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Volume 1 of 1

Antenatal vitamin D supplementation and offspring body composition and muscle strength: a translational approach

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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ANTENATAL VITAMIN D SUPPLEMENTATION AND OFFSPRING BODY COMPOSITION AND MUSCLE STRENGTH: A TRANSLATIONAL APPROACH

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The in utero environment to which a fetus is exposed might influence body composition and muscle strength in later life. Modulation of this environment could therefore represent an approach to addressing the increasing burden of obesity and sarcopenia. One potential modifiable exposure is vitamin D. The aim of this work was to explore the determinants of maternal serum 25-hydroxyvitamin D [25(OH)D] status in pregnancy and the use of antenatal vitamin D supplementation to improve offspring growth, body composition and muscle strength.

The Southampton Women's Survey (SWS) is a prospective birth cohort study that included assessment of maternal serum 25(OH)D at 11 (n=2019) and 34 weeks (n=2328) of gestation. Marked seasonal variation in serum 25(OH)D was observed at both gestations ($p < 0.001$ for both). After adjustment for season, 25(OH)D tracked moderately from early to late pregnancy ($r=0.53$), but supplementation use and pregnancy weight gain were significantly associated with changes in 25(OH)D status. The offspring of 678 women who had a late pregnancy 25(OH)D measurement were reviewed at 4 years of age. There were no significant associations between maternal 25(OH)D and offspring lean mass (LM) measured by dual-energy x-ray absorptiometry (DXA), but a positive association with grip strength was found ($\beta=0.10$ SD/SD, $p=0.01$).

These findings were translated to an intervention study using the MAVIDOS trial, a randomised placebo-controlled trial of antenatal vitamin D supplementation (1000 IU/day cholecalciferol from 14 weeks of gestation until delivery) in women with a baseline 25(OH)D of 25-100 nmol/l. Offspring anthropometry was assessed at birth (n=768), 1 year (n=594) and 2 years (n=577) of age. At 4 years (n=378), body composition was assessed by DXA and grip strength by hand dynamometry. Weight, length/height and measures of adiposity (skinfold thicknesses at birth, 1 and 2 years of age; fat mass measured by DXA at 4 years) did not differ between the randomisation groups at any age ($p > 0.05$ for all) despite a significantly greater maternal 25(OH)D in the cholecalciferol supplementation group at 34 weeks of gestation (mean difference 24.7 nmol/l, $p < 0.001$). LM and grip strength at 4 years were also similar, but in women with baseline 25(OH)D < 30 nmol/l, offspring grip strength was greater in those randomised to cholecalciferol (0.70 SD [95% CI 0.02, 1.38], $p=0.04$). LM did not differ in this subgroup.

These findings suggest that 1000 IU/day cholecalciferol supplementation during mid and late pregnancy in women with baseline 25(OH)D 25-100 nmol/l does not improve offspring body composition or muscle strength despite an increase in maternal 25(OH)D status. Supplementation increased muscle strength in offspring of women with the lowest 25(OH)D levels, highlighting the need for further trials of vitamin D supplementation in deficient women.

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Declaration of Authorship

I, Rebecca Jane Moon, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

ANTENATAL VITAMIN D SUPPLEMENATION AND OFFSPRING BODY COMPOSITION AND MUSCLE STRENGTH: A TRANSLATIONAL APPROACH

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
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Signed: .....

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Project Outputs

Findings presented in this work

- Chapter 5: **Moon RJ**, Crozier SR, Dennison EM, Davies JH, Robinson SM, Inskip HM, Godfrey KM, Cooper C, Harvey NC. Tracking of 25-hydroxyvitamin D status during pregnancy: the importance of vitamin D supplementation. *American Journal of Clinical Nutrition*. 2015 Nov;102(5):1081-7

- Chapter 6: Harvey NC, **Moon RJ**[1], Sayer AA, Ntani G, Davies JH, Javaid MK, et al. Maternal antenatal vitamin D status and offspring muscle development: findings from the Southampton Women's Survey. *Journal of Clinical Endocrinology & Metabolism*. 2014 Jan;99(1):330-7

- Chapter 9: **Moon RJ**, Harvey NC[1], Cooper C, D'Angelo S, Crozier SR, Inskip HM, Schoenmakers I, Prentice A, Arden NK, Bishop NJ, Carr A, Dennison EM, Eastell R, Fraser R, Gandhi SV, Godfrey KM, Kennedy S, Mughal MZ, Papageorghiou AT, Reid DM, Robinson SM, Javaid MK. Determinants of the Maternal 25-Hydroxyvitamin D Response to Vitamin D Supplementation during Pregnancy. *Journal of Clinical Endocrinology & Metabolism*. 2016 Dec;101(12):5012-5020

Related publications to which the candidate has contributed

Original Research

- **Moon RJ**, Harvey NC, Cooper C, D'Angelo S, Curtis EM, Crozier SR, Barton SJ, Robinson SM, Godfrey KM, Graham NJ, Holloway JW, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I, Fraser R, Gandhi SV, Prentice A, Inskip HM, Javaid MK; MAVIDOS Trial Group. Response to antenatal cholecalciferol supplementation is associated with common vitamin D related genetic variants. *Journal of Clinical Endocrinology & Metabolism*. 2017. Epub ahead of print. DOI doi: 10.1210/jc.2017-00682

- Cooper C, Harvey NC, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I, Fraser R, Gandhi SV, Carr A, D'Angelo S, Crozier SR, **Moon RJ**, Arden NK, Dennison EM, Godfrey KM, Inskip HM, Prentice A, Mughal MZ, Eastell R, Reid DM, Javaid MK. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Diabetes & Endocrinology*. 2016 May;4(5):393-402.

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[1] Joint first authorship

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Definitions and abbreviations

$1,25(OH)_2D$	1, 25-dihydroxyvitamin D; calcitriol
25(OH)D	25-hydroxyvitamin D; calcidiol
%FM	Percent fat mass
%LM	Percent lean mass
ABCD	Amsterdam Born Children and their Development
aBMD	Areal bone mineral density
ADP	Air displacement plethysmography
ALSPAC	Avon Longitudinal Study of Parents and Children
AR	Adiposity rebound
AViDD	Antenatal Vitamin D in Dhaka
BIA	Bioelectrical impedence analysis
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
Ca^{2+}	Ionised calcium concentration
CHL	Crown-heel length
CI	Confidence interval
CPP	Collaborative Perinatal Project
CRL	Crown-rump length
CSA	Cross-sectional area
CT	Computed tomography

CV	Coefficient of variation
DBP	Vitamin D binding protein
DEQAS	Vitamin D External Quality Assessment Scheme
DH	Department of Health
DOHAD	Developmental origins of health and disease
DXA	Dual-energy X-ray Absorptiometry
EU	European Union
FFM	Fat free mass
FGF-23	Fibroblast growth factor-23
FM	Fat mass
GDM	Gestational diabetes mellitus
GUSTO	Growing up in Singapore Towards Healthy Outcomes
IMAT	Intramuscular adipose tissue
IQR	Interquartile range
IU	International units
LM	Lean mass
LC-MS/MS	Liquid chromatography and tandem mass spectrometry
LS	Lumbar spine
MAVIDOS	Maternal Vitamin D Osteoporosis Study
MPH	Mid-parental height
MPS	Mysore-Parthenon Study
MSC	Mesenchymal stem cell
MUAC	Mid-upper arm circumference
MVPA	Moderate-vigorous physical activity

OFC	Occipito-frontal circumference
OR	Odds ratio
PBM	Peak bone mass
PTH	Parathyroid hormone
pQCT	Peripheral quantitative computed tomography
RCT	Randomised controlled trial
ROI	Region of interest
RR	Relative risk
RXR	Retinoid-X receptor
SD	Standard deviation
SFT	Skinfold thickness
SGA	Small for gestational age
SNP	Single nucleotide polymorphism
SWS	Southampton Women's Survey
T2DM	Type 2 diabetes mellitus
UK	United Kingdom
USA	United States of America
UVB	Ultraviolet B
VAT	Visceral adipose tissue
vBMD	Volumetric bone mineral density
VDD	Vitamin D deficiency
VDDR	Vitamin D dependent rickets
VDR	Vitamin D receptor
WBLH	Whole body less head

WHO	World Health Organisation

Chapter 1: Literature Review

1.1 Introduction

Globally, improvements in health care and increasing life expectancy have led to a growing burden of chronic non-communicable diseases, including cardiovascular disease, type 2 diabetes mellitus (T2DM), malignancy, chronic respiratory and musculoskeletal diseases. Traditional approaches to management of these conditions have involved targeting those at highest risk in later life. However, emerging evidence suggests that there are opportunities for the prevention of these diseases much earlier in the lifecourse. The developmental origins of health and disease (DOHAD) hypothesis suggests that exposure to environmental and nutritional factors during sensitive periods in development, including in utero or during early childhood, programmes the risk for specific diseases in adult life. As such, identification of critical factors which might influence developmental trajectories could represent a population-based approach to reducing the non-communicable disease burden. This work will address whether maternal vitamin D supplementation during pregnancy might influence offspring body composition and muscle strength, and thus represent a novel strategy to address obesity, sarcopenia and/or osteoporosis.

1.1.1 The importance of obesity, sarcopenia and osteoporosis

Obesity, sarcopenia and osteoporosis are important public health problems. Not only are they highly prevalent, but secondary health complications, for example T2DM and the metabolic syndrome in obesity, or fracture in osteoporosis, result in significant health care utilisation and expenditure, highlighting the need for primary preventative strategies.

1.1.1.1 Obesity

Obesity is a major public health concern in both adults and children. The prevalence of adult obesity in England increased dramatically in the late 20th and early 21st centuries (Figure 1.1), such that in 2014 over a quarter of adults were considered to be obese (1). A similar picture is also evident for childhood obesity; worldwide, the estimated prevalence of overweight or obesity in pre-school children (under 5 years) increased from 4.2% in 1990, to 6.7% in 2010 and is predicted to reach 9.1% in 2020 (2). However, the prevalence in developed countries is nearly twice that of

developing countries (2); in England during 2013-2015, over a fifth of 4-5 year olds and one third of 10-11 years were overweight or obese (3). The prevalence of obesity is higher in boys, in more socially deprived areas and in Black and Asian ethnicities compared with White British children (3). Notably, children who are obese are at high risk of remaining overweight or obese in adulthood (4), highlighting the importance of preventing excessive weight gain from an early age.

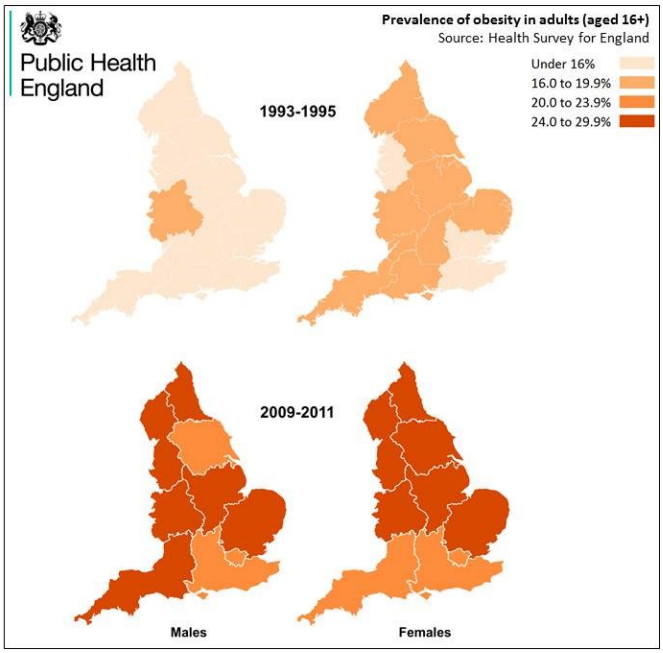


Figure 1.1: Prevalence of adult obesity in England 1993-1995 and 2009-2011
Reproduced from Public Health England (5)

Obesity is associated with a wide ranging burden of chronic complications including hypertension, T2DM, cardiovascular diseases, mental health disorders, malignancy and osteoarthritis. The direct costs of treating obesity and its health consequences in the United Kingdom (UK) was estimated to be approximately £1000 million in 2002, with indirect costs from loss of earnings estimated to represent up to a further £2500 million (6). However as the prevalence of adult and childhood obesity continues to rise, these costs will also increase.

1.1.1.2 Sarcopenia

Sarcopenia is the loss of muscle mass and strength with aging. Currently, there is no universally accepted operational definition for sarcopenia, although a number of definitions have been proposed and each includes a measurement of both muscle size and muscle function (strength

and/or physical performance, for example gait speed) (7). Due to the lack of universal consensus, the exact prevalence of sarcopenia is unknown and is highly variable depending on the definition chosen and the age of those studied. This was highlighted by a study using a free-living community cohort of older individuals with a mean age of 76 years in the UK, in which the prevalence of sarcopenia ranged from 2.0-8.3% when three definitions were considered (8). Nonetheless, there is evidence to demonstrate that these measures are associated with adverse clinical outcomes in later life; low muscle mass is associated with an increased risk of T2DM, and low muscle strength is a predictor of mortality independent of muscle mass (9). Without a consensus definition it is also difficult to determine the economic burden, but a recent study in the UK using a single definition for sarcopenia based on hand grip strength estimated the excess healthcare costs in an adult with sarcopenia compared to a non-sarcopenic adult to be £2646 per year (10).

1.1.1.3 Osteoporosis

Osteoporosis is characterised by low bone mass and microarchitectural deterioration in bone tissue. In adults, a diagnosis of osteoporosis is based on a measurement of bone mineral density (BMD) at the femoral neck by dual-energy X-ray absorptiometry (DXA). For all adults, independent of age and sex, an individual's measured BMD is related to data from a reference population comprised of healthy young adult females to generate a standard deviate "T-score". A BMD that is 2.5 standard deviations (SD) or more below the young adult female mean defines osteoporosis; a T-score between -1 and -2.5 is osteopenia (11). The definition of osteoporosis in childhood differs to that in adulthood due to the increase in BMD during growth. Thus, the definition includes a clinical component: one or more non-traumatic vertebral fractures is considered to represent osteoporosis independent of BMD, or sustaining multiple long-bone fractures (two or more below the age or 10 years or 3 or more above 10 years of age) in conjunction with low BMD on DXA (12).

Clinically, osteoporosis is important due to the increased propensity to fracture. In 2010, it was estimated that 6.6% of men and 22.1% of women aged over 50 years in the European Union (EU) had osteoporosis, and that 3.5 million fragility fractures had occurred (13). This impacts significantly at both the individual and societal level; mortality risk is elevated by 5-8 times in the first three months following a hip fracture (14), and whilst this risk does decrease with time, at 10 years post-fracture it still remains above baseline (14). Functional decline is also common, with fewer than 40% of individuals who sustain a hip fracture regaining their pre-fracture ambulatory status within two years (15). Furthermore, the annual costs of direct fracture treatment and

indirect costs of osteoporosis care, including fracture prevention and long-term post-fracture care in the EU have been estimated to be €37 billion (13).

1.1.1.4 Osteosarcopenic obesity

Obesity, sarcopenia and osteoporosis can occur in isolation but it is increasingly recognised that the three conditions commonly co-exist (16). Sarcopenic obesity is a disproportion between the amount of lean tissue and body fat such that there is greater fat mass (FM) than can be supported by the muscle mass. Similarly to sarcopenia, there is currently no standard definition for sarcopenic obesity and hence exact prevalence and its reported relationships with clinical outcomes are highly variable. However, sarcopenic obesity has been associated with a higher incidence of osteoporosis than observed in non-sarcopenic obesity and non-sarcopenic non-obese individuals, but not when compared to sarcopenia alone (17). The concept of osteosarcopenic obesity highlights the interactions between fat, muscle and bone. This will be discussed in more detail in section 1.2.9.

1.1.2 Summary

It is clear that these three chronic conditions can have a massive impact on quality of life and health outcomes at the individual level, but also contribute significantly to healthcare expenditure. This highlights the necessity for novel primary prevention strategies to be developed and implemented.

1.2 Body composition through the lifecourse

1.2.1 Overview of body composition models

An individual's body composition reflects their lifetime accumulation and turnover of nutrients and substrates. The composition of total body mass can be considered at a number of different levels and these have been divided according to the types of component considered, as proposed by Wang et al in 1992, and summarised in Figure 1.2 (18).

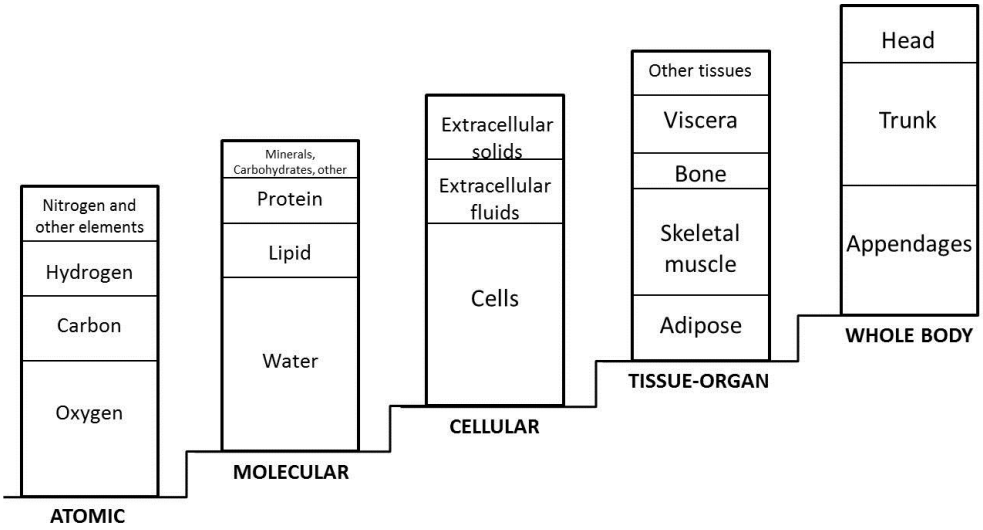


Figure 1.2: The five body composition levels
Redrawn from Wang et al, 1992 (18)

In clinical practice, variations on the molecular level are typically used to assess body composition by dividing these into a number of compartments. The simplest of these is the two compartment model, which divides the body into FM and fat-free mass (FFM) (Figure 1.3) (18). It is important to recognise that within this model, FM includes not only adipose tissue, but also intracellular fat pools, such as those found in the liver and skeletal muscle. Furthermore, this model provides no information on the relative contributions of each of the other molecular components (water, protein, mineral) to FFM, and is therefore particularly vulnerable to inaccuracies due to differences in hydration. The FFM compartment can therefore be further divided into bone mineral content (BMC) and lean mass (LM) (Figure 1.3). The latter compartment includes skeletal muscle mass, viscera and water.

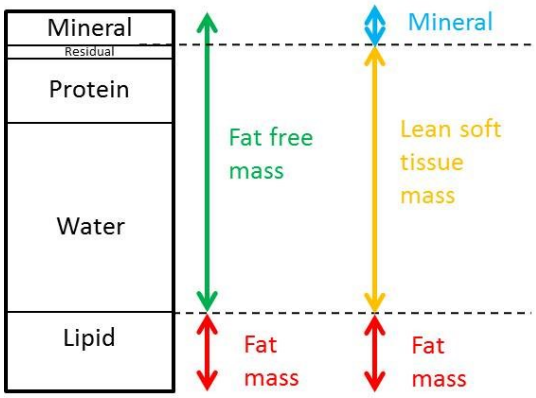


Figure 1.3: Components of the molecular level of body composition
Adapted from Wang et al, 1992 (18)

1.2.2 Assessment of body composition in clinical research

The gold standard and only direct method for assessment of body composition is chemical analysis of cadavers, which is of limited use in clinical research of human subjects. In vivo methods for body composition assessment can be broadly divided into simple indices (such as body mass index (BMI) and waist circumference), predictive equations and compartment models.

The predictive models use a direct measurement, for example size of a skinfold, and from this utilise a predictive algorithm to derive a single component of body composition, such as fat percentage.

Compartment models undertake a measurement of one of more compartments of body composition, for example total body water, and from this predict the size of the other body compartments. It has been demonstrated that methods for analysis of body composition are not interchangeable (19), which highlights measurement inaccuracies and errors introduced by predictive models.

1.2.2.1 Anthropometric measurements

Anthropometric indices, including height (or length up to age 2 years), weight and circumferences are inexpensive and easily obtained measures. However, a measurement of weight alone conveys little information on health risk. Weight in is part related to height; a taller individual of identical body proportions to a shorter individual would be expected to have a higher weight. BMI, calculated by weight (in kg) divided by height (in metres) squared, is the most widely used index to adjust weight for height. The risk between BMI and morbidity, such as cardiovascular disease, and mortality is continuous but the World Health Organisation (WHO) has also used BMI to define overweight (BMI ≥ 25.0 kg/m^2) and obesity (BMI ≥ 30.0 kg/m^2) in adults (20). This dichotomisation is useful in clinical practice for quantifying risk, but also enables comparison of epidemiological trends.

During childhood BMI varies with age. It increases over the first year of life followed by a decline to a nadir at approximately age 6-8 years in females and 7-9 years in males. Thereafter BMI increases through puberty into early adulthood (21). Absolute BMI values cannot therefore be used to define overweight or obesity in childhood. A number of different definitions have been proposed, which typically label the upper segment of the population distribution as overweight and obese, rather than being based on associations with a defined clinical outcome. For example, the WHO suggests an approach based on centiles for the geographic area (Table 1.1). The

International Obesity Foundation Taskforce (IOFT) adopted a different approach; BMI data from six cross-sectional surveys in Brazil, Great Britain, Hong Kong, the Netherlands, Singapore and the United States of America (USA) were used to determine the standard deviation scores (or z-scores) which correspond to the absolute adult BMI values at 18 years of age used to define overweight and obesity (22, 23) (Table 1.1). Due to the varying definitions used, care needs to be taken when comparing the reported prevalence of overweight and obesity between studies.

Table 1.1: Definitions for overweight and obesity in childhood using body mass index (BMI) standard deviation scores

		Thinness	Healthy	Overweight	Obesity
WHO (20)	**< 5 years**	< -2	-2 to 1 (1-2 "at risk of overweight")	> 2	≥ 3
	5- 19 years	< -2	-2 to 1	1 to 2	≥ 2
IOTF (23)	**Boys**	< -1.014	-1.014 to 1.310	1.310 to 2.288	> 2.288
	Girls	< -0.975	-0.975 to 1.244	1.244 to 2.192	> 2.192
US CDC (24)			< 1.645 (1.036-1.645 "at risk of overweight")	≥ 1.645	

(WHO: World Health Organisation; IOTF: International Obesity Task Force; US CDC: United States Center for Disease Control)

BMI provides no information on the relative proportion of FM, LM and BMC, and some studies have suggested that there may be residual correlation with height (25). Although BMI shows a moderate-strong correlation with measurements of FM by DXA in children, there is marked variability in the body fat mass percentage (%FM) for a given BMI, and some children who have a BMI within the healthy range have similar %FM to those categorised as overweight and obese (26-28). Furthermore, ethnic differences in this relationship exist; for a given BMI, %FM is greater in Hispanic than White children, and lowest in Black children (27). Nonetheless despite of these limitations, there is consistent evidence from longitudinal cohort studies that higher BMI in childhood is associated with increased long term morbidity (29) and thus it remains an important measurement and outcome in epidemiological studies.

Mid-upper arm circumference (MUAC) and waist circumferences are also easily obtained measurements. MUAC provides a composite measurement of bone (humerus) cross-sectional area, muscle size and subcutaneous fat, and has typically been used in the assessment of undernutrition. Recent studies have also demonstrated a correlation between MUAC and %FM

measured by bioelectrical impedance analysis (BIA) in children aged 9-11 years, although the accuracy of MUAC to detect high %FM in younger children was low (30, 31). Waist circumference is a proxy measure for abdominal adiposity, and has been positively associated with cardiometabolic risk factors in children (32).

1.2.2.2 Skinfold thickness measurements

Measurements of a fold of skin and subcutaneous fat are easily and cheaply performed and can be undertaken outside of the laboratory environment on any age group. Their use is limited by the potential for inter-observer variation, and measurement of skinfold thickness (SFT) is not possible in severely obese individuals. In children, measurements are most commonly obtained on the back of the arm overlying the posterior triceps ("triceps skinfold thickness") and at the angle of the scapula ("subscapular skinfold thickness"). In adults a further two sites are typically measured overlying the biceps and in the suprailiac region. These four sites are usually chosen due to the ability to separate a true fold of skin and subcutaneous fat from the underlying tissues at these sites (33).

Skinfold thickness data can be used in the raw form, representing a measurement of regional subcutaneous adiposity, or predictive equations can be used to combine several sites of measurement and calculate body fat percentage, but age and sex specific equations are required (34). The use of SFT to estimate %FM does assume that the relationship between subcutaneous fat and other fat depots is constant. Furthermore SFT assessment assumes constant skin thickness and adipose tissue compressibility between subjects.

1.2.2.3 Bioelectrical impedance (BIA)

Bioelectrical impedance analysis is a technique used to estimate total body water by measuring the impedance of the body to a flow of current. Whilst easy, quick and relatively cheap to perform, the calculation of FM from total body water measurement is based on a series of equations, which are population and age specific (35). Furthermore, accurate assessment of FM by BIA is highly influenced by hydration status, which is particularly variable during early infancy, and therefore should not be used in that age group (36).

1.2.2.4 Air displacement plethysmography (ADP)

Air displacement plethysmography estimates FM and FFM from the calculation of body density (body mass/volume). Body volume is measured from air pressure differences when the subject is placed within an air tight chamber. Density coefficients for FM and FFM, which vary with age and sex, are used to calculate the relative contributions of each to total body weight. Two instruments are commercially available for ADP assessment of body composition: the *PEAPOD* (Figure 1.4) has been shown to be accurate in comparison to underwater weighing for assessment of infants weighing less than 8kg (36), and the *BODPOD* (Figure 1.5) is suitable for older children who are able to remain still in an enclosed chamber (from approximately 4 years of age) (36). Thus, ADP cannot be used in late infancy and early childhood.

Figure 1.4: PEAPOD
Image from www.bodpod.com

Figure 1.5: BODPOD
Image from www.bodpod.com

1.2.2.5 Dual-energy X-ray Absorptiometry (DXA)

The primary clinical use of DXA is to assess BMD and diagnose osteoporosis. However it is also considered the gold standard for assessing body composition at the molecular level. DXA assessment is based on the differential attenuation of X-ray by different tissue types. The DXA instrument generates two X-ray beams of high and low energies. The principle manufacturers of DXA instruments are Hologic and GE Lunar and each uses different technology to generate the two energy beams: Hologic instruments utilise a switching pulse system that rapidly alternates the

voltage of the X-ray generator to produce the two energy levels, whereas Lunar instruments create an initial beam which passes through a "K-edge filter" that absorbs X-rays in the middle range of energies but allows those of high and low energy to pass through to the subject (37). The attenuation of these X-ray beams by body tissues is dependent on the density and thickness of the tissue. Soft tissues, such as fat, are of low density and attenuate the X-ray beams less than high density tissues, such as bone. As only two different X-ray energies are used, theoretically the body can only be divided into two compartments: bone mineral and soft tissue. In the analysis, the DXA instrument generates a pixel-by-pixel map of the X-ray attenuation coefficients and the ratio of the attenuation of the two energy beams are compared to known constants for BMD and soft tissue to determine the content of each pixel. Algorithms are used for bone edge detection, and the total projected area of bone is then derived by summing the pixels within the bone edges. The reported value of BMD is the mean BMD measured within the pixels identified as bone. BMC is calculated by multiplying the mean BMD by the projected area. Soft tissue composition can only be assessed in pixels which do not contain bone mineral. In these pixels, the proportion of fat is linearly related to the ratio of attenuation of the two energies (38). In a whole body scan, approximately one third of pixels contain bone and therefore soft tissue composition of these pixels is estimated from the surrounding tissues. DXA can therefore report measurements of BMC, FM and non-bone fat-free mass (commonly referred to as lean mass).

The accuracy of DXA in assessing body composition has been demonstrated by comparison with chemical analysis of carcass composition in small animals including piglets and monkeys (39, 40). However, DXA is not without limitations, and in particular it has been investigated whether the anteroposterior thickness of the subject has an effect on X-ray attenuation. Greater tissue depths result in higher attenuation of the lower energy photons with a subsequent overestimation of FM. This can result in errors at tissue depths greater than 25cm (41). Furthermore, the proportion of soft tissue cannot be measured in any pixels that contain bone mineral, and therefore this is estimated from the surrounding tissues. This could lead to over or under estimation of the true soft tissue composition. DXA is also unable to differentiate intramuscular fat from lean tissue, potentially leading to underestimation of whole body FM. Additionally, the attenuation coefficients for soft tissue are calibrated to assume a constant hydration of fat-free tissue of 73%. Exercise, dehydration and pathological processes can result in alterations to the degree of lean tissue hydration, and variation with age also occurs. Lean tissue hydration may be up to 90% in early infancy and gradually decreases with age. Whilst theoretically over-hydration will result in overestimation of FM, the effect size is very small (typically < 1%) (42). Bone edge detection is more difficult in children due to the lower BMD relative to adults, which can lead to

underestimation of the bone area and false identification of bone as soft tissue. However, specific paediatric software is now available, which has largely overcome this issue.

Although the primary clinical use of DXA is in the measurement of bone mass and BMD is used in the definition of osteoporosis, DXA does not provide a true measurement of volumetric bone mineral density (vBMD). In the acquisition of a DXA scan, a three dimensional (3D) structure is converted into a two dimensional (2D) image that is a combination of the high and low energy attenuations. BMD assessed by DXA is therefore reported in g/cm^2 and is typically referred to as an areal BMD (aBMD). However, a thick low density bone and a thin high density bone might have the same aBMD. In adults it has been shown that BMC and aBMD similarly predict fracture risk (43). Nonetheless, aBMD will overestimate true vBMD in larger bones and underestimate this in smaller bones, and thus care should be taken when using aBMD as an outcome in children. A number of mathematical models have been developed to adjust aBMD for this proving an estimate of vBMD. One of the most commonly used in children is bone mineral apparent density (BMAD) (44).

In young children, the main limitation to the use of DXA is scan acquisition. As the instrument is open (Figure 1.6) and radiation exposure is low, a parent can remain in the scanning room, but movement artefact limits the age at which a scan can be successfully acquired. Acquisition times for a whole body DXA scan in a preschool child and an adult are approximately 5 and 8 minutes, respectively.

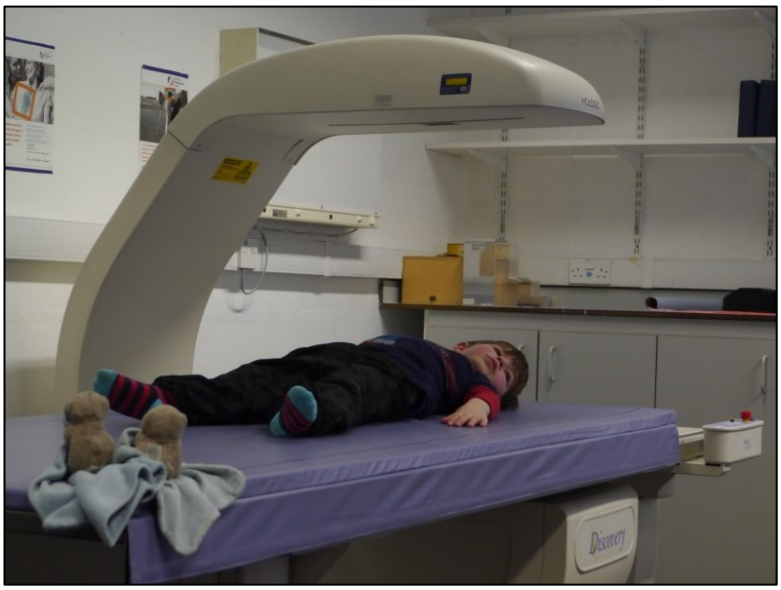


Figure 1.6: Hologic Discovery Dual-Energy X-ray Absorptiometer
Photo credit: Author's own. Parental permission was granted for publication

DXA does require exposure to a low dose of radiation. The effective dose of radiation in part depends on the type of scanner (fan beam or pencil beam), scanning mode and the area being scanned. A whole body, lumbar spine (LS) and hip DXA scan exposes a pre-school child to an effective radiation dose of approximately 21 microsieverts of radiation, and an adult to 16 microsieverts. This is approximately equal to 2-3 days background radiation in most parts of the UK, or one day in Cornwall where background radiation is higher (45). A transatlantic flight exposes an individual to a radiation dose of approximately 80 microsieverts (45). As such this additional radiation exposure is of negligible risk.

1.2.2.6 Computed Tomography

Computed tomography (CT), similarly to DXA, utilises the differential attenuation of X-ray by tissue types. An image is formed of pixels on a grey-scale, the relative colour of each reflecting the density, and therefore composition, of the tissue. Whilst CT can therefore provide more information on tissue types than DXA, its use in clinical research is limited by cost, high radiation exposure and particularly in young children compliance with scan acquisition.

Peripheral quantitative computed tomography (pQCT) is a form of computed tomography obtained with a smaller instrument that can accommodate a single limb rather than the whole body (Figure 1.7). Cross-sectional images obtained through a limb (Figure 1.8) can be used to assess true volumetric BMD, in addition to muscle cross-sectional area and intramuscular adipose tissue (IMAT) depots. This technique is limited by availability of the scanning instrument and is extremely sensitive to movement artefact, but data can be successfully obtained in children (46, 47). Radiation exposure is low and therefore does not prohibit its use in the research setting; scanning a single limb at 4 sites exposes the child to approximately 1.5 microsieverts of radiation compared to 5 microsieverts for a whole body DXA scan.

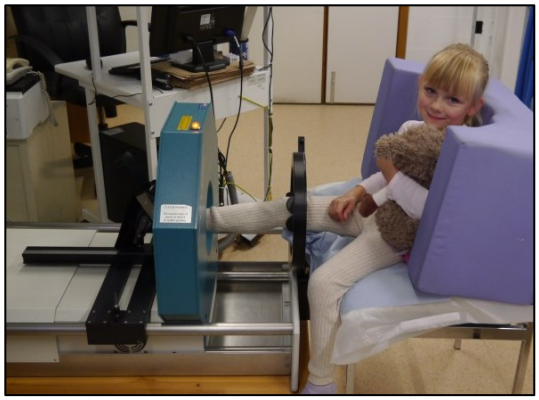
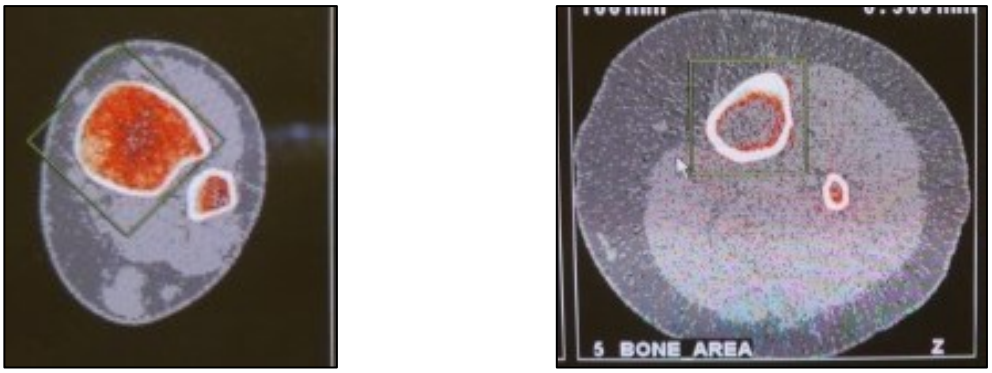


Figure 1.7: Peripheral quantitative computed tomography using a Stratec XCT instrument
Photo credit: Author's own. Parental permission was granted for publication.



Distal lower leg

Proximal lower leg

Figure 1.8: Examples of pQCT images from the right lower leg of a 4 year old
Photo credit: Author's own. Parental permission was granted for publication. Lower leg scans are not from the participant photographed in Figure 1.7.

1.2.3 Assessment of muscle strength

The assessment methods discussed above have focussed on measuring the size of body compartments, yet muscle quality and/or strength might not be directly proportional to muscle size, but is clinically important. There are a number of methods available to assess muscle strength including jumping mechanography, fitness batteries and isokinetic dynamometry. Many of these are limited by the cost and size of equipment, time required for assessment and, particularly in young children, the ability to understand and follow the protocol. Furthermore, it can be difficult to distinguish muscle strength from cardiovascular fitness, neurological function and intellectual capacity to follow instructions. In contrast, hand grip strength is an easily performed measured of isometric muscle strength; grip strength in children aged 7-12 years is highly correlated with isokinetic quadriceps strength (48) and high test-retest reliability has been demonstrated in children aged 4-11 years (49). Furthermore lower grip strength in adulthood has been prospectively associated with worse clinical outcomes including all-cause mortality, non-cardiovascular mortality and myocardial infarction (50), and in childhood and adolescence grip strength has been negatively associated with blood pressure and positively associated with insulin sensitivity after adjustment for cardiorespiratory fitness (51).

1.2.4 Embryological development of adipose tissue, muscle and bone

Adipose tissue, muscle and bone all have a common embryonic origin: the mesenchymal stem cell (MSC). These cells have the potential to differentiate into a number of different tissue types, as shown schematically in Figure 1.9.

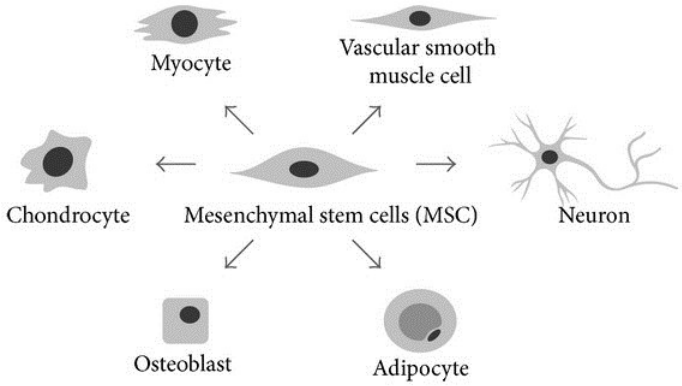


Figure 1.9: Cell types derived from the common mesenchymal stem cell
Reproduced with permission from James et al, 2013 (52)

There are a number of signalling pathways which are involved in determining the cell lineage into which MSCs differentiate, and the relative balance of these might promote differentiation into one cell type, and inhibit differentiation into another. For example, the Wnt signalling pathway stimulates myogenesis and osteogenesis whilst inhibiting differentiation of pre-adipocytes to mature adipocytes (53). As such, the relative exposure of MSCs to nutrients and growth factors might alter the commitment to myogeneic or adipogenic lineage. Indeed, in vitro, adipogenic inducers can convert the differentiation of myoblasts into adipoblasts, thus promoting adipogenesis (54).

Adipogenesis begins in the human fetus from 14 to 16 weeks of gestation (55) and requires a complex interplay of growth and transcription factors to stimulate the differentiation of MSC into pre-adipocytes. Subsequent hyperplasia and hypertrophy of adipocytes with lipid accumulation result in a gradual increase in FM through late gestation. Indeed, using ADP to assess FM in preterm infants, FM increased linearly by an average of 46g for each additional week of in utero development from 30 to 36 weeks of gestation (56).

Skeletal muscle is formed from multinucleated myofibres. Myogenesis begins from 8-10 weeks of gestation with the fusion of embryonic myoblasts, derived from the MSC, into primary muscle

fibres. These primary myofibres provide the scaffolding for the formation of secondary myofibres in mid to late gestation. In animal models it has been shown that myofibre number is almost complete by full gestation, and thereafter postnatal muscle growth occurs primarily by myofibre hypertrophy (57).

Bone formation occurs through two mechanisms: endochondral ossification to form long bones, and intramembranous to form flat bones, such as the skull. Endochondral ossification requires the formation of a cartilaginous framework onto which the bone develops. This cartilage model begins to develop at around 5 weeks of gestation with the differentiation of MSCs into chondrocyte precursors, followed by rapid proliferation, hypertrophy and subsequent apoptosis of chondrocytes. Death of the chondrocytes enables the entry of blood vessels and deposition of osteoblasts. These osteoblasts deposit osteoid onto the degrading cartilage (58). In contrast, intramembranous ossification is not preceded by a cartilaginous model. Instead, MSCs differentiate directly into osteoblasts which begin to secrete bone matrix, which becomes mineralised. The deposition of mineral within the bone matrix formed by both types of ossification is at its maximum in the third trimester of pregnancy (59).

1.2.5 Body composition at birth

The mean birth weight for a white male infant in the UK is 3.55 kg and for a white female infant is 3.41 kg (60). Approximately 10-14% of the body weight of a newborn infant is comprised of FM (61-65), although in the neonatal period measures of FM by DXA are typically higher than values obtained using ADP. Furthermore, %FM increases with gestational age even in term infants (64), and sexual dimorphism in body composition is evident at birth. Proportionally, males have greater FFM and lower FM than females and this difference persists through childhood and adulthood (64, 66). Additionally, a number of maternal factors have been associated with offspring neonatal body composition; thus neonatal %FM is positively associated with maternal height and maternal fat stores in pregnancy (66). Faster walking speed in late pregnancy is negatively associated with neonatal adiposity, as are smoking and nulliparity (66). Obstetric complications also influence body composition at birth; for example macrosomia and increased %FM is common in infants born to women with gestational diabetes (GDM) (67).

Whole body BMC in an infant with a weight of 3.0-3.5 kg is approximately 66 g, therefore representing around 2% of body weight (68). Similarly to soft tissues, whole body and LS BMC and BMD measured in the neonatal period increase with gestational age (69), and some, but not all, studies suggest these are higher in males than females (69-71). Up to 95% of the variance in

whole body BMC and 86% of whole body BMD is explained by birth weight (68), and whilst Godfrey et al additionally demonstrated that maternal fat stores and height are positively associated with neonatal whole body BMC and BMD, whereas smoking and vigorous physical activity in pregnancy displayed a negative association (69), it is unclear if these factors are acting through differences in birth weight or directly on bone. Furthermore, several of these maternal factors are positively associated with placental volume, which is also associated with offspring neonatal size, adiposity and bone mass (72).

1.2.6 Body composition during infancy, childhood and adolescence

Growth is a complex biological process influenced by genetic predisposition, nutrition, health, illness and endocrine factors. During childhood growth, there are both quantitative increases and qualitative changes in the relative proportions of FM, LM and BMC. Between birth and 6 months of age, FM increases approximately five to six-fold, whereas the FFM compartment only doubles in size (65). Thus, %FM increases approximately 3-fold in males and doubles in females during this period (65). Between 6 months and 2 years of age, the FFM compartment expands relatively more quickly than FM, resulting in a subsequent decline in %FM to 2 years of age (73). The precise trajectory of FM accrual is complex, but in early postnatal life does appear to involve increases in both adipose cell number and size. It is in part dependent on infant feeding mode: formula fed infants have lower FM than breast-fed infants at 3-4 and 6 months of age, but by 12 months this pattern has reversed. FFM is consistently higher in formula fed infants throughout the first year of life and thus %FM follows a similar pattern to total FM, being higher in breastfed infants at 6 months, but lower at one year of age compared with formula fed infants (74).

Total FM, LM and BMC increase throughout early childhood due to linear growth. In pre-pubertal males, the relative increase in each of the components of body composition remains relatively static such that percentage FM, LM and BMC change very little. In contrast, in pre-pubertal females, the gain in FM exceeds that of FFM, such that there is a steady increase in %FM throughout childhood (75-78). During puberty, there is a more rapid increase in total FM, LM and BMC in line with the pubertal growth spurt. At the population level, puberty occurs at an earlier age in females than males, and as such, at a chronological age of 11-12 years, there is temporarily little difference in total LM between males and females as shown in Figure 1.10 (79). The accumulation of soft tissue during puberty differs markedly in males and females: girls gain relatively more FM, whereas males accumulate relatively greater LM (75), probably in response to increasing testosterone. Thus at the end of puberty, females have significantly greater %FM than

males, and there is a marked discrepancy in total LM between males and female (Figure 1.10). Furthermore, the sites of fat accumulation differ by sex. These differences are evident in pre-pubertal children but become more marked after puberty. Total subcutaneous fat is greater in girls than in boys and tends to be accrued in the gluteal-femoral depots in females (gynoid distribution) but in the trunk in males (android distribution) (80, 81). During childhood, visceral adipose tissue (VAT) depots, which are more strongly associated with insulin resistance and metabolic risk than subcutaneous adiposity, increase with age and the majority of studies have found visceral adiposity to be higher in boys than girls, with an increase in this disparity during puberty due to greater accumulation of VAT in males than females during this time (81).

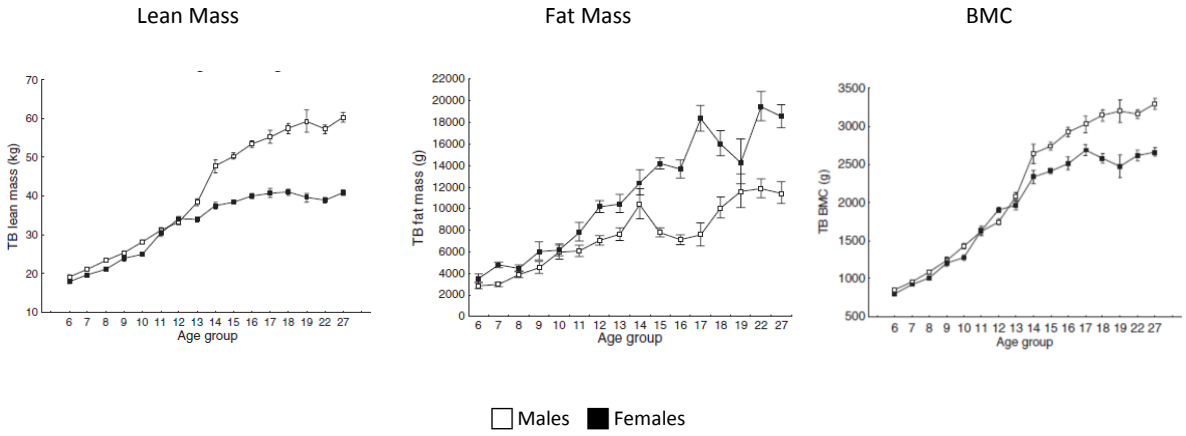


Figure 1.10: Total body (TB) Lean mass, fat mass and BMC measured by dual-energy X-ray absorptiometry in males and females
Reproduced with permission from Alwis et al, 2010 (79)

The increase in BMC during childhood and adolescence follows a similar pattern to that observed with LM. There is a steady increase in BMC in both sexes during pre-pubertal growth, followed by acceleration during puberty. Due to the completion of linear growth at an earlier age in females, accretion of bone mass begins to plateau earlier in females than in males (Figure 1.10) (75, 79).

1.2.7 Body composition in adulthood

Total body FM continues to accumulate during adulthood until the age of 60-65 years, thereafter showing a mild decline (76). Visceral adiposity typically increases with age, and most markedly during the menopause in women. In older adults, the total mass of VAT is relatively stable and therefore it represents a larger proportion of total FM.

Peak muscle mass occurs earlier in the life course than peak FM at approximately 18-20 years of age (82), followed by a subsequent decline due to myofibre loss. A similar pattern to LM is evident for grip strength, increasing through childhood to a peak in the mid-late 3rd decade of life followed by a decline thereafter (83). Owing to the differences in ages at which LM and FM are typically gained and lost during adulthood, %FM reaches a peak at approximately 80 years of age in males and 65 years in women (76).

Peak bone mass (PBM) also occurs in the 3rd decade of life in both men and women, followed by a gradual decline in whole body BMC, which accelerates after the menopause in females. Accordingly both the PBM achieved and the rate of decline are important determinants of bone mass in later life. Mathematical modelling has demonstrated that a 10% increase in PBM will delay the onset of osteoporosis by 13 years (84). Whilst similar modelling has not been undertaken with regards to LM or muscle strength, the similarity in lifecourse pattern would suggest that interventions early in life to increase BMC, LM and muscle strength could reduce the incidence of clinical outcomes secondary to osteoporosis and sarcopenia.

1.2.8 Tracking of body composition and muscle strength

Tracking refers to the maintenance of a relative rank of an individual within a group when a measurement is repeated at two or more time intervals, and thus can be used to determine the ability of a single measurement to predict a future measurement in the same individual. If interventions early in life are undertaken to improve later life outcomes, evidence for high levels of tracking of the characteristic would support an increased likelihood of a sustained positive effect of the intervention.

There is strong evidence that children who are overweight or obese are at high risk of obesity in adulthood (4). However there are few studies that have investigated the tracking of individual components of body composition, and none which have undertaken the initial assessment in the neonatal period or early infancy. In the Avon Longitudinal Study of Parents and Children (ALSPAC), 6066 children had body composition assessed by BIA at 7 and 11 years of age. Both FM (r=0.70) and FFM (r=0.73) showed moderate tracking despite being assessed in an age group when the subjects are likely to be in varying stages of puberty (85). Cheng et al performed DXA in 101 girls aged 9-13 years and again after 7 years, therefore encompassing pubertal changes; 69% and 66% of the girls remained in the same decile for LM and FM, respectively (86).

There are few data on the tracking of LM, but tracking coefficients for measures of muscle strength have been reported. Trudeau et al assessed hand grip strength at 10, 11, 12 and 35 years of age in 106 Canadian individuals (87). Tracking of grip strength at yearly intervals between 10 and 12 years was high in both males (r=0.84-0.92) and females (r=0.87-0.91). The tracking coefficients between childhood and 35 years of age were more modest (r=0.45-0.61) and tended to be higher for the measurement at 12 years than at 10 years, and stronger in girls than boys.

The tracking of BMD and BMC has been more frequently reported. In the largest study, 1554 children aged 6-16 years in the USA had DXA at baseline and at yearly intervals for three years (88). Measurements of bone mineral status at baseline accounted for 86-96% of the variability one year later. The correlation coefficient did gradually decline with increasing time from baseline, but even at three years at least 80% (depending on sex and ethnicity) of the variation in whole body BMC z-score could be explained by the baseline measurement. Furthermore, 52% and 61% of children who had a low (z-score < -1.5) and high (z-score > 1.5) whole body BMC at baseline remained in the low or high group, respectively, at the 3 year follow-up. In this study, the tracking coefficients were highest for children who were either younger (6-7 years) or older (14-16 years) at baseline, compared with the intermediate years, suggesting that the timing of pubertal development might confound the tracking of bone mass. However, studies including smaller numbers of children, but followed up after a longer time interval and including spanning the peripubertal period, have similarly demonstrated moderate-high levels of tracking of BMC and aBMD (86, 89-91). There is only one study, which has examined the tracking of bone mass from childhood into adulthood; Buttazzoni et al assessed radial BMC, BMD and bone area using single photon absorptiometry in 214 individuals at age 3-17 years and subsequently at a mean of 28 years later (follow up age 28-44 years). The correlation coefficients were more modest than those observed for shorter intervals, but still demonstrated moderate tracking (BMC r=0.56, aBMD r=0.42, bone area r=0.58). When stratified by age at baseline measurement, correlation coefficients were stronger for those who were older than 10 years at baseline compared to the younger participants, but were statistically significant for both age groups (92).

Overall, these studies would therefore support the notion that interventions to increase LM, BMC and muscle strength and/or reduce FM in childhood might have sustained effects into adulthood.

1.2.9 Interactions between adipose tissue, muscle and bone

The sizes of fat, muscle and bone compartments are closely related due to direct interactions between the three compartments and as a result of each compartment responding to hormones,

as will be discussed briefly in the subsequent paragraphs. Importantly, this would therefore suggest that each compartment should not be considered in isolation; indeed, the increased FM observed in nutritional obesity is associated with greater total LM and bone mass relative to a non-obese individual (93) although the relative proportions of each tissue compartment will be altered. Conversely, greater LM will be associated with higher resting metabolic rate and energy expenditure, and hence it is hypothesised that this could be protective against obesity.

1.2.9.1 Mechanical

It is well recognised that muscle function is important to bone mineralisation. Frost's mechanostat theory suggests that bone has a homeostatic mechanism which enables mineralisation and geometric properties to adapt in response to mechanical strains and loading (94). This theory is supported by reductions in BMC and BMD in astronauts experiencing a period of weightlessness (95) and higher BMC and bone cross-sectional area (CSA) in the dominant limb of individuals partaking in racquet sports (96). Furthermore, some (97), but not all (98), studies have shown that peak LM precedes PBM, further supporting the notion that skeletal muscle function may influence bone acquisition. Thus, although obesity is typically associated with greater bone CSA and BMD in children (93), the mechanostat theory would suggest that this is the result of higher LM and the greater strains that higher body weight imposes on bone.

1.2.9.2 Hormonal

Many hormones that are essential for linear growth and pubertal development have actions on bone, muscle and/or adipose tissue. Growth hormone is an important anabolic hormone and in addition to stimulating linear growth, it has positive effects on the development of LM and adipose tissue function. Children with growth hormone deficiency typically have low muscle mass and strength and increased central adiposity in addition to short stature. These features can be corrected by exogenous growth hormone administration (99).

Sex steroids similarly influence both bone and lean tissue development. At puberty, the increase in testosterone in males results in increased BMD and expansion of muscle mass. Androgen deficiency results in low BMD and LM which corrects with testosterone replacement. Oestrogen similarly has important roles in bone mineralisation and maintenance of soft tissue body composition, as clearly demonstrated by the loss of BMD and increase in adiposity occurring after the menopause.

Glucocorticoid excess, both endogenous and when used for therapeutic reasons, is associated with lower BMD, increased adiposity and muscle weakness.

The role of vitamin D in bone, fat and muscle function will be discussed in detail in section 1.4.5.

1.2.9.3 Adipokines, osteokines and myokines

There is also increasing recognition that adipose tissue, bone and skeletal muscle interact through hormones derived from within these tissue compartments, as summarised in Figure 1.11. This cross-talk between cell types might be important to modulating body composition and balancing the expansion of each tissue type.

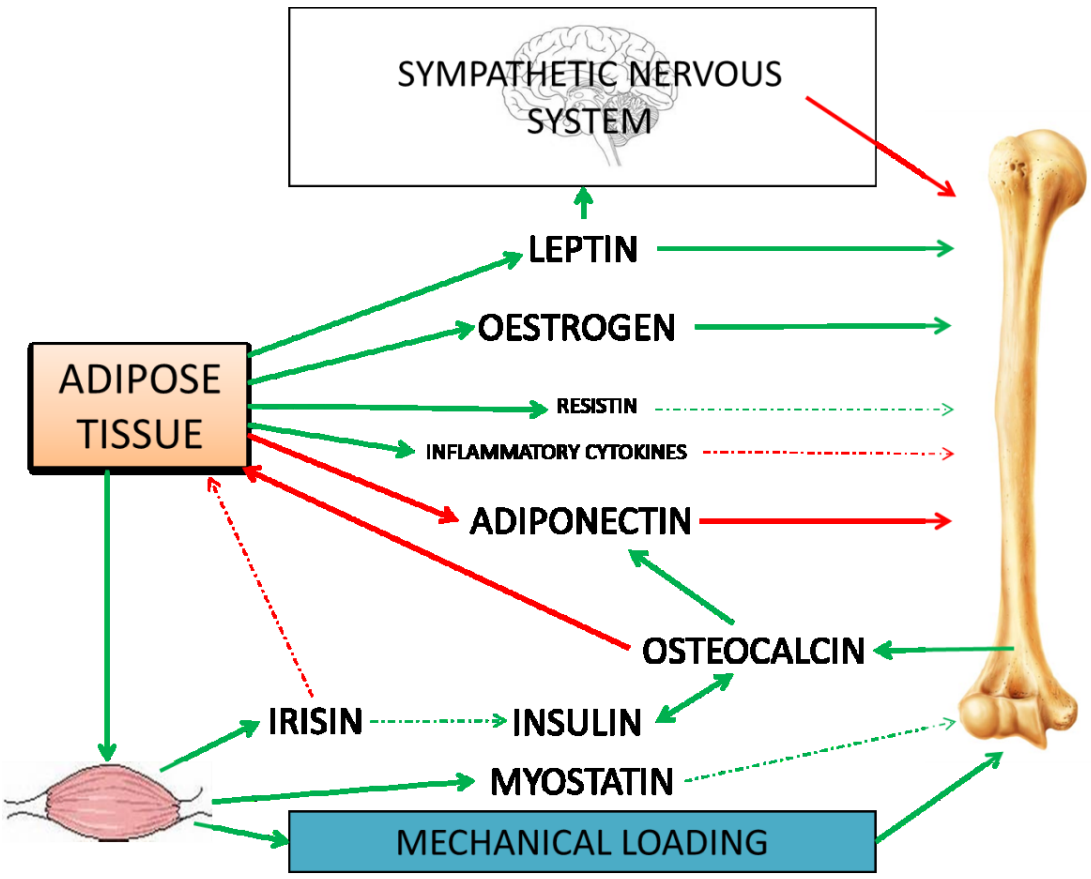


Figure 1.11: Interactions between bone, adipose tissue and skeletal muscle
Red arrows represent inhibitory effects, green arrows stimulatory effects and dotted arrows show possible pathways, which require further characterisation.

Adipocytes are active endocrine organs and the most well characterised adipocyte-derived hormones are leptin and adiponectin. Leptin has key roles in regulation of appetite and body weight. Serum leptin levels are positively correlated with FM, and the actions of leptin via the hypothalamus to suppress appetite and increase energy expenditure should therefore homeostatically maintain FM levels. Moreover, leptin can also influence bone metabolism; ob/ob leptin deficient mice have high bone mass despite hypogonadism, but intracerebroventricular infusion of leptin reverses this phenotype and in wild-type mice induces bone loss (100). Conversely, in vitro leptin can increase osteoblast differentiation, which is likely to stimulate bone formation (101). Adiponectin is also secreted by adipose tissue, but in contrast to leptin, serum levels are inversely correlated with FM and therefore levels are low in obesity. It has a major role in increasing insulin sensitivity, but adiponectin concentrations also appear to be negatively associated with BMD in adults independent of FM (102), and the adiponectin receptor has been identified on both osteoblasts and osteoclasts (103). Adipose tissue is additionally a site of peripheral synthesis of oestrogen through aromatization of androgens, and increased oestrogen will have positive effects on BMD.

Osteocalcin is secreted by osteoblasts, but influences adiposity. Osteocalcin knockout mice have increased FM, reduced insulin secretion and impaired glucose tolerance. In vitro and in vivo work supports a role for osteocalcin in insulin secretion, and osteocalcin infusions decrease FM in wild-type mice. The exact mechanism for this is currently unclear, although alterations in energy expenditure might be involved (104). Furthermore, osteocalcin can induce adiponectin expression, and through this mechanism might therefore have a negative feedback on bone formation (105).

Myokines released by skeletal muscle are the least well characterised. However one candidate myokine is myostatin, which has a role in switching muscle fibres from slow twitch to fast twitch type. Moreover, myostatin knockout mice have a phenotype characterised by high muscle mass, reduced white but increased brown adipose tissue and increased insulin sensitivity. Conversely, overexpression of myostatin is associated with low muscle mass and increased BMD and BMC (106). However correlations between serum myostatin and muscle and bone mass in humans are less conclusive (106). Irisin is a second emerging myokine, which appears to influence adipose tissue, resulting in increased energy expenditure through the conversion of white to brown adipocytes (107), but similarly, further characterisation is needed.

1.2.10 Summary

Body composition is an important determinant of health and disease. The relative size of the FM, LM and BMC is constantly changing throughout the lifecourse in response to the hormonal stimulants of linear growth and aging and will be influenced by cross-talk between these compartments. Despite of this, there is evidence to demonstrate the tracking of each of these compartments, therefore suggesting that manipulation of the size of one compartment in early life might have long-lasting effects. I will now discuss the concept of developmental programming and how this might be used to address the increasing burden of obesity, sarcopenia and osteoporosis.

1.3 Development Programming

1.3.1 Developmental plasticity

Development plasticity is the ability of a single genotype to give rise to multiple different phenotypes. The phenomenon allows organisms to adapt to prevailing environmental conditions during critical periods of development, thereby conferring a survival advantage. It is observed in many animal species. One example is the meadow vole (*Microtus pennsylvanicus*), in which the thickness of the coat in the offspring is determined by the photoperiod experienced by the mother during gestation. Pups born in autumn have a thicker coat than those born in spring (108). In this example, maternal signals regarding the environmental conditions, most likely melatonin (109), indicate to the pup to adopt a developmental trajectory which is appropriate to the postnatal environment to which it is likely to be exposed after birth. However, a mismatch between the expected postnatal environment and that to which the pup has been developmentally programmed, for example due to a change in the postnatal environment or inappropriate maternal cues, would be at detriment to survival. This is shown schematically in Figure 1.12.

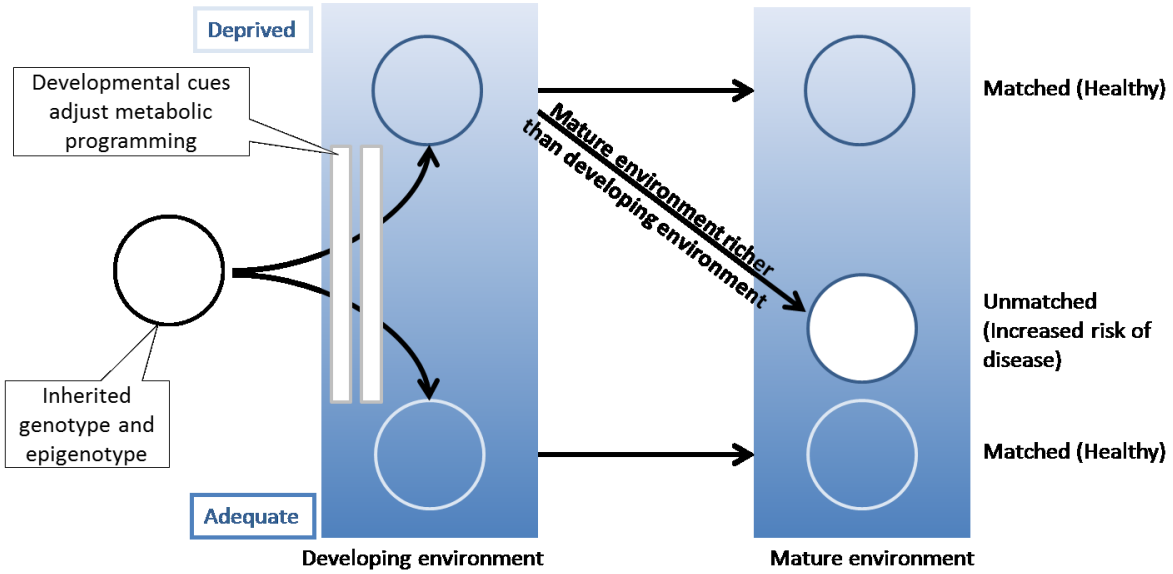


Figure 1.12: Developmental plasticity
The default trajectory of development is modified in response to environmental cues. In the event that the mature environment mismatches with the developing environment, there is increased risk of disease.
Reproduced with permission from Gluckman et al, 2007 (110)

1.3.2 Developmental origins of health and disease hypothesis

It is now recognised that developmental programming in the human fetus and infant might have a role in long-term non-communicable disease risk. The DOHAD hypothesis was first proposed by Barker et al who recognised a close geographical relationship between infant mortality rates and standardised mortality rates from cardiovascular disease 65 years later (111). Subsequent work has demonstrated that birth weight, even within the normal range, is inversely associated with cardiovascular and all-cause mortality (112) and risk factors for cardiovascular disease including T2DM (113) and hypertension (114). Low birth weight can be a marker of fetal undernutrition, which can occur either due to poor placental function or maternal nutritional deprivation. Adaptations to physiological and metabolic processes will prepare the fetus for a nutritionally deplete postnatal environment to match that experienced in utero. For example insulin resistance will be increased. This differs from the currently nutritionally abundant society, and can potentially result in pathological outcomes. Indeed accelerated growth in infancy and childhood, particularly in those with low birth weight ("catch up growth") is also associated with an increased risk of cardiovascular outcomes (115), further supporting this hypothesis. There is now evidence to suggest that the DOHAD hypothesis also extends to other diseases, including obesity, osteoporosis and sarcopenia.

1.3.3 Developmental programming of body composition

Epidemiological studies have shown that both high and low birth weights are associated with an increased risk of obesity in childhood and adulthood (116). In the Southampton Women's Survey (SWS) mother-offspring birth cohort study in Southampton, UK, higher maternal fat stores as measured by triceps SFT, were positively associated with offspring birth weight (66). Thus, for high birth weight infants, it is possible that shared obesogenic traits in the postnatal environment, particularly diet and physical activity, or shared genetic determinants of obesity, might underlie the relationship between birth weight and later BMI. For infants with low birth weight, the pathway to later life obesity, similarly to the observations with cardiovascular disease, is likely to reflect rapid catch up growth in the early postnatal period due to a mismatch between nutritional expectation and availability. Indeed, greater infancy weight gain, particularly in those who were born small for gestational age (SGA), has also been associated with a higher risk of obesity in childhood and adolescence (117).

As discussed previously, BMI is a poor indicator of body composition, yet there is also evidence accruing that the relative proportions of LM and FM in addition to fat distribution and muscle strength are influenced by the in utero environment. Many studies have demonstrated that birth weight is positively associated with LM and muscle strength in childhood and adulthood (118-122). Dodds et al included 14 studies in a meta-analysis and despite a wide range of ages included in these studies, they found that for every 1 kg increase in birth weight, age and height adjusted grip strength increased by 0.86 kg (95% confidence interval (CI) 0.58, 1.15) (119). Although the relationship with grip strength might partly reflect the positive association between birth weight and LM, differences in muscle composition in late adulthood in those with lower birth weight have also been reported (123).

Low birth weight has also been associated with higher FM, although the findings are less consistent than for LM. For example, Kensara et al studied 32 men aged 64-72 years, half of whom had a birth weight ≤ 25th centile and half greater than ≥ 75th centile; adult height and weight were significantly greater in those with the higher birth weights, and after adjustment for these, %FM measured by DXA was approximately 4% lower in the men with higher birth weights than the low birth weight group (120). Gale et al similarly found in 132 men and women of similar age to those included in the study by Kensara et al that total FM measured by DXA was nearly 2 kg higher in those with birth weight < 3.15kg compared to > 3.64 kg after adjustment for age, sex and adult height and weight (121). In contrast, analysis of the ALSPAC cohort at 9-10 years of age found a positive association between birth weight and total FM measured by DXA and adjusted for sex, age, gestational age at birth, height, height^2 and a number of maternal covariates (122).

Only in the girls and not the boys was there a suggestion of a J-shaped association between birth weight and FM. There was a small increase in FM in the girls in the lowest decile of birth weight compared to other deciles at the lower end of the birth weight range but even in those with the lowest birth weights FM at age 9-10 years remained lower than those with the highest birth weights. Notably there are many differences between these studies, in particular the age of the participants, the methods of analysis (categorical versus continuous) and covariates included which might account for the different findings. Additionally the large size of the ALSPAC cohort will have enabled the inclusion of more participants with very low and high birth weights. However, it has also been suggested that the relationship between low birth weight and later life adiposity is modified by catch-up growth in infancy. Indeed, again using the ALSPAC cohort, Ong et al demonstrated that children who experienced clinically significant catch-up growth from birth to 2 years of age had lower birth weights, but also had greater FM and %FM assessed by 4 site SFT at 5 years of age (124). Similarly, using DXA to assess body composition, Leunissen et al found that %FM was greater in early adulthood in those born SGA who displayed catch-up growth in infancy compared to those of normal birth weight. In contrast, being born SGA but not experiencing catch-up growth did not result in a significant difference in %FM compared to the normal birth weight group (125). Furthermore, SGA and catch-up growth have been associated with increased central adiposity measured by either waist:hip ratio or DXA in several studies (126-128), which is further associated with increased risk of insulin resistance, T2DM and the metabolic syndrome. Overall, these findings would support the DoHAD theory that a mismatch between prenatal and postnatal environment increases the risk of later disease.

Whilst the observations between birth weight and BMI and body composition are interesting, identification of modifiable factors which determine birth weight and/or long term body composition would enable clinical intervention to improve long-term outcomes. There is now increasing evidence from observational studies that maternal lifestyle, anthropometric and dietary factors are associated with offspring body composition at birth (66, 129), and that these relationships persist into later childhood (130). One such example of the potential effect of an adverse in utero environment on the development programming of body composition is the relationships with maternal smoking; in the SWS we have demonstrated that infants born to mothers who smoked in late pregnancy have symmetric growth restriction with proportionally lower FM at birth than infants of non-smokers (131). However, by 6 months of age, infants of mothers who smoked have a higher BMI z-score, which persisted at 6 years. In contrast to at birth, at 6 years of age being born to a mother who smoked in pregnancy was associated with significantly greater BMI z-score and whole body %FM measured by DXA (Figure 1.13) (131). This finding remained statistically significant after adjustment for maternal age, educational

attainment, parity, maternal height, pre-pregnancy BMI, weight gain during pregnancy, walking speed and alcohol consumption in late pregnancy, duration of breastfeeding and the child's dietary quality, but other differences in post-natal environment might have also confounded the relationship. Undertaking a randomised controlled trial (RCT) of smoking in pregnancy to confirm the relationship with offspring adiposity is clearly not feasible, but trials of nutritional supplements are possible. For example, observational data suggest that maternal serum polyunsaturated fatty acid (PUFA) concentrations during pregnancy are associated with offspring adiposity (130), yet the few trials published to date have not demonstrated positive effects of gestational fish oil supplementation on this outcome (132-134). This highlights the need to undertake RCTs, where possible, before implementing public health policy.

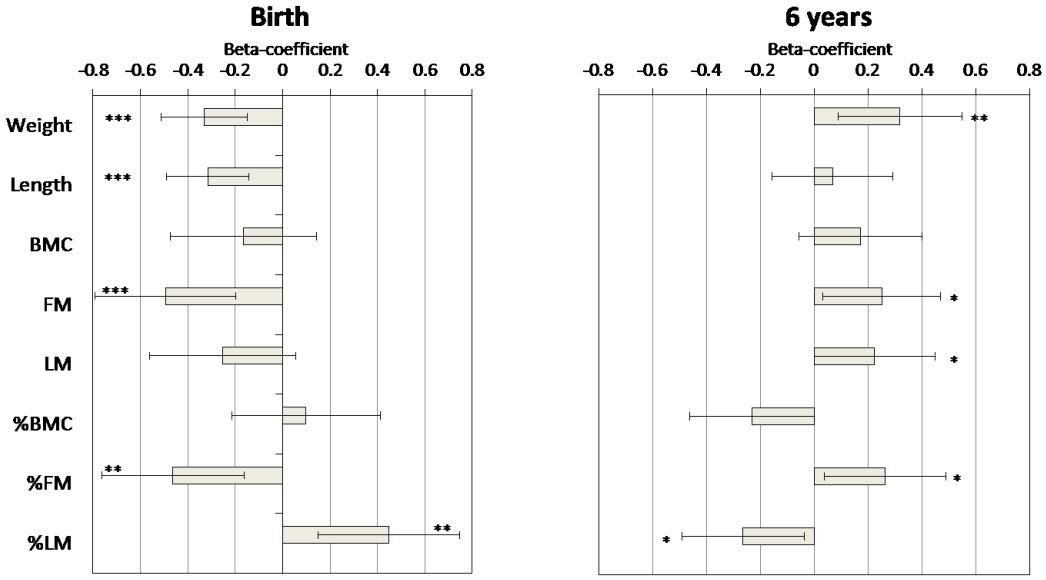


Figure 1.13: Effect of maternal smoking on offspring body composition assessed by DXA at birth and 6 years of age

Shown as standard deviation change (95% CI) for smoking vs non-smoking in late pregnancy. Adjusted for maternal age, educational attainment, parity, maternal height, pre-pregnancy BMI, weight gain during pregnancy, walking speed and alcohol consumption in late pregnancy, duration of breastfeeding and the child's dietary quality at 6 years.
*p < 0.05, **p < 0.01, ***p < 0.001

1.3.4 Developmental programming of bone mineralisation

Birth weight has also been associated with bone mass: in meta-analysis of six studies, for every 1 kg increase in birth weight, lumbar spine BMC in adulthood increased by 1.49 g (95% CI 0.77, 2.21)

(135). Similarly, meta-analysis of five studies demonstrated that adult hip BMC increased by 1.41 g (95% CI 0.91, 1.91) for every kg increase in birth weight (135). However, birth weight was not significantly associated with LS or hip BMD (135). As BMC is a composite measure of bone size and true volumetric BMD, the associations with BMC but not BMD would suggest that this relationship reflects associations between birth weight and skeletal size. Although BMD is used in the definition of adult osteoporosis, skeletal size is also an important determinant of fracture risk and extrapolation of the BMC data suggest there is a 12% increased risk of hip fracture for every 1 kg decrease in birth weight (135). Furthermore, a recent study demonstrated that birth weight was positively associated with radial CSA and strength strain index, a measure of bone strength, at 60-64 years (136). Both smaller bone area and lower strength strain index are also associated with increased fracture risk (137, 138)

There is also now increasing evidence that maternal antenatal characteristics are associated with offspring bone development: maternal smoking in pregnancy, faster walking speed and lower fat stores in late pregnancy were associated with lower offspring neonatal whole body BMC and BMD (69). One study suggested that the negative association between maternal smoking and offspring bone mineralisation persists into later childhood (139), although this is not consistent in all cohorts (140, 141). However, the greater BMI observed in children born to mothers who smoke (131), which is associated with a higher BMC and BMD, could confound this relationship. Maternal diet during pregnancy has also been associated with offspring BMC and aBMD during later childhood. In 198 women participating in the Princess Ann Hospital Study, maternal diet was assessed using a food frequency questionnaire at 15 and 32 weeks of pregnancy. A dietary score was calculated from this to quantify the consistency of the woman's intake with recommendations for a healthy diet. A positive association was observed between a more "prudent" diet (characterised by higher intakes of fresh fruit and vegetables and lower processed foods) in late pregnancy and offspring whole body and LS BMC and aBMD at 9 years of age (142). Several studies have also examined the relationships between individual dietary components, including macronutrients and micronutrients (e.g. phosphorus, folate, magnesium) in pregnancy and offspring bone mass, and whilst the findings are inconsistent, this could reflect the inaccuracies in estimating intake from food frequency questionnaires and differences in the populations studied (143-147). The role of maternal vitamin D status in offspring bone development has been investigated in several observational studies and will be discussed in section 1.4.7.3.

1.3.5 Summary

There is evidence to support the theory that the prenatal environment to which an individual is exposed might have long-lasting effects on body composition. Currently, the exact mechanisms and potential nutrients involved in this programming are not well understood, but identification of such factors could enable the development of clinical interventions. One potential candidate is vitamin D, and in the next section I will briefly review the biology of vitamin D and its known functions, discuss the existing evidence that relates maternal vitamin D status to developmental programming of growth and body composition and highlight the gaps in the current literature in relation to this.

1.4 Vitamin D and developmental programming

1.4.1 Vitamin D metabolism

Vitamin D can be derived from the diet, as ergocalciferol (vitamin D_2) from plant sources, or cholecalciferol (vitamin D_3) from animal sources. However, the majority is formed endogenously within the skin from the action of ultraviolet B (UVB) (290-315nm wavelength) to convert 7-dehydrocholesterol to pre-vitamin D_3. This pro-hormone is hydroxylated in the liver to calcidiol (25-hydroxyvitamin D [25(OH)D]) by a cytochrome-p450 dependent enzyme (25-hydroxylase). 25(OH)D is the predominant circulating form of vitamin D, either bound to vitamin D binding protein (DBP), albumin, or in the free form. This circulating 25(OH)D acts as a reservoir for conversion to the active metabolite, calcitriol (1,25-dihydroxyvitamin D [1,25$(OH)_2$D]).

25(OH)D is converted primarily in the renal proximal tubular cells, although additionally to a lesser degree in extra-renal tissues including bone and the parathyroid gland, by the 1α-hydroxylase enzyme, to the active form 1,25$(OH)_2$D. From the kidney, calcitriol is secreted into the circulation and acts in an endocrine manner, whereas the extra-renally synthesised 1,25$(OH)_2$D has autocrine and paracrine functions. 25(OH)D is also converted to the inactive metabolite, 24,25-dihydroxyvitamin D, primarily within the kidney, in the first step of the degradation pathway.

1.4.2 Classical actions of vitamin D

The classical function of $1,25(OH)_2D$ is in calcium and phosphate homeostasis. Synthesis of calcitriol within the kidney is tightly regulated in response to serum ionised calcium (Ca^{2+}) levels. This occurs in conjunction with parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23). Low Ca^{2+} stimulates the release of PTH from the parathyroid gland. PTH simultaneously increases renal calcium reabsorption in the distal tubule of the kidney, decreases proximal tubule phosphate reabsorption, and upregulates 1α-hydroxylase activity to increase calcitriol synthesis. The main action of $1,25(OH)_2D$ is to increase uptake of dietary calcium through the intestinal enterocytes. PTH also promotes bone resorption by osteoclasts, thereby mobilising calcium and phosphate from bone mineral. However, the presence of calcitriol is required for this to occur. Completion of the negative feedback loop occurs directly with the calcium sensing receptor in the parathyroid gland reducing release of PTH in response to increased serum Ca^{2+}, but also through FGF-23. The production of FGF-23 is increased by calcitriol and high circulating phosphate and it acts to increase urinary phosphate excretion and down-regulate 1α-hydroxylase activity and PTH release (Figure 1.14).

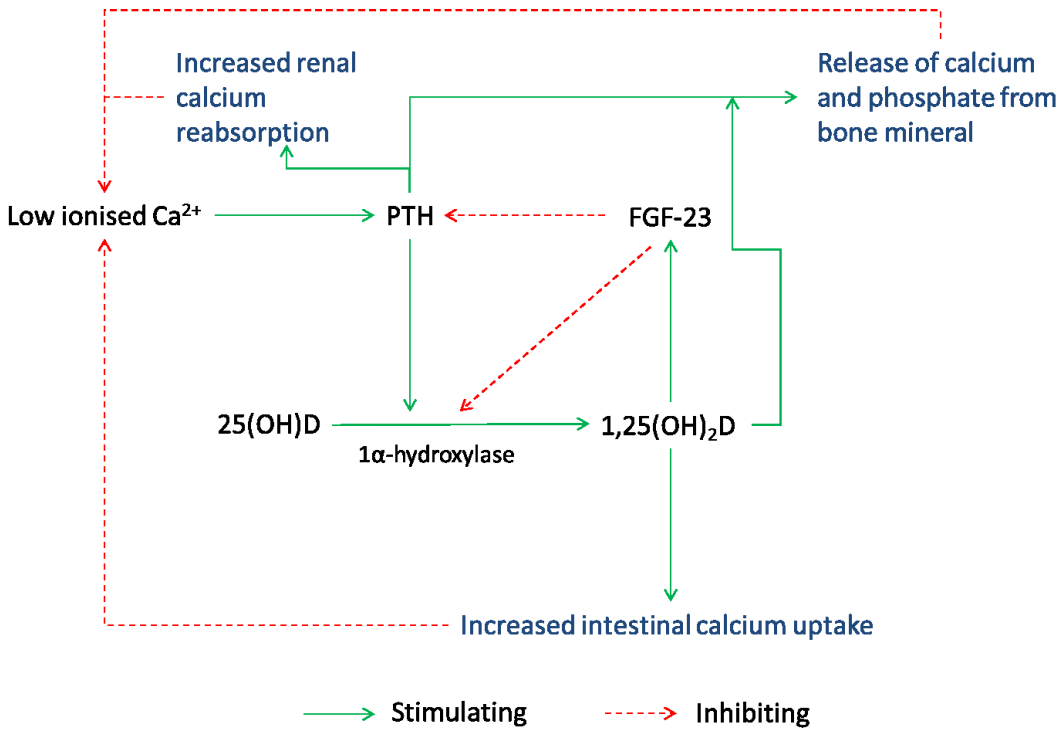


Figure 1.14: Pathways involved in calcium homeostasis

Low levels of 25(OH)D can result in low intestinal calcium absorption, and subsequently a reduction in serum Ca^{2+}. This leads to secondary hyperparathyroidism, with subsequent mobilisation of bone mineral, increased renal calcium re-absorption, but also increased urinary phosphate wasting. As such, in early vitamin D deficiency (VDD), serum calcium concentration is usually maintained in the normal range, but phosphate is often low. Hypocalcaemia will ensue when skeletal calcium stores are depleted.

1.4.3 The vitamin D receptor

Vitamin D has both slow genomic actions, such as transcription of intestinal calcium channels and FGF-23, and more rapid non-genomic effects, including rapid intestinal calcium uptake (148). Vitamin D is a secosteroid, thus the genomic actions are mediated through the binding of $1,25(OH)_2D$ to the nuclear vitamin D receptor (VDR). The $1,25(OH)_2D$-VDR complex can heterodimerise with a retinoid X receptor (RXR) (usually the RXR-A), which binds to vitamin D response elements in promoter regions of target genes, resulting in activation or repression of gene transcription. The mechanisms of the non-genomic effects remain to be fully elucidated, but likely involves the VDR, but located within the plasma membrane (148).

Although the classical function of vitamin D is in calcium and phosphate homeostasis, the nuclear VDR has been identified in a wide variety of cell types including osteoblasts, keratinocytes, macrophages, pancreatic β cells, adipose tissue and skeletal muscle (149, 150). This supports the hypothesis that vitamin D might have diverse actions apart from in bone and calcium metabolism.

1.4.4 Epidemiology of vitamin D deficiency

1.4.4.1 Assessment of vitamin D status

Hepatic 25-hydroxylation of cholecalciferol to 25(OH)D is not physiologically regulated but is dependent of substrate availability. In contrast, the conversion of 25(OH)D to $1,25(OH)_2D$ is tightly regulated in response to serum Ca^{2+} and PTH. 25(OH)D has a half-life of approximately 2-3 weeks (151), whereas $1,25(OH)_2D$ has a significantly shorter half-life of 4 to 6 hours (151). As such, 25(OH)D is currently considered the best biochemical markers of vitamin D status.

The two most common methods to measure 25(OH)D concentration are immunoassay and chromatography based, typically combined with tandem mass spectrometry (LC-MS/MS). The

analytical method used must be able to measure both 25(OH)D_2 and 25(OH)D_3. As such LC-MS/MS is considered the gold standard due to high specificity for each metabolite enabling quantification of 25(OH)D_2 and 25(OH)D_3 separately, which can be combined for a total 25(OH)D result. LC-MS/MS is however time consuming, expensive and requires well-trained operators, and therefore automated immunoassays are commonly used in clinical settings. Their major disadvantage is a lower ability to detect 25(OH)D_2 compared with 25(OH)D_3. The Diasorin Liaison^{TM} chemiluminescence assay is able to measure 25(OH)D_2 and 25(OH)D_3 equally (152), whereas the Immunodiagnostic Systems (IDS) radioimmunoassay only has approximately 75% cross-reactivity for 25(OH)D_2 and the Roche immunoassay does not detect 25(OH)D_2 (152). As such, care should be taken in interpreting studies using these latter assays, particularly if high levels of dietary ergocalciferol is likely or ergocalciferol supplementation has been taken. Variability between analytical techniques has also been reported. The Diasorin Liaison platform typically reports a lower value for serum 25(OH)D for the same sample than LC-MS/MS (152). The Vitamin D External Quality Assurance scheme (DEQAS), in which over 1200 laboratories from 54 countries participates, aims to maintain the reliability of 25(OH)D analysis (www.deqas.org).

1.4.4.2 Definition of vitamin D deficiency

Although vitamin D status has been related to a wide range of biochemical and clinical outcomes in observational studies (153), the serum concentration of 25(OH)D which is felt to constitute adequacy is a subject of much debate. The recommended threshold for a definition of VDD is highly variable between guidelines and consensus statements. A number of these are summarised in Table 1.2. The great variability in these definitions partly reflects that there does not appear to be a single threshold below which secondary hyperparathyroidism or clinical outcomes, such as metabolic bone disease, always occurs in either adults (154, 155) or children (156). This is likely due to the interaction between vitamin D and dietary calcium intake, which will modify the association between 25(OH)D and PTH (157). Nonetheless, variations in the definitions used limits comparison of the prevalence of VDD reported in different studies.

1.4.4.3 Incidence and risk factors for low serum 25-hydroxyvitamin D

There are few data which document the epidemiology of serum 25(OH)D concentrations across the general population, but studies in selected groups often report a high prevalence of biochemical VDD. For example, two recent large studies in the UK demonstrated that around a third of children had a serum 25(OH)D < 50 nmol/l (158, 159). A number of risk factors have been

Table 1.2: Definitions of vitamin D deficiency, insufficiency and sufficiency according to a number of recent guidelines and consensus statements

Guideline	*Deficiency (nmol/l)*	*Insufficiency (nmol/l)*	*Sufficiency (nmol/l)*
Institute of Medicine (IOM)(160)	< 30	30-50	≥ 50
Endocrine Society Practice Guidelines (161)	< 50	50-75	≥ 75
British Paediatric and Adolescent Bone Group (162)	< 25	25-50	≥ 50
Global Consensus Recommendations on Prevention and Management of Nutritional Rickets (163)	< 30	30-50	≥ 50
National Osteoporosis Society (UK) (164)	< 30	30-50	≥ 50
Canadian Paediatric Society (165)	< 25	25-75	75-225
Working group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia (166)	< 50		≥ 50 At the end of winter (level may need to be 10-20 nmol/l higher at the end of summer)

identified for VDD, many of which are related to reduced UVB exposure and limited cutaneous synthesis of cholecalciferol. In the UK and other high latitude countries, there is marked seasonal variation in the prevalence of VDD (158, 159, 167, 168). 25(OH)D levels are typically lowest in late winter and peak in mid-late summer months. Furthermore the effect of latitude on 25(OH)D status is even observed within the UK with children and post-menopausal women residing in Northern England and Scotland having lower 25(OH)D levels than those living in the South of England (158, 168, 169). Figure 1.15 clearly shows the higher 25(OH)D levels in postmenopausal women living in Surrey compared to Aberdeen. As such, at northern latitudes, individuals are more reliant on dietary sources and supplementation to prevent VDD during winter months (158, 168, 170). Furthermore, even at the same latitude, serum 25(OH)D levels are consistently higher in white compared with dark skinned individuals (Figure 1.15) (158, 169-171). Greater outdoor time and play is protective against VDD (158, 159), whereas extensive skin covering for religious or cultural reasons and liberal use of sun protection prevent cutaneous vitamin D synthesis (172).

A number of studies have suggested that markers of greater social deprivation, including lower parental educational achievement and income and living in rented housing, are associated with poorer 25(OH)D status in childhood (158, 159, 170, 171). The mechanisms for this have not been

elucidated, but could include lower outdoor leisure time, sunlight exposure or poorer dietary intake.

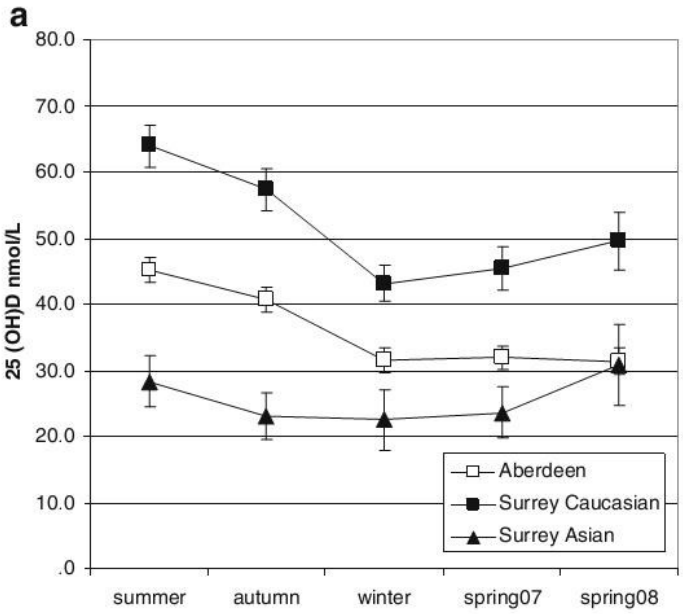


Figure 1.15: Seasonal variation in 25-hydroxyvitamin D status in post-menopausal women living in Aberdeen and Surrey
Reproduced with permission from Macdonald et al, 2011 (169)

1.4.5 Vitamin D, bone and body composition

1.4.5.1 Bone health

1.4.5.1.1 Rickets and osteomalacia

Rickets and osteomalacia are the bony consequences of severe VDD. Rickets is a disorder of growth plate ossification and mineralisation, which occurs only in growing bone. VDD is one of a number of biochemical and/or hormonal disturbances (eg calcium deficiency, phosphate deficiency) which result in the clinical phenotype of rickets. Hypophosphatemia is the common end pathway in these biochemical abnormalities, and is postulated to be the cause of the rachictic changes in the growth plate (173). The clinical features of rickets are variable and in part dependent on the developmental age of the child. The classical skeletal features include bony swelling at the wrists, knees, ankles and costochondral junction (rachictic rosary), and in mobile

children bending of the limbs might be observed. Linear growth, dentition and motor development are also commonly delayed. In the neonatal period and in adolescence, when calcium demands are higher to meet the demands for faster linear growth, hypocalcaemic seizures can be the first presenting feature of rickets.

Following fusion of the growth plates, VDD can result in osteomalacia, in which there is undermineralisation of the protein matrix (osteoid) of bone. This is a histological diagnosis that can only definitively be made by bone biopsy. Clinical features of osteomalacia include bony pains, muscle weakness and pathological fractures, and hence presence of these symptoms in the context of a low serum 25(OH)D level would be clinically suspicious of osteomalacia and treatment would be warranted without bone biopsy. Osteomalacia contrasts with osteoporosis in which there is normal mineral:osteoid ratio but overall bone mass is reduced.

1.4.5.1.2 Subclinical vitamin D deficiency and bone health

There is increasing interest as to whether low levels of serum 25(OH)D, which are not sufficiently low to cause rickets or osteomalacia, can still have detrimental effects on bone development and BMD.

Early infancy is a period of particular interest due to the low vitamin D content in maternal breast milk. However, three interventional studies of vitamin D supplementation in breast fed infants found no differences in whole body or LS BMC or aBMD compared to either placebo or increasing doses of vitamin D supplementation at 3 months (174), 6 months (175) or 12 months of age (175, 176). In a fourth small study distal radius BMC measured by single photon absoprtiometry was significantly greater at 12 weeks of age in infants who received 400 IU/day vitamin D compared to the placebo group. However, no difference in this outcome was observed between the two groups at 26 weeks of age despite a persistent difference in serum 25(OH)D, but the small number of study participants (n=13) would have lower power to detect a difference (177).

A number of cross-sectional studies in children and adolescents have examined the association between 25(OH)D status and aBMD, BMC or BMAD assessed by DXA. When 25(OH)D has been considered as a continuous variable, the findings of these studies are inconsistent, but variation in the age of the study participants, geographic locations and confounding factors considered limits comparison (178-194). However, when 25(OH)D status has been categorised, children and adolescents with the lowest levels of serum 25(OH)D consistently had significantly lower BMD at one or more skeletal sites (178, 182, 183, 185, 195, 196), except in studies based on cohorts of ballet dancers in which high levels of indoor physical activity might have confounded the findings

and/or caused reverse causality (194, 197). A non-linear association between vitamin D status and BMD is also suggested by a meta-analysis of RCTs of vitamin D supplementation in children. Winzenberg et al included 6 studies with a total of 343 participants randomised to placebo and 541 to cholecalciferol. Vitamin D doses in the included studies ranged from 132 IU/day to 14 000 IU/week for one to 2 years. Overall, vitamin D supplementation did not lead to significant gains in whole body BMC, forearm, hip or LS aBMD, but in the studies with a baseline 25(OH)D < 35 nmol/l, vitamin D supplementation had a significant effect on whole body BMC and LS BMD, which equated to approximately a 2.6% and 1.7% percentage greater change in participants randomised to cholecalciferol (198). This effect was not observed in those with a baseline 25(OH)D > 35 nmol/l, therefore supporting the notion that very low levels of 25(OH)D might have subclinical effects on bone development.

Evidence for a role of vitamin D supplementation in fracture and bone loss prevention in adults is equally inconsistent. Meta-analysis of 23 RCTs of vitamin D supplementation on aBMD assessed by DXA did not show a significant effect on aBMD at the LS, hip, whole body or forearm, but femoral neck aBMD was significantly increased (weighted mean difference 0.8% [95% CI 0.2, 1.4], p=0.005) (199). There was marked heterogeneity in study design, type and dose of vitamin D supplementation, and age, sex and ethnicity of study participants. In contrast to the aforementioned meta-analysis in children, stratification of studies by mean baseline 25(OH)D did not alter these findings, but this could reflect the higher cut-point chosen to define low 25(OH)D (50nmol/l) (199). However, there is evidence to support a role for vitamin D in fracture prevention in older individuals, but only when taken in combination with calcium supplementation (200). Indeed, in a Cochrane meta-analysis of low risk community dwelling post-menopausal women in whom hip fracture incidence is approximately 8 per 1000/year, vitamin D and calcium supplementation reduced this by 1 per 1000/year. In institutionalised individuals, for whom hip fracture rate is substantially higher, vitamin D with calcium supplementation can reduce hip fracture rate by 9 per 1000/year (200). As the effect of vitamin D supplementation on BMD is less clear, it is therefore possible that the reduction in fracture risk is occurring due to extraskeletal actions of vitamin D, which might include effects on muscle function and subsequently falls incidence or adiposity/obesity, which is additionally associated with fracture risk.

1.4.5.2 Muscle mass and function

It is well recognised that myopathy, muscle pains and, in young children, developmental delay can be presenting features of severe VDD. These symptoms typically improve following vitamin D

supplementation (201-204), but whether they occur as a direct result of low 25(OH)D or due to other biochemical disturbances secondary to this, for example hypophosphataemia or hypocalcaemia, is not currently understood. However, histological studies have demonstrated atrophy of the type II muscle fibres in VDD (205), and an increase in type II fibre size and number following supplementation with 1000 IU/day ergocalciferol in the non-paretic limb of elderly Japanese women with post-stroke hemiparesis (206). These fibres are required for rapid bursts of muscle power.

Several observational studies have demonstrated positive associations between 25(OH)D status and measures of muscle mass (207), muscle strength and power in adolescents (208, 209), but interpretation of these studies is limited by the potential for reverse causality and confounding as more physically active individuals are probably more likely to spend greater lengths of time outside. There is only one trial of vitamin D supplementation in adolescence examining the effect on muscle function: Ward et al randomised 72 ethnically diverse post-menarchal girls aged 12-14 years to 150 000 IU oral ergocalciferol or placebo every 3 months for 1 year. At the end of the study, jumping height and velocity and hand-grip strength were numerically greater in the girls randomised to vitamin D but these did not reach statistical significance (210).

Meta-analysis of RCTs of vitamin D supplementation in adults have demonstrated a small, but statistically significant, beneficial effect of vitamin D supplementation on muscle strength (29 studies, standardised mean difference of 0.17 [95% CI 0.03, 0.31], p=0.02) but not muscle mass (6 studies) or muscle power (5 studies) (211). The included studies were markedly heterogeneous in terms of population studied and degree of supplementation, and varied in the methods of outcome assessment, however, similarly to meta-analyses of bone outcomes when studies were stratified on participant characteristics, vitamin D supplementation had a greater effect on muscle strength in individuals with a serum 25(OH)D < 30 nmol/l, aged over 65 years and female. Furthermore, when studies using grip strength and lower limb muscle strength as the outcome were considered separately, the effect was only significant for lower limb strength (211).

1.4.5.3 Obesity and adiposity

There is an ever expanding field of data demonstrating that overweight and obese individuals have a higher prevalence of VDD than those of healthy weight (171, 212). Moreover in observational studies, FM measured by DXA is consistently negatively associated with 25(OH)D status (213, 214). As with the relationships between 25(OH)D and BMD and muscle mass/strength, a confounding effect of outdoor physical activity might also underlie this

relationship, and indeed, objectively assessed physical activity using accelerometry is positively associated with 25(OH)D status in children and adolescents (215, 216).

PTH is positively correlated with BMI and total FM (217), suggesting a functional effect of VDD in obesity that warrants treatment with vitamin D supplementation. Furthermore, chronic excess PTH can lead to insulin resistance and has been hypothesised to promote adipogenesis (218). Despite this, there is currently limited evidence from intervention studies to support a causal role for vitamin D in the prevention of excess adiposity. To date, there is only one intervention study in childhood and adolescence. Belenchia et al randomised 35 obese adolescents to either 4000 IU/day cholecalciferol or placebo in a double-blind RCT (219). There was no significant difference in BMI, BMI z-score or waist circumference at 3 or 6 months despite a significant increase in 25(OH)D and insulin sensitivity in the intervention group (219). A greater number of intervention studies of vitamin D supplementation focussing on body composition have been conducted in adult populations. This has allowed for a recent meta-analysis, which included 12 RCTs. No significant effect of vitamin D supplementation on BMI, FM or %FM was observed (220). However, it was observed that studies in older individuals were less likely to find a significant effect (220), highlighting that further RCTs in childhood should be considered.

The lack of change in adiposity in response to vitamin D supplementation would suggest that the observed negative association between 25(OH)D and FM is a cause rather than consequence of VDD. Indeed, 25(OH)D is lipophilic and sequestration in adipose tissue will decrease its bioavailability. Serum 25(OH)D increased to a lesser extent in obese compared to non-obese individuals following total body UVB irradiation despite a similar capacity of the skin to synthesise vitamin D, suggesting sequestration outside of the circulating pool (221). This is supported by intervention studies in which a lower increase in 25(OH)D was observed in response to the same dose of vitamin D in obese compared with non-obese individuals (212, 222). Furthermore, Reinehr et al assessed 25(OH)D status at baseline and after 1 year in 67 participants of an adolescent obesity weight loss intervention programme. A significant negative relationship was identified between change in BMI z-score and change in 25(OH)D status (223), further suggesting that the low 25(OH)D might be a consequence rather than a cause for obesity. Similar results have also been observed following weight loss in adult women (224, 225). Mendelian randomisation has also been used to demonstrate that single nucleotide polymorphisms (SNP) associated with higher BMI are also associated with lower 25(OH)D, whereas SNPs known to determine 25(OH)D status were not associated with BMI (226), further suggesting that VDD occurs secondary to obesity.

In summary, there is evidence from observational studies to suggest VDD is associated with low BMD and impaired muscle mass and function, but data from meta-analysis of RCTs supports a beneficial effect of vitamin D supplementation on these outcomes only in individuals with the lowest levels of 25(OH)D. There is little evidence to support a causal role for low 25(OH)D in increased adiposity or to support the use of vitamin D supplementation to prevent or treat obesity.

1.4.6 Vitamin D and pregnancy

VDD is common in pregnancy: in the Princess Anne Hospital study, which included 198 predominantly Caucasian Women in Southampton, UK, 31% had a serum 25(OH)D < 50 nmol/l and 18% < 25 nmol/l at 34 weeks of gestation (227). However, in a more ethnically diverse population in London, 36% of women had a 25(OH)D < 25 nmol/l at pregnancy booking (228).

Alterations to maternal calcium and phosphate metabolism occur during pregnancy to meet the demands for fetal mineral accretion, as summarised in Figure 1.16 (229). The fetal skeleton contains approximately 30 g of calcium by the end of pregnancy, the majority of which is obtained during the last trimester (59). This occurs through both increased maternal intestinal calcium absorption (230) and mobilization of the maternal skeleton (231), but without alteration to maternal serum Ca^{2+} concentration. Maternal calcitropic hormones are likely to have an important role in these adaptations. Total $1,25(OH)_2D$ increases early in pregnancy (230, 232, 233). DBP also increases early in pregnancy, but unlike $1,25(OH)_2D$ does not continue to increase in late pregnancy, thus free $1,25(OH)_2D$ is increased in the third trimester relative to earlier in pregnancy (233). This increase in $1,25(OH)_2D$ appears to be independent of PTH, which remains within the normal adult range throughout pregnancy (59). However PTH-related protein is elevated in the maternal circulation from early pregnancy (232) and might contribute to the rise in $1,25(OH)_2D$.

Despite the increase in $1,25(OH)_2D$, the effect of pregnancy on 25(OH)D status is less well understood. There are few longitudinal data and most are from small studies including between 10 and 40 women. Moreover, the findings are contradictory. Zhang et al observed a reduction in 25(OH)D in late compared with early pregnancy, but as all thirty subjects were recruited in Cork, Ireland during the summer months and delivered during autumn and winter this finding might simply reflect seasonal variation (234). In contrast, Ritchie et al reported no significant differences in 25(OH)D measured in 14 women before pregnancy, in each trimester and during lactation (235) and More et al found no significant differences in 25(OH)D assessed within 3

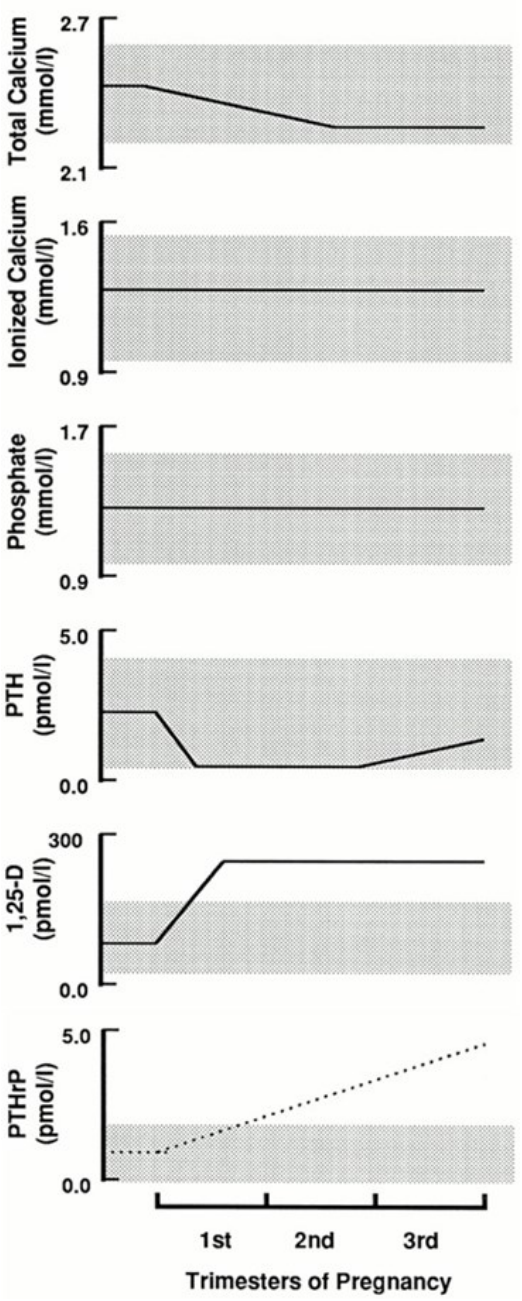


Figure 1.16: Schematic illustration of the longitudinal changes in calcium, phosphate, and calciotropic hormone levels that occur during pregnancy
Normal adult ranges are indicated by the shaded areas.
Reproduced with permission from Kovacs et al 1997 (229)

months of conception, at 22-24 weeks of gestation and within 6 days of delivery in 20 women (231). However, Cross et al did find a significantly higher 25(OH)D in the third trimester compared to the first trimester or pre-pregnancy (230), but season of recruitment and sampling is not reported. As such the effect of pregnancy on 25(OH)D remains to be elucidated and would require longitudinal data including each trimester in all seasons. Furthermore, whilst there is some evidence to suggest that an individual's 25(OH)D status remains relatively stable compared to the population in non-pregnant adults over one to five year intervals (236-238) (ie an individual

with a high 25(OH)D for the population remains within the upper end of the population distribution when 25(OH)D status is repeated at a later date), there is no data on the tracking of 25(OH)D status during pregnancy.

Nonetheless, maternal VDD in pregnancy is an important consideration as the fetus is dependent on the mother for 25(OH)D. 25(OH)D will readily cross the placenta, and maternal and umbilical cord venous blood 25(OH)D are moderate-highly correlated (239-241). Furthermore, it has been clearly demonstrated in RCTs that vitamin D supplementation in pregnancy can increase umbilical cord venous and neonatal serum 25(OH)D compared to placebo (240, 242-245), and that larger oral doses of cholecalciferol (1000 IU/day-4000 IU/day) result in higher umbilical cord venous or neonatal serum 25(OH)D when compared to supplementation with 400 IU/day (246, 247).

1.4.7 Maternal vitamin D deficiency and offspring health and development

Currently the UK Department of Health (DH) recommends supplementation with 400 IU/day cholecalciferol to all pregnant and lactating women (248). Maternal VDD has been associated with neonatal hypocalcaemia. Clinically, this can result in seizures, and has been associated with craniotabes (249), and rarely dilated cardiomyopathy (250). Symptomatic neonatal hypocalcaemia due to maternal VDD is rarely reported in infants of White mothers, and most commonly occurs in infants of mothers with dark skin pigmentation, extensive skin covering and profound VDD.

Several intervention studies assessing the effect of vitamin D supplementation on umbilical cord and neonatal serum Ca^{2+} have been reported. The results of these are inconsistent, although all but two of these studies were conducted over 20 years ago. The differences in findings might reflect the variations in supplementation used; indeed three of the four studies that used high dose weekly or monthly oral supplementation reported positive effects of vitamin D supplementation on umbilical venous cord blood Ca^{2+} (244, 251-253), whereas the trials using daily oral vitamin D supplementation tended to demonstrate a null effect (242-244, 247, 252, 254). Conversely, vitamin D supplementation consistently reduced the incidence of symptomatic hypocalcaemia in the three studies in which this outcome was reported (242, 253, 254). Thus, although the evidence for biochemical changes to calcium homeostasis with antenatal vitamin D supplementation are variable, the clinical outcome in relation to this, which is perhaps more important, is more consistent and justifies the routine use of antenatal vitamin D supplementation. However, considering that studies in non-pregnant adults have demonstrated a variable response to supplementation according to adiposity, age and baseline 25(OH)D status

(255, 256), it is possible that women with certain characteristics might require higher supplement doses to achieve vitamin D replete status. This has not been examined in pregnant women, but such knowledge could enable individualised antenatal counselling.

1.4.8 Vitamin D and development programming of growth, body composition and bone mineralisation

There is increasing evidence to suggest that vitamin D might have a more diverse role in the fetal programming of offspring development, and here I will review the existing evidence for this, and discuss whether this provides further evidence for population wide antenatal vitamin D supplementation.

1.4.8.1 Vitamin D dependent rickets

Vitamin D dependent rickets (VDDR) type I and type II are two rare inborn errors of vitamin D metabolism and function. These two genetic abnormalities represent the severest form of developmental vitamin D deficiency but do potentially provide additional clues to the roles of vitamin D in early development.

VDDR I is the result of a genetic mutation in the *CYP27B1* gene which encodes the 1α-hydroxylase enzyme. It therefore results in incomplete or reduced capacity to convert 25(OH)D to $1,25(OH)_2D$. Children with VDDR-I usually present by 2 years of age, with clinical manifestations of growth retardation, rickets, dental hypoplasia and bone pain. Biochemical evidence of rickets is also present with hypocalcaemia, hypophosphatemia, secondary hyperparathyroidism and a low $1,25(OH)_2D$. Although in utero development is reported to be grossly normal (257), due to the rarity of this condition, subclinical changes, for example BMD or body composition in the neonatal period and prior to diagnosis have not been examined. Treatment with alphacalcidol heals the rickets.

VDDR-II is due to a genetic mutation in the gene encoding the VDR, which can result in either complete or partial non-functioning of the VDR. Children typically present within the first month of life with symptomatic hypocalcaemia in addition to severe rickets, hypotonia and growth retardation. Alopecia or sparse hair is also a common feature of VDDR-II. Often these children respond poorly to calcitriol, even at supraphysiological doses, and require treatment with high dose oral or intravenous calcium supplementation (257).

1.4.8.2 Anthropometry

1.4.8.2.1 Birth weight, length and occipito-frontal (head) circumference

Many observational studies have examined the relationships between a single measurement of maternal or umbilical cord venous 25(OH)D and birth weight, length and/or occipito-frontal circumference (OFC). These studies have varied considerably in study size (n=50 to 3730), maternal gestation at assessment of 25(OH)D and the populations studied (North America (258-260), Europe (261-264), Australia (265-268), Middle Eastern countries (239, 269), Africa (270), India (271), China (241), Vietnam (272) and Singapore (273)).

When 25(OH)D was analysed as a continuous variable, a statistically significant relationship between 25(OH)D and birth weight was only reported in one study (239, 241, 258, 261, 262, 265, 266, 268-272). This study, which included 84 Arab and South Asian women in the United Arab Emirates with assessment of 25(OH)D at delivery, reported a statistically significant association with birth weight (β=11.6 g per nmol/l [95% CI 3.0, 20.1]) (269). The incidence of biochemically low serum 25(OH)D in this cohort of women was very high (median 25(OH)D 18.4 nmol/l [Interquartile range (IQR) 11.0-25.4 nmol/l]), and was the lowest reported of the studies in which birth weight was reported as an outcome (239, 241, 258, 261, 262, 265, 266, 268-272). However, multivariable regression analysis to account for other potential confounding factors was not performed in this study (269). Similarly, only one study, authored by Song et al, identified a significant association between maternal 25(OH)D and birth length (241); in this cohort 70 women in Beijing had serum 25(OH)D assessed prior to labour (exact gestation not reported) and offspring length measured at birth (r=0.25, p=0.04). The correlation between umbilical venous blood 25(OH)D and birth length in 53 of the mother-offspring pairs was similar but did not reach statistical significance (r=0.24, p=0.07). Again, multivariate analysis including maternal covariates was not undertaken. Two of seven studies considering OFC at birth as the outcome found significant associations with maternal 25(OH)D. However, Hanieh et al reported a negative association between maternal 25(OH)D at 32 weeks of pregnancy and offspring birth OFC in women in rural Vietnam (adjusted β=-0.35 cm per 25 nmol/l [95% CI -0.62, -0.09]) (272), whereas Song et al found a positive correlation between maternal 25(OH)D at delivery and neonatal OFC ($r=0.23$, $p < 0.05$), but did not perform adjustment for potential confounding factors.

In contrast, when maternal 25(OH)D status was dichotomized into two or more groups, the findings are more conflicting. Notably, the studies that identified a significant difference in birth weight typically used a lower threshold level to define VDD (25-37.5 nmol/l) compared to those that did not identify a difference between the groups (28-80 nmol/l) (241, 260, 263-265, 267, 270, 273, 274). The largest of these studies included historical data from 2146 participants in the

Collaborative Perinatal Project (CPP) in the USA and assessed 25(OH)D before 26 weeks of pregnancy. When maternal 25(OH)D was less than 37.5 nmol/l, 25(OH)D was positively associated with offspring birth weight (β=3.6 g per nmol/l [95% CI 1.1, 6.1]) and OFC (0.01 cm per nmol/l [95% CI 0.002, 0.018]), but there was no significant association between maternal 25(OH)D and offspring size for 25(OH)D concentrations above 37.5 nmol/l. This is shown in Figure 1.17 (260). Although the effect sizes for both birth weight and OFC are clinically small, this would suggest that fetal size is only affected by the lowest levels of maternal 25(OH)D. This finding is also consistent with the aforementioned study of women in the United Arab Emirates in which the majority of women had 25(OH)D < 37.5 nmol/l (269).

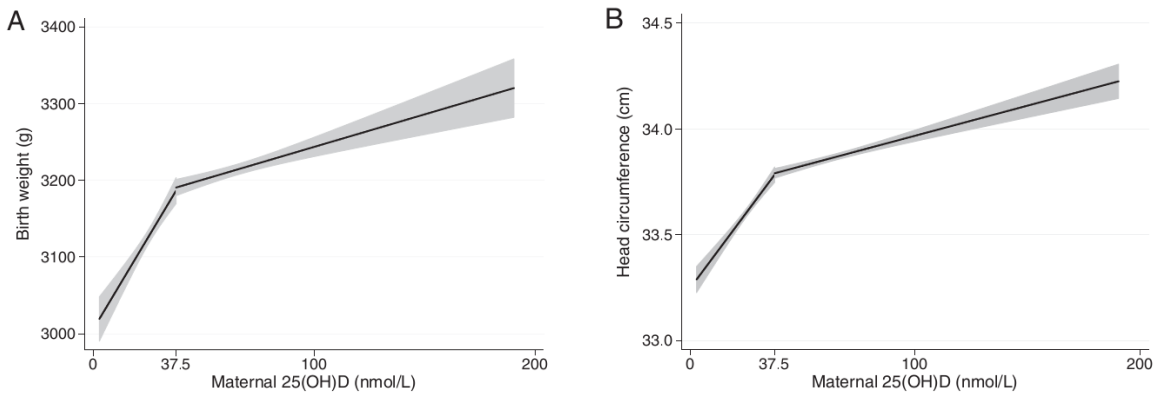


Figure 1.17: Nonlinear association between maternal 25(OH)D and (A) birth weight and (B) head circumference at birth

Adjusted for trimester at maternal blood draw, maternal race (white/other), pre-pregnancy BMI, height, smoking, season and study site

Reproduced with permission from Gernand et al, 2013 (260)

In addition to analysis of birth weight as a continuous variable, a number of studies have investigated the relationship between maternal 25(OH)D and the risk of delivering a small for gestational age (SGA) infant, but there is inconsistency in the definition used, with either the 3rd or 10th centile for sex and gestational age being chosen. A meta-analysis, which combined studies using both of these definitions to include a total of 6013 mother-offspring pairs demonstrated that maternal 25(OH)D < 50nmol/l was associated with a 1.52 (95% CI 1.08, 2.15) increased odds of SGA (275). However, when the different definitions for SGA were analysed separately, maternal 25(OH)D < 50 nmol/l was not associated with an increased risk of SGA < 3rd centile for gestational age and sex (odds ratio (OR) 0.97 [95% CI 0.15, 6.22]), but was significantly associated with higher odds of SGA less than 10th centile (OR 1.60 [95% CI 1.15, 2.22]) (275). This finding was

supported by a recent large study from Australia, in which retrospective analysis of 25(OH)D in stored serum samples from early pregnancy (10-14 weeks of gestation) in 5109 women, found those with 25(OH)D < 25nmol/l had an increased incidence of SGA less than the 10th centile compared to women with 25(OH)D 50-75 nmol/l, including after adjustment for potential maternal confounding factors (OR 1.58 [95% CI 1.06, 2.35]). The risk of SGA < 3rd centile for sex and gestational age was however not increased (OR 1.80 [95% 0.82, 3.95]) (276). These findings could represent reduced power to detect the increased risk of SGA less than 3rd centile, or that there is a higher probability of an alternative underlying aetiology with increasing degree of SGA.

Several interventional trials investigating the effect of vitamin D supplementation on birth anthropometry have been reported. Dose and timing of introduction of vitamin D supplementation varied widely. Two studies conducted in India in the 1980s found a positive effect of vitamin D supplementation on birth weight, and length and OFC in one of these studies (251, 252). Similarly, more recently, Kalra et al demonstrated in a group of 140 Indian women that supplementation with either a single dose of 60 000 IU oral cholecalciferol at mid gestation, or two doses of 120 000 IU oral cholecalciferol at mid gestation and 28 weeks resulted in higher birth weight, length and OFC compared to usual care (277). Hashemipour et al also found in 109 pregnant Iranian women with a baseline 25(OH)D < 75nmol/l that supplementation with 50 000 IU/week of cholecalciferol for 8 weeks from 24-26 weeks of gestation in an open-labelled trial was associated with significantly increased birth weight (mean difference 170 g, p=0.01), length (mean difference 0.8 cm, p=0.01) and OFC (mean difference 0.6 cm, p=0.001) (278). Interestingly, in both these studies, all women were additionally provided with calcium supplementation. In contrast, Roth et al randomised women in Dhaka, Bangladesh to only 35 000 IU/week cholecalciferol from 26-30 weeks of pregnancy, and despite achieving a similar mean 25(OH)D level at delivery in the supplementation group to that observed in the study of Iranian women, no differences in birth weight, length or OFC compared to their placebo group were observed (279). These differing findings might therefore suggest that the effect of vitamin D is dependent on the availability of calcium, or could result from genetic/racial variation in response to vitamin D supplementation. However, a further 7 studies, including two assessing cholecalciferol doses of 4000 IU/day, and from both developed (240, 242, 244, 245, 280) and developing countries (247, 281), did not identify any effect of supplementation on birth anthropometry.

1.4.8.2.2 Anthropometry and growth in infancy and childhood

Data relating maternal 25(OH)D status to offspring anthropometry in infancy or childhood are interesting as whilst findings from observation studies are conflicting, intervention studies have

consistently demonstrated an effect of vitamin D supplementation on postnatal growth, but are limited by study design, reporting of details and lack of generalisability to non-Asian ethnic groups.

Two large observational cohort studies have been used to demonstrate that infants born to mothers with VDD in pregnancy experience catch-up growth in infancy. The first study included data from the Amsterdam Born Children and their Development (ABCD) cohort, a multi-ethnic cohort of 3730 children born in Amsterdam, The Netherlands. Maternal 25(OH)D was measured in the first trimester and offspring weight and length assessed at 1, 3, 6, 9 and 12 months of age. There were significant differences in a number of maternal characteristics across categories of serum 25(OH)D concentration, but after adjustment for gestational age at birth, season of vitamin D measurement, infant sex, maternal height, parity, maternal age, smoking, pre-pregnancy BMI, educational level, duration of exclusive breastfeeding, and ethnicity, infants born to mothers who were vitamin D deficient (25(OH)D ≤ 29.9 nmol/l, n=861) were shorter at birth, but longer at 9 and 12 months of age than infants born to mothers who had adequate vitamin D status (25(OH)D ≥ 50 nmol/l, n=2072), as shown in Figure 1.18(b) (264). Although a similar pattern was seen for the relationship between infant weight z-score and maternal vitamin D status, this did not reach statistical significance. This is shown in Figure 1.18 (a). Similarly, Eckhardt et al using data from 2125 mother-offspring pairs in the Collaborative Perinatal Project found infants born to mothers with 25(OH)D < 30nmol, had lower birth weight and length z-scores that those born to mothers above this threshold, but at one year of age length z-score remained lower whereas weight z-score was similar in the two groups, therefore suggesting faster weight gain during infancy (282).

In contrast to the findings from the ABCD and CPP studies, in the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) cohort study, infants born to mothers with a either a 25(OH)D < 50 nmol/l or 50-75 nmol/l in late pregnancy did not differ in weight, length or OFC z-scores at birth, 3, 6, 9, 12, 15, 18 or 24 months when compared with infants born to mothers with 25(OH)D > 75 nmol/l (273). There are a number of methodological and cohort differences between these studies which could explain the different findings, including the 25(OH)D threshold used, timing of 25(OH)D assessment (earlier in pregnancy in ABCD and CPP compared with GUSTO) and differences in ethnicities included, or the lower power in GUSTO to detect a difference (n=807). Indeed the notion that postnatal growth is only affected by a very low maternal 25(OH)D is supported by the null findings of a number of other studies in which median maternal 25(OH)D was higher than in the previously mentioned cohorts. Firstly, in a study maternal serum 25(OH)D at 20 and 36 weeks of gestation was not associated with weight, length or OFC at 2, 13 or 52 weeks of age in 125 mother-offspring pairs in the Gambia (270). All women in that cohort were vitamin D replete (25(OH)D ≥ 50 nmol/l) and only 20% and 16% had

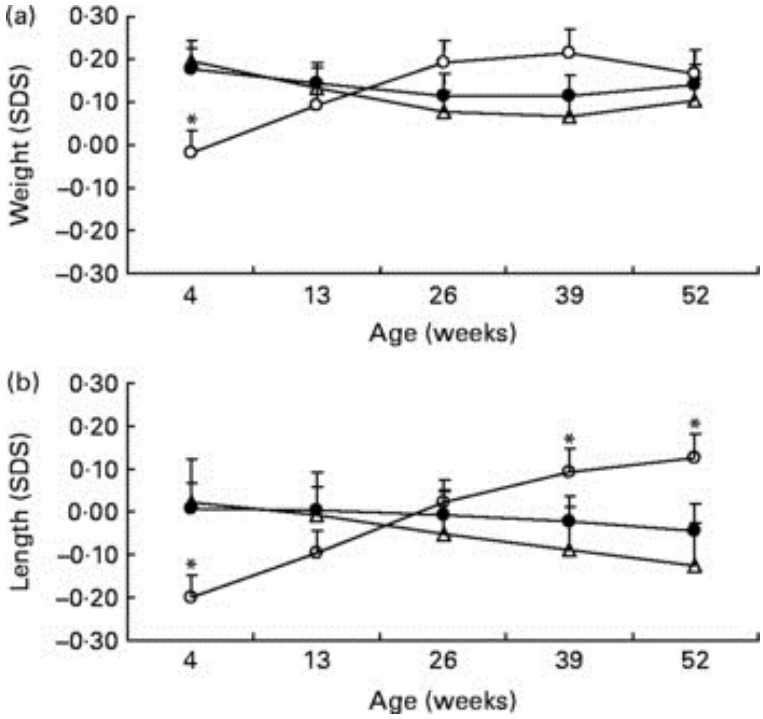


Figure 1.18: Weight and length standard deviation scores (z-scores) in infancy according to maternal serum 25(OH)D status in pregnancy
○, deficient (25(OH)D ≤ 29.9 nmol/l); ●, insufficient (25(OH)D 30-49.9 nmol/l); Δ, adequate (25(OH)D ≥ 50 nmol/l). *significantly different from "adequate" group, $p < 0.01$
Reproduced with permission from Leffelaar et al 2010 (264)

25(OH)D < 80 nmol/l at 20 and 36 weeks, respectively. Secondly, when the children born to only the ethnic Danish women participating in the ABCD study (n=1208) were followed up at 5-6 years of age, maternal 25(OH)D in the first trimester was not associated with offspring height or leg length (283). In contrast to the infancy study, median 25(OH)D concentration for all included women in the follow-up study was higher (65.9 nmol/l compared with 54.4 nmol/l) and the analysis was performed with 25(OH)D as a continuous variable rather than categorized into "deficient", "insufficient" and "adequate". As such, it remains possible that follow-up of the whole cohort might reveal differing findings. However, using the Mysore-Parthenon Study (MPS) cohort study in India, Krishnaveni et al also did not identify an association between late pregnancy 25(OH)D and offspring height or weight at 5 or 9.5 years of age, despite low levels of 25(OH)D amongst the cohort (median 25(OH)D 39.0 nmol/l [IQR 24.0-58.0]). Similarly, late pregnancy 25(OH)D status was not associated with height or weight at 9 months or 9 years of age in children born to mothers participating in the Princess Anne Hospital Cohort Study in Southampton, UK (227, 261) and umbilical venous blood 25(OH)D was not associated with height or weight at 18 months or 4 years of age in an Australian Study (268). In both of these cohorts median 25(OH)D measurement was > 50 nmol/l. However, it should also be noted that the studies reporting null

associations have typically been smaller in size (n=125 to 1208) than the CPP and ABCD infancy study.

To date, intervention studies, in contrast to the observational findings, do consistently suggest a positive effect of vitamin D supplementation on postnatal growth. The earliest study was conducted by Brooke et al, who showed that despite no differences in birth weight or length, infants of Asian mothers living in the UK who received 1000 IU/day ergocalciferol during the last trimester of pregnancy were significantly heavier at 3, 6, 9 and 12 months of age, and had longer crown-heel length at 9 and 12 months of age than infants born to the mothers in the control group (placebo was not given) (284). Infants born to mothers who received vitamin D supplementation gained 27.9 cm (SD 2.0) in length and 6.39 kg (SD 0.78) in weight over the first year of life, compared with 24.6 cm (SD 5.7) and 5.92 kg (SD 0.92), respectively ($p < 0.01$ for both). Maternal characteristics are not presented and therefore it is not certain that these were similar between the two groups.

More recently, Kalra et al conducted an open-label study in which women in India were randomised to either 60 000 IU oral cholecalciferol in mid-pregnancy (n=36) or two doses or 120 000 IU oral cholecalciferol in mid-pregnancy and at 28 weeks (n=35). These women all additionally received 1 g/day elemental calcium supplementation from enrollment. Each group was compared to a third group of women identified in the third trimester who received only the calcium supplement (n=38). Interestingly maternal serum 25(OH)D at delivery was lower in the group randomised to the single dose of cholecalciferol compared to the usual care group, but offspring weight, length and OFC were significantly greater in each of the vitamin D supplemented groups compared to the usual care group at birth, 3, 6, 9 and 12 months of age (277). It should however be noted that the calcium supplementation was commenced later in pregnancy in the usual care group and as the baseline calcium intake of this cohort was low, this might have contributed to the differences observed.

Finally, in infants born to mothers who participated in the Antenatal Vitamin D in Dhaka (AViDD) trial in Bangladesh, supplementation with 35 000 IU/week oral cholecalciferol from 26-30 weeks of gestation until delivery was associated with accelerated linear growth in the first 4 weeks of postnatal life compared to placebo despite no significant difference in birth length (279). The difference in length z-score persisted until 1 year of age, but without further increase in the mean difference between the two groups, as shown in Figure 1.19. This translated to a difference in sex-adjusted length of 1.1 cm (95% CI 0.06, 2.04) between the two groups at 1 year of age. No difference in weight z-score was observed at either age. Assessment of infant serum 25(OH)D at 2 and 4 months of age found that the higher 25(OH)D in the supplementation group observed at

birth persisted at 2 months of age, but not at 4 months of age. It is therefore possible that this contributed to the accelerated post-natal growth, although previous trials of vitamin D supplementation in infancy have not demonstrated positive effects on linear growth (175, 176).

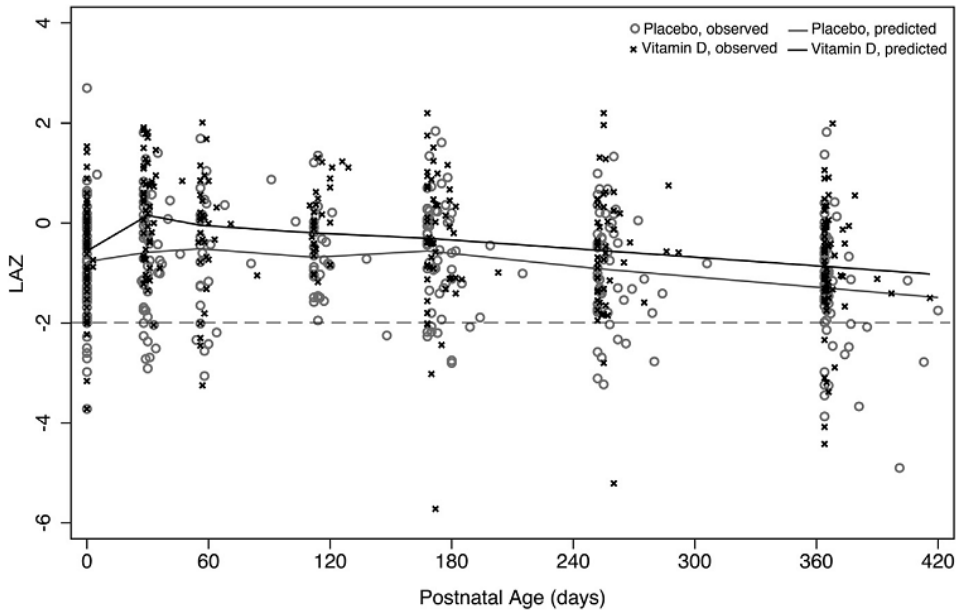


Figure 1.19: Length z-score (LAZ) in infants born to mothers participating in the AViDD trial of vitamin D supplementation in pregnancy in Bangladesh

Length z-score (LAZ) by postnatal age, among infants whose mothers had received either vitamin D (35 000 IU/week) or placebo during the third trimester of pregnancy (n=145). Lines illustrating predicted LAZ in each group were derived from piecewise linear regression spline models. The horizontal dashed line denotes the threshold below which infants are conventionally considered stunted.

Reproduced with permission from Roth et al, 2013 (279)

It is currently unclear why the intervention studies suggest vitamin D supplementation is beneficial to infant growth whereas findings from observational cohort studies are, on the whole, not consistent with a higher maternal 25(OH)D promoting improved offspring growth, and in contrast maternal VDD was associated with accelerated postnatal linear growth or weight gain in two large observational studies. Differences in the study populations including ethnicity, distribution of maternal 25(OH)D status and calcium status and duration of follow-up in the supplementation trials might be key to these inconsistencies. Indeed, to date there are no intervention studies examining the effect of antenatal vitamin D supplementation on growth in non-Asian ethnic groups or beyond the first year of life.

1.4.8.3 Bone development

The consistent finding from the published intervention studies that gestational vitamin D supplementation increases infant length would support the notion that in utero vitamin D exposure does affect skeletal development through at the very least an increase in the size of the skeletal envelope. There are several case reports of infants born to mothers with VDD, who displayed clinical signs of rickets including bony abnormalities in addition to low serum 25(OH)D from day 1 of life (285). Whilst these rare cases represent the most extreme descriptions of in utero VDD, there is increasing evidence that subclinical maternal vitamin D insufficiency in pregnancy might also influence offspring bone mineral accrual. The majority is, as with other outcomes, observational in nature, although in recent years trials of antenatal vitamin D supplementation with assessment of offspring bone mineralisation have been reported.

Some of the earliest data to suggest that vitamin D exposure might influence in utero bone mineral accrual used season as a proxy marker of vitamin D status. Namgung et al found that in 71 Korean neonates, those born in summer months had 8% higher whole body BMC after adjustment for weight than infants born in winter. Furthermore, in that cohort, neonatal 25(OH)D measured at delivery was positively correlated with whole body BMC (286). In contrast, the same authors found that infants in the USA who were born in the summer had lower whole body BMC than winter-born infants (287). The authors proposed that these differences reflect the use of vitamin D supplementation in the two populations; the uptake of supplementation is low throughout pregnancy in Korea but standard practice after the first trimester in the USA, where differences in maternal 25(OH)D by season of birth were not observed (286). This would suggest that early pregnancy 25(OH)D status is crucial to vitamin D mineralisation, which is in contrast to several later studies with assessment of serum 25(OH)D.

Subsequent studies have used measurements of maternal or cord blood 25(OH)D as the exposure variable. Weiler et al studied 50 neonates born in Canada between April and August. 25(OH)D was measured in venous cord blood and used to divide the infants into two groups using a cut point of 37.5 nmol/l. The infants in the low 25(OH)D group tended to be heavier and longer, but this might have reflected the greater ethnic diversity in the group compared to the group with a high 25(OH)D level. However, whole body and femur BMC relative to body weight were significantly lower in the 18 neonates with a cord blood 25(OH)D < 37.5 nmol/l compared with 32 infants with a 25(OH)D above this cut point (259). Similarly, Viljakainen et al, using the mean of two maternal serum 25(OH)D measurements from early pregnancy and 2 days postpartum as the assessment of maternal vitamin D status, found neonatal tibial BMC and cross-sectional area measured by pQCT were 14% and 16% higher, respectively, in infants born to mothers with

25(OH)D above the median for the cohort. Although vBMD of the tibia did not differ between the two groups, the difference in BMC and CSA did persist after adjustment for weight (262). When a subset of these children were reassessed at 14 months of age, the difference in tibial BMC was no longer present, but tibial CSA remained significantly higher in those born to mothers with higher vitamin D status in pregnancy (288). Conversely, in 125 Gambian mother–offspring pairs, no significant relationships were observed between maternal 25(OH)D at either 20 or 36 weeks of gestation and offspring whole body BMC or bone area at 2, 13 or 52 weeks of age (270). In contrast to the other studies, none of the mothers had a 25(OH)D below 50 nmol/l, which is consistent with the notion that poorer skeletal mineralisation might only occur in fetuses of mothers with the lowest 25(OH)D levels.

There is some evidence to suggest that these relationships persist into childhood, although study findings are less consistent than in the neonatal period. Positive relationships between maternal 25(OH)D measured in late pregnancy and offspring whole body and LS bone area, BMC and aBMD at 9 years of age in 198 mother-offspring pairs in the Princess Anne Hospital Study (Southampton, UK) were reported by Javaid et al (Figure 1.20) (227). Beneficial effects of vitamin D supplementation were also suggested by this study as children born to women who consumed vitamin D containing supplements had higher whole body BMC and bone area, but not aBMD. Although the women who took supplements were self-selected, this finding was not changed by adjustment for socioeconomic status. These findings were replicated in the Southampton Women's Survey (SWS), in which 1030 mother-offspring pairs had measurement of 25(OH)D at 34 weeks of gestation and whole body less head (WBLH) and LS DXA at 6-7 years. WBLH BMC, bone area, BMD and LS BMC were all significantly lower in children born to mothers with 25(OH)D < 25 nmol/l in late pregnancy, including after adjustment for maternal age, ethnicity, height, pre-pregnancy BMI, smoking in late pregnancy, social class, maternal educational attainment and duration of breast feeding (289). Similarly, Zhu et al found a positive relationship between maternal 25(OH)D status at 18 week gestation and bone mass in young adulthood in the Raine cohort in Western Australia. Thus, after adjustment for sex, age, height and body composition at age 20 years, maternal height and pre-pregnancy weight, maternal age at delivery, parity, education, ethnicity, smoking during pregnancy and season of maternal blood sampling, whole body BMC and aBMD were 2.7% and 1.7% lower at 20 years of age in offspring of mothers with 25(OH)D < 50 nmol/l compared to those above this level (290).

In contrast, analyses using the ALSPAC cohort study do not support these studies. Initially, using 6995 mother-offspring pairs, Sayer et al reported a positive relationship between estimated maternal UVB exposure in late pregnancy and offspring WBLH BMC, bone area and BMD at 9.9 years of age (291). However, further analysis in a subset of 3960 of the children for whom

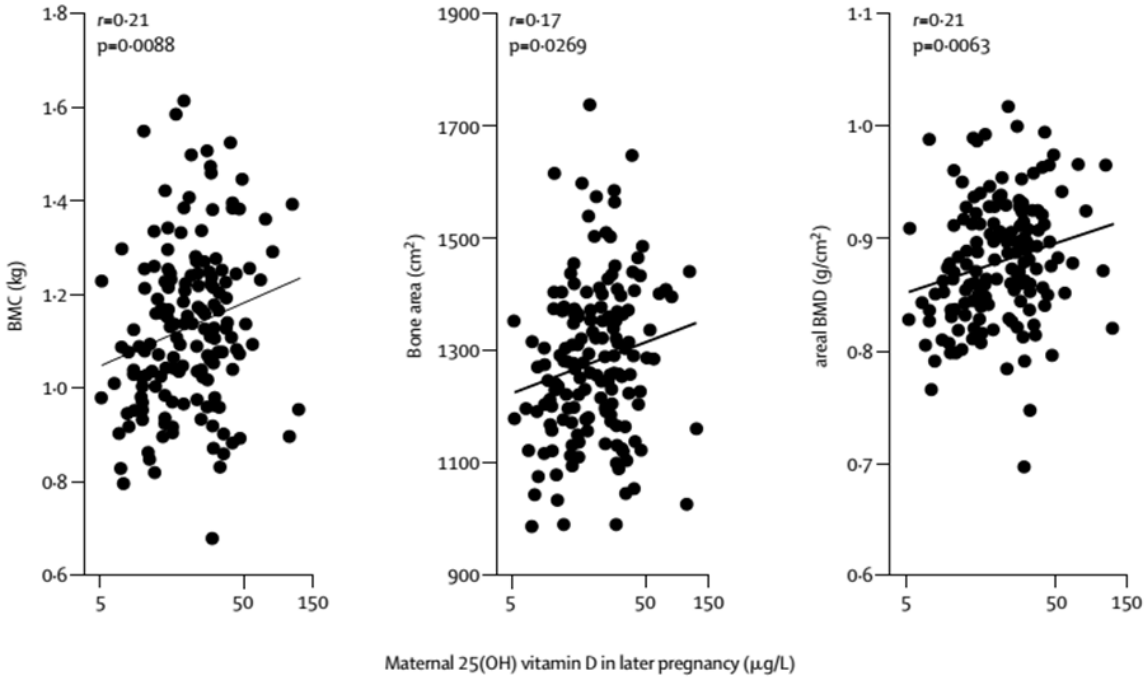


Figure 1.20: Relationships between maternal 25(OH)D and offspring bone mineral content (BMC), bone area and areal bone mineral density (aBMD) at 9 years of age
Reproduced with permission from Javaid et al, 2006 (227)

maternal serum 25(OH)D measurement was available in the first (n=1035), second (n=879) or third (n=2046) trimester did not reveal any significant associations between maternal 25(OH)D and offspring bone mineralisation (292). Collinearity of the estimated maternal UVB measurement with age at assessment might have confounded the relationships reported in the initial study.

The first intervention study to assess the effect of antenatal vitamin D supplementation on offspring bone mineralisation was undertaken by Congdon et al and published in 1983. Sixty-four women of Asian ethnicity living in the UK participated in a non-randomised study; 19 received a daily supplement containing 1000 IU vitamin D and calcium (of unknown strength) during the last trimester, whereas 45 received no supplementation. There was no significant difference in forearm BMC of the offspring at birth assessed using single photon absorptiometry (293), but the study size, lack of randomisation and technology used limits the interpretation of the findings.

There are three more recently published studies of gestational vitamin D supplementation, of which the largest is the Maternal Vitamin D Osteoporosis Study (MAVIDOS). Detailed methodology for the MAVIDOS study is described in Chapter 3 (page 100) as this study forms the basis for this thesis. However, in brief, MAVIDOS is a randomised double-blind placebo-controlled

trial of antenatal vitamin D supplementation from 14 weeks of gestation until delivery conducted in three centres in the UK. The primary outcome was neonatal bone mass (294). 1134 women with a baseline 25(OH)D between 25 and 100 nmol/l were randomised to 1000 IU/day cholecalciferol or placebo; 965 remained in the study until delivery, and 736 infants had DXA of the whole body and/or LS. Although there were no differences in whole body or LS BMC, bone area or aBMD between the two groups overall, a significant interaction was observed between season of birth and maternal randomisation group, as shown in Figure 1.21 (p for interaction for BMC 0.04) (295). Thus, whole body BMC and BMD were approximately 9% and 5% higher, respectively, in the children born in winter to mothers randomised to cholecalciferol compared to those randomised to placebo. This effect size is substantially larger than those observed between children with and without fractures (296), and hence if persisting into later childhood is likely to be clinically relevant.

Two small intervention studies from India and Iran have also assessed bone mass in infants born to mothers randomised to vitamin D supplementation or placebo. Sahoo et al randomised 300 women to three groups, which received 400 IU/day cholecalciferol daily ("placebo"), 60 000 IU cholecalciferol every 4 weeks or 60 000 IU cholecalciferol every 8 weeks from the second trimester. All women also received daily calcium supplementation. Only 160 women were followed up until delivery, and 52 children (17% of the original cohort) underwent DXA at 12-16 months of age. The children in the placebo group were significantly older at DXA scan and had higher measurements of whole body BMC and BMD, but in multivariate analysis randomisation group was not a significant predictor of BMC or BMD (297). Vaziri et al randomised 153 women to placebo or 2000 IU/day cholecalciferol from 26-28 weeks until delivery, but only 25 infants (16% of the original cohort) had DXA assessment. No significant difference in whole body BMC, BMD or bone area was found (298), but as with the study by Sahoo et al, the small numbers included are unlikely to have sufficient power to detect a difference in the outcomes studied.

Evidence from observational studies does therefore suggest that achieving higher levels of serum 25(OH)D in pregnancy might have beneficial effects on offspring bone development, but further high quality RCTs are required to assess this. Moreover, long-term follow up of children born to participants of these trials is required to determine whether any effects observed in the neonatal period, such as the effect of gestational vitamin D supplementation on increased bone mineralisation in children born in winter, does persist beyond the neonatal period.

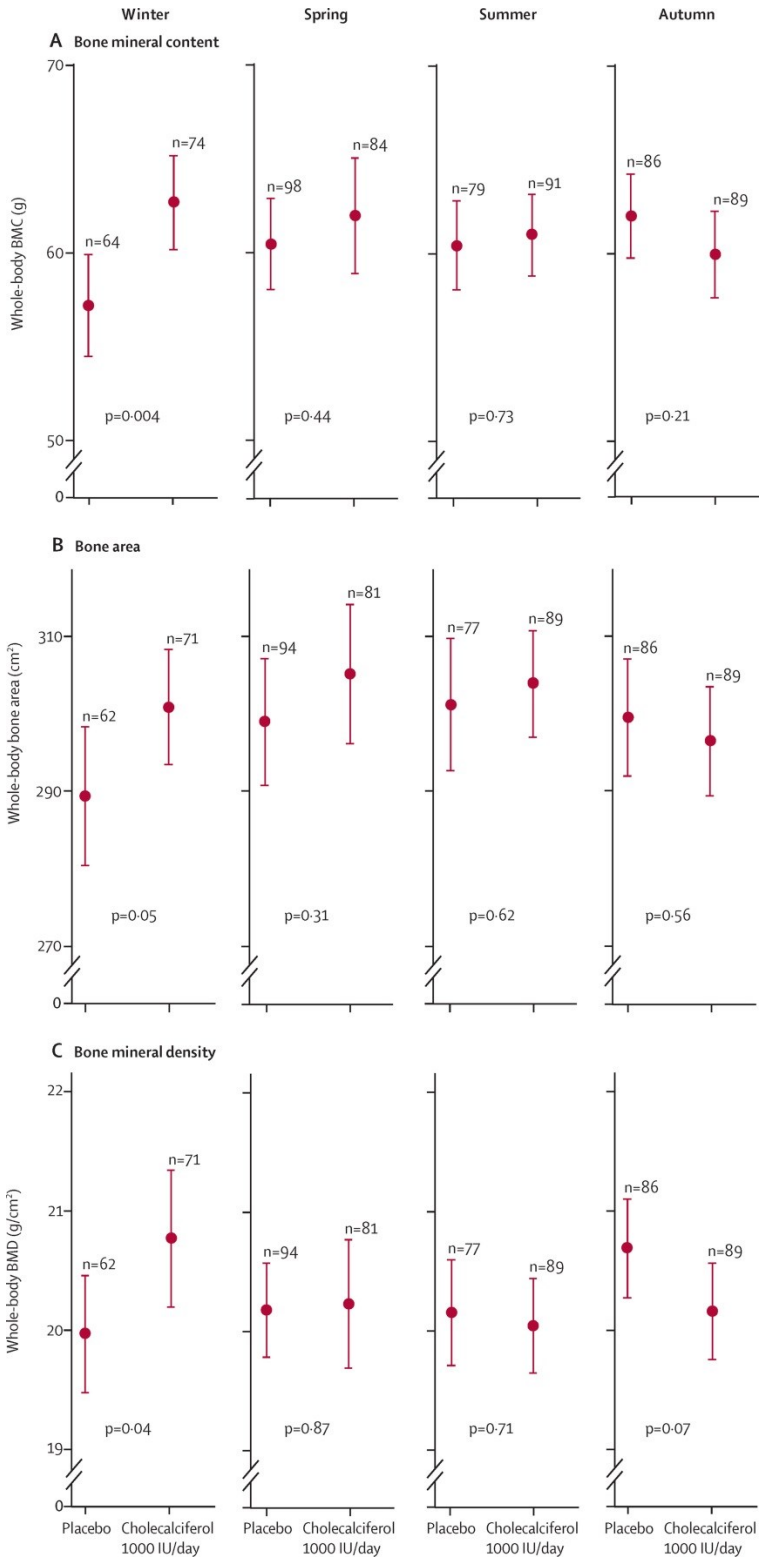


Figure 1.21: Neonatal whole body bone mineral content (BMC), bone area and bone mineral density (BMD) by intervention group and season of birth in the MAVIDOS trial

Data are shown as mean and 95% confidence interval. Winter is December to February, spring is March to May, summer is June to August and autumn is September to November. Reproduced with permission from Cooper et al, 2016 (295)

1.4.8.4 Adiposity

The effects of in utero vitamin D exposure on fat and lean tissue development has been reported in far fewer studies, and of these, various methods of body composition assessment have been used, including measurement of SFT, limb circumferences, BIA and DXA, which limits comparison of the findings.

At birth, Morley et al, found a negative association between maternal serum 25(OH)D at 28-32 weeks of gestation and offspring subscapular and triceps SFT after adjustment for gestational age (which was positively associated with maternal 25(OH)D) in 374 mother-offspring pairs (265). However, after adjustment for sex, maternal height, parity, smoking in pregnancy and season of blood sampling, only the association with subscapular SFT remained statistically significant; Doubling of maternal 25(OH)D was associated with a 0.2 mm (approximately 0.1 SD) decrement in subscapular SFT. No significant associations between maternal 25(OH)D and offspring MUAC or calf circumferences were identified in this cohort (265). In contrast to these findings, Farrant et al reported no significant associations between maternal 25(OH)D measured at the same gestation (28-32 weeks) and offspring subscapular or triceps SFT in the MPS cohort of mother-offspring pairs in India (271). Similarly, Ong et al did not find statistically significant differences in subscapular or triceps SFT at birth, 18 months or 2 years of age in offspring born to mothers with serum 25(OH)D < 50 nmol/l or 50-75 nmol/l compared to > 75 nmol/l at 26-28 weeks of gestation in the GUSTO observational study (273).

Three studies have examined the relationships between maternal 25(OH)D and offspring body composition at birth using DXA. Crozier et al identified a positive relationship between maternal 25(OH)D in late pregnancy and offspring FM measured by DXA within two weeks of birth after adjustment for maternal educational attainment, smoking in pregnancy, pre-pregnancy BMI, pregnancy weight gain, height, parity and social class (β=0.08 SD/SD [95% CI 0.02, 0.15]) in 574 participants of the SWS (Figure 1.22(A)) (167). Godang et al also reported a positive relationship between umbilical cord blood 25(OH)D and total FM and %FM measured by DXA in 202 infants, although the latter was of borderline statistical significant after adjustment for maternal BMI. Associations between maternal 25(OH)D measured at 30-32 weeks of pregnancy and neonatal FM and %FM were not significant (299). In contrast to the findings of Crozier et al and Godang et al, Weiler et al reported a statistically non-significant greater %FM in 18 infants of mothers with 25(OH)D status < 37.5 nmol/l compared to 24 infants of mothers above this threshold (12.7% vs 10.6%, p=0.09) (259). Differences in study size and therefore power might account for the statistically significant finding in the larger SWS cohort, compared to the borderline statistically significant finding of Godang et al, and null result of Weiler et al.

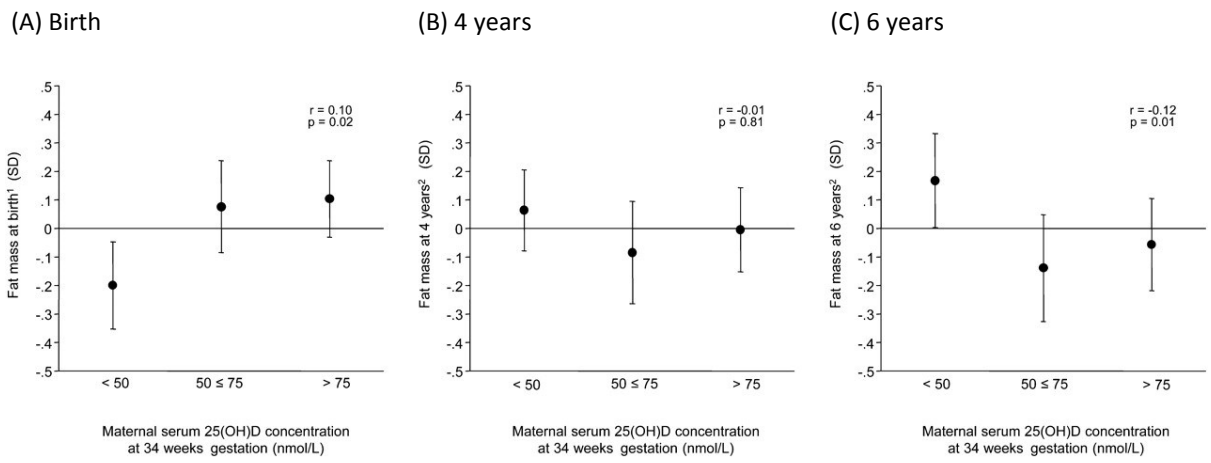


Figure 1.22: Offspring fat mass measured by dual-energy X-ray absorptiometry grouped by maternal serum 25(OH)D concentration at 34 weeks of gestation in the Southampton Women's Survey

Displayed as mean (95% CI). [1]Adjusted for sex, gestation, age at measurement, age squared, length, maternal educational attainment, smoking in pregnancy, pre-pregnancy BMI, height, parity, social class, and Institute of Medicine weight-gain categories. [2]Adjusted for child's sex, age, child's height, maternal educational attainment, smoking in pregnancy, pre-pregnancy BMI, maternal height, parity, social class, Institute of Medicine weight-gain categories, breastfeeding duration, vitamin D intake at age 3 years, and physical activity at age 3 years. Reproduced with permission from Crozier et al, 2012 (167)

Follow-up of children in the SWS using DXA at age 4 and 6 years demonstrated a "flipping" of the previously described positive association between maternal 25(OH)D and FM at birth. Thus, whilst no significant associations were observed at 4 years of age, at 6 years of age, maternal 25(OH)D status in late pregnancy was negatively associated with FM (Figure 1.22 (B) and (C)). This relationship persisted after adjustment for potential confounding factors (β=-0.10 SD/SD [95% CI -0.17, -0.02]).

Krishnaveni et al assessed body composition using BIA at age 5 and 9.5 years in the MPS cohort (300). Similarly to the findings in SWS, at 5 years of age boys born to mothers with 25(OH)D < 50 nmol/l at 28-32 weeks of gestation had greater %FM by 1.7% (95% CI 0.4, 3.0) than children born to vitamin D replete mothers. This persisted after adjustment for age, maternal BMI, gestational diabetes, socioeconomic status, parity and religion. However, this difference was no longer present at 9 years of age. No significant differences in body composition by maternal vitamin D status were observed in girls. Similarly, no linear association between maternal 25(OH)D in late pregnancy and offspring FM assessed by DXA at 9 years of age was observed in the Princess Ann Hospital Study (261). Importantly, neither study assessed pubertal development at age 9 years. It is likely that a proportion of the children, and a greater percentage of females than males, would have commenced puberty at this age. As such, body composition changes in response to sex

steroids and pubertal acceleration in growth velocity, in some but not all children, might have masked any relationships with in utero vitamin D exposure. However, at completion of growth, no statistically significant relationships were observed between FM and LM assessed by DXA and maternal 25(OH)D at 18 weeks of gestation in the Western Australian Pregnancy Cohort (Raine) study (290).

Two intervention studies of vitamin D supplementation in pregnancy included assessment of offspring skinfold thicknesses at birth. Both studies were reported in the 1980s and the interpretation of them is limited by a lack of reporting of study details and participant characteristics. Marya et al found a significantly greater triceps SFT (7.72 mm [SD 0.67] vs 7.30 mm [SD 0.83], $p < 0.001$), subscapular SFT (7.82 mm [SD 0.67] vs 7.49 mm [SD 0.89], $p < 0.01$) and MUAC (9.82 cm [SD 0.72] vs 9.44 cm [SD 0.85], $p < 0.001$) in infants born to Indian women randomised to two doses of 600 000 IU cholecalciferol in the 7^{th} and 8^{th} months of pregnancy (n=100) compared to a control group (n=100) (251). These infants also had significantly higher birth weight, length and OFC (251). In contrast, supplementation of 59 Asian women living in the UK with 1000 IU/day ergocalciferol in the last trimester had no significant effect on triceps SFT at birth in their infants compared to the infants of 67 women who received placebo in a double blind RCT despite greater gestational weight gain in the mothers (242). No intervention studies have assessed the effect of antenatal vitamin D supplementation on offspring adiposity using more detail assessment techniques, such as DXA, in non-Asian populations or outside of the neonatal period.

1.4.8.5 Lean mass, motor development & muscle function

Despite the aforementioned relationships between VDD and muscle function in adulthood, there are few previous data relating maternal 25(OH)D during pregnancy to offspring lean mass development. Children born to vitamin D replete (25(OH)D > 50 nmol/l) mothers participating in the MPS in India, had greater arm muscle area (determined from a measurement of MUAC and triceps SFT) at 5 and 9.5 years than those born to vitamin D deficient mothers (25(OH)D < 50 nmol/l) (300). Consistent with this, a positive association between maternal estimated UVB in the third trimester and offspring LM measured by DXA at 9 years was observed in 6995 mother-offspring pairs in ALSPAC (291). However, reassessment of this relationship using measured maternal 25(OH)D as has been undertaken with regards to bone density assessment following the recognition of collinearity between estimated UVB and age at DXA (292) has not been reported.

The relationship between maternal 25(OH)D and muscle strength has only been examined in the MPS study, in which no difference in grip strength was identified at 9 years of age between children born to mothers defined as vitamin D deficient and replete at 28-32 weeks of gestation (300). As previously mentioned, the lack of pubertal assessment however limits the interpretation of this study. Thus further data examining the relationship between in utero vitamin D exposure and muscle development are needed.

1.4.9 Summary

Biochemically low levels of 25(OH)D in pregnancy are common but currently it remains unclear as to whether this affects offspring birth size, growth and body composition. There is a wealth of observational evidence relating maternal 25(OH)D to these outcomes, but the findings of these studies are inconsistent. This likely reflects the wide heterogeneity in the populations studied (including the prevalence of VDD, calcium status, ethnicity), timing of assessment of 25(OH)D status, statistical methods and adjustments and definition used for the outcomes. Furthermore, seasonal variation in 25(OH)D and the potential for confounding and reverse causality when using a primarily environmentally determined exposure needs to be considered. Reviewing these observational studies simply highlights the need for high quality RCTs to be conducted to support the need for routine antenatal supplementation. The few intervention studies that have been performed to date are typically limited by their generalisability as many have been performed in Asian ethnic groups, and are often too small and thus underpowered to demonstrate a clinically detectable effect. Many are open-label studies, do not provide a placebo to the control group or deliver other unmatched interventions in addition to vitamin D thus potentially increasing the likelihood of bias. Further large rigorously conducted RCTs are now required.

1.5 A summary of the knowledge gaps

New approaches to addressing the increasing burden of non-communicable diseases are urgently needed, and the DOHAD hypothesis presents a potential approach to targeting the population, rather than individual, level. There is enough evidence from observational studies to suggest that antenatal vitamin D supplementation might be a useful approach to addressing the burden of osteoporosis and obesity, but currently RCTs of these outcomes are lacking. Furthermore, despite

some evidence of a role for vitamin D in increasing muscle strength in adult life, there are few data available examining the relationships between fetal vitamin D exposure and LM or muscle strength. This outcome has not been examined in a RCT of antenatal vitamin D supplementation. It is therefore currently unknown whether addressing biochemically low 25(OH)D levels in pregnancy might be a useful approach to tackling the burden of sarcopenia.

The UK DH currently recommends routine antenatal vitamin D supplementation with 400 IU cholecalciferol daily throughout pregnancy for all women, independent of ethnicity and other risk factors for VDD (248). Supplementation is important to reduce the incidence of neonatal hypocalcaemia and increase neonatal 25(OH)D status, but it is not currently understood how women respond to supplementation and whether maternal characteristics would suggest that higher level supplementation is necessary for some individuals. Avoidance of vitamin D toxicity by over-supplementation, which could cause harm, is also important. It is currently not well understood how 25(OH)D status changes during pregnancy or whether vitamin D status tracks during this period. This knowledge could be useful to identify women with very low 25(OH)D in early pregnancy, and if tracking is high, would enable individualised counselling as to whether supplementation is necessary.

Chapter 2: Aims and objectives

2.1 Aims

This overarching aim of this work is to increase the understanding of maternal 25(OH)D status in pregnancy and its relationships with offspring growth, body composition and muscle strength to determine whether an intervention with antenatal vitamin D supplementation might affect these outcomes.

2.2 Objectives

This work will address a number of research questions, as follows:

1. Does 25(OH)D status track during pregnancy?
2. Is maternal 25(OH)D concentration in late pregnancy associated with offspring lean mass and muscle strength in early childhood?
3. Does antenatal vitamin D supplementation affect offspring size and/or growth in infancy?
4. Does antenatal vitamin D supplementation influence offspring body composition and/or muscle strength in early childhood?
5. Do maternal characteristics affect the 25(OH)D response to antenatal vitamin D supplementation?

Chapter 3: Methods

The objectives of this study have been addressed using two unique mother-offspring studies: The Southampton Women's Survey (SWS), an observational birth cohort study (301), and the Maternal Vitamin D Osteoporosis Study (MAVIDOS), a randomised placebo-controlled trial of antenatal cholecalciferol supplementation (294).

3.1 The Southampton Women's Survey (SWS)

The SWS is a prospective pre-conception mother-offspring birth cohort study in Southampton, UK (latitude 50.9°N). The primary objective of the SWS was to examine how maternal factors before and during pregnancy influence offspring growth and development (301). An overview of the SWS is shown in Figure 3.1.

The SWS was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Southampton and South West Hampshire Research Ethics Committee approved the initial study on 28/11/1997 (Appendix A). Written informed consent was obtained from all participating women. Ethical approval has been granted for all subsequent offspring follow-up. Appendix A includes the ethical approvals for the phases of data collection that have been used in this work. Parental consent for ongoing participation in the study was obtained at each offspring assessment.

3.1.1 Identification of participants and pre-pregnancy data collection

Women aged 20-34 years were recruited into the study during 1998-2002. Potential participants were identified through general practices in the city of Southampton. Each general practitioner provided a list of all eligible women, and women were subsequently approached through postal contact, telephone calls and home visits. Additional participants were identified through local publicity, including at events and supermarkets.

12579 women, which represented 75% of those contacted about the study, agreed to participate and were interviewed between April 1998 and October 2002 by a trained research nurse. Questionnaire data collected included demographics (education, ethnicity, marital status, employment, income and benefits, and housing arrangements), general health, medications and

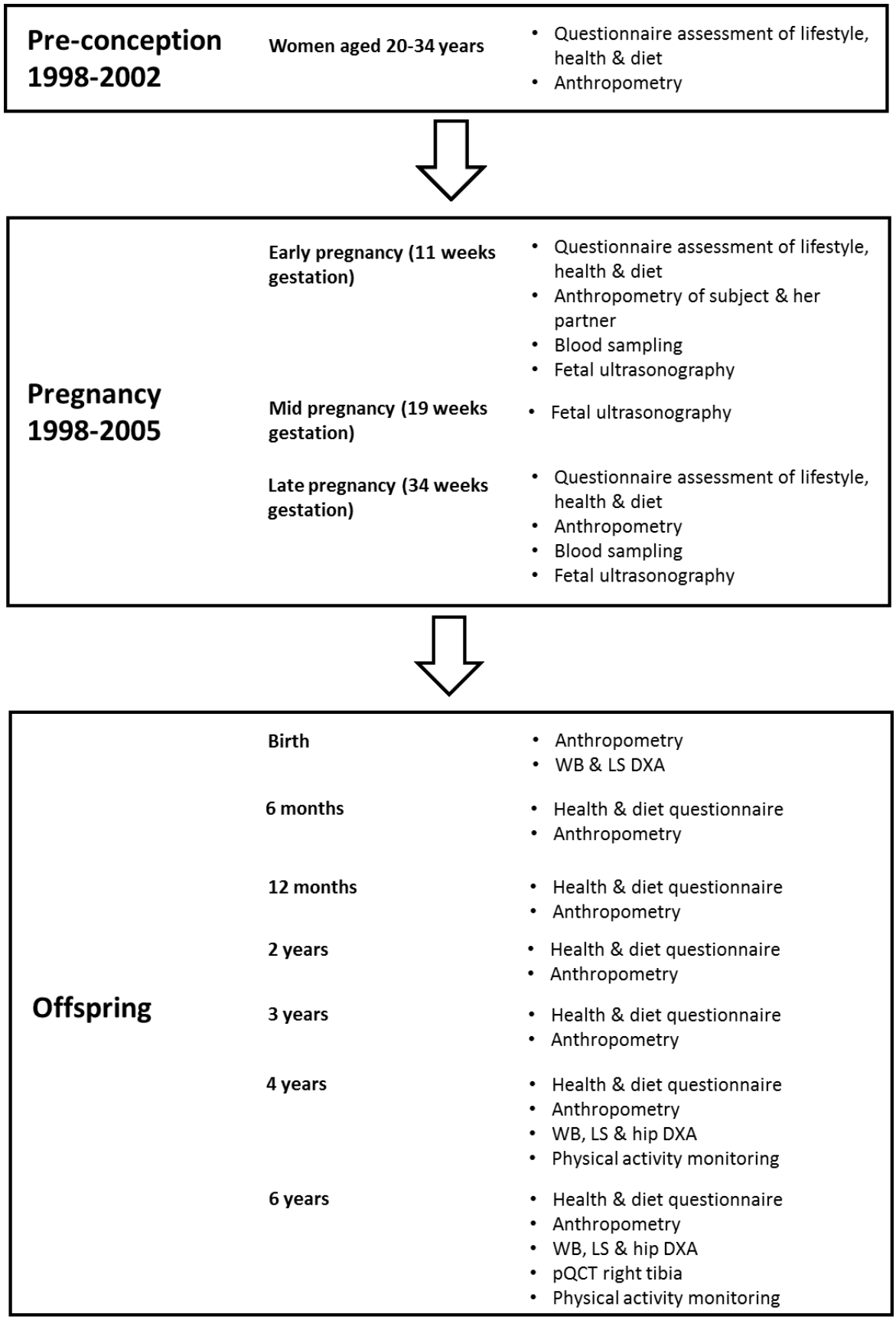


Figure 3.1: The Southampton Women's Survey

(DXA, Dual Energy Xray Absorptiometry; LS, lumbar spine; pQCT, peripheral quantitative computed tomography; WB, whole body)

lifestyle (smoking, alcohol consumption, physical activity) and previous pregnancies. A 100 item questionnaire was used to assess dietary intake (302).

3.1.1.1 Pre-pregnancy anthropometry

Height, weight, waist, hip and MUAC and four site SFT were measured by a trained research nurse.

Height, without shoes, was measured using a portable stadiometer (Harpenden, CMS Weighing Equipment Ltd, London, UK) to the nearest 0.1 cm. The head was placed in the Frankfurt plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye was horizontal.

Weight was measured using calibrated electronic scales (Seca Ltd, Birmingham, UK) to the nearest 0.1 kg. Weight was obtained without shoes, and participants were asked to remove any heavy items of clothing or jewellery. BMI was calculated from height and weight measurements.

MUAC was measured with a cloth tape to the nearest 0.1 cm on the non-dominant side. With the participant standing with her back to the measurer and arms hanging by her side, the tip of the acromion was palpated and marked. The participant's arm was then flexed at the elbow to 90° and the olecranon palpated. The mid-point between the acromion and olecranon was marked. The MUAC measurement was obtained at this level with the participants arm relaxed and hanging by her side.

SFTs were measured to the nearest 0.1 mm on the non-dominant side of the body using a Harpenden skinfold calliper. Three measurements were obtained at each site, releasing the skinfold between measurements, and the average calculated. The triceps and biceps SFT were measured posteriorly and anteriorly, respectively, on a relaxed arm at the level at which the tape measure was placed for the MUAC measurement. The skinfolds are picked up vertically. The subscapular SFT was measured at the lowest point of the scapula when stood in a relaxed position. The skinfold was picked up obliquely in the natural cleavage of the skin. The upper suprailiac SFT was measured at the intersection between an imaginary vertical line from the mid-axillary point and a horizontal line along the iliac crest.

3.1.2 Pregnancy assessments

Women were provided with contact details for the research centre and asked to contact the centre in the event of pregnancy. 3219 women informed the research centre of a pregnancy.

In early pregnancy (approximately 11 weeks of gestation) and late pregnancy (approximately 34 weeks of gestation), the women completed an interviewer directed questionnaire on health, lifestyle, physical activity and diet. Anthropometric measurements were also obtained, including height, weight and four site SFT, using the same protocol as used in pre-pregnancy (section 3.1.2). Pregnancy weight gain was calculated as the difference between the weight measurements in early and late pregnancy. Details of pregnancy complications, for example, GDM and pregnancy induced hypertension, were recorded.

3.1.2.1 Assessment of 25(OH)D in pregnancy

Non-fasted blood samples were obtained at both the early and late pregnancy assessments and serum stored at -70°C until analysis. These samples were used to determine 25(OH)D status. Different analytical techniques were used for the early and late pregnancy samples, however all samples from the same stage of pregnancy were analysed using the same method and in a single batch.

The early pregnancy samples were analysed in 2013 using LC-MS/MS. Serum samples had an internal standard added, which was followed by protein denaturation by the addition of zinc sulphate and methanol. The internal standard and both 25(OH)D_2 and 25(OH)D_3 were extracted into hexane, which was dried and reconstituted in the mobile phase. The extracts were analysed with the use of liquid chromatography with detection by tandem mass spectroscopy (Waters Corporation, Milford, MA, USA). For the late pregnancy samples, 25(OH)D concentration was measured in 2008 with the use of chemiluminescent immunoassay (Diasorin Liaison, Stillwater, MN, USA). This assay measures both 25(OH)D_2 and 25(OH)D_3. For both early and late pregnancy, total 25(OH)D was calculated from the sum of 25(OH)D_2 and 25(OH)D_3. The laboratories that undertook both analyses are members of DEQAS, and both assays met the requirements of this scheme. Intra-assay and interassay coefficients of variation (CV) for both methods were < 10%.

3.1.3 Offspring assessments

3.1.3.1 Neonate

At birth, anthropometric measurements were obtained. Weight, whilst naked, was measured using calibrated digital scales to the nearest 0.001 kg (Seca Ltd, Birmingham, UK). Crown-heel length (CHL) was measured using a neonatometer (Harpenden, Wrexham, UK) to the nearest 0.1 cm. The infant was placed supine on the neonatometer and their head held in the Frankfurt plane against the head plate by one research nurse. The left leg was extended until the baby was lying flat, and the foot plate brought up the heel of the left foot. The measurement was taken three times, and an average of the measurements calculated.

A subset of mothers was invited to participate in a study of offspring body composition. These infants attended for a whole body and LS DXA scan. This data has not been used in this thesis, and therefore details of the methods have not been included.

3.1.3.2 Home visits at 6 months, 1, 2 and 3 years of age

At 6 months, 1, 2 and 3 years of age, a research nurse visited the participant at home. A questionnaire detailing the health, diet (including duration of breastfeeding) and physical activity of the child was completed. Anthropometric measurements were also obtained, but as this data has not been used in the analyses included here, details of the methods have not been included.

3.1.3.3 Four year assessment

At 4 years of age, the children were invited to attend the Osteoporosis Centre at Southampton General Hospital for a detailed assessment of body composition. Health, diet and lifestyle were determined using an interviewer administered questionnaire.

3.1.3.3.1 Anthropometry

Standing height was measured to the nearest 0.1 cm using a Leicester height measurer (Seca Ltd, Birmingham, UK). Height was measured with no shoes and standing with feet together and heels in contact with the ground and arms held relaxed by their sides. The head was placed in the Frankfurt plane. The spine was stretched by gentle upward pressure applied beneath the mastoid

processes. Height was measured three times and the average calculated. Weight was measured in light clothing to the nearest 0.1 kg using calibrated electronic scales (Seca Ltd, Birmingham, UK).

3.1.3.3.2 Dual-energy X-ray Absorptiometry

A whole body, LS and left hip DXA scan was obtained using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA) in paediatric scan mode (Apex 3.1 software). Before scanning, the child was asked to remove any clothing containing metal elements (eg, zips, buttons) and metal jewellery as these would falsely elevate BMC measurement. The child lay supine on the scanning instrument and was asked to remain as still as possible. A DVD was played to reduce movement artefact. A whole body scan took approximately 5 minutes, and LS and left hips scans approximately 30 seconds each. The total radiation exposure was approximately 5.2 microsieverts for the whole body scan, 8.8 microsieverts for the LS scan and 4.9 microsieverts for the hip scan (total 18.9 microsieverts). The DXA instrument underwent daily calibration using a spine phantom. The manufacturer's CV for the instrument was 0.75% for whole body scans (for adults), and the experimental CV when a spine phantom was repeatedly scanned in the same position 16 times was 0.68%. Precision assessment for body composition in children has not been undertaken in this research centre, but a previously reported study has shown high precision for body composition assessment in children under the age of 10 years when scans obtained using a Hologic Discovery instrument were repeated three times on the same day (coefficient of variation for BMC, LM and FM were 1.3%, 0.9% and 2.2%, respectively) (303).

All scan images were reviewed by two researchers and any scans with excess movement artefact (eg duplication or missing parts of limbs) or other artefact (eg metal object) were excluded from the dataset. When movement of one leg or arm was present, data from the contralateral region of interest (ROI) was imputed into this area (304). The arm ROI was defined by a line through the centre of the shoulder joint and separating the soft tissues of the arm from that of the trunk. The leg ROI was defined by a line passing diagonally downwards through the femoral neck to below the pubis (Figure 3.2). The ROIs are automatically placed by the scanning technology, but were reviewed by trained densitometry technicians and adjusted if necessary. Positioning and content of the ROIs were reviewed by the researchers when the scan images were assessed to ensure the ROIs contained the correct anatomical regions.

Measures of body composition obtained from the whole body scan were FM, LM and BMC. %FM, %LM and %BMC were calculated as a proportion of body weight. In addition to data

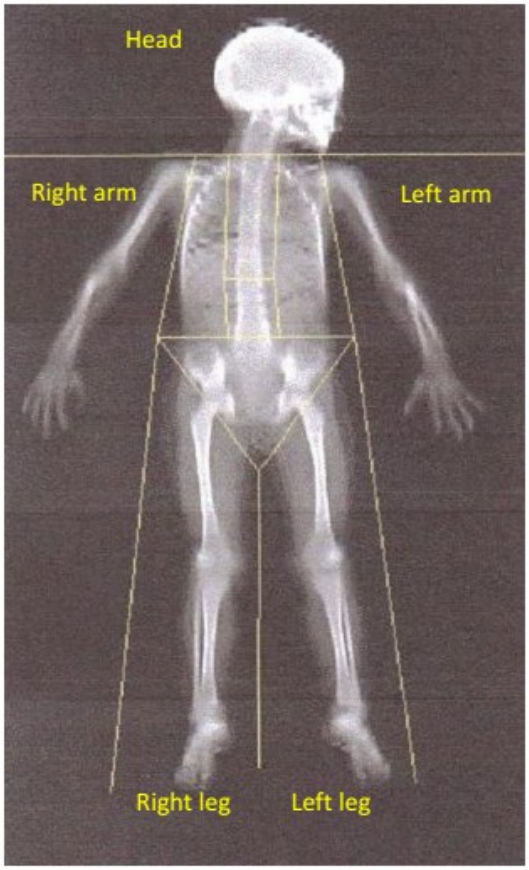


Figure 3.2: An example DXA scan from a 4 year old showing the arm and leg regions of interest

for the whole body, appendicular measurements were obtained using the four arm and leg ROIs on the whole body scan. Total appendicular LM, FM and BMC were calculated by totalling the respective compartments for all four limbs.

3.1.3.3.3 Hand grip strength

Grip strength was measured using a Jamar handgrip dynamometer (Promedics, Blackburn, UK) with a standardised approach (262). The child was seated in a standard chair with back support and a fixed arm and asked to rest their forearm on the arm of the chair with their wrist extending just beyond the arm of the chair. The dynamometer was adjusted to fit the hand size of each individual. The researcher rested the dynamometer on the palm of their hand to support the weight of the dynamometer without restricting movement (Figure 3.3). The child was verbally encouraged to squeeze as tightly as possible. Three measurements of each hand were taken, alternating the hand used. Measurements were made to the nearest 0.5 kg. Due to the learning and tiring effect in grip strength assessment, which can lead to some variability across

measurements (305), the maximum of six measurements was used as the main outcome. Test-retest reliability has previously been demonstrated in this age group (49).

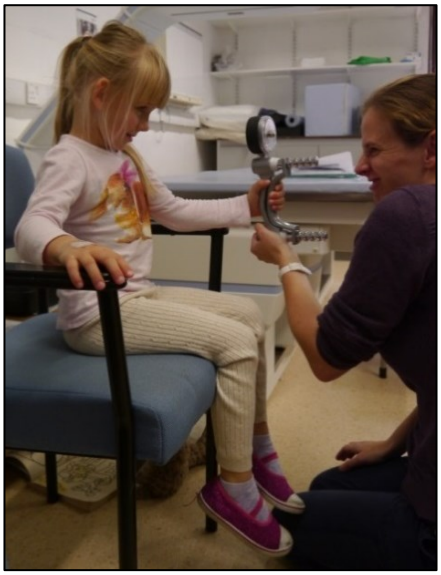


Figure 3.3: Assessment of hand-grip strength at age 4 years using a standard armed chair with the researcher supporting the weight of the Jamar dynamometer and providing verbal encouragement
Photo credit: Author's own. Parental permission was granted for publication

3.1.3.3.4 Physical activity

In a subset of children, habitual physical activity was assessed using an Actiheart monitor (Cambridge Neurotechnology Ltd, Cambridge, UK). This is a combined accelerometer and heart rate monitor. The device was worn on the chest, connected to the skin by two ECG electrodes (Figure 3.4). The children were asked to wear the device continuously for 7 days except during bathing and swimming.

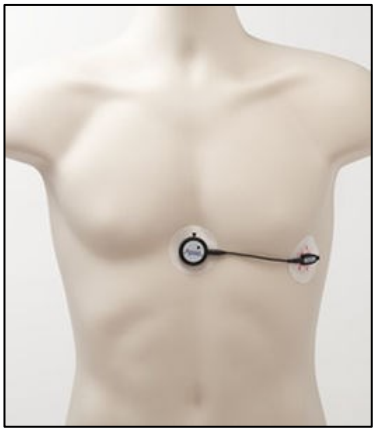


Figure 3.4: Positioning of the Actiheart device
(Image from http://www.cephalon.dk/products/activity-monitors/actiheart)

Data were downloaded from the device and analysed by the MRC Epidemiology Unit, Cambridge. Only the accelerometer data was used in the analyses included in this work as algorithms for the interpretation of the heart rate data in this age group have not yet been established. Pre-defined cut-points were used to determine the average number of minutes per day each child spent in sedentary, light, moderate, vigorous and very vigorous activity (306). Moderate, vigorous and very vigorous activity levels were combined to give the exposure measure (MVPA).

3.1.3.4 Ongoing assessments at 6-7, 8-9 and 10-12 years of age

There is ongoing follow up of the children as outlined in Table 3.1. Data from these assessments will not be included in this work, but might enable future hypotheses that arise from this work to be explored.

Table 3.1: Offspring assessments in the Southampton Women's Survey at 6-7, 8-9 and 10-12 years of age

6-7 years	*8-9 years*	*10-12 years*
Health, diet and lifestyle questionnaire	Health, diet and lifestyle questionnaire	Health, diet and lifestyle questionnaire
Anthropometry	Anthropometry	Anthropometry
DXA (whole body, lumbar spine and left hip)	DXA (whole body, lumbar spine and left hip)	DXA (whole body, lumbar spine and left hip)
Peripheral quantitative computed tomography of the right leg	Cardiac and vascular resistance examination including echocardiography and blood pressure	High-resolution peripheral quantitative computed tomography (HR-pQCT) of the right leg
Objective physical activity assessment by accelerometry		Pubertal staging
		Cardiovascular fitness assessment by step-test
		Venous blood sampling

3.2 Maternal Vitamin D Osteoporosis Study (MAVIDOS)

MAVIDOS is a double-blind, randomised placebo-controlled trial of vitamin D supplementation in pregnancy (1000 IU cholecalciferol per day). The primary outcome was whole body BMC assessed by DXA at birth. These findings were published in 2016, and were discussed in section 1.4.8.3 (page 78) (295). An overview of the MAVIDOS study is shown in Figure 3.5.

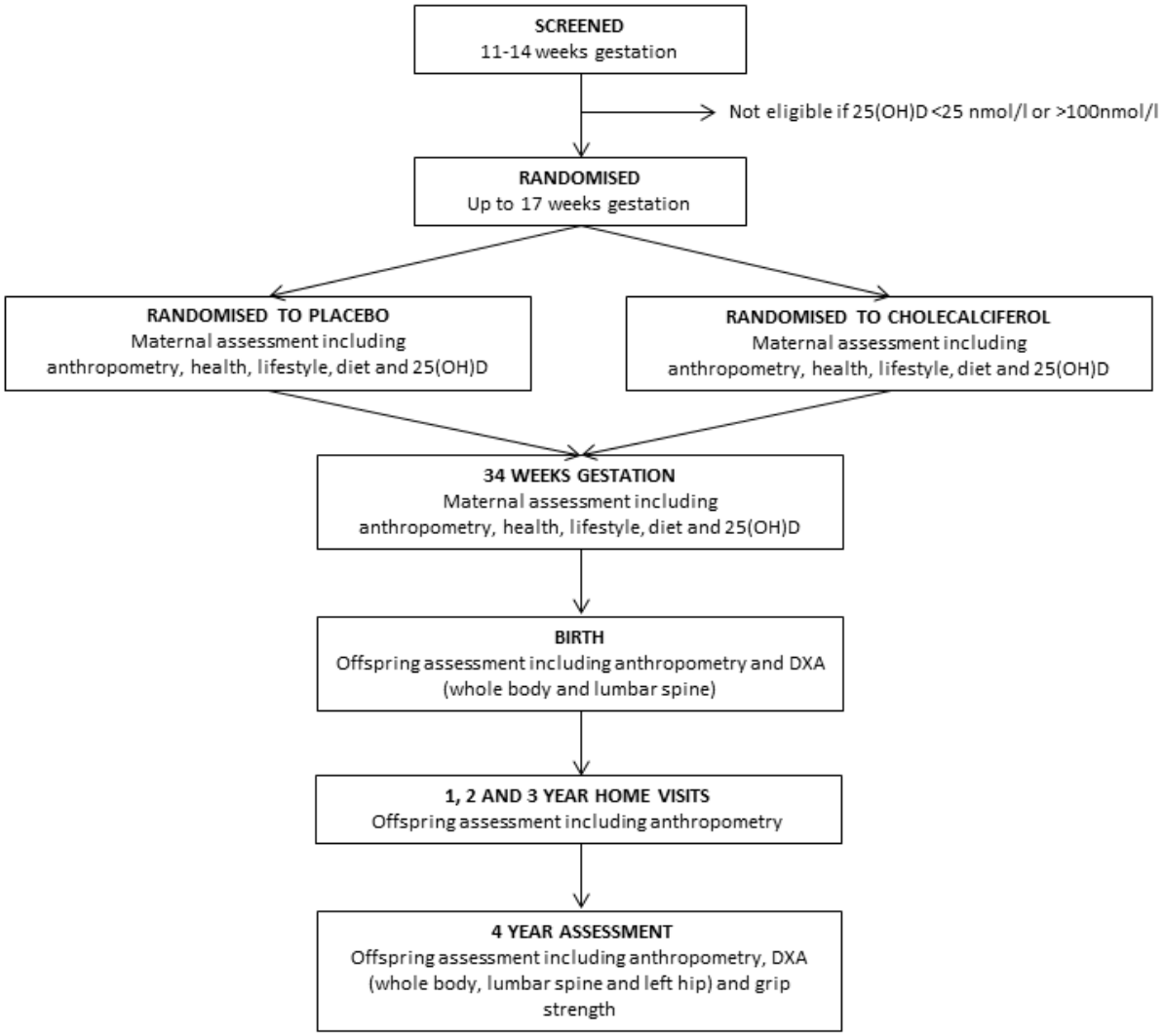


Figure 3.5: Overview of the MAVIDOS trial

The study was approved by the Southampton and South West Hampshire Research Ethics Committee (Appendix B) and full approval was granted by the UK Medicines and Healthcare Products Regulatory Agency. Written informed consent was obtained from all participants, and written parental consent was obtained for follow-up of the offspring at 4 years of age.

3.2.1 Identification and recruitment of women into the study

Women attending one of three hospitals in the UK (Princess Anne Hospital, University Hospital Southampton NHS Foundation Trust, Southampton [latitude 50.9°N]; John Radcliffe Hospital, Oxford [latitude 51.8°N]; Sheffield Hospitals NHS Trust, Sheffield [latitude 53.4°N]) for early pregnancy ultrasonography were invited to participate. An information sheet about the study was sent with their ultrasound scan appointment (Appendix C). Women were subsequently approached by a research nurse when attending for this scan. Informed consent was obtained and a blood sample taken and measured for 25(OH)D and serum calcium in the local hospital laboratory. Women with a 25(OH)D between 25 and 100 nmol/l were eligible to continue in the study. Ethical approval was not granted for women with 25(OH)D < 25 nmol/l or 25(OH)D > 100 nmol/l to participate. Women with a 25(OH)D below the lower threshold were advised to see their general practitioner for advice regarding vitamin D supplementation. Other exclusion criteria are listed in Figure 3.6.

MAVIDOS Exclusion Criteria

- Baseline 25(OH)D less than 25 nmol/l or greater than 100 nmol/l
- Age <18 years at recruitment
- Twin or other multiple pregnancy
- >17 weeks gestation at recruitment
- Known metabolic bone disease, previous renal stones, hyperparathyroidism or hypercalciuria
- Medications known to interfere with fetal growth (eg corticosteroids, anticonvulsants, bisphosphonates)
- Current daily vitamin D supplementation > 400 IU
- Fetal anomaly on early pregnancy USS or subsequently on mid pregnancy anomaly scan
- Cancer diagnosis within the last 10 years
- Serum corrected calcium > 2.75mmol/l

Figure 3.6: MAVIDOS exclusion criteria

3.2.2 Investigational medicinal product and randomisation

Women were randomly assigned to receive either 1000 IU/day cholecalciferol or matched placebo (Merck KGaA, Darmstadt, Germany). Both capsules were identical in appearance, and provided in a blister pack in a single box containing all medication for the whole pregnancy. The study medication was pre-randomised by the manufacturer (Sharp Clinical Services, Powys, UK) in a 1:1 ratio and was not stratified by research centre. Medication packs were sequentially allocated to study participants. Study medication was commenced at 14 weeks of gestation, or as soon as possible up to a maximum of 17 weeks of gestation in women enrolled after this time point, and continued until delivery.

3.2.3 Maternal assessments in pregnancy

Before starting the study medication (approximately 14 weeks of gestation) and again at 34 weeks of gestation, an interviewer administered health, diet and lifestyle questionnaire was completed. Height, weight and four site SFT measurements were obtained by a trained research nurse following standard protocols identical to that used in the SWS (section3.1.2, page94).

At 18-21 weeks of gestation, the women attended for their routine fetal anomaly ultrasound scan. In the event of significant fetal anomalies, the participant was referred for further review in the fetal medicine department of their hospital, and withdrawn from further participation in the study.

Where possible a measurement of the height of the baby's father was also obtained at one of the pregnancy visits. If the father did not attend either visit, reported paternal height was documented.

3.2.3.1 Assessment of 25(OH)D in pregnancy

Non-fasted venous blood samples were drawn on the day that the study medication was collected and at 34 weeks of gestation. Serum was stored at -70°C until analysis. 25(OH)D concentration was assessed by chemiluminescence immunoassay (Liaison automated platform, Diasorin, Minnesota, USA). All samples were analysed in a single batch at the end of the study at MRC Human Nutrition Research, Cambridge, UK. The laboratory participates in the DEQAS scheme.

3.2.3.2 Assessment of compliance with study medication

Participants were asked to bring any remaining study medication to each assessment. The pills were counted and compliance calculated as number consumed divided by the expected consumption based on number of days since the medication was dispensed, and expressed as a percentage. When available, compliance was calculated from the visit at 34 weeks of gestation, but when this was not available, the count at 18-21 weeks was used.

3.2.4 Neonatal anthropometry and DXA

Birth weight, measured with electronic scales to the nearest 0.001 kg on the day of birth, was extracted from the hospital records.

All other measurements were taken by a trained research nurse within 14 days of birth. All measurements were taken in triplicate and the average calculated. CHL were measured using a neonatometer to the nearest 0.1 cm and following the same protocol as used in the SWS (section 3.1.3.1, page 95). Crown-rump length (CRL) was also measured with the infant lying naked on the neonatometer and the legs flexed to 90° at the hips. OFC was measured using a cloth tape positioned at the widest point of the skull anteriorly and posteriorly.

MUAC was measured with an unmarked tape at the point judged by eye to be the mid-point of the upper arm with the infant's arm as relaxed as possible. The tapes were marked and measured against a fixed rule. The level at which the MUAC was taken was marked on the baby, and triceps SFT assessed on the posterior aspect of the arm at the same level. Subscapular SFT was measured at the angle of the scapula, similarly to in the adult.

A whole body and LS DXA scan was obtained using a Hologic Discovery instrument (Hologic Inc. Bedford, MA, USA) or GE-Lunar iDXA (GE-Lunar, Madison, WI, USA) depending on the research centre. This data has not been used in the analyses included in this work and therefore the methods will not be reported in detail.

3.2.5 Offspring assessments at 1, 2 and 3 years of age

Mothers recruited in Southampton were invited to participate in further follow-up of their offspring. At 1 and 2 years of age the children were reviewed during a home visit. This included an interviewer-led questionnaire assessment of diet, health and lifestyle and anthropometry. At 1

year of age, CHL was assessed using the same method as at birth. At 2 years of age, standing height was assessed following the same protocol previously described for children participating in the SWS at age 4 years (section 3.1.3.3.1, page 95). MUAC, triceps and subscapular SFT was performed following the same protocol as used in adult study participants (section 3.1.2, page 94).

Follow-up of the children at 3 years of age with a home visit is ongoing and expected to complete in September 2017.

3.2.6 Offspring assessment at 4 years of age

At 4 years of age, the children born in Southampton were invited to attend the Osteoporosis Centre at Southampton General Hospital for a detailed assessment. At this visit, a diet, health and lifestyle questionnaire was completed (Appendix E) and anthropometric measurements obtained including weight, height, OFC, MUAC, triceps and subscapular SFT following standard protocols as described previously. All measurements were performed in triplicate by a trained researcher and the average calculated.

Body composition was assessed by whole body DXA scan obtained using a Hologic Discovery DXA instrument (Hologic Inc., Bedford MA, USA) in paediatric scan mode (Apex 5.5.3.1 software), as described in section 3.1.3.3.2 for children participating in the SWS at 4 years of age (page 96).

Hand grip strength was measured using a Jamar hand dynamometer (Promedics, Blackburn, UK) and following the same standardised protocol as used in the SWS (section 3.1.3.3.3, page 97).

A pQCT scan of the right tibia was obtained using a Stratec XCT 2000 instrument (Stratec Inc, Pforzhein, Germany), and when consent was given a venous blood sample was collected from the child. Serum samples have been stored at -70°C. The data obtained from the pQCT scans and the blood samples has not been used in the analyses included in this thesis and therefore the methods for these will not be described in detail. However these data will enable further hypotheses to be explored in the future.

3.3 Statistical Methods

All outcomes were assessed for normality using visual inspection. Comparisons between groups were performed using t-test, Mann-Whitney U test and χ^2 test for normally distributed, non-

normally distributed and categorical variables, respectively. Correlations were assessed using Pearson's correlation coefficient (denoted r) and Spearman's rank correlation coefficient (denoted r_s) for normally distributed and non-normally distributed variables, respectively. Further details of statistical methods relevant to the individual analyses performed will be provided in each of the results chapters. All women who remained in the study were included in the analysis in the group to which they were randomised. Participants who reported low compliance with the study medication were not excluded from the analysis.

Due to substantial collinearity among the outcomes considered, testing for multiple comparisons was felt to inappropriate (307). All analyses were performed in Stata v14.2 (Statacorp, College Station, Texas, USA). A p value of < 0.05 was considered statistically significant.

3.3.1 Power calculation for MAVIDOS

A power calculation for this work was based on the knowledge that the MAVIDOS study had recruited 965 women. The SWS achieved a follow up rate of 84% of those invited and therefore, based on a conservative estimate, it would be expected that 700 children would participate at 4 years. This sample size would have an 80% power to detect a 0.21 standard deviation difference in outcome between the treatment and placebo groups at the 5% significance level.

3.4 Author's contribution to this project

All hypotheses and analysis ideas included in this thesis are my own. The SWS and MAVIDOS studies were both established studies before I started my research at the MRC Lifecourse Epidemiology Unit, Southampton. The data collection in pregnancy and at 4 years of age in SWS had been completed. I recruited a number of women to the MAVIDOS trial, but assessments in pregnancy, at birth and 1 and 2 years were completed by trained research nurses.

I developed and submitted an ethics application to collect additional data in the follow-up at 4 years of age in MAVIDOS. This included assessment of grip strength, pQCT and venesection. The ethics approval for this follow-up is shown in Appendix B, and the participant information sheets produced by me for this additional follow-up are shown in Appendix D. During my candidature I have collected the data at this visit, including obtaining informed consent for participation, questionnaire administration, anthropometry, assessment of grip strength and venesection. The DXA and pQCT scans have been performed by a trained DXA technician.

Chapter 3

I reviewed all DXA scan images for the MAVIDOS assessments at birth and at 4 years of age, and cleaned the MAVIDOS data collected at 1, 2 and 4 years of age. All statistical analysis in this thesis was undertaken by me and subsequently reviewed by a statistician from the MRC LEU.

The interpretation of the data is all my own work. The three journal papers (see Project Outputs) that have been published including the results included in this thesis were primarily written by me, with subsequent comments on the drafts from my supervisors and co-authors.

Chapter 4: Cohort profiles

4.1 Aims

To describe the characteristics of the women included in the studies used in these analyses.

4.2 Participant characteristics

4.2.1 The Southampton Women's Survey

4.2.1.1 Demographics of the mothers

12,579 women were initially recruited into the SWS, of whom 3219 reported a pregnancy to the research centre before the end of 2005. 3158 were known to deliver a liveborn singleton infant. Demographic characteristics of these mothers are presented in Table 4.1.

4.2.1.2 Maternal 25(OH)D status

Serum 25(OH)D concentration was measured at a median 11.7 weeks of gestation (IQR 11.4-12.1) in early pregnancy and 34.6 weeks of gestation (IQR 34.3-34.9) in late pregnancy. Median 25(OH)D in early pregnancy was 60.5 nmol/l (IQR 42.5-79.5, n=2019) and in late pregnancy was 58.2 nmol/l (IQR 40.2-83.7, n=2328). Distributions of serum 25(OH)D status in early and late pregnancy are shown in Figure 4.1, which demonstrates the full range of 25(OH)D concentrations included in this cohort.

4.2.1.3 Characteristics of the offspring at birth

51.8% of the offspring were male. Median gestation at delivery was 40.0 weeks (IQR 39.0-41.0). 197 infants (6.3%) were born preterm (< 37 weeks of gestation). Mean birth weight was 3431 g [SD 563], but males were significantly heavier at birth than females (3490 g [SD 573] vs 3369 g [SD 546], $p < 0.001$).

Table 4.1: Characteristics of the mothers in the Southampton Women's Survey

Maternal Characteristic	***Number in cohort with data***	***Result***
Age at delivery (years), mean (SD)	3156	30.7 (3.8)
White ethnicity, %	3157	95.5
Height (cm), mean (SD)	3141	163.2 (6.5)
Pre-pregnancy BMI (kg/m^2), median (IQR)	3130	24.1 (21.9-27.4)
Nulliparous, %	3155	51.1
Smoking, %		
Pre-pregnancy	3155	27.9
Early pregnancy	2843	16.0
Late pregnancy	2643	15.1
Educational achievement (%)	3149	
None		3.1
CSE/O Level/GCSE (high school)		38.3
A-levels		30.3
Diploma		6.3
Degree or higher		22.0

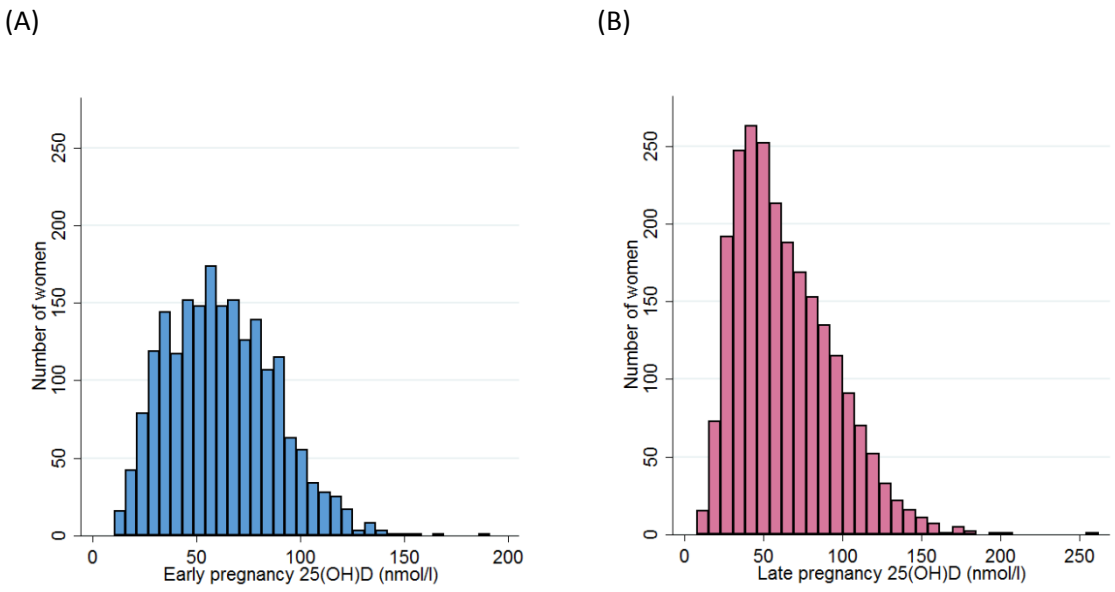


Figure 4.1: Distribution of 25(OH)D status in (A) early pregnancy and (B) late pregnancy in women participating in the Southampton Women's Survey

4.2.2 MAVIDOS

4.2.2.1 Characteristics of the participants

Women were recruited into the MAVIDOS study between 6th October 2008 and 11th February 2014. 1449 women initially agreed to participate in the MAVIDOS study and underwent screening of 25(OH)D. Of these, 148 were ineligible to participate due to 25(OH)D < 25 nmol/l (n=89) or 25(OH)D > 100 nmol/l (n=59), and a further 167 women withdrew prior to randomisation. 1134 women were therefore randomised to either placebo or cholecalciferol (Figure 4.2). Characteristics of the women at randomisation are shown in Table 4.2.

Table 4.2: Characteristics of the mothers at randomisation to either placebo or 1000 IU/day cholecalciferol during pregnancy

	Placebo	***Cholecalciferol***
n	569	565
Age (years), mean (SD)	30.4 (5.2)	30.5 (5.2)
Gestation (weeks), mean (SD)	16.0 (1.5)	15.9 (1.5)
White ethnicity, %	94.3	94.0
Height (cm), mean (SD)	165.8 (6.6)	165.6 (6.4)
BMI (kg/m^2), median (IQR)	25.7 (23.0-30.0)	24.7 (22.3-28.6)
Nulliparous, %	43.9	43.6
Smoking, %	8.2	8.3
Education to degree level or above, %	44.3	47.1
25(OH)D (nmol/l), mean (SD)	45.9 (17.0)	46.7 (17.7)

965 women remained in the study until delivery (Southampton n=767, Sheffield n=56, Oxford=142). Women who did not continue in the study until delivery tended to be younger ($p < 0.001$), were more likely to be of non-white ethnicity ($p=0.01$) and less likely to have degree level education ($p < 0.001$).

Figure 4.2: Consort diagram for the follow-up of mothers and their offspring in MAVIDOS

4.2.2.2 Compliance with study medication

Compliance with the study medication was high in both groups (placebo median 95.0% [IQR 88.2-98.8]; cholecalciferol median 96.2% [IQR 88.9-99.2]).

4.2.2.3 Maternal 25(OH)D status

At randomisation, serum 25(OH)D was similar between the two groups (p=0.35) with a mean of 46.2 nmol/l (SD 17.1) across the whole cohort. At 34 weeks of gestation 25(OH)D was significantly higher in the women randomised to cholecalciferol (p < 0.001), as shown in Figure 4.3. The mean difference between the two groups was 24.7 nmol/l (95% CI 21.7, 27.7).

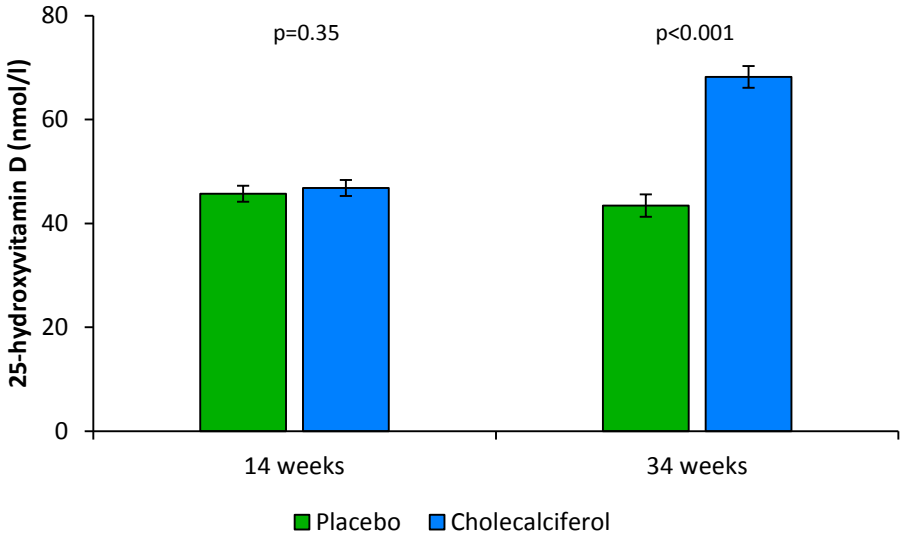


Figure 4.3: Maternal serum 25(OH)D at randomisation (14 weeks) and 34 weeks of gestation in women randomised to placebo and cholecalciferol

Displayed as mean (95% confidence interval)

4.2.2.4 Characteristics of the offspring at birth

Overall, 52.8% of the infants were male. The proportion of boys and girls was similar in each treatment group (p=0.28). The median gestation at delivery was 40.3 weeks (IQR 39.3-41.1) in women randomised to placebo and 40.3 weeks (IQR 39.1-41.0) in women randomised to

cholecalciferol (p=0.22). 49 infants (5.1%) were born preterm, but this did not differ between the two groups (placebo 4.5%, cholecalciferol 5.7%, p=0.43).

4.2.2.5 Characteristics by recruitment centre

Only offspring of the women recruited in Southampton were invited for follow up at 1, 2, 3 and 4 years of age. Women recruited in Southampton and continuing in the study until delivery were of similar age, height, early pregnancy BMI, ethnicity, smoking status, parity and educational achievement to those recruited and delivering an infant in Oxford and Sheffield.

4.3 Summary

Both the SWS and the MAVIDOS trial encompass a wide range of maternal characteristics, but it is important to note the lack of ethnic diversity in the participants of these studies, which might limit the generalisability of the study findings. Furthermore, despite the majority of participants in MAVIDOS being recruited from the same geographical area, the proportion of women educated to degree level was higher and the number of women who smoked was lower than in the SWS. This could reflect volunteer biases for participation in an intervention study or might reflect a demographic change during the time interval between the two studies.

Chapter 5: Tracking of 25-hydroxyvitamin D status during pregnancy

5.1 Background and aims

Most observational studies assessing the relationship between maternal 25(OH)D status and offspring bone development and body composition have used only a single measurement of 25(OH)D, and the timing of this varies between studies from the first trimester to umbilical cord blood obtained at delivery. Pregnancy crosses several seasons and therefore the 25(OH)D status of an individual is likely to vary during pregnancy but how a single measurement of 25(OH)D relates to 25(OH)D status at other time points in pregnancy is not currently known.

Tracking describes the stability of a measurement relative to the population distribution over time. As such, if a biological marker is known to track highly, one measurement can be used to predict future measurements, and therefore inform the need for interventions to prevent high or low levels. The tracking of 25(OH)D is not currently well understood. High correlation between 25(OH)D status in samples obtained in the same month at one to 5 year intervals has been demonstrated in non-pregnant adults (236-238), but the tracking of 25(OH)D between seasons, or during pregnancy, has not previously been investigated.

Currently, the UK Department of Health recommends antenatal vitamin D supplementation for all pregnant women (248). However, further knowledge on the stability of 25(OH)D status and factors that influence this during pregnancy, might enable individualised advice on the need for supplementation following a single measurement of 25(OH)D.

The aim of this analysis was therefore to assess the tracking of 25(OH)D from early to late pregnancy in an observational birth cohort, and to explore maternal factors which are associated with deviations in 25(OH)D tracking.

5.2 Methods

Observational data collected during the pre-pregnancy and pregnancy phases of the SWS were used in this analysis, as described in Chapter 3.

5.2.1 Statistical analysis

Maternal characteristics for women who participated in SWS but did and did not have measurements of serum 25(OH)D status in pregnancy were compared using t-test, Mann-Whitney and χ^2 test for normally distributed, non-normally distributed and categorical outcomes. All women with a measurement at either early and/or late pregnancy were included in the seasonal modelling.

Fourier transformations were used to model the seasonal variation in 25(OH)D for early and late pregnancy separately. The date of collection of each sample was converted to a numerical value (A) which described the number of days the sample was taken after the first sample in the study. This was converted to radians using the formula $\theta=[2\pi A]/365$. As 25(OH)D was not normally distributed, $\log_e$[25(OH)D] was regressed on sinθ and cosθ, and subsequently sinθ, cosθ, sin 2θ and cos2θ, and sinθ, cosθ, sin 2θ, cos2θ sin3θ and cos3θ to determine the best model. From this, equations predicting $\log_e$[25(OH)D] for each day of blood sampling in early and late pregnancy were determined. The difference between measured 25(OH)D and the seasonally modelled 25(OH)D for the exact date of sampling was calculated for each participant for early and late pregnancy separately, to generate a season-corrected 25(OH)D. Thus a positive season-corrected 25(OH)D value represents a measured 25(OH)D higher than that modelled for the day of sampling, and a negative value a lower measured 25(OH)D than the modelled value. Maternal characteristics associated with season-corrected 25(OH)D were explored using linear regression, and all characteristics with $p < 0.20$ were included in multivariate linear regression.

Tracking of both $\log_e$[25(OH)D] and season-corrected 25(OH)D were assessed using the Pearson's correlation coefficient (308). Maternal factors which were associated with the change in season-corrected 25(OH)D were assessed using simple linear regression, and predictors with $p < 0.20$ were included in a multiple linear regression model. Finally, differences in maternal characteristics according to vitamin D supplement usage were determined using ANOVA and χ^2 test.

5.3 Results

5.3.1 Characteristics of the participants

Of the 3158 women participating in the SWS who were known to deliver a live-born infant, serum 25(OH)D concentration was assessed in 2019 (64.0%) and 2328 (73.7%) women in early and late

pregnancy, respectively. 1753 (55.5%) women had a measurement of serum 25(OH)D in both early and late pregnancy. Women with a 25(OH)D measurement at both time points tended to be younger, were more likely to be in their first pregnancy and less likely to smoke in early pregnancy than women who did not have blood sampling at both stages of pregnancy ($p < 0.001$ for all, table 5.1). Although the SWS cohort is compromised predominantly of women of White ethnicity, the proportion of women of non-white ethnicity (3.2% vs 6.0%, $p < 0.001$) was also lower in the women with 25(OH)D measured in both early and late pregnancy.

Table 5.1: Characteristics of the women participating in SWS who did and did not have 25-hydroxyvitamin D measured in both early and late pregnancy

	25(OH)D measured in both early and late pregnancy	***25(OH)D not measured in both early and late pregnancy***	***p***
N (%)	1753 (55.5)	1405 (44.5)	
Maternal age at delivery (years), mean (SD)	30.4 (3.7)	31.0 (4.0)	< 0.001
White ethnicity (%)	96.8	94.0	< 0.001
Pre-pregnancy BMI (kg/m2), median (IQR)	24.2 (21.9-27.4)	24.1 (21.8-27.3)	0.28
Nulliparous (%)	48.3	54.6	< 0.001
Education at or above degree level (%)	22.2	21.7	0.74
Smoked in early pregnancy (%)	13.9	19.2	< 0.001

The median (IQR) 25(OH)D of women included in the analysis in early and late pregnancy were 61 nmol/l (IQR 43-81) and 59 nmol/l (IQR 41-84), respectively. 37.3% and 22.2% of the women reported taking vitamin D supplementation in early and late pregnancy, respectively.

5.3.2 Seasonal modelling of 25-hydroxyvitamin D

In both early and late pregnancy, 25(OH)D displayed statistically significant seasonal variation. This was best modelled by the regression of $\log_e$[25(OH)D] on $\sin\theta$, $\cos\theta$, $\sin 2\theta$ and $\cos 2\theta$ (Figure 5.1). The Fourier series model explained 17% ($p < 0.0001$) and 30% ($p < 0.0001$) of the variance in 25(OH)D in early and late, respectively.

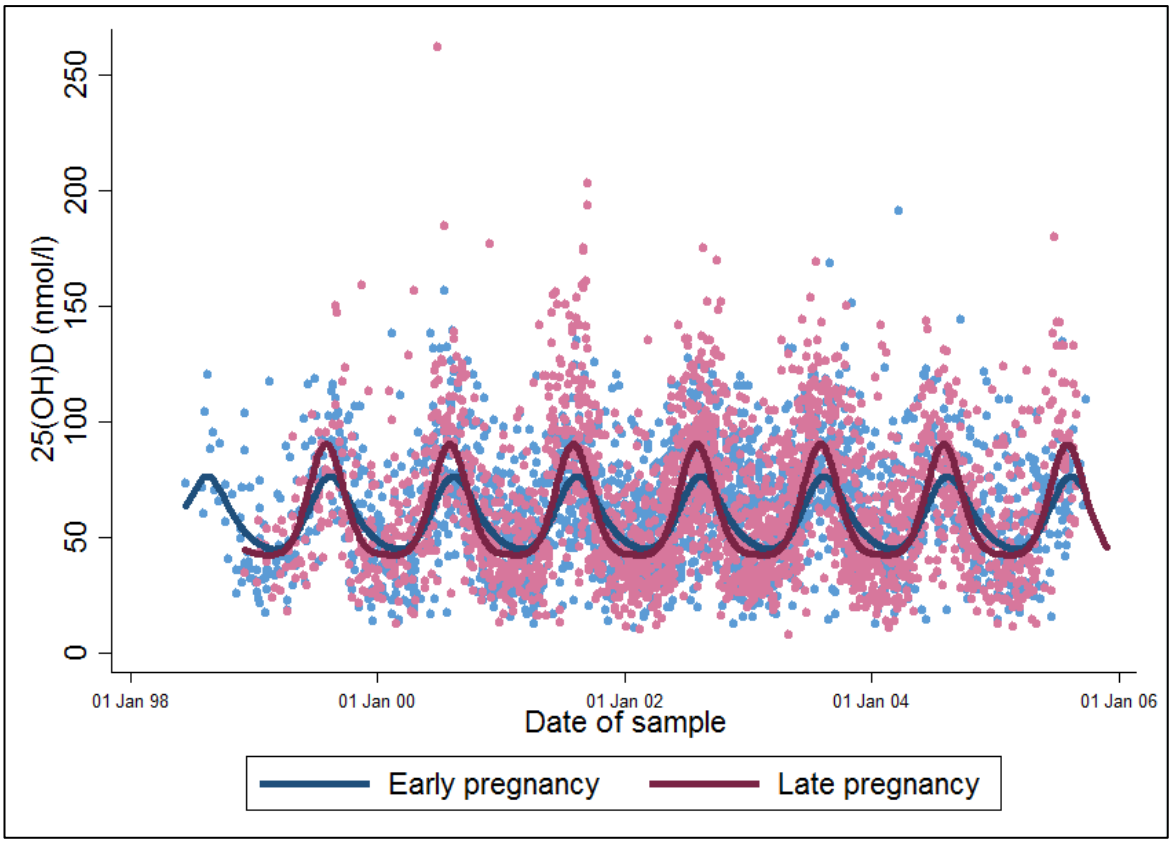
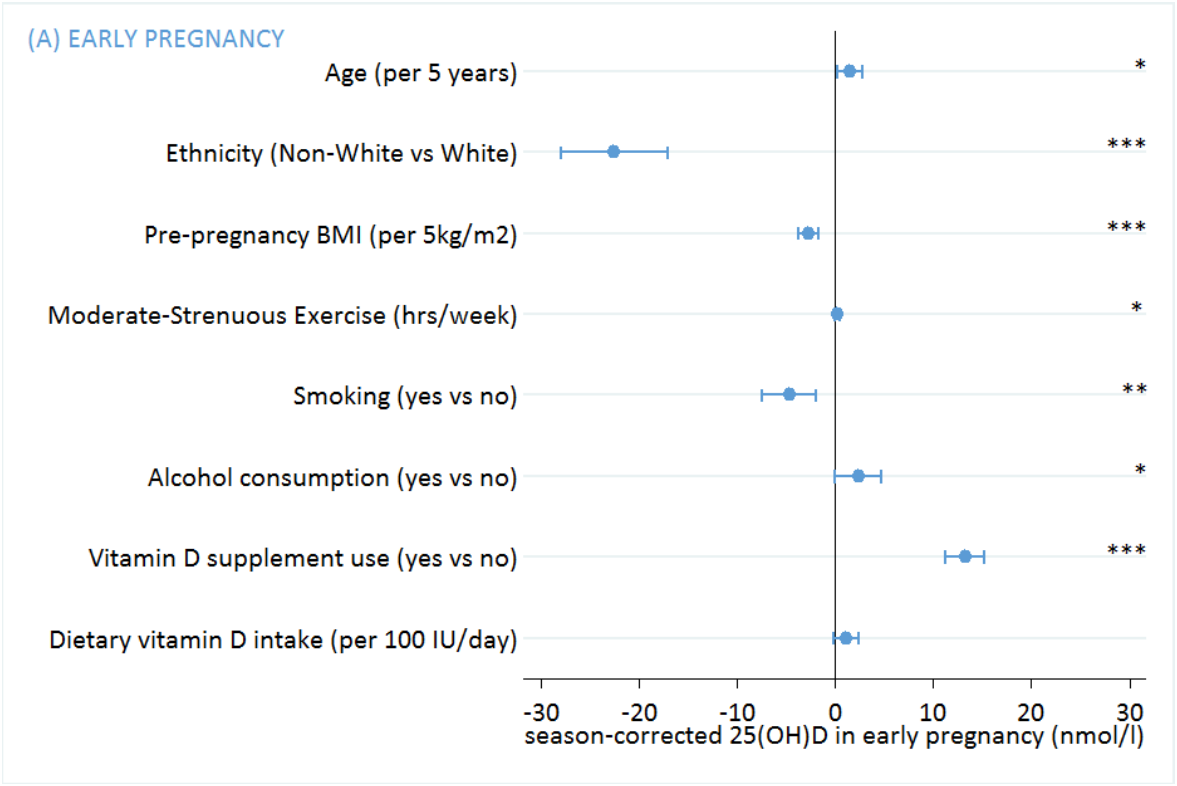


Figure 5.1: Seasonal modelling of 25(OH)D in early and late pregnancy

5.3.3 Determinants of season-corrected 25(OH)D

The mean difference between measured and seasonally modelled 25(OH)D ("season-corrected 25(OH)D") in early pregnancy was 4.6 nmol/l (SD 23.3, range -61.2 to 145.9) and in late pregnancy was 4.8 nmol/l (SD 25.7, range -66.2 to 182.7).

Using univariate linear regression, maternal age, ethnicity, pre-pregnancy BMI, participation in moderate-strenuous exercise during early pregnancy, smoking status, alcohol consumption, dietary vitamin D intake and vitamin D supplement use were all significantly associated with season-corrected 25(OH)D in early pregnancy, whereas parity and educational achievement were not. When all statistically significant factors were included in multivariate linear regression, all remained significantly associated with season-corrected 25(OH)D, except dietary vitamin D intake (Figure 5.2 (A)). The maternal characteristics with the largest effect sizes were non-White ethnicity (β=-22.5 nmol/l [95% CI -27.8, -17.1], $p < 0.001$) and use of vitamin D supplementation (β=13.2 nmol/l [95% CI 11.3, 15.2], $p < 0.001$). The pattern and effect sizes of factors associated with late pregnancy season-corrected 25(OH)D in multivariate analysis were similar (Figure 5.2 (B)).



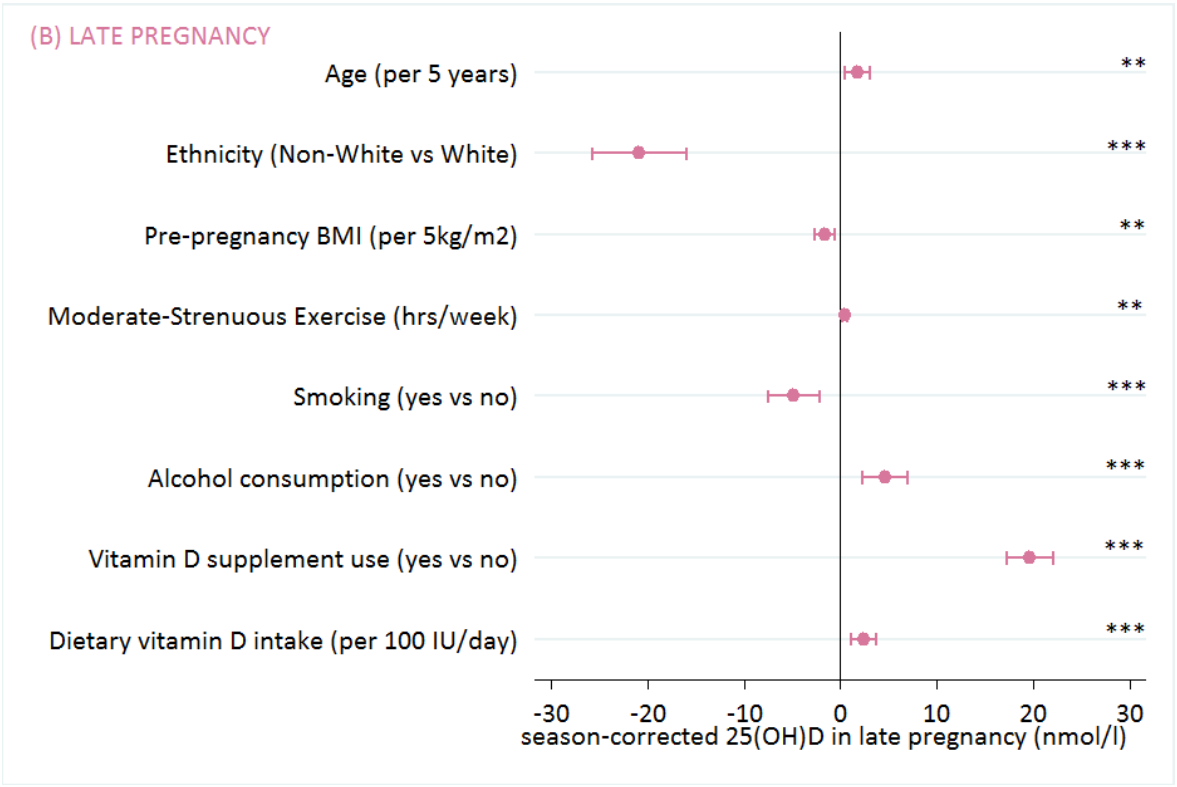


Figure 5.2: Independent associations between maternal characteristics and season-corrected 25(OH)D in (A) early pregnancy and (B) late pregnancy

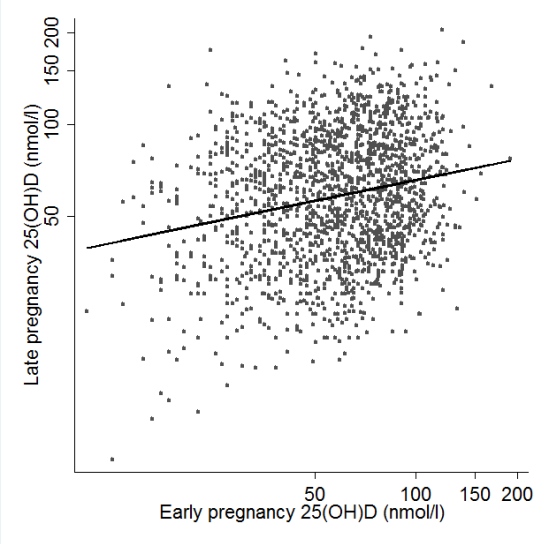
Shown as beta coefficient (95% confidence interval) per unit predictor

*p < 0.05, **p < 0.01, ***p < 0.001

5.3.4 Tracking of 25(OH)D status from early to late pregnancy

The correlation coefficient between measured $\log_e$[25(OH)D] in early and late pregnancy was 0.21 (95% CI 0.17, 0.26, Figure 5.3 (A)). However, season-corrected 25(OH)D was more highly correlated from early to late pregnancy (r=0.53 [95% CI 0.50, 0.57], Figure 5.3(B)).

(A) r=0.21



(B) r=0.53

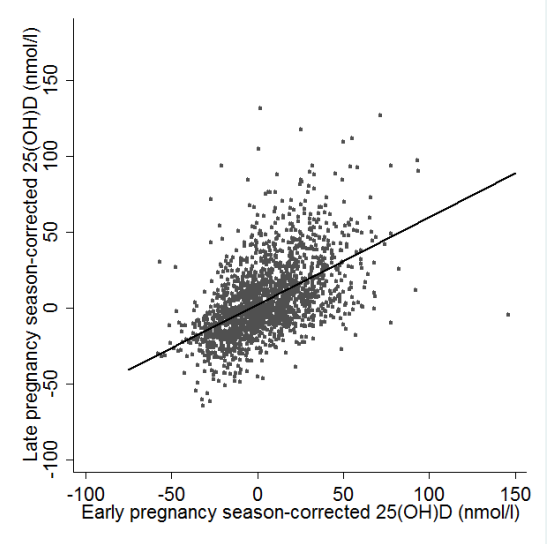


Figure 5.3: Correlation between (A) measured 25(OH)D and (B) season-corrected 25(OH)D in early and late pregnancy

5.3.5 Maternal factors and change in season-corrected 25(OH)D

The mean difference between season-corrected 25(OH)D in early and late pregnancy was 0.3 nmol/l (SD 23.5). A number of maternal factors were associated with change in season-corrected 25(OH)D in univariate analysis (Table 5.2). However, in multivariate analysis, only the timing of use of vitamin D supplements, exercise in late pregnancy and pregnancy weight gain remained statistically significant (Table 5.2). Thus, in comparison to women who never took vitamin D supplementation, discontinuation of vitamin D supplements after early pregnancy blood sampling was negatively associated with change in season-corrected 25(OH)D (β=-7.3 nmol/l, $p < 0.001$), whereas starting (β=12.6 nmol/l, $p < 0.001$) or continuing supplementation (β=6.6 nmol/l, $p < 0.001$) was positively associated. There was no statistical interaction between pregnancy weight gain and late pregnancy exercise (p=0.94).

Table 5.2: Associations between maternal demographic and lifestyle characteristics and the tracking of season-corrected 25(OH)D during pregnancy

	Change in season-corrected 25(OH)D (nmol/l)					
	Univariate			Multivariate		
	β	95% CI	p	β	95% CI	p
Age at delivery (years)	-0.0	-0.3, 0.3	0.86			
Education to degree level or higher (yes vs no)	2.6	-0.1, 5.3	0.06	1.8	-0.8, 4.5	0.18
Parity (Multiparous vs Nulliparous)	-0.7	-2.9, 1.5	0.54			
Ethnicity (Other vs White)	4.3	-1.9, 10.5	0.17	2.4	-4.0, 8.7	0.46
Moderate/Strenuous exercise (hrs/wk)						
Early pregnancy	0.1	-0.1, 0.4	0.25			
Late pregnancy	0.4	0.1, 0.8	0.01	0.4	0.1, 0.7	0.01
ΔEarly Pregnancy to Late Pregnancy	0.9	-0.1, 0.3	0.46			
Vitamin D supplement use (compared to never in pregnancy)						
Early pregnancy only	-7.4	-10.3, -4.5	< 0.001	-7.3	-10.1, -4.4	< 0.001
Late pregnancy only	11.9	6.8, 17.0	< 0.001	12.6	7.5, 17.6	< 0.001
Early and late pregnancy	6.5	3.5, 9.5	< 0.001	6.6	3.6, 9.7	< 0.001
Dietary vitamin D intake (per 100 IU/day)						
Early pregnancy	0.3	-1.2, 1.8	0.73			
Late pregnancy	1.3	-1.3, 2.8	0.08	1.1	-0.6, 2.8	0.21
ΔEarly Pregnancy to Late Pregnancy	1.2	-0.3, 2.6	0.12	0.9	-0.8, 2.6	0.29
Pre-pregnancy BMI (kg/m2)	0.1	-0.2, 0.3	0.52			
Weight gain early to late pregnancy (kg)	-0.4	-0.7, -0.1	0.003	-0.4	-0.7, -0.1	0.003
Smoking (yes vs no)						
Early pregnancy	-1.7	-4.9, 1.5	0.30			
Late pregnancy	-2.1	-5.3, 1.2	0.21			
Alcohol consumption (yes vs no)						
Early pregnancy	-2.0	-4.8, 0.8	0.15	-2.1	-4.9, 0.6	0.13
Late pregnancy	-0.1	-2.6, 2.7	0.96			

5.3.6 Maternal characteristics and vitamin D supplementation use

Given the importance of vitamin D supplementation use to the stability of maternal 25(OH)D status during pregnancy, maternal characteristics associated with vitamin D supplementation were explored as this information might enable targeted public health approaches. Women who either never started or discontinued vitamin D supplementation during pregnancy were younger, less well educated, more likely to smoke, less likely to be in their first pregnancy and had a higher pre-pregnancy BMI than women who continued supplementation throughout pregnancy (Table 5.3).

Table 5.3: Characteristics of mothers according to timing of vitamin D supplementation use in pregnancy

	Vitamin D supplementation use				
	Never	Early Pregnancy Only	Late Pregnancy Only	Early and Late Pregnancy	p across groups
N (%)	1018 (59.1)	327 (19.0)	84 (4.9)	293 (17.0)	
Early pregnancy season-corrected 25(OH)D (nmol/l), mean (SD)	-0.1 (22.3)	9.4 (21.2)	1.6 (20.1)	18.5 (23.0)	< 0.0001
Late pregnancy season-corrected 25(OH)D (nmol/l), mean (SD)	0.0 (21.8)	2.0 (22.2)	13.6 (27.8)	25.0 (28.7)	< 0.0001
Maternal age at delivery (years), mean (SD)	30.3 (3.8)	30.2 (3.6)	30.3 (3.4)	31.2 (3.5)	0.002
White Ethnicity (%)	96.7	97.3	100	96.6	0.37
Education to degree level or higher (%)	17.3	22.6	28.6	36.9	< 0.001
Smoked in early pregnancy (%)	18.0	11.3	3.6	4.8	< 0.001
Nulliparous (%)	40.6	50.5	61.9	70.0	< 0.001
Pre-pregnancy BMI (kg/m^2), median (IQR)	24.4 (22.2-27.8)	24.3 (22.0-27.2)	24.0 (22.2-27.8)	23.7 (21.2-25.2)	< 0.001

5.4 Summary of findings

In this large cohort of women in Southampton, UK, 25(OH)D status in both early and late pregnancy displayed seasonal variation. After correcting for this seasonal variation, maternal age, participation in moderate-strenuous exercise, dietary vitamin D intake, vitamin D supplement usage and consumption of alcohol were positively associated with 25(OH)D status, whereas pre-pregnancy BMI, smoking and non-White ethnicity were negatively associated with 25(OH)D.

There was moderate tracking of 25(OH)D status from early to late pregnancy after correcting for season. This suggests that an individual with a high 25(OH)D for the season in early pregnancy is likely to have a high 25(OH)D for the season in late pregnancy although absolute 25(OH)D level is likely to change due to the seasonal variation. Furthermore, change in 25(OH)D status of an individual relative to the population in pregnancy is associated with changes in the use of vitamin D supplementation, weight gain and participation in physical activity in late pregnancy. This suggests that women with high levels of weight gain in pregnancy might require higher supplementation doses to maintain their 25(OH)D status.

Finally, as the use of vitamin D supplementation was significantly associated with both 25(OH)D status and the tracking of 25(OH)D during pregnancy, maternal characteristics associated with supplementation use were explored. Women who did not take vitamin D supplements in pregnancy tended to be younger, less well educated, more likely to smoke and have a higher BMI than women who took vitamin D supplements throughout their pregnancy. These women might require additional advice and support with supplementation use.

Chapter 6: Maternal 25-hydroxyvitamin D status in late pregnancy and offspring muscle development

6.1 Background and aims

There are a large number of observational studies assessing the association between maternal 25(OH)D status and offspring bone development (227, 289, 290, 292), but despite of evidence to suggest that vitamin D supplementation can increase muscle strength in adults (211), there are few previous data on the associations between antenatal vitamin D status and offspring LM or muscle strength. The aim of this analysis was therefore to explore the associations between maternal vitamin D status in late pregnancy and offspring LM and grip strength at 4 years of age in an observational cohort study.

6.2 Methods

This analysis used observational data collected in the SWS, including maternal serum 25(OH)D concentration in late pregnancy and assessment of the offspring at 4 years of age. This included anthropometry, whole body DXA, grip strength by hand dynamometry and an objective assessment of physical activity. The methodology for this assessment is described in detail in section 3.1.3.3, page 95.

6.2.1 Body composition data from DXA

Measurements of FM and LM were obtained from whole body DXA. As children with greater adiposity also tend to have higher absolute LM (309), %FM and %LM were subsequently derived as a proportion of total body weight to provide an indication of a more favourable body composition. Furthermore, the variable LM adjusted for FM was generated to remove the effect of LM increasing with FM.

6.2.2 Statistical analysis

Mother-offspring pairs that had measurement of 25(OH)D in late pregnancy, in addition to offspring DXA and grip strength assessment at age 4 years, were included in the analysis. Differences in demographic characteristics and body composition of the children by sex were explored using t-tests and Mann-Whitney U tests for normally and non-normally distributed variables, respectively. Owing to differences between boys and girls, the body composition variables were adjusted for the sex of the child. In order to allow for subsequent comparison of effect sizes in univariate and multivariable linear regression models, the exposure (maternal 25(OH)D in late pregnancy) and outcomes (offspring body composition, grip strength and physical activity) were standardised using a Fisher-Yates transformation to a normally distributed variable with a mean of 0 and a standard deviation of 1. These analyses therefore yielded standardised regression coefficients (SD per SD). The first multivariable model (Model 1) included a number of child (sex, age, height, milk intake at 4 years, duration of breastfeeding) and maternal factors (parity, late pregnancy walking speed, late pregnancy smoking status, triceps SFT at 34 weeks of gestation, age at delivery and social class). It was additionally explored whether substitution of late pregnancy walking speed for participation in moderate-strenuous activity (hours per week) in late pregnancy and pre-pregnancy BMI for triceps SFT at 34 weeks of gestation altered the findings. In further analyses, offspring physical activity, measured as time in MVPA, was subsequently added to the model (Model 2).

6.3 Results

6.3.1 Maternal characteristics

678 mother-offspring pairs were included in this analysis. The characteristics of the mothers are presented in Table 6.1. The mothers included in this study were of similar age at delivery (30.7 years [SD 3.8] vs 30.6 years [SD 3.9], p=0.69) and parity (51.3% vs 51.0% nulliparous, p=0.88), but had achieved a higher educational level (25% vs 21% had a degree, $p < 0.001$) compared with mothers in the SWS cohort whose children did not participate in this study. Additionally fewer mothers included in this analysis smoked in late pregnancy (9.9% vs 16.9%, p=0.001).

Table 6.1: Characteristics of the mothers

Displayed as median (IQR) unless otherwise stated.

Maternal Characteristic	
N	678
Age at delivery (years), mean (SD)	30.7 (3.8)
Height (cm), mean (SD)	164.0 (6.5)
Pre-pregnancy BMI (kg/m^2)	24.2 (22.2-27.3)
Triceps skinfold thickness in late pregnancy (mm)	20.8 (16.9-25.7)
Smoking in late pregnancy, % (n)	9.9 (67)
Nulliparous, % (n)	51.3 (348)
Duration of breastfeeding, % (n)	
Never tried	13.6 (89)
< 1 month	21.0 (138)
1-3 months	19.7 (129)
4-6 months	20.3 (133)
7-11 months	15.6 (102)
12 or more months	9.9 (65)
Serum 25(OH)D at 34 weeks of gestation (nmol/l)	61 (43-88)
Dietary vitamin D intake (excluding supplements) at 34 weeks of gestation (IU/day)	136 (100-178)
% taking 400 IU/day vitamin D supplement in late pregnancy, % (n)	9.2 (62)

6.3.2 Characteristics of the children

6.3.2.1 Anthropometry and body composition

The boys and girls were of similar age, height and weight, but the girls had lower whole body and appendicular total and percent LM and higher FM (Table 6.2). Age at measurement was positively correlated with height in females, but not with any body composition outcomes in either boys or girls (Table 6.3). Thus, body composition variables were adjusted for sex, but not age.

Table 6.2: Characteristics of the children at 4 years of age

Displayed as mean (SD), unless otherwise stated.

	Boys	***Girls***	***p value***
N	345	333	
Age (years), median (IQR)	4.11 (4.08-4.16)	4.10 (4.07-4.15)	0.23
Height (cm)	104.6 (3.6)	104.1 (4.1)	0.10
Weight (kg)	17.5 (2.0)	17.4 (2.3)	0.28
Whole body lean mass (kg)	12.4 (1.4)	11.4 (1.4)	< 0.001
Whole body fat mass (kg), median (IQR)	4.3 (3.8-4.9)	5.1 (4.4-5.9)	< 0.001
Whole body percentage lean mass (%)	71.1 (3.9)	66.0 (4.5)	< 0.001
Whole body Percentage fat mass (%)	25.4 (4.0)	30.5 (4.7)	< 0.001
Appendicular lean mass (kg)	4.3 (0.6)	4.0 (0.7)	< 0.001
Appendicular fat mass (kg), median (IQR)	2.3 (1.9-2.6)	2.7 (2.3-3.2)	< 0.001
Appendicular percentage lean mass (%)	63.2 (5.7)	56.9 (6.1)	< 0.001
Appendicular percentage fat mass (%)	33.7 (5.8)	40.0 (6.3)	< 0.001

Table 6.3: Spearman's rank correlation coefficients between age at measurement and anthropometry and body composition in boys and girls

	Boys		***Girls***	
	r_s	p	r_s	p
Height	0.01	0.84	0.15	0.007
Weight	-0.01	0.90	0.04	0.44
Whole body lean mass	0.00	1.00	0.09	0.12
Whole body fat mass	0.01	0.92	-0.02	0.66
Whole body percentage lean mass	-0.03	0.54	0.06	0.27
Whole body percentage fat mass	0.03	0.59	-0.06	0.29
Appendicular lean mass	-0.03	0.58	0.08	0.12
Appendicular fat mass	0.02	0.76	-0.04	0.51
Appendicular percentage lean mass	-0.02	0.77	0.06	0.28
Appendicular percentage fat mass	0.02	0.76	-0.06	0.29

6.3.2.2 Grip strength

Mean maximum grip strength across the cohort was 8.4 kg (SD 1.7). However, this was greater in the boys (8.5 kg [SD 1.7]) than in the girls (8.2 kg [SD 1.7], p=0.023) and was positively associated with height (r=0.36, p < 0.0001, Figure 6.1) and weight (r=0.28, p < 0.0001), but not age (r_s=0.06, p=0.10).

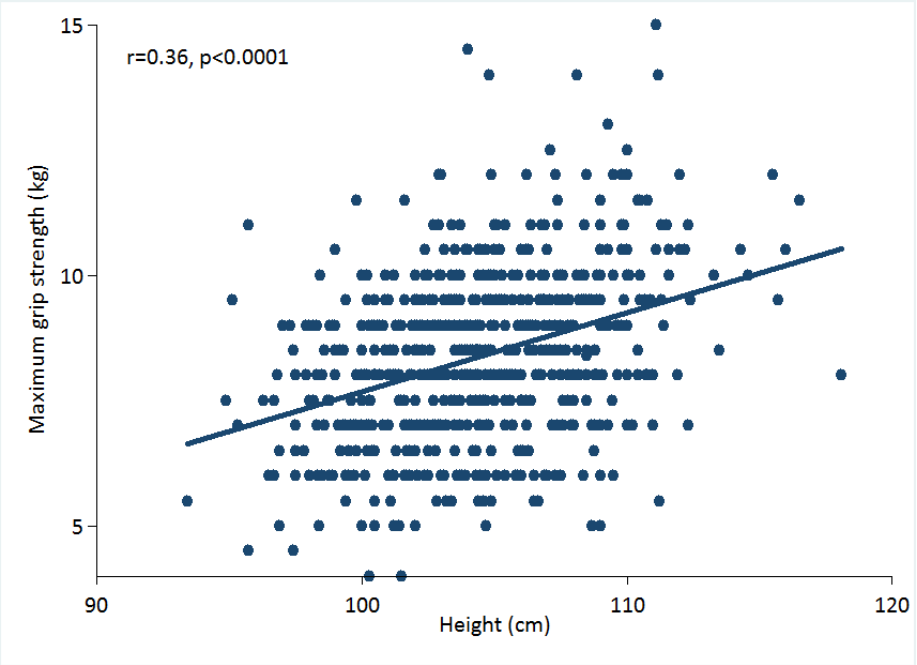


Figure 6.1: Relationship between height and maximum grip strength in children at age 4 years

After adjustment for the child's height using linear regression, the difference between boys and girls was attenuated and became statistically non-significant (8.5 kg [SD 1.5] vs 8.3 kg [SD 1.6], p=0.072), and the association with weight was no longer statistically significant (r=0.02, p=0.57).

Maximum grip strength and the average of the six grip strength measurements were highly positively correlated (r=0.94, p < 0.0001).

6.3.2.3 Physical activity

326 (48% male) of the children participated in the physical activity monitoring. These children had similar height, weight and body composition to the remaining 352 children ($p > 0.05$ for all).

The median number of minutes per day spent in moderate, vigorous or very vigorous activity was 64 minutes (IQR 45-84 minutes). This was similar for the boys and the girls ($p=0.31$), and was not associated with age ($r_s=-0.01$, $p=0.89$).

Girls with higher levels of physical activity had lower whole body and appendicular %FM and higher height-adjusted grip strength (Table 6.4). These associations between physical activity and body composition or grip strength were not observed in the boys (Table 6.4).

Table 6.4: Correlations between objectively measured physical activity and body composition and grip strength at 4 years of age

	Boys		***Girls***	
	r_s	p	r_s	p
n	156		170	
Height	0.06	0.47	0.02	0.82
Weight	0.02	0.84	-0.04	0.58
Whole body lean mass	0.04	0.59	0.07	0.38
Whole body fat mass	-0.04	0.63	-0.13	0.08
Whole body percentage lean mass	0.04	0.61	0.18	0.02
Whole body percentage fat mass	-0.04	0.64	-0.18	0.02
Appendicular lean mass	0.11	0.19	0.07	0.35
Appendicular fat mass	-0.02	0.85	-0.17	0.03
Appendicular percentage lean mass	0.06	0.46	0.21	0.007
Appendicular percentage fat mass	-0.06	0.46	-0.21	0.007
Maximum grip strength (adjusted for height)	0.14	0.08	0.18	0.02

6.3.3 Maternal 25(OH)D and offspring whole body lean mass

Maternal serum 25(OH)D concentration in late pregnancy was positively associated with offspring whole body %LM (β=0.11 SD/SD [95% CI 0.03, 0.18], p=0.006, Figure 6.2) but not total LM (β=0.06 SD/SD [95% CI -0.02, 0.13], p=0.15, Figure 6.3). There was a negative relationship with total FM of borderline significance (β=-0.07 SD/SD [95% CI -0.15, 0.00], p=0.064). As LM was positively associated with FM in these children (β=0.29 SD/SD, p < 0.001), the potential confounding effect of FM was addressed by using LM adjusted for FM. This was positively associated with maternal 25(OH)D (β=0.08 SD/SD [95% CI 0.01, 0.16], p=0.035).

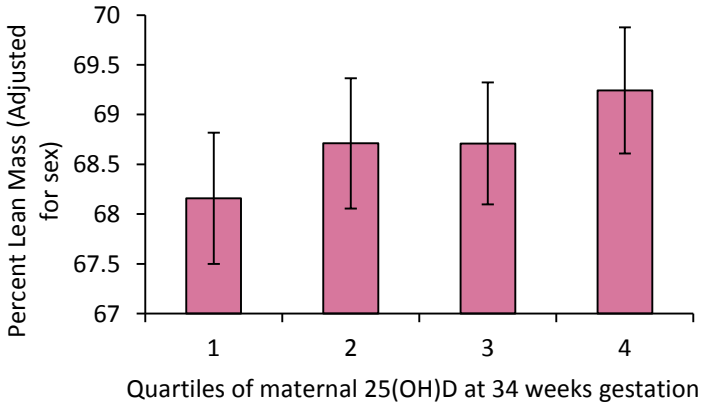


Figure 6.2: Maternal 25(OH)D in late pregnancy and offspring percent lean mass at age 4 years
Displayed as mean (95% confidence interval)

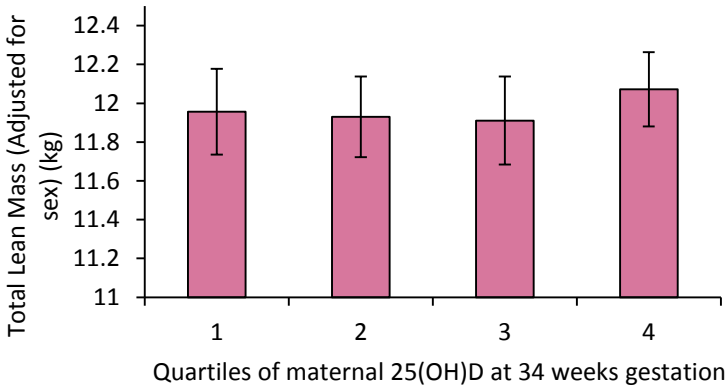


Figure 6.3: Maternal 25(OH)D in late pregnancy and offspring total lean mass at age 4 years
Displayed as mean (95% confidence interval)

The associations with %LM and LM adjusted for FM however were attenuated after the addition of potential confounding maternal and child factors (Model 1), and just failed to achieve statistical significance (β=0.07 SD/SD, p=0.062 and β=0.05 SD/SD, p=0.051, respectively). These relationships were further attenuated by the inclusion of physical activity in the model.

6.3.4 Maternal 25(OH)D and offspring appendicular lean mass

There was no significant association between maternal 25(OH)D and offspring appendicular LM (β=0.03 SD/SD [95% CI -0.04, 0.11], p=0.43) or appendicular FM (β=-0.05 SD/SD [95% CI -0.13, 0.02], p=0.16), but a positive association with appendicular percentage LM was identified (β=0.09 SD/SD [95% CI 0.02, 0.17], p=0.015). However, this relationship was attenuated in multivariate analysis (Model 1: β=0.07 SD/SD [95% CI -0.01, 0.15] p=0.09; Model 2: β=0.10 SD/SD [95% CI -0.01, 0.21], p=0.08).

6.3.5 Maternal 25(OH)D and offspring grip strength

A significant positive correlation was identified between maternal serum 25(OH)D concentration in late pregnancy and offspring height-adjusted grip strength at 4 years (β=0.10 SD/SD [95% CI 0.02, 0.17], p=0.013; Figure 6.4). This equated to an increase in offspring height-adjusted grip strength of 0.15 kg (95% CI 0.03, 0.27) for every standard deviation increase in maternal serum 25(OH)D. This association persisted in multivariate linear regression (Model 1: β=0.08 SD/SD [95% CI 0.00, 0.16], p=0.040).

In the 326 children who had physical activity monitoring, the child's mean daily time in MVPA was positively associated with height-adjusted hand grip strength (β=0.13 SD/SD, p=0.011). However, inclusion of time in MVPA in the model did not change the association between maternal 25(OH)D and offspring grip strength (Model 2: β=0.13 SD/SD [95% CI 0.03, 0.23], p=0.014).

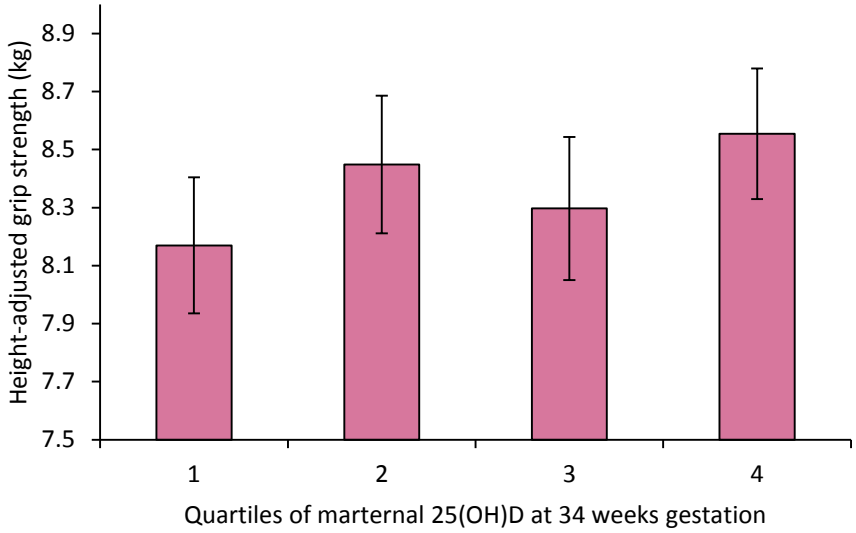


Figure 6.4: Offspring grip strength (adjusted for height) at age 4 years by quartiles of maternal 25(OH)D in late pregnancy.
Shown as mean (95% confidence interval)

In further sensitivity analysis, height-adjusted average grip strength instead of maximum grip strength was used. The relationship with maternal 25(OH)D in late pregnancy was almost identical (β=0.10 SD/SD, p=0.012; Model 1: β=0.08 SD/SD, p=0.042; Model 2: β=0.12 SD/SD, p=0.016).

6.3.6 Differences in boys and girls

The relationships between maternal 25(OH)D and offspring grip strength and whole body LM for the boys and girls separately are shown in Table 6.5. Although the associations between maternal 25(OH)D and offspring grip strength appeared somewhat more robust in the girls than the boys, the test for a statistical interaction between maternal serum 25(OH)D concentration and grip strength by sex did not achieve statistical significance (p=0.30).

Table 6.5: Associations between maternal 25(OH)D in pregnancy and offspring grip strength and lean mass at 4 years of age after addition of confounding factors

Presented as beta coefficients (95% confidence interval) for standardised variables (SD/SD). *p < 0.05, **p < 0.01.

	Unadjusted			***Model 1***			***Model 2***		
	All	**Boys**	**Girls**	**All**	**Boys**	**Girls**	**All**	**Boys**	**Girls**
n	678	345	333	636	316	320	309	145	164
Height-adjusted grip strength	0.10* (0.02, 0.17)	0.06 (-0.05, 0.17)	0.14* (0.03, 0.24)	0.08* (0.00, 0.16)	0.03 (-0.08, 0.15)	0.14* (0.03, 0.26)	0.13* (0.03, 0.23)	0.09 (-0.07, 0.24)	0.18* (0.04, 0.32)
% lean mass	0.11** (0.03, 0.18)	0.10* (0.00, 0.19)	0.12 (-0.00, 0.12)	0.07 (-0.00, 0.15)	0.06 (-0.05, 0.16)	0.09 (-0.04, 0.20)	0.09 (-0.02, 0.20)	0.09 (-0.06, 0.24)	0.09 (-0.08, 0.25)
Total lean mass	0.06 (-0.02, 0.13)	0.12* (0.02, 0.22)	-0.01 (-0.12, 0.10)	0.04 (-0.01, 0.08)	0.04 (-0.03, 0.11)	0.03 (-0.04, 0.09)	-0.00 (-0.07, 0.06)	0.01 (-0.09, 0.12)	-0.03 (-0.11,-0.06)
Lean mass adjusted for Fat Mass	0.08* (0.01, 0.16)	0.14** (0.03, 0.24)	0.02 (-0.09, 0.13)	0.05 (0.00, 0.11)	0.05 (-0.02, 0.13)	0.05 (-0.03, 0.12)	0.03 (-0.05, 0.10)	0.04 (-0.07, 0.15)	0.00 (-0.10, -0.11)

Model 1: child sex, age, height, current milk intake, duration of breastfeeding; maternal age and parity at delivery, maternal social class, smoking status, walking speed and triceps skinfold thickness in late pregnancy; Model 2: Model 1 + child's physical activity (minutes per day spent in moderate-vigorous physical activity) at 4 years of age.

6.3.7 Changing confounders in the multivariate models

Substitution of maternal pre-pregnancy BMI for late pregnancy triceps SFT and/or hours per week spent in moderate-strenuous physical activity in late pregnancy instead of walking speed did not appreciably alter the relationships.

6.4 Summary of findings

Maternal serum 25(OH)D concentration in late pregnancy was positively associated with offspring grip strength at 4 years. This relationship was robust to a number of confounding factors, including objectively measured physical activity. The association appeared to be stronger in girls than boys although the statistical test for an interaction between maternal 25(OH)D and offspring sex with grip strength as the outcome was not significant.

The relationships between maternal serum 25(OH)D and offspring whole body and appendicular LM measured by DXA and measures of relative LM to body size and adiposity were much weaker, and were non-significant after adjustment for covariates. This would suggest that in utero vitamin D exposure might influence muscle function independent of an effect on muscle size.

Chapter 7: Maternal antenatal vitamin D supplementation and infant anthropometry

7.1 Background and aims

Intervention studies in women in India (277), Bangladesh (279) and Asian women living in the UK (284) have shown antenatal vitamin D supplementation can increase offspring weight, CHL and/or OFC in the first year of life. To date, there are no studies assessing the effect of vitamin D supplementation in pregnancy on offspring growth in women of non-Asian ethnicity.

The aim of this study was therefore to assess the effect of supplementation with 1000 IU/day cholecalciferol during pregnancy in women in the UK on offspring anthropometry at birth, and 1 and 2 years of age.

7.2 Methods

Children born to mothers participating in the MAVIDOS randomised placebo controlled trial of 1000 IU/day cholecalciferol supplementation in pregnancy had anthropometric measurements obtained within 14 days of birth, and those born to mothers recruited in Southampton were measured again during a home visit at 1 and 2 years of age. Full study methodology is described in chapter 3.

Children who were preterm (< 37 weeks of gestation) were excluded from the analysis due to the potential effects of prematurity and its management on growth. Sensitivity analysis was additionally performed excluding children whom at each assessment were reported by the parent(s) to have been diagnosed with a chronic medical condition that requires on-going monitoring by a paediatrician.

7.2.1 Statistical Analysis

In order to compare data between children independent of sex and exact age, anthropometric data was converted to z-scores. Length/height, weight, OFC and BMI were converted using Stata v14.2 (Statacorp, College Station, Texas, USA)(310) to z-scores for sex and age using the UK-WHO growth standards. This reference database incorporates the British 1990 growth data at birth, the 2006 WHO multicentre growth standards from 2 weeks to 4 years of age and the British 1990 growth data from 4 to 20 years (311). Mid-parental height (MPH) was calculated as the average parental height in centimetres plus 7cm for boys or minus 7cm for girls. A MPH z-score was calculated using age 20 years on the sex-appropriate UK-WHO growth standards. The difference between the child's length z-score and the MPH z-score was calculated as an indication of achievement of growth potential.

MUAC, triceps and subscapular SFT measured at the 1 and 2 year assessments were converted to z-scores for sex and age using the WHO growth standards (312). Externally standardised data for MUAC, triceps and subscapular SFT in the neonatal period are not available. Therefore to account for sex differences and the effect of age on these measures, the data were adjusted for age and sex using linear regression.

Anthropometric data were compared between the groups using t-test and Mann-Whitney U test for normally and non-normally distributed data, respectively. Linear regression was used to adjust for maternal age, parity (categorised as nulliparous or multiparous), BMI at randomisation, maternal educational achievement (categorised as at or above degree level or below degree level), smoking status in late pregnancy (yes/no) and exercise in late pregnancy (reported hours per week of moderate-strenuous activity). Additionally at age 1 and 2 years, duration of breastfeeding (established by questionnaire at 1 year of age) was included.

The effect of maternal cholecalciferol supplementation on infant anthropometry was examined stratified by sex, maternal 25(OH)D at randomisation and by season of birth. Seasons were defined according to the UK Meteorological office meteorological seasons as winter (December-February); spring (March-May); summer (June-August); and autumn (September-November) (www.metoffice.gov.uk). Linear regression was used to examine for an interaction between randomisation group and the stratification variable.

Growth in length/height, weight and OFC between birth, 1 and 2 years of age was explored using conditional growth modelling. This approach takes in to account that rates of growth between

time points are likely to be correlated. Thus, mutually uncorrelated measures of growth between birth to 1 year and 1 to 2 years were generated using linear regression. The z-score at birth was used as the baseline measure, and the conditional change in z-score from birth to 1 year equated to the residual of the linear regression of z-score at 1 year on the z-score at birth. This therefore represents the amount by which the z-score at 1 year exceeds that which would have been predicted from the z-score at birth. The conditional growth from 1 to 2 years was generated as the residuals of the regression of z-score at 2 years on both the z-score at 1 year and at birth.

7.3 Results

A consort diagram for the follow-up of children born into the MAVIDOS study is shown in Figure 4.2, page 110. Birth weight was obtained from hospital records for all 965 infants born to mothers participating in the MAVIDOS study. 49 infants were preterm and therefore excluded from the analysis. Anthropometric measurements obtained within 14 days of birth were available for 758 term infants. At 1 year, 603 home visits were undertaken and 594 children had anthropometry (82.1% of term infants recruited in Southampton). At 2 years, there were 599 home visits and 577 children had anthropometric measurements taken (79.8% of eligible children). At each age of follow-up, maternal characteristics for those children that had at least one measurement taken remained similar between the two randomisation groups, except for maternal height. This was significantly greater in mothers randomised to placebo for children with measurements at 1 year of age, and a similar trend was observed at 2 years of age (Table 7.1)

7.3.1 Birth weight

There was no significant difference in birth weight or birth weight z-score in term infants born to mothers randomised to placebo or cholecalciferol, as displayed in Figure 7.1.

The incidence of SGA did not differ between the randomisation groups when defined as either birth weight < 10^{th} percentile for gestational age (placebo 5.2%, cholecalciferol 4.5%, p=0.94) or < 3^{rd} percentile for gestational age (placebo 1.3%, cholecalciferol 0.8%, p=0.78).

Table 7.1: Maternal characteristics by randomisation group for infants with anthropometry at birth, 1 year and 2 years of age

	Birth			***1 year***			***2 years***		
	Placebo	***Chole-calciferol***	***p***	***Placebo***	***Chole-calciferol***	***p***	***Placebo***	***Chole-calciferol***	***p***
n	385	373		296	298		290	287	
Age at delivery (years), mean (SD)	31.4 (5.2)	31.5 (5.0)	0.70	31.6 (5.1)	31.7 (4.7)	0.88	31.5 (5.2)	31.6 (5.0)	0.87
Smoking in late pregnancy (%)	6.6	6.4	0.91	5.6	3.8	0.32	8.5	5.0	0.11
Nulliparous (%)	45.3	42.8	0.50	42.3	42.0	0.93	42.9	42.9	0.99
White ethnicity (%)	95.8	94.6	0.50	97.0	95.5	0.34	97.7	95.4	0.15
Height (cm), mean (SD)	166.1 (6.7)	165.5 (6.2)	0.24	166.4 (6.5)	165.3 (6.3)	0.05	166.3 (6.4)	165.2 (6.3)	0.06
Early pregnancy BMI ($kg/m^{2)}$), median (IQR)	25.7 (23.0-29.7)	24.9 (22.4-28.9)	0.09	25.5 (23.0-29.6)	24.6 (22.3-28.2)	0.07	25.5 (23.0-29.6)	24.9 (22.3-28.3)	0.25
Education to degree level (%)	49.0	51.5	0.52	48.1	51.5	0.44	47.7	51.0	0.45
Exercise in late pregnancy (hrs/wk), median (IQR)	1.0 (0-2.5)	0.5 (0-3.0)	0.75	1.0 (0-3.0)	0.8 (0-3.0)	0.51	1.0 (0-2.5)	1.0 (0-3.0)	0.94
Early pregnancy 25(OH)D (nmol/l), mean (SD)	45.7 (16.3)	46.5 (16.8)	0.52	45.1 (15.9)	46.3 (16.2)	0.36	44.4 (15.7)	45.4 (16.4)	0.44
Late pregnancy 25(OH)D (nmol/l), mean (SD)	43.0 (22.1)	68.5 (21.0)	< 0.001	42.8 (20.9)	66.9 (19.6)	< 0.001	41.4 (20.5)	67.1 (19.7)	< 0.001

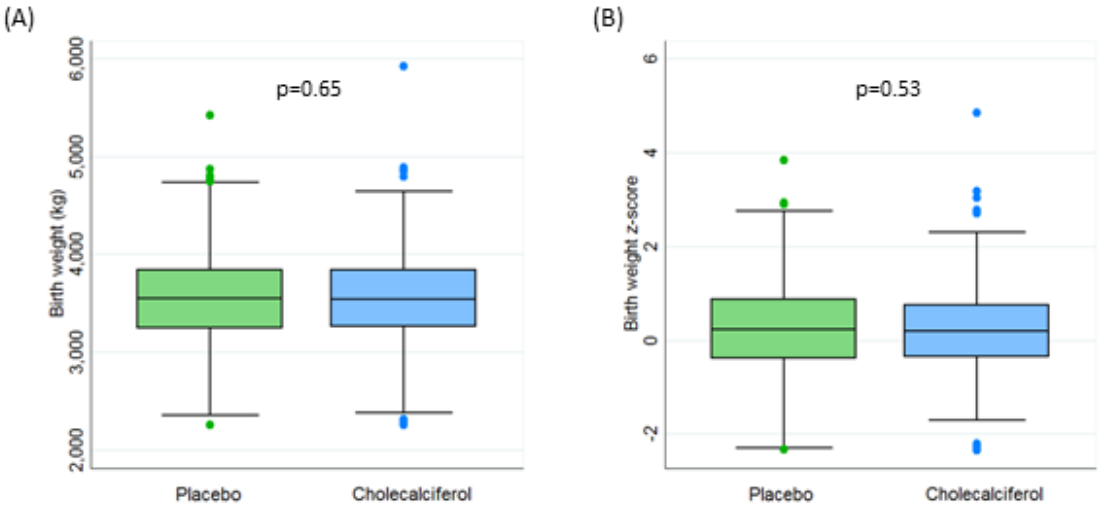


Figure 7.1: (A) Birth weight and (B) birth weight z-score in term infants born to mothers randomised to placebo or 1000 IU/day cholecalciferol during pregnancy

7.3.2 Birth length, occipito-frontal circumference and measures of adiposity

758 term infants had anthropometric measurements obtained at a median age of 2 days (IQR 2-9). The distribution of age at measurement is shown in Figure 7.2. BMI was not calculated in the neonatal period as length measurement was not obtained on the same day as birth weight for the majority of infants.

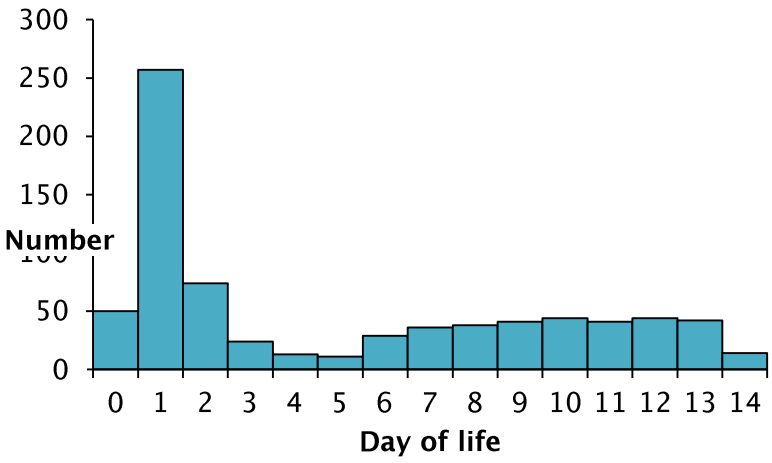


Figure 7.2: Age at anthropometric assessment in infants in the MAVIDOS study

Age at measurement was significantly correlated with CHL (r_s=0.29, p < 0.001), CRL (r_s=0.12, p=0.003), OFC (r_s=0.33, p < 0.001), MUAC (r_s=-0.14, p < 0.001), triceps SFT (r_s=0.20, p < 0.001) and subscapular SFT (r_s=0.28, p < 0.001). Boys had longer CHL (mean 51.2 cm [SD 1.9] vs 50.4 cm [SD 1.9], p < 0.001) and CRL (mean 34.3 cm [SD 1.6] vs 33.8 cm [SD 1.4], p < 0.001), larger OFC (mean 35.8 cm [SD 1.3] vs 35.1 cm [SD 1.2], p < 0.001) and greater MUAC (mean 11.5 cm [SD 0.9] vs 11.3 cm [SD 0.8], p=0.03), but similar triceps and subscapular SFT compared to the girls. As such, the measurements were internally adjusted for sex and age at measurement.

There were no significant differences between the randomisation groups in any anthropometric measurement (Table 7.2). This persisted after adjustment for maternal age, parity, physical activity, smoking status in late pregnancy, pre-pregnancy BMI and educational achievement (data not shown). When the analysis was limited to only infants with measurements obtained at age ≤ 10 days (n=618), ≤ 7 days (n=494) or ≤ 3 days (n=405), there were similarly no significant differences between the two groups.

Table 7.2: Anthropometry (adjusted for age and sex) in infants born to mothers randomised to placebo or 1000 IU/day cholecalciferol in pregnancy

Data shown as mean (SD), unless otherwise stated

	Placebo		***Cholecalciferol***		***p value***
	n	**Mean (SD)**	**n**	**Mean (SD)**	
Age (days), median (IQR)	385	2 (1-9)	375	3 (1-9)	0.77
Male (%)	385	52.7	375	54.9	0.54
Crown-heel length (cm)	347	50.8 (1.8)	317	50.8 (1.8)	0.68
z-score	347	0.11 (0.93)	317	0.10 (0.89)	0.83
Crown-rump length (cm)	338	34.1 (1.5)	310	34.1(1.5)	0.96
OFC (cm)	361	35.5 (1.2)	356	35.4 (1.1)	0.53
z-score	361	0.75 (1.07)	356	0.71 (0.98)	0.57
MUAC (cm)	358	11.4 (0.9)	353	11.4 (0.9)	0.65
Triceps SFT (mm)	335	5.0 (1.1)	338	5.0 (1.1)	0.62
Subscapular SFT (mm)	332	5.6 (1.2)	334	5.6 (1.2)	0.96

7.3.2.1 Adjustment for gestational age

Gestation at birth did not differ between the two groups for term infants (p=0.30). Even in the term infants, there were positive associations between gestational age at birth and weight z-score (r_s=0.40, p < 0.001), length z-score (r_s=0.44, p < 0.001) and OFC z-score (r_s=0.41, p < 0.001) and MUAC adjusted for sex and age at measurement (r_s=0.32, p < 0.001). The correlations between gestation at birth and SFT measurement adjusted for sex and age at measurement were less strong but remained statistically significant (triceps SFT r_s=0.11, p=0.005; subscapular SFT r_s=0.12, p=0.001). However, there were no significant differences in any of the anthropometric outcomes between the randomisation groups after adjustment for gestational age.

7.3.3 Anthropometry at 1 and 2 years of age

594 and 577 children had measurements at 1 and 2 years, respectively. At both 1 and 2 years of age, weight, length/height and OFC were positively correlated with age, but measures of adiposity were not significantly correlated with age, except triceps SFT at 1 year (Table 7.3). Differences in anthropometry by sex were also observed: boys were taller and heavier at both 1 and 2 years of age, but girls had larger triceps and subscapular SFTs at 2 years (Table 7.4). To account for these differences, measurements were converted to z-scores for age and sex using the UK-WHO growth standards.

There were no significant differences between the randomisation groups in z-scores for any of the anthropometric measures at either 1 year (Table 7.5) or 2 years of age (Table 7.6). Adjustment for maternal age, parity, pre-pregnancy BMI, educational achievement, smoking status in late pregnancy, exercise in late pregnancy and duration of breastfeeding did not alter the findings.

Mid-parental height z-score could be calculated for 419 (86.2%) children with a length measurement at 1 year of age, and 450 (86.9%) children with a height measurement at 2 years of age. MPH z-score was greater for children born to mothers randomised to placebo than cholecalciferol at 1 year of age (mean difference 0.18 [95% CI 0.02, 0.34], p=0.02), but not significantly different for children with a height measurement at age 2 years (mean difference 0.11 [95% CI -0.05, 0.26], p=0.18). Thus, children in the placebo group would be expected to be taller at age 1 year. The difference between the child's length z-score and MPH z-score did not differ between the two randomisation groups at either 1 year (p=0.72) or 2 years (p=0.28) of age.

Table 7.3: Correlations between age at measurement and anthropometry in children at 1 and 2 year assessments

	1 year visit			*2 year visit*		
	n	**r_s**	**p**	**n**	**r_s**	**p**
Weight	574	0.16	< 0.001	567	0.11	0.008
Length/Height	486	0.27	< 0.001	518	0.21	< 0.001
BMI	481	-0.53	0.26	513	-0.05	0.30
OFC	581	0.13	0.002	532	0.09	0.05
MUAC	583	0.01	0.87	512	0.03	0.50
Triceps SFT	469	-0.09	0.04	418	0.02	0.67
Subscapular SFT	492	-0.07	0.11	406	-0.0	0.73

Table 7.4: Differences in anthropometry in boys and girls at the 1 and 2 year assessments

	1 year visit			*2 year visit*		
	Boys	**Girls**	**p**	**Boys**	**Girls**	**p**
Weight (kg)	10.4 (1.1)	9.8 (1.1)	< 0.001	13.0 (1.5)	12.4 (1.5)	< 0.001
Length/Height (cm)	77.0 (2.7)	75.5 (3.1)	< 0.001	87.2 (3.1)	86.2 (3.4)	< 0.001
BMI (kg/m^2)	17.6 (1.4)	17.0 (1.3)	< 0.001	17.0 (1.3)	16.7 (1.2)	0.008
OFC (cm)	47.4 (1.4)	46.2 (1.3)	< 0.001	49.6 (1.4)	48.5 (1.3)	< 0.001
MUAC (cm)	15.9 (1.2)	15.6 (1.2)	< 0.001	16.3 (1.1)	16.2 (1.1)	0.22
Triceps SFT (mm)	10.7 (2.5)	10.9 (2.3)	0.36	9.8 (2.1)	10.4 (2.3)	0.009
Subscapular SFT (mm)	7.3 (1.8)	7.3 (1.8)	0.95	6.6 (1.6)	7.0 (1.9)	0.03

Table 7.5: Anthropometry at age 1 year in children born to mothers randomised to placebo or 1000 IU/day cholecalciferol in pregnancy

Data presented as mean (SD), unless otherwise stated

	Placebo		***Cholecalciferol***		***p value***
	n		**n**		
Age (years), median (IQR)	296	1.07 (1.03-1.11)	298	1.06 (1.03-1.11)	0.33
Male (%)	296	51.4	298	57.4	0.14
Weight (kg)	288	10.1 (1.1)	286	10.1 (1.2)	0.78
z-score	288	0.47 (0.90)	286	0.46 (0.94)	0.90
Crown-heel length (cm)	241	76.5 (3.0)	245	76.3 (3.0)	0.40
z-score	241	0.18 (1.08)	245	0.05 (1.10)	0.20
BMI (kg/m^2)	239	17.3 (1.4)	242	17.4 (1.4)	0.57
z-score	239	0.42 (0.93)	242	0.57 (0.94)	0.49
OFC (cm)	289	46.8 (1.4)	292	46.9 (1.5)	0.39
z-score	289	0.79 (0.99)	292	0.84 (1.02)	0.57
MUAC (cm)	291	15.8 (1.2)	292	15.8 (1.2)	0.45
z-score	291	1.03 (0.93)	292	1.08 (0.93)	0.54
Triceps SFT (mm)	238	10.8 (2.3)	231	10.8 (2.5)	0.92
z-score	238	1.37 (1.06)	231	1.35 (1.11)	0.87
Subscapular SFT (mm)	247	7.3 (1.7)	245	7.3 (1.9)	0.92
z-score	247	0.47 (1.18)	245	0.41 (1.31)	0.63

Table 7.6: Anthropometry at age 2 years in children born to mothers randomised to placebo or 1000 IU/day cholecalciferol in pregnancy
Data presented as mean (SD), unless otherwise stated

	Placebo		***Cholecalciferol***		***p value***
	n		n		
Age (years), median (IQR)	290	2.04 (2.01-2.08)	287	2.03 (2.01-2.08)	0.25
Male (%)	290	51.7	287	57.2	0.19
Weight (kg)	283	12.7 (1.5)	284	12.7 (1.5)	0.87
z-score	283	0.42 (0.90)	284	0.43 (0.97)	0.94
Height (cm)	261	86.8 (3.3)	257	86.8 (3.2)	0.96
z-score	261	-0.13 (0.97)	257	-0.13 (1.00)	1.00
BMI (kg/m^2)	258	16.8 (1.2)	255	16.9 (1.3)	0.44
z-score	258	0.70 (0.87)	255	0.74 (0.95)	0.61
OFC (cm)	258	49.1 (1.5)	274	49.2 (1.5)	0.40
z-score	258	0.89 (0.99)	274	0.95 (0.97)	0.52
MUAC (cm)	251	16.3 (1.1)	261	16.3 (1.1)	0.90
z-score	251	0.92 (0.84)	261	0.93 (0.88)	0.92
Triceps SFT (mm)	199	10.3 (2.2)	229	9.9 (2.2)	0.12
z-score	199	1.18 (1.02)	219	1.04 (1.06)	0.15
Subscapular SFT (mm)	191	6.8 (1.8)	215	6.9 (1.7)	0.57
z-score	191	0.35 (1.20)	215	0.45 (1.20)	0.42

Similarly, after adjustment using linear regression to adjust the effect of cholecalciferol supplementation on length z-score for MPH z-score, there remained no significant effect of cholecalciferol supplementation on length z-score at 1 year of age (β=-0.05 (95% CI -0.24, 0.15), p=0.63) or height z-score at 2 years of age (β=0.05 (95% CI -0.12, 0.21), p=0.56). Furthermore, the number of children with a length (height at 2 years) z-score greater than 2 standard deviations below that of their MPH z-score was similar between the randomisation groups at 1 year (placebo 3.9%, cholecalciferol 4.2%, p=0.86) and 2 years of age (placebo 5.6%, cholecalciferol 4.4%, p=0.52).

7.3.4 Stratification by offspring sex

As previous analyses assessing the relationship between maternal 25(OH)D and offspring lean mass and grip strength showed differences by sex of the offspring (Chapter 6), the effect of antenatal vitamin D supplementation on offspring anthropometry was examined stratified by sex. There were no significant differences in any anthropometric measurement by randomisation group at birth, 1 year or 2 year when each sex was analysed separately.

7.3.5 Stratification by maternal baseline serum 25(OH)D status

Published literature, and in particular meta-analyses, relating 25(OH)D status to a number of outcomes, including BMD in childhood (198) and offspring birth size (260, 275), have suggested that supplementation with vitamin D might only be of benefit to those with the lowest levels of serum 25(OH)D. The effects of gestational cholecalciferol supplementation on infant anthropometry were therefore explored stratified by maternal baseline 25(OH)D. Firstly, the median 25(OH)D (45.3 nmol/l) for all participants who remained in the study until delivery was used to divide the participants into two groups. There was no significant difference in any anthropometric measurement by randomisation group for infants born to mothers with a baseline 25(OH)D either above or below this level ($p > 0.05$ for all).

Secondly, a threshold of < 30 nmol/l was used to define a group more representative of VDD. This is the definition for VDD suggested by the Institute of Medicine (160). There was no differences in birth anthropometry by randomisation group in women with baseline 25(OH)D < 30 nmol/l, but in this subset, offspring height z-score at 1 year of age was significantly lower in those randomised to cholecalciferol (mean difference -0.48 [95% CI -0.91, -0.05] p=0.03) but not at 2 years of age (mean difference =0.26 [95% CI -0.65, 0.13], p=0.19). However MPH z-score was non-significantly greater for the children randomised to placebo in this subset, and after adjustment for MPH z-score, the significant difference between the two groups at 1 year of age was no longer present (β=-0.35 [95% CI -0.78, 0.08], p=0.11). Statistically significant differences were not found in the other anthropometric measurements between groups in either subset of women when stratified by baseline 25(OH)D < 30 nmol/l.

7.3.6 Stratification by season of birth

In the analyses of the primary outcome of MAVIDOS (neonatal bone mass), a statistically significant interaction between randomisation group and season of birth was identified, such that infants born in winter months (December-February) and randomised to cholecalciferol had significantly greater BMC and BMD than those randomised to placebo (Figure 1.21, page 82). This difference was not observed in the other seasons of birth (295). As such, the effect of cholecalciferol supplementation on infant anthropometry was also examined stratified by season.

Birth weight z-score, OFC, MUAC and SFTs (adjusted for age and sex) in the neonatal period were significantly greater in infants born in winter months to mothers randomised to cholecalciferol compared to infants born in winter to mothers randomised to placebo (Figure 7.3, Figure 7.4). In contrast, for infants born in autumn, MUAC (adjusted for age and sex) was significantly smaller in the cholecalciferol supplemented group (p=0.02, Figure 7.4). There were no significant differences between randomisation groups in any measurement for infants born in spring or summer.

When stratified by season, maternal characteristics for those with infant anthropometry within 14 days of birth were similar between the randomisation groups (Table 7.7). After adjustment for maternal covariates, infants born in winter to mothers randomised to cholecalciferol were 190.0 g (95% CI 62.1, 318.0) heavier (equating to a z-score difference of 0.36 [95% CI 0.10, 0.62]), had 0.5 cm (95% CI 0.1, 0.8) larger OFC and 0.5 cm (95% CI 0.2, 0.7) greater MUAC. The interaction for season of birth*randomisation remained statistically significant for birth weight z-score ($p_{interaction}$=0.02), OFC ($p_{interaction}$ =0.03) and MUAC ($p_{interaction}$ =0.007) after adjustment for maternal covariates.

Maternal serum 25(OH)D concentration in late pregnancy was significantly higher in the women randomised to cholecalciferol than placebo for each season of offspring birth ($p < 0.0001$), but the difference between the two randomisation groups was greatest for women delivering in winter months (Table 7.8).

When winter season was changed to November-January or January-March, the previously observed significant differences in neonatal anthropometry between randomisation groups were no longer present.

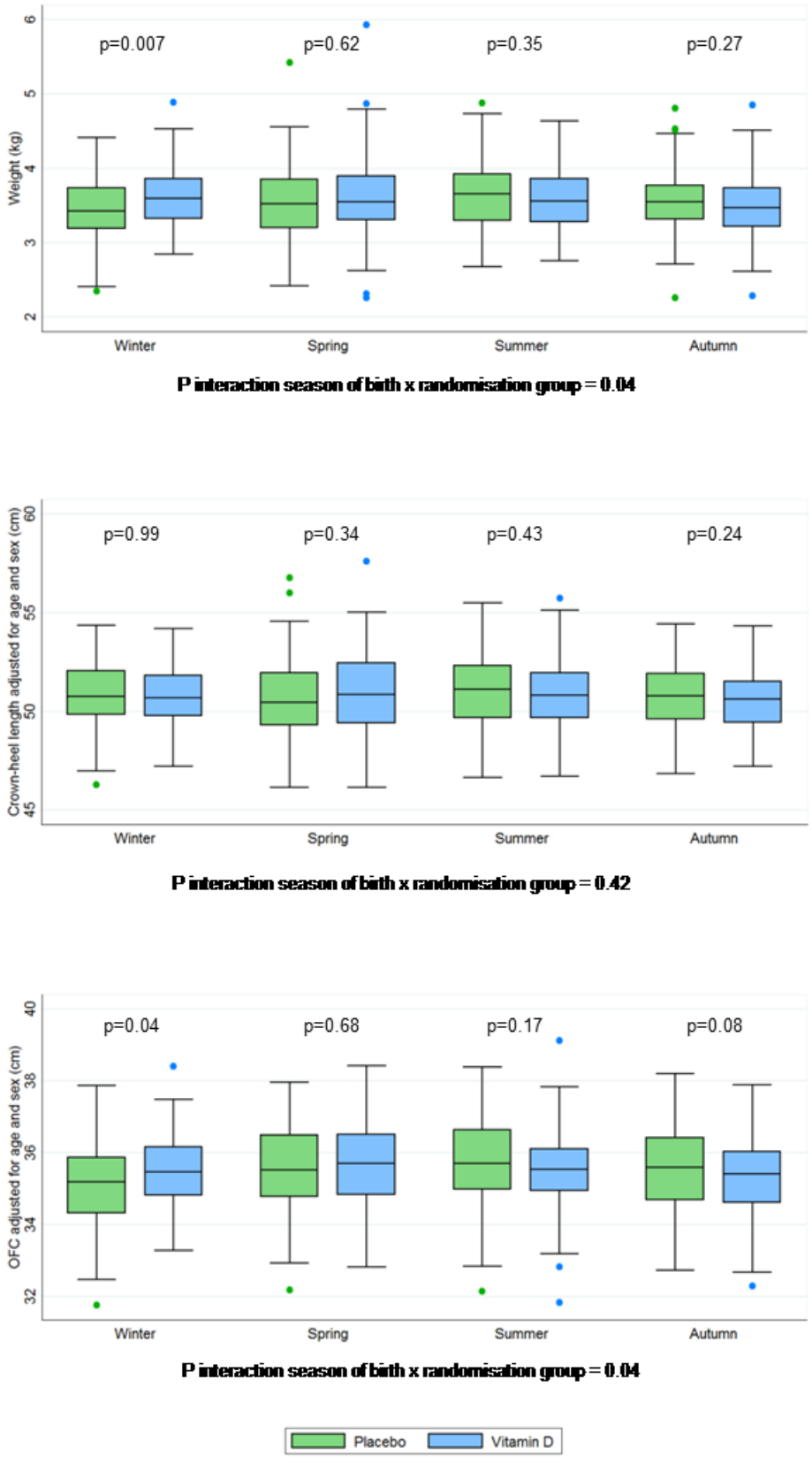


Figure 7.3: Neonatal anthropometry stratified by season of birth and maternal randomisation to placebo or 1000 IU/day cholecalciferol during pregnancy

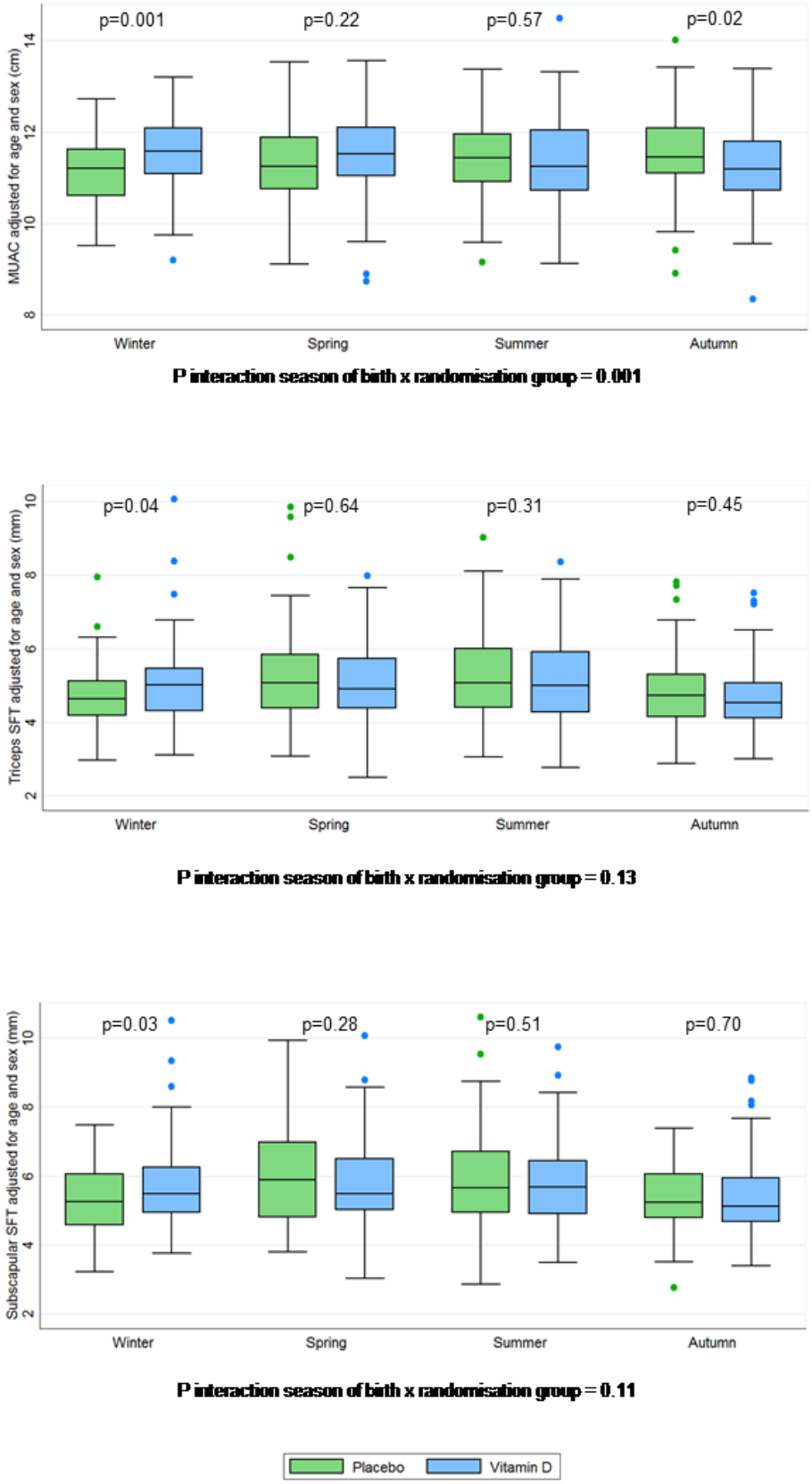


Figure 7.4: Neonatal MUAC, triceps and subscapular skinfold thicknesses stratified by season of birth and maternal randomisation to placebo or 1000 IU/day cholecalciferol during pregnancy

Table 7.7: Maternal characteristics stratified by season of offspring birth and randomisation group

Only mothers of infants who had anthropometry within 14 days of birth are included. *p < 0.05 ǂ p < 0.01 compared with mothers randomised to placebo who delivered in autumn months

Season of offspring birth	***Winter (December-February)***		***Spring (March-May)***		***Summer (June-August)***		***Autumn (September-November)***	
	Placebo	**Cholecalciferol**	**Placebo**	**Cholecalciferol**	**Placebo**	**Cholecalciferol**	**Placebo**	**Cholecalciferol**
n	75	77	105	93	104	97	101	108
Age at delivery (years), mean (SD)	31.4 (5.1)	31.6 (5.3)	31.3 (5.3)	30.9 (5.4)	30.9 (5.0)	31.9 (4.4)	32.1 (5.3)	31.7 (4.9)
Smoking in late pregnancy (%)	7.9	4.6	9.7	10.0	5.3	3.5	4.1	7.1
Nulliparous (%)	46.0	46.7	48.5	46.3	40.6	38.9	46.4	40.3
White ethnicity (%)	93.2	94.7	94.9	95.1	94.9	95.6	100.0	93.4*
Height (cm), mean (SD)	165.9 (7.2)	165.0 (6.2)	166.2 (7.1)	166.3 (7.0)	166.3 (6.5)	165.1 (5.3)	165.9 (6.1)	165.5 (6.1)
Early pregnancy BMI ($kg/m^{2)}$), median (IQR)	24.7 (22.7-29.3)	25.3 (22.6-30.4)	25.7 (22.5-29.5)	24.8 (22.1-28.9)	25.8 (22.9-29.2)	24.9 (22.7-28.3)	26.5 (23.2-30.4)	24.6 (22.0-27.8)ǂ
Education to degree level (%)	50.0	54.7	48.5	42.7	49.5	56.7	48.2	52.7
Exercise in late pregnancy (hrs/wk), median (IQR)	0.5 (0.0-3.7)	1 (0.0-3.0)	1 (0.0-3.0)	0.5 (0.0-3.0)	0.9 (0.0-2.0)	0.5 (0.0-3.0)	1 (0.3-2.0)	0.6 (0.0-2.0)

Table 7.8: Maternal serum 25(OH)D concentration at 34 weeks of gestation stratified by randomisation group and season of delivery

Season of delivery	***Placebo*** Mean (SD)	***Cholecalciferol*** Mean (SD)	***Mean difference between placebo and cholecalciferol*** Mean (95% CI)	***p value***
Winter	29.6 (15.2)	64.0 (18.7)	34.4 (28.7, 40.0)	< 0.001
Spring	31.1 (19.7)	65.1 (24.0)	33.9 (27.2, 40.6)	< 0.001
Summer	49.1 (18.1)	71.3 (20.0)	22.2 (16.6, 27.7)	< 0.001
Autumn	57.6 (20.6)	72.2 (20.3)	14.6 (8.8, 20.4)	< 0.001

The differences in anthropometry by randomisation group in babies born in winter were no longer present at 1 or 2 years of age ($p > 0.05$ for all). At 1 year of age BMI z-score was significantly greater in infants born in winter to mothers randomised to cholecalciferol (mean 0.74 [SD 0.85]) compared to placebo (mean 0.37 [SD 0.72], p=0.03), but differences were not observed in the other seasons. However the interaction between randomisation group and season of birth was not statistically significant ($p_{interaction}$ =0.33). This difference in BMI z-score between randomisation groups in winter born infants did not persist at 2 years of age (p=0.88).

Height z-score at 2 years of age was significantly greater in infants born in spring to mothers randomised to cholecalciferol (Figure 7.5), and the interaction between randomisation group and season of birth was also statistically significant (p=0.02). This difference in height z-score remained significant in spring-born infants after adjustment for MPH z-score, but with inclusion of maternal age at delivery, parity, educational achievement, pre-pregnancy BMI, late pregnancy smoking status, exercise in late pregnancy and duration of breastfeeding, the interaction was no longer statistically significant (p=0.16).

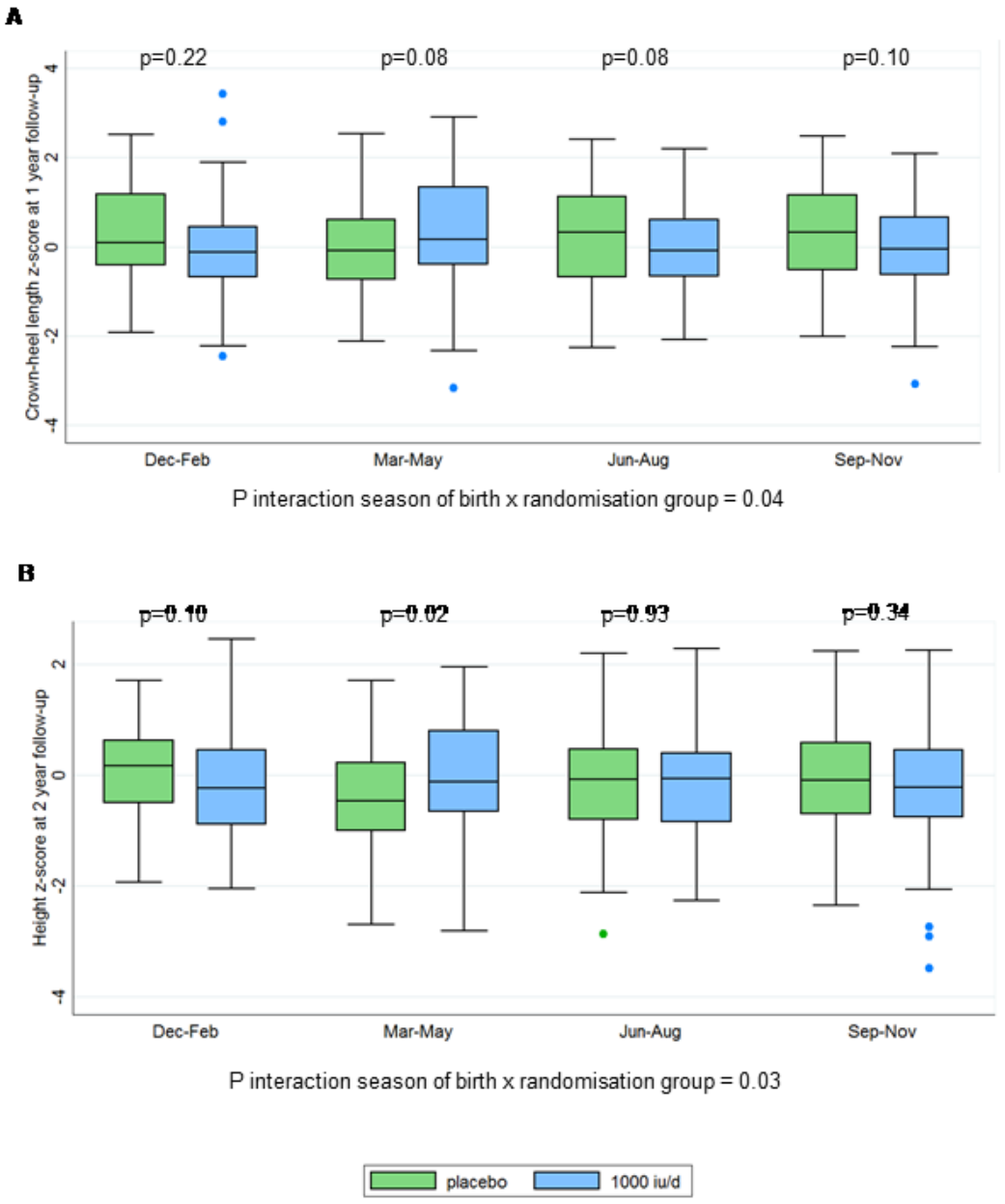


Figure 7.5: (A) Length z-score at 1 year of age and (B) height z-score at 2 years of age by season of birth and maternal randomisation to placebo or 1000 IU/day cholecalciferol in pregnancy

7.3.7 Growth between birth, 1 and 2 years

490 and 303 children had measurements of weight and height, respectively, at all three time points. Conditional change in z-score for weight or height between birth and 1 year and 1 and 2 years of age did not differ between randomisation groups ($p > 0.05$ for all). No statistically significant differences emerged when the analyses were repeated stratified by sex, maternal baseline 25(OH)D status or season of birth.

7.3.8 Exclusion of children requiring long-term paediatric follow-up

Exclusion of 6 children (4 placebo) with chronic medical conditions at age 1 year (α1-antitrypsin deficiency [n=1], congenital cardiac defect [n=2], structural abnormality of the renal tract [n=3]), and 11 children (6 placebo) with chronic medical conditions at 2 years of age (α1-antitrypsin deficiency [n=1], congenital cardiac defect [n=2], structural abnormality of the renal tract [n=3], nephrotic syndrome [n=1], multiple food allergies [n=2], severe eczema [n=1], persistent vomiting following surgical correction of malrotation [n=1]) did not alter the findings.

7.4 Summary of findings

Supplementation with 1000 IU/day cholecalciferol from the end of the first trimester until delivery did not affect offspring size at birth, 1 or 2 years of age in a group of pregnant women in the UK with baseline 25(OH)D from 25-100 nmol/l. Similarly linear growth or rate of weight gain between these ages did not differ between the randomisation groups. At birth, there was evidence of an interaction between season of birth and randomisation group; infants born in winter months to mothers randomised to cholecalciferol had higher birth weight, OFC and MUAC than those born to mothers randomised to placebo. This could reflect a greater difference in 25(OH)D achieved in late pregnancy between the randomisation groups in winter births compared to the other seasons, but these differences were not observed at 1 or 2 years of age.

A number of other subgroup analyses found effects of cholecalciferol in univariate analysis, including greater height at 2 years of age in spring born infants and higher BMI at 1 year but not 2 years of age in winter born infants. However, these findings were not robust to adjustment for maternal covariates.

Chapter 8: Maternal antenatal vitamin D supplementation and offspring body composition and muscle strength at 4 years

8.1 Background

Associations between maternal serum 25(OH)D status in pregnancy and measures of adiposity in the offspring have been reported using data from observational studies (167, 265, 300) although the findings are not consistent across all published work (271, 273, 290, 299). Using data from the SWS, a positive association between maternal 25(OH)D in late pregnancy and offspring grip strength but not LM in early childhood was found (Chapter 6). However, there are few intervention studies that have been used to examine the effect of vitamin D supplementation on offspring soft-tissue body composition. Both previously reported studies measured adiposity using SFT rather than a more detailed assessment technique (251, 284) and only assessed the offspring at birth. There are no RCTs which have determined the effect of antenatal vitamin D supplementation on body composition in later childhood. Similarly muscle strength has not been considered as an outcome in any intervention study of gestational vitamin D supplementation. The aim of this study was therefore to determine whether 1000 IU/day cholecalciferol in pregnancy alters offspring body composition and increases grip strength at 4 years of age.

8.2 Methods

The methodology for the MAVIDOS study has been described in detail in Chapter 3.

Follow-up at 4 years of age commenced in March 2013 and is ongoing as the last birth to a mother participating in the MAVIDOS study was in September 2014. For the purpose of this thesis a cut-off date for visit attendance by 31st October 2016 was chosen. This date was selected to allow sufficient time for data entry, extraction of DXA data from the instrument, building of the data set, data cleaning and statistical analysis.

In this analysis, WBLH scans were used to maximise the number of scans included as head movement was common. Appendicular FM, LM and BMC were calculated by summing the data

for each of the limb ROIs, as described previously (section 3.1.3.3.2, page 96). Additionally trunk FM was included as an outcome variable. This was calculated from the sum of all ROIs except the arms, legs and head (Figure 3.2, page 97). The percentage of subtotal FM located within the trunk region and the trunk:appendicular FM ratio were also calculated as these measures have been associated with visceral adiposity assessed by magnetic resonance imaging (313). Trunk LM was not included as LM in this region predominantly represents viscera.

8.2.1 Statistical analysis

Comparisons were made between randomisation groups using t-test, Mann-Whitney U test and χ^2 test for normally distributed, non-normally distributed and categorical variables, respectively.

Height, weight, BMI, OFC, MUAC and SFT measurements were converted to z-scores for age and sex using external reference data. The UK-WHO growth standards uses the WHO multicentre growth standards from 2 weeks until 4 years of age and then the British 1990 reference data from 4 years for height, weight, BMI and OFC. A step is apparent in the UK-WHO growth chart at 4 years, which reflects the change in reference data. Some children participated in the study just before their fourth birthday and therefore to maintain consistency with the reference data used, the British 1990 growth data was used for all children for these outcomes. Z-scores for MUAC and SFTs were calculated using the WHO multicentre growth standards. All z-score were generated in Stata v14 using the *zanthro* programme (310). MPH and MPH z-scores were calculated where both parental heights were available using the methods previously described in section 7.2.1 (page 136). The difference in height z-score and MPH z-score was used as a marker of achievement of growth potential.

Owing to differences in body composition between boys and girls, and correlations between body composition variables and age in the girls, body composition outcomes were adjusted for age and sex using linear regression. Grip strength was adjusted for both height and sex.

Differences in outcomes between the groups were also examined following adjustment for maternal covariates including maternal age at delivery, parity (categorised as nulliparous or multiparous), BMI at randomisation, maternal educational achievement (categorised as at or above degree level or below degree level), smoking status in late pregnancy (yes/no), exercise in late pregnancy (reported hours per week of moderate-strenuous activity) and duration of breast feeding (established by questionnaire at 1 year of age). A Fisher-Yates transformation was used to transform non-normally distributed variables to a normally distributed variable with a mean of

0 and SD of 1. Linear regression was then used to examine the association between the transformed variable and randomisation group including the maternal covariates.

The effect of antenatal cholecalciferol supplementation on the outcomes was also assessed stratified by offspring sex, maternal serum 25(OH)D at randomisation and season of birth. Seasons were defined as described previously: winter (December-February); spring (March-May); summer (June-August); and autumn (September-November). Linear regression was used to examine for an interaction between randomisation group and stratification variable.

8.3 Results

Of the 723 babies born at term to mothers in the MAVIDOS study and recruited in Southampton, 380 had attended the 4 year follow-up visit by 31st October 2016 (52.6% of eligible children) (see Figure 4.2, page 110 for consort diagram). Maternal age at delivery, ethnicity, late pregnancy smoking status, parity, height, early pregnancy BMI, educational achievement and 25(OH)D at randomisation did not differ between the mothers who had attended this visit and the other women who remained in the study until delivery ($p > 0.05$ for all). Moreover, these characteristics were also similar between the two randomisation groups for those that had attended this visit ($p > 0.05$ for all).

8.3.1 Anthropometry

Overall, more boys (52.9%) attended the 4 year visit than did girls, but the distribution of sexes between the randomisation groups was similar (Table 8.1). Age was also similar between the two groups. There were no statistically significant differences in height, weight, BMI, OFC, MUAC, triceps SFT or subscapular SFT z-scores between the two treatment groups (Table 8.1).

8.3.2 Body composition assessed by DXA

334 children (87.9% of attendees) agreed to a DXA scan. 34 (10.2%) of the WBLH scans were excluded due to movement artefact and 47 (14.1%) had movement in a limb but no movement in the contralateral limb. In the 253 scans with no movement artefact, there were small, but significant differences in FM, LM and BMC between the two arms or between the two legs, as

Table 8.1: Offspring anthropometry at 4 years of age by maternal randomisation to placebo or 1000 IU/day cholecalciferol during pregnancy
Shown as mean (SD), unless otherwise stated

	Placebo		***Cholecalciferol***		***p value***
	n	**Mean (SD)**	**n**	**Mean (SD)**	
Age (years), median (IQR)	192	4.05 (4.02-4.16)	188	4.06 (4.02-4.11)	0.94
Male (%)	192	49.0	188	56.9	0.12
Weight z-score	174	0.20 (0.91)	173	0.25 (0.98)	0.66
Height z-score	189	0.59 (1.09)	184	0.66 (1.03)	0.48
Height z-score-MPH z-score	164	0.29 (0.94)	168	0.45 (0.97)	0.13
BMI z-score	171	-0.21 (0.99)	172	-0.21 (0.94)	0.96
OFC z-score	186	-0.67 (1.16)	179	-0.59 (1.09)	0.53
MUAC z-score	186	0.76 (0.77)	181	0.79 (0.80)	0.85
Triceps SFT z-score	170	0.79 (0.83)	160	0.73 (0.93)	0.53
Subscapular SFT z-score	162	0.30 (1.02)	158	0.15 (1.06)	0.21

shown in Table 8.2. As these differences were small, cross-imputation was still performed to give body composition data from DXA for 300 children, but sensitivity analysis was undertaken excluding the 47 children in which imputation of limb data had been performed.

Table 8.2: Comparison of limb fat mass, lean mass and BMC in scans without movement artefact

	Left Mean (SD)	***Right*** Mean (SD)	***Mean difference (95% CI)***	***p value***
Arm				
Fat mass (g)	336.7 (112.3)	340.6 (122.6)	3.9 (-8.2, 16.1)	0.52
Lean mass (g)	539.6 (121.7)	578.5 (114.6)	38.8 (26.7, 50.9)	< 0.001
BMC (g)	29.9 (5.2)	32.0 (5.6)	2.1 (1.6, 2.6)	< 0.001
Leg				
Fat mass (g)	1008.3 (253.4)	1025.0 (264.0)	16.6 (7.6, 25.6)	< 0.001
Lean mass (g)	1427.7 (262.7)	1473.4 (269.4)	45.7 (34.6, 56.7)	< 0.001
BMC (g)	74.8 (10.3)	76.3 (10.5)	1.6 (0.9, 2.2)	< 0.001

Age and sex of the children with and without DXA data were similar (p=0.95 and p=0.50, respectively).

Both WBLH and appendicular LM and %LM were higher, whereas WBLH and appendicular FM and %FM were lower in the boys than the girls ($p < 0.001$ for all). WBLH and appendicular BMC were both similar between the two sexes (p=0.68 and p=0.31, respectively). Soft tissue body composition variables were weakly, but significantly, correlated with age at DXA in the girls, but not the boys. As such, all variables were adjusted for sex and age of the child.

There were no statistically significant differences in any of the body composition measurements by randomisation group (Table 8.3). Inclusion of maternal age at delivery, parity, early pregnancy BMI, smoking status in late pregnancy, exercise in late pregnancy, educational achievement and duration of breastfeeding in a linear regression model did not alter the findings.

Table 8.3: Body composition assessed by DXA at age 4 years in children born to mothers randomised to placebo or 1000 IU/day cholecalciferol in pregnancy.
All outcomes have been adjusted for sex and age of the child. Shown as mean (SD), unless otherwise stated.

	Placebo	***Cholecalciferol***	***p value***
Whole body less head			
Fat mass (kg), median (IQR)	4.45 (3.87-5.21)	4.46 (3.91-5.13)	0.68
Lean mass (kg)	9.15 (1.28)	9.17 (1.29)	0.92
BMC (kg)	0.358 (0.043)	0.362 (0.042)	0.42
% Fat mass, median (IQR)	32.3 (29.2-35.6)	31.9 (29.2-34.9)	0.76
% Lean mass, median (IQR)	65.2 (62.0-68.2)	65.4 (62.6-68.2)	0.77
Appendicular			
Fat mass (kg), median (IQR)	2.66 (2.26-3.05)	2.67 (2.21-3.05)	0.91
Lean mass (kg)	4.05 (0.69)	4.01 (0.65)	0.65
BMC (kg)	0.213 (0.027)	0.215 (0.029)	0.50
% Fat mass	38.8 (6.0)	38.9 (5.7)	0.78
% Lean mass	58.1 (6.0)	58.0 (5.6)	0.88
Trunk			
Fat mass (kg), median (IQR)	1.80 (1.58-2.15)	1.79 (1.60-2.12)	0.87
Trunk fat (% of subtotal fat)	40.7 (3.4)	40.8 (3.9)	0.93
Trunk:appendicular fat ratio	0.69 (0.10)	0.70 (0.12)	0.80

8.3.3 Grip strength

Grip strength was measured in 349 children. Maximum grip strength was highly correlated with average grip strength (r=0.94, p < 0.001). As had also been observed in the children at 4 years of age in the SWS, maximum grip strength was greater in boys than girls (mean difference 0.51 kg [95% CI 0.1, 0.9], p=0.009) and was positively correlated with height (r=0.34, p < 0.001) and weight (r=0.32, p < 0.001), but not age (r_s=0.09, p=0.10). After adjusting grip strength for height, the difference by sex remained (p=0.02), and therefore grip strength was additionally adjusted for sex.

Maximum grip strength did not differ significantly between the two randomisation groups (p=0.45) (Figure 8.1). Adjustment for potential maternal confounders and duration of breastfeeding did not change the finding. The findings were similar when average grip strength adjusted for height and sex was used as the outcome.

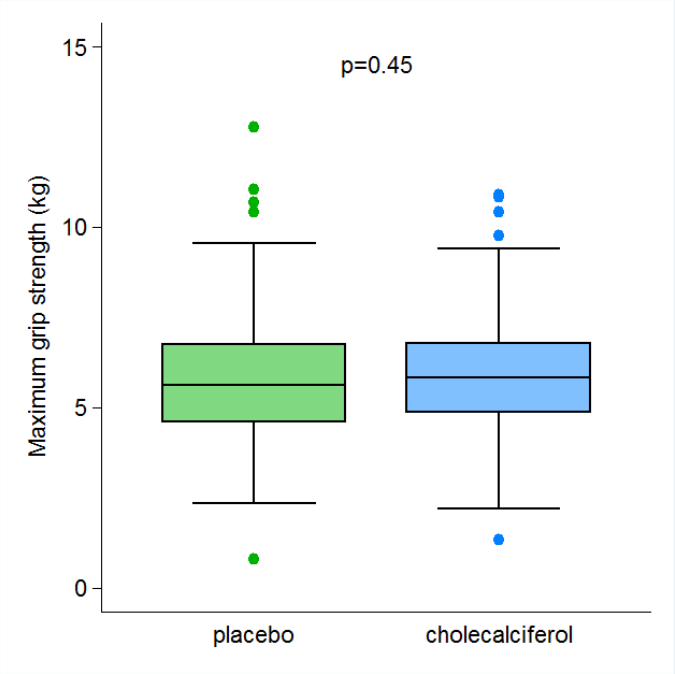


Figure 8.1: Maximum grip strength (adjusted for height and sex) at 4 years of age in children born to mothers randomised to placebo or 1000 IU/day cholecalciferol in pregnancy

8.3.4 Stratification by offspring sex

No significant differences in anthropometry or body composition between randomisation groups were observed when the children were stratified by sex ($p > 0.05$ for all).

Maximum grip strength (adjusted for height) was numerically greater in girls born to mothers randomised to cholecalciferol compared with placebo, but this did not reach statistical significance, as shown in Figure 8.2.

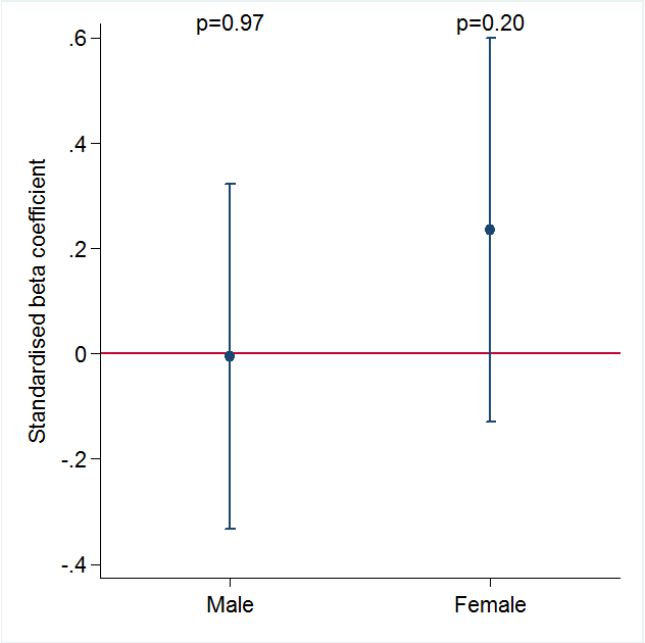


Figure 8.2: The effect of gestational cholecalciferol supplementation compared with placebo on offspring maximum grip strength at age 4 years stratified by sex

Results are shown as standardised beta coefficient (95% CI) for the effect of cholecalciferol in comparison to placebo (SD difference between cholecalciferol and placebo groups). Grip strength was adjusted for height. Maternal age at delivery, parity, early pregnancy BMI, smoking status in late pregnancy, hours of moderate-strenuous exercise in late pregnancy, educational achievement and duration of breastfeeding have been included as covariates in the model.

8.3.5 Stratification by maternal baseline serum 25(OH)D status

When the median maternal baseline serum 25(OH)D for the whole cohort (45.3 nmol/l) was used to stratify the mothers into two groups, there was no difference in offspring anthropometry, body composition or grip strength between the randomisation groups in either those with serum 25(OH)D above or below this threshold ($p > 0.05$ for all).

However, when maternal baseline 25(OH)D was stratified using a threshold of 30 nmol/l, in the offspring born to mothers with 25(OH)D ≤ 30nmol/l (n=47), maximum grip strength adjusted for height, sex and maternal covariates was significantly greater in those born to mothers randomised to cholecalciferol. Thus, maximum grip strength was 0.70 SD (95% CI 0.02, 1.38) greater in these children than those in the placebo group (p=0.043, Figure 8.3). Body composition did not differ between the two groups in children born to mothers with baseline 25(OH)D ≤ 30 nmol/l. However, the interaction between baseline 25(OH)D and randomisation group with grip strength as the outcome was not statistically significant ($P_{interaction}$=0.32)

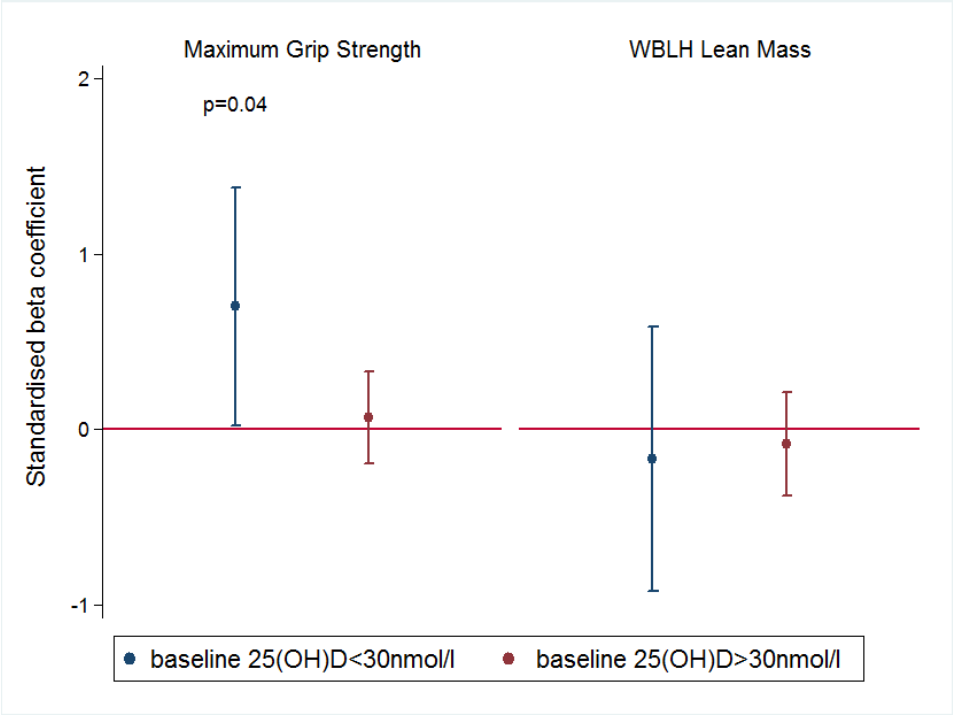


Figure 8.3: The effect of gestational cholecalciferol supplementation on offspring maximum grip strength and whole body less head lean mass when stratified by maternal baseline serum 25(OH)D level

Results are shown as standardised beta coefficient (95% CI) for the effect of cholecalciferol in comparison to placebo (SD difference between cholecalciferol and placebo groups). Maximum grip strength is adjusted for height and sex. Both models are adjusted for maternal age at delivery, parity, early pregnancy BMI, smoking status in late pregnancy, hours of moderate-strenuous exercise in late pregnancy, educational achievement and duration of breastfeeding.

8.3.6 Stratification by season of birth

Children born in winter months (December-February) to mothers randomised to cholecalciferol had significantly greater appendicular FM than the placebo group and there were also trends towards higher BMI z-score, WBLH FM and %FM and appendicular %FM in the cholecalciferol group, but no significant differences in WBLH LM or appendicular LM (Table 8.4). However, maternal early pregnancy BMI was also greater in the cholecalciferol group (median 25.1 kg/m^2 [IQR 22.3-29.8]) compared with the placebo group (23.3 kg/m^2 [IQR 21.8-24.5], p=0.05), and after adjustment for maternal covariates, these differences were attenuated (Table 8.4). Differences in anthropometry and body composition between randomisation groups were not observed for the other seasons of birth.

There was also a trend towards increased grip strength in the children born in winter to mothers in the cholecalciferol groups; however this was attenuated by adjustment for maternal covariates (Table 8.5).

8.3.7 Exclusion of DXA data for children with limb movement and cross-imputation of limb data

When the 47 children for whom DXA data obtained from a limb without movement artefact was used to replace data from a limb with movement artefact were excluded, there were similarly no statistically significant differences in body composition assessed by DXA between the two groups.

8.3.8 Exclusion of children requiring long-term paediatric follow-up

14 children required regular review in the paediatric outpatient department (nephrotic syndrome [n=1], asthma/respiratory conditions [n=4], food allergies [n=2], immunodeficiency [n=1], α1-antitrypsin deficiency [n=1], type 1 diabetes mellitus [n=1], epilepsy [n=1], congenital cardiac disease [n=1], faltering grown of unknown origin [n=2]). Exclusion of these children from the dataset did not alter the overall findings.

Table 8.4: Anthropometry and body composition at age 4 years in children born in winter (December-February) by maternal randomisation to placebo or 1000 IU/day cholecalciferol in pregnancy

All body composition outcomes are adjusted for age and sex. In the adjusted model maternal age at delivery, parity, early pregnancy BMI, smoking status in late pregnancy, hours of moderate-strenuous exercise in late pregnancy, educational achievement and duration of breastfeeding were included as covariates. Shown as mean (SD), unless otherwise stated

	Placebo	***Cholecalciferol***	***p value***	***Adjusted p value***
Height z-score	0.79 (0.21)	0.70 (0.18)	0.76	0.99
Weight z-score	0.17 (0.97)	0.42 (0.89)	0.30	0.50
BMI z-score	-0.45 (0.84)	-0.03 (0.87)	0.07	0.37
Whole body less head				
Fat mass (kg), median (IQR)	4.38 (3.81-4.82)	4.78 (4.24-5.63)	0.07	0.12
Lean mass (kg)	9.22 (1.46)	9.26 (1.22)	0.93	0.96
BMC (kg)	0.361 (0.049)	0.371 (0.034)	0.40	0.94
% Fat mass, median (IQR)	30.7 (28.8-33.8)	33.2 (30.7-35.6)	0.07	0.09
% Lean mass, median (IQR)	66.8 (63.5-68.5)	64.4 (61.7-66.2)	0.07	0.10
Appendicular				
Fat mass (kg), median (IQR)	2.49 (2.21-2.90)	2.84 (2.62-3.39)	0.02	0.08
Lean mass (kg)	4.10 (0.72)	4.15 (0.59)	0.77	0.90
BMC (kg)	0.214 (0.027)	0.222 (0.021)	0.30	0.60
% Fat mass	37.4 (6.0)	40.5 (5.6)	0.09	0.11
% Lean mass	59.4 (6.0)	56.5 (5.4)	0.09	0.12
Trunk				
Fat mass (kg), median (IQR)	1.71 (1.57-1.97)	1.88 (1.61-2.27)	0.17	0.21
Trunk fat (% of subtotal fat)	41.1 (3.4)	39.7 (3.0)	0.16	0.27
Trunk:appendicular fat ratio	0.70 (0.10)	0.66 (0.08)	0.14	0.27

Table 8.5: Grip strength by season of birth and maternal randomisation to placebo or 1000 IU/day cholecalciferol in pregnancy

Shown as mean (SD). Grip strength adjusted for height and sex. In the adjusted model maternal age at delivery, parity, early pregnancy BMI, smoking status in late pregnancy, hours of moderate-strenuous exercise in late pregnancy, educational achievement and duration of breastfeeding were included as covariates.

Season of birth	***Placebo***	***Cholecalciferol***	***p value***	***Adjusted p value***
Winter (December-February)	5.6 (1.7)	6.4 (1.7)	0.06	0.12
Spring (March-May)	5.8 (1.9)	5.9 (1.5)	0.85	0.77
Summer (June-August)	5.7 (1.4)	5.8 (1.6)	0.78	0.69
Autumn (September-November)	5.7 (1.8)	5.6 (1.8)	0.68	0.90

8.4 Summary of findings

In summary, 1000 IU/day cholecalciferol from 14 weeks of gestation until delivery in women with a baseline 25(OH)D of 25-100 nmol/l did not affect offspring height, weight, body composition assessed by DXA or grip strength at 4 years of age when compared with placebo.

Supplementation with cholecalciferol did however appear to increase grip strength compared to placebo in children whose mothers had a 25(OH)D < 30 nmol/l at randomisation, but the formal interaction coefficient was not statistically significant.

The findings suggested a possible trend towards an effect of cholecalciferol supplementation on increasing FM in children born in winter months. However this may reflect the higher BMI of the mothers who received the cholecalciferol supplement compared to the placebo in winter born children. Reanalysis of these outcomes in the whole cohort once the data are available will be important to confirm or refute these early findings.

Chapter 9: Determinants of the maternal response to antenatal vitamin D supplementation

9.1 Background and aims

Analysis of the observational data from the SWS cohort demonstrated that vitamin D supplement usage, weight gain during pregnancy and exercise participation were associated with changes in 25(OH)D status relative to other pregnant women during pregnancy (Chapter 5). Uptake of vitamin D supplementation in that group of women was low, but many of the pregnancies were at a time when antenatal vitamin D supplementation was not routinely advised. Many guidelines now suggest universal vitamin D supplementation in pregnancy (161, 248, 314, 315), which is important to prevent neonatal hypocalcaemia. Understanding which characteristics influence the response to supplementation might enable individualised antenatal counselling regarding vitamin D supplementation to ensure vitamin D repletion is achieved without increasing the risk of vitamin D toxicity.

Studies in non-pregnant adults have demonstrated that baseline 25(OH)D concentration, body weight or adiposity and age are important determinants of the incremental rise in 25(OH)D following vitamin D supplementation (255, 256). However, as pregnancy is associated with physiological changes to both vitamin D metabolism and maternal body composition, care should be taken in extrapolating these findings to pregnant women. The aim of this analysis was therefore to determine maternal characteristics associated with the achieved 25(OH)D following antenatal vitamin D supplementation in the context of an intervention study.

9.2 Methods

This analysis was based on data from the MAVIDOS intervention study, full details of which can be found in Chapter 3. Briefly, pregnant women had 25(OH)D assessed at randomisation to either placebo or cholecalciferol (14-17 weeks of gestation) and again at 34 weeks of gestation. Women who had a measurement of 25(OH)D at both time points and delivered a live born infant were included in the analysis.

The primary outcome in this analysis was achieved 25(OH)D at 34 weeks of gestation. Clinically it was felt that the achieved 25(OH)D was more important than the change in 25(OH)D from 14 to 34 weeks of gestation as women with a very low 25(OH)D at baseline may have a large rise in 25(OH)D but still not achieve a recommended level and conversely a small rise may be sufficient to achieve vitamin D replete status in some women with a moderate-low 25(OH)D. Thus, additionally factors associated with the likelihood of achieving 25(OH)D replete status, defined as > 50 nmol/l as suggested by the IOM (160), were also explored. Additionally, we considered a 25(OH)D > 125 nmol/l as indicating risk of toxicity, as suggested by the IOM (160).

9.2.1 Statistical analysis

Maternal characteristics were compared between the women included in this analysis and those who remained in the MAVIDOS study until delivery but who did not have assessment of 25(OH)D status at both randomisation and 34 weeks of gestation using t-tests, Mann-Whitney U tests and $\chi 2$ tests for normally distributed, non-normally distributed and categorical variables, respectively. Linear regression was used to assess the association between maternal characteristics and 25(OH)D at 34 weeks of gestation for each treatment group separately. Multivariate linear regression was subsequently performed including all variables with a $p < 0.2$ from the univariate linear regression. Maternal factors associated with achieving a vitamin D replete status (> 50 nmol/l) were determined using Poisson regression with robust standard errors (316).

In the primary trial analysis (295) and previous analyses with offspring outcomes, season of birth was classified into 4 seasons. Since 25(OH)D concentrations are non-linearly associated with season, to facilitate ready comparison, for this analysis season of birth was classified into 2 groups with a notional “winter” (the months in which 25(OH)D concentrations tended to be lowest: December-May) and a “summer” (the months in which 25(OH)D concentrations tended to be highest: June-November).

Finally, in sensitivity analysis, women who reported having taken any additional vitamin D-containing supplements within 90 days of the late pregnancy blood sampling were excluded.

9.3 Results

9.3.1 Study participants

829 (85.9%) of the 965 women participating in the study who delivered a live born infant had measurements of 25(OH)D at both randomisation and 34 weeks gestation. Women with missing 25(OH)D were of similar age, parity, height, ethnicity, educational achievement, early pregnancy BMI and smoking status to those included in this analysis ($p > 0.05$ for all).

There were no significant differences in baseline characteristics between the randomisation groups for women included in this analysis (Table 9.1). Compliance with study medication was high in both treatment groups (placebo: median 95% [IQR 88-98], cholecalciferol: median 96% [IQR 89-99], p=0.11].

Table 9.1: Maternal characteristics at randomisation

	Placebo	***Cholecalciferol***	***p***
n	422	407	
Age (years), mean (SD)	30.7 (5.4)	30.7 (5.0)	0.94
Gestation (weeks), mean (SD)	15.9 (1.5)	15.9 (1.5)	0.66
White ethnicity, %	94.8	95.6	0.58
Height (cm), mean (SD)	165.6 (6.6)	165.5 (6.3)	0.73
BMI (kg/m^2), median (IQR)	25.4 (22.7-29.7)	24.6 (22.2-28.6)	0.07
Nulliparous, %	44.8	42.7	0.56
Smoking, %	7.7	7.7	0.98
Education to degree level or above, %	46.5	49.0	0.49
25(OH)D (nmol/l), mean (SD)	45.5 (16.8)	46.8 (17.4)	0.27
25(OH)D > 50nmol/l, %	36.5	41.3	0.16

9.3.2 Maternal 25(OH)D status at 34 weeks of gestation

Maternal 25(OH)D at 34 weeks of gestation was greater in the women randomised to cholecalciferol (mean 67.7 nmol/l [SD 21.3]) compared with the placebo group (mean 43.1 nmol/l [SD 22.5 nmol/l], $p < 0.0001$). 83.3% of women randomised to cholecalciferol achieved vitamin D replete status (> 50 nmol/l) at 34 weeks of gestation compared with 35.6% in the placebo group ($p < 0.001$). In both treatment groups, the proportion of women who were vitamin D replete at 34 weeks of gestation was lower in those who delivered in winter, as shown in Figure 9.1.

Figure 9.1: Proportion of women achieving vitamin D replete status (25(OH)D > 50 nmol/l) in late pregnancy stratified by randomisation to placebo or 1000 IU/day cholecalciferol and season of delivery
(Winter was defined as December-May)

No participant reported symptoms suggestive of vitamin D toxicity. Two participants (0.5%) randomised to placebo and one to cholecalciferol (0.3%, p=0.58) had a 25(OH)D ≥ 125 nmol/l at 34 weeks of gestation, with the maximum value being 139 nmol/l.

9.3.3 Determinants of achieved maternal 25(OH)D at 34 weeks of gestation

In univariate analysis, maternal age, baseline 25(OH)D, season of delivery and compliance with study medication were significantly associated with 34 week 25(OH)D in both the placebo and

vitamin D supplementation groups (Table 9.2). Additionally, women who reported smoking in late pregnancy had significantly lower 25(OH)D in the placebo group, but this association was not observed amongst women randomised to cholecalciferol. Conversely, markers of maternal weight and adiposity were significantly inversely associated with maternal 25(OH)D in the cholecalciferol group, but not the women randomised to placebo (Table 9.2).

In multiple linear regression analysis, maternal factors significantly associated with greater 25(OH)D at 34 weeks of gestation in the women who received vitamin D supplementation were pregnancy weight gain (β=-0.8 nmol/l per kg [95% CI -1.4, -0.2], p=0.007), compliance (β=0.3 nmol/l per % [95% CI 0.1, 0.5], p=0.008), early pregnancy 25(OH)D (β=0.3 nmol/l per nmol/l [95% CI 0.2, 0.4], p < 0.001) and summer delivery (β=10.5 nmol/l summer vs winter [95% CI 6.4, 14.6], p < 0.001). This is shown graphically in Figure 9.2 (A). In the placebo group (Figure 9.2 (B)), early pregnancy 25(OH)D (β=0.6 nmol/l per nmol/l [95% CI 0.5, 0.7], p < 0.001), summer delivery (β=25.0 nmol/l summer vs winter [95% CI 21.9, 28.2], p < 0.001), and maternal age (β=0.3 nmol/l per year [95% CI: 0.0, 0.6], p=0.04) remained significantly associated with 25(OH)D at 34 weeks' gestation.

9.3.4 Determinants of replete maternal 25(OH)D status at 34 weeks of gestation

When achievement of vitamin D replete status at 34 weeks of gestation was considered instead of absolute achieved 25(OH)D concentration, in multivariate analyses, delivery in summer (RR=1.20 [95% CI 1.09, 1.33], p < 0.001), white ethnicity (RR=1.27 [95% CI 1.17, 1.37], p < 0.001), compliance with medication [%] (RR=1.01 [95% CI 1.00, 1.02], p=0.03), and early pregnancy 25(OH)D concentration [nmol/l] (RR=1.003 [95% CI 1.001, 1.006], p=0.007) were significantly associated with achieving 25(OH)D > 50 nmol/l in the women randomised to cholecalciferol.

9.3.5 Interaction between baseline 25(OH)D and randomisation group

When comparing achieved 25(OH)D at 34 weeks of gestation between placebo and cholecalciferol groups, it was apparent that there was a statistically significant interaction between baseline 25(OH)D and randomisation group (p < 0.001). Thus there was a smaller difference in 25(OH)D concentrations at 34 weeks of gestation between the placebo and treatment arms with increasing 25(OH)D at 14 weeks of gestation (Figure 9.3).

Table 9.2: Associations between maternal characteristics and achieved 25(OH)D status at 34 weeks of gestation in women randomised to placebo or vitamin D supplementation from 14 weeks of gestation until delivery.

Shown as nmol/l change in 25(OH)D per unit predictor

	Placebo		***Cholecalciferol***	
	Beta (95% CI)	p	Beta (95% CI)	p
Maternal age (years)	0.7 (0.3, 1.1)	0.001	0.7 (0.3, 1.1)	0.001
Parity (yes vs no)	-1.3 (-5.7, 3.2)	0.58	-0.3 (-4.6, 4.0)	0.90
Smoking at 34 weeks of gestation (yes vs no)	-13.5 (-22.1, -4.8)	0.002	-1.5 (-9.5, 6.5)	0.72
Ethnicity (other vs white)	-8.7 (-18.6, 1.2)	0.09	2.0 (-8.5, 12.5)	0.71
Height (cm)	0.2 (-0.2, 0.5)	0.39	-0.07 (-0.4, 0.3)	0.68
BMI at 14 weeks of gestation (kg/m^2)	-0.2 (-0.7, 0.2)	0.28	-0.5 (-0.9, -0.0)	0.03
Weight at 34 weeks of gestation (kg)	-0.1 (-0.2, 0.1)	0.49	-0.2 (-0.4, -0.1)	0.002
Weight gain early to late pregnancy (kg)	-0.2 (-0.9, 0.4)	0.47	-0.7 (-1.3, -0.0)	0.04
Triceps SFT at 34 weeks of gestation (mm)	-0.1 (-0.4, 0.3)	0.72	-0.4 (-0.7, -0.1)	0.01
Moderate/strenuous exercise in late pregnancy (hrs/week)	1.4 (-1.8, 4.5)	0.39	-0.8 (-3.6, 2.1)	0.60
25(OH)D at 14 weeks of gestation (nmol/l)	0.5 (0.4, 0.6)	< 0.001	0.2 (0.1, 0.3)	0.001
Season of delivery (summer vs winter)	22.8 (19.1, 26.5)	< 0.001	10.1 (6.0, 14.2)	< 0.001
Compliance (%)	0.2 (0.0, 0.4)	0.02	0.4 (0.2, 0.6)	< 0.001

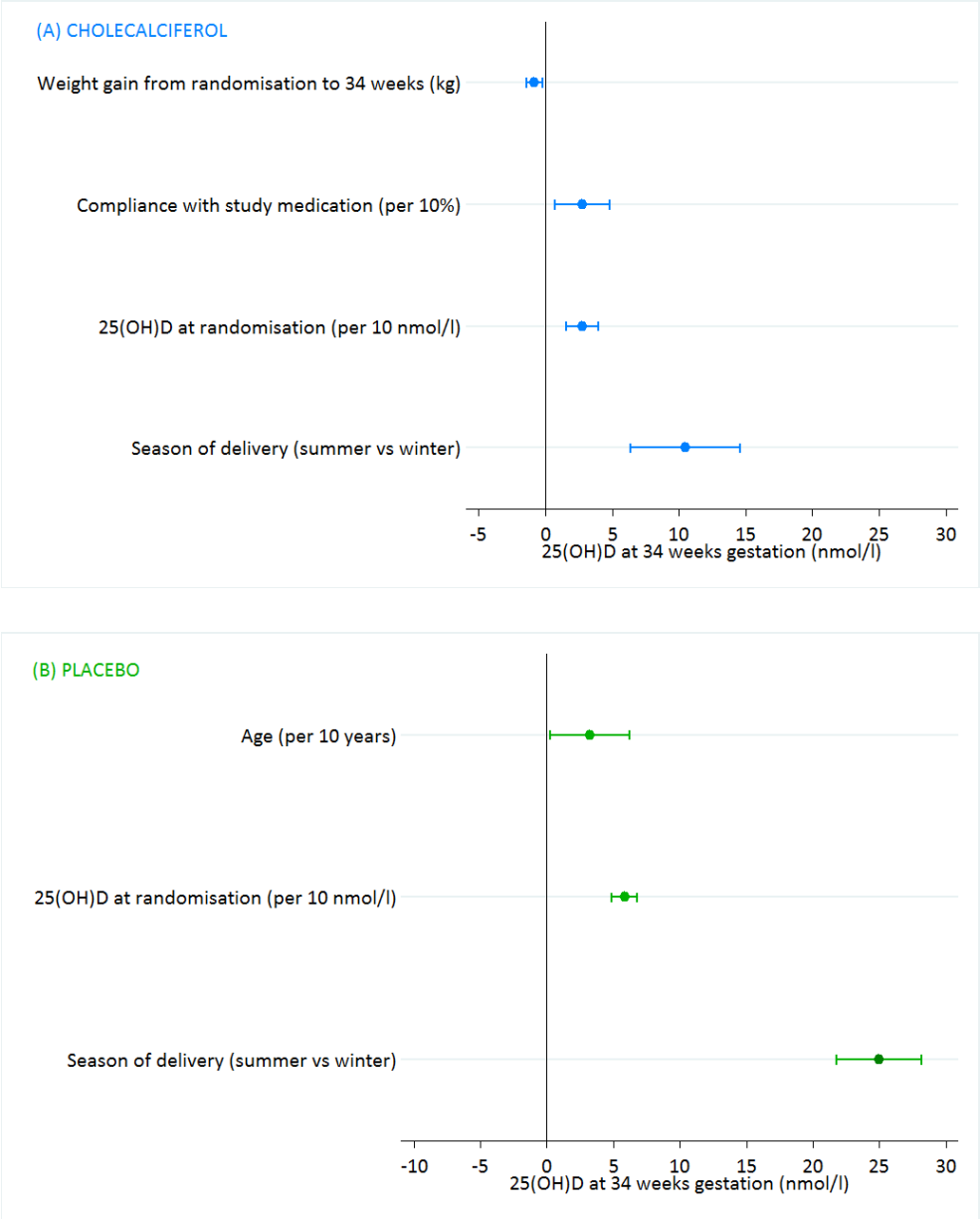


Figure 9.2: Independent determinants of maternal 25(OH)D at 34 weeks of gestation in women randomised to (A) 1000 IU/day cholecalciferol and (B) placebo.

Shown as change in 25(OH)D per unit predictor (Beta and 95% confidence interval).

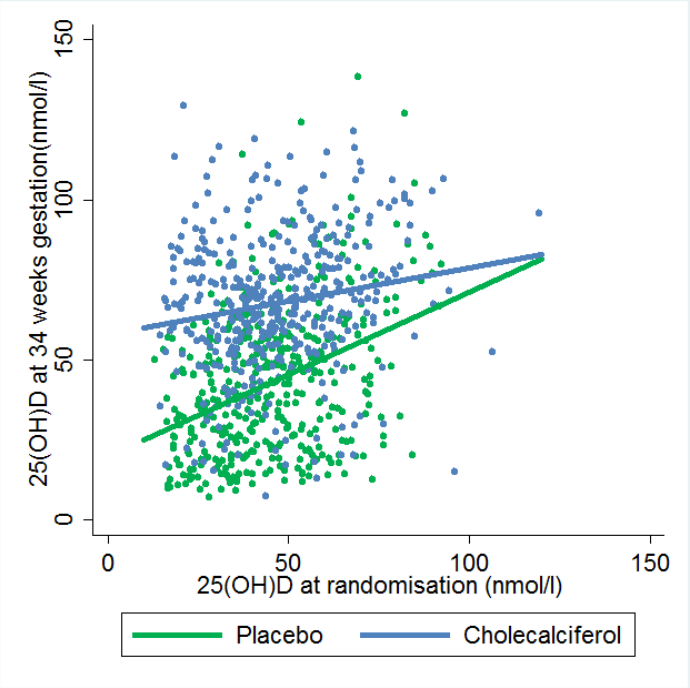


Figure 9.3: Relationship between 25(OH)D at randomisation and at 34 weeks of gestation in women randomised to placebo and 1000 IU/day cholecalciferol during pregnancy

9.3.6 Sensitivity analyses

In sensitivity analysis, it was explored whether altering the definition of the seasons changed the determinants of achieved 25(OH)D. Thus, the analysis was repeated defining winter firstly as November-April and secondly as October-March. Using both these definitions, in women randomised to cholecalciferol season of birth, 25(OH)D at randomisation, pregnancy weight gain and compliance remained significant determinants of achieved 25(OH)D. Additionally, when winter was defined as October-March, maternal age was positively associated with achieved 25(OH)D in the multivariate model (β=4.5 nmol per year [95% CI 0.4, 8.7], p=0.03).

As participants were permitted to continue taking daily vitamin D supplements containing up to 400 IU/day, in a sensitivity analysis 229 women (n=117 randomised to cholecalciferol) who reported taking other vitamin D containing dietary supplements at the late pregnancy interview were excluded. Similarly to that observed in all women, 81.0% of women randomised to cholecalciferol were vitamin D replete at 34 weeks of gestation, compared with 29.4% of women randomised to placebo ($p < 0.001$). The maternal characteristics associated with 25(OH)D at 34 weeks of gestation and achieving vitamin D replete status were similar to those observed in the whole cohort.

9.4 Summary of findings

Maternal supplementation with 1000 IU/day cholecalciferol increased maternal 25(OH)D concentration in late pregnancy, but the proportion of supplemented women who were vitamin D replete was still lower for those who delivered in winter months compared to summer, highlighting that this degree of supplementation does not abolish seasonal variation in 25(OH)D status. In addition to season of delivery, having a low baseline 25(OH)D and gaining more weight during pregnancy were associated with achieving a lower 25(OH)D in late pregnancy. Women with these characteristics might therefore require greater supplementation doses.

Chapter 10: Discussion

10.1 Main findings

This work has explored a number of objectives in relation to vitamin D status in pregnancy and its relationships with offspring size, body composition and muscle strength using an observational mother-offspring cohort study and a randomised controlled trial of antenatal vitamin D supplementation. There are a number of novel findings.

1. After adjustment for season, 25(OH)D status tracks moderately from early to late pregnancy.

2. Gestational weight gain, use of vitamin D supplementation during pregnancy and exercise participation in late pregnancy are associated with deviations in the tracking of seasonally adjusted 25(OH)D status during pregnancy.

3. Supplementation with 1000 IU/day cholecalciferol during pregnancy in women with an early pregnancy baseline 25(OH)D of 25-100 nmol/l did not affect offspring size or adiposity (measured by MUAC and SFT) at birth in the full cohort of births. However, in infants born in winter months, maternal cholecalciferol supplementation did result in higher birth weight, OFC and MUAC.

4. 1000 IU/day antenatal cholecalciferol supplementation in women with baseline 25(OH)D of 25-100 nmol/l did not affect infant size or adiposity at 1 or 2 years of age, or linear growth or weight gain during this period.

5. In an observational cohort, maternal serum 25(OH)D status in late pregnancy was positively associated with offspring grip strength at age 4 years. Associations between maternal serum 25(OH)D and offspring LM and %LM were weaker and less robust to adjustment for maternal covariates. When stratified by sex, the relationships between maternal 25(OH)D in late pregnancy and offspring grip strength were only significant in females, but the statistical interaction between sex and maternal 25(OH)D was not significant for this outcome.

6. Antenatal supplementation with 1000 IU/day cholecalciferol in women with a baseline 25(OH)D of 25-100 nmol/l did not result in higher offspring grip strength or LM, FM or

relative body composition measured by DXA at age 4 years. The difference in grip strength between children randomised to cholecalciferol and placebo was numerically greater in girls than boys, but did not reach statistical significance in either sex. In women with a baseline 25(OH)D < 30 nmol/l, cholecalciferol supplementation did result in a higher grip strength at age 4 years, but no differences in body composition. However, again the statistical interaction was not significant.

7. In an observational cohort study of women who were pregnant between 1998 and 2005, use of vitamin D supplementation during pregnancy was low, and uptake was less likely in women who were younger, less well educated, smokers and those not in their first pregnancy.

8. Supplementation with 1000 IU/day cholecalciferol during pregnancy increases maternal 25(OH)D but seasonal variation in 25(OH)D status in women in the UK remained present after supplementation. Women who received the vitamin D supplement achieved a higher 25(OH)D when they delivered in summer months, had a higher pre-supplementation serum 25(OH)D level, had greater compliance with the study medication and gained less weight during pregnancy.

10.2 Tracking of 25(OH)D status from early to late pregnancy

Understanding the tracking of 25(OH)D during pregnancy is important for both the interpretation of research in which a single measurement of 25(OH)D has been used, and clinically to be able to provide appropriate advice on the need for supplementation. In the SWS, a large observational cohort in the south of the UK, there was marked seasonal variation in 25(OH)D status in both early and late pregnancy. This finding is consistent with other population studies from similar latitudes (228, 292) and the known biology of UVB mediated vitamin D biosynthesis in the skin. The correlation between measurements of 25(OH)D in early and late pregnancy was low. This will partly reflect this seasonal variation as the measurements in early and late pregnancy were taken 5-6 months apart and therefore will have occurred in differing seasons. After adjustment for date of measurement, there was moderate tracking of 25(OH)D concentration from 11 to 34 weeks of gestation.

Prior to this analysis and its publication in 2015 (317), there were no previous studies investigating the tracking of 25(OH)D status during pregnancy. Moderate-high levels of tracking

of 25(OH)D concentration in non-pregnant adults at intervals of one to five years (r=0.53-0.90) have previously been demonstrated (236-238). In those studies, the correlation coefficient did reduce with increasing number of years from baseline measurement (237, 238), which is likely to reflect more substantial changes in lifestyle and dietary intake over longer periods of time coupled with the reduction in epidermal previtamin D_3 biosynthesis with aging (318). Nonetheless, the comparison of those findings to pregnancy are limited as the assessment of 25(OH)D concentration was undertaken in the same month/season at baseline and follow-up rather than adjusting for seasonal variation. Jorde et al did examine the tracking of 25(OH)D status across seasons in 2668 adults in Norway, but also had a 14 year interval between baseline and follow-up (236). In that study, 25(OH)D z-scores were calculated for each month of sampling at baseline and follow-up and compared between the two time-points. The correlation coefficient between the 25(OH)D z-scores was 0.42, therefore lower than in our cohort, but this could reflect the 14 year time interval between measurements.

Bärebring et al have also reported on the tracking of 25(OH)D in pregnancy (319) since our findings were published. Similar statistical methods to those used in our analysis were performed to adjust measurements of 25(OH)D in the first and third trimester of pregnancy for season in 1829 women in south-west Sweden (latitude 57.7-58.1°N). The correlation between early and late pregnancy season-corrected 25(OH)D was higher (r=0.68) than in the SWS cohort (r=0.53). This might reflect more frequent food fortification in Sweden compared with the UK and the greater use of vitamin D supplementation, which was higher than in the SWS: 43% and 42% of women in the Swedish cohort took a vitamin D containing supplement in early and late pregnancy, respectively, compared with 36% and 22% in the SWS. Indeed, in the SWS, changes in vitamin D supplement use strongly influenced the stability of season-corrected 25(OH)D concentration relative to the population distribution, highlighting the importance of this source of vitamin D to the maintenance of maternal 25(OH)D status. Bärebring et al similarly found that use of vitamin D supplements during the third trimester was positively associated with change in season-corrected 25(OH)D from early to late pregnancy (319), and Jorde et al found that change in supplement use was a determinant of change in 25(OH)D z-score over 14 years in older adults (236).

Greater weight gain during pregnancy and less exercise in late pregnancy were independently associated with downward tracking of 25(OH)D status, although the effect sizes were small: for each additional kilogram of weight gained during pregnancy season-corrected 25(OH)D reduced by 0.4 nmol/l from early to late pregnancy, and for every additional hour of exercise per week in late pregnancy, season-corrected 25(OH)D increased by 0.4 nmol/l. Adiposity is negatively associated with 25(OH)D status in non-pregnant populations, and in this cohort pre-pregnancy

BMI was negatively associated with season-corrected 25(OH)D in both early and late pregnancy. It is hypothesised that this results from sequestration of 25(OH)D in adipose tissue. Indeed the increase in 25(OH)D is lower in obese than non-obese individuals following oral supplementation (212, 222) or UVB irradiation (221), whereas conversely weight loss is associated with positive changes in 25(OH)D status (223, 224). In a study comparing the change in 25(OH)D status in mice following discontinuation of vitamin D supplementation, the rate of decline in 25(OH)D was slower in obese compared to lean mice (320), suggesting the increased adipose tissue in obesity could act as a buffer for serum 25(OH)D status, but this would be dependent on having previously achieved adequate repletion of the 25(OH)D in adipose tissue. Despite this the lower 25(OH)D in obesity might be of functional significance. PTH is positively associated with body weight and FM in eucalcaemic individuals, but the importance of 25(OH)D status to this relationship is uncertain (321). Nonetheless, as 25(OH)D in the fetal circulation mirrors that in maternal serum, the effect of adiposity and pregnancy weight gain might be important to the risk of neonatal hypocalcaemia and, if demonstrated, fetal development.

Whilst it cannot be certain that greater weight gain during pregnancy represents increased fat mass as opposed to feto-placental tissues or the increase in circulating blood volume and extracellular fluid that occurs during pregnancy (322), it is likely that the downward tracking of season-corrected 25(OH)D associated with greater gestational weight gain does reflect a larger volume of dilution. Jorde et al similarly found that change in BMI was a significant determinant of change in 25(OH)D z-score over 14 years (236), but in contrast to our findings, gestational weight gain was not a significant determinant of change in season-corrected 25(OH)D during pregnancy in women in Sweden (319). It is possible that the more prevalent use of supplementation in that cohort prevented the downward tracking with excess weight gain. Nonetheless our finding would suggest that women who gain greater weight during pregnancy might require higher supplementation doses to prevent vitamin D deficiency, and this is indeed supported by the analysis of data from the MAVIDOS study, which showed greater gestational weight gain was associated with lower achieved 25(OH)D following supplementation with 1000 IU/day cholecalciferol.

There are a number of factors which are likely to influence the tracking of 25(OH)D status that were not assessed in this study. In particular, we did not ask about holidays to a sunny destination, which have been shown in older women to reduce the prevalence of VDD for up to 3 months after the holiday (323). The use of sun protection and sun-seeking behaviour and changes in this during the pregnancy were also not determined in the SWS.

Overall the mean difference in season-corrected 25(OH)D status between early and late pregnancy was very small, but care must be taken in the interpretation of this finding as different analytical techniques were used to determine 25(OH)D concentrations at each stage of pregnancy. A chemiluminescent immunoassay (Diasorin Liaison) was used to analyse the late pregnancy samples. This method tends to underreport 25(OH)D concentrations in comparison to LC-MS/MS (152), which was used for the early pregnancy samples. The small increase in season-corrected 25(OH)D from early to late pregnancy (0.3 nmol/l) therefore might not be a true finding. Bärebring et al found season-corrected 25(OH)D increased by a mean of 11 nmol/l during pregnancy in Sweden (319). However, it is interesting that in the SWS, late pregnancy 25(OH)D concentration appeared to have a higher summer peak than early pregnancy 25(OH)D, despite a reduction in vitamin D supplementation use from early to late pregnancy. A similar higher summer peak in 25(OH)D in the third trimester compared with the first trimester was also present in the cohort in Sweden (Figure 10.1). In cross-sectional analysis of that cohort, serum 25(OH)D concentration was higher in late pregnancy than early pregnancy in all seasons apart from winter in which there was no significant difference (319). The interpretation of earlier studies that have attempted to describe the longitudinal changes in 25(OH)D status during pregnancy are limited by lack of adjustment for seasonal variation. Reducing (234), increasing (230) and stable (231, 235) 25(OH)D concentrations during pregnancy have all been reported. More recently in a study of women in the Gambia, $25(OH)D_3$ was found to be significantly higher in late pregnancy than in non-pregnant non-lactating women who were matched for the day of sampling as the method to eliminate seasonal variation (324). No participants were regularly consuming vitamin D supplementation. This therefore supports the notion of physiological changes to increase 25(OH)D status in late pregnancy although additional studies are needed to confirm this, in particular comparing early and late pregnancy 25(OH)D measurements from the same individuals following deseasonalisation of the 25(OH)D data. This would require a large cohort with early and late pregnancies occurring throughout the calendar year. Re-analysis of our data following harmonisation of the 25(OH)D assays could contribute further to this understanding.

The tracking of 25(OH)D is an important finding. Firstly, it suggests that a single measurement of 25(OH)D in early pregnancy could be used to identify women who are at high risk of low levels of 25(OH)D at other stages of pregnancy. This could enable appropriate counselling regarding the need for supplementation to be delivered. Such an approach would however be limited by the need for background data to generate a population distribution for the time of year and latitude at which the 25(OH)D measurement was to be performed. Secondly, it suggests that a single measurement of 25(OH)D status in pregnancy, might be a useful, although not perfect, biomarker of overall 25(OH)D status throughout pregnancy when adjusted for season. Care therefore needs

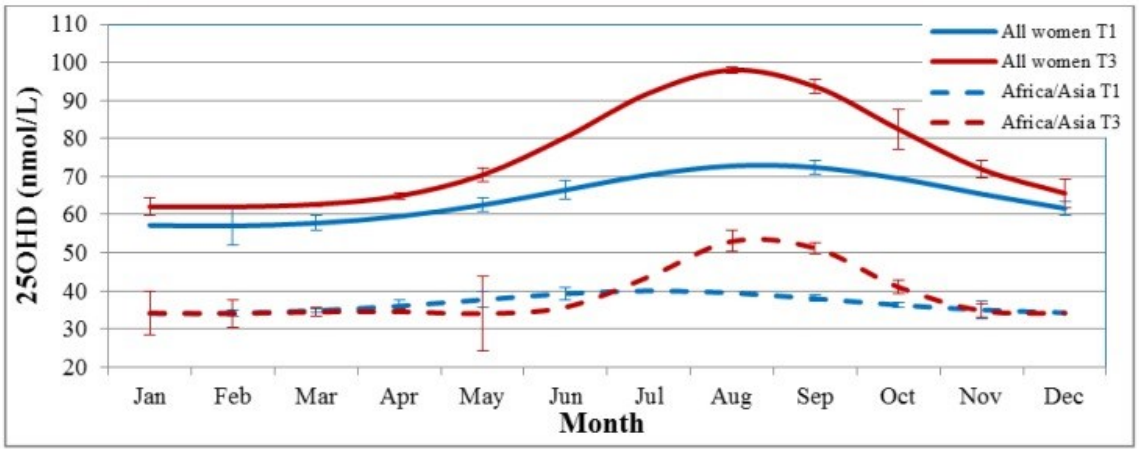


Figure 10.1: Serum 25(OH)D concentrations in trimester 1 (T1) and trimester 3 (T3) in a cohort of women in Sweden

Solid lines show the whole cohort, dashed lines represent women of African and Asian ethnicity.

Reproduced with permission from Barebring et al, 2016 (319)

to be taken in interpretation of observational studies in which adjustment for season has been included. For example, if a significant association is observed between an outcome and 25(OH)D status obtained during a narrow window of gestations in pregnancy but an adjustment for season of sampling has been included, the gestation at which 25(OH)D was measured might not be the critical window for any potential effect of vitamin D on the outcome due to the moderate correlation between season-adjusted 25(OH)D at the point of measurement and at other stages of pregnancy. Translation of such findings to an intervention study would therefore need supplementation extending beyond the gestations at which 25(OH)D measurement was performed in the observational work.

10.3 Vitamin D supplementation in pregnancy and offspring size

10.3.1 Size at birth

In a rigorously conducted randomised placebo-controlled trial, supplementation with 1000 IU/day cholecalciferol from 14 weeks of gestation until delivery in women with a baseline 25(OH)D of 25-100 nmol/l did not affect birth size despite a significant difference in late pregnancy 25(OH)D concentration between the two groups.

This finding is consistent with other small intervention studies comparing antenatal vitamin D supplementation to either placebo or between doses of cholecalciferol and undertaken in

developed countries despite methodological differences in the timing of the intervention, doses of vitamin D studied and including a more ethnically diverse group of women than included in our study (240, 245, 325). However, an effect of gestational vitamin D supplementation on offspring birth weight, length and OFC has been reported in three studies, two of which were carried out in India and one in Iran (251, 277, 278). There are a number of differences between these studies and the MAVIDOS study that could account for the inconsistent findings. Firstly, the three studies which reported an effect of vitamin D supplementation were all open-labelled trials and therefore are potentially subject to more bias than our double-blind trial. The double-blinded RCTs of Wagner et al (325), Grant et al (240) and Roth et al (279), similarly to our study, found a null effect of cholecalciferol supplementation on birth weight.

Secondly, the dosing schedules also differed between MAVIDOS and the studies that identified an effect of vitamin D supplementation on birth size. High dose oral vitamin D given between one and eight times in mid-late pregnancy were used in the three studies that reported an effect on birth weight as opposed to the daily dosing schedules used in our study and several others in which no effect on birth weight has been observed (240, 281, 325). This could reflect differences in compliance in directly observed intermittent dosing compared with daily supplementation, although compliance in our study was assessed to be high. The total vitamin D dose received in pregnancy was considerably greater than in MAVIDOS in two of the studies: Marya et al gave a total of 1 200 000 IU ergocalciferol (251) and Hashemipour et al gave 439 200 IU cholecalciferol (278) compared to approximately 182 000 IU cholecalciferol in MAVIDOS (based on a woman randomised at 14 weeks and delivering at 40 weeks with 100% compliance). However the total dose of vitamin D in the study of Kalra et al (60 000 IU or 240 000 IU cholecalciferol), who still found a positive effect of supplementation on birth size, was more similar to MAVIDOS suggesting that perhaps such high total vitamin D doses are not necessary to observe a clinical effect. This is also supported by the null effect of 4000 IU/day cholecalciferol from 12-16 weeks of gestation (equating to a total dose of approximately 784 000 IU over the duration of the pregnancy) on birth weight compared to 2000 IU/day or 400 IU/day in a study in the USA (325). It is however likely that the bolus dosing schedules achieved a higher peak 25(OH)D concentration (326). Indeed Hashemipour et al reported a 170 g difference in offspring birth weight, 0.8 cm difference in birth length, 0.6 cm difference in OFC and 79.5 nmol/l difference in 25(OH)D concentration at delivery between their intervention (500 000 IU cholecalciferol eight times in mid-late pregnancy 1-2 weeks apart) and control (400 IU/day from 24-26 weeks of gestation) groups (278). This difference in 25(OH)D is considerably greater than the mean difference of 24.7 nmol/l between the intervention and placebo group in our study. In contrast, Kalra et al reported significantly greater offspring birth weight, OFC and length in infants born to mothers randomised to either a

single dose of 60 000 IU cholecalciferol at 12-24 weeks of gestation (Group 1) or two doses of 120 000 IU cholecalciferol at 12-24 and again at 28 weeks of gestation (Group 2) compared to "usual care" (all women in fact received supplemental calcium daily although this was started later in the "usual care" group) despite a lower median maternal 25(OH)D and higher incidence of VDD at delivery in group 1 compared to the usual care group (277). In a study of older adults, 25(OH)D level had returned to baseline by approximately 112 days after a single oral dose of 100 000 IU cholecalciferol (326), and one study has shown that the half-life of 25(OH)D is similar in pregnant and non-pregnant women (324). As such, it remains likely that a higher 25(OH)D was achieved earlier in pregnancy in Group 1 but that the difference was no longer detectable at delivery, which could have been up to 30 weeks after cholecalciferol dosing in some women in group 1.

Finally, the greater birth size in the intervention groups in the study by Kalra et al might have occurred due to differences in calcium supplementation. Although all women were given 1g/day elemental calcium from study entry, this was commenced at 12-24 weeks in women randomised to the cholecalciferol treatment protocols but started in the third trimester in the "usual care" group. This inconsistency raises uncertainty as to whether the differences in vitamin D or calcium supplementation were actually responsible for the effect reported, as antenatal calcium supplementation has also been shown to increase offspring birth weight (327). Hashemipour et al also provided calcium supplementation to all women and therefore it is possible that the effect of vitamin D supplementation on birth size is dependent on adequate calcium intake. Trials of vitamin D+calcium supplementation compared to vitamin D alone and placebo are needed to assess this, but combined calcium (500-600 mg/day) and vitamin D supplementation (200 IU/day) has been shown to increase the risk of preterm birth despite reducing the risk of preeclampsia (328), potentially limiting the feasibility of it as a clinical intervention to increase birth weight.

10.3.2 Size and growth in infancy

Similarly to the findings in relation to birth size, maternal supplementation with 1000 IU/day cholecalciferol during pregnancy did not affect offspring height, weight or OFC at 1 or 2 years of age, or the rate of growth in these parameters between birth, 1 and 2 years of age. This finding is in contrast to three previous intervention studies examining the effect of gestational vitamin D supplementation on offspring growth in the first year of life (277, 279, 284). Interestingly, those studies were all performed in women of Asian ethnicity, albeit one included women of Asian ethnicity living in the UK in 1978-1979 (284). In contrast, the MAVIDOS study was comprised

predominantly of women of white ethnicity, reflecting the local populations from which the participants were drawn. This could suggest that the effect of vitamin D supplementation on offspring early postnatal growth is dependent on ethnicity/racially determined genetic profiles.

As previously discussed with regards to the birth weight findings, care should be taken in the interpretation of the findings of Kalra et al who found greater weight, length and OFC at 3, 6 and 9 months of age in infants born to mothers randomised to one of two high dose intermittent oral cholecalciferol regimens or "usual care" due to both the open-label nature of the study design, and more importantly, the unmatched use of calcium supplementation in the three randomisation groups (277). Additionally the exact age of the offspring at each follow-up is not reported or compared between groups and the data is presented without adjustment for age, which is likely to contribute considerably to anthropometric measurements in the first year of life.

Roth et al in the AViDD double-blind RCT in Bangladesh found a 0.44 greater length z-score at 1 year in infants of mothers randomised to cholecalciferol supplementation compared to placebo, despite similar lengths at birth (279). There was no difference in weight, weight for length or OFC z-score between the groups at 1 year. In similarity to the studies with positive findings related to birth size, a cholecalciferol dose of 35 000 IU/week for a median of 10 weeks was used in that study, which is five times higher than the total weekly dose in the MAVIDOS trial. This achieved a 25(OH)D concentration after treatment nearly twice that of our study, with similar 25(OH)D concentration in the placebo arms of both studies (mean 38.3 nmol/l [SD 18.1] in AVIDD and 43.3 nmol/l [SD 22.3] in MAVIDOS). Although this might suggest that higher cholecalciferol doses than were given in MAVIDOS are required to affect offspring growth in infancy, Brooke et al did report a significant effect of vitamin D supplementation in the last trimester on offspring weight and length at 9 and 12 months in Asian women in the UK using a similar dose to MAVIDOS (284). However, Brooke et al supplemented the women using ergocalciferol, which has been suggested to increase 25(OH)D to a lower extent than the same dose of cholecalciferol (329), but mean maternal 25(OH)D at delivery was 168.0 nmol/l in the supplemented women compared to 16.2 nmol/l in the placebo group, raising some doubt over either the actual dose of ergocalciferol used or the 25(OH)D analysis (242).

A number of other differences between the AViDD and MAVIDOS cohorts, in addition to ethnicity and vitamin D dose, might have contributed to the contrasting findings. Mothers in the AViDD trial were on average 8 years younger than in MAVIDOS, but it is unclear how this would affect the outcome. Perhaps more importantly are differences in the expected size of the offspring. In both the AViDD study, and this analysis of the MAVIDOS cohort, the WHO growth standards were used to generate length and weight z-scores for age and sex, hence enabling a direct comparison

of the two cohorts. Stunting was common in the AViDD cohort in both the placebo and supplemented arms, and overall the mean length z-score at 1 year of age in the placebo group of AViDD (-1.33 [SD 1.2]) was considerably lower than in MAVIDOS (-0.13 [SD 0.97]). This may reflect the genetic growth potential as maternal height was approximately 15 cm shorter in AViDD compared to MAVIDOS, but dietary differences and other micronutrient deficiencies, although not assessed in either study, could contribute to the different findings. Moreover, maternal 25(OH)D in pregnancy has been negatively associated with the incidence of acute respiratory illnesses in the offspring during infancy, although RCTs assessing the effect of supplementation on this outcome are also inconsistent (330). As such, the positive effect of vitamin D supplementation on growth in the study in Bangladesh could be secondary to a reduction in acute infectious diseases in infancy, although this outcome was not assessed in either study.

10.3.2.1 Subgroup analyses and size at birth and in infancy

Observational studies assessing the relationship between maternal 25(OH)D concentration and offspring weight, length, OFC at birth and in infancy have suggested that the effects are particularly evident at very low levels of 25(OH)D (260, 264, 275, 276, 282). Due to stipulations from the research ethics committee, women with baseline 25(OH)D < 25 nmol/l were not eligible to participate in the MAVIDOS study. This could contribute to the null findings, although stratification of the women by either median baseline 25(OH)D (45.3 nmol/l) or 25(OH)D < 30 nmol/l did not reveal any differences between the intervention and placebo group in women below these thresholds. These analyses were however limited by a lower power to detect a significant difference.

Significantly greater birth weight z-score, OFC and MUAC were identified in infants born in winter months to mothers randomised to cholecalciferol. It should be noted that this finding of a statistical interaction between season of birth and maternal cholecalciferol supplementation in determining birth weight differed to that in the published report of the primary outcome of the MAVIDOS trial (295). This will reflect the a priori decision to exclude infants born preterm in the analysis included here, which is in contrast to the primary trial analysis, which included all births independent of gestation at delivery. The different methodological approaches reflect the varying hypotheses assessed; the analyses included here concentrated on size, growth and body composition in infancy and early childhood in addition to at birth, and these outcomes are known to be altered by prematurity and its management (331, 332). Therefore exclusion of these children from the analysis was considered appropriate. As those born preterm were exposed to the intervention for less time, exclusion of these children might account for the significant effect

of cholecalciferol on birth weight in winter-born term infants, or this difference might suggest that late pregnancy is a critical period for the effect of antenatal vitamin D exposure on offspring size.

The reasons for an effect of cholecalciferol supplementation on birth anthropometry only in winter-born infants are unclear; the mean difference in late pregnancy 25(OH)D concentration between the intervention and placebo groups was highest for winter births. Whilst this might have contributed to an effect, the mean 25(OH)D difference between the groups in winter was only 0.5 nmol/l higher than the difference between the groups for spring births, in which an effect of cholecalciferol on birth size was not observed. It has also been previously shown in the MAVIDOS study that supplementation with 1000 IU/day cholecalciferol prevents the decline in 25(OH)D from early to late pregnancy that was evident in women who delivered in winter and spring months in the placebo group (Figure 10.2). Prevention of the decline in 25(OH)D might be important to the observed effect of vitamin D supplementation on birth size in winter-born infants, but again it is unclear why a similar difference was not observed between the two groups in the spring born children. When the definition of winter was shifted to one month earlier or later, the significant differences in these outcomes between the randomisation groups were no longer present, and these differences did not persist into later infancy, therefore raising the possibility that this was a chance finding (type 1 error) and the need for replication of this finding in further high quality intervention studies. But, if this is indeed a true finding, then the clinical relevance of the difference does need to be considered. Birth weight has been associated with LS and hip BMC (135) and grip strength (119) in adulthood: in meta-analysis a 1 kg increase in birth weight was associated with a 1.41 g increase in hip BMC in adulthood. Using this, it was estimated to equate to a relative risk of hip fracture of 1.12 for every 1 kg decrease in birth weight (135). Thus, a 0.19 kg increase in birth weight as a result of cholecalciferol treatment could result in a small reduction in hip fracture incidence (relative risk of hip fracture=0.98).

10.4 Vitamin D supplementation in pregnancy and offspring body composition in childhood

10.4.1 Adiposity

The two previously reported trials assessing the effect of vitamin D supplementation during pregnancy on offspring body composition assessed adiposity in the neonatal period (242, 251). This is the first study to examine the effect on body composition in later infancy and early

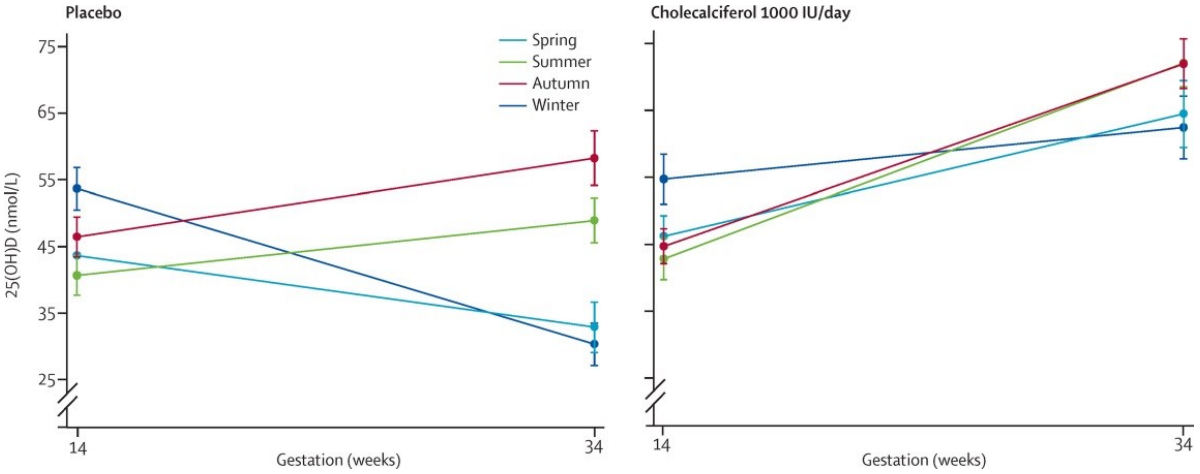


Figure 10.2: Maternal 25(OH)D status at randomisation (14 weeks) and 34 weeks of gestation by randomisation group and season of delivery
Data shown are mean and 95% CI. Winter is December to February, spring is March to May, summer is June to August, and autumn is September to November
Reproduced with permission from Cooper et al, 2016 (295)

childhood. In this study, 1000 IU/day cholecalciferol during pregnancy did not affect offspring adiposity assessed by MUAC or SFT at birth, 1 or 2 years of age, or in a smaller subset of children WBLH FM, LM or BMC measured using DXA at 4 years of age. Trunk FM used as a measure of central adiposity also did not differ between the two groups. Previous analysis of whole body DXA scans obtained within 14 days of birth in this study also found no significant difference in FM,LM or BMC between the randomisation groups overall. Similarly to birth size and BMC, FM but not LM was significantly greater in neonates born to mothers randomised to cholecalciferol and born in winter months. Differences in soft tissue body composition by randomisation group were not evident for the other birth seasons (295). The difference observed between the randomisation groups in winter births also did not persist at 4 years of age.

Several lines of evidence suggest a possible role for vitamin D in adipogenesis. Firstly, the VDR and vitamin D metabolizing enzymes are expressed in human adipose tissue (333), suggesting a likely functional role for vitamin D. Secondly, in the 3T3-L1 cell line (mouse embryonic fibroblast cell line, which is responsive to lipogenic hormones and can be induced to differentiate into an adipocyte-like cell), VDR expression reduces with progression of differentiation. Furthermore, $1,25(OH)_2D$ can inhibit the differentiation into adipocytes, albeit only at a very early stage of differentiation (334). However, such an effect is not consistent across all cell types. Both $25(OH)D_3$ and $1,25(OH)_2D_3$ promoted adipogenesis in cultured human pre-adipocytes derived from adipose tissue from middle-aged adults (335). These inconsistencies highlight the need for *in vivo* data

Previous observational data on the relationships between maternal 25(OH)D and offspring adiposity (167, 259, 265, 271, 273, 299) and findings from intervention studies of vitamin D supplementation in pregnancy with regards to this outcome are inconsistent (242, 251), but justified the need for a large intervention study. Moreover the two previous trials were both conducted in women of Asian ethnicity and are limited by their size and assessment of adiposity using SFT and MUAC (251, 284). In the work of Marya et al, two high doses of cholecalciferol in late pregnancy did result in a significant increase in offspring triceps and subscapular SFT and MUAC at birth (251), whereas Brooke et al, similarly to MAVIDOS, found that after supplementation with 1000 IU/day ergocalciferol that neonatal triceps SFT was not increased (242, 284).

Interestingly the null results of the MAVIDOS study in relation to neonatal adiposity, measured either by SFT or MUAC in this analysis or by DXA in the previously published analysis (295), are not consistent with the observational data from the SWS (167), despite both studies being conducted in the same geographic location, and having similar cohort demographics. Indeed, in the SWS maternal 25(OH)D in late pregnancy was negatively associated with offspring FM measured by DXA at birth. The difference in findings between the SWS and MAVIDOS could result from residual confounding in the observational associations from the SWS, or insufficient power to detect a small difference in the MAVIDOS study. In the SWS, neonatal FM was 8% and 10% lower in infants born to mothers with 25(OH)D < 50 nmol/l compared to 50-75 nmol/l and > 75 nmol/l, respectively (167). As the mean difference in 25(OH)D in late pregnancy between the two treatment groups in MAVIDOS was approximately 25 nmol/l, this might have been insufficient to result in a clinically detectable difference in FM.

At 4 years of age in the SWS, there was no significant relationship between maternal 25(OH)D status in late pregnancy and offspring FM, but at 6 years of age, the relationship was inverse to that observed in the neonatal period (167). Adiposity (or BMI) rebound is the age at which the decline in BMI during late infancy reaches a nadir and begins to increase again, typically occurring between 5 and 7 years of age. Earlier age at adiposity rebound (AR) is a well-recognised risk factor for adult obesity. The shift in direction of the association between maternal 25(OH)D in late pregnancy and offspring adiposity in the SWS has occurred at a similar age to the AR. Catch-up growth in the first year of life, but not birth weight or length, has been associated with earlier AR (336), suggesting that in utero nutritional deficiencies might be important. Diabetes in pregnancy (gestational or type 1) has also been associated with an earlier age at AR (337) further highlighting a possible role of fetal programming in the timing of AR. Whilst there are no studies that have assessed the association between maternal 25(OH)D and AR, an effect on AR could potentially mediate the changing relationships with FM observed in the SWS.

In the MAVIDOS study assessments of offspring adiposity were performed at 1 and 2 years of age with measurements of SFT, and at 4 years by DXA. The timings of these measurements therefore coincide with the transition period for the relationship between maternal 25(OH)D and offspring FM that was observed in the SWS and this could account for the null findings. A recent animal study found that maternal VDD during pregnancy in mice increased expression of peroxisome proliferator-activated receptors-γ (PPARγ), a key regulator of adipogenesis, in male offspring at 75 days of age despite no difference in the size of fat depots (338). This suggests potential long term effects of in utero VDD on metabolic programming, which could result in clinical findings emerging with aging. Further follow-up of the MAVIDOS children in later childhood is therefore needed to determine if any effect on adiposity becomes apparent.

10.4.2 Lean mass

This work included both analysis of observational data from the SWS and interventional data from MAVIDOS assessing an effect of in utero vitamin D exposure on muscle size and strength. There are few previous data relating maternal vitamin D status to offspring LM, despite growing evidence that vitamin D might be related to muscle mass and function, including isolation of the VDR in skeletal muscle (150), myopathy and delays in motor development as a feature of severe VDD (203, 204) and increased muscle strength following supplementation in adults (211).

In the SWS, maternal 25(OH)D concentration in late pregnancy was positively associated with offspring %LM and LM adjusted for FM at 4 years of age in unadjusted analyses. %LM and LM adjusted for FM were used as the outcome to account for higher absolute LM observed in more adipose children. The effect sizes were, however, small and the relationships were no longer statistically significant after adjustment for maternal covariates. The relationship with appendicular LM was also considered to provide a clearer estimate of the effect on muscle compartment size as whole body LM includes both muscle and visceral tissue. However a positive association in univariate analysis with offspring appendicular %LM was similarly attenuated by the addition of maternal covariates and the child's physical activity in a multivariate model. These findings are in contrast to two previous observational studies. In the MPS in India, maternal 25(OH)D status at 28-32 weeks of gestation was positively associated with arm muscle area across the whole cohort at ages 5 and 9 years and with percentage FFM measured by BIA in boys at 5 but not 9 years after adjustment for age, sex, maternal BMI, GDM, parity, socioeconomic status and religion (300). Consistent with this, in the ALSPAC cohort a positive association between maternal estimated UVB exposure in the third trimester used as a proxy marker of maternal 25(OH)D

concentration and offspring WBLH LM assessed by DXA at 9 years of age was reported (291), but subsequent analysis has suggested collinearity between estimated UVB exposure and age at DXA and therefore this finding should be interpreted with caution (292). There are marked differences between the SWS and these two studies, including the exposure definition (25(OH)D concentration or estimated sunlight exposure), the method used to assess LM and covariates included in adjustments. Furthermore, the interpretation of observational data in relation to vitamin D status is complicated by the high risk of confounding and the potential for reverse causality. As 25(OH)D status is primarily environmentally determined, in developed countries it is typically higher in individuals who do not have chronic illness, are more physically active and able to spend time outdoors. Indeed in the SWS, women who participated in more exercise in pregnancy had higher 25(OH)D, but such relationships might differ in developing countries where exposure to outdoor manual work is more common. This could result in differing directions of associations in different population groups. Furthermore, physical activity and time spent outdoors also typically correlate highly between parents and pre-school children in developed countries (339), such that disentangling whether the maternal 25(OH)D is causally related to an outcome such as LM, or simply co-linear with other related characteristics is difficult.

In the MAVIDOS trial, in agreement with the findings in the SWS, antenatal supplementation with 1000 IU/day cholecalciferol did not result in greater offspring WBLH LM, %LM or appendicular LM at 4 years of age. Meta-analysis of trials has also suggested that vitamin D supplementation does not affect LM in adults (211). This analysis of offspring body composition in MAVIDOS does however only represent approximately half of those eligible for follow-up, and data collection at 4 years in ongoing.

10.4.3 Bone mass

MAVIDOS is the first study to examine the relationship between maternal vitamin D supplementation in pregnancy and offspring bone mass in childhood. In the subset of children included in this analysis at 4 years of age, there was no significant difference in WBLH BMC by randomisation group. This is consistent with the null finding at birth (295). Detailed analysis of the data with regards to bone outcomes, including WBLH, hip and LS bone area, BMD, BMC and BMAD in addition to other adjustments for body size will be undertaken once the complete dataset is available.

10.5 Vitamin D supplementation in pregnancy and offspring muscle strength in childhood

In the SWS, despite no statistically significant association with LM, maternal 25(OH)D status in late pregnancy was positively associated with offspring grip strength at age 4 years. This relationship was robust to adjustment for a number of maternal covariates, including reported maternal physical activity in late pregnancy, and objectively measured physical activity in the child. However, in the MAVIDOS trial, grip strength was not significantly greater in children born to mothers who received the cholecalciferol supplementation across the full subset of children who have been followed-up to date. A 0.7 SD difference in grip strength was observed between the vitamin D and placebo groups in mothers with a 25(OH)D < 30 nmol/l at randomisation, suggesting that supplementation might have beneficial effects on offspring muscle strength in those with lower levels of 25(OH)D. Further studies including large numbers of women with low levels of 25(OH)D are needed to support this finding for two reasons. Firstly although a threshold of 30 nmol/l has been advised by the Institute of Medicine (160) and the Global Consensus Recommendations on the Prevention and Management of Rickets (163) to define VDD, this definition was created in relation to bone health and not other outcomes, and remains hotly debated (161). Secondly, women with a 25(OH)D < 25 nmol/l at screening were excluded from participation in the MAVIDOS study and therefore the number of women with 25(OH)D < 30 nmol/l at randomisation is small and likely to reflect the upper range within this threshold, although due to the time lag between screening and randomisation some women did have 25(OH)D < 25 nmol/l at randomisation.

Only the MPS has previously investigated the relationship between maternal vitamin D status and offspring muscle strength in an observational study. No significant association was found between maternal 25(OH)D at 28-32 weeks of gestation and grip strength at 9 years of age in this cohort in India (300). Pubertal status was not assessed in that study, but given the age of participants, sex hormone exposure in early puberty in some children might have obscured any relationship.

The lack of association with LM, as discussed previously, would suggest that the relationship between in utero vitamin D exposure and grip strength might be mediated via an effect on muscle function independent of an increase in muscle mass. This is consistent with the increase in muscle strength but not LM as a result of vitamin D supplementation in older adults (211). There are a number of potential mechanisms that could account for this. Firstly, muscle fibre number is largely set in utero and muscle size increases by hypertrophy postnatally (340, 341) so it is possible that maternal 25(OH)D concentrations in some way influences fibre number more than

overall mass. A number of lines of evidence support this theory: VDD in later life has been shown to result in selective atrophy of type II muscle fibers (205, 206). These fibres are necessary for rapid bursts of speed and power and are therefore likely to be involved in the action required for grip strength assessment. In the VDR knock out mouse model, muscle fibre size is approximately 20% smaller than in the $VDR^{+/+}$ mouse, with increased spacing of fibres, although this was evident for both type I and type II muscle fibres (342). These effects were seen despite no differences in serum calcium and phosphate status. Similarly, Zhou et al have recently shown that a high vitamin D diet in pigs during pregnancy increases offspring muscle fibre number and density at birth compared to a normal vitamin D diet (343). In contrast to our findings in later childhood, at weaning muscle weight and cross-sectional area were significantly increased in the piglets from the maternal high vitamin D diet group despite no differences at birth. Differences in expression of myogenic regulatory factors between the two groups were also observed.

Secondly, the association between maternal 25(OH)D and offspring grip strength but not LM might reflect intramuscular adipose tissue (IMAT) accumulation. Anthropometric measurements including arm muscle area and LM measured by DXA are unable to distinguish LM from IMAT, which could account for the discrepancy in relationships. In young women IMAT is inversely associated with 25(OH)D concentration independent of BMI and muscle area (344) and muscle adiposity has been negatively related to muscle strength (345, 346). An in vivo study has suggested that exposure to low $1,25(OH)_2D_3$ results in transdifferentiation of a muscle cell line to adipocytes (347). Although the translation of this to clinical studies is limited by the use of $1,25(OH)_2D_3$, which is tightly regulated in the human body and unlikely to fall to very low levels unless 25(OH)D deficiency is extremely severe, it does highlight the need for further clinical studies. A measure of IMAT can be obtained from pQCT, which has been undertaken in the MAVIDOS study at the 4 year follow-up visit. This data was not available for inclusion in this thesis but will be analysed in due course.

Stratification by sex revealed that the association between maternal 25(OH)D and offspring grip strength was only statistically significant in girls but the statistical interaction was not significant. A similar pattern was observed in MAVIDOS although the difference between cholecalciferol and placebo did not reach statistical significance in either sex. Previous studies have suggested pre-school boys are more physically active than girls (339), although objectively measured moderate-vigorous physical activity at 4 years was similar in the boys and girls in the SWS. Greater physical activity in boys could obscure the relationship between in utero vitamin D exposure and grip strength in males. Alternatively true sex differences in the effect of vitamin D on the fetal programming of muscle strength may be present. One of the key mechanisms believed to mediate the interaction between the in utero environment and clinical characteristics is

epigenetics; epigenetic modifications, including DNA methylation and histone modifications, are stable heritable changes, which can influence gene transcription, but do not affect the DNA sequence. Sex differences in methylation have been observed in cord blood in relation to in utero environmental exposures, including maternal smoking status (348) and cadmium exposure (349). To date there are very few published data assessing whether vitamin D can influence methylation status. These are inconsistent. Maternal free vitamin D index (ratio of serum 25(OH)D to DBP) in late pregnancy was negatively associated with methylation at one of 5 studied CpG sites in the promoter region of the RXR-A in umbilical cord tissue in the SWS (350). In contrast maternal 25(OH)D status at either 18 or 28 weeks of gestation were not associated with offspring genome wide methylation in cord blood in a different observational cohort (351). Stratification by sex was not reported in either study, and it is possible that methylation differs between tissue types. In the MAVIDOS study, differences in methylation at the RXR-A promoter region in umbilical cord tissue samples by randomisation group has been observed (352). Genome wide methylation and stratification by sex has not been undertaken, but cord blood samples and, in some children, venous blood samples at age 4 years are available and would enable future studies of this.

Overall, it is evident that the observational relationships between maternal 25(OH)D and offspring body composition and muscle strength do not translate to differences in these outcomes following supplementation with 1000 IU/day cholecalciferol during the second and third trimester. I have already discussed a number of possible reasons for these differences including residual confounding in the observational relationships, the exclusion of women with very low levels of 25(OH)D from the study, the statistical power of both MAVIDOS and other studies to detect a clinical difference and the size of the supplementation dose. One final possibility for these differences is the timing of the introduction of supplementation. Intervention studies of antenatal vitamin D supplementation to date, including MAVIDOS, have typically initiated supplementation during the second or third trimester. As vitamin D status tracks moderately during pregnancy, women with a low 25(OH)D level in mid to late pregnancy are also likely to have a low level at pre-conception and early pregnancy. Myogenesis begins at 8-10 weeks of gestation and adipogenesis from approximately 14 weeks of gestation. It is therefore possible that the vitamin D supplement was commenced after a potential critical window to alter development. Indeed in animal models, maternal nutritional depletion limited only to the peri-implantation period results in long-term changes to growth, cardiovascular and metabolic health (353). A pre-conception intervention study is necessary to explore whether maternal 25(OH)D status around conception and in early pregnancy is key to affecting offspring health.

10.6 Determinants of the 25(OH)D response to vitamin D supplementation in pregnancy

Although these analyses have not demonstrated statistically significant effects of gestational vitamin D supplementation on offspring growth and body composition, optimising maternal 25(OH)D status is important for the prevention of neonatal hypocalcaemia, and in the MAVIDOS study, 1000 IU/day cholecalciferol increased neonatal bone mass and birth weight in those born in winter months (295). Furthermore, recent meta-analyses of data from RCTs have suggested that antenatal vitamin D supplementation reduces the risk of pre-term birth (354) and low birth weight (defined as < 2.5kg) (328). As such, understanding the uptake of supplementation and how maternal characteristics modify the response to vitamin D supplementation remains clinically important.

10.6.1 Uptake of vitamin D supplementation in the SWS

In the SWS, demographic differences were observed between women who did and did not take vitamin D supplementation during pregnancy. Women who either never started or stopped vitamin D supplementation after early pregnancy were younger, less well educated, more likely to smoke, less likely to be in their first pregnancy and had higher pre-pregnancy BMI than women who continued supplementation throughout pregnancy. Although the interpretation of this finding is limited by the majority of women experiencing pregnancy before the publication of the UK DH guidelines, which suggest that all women should receive supplementation (248), similar demographic factors have also been associated with reduced likelihood of folic acid supplementation during pregnancy (355, 356). Moreover, younger maternal age, higher pre-pregnancy BMI and smoking were all significantly associated with lower season-corrected 25(OH)D status in the SWS independent of vitamin D supplement use, further highlighting that women with these characteristics might require additional health education during early pregnancy.

10.6.2 Maternal characteristics and the 25(OH)D response to vitamin D supplementation in the MAVIDOS trial

1000 IU/day cholecalciferol achieved vitamin D repletion (> 50 nmol/l) in over 80% women in the MAVIDOS trial, without causing 25(OH)D levels potentially associated with vitamin D toxicity.

However, women with biochemically high 25(OH)D at baseline (> 100 nmol/l) were excluded from participation. These women might have been at higher risk of toxicity with supplementation, although in a previous study in pregnant women 4000 IU/day did not result in hypercalcaemia, hypercalciuria (based on a spot urine sample rather than 24 hour collection) or clinical side effects (246). Similarly, women with 25(OH)D < 25 nmol/l were excluded from the study. As more women were excluded due to low compared with high 25(OH)D, the repletion rate across the whole population would be expected to be lower than observed in the trial population.

Gaining less weight during pregnancy, having a higher 25(OH)D in early pregnancy, delivering in summer and having higher compliance with supplementation were independently associated with achieving a greater 25(OH)D concentration in late pregnancy amongst women randomised to vitamin D supplementation. Thus, those women who are at risk of vitamin D insufficiency in early pregnancy, gain more weight, and deliver in winter might need supplementation with a higher dose of cholecalciferol to achieve similar 25(OH)D concentrations.

To my knowledge, the factors which determine the response to vitamin D supplementation in pregnancy have not previously been assessed. However, these findings are consistent with previous observations in non-pregnant adults (255, 256). Studies in non-pregnant adults have also shown that obese individuals achieve a lower 25(OH)D with the same dose of supplementation as non-obese individuals (256). Meta-analysis of vitamin D supplementation studies has suggested that over 50% of the variance in 25(OH)D increment in response to supplementation is explained by body weight (255). In this study, pre-pregnancy BMI and late pregnancy triceps skinfold thickness (as a marker of adiposity) were not associated with 25(OH)D after supplementation in multivariate analysis, but pregnancy weight gain was negatively associated. This is consistent with the findings in the SWS in which greater gestational weight gain was negatively associated with the tracking of 25(OH)D from early to late pregnancy independent of supplement use, and therefore similarly suggests that overall volume of dilution and not just adiposity may be important for the response to vitamin D supplementation in pregnancy. However, interestingly when using a threshold of 25(OH)D > 50 nmol/l as a definition for repletion, pregnancy weight gain was not an independent predictor of achieving vitamin D repletion. This could reflect the relatively small effect size of 0.8 nmol/l decrease in achieved 25(OH)D for every kilogram of weight gain or a non-linear relationship between weight gain and achieved 25(OH)D.

Despite receiving 1000 IU cholecalciferol per day, 17% of women overall and 25% of mothers who delivered in winter had a 25(OH)D less than 50 nmol/l in late pregnancy. This is a higher non-repletion rate that that reported in other recent pregnancy supplementation studies, although

variation in dose might contribute to this difference. For example, Grant et al, reported 91% of women who received 1000 IU/day cholecalciferol in pregnancy in New Zealand achieved 25(OH)D > 50 nmol/l at 36 weeks of gestation (240). There are several possible reasons for the difference in finding. Firstly, that study was conducted at 36°South, compared with 50.9-53.4°North for MAVIDOS so greater cutaneous production of vitamin D might have contributed to the higher repletion rate. However, interestingly in Grant et al's study the vitamin D repletion rate in women who received 1000 IU/day was not significantly different from that for women who received 2000 IU/day cholecalciferol, potentially suggesting a ceiling effect. Secondly, maternal 25(OH)D at randomisation was higher in Grant et al's study. In MAVIDOS, the baseline 25(OH)D was positively associated with both the likelihood of achieving vitamin D replete status and absolute 25(OH)D achieved at 34 weeks of gestation, hence the higher 25(OH)D at randomisation in the study in New Zealand might account for the higher repletion rate. Alternatively, ethnic variation in common genetic variation in components of the vitamin D metabolism pathway could account for a greater repletion rate in the multi-ethnic study population of Grant et al compared to MAVIDOS (357).

We observed that the difference between the 25(OH)D achieved at 34 weeks of gestation in women randomised to placebo compared with cholecalciferol decreased with increasing baseline 25(OH)D (Figure 9.3, page 172). This is consistent with previous studies in adults, which have shown that the incremental response to vitamin D supplementation is higher in vitamin D insufficient than replete subjects (255, 256) and that the increase in 25(OH)D relative to supplementation dose is negatively associated with dose of vitamin D supplement (358). This suggests that physiological processes such as saturation of the hepatic 25-hydroxylase limit attainment of very high 25(OH)D concentrations (326).

It is evident from these findings that 1000 IU/day cholecalciferol does not eliminate the seasonal variation in 25(OH)D status that was observed in women in the SWS during pregnancy, and therefore from a comparable latitude. Non-white ethnicity was also associated with a higher risk of not achieving vitamin D replete status in the supplemented women. Hollis et al similarly found that even with 4000 IU/day cholecalciferol during pregnancy that women of African-American ethnicity had lower 25(OH)D than Caucasian or Hispanic women (246). Possible explanations for this include, firstly, that even with this degree of supplementation cutaneous biosynthesis of vitamin D still contributes to 25(OH)D status and this contribution is lower in both winter and in non-White women. Secondly, a lower baseline 25(OH)D could also account for the lower achieved 25(OH)D in non-White women. Thirdly genetic variation might underlie the findings. Both common genetic variation in DBP and a number of single nucleotide polymorphisms (SNP) within the vitamin D metabolism pathway, including in genes encoding 7-dehydrocholesterol

reductase in the skin, 25-hydroxylase, 24-hydroxylase and DBP have been identified as significantly associated with 25(OH)D status (359, 360). In women of white ethnicity in the MAVIDOS study, SNPs in genes encoding 25-hydroxylase (*CYP2R1*) and DBP (GC) are associated with the achieved 25(OH)D after supplementation (357). Due to the small number of non-white participants in the MAVIDOS cohort, they were not included in this analysis, but ethnic clustering of genotypes associated with lower 25(OH)D might therefore contribute to the lower likelihood of vitamin D repletion in non-white women. Future studies should also aim to establish the dose required to achieve optimal 25(OH)D status amongst women of non-white ethnicity and amongst those who deliver in winter months.

10.7 Strengths and limitations of this work

10.7.1 Study cohorts

The studies used in these analyses are large and rigorously conducted with comprehensive phenotyping of the mothers and offspring. However, they are not without limitations. The strengths and limitations of the methods and assessment techniques will be discussed here.

10.7.1.1 The SWS

The SWS is a prospective birth cohort. It is unique in that mothers were recruited and assessed before they were pregnant. This approach has the advantage of characterising the mothers, particularly with regards to BMI and measures of adiposity, before there were changes as a result of pregnancy. Conversely, the time-lag between the pre-pregnancy and early pregnancy assessments does provide the opportunity for these characteristics to change.

75% of women invited to participate in the SWS consented to take part in the pre-pregnancy phase of the study. These women are self-selected and therefore more likely to be healthy, but they do still encompass a wide range of demographic characteristics and, importantly for these analyses, 25(OH)D concentrations. Additionally there was reliance on the participant to inform the research centre of a pregnancy, which may have resulted in further self-selection to the pregnancy phase of the study. Furthermore, those included in the analysis at 4 years of age tended to be older, better educated and less likely to smoke. This needs to be considered in the

generalisability of the data to the wider population, but as all the relationships were internal it is unlikely to affect the findings.

The SWS is an observational study and causality cannot be inferred from the observed relationships between maternal 25(OH)D status and offspring grip strength due to the potential for confounding. The wealth of data collected in the SWS did enable a range of maternal and child-related covariates to be included in the models but cannot eliminate all potential sources of confounding. Nonetheless the associations are biologically plausible, and moreover the MAVIDOS has provided a unique opportunity to translate the observational findings to an intervention study.

10.7.1.2 MAVIDOS

The MAVIDOS trial is currently the largest completed RCT of vitamin D supplementation in pregnancy. The double-blind placebo controlled nature of the study generates high quality evidence with low risk of bias, and the large number of participants provides higher statistical power than some of the earlier much smaller studies of gestational vitamin D supplementation. However, due to the five year timeframe required to recruit such large numbers of women into the study, the analyses presented here on body composition and grip strength at 4 years of age do not include the full cohort. Analysis of the full cohort will be undertaken once data collection has been completed, and will increase the statistical power and reduce the likelihood of falsely accepting the null hypothesis.

The main limitation of the MAVIDOS study is the exclusion of women with very low levels of 25(OH)D at screening. This was based on recommendation from the ethics committee that these women should receive supplementation despite allowing all participants to take up to 400 IU/day vitamin D, as advised by the DH (248), if they wished. As many observational studies have suggested that any detrimental effects of low vitamin D only become apparent at very low levels of 25(OH)D, women with 25(OH)D < 25 nmol/l are potentially more likely to benefit from supplementation (260, 264, 275, 276, 282). This stipulation might therefore have increased the likelihood of a null finding.

Over 95% of the participants of MAVIDOS were of White ethnicity. This reflects the local populations from which the women were recruited and does give more homogeneity to the study population, but also limits the generalisability of the study to women from non-White ethnic groups.

10.7.2 25-hydroxyvitamin D assays

In the SWS, 25(OH)D was measured using different assays in early and late pregnancy. Differences in absolute 25(OH)D concentrations between assays is well recognised (152). Serum samples were stored at -70°C, and all samples from each of early and late pregnancy were analysed at the same time in a single batch. Thus each of the seasonal models was generated on the basis of a single assay, which was performed in a laboratory that is a member of the DEQAS scheme. It is therefore unlikely that the use of different assays in early and late pregnancy would have affected the rank change or tracking coefficient, but it cannot be concluded with certainty that the differing shapes of the graphs of 25(OH)D status by date of sampling in early and late pregnancy is due to a pregnancy-induced effect or secondary to differences in the assays.

In the MAVIDOS trial, both the baseline and 34 week gestation serum samples were analysed by chemiluminescence assay (Diasorin Liaison), although the screening 25(OH)D analysis was performed on the local hospital platform. Differences between the local hospital laboratory assay and measurements by the Diasorin Liaison method could contribute to some women having a 25(OH)D < 25 nmol/l at randomisation. Again, stored frozen serum samples were used for analysis in the MAVIDOS study, but it has been previously shown that the storage of serum at -80°C does not affect the stability of 25(OH)D (361).

10.7.3 Questionnaire data

Using interviewer-led questionnaires allows large amounts of data on demographic, dietary and health characteristics to be collected, but self-reported data relies on the accuracy and honesty of the participants to provide the correct information. There might be a tendency for women to under-report some health-related parameters, such as smoking, alcohol use and dietary intakes. Whilst this can lead to biases in the SWS data and other observational studies, the randomisation in MAVIDOS should minimise the effects of such reporting inaccuracies.

10.7.4 Anthropometry

Anthropometric measurements are subject to inter-observer error. All measurements in both the SWS and MAVIDOS were performed by a trained research nurse or doctor and following detailed protocols to improve the accuracy of the measurements. Regular training sessions with calculations of coefficients of variation were undertaken for research staff.

Due to the tendency for children to move, all measurements in the children (except weight) were repeated three times and an average taken in an attempt to obtain as precise a measurement as possible. In the mothers, SFT and circumferences were also repeated three times.

Mid-parental height was used as an indication of genetic growth potential. This relies on the accuracy of reported paternity. Height was measured for all the mothers, but in many cases, reported paternal height had to be used. Reported measurements tend to be higher than actual measurements (362), which will lead to a small discrepancy in calculated MPH.

10.7.5 Dual-energy X-ray Absorptiometry

DXA is the gold standard for assessment of body composition, but its use in children can be technically challenging. Some children simply refused to participate in this element of the study, and for others remaining still for the duration of the scan proved difficult. Any scans with excessive movement were excluded from the analysis. Micklesfield et al have previously demonstrated in adults that significant side to side differences in limb FM, FFM and BMC measured by DXA were not present (304) and this was the planned approach to manage movement on the DXA scans. In the MAVIDOS cohort small but statistically significant differences between limbs were present in the children. This could result from more subtle movement than is visible on the scan image, or from the placing of the ROIs. Nonetheless sensitivity analysis excluding the data from the children in which limb cross-imputation had been performed did not alter the overall findings.

Inaccuracies in soft tissue composition measurement due to difficulties with bone edge detection and/or large tissue depths were not largely an issue in these studies due to the use of specific paediatric software in the DXA instruments and the size of the children at 4 years of age.

10.7.6 Hand dynamometry to assess grip strength

Grip strength is an easily obtained measurement of muscle strength in large epidemiological settings, but in pre-school children is less straightforward than in adults as it additionally requires the child to be able to understand and follow instructions. Despite this, the children were able to cooperate, and the validity and reproducibility of grip strength measurements has been demonstrated in this age group previously (49, 363). Maximum grip strength was used as the primary outcome. For some children, it is evident that they become more aware of what they

need to do after the first attempt and subsequently put in more effort and obtain a higher reading. Conversely other children loose interest after multiple measurements. Use of the maximum reading will account for these events, although average grip strength was highly correlated with the maximum reading in both the SWS and MAVIDOS and the findings did not differ when average grip strength was used.

The hydraulic Jamar dynamometer used in the SWS and MAVIDOS at 4 years of age can be read to the nearest 0.5 kg. It is possible that the sensitivity of this instrument is insufficient to detect a small effect of cholecalciferol. After the follow-up of the MAVIDOS offspring at 4 years was commenced, an electronic Jamar instrument was sourced. This digitally reports grip strength to the nearest 0.1 kg, but lack of interchangeability between the two devices has been demonstrated (364), and therefore the study protocol was not changed to use this instrument. Follow-up of the MAVIDOS offspring at 6 years of age began in December 2016 and the electronic Jamar dynamometer is being used at that visit.

Alternative measures, including jumping mechanography and isokinetic dynamometry, can provide more detailed assessment of muscle function and power than isometric grip strength, but the equipment are more costly and procedures more time consuming to perform. In pre-school children the use of these methods is also likely to be limited by their ability to follow more complex instructions. Nonetheless, such assessments could be considered in future follow-up at an older age.

10.7.7 Physical activity assessment

Objective assessment of physical activity was undertaken in a subset of children in the SWS using the Actiheart device. A number of children had reactions to the stickers used to attach the electrodes to the skin, which limited the availability of the data. Nonetheless, the children with physical activity data were representative of those without this data.

10.7.8 Statistical methods

The statistical methods used in these analyses did not account for multiple testing. This approach was chosen due to substantial collinearity between outcomes (307), as correction for multiple testing can lead to falsely accepting the null hypothesis. Importantly, the findings are biologically plausible, and based on a priori hypothesis.

A number of subgroup analyses have been undertaken. Again, these were chosen scientifically based on my review of the literature, but subgroup analysis does increase the risk of a type 1 statistical error due to the number of analyses performed. Tests for statistical interaction have also been undertaken, and when statistically significant reduces the likelihood of the finding being significant due to chance. Subgroup analysis can also lead to an imbalance in the baseline characteristics that would be expected following randomisation, and hence adjustment for maternal characteristics that have previously been associated with the outcomes was undertaken. No other subgroup analyses were undertaken apart from those reported in the results section.

Finally, there is a specific limitation of the Fourier model used to model the seasonal variation in 25(OH)D during pregnancy in the SWS. The pregnancies spanned an 8 year time period, but the Fourier modelling approach assumes the seasonal pattern is identical each year. However, given year to year differences in factors which may determine cutaneous vitamin D synthesis, including sunlight, ambient temperature and cloud cover, it is unlikely that the pattern is identical every year. Nonetheless, the fit of the model was highly statistically significant.

10.8 Implications for clinical practice

The UK DH recommends that women should take a vitamin D supplement containing 400 IU/day throughout pregnancy (248). The findings of the MAVIDOS trial with regards to offspring size, growth, body composition and muscle strength do not support increasing the recommended supplementation dose to 1000 IU/day in all women with a 25(OH)D level in early pregnancy of 25-100 nmol/l in the UK. However, effects of cholecalciferol on birth size and bone mass (295) in infants born in winter would suggest increasing supplementation dose for that subgroup of women might be of benefit to offspring development. It remains possible that women who are more deficient at baseline than those included in the trial would require higher doses of cholecalciferol but this was not examined in this study. Indeed, the findings of these analyses suggest further investigation into the effect of antenatal cholecalciferol on offspring muscle strength is needed in vitamin D deficient women.

It is clear from the MAVIDOS trial that 1000 IU/day cholecalciferol does not abolish the seasonal variation in 25(OH)D status during late pregnancy and that a large proportion of women who were supplemented with this dose, and in particular those who delivered in winter months, will still have a 25(OH)D level in late pregnancy less than 50 nmol/l. As such, if the aim of

supplementation is to increase maternal 25(OH)D to > 50 nmol/l, which is often considered the definition for repletion, then it is likely that 400 IU/day will not achieve this in many women. However, whilst many observational studies do suggest that achieving higher 25(OH)D levels in pregnancy might have beneficial effects on offspring physical development, such a change in public health policy should be based on established benefits in high quality RCTs. It is also important to be certain that in addition to benefits, that a higher dose will not be harmful. The literature with regards to falls risk in older individuals suggests that moderate doses of vitamin D (600-1000 IU/day) may have a beneficial effect whilst high bolus doses increase the risk of falls (365). Whilst there were no obvious side effects of 1000 IU/day during pregnancy in MAVIDOS or up to 4000 IU/day in another pregnancy study (366) and there appears to be a ceiling effect to the achievable 25(OH)D following this level of supplementation when baseline 25(OH)D levels are high, until a clear benefit of higher dose antenatal supplementation has been demonstrated, it should not be recommended. Additionally, the lack of significant findings in the MAVIDOS study for some outcomes (eg offspring growth) for which significant effects of vitamin D supplementation were observed in other population groups highlights the need for public health policy to be based on data from a population similar to that in which the policy will be implemented.

A public health message needs to be clearly interpretable and easily implemented for all women. There is firstly a need to replicate the seasonal variation in the effect of antenatal cholecalciferol supplementation on offspring neonatal bone mass and size, but if demonstrated, public health approaches could be to recommend higher dose supplementation to all women, or just those with expected delivery dates in winter. The former would ensure an uncomplicated message for all women and avoid confusion about who to supplement, but would potentially expose around three quarters of pregnant women to higher than necessary doses of vitamin D supplementation. However, whilst the seasons have been distinctly divided for the subgroup analyses, the exact divisions are arbitrary and women delivering in late autumn or early spring might similarly benefit to those delivering in winter. These women would be missed by targeted supplementation, although such an approach would be at approximately a quarter of the cost of widespread supplementation.

This work has confirmed that a number of maternal factors are associated with lower 25(OH)D levels in pregnancy independent of supplementation usage, including younger age, non-White ethnicity, higher maternal BMI, lower exercise participation and smoking. Many of these characteristics were also associated with lower uptake of vitamin D supplementation. Furthermore, women with low 25(OH)D in early pregnancy are highly likely to continue to have low 25(OH)D levels for the population in late pregnancy, and low baseline 25(OH)D was

associated with lower achieved 25(OH)D following supplementation. There are several possible approaches to managing this in clinical practice. To some extent those with low 25(OH)D levels can be predicted from the characteristics mentioned above and therefore these women should receive appropriate counselling on the risk of VDD and be informed that supplementation is recommended. This should be routinely reviewed at every antenatal appointment. Alternatively 25(OH)D could be assessed as part of the routine early pregnancy screening and those with biochemically low levels again provided with the above advice. Such an approach would however come at an additional economic cost and might be difficult to justify in light of the low likelihood of harm from low dose cholecalciferol supplementation in those with higher levels of 25(OH)D. It perhaps needs to be demonstrated in research studies that dosing schedules based on baseline 25(OH)D will achieve higher vitamin D repletion in a greater number of women and improved clinical outcomes (eg neonatal hypocalcaemia) before measurement of 25(OH)D in early pregnancy could be deemed necessary. Similarly, it is clear that the degree of weight gain during pregnancy is associated with 25(OH)D status and the response to supplementation, highlighting the need for this to be reviewed regularly during pregnancy and for women with higher than recommended weight gain to be counselled on the need for vitamin D supplementation to maintain their 25(OH)D status.

10.9 Future research

This analysis did not include all the MAVIDOS offspring at 4 years of age due to ongoing data collection. This is likely to be completed in late 2018/early 2019 and thereafter reanalysis of the full data set will be undertaken. Additionally pQCT has been performed in the MAVIDOS offspring at 4 years of age and will be analysed once all data has been collected. This will provided a more detailed assessment of bone geometry, volumetric BMD and lower leg soft tissue parameters including muscle cross-sectional area and muscle density. Venous blood samples obtained at 4 years of age and currently stored at -80°C will enable further studies of epigenetic markers to be conducted.

The uniqueness of the MAVIDOS study also lends its hand to undertaking other hypothesis-generating analyses, in particular with regards to obstetric health and outcomes. Observational studies have related maternal 25(OH)D status to timing and mode of delivery, occurrence of gestational hypertension, pre-eclampsia and GDM (367) but similarly RCTs are lacking. Whilst this

thesis has concentrated on offspring physical development, analyses related to other outcomes are being explored.

It is clear that a future study is required in women who are vitamin D deficient (< 25 nmol/l) at baseline. Firstly, based on the observational data these are the women who are most likely to benefit from vitamin D supplementation, and the exclusion of these women from the MAVIDOS study might have contributed to the null findings. Secondly, the subgroup analysis of women with lower 25(OH)D concentration at randomisation found a significantly greater offspring grip strength in those randomised to cholecalciferol. Whilst the formal interaction term was not significant, this finding highlights the need to assess this outcome further specifically in this group of women.

10.10 Conclusions

In conclusion, 1000 IU/day cholecalciferol supplementation during pregnancy in women with 25(OH)D of 25-100 nmol/l at baseline increases maternal 25(OH)D status in late pregnancy but seasonal variation in 25(OH)D status remains evident. Despite this, supplementation at this dose in women in the UK did not affect offspring size at birth, growth or size in infancy, or soft-tissue body composition and muscle strength in early childhood across the whole cohort. In infants born in winter, birth weight was significantly higher following maternal vitamin D supplementation. Although this effect did not persist into early childhood, birth weight has been associated with long term clinical outcomes independent of later size. This finding therefore warrants replication in further studies. A significant effect of cholecalciferol supplementation on offspring grip strength in those born to mothers with the lowest levels of 25(OH)D in early pregnancy also highlights the need for further RCTs of antenatal vitamin D supplementation in women with vitamin D deficiency.

Appendices

Appendix A Ethical approvals for the Southampton Women's Survey

Appendix B Ethical approvals for the MAVIDOS trial and 4 year follow-up

Appendix C Participant information sheet for the MAVIDOS trial

Appendix D MAVIDOS 4 year follow up additional participant information sheets

Appendix E MAVIDOS 4 year questionnaire

Appendix A: Ethical approvals for the Southampton Women's Survey

Southampton University Hospitals NHS Trust

Southampton & S.W. Hants
Joint Research Ethics Committee
Trust Management Offices
Mailpoint 18
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Tel 01703 794912
Fax 01703 798678

Ref: TEW/TMD

cc Mayer

28 November 1997

Professor D Barker
MRC Epidemiology Unit
SGH

Dear Professor Barker

Submission No: 276/97 - Survey of diet, body composition and hormone levels in young women in Southampton.

Following the conditional approval and in response to your letter dated 12 November 1997, I am pleased to confirm **full approval** having received the information requested.

This will be brought to the attention of the Committee at their meeting on 16 December.

Yours sincerely,

pp **Dr T E Woodcock**
Honorary Secretary

Appendix A

Southampton University Hospitals NHS Trust

Southampton & S.W. Hants
Joint Research Ethics Committee
Trust Management Offices
Mailpoint 18
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Tel 01703 794912
Fax 01703 798678

Ref: TEW/CPW

cc Hazel

28 November 1997

Professor D Barker
MRC Epidemiology Unit
University of Southampton
SGH

Dear Professor Barker

RE: 307/97 - The effects of maternal nutrition, body composition and cardiovascular status on fetal development & metabolic programming.

The Joint Ethics Committee considered your application for the above study at its recent meeting and I am pleased to inform you that **approval was given**. May I draw your attention to the enclosed conditions of approval which must be complied with.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an Independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

The composition of the committee is enclosed for your files and confirms which members were present at the meeting. Most pharmaceutical companies request this information and we would be grateful if you could forward this to them if appropriate.

Should any unforeseen problem of either an ethical or procedural nature arise during the course of this research and you feel the Joint Ethics Committee may be of assistance, please do not hesitate to contact us.

Yours sincerely,

Dr T E Woodcock
Honorary Secretary
Joint Ethics Committee

Southampton University Hospitals NHS Trust

→ Hazel

Southampton & S.W. Hants
Joint Research Ethics Committee
Trust Management Offices
Mailpoint 18
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Tel 01703 794912
Fax 01703 798678

Ref: CPW/DBL

30
~~26~~th April 1999

Professor D J P Barker - Director
MRC Environmental Epidemiology Unit
(University of Southampton)
Southampton General Hospital

Dear Professor Barker

Submission No:089/99 -Follow-up of infants born to women in the Southampton Women's Survey.

Following the conditional approval and in response to your letter dated 13th April 1999, I am pleased to confirm **full approval** having received the amended consent forms.

This approval was granted on Chairmans action and was brought to the attention of the Committee at their meeting on 28th April 1999.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an independent EthicsCommittee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

Yours sincerely,

C Wilkinson

Clair Wilkinson (Ms)
Research Ethics Administrator

Appendix A

Hampshire and Isle of Wight NHS
Strategic Health Authority

Ref: CPW/HPH

SOUTHAMPTON & SOUTH WEST HAMPSHIRE
LOCAL RESEARCH ETHICS COMMITTEES
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
SO16 4 RJ

Tel: 023 8036 2466
023 8036 3462
Fax: 023 8036 4110

General Enquiries: sharon.atwill@gp-j82203.nhs.uk
clair.wright@gp-j82203.nhs.uk

21 March 2003

Dr M K Jarvaid
ARC Clinical Research Fellow to Professor C Cooper
MRC Environmental Epidemiology Unit
MP 95
SGH

Dear Dr Jarvaid,

Submission No: 005/03/t – Parental determinants of skeletal growth: a longitudinal study.

Following conditional approval and in response to your letter dated 4th March 2003, I am please to confirm **full approval** having responded satisfactorily to the committees concerns.

The following document(s) were re-considered:

- **Letter dated 4th March 2003**
- **Information Sheet**
- **Consent Form**

Please note that all paperwork i.e. Information Sheet etc. should be on departmental headed paper and must carry identification version number and date.

This approval was granted under Chairman's action by Vice Chairman Mr Mervyn Griffiths, and will be recorded by the Committee at their meeting in April.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

Yours sincerely

C Wright

Mrs Clair Wright
Research Ethics Manager

Chairmen: Dr Audrey Kermode/ Dr David Briggs
Manager: Mrs Clair Wright

Appendix B: Ethical approvals for the MAVIDOS trial and four year follow-up

NHS

National Research Ethics Service

VY/STA/hph

03 December 2007

SOUTHAMPTON & SOUTH WEST HAMPSHIRE
RESEARCH ETHICS COMMITTEE (A)
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
Hampshire
SO16 4RJ

Tel: 023 8036 2466
023 8036 3462
Fax: 023 8036 4110

Email: scsha.SWHRECA@nhs.net

Professor Cyrus Cooper
Professor of Rheumatology, Director of MRC ERC
MRC Epidemiology Resource Centre
MRC ERC
Southampton General Hospital
Southampton
SO16 6YD

Dear Professor Cooper,

Full title of study: **A double blind randomised placebo controlled trial of vitamin D supplements for pregnant women with low levels of vitamin D in early pregnancy**

REC reference number: **07/H0502/113**
Protocol number: **1.5**
EudraCT number: **2007-001716-23**

Thank you for your letter of 20 November 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

In the second page of your response, the Committee have picked up that you have made a few minor changes to the pregnancy questionnaire and it is possible that further minor changes may need to be made during the pilot phase, so The Committee requests to see the final versions at the end of pilot.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form. Confirmation of approval for other sites listed in the application will be issued as soon as local assessors have confirmed they have no objection.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application		26 July 2007
Investigator CV		
Protocol	1.5	10 November 2007
Covering Letter		25 July 2007
Compensation Arrangements		08 June 2007
Questionnaire: Child Follow Up	1.0	25 July 2007
Questionnaire: 34 Week	1.4	19 November 2007
Questionnaire: 14 Week	1.7	19 November 2007
Letter of invitation to participant	1.1	11 October 2007
GP/Consultant Information Sheets	1.0	07 June 2007
Participant Information Sheet	1.15	19 November 2007
Participant Consent Form: 4 Year	1.1	19 November 2007
Participant Consent Form: Neonatal	1.2	19 November 2007
Participant Consent Form: Initial	1.7	19 November 2007
Response to Request for Further Information		20 November 2007
Obstetric Information Letter	1.0	07 June 2007
Midwife Information Letter	1.0	07 June 2007
Request for Authorisation form MHRA		31 July 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

07/H0502/113	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Mrs Vikkie Yule
Vice-Chair

Email: scsha.SWHRECA@nhs.net

Enclosures: Standard approval conditions

Site approval form

Copy to: Ms Christine McGrath
Southampton University Hospital NHS Trust

Clinical Trials Unit, MHRA

Southampton & South West Hampshire REC (A)

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	07/H0502/113	**Issue number:**	0	**Date of issue:**	03 December 2007
Chief Investigator:	Professor Cyrus Cooper				
Full title of study:	A double blind randomised placebo controlled trial of vitamin D supplements for pregnant women with low levels of vitamin D in early pregnancy				

This study was given a favourable ethical opinion by Southampton & South West Hampshire REC (A) on 30 November 2007. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* (1)
Professor Cyrus Cooper	**Professor of Rheumatology, Director of MRC ERC**	**Southampton University Hospitals NHS Trusts Tremona Road Southampton**	**Southampton & South West Hampshire REC (A)**	**03/12/2007**	

Approved by the Chair on behalf of the REC:

..... (Signature of Co-ordinator)

Mrs. Sharon Atwill

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

NHS
Health Research Authority

NRES Committee South Central - Hampshire A
Bristol Research Ethics Committee Centre
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Tel: 0117 342 1381

26 November 2013

Ms Christine McGrath
University Hospital Southampton NHS Foundation Trust
R & D Department,SUHT
Ground Floor, Duthie Building,MP138
Southampton General Hospital
SO16 6YD

Dear Ms McGrath

Study title:	**A double blind randomised placebo controlled trial of vitamin D supplements for pregnant women with low levels of vitamin D in early pregnancy**
REC reference:	**07/H0502/113**
Protocol number:	**1.3**
EudraCT number:	**2007-001716-23**
Amendment number:	**2.2**
Amendment date:	**03 October 2013**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Protocol	2.2	20 September 2013
Participant Information Sheet: Bone density scans in mothers and children	1.0	06 September 2013
Participant Consent Form: 4yr - additional maternal investigations	1.0	09 September 2013
Questionnaire: Child Follow up 4 years old		15 February 2013
Participant Consent Form: 4yr - additional assessments	1.0	09 September 2013

Letter from radiation expert		25 September 2013
Covering Letter		10 October 2013
Letter from Pat Taylor		
Participant Information Sheet: Blood tests in children at 4 years of age	1.0	06 September 2013
European Commission Notification of Substantial Amendment Form	2.2	03 October 2013
Participant Information Sheet: Physical activity monitoring	1.0	06 September 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

07/H0502/113:	**Please quote this number on all correspondence**

Yours sincerely

Dr Iain MacIntosh
Chair

E-mail: nrescommittee.southcentral-hampshirea@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Prof Cyrus Cooper, MRC Lifecourse Epidemiology Unit*

A Research Ethics Committee established by the Health Research Authority

NRES Committee South Central - Hampshire A

Attendance at Sub-Committee of the REC meeting on 12 November 2013

Name	*Profession*	*Capacity*
Dr Ronja Bahadori	Clinical Trial Coordinator	Expert
Dr Mary Lanyon	Retired Veterinarian	Lay Plus
Dr Iain MacIntosh	Consultant Paediatric Intensive Care	Expert

Also in attendance:

Name	*Position (or reason for attending)*
Mrs Vicky Canfield-Duthie	REC Manager

What are the possible benefits of taking part?

We will perform the NHS ultrasound scan at 19 weeks in our dedicated suite. You will be given a picture of your baby to take home. Your child will have a measurement of their bone mass and you will find out if your vitamin D levels are very low, so that you can receive supplements if needed.

Will my taking part in this study be kept confidential?

All information collected about you will be kept strictly confidential. Your GP and obstetric team will be informed of your participation and we will inform your GP if your vitamin D levels are very low and require treatment.

What will happen to the results of the research?

We will see how taking vitamin D supplements in pregnancy affects bone mass in the baby. These findings will be published in medical journals, and possibly in the local and national press. You will not be identified in these reports/publications in any way. Ultimately, we hope that this study will inform government policy makers.

What if there is a problem?

If you have a concern about any aspect of this study, please ask to speak to the researchers who will do their best to answer your questions (023 8079 4186).

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (via Southampton General Hospital). In the unlikely event that you are harmed due to someone's negligence, you may have grounds for compensation but you may have to pay your own legal costs.

Who has reviewed the study?

This study has been reviewed and approved by Southampton and South West Hampshire Research Ethics Committee. Ethics No.: 07/H0502/113

Who is organizing and funding the research?

This study is funded by the MRC and Arthritis Research Campaign, and organized by the MRC Lifecourse Epidemiology Unit and University of Southampton.

Contact for further information

For further information please contact Professor Cyrus Cooper (Chief Investigator) or Dr Nick Harvey (Principal Investigator) at the MRC Lifecourse Epidemiology Unit at Southampton on 023 8077 7624

Information Sheet V1.29 28/03/12

MRC Lifecourse Epidemiology Unit

You are invited to take part in

Mavidos

Maternal Vitamin D Osteoporosis Study

A Vitamin D trial in pregnant women:

A double-blind randomised placebo-controlled trial of vitamin D supplements for pregnant women with low levels of vitamin D in early pregnancy.

If you are interested in taking part, please telephone us at the earliest opportunity on:

Telephone: 023 8120 4186

Email: mavidos@mrc.soton.ac.uk

UNIVERSITY OF Southampton

You are being invited to take part in a trial of Vitamin D supplementation in pregnancy. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with anyone you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. A nurse may approach you when you attend for your ultrasound scan.

What is the purpose of this study?

To find out whether giving mothers vitamin D supplements in pregnancy might improve the strength of their child's bones. We also aim to find out if vitamin D affects the immune defence system in the child.

Why might I be approached?

You are currently in early pregnancy and are due to attend hospital for an ultrasound scan.

Do I have to take part?

You decide whether or not to take part. If you do take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

At 12 weeks: After your NHS ultrasound scan, a blood sample will be taken to measure the level of vitamin D in your blood. If this is below the normal level you will not be eligible to take part in the trial and we will arrange for you to receive a vitamin D supplement through your GP. If the level is sufficiently high you will also not be eligible to take part in the trial.

At 14 weeks: If the vitamin D level is intermediate, we will arrange an appointment either at your home or at Princess Anne Hospital (PAH) in the 14th week of your pregnancy. At this visit a nurse will ask you questions about your diet and lifestyle, and take some body measurements, including your partner's height, if they are present. The nurse will take a blood sample (which will include a sample for genetic studies: this will not identify any genetic conditions), and ask for a urine sample. You will be given a supply of study medication for the duration of the trial. You will be randomly allocated to receive either vitamin D tablets 1000 units daily or placebo (dummy) tablets; neither you nor the nurse will know which. You may also be given a light sensitive badge to wear.

At 19 weeks: At PAH, NHS routine ultrasound scan, research ultrasound scan.

***At 34 weeks*:** At PAH, the measurements taken at 14 weeks will be repeated.

At birth: When you have given birth, the midwife will collect samples from the afterbirth (tissue from the placenta and tissue and blood from the cord). This will have no effect on the baby whatsoever. We will also record some information from your hospital notes.

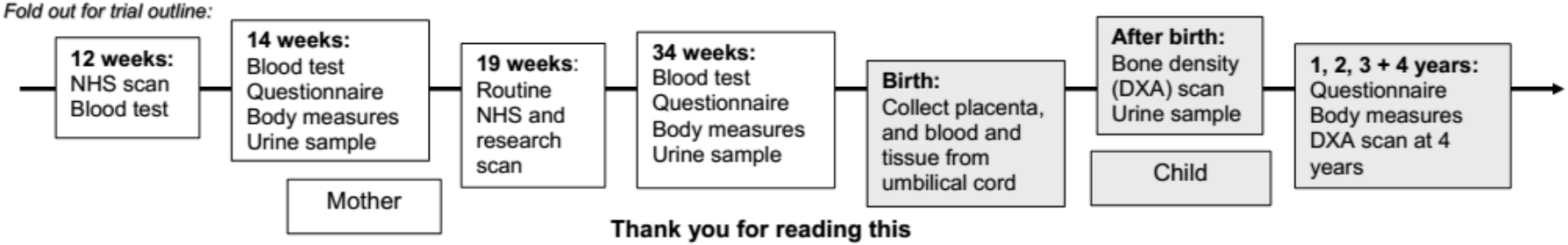
After birth: We will collect a urine sample from your baby and perform a bone density (DXA) scan, and body measurements.

In childhood: When your child is 1, 2, 3 and 4 years old, we will assess your child's height, weight, body build, diet and health. At 4 years we will perform another DXA scan of your child.

What are the possible disadvantages and risks in taking part?

The bone density scan involves exposing your child to a very low dose of x-rays, similar to the x-rays experienced by spending a week outdoors. The dose of vitamin D has been chosen to keep levels in the **normal** range, as there is speculation that high vitamin D levels in pregnancy may make the child slightly more prone to eczema and asthma.

Fold out for trial outline:



Thank you for reading this

Appendix D: MAVIDOS 4 year follow-up participant information sheets

MRC Lifecourse Epidemiology Unit
Southampton General Hospital
Southampton S016 6YD

Telephone: 023 8079 4186

Bone density scans in mothers and children

Introduction

You are currently participating in the MAVIDOS study. As part of this study your child will have had his/her bone density measured soon after they are born using a DXA bone density scanner. Some mothers also had a DXA scan at the same time.

Your child is invited to have a repeat DXA at 4 years of age. At this visit, we would also like to invite you to have a DXA scan, and for both you and your child to both have another type of bone scan (pQCT).

What if I would prefer not to have these additional scans?

You can decide whether or not you would like you and/or your child to have these additional scans. If you decide not to have it then this will not affect your participation in the MAVIDOS trial in any way, or the medical care you receive.

What do the scans involve?

DXA scan - mother

The DXA scan is similar to the scan your child had just after he or she was born, and is the same scan as your child will have at the 4 year visit. A small scanning arm passes over you about two feet in the air; it does not touch you. The scans are quick: The main scan of the whole body will take around 5 minutes and then scans of your lower spine and both hips will take around 15 seconds each.

pQCT scan – mother and child

The pQCT scan involves sitting on a chair and putting your leg into an open metal tube; it does not touch you. This scan takes around 5 minutes.

Neither of the scans will hurt and the dose of X-rays used in these scans is less than one week's background radiation. You would not be eligible to participate in these studies if you are pregnant. The two scanners are located in the same room and you and your child will both be able to stay in the room whilst the scans are being performed.

It is important that you are wearing light clothing (eg T shirt and tracksuit bottoms) with no metal fixings as these will show on the scan.

If you have any questions about this, please call 023 8079 4186

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MRC Lifecourse Epidemiology Unit
Southampton General Hospital
Southampton S016 6YD

Telephone: 023 8079 4186

Mavidos
Maternal Vitamin D Osteoporosis Study

Blood tests in children at 4 years of age

Introduction

You and your child are currently participating in the MAVIDOS study. As part of this study you and your child will be invited to attend Southampton General Hospital for a repeat bone scan (similar to the scan you child had shortly after birth) when your child is 4 years of age.

We would also like to invite your child to donate a blood sample to determine your child's vitamin D level and how this affects your child's bone development. We may also use this sample to investigate other markers of your child's health and to look at genes that might be important to bone development, but we will not be testing for individual genetic disorders.

What will happen to my child if I agree to blood sampling?

Some local anaesthetic cream ("magic cream") will be put on your small areas of your child's hands and/or arms. This numbs the skin so that any discomfort is minimised. The blood sample will be taken by a doctor or nurse, who has been specifically trained in taking blood samples from children. Distraction techniques will also be used, which often means children of this age do not realise they have had blood taken. Approximately 10ml (equivalent to two teaspoons) of blood will be taken.

What will happen with the blood sample?

The samples will be stored securely in freezers at the MRC Lifecourse Epidemiology Unit. We will not be testing for any specific disorders and we will not be able to inform you about any blood results for your child. Some samples may be stored for future research.

Does my child have to take part in this additional study?

It is up to you to decide if you would like your child to participate in these studies. If you do not wish to take part in the blood sampling, this will not affect you and your child's participation in the MAVIDOS study or your health care.

If you have any questions about this, please call 023 8079 4186

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Appendix E: MAVIDOS 4 year questionnaire

Study No: ☐☐☐☐

Child Follow-up Questionnaire **4 Years Old** ☐

Date of Birth _____

Date Questionnaire Completed _____

Nurse Number ☐☐

Mavidos

V1.0 15.02.2013 07/H0502/113

1. **Food Frequency** Now I am going to ask you about **a few** of the **foods** your child has eaten in the **past 3 months**. I will ask you how often he/she has eaten certain foods and also the amount of food eaten. For some foods, I will show you drawings and models to help you estimate the amount of food. Your child may sometimes have eaten food away from home. If you know the type of food and approximate amount eaten at these times please include them.
Explain the use of spoons, cups, bowl and diagrams.

	Food	never	less than once per month	1-3 times per month	number of times per week 1	2	3	4	5	6	7	more than once per day	no. of times per day	Average amount per serving
bread and crackers														
1.1	white bread, rolls, toast	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of slices ☐•☐ *(1 roll/bagel/croissant = 2 slices bread) (if all crusts gone=0.7 slice)*
1.2	brown bread, rolls, toast	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of slices ☐•☐
1.3	cakes, scones biscuits	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of portions *(1 portion = 2 biscuits, 1 scone, 1 slice of cake)* ☐•☐
1.4	breakfast cereals	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of tbsp (1 weetabix = 4 tbsp 1 minibix = 1 tbsp) ☐•☐
1.5	What are the main types of breakfast cereal used?	Type			Brand									☐☐☐☐☐
		Type			Brand									☐☐☐☐☐
		Type			Brand									☐☐☐☐☐

	Food	never	less than once per month	1-3 times per month	number of times per week							more than once per day	no. of times per day	Average amount per serving
					1	2	3	4	5	6	7			
1.6	oily fish	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of portions ☐.☐ *1 sm can tuna = 2, med= 4; 1 tbsp= 0.25 portions salmon in sandwich = 1*
1.7	eggs	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of eggs ☐.☐ *yoke=0.4, white= 0.6*
1.8	cheese	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	*tbsp grated = 0.5* ☐.☐ *small triangle = 1 cheese per slice = 1 see drawing*
1.9	What are the main types of cheese used?	Type			Brand									☐☐☐☐☐
		Type			Brand									☐☐☐☐☐
		Type			Brand									☐☐☐☐☐
1.10	baked beans	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of small tins ☐.☐ *. small tin = 200g*
1.11	yoghurt & fromage frais	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of grams ☐☐☐ *tiny pot 50g, bigger 100g*

	Food	never	less than once per month	1-3 times per month	number of times per week 1	2	3	4	5	6	7	more than once per day	no. of times per day	Average amount per serving
1.12	What are the main types of yoghurt and fromage frais used?	Type			Brand									
		Type			Brand									
		Type			Brand									
1.13	ice-cream	0	0.3	0.5	1	2	3	4	5	6	7	8		no. of tablespoons *1scoop = 4* *1 choc ice/ Fab/ Mars i/c etc = 4*
1.14	custard and sweet white sauce	0	0.3	0.5	1	2	3	4	5	6	7	8		no. of tablespoons
1.15	butter & margarine	0	0.3	0.5	1	2	3	4	5	6	7	8		no. of teaspoons *1 sl bread = 1.5 tsp* *1 pat =2 tsp*
1.16	What are the main types of spread?	1/			2/				3/					1 2 3
1.17	milky drinks	0	0.3	0.5	1	2	3	4	5	6	7	8		No. standard beakers 7 oz/ 200 ml
1.18	What are the main types of milky drink?	1/			2/				3/					1 2 3

1.19 Now I would like to ask in more detail about your child's milk intake over the past 3 months.

Which types of milk has your child used regularly in drinks and added to breakfast cereals?
(list up to 3 below)

0. None
1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6. Skimmed UHT
7. Breast milk
8. Formula milk, toddler milks, 'growing up' milk etc
9. Other

Milk 1 ☐ If "Other" *specify* _____

Milk 2 ☐ If "Other" *specify* _____

Milk 3 ☐ If "Other" *specify* _____

1.20 On average over the last 3 months how much of each milk has he/she consumed per day?
(1 average beaker = 0.35 pints; 1 pint = 20oz / 568 mls)

Milk 1 ☐ . ☐☐ Pints

Milk 2 ☐ . ☐☐ Pints

Milk 3 ☐ . ☐☐ pints

1.21 During the past 3 months have you given him/her any vitamins or minerals, including vitamin D, iron and fluoride drops or tablets?

0. No go to Q2
1. Yes

☐

1.22 Please state which:

Supplement Name	Code	How many days in the last 90?	Is It: 1) Tablet 2) Drops 3) Liquid 4) Other? (State)	No. of stated units per day
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			

1.23* On average over the last 3 months how many beakers of water has your child drunk each day? Include plain water and drinks made mostly from water, such as squash, tea and coffee.
Number of beakers per day ☐☐ • ☐

1.24* Is this water mainly

1. Tap Water,
2. Filtered Tap Water,
3. Ordinary mineral water,
4. Mineral water with added calcium (eg Danone variety)
5. Other *(Please specify)*

☐

_____

2. SLEEP, ACTIVITY AND EXERCISE

Now I'm going to ask you about your child's sleeping, activity and exercise patterns over the last three months. *[We are trying to get figures that eventually total approximately 24 hours, so rounding to nearest hour is OK – best guess is acceptable]*

2.1 What time does the study child generally go to sleep at night (24 hour clock) ☐☐☐☐

2.2 How many times **per night** does he/she generally wake for any reason? ☐☐ • ☐ **Per night**
Please answer this in relation to the last month.

If (0) please go to Question 2.4

2.3 In total, how long is he/she awake? ☐☐ **hrs** ☐☐ **mins per night**
(Only record if regularly over 30 mins)

2.4 What time does he/she generally wake up in the morning (24 hour clock) ☐☐☐☐

2.5 **This means that he sleeps for about** ☐☐ **hrs** ☐☐ **mins per night**

2.6 How many **days per week** does he/she take a daytime nap? ☐
Please answer this in relation to the last month
If "0" deduct 2.5 from 24 and insert at 2.9

2.7 On the days he/she naps, what is the **total time** spent napping during the day? ☐☐ **hrs** ☐☐ **mins**

Using responses to 2.6 and 2.7 consult "Daily Averages" grid

2.8 Average daily nap time ☐ **hrs** ☐☐ **mins**

Add 2.5 to 2.8 and deduct from 24

2.9 This would indicate that he/she is awake on average each day? ☐☐ **hrs** ☐☐ **mins**

2.10* On a typical day, how many hours does he/she generally spend watching television/computer games (i.e. DS/iPad or anything requiring 'screen time')?

1. More than 5 hours ☐ 2. 4-5 hours ☐ 3. 3-4 hours ☐
4. 2-3 hours ☐ 5. 1-2 hours ☐ 6.Less than one hour ☐
7. None ☐

YOUR CHILD'S HEALTH

I would like to ask you some questions about your child's skin and any illnesses your child might have had **since we last visited you when he/she was about three years old (since three years old if not seen for the thirty-six month follow-up)**

3 SKIN CONDITIONS

3.1 Has he/she had an itchy skin condition at any time **-** by itchy we mean scratching or rubbing the skin a lot? *(exclude chicken pox)* ☐

No *go to 2.4* 1. Yes

3.2 *Has thi*s skin condition affected the cheeks, the outer arms or legs, or the skin creases in the past **-** by skin creases we mean the folds of the elbows, behind the knees, the fronts of the ankles, or around the eyes? ☐

No 1. Yes

3.3 How old was he/she when the rash first appeared? – *Clarify first* ☐ Yrs ☐☐ Mths ☐ Wks ☐ Days

3.4 In the past twelve months, has he/she suffered from a generally dry skin? ☐

0. No 1. Yes 8. To a minor degree

3.5* **In the past twelve months**, has he/she had a **scaly**, or **red and weeping** skin rash affecting any of the following areas: ☐

a) the scalp or behind the ears (including "cradle cap")	0.No	1. Yes	☐
b) around the neck	0.No	1. Yes	☐
c) the cheeks or forehead	0 No	1. Yes	☐
d) either the folds of the elbows or behind the knees	0 No	1. Yes	☐
e) the forearms, wrists, shins or ankles	0 No	1. Yes	☐
f) the shoulders, chest, tummy or back	0 No	1. Yes	☐
g) in the armpits	0 No	1. Yes	☐
h) the nappy area (including nappy rash)	0 No	1. Yes	☐

4 Has your child had a problem with sneezing or a runny, or blocked nose when s/he did not have a cold or the flu? *If No go to Q6* 0. No 1. Yes ☐

5 Has this nose problem been accompanied by itchy-watery eyes? 0. No 1. Yes ☐

6 Has your child had an allergic reaction when in contact with animals? 0. No 1. Yes ☐

7 Has your child had wheezing or whistling in the chest 0. No 1. Yes ☐
If no, go to Question 11a

If yes, when did it start? ☐ Yrs ☐☐ Mths ☐ Wks ☐ Days

8 Does your child wheeze in association with chest infection? (chesty cough and fever) 0. No 1. Yes ☐

9 Has your child had wheezing in association with a cold? (nasal congestion and discharge) 0. No 1. Yes ☐

10 Does your child wheeze in between colds or a chest infection? 0. No 1. Yes ☐

11a Has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? 0. No 1. Yes ☐

If yes, when did it start? ☐ Yrs ☐☐ Mths ☐ Wks ☐ Days

11b Has your child had chest infection/s (chesty cough and fever) 0. No 1. Yes ☐

If so, how many since last visit? ☐☐

12 Has your child had any colds (nasal congestion and discharge) 0. No 1. Yes ☐

If so, how many since last visit (or in the last 12 months if seen for the first time)? ☐☐

13 Has a doctor ever diagnosed asthma in your child? 0. No 1. Yes ☐

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14* Have any of the following medications been used? 0. No 1. Yes ☐

a) Bronchodilators (e.g. Ventolin/Salbutamol) 0. No 1. Yes ☐

If yes, how long ago was it last given? ☐☐ Mths ☐ Wks ☐ Days

b) Antihistamines 0. No 1. Yes ☐

If yes, how long ago was it last given? ☐☐ Mths ☐ Wks ☐ Days

c) Corticosteroids 0. No 1. Yes ☐

Oral 0. No 1. Yes ☐

If yes, how many courses? ☐☐

If yes, how long ago was it last given? ☐☐ Mths ☐ Wks ☐ Days

Inhaled 0. No . 1. Yes ☐

If yes, age when prescribed? ☐☐ Mths ☐ Wks ☐ Days

Any other medication for wheeze? (*Please specify below)*

_____

_____

15 Does your child take any regular medicines (either from the chemist, doctor, or alternative therapies)? *Please include inhalers for asthma.* 0. No . 1. Yes ☐

If no, please go to Question 21.
If yes, please list them in the table below

Medicine Name	**Code**	**How many days in the last 90?**	**Is it: 1) Tablet 2) Drops 3) Liquid 4) Other *(specify)***	**Dose per day**
	☐☐☐☐			
	☐☐☐☐			

16* How is the study child's health in general? Would you say it was:-

1. Very good ☐ 2. Good ☐ 3. Fair ☐

4. Bad ☐ 5. Very bad ☐

17 Does he/she have any long-standing medical conditions? By long standing I mean anything that has troubled him/her over a period of time, or that is likely to effect him/her over a period of time.

0. No 1. Yes ☐

If no, go to Q21

18 What is this condition?

_____

19 Does this condition limit his/her activities in any way?

0. No 1. Yes ☐

If no, go to Q20

20 If yes, in what way does it limit his/her activities?

_____

_____

FOOD ALLERGIES

21 Does your child have adverse reactions to any foods, such as eczema, breathing problems or gastrointestinal problems? 0. No . 1. Yes ☐

22* *Has your child suffered from a food allergy? 0. No . 1. Yes ☐

If no, go to question 23

Suspected food 1

_____

How many reactions to this food? ☐☐

a) How long did it take to develop the symptoms?

1. 30 mins 2. 30 mins – 2 hours 3. > 2 hours ☐

b) What symptoms? Please tick (✓)

Urticaria/ hives ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐ Wheezing/ SOB ☐

Vomiting ☐ Diarrhoea ☐ Colic ☐ Sysemic ☐

Other (Please specify)

_____

c) ***Suspected food 2***

_____

How many reactions to this food? ☐☐

d) How long did it take to develop the symptoms?

1. 30 mins 2. 30 mins – 2 hours 3. > 2 hours ☐

e) What symptoms? Please tick (✓)

Urticaria/ hives ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐ Wheezing/ SOB ☐

Vomiting ☐ Diarrhoea ☐ Colic ☐ Sysemic ☐

Other (Please specify)

_____

f) ***Suspected food 3***

How many reactions to this food? ☐☐

g) How long did it take to develop the symptoms?

1. 30 mins 2. 30 mins – 2 hours 3. > 2 hours ☐

h) What symptoms? Please tick (✓)

Urticaria/ hives ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐ Wheezing/ SOB ☐

Vomiting ☐ Diarrhoea ☐ Colic ☐ Sysemic ☐

Other (Please specify)

YOU AND YOUR FAMILY

23 Do you (mother) smoke?
0. No
1. Yes, Occasionally
2. Yes Daily ☐

24 Does anyone else smoke <u>inside</u> your home?
0. No
1. Yes, Occasionally
2. Yes Daily ☐

25 Is your child exposed to tobacco smoke outside the home? (for example at grandparents or other relatives, baby sitter)
0. No
1. Yes, Occasionally
2. Yes Daily ☐

26 Do you work in paid employment at the moment?
0. No
1. Yes, but currently on maternity leave
2. Yes ☐

27 How old was your child when you first went back to work? ☐☐ Mths ☐ Wks ☐ Days ☐ n/a

OR

On what date did you go back to work? d d m m y y ☐☐ ☐☐ ☐☐

CHILD CARE QUESTIONS

NURSERY

28 Has your child attended day care, a nursery, pre-school, or childminder? (Day care could include going to Grandparents) 0. No *(go to Q37)* 1. Yes *(go toQ29)* ☐

29 How old was your child when she/he first started day care? ☐☐ Mths ☐ Wks ☐ Days ☐ n/a

30 Has your child now stopped attending day care, a nursery, pre-school or childminder? 0. No (go to Q32 1. Yes (go toQ31) ☐

31 How old was your child when she/he stopped attending day care? ☐☐ Mths ☐ Wks ☐ Days ☐ n/a

32 How many hours a week on average does/ did your child attend day care, a nursery, pre-school or childminder? ☐☐ hrs ☐☐ mins

33 What type of day care does your child attend? *Please tick (✓)*

Child minder ☐ Nursery/Creche ☐ Pre-school ☐ Grandparents ☐

34 Approximately how many other children are/ were cared for by the childminder or attend/ attended the nursery (number in child's nursery/ pre-school room)/crèche ☐☐ children

35* Does/ did the childminder OR nursery/crèche/ pre-school have a pet(s)? 0. No 1. Yes ☐

36* If yes, please specify what pet(s) and where they are allowed
0. No,
1.Yes, allowed in bedroom (room where child sleeps)
2. Yes, not allowed in bedroom,
3. Yes, not in the house

Dog ☐ Cat ☐

Other *Please specify* ..... ☐

Other Please specify ..... ☐

Other Please specify ..... ☐

HOME

37* What main type of flooring is in the room where your child sleeps?

1. Carpet ☐ 2. Wooden, laminate,parquet ☐ 3. Linoleum or vinyl tiles ☐

4. Ceramic/terracotta tiles or stone ☐ 5. Sea-grass or coir-type matting ☐ 6. Other (Please specify) ☐

---

38 Does your child's mattress have a plastic surface or cover? 0. No . 1. Yes ☐

PETS

39 Do you have a pet at home? 0. No . 1. Yes ☐

40* If yes, please specify what pet(s) and where they are allowed
0. No,
1.Yes, allowed in bedroom (room where child sleeps)
2. Yes, not allowed in bedroom,
3. Yes, not in the house

Dog ☐

Cat ☐

Other *Please specify*..... ☐

Other Please specify..... ☐

Other Please specify..... ☐

41a* Do you have regular *(i.e. more than once a week)* exposure to animals elsewhere? ☐

0. No . 1. Yes

1. Dog ☐ 2. Cat ☐ 3. Other *(Please specify)* ☐

41b* Does your child have regular *(i.e. more than once a week)* exposure to animals elsewhere? ☐

0. No . 1. Yes

1. Dog ☐ 2. Cat ☐ 3. Other *(Please specify)* ☐

_____

42 How many adults live in the household? ☐☐

43 How many children live in the household (including this child)? ☐☐

44 How many bedrooms does your home have, including the child's room and guest room? ☐☐

QUESTIONS ABOUT YOUR HOME

45* Is your home

1. Owned Privately ☐ 2. Rented Privately ☐

3. Rented–Council/Housing Association ☐ 4. Other ☐

46* Do you cook on

1. Gas ☐ 2. Electricity ☐ 3. Other (specify) ☐

_____

47 Does your home have damp spots on the walls or ceilings? 0. No 1. Yes ☐

48 Does your home have visible moulds or fungus on the walls or ceiling? 0. No 1. Yes ☐

49* How often do you keep the windows open in your home?

1. Every day 2. Once a Week 3. Once a Month 4. Seldom 5. Never ☐

50* How often do vehicles pass your house or on the street less than 100 metres away?

1. ≥10 per hour 2. 1-9 per hour 3. 10 per day 4. Seldom 5. Never ☐

51* What term best describes where you live now?

1. Farm *(Go to Q59)* 2. Small town e.g. Romsey
3. City Centre e.g. Southampton
4. Rural village e.g. Exbury 5. Suburbs of a city e.g. Bassett ☐

52 If you live on a smallholding, is there contact with farm animals (including animals kept in the garden)? (0. No. 1. Yes) please mark each box

Dairy Cattle .	☐	Beef Cattle	☐	Poultry	☐
Sheep	☐	Pigs	☐	Horse	☐
Other *(Please specify)* _____			0. No 1. Yes		☐

53 Has your child received any vaccinations? 0. No 1. Yes ☐

Since the 3 year visit, *(or since the age of 3 years if the 3 year visit was omitted)*

54 **VACCINATIONS**

What vaccinations has your child received and dates received? (from vaccination record)
(Insert date if "Yes", otherwise tick under "No" or "Don't know")

	Routine Immunisations	Yes – Date given	0. No	9. Don't know
3 years 4 months	Diphtheria, tetanus, pertussis and polio Measles, mumps and rubella	d d m m y y	☐	☐

Non-Routine Immunisations	Yes – Date given	0. No	9. Don't know
Tuberculosis	d d m m y y	☐	☐
Hepatitis B	d d m m y y	☐	☐
Other (please specify) _____	d d m m y y	☐	☐
Other (please specify) _____	d d m m y y	☐	☐

55 In the past twelve months has your child broken any bones? 0. No 1. Yes ☐

If Yes which bone was broken? *(Please specify)* _____

56 Has your child had any illnesses requiring admission or assessment in hospital? 0. No 1. Yes ☐

Details:

57 Assessed (including outpatient appointments, and GP assessments)? 0. No 1. Yes ☐

Details:

58 Does your child do any organised physical activities e.g. swimming? 0. No 1. Yes ☐

(Please specify)

59 On an average day how many hours does your child spend outdoors:
a) in the winter ☐☐.☐☐

b) in the summer ☐☐.☐☐

60 Does your child use sunblock in sunny weather? 0. No 1. Yes ☐

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61 CHILD EXAMINATION

61.1 Measurement Date d d m m y y

61.2 Time (24 hr clock)

61.3 Measurer

61.4 Helpers (Parent 90)

61.5 Occipito-frontal circumference

cm cm

cm cm

cm

Wriggling 0. No, 1. Yes

61.6 Left mid-upper arm circumference (arm straight)

cm cm

cm cm

cm

Wriggling 0. No, 1. Yes

61.7 Chest circumference

cm cm

cm cm

cm

Wriggling 0. No, 1. Yes

61.8 Waist circumference (standing)

cm cm

cm cm

cm

Wriggling 0. No, 1. Yes

61.9 |Hip circumference (standing)

cm

Wriggling 0. No, 1. Yes

Skinfold thicknesses

61.10 Triceps skinfold

☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	

Wriggling 0. No, 1. Yes ☐

61.11 Subscapular skinfold

☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	

Wriggling 0. No, 1. Yes ☐

61.12 Skinfold callipers used ☐☐☐☐

61.13 Height (barefoot) Leicester H/M

☐☐☐.☐ cm	☐☐☐.☐ cm
☐☐☐.☐ cm	☐☐☐.☐ cm
☐☐☐.☐ cm	

Wriggling 0. No, 1. Yes ☐

61.14 Sitting height Leicester H/M

☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	☐☐.☐ cm
☐☐.☐ cm	

Wriggling 0. No, 1. Yes ☐

61.15 Stadiometer used ☐☐

61.16 Child's weight ☐☐.☐☐ kg
(preferably in underwear only, with no nappy)

61.17 Weight of any clothes/nappy ☐.☐☐ kg

61.18 Weighing scales used ☐☐☐☐

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61.19 **RIGHT SIDE** **LEFT SIDE**

GRIP STRENGTH
(Record to nearest 0.5 kg)

□□ • □ □□ • □ □□ • □

□□ • □ □□ • □ □□ • □

61.20 Which hand does your child mostly use to write or hold a pencil with?

Left □ Right □

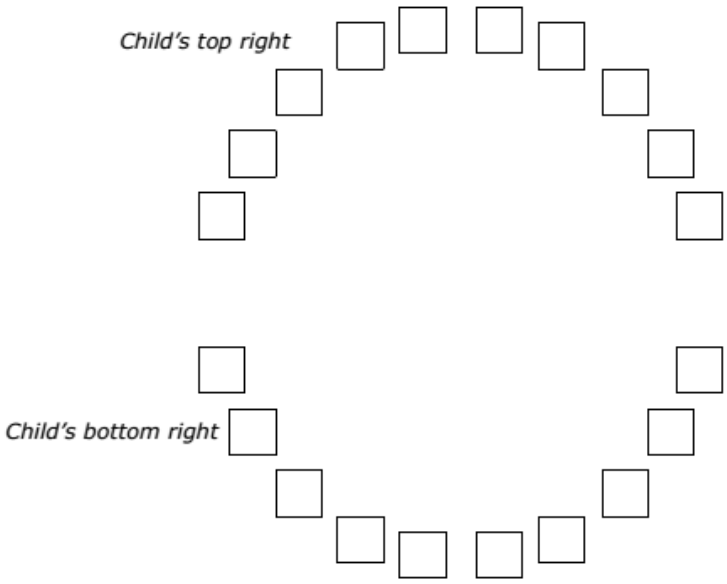
Ambidextrous □

(Writes with both hands)

62 TEETH

62.1 Number of teeth □□

62.2 Position of teeth
(Mark with a cross for each tooth present) □□



62.3 Has your child lost any teeth? □
0. No.
Yes – number of teeth

63 SKIN EXAMINATION

Eczema = poorly defined redness with scaling, crusting, vesicles or accentuated skin markings (lichenification).

		Eczema Please tick box 0. No 1 Yes
63.1	Is/are there any?	☐
63.2	Scalp/Behind ears	☐
63.3	Face – cheeks and forehead	☐
63.4	Face – around the mouth	☐
63.5	Neck	☐
63.6	Arms – palms of the hands	☐
63.7	Arms – antecubital fossae	☐
63.8	Arms – remainder (backs of hands, forearms, upper arms)	☐
63.9	Arms – axillae	☐
63.10	Trunk – back	☐
63.11	Trunk – front (chest and abdomen)	☐
63.12	Legs – soles of feet	☐
63.13	Legs – popliteal fossae (behind knees)	☐
63.14	Legs – remainder of (i.e. thighs, lower leg, dorsa feet)	☐
63.15	Nappy area (including nappy rash)	☐

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