	.950	0.948 ± .127	.593	0.776 ± .100	.964	1.093 ± .090	.590
	Yes	30 (57.7)	0.993 ± .123		0.928 ± .135		0.777 ± .112		1.109 ± .109	
Current INI	No	46 (88.5)	1.003 ± .124	.155	0.950 ± .127	**.037**	0.791 ± .100	**.007**	1.106 ± .107	.488
	Yes	6 (11.5)	0.928 ± .056		0.833 ± .119		0.668 ± .100		1.075 ± .017	
Ever INI	No	45 (86.5)	1.002 ± .125	.219	0.946 ± .124	.228	0.788 ± .099	**.055**	1.101 ± .105	.966
	Yes	7 (13.5)	0.941 ± .063		0.881 ± .168		0.705 ± .133		1.104 ± .078	

Table A1.47. Differences in lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52) according to current or ever exposure to at least one protease inhibitor (PI) or integrase inhibitor (INI)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on PI	-0.116	.413	-0.144	.310	-0.126	.373	0.007	.959
Cumulative number of months ever on PI	-0.138	.331	-0.232	.097	-0.213	.130	-0.077	.585
Continuous number of months on INI	-0.226	.107	-0.307	**.027**	-0.399	**.014**	-0.098	.491
Cumulative number of months ever on INI	-0.180	.203	-0.199	.157	-0.238	.089	-0.003	.985

Table A1.48. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one protease inhibitor (PI) or integrase inhibitor (INI) with lumbar spine, total hip, femoral neck and total body BMD in white male patients (n = 52)

HIV antiretroviral therapy-related factor	Exposure or presence	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current ART	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Ever ART	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Plasma HIV RNA <40 copies/ml	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Plasma HIV RNA <200 copies/ml	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	

Table A1.49. Differences in lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10) according to exposure to or presence of general HIV antiretroviral therapy (ART)-related factors

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on ART	-0.042	.907	0.067	.855	0.139	.701	0.115	.751
Cumulative number of months ever on ART	-0.042	.907	0.067	.855	0.139	.701	0.115	.751

Table A1.50. Relationship between continuous number of months on antiretroviral therapy (ART) at time of BMD measurement or cumulative number of months ever on ART with lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NRTI	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Ever NRTI	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Current TDF	No	1 (10.0)	0.916	.654	0.852	.654	0.726	.719	1.034	.887
	Yes	90 (90.0)	0.998 ± .167		0.936 ± .170		0.795 ± .175		1.057 ± .152	
Ever TDF	No	0 (0.0)	-	-	-	-	-	-	-	-
	Yes	10 (100.0)	-		-		-		-	
Current ABC	No	7 (70.0)	1.028 ± .172	.264	0.954 ± .186	.467	0.820 ± .192	.383	1.068 ± .172	.693
	Yes	3 (30.0)	0.899 ± .091		0.866 ± .077		0.713 ± .039		1.025 ± .048	
Ever ABC	No	7 (70.0)	1.028 ± .172	.264	0.954 ± .186	.467	0.820 ± .192	.383	1.068 ± .172	.693
	Yes	3 (30.0)	0.899 ± .091		0.866 ± .077		0.713 ± .039		1.025 ± .048	
Current AZT	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Ever AZT	No	8 (80.0)	1.016 ± .163	.318	0.935 ± .175	.775	0.792 ± .180	.901	1.068 ± .159	.593
	Yes	2 (20.0)	0.883 ± .116		0.895 ± .139		0.774 ± .147		1.003 ± .040	

Table A1.51. Differences in lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10) according to current or ever exposure to at least one nucleos(t)ide reverse transcriptase inhibitor (NRTI), tenofovir disoproxil fumarate (TDF), abacavir (ABC) and zidovudine (AZT)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NRTI	-0.042	.907	0.067	.855	0.139	.701	0.115	.751
Cumulative number of months ever on NRTI	-0.042	.907	0.067	.855	0.139	.701	0.115	.751
Continuous number of months on TDF	0.127	.726	0.055	.881	0.188	.603	-0.018	.960
Cumulative number of months ever on TDF	-0.036	.920	0.043	.907	0.146	.688	0.079	.828
Continuous number of months on ABC	-0.425	.221	-0.231	.521	-0.291	.415	0.142	.696
Cumulative number of months ever on ABC	-0.425	.221	-0.231	.521	-0.291	.415	0.142	.696
Continuous number of months on AZT	-	-	-	-	-	-	-	-
Cumulative number of months ever on AZT	-0.311	.381	-0.138	.703	-0.061	.868	-0.104	.775

Table A1.52. Relationship between continuous number of months on or cumulative number of months ever on either at least one NRTI (nucleos(t)ide reverse transcriptase inhibitors), tenofovir disoproxil fumarate (TDF), abacavir (ABC) or zidovudine (AZT) with lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current NNRTI	No	5 (50.0)	0.915 ± .070	.149	0.845 ± .083	.110	0.721 ± .030	.222	0.996 ± .053	.211
	Yes	5 (50.0)	1.064 ± .196		1.010 ± .188		0.855 ± .224		1.114 ± .187	
Ever NNRTI	No	5 (50.0)	0.915 ± .070	.149	0.845 ± .083	.110	0.721 ± .030	.222	0.996 ± .053	.211
	Yes	5 (50.0)	1.064 ± .196		1.010 ± .188		0.855 ± .224		1.114 ± .187	
Current EFV	No	7 (70.0)	0.941 ± .085	.148	0.882 ± .124	.193	0.726 ± .072	.068	1.019 ± .080	.247
	Yes	3 (30.0)	1.103 ± .254		1.033 ± .219		0.932 ± .254		1.139 ± .241	
Ever EFV	No	7 (70.0)	0.941 ± .085	.148	0.882 ± .124	.193	0.726 ± .072	.068	1.019 ± .080	.247
	Yes	3 (30.0)	1.103 ± .254		1.033 ± .219		0.932 ± .254		1.139 ± .241	
Current NVP	No	10 (100.0)	-	-	-	-	-	-	-	-
	Yes	0 (0.0)	-		-		-		-	
Ever NVP	No	9 (90.0)	0.992 ± .169	.882	0.920 ± .170	.692	0.778 ± .173	.599	1.058 ± .152	.871
	Yes	1 (10.0)	0.965		0.994		0.878		1.031	

Table A1.53. Differences in lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10) according to current or ever exposure to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) and nevirapine (NVP)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on NNRTI	0.588	.074	0.537	.110	0.394	.259	0.407	.243
Cumulative number of months ever on NNRTI	0.588	.074	0.537	.110	0.394	.259	0.407	.243
Continuous number of months on EFV	0.455	.187	0.440	.203	0.425	.221	0.246	.493
Cumulative number of months ever on EFV	0.455	.187	0.440	.203	0.425	.221	0.246	.493
Continuous number of months on NVP	-	-	-	-	-	-	-	-
Cumulative number of months ever on NVP	0.174	.631	0.290	.416	0.406	.244	0.058	.873

Table A1.54. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV) or nevirapine (NVP) with lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10)

HIV antiretroviral therapy-related factor	Exposure	n (%)	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
			Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value	Mean ± s.d.	*p*-value
Current PI	No	6 (60.0)	1.050 ± .179	.150	1.000 ± .170	.081	0.836 ± .205	.285	1.106 ± .168	.180
	Yes	4 (40.0)	0.899 ± .068		0.819 ± .068		0.715 ± .031		0.978 ± .039	
Ever PI	No	7 (70.0)	0.999 ± .075	.908	0.982 ± .141	.515	0.814 ± .091	.766	1.058 ± .104	.966
	Yes	3 (30.0)	0.985 ± .190		0.904 ± .175		0.777 ± .195		1.054 ± .165	
Current INI	No	9 (90.0)	0.991 ± .169	.959	0.925 ± .172	.898	0.793 ± .176	.798	1.054 ± .152	.931
	Yes	1 (10.0)	0.981		0.949		0.744		1.068	
Ever INI	No	8 (80.0)	0.994 ± .180	.862	0.929 ± .183	.948	0.800 ± .187	.686	1.065 ± .158	.681
	Yes	2 (20.0)	0.971		0.920 ± .041		0.742 ± .003		1.015 ± .076	

Table A1.55. Differences in lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10) according to current or ever exposure to at least one protease inhibitor (PI) or integrase inhibitor (INI)

HIV antiretroviral therapy-related factor	Lumbar spine BMD *g cm^{-2}*		Total hip BMD *g cm^{-2}*		Femoral neck BMD *g cm^{-2}*		Total body BMD *g cm^{-2}*	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
Continuous number of months on PI	-0.792	**.006**	-0.601	.066	-0.430	.215	-0.464	.176
Cumulative number of months ever on PI	-0.138	.331	-0.485	.156	-0.387	.270	-0.178	.623
Continuous number of months on INI	0.290	.416	0.174	.631	0.174	.631	0.290	.416
Cumulative number of months ever on INI	0.242	.500	0.164	.650	0.164	.650	-0.138	.703

Table A1.56. Relationship between continuous number of months on or cumulative number of months ever on, at time of BMD measurement, at least one protease inhibitor (PI) or integrase inhibitor (INI) with lumbar spine, total hip, femoral neck and total body BMD in white female patients (n = 10)

A1.2 Multivariate analysis of clinical determinants of BMD backward elimination

The backward elimination process to identify covariates and factors with significant association with BMD are detailed in Tables A1.57a-f, A1.58a-e, A1.59a-f and A1.60a-c for lumbar spine, total hip, femoral neck and total body BMD respectively.

Covariate / factor	Wald Chi-Square	*p*-value
Age	1.207	.272
BMI	18.677	.000
Fragility fracture history	4.202	.040
Current AZT	0.329	.566
Cumulative no. of months on PI	1.397	.237
Other recreational drug use	0.073	.788
Cumulative no. of months on NNRTI	**0.000**	**.991**
MSM route of HIV acquisition	0.218	.641

Akaike's Information Criterion (AIC) = -160.090

Table A1.57a. Backward elimination step 1 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	1.318	.251
BMI	19.089	.000
Fragility fracture history	4.261	.039
Current AZT	0.329	.566
Cumulative no. of months on PI	1.521	.217
Other recreational drug use	**0.073**	**.788**
MSM route of HIV acquisition	0.221	.638

Akaike's Information Criterion (AIC) = -162.090

Table A1.57b. Backward elimination step 2 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	1.247	.264
BMI	19.062	.000
Fragility fracture history	4.754	.029
Current AZT	0.364	.546
Cumulative no. of months on PI	1.713	.191
MSM route of HIV acquisition	**0.169**	**.681**

Akaike's Information Criterion (AIC) = -164.017

Table A1.57c. Backward elimination step 3 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	1.209	.271
BMI	20.117	.000
Fragility fracture history	4.757	.029
Current AZT	**0.314**	**.575**
Cumulative no. of months on PI	1.779	.182

Akaike's Information Criterion (AIC) = -165.849

Table A1.57d. Backward elimination step 4 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	1.241	.265
BMI	22.075	.000
Fragility fracture history	5.987	.014
Cumulative no. of months on PI	**1.773**	**.183**

Akaike's Information Criterion (AIC) = -167.535

Table A1.57e. Backward elimination step 5 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.051	.152
BMI	**27.964**	**.000**
Fragility fracture history	**8.069**	**.005**

Akaike's Information Criterion (AIC) = -167.776

Table A1.57f. Backward elimination step 6 for lumbar spine BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.346	.556
BMI	24.182	.000
Fragility fracture history	3.809	.051
Current alcohol ≤ 3 units d^{-1}	0.310	.578
Rheumatoid arthritis	4.805	.028
Other disorder	4.430	.035
Other recreational drug use	**0.001**	**.974**
Cumulative no. of months on AZT	2.113	.146
Cumulative no. of months on PI	4.863	.027
Cumulative no. of months on NNRTI	0.022	.882
Current INI	0.425	.515

Akaike's Information Criterion (AIC) = -163.190

Table A1.58a. Backward elimination step 1 for total hip BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.374	.541
BMI	24.351	.000
Fragility fracture history	4.054	.044
Current alcohol ≤ 3 units d^{-1}	0.325	.568
Rheumatoid arthritis	4.824	.028
Other disorder	4.429	.035
Cumulative no. of months on AZT	2.115	.146
Cumulative no. of months on PI	4.881	.027
Cumulative no. of months on NNRTI	**0.023**	**.880**
Current INI	0.429	.512

Akaike's Information Criterion (AIC) = -165.189

Table A1.58b. Backward elimination step 2 for total hip BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.353	.553
BMI	25.089	.000
Fragility fracture history	4.237	.040
Current alcohol ≤ 3 units d^{-1}	**0.327**	**.567**
Rheumatoid arthritis	4.832	.028
Other disorder	4.426	.035
Cumulative no. of months on AZT	2.433	.119
Cumulative no. of months on PI	5.768	.016
Current INI	0.448	.503

Akaike's Information Criterion (AIC) = -167.166

Table A1.58c. Backward elimination step 3 for total hip BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.409	.523
BMI	26.856	.000
Fragility fracture history	4.393	.036
Rheumatoid arthritis	4.702	.030
Other disorder	4.563	.033
Cumulative no. of months on AZT	2.398	.121
Cumulative no. of months on PI	6.337	.012
Current INI	**0.490**	**.484**

Akaike's Information Criterion (AIC) = -168.139

Table A1.58d. Backward elimination step 4 for total hip BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.398	.528
BMI	**26.669**	**.000**
Fragility fracture history	**4.286**	**.038**
Rheumatoid arthritis	**4.687**	**.030**
Other disorder	**6.086**	**.014**
Cumulative no. of months on AZT	3.404	.065
Cumulative no. of months on PI	**8.484**	**.004**

Akaike's Information Criterion (AIC) = -170.350

Table A1.58e. Backward elimination step 5 for total hip BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.465	.116
BMI	20.224	.000
Current tobacco smoking	0.024	.878
Current alcohol ≤ 3 units d^{-1}	2.516	.113
Rheumatoid arthritis	3.334	.068
Other disorder	3.410	.065
MSM route of HIV acquisition	0.017	.897
Cumulative no. of months on AZT	1.569	.210
Cumulative no. of months on PI	2.894	.089
Cumulative no. of months on NNRTI	**0.000**	**.999**
Current INI	1.770	.183

Akaike's Information Criterion (AIC) = -162.405

Table A1.59a. Backward elimination step 1 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.678	.102
BMI	20.615	.000
Current tobacco smoking	0.024	.878
Current alcohol ≤ 3 units d^{-1}	2.516	.113
Rheumatoid arthritis	3.338	.068
Other disorder	3.410	.065
MSM route of HIV acquisition	**0.017**	**.896**
Cumulative no. of months on AZT	1.692	.193
Cumulative no. of months on PI	3.285	.070
Current INI	1.782	.182

Akaike's Information Criterion (AIC) = -164.405

Table A1.59b. Backward elimination step 2 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.661	.103
BMI	22.370	.000
Current tobacco smoking	**0.031**	**.861**
Current alcohol ≤ 3 units d^{-1}	2.504	.114
Rheumatoid arthritis	3.321	.068
Other disorder	3.433	.064
Cumulative no. of months on AZT	1.706	.191
Cumulative no. of months on PI	3.366	.067
Current INI	1.767	.184

Akaike's Information Criterion (AIC) = -166.388

Table A1.59c. Backward elimination step 3 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.747	.097
BMI	22.647	.000
Current alcohol ≤ 3 units d^{-1}	2.481	.115
Rheumatoid arthritis	3.378	.066
Other disorder	3.402	.065
Cumulative no. of months on AZT	1.750	.186
Cumulative no. of months on PI	3.335	.068
Current INI	**1.749**	**.186**

Akaike's Information Criterion (AIC) = -168.358

Table A1.59d. Backward elimination step 4 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	2.635	.105
BMI	22.150	.000
Current alcohol ≤ 3 units d^{-1}	**2.666**	**.103**
Rheumatoid arthritis	3.340	.068
Other disorder	5.463	.019
Cumulative no. of months on AZT	3.269	.071
Cumulative no. of months on PI	5.553	.018

Akaike's Information Criterion (AIC) = -168.622

Table A1.59e. Backward elimination step 5 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	3.026	.082
BMI	**24.874**	**.000**
Rheumatoid arthritis	2.991	.084
Other disorder	**5.994**	**.014**
Cumulative no. of months on AZT	3.154	.076
Cumulative no. of months on PI	**7.278**	**.007**

Akaike's Information Criterion (AIC) = -167.988

Table A1.59f. Backward elimination step 6 for femoral neck BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.556	.456
BMI	3.848	.050
Fragility fracture history	5.275	.022
Significant steroid exposure	8.764	.003
Other disorder	6.427	.011
MSM route of HIV acquisition	**0.005**	**.946**
Continuous no. of months on TDF	1.423	.233

Akaike's Information Criterion (AIC) = -197.674

Table A1.60a. Backward elimination step 1 for total body BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.552	.458
BMI	4.383	.036
Fragility fracture history	5.284	.022
Significant steroid exposure	9.416	.002
Other disorder	6.458	.011
Continuous no. of months on TDF	**1.441**	**.230**

Akaike's Information Criterion (AIC) = -199.670

Table A1.60b. Backward elimination step 2 for total body BMD

Covariate / factor	Wald Chi-Square	*p*-value
Age	0.742	.389
BMI	**4.727**	**.030**
Fragility fracture history	**5.549**	**.018**
Significant steroid exposure	**9.277**	**.002**
Other disorder	**6.242**	**.012**

Akaike's Information Criterion (AIC) = -200.238

Table A1.60c. Backward elimination step 3 for total body BMD

Appendix 2. Chapter 5 supplementary results

A2.1 Multivariate analysis of determinants of 25-hydroxyvitamin D backward elimination

The backward elimination process to identify covariates and factors with significant association with 25-hydroxyvitamin D are detailed in Tables A2.1a-d.

Covariate / factor	Wald Chi-Square	*p*-value
White race	57.938	<.001
Summer sampling	8.660	.003
Female gender	2.797	.094
Current efavirenz exposure	7.678	.006
Age	**0.020**	**.888**
Nadir CD4 cell count	0.795	.372

Akaike's Information Criterion (AIC) = 4872.364

Table A2.1a. Backward elimination step 1

Covariate / factor	Wald Chi-Square	*p*-value
White race	59.362	<.001
Summer sampling	8.677	<.001
Female gender	2.831	.092
Current efavirenz exposure	7.671	.006
Nadir CD4 cell count	**0.776**	**.378**

Akaike's Information Criterion (AIC) = 4870.384

Table A2.1b. Backward elimination step 2

Covariate / factor	Wald Chi-Square	*p*-value
White race	63.083	<.001
Summer sampling	8.426	.004
Female gender	**2.623**	**.105**
Current efavirenz exposure	9.174	.002

Akaike's Information Criterion (AIC) = 4869.159

Table A2.1c. Backward elimination step 3

Covariate / factor	Wald Chi-Square	*p*-value
White race	**98.189**	**<.001**
Summer sampling	**8.011**	**.005**
Current efavirenz exposure	**7.825**	**.005**

Akaike's Information Criterion (AIC) = 4869.776

Table A2.1d. Backward elimination step 4

A2.2 Multivariate analysis of determinants of serum phosphate backward elimination

The backward elimination process to identify covariates and factors with significant association with serum phosphate are detailed in Tables A2.2a to A2.2d.

Covariate / factor	Wald Chi-Square	*p*-value
White race	**0.285**	**.594**
Female gender	2.938	.087
Race-adjusted eGFR <90 ml min^{-1}	2.014	.156
Current CD4 cell count	6.854	.009
Cumulative exposure to NRTI	0.749	.387
Cumulative exposure to NNRTI	1.460	.227

Akaike's Information Criterion (AIC) = -74.083

Table A2.2a. Backward elimination step 1

Covariate / factor	Wald Chi-Square	*p*-value
Female gender	5.128	.024
Race-adjusted eGFR <90 ml min^{-1}	2.943	.086
Current CD4 cell count	6.559	.010
Cumulative exposure to NRTI	**0.787**	**.375**
Cumulative exposure to NNRTI	1.270	.260

Akaike's Information Criterion (AIC) = -75.299

Table A2.2b. Backward elimination step 2

Covariate / factor	Wald Chi-Square	*p*-value
Female gender	5.209	.022
Race-adjusted eGFR <90 ml min^{-1}	3.676	.055
Current CD4 cell count	6.059	.014
Cumulative exposure to NNRTI	**3.276**	**.070**

Akaike's Information Criterion (AIC) = -75.299

Table A2.2c. Backward elimination step 3

Covariate / factor	Wald Chi-Square	*p*-value
Female gender	**5.436**	**.020**
Race-adjusted eGFR <90 ml min^{-1}	**5.201**	**.023**
Current CD4 cell count	**4.928**	**.026**

Akaike's Information Criterion (AIC) = -74.089

Table A2.2d. Backward elimination step 4

A2.3 Multivariate analysis of determinants of tubular reabsorption of phosphate backward elimination

The backward elimination process to identify covariates and factors with significant association with tubular reabsorption of phosphate are detailed in Tables A2.3a and A2.3g.

Covariate / factor	Wald Chi-Square	*p*-value
White race	0.871	.351
Female gender	1.428	.232
Age	1.481	.224
BMI	0.462	.496
Race-adjusted eGFR <90 ml min^{-1}	13.300	<.001
25-hydroxyvitamin D	0.223	.637
Parathyroid hormone	**0.003**	**.955**
Current exposure to efavirenz	2.814	.093
Continuous exposure to PI	0.010	.919

Akaike's Information Criterion (AIC) = 525.453

Table A2.3a. Backward elimination step 1

Covariate / factor	Wald Chi-Square	*p*-value
White race	0.945	.331
Female gender	1.426	.232
Age	1.538	.215
BMI	0.492	.483
Race-adjusted eGFR <90 ml min^{-1}	13.693	<.001
25-hydroxyvitamin D	0.245	.621
Current exposure to efavirenz	2.911	.088
Continuous exposure to PI	**0.011**	**.918**

Akaike's Information Criterion (AIC) = 523.456

Table A2.3b. Backward elimination step 2

Covariate / factor	Wald Chi-Square	*p*-value
White race	0.946	.331
Female gender	1.547	.214
Age	1.543	.214
BMI	0.544	.461
Race-adjusted eGFR <90 ml min^{-1}	13.991	<.001
25-hydroxyvitamin D	**0.245**	**.620**
Current exposure to efavirenz	2.956	.086

Akaike's Information Criterion (AIC) = 521.467

Table A2.3c. Backward elimination step 3

Covariate / factor	Wald Chi-Square	*p*-value
White race	1.305	.253
Female gender	1.388	.239
Age	1.885	.170
BMI	**0.668**	**.414**
Race-adjusted eGFR <90 ml min^{-1}	14.494	<.001
Current exposure to efavirenz	3.166	.075

Akaike's Information Criterion (AIC) = 519.712

Table A2.3d. Backward elimination step 4

Covariate / factor	Wald Chi-Square	*p*-value
White race	1.930	.165
Female gender	2.560	.110
Age	**1.439**	**.230**
Race-adjusted eGFR <90 ml min^{-1}	14.036	<.001
Current exposure to efavirenz	4.189	.041

Akaike's Information Criterion (AIC) = 518.377

Table A2.3e. Backward elimination step 5

Covariate / factor	Wald Chi-Square	*p*-value
White race	**1.652**	**.199**
Female gender	2.878	.090
Race-adjusted eGFR <90 ml min^{-1}	22.227	<.001
Current exposure to efavirenz	4.548	.033

Akaike's Information Criterion (AIC) = 518.377

Table A2.3f. Backward elimination step 6

Covariate / factor	Wald Chi-Square	*p*-value
Female gender	**6.218**	**.013**
Race-adjusted eGFR <90 ml min^{-1}	**28.504**	**<.001**
Current exposure to efavirenz	**4.649**	**.031**

Akaike's Information Criterion (AIC) = 517.437

Table A2.3g. Backward elimination step 7