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The Influence of Early Nutrition and Growth on Body Composition in Childhood and Early Adult Life

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Date..... 29/11/06 .....

Abstract

There is increasing evidence that events early in life may 'programme' later body composition (BC) and health outcomes. The aim of the work presented in this thesis was to test the following hypotheses:(1) birthweight and growth in infancy programme specific components of BC in later life and therefore have differential effects on health outcomes; (2) infant BC is differentially related to later BC, and is potentially involved in the programming process. These hypotheses were investigated in 2 cohorts (1) prospective follow-up during adolescence of subjects who had BC measurements during infancy (by stable isotope) as part of nutrition intervention studies; (2) healthy children who participated in a BC reference study, with retrospective collection of early growth records. The main outcome was BC assessed by the four-component model. Secondary outcomes were cardiovascular risk factors (blood pressure, lipid profiles, and insulin resistance markers).

308 subjects aged 4.22-20.36 years were studied. Birthweight (regarded as a proxy for prenatal growth) was positively related to later height in both sexes, and positively associated with fat free mass (FFM) in boys. In contrast, postnatal growth during the first 6 months of life positively influenced later fat mass (FM) and central fat distribution, with a weaker effect on FFM. Whilst FM showed a consistent association with most cardiovascular risk factors except HDL-C, FFM showed a strong negative association with HDL-C, independent of FM and central fat distribution. In a smaller group(n=41), BC at 12 weeks of age showed no significant association with adolescent BC; but SF during very early postnatal life (3 and 6 weeks) was related to later FFM and central fat distribution. Infant nutrition affected infant body composition, but I was unable to detect effects on later BC.

Conclusion: I found differential effects of growth during different periods on later BC, measured using the 'gold standard' four-component model. The mechanism by which early growth 'programs' later BC possibly involves both prenatal and postnatal nutrition

and warrants further investigation, since it could be useful in terms of designing effective early intervention to reduce obesity prevalence in childhood and adolescence. BC during adolescence has a differential impact on cardiovascular risk factors. Therefore, separate measurements of FM, FFM, and central fat distribution may offer more insight into the impact of body composition programming on health outcomes.

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Abbreviations

4C model	4-component model
2C model	2-component model
AFA	arm fat area
AGA	average for gestational age
AMA	arm muscle area
BC	body composition
BMC	bone mineral content
BMI	body mass index
BP	blood pressure
CT	computerised tomography
CVD	cardiovascular disease
D_2O	deuterium
DBP	diastolic blood pressure
DXA	Dual X-ray absorptiometry
DXA ROI	Dual X-ray absorptiometry, region of interest
FM	fat mass
FFM	fat free mass
FLR	fat: lean ratio
FMI	fat mass index
FFMI	fat free mass index
GA	gestational age
HDL-C	high density lipoprotein cholesterol
HT	height
HOMA IR	homeostasis model assessment, insulin resistance
LBW	low birthweight
LDL-C	low density lipoprotein cholesterol
LGA	large for gestational age
LM	lean mass

LMI	lean mass index
MAP	mean arterial pressure
MRI	magnetic resonance imaging
MUAC	mid-upper arm circumference
NS	non-significant
OFC	occipito-frontal circumference
OR	odds ratio
PI	ponderal index
RCT	randomised controlled trial
RE formula	reduced energy formula
SAD	sagittal abdominal diameter
SBP	systolic blood pressure
SDS	standard deviation score
SES	socioeconomic status
SF	skinfold thickness
SGA	small for gestational age
SISF	suprailiac skinfold
SSSF	suscapular skinfold
S:T	subscapular: triceps ratio
TBW	total body water
TG	triglycerides
TMA	thigh muscle area
TFA	thigh fat area
TF formula	standard term formula
TSF	triceps skinfold
Δweight SDS	weight SDS change
WC	waist circumference
WHR	waist: hip ratio
WT	weight

Chapter 1

Introduction

Chapter 1 Introduction

There is a rising trend of childhood overweight and obesity worldwide with increasing evidence of short term health consequences and long term morbidity in adulthood (1-8). A great deal of research has focused on potential explanations for the obesity epidemic; most agree that a western lifestyle consisting of physical inactivity and excessive energy intake combined with genetic susceptibility are contributing factors to childhood and adult overweight (7-9). However, there is also increasing interest in the concept that obesity risk may at least in part be 'programmed'[#] by events early in life (11). Early studies in animal models showed that overnutrition and excessive weight gain during the postnatal period are predisposing factors for obesity (11-14). In humans, the concept that events operating in early life influence or 'programme' later health has been extensively investigated over the past 15 years. Early studies tended to focus on intrauterine life as the main critical period for programming effects (15), however, increasing evidence points to the importance of growth and nutrition during the postnatal period (16;17) and the possibility that this may interact with subsequent lifestyle factors to cause overweight and the metabolic syndrome (16).

The aim of the work presented in this thesis was to investigate the programming of specific components of body composition by growth and nutrition during early life, and to examine the relationship between body composition outcomes and cardiovascular risk factors in healthy children and adolescents, using the most accurate body composition techniques currently available.

The organisation of this thesis is as follow: Chapter 2 covers background to my research and review of the existing research works; issues that require further research are highlighted. Chapter 3 outlines the hypotheses and describes methodology including

[#] The term "programming" refers to the idea that stimuli or insults during critical or sensitive periods in early life can have lifetime consequences (10).

statistics and details of outcomes measured. The next 5 chapters present results and discussion, within each chapter the results are presented and immediately followed by relevant discussion about validity of the results and comparison with other studies. Chapter 9 provides an overall discussion about potential mechanism of body composition programming and broader implication for public health as well as recommendation for future research.

Chapter 2

Background

Chapter 2 Background

This chapter is divided into 3 parts: 1) a general background section giving an overview of growth and the basic principles of body composition measurement in children, 2) a review of the literature on the programming of body composition, and 3) a review of the literature on the relationship between body composition and health outcomes.

2.1 General background

In this general background section, I will briefly summarize the physiology of fetal and infant growth (particularly in relation to nutrition). Since my study involves body composition data during different stages of development, I also provide a review of the body composition changes seen during growth, followed by a discussion of body composition measurement techniques available for use in the paediatric population.

2.1.1 Physiology of growth

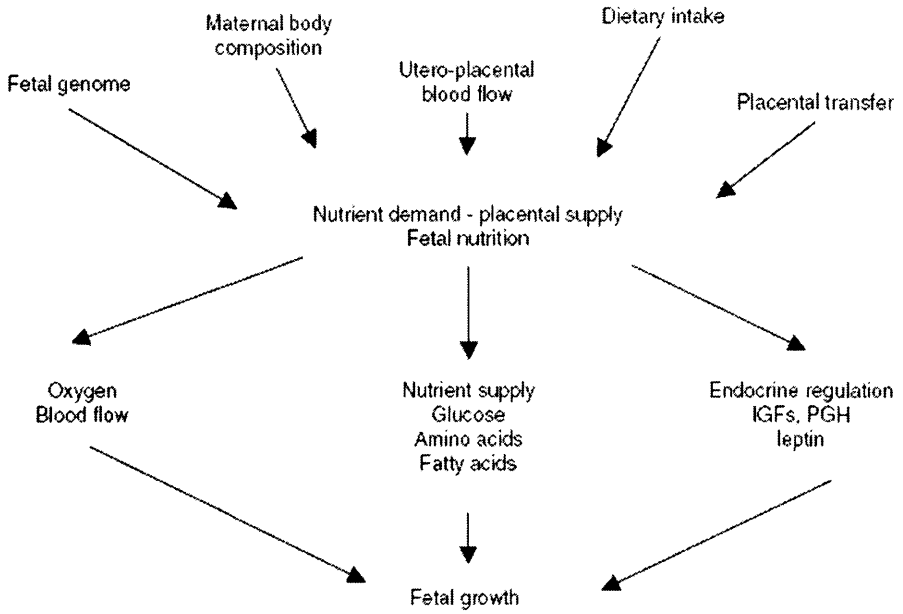
"Growth is the maturation process characterized by change, compensation, and adaptation; it is genetically predetermined and significantly affected by hormonal, nutritional, and environmental factors"(18). For the purpose of this thesis, it is most relevant to focus on the influence of hormonal, nutritional, and environmental factors that affect prenatal and early postnatal growth.

Prenatal growth

Fetal growth and development are limited both by the intrauterine environment and by genetic potential. Many factors influence fetal growth throughout pregnancy; during this period nutrition and hormonal regulation may alter the expression of genes and have life-long consequences through a process that has been defined as 'fetal

programming'(19). The ability of the maternal-placental system to transfer nutrients to the fetus, and the endocrine environment, also determine whether the fetus will achieve its growth potential during intrauterine life (see **Figure 2-1**)

Figure 2-1 Main determinants of fetal nutrition and growth[#]



The three main intrauterine factors that affect fetal growth are briefly summarised below:

1) Hormonal regulation (20;21): the most important one are the insulin-like growth factors (IGFs—which induce cell proliferation and differentiation together with DNA synthesis), insulin (regulating the transport of nutrients across cell membrane and the synthesis of the peptide growth factors), placental growth hormone (PGH—which controls maternal IGF-1 level), thyroid hormone (critical for neural development), glucocorticoids (for maturation of lungs, liver, pancreas, gastrointestinal tract), and

[#] Reproduced from Cetin et al (20)

leptin. The function of leptin for fetal growth is still under investigation, however, its level in cord blood is related to fetal weight and fat mass (22).
2) The nutrient environment; which can modulate the activity of IGF-1 and its binding protein (21).
3) Maternal constraints; i.e. maternal-placental factors such as multiple pregnancy, maternal nutrition.

Birthweight is a convenient measurement of pregnancy outcome which has been used widely in clinical and research settings. It is clearly affected by multiple endogenous and environmental factors. Kramer (23) has identified the important factors associated with low birthweight in developed countries as follows: cigarette smoking, low maternal energy intake during gestation, and low prepregnancy weight, primiparity, female infant sex, maternal short stature, non-European ethnicity, and previous history of low birthweight. Such knowledge is important for targeting public health interventions aimed at improving prenatal growth.

Postnatal growth

Growth is a complex process that is sustained throughout intrauterine life, infancy, childhood and puberty until early adult life. During infancy, nutrition is a main determinant of growth, whereas in childhood and adolescence, growth hormone and sex steroids play a vital role. Indeed, infant growth rate is highly variable and its regulation is not well understood. It is likely that early postnatal growth is controlled by nutritional intake and by appetite, which might be influenced by feeding practices as well as by prenatal growth restraint (24;25). However, although there is increasing evidence from animal models that appetite and food preference can be altered by manipulating nutrient intakes and growth in the early post-natal period (26;27), this area has not so far received much attention in humans. A limited number of studies have suggested that during the first weeks of life, infant appetite regulation may not be well developed, so it is possible to manipulate infant nutrient intake to a significant degree – for example using formulas with different energy or protein contents. However, beyond 6-8 weeks of

age, infants seem better able to adjust their intake and can effectively sabotage attempts to manipulate their intake by up or down-regulating the volume taken (28-30)—see later review in this chapter. The significance of these observations is poorly understood, although likely to be important, particularly in relation to infants who suffer poor early growth.

It is well-recognized, for example, that infants born small for their gestational age are likely to show rapid postnatal growth, or 'catch-up' growth. Low birthweight may occur as a result of poor fetal growth throughout pregnancy or just in the last trimester. The underlying causes and outcomes differ in the two situations. The latter scenario, which is more common, arises as a result of maternal constraints such as placental failure as mentioned previously. An otherwise normal fetus is starved of nutrients and fails to gain weight, but grows in length near normally; so-called asymmetrical growth retardation. After birth, these infants almost always show complete catch-up growth with rapid weight gain within the first 6–12 months (31). Whilst these adaptations certainly benefit short-term infant survival there is evidence from epidemiological studies that this pattern of growth is associated with an increased risk of adult chronic disease such as type II diabetes and cardiovascular disease (32;33); similar effects of rapid postnatal growth seen even in non-small for gestational age (SGA) babies (34). Consequently, the mechanisms that regulate infancy growth or catch-up growth are under investigation.

2.1.2 Body composition changes during growth[$]

In general, the body can be divided into fat mass (FM) and fat-free mass (FFM). The FM includes all ether-extractable lipids and the FFM consists of 4 major constituents: water, protein, glycogen, and minerals (see **Figure 2-2**). The study of body composition allows better comprehension of physiologic changes during growth and the effects of factors such as hormones, nutrition, and physical activity. **Figure 2-3** and **Table 2-1** illustrate the changes in body composition seen during different periods of growth.

[$] Adapted from Bechard and Puig (18)

Figure 2-2 Body composition models.[#]

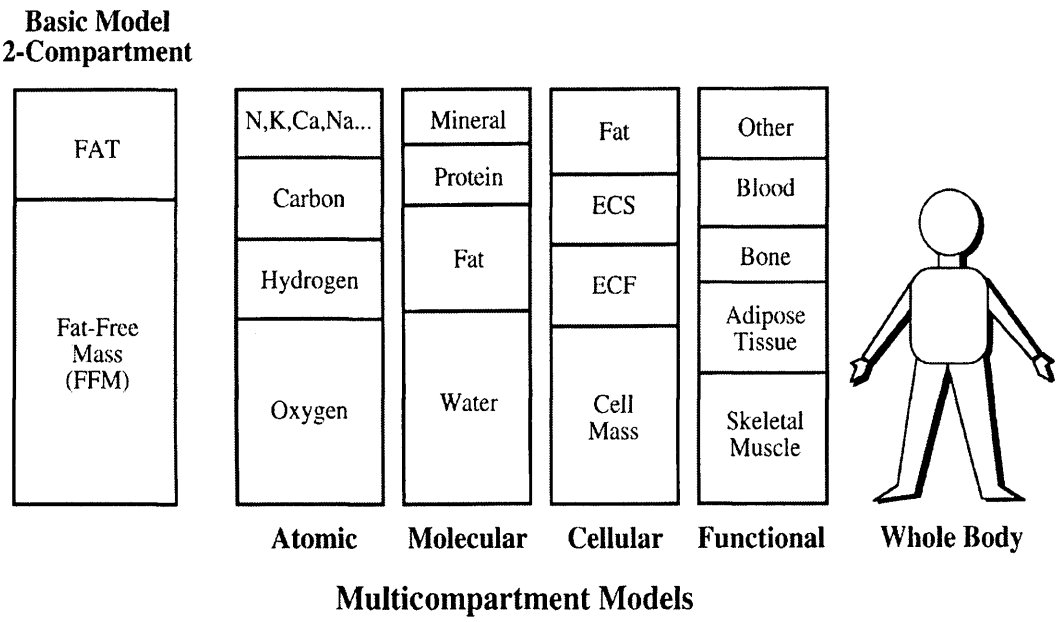
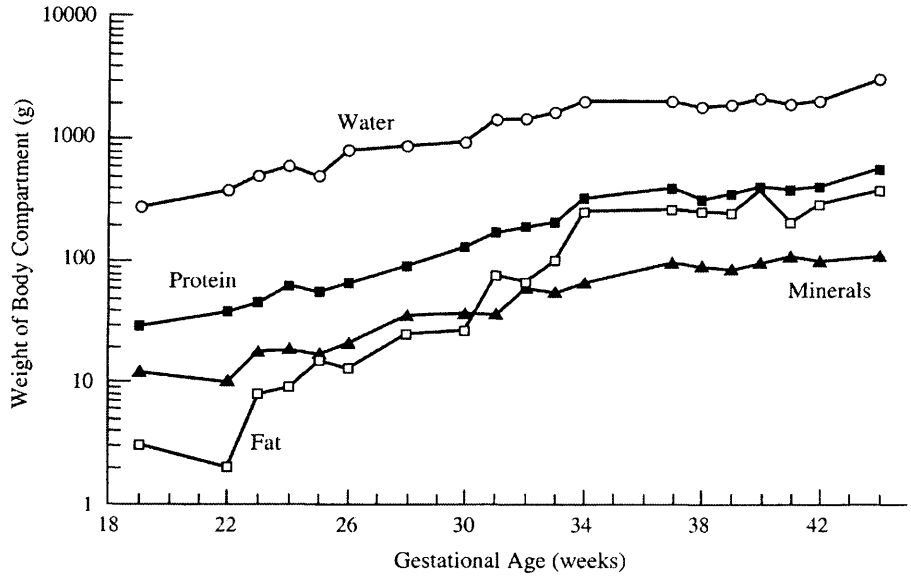


Figure 2-3 Changes in body composition during fetal development and early life.[#]



[#] Reproduced from Ellis (35)

Table 2-1 Body composition of reference children and adolescents[#]

Age (yr)	Length (cm)	Weight (kg)	Percentage of Body Weight						
			Fat	Protein	Mineral	Carbohydrate	TBW	ECW	ICW
Boys									
Birth	51.6	3.5	13.7	12.9	3.2	0.5	69.6	42.5	27.0
0.5	67.9	8.04	29.1	10.9	2.4	0.5	57.2	32.9	24.3
1	76.1	10.03	25.6	12.3	2.7	0.5	59.0	31.6	27.4
1.5	82.6	11.43	24.5	12.9	3.0	0.5	59.1	30.1	29.4
2	87.6	12.46	25.4	13.5	3.1	0.5	58.1	26.6	30.7
5	109.9	18.7	14.6	15.8	3.7	0.5	65.4	30.0	35.4
10	137.5	31.4	13.7	16.8	4.1	0.5	64.8	26.7	38.0
12.5	153.0	42.3	16.3	16.4	4.1	0.6	62.7	26.4	36.4
15.5	171.5	59.5	13.0	17.4	4.5	0.6	64.6	25.8	38.8
18.5	177.0	69.9	12.9	17.7	4.8	0.7	64.1	24.7	39.4
Girls									
Birth	50.5	3.3	14.9	12.8	3.2	0.5	68.6	42.0	26.7
0.5	66.5	7.6	32.0	10.4	2.3	0.5	54.9	31.7	23.2
1	75.3	9.5	27.6	12.2	2.7	0.5	56.9	29.7	27.4
1.5	82.0	10.94	26.3	12.7	2.9	0.5	57.8	29.0	28.5
2	87.7	12.02	25.4	13.1	3.0	0.5	57.7	29.0	29.4
5	108.4	17.7	16.7	15.0	3.1	0.5	64.6	31.0	33.6
10	138.3	32.6	19.4	15.0	3.1	0.5	62.0	28.1	33.9
12.5	154.6	43.8	21.5	15.4	4.2	0.5	58.5	25.6	32.9
15.5	162.1	55.0	24.7	14.9	4.5	0.5	55.5	23.7	31.8
18.5	164.0	57.0	25.0	14.9	4.4	0.7	55.2	23.5	31.7

Adapted from Fomon SJ et al,[3] Haschke F[37,38] and Butte NF et al.[39]
ECW = extracellular water; ICW = intracellular water; TBW = total body water

[#] Reproduced from Bechard and Puig (18); this table was adapted from Fomon et al (36), Haschke (37;38), and Butte et al (39).

Changes in the fat component

Fat is the most variable of body components. FM increases most rapidly during the 3rd trimester, and comprises 13.7% of birthweight in healthy full-term boys and 14.9% in girls (36). From birth, the accumulation of fat progresses rapidly to a peak of around 29.1% in boys and 32% in girls at age 6 months (39). After infancy, the fat content drops to a minimum at around 6-7 years of age and then increases steadily to adolescence. Although gender differences in body composition are present at birth (40), they become more obvious during the adolescent years. During puberty, adipocyte size and number increase substantially and fat becomes more centrally distributed, especially in males (41). The tendency for fat accumulation in the abdomen for males and around the hips for females can be attributed to the effects of hormonal change; androgens significantly reduce adipocyte volume and number around the hips, whereas estrogen and progesterone induce a slight increase (42). During and after puberty, the relative amount of fat diverges in boys and girls; the former tend to have less fat while the latter demonstrate an increase in fat as a proportion of weight.

Changes in body water

Water content is highest during fetal life – about 88.6% of body weight at 24 weeks gestation and around 70% at term (18). After birth, it drops rapidly to around 60% at the age of 6 months before stabilizing between 60-65% during childhood and adolescence.

Changes in bone mineral content

Bone is constantly being formed and resorbed throughout life; bone modelling followed by bone remodelling, takes place continually from birth until late adolescence. The skeleton grows through infancy, childhood, and puberty, reaching maturity by late adolescence. Mineral accretion is particularly rapid during the third trimester and infancy, and again during the pubertal growth spurt. The period of

maximal mineral accretion during puberty occurs after peak height velocity has been reached.

Changes in skeletal muscle mass

Skeletal muscle represents 22-25% of the mass of a newborn infant and approximately 40-54% of body weight in adolescence, with a higher figure in males than females. The number of muscle fibres in humans is probably set before birth so growth afterward occurs by hypertrophy without hyperplasia (43). Total muscle mass develops slowly during childhood with a growth spurt in the adolescent years, which is more intense and prolonged in boys than in girls. The development of muscle mass is influenced by several factors including age, sex, nutrition, hormonal, and physical activity.

Changes in visceral mass

In general, the growth and development of the viscera are associated with the same changes in chemical composition as those seen in muscle mass: a decrease in the proportion of water and an increase in the proportion of protein.

Table 2-2 Contributions of organs and major tissues to body weight[#]

	Percentage of Body Weight			
			Adult	
Organ or Tissue	Fetus (20–24 wk)	Full-Term Newborn	Male	Female
Skeletal muscle	25.0	25.0	40.0	29.3
Skeleton	22.0	18.0	17.1	17.1
Skin	13.0	15.0	3.7	3.1
Liver	4.0	5.0	2.6	2.4
Brain	13.0	13.0	2.0	2.1
Lungs	3.3	1.5	1.4	1.4
Kidneys	3.1	1.6	0.4	0.5
Heart	0.6	0.5	0.5	0.4
Spleen	—	0.2	0.3	0.3

Adapted from White DR et al.[45]

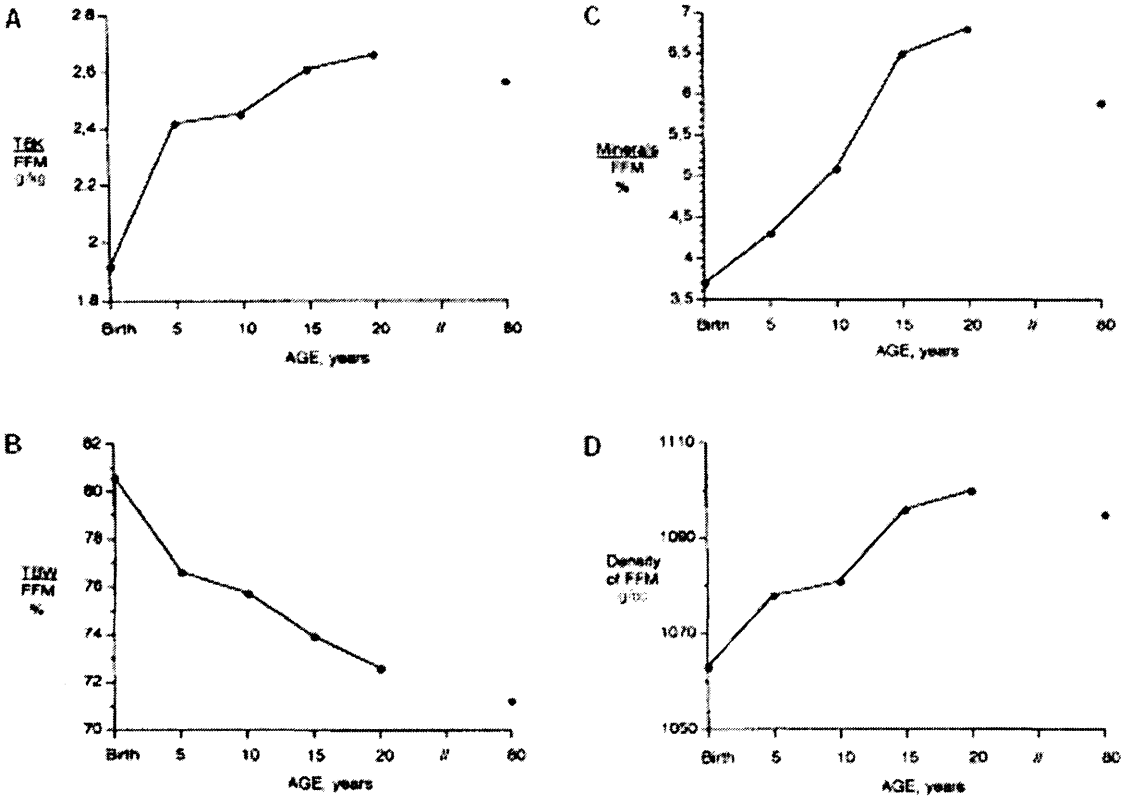
[#] Reproduced from Bechard and Puig (18); this table was adapted from White et al (44)

Chemical changes in fat-free mass

FFM increases rapidly during the late fetal and neonatal period, followed by a rapid deceleration between the 1st and 3rd year of life until the adolescent growth spurt begins (18). There is a slight predominance of FFM in boys compared to girls, which becomes apparent during the adolescent period.

The concept of chemical maturity of FFM was introduced by Moulton in 1923 (45); he found that the body composition of most species approached that of an adult when puberty was reached. The chemical composition of FFM varies greatly during growth since the chemical maturity of each component is reached at different stages. See **Figure 2-4**. For example, in children, FFM contains relatively more water than adults; the hydration fraction ranges from 80.2% in the newborn to 75% in early puberty and 73% in adults (**Figure 2-4B**). During growth, as the water content decreases and protein and mineral content rise, there is an increase in body density (**Figure 2-4D**). As a result, paediatric body composition cannot be correctly estimated if adult constants are used.

Figure 2-4 Changes in FFM composition as a function of age. **A** Total body potassium (TBK) in relation to FFM. **B** Total body water (TBW) in relation to FFM. **C** Bone mineral content in relation to FFM. **D** Density of the FFM. [#]



[#] Reproduced from Bechard and Puig (18). This table was adapted from Fomon et al (36), Haschke (37), Lohman (45), and Heymsfield et al (46;47).

2.1.3 Paediatric body composition measurement techniques[#]

The gold standard for measuring body composition is cadaver analysis; in vivo techniques do not *measure* body composition directly, but rather *predict* it from measurements of body properties. Therefore, methodological error when obtaining the raw data and assumption errors when converting raw data to final values need to be considered. There are a number of available techniques for measuring body composition; however, in this review, I have only addressed the measurement techniques used in my study or comparable studies.

Simple measurements

Body mass index (BMI, calculated as weight/height^2) is widely used as an index of relative weight and used in both adult and children populations to categorise overweight and obesity. Some studies reported high correlations between BMI and FM in children (49); however, BMI cannot distinguish fat and lean masses and cannot represent fat distribution. FM or percentage fat (in relation to weight) can vary greatly within individual children (50), with ethnicity (51), and within a population over time (52). Moreover, in paediatric populations, the predictive value of BMI for clinical outcomes such as cardiovascular risk factors is less clear than in adult populations (see later).

Waist circumference (WC) is used as a simple measurement of central fatness, which may be more predictive of clinical outcomes than total fatness. Some recent studies in children have suggested that WC might be a better predictor of cardiovascular risk factors than BMI (53-55).

Skinfold thickness (SF) measurements have traditionally been used as an indicator of fatness to rank individuals or to assess the size of specific subcutaneous fat depots (56). However, they can also be used to 'predict' total body composition as discussed

[#] Adapted from Wells and Fewtrell (48)

below. SF measurements are fairly quick and simple to obtain, but are subject to greater inter- observer errors than WC or BMI.

Predictive techniques

SF equations

SF measurements can also be used to predict percentage body fat or body density by equations based on two-component models (57). However, these equations have limited use beyond the population from which they were derived and inevitably confound accurate raw values with predictive error. Indeed, it has been suggested that for assessment of fatness, it is better to rank people according to raw or SDS values of SF (48). Moreover, for assessment of FFM, the use of SF equations is particularly inappropriate since no index of this component is directly measured.

Bioelectrical impedance analysis (BIA)

BIA measures the impedance (resistance) of the body to a small electric current. It assumes that the body is a single cylinder and the lean tissue contains a high level of water and electrolytes and therefore acts as an electrical conductor. Adjustment of bioelectrical data for cylinder length (height) then allows estimation of cylinder volume (total body water—TBW), by using the appropriate regression equations relating the square of height divided by impedance to TBW. The predicted TBW is then converted to FFM. This principle relies on several assumptions which can be affected by the age range and characteristics (e.g. ethnicity, disease state) of the population. Published BIA equations are population specific and perform poorly in healthy individuals, with errors typically ± 8% fat (58).

Two-component models (2C models)

The 2C model divides the body into FM and FFM; the accuracy mainly depends on the assumption of constancy of FFM composition. As previously mentioned, the

chemical composition of FFM varies with age, sex, and pubertal status; these changes are generally accounted for by assuming constant FFM characteristics for a given age and sex.

Dual Energy X-ray absorptiometry (DXA)

DXA was introduced as a technique for measuring bone mineral mass from the differential absorption of 2 different energy X-rays. The technique only estimates the relative proportions of fat and fat-free tissue in pixels without bone. In the limbs, this allows the majority of pixels to undergo soft tissue analysis, but in the trunk, where the pelvis, spine, ribs obscure significant numbers of pixels, soft tissue composition is predicted rather than measured. Due to these limitations, total body FM and FFM derived from DXA need to be interpreted with caution. In a recently published study (59) evaluating DXA against the 4-component model (see later), the researchers reported that the bias of DXA for measuring body composition varies according to the sex, size, fatness, and disease state of the subjects.

Densitometry

The densitometric approach uses Archimedes' principle to distinguish fat and fat-free components of weight assuming specific densities of these two tissues. It thus requires the measurement of total body density (mass/volume). FM density is relatively constant whereas that of FFM varies, so age and sex specific equations (based on FFM density in reference children(36)) are used to convert body density to final body composition values (60). Wells et al (58) have shown that these values are biased in contemporary children and inter-individual variability is significant even in normal healthy children.

There are two available methods for body volume measurements: hydrodensitometry and air displacement plethysmography. The latter is the newer method which is reported to have a better precision and to be more readily acceptable in children (61) (measurement details are shown in **Chapter 3**).

Isotope dilution

The deuterium (a non-radioactive stable isotope of hydrogen) dilution technique is used to measure TBW, allowing the estimation of FFM. A dose of water labelled with deuterium (D_2O) is given and, following equilibration, the enrichment of deuterium in the body water pool is measured using samples of saliva (in children) or urine (preferable in infants). In most children, 4 hours is sufficient for equilibration of deuterium in saliva. In infants, who have rapid water turnover, collecting post-dose samples (urine) over several days and calculating the maximum post-dose enrichment through back extrapolation is a better approach (62). For calculating TBW, it is assumed that the deuterium dilution space overestimates TBW by a factor of 1.044 (63), although in infancy this factor varies in relation to age (64).

An alternative isotope, 18-oxygen (^{18}O), can also be used and the protocol is identical to that for deuterium except the overestimation factor is 1.01 (63). However, it is substantially more expensive than deuterium.

Estimation of FFM from TBW requires an assumption of the hydration of fat-free tissue. Published reference values are relatively consistent with measured values in healthy children and infants (65) with relatively low inter-individual variability (58). Since deuterium dilution is simple to perform and requires minimal subject cooperation, it can easily be used in field studies, particularly in populations where the normality of hydration is known or can be assumed.

Magnetic resonance imaging (MRI)

MRI is an imaging technique that estimates the volume rather than the mass of adipose tissue. It primarily detects hydrogen nuclei, located either in water or fat, and uses these data to discern tissue types in imaging slices which can then be summed to calculate regional tissue volumes. The main advantage is that currently it is the only accurate and viable approach for the estimation of intra-abdominal adipose tissue, despite its relatively high cost and limited availability. An alternative imaging technique is computed tomography (CT), based on X-rays, as opposed to

electromagnetic radiation in MRI. However, the high radiation dose makes it essentially unsuitable for routine use in paediatric populations.

Multicomponent models

The greatest advantage of multicomponent models lies in minimizing the assumptions made in simpler models. Bone mineral content and TBW are measured by techniques specifically designed for that purpose. It therefore provides a greater degree of accuracy. The three-component model (3C) divides the body into fat, water by measuring TBW (by the isotope dilution), and remaining fat-free dry tissue, and thus avoids the need to assume a value for the hydration of fat-free tissue. However, it assumes a constant ratio of protein to mineral (66). The four-component model (4C) further divides fat-free dry tissue into protein and mineral, by measuring total body mineral (by DXA). This avoids the assumption of a constant protein to mineral ratio, but still assumes a constant ratio of bone mineral to total mineral (66). The practical measurement details and equations used in my study are shown in **Chapter 3**.

***Summary*:**

Growth and body composition vary greatly in paediatric populations, both between individuals and in the same individual over time. A basic understanding of the physiology of growth and body composition changes during growth is fundamental to research in this area. Advances in *in vivo* techniques for measuring body composition allow researchers to explore growth and its outcomes in more detail, and to investigate the role of specific components in the development of obesity and other diseases.

The next part of this background section is a review of published work on the programming of body composition.

2.2 Body composition programming

Research on the long-term effects of birthweight and infant growth on later health, including obesity and cardiovascular disease, has increased rapidly during the past 10-15 years, with publications from both developed and developing countries. The majority of studies investigating the possible programming of obesity have used BMI as the main outcome, since it has been shown to correlate well with morbidity and mortality from cardiovascular disease in adults. However, in paediatric populations the evidence that any BMI 'cut-off' relates to the outcome of interest (e.g. the metabolic syndrome, type II diabetes, or early signs of cardiovascular disease) is less strong. More recent studies have included more detailed measurements of the components of BMI i.e. FM and FFM, mostly in relation to birthweight. Fewer studies have looked into the effects of postnatal growth on later body composition.

Most of the papers cited in this section were published before I proposed my hypotheses and started my research, and these formed the background to my work. More recent research and work in progress will be included in the discussion sections of the relevant chapters.

Here I will review: 1) the influence of birthweight and early growth in infancy on later body composition; 2) the effect of early nutrition (breast vs. formula feeding and different infant formulas) on growth and body composition; and 3) the long term effect of *early* body composition on later outcomes.

2.2.1 Long term effects of early growth on later body composition

Animal data

Early studies in animals demonstrated that nutrition and growth in the early postnatal period have a long term effect on later growth trajectory and also later body fat. McCance (67) manipulated litter size in rats, so that rats from larger litters received less breast milk than those from smaller litters during the 3-week suckling period, by

which time rats from larger litters were substantially smaller. These smaller animals continued to diverge in body size even though they were fed normally after weaning. The same researcher also showed that equivalent dietary manipulation for a 3-week period a few weeks later (post-weaning) had no lasting effect: the underfed animals showed catch-up growth when they were re-fed. This showed that 3-weeks of intervention in a critical period had resulted in a lifetime programming of the growth trajectory. Knittle and Hirsch reported that the preweaning nutrition of rats also markedly influenced the number of fat cells of these animals during adult life (68).

Lewis et al tested the fat cell number hypothesis in primates by feeding newborn baboons with different caloric density formulas (40.5, 67.5, 94.5 kcal/100ml-underfed n=8, normally fed n=12, overfed n=12, respectively) in similar volumes until they were weaned at 4 months of age (11-14). In the first experiment (11), overfed baboons at 4 months of age (at weaning) had greater absolute FM and fat cell size, but not more fat cells, than baboons that were underfed. The second experiment (13) examined the long-term effect of infant overnutrition by follow up of the baboons that were overfed, normally fed, or underfed until 4 months of age and then fed an identical diet until they were young adults at 5 years of age. Overfeeding during infancy markedly increased FM in the 10 fat depots measured in 5-yr-old female baboons and marginally increased FM in 4 of 10 fat depots in 5-yr-old male baboons. On the contrary, underfeeding did not affect body weight or adipose mass of either sex. The increased FM resulted primarily from an increase in fat cell size but not fat cell number.

These studies in baboons thus demonstrated that 1) overfeeding during the early postnatal period increased body mass and FM at weaning compared with underfeeding; 2) the effect of preweaning diet on body mass disappeared before 1 year, but the effect of the exposure was 'remembered': female baboons overfed as infants began to gain weight at a greater rate than those underfed or normally fed especially between 3-5 years of age (female baboons enter puberty around 3 years of age)(13) and the majority of the excess weight gained was fat (14). These results therefore provide evidence in support of the hypothesis that rapid growth during infancy can predispose to obesity in later life, even when the early effects are transitory. The findings were confined to females, and the researchers suggested that

the rapid increase in lean body mass during the pubertal growth spurt in males (age 5 years) may have masked an effect of infant overfeeding on FM (13). Consequently they might develop obesity at a later age than females. Alternatively, the obesity response to infant overfeeding may be sexually dimorphic, at least in the baboon. They also found that much of the body fat in overfed male and female baboons was located in the intra-abdominal fat region (omentum, perirenal, and mesentery). However, it is difficult to determine whether this type of obesity is similar to the truncal obesity reported in human, as defined by SF ratios, circumferences, and waist-to-hip ratios, which do not differentiate between subcutaneous and intra-abdominal fat.

Human data

The following is a review of evidence supporting the programming of body composition in humans, divided into 'prenatal' and 'postnatal' periods of growth.

Birthweight or 'prenatal' growth

A large number of studies have addressed the relationship between birthweight and later BMI. Almost all have found direct positive associations although of differing magnitudes- typically 0.5 to 0.7 kg/m^2 per 1 kg increase in birthweight (69;70). As mentioned previously, although a higher BMI is traditionally interpreted as indicating greater 'fatness', it is also influenced by lean mass or FFM. Thus for instance, a tall athletic individual with high muscle mass and a tall individual with excess fat might both have a high BMI but for entirely different reasons and with very different implications for health.

I will focus on the limited number of recent studies attempting to look at more specific measures of body composition in relation to birthweight. These are summarized below:

Allison et al (71) reported a positive association between birthweight and adult height but not weight, and Phillips (72) reported a similar association between

birthweight and later muscle mass (estimated by urinary creatinine excretion) but not later weight.

Stettler et al (73) studied 447 African Americans followed to 20 years of age. 'Adiposity' was defined as the sum of triceps plus subscapular SF >85th centile for age and sex. Three predictors for adiposity were identified: being a first-born child, female sex, and high maternal BMI. After adjusting for these factors, there was no relationship between birthweight and later adiposity.

Hediger et al (74) studied 3192 children at 3-6 years. Those born small for gestational age (SGA) remained shorter and lighter at follow-up. Those born large for gestational age (LGA) remained taller and heavier with a significant increase in mid-upper arm circumference (MUAC) SDS with age. The authors suggested that they may be at risk for increasing accumulation of fat. However, the same group of researchers studied arm fat and muscle mass, calculated from MUAC, in 4431 children aged 2-47 months and reported that those born SGA had a deficit in arm muscle, but less of a deficit in arm fat, whereas LGA infants showed a greater excess of arm muscle than arm fat (75).

Weyer et al (76) studied 272 adult non-diabetic Pima Indians. Underwater weighing or DXA was used to measure body composition. Birthweight was positively related to adult height and to FFM, but not FM.

Gale et al (77) reported data from 143 women aged 70-75 years from Sheffield. DXA was used to measure body composition. Birthweight was positively related to weight, lean mass and bone mass. The association with lean mass persisted after adjustment for weight and height in adult life. There was no significant association between birthweight and adult FM. On the basis of these findings, the authors suggested that bone and muscle growth are programmed by intrauterine factors.

Loos et al (78;79) studied 229 male twins pairs and 238 female twins pairs. Twins who were heavier at birth were taller and heavier as adults, with more lean body mass (from BIA) and less subcutaneous and abdominal fat (measured using waist-hip ratio and sum of SF) when adjusted for current body weight.

Singhal et al (80) used BIA and DXA to assess body FM and FFM in children and adolescents aged 8-12 years and 13-16 years old respectively and found that higher birthweight was associated with greater FFM, but not with greater FM. Moreover, this association was independent of age, sex, pubertal stage, socioeconomic status, physical activity and height.

To summarise, data are quite consistent in showing a positive association between birthweight and later BMI, although the size of the effect varies. Nevertheless, more recent studies that have examined body composition in detail suggest that this relationship may predominantly reflect greater height and lean body mass rather than excess adiposity.

Postnatal growth

There is increasing evidence from longitudinal studies that a pattern of rapid weight gain during early infancy is associated with obesity (defined by BMI) not only in childhood but also in adulthood (81-87). For example, Stettler et al (82) reported that rate of weight gain during the first 4 months of life was associated with being overweight (defined by BMI over 95th centile) at age 7 years in 19,397 US children; Odds ratio (OR): 1.38 (1.32-1.44). This association was present in each birthweight quintile, and remained significant after adjustment for the weight attained at age 1 year. The same authors (84) presented data from a longitudinally followed cohort of 300 term-born African Americans from birth to 20 years suggesting that high infancy weight gain during the first 4 months of life (defined as an increase of $\geq$ 1 SD in weight-for-age SDS from birth to age 4 months) can help to predict the likelihood of developing obesity in early adulthood (defined by BMI; OR 5.22 (1.55-17.6, p 0.008)).

Ong et al (85) studied 848 healthy term infants from the ALSPAC study (Avon Longitudinal Study of Pregnancy and Childhood) and showed that infants who gained greater than 0.67 SD in weight SDS between birth and 2 years (30.7 % of the cohort), indicating clinically significant catch-up growth, were heavier, taller and

fatter (by BMI, percentage body fat and total FM which was calculated from SF) at 5 years than those who showed no change (remained within the same weight percentile band between birth and two years - 44.8%) or catch-down growth (lost more than 0.67 SD - 24.5%). Moreover, they also had larger waist circumference at 5 years even after allowing for BMI or FM. Infants showing the 'catch-up' pattern of growth were also smaller at birth, more likely to be first-born, had taller fathers, and their mothers had lower birthweight and were more likely to have smoked; all of these factors are thought to result in intrauterine constraint of fetal growth which is then followed by post-natal catch-up due to the effect of parental genes on childhood growth (e.g. taller fathers). In contrast, catch-down growth in babies of large birthweight was related to small size at five years. Even if catch-up growth was defined on the basis of gain in SDS for length, those who showed catch-up growth also had greater BMI SD scores and total FM. In addition, these results were unaffected by feeding mode (breast or bottle feeding).

Parsons et al (86) reported data from 10,683 members of the 1958 birth cohort. The risk of adult obesity was highest in those who had grown to a greater proportion of their adult height by 7 years of age. In men, the effect of childhood growth was strongest in those with lower birthweight, and, to a lesser extent, those born to lighter mothers.

In addition, central fat distribution may be of greater clinical significance than the total amount of fat in terms of its association with the metabolic syndrome (9;55;88-90). Several studies (78;91-95) have shown that, after adjusting for current BMI or weight, there has been a negative association between birthweight and later central fatness as measured either by S:T ratio or waist: hip ratio. This finding suggests that there is actually an association between postnatal growth and later central fatness; in other words, those who show the greatest change in size between birth and the later measurement have the greatest central fatness.

More recently, there have been a limited number of studies that have directly measured central fatness using more sophisticated body composition techniques. Garnett et al (96) studied 255 healthy children aged 7-8 years using DXA and found a significant negative relationship between birthweight SDS and abdominal fat in

childhood. The greatest abdominal fat was seen in those with lowest birthweight SDS who had gained weight centiles, and increased abdominal FM in these children was associated with higher total cholesterol, triglycerides and diastolic blood pressure. However, conflicting results were reported from a study that used a more direct technique for in vivo abdominal fat measurement in a smaller population. Choi et al (97) studied the relationship between birthweight, visceral fat area (by computerized tomography) and insulin sensitivity in 22 healthy young Korean adults. Without adjustment for current BMI or weight, they could not find a significant correlation between birthweight and any of the abdominal obesity measurements (waist: hip ratio, visceral fat area and visceral: subcutaneous fat ratio).

2.2.2 The long term influence of early nutrition

Many studies looking at associations between events during early life and later outcome have also examined the influence of infant nutrition, particularly the effect of breast versus formula feeding, but there are also some studies examining the effects of infant formulas with different composition. These studies are relevant to the study of early growth and later outcomes since the growth pattern in infancy is strongly influenced by early nutrition.

Breast versus formula feeding

There are a large number of publications from studies investigating the effect of breastfeeding on later obesity risk. Because it is unethical to randomly assign healthy term infants to be breast or formula-fed, the large majority of data come from observational studies. These may demonstrate associations between breastfeeding and later outcome but cannot, individually, prove causation. The odd ratios (OR) for the protective effect of breastfeeding against obesity are remarkably similar across several studies, ranging from 0.75-0.84 (98;99). For example, Von Kries et al. (99) studied 9357 children from Germany and found that the prevalence of obesity (defined as BMI above the 97th centile for the population) at age 5-6 years was 4.5% in those never breast-fed and fell with increasing duration of exclusive breast-fed (2 months, 3.8%; 3-5 months, 2.3%; 6-12 months, 1.7% and more than 12 months,

0.8%). Furthermore, the effect of breastfeeding remained after adjusting for potential confounding factors such as social class or lifestyle. After adjustment for confounders, the OR for development of obesity in breast-fed children was 0.75 (95%CI 0.57-0.98), and for being overweight, 0.79 (95%CI 0.68-0.93). It is possible that the apparent protective effect of breastfeeding on obesity is related to the lower nutrient intakes and slower weight gain of breast-fed infants early in life, and this is currently being investigated by a number of groups. Singhal et al (100) reported lower plasma leptin concentrations in relation to FM during adolescence in preterm infants randomised to receive breast milk when compared with those fed a nutrient-enriched preterm formula. These findings might suggest that early diet may program leptin physiology with the potential for influencing fatness, although no differences in body composition were apparent between diet groups at this follow-up.

In contrast, Li et al. (101) have published data from 2631 offspring of the 1958 British birth cohort (age 4-18 years) suggesting that there is no supportive evidence for a protective effect of breast feeding on obesity (defined by BMI above 95th percentile). Parsons et al. (102) also examined the associations between infant feeding and obesity at 33 year follow-up of the 1958 British birth cohort and found that breastfeeding and BMI were unrelated in childhood, but breastfeeding was protective against increased BMI at 16 and 33 years in women and at 33 years in men. However, after adjusting for social class, mother's BMI and smoking during pregnancy, the effects became non-significant. It is difficult to reconcile this finding with the fairly consistent results of more recent studies relating infant feeding to obesity risk during childhood, and further follow-up of these and other cohorts into adult life is clearly required.

Infant formulas with different composition

During the 1970's and 1980's, there were several studies looking at the effects on growth of different infant formulas, generally with different protein contents or protein: energy ratios - only a few studies concentrated specifically on energy content.

Fomon et al found that healthy term infants receiving a more concentrated formula (133 vs. 67 kcal/100ml (29), 100 vs. 54 kcal/100ml (30)) had a higher caloric intake and rate of growth (gain in weight and length) in the interval 8 through 41 days of age but similar caloric intake and rate of growth from 42 through 112 days of age. This was because during this later period the groups that received lower energy formulas consumed a relatively higher volume of milk and considerably greater quantity of other foods. Brooke and Kinsey (28) reported similar findings in term SGA fed different energy formulas. These studies suggest that it is possible to manipulate infant nutrient intake during the first few weeks of post-natal life but that at least some degree of appetite regulation becomes apparent by about 8 weeks, as demonstrated by the infant's ability to up or down regulate intake during this period.

A few studies have reported in greater detail on the *composition* of growth obtained using different energy formulas. Fomon et al (103) showed that a high energy intake between 8 and 55 days of age led to increased gain in weight but not in length, resulting in greater BMI. It was suggested that this represented increased fat deposition. Similar and more obvious findings were reported in studies in preterm infants (104-106). For example, Van Goudoever et al (106) showed that weight gain and percentage body fat (by SF) were significantly lower in very low birthweight infants fed with lower energy formula (67 vs. 80 kcal/ 100 ml).

Most studies of formula composition and body composition used basic anthropometry as outcome measures. More recently, DXA has been used to assess body composition and bone health in preterm infants. These studies found that infants fed diets with higher nutrient contents (preterm vs. term formula, formula vs. fortified human milk) have greater lean mass, FM and bone mineral mass (107;108) at the time of hospital discharge and age 6 months.

These studies therefore agree that body composition and growth pattern can be altered by changing the composition of the formula fed in early infancy. However, very few studies have followed the infants for a longer period in order to look into longer term outcomes of different early growth patterns resulting from different formula composition. Some follow-up studies in these preterm infants showed that the effects of diets during the post-discharge period on the growth of preterm infants

persisted up to 18 months of age, which was beyond the period when the formulas were fed (109;110). In contrast to post-discharge nutrition, studies examining the effect of *pre-discharge* nutrition in preterm infants generally show neither apparent persisting effects on growth at 18 months nor long term effects at age 7.5-8 years (111) - despite marked dietary effects on growth during the early post-natal period. Similarly, Fewtrell et al (112) demonstrated that term SGA infants fed nutrient-enriched formula were longer and had greater gains in length and OFC by 9 and 18 months than those fed a standard term formula, but a 6 year follow-up showed no differences in growth parameters. Worryingly however, the enriched formula group had higher FM (assessed by BIA) at 6 years of age (Singhal et al., Unpublished data) and the researchers suggested that these findings might be explained by the results of early rapid growth rather than the effects of different formula per se. It should be noted that there is the possibility of delayed emergence of the long term effects of early nutrition and growth on later body composition until a certain age (e.g. around puberty) as seen in the baboons (13;14). Therefore, longer term follow-up in adolescents and early adulthood in these cohorts is still required.

2.2.3 Relationship between infant body composition and later health outcomes

The data discussed above suggest that early 'growth' has significant effects on later body composition. However, it is important to emphasize that most studies on the later consequences of early growth pattern use only crude indices to assess growth or catch-up growth such as weight, height or BMI (absolute value, gain or SDS change), mainly because of the limitations of growth records and infant body composition measurement techniques.

There are a few longitudinal studies looking at the tracking of BMI from *infancy* through to adolescence and adult life. Rolland-Cachera et al (113) found that only 42% of 162 subjects followed up from 1 year to 21 years remained in their original category of lean, medium, and fat (defined using the 25^{th} and 75^{th} centiles of BMI as cut-offs), and the relative risk of being in the fat group at age 21 was 1 for the lean, 1

for the medium, and 2 for the infants categorised as fat at 1 year. Gasser (83) showed that correlations between BMI and SF measurements at 1 month and in adult life were very weak compared to the correlation from childhood to adolescence and from adolescence to adulthood. Due to the limitations of BMI mentioned previously, these findings cannot provide information on whether infant FM or FFM have any differential effect on later BC.

Available techniques for infant body composition measurements such as stable isotopes, total body potassium, total body electrical conductivity, DXA, and densitometry have been used more recently (39;114-116) and a few studies have followed up these infants to 2-3 years of age. Butte et al (117) found that differences in body composition (by multicomponent model; deuterium dilution, total body potassium, and DXA) in early infancy (up to 6 months), which were associated with infant feeding mode (breast vs. formula), did not persist into the second year of life. In contrast, Wells et al.(118) reported that infant fatness (by doubly labelled water study) showed a strong relationship with childhood fatness at the age 2.5-3.5 years. However, there have been no longitudinal follow-up studies of the effects of alterations in infant body composition on later obesity. Follow-up studies of cohorts that have detailed anthropometry and body composition measurements in infancy would be very useful to examine which components of growth (e.g. lean mass, or FM) are important for long term health outcomes. This could in turn provide clues about the potential mechanisms behind the programming effects of early growth.

Summary:

Available evidence supports the hypothesis that the growth pattern early in life, which can be manipulated by nutrition, affects different components of body composition in later life. Collectively, the data suggest that birthweight predicts later lean mass whereas postnatal growth may predict FM and possibly the development of central fat stores. However, the majority of studies have used proxy measures of 'fatness' such as BMI, and very few have attempted to directly measure specific components of body composition. In addition, the effects of body composition changes during infancy (which have actually been measured, rather than using crude indices as a proxy of fatness) on health outcomes have not been explored and warrant

further investigation. Such data could be important in determining the direction of infant feeding policy, in order to make infants 'grow' in a way that might be more beneficial for long term health outcome e.g. reducing the risk of obesity.

2.3 Body composition and health outcomes

Although there was no commonly accepted definition of overweight and obesity in children until the International Obesity Task Force (IOTF) recently recommended using the BMI cut-offs proposed by Cole et al (119), there is a clearly rising trend of childhood overweight and obesity worldwide (1;3-5). Moreover, there are a number of studies showing that overweight children and adolescents are likely to become overweight adults (8;120-122) and some (123), but not all (124;125), studies suggest that childhood obesity is associated with an increased risk of cardiovascular disease even when obesity does not itself persist. Both short and long term health consequences of childhood obesity are under extensive investigation.

In adults, WHO criteria for overweight (BMI >25 kg/m^2) and obesity (BMI > 30 kg/m^2) are widely accepted and used as health risk markers for cardiovascular disease and the metabolic syndrome (consisting of insulin resistance, hypertension, dyslipidemia and several abnormalities in clotting and inflammatory markers) (89;126;127). In adults, studies comparing the relative value of BMI and percentage fat (relative to weight) in the prediction of metabolic risk are contradictory, with some reporting that BMI has similar, or even better correlation, with cardiovascular risk factors (128), and others reporting that the value of %FM exceeds that of BMI (129).

Although BMI has been shown to be associated with cardiovascular risk factors in children to a certain extent (130-132), the use of BMI as a 'proxy' for adiposity in children and adolescents might have limitations due to variation in growth rates and maturity. For example, it has been reported that annual increases in BMI during childhood are generally attributable to the lean rather than the fat component of BMI until late adolescence (133;134). Furthermore, there is evidence that in obese children, FFM increases as well as FM (135). The mechanism by which FM regulates a host of physiological processes related to the development of insulin

resistance and cardiovascular disease has been extensively investigated (136-138). In contrast, the impact of increased FFM on health outcomes has rarely been explored. Differential effects of these two components of BMI on cardiovascular health risks warrant further exploration.

There is increasing evidence that enlarged adipocytes in the visceral fat depot synthesize and release biologically active molecules that may affect cardiovascular risk factors such as circulating free fatty acid molecules, inflammatory cytokines (TNF-α, IL-6), adiponectin, resistin and leptin (9;89;90). Therefore, central fat distribution may be of greater clinical significance than the total amount of fat in terms of its association with the metabolic syndrome (88;96;97;139). Since MRI or CT, which are considered to be the 'gold standard' for visceral adipose tissue assessment, are not always practical in clinical and research settings, WC has been proposed as a better marker for cardiovascular disease and insulin resistance than BMI (53-55) in children as well as adults. For example, Maffeis et al (55) studied 818 prepubertal children aged 3-11 years and showed that ApoA1: ApoB, HDL cholesterol, total cholesterol: HDL cholesterol, and systolic as well as diastolic BP were significantly associated with WC, triceps and subscapular SF, independently of age, gender, and BMI. Moreover, WC had a higher correlation with systolic and diastolic BP than triceps, subscapular SF and relative body weight.

Although designed to measure bone mineral content, DXA has also recently been used to assess soft tissue composition. Increasing number of studies investigating the programming of body composition have used trunk FM from DXA as an index for central fat distribution (140;141). Therefore, its relationship with cardiovascular risk factors is relevant in my study. As previously mentioned, DXA has some limitations in soft tissue assessment since it predicts rather than measures soft tissue composition in pixels that contain bone; thus, trunk soft tissue measurements (which include the thoracic cage) are likely to be predicted to a greater extent than limb measurements (which only include the long bones). For this reason, a DXA manual region of interest (excluding the thoracic cage and defining the abdominal area from L1-L4) has been introduced and validated against CT in an adult population (142). The same researchers reported that abdominal FM by this method provided a sex-independent predictor of insulin sensitivity index in a group of older adults (143).

Therefore, its relationship with cardiovascular markers in children and adolescents, especially in comparison with other body composition variables, deserves further investigation.

Summary:

In children, BMI is not a good index of body fat, which may be the component that is most associated with health risks. Separate measurements of FM and FFM would be desirable in further defining the health effects of increasing body weight. Simple techniques for assessing central fat distribution in paediatric populations warrant further investigation.

The next chapter is an outline of my hypotheses and research plan, beginning with a description of the study populations and followed by details of the measurement techniques used for the main outcomes and potential confounding factors.

Chapter 3

Hypotheses, Study plan and Outcome measures

Chapter 3 Hypotheses, Study plan and Outcome measures

3.1 Hypotheses

Base on the findings discussed in **Chapter 2**, I proposed 2 main hypotheses which have been tested in this dissertation:

(I) Early growth patterns program later specific component of body composition in adolescence and early adulthood, and could thereby influence the development of obesity and cardiovascular health risks.

This first hypothesis can then be divided as follows:

I-1 Different periods of growth, i.e. 'prenatal' (represented by birthweight) and 'postnatal', have differential influences on later body composition.

I-2 Different components of body composition in adolescence and early adulthood have differential effects on cardiovascular health risks.

The research and specific analyses addressing this hypothesis are shown in **Chapter 4**, **Chapter 5**, and **Chapter 6**.

(II) Specific components of body composition (e.g. lean body mass or FM) during infancy are differentially related to later body composition and cardiovascular risk factors; and are, therefore, potentially involved in the programming process. Infant nutrition may have long-term consequences for later body composition and health that are mediated by effects on infant body composition.

Research and specific analyses addressing this hypothesis are shown in **Chapter 7** and **Chapter 8**.

The remaining part of this chapter will provide an overview of the research project and outcome measures which apply broadly to all of the subsequent chapters. Additional measurements or details of more specific statistical analyses will be discussed in greater detail in the relevant chapters.

3.2 Study populations

The hypotheses were tested using 4 cohorts of healthy UK children and adolescents. Three cohorts had body composition measurements during infancy and were prospectively followed-up during adolescence. The fourth cohort consisted of healthy children and adolescents who are a part of body composition reference study; birthweight and growth records were retrieved retrospectively from infant records and maternal recall in this group.

3.2.1 Prospective follow-up of healthy term infants

A follow-up study was carried out in three different cohorts of healthy term infants who had early growth and body composition measurements using the doubly-labelled water technique (144-147). A summary of each of these three cohorts is given below:

Cohort A: Randomised controlled trial of different energy infant formulas

Based on the observation that formula-fed infants might effectively be overfed compared to breast-fed infants when consuming current infant formulas, a randomised controlled trial was set up in 1990 to test the hypothesis that the use of a formula with an energy content similar to that in breast milk (metabolisable energy density around 60

kcals/100ml, as determined by doubly-labelled water measurements) would result in the pattern of growth, body composition and energy metabolism seen in breast-fed babies. Between 1990 and 1992, 107 healthy term infants with birthweight >10th centile by local growth standards were recruited from the maternity hospital in Cambridge. Infants were eligible if their mother intended to formula feed. After informed parental consent was obtained, the infants were randomly assigned to receive a standard (67kcal/100ml) or a low energy (60kcal/100ml), lower fat formula (both manufactured by Ross Products, Ohio, US; detailed composition shown in **Appendix A**). The formulas were otherwise identical. Formulas were fed as sole diet until 12 weeks and as a supplement to weaning foods thereafter. Anthropometry was performed at recruitment (age 2-3 days), 3, 6 weeks, and at 3, 6, 9, and 12 months of age. Energy expenditure, milk volume intake and body composition were measured using the doubly-labelled water technique at 3 months.

Results (unpublished data, further details of the study are shown in **Appendix A** and discussed in **Chapter 8**)

Infants who received the reduced energy (RE) formula showed similar weight and length gains to those fed standard formula. However, at 6 weeks and 3 months they had evidence of reduced adiposity with lower SF, lower FM and higher FFM. From 3 to 12 months of age, when both groups were receiving solids as well as trial formulas, there were no differences between the groups. Milk intake at 3 months was greater in infants receiving the RE formula, indicating some attempt to 'up regulate' intake, consistent with previous studies (28;30). However, their fat intakes were still significantly lower than those of the standard formula group, whilst their protein intakes per kg body weight were higher. This finding corresponded with the difference in body composition at 3 months. No differences were detected in energy expenditure between the two groups at the age of 3 months.

Cohort B: Study of energy metabolism in infancy

50 healthy full-term infants (25 breast-fed and 25 formula-fed) were recruited from the Maternity Hospital in Cambridge in 1992-1993 for a comprehensive study of energy metabolism in infancy. Anthropometry, doubly-labelled water study and indirect calorimetry were performed at 3 months of age and part of the cohort was followed up at 2-3.5 years.

Results

The detailed description and results from this cohort are published elsewhere (118;148-153). Briefly, there were no significant differences in anthropometry (weight, length, SF) and body composition (FM and FFM) between breast-fed and formula-fed infants in this cohort, although FFM was slightly greater in the formula-fed group. Sleeping metabolic rate was higher in the formula-fed group, which was accounted for by the difference in body composition (149). The researchers found no relationship between energy expenditure and energy intake at 3 months and fatness at 2-3.5 years (152). However, infant fatness at 3 months of age influenced both later fatness and activity pattern at 2-3.5 years (118).

Cohort C: Infant feeding study

52 healthy full-term infants (21 breast-fed and 31 formula-fed) were recruited from the Maternity Hospital in Cambridge in 1985-1987 for a study of energy requirements and expenditure in healthy term infants. Anthropometry was performed at 5 and 11 weeks, 6 and 9 months and at 2 years of age. Total energy expenditure and body composition was measured at 1.5, 3 and 6 months of age using doubly-labelled water technique. Part of this cohort was followed up at 2-3.5 years of age.

Results

The detailed description and results from this cohort are published elsewhere (154-158). Briefly, the researchers presented the centiles for total energy expenditure in early infancy and suggested the most appropriate method of expressing energy expenditure relative to body weight (expressed as KJ/square root of body weight)(154;155). In terms of infant body composition, the researchers showed that BMI (weight/height^2) and SF were poorly predictive of body fatness in young infants (156;157). Moreover, the follow-up study at 2-3.5 years of age showed no relationship between the level of energy expenditure at age 12 weeks and later indices of body fatness (by BMI, SF, and FM from stable isotope measurements)(158).

These 3 cohorts provide the opportunity to test the hypothesis (I), concerning the programming of body composition by early growth, and hypothesis (II), concerning the relationship between body composition in infancy and later outcomes, in normally-grown term infants with prospective early growth and body composition measurements. They also provide the opportunity to investigate the relationship between different feeding modes (breast vs. formula or different infant formulas) in early infancy and later body composition and cardiovascular risk factors (hypothesis II).

3.2.2 Retrospective study of infant growth data (Cohort D)

Healthy term-born children aged 4-19 years were recruited for a body composition reference study using the 4 component model of body composition, at the same study centre. The subjects were eligible for the study if they were born full-term and had no condition that might affect growth and body composition. This study started in February 2002 and data collection is ongoing. The aim was to recruit a minimum of 320 healthy children and adolescents to form a reference for body composition data. Initially, recruitment was done through advertisements in schools and sport clubs and by word of mouth, followed by advertisement on the intranet within the Institute of Child Health, University College London, and Great Ormond Street Hospital. From March 2005,

recruitment was also via local newspapers. The studied population were based in Greater London and Cambridgeshire (some of the baseline characteristics are shown in **Table 3-1**).

Collection of the retrospective growth records was started in March 2004. Birthweight and early growth data were collected by parental recall and from parent-held baby record books. The families were asked to bring their child's baby record book on the study day. Subjects who took part in the study before this time (85 subjects) were contacted and asked for a copy of growth records to be sent by post. Overall, around 58 % of subjects provided baby record books that could be analysed. This cohort provides the opportunity to test hypothesis (I), concerning the relationship between early growth and later body composition in healthy children at different ages.

Table 3-1 Characteristics of subjects from cohort D[++]

	Boys (n=125)	Girls (n=131)	p
Age (y)	11.1 ± 3.9	11.0 ± 3.8	0.9
Birthweight (kg)	3.57 ± 0.53	3.42 ± 0.52	***0.03***
Gestation (wk)	40.1 ± 1.3	40.0 ± 1.5	0.6
Birthweight SDS	-0.02 ± 1.06	0.04 ± 1.06	0.7
Height (cm)	145.2 ± 22.5	142.9 ± 18.6	0.4
Height SDS	0.22 ± 0.96	0.22 ± 0.96	1.0
BMI (kg/m^2)	18.6 ± 4.2	19.0 ± 3.5	0.4
BMI SDS	0.28 ± 1.28	0.37 ± 1.15	0.6
Maternal BMI (kg/m^2)	25.1 ± 4.8	24.6 ± 4.9	0.5
Paternal BMI (kg/m^2)	26.5 ± 3.8	25.4 ± 3.6	***0.02***
Pubertal status (n, %)			
Prepubertal	65, 52.8%	69, 52.7%	0.7
Early pubertal	30, 24.4%	27, 20.6%	
Late pubertal	28, 22.8%	35, 26.7%	
Social class (n, %)			
Class 1	29, 23.8%	26, 19.8%	
Class 2	54, 44.3%	61, 46.6%	0.4
Class 3	7, 5.7%	14, 10.7%	
Class 4 or more	32, 26.2%	30, 22.9%	
Ethnicity (n, %)			
White	89, 72.4%	92, 70.8%	
African-Caribbean	29, 23.6%	27, 20.7%	0.7
Asian	3, 2.4%	3, 2.3%	
Chinese	1, 0.8%	3, 2.3%	
Other	1, 0.8%	5, 3.8%	

[++] Continuous variables were expressed as mean ± SD. Independent sample t-test was used to test differences between genders. Categorical variables were expressed as n (%). Chi-square test was used to test differences between genders.

3.3 Outcome measures

3.3.1 Primary outcome: Body composition measurements

Detailed body composition measurements in children and adolescents are important for two main reasons. Firstly, they enable more specific testing of the programming of body composition, using measured components rather than proxies of fatness such as BMI or SF. Secondly, although it is widely accepted that BMI in adults predicts cardiovascular health risks (89;126;127), in children and adolescents there is less available evidence relating BMI, or indeed other measures of body composition to clinical outcome. Hence, one important aspect of research using body composition measurements in children and adolescents might be to help identify which elements of body composition are most related to obesity-related health risk (e.g. whilst most interest is focused on FM, it is also interesting to determine whether FFM shows any association with cardiovascular risk factors). It would also be possible to determine if body composition measurements obtained from the 'gold standard' method (four-component model) are better predictors of the risk of cardiovascular disease than simple methods such as BMI, WC or SF which would have practical implications for research and clinical practice.

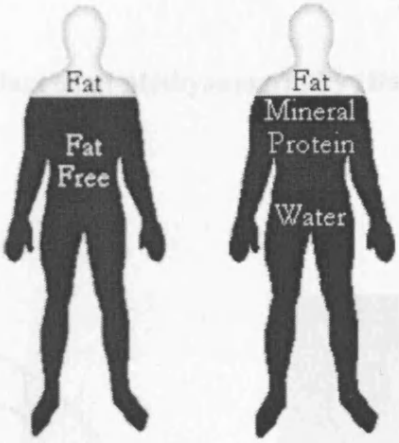
The next section is a brief explanation of the body composition measurement procedures used, which are standardized in all of the populations previously mentioned.

3.3.1.1 The four-component model

The four-component model of body composition (4C model) divides the body into fat, water, protein, and mineral (see **Figure 3-1**). As described in **Chapter 2**, it minimises the assumptions made in the 2C model by directly evaluating several presumed constant relations (e.g. hydration, density, and BMC of FFM) that is fundamental to the 2C model. It has been accepted to be the most robust technique to detect inter-individual

variability in the composition of FFM, as well as showing consistent accuracy across a range of body fat (159).

Figure 3-1 Picture illustration of 2-component and 4-component models of body composition measurement



The minimal assumptions[#] for density of fat, water, protein, and mineral are used to calculate FM from the basic measurements.

$$FM\ (kg) = 2.747BV - 0.710TBW + 1.460BMC - 2.050BW$$

BV = actual body volume in litres (from Air-displacement plethysmography)
TBW = total body water in litres (from Deuterium dilution)
BMC = total body bone mineral content in kg (from DXA)
BW = body weight in kg

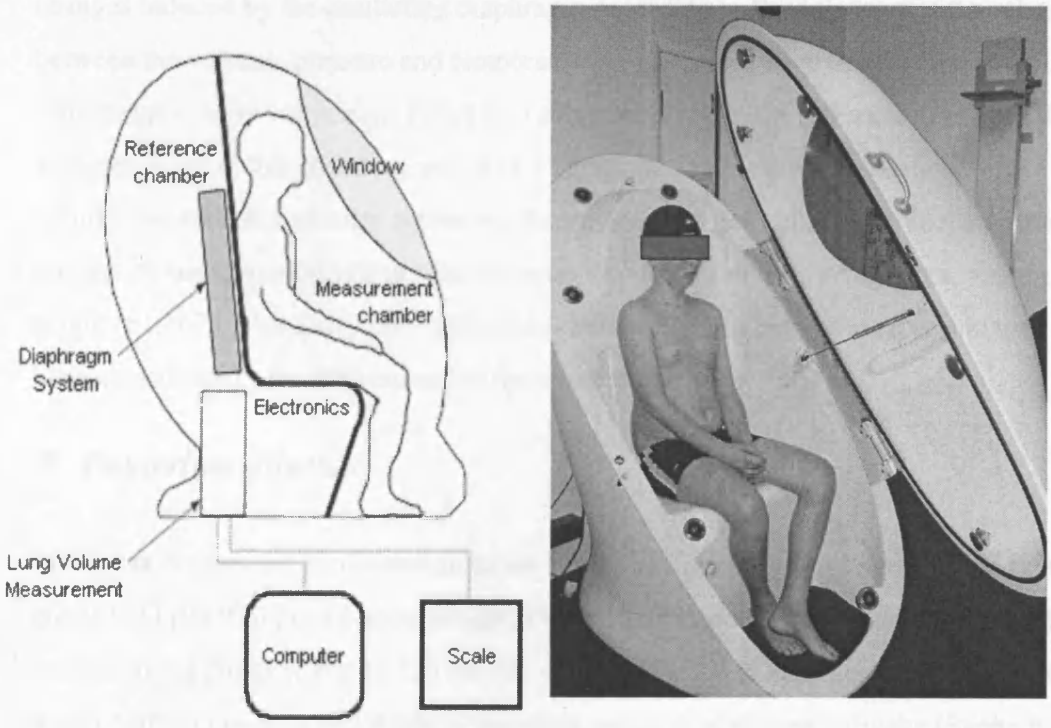
This calculation is based on the equation of Keys and Brozek (161) and is described in detail elsewhere (58;66). In order to calculate the 4C model, measurements were obtained using the following methods:

[#] From Brozek et al (160): density of body fat = 0.9007 kg/l, density of water at 36°C = 0.99371 kg/l, density of protein = 1.34 kg/l, density of total body mineral = 3.0375 kg/l (assumed constant ratio (0.8191:0.1809) of osseous to non-osseous mineral)

A. Air displacement plethysmography

Whole body air displacement plethysmography was performed using the Bodpod body composition system (Life measurement Instruments, Concord, California, USA), with the subject wearing a tight fitting swimming costume and a swimming cap (see **Figure 3-2**).

Figure 3-2 Air displacement plethysmography (Bodpod) body composition system[##]



[##] Photograph presented with consent from the subject and parents

The procedure (61) involved a volume calibration with and without a 50 litre metal cylinder. The subject entered the Bodpod and sat inside the anterior chamber (450 litres), which was connected to a rear measuring chamber (300 litres) via oscillation diaphragms (used to induce pressure change in anterior chamber), and breathed normally (relaxed tidal breathing). The recommended procedure, consisting of two measurements of body volume (50 seconds each), was adopted and when, occasionally, body volume differed by more than 150 ml, the system required that a third measurement be performed. To improve precision, the procedure was repeated until 2 values for raw density of within 0.007 kg/L were obtained (162).

The principle is to measure the volume of air in the anterior chamber, using pressure changes induced by the oscillating diaphragm according to Boyle's laws of the relations between the volume, pressure and temperature of gases (see **Figure 3-2**). The machine provides raw body volume (in litres) for each subject, from the difference between the volumes of air in this chamber, with and without the subject present. Actual body volume can be obtained after correction for the thoracic gas volume and surface area artefact by using appropriate prediction equations for children from age, sex, weight, and height (61;163). This fairly new method has been shown to be more acceptable than hydrodensitometry in children, and to have better precision (61;164).

B. Deuterium dilution

TBW was determined by deuterium oxide (D_2O) dilution with a dose equivalent to 0.05 gm of D_2O (99.9%) per kg body weight. Doses were made up with water to around 100 mL for young children and to 150 mL for older children and adolescents. Saliva samples were obtained pre-dose and 4-hours post-dose using an absorbent salivette (Sarstedt, Rommelsdorf, Germany) at least 30 minutes after the last food or drink. The samples were frozen at -30°C and then analyzed in duplicate by Iso-Analytical Ltd (Sandbach, UK) using the equilibration method (165) and continuous-flow isotope ratio mass spectrometry (Geo20-20; Europa Scientific, Crewe, UK). The accuracy of the analyses was checked by measuring an intermediate water standard within each batch of samples. The coefficient of variation of deuterium analyses by this technique in the laboratory is

< 2.5%. For calculating TBW, it was assumed that the D_2O dilution space overestimates TBW by a factor of 1.044 (63;64). Correction was made for dilution of the dose by water intake during the 4-hour equilibration period (166).

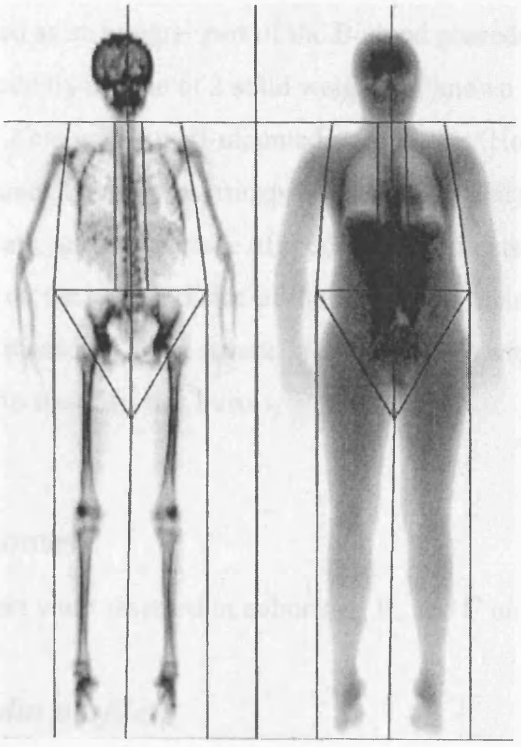
C. Dual-energy X-ray absorptiometry (DXA)

Bone mineral content (BMC), FM and lean soft tissue mass was determined using a GE Lunar Prodigy whole-body scanner (GE Medical Systems, Madison, Wisconsin, USA) in conjunction with Encore 2002 software. All DXA scan and analyses, including the manual ROI, were performed by a single operator. The instrument automatically alters scan depth depending on the thickness of the subject, as estimated from age, height, and weight. A whole body scan was performed while the subject was wearing light indoor clothing and no metal objects. The typical scan duration was 5-10 minutes, depending on the subject's height. The radiation exposure per whole body scan is estimated to be 2.2 μSv, which is lower than the daily background radiation in the UK (around 7μSv). The coefficient of variation (CV, %) for a Lunar DPX-L instrument (regarded by the manufacturers to be similar to the Lunar Prodigy) has been reported as 1.10, 2.0, 1.11 % for total body BMC, FM, and lean mass, respectively(167). Regional measurements (arm, leg, and trunk) were less precise than total body measurements, with CVs in the range of 1% to 3%.

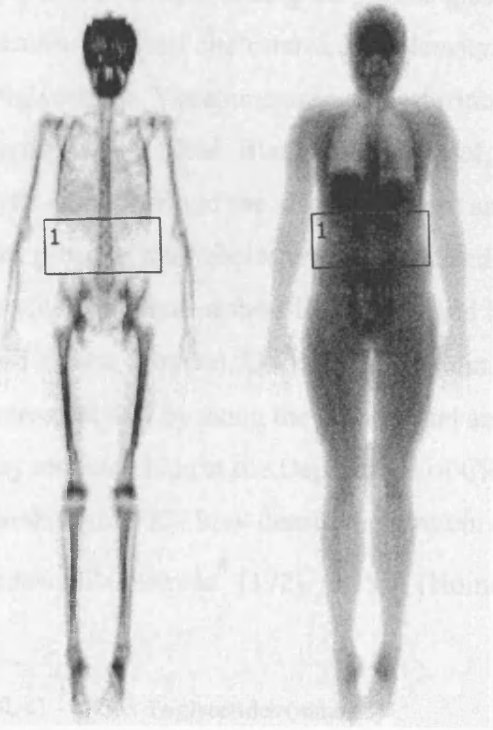
Due to its limitations in measuring soft tissue composition (59;168;169) as mentioned previously, only total body BMC was used in the analysis (as a part of the 4-component model). However, for regional body composition, DXA is still a practical tool when compared to gold standards such as MRI and CT which are more complicated, expensive, and, in the case of CT, involve more radiation exposure. Therefore, in this dissertation, DXA-derived trunk and limb composition were also used in some analyses. In addition, abdominal FM by manual ROI between L1-L4 was analyzed. A validation study in adults showed good agreement between this method and CT for abdominal total tissue mass and FM (142). Examples of a DXA scan and the manual ROI L1-L4 are shown in **Figure 3-3**.

Figure 3-3 Examples of DXA scans

a) Whole body scan



b) Manual region of interest L1-L4



3.3.1.2 Anthropometry

Body weight was measured as an integral part of the Bodpod procedure to within 0.01 kg. Accuracy was confirmed by the use of 2 solid weights of known mass. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Holtain, Dyfed, United Kingdom). SF was measured at the biceps, triceps, subscapular, and suprailiac sites with Holtain callipers in triplicate, and the average of the 3 measurements was used. All measurements were done on the left hand side of the body, with the subject standing. Waist circumference was measured at the natural waist site (170) with a non-stretchable fibre glass insertion tape, to the nearest 0.1 cm.

3.3.2 Secondary outcomes

Cardiovascular risk markers were assessed in cohorts A, B, and C only.

3.3.2.1 Lipid and insulin profiles

A blood sample was obtained after overnight fasting for plasma glucose, insulin (total, proinsulin and 32-33 split proinsulin), total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides. Venepuncture was performed at an antecubital vein after local anaesthetic cream was applied. Blood was kept cool, separated within 40 minutes (to prevent proteolytic degradation of the insulin peptide) and plasma stored at -80°c prior to analysis. Plasma glucose, total cholesterol, HDL-C and triglycerides were measured using standard enzymatic method at the Biochemical and Nutrition Department, Institute of Child Health (London, UK). Plasma insulin, intact proinsulin and 32-33 split proinsulin were analysed by using the monoclonal antibody-based two-site immunoradiometric assay method (171) at the Department of Clinical Biochemistry, Addenbrooke's Hospital (Cambridge, UK). Low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula[#] (172). HOMA (Homeostasis model

[#] LDL-C = total cholesterol – HDL-C – 0.456x Triglycerides (mmol/L)

assessment) was calculated by the HOMA Calculator ©The University of Oxford 2004 (173;174) from fasting glucose and insulin.

Surrogate markers of insulin resistance, rather than a direct measure (the glucose clamp (175)), were used because of technical difficulties of the latter method in children. The HOMA index has been reported to be strongly correlated with the glucose clamp, and proposed to be suitable for assessing insulin sensitivity in situations where only a fasting blood sample is available (176). Proinsulin and split-proinsulin have been shown to be strongly related to the development of insulin resistance, and the latter has been reported to predict progression to type II diabetes more strongly than total insulin concentrations (177).

3.3.2.2 Blood pressure

Systolic, diastolic and mean arterial pressure were recorded in triplicate using an automated oscillometric device with appropriate cuff size (Accutorr, Datascope Corp., New Jersey, USA) (178). The subject rested on a couch for at least 15 minute before the measurement was made.

3.3.3 Assessment of confounders

Questionnaires (**Appendix B**) were used to determine socioeconomic status, previous medical history, physical activity, family history of cardiovascular disease, and parental body size. Pubertal stage, which has a significant effect on body composition, was evaluated using a pubertal status self assessment form (line drawings of the different Tanner pubertal stages with written explanation) for subjects to complete by themselves in private and return in a sealed envelope to avoid the need for undressing. This method has been shown to have a good agreement with physician's assessment (179). Social class was assessed using the Standard Occupational Classification (180), according to primary earner of the family (either mother or father or both). For older subjects, social

class was still based on parents' occupation since it provides information on family background and environment they were brought up. A simple assessment of subjects' physical activity level was made by asking parents to rate their child (or asking young adults to rate themselves) compared with his or her peers on a 5-point scale ranging from "much less active" to "much more active". This simple method has been shown to correlate well with physical fitness and BMI in children (181).

3.4 Statistical methods

3.4.1 Sample size

In published studies, birthweight explained between 2.7% (182) and 5.4% (79) of the variance in BMI and FFM; therefore, to test hypothesis I, I estimated that the sample size required to detect a similar correlation (r=0.16-0.23) would be 143-300 subjects (at 5% significance with 80% power[#] (183)). For early infancy growth, the results from a similar study showed that weight gain from birth to 3 months explained around 4.9% of the variance in BMI SDS at age 19 years (182); therefore, I estimated that the sample size required to detect a similar correlation (r=0.22) would be around 157 subjects.

There were no published studies from which to calculate sample size for the relationship between infant BC and adolescent BC (hypothesis II). The study by Wells et al (118) showed that the correlation between infant FM at 3 months and childhood FM at 3 years (n=30) was around 0.55 (p 0.006); however, I appreciate that the correlation at follow-up 10 years later during adolescence could be much weaker and require a bigger sample size. For example, if half of the original cohort who completed the isotope study at 3

[#] From Cole TJ (183) $n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^2/(1-d^2)} + 5$

when d=correlation, $(Z_{1-\alpha/2} + Z_{1-\beta})^2 = 7.85$ for $\alpha = 0.05$ and $1-\beta = 0.8$

months (n=75) agreed to take part, I would be able to detect a correlation of around 0.31 with 80% power and 5% significance.

For the comparison between groups in the randomised trial of different energy formulas, 40 subjects per randomised group would allow detection of 0.63 SD difference in body composition outcomes (80% power, 5% significance)[$]. It was recognized that there might be a limitation of the sample size since the follow-up study took place around 12 years after the last contact had been made with these families, and a significant loss to follow-up might therefore be anticipated. However, the main reason for following up this cohort in my thesis was not to examine the effect of early diet but to combine the subjects with those from cohort B and C to examine the effects of infant growth and body composition.

Combining the 3 infant cohorts (A, B, and C), the number of potential research participants was 199 (excluding 10 early drop-outs from cohort A), which was considered a reasonable number for a follow-up study to look at the association between early growth (hypothesis I) and infant body composition (hypothesis II) in relation to later outcomes. Moreover, these cohorts are unique in that they all had detailed anthropometric measurements at different periods in infancy, and 150 of them also completed the body composition measurement using stable isotopes at 3 months. In fact, it is inevitable that in a study with such detailed measurements of infant body composition the sample size cannot be comparable with a study using only weight or length as a proxy of growth. A further consideration here is that the primary outcome measure for the follow-up study is body composition using the 4C model which also cannot be achieved in a very large population. These issues are discussed further in the relevant chapters and in the general discussion (**Chapter 9**).

[$] From van Belle (184) $n = \frac{16}{sd^2}$ for $\alpha = 0.05$ and $1-\beta = 0.8$,

when sd = standardized difference (the difference to be detected in the unit of standard deviation)

Since a relatively large (although unknown) loss to follow-up was expected from cohorts A, B, and C, and the sample size from these 3 cohorts alone might not be sufficient to test hypothesis I, retrospective growth data were collected from cohort D at the same time as the follow-up study.

3.4.2 General

Standard deviation scores (SDS) were derived for weight, height, and BMI using the 1990 British reference (185;186) and calculated by the lmsGrowth program© (Copyright 2002-2005, Medical Research Council, UK). Triceps, and subscapular SF SDS were derived using Tanner-Whitehouse reference data and calculated manually using age and sex-specific L, M, and S values (187). Waist circumference was converted to SDS using a nationally representative sample of UK children in 1988 (170) and calculated by the lmsGrowth program© (Copyright 2002-2005, Medical Research Council, UK). One sample t-test was used to compare the SDS of the study population with reference data.

To describe the characteristics of study subjects, continuous variables were compared between genders using independent sample t-test. Categorical variables were compared using Chi-square or Fisher's exact test as appropriate.

To test the association between birthweight, early growth, and early body composition and later outcomes (**Chapter 4**, **Chapter 5**, and **Chapter 7**), multiple linear regression analysis was used to adjust for potential confounders (pubertal staging, physical activity, socioeconomic status, ethnicity, and parental size). Continuous variables which were not normally distributed were transformed to natural logarithms and the data were interpreted in the form of sympercents described in details by Cole et al (188).

> "A difference of natural logs corresponds to a fractional difference on the original scale. The agreement is exact if the fractional difference is based on the logarithmic mean. The transform y = 100 log(e)x leads to differences, standard deviations and regression coefficients of y that are equivalent to symmetric percentage differences, standard deviations and regression coefficients of x. The

100 log(e) scale is the natural scale on which to express percentage differences. The term sympercent or s% is proposed for them."

Specific details of the analyses are given in the relevant results chapters. General linear model (GLM) was use to test the interaction between gender and birthweight or early growth in predicting later body composition. Partial correlation was used to 1) test the relationship between different confounders and body composition outcomes (**Chapter 4**), and 2) compare the correlation between different body composition variables and cardiovascular risk factors (**Chapter 6**). Continuous variables which were not normally distributed were transformed to natural logarithms. To test the mean difference between diet groups (**Chapter 8**), one way ANOVA and GLM were used where appropriate.

All statistical analyses were performed using SPSS version 13 (SPSS Inc, Chicago, USA).

3.4.3 Expression of body composition data

Since paediatric populations vary in body size much more than adult populations, meaningful interpretation of body composition data depends greatly on size adjustment. In this dissertation, body composition data (e.g. FM, FFM, trunk FM) were adjusted for body size using height or height^2 (189;190). Alternative adjustments by weight or BMI are discussed in **Chapter 4**.

Due to the wide age range of the subjects, internal SDS for body composition variables (FMI, FFMI, trunk FMI) were calculated using the LMS method (191) by lmsChartMaker program© (Copyright 1997-2005, Medical Research Council, UK). A detailed description and discussion is included in **Chapter 5** and curve fits for each variable are shown in **Appendix C**.

Different indices for central fat distribution (e.g. waist circumference, triceps and subscapular SF, trunk FM, abdominal FM) were used in the analyses for the purpose of

comparison since the gold standard for visceral fat measurement (MRI) was not available. The magnitude of association between different body composition variables and cardiovascular risk factors is analyzed and discussed in **Chapter 6**.

3.4.4 Longitudinal growth data

I considered several options for analyzing longitudinal growth data, with statistician input. Multiple linear regression analysis of body composition outcomes on change in weight SDS during different periods in infancy was adopted (Cole TJ, personal communication). Because the early growth measurements in the retrospective study (Cohort D) were not all made at standardised ages, imputation of the missing data points was also considered. Specific details are discussed in **Chapter 5**.

3.5 Ethical and practical considerations for cohorts A, B, C

The protocol was approved by Research Ethic Committee at the Institute of Child Health. Informed consents and assents were obtained from parents and study subjects accordingly before the study. The important ethical and practical issues are highlighted below. Examples of the information sheets and consent forms are shown in **Appendix D**.

3.5.1 Recruitment

The procedure was as follows:

a) Research participants were identified using existing information from the original study (addresses and GP names from that time were kept by the research team). The parents of subjects who participated in the study expected to be contacted for further follow-up after the trial finished, although written consent for this was not obtained (this

was not standard practice at the time); if any parent requested that they were not contacted again, this was recorded at the time.

b) Parent and subject information sheets were sent to the subject's last known address. If this was unsuccessful, the family was traced via the GP. If the potential research participants had moved, the GP (or new GP if appropriate) was asked to forward an information sheet to the new address.

c) Subjects and parents who wished to participate or find out more about the study were invited to return a reply slip to me. I then telephoned them to discuss the project, and, if they wished to participate, I arranged an appointment. All consenting participants who were clinically well at the time of the study were recruited.

3.5.2 Informed consent

Both written consent and assent from the subject and his/her parents was obtained on the day of the study visit after I had explained the procedures, shown all the equipments to the subjects and their parents, and answered any question. Separate written consent was obtained from the parent (or subject if over 16 years) for the blood test, before taking the blood sample and after the subject and their parents had been informed of how the samples were to be used and stored.

3.5.3 Data protection

All procedures and data storage were compliant with the Data Protection Act. Individuals were identified on computer databases by their code number; this was also used on blood and saliva samples. The papers files containing data were kept in a locked cabinet at the Childhood Nutrition Research Centre with the personal information section kept separately. Computerised data were kept password protected on my computer at the Childhood Nutrition Research Centre. Master copies of computer data

files will be kept locked in the fire-proof safe within the department for at least the next 15 years.

3.5.4 Overview of the study day at the MRC Childhood Nutrition Research Centre (half-day visit)

The study took place at Great Ormond Street Hospital and the Childhood Nutrition Research Centre, Institute of Child Health, London, UK. The subject was asked to arrive at 9am, having fasted overnight. Informed consent was obtained as described above. Breakfast was provided after the blood test had been performed and further refreshments throughout the day as required.

The following investigations were performed:

1. A blood sample was taken for plasma insulin and lipid profiles. Local anaesthetic cream was used to minimize discomfort.

2. Blood pressure was measured using an automated device while the subject was resting on a couch for at least 15 minutes.

3. Body composition was measured in the Radiology Department in Great Ormond Street Hospital as follows:
a) TBW was measured by deuterium kinetics. The subject was asked to drink some water containing a known amount of deuterium. A saliva sample was collected before (early on the study day) and 4 hours after dosing (before the study day finished) using a chewable cotton swab after the subject had cleared their mouth 30 minutes before the sample collection. The volume of drinks consumed between the two saliva samples was recorded.
b) DXA was used to measure whole-body bone mineral mass.
c) Air-displacement plethysmography (Bodpod) was used to measure body volume

4. Anthropometric measurements were performed.

5. Questionnaires were used to determine general health, life style, physical activity, socioeconomic status of the family and pubertal status (the latter was self-rated in private using line drawings of the different Tanner stages and given back in a sealed envelope).

The study took approximately 4 hours on the day of the visit to London (a single occasion). Travelling expenses were reimbursed for both subjects and parents to come from Cambridge to London. The subject was given a £10 gift token after completing the study day as a thank you for their time, together with picture of their skeleton (from whole body DXA).

Subjects will, in due course, receive a description of the results in lay terms, and they can also contact me by telephone to discuss particular research results. In the event of an abnormal test result (such as lipid profiles, blood pressure, bone density), both the family and GP was informed so that further investigation could be arranged if necessary.

3.5.5 Recruitment and follow-up rates for cohorts A, B, and C

The research proposal was written and the research ethics committee application submitted in March 2004. After minor changes to the protocol and information sheets, the research project was approved in May 2004. Information sheets and invitation letters were sent out to Cohort A in June 2004 and the study started in August 2004. The follow-up rate from Cohort A (subjects were around 12-14 years of age) was about 30%. This was possibly due to the requirement to travel to the study centre in London, and the long time period between the original study and the follow-up. I considered several possible ways of improving the follow-up rate: 1) sending a second invitation letter, in case the first letter did not get through to the potential participants and their parents; 2) instead of asking the GP to forward the information sheet and invitation letter to

potential research participants who had moved from the last known address, I obtained permission from the Research Ethics Committee to access their new address and send the invitation to them directly; 3) I offered a home visit in Cambridge for some parts of body composition measurements, if the subject was unwilling to travel to London (deuterium dilution and anthropometry—the home visit protocol is shown below).

Cohorts C and B were sent the information sheets and invitation letters (which offered a home visit if they could or would not travel to the study centre in London) in February and September 2005, respectively.

3.5.6 Overview of the home visit

The study took place at the subject's home in the Cambridge area. Informed consent was obtained as described above. The following investigations were performed:

1. Blood pressure was measured using an automated device while the subject was resting on a couch for at least 15 minutes.

2. TBW was measured by deuterium kinetics with the same standard procedures as the centre visit. Both saliva samples and the sheet record the amount of drinks were posted back to the researcher by prepaid envelope.

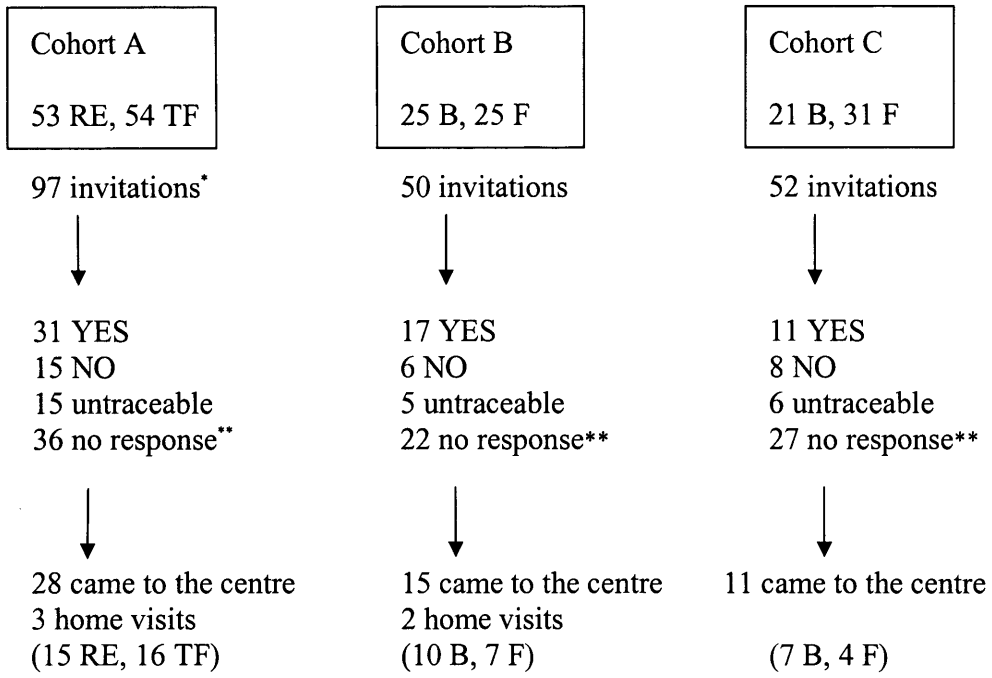
3. Anthropometric measurements were performed with the same standard procedures as the centre visit. Height was measured by a portable stadiometer.

4. The same questionnaires were used to determine general health, life style, physical activity, socioeconomic status of the family and pubertal status.

The follow-up study finished in May 2006. In total, 54 subjects out of 199 potential participants agreed to come to the study centre in London and completed all the

measurements. 5 subjects agreed to take part in the study by home visit only (see **Figure 3-4**). Therefore, the overall follow-up rate was around 30%, which is comparable to other similar cohorts that have been followed-up at the same centre over the past two years.

Figure 3-4 Recruitment and follow-up rate of the prospective cohorts[#]



3.6 Key study design features

Since there were a substantive number of subjects who were not successfully followed-up in cohorts A, B, and C, comparison of baseline characteristics of these cohorts for those seen or not seen is presented here (**Table 3-2a, b, c**). For cohort A, those who agreed to come for the follow-up study were lighter at birth (mean difference 0.15 kg; 95%CI 0.01-0.29) and had lower FMI at the age of 12 weeks (mean difference 0.84

RE = reduced energy formula, TF = standard term formula, B = breast-fed, F = formula-fed

* 5 early drop-out (before 3 wks visit) in each group

** at least 3 letters were sent to the last known address

kg/m2; 95%CI 0.16-1.52). For cohort B and C, there was no significant difference in baseline characteristics between the subjects who were followed-up and those were not. When all three cohorts were analysed together, there was no statistically significant difference in any of these baseline characteristics between those who were successfully followed-up and those who were not (data not shown).

Table 3-2 Comparison of baseline characteristics between the subjects who were and were not followed-up. [++]

a) Cohort A

	Follow-up (n=31)	**Lost to follow-up (n= 66)**	**p**
Boys (n, %)	11, 35.5%	39, 51.3%	0.2
First-born (n, %)[#]	11, 37.9%	20, 29.0%	0.5
Reduced energy formula (n, %)	15, 48.4%	38, 50%	1.0
Mothers with A level/higher qualification (n, %)[#]	3, 18.8%	7, 19.4%	1.0
Maternal BMI (kg/m^2)	24.3 ± 4.1	24.5 ± 5.5	0.9
Paternal BMI (kg/m^2)	24.7 ± 3.0	24.6 ± 3.2	0.9
Birthweight (kg)	3.42 ± 0.33	3.57 ± 0.32	***0.03***
Gestation (wk)	40.1 ± 1.3	40.0 ± 1.3	0.7
Birthweight SDS	-0.11 ± 0.58	0.22 ± 0.68	***0.02***
FMI at 12 weeks (kg/m^2)[*]	3.4 ± 1.1	4.3 ± 1.2	***0.02***
FFMI at 12 weeks (kg/m^2)*	12.0 ± 1.1	12.0 ± 1.1	1.0

[++] Continuous variables were expressed as mean ± SD. Independent sample t-test was used to test differences between those who were and were not followed-up. Categorical variables were expressed as n (%). Chi-square test was used to test differences between the two groups.

[#] parity data was missing in 9 subjects, maternal education was missing in 55 subjects

[*] n=16 for follow-up and 42 for lost to follow-up subjects

b) Cohort B

	Follow-up (n=17)	Loss follow-up (n= 33)	p
Boys (n, %)	8, 47.1%	15, 45.5%	0.6
First-born (n, %)	9, 52.9%	15, 45.5%	0.8
Breast-fed (n, %)	10, 58.8%	15, 45.5%	0.6
Social class 1 or 2 (n, %)	8, 47.1%	23, 69.7%	0.14
Maternal BMI (kg/m^2)	23.1 ± 3.7	23.1 ± 3.1	0.9
Paternal BMI (kg/m^2)	24.6 ± 4.1	24.3 ± 3.5	0.8
Birthweight (kg)	3.54 ± 0.35	3.55 ± 0.37	1.0
Gestation (wk)	40.1 ± 1.5	40.2 ± 1.2	0.9
Birthweight SDS	0.04 ± 0.34	0.04 ± 0.56	1.0
FMI at 12 weeks (kg/m^2) **	4.0 ± 1.5	4.1 ± 1.2	0.7
FFMI at 12 weeks (kg/m^2)**	12.1 ± 1.0	11.8 ± 0.9	0.3

c) Cohort C

	Follow-up (n=11)	Loss follow-up (n= 41)	p
Boys (n, %)	4, 36.4%	18, 43.9%	0.7
First-born (n, %)	5, 45.5%	16, 39.0%	0.7
Breast-fed (n, %)	7, 63.6%	14, 34.1%	0.10
Social class 1 or 2 (n, %)	5, 45.5%	24, 58.5%	0.5
Birthweight (kg)	3.48 ± 0.30	3.40 ± 0.48	0.6
Gestation (wk)	40.5 ± 0.9	39.7 ± 1.4	0.09
Birthweight SDS	-0.13 ± 0.86	0.01 ± 0.97	0.7
FMI at 12 weeks (kg/m^2) ***	3.5 ± 1.4	3.7 ± 0.9	0.5
FFMI at 12 weeks (kg/m^2)***	12.8 ± 1.4	12.9 ± 1.1	0.8

** n=15 for follow-up and 27 for loss follow-up subjects

*** n=10 for follow-up and 40 for loss follow-up subjects

Comparison of the key design features for all 4 cohorts are summarised in **Table 3-3**. Selected baseline and BC outcomes of the 4 cohorts are shown in **Table 3-4**. There were no significant differences between birthweight and gestation between the 4 cohorts. Infant FMI and FFMI did not differ among those followed-up in cohorts A, B, and C. There were no significant differences in the SDS of BC outcomes between cohorts. Therefore, in the subsequent chapters, all relevant cohorts were pooled together for the analyses of the association between early growth or BC and later outcomes.

The next 5 chapters contain the results of my study; each provides a short introduction and description of methods specific to the chapter, followed by the main analyses and discussion. A general discussion on the programming of body composition and the broader implication of my findings is then presented in **Chapter 9**.

Table 3-3 Key design features of the 4 cohorts.

	Cohort A	Cohort B	Cohort C
Rationale for setting up original study	Randomised controlled trial of reduced energy and standard term formulas (details in Appendix A)	To study energy metabolism of breast-fed and formula-fed infants	To study energy req of breast-fed and fo infants
Source of cohort	Cambridge, all Caucasian	Cambridge, all Caucasian	Cambridge, all Cau
Eligibility criteria	Full-term, healthy, birthweight between 10th and 90th centile.	Full-term, healthy, breast-fed or formula-fed until 12 weeks old.	Full-term, healthy, b or formula-fed until old.
Period of original study	1990-1992	1992-1993	1985-1987
Time period of current BC measurements at the study centre	August 2004-February 2005	October 2005-April 2006	March 2005-Noven
Time period of home visit	May 2006	May 2006	N/A
Time period of lipid and insulin essays	May-June 2006	May-June 2006	May-June 2006
Source of growth data:			
- **Birthweight**	Hospital records	Parental recall in infancy	Parental recall in in
- **Post natal growth**	Collected prospectively from the original study	Collected prospectively from the original study	Collected prospecti the original study
Age at which prospective post natal growth data were available	3, 6, 12 weeks, 6 and 12 months	12 weeks only	6, 12 weeks, 6, 12 r

Table 3-4 Comparison of some baseline characteristics and BC outcomes among the subjects who w up in cohort A, B, C and the subjects who were from cohort D.

	Cohort A (n=31)		Cohort B (n=17)		Cohort C (n=11)	
	Mean ± SD	**range**	**Mean ± SD**	**range**	**Mean ± SD**	**range**
Birthweight (kg)	3.42 ± 0.33	2.87 – 4.02	3.54 ± 0.35	2.89 – 4.17	3.48 ± 0.30	2.90 – 3.
Gestation (weeks)	40.1 ± 1.3	38.0 – 42.0	40.1 ± 1.5	38.0 – 42.0	40.5 ± 0.9	39.0 – 4
Birthweight SDS	-0.11 ± 0.58	-1.05 – 0.98	0.04 ± 0.34	-0.57 – 0.56	-0.13 ± 0.86	-2.14 – 0
Infant FMI (kg/m^2)	3.4 ± 1.1	1.4 – 6.0	3.9 ± 1.5	2.3 – 7.1	3.5 ± 1.4	1.6 – 6.
Infant FFMI (kg/m^2)	12.0 ± 1.1	9.5 – 13.7	12.1 ± 1.0	11.0 – 14.0	12.8 ± 1.4	11.4 – 1
Age at follow-up (yr)	13.2 ± 0.8	12.0 – 14.8	13.3 ± 0.4	12.7 – 13.9	19.6 ± 0.6	18.2 – 2
Boys (n, %)	11, 35.5%		8, 47.1%		4, 36.4%	
Height (cm)	157.3 ± 7.5	142.1 - 175	158.7 ± 6.6	146.1 – 174.9	173.8 ± 9.0	161.5 – 1
BMI (kg/m^2)	20.0 ± 2.9	14.4 – 25.0	19.6 ± 4.4	14.7 – 32.9	23.1 ± 2.9	18.7 – 2
FMI (kg/m^2)	5.5 ± 2.3	1.4 – 10.8	4.6 ± 2.7	1.8 – 13.5	6.7 ± 2.8	2.3 – 12
FFMI (kg/m^2)	14.6 ± 1.2	12.2 – 16.9	15.0 ± 2.2	12.0 – 19.9	16.4 ± 2.16	13.5 – 1
Height SDS	0.24 ± 0.93	-1.44 – 1.83	0.28 ± 0.81	-1.16 – 2.28	0.82 ± 1.07	-0.35 – 2
BMI SDS	0.38 ± 1.07	-2.33 – 1.98	0.03 ± 1.39	-2.01 – 3.05	0.34 ± 1.08	-1.64 – 1
FMI SDS	0.19 ± 0.83	-1.57 – 1.76	-0.14 ± 0.91	-1.17 – 2.30	0.31 ± 0.81	-0.89 – 1
FFMI SDS	-0.02 ± 0.80	-1.50 – 1.55	-0.01 ± 1.38	-2.31 – 2.73	-0.30 ± 1.01	-1.61 – 1

Chapter 4

The Influence of Birthweight on Later Body Composition

Chapter 4 The Influence of Birthweight on Later Body Composition

4.1 Introduction

Studies of birthweight and later body composition to date suggest that birthweight might influence FFM rather than FM (see **Chapter 2**). Nevertheless, there has been no study using the 4- component model to test this hypothesis.

4.2 Methods

4.2.1 Subjects

All children who completed the four-component model and had birthweight data were included in this analysis. 52 children were from the prospective follow-up study (cohort A, B, C) and 256 children were from the body composition reference study (cohort D) (description in **Chapter 3**). All were full-term born, singleton and did not have any disease that might affect growth or body composition.

4.2.2 Birthweight data

In cohorts A, B, and C, birthweight and gestation were documented from the hospital record. In cohort D, birthweight and gestational age data were collected from parental recall and verified with parent-held baby book where available (58.44%). There was no significant difference between parental recall and baby book record for birthweight (mean difference 0.004 kg; 95% CI -0.008, 0.017). Birthweight SDS was calculated according to gestation and sex using the British 1990 reference.

4.2.3 Measurement of body composition

Body composition variables derived from the 4C model were used in this analysis together with the anthropometry data and regional DXA FM to assess central fat distribution. For more details see **Chapter 3**. In addition, total FM and FFM derived from 2C model (DXA and SF equations (57)) were used for the purpose of comparison with other published data.

4.2.4 Confounding variables

Factors that might confound the relationship between birthweight and body composition were assessed using the structured questionnaire as mentioned in **Chapter 3** and **Appendix B**. Pubertal status was self-assessed using pictures of the Tanner stages (179) and recoded as prepubertal (stage 1), early pubertal (stage 2,3) and late pubertal (stage 4,5). A simple assessment of subjects' physical activity level was made by a 5-point scale ranging from 'much less active', 'less active', 'same as peers', 'more active', and 'much more active'. Social class was assessed using the Standard Occupational Classification (180) and classified as class 1 (high), 2, 3 and ≥4. Ethnicity was coded as white and non-white since in this dataset there were too few subjects for each of the other ethnicities to analyse separately. Reported parental height and weight was used in this analysis.

4.2.5 Statistical methods

Body size data were converted to SDS using the British 1990 reference where possible since relative body size i.e. BMI changes substantially with age. Multiple linear regression was used to assess associations between birthweight SDS and later body size and composition adjusting for age and potential confounders. This association was also explored after correction for variation in body size by adjusting for current height. Variables which were not normally distributed were Log_e transformed to reduce skewness in distribution. In this case, the degree of association was interpreted as sympercents change in body composition variables per SDS change in birthweight (188).

General linear models were used to test the interaction between gender or pubertal status and birthweight SDS in predicting later body composition.

4.3 Results

4.3.1 Characteristics of study subjects

Descriptive statistics of the subjects (age range 4.22-20.36 years) are summarized in **Table 4-1**. Boys and girls in this analysis were heavier, taller, and had higher BMI than the British 1990 reference. Compared with reference data (in the form of SDS), both boys and girls had higher waist circumference and triceps SF while girls also had higher subscapular SF.

Not surprisingly, boys were heavier than girls at birth, but there was no difference in gestation and birthweight SDS which was comparable to the British 1990 reference. In general, boys had higher FFM and lower FM compared to girls; however, both sexes had the same BMC. Girls also had higher waist circumference and SF than boys even after difference between sexes were adjusted by SDS. In subgroup analysis (data not shown), this difference was significant only in late pubertal subjects. Since body composition was significantly different in boys and girls, and there was a significant interaction between birthweight and gender in predicting some of the body composition variables (see later), all the analyses were performed separately for boys and girls.

There was no difference in any of the potential confounders (parental height, pubertal status, physical activity, social class, and ethnicity) between boys and girls except for higher paternal BMI in boys (mean difference 0.98 kg/m^2; 95%CI 0.11, 1.84).

Table 4-1 Characteristics of study subjects[++]

	Boys (n=145)	Girls (n=163)	p
Age (y)	11.5 ± 3.9	11.7 ± 3.9	0.65
Birthweight (kg)	3.55 ± 0.51	3.43 ± 0.49	***0.03***
Gestation (wk)	40.1 ± 1.3	40.0 ± 1.4	0.55
Birthweight SDS	-0.06 ± 1.02	0.04 ± 0.99	0.38
Height (cm)	147.4 ± 22.1	146.3 ± 18.4	0.65
Weight (kg)	42.8 ± 19.7	43.4 ± 15.9	0.71
BMI (kg/m^2)	18.7 ± 4.1	19.5 ±3.6	0.05
Height SDS	0.21 ± 0.94	0.26 ± 0.96	0.66
Weight SDS	0.31 ± 1.17	0.45 ± 1.08	0.29
BMI SDS	0.23 ± 1.29	0.40 ± 1.13	0.20
FM from 4C (kg)	8.8 ± 7.1	12.5 ± 7.2	***<0.001***
FFM from 4C (kg)	34.0 ± 15.2	31.0 ± 9.9	***0.04***
Trunk FM from DXA (kg)	3.8 ± 4.0	5.8 ± 4.0	***<0.001***
DXA lean soft tissue (kg)	32.5 ± 13.9	28.6 ± 8.6	***0.003***
DXA BMC (kg)	1.62 ± 0.78	1.62 ± 0.78	0.99
Waist circumference (cm)	65.5 ± 11.4	65.2 ± 9.2	0.81
Triceps SF (mm)	10.8 ± 5.0	15.3 ± 5.1	***<0.001***
Subscapular SF (mm)	8.2 ± 5.5	11.2 ± 6.4	***<0.001***
WC SDS[#]	0.49 ± 1.06	0.95 ± 1.06	***<0.001***
Triceps SDS	0.20 ± 1.01	0.56 ± 0.89	***0.001***
Subscapular SDS	-0.02 ± 1.05	0.23 ± 1.01	***0.04***
Maternal height (cm)	164.1 ± 6.6	163.8 ± 6.0	0.62
Paternal height (cm)	178.3 ± 7.3	178.9 ± 7.6	0.51
Maternal BMI (kg/m^2)	25.1±4.6	25.0±5.2	0.82
Paternal BMI (kg/m^2)	26.6±3.8	25.6±3.6	***0.027***
Pubertal status (n, %)			
Prepubertal	67 (46.2%)	70 (42.9%)	
Early pubertal	39 (26.9%)	42 (25.8%)	0.58
Late pubertal	37 (25.5%)	51 (31.3%)	

[++] Continuous variables were expressed as mean ± SD. Independent sample t-test was used to test differences between genders. Categorical variables were expressed as n (%). Chi-square test was used to test differences between genders.

[#] n=130 in boys and 147 in girls since the database for waist SDS was available only up to the age of 17

Table 4-1 (cont.) Characteristics of study subjects [##]

	Boys (n=145)	Girls (n=163)	p
Physical activity (n, %)			
Much less or less active	13 (9%)	17 (10.4%)	
Same as peers	60 (41.4%)	76 (46.6%)	0.62
More active than peers	54 (37.2%)	54 (33.1%)	
Much more active than peers	15 (10.3%)	12 (7.4%)	
Social class (n, %)			
Class 1	33 (22.8%)	29 (17.8%)	0.49
Class 2	60 (41.4%)	74 (45.4%)	
Class 3	12 (8.3%)	20 (12.3%)	
Class 4 or more	37 (25.5%)	40 (24.5%)	
Ethnicity (n, %)			
White	109 (75.2%)	124 (76.1%)	0.95
Non-white	34 (23.4%)	38 (23.3%)	

[##] Numbers of missing data are as follow (boys, girls): WC (2, 0), Triceps SF (4, 3), Subscapular SF (4, 2), Maternal height (2, 4), Paternal height (3, 5), Maternal BMI (6, 6), Paternal BMI (10, 11), Pubertal status (2, 0), Physical activity (3, 4), Social class (3, 0), Ethnicity (2, 1)

4.3.2 Relationship of potential confounding factors with birthweight and body composition

After controlling for age, the effect of potential confounders was assessed (**Table 4-2**). Puberty showed a significant positive correlation with birthweight SDS in boys i.e. at the same age, boys with higher birthweight SDS were at a more advanced pubertal stage. Pubertal status had a significant positive relationship with FFM in both sexes but correlated in an opposite direction with FM (weak positive in girls and negative in boys). A higher level of physical activity had a significant negative association with nearly all indices of fatness in both sexes and had a positive association with FFM in girls. The other environmental factor, social class, also showed a significant association with the indices of fatness in boys (tendency for greater fatness with lower social class). Parental height had a significant positive relationship with birthweight SDS and later height in both sexes; taller parents were associated with higher birthweight and taller offspring. Maternal height was positively related to a higher amount of FFM in both sexes. Only maternal BMI had a significant positive association with her offspring's birthweight SDS. Parental BMI was significantly correlated with most of the later body composition variables.

4.3.3 Relationship between current body size and body composition

Partial correlation coefficients (controlled for age) are shown in **Table 4-2**. As expected, height had a strong positive correlation with all body composition variables (except SF) and correlated with FFM more strongly than FM. BMI showed a stronger relationship with all indices of fatness than with FFM. Therefore, in the subsequent regression analysis, body composition variables were adjusted for body size by adjusting for height and, alternatively, for BMI. FM explains a high proportion of variability (90-92%) in trunk fat; therefore, regression models with height and non-trunk FM are shown for the analysis of trunk FM, to demonstrate any effect of birthweight on central fat that is beyond its association with total FM.

Table 4-2 Partial correlation coefficients (r) controlled for age[@]

Boys	BirthWT SDS	Height	FM	FFM	WC	Triceps SF	Subsc SF	TrunkFM
Puberty	***0.17***[a]	***0.27***[b]	***-0.19***[a]	***0.30***[c]	-0.08	***-0.23***[a]	-0.16	***-0.17***[a]
Physical activity	-0.12	0.07	***-0.28***[b]	0.10	-0.15	***-0.25***[b]	***-0.33***[c]	***-0.22***[a]
Social class	0.02	-0.01	***0.22***[a]	0.01	***0.18***[a]	***0.19***[a]	***0.19***[a]	***0.20***[a]
Maternal height	***0.17***[a]	***0.29***[c]	-0.04	***0.18***[a]	0.09	-0.07	-0.03	-0.04
Paternal height	***0.20***[a]	***0.33***[c]	-0.06	0.14	0.00	-0.03	-0.10	-0.05
Maternal BMI	***0.21***[a]	-0.001	***0.37***[c]	0.13	***0.31***[c]	***0.27***[b]	***0.35***[c]	***0.39***[c]
Paternal BMI	0.05	0.09	***0.36***[c]	***0.20***[a]	***0.29***[b]	***0.36***[c]	***0.37***[c]	***0.39***[c]
Height	***0.27***[b]	-	***0.22***[a]	***0.80***[c]	***0.31***[c]	0.05	0.09	***0.23***[a]
BMI	***0.23***[a]	***0.20***[a]	***0.84***[c]	***0.59***[c]	***0.93***[c]	***0.75***[c]	***0.84***[c]	***0.88***[c]
FM	0.11	***0.22***[b]	-	***0.35***[c]	***0.82***[c]	***0.90***[c]	***0.84***[c]	***0.96***[c]

Girls	BirthWT SDS	Height	FM	FFM	WC	Triceps SF	Subsc SF	TrunkFM
Puberty	-0.14	***0.25***[b]	0.15	***0.37***[c]	0.15	0.05	0.09	***0.17***[a]
Physical activity	-0.07	***0.23***[b]	***-0.25***[b]	***0.24***[b]	-0.10	***-0.32***[c]	***-0.27***[b]	***-0.19***[a]
Social class	-0.14	-0.01	0.01	0.11	0.08	<0.001	0.02	0.02
Maternal height	***0.16***[a]	***0.32***[c]	0.07	***0.20***[a]	0.09	-0.14	-0.08	0.10
Paternal height	***0.20***[a]	***0.19***[a]	0.02	-0.01	-0.04	-0.03	-0.04	-0.02
Maternal BMI	***0.17***[a]	0.08	***0.32***[c]	***0.23***[b]	***0.24***[b]	***0.30***[c]	***0.22***[b]	***0.29***[c]
Paternal BMI	-0.04	0.16	***0.24***[b]	***0.23***[b]	***0.22***[b]	0.15	0.10	***0.26***[b]
Height	0.11	-	***0.38***[c]	***0.82***[c]	***0.40***[c]	0.04	0.13	***0.41***[c]
BMI	0.03	***0.20***[b]	***0.88***[c]	***0.58***[c]	***0.90***[c]	***0.78***[c]	***0.81***[c]	***0.89***[c]
FM	0.12	***0.38***[c]	-	***0.51***[c]	***0.86***[c]	***0.79***[c]	***0.80***[c]	***0.95***[c]

[@] [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$

All variables which were not normally distributed (FM, FFM, BMI, WC, Triceps SF, Subscapular SF, Trunk FM, Maternal BMI, Paternal BMI) were natural log transformed before analysis.

Puberty was coded as: 1 = prepubertal, 2 = early pubertal, 3 = late pubertal
Physical activity was coded as: 2= much less or less active than peers, 3= same as peers, 4= more active than peers, 5= much more active than peers
Social class ranged from 1 which is the highest to ≥ 4 which is the lower social class

4.3.4 Birthweight and later body size

The results of regression analyses are shown in **Table 4-3.** Birthweight was positively associated with later height in both sexes; at any given age, a 1 SDS increase in birthweight corresponded to a 0.24 and 0.17 SDS increase (95%CI: 0.09-0.38 for boys; 0.02-0.32 for girls) in height in boys and girls respectively. However, this association was attenuated substantially and became statistically non-significant after adjusting for parental height. Birthweight SDS positively predicted later weight and BMI SDS only in boys; a 1 SDS increase in birthweight predicted a 0.30 SDS increase (95%CI: 0.09-0.50) in later BMI at any given age. The association remained in the same direction after adjusting for potential confounders.

Table 4-3 Regression of body size on birthweight SDS[&]

	Adjusted for age			**Adjusted for age, puberty, physical activity, social class, ethnicity, parental height**			**Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI**		
Boys	B	SE	p	B	SE	p	B	SE	p
Weight SDS	0.325	0.09	***0.001***	0.312	0.102	***0.003***	0.245	0.093	***0.010***
Height SDS	0.237	0.075	***0.002***	0.116	0.073	0.115	0.215	0.081	***0.009***
BMI SDS	0.297	0.104	***0.005***	0.366	0.109	***0.001***	0.191	0.100	0.058
Girls									
Weight SDS	0.125	0.086	0.149	0.117	0.090	0.193	0.092	0.089	0.303
Height SDS	0.167	0.076	***0.031***	0.07	0.071	0.320	0.167	0.083	***0.045***
BMI SDS	0.056	0.091	0.541	0.108	0.095	0.254	0.009	0.092	0.926

[&] From multiple regression analysis, each row represents a different model with body size in the left hand column as a dependent variable; birthweight SDS was an independent variable; age, puberty, physical activity, social class, ethnicity, and parental height or BMI were covariates.

B = the coefficient of birthweight SDS i.e. the change in body size or body composition per SDS increase in birthweight, SE = standard error, highlighted p value indicate significant at $p<0.05$

4.3.5 Birthweight and body composition (FFM and FM)

The results of the regression analyses are shown in **Table 4-4**. Birthweight had a strong positive association with FFM in boys. This association was equivalent to around a 5.2% increase (95%CI: 2.8-7.6%) in FFM per 1 SDS increase in birthweight. The relationship was attenuated slightly after adjusting for current height and parental BMI but unaffected by adjustment for other confounders. In girls, there was no association between birthweight and FFM. The associations between birthweight and FFM differed between boys and girls (p for interaction =0.02). In a subgroup analysis according to puberty; this interaction was nearly significant (p=0.07) in prepubertal children only. There was no evidence that the associations for FM differed between boys and girls (p for interaction = 0.96). Birthweight was not significantly related to FM in either sex.

Table 4-4 Regression of body composition on birthweight SDS[&]

	Adjusted for age			**Adjusted for age, puberty, physical activity, social class, ethnicity, parental height**			**Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI**		
Boys	B	SE	p	B	SE	p	B	SE	p
FM	0.065	0.048	0.179	0.081	0.049	0.101	0.026	0.045	0.564
FM[#]	0.034	0.049	0.485	0.052	0.046	0.26	-0.012	0.044	0.787
FFM	0.052	0.012	***<0.001***	0.042	0.012	***0.001***	0.043	0.012	***0.001***
FFM[#]	0.021	0.008	***0.008***	0.027	0.008	***0.001***	0.017	0.008	***0.036***
Girls									
FM	0.056	0.036	0.125	0.054	0.038	0.159	0.028	0.037	0.451
FM[#]	0.037	0.034	0.285	0.037	0.035	0.289	<0.001	0.034	0.994
FFM	0.009	0.013	0.504	0.016	0.013	0.200	0.016	0.013	0.222
FFM[#]	-0.007	0.008	0.379	0.006	0.008	0.458	-0.003	0.008	0.746

[&] From multiple regression analysis, each row represents a different model with body composition in the left hand column as a dependent variable; birthweight SDS was an independent variable; age, puberty, physical activity, social class, ethnicity, and parental height or BMI were covariates. FM and FFM were natural log transformed and presented in $\log_e$scale.

[#] Adjusted for current height (m)

4.3.6 Birthweight and fat distribution

Regression analyses of different proxies for fat distribution on birthweight SDS are shown in **Table 4-5.** In boys, there was a strong positive association between birthweight and waist circumference; equivalent to around a 2.8% increase (95%CI: 1- 4.6%) in waist circumference per SDS increase in birthweight. This association was generally unaffected after adjusting for potential confounders. There was also a weak positive association between birthweight and trunk FM which was slightly stronger after adjusting for non-trunk FM; however, the association was attenuated after adjusting for parental BMI. There was a weak interaction between gender and birthweight SDS in predicting WC and subscapular SF SDS (p for interaction = 0.07 and 0.06, respectively).

Among girls, birthweight was not associated with fat distribution indices in any of the models.

Table 4-5 Regression of fat distribution indices on birthweight SDS [&]

	Adjusted for age			Adjusted for age, puberty, physical activity, social class, ethnicity, parental height			Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI		
Boys	B	SE	p	B	SE	p	B	SE	p
Triceps SDS	0.139	0.085	0.103	0.172	0.088	0.052	0.096	0.085	0.261
Subscapular SDS	0.09	0.089	0.299	0.115	0.091	0.205	0.006	0.085	0.945
WC SDS	0.268	0.089	***0.003***	0.301	0.096	***0.002***	0.207	0.089	***0.022***
WC[#]	0.028	0.009	***0.025***	0.024	0.009	***0.009***	0.015	0.009	0.104
WC[∞]	0.015	0.005	***0.004***	0.016	0.005	***0.005***	0.016	0.006	***0.004***
Trunk FM	0.112	0.057	0.052	0.142	0.06	***0.019***	0.067	0.054	0.216
Trunk FM[#]	0.075	0.059	0.203	0.107	0.056	0.06	0.023	0.053	0.672
Trunk FM[∞∞]	0.067	0.032	***0.036***	0.090	0.033	***0.008***	0.060	0.034	0.079
Girls									
Triceps SDS	0.083	0.072	0.249	0.079	0.073	0.279	-0.005	0.071	0.939
Subscapular SDS	-0.007	0.081	0.932	-0.019	0.083	0.82	-0.108	0.082	0.191
WC SDS	0.01	0.089	0.913	0.023	0.095	0.805	-0.033	0.096	0.729
WC[#]	-0.001	0.008	0.875	0.001	0.008	0.919	-0.006	0.008	0.480
WC[∞]	-0.008	0.004	0.058	-0.006	0.005	0.159	-0.006	0.005	0.210
Trunk FM	0.051	0.044	0.251	0.05	0.046	0.285	0.022	0.045	0.622
Trunk FM[#]	0.025	0.041	0.537	0.029	0.042	0.494	-0.012	0.042	0.783
Trunk FM[∞∞]	-0.018	0.034	0.593	-0.007	0.036	0.842	-0.029	-0.036	0.430

[&] From multiple regression analysis, each row represents a different model with fat distribution indices in the left hand column as a dependent variable; birthweight SDS was an independent variable; age, puberty, physical activity, social class, ethnicity, and parental height or BMI were covariates.

WC and Trunk FM were natural log transformed and presented in $\log_e$ scale

[#] Adjusted for current height (m)

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

[∞∞] Adjusted for both current height (m) and non-trunk FM ($\log_e$ scale)

4.3.7 Interaction between pubertal status and birthweight SDS

There was no evidence that the associations between birthweight and FFM differed between pubertal groups; however, the association between birthweight and FM did differ between pubertal groups (p for interaction =0.03). This interaction was not significant in a subgroup analysis according to gender. There was also a significant interaction between puberty and birthweight SDS in predicting triceps SF SDS, and waist circumference SDS (p for interaction =0.02, 0.05, respectively). Since there was a significant interaction between pubertal status and birthweight SDS in predicting some of the later body composition variables, sub-group analysis was performed according to pubertal group.

Boys: Birthweight SDS was positively associated with FFM and central fat, persisting after adjusting for current size in the prepubertal group (n=67). The relationship was weaker (although in the same direction) in early pubertal boys (n=39). None of the associations were significant in late pubertal boys (n=37). Pubertal stage was missing in 2 boys.

***Girls*:** There was a weak positive association between birthweight SDS and FFM (p=0.064), which was largely attenuated after adjusting for current height in prepubertal girls (n=70). None of the associations between birth SDS and FFM, FM, and fat distribution were significant in the other 2 groups (early pubertal, n=42; late pubertal, n=51).

4.3.8 Effects of using different body composition techniques

Most published studies use 2C techniques to determine body composition in later life. The two most commonly used are different SF equations and DXA. Therefore, for the purpose of comparison, the same analyses were repeated within the same dataset using FM and FFM derived from DXA and from the SF equation of Slaughter et al.(57;192). The results are shown in **Table 4-6** and **Table 4-7.** There was a significant positive association between birthweight and FFM in boys, which

was slightly weaker after adjusting for confounders. Interestingly, if FFM in the analysis was derived from the SF equation, a weak positive association was also seen in girls. However, it became statistically non-significant after adjusting for height. From these 2 methods, there was a trend for a positive relationship between birthweight and FM in boys which was also substantially attenuated after adjusting for height.

Table 4-6 Regression of body composition by DXA on birthweight SDS [&]

	Adjusted for age			Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI		
Boys	B	SE	p	B	SE	p
FM	0.097	0.051	0.059	0.058	0.048	0.233
FM[#]	0.061	0.052	0.245	0.016	0.047	0.740
FFM	0.047	0.012	***<0.001***	0.037	0.012	***0.002***
FFM[#]	0.016	0.007	***0.027***	0.011	0.007	0.129
Girls						
FM	0.058	0.038	0.132	0.032	0.039	0.413
FM[#]	0.035	0.035	0.320	0.001	0.035	0.970
FFM	0.009	0.013	0.504	0.016	0.012	0.194
FFM[#]	-0.007	0.008	0.379	-0.002	0.007	0.794

[&] From multiple regression analysis, each row represents a different model with body composition in the left hand column as a dependent variable; birthweight SDS was independent variable; age, puberty, physical activity, social class, ethnicity, and parental BMI were covariates.

FM and FFM were natural log transformed and presented in $\log_e$ scale
DXA FFM = Fat free soft tissue+ BMC

[#] Adjusted for current height (m)

Table 4-7 Regression of body composition calculated by SF equations (Slaughter's) on birthweight SDS [&]

	Adjusted for age			Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI		
Boys	B	SE	p	B	SE	p
FM	0.112	0.047	***0.019***	0.080	0.046	0.087
FM[#]	0.079	0.048	0.105	0.042	0.046	0.355
FFM	0.051	0.012	***<0.001***	0.044	0.012	***<0.001***
FFM[#]	0.022	0.007	***0.003***	0.020	0.008	***0.012***
Girls						
FM	0.035	0.036	0.324	0.008	0.036	0.813
FM[#]	0.016	0.033	0.634	-0.017	0.033	0.601
FFM	0.022	0.013	0.097	0.027	0.013	***0.034***
FFM[#]	0.006	0.007	0.391	0.008	0.007	0.240

[&] From multiple regression analysis, each row represents a different model with body composition in the left hand column as a dependent variable; birthweight SDS was independent variable; age, puberty, physical activity, social class, ethnicity, and parental BMI were covariates.

FM and FFM were natural log transformed and presented in $\log_e$ scale

[#] Adjusted for current height (m)

4.3.9 Effects of using different methods for size adjustment and expression of BC

To be comparable to other studies, the results of my analysis using alternative body size adjustment by BMI are shown in **Table 4-8** and **Table 4-9**. The association between birthweight and FFM remained the same in this analysis; yet, there was a negative association between birthweight and fat percentage (in relation to body weight) in boys which was apparent only after adjusting for BMI (see later discussion). There was a significant negative association between birthweight SDS and subscapular SF in girls and a trend in the same direction in boys which was slightly stronger after adjusting for BMI. The association remained in the same direction in girls if the ratio of subscapular and triceps SF was used. Indeed, S:T ratio showed a significant negative association 'before' BMI adjustment but the relationship was stronger 'after' the adjustment.

Table 4-8 Regression of body composition on birthweight SDS (Alternative size adjustment by BMI) [&]

	Adjusted for age			Adjusted for age, puberty, physical activity, social class, ethnicity, parental height		
Boys	B	SE	p	B	SE	p
FM[$]	-0.049	0.027	0.069	-0.043	0.027	0.115
%Fat	0.478	0.724	0.510	-0.007	0.655	0.992
%Fat[$]	-1.105	0.471	***0.020***	-0.936	0.448	***0.039***
FFM[$]	0.033	0.01	***0.002***	0.029	0.010	***0.004***
Girls						
FM[$]	0.045	0.017	***0.011***	0.028	0.019	0.135
%Fat	0.739	0.597	0.217	0.083	0.595	0.889
%Fat[$]	0.573	0.367	0.120	0.092	0.364	0.800
FFM[$]	0.009	0.013	0.504	0.016	0.011	0.133

[&] From multiple regression analysis, each row represents a different model with body composition in the left hand column as a dependent variable; birthweight SDS was independent variable; age, puberty, physical activity, social class, ethnicity, and parental BMI were covariates.

FM, FFM were natural log transformed and presented in $\log_e$ scale

[$] Adjusted for BMI ($\log_e$scale)

Table 4-9 Regression of fat distribution indices on birthweight SDS (Alternative size adjustment by BMI) [&]

	Adjusted for age			Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI		
Boys	B	SE	p	B	SE	p
WC[$]	0.005	0.004	0.186	0.007	0.004	0.071
Triceps SF[$]	-0.023	0.023	0.331	-0.011	0.023	0.638
Subscapular SF[$]	-0.037	0.021	0.088	-0.037	0.021	0.073
S:T	0.004	0.022	0.871	-0.018	0.023	0.438
S:T[$]	-0.015	0.021	0.486	-0.027	0.023	0.236
Trunk FM[$]	-0.03	0.028	0.278	-0.022	0.028	0.438
Girls						
WC[$]	0.001	0.004	0.799	0.001	0.004	0.883
Triceps SF[$]	0.025	0.016	0.126	0.005	0.017	0.765
Subscapular SF[$]	-0.036	0.021	0.085	-0.061	0.021	***0.005***
S:T	-0.053	0.024	***0.026***	-0.063	0.026	***0.017***
S:T[$]	-0.056	0.022	***0.011***	-0.063	0.024	***0.010***
Trunk FM[$]	0.037	0.02	0.065	0.023	0.022	0.283

[&] From multiple regression analysis, each row represents a different model with central fat distribution indices in the left hand column as a dependent variable; birthweight SDS was independent variable; age, puberty, physical activity, social class, ethnicity, and parental BMI were covariates.

WC, Triceps, Subsc, S:T, TrunkFM were natural log transformed and presented in $\log_e$ scale

[$] Adjusted for BMI ($\log_e$scale)

4.4 Discussion

This study shows the association between birthweight and later body size and composition using the 4-component model which is the best available in vivo body composition technique to date. It confirms that higher birthweight is associated with higher later height in both sexes, and with higher weight and BMI in boys.

Gender differences in the programming effect of birthweight on body composition were demonstrated. In boys, the data showed that a higher birthweight was associated with higher FFM and central fat distribution. These associations were independent of current body size, age, pubertal stage, physical activity, social class, ethnicity, and parental size. In girls, there was no association between birthweight and later body composition except for a negative association with subscapular SF that became statistically significant after adjusting for current BMI.

Comparison between published studies exploring the relationship between birthweight and later body composition is difficult because they have used different body composition techniques and different methods of expression of body composition data. Although the sample size of my study was not comparable to the large-scale epidemiologic studies, the use of the 4-component model can give a more accurate answer regarding the programming of body composition and demonstrate some critical points of body composition data interpretation. In this discussion, I will focus on 1) comparison of my main findings with other studies; a summary is shown in **Table 4-10**, 2) effects of the use of different body composition techniques on the apparent relationship between birthweight and later body composition, 3) effects of the use of different methods for size adjustment and expression of body composition data on this relationship, and 4) criticisms of my study. The potential mechanism of body composition programming and broader implication of my findings will be discussed in more detail later in **Chapter 9.**

Table 4-10 Summary of studies examining the association between birthweight and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Birth variables	Outcomes	Covariates	Results
Hediger et al, 1998 (75)	US infants& children, Mixed term/preterm	2-47 m	4431	Anthropometry	Both sexes included	Regression	Birthweight categories – SGA (<10th%), AGA, LGA (>90th%)	SDS of Weight, MUAC, AMA, AFA, AFI (%arm fat), TSF, SSSF, sumSF	6 current age cohorts, race, sex, birth order, maternal smoking, gestation in SGA vs. LGA	- SGA smaller size (Wt., MUAC) - SGA deficit in muscularity (MUAC, AMA) approximately -0.5 SDS, less deficit in fat - SGA had higher AFI in 1st yr than AGA - LGA surfeit in muscularity (0.45 SDS) but less surfeit in fat - NS for SF
Mulligan et al, 2005 (193)	UK children	6.6-9.1y	85	DXA, Bodpod (n=63)	Both sexes included	Regression	Birthweight SDS	% Fat	BMI, age, sex, SES, activity	- Negative association (p=0.046, 0.006 for DXA, Bodpod), weaken (p=0.038, 0.022) after adjustment for covariates
Garnett et al, 2001(96)	Australian children, term born	7-8 y	255	Anthropometry, DXA abdominal fat by ROI (lower ribs-supra iliac crest)	Both sexes included	ANOVA, Regression, Partial correlation for CVD risks	Birthweight (gm) 3 categories (<3000, 3000-4000, >4000); Birthweight SDS	Weight SDS at 7-8y, %Abdominal fat (from total fat), % total fat (from weight); CVD risks (Total chol:HDL, TG, Insulin, Insulin: Glucose, BP)	age, gender, %fat (for partial correlation with CVD risks) Weight SDS at7-8y	- Lowest birthweight (<3000gm) and highest current weight SDS group (>0.67SDS) had significantly higher %abdominal fat (6.53±1.3%, p<0.001); similar results if birthweight SDS used - Birthweight SDS was negatively associated with %abdominal Fat (p=0.009), stronger if adjusted for current weight SDS (p<0.001); negatively associated with %fat only after adjusting for current weight SDS (p=0.007) - NS interaction between birth and current weight SDS in predicting %abdominal fat or %fat - NS birthweight SDS and CVD risks - Insulin, SBP associated with % fat; Total chol: HDL ratio, TG associated with % abdominal fat

Table 4-10 (cont.) Summary of studies examining the association between birthweight and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Birth variables	Outcomes	Covariates	Results
Duran-Tauleria et al,1995 (194)	UK children (various ethnicity)	5-11y	8374	Anthropometry	Both sexes included	Regression	Birthweight	Weight for height, TSF, SSSF, sumSF	Age, sex, SES, parental BMI, ethnic	- Positive association with all indices
Okosun et al, 2000 (195)	US children (Black, White, Hispanic)	5-11y	2488	Anthropometry	Both sexes included	Regression, Partial correlation	Birthweight	TSF,SSSF, SISF, Thigh SF, S:T,	Age, sex, BMI	- NS for TSF/sumSF - Positive association for thigh SF - Negative association for SSSF, SISF, S:T
Bavdekar et al, 1999 (94)	Indian children	8y	477	Anthropometry	Both sexes included	Regression	Birthweight	BMI, SF, S:T, WHR	Age, sex, weight	- Positive association (j-shape) with BMI - Positive association with TSF, NS with SSSF - Negative association with S:T after adjusting for weight
Wells et al, 2005(196)	Brazilian boys	9-10.1y	172	BIA, Anthropometry	Boys	ANOVA, Regression	Birthweight/PI SDS quartile	Height, BMI, LMI (height^n), FMI, FM/LM^n	SES, maternal BMI	- Positive association with Height, BMI, LMI
Rogers I, 2006 ((141))	UK children	9-10y	6086	DXA	Separate sex	Regression	Birthweight/length/PI SDS	LM, FM, FLR (FM/LM^n), truncal fat, BMI, WHR	GA, age, height, height^2, SES, stage of puberty (girls),FM (trunk fat), LM (trunk fat)	- Birthweight positive association with LM both sexes/ positive association with FM (not with FLR) only in girls - PI positive association with LM, FM, FLR in both sexes - NS birth size with central adiposity except PI positive association with trunk fat (NS after adjusted for total FM) - Weak sex-birthweight SDS interaction in predicting LM (p=0.097)

Table 4-10 (cont.) Summary of studies examining the association between birthweight and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Birth variables	Outcomes	Covariates	Results
Walker et al, 2002 (197)	Jamaican children	7 and 11 y	116 stunted, 190 non-stunted	Anthropometry (SF equation for black children)	Both sexes included	Regression	Birthweight	BMI, SF, S:T, %Fat	Age, sex, pubertal status, stunted groups (chronic/ previously/ non-stunted) BMI	- Positive association BMI - Negative association S:T ratio at 11y, change from 7-11 y (not with S:T at 7y) - BMI NS with S:T, not change negative association after adjustment
Malina et al, 1996(92)	White US adolescents	7-12y	237	Anthropometry	Separate sex	Partial correlation	Birthweight	BMI, sum SF, S:T	age	- Negative association with S:T only after adjusting for BMI or sumSF; stronger in girls than boys
Fewtrell et al, 2004 (198)	UK children (preterm born) UK Children (term born)	8.8-12.7y 8-12y	497 95	DXA	Both sexes included Term vs. Preterm	Regression T-test to compare term vs. preterm	Birthweight SDS (with GA)	% Fat, FM, FFM, FMI (height^{2}or height^n), FFMI, SF, WC, AFA, AMA	Age, sex, puberty, activity	- NS with FMI, FFMI (preterm group) - Preterm had lower FM, FFM, FMI, SF, WC, Weight., BMI than term - FMI lower in boys/ higher activity level
Singhal et al, 2003 (80)	UK adolescents UK children	13-16y 7.4±1.9y	78 86	BIA, DXA, Anthropometry (SF equations)	Both sexes included	Regression	Birthweight SDS (with GA)	FM, FFM, BMI Not mention fat distribution	Age, sex, SES, Tanner stage, physical activity, height^2	- Positive association FFM, weaken after adjusting for height^2 in adolescents; only positive association FFM after adjustment in children. - Weak positive association BMI in children - NS with FM - FFM in adolescent higher in top half of birthweight SDS - No significant sex-birthweight SDS in predicting FFM

Table 4-10 (cont.) Summary of studies examining the association between birthweight and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Birth variables	Outcomes	Covariates	Results
Barker et al, 1997 (93)	Southampton girls	14-16y	216	Anthropometry	Girls	Regression	Birthweight (kg), divide to 3 groups (<=3000, 3001-3500, >3500)	BMI (divided to >,<median), WHR, S:T, SSSF, TSF	BMI, social class BMI, SF adj. to age 15y	- Weak positive association BMI (p=0.08) - Negative association SSSF (p=0.02), S:T ratio (p=0.05) only after adjusting for BMI - Include GA in regression weaken relationship
Matthes et al, 1996 (199)	UK adolescents	mean 15.7y	165 pairs	Anthropometry	Both sexes included	Case-control (low birthweight vs. normal)	LBW (<2500gm, term) vs. normal (3000-3800)	BMI, TSF, SSSF, T:S	Matches for sex, parity, gestation, DOB	- LBW had lower BMI - NS different in SF
Labayen et al, 2006 (200)	Spanish adolescents	13-18y	234	DXA, Anthropometry (SF equation)	Separate sex	Regression, Compare geometric means in different group	Birthweight SDS	FM, FFM by DXA/SF, BMC, %fat, S:T	Age, gestation, Tanner stage, SES, physical activity, height^2,	- Significant sex-birthweight SDS interaction - Positive association height, BMC/ Negative association S:T (before height^2 adjustment), with %FM (after height^2 adjustment) for both sexes combine - Negative association with S:T after adjusting for height^2 in boys - Positive association with FFM, BMC (persist after adjusting for height^2) in girls
Frisancho et al, 2000 (201)	White US adolescents	15-17y	1993	Anthropometry	Separate sex	ANOVA compare between group, Regression	Birthweight (SGA, AGA, LGA)	BMI (high, low), TSF(high, low)	Maternal BMI, GA, sex, family income	- NS different between SGA, AGA, LGA

Table 4-10 (cont.) Summary of studies examining for the association between birthweight and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Birth variables	Outcomes	Covariates	Results
Kahn et al, 2000 (202)	US black/white men	17-22y	192	Anthropometry	Men (military recruits)	Regression	Birthweight (kg)	BMI, height, midthigh circ, thigh muscle area (TMA), thigh fat area (TFA), WC, sagittal abdominal diameter (SAD)	Race, height	- Positive association with BMI, TMA, WC, SAD - BMI-birth association reduce more (68%) with adjustment for TMA than TFA (30%) → larger BMI associate with high birthweight reflect increase lean > fat
Euser et al, 2005 (182)	Dutch adult Preterm born <32 wk, BW<1500 gm	19y	403	Anthropometry (SF equation)	Both sexes included	Regression, compare R^2, R^2 change	Birthweight SDS (with GA)	Weight/ Height/ BMI/ WC/SumSF SDS, WHR, S:T,%Fat, FM, FFM	Height SDS, sex, race, SES, activity	- Positive association with HTSDS, WTSDS, BMISDS, WC SDS, FM, FFM - Weak positive association with FFM, NS with WC SDS,FM after adj. for Height SDS

4.4.1 Comparison with other studies

4.4.1.1 Birthweight and body composition (FFM and FM)

The finding that birthweight has a positive association with FFM in later life is supported by several studies. The majority of them used anthropometric calculations to determine proxies for FFM; for example, arm or thigh muscle area (75;202) and FFM calculated by subtracting FM (from SF equation) from weight (182;203-205). Recently, there have been an increasing number of studies trying to measure FFM with different body composition techniques. More of them were done in adults and elderly populations (76-79;140) but a few studies in children and adolescents also found the same positive relationship (80;141;196;200). My study confirmed that this association persists from childhood to young adulthood but was more prominent in prepubertal boys. Indeed, in the subgroup analysis there was no significant association in the late pubertal group; although this may arguably reflect the smaller sample size. However, my study demonstrated this association only in boys, which has not been generally reported in other studies (see later discussion).

In contrast, the relationship reported between birthweight and FM is less consistent in all age groups. A few studies using anthropometry found a weak positive association of birthweight with FM (141;182;203;204). These associations seemed to be more prominent in females(141;203;204). Interestingly, a number of studies found a negative association of birthweight and proxies of fatness only after adjusting for current body size (in the form of weight, height, or BMI) (77;96;140). Therefore, it is not surprising that in my dataset, there was no significant relationship of birthweight and FM without adjustment for later weight or BMI.

4.4.1.2 Birthweight and fat distribution

Most studies looking at the relationship of birthweight and central fat distribution defined by subscapular SF, subscapular:triceps (S:T) ratio, waist circumference, or waist: hip ratio reported a negative association after adjusting for current weight or

BMI in prepubertal children (92;94;141;195), adolescents (93;200), and adults (78;204;206) although a few studies found this negative association without size adjustment in children and adolescents (197;200). In my study, after adjusting for current BMI, a negative relationship between birthweight SDS and subscapular SF and subscapular: triceps ratio in girls was also found. This finding has generally been interpreted as the programming of later central adiposity by low birthweight. However, an alternative explanation for this is either that postnatal growth actually plays an important role in the programming of body fat and central fat, or the prenatal constraint of growth (represented by low birthweight) interacts with rapid post natal growth to predict later central fatness. A similar interpretation of data regarding low birthweight and coronary heart disease risk in later life was discussed by Cole et al (207).

There are few studies that have attempted to directly measure central fatness, and the results are not consistent. Garnett et al. studied 255 healthy children aged 7-8 year using a DXA region of interest (from lower rib cage to upper iliac crest) to determine central fatness, and confirmed a negative association with birthweight either with or without considering current weight SDS. On the other hand, the ALSPAC study (141) which had a very large sample size of 3006 boys and 3080 girls aged 9-10 years reported a positive association of ponderal index at birth and trunk FM (by DXA), which seems to correspond with my study; however, in their study the association depended on total FM. In a small study that assessed visceral fat area by computerized tomography (CT), Choi et al (97) reported no relationship between birthweight and visceral fat area in 22 healthy young Korean adults.

The other interesting point in my dataset is that higher birthweight SDS was related to higher trunk FM in boys both with and without 'current size' adjustment; this might imply that there is a genuine relationship between prenatal growth and later risk of central adiposity in boys, which would be difficult to reconcile with the widely-accepted low birthweight and increased later cardiovascular risk hypothesis. Alternatively, the 'trunk FM' measured by DXA may not be a good proxy for central adiposity and metabolic risk. Yet, the relationship of birthweight SDS and WC (which is a good indicator of metabolic risk in adults(208) and children(55) was in the same direction. It could be argued that both trunk FM and WC are in fact not an

'actual' measurement of visceral adipose tissue, which is important in predicting the metabolic syndrome. In other words, among adolescent boys with the same height and total FM, those who have higher birthweight tend to have bigger WC or 'trunk FM' – both of which have a 'lean' component, and do not simply represent fat; i.e. they could be bigger around the central area just because they have more FFM which may track from birth. This issue is difficult to resolve unless we have a valid method for measuring 'central fat distribution' in a large sample size.

4.4.1.3 Gender difference in the relationship between birthweight and body composition

Differences in body composition between males and females are well-known. However, interactions between gender and birthweight in predicting later body composition are not well established. Most studies of birthweight and later body composition in children were done in prepubertal children where gender difference in body composition is less prominent. A few studies have been conducted in adolescents when pubertal status is the main confounder. Singhal et al. (80) found a positive association between birthweight and FFM in 78 adolescents aged 13-16 (using BIA and SF). In a younger age group (age 7.4±1.9 year) from the same study (using DXA and SF), birthweight was only positively related to FFM after adjusting for age, sex, puberty and physical activity. However, there was no interaction between gender and birth SDS in predicting FFM in either adolescents or children. This finding seems to support the relationship of birthweight and FFM seen in my dataset; however, it does not support the gender difference. Barker et al.(93) found a negative association (weakened if gestation was included in the model) between birthweight and subscapular SF only after adjusting for BMI in 216 adolescent girls aged 14-16. This finding also corresponds with my data in that a similar association occurred in girls. In this study the authors did not mention the association with FFM so it is uncertain whether or not there is any association or gender difference in the association. There is one published study that showed a gender difference in the association of birthweight and later body composition in an adolescent population. Labayen et al (200) reported a significant interaction between sex and birth SDS in predicting FM, FFM (by DXA) and S:T in 234 Spanish adolescents (age 13-18

years). However, the association of birthweight and FFM appeared in the opposite sex compared to my data. They demonstrated that birthweight was inversely associated with the S:T ratio in boys and directly associated with FFM in girls. One possible explanation might be that their subjects are older and the 'noise' of puberty that might interfere with the results is more obvious in boys, while in my dataset more of the subjects were younger (mean age 11.6 years) and the 'noise' of puberty might be more prominent in girls.

In my opinion, it is not surprising that we found a gender difference in the relationship at this age range. My study showed that at a given age, greater pubertal maturity positively predicted more FFM and less FM in boys while it was positively associated with both FFM and FM in girls. This can probably be explained by hormonal changes during pubertal maturation where the estrogen surge in girls could result in more FM accumulation, whereas testosterone predominantly affects FFM in boys. That is probably one explanation why the association with FFM was different between sexes in this dataset when more than half of the subjects had already entered puberty. One could argue that if this is the only explanation, the subset of prepubertal girls should show the same association with FFM as boys. Nevertheless, there was only a weak positive association of birthweight and FFM in prepubertal girls, which depended on height.

In my subgroup analyses, the gender and birthweight interaction was only significant in the prepubertal group which may seem inconsistent with the above explanation. However, I think it is probably due to the smaller sample size in the pubertal and late pubertal groups. Most studies of birthweight and later body composition in the prepubertal age group included both sexes in the same analysis and adjusted for sex as a confounder. However, among the studies that analysed boys and girls separately, it is not clear whether there is a gender difference in the prepubertal age group. Malina et al (92) reported the same negative association of birthweight and SF in both sexes. In the study of Garnett et al (96) where a negative association between birthweight and abdominal fat (by DXA) was found they did not mention FFM or any sex and birthweight interaction. The other published study in 172 Brazilian boys aged 9-10.1 years by Wells et al (using BIA) showed a similar association of birthweight and FFM in prepubertal boys (196), but unfortunately no data in girls

was shown. In the ALSPAC study (141) which has an exceptionally large sample size, there was no evidence that the association between birthweight and FM or trunk FM differed between the sexes. However, they reported a weak interaction of sex and birthweight for the association with FFM (p=0.097). The significance of the interaction was stronger if ponderal index was used as a proxy for birth size (p=0.04). The effect size on lean mass was larger in boys (350 and 280 gm increase in LM per SDS increase in birthweight in boys and girls). This may support my finding that the association was only significant in boys in a smaller sample size.

4.4.2 Effects of using different body composition techniques

In the large scale published studies, anthropometry has been used to derive body fat from SF measurements. More recently, DXA has been used widely to assess body composition in children. Both methods are not ideal and involve several assumptions in calculating body composition (see **Chapter 2**). My finding of an association between birthweight and FFM in boys did not change with the use of different body composition techniques. However, there was a height-dependent positive association of birthweight and FFM in girls when the SF equation was used. Moreover, my data showed that if DXA or SF equation derived FM was used in the analysis; one could see a weak positive association of birthweight and FM which was not statistically significant if 4C model FM was used. This is a good example of the need for more caution in the interpretation and comparison of different studies that use different body composition techniques, since they can give us different answers.

4.4.3 Effects of using different methods for size adjustment and expression of BC

The interpretation of body composition data in children and adolescents in whom body size varies greatly is difficult without appropriate adjustment for individual body size. In the regression analysis of later body composition parameters where SDS were not available, I chose to adjust these body composition variables by height to look at the effect of birthweight on different body composition parameters having taken out the effect on body size. In my opinion, adjusting for body size using height

is a more appropriate option than using weight (e.g. using percentage of body fat) which precludes individual consideration of fat and lean mass since one is the inverse of the other. This issue has recently been discussed elsewhere (189;190). For instance, a child with high percentage fat could either have high FM or low lean body mass in relation to total weight. Therefore, the use of percentage fat might not be an ideal way of expressing body composition data. However, to be comparable to other studies and to evaluate the importance of size adjustment method to the interpretation of results, I have also shown the results of the alternative analyses using fat percentage and body size adjustment by BMI. Interestingly, a negative association between birthweight and FM (in the form of fat percentage) appeared in boys after adjusting for BMI. This was also reported by other studies (96;193;200). The challenge here is how to interpret such findings. Some authors would interpret this as indicating that a small size at birth was associated with higher fatness in a person with the same BMI. Conversely, another approach would be to interpret it as indicating that a greater increase in body size from birth is related to higher fatness, as mentioned previously. A third interpretation could be that a lower birthweight was associated with lower FFM in later life (since lower FFM with same FM could result in higher percentage fat). This is an important issue, since it can change the conclusions drawn from the same findings.

Another consideration when using size adjustment is the interpretation of the relationship between birthweight and later fat distribution. Most studies (including my dataset) only found a negative association after 'current size' adjustment, which may imply that this association depends on postnatal growth. The issue of which 'body size' parameter should be used to adjust for central fat distribution depends on the research question. For example, birthweight SDS was positively associated with trunk FM in boys (**Table 4-5**). Without adjustment, it would mean that higher birthweight is related to a higher absolute trunk FM, but after adjustment for total FM, the interpretation would be that a higher birthweight is related to a tendency to accumulate central fat despite the same total amount of fat in the body - which possibly has different consequences in terms of cardiovascular health outcomes. In my opinion, there is no 'right' or 'wrong' in this matter provided that we understand these different meanings and justify the preference according to the question we are interested in.

4.4.4 Criticisms of the study

4.4.4.1 Characteristics of the dataset

I used the British 1990 reference data to calculate SDS. My subjects tend to be bigger compared to 1990 reference data (which was constructed using growth data from an 'earlier' period). I presume that this might result from changes in life style and environmental factors (e.g. more sedentary life style, higher caloric density diet) over time. In general, girls usually have more FM and less FFM than boys especially when they reach puberty, but this group of girls had higher waist circumference and SF 'SDS' than boys, suggesting that their higher 'fatness' is probably beyond the typical gender difference. In subgroup analysis, this difference was only significant in late pubertal subjects (data not shown). One possible explanation might be that both boys and girls in my study entered puberty earlier than the children that formed the 1990 reference, but the difference over time is probably larger in girls than boys. This might partly contribute to gender differences in the association between birthweight and body composition found in this study.

4.4.4.2 Reliability of birthweight data

Since this dataset came partly from retrospective records of birthweight, the accuracy of the findings depends greatly on the reliability of the information recorded. I have tried to verify parental recall and records in baby books; the result seems to be satisfactory for birthweight in that there was no difference between the two sources. However, gestational age from parental recall was different (mean difference 0.13 week, 95% CI 0.02, 0.23) from what was recorded in baby books (45.5% available). This probably contributes to some inaccuracy in SDS calculation.

4.4.4.3 Potential confounders

In theory, there are several confounders that influence both birthweight and later body composition; for example, parental size, social class, and ethnicity. However, in

this study, I only found a significant relationship between parental size and offspring birthweight. The lack of an apparent association of ethnicity and birthweight is probably due to small sample size for non-white ethnicity (23%). In addition, I do not have data on some factors that have been shown to affect birthweight such as parity or maternal smoking; therefore, I cannot exclude these confounding effects in my results.

In some analyses I have shown that parental height and BMI have differential effects on the relationship between birthweight and later body size. For instance, the positive association between birthweight and later height disappeared after adjusting for parental height but persisted with the adjustment for parental BMI. Given that parental height influences both the birth size of offspring and later height, which may be tracked from birth, it is not surprising that association between birthweight and later height disappears after adjusting for parental height. This finding suggests that later height is more inheritable (partly mediated through birthweight) whereas for later body composition e.g. fatness, the mechanism is less obvious since its association with parental BMI could be interpreted as either 'family lifestyle' or an 'inheritable factor'. However, the correlation table (**Table 4-2**) shows that parental BMI has more influence on later BC than parental height (except for offspring height); therefore, in the subsequent analysis I have only shown the results from models with parental BMI adjustment.

After adjusting for age, boys who were bigger at birth were in a more advanced pubertal stage at the outcome measurement so it might be plausible that the positive association between birthweight and FFM in boys is mediated through earlier puberty (e.g. higher IGF-1). Nevertheless, the association remained in the same direction after adjusting for pubertal staging and was even stronger in prepubertal boys which supports an alternative explanation.

From the correlation table (**Table 4-2**) the strongest predictor for higher indices of fatness is a lower physical activity level. However, the data did not show any trend for an association between birthweight and physical activity, and the relationship of birthweight and later BC did not change after adjusting for physical activity. Therefore, my data do not support physical activity as a mediator of the association

between birthweight and body composition programming. Although, I accept that the physical activity rating used in my analysis is relatively crude and my sample size is not large, a bigger study recently published by Hallall et al (209) also did not find any relationship between birthweight and physical activity in 4453 Brazilian adolescents.

4.4.4.4 Wide age range and inclusion of children with different pubertal stages

Due to the wide age range of my study population, that covers pubertal maturation, it is inevitable that the results may be confounded by changes of body composition during this period. The ideal method would be to separate the analyses according to different pubertal stages; unfortunately, the sample size is not big enough. However, this study can at least show the different associations of birthweight and later body composition (by the same 'gold standard' technique) in different pubertal groups (more obvious results in prepubertal).

Another way to overcome this problem would be to use body composition SDS calculated from a bigger sample size of normal children, which (to the best of my knowledge) is not available yet. However, in order to confirm the consistency of the findings, I have created age and sex-specific internal SDS for body composition variables using the LMS method which allows adjustment for the non-linear relationship of these variables with age (details in **Appendix C**). FMI ($FM/Height^2$) and FFMI ($FFM/Height^2$) were used to create the SDS in order to take body size into account and to be comparable with BMI. The results (**Table 4-11)** confirmed the findings discussed previously. Nevertheless, I still think that it is important to present the other methods of body composition data expression, in order to compare with other studies and to illustrate that they can make a difference to the study results. Having established this point, for the following results chapters (covering the relationship between growth and body composition in infancy and later body composition), most of the results are shown using body composition SDS where possible to simplify the expression of body composition variables and to address the issue of the wide age range over which body composition outcomes were measured.

Table 4-11 Regression of body composition variables expressed in SDS on birthweight SDS [&]

	No adjustment				Adjusted for age, puberty, physical activity, social class, ethnicity, parental BMI			
Boys	B	SE	p	r^2	B	SE	p	r^2
BMI SDS	0.295	0.103	***0.005***	0.055	0.191	0.100	0.058	
FMI SDS	0.076	0.082	0.357		-0.013	0.075	0.861	
FFMI SDS	0.270	0.080	***0.001***	0.075	0.218	0.083	***0.010***	0.052
Trunk FMI SDS	0.140	0.082	0.088		0.074	0.078	0.349	
Trunk FMI SDS[∞]	0.110	0.049	***0.026***	0.034	0.033	0.035	0.350	
Girls								
BMI SDS	0.043	0.081	0.593		0.009	0.092	0.926	
FMI SDS	0.095	0.080	0.239		0.009	0.079	0.914	
FFMI SDS	-0.033	0.081	0.685		-0.026	0.085	0.762	
Trunk FMI SDS	0.060	0.080	0.452		-0.011	0.081	0.890	
Trunk FMI SDS[∞]	0.026	0.059	0.662		-0.019	0.029	0.516	

[&] From multiple regression analysis, each row represents a different model with body size or composition in the left hand column as a dependent variable; birthweight SDS was independent variable; age, puberty, physical activity, social class, ethnicity, and parental BMI were covariates.
r^2 = coefficient of determination (calculated from partial r of birthweight SDS)

[∞] Adjusted for total FM (log_e scale)

4.5 Summary

- My analysis confirmed that higher birthweight is associated with higher later height in both sexes, and with higher weight and BMI in boys.

- A gender difference in the influence of birthweight on later body composition was shown. In boys, higher birthweight was associated with higher FFM which was more prominent in the prepubertal group.

- Although the sample size of my study was not comparable to the large-scale epidemiologic studies, the use of the 4-component model can give a more accurate answer regarding the programming of body composition and demonstrates some interesting points about the interpretation of body composition data.

Chapter 5

The Influence of Infant Growth on Later Body Composition

Chapter 5 The Influence of Infant Growth on Later Body Composition

5.1 Introduction

Most of the earlier publications investigating postnatal growth and later outcome used BMI as a proxy for obesity. They demonstrated a positive relationship between rapid infancy and childhood weight gain and higher BMI in childhood and adult life. See **Chapter 2**. However, a limited number of recent publications that have attempted to measure FM and FFM separately have reported different associations between postnatal growth and these individual components (see the discussion section of this chapter). There has been no study using the 4C model of body composition to explore the longer term effects of growth during different periods of infancy.

5.2 Methods

5.2.1 Subjects

Children included in this analysis were those (as in **Chapter 4**) who completed four-component model measurements and had early growth records available from their baby books for at least one time point at 3, 6, 12 weeks, 6 and 12 months. 54% (139 out of 256) of the BC reference study research participants provided growth records (from parent-held baby books) in the first year of life that could be analyzed. 52 children from the prospective follow-up study (cohorts A, B, C) were also included. All were born full-term, singletons and did not have any disease that might affect growth or body composition.

5.2.2 Early growth data

In the prospective follow-up study, growth measurements were performed prospectively at specific ages (3, 6, 12 weeks, 6 and 12 months). In the retrospective study using weights obtained from parent-held baby record books, weight measurements performed as close as possible to these ages were selected. A description of the age distributions is shown in **Appendix E, Table 10-6**. Weight SDS at different ages were calculated (for gestation, sex and exact age) using the British 1990 reference.

5.2.3 Measurement of body composition

Body composition variables derived from the 4C model were used in this analysis together with anthropometry and DXA regional FM to assess central fat distribution. For more details see **Chapter 3**.

5.2.4 Confounding variables

Factors that might confound the relationship between early growth and body composition were assessed using the structured questionnaire as mentioned in the previous chapter and in **Chapter 3** and **Appendix B**.

5.2.5 Statistical methods

Early growth data

Changes in weight SDS (ΔweightSDS) between two time points were calculated by subtracting the earlier weight SDS from the later measurement. The association between early growth and later body composition was explored using multiple regression models.

Body composition outcomes were regressed on ΔweightSDS with adjustment for birthweight SDS (Cole TJ, personal communication).

Body composition outcomes

The importance of body size adjustment in the interpretation of body composition data was addressed in the previous chapter. In this chapter, dealing with early growth data which is complicated to analyze and interpret, I chose to simplify the body composition outcome variables by using the SDS which provides body composition measurements adjusted for age and gender. In the SDS calculation using the LMS method (details shown in **Appendix C**), FMI, FFMI, and trunk FMI (kg/m^2) were chosen in order to adjust for height in a comparable way to BMI. In the previous chapter, the main findings from analyses using body composition SDS were similar to those from analyses adjusting for height in regression models. A second reason is that this method allows direct comparison of the effect size of early growth on later FM or FFM since they both are in SDS, avoiding the problem of unequal variance between different body composition variables. Lastly, using body composition SDS has another advantage in this chapter since it reduces the number of variables for body composition adjustment in the regression model and, therefore, allows for more 'early growth' and confounding variables to be included in the model. This is even more relevant for analyses presented in the subsequent chapters, which had smaller sample sizes.

5.3 Results

5.3.1 Characteristics of study subjects

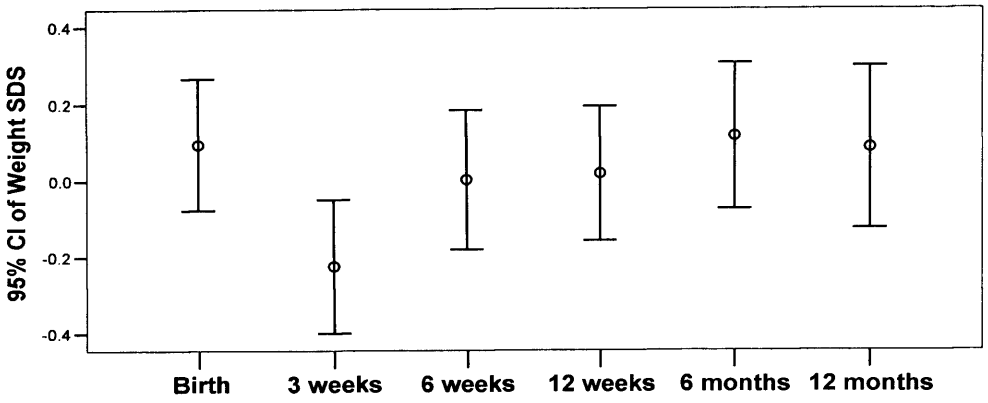
5.3.1.1 Early growth parameters

Weight SDS at different periods in infancy are shown in **Figure 5-1**. In general, mean weight SDS in this dataset was not different from zero apart from at the age of 3 weeks when there was a drop in weight SDS. The mean difference was significant for boys only (mean difference -0.32; 95%CI -0.56, -0.09). Boys were heavier than girls at birth but had the same birth SDS. Boys also had higher absolute weight than girls throughout infancy (data not shown) but gender differences were no longer apparent when SDS were used. ΔweightSDS was different between boys and girls only during the period 6-12 weeks (mean difference 0.15; 95%CI 0.006, 0.295).

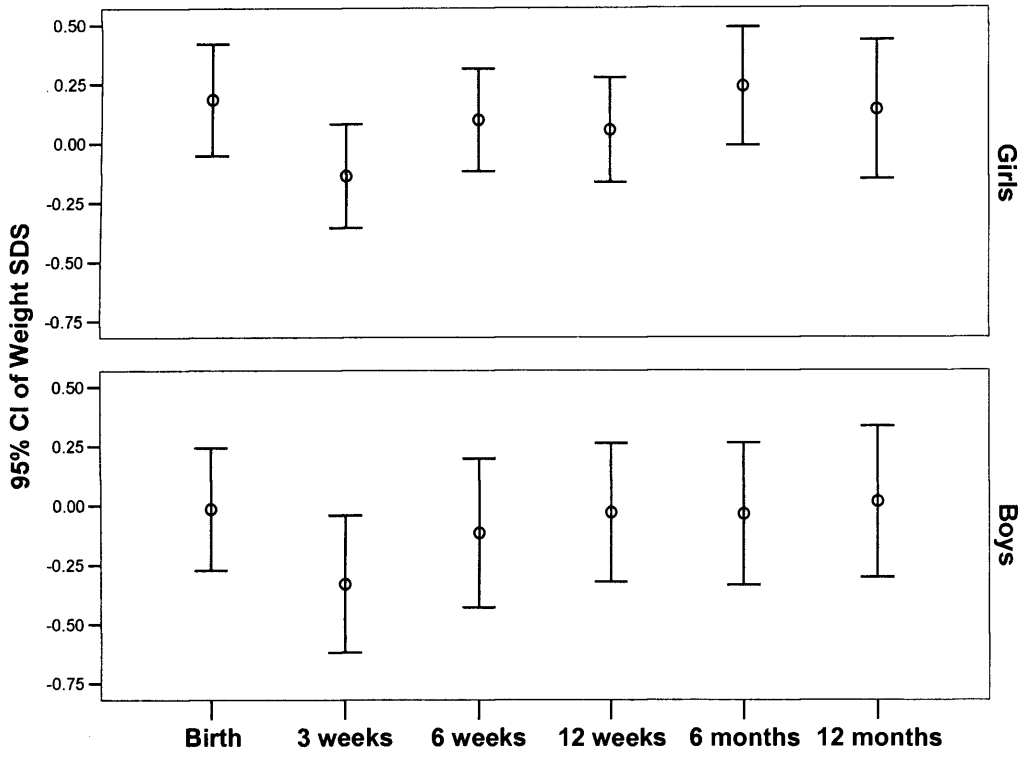
From the GLM, there was a significant interaction between gender and ΔweightSDS from 6-12 weeks and 3-6 months in predicting later FM (p for interaction 0.06 and 0.04, respectively). However, when FMI SDS, FFMI SDS, and trunk FMI SDS were used as outcomes, no interaction was seen. Therefore, the analyses were performed without separating the sexes. This is also preferable since separating the sexes in the analysis might further reduce statistical power in detecting associations. However, I included sex in the multiple regression models since there was a difference in ΔweightSDS from 6-12 weeks between boys and girls, and to adjust for differences in body composition that are beyond normal gender differences (e.g. waist SDS, triceps SDS were significantly different between boys and girls as discussed in the previous chapter).

Figure 5-1 Mean and 95% confidence interval for weight SDS at different ages[#]

a) All subjects



b) Separate plots for boys and girls



[#] Different sample size at different ages:

N=191, 149, 172, 173, 161, 136 for birth, 3, 6, 12 weeks, 6 and 12 months

Since weight SDS during infancy are likely to be strongly related, partial correlations (adjusting for gender) between the measurements periods are shown in **Table 5-1a**. As expected, weight SDS for the different periods between birth and 12 months were strongly correlated with each other and the two nearest measurements showed the highest correlation. Lower birthweight SDS correlated with later upward ΔweightSDS up to 6 months of age (**Table 5-1b**). Low ΔweightSDS during the first 3 weeks correlated with higher ΔweightSDS during 6-12 weeks of age. ΔWeightSDS from 6-12 weeks was positively related to the change in the next period, 3-6 months, but negatively related to the change in the second 6 months of life.

Additional analyses showed that there was no significant interaction between birth SDS and ΔweightSDS over the 5 periods in predicting FM or FFM (data not shown). Therefore, I did not separate birthweight SDS categories in this analysis but added birthweight SDS as a covariate in the multiple regression analyses. In each regression analysis, ΔweightSDS for particular periods that were significantly correlated with the period of interest were adjusted for in some models (see later results).

Table 5-1 Partial correlation coefficients (r) controlled for gender

a) Between weight SDS at different ages[#]

	Birth SDS	WT3wk SDS	WT6wk SDS	WT12wk SDS	WT6mo SDS	WT12mo SDS
Birth SDS	1					
WT 3 wk SDS	0.80	1				
WT 6 wk SDS	0.70	0.93	1			
WT 12 wk SDS	0.62	0.77	0.88	1		
WT 6 mo SDS	0.46	0.54	0.63	0.82	1	
WT 12 mo SDS	0.48	0.49	0.55	0.66	0.85	1

b) Between Δweight SDS at different ages[##]

	Birth SDS	ΔSDS0-3wk	ΔSDS3-6wk	ΔSDS6-12wk	ΔSDS 3-6mo	ΔSDS6-12mo
Birth SDS	1					
ΔSDS 0-3wk	***-0.32[c]***	1				
ΔSDS 3-6wk	***-0.19[a]***	0.11	1			
ΔSDS 6-12wk	-0.13	***-0.37[c]***	0.12	1		
ΔSDS 3-6mo	-0.17[a]	-0.16	-0.11	***0.24[b]***	1	
ΔSDS 6-12mo	0.005	-0.07	-0.04	***-0.19[a]***	0.13	1

[#] All correlation coefficients were significant at $p<0.001$

[##] [a]$p < 0.05$, [b]$p < 0.01$, [c]$p<0.001$

5.3.1.2 Current body composition outcomes

Descriptive statistics of 191 subjects (age range 4.2 to 20.4 years) are summarized in **Table 5-2**. Boys were comparable to the British 1990 reference in terms of height, weight, and BMI whereas girls in this dataset were heavier, taller and had higher BMI than the reference children. Both boys and girls had significantly higher WC compared to the British 1990 reference while girls also had higher triceps and subscapular SF.

Gender differences in body composition in this dataset were in the same direction as described previously in the previous chapter except that boys did not show significantly higher FFM compared to girls. Using age and sex specific internal SDS for body composition outcomes, no gender differences were apparent.

There was no difference in other potential confounders between boys and girls.

5.3.2 Potential confounders

Partial correlation (controlling for age and sex) was used to test the influence of the potential confounders on early growth variables (**Appendix E, Table 10-7**). Lower social class was associated with higher Δ weight SDS between 3-6 months. This could either be explained by a tendency toward earlier weaning or a higher rate of formula feeding in lower social class groups. Maternal height and paternal BMI were positively associated with ΔweightSDS between 3-6 wk and 3-6 months, respectively.

Table 5-2 Characteristics of study subjects[&]

	All (n=191)	Boys (n=88)	Girls(n=103)	p
Age (y)	11.8 ± 3.8	11.3 ± 3.7	12.2 ± 3.9	0.13
Height (cm)	147.5 ± 19.2	146.1 ± 20.1	148.8 ± 18.5	0.34
Weight (kg)	42.8 ± 16.2	40.2 ± 16.0	44.9 ± 16.1	***0.04***
BMI (kg/m^2)	18.9 ± 3.5	18.1 ± 3.2	19.5 ± 3.6	***0.004***
Height SDS	0.24 ± 0.94	0.17 ± 0.94	0.30 ± 0.94	0.35
Weight SDS	0.31 ± 1.09	0.20 ± 1.16	0.40 ± 1.03	0.20
BMI SDS	0.21 ± 1.21	0.10 ± 1.32	0.31 ± 1.11	0.23
FMI (kg/m^2)	4.6 ± 2.5	3.6 ± 2.2	5.5 ± 2.3	***<0.001***
FFMI (kg/m^2)	14.2 ± 1.9	14.4 ± 2.1	14.0 ± 1.7	0.15
Trunk FMI (kg/m^2)	2.0 ± 1.3	1.5 ± 1.1	2.5 ± 1.4	***<0.001***
FMI SDS	-0.05 ± 0.98	-0.05 ± 1.03	-0.05 ± 0.93	0.96
FFMI SDS	-0.08 ± 1.00	-0.09 ± 1.02	-0.07 ± 0.99	0.90
Trunk FMI SDS	-0.04 ± 0.98	-0.04 ± 1.04	-0.03 ± 0.94	0.91
WC(cm)	65.2 ± 9.2	64.3 ± 9.0	65.9 ± 9.3	0.23
Triceps SF (mm)	13.3 ± 5.3	10.8 ± 4.7	15.4 ± 4.9	***<0.001***
Subscapular SF (mm)	10.0 ± 6.3	7.9 ± 5.0	11.8 ± 6.8	***<0.001***
WCSDS[#]	0.70 ± 1.07	0.45 ± 1.09	0.91 ± 1.02	***0.004***
Triceps SDS	0.40 ± 0.95	0.22 ± 1.03	0.56 ± 0.85	***0.01***
Subscapular SDS	0.12 ± 1.06	-0.04 ± 1.11	0.26 ± 1.00	0.05

[&] Continuous variables were expressed as mean ± SD and independent sample t-test was used to test the difference between gender; categorical variables were expressed as n (%) and Chi-square test was used to test the difference between gender.

[#] n= 80 in boys and 93 in girls since the database for waist SDS was available only up to the age of 17

Table 5-2 Characteristics of study subjects (cont.) [@]

	All (n=191)	Boys (n=88)	Girls(n=103)	p
Maternal height (cm)	163.9 ± 6.6	164.8 ± 6.9	163.1 ± 6.2	0.06
Paternal height (cm)	178.6 ± 7.0	178.1 ± 7.1	179.0 ± 6.9	0.37
Maternal BMI (kg/m^2)	24.8 ± 4.8	25.0 ± 4.6	24.7 ± 4.9	0.66
Paternal BMI (kg/m^2)	26.5 ± 3.9	27.1 ± 4.0	26.1 ± 3.7	0.08
Pubertal status (n, %)				
Prepubertal	81, 42.6%	44, 50%	37, 35.9 %	
Early pubertal	51, 26.8 %	22, 25%	29, 28.2 %	0.10
Late pubertal	58, 30.5 %	21, 23.9%	37, 35.9 %	
Physical activity (n, %)				
Much less or less	15, 8.0%	8, 9.0%	7, 6.8%	
Same as peers	86, 45.7 %	38, 43.2%	48, 46.6%	0.86
More	68, 36.2 %	32, 36.4%	36, 35.0%	
Much more	19, 10.1%	10, 11.4%	9, 8.7%	
Social class (n, %)				
Class 1	36, 18.9 %	20, 22.7%	16, 15.5%	
Class 2	90, 47.4 %	38, 43.2%	52, 50.5%	0.40
Class 3	22, 11.6%	8, 9.0%	14, 13.6%	
Class 4 or more	42, 22.1 %	21, 29.3 %	21, 20.4%	
Ethnicity (n, %)				
White	172, 90.5%	80, 90.9%	92, 89.3%	0.87
Non-white	18, 9.5%	8, 9.1 %	10, 9.7%	

[@] Numbers of missing data (boys, girls) are as follow: Maternal height (1, 2), Paternal height (2, 2), Maternal BMI (2, 3), Paternal BMI (8, 6), Pubertal status (1, 0), Physical activity (0, 3), Social class (1, 0), Ethnicity (0, 1).

5.3.3 Early growth and later body size

Regression analyses of later body size and composition on ΔweightSDS during different periods of infancy are shown in **Table 5-3**. In the first model (**Table 5-3a**), only birthweight SDS and gender were adjusted for. ΔWeightSDS from birth to 3 weeks and 3 to 6 weeks showed a positive association with height SDS, but not weight or BMI, in childhood and adolescence. A 1 ΔweightSDS increase during these respective periods was associated with a 0.37 SDS (95%CI 0.09, 0.64) and 0.55 SDS (95%CI 0.10, 1.01) increase in later height. The association remained significant in the same direction after adjusting for confounding factors, except for parental height (data shown in **Appendix E, Table 10-8**).

ΔWeightSDS during later infancy tended to be positively associated with later weight SDS and BMI SDS. The association was most prominent for the period 3-6 months; a 1 ΔweightSDS increase in early growth corresponded to a 0.68 SDS increase (95%CI: 0.38, 0.98) in later weight and a 0.60 SDS increase (95%CI: 0.26, 0.95) in later BMI. ΔWeightSDS during this period explained around 11.8% and 7.4% of variability in weight SDS and BMI SDS respectively. This association was robust to the adjustment for potential confounders (**Table 5-3b**). These results did not change considerably after adjusting for ΔweightSDS during other periods that were significantly correlated with the period of interest (data not shown).

There was no significant association between ΔweightSDS in the second half of infancy and later body size.

Table 5-3 Regression of current body size and composition on Δ weight SDS during different infancy periods[&]

a) Adjusted for birthweight SDS and gender

	Δweight SDS 0-3wk (n=149)				Δweight SDS 3-6wk (n=139)				Δweight SDS 6-12wk (n=157)				Δweight SDS 3-6mo (n=150)				Δweight SDS 6-12mo (n=125)			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.22	0.17	0.18		0.57	0.27	***0.04***	0.032	0.55	0.20	***0.006***	0.048	0.68	0.15	***<0.001***	0.118	0.20	0.17	0.26	
Ht SDS	0.37	0.14	***0.01***	0.045	0.55	0.23	***0.02***	0.041	0.25	0.17	0.15		0.44	0.14	***0.001***	0.067	0.17	0.15	0.26	
BMI SDS	0.03	0.19	0.88		0.38	0.30	0.21		0.58	0.22	***0.01***	0.043	0.60	0.18	***0.001***	0.074	0.14	0.19	0.47	
FMI SDS	0.05	0.15	0.73		0.33	0.25	0.18		0.45	0.18	***0.01***	0.039	0.48	0.14	***0.001***	0.071	0.11	0.16	0.47	
FFMI SDS	0.08	0.15	0.62		0.07	0.25	0.77		0.17	0.19	0.36		0.29	0.15	0.06		0.11	0.16	0.47	
Triceps SDS	0.04	0.15	0.77		0.56	0.23	***0.02***	0.041	0.45	0.17	***0.01***	0.043	0.40	0.14	***0.005***	0.052	-.05	0.15	0.73	
Subscapular SDS	0.01	0.16	0.95		0.45	0.26	0.09		0.37	0.20	0.06		0.37	0.16	***0.02***	0.036	0.18	0.17	0.28	
WC SDS[#]	0.21	0.16	0.20		0.48	0.27	0.07		0.30	0.21	0.15		0.52	0.16	***0.002***	0.072	-.004	0.18	0.98	
WC[$]	0.01	0.02	0.47		0.02	0.02	0.47		0.02	0.02	0.22		0.04	0.01	***0.006***	0.051	0.002	0.02	0.88	
WC[∞]	0.01	0.01	0.29		-.01	0.02	0.62		-.02	0.01	0.15		0.004	0.01	0.69		0.001	0.01	0.92	
TrunkFMISDS	0.06	0.15	0.69		0.49	0.25	***0.05***	0.028	0.48	0.18	***0.009***	0.044	0.51	0.14	***0.001***	0.080	0.12	0.16	0.46	
TrunkFMISDS[ε]	0.04	0.06	0.56		0.21	0.10	***0.04***	0.031	0.06	0.08	0.46		0.12	0.06	0.06		0.10	0.06	0.13	

[&] From multiple regression analysis, each row represents a different model with body size or BC variables in the left hand column as a dependent variable; Δweight SDS was an independent variable; birthweight SDS and gender were covariates; B = the coefficient of Δweight SDS i.e. the change in current body size or body composition per early Δweight SDS (weight SDS change between 2 time points), SE = standard error, highlighted p value indicate significant at $p<0.05$, r^2 = coefficient of determination (calculated from partial r of Δweight SDS)

[#] n=144, 134, 142, 136, and 116 for Δweight SDS 0-3wk, Δweight SDS 3-6wk, Δweight SDS 6-12wk, Δweight SDS 3-6mo, and Δweight SDS 6-12mo, respectively since the database for waist SDS was available only up to the age of 17.

[$] Adjusted for current height (m) WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.
[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

b) Adjusted for birthweight SDS, gender, puberty, physical activity, social class, ethnicity, and parental BMI [&]

	Δweight SDS 0-3wk				Δweight SDS 3-6wk				Δweight SDS 6-12wk				Δweight SDS 3-6mo				Δweight SDS 6-12mo			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.17	0.16	0.29		0.54	0.25	***0.03***	0.036	0.44	0.19	***0.02***	0.038	0.55	0.15	***<0.001***	0.090	0.13	0.17	0.45	
Ht SDS	0.31	0.15	***0.04***	0.032	0.53	0.24	***0.03***	0.039	0.23	0.18	0.20		0.47	0.15	***0.001***	0.075	0.20	0.16	0.20	
BMI SDS	-.01	0.17	0.96		0.36	0.27	0.19		0.45	0.20	***0.03***	0.034	0.41	0.17	***0.02***	0.042	0.02	0.18	0.93	
FMI SDS	0.04	0.14	0.77		0.32	0.22	0.16		0.33	0.17	***0.05***	0.028	0.32	0.14	***0.02***	0.038	-.01	0.15	0.93	
FFMI SDS	0.03	0.15	0.85		0.07	0.24	0.77		0.13	0.18	0.46		0.21	0.15	0.16		0.07	0.16	0.64	
Triceps SDS	0.03	0.14	0.84		0.53	0.22	***0.02***	0.045	0.34	0.17	***0.04***	0.029	0.28	0.14	***0.05***	0.029	-.17	0.15	0.24	
Subscapular SDS	0.002	0.15	0.99		0.43	0.25	0.08		0.25	0.18	0.17		0.22	0.15	0.16		0.04	0.16	0.82	
WC SDS	0.19	0.16	0.24		0.46	0.25	0.07		0.19	0.20	0.33		0.39	0.16	***0.02***	0.046	-.10	0.17	0.57	
WC[$]	0.01	0.02	0.53		0.02	0.02	0.48		0.01	0.02	0.43		0.03	0.01	0.07		-.01	0.02	0.65	
WC[∞]	0.01	0.01	0.28		-.01	0.02	0.71		-.02	0.01	0.15		0.003	0.01	0.78		0.001	0.01	0.89	
TrunkFMISDS	0.04	0.14	0.80		0.47	0.23	***0.04***	0.034	0.36	0.17	***0.03***	0.032	0.36	0.14	***0.01***	0.047	0.001	0.15	0.99	
TrunkFMISDS[∞]	0.04	0.07	0.57		0.23	0.11	***0.03***	0.038	0.05	0.08	0.58		0.11	0.07	0.09		0.10	0.07	0.13	

[&] From multiple regression analysis, each row represents a different model with body size and BC in the left hand column as a dependent variable; Δweight SDS was an independent variable; birthweight SDS, gender, puberty, physical activity, social class, ethnicity, and parental BMI were covariates. See footnote to previous table.

[$] Adjusted for current height (m) WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.
[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

5.3.4 Early growth and later body composition

Regression analyses results are shown in **Table 5-3a** and **b**. ΔWeightSDS 6-12 weeks and 3-6 months showed a positive relationship with FMI SDS (1 ΔweightSDS increase in early growth was associated with 0.45 SDS (95% CI 0.09, 0.80) and 0.48 SDS (95% CI 0.20, 0.76) increases in later FMI. ΔWeightSDS 3-6 months explained more of the variability in FMI SDS than ΔweightSDS 6-12 weeks (7.1% vs. 3.9%). The association remained significant but the magnitude was decreased after adjusting for potential confounders (**Table 5-3b**). The results for other indices of fatness such as triceps and subscapular SF were similar.

There was a weak trend toward a positive association between ΔweightSDS at 3-6 months and later FFMI SDS; however, it was diminished after adjusting for potential confounders. Interestingly, this trend strengthened slightly after adjusting for parental height (**Appedix E, Table 10-8**) instead of parental BMI in the model. However, the effect size was smaller than for FMI SDS at the same period (1ΔweightSDS increase in early growth was associated with a 0.29 SDS increase in later FFMI; 95% CI -0.003, 0.58). The main findings did not change when birthweight SDS was removed from the models (data not shown).

5.3.5 Early growth and fat distribution

Higher ΔweightSDS 3-6 months was associated with a tendency for central fat distribution assessed by WC, and trunk FMI; the effect size was equivalent to a 0.52 SDS (95%CI 0.20, 0.84) increase in WC and 0.51 SDS (95%CI 0.23, 0.80) increase in trunk FMI per 1ΔweightSDS increase in early weight (**Table 5-3a**). Since WC SDS was not available for subjects older than 17, analyses using WC adjusted for height were also performed; the association was similar to that using WC SDS. After adjusting for potential confounders (**Table 5-3b**), this association remained significant but the strength was attenuated.

Moreover, there was also a weak positive association between Δ SDS 3-6wk and 6-12wk and trunk FMI SDS.

To separate the effects of early growth on the tendency to store fat centrally from effects on total fatness, further adjustment for total FM was performed. The positive association between ΔweightSDS 3-6 months and waist circumference was no longer statistically significant after adjusting for total FM (from 4C model) whereas the relationship with trunk FMI SDS reduced substantially but a positive trend still remained. This finding suggests that the association between ΔweightSDS 3-6 months and WC was not beyond its relationship with total fatness, whereas the association with trunk FMI might be to some degree.

5.3.6 Additional analyses

There was no significant association between ΔweightSDS in the second half of infancy with later body composition and fat distribution. Since the sample size in this period was smaller than for the other periods, analyses were repeated with imputed data (n=178). ΔWeightSDS during this period did not show any significant association with body composition outcomes except for a weak positive association with later weight and height (data shown in **Appendix E, Table 10-9**). In addition, the results of the same analyses with imputed data for other periods of growth were comparable to those described in the previous sections.

The results table for the subgroup analysis in boys and girls for the 2 periods when the interaction between sex and ΔweightSDS was significant, and when there was a difference in ΔweightSDS between boys and girls is shown in **Appendix E, Table 10-10**. Results were comparable to those shown in **Table 5-3** except that: 1) the period of ΔweightSDS that correlated with later fatness was confined to 3-6 months in girls and 2) boys showed a significant positive association between ΔweightSDS 3-6 months and FFMI SDS which

persisted after adjusting for the potential confounders. There was no significant relationship between any other period of ΔweightSDS and FFMI SDS in boys (data not shown).

To compare my results with those from other published studies, the same analysis was repeated on using ΔweightSDS 0-6 months (**Table 5-4**). Weight SDS gain during the first half of infancy was positively associated with later FM, FFM, and central fat distribution. The effect size was slightly larger for FM than FFM; 1ΔweightSDS increase in early growth was associated with a 0.34 SDS (95%CI 0.16, 0.51) increase in FMI and a 0.24 SDS (95%CI 0.06, 0.42) increase in FFMI. Δ SDS 0-6 months explained 8.4% and 4.2% of the variability in FMI SDS and FFMI SDS, respectively.

Another issue is whether, using short periods of weight gain (e.g. Δ SDS 0-3wk, 3-6wk, 6-12wk) compared to a 3 month period (3-6 months) makes associations with later body composition more difficult to detect. To investigate this, the same analysis was repeated using weight gain over the 3 month period from birth to 3 months (Δ SDS 0-3 months) (**Table 5-4**). A similar positive association with FMI SDS and various indices of central fatness was found; however, the strength of the association and the effect size were generally smaller than seen for Δ SDS 3-6 months. For example, 1Δ SDS increase from 0-3 months was associated with a 0.28 SDS (95%CI 0.07, 0.49) increase in FMI whereas 1Δ SDS increase from 3-6 months was associated with a 0.48 SDS (95% CI 0.20, 0.76) increase in later FMI. The percentages of variance in FMI SDS explained by Δ SDS 0-3 months and 3-6 months were 3.8% and 7.1%, respectively. There was no significant association between Δ SDS 0-3 months and FFMI SDS.

Table 5-4 Regression of current body size and composition on Δweight SDS from birth to 3 months and birth to 6 months[&]

	Δweight SDS 0-3 mo (n=173)				Δweight SDS 0-6 mo (n=161)			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.43	0.12	***<0.001***	0.075	0.57	0.09	***<0.001***	0.200
Ht SDS	0.34	0.10	***0.001***	0.063	0.44	0.08	***<0.001***	0.162
BMI SDS	0.34	0.13	***0.01***	0.038	0.38	0.12	***0.001***	0.064
FMI SDS	0.28	0.11	***0.01***	0.038	0.34	0.09	***<0.001***	0.084
FFMI SDS	0.17	0.11	0.13		0.24	0.09	***0.01***	0.042
Triceps SDS	0.33	0.10	***0.002***	0.057	0.35	0.08	***<0.001***	0.098
Subscapular SDS	0.25	0.12	***0.03***	0.026	0.30	0.10	***0.003***	0.056
WC SDS[#]	0.34	0.12	***0.01***	0.048	0.46	0.10	***<0.001***	0.135
WC[$]	0.03	0.01	***0.01***	0.035	0.03	0.01	***0.001***	0.075
WC[∞]	0.004	0.01	0.53		0.01	0.01	0.34	
TrunkFMISDS	0.32	0.11	***0.004***	0.048	0.38	0.09	***<0.001***	0.104
TrunkFMISDS[∞]	0.06	0.05	0.21		0.10	0.04	***0.011***	0.041

[&] From multiple regression analysis, each row represents a different model with body size and BC in the left hand column as a dependent variable; Δweight SDS was an independent variable; birthweight SDS and gender were covariates.

[#] n=157, and 145 for Δweight SDS 0-3 mo, and Δweight SDS 0-6 mo, respectively since the database for waist SDS was available only up to the age of 17.

[$] Adjusted for current height (m) WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

5.4 Discussion

My analyses showed an association between early growth and later body size and body composition using the 4-component model. A summary of the findings is shown in **Table 5-5**. To recap, I have demonstrated that growth during early infancy (first 6 months) was associated with FMI and central fat distribution whereas growth in the second half of infancy did not significantly relate to any of the body composition outcomes. These associations were independent of birthweight, current body size, sex, age, pubertal stage, physical activity, social class, ethnicity, and parental size. This finding might be of importance since early infancy is a period when infant growth can change considerably depending on infant feeding and weaning practice.

Interestingly, growth during 3-6 months of age also showed a weak positive relationship with FFMI in boys. This gender difference is in agreement with the findings from the previous chapter in that the association of birthweight and FFM was apparent only in boys. However, if the period of growth in the analysis was extended from birth to 6 months, a positive association with FFMI was statistically significant in both boys and girls. Indeed, gender differences in this early growth analysis were not as obvious as in the birthweight analysis; the effect of early growth on later FMI and central fat distribution was substantial in both boys and girls.

In this discussion, I will focus on three specific issues: 1) comparison of my study with other studies investigating infant growth and later body composition outcomes, 2) different methods of analysis of early growth, and 3) criticisms of my study. Again, the potential mechanism of body composition programming and broader implication of my findings will be discussed in more detail later in **Chapter 9**.

Table 5-5 Summary of the effect of early growth on later body size and composition[&]

	Early growth period						
Outcomes	0-3weeks	3-6 weeks	6-12 weeks	3-6 months	6-12 months	0-3months	0-6months
Height	↑↑	↑↑	–	↑↑	–	↑↑	↑↑
BMI	–	–	↑↑	↑↑	–	↑↑	↑↑
FMI	–	–	↑↑	↑↑	–	↑	↑↑
FFMI	–	–	–	↑?	–	–	↑
Central fat distribution[#]	–	↑?	↑?	↑↑	–	↑↑	↑↑

[&] – indicates no statistically significant association; ↑ indicates weak positive association, ↑↑ indicates strong positive association (i.e. B coefficient > 0.3 SDS increase in BC outcomes per 1Δ weight SDS in infancy; ? = the results were only statistically significant in some models with adjustment for different confounders.

[#] Considered from WC, subscapular SF, and trunk FMI; however, most of the association with waist circumference or trunk FMI SDS were attenuated substantially after adjusting for total FM.

5.4.1 Comparison with other studies

5.4.1.1 Early growth and later body size

A number of publications showed that rapid growth in early infancy was positively associated with later height, weight, and BMI in childhood and early adult life (82;84;85;87;182;196;210). My findings agree with these studies in that early infancy weight gain was positively related to later height and BMI; however, it gives more information about the possible 'sensitive' period of infant growth. Growth (defined by weight SDS change) in very early postnatal life i.e. the first 6 weeks, seems to have a long term association with later height. However, this relationship depends on parental height, suggesting that early ΔweightSDS may mediate the relationship between parental height and offspring height as well as birthweight, i.e. infants of taller parents grow more quickly during this period and have higher height SDS in later life. The positive correlation between parental height and ΔweightSDS 3-6 weeks seems to support this idea.

Most studies exploring the relationship between infant growth and later BMI (as a proxy for 'adiposity' or 'obesity') only present infant growth over a 6-month or 1-year period (see **Chapter 2**). This is probably because more detailed, reliable growth records during infancy are difficult to obtain. Stettler et al (211) recently published an article using detailed early growth data (weight at 8, 14, 28, 42, 56, 84, and 112 days) from several infant formula studies in relation to later BMI. They proposed that the period between birth and the first week as well as the first 112 days of life were potentially critical in predicting overweight status (defined by reported BMI $\geq$ 25 kg/m^2) in adults aged 20-32 years. In my opinion, the early growth data in this study seem to be sound but, unfortunately, the outcome measure was quite crude since it was the 'reported' usual weight and height obtained by telephone interview which may be subject to underreporting in the case of overweight subjects. In my data, I did not have measurements at the age of 1 week, but growth from birth to 3 weeks did not show

correlation with later BMI. However, my data agreed that growth in the first 3 months was positively associated with later BMI in childhood and adolescence. Again, it is difficult to directly compare the studies since available data on early growth and outcome measures were at different ages.

5.4.1.2 Early growth and later body composition and fat distribution

There have been considerably fewer studies looking into the association between infant growth and later body composition that use measurements beyond BMI i.e. that consider FM and FFM separately (**Table 5-6**). Most have been published in the last few years. Wells et al (196) studied 172 Brazilian boys at the age of 9 years (with BIA) and found that ΔweightSDS from birth to 6 months was associated with later height, BMI and LM (not FM) whereas ΔweightSDS 6-12 months showed no relationship with later body size and composition. The association with LM found in their study was consistent with my study as I also found a positive association between ΔweightSDS 3-6 months and FFMI in boys. However, the main difference is that growth in early infancy in their study did not seem to be associated with later FM (adjusted for height). It should be noted that the two studies were different in that 1) the population studied by Wells et al were from a non-western country, so the pattern of growth during infancy and childhood might be different from the western children in my study (supported by the fact that their birthweight and weight SDS during infancy were lower than the British reference), 2) Wells et al measured outcomes at a prepubertal age when the effect of early growth on FM might not be so apparent, whereas in my study the dataset includes both early and late pubertal subjects; and 3) the body composition technique used was different (BIA vs. 4C model). Indeed, an exclusive association between postnatal growth (birth to 2 years) and FFM in adults was also found by Li et al (203) in a Guatemalan population. Unfortunately, their results cannot be directly compared with mine because the researchers used length gain as a proxy for postnatal growth. Their findings are perhaps not surprising since linear growth is usually highly correlated with adult height (which in turn is more strongly related to FFM than FM).

Table 5-6 Summary of the studies investigating the association between early infant growth and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Early growth variables	Outcomes	Covariates	Results
Wells et al, 2005(196)	Brazilian boys	9-10y	172	BIA, Anthropometry	Boys	ANOVA, Regression	Quartile of ΔweightSDS 0-6, 6-12mo, 1-4,4-9 yr	Height, BMI, LMI (height^n), FMI, FM/LM^n	SES, maternal BMI	- ΔweightSDS 0-6 mo positively associated with later height, BMI and LMI - ΔweightSDS 6-12 mo show no significant association -ΔweightSDS 1-4 yr positively associated with both FMI, LMI but ΔweightSDS 4-9 yr associated with only FMI
Ekelund et al, 2006 (210)	Swedish adolescents	17y	248	Bodpod	Both sexes included	GLM (ANCOVA)	ΔweightSDS 0-6mo, 3-6y as continuous variable vs. divided into rapid, slow, no change (ΔweightSDS >0.67, <-0.67, and between)	Height, BMI, WC, %fat, FM, FFM	Sex, gestation, height, SES, maternal FM, ***birthweight***	- ΔweightSDS 0-6 mo and 3-6y were positively associated with later BMI, WC, %fat, FM, FFM - only ΔweightSDS 0-6 mo was positively associated with height - No significant interaction between birthweight SDS and ΔweightSDS with regard to any outcomes - the effect size on both FM and FFM of ΔweightSDS 3-6y was bigger that that of 0-6 mo
Euser et al, 2005 (182)	Dutch adult Preterm born <32 wk, BW<1500 gm	19y	403	Anthropometry (SF equation)	Both sexes included	Regression	ΔweightSDS 0-3 and 3-12 mo	Weight/ Height/ BMI/ WC/SumSF SDS, WHR, S:T,%Fat, FM, FFM	Height SDS, sex, race, SES, activity, ***birthweight SDS and earlier ΔweightSDS***	- Both ΔweightSDS 0-3mo and 3-12 mo positively associated with HTSDS, WTSDS, BMISDS, WC SDS, FM, FFM, %fat - No association between ΔweightSDS and WHR or S:T ratio - No significant interaction between birthweight SDS and ΔweightSDS with regard to any outcomes -ΔweightSDS 0-3 mo contribute to larger r^2 for BMI, WC, FM, FFM than ΔweightSDS 3-12 mo/ r^2 for FM was larger than FFM

Table 5-6 (cont.) Summary of the studies investigating the association between early infant growth and later body composition in children, adolescents and young adults

Reference	Population	Age	n	BC technique	Gender	Statistics	Early growth variables	Outcomes	Covariates	Results
Li et al, 2003 (180)	Guatemalan adults	21-27y	267	Anthropometry (SF equation)	Both sexes included	Regression 2-stage least-squares analyses	Birthweight, length, PI, Length at 2 yr	Height, weight, FFM, FM, %fat, WHR	SES, sex, maternal height, physical activity, residency, smoking, ***birth length***	-Birthweight, length, length at 2yr were positively associated with height, weight, FFM in both sexes - Birth length and change in length 0-2yr equally determined height, weight, and FFM
Sachdev et al, 2005 (204)	Indian adults	26-32y	1526	Anthropometry (SF equation)	Both sexes included	Regression, Partial correlation, conditional SDS of BMI change	Birthweight, length, PI, BMI at 6mo, 1y, 2y, 5y, 8y, 11y, and 14y	Height, weight, BMI, WC, WHR, sum SF, lean residual, arm muscle area,	Age, sex, adult life styles, ***birth BMI***	- BMI at all age correlated with lean residual > sum SF, differences were more significant in males - BMI change (by conditional BMI SDS) 0-6 mo and 2-8 y showed a strong positive association with later lean residual - BMI change 0-6 mo showed a weak positive association with later sum SF, strength of association increased steeply from 2-8 y and sustained.

Sachdev et al. (204) studied another population from a developing country (New Delhi birth cohort) and reported a positive association between change in BMI SDS from birth to 6 mo and later lean residual (calculated from SF equation and adjusted for height in the regression model) in young adults. They also reported a weak positive association between growth in the same period and later sum of SF. This study, however, predicted fat and lean mass from anthropometry and used BMI SDS change as measure of growth rather than weight SDS change as in my study.

Euser et al (182) studied preterm-born Dutch young adults presenting FM and FFM calculated from a SF equation. Their findings support my results in that ΔweightSDS 0-3 months and 3-12 months were both associated with FM and FFM (adjusted by height SDS) independent of birthweight. Moreover, the percentages of variance explained by ΔweightSDS in both periods were larger for adult FM than FFM. They also found a positive association between ΔweightSDS in both periods and later WC SDS which agrees with my data. The small inconsistency is that ΔweightSDS 0-3 months in their study contributed to a larger r^2 for body composition outcomes than ΔweightSDS 3-12 months. It could be argued that, although the population were from a western country, they were born very preterm (GA<32 weeks) and these results might not be generalisable to term-born populations. Also the body composition measurements were derived from SF which predisposes to interobserver variation, and FFM was not directly measured.

A recent study from Sweden by Ekelund et al (210) showed that increase in weight SDS from birth to 6 months was positively associated with FM, FFM, and WC in late adolescence (17yr). The setting of this study is quite similar to my study in that it was conducted in a western population using a more precise body composition technique than SF (Air displacement plethysmography); their results support the findings in my study.

In short, there are some discrepancies between my results and the findings from 3 non-western countries in that they found a stronger or exclusive association of early infant

growth and later FFM whereas I found a positive correlation with both components (stronger for FM). However, another 2 studies from western countries seem to support my findings. This inconsistency might be due to the fact that infants in a developing country (where prenatal nutrition might be restricted) are born with a 'suboptimal' muscle mass compared to those from a developed country, and therefore have a higher potential to develop more FFM in early postnatal life. Another explanation could be differences in infant feeding practice in non-western and western countries, where the latter tend to have an 'excess' nutrition compared to the former. See more detail discussion in **Chapter 9**.

5.4.2 Analysis of early growth data

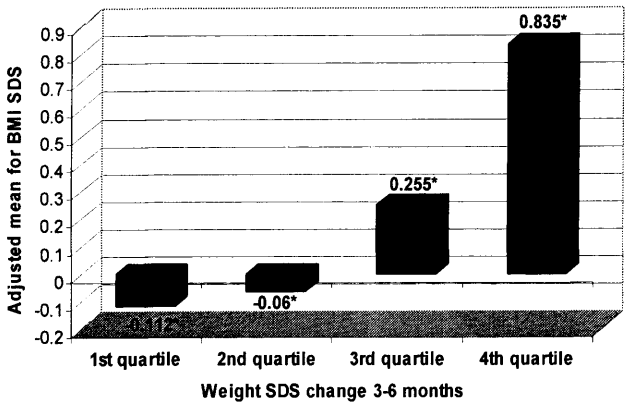
5.4.2.1 Different statistical approaches

In this study, I used multiple regression analysis of body composition outcomes on ΔweightSDS during different periods of infancy, adjusted for birthweight since the aim was to look at the effects of 'postnatal growth' beyond the effect of 'prenatal growth' represented by birthweight. In my view this approach has the advantage of being statistically powerful and using the continuous nature of the early growth variables. However, it is more difficult to illustrate the findings in an easily understandable form such as a plot or graph. Data can be more easily presented if early growth data are analysed as categories (e.g. catch-up, no change, catch-down or dividing ΔweightSDS into quartiles or quintiles). In this dataset, the main findings of the association between ΔweightSDS 3-6 months quartiles and later FMI SDS or FFMI SDS were similar to the regression methods. For example, the adjusted mean (for birth SDS and gender) of FMI SDS was significantly higher in the 4th quartile of Δ SDS 3-6 months compared to the 1st and 2nd quartile of ΔweightSDS (p=0.011 for trend). See **Figure 5-2**.

Figure 5-2 Adjusted mean for BMI SDS, FMI SDS, FFMI SDS, stratified according to Δ weight SDS 3-6 months quartiles[#]

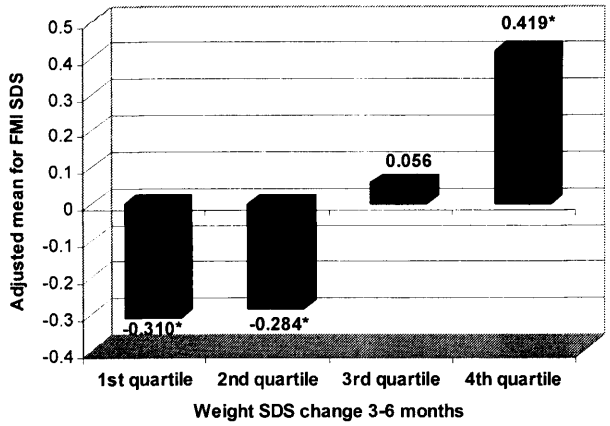
a) BMI SDS,

p = 0.007 for trend



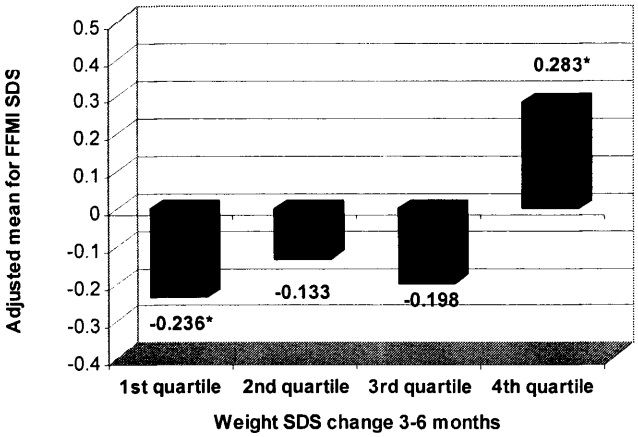
b) FMI SDS,

p = 0.011 for trend



C) FFMI SDS,

p = 0.143 for trend



[#] From general linear model, adjusted for birthweight SDS and gender; BC variables were dependent variable, gender and Δ weight SDS 3-6 months quartile were fixed factors, birthweight SDS was a covariate. Arterisk show a significant difference from the 4th quartile at p <0.05. n=37, 38, 38, 37 in 1st, 2nd, 3rd, 4th quartile, respectively.

Another approach is the life course plot proposed by Cole TJ (212) in which the regression coefficients of size (e.g. weight SDS) at different periods are plotted against age. In my dataset, weight was measured at 6 ages over a relatively short period of time (the first year) and weight SDS were highly correlated (see **Table 5-1a**); therefore, when weight SDS at combinations of the 6 different ages were put into the same regression model, the significance and regression coefficients changed dramatically (how they changed depended on which set of measurements were in the model). However, when ΔweightSDS were used instead of weight SDS (adjusting for each other and birthweight SDS at the same time), the result was consistent with my main analyses in that ΔweightSDS 3-6 mo was the most significant period for the association with later FMI or central fatness.

As mentioned previously, I also considered imputing the missing weight SDS data in order to increase the sample size and statistical power in the analysis. However, imputation relies on the assumption of linearity of weight SDS and data being missing in an unsystematic manner. The main findings of my analyses using imputed data were not substantially different from those using the original data (results are shown in **Appendix E, Table 10-9**). However, I decided to present non-imputed data since the main period of interest was the first 6 months when weight SDS can change dramatically; thus the assumption of linearity might not always be applicable.

5.4.2.2 Critical windows of early growth

The attempt to find 'critical windows' for body composition programming also leads to difficulty in the selection of growth periods for the analysis. If the period of ΔweightSDS chosen is too short, there might be more 'noise' from measurement error at both ends, and the influence of growth over a short period might be too trivial to be significant. On the other hand, birth to 1 year might be too long a period to detect any meaningful 'sensitive' period of early growth. Therefore, in my analysis I presented

ΔweightSDS for all available periods of growth to examine the different influence of each, as well as the sum of the smaller windows.

Another interesting issue is why growth in the second 6 months of life was not associated with any of the later outcomes. The generally slower relative growth rate during this period could make it less likely to be (statistically) significant or it may not be the 'right' window for body composition programming. In my opinion, both explanations could apply but the latter idea seems to make sense biologically as well, since it seems logical that the 'critical' period for programming later outcomes should be the period of greatest change (i.e. early infancy).

However, most of the studies published so far (including mine) use different time points in infancy and childhood depending on which data were available rather than selected for a biological reason. Therefore, it is even more difficult to draw a firm conclusion regarding any critical window(s) of early growth for different body composition outcomes.

5.4.3 Criticisms of the study

5.4.3.1 Characteristics of the dataset

This dataset was a subset of the population analyzed in **Chapter 4**; therefore, their physical characteristics were similar—girls generally had higher SDS for body size and SF than the British 1990 reference.

In general, the infant growth pattern of my subjects was representative of the reference population, except for age 3 weeks when their weight SDS was significantly less than zero. This might be explained by: 1) the smoothing of reference growth data by the LMS method and 2) the fact that reference growth data were collected cross-sectionally;

growth charts therefore cannot reflect the physiology of slower weight gain in this period (i.e. after the normal physiologic weight loss in the first 1-2 weeks); rather than indicating that the early growth pattern of my subjects was unusual.

5.4.3.2 Reliability of early growth data

The majority of infant weight data in my dataset (139 out of 191, 73%) came from parent-held baby record books, and weight was generally not recorded at the precise times specified by the protocol for the prospective study. Although I have tried to overcome this problem by using SDS (which were calculated using the exact age at measurement) and selecting the measurement that was nearest to the age in the prospective cohort, the shorter periods of ΔweightSDS (3, 6, 12 weeks) inevitably suffered more noise. This could lead to some inaccuracies in the data analysis; however, when these short windows were added together, the main findings did not change.

5.4.3.3 Potential confounders

The effects of potential confounders on body composition outcomes were already discussed in the previous chapter. In fact, the main confounder that could significantly influence infant growth is the nutritional intake or at least infant feeding mode. Unfortunately, feeding mode data were only available in the prospective study (only 27%); therefore, I could not adjust for the feeding mode in this analysis. Further analyses of the influence of early nutrition on body composition are shown in **Chapter 8**.

5.4.3.4 Wide age range and inclusion of children at different pubertal stages

I have tried to rectify the criticism that my body composition outcomes were measured over a wide range of age and pubertal maturation (previously mentioned in **Chapter 4**) by using age and sex specific SDS for the body composition outcomes. Moreover, the results did not change after adjusting for pubertal maturation in the model. Therefore, I believe that the results from this analysis are at least not significantly clouded by the wide age range of outcomes measured. However, I have to accept that there is a possibility that some associations, which might only be seen at some stages of maturation i.e. prepubertal or late pubertal, might be missed in this analysis. The ideal solution would be to conduct a study with a big enough sample size to perform subgroup analysis according to stage of maturation, which might not be possible with more detailed body composition techniques such as those used in my study. See **Chapter 9**.

5.5 Summary

- In western populations, growth in the first 6 months of life is associated with both FM and FFM in adolescence and adult life (with a stronger effect on FM).

- My study provides supporting evidence for the association between early growth and central fat distribution using a more precise body composition technique (regional FM by DXA).

- The clinical importance of these findings needs further investigation in terms of the relative importance of FM and FFM for health outcomes such as cardiovascular risk factors. Furthermore, although both FM and FFM were associated with early growth, the mechanism by which they are programmed could be dissimilar and warrants further investigation.

Chapter 6

The Relationship between Body Composition and Cardiovascular Risk

Chapter 6 The Relationship between Body Composition and Cardiovascular Risk

6.1 Introduction

In this chapter, I have analyzed the relationship between different body composition variables and several cardiovascular risk markers. The aims were 1) to test hypothesis I-2 that different components of body composition (which may themselves be programmed by early growth) have differential effects on cardiovascular risk factors; and 2) to compare associations of different 'indices' of central fat distribution from different body composition techniques with cardiovascular risk factors.

6.2 Methods

6.2.1 Subjects

Blood results were available in the prospective cohorts (cohort A, B, and C) only. Of the 54 subjects who agreed to come to the study centre; 48 subjects had a blood test after an overnight fast. All completed the 4C model and had abdominal FM calculated from the DXA scan by manual region of interest analysis (details in **Chapter 3**).

6.2.2 Body composition data

FM and FFM were derived from the 4C model. The following regional DXA soft tissue measurements were used: trunk FM, trunk LM, and limb LM (both arms combined with both legs). These variables were adjusted for body size by height squared giving FMI, FFMI, trunk FMI, trunk LMI, and limb LMI. SDS was calculated (from 308 subjects who form the database in **Chapter 4**) for each variable using the LMS methods as

described previously in **Chapter 5** (details in **Appendix C**). Abdominal FM was assessed by the DXA manual region of interest (142)(see **Chapter 3**, **Figure 3-3** Examples of DXA scans)

WC, triceps SF, subscapular SF, trunk FM, and abdominal FM, which are generally used as proxies for central fat distribution, were compared. All were adjusted for height to take account of differences in body size.

6.2.3 Assessment of cardiovascular risk factors

Well-known risk factors for cardiovascular disease were measured. Details are described in **Chapter 3**. Briefly, triglycerides, total cholesterol, HDL cholesterol (HDL-C), glucose, insulin, proinsulin, and split-proinsulin were measured in fasting blood samples. LDL cholesterol (LDL-C) was calculated using Friedewald's formula (172). HOMA (Homeostasis model assessment) was calculated by the HOMA Calculator ©The University of Oxford 2004 (173;174). Blood pressure was measured in triplicate while the subject was resting on a couch.

6.2.4 Statistical methods

Partial correlation (adjusting for age, sex and/or height) was used in the analyses. In addition, in order to test if the association between several indices for central fatness and cardiovascular risks were beyond their relationship with total fatness, total FM was also controlled for in some analyses.

All variables that were not normally distributed (Insulin, Proinsulin, 32-33 Split proinsulin, Insulin: glucose, HOMA, WC, Triceps, Subscapular, Trunk FM, Abdominal FM) were natural log transformed before analysis.

6.3 Results

6.3.1 Characteristics of subjects and cardiovascular risk factors

Characteristics of study subjects are shown in **Table 6-1**. Twelve (25%) subjects were overweight and one (2.1%) subject was obese according to the IOTF cut offs (119). Boys were comparable with the British 1990 reference whereas girls in this dataset had higher BMI, WC, triceps, and subscapular SF than the reference children. As expected, girls had more absolute trunk FM, abdominal FM, and SF than boys. However, this gender difference was no longer apparent when SDS were used, except for subscapular SF and WC.

In general, girls demonstrated higher triglycerides, insulin, 32-33 split proinsulin and HOMA IR than boys.

Table 6-1 Characteristics of study subjects and cardiovascular risk factors

	All (n=48)	Boys (n=18)	Girls(n=30)	p
Age (y)	14.5 ± 2.7	14.0 ± 2.4	14.7 ± 2.8	0.40
BMI SDS	0.40 ± 1.17	0.02 ± 1.30	0.63 ± 1.04	0.08
FMI SDS	0.20 ± 0.89	0.11 ± 0.90	0.25 ± 0.90	0.60
FFMI SDS	-0.02 ± 1.09	-0.33 ± 1.09	0.16 ± 1.07	0.14
Trunk FMI SDS	0.18 ± 0.94	0.08 ± 1.00	0.23 ± 0.91	0.60
Limbs LMI SDS	-0.01± 1.17	-0.38 ± 1.15	0.22 ± 1.15	0.09
Trunk LMI SDS	-0.05 ± 1.05	-0.32 ± 1.15	0.11 ± 0.97	0.18
Trunk FM (kg)	7.2 ± 4.2	4.8 ± 3.0	8.6 ± 4.2	***0.002***
Abdominal fat by DXA(kg)[$]	1.5 ±1.1	1.0 ± 0.7	1.9 ± 1.2	***0.01***
WC (cm)	71.3 ± 8.6	69.9 ± 9.3	72.2 ± 8.2	0.36
Triceps SF (mm)	15.1 ± 5.5	11.6 ± 4.8	17.3 ± 4.8	***<0.001***
Subscapular SF (mm)	13.3 ± 7.9	9.5 ± 5.8	15.5 ± 8.1	***0.008***
WC SDS[#]	0.89 ± 1.11	0.31 ± 1.09	1.27 ± 0.97	***0.008***
Triceps SDS	0.60 ± 0.88	0.26 ± 1.13	0.81 ± 0.64	0.07
Subscapular SDS	0.42 ± 1.09	-0.11 ± 1.37	0.74 ± 0.73	***0.02***
Glucose (mmol/L)	5.1 ± 0.3	5.2 ± 0.3	5.0 ± 0.3	0.05
Total cholesterol (mmol/L)	4.7 ± 0.7	4.6 ± 0.7	4.7 ± 0.7	0.40
Triglycerides (mmol/L)	0.8 ± 0.3	0.7 ± 0.2	0.9 ± 0.4	***0.02***
HDL cholesterol (mmol/L)	1.5 ± 0.3	1.5 ± 0.3	1.4 ± 0.2	0.23
LDL cholesterol (mmol/L)[§]	2.8 ± 0.6	2.7 ± 0.5	2.9 ± 0.6	0.29
LDL: HDL ratio	2.0 ± 0.6	1.9 ± 0.6	2.1 ± 0.6	0.18
Insulin (pmol/L)	55.2 ± 31.1	41.7 ± 21.0	63.3 ± 33.6	***0.02***
Proinsulin (pmol/L)	3.8 ± 2.1	3.1 ± 1.2	4.1 ± 2.5	0.12
32-33 Split proinsulin (pmol/L)	5.8 ± 3.9	4.3 ± 3.3	6.7 ± 3.9	***0.03***
Insulin: glucose ratio	10.9 ± 6.2	7.9 ± 3.7	12.6 ± 6.8	***0.01***
HOMA IR[&]	1.0 ± 0.6	0.8 ± 0.4	1.2 ± 0.6	***0.02***
SBP (mmHg)[@]	109.6 ± 6.4	109.6 ± 6.7	109.5 ± 6.4	0.98
DBP (mmHg)[@]	60.8 ± 6.5	60.1 ± 6.8	61.2 ± 6.4	0.57
MAP (mmHg)[@]	79.9 ± 5.7	78.7 ± 6.7	80.5 ± 5.1	0.29

[$] Measured by DXA manual region of interest (upper border of L1 - lower border of L4)

[#] n=38 since the database for waist SDS was available only up to the age of 17

[§] calculated by Friedewald formula (172): LDL = total cholesterol-HDL-0.456 x Triglycerides (mmol/L)

[&] HOMA = Homeostasis Model Assessment, calculated by the HOMA Calculator ©The University of Oxford 2004 from fasting glucose and insulin, IR = insulin resistance

[@] Blood pressure was available in 51 subjects, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure

6.3.2 Differences in the relationship between FM vs. FFM and cardiovascular risk factors

Partial correlations (adjusting for age and gender) between SDS for BMI, FMI, Trunk FMI, FFMI, Limb LMI, Trunk LMI and cardiovascular risk factors are shown in **Table 6-2**. FMI was more strongly and positively related to total cholesterol, triglycerides, and LDL-C (correlation coefficients—r were 0.33, 0.41, and 0.37; $p < 0.05$) than BMI. BMI showed a stronger correlation with LDL:HDL ratio than other BC indices. Higher FMI also correlated well with unfavourable insulin profiles and higher blood pressure ($r = 0.37$-0.57); however, the correlations in general were not stronger than those for BMI ($r = 0.45$-0.57). FMI and Trunk FMI showed similar relationships with lipid and insulin profiles.

Interestingly, higher FFMI was associated with lower HDL-C ($r = -0.54$, $p<0.001$—scatter plots are shown in **Figure 6-1**) and the correlation was stronger than that for BMI; FFMI explained 29 % of variability in HDL-C whereas BMI only explained 19%. Although higher FFMI was also associated with various indices indicating a tendency towards insulin resistance and higher blood pressure, the correlations were lower than those for FMI or Trunk FMI. Regional LMI showed the same trends for association with cardiovascular risk factors as FFMI; generally, limb LM showed a stronger relationship with these markers than trunk LM, except for HDL-C, where trunk LM showed a greater correlation.

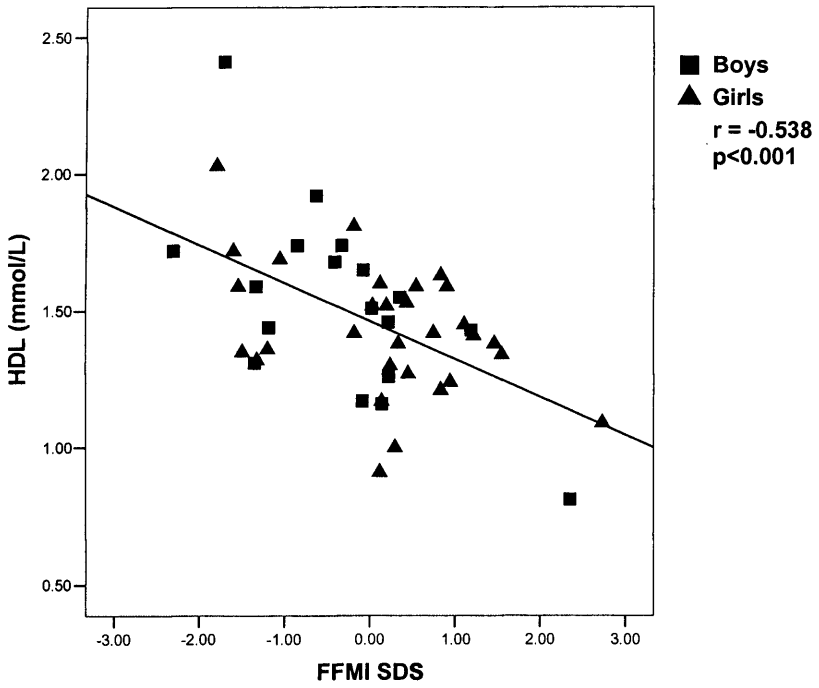
Table 6-2 Partial correlation (r) between BC and cardiovascular risk factors [++]

	BMI	FMI	Trunk FMI	FFMI	Limbs LMI	Trunk LMI
Glucose	0.23	0.10	0.15	0.15	0.21	0.07
Total cholesterol	0.09	***0.33*** [a]	0.26	-0.14	-0.10	-0.27
Triglycerides	0.29	***0.41*** [b]	***0.40*** [b]	0.09	0.07	0.10
HDL cholesterol	***-0.44*** [b]	-0.19	-0.25	***-0.54*** [c]	***-0.46*** [b]	***-0.58*** [c]
LDL cholesterol	0.25	***0.37*** [a]	***0.33*** [a]	0.08	0.09	-0.06
LDL: HDL ratio	***0.54*** [c]	***0.41*** [b]	***0.41*** [b]	***0.44*** [b]	***0.41*** [b]	***0.36*** [a]
Insulin	***0.56*** [c]	***0.57*** [c]	***0.61*** [c]	***0.33*** [a]	***0.32*** [a]	0.24
Proinsulin	***0.51*** [c]	***0.48*** [b]	***0.51*** [c]	***0.38*** [b]	***0.38*** [b]	0.27
32-33 Split proinsulin	***0.45*** [b]	***0.37*** [b]	***0.43*** [b]	***0.35*** [b]	***0.32*** [a]	***0.30*** [a]
Insulin: glucose	***0.53*** [c]	***0.56*** [c]	***0.59*** [c]	***0.31*** [a]	0.29	0.23
HOMA IR	***0.57*** [c]	***0.57*** [c]	***0.61*** [c]	***0.33*** [a]	***0.32*** [a]	0.24
Systolic BP	***0.54*** [c]	***0.50*** [c]	***0.54*** [c]	***0.36*** [a]	***0.34*** [a]	0.24
Diastolic BP	***0.56*** [c]	***0.56*** [c]	***0.54*** [c]	***0.31*** [a]	***0.38*** [b]	0.21
MAP	***0.56*** [c]	***0.49*** [c]	***0.51*** [c]	***0.39*** [b]	***0.43*** [b]	***0.29*** [a]

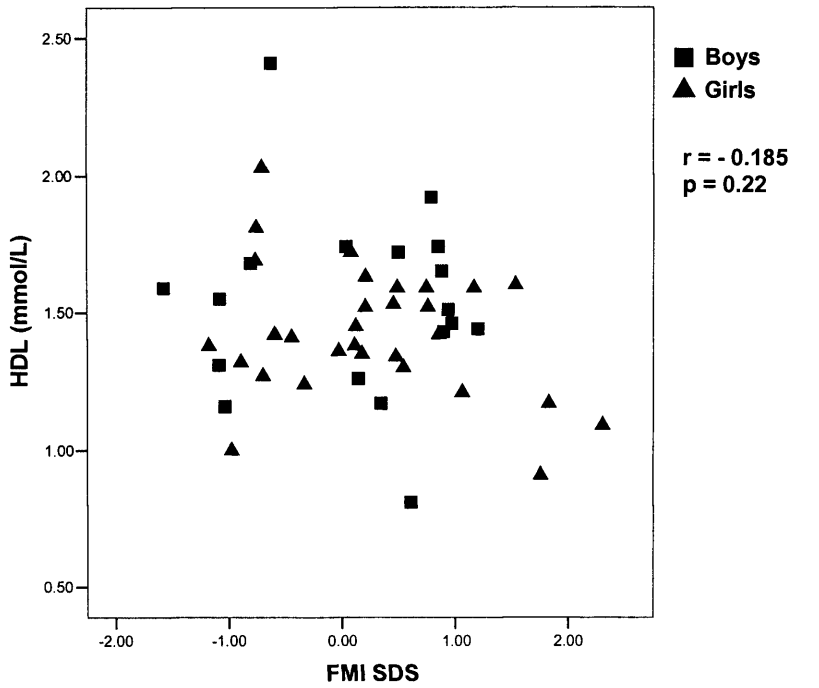
[++] Controlled for age and gender, all BC variables were analyzed in SDS form; [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$

Figure 6-1 Scatter plots of body composition and later HDL cholesterol

a) FFMI SDS



b) FMI SDS



Since FMI and FFMI were strongly correlated with each other, further analysis was performed by adjusting for FFMI in the partial correlation analysis of FMI and vice versa. The results are shown in **Table 6-3**. The relationships between FMI or trunk FMI and various cardiovascular risk factors were hardly affected while those of FFMI or regional LMI was substantially attenuated and became non-significant except for the association with HDL-C (which was a strong negative association, r range from-0.43 to -0.57) and LDL:HDL ratio (which was a weak positive association, r range from 0.30-0.35). This confirms that FM and FFM have differential effects on lipid and insulin profiles. The association between higher FFMI and an unfavourable insulin profile in **Table 6-2** is mostly due to its association with FMI. However, the negative association between FFMI and HDL-C was independent of FMI.

Table 6-3 Comparison of partial correlation (r) between FMI vs. FFMI and cardiovascular risk factors adjusted for each other[++]

	FMI [1]	Trunk FMI[1]	FFMI [2]	Limbs LMI[2]	Trunk LMI[2]
Glucose	0.05	0.10	0.13	0.18	0.06
Total cholesterol	***0.41***[b]	***0.35***[a]	-0.29	-0.26	***-0.32***[a]
Triglycerides	***0.40***[b]	***0.40***[b]	-0.07	-0.10	0.06
HDL cholesterol	0.01	-0.03	***-0.51***[c]	***-0.43***[b]	***-0.57***[c]
LDL cholesterol	***0.37***[a]	***0.33***[a]	-0.06	-0.06	-0.10
LDL: HDL ratio	***0.30***[a]	0.28	***0.35***[a]	***0.30***[a]	***0.35***[a]
Insulin	***0.51***[c]	***0.55***[c]	0.16	0.14	0.22
Proinsulin	***0.40***[b]	***0.42***[b]	0.26	0.25	0.26
32-33 Split proinsulin	0.27	***0.33***[a]	0.26	0.21	0.28
Insulin: glucose	***0.51***[c]	***0.53***[c]	0.14	0.10	0.21
HOMA IR	***0.51***[c]	***0.55***[c]	0.17	0.14	0.22
Systolic BP	***0.43***[b]	***0.47***[b]	0.23	0.19	0.22
Diastolic BP	***0.51***[c]	***0.47***[b]	0.14	0.22	0.18
MAP	***0.41***[b]	***0.42***[b]	0.27	***0.30***[a]	0.28

[++] Controlled for age, gender, and either [1]FFMI SDS or [2]FMI SDS, all BC variables were analyzed in SDS form

Further adjustment for WC reduced the association between FMI and these outcomes substantially; FMI and trunk FMI remained only weakly correlated with insulin and HOMA IR ($r = 0.31$, $p < 0.05$) (**Table 6-4**). On the other hand, FFMI and trunk LMI still showed a strong negative association with HDL-C, suggesting that the association was independent of WC.

Table 6-4 Comparison of partial correlation (r) between FMI vs. FFMI and cardiovascular risk factors adjusted for waist circumference[++]

	FMI	Trunk FMI	FFMI	Trunk LMI
Glucose	0.12	0.19	0.12	0.03
Total cholesterol	0.28	0.19	-0.30	***-0.37***[a]
Triglycerides	0.15	0.12	-0.20	-0.08
HDL cholesterol	0.26	0.15	***-0.42***[b]	***-0.50***[b]
LDL cholesterol	0.18	0.13	-0.10	-0.19
LDL: HDL ratio	0.02	0.02	0.26	0.22
Insulin	***0.31***[a]	***0.32***[a]	-0.09	-0.05
Proinsulin	0.02	0.02	-0.01	-0.01
32-33 Split proinsulin	0.03	0.10	0.06	0.10
Insulin: glucose	0.28	0.28	-0.11	-0.06
HOMA IR	***0.31***[a]	***0.33***[a]	-0.08	-0.05
Systolic BP	0.20	0.27	0.08	0.03
Diastolic BP	0.28	0.21	0.002	-0.01
MAP	0.19	0.21	0.13	0.10

[++] Controlled for age, gender, waist circumference and height, all BC variables were analyzed in SDS form

6.3.3 Relationship between different indices of central fat distribution and cardiovascular risk factors

To compare the various proxies of central fat distribution, partial correlations adjusted for age, gender, and height are shown in **Table 6-5**. Among the 'central fat distribution' indices used in my analysis, WC seemed to perform as well as trunk FM and abdominal FM (by DXA ROI) in relation to the association with cardiovascular risk factors. Interestingly, WC was negatively correlated with HDL-C, whereas trunk FM and abdominal FM were not significantly correlated; therefore, it also showed a stronger positive association with LDL:HDL ratio. Higher subscapular SF was more strongly related to an unfavourable insulin profile than higher triceps SF.

Table 6-5 Partial correlation (r) between proxies for central fat distribution and cardiovascular risk factors[+++]

	WC	Triceps	Subscapular	Trunk FM	Abdominal FM
Glucose	0.04	0.13	0.23	0.17	0.18
Total cholesterol	0.27	0.28	0.20	0.26	0.27
Triglycerides	***0.38***[b]	***0.33***[a]	***0.39***[b]	***0.33***[a]	***0.31***[a]
HDL cholesterol	***-0.33***[a]	-0.01	-0.22	-0.19	-0.18
LDL cholesterol	***0.37***[a]	0.24	0.24	***0.31***[a]	***0.32***[a]
LDL: HDL ratio	***0.47***[b]	0.19	***0.31***[a]	***0.34***[a]	***0.34***[a]
Insulin	***0.56***[c]	***0.53***[c]	***0.59***[c]	***0.59***[c]	***0.58***[c]
Proinsulin	***0.58***[c]	0.29	***0.46***[b]	***0.45***[b]	***0.42***[b]
32-33 Split proinsulin	***0.43***[b]	0.26	***0.36***[b]	***0.39***[b]	***0.35***[a]
Insulin: glucose	***0.55***[c]	***0.51***[c]	***0.56***[c]	***0.57***[c]	***0.55***[c]
HOMA IR	***0.56***[c]	***0.53***[c]	***0.60***[c]	***0.60***[c]	***0.58***[c]
Systolic BP	***0.47***[b]	***0.50***[c]	***0.53***[c]	***0.51***[c]	***0.51***[c]
Diastolic BP	***0.50***[c]	***0.49***[c]	***0.59***[c]	***0.53***[c]	***0.53***[c]
MAP	***0.46***[b]	***0.42***[b]	***0.54***[c]	***0.48***[b]	***0.50***[c]

[+++] Controlled for age, gender, and height

After adjusting for total fatness (by 4C FM, and in the case of trunk and abdominal FM by non-trunk or non-abdominal FM accordingly) (**Table 6-6**), the proxies that remained significantly correlated with lipid and insulin profile were WC (negatively associated with HDL-C and positively associated with LDL:HDL ratio and proinsulin), triceps SF (negatively associated with LDL:HDL ratio and proinsulin), and trunk FM (positively associated with SBP).

Table 6-6 Partial correlation (r) between proxies for central fat distribution and cardiovascular risk factors adjusted for total fatness[+++]

	WC[3]	Triceps[3]	Subscapular[3]	Trunk FM[4]	Abdominal FM[5]
Glucose	-0.13	0.03	0.24	0.17	0.19
Total cholesterol	0.03	0.01	-0.13	-0.03	-0.02
Triglycerides	0.19	0.06	0.19	0.12	-0.02
HDL cholesterol	***-0.41***[b]	0.29	-0.20	-0.26	-0.15
LDL cholesterol	0.19	-0.15	-0.10	0.07	0.06
LDL: HDL ratio	***0.39***[a]	***-0.32***[a]	0.05	0.20	0.13
Insulin	0.15	-0.02	0.21	0.27	0.11
Proinsulin	***0.42***[b]	***-0.34***[a]	0.13	0.21	-0.01
32-33 Split proinsulin	0.27	-0.17	0.11	0.26	0.05
Insulin: glucose	0.18	-0.02	0.16	0.24	0.07
HOMA2 IR	0.14	-0.01	0.22	0.28	0.13
Systolic BP	0.14	0.17	0.25	***0.34***[a]	0.21
Diastolic BP	0.06	-0.07	0.25	0.13	0.03
MAP	0.13	-0.04	0.29	0.23	0.19

None of the relationships between body composition and cardiovascular risk factors shown in this chapter were considerably changed after adjusting for physical activity

[+++] Controlled for age, gender, height, and total fatness (by adjusting for [3]4C FM and [4]non-trunk FM or [5]non-abdominal FM, accordingly)

assessed by parents or self- rating from 1 (much less active than peers) to 5 (much more active than peers).

6.4 Discussion

6.4.1 Body composition and cardiovascular risk factors

Despite a relatively small sample size, my dataset was reasonably representative of contemporary UK children with a similar proportion of overweight children to that reported by Lobstein et al (4). Gender difference in the indices of insulin resistance in my data corresponds with recent studies of type 2 diabetes in children that show a predominance of affected girls than boys, and also the finding that healthy girls might be intrinsically more insulin resistant than boys (213).

Although my data supports the increasing evidence from paediatric populations that BMI is significantly related to cardiovascular risk factors (see **Chapter 2**), it also showed that separate measurements of FMI and FFMI might provide further information for health outcomes. Compared to BMI, FMI had a stronger association with total cholesterol, triglycerides, and LDL-C whereas FFMI had a stronger association with HDL-C. The latter correlation was independent of FMI and waist circumference. On the other hand, FMI and FFMI showed the same correlation with LDL:HDL ratio which might be a better predictor of cardiovascular risk than absolute LDL-C or HDL-C. However, in this dataset, the relationship between FMI and LDL:HDL ratio arises from its positive correlation with LDL-C whereas the same association of FFMI was mediated by its negative correlation with HDL-C.

The findings presented here are quite interesting, in that most published literature regarding body composition and cardiovascular risk factors only reports the relationship between fat or central fat and metabolic outcomes rather than the effect of FFM. However, there are a few studies comparing the effects of the FM and FFM components of BMI on cardiovascular risk factors. Schubert et al studied middle-age men and

women from the Fels Longitudinal Study (214) and also reported this negative association between FFMI and HDL-C (independent of FMI) in 126 men. Pietrobelli et al (215) also reported that there is an inverse correlation between HDL-C and the nonadipose component of the body (i.e. adipose tissue-free mass and skeletal mass by whole body MRI, and body cell mass by ^{40}K counting) but not between HDL-C and any adipose tissue component, in an adult population (using height^2-normalised indices). Indeed, other studies (that did not measure body composition directly) also found that HDL-C was lower in power athletes (e.g. weight lifters) compared to normal or endurance-trained persons (216;217). This leads to the idea that skeletal muscle might be important in HDL-C regulation as supported by evidence from animal studies showing that HDL-C concentrations may be altered by increased HDL-C catabolism through skeletal muscle lipoprotein lipase (218). Pietrobelli et al (215) also proposed in their study that aerobic physical activity is associated with increase in HDL-C whereas skeletal muscle hypertrophy per se might not be beneficial for health.

Even fewer studies have actually reported the relationship of FFM and cardiovascular risk factors in children or in the context of body composition programming. There is a recent report from 234 prepubertal children from the Early Bird study showing that FFM (assessed by BIA) had a negative association with triglycerides and total cholesterol/HDL-C ratio after controlling for FM in boys (219). The authors suggested that this finding supports the argument proposed by Singhal et al (80) that higher birthweight predicts lower metabolic risk because it programs more lean mass. I did not find a negative association between FFM and triglycerides (which would be regarded as a 'desirable' effect), but found that higher FFMI was strongly associated with lower HDL-C and hence higher LDL:HDL ratio, which would be regarded as an 'undesirable' effect. A similar effect has been found in a large cohort (n=2000) of Brazilian young adult males (age 19-20) and in a smaller group (n=250) of 8-12 year old preterm-born children (Fewtrell MS, unpublished data). Although, it is still not clear what the underlying mechanism for such an effect might be, further investigation is clearly warranted, especially given the apparent positive relationship between birthweight and later FFM in males. Moreover, Toikka et al showed that constantly low HDL-C alone

(for 2 years) without other cardiovascular risk factors was associated with endothelial dysfunction and increased in vivo LDL-oxidation in healthy young men (220) suggesting that HDL-C may act as an antioxidant and inhibit LDL oxidation (which causes arterial dysfunction). Therefore, lower HDL-C in children might be of clinical importance.

This is a very good example of the importance of considering the FM and FFM components of BMI separately, and preferably by normalising for height since the effect of these two components cannot be distinguished when using only percent body fat as a representative of body composition. In fact the findings presented here can explain the weak negative correlation between BMI and HDL-C found in some large-scale studies (221), and suggest that it was perhaps explained by the FFM component rather than FM.

My study confirmed in a non-obese adolescent population the consistent findings from other studies showing a strong positive association between total FM or visceral fat and triglycerides and LDL in obese populations (222-224) and non-obese adults (129;214;215;225). Moreover, the association between FM and insulin resistance indices is consistent with a number of publications (138;226;227). There are several possible mechanisms that link adipose tissue with the metabolic syndrome. Recent investigations have shown that although adipose tissue was once considered metabolically inert, it is in fact metabolically active. For example, adipose tissue produces leptin (which is a critical hormone in energy balance), a family of cellular mediators (adipocytokines) e.g. TNF-α, IL-6 (228) (which play a role in inflammatory process), resistin and adiponectin (which is related to insulin resistance). There is evidence that the increase in production of these adipocyte factors leads to the metabolic and cardiovascular complication of obesity (9;136;228;229).

My results also correspond with other studies in that higher BMI during the adolescent period was associated with higher blood pressure (96;131;132;230), but it also suggests that the FM component of BMI is responsible for this association. Murphy et al studied a bigger number of prepubertal children (133 boys, and 101 girls) and also showed a

positive correlation between systolic blood pressure and FM (219). One potential explanation could be an increase in metabolically active factors produced from adipocytes e.g. angiotensinogen, the substrate for renin in the renin-angiotensin system which plays a central role in blood pressure regulation (231).

In short, my analyses agree with the general assumption that FM contributes to most of the adverse cardiovascular risk factors; however, I also demonstrate that FFM also shows a significant 'undesirable' effect on HDL-C. Therefore, the 'ideal' body composition to minimize cardiovascular risk factors could be both low FM and proportionate FFM. I propose that one might need to bear this in mind when selecting the interventions for a 'healthier' lifestyle in children since anaerobic exercise that will put on disproportionate muscle mass might not always be beneficial for health.

6.4.2 Central fat distribution indices

The importance of visceral adipose tissue and central fat distribution for cardiovascular risk factors was reviewed in **Chapter 2**. All the central fatness indices are associated with cardiovascular risk factors but with differing magnitudes. For central fat distribution indices presented here, I showed that subscapular SF (which is a more stable trunk depot) is a better predictor of insulin profile than triceps SF. WC seems to be the best predictor of metabolic profiles in this analysis (or at least not worse than trunk or abdominal FM). This finding is supported by other studies in children, showing that WC correlated well with visceral adipose tissue and performed better than BMI in predicting abdominal fat (by CT) and metabolic profiles (53;232;233). A study in middle-aged women comparing anthropometric measurements of central fat distribution and DXA abdominal fat (by region of interest L1-L4) also found that the latter did not show a stronger association with metabolic variables (234). Despite being assessed using a more sophisticated technique, either trunk FM or abdominal FM assessed by DXA are not ideal for studying central fat distribution (see explanation in **Chapter 2** and **Chapter 3**). Moreover, this technique has limitation for the measurement of soft tissue composition

(particularly at the extremes of body mass) as well as lack of ability to distinguish subcutaneous from intra-abdominal fat. In my opinion, WC is a good screening tool for central fat distribution and risk; however, for a medium scale study (in a non-obese population) where MRI is not suitable, further research in a larger sample of children and adolescent is still needed to see whether DXA regional FM is valuable in estimating central fat distribution. In the context of body composition programming, my results in **Chapter 4** and **Chapter 5** show consistent results either when using WC or trunk FM as a proxy for central fat distribution.

However, it is worth noting that there was a discrepancy between the magnitude of the association of WC vs. trunk FM and HDL-C. WC showed a strong negative association with HDL-C whereas trunk FM showed a non-significant relationship with HDL-C (both before and after adjusting for total or non-trunk FM). I suggest that this discrepancy might be explained by the strong negative association between trunk LM and HDL-C i.e. the negative association between WC and HDL-C could be due to its lean component rather than the fat component. This example suggests that in order to fully understand the relationship between BC and health outcomes, specific measurement of BC is needed even though WC seems to be a good predictor of the well-known risks at the population level.

To address the issue of whether total fatness or central fat has more influence on health outcomes, I have also further analysed these associations by adjusting for WC in the relationship between total FM and cardiovascular risk factors (**Table 6-4**) and adjusting for total fatness in the analysis of proxies of central fatness and cardiovascular risk factors (**Table 6-6**). The positive association between FMI and lipid profile or blood pressure was significantly attenuated after adjusting for WC; however, among children with the same WC, higher FMI still predicted higher insulin and HOMA IR. In a similar manner, for children with the same amount of FM, a larger WC was still associated with lower HDL-C and higher proinsulin. Moreover, despite general agreement that visceral fat is more metabolically active than subcutaneous fat, there is also some evidence that elevated leptin in childhood obesity might be more closely link to subcutaneous than

visceral FM (235). For these reasons, I believe that good measurements of total fatness as well as central fat distribution are just as important.

6.4.3 Criticisms of the study

The sample size in this dataset is rather small, therefore I cannot perform the analyses separately in boys and girls (who have been shown to have differences in insulin resistance markers and body composition). Moreover, I do not have the gold standard for central fat distribution (MRI or CT) data to compare with other simpler measurements of central fat distribution. The findings should therefore be interpreted with caution and a bigger sample size is needed to confirm the findings.

It is important to consider, for practical purposes, whether more detailed BC measurements have an advantage over 'simple' measurements in terms of predicting cardiovascular risk. My finding that different components may be differentially related to cardiovascular risk suggests that this may be the case. However, since it is more difficult, time-consuming and expensive to obtain more detailed measurements, the decision may depend on the situation. From the findings discussed here, I think that for screening purposes in a normal population, BMI and WC are valid tools for identifying children who might be at a higher risk for developing cardiovascular disease in later life. On the other hand, more detailed measurements of BC may be useful for 1) research purposes e.g. investigating the long-term health outcomes of BC programming or investigating health outcomes in populations (e.g. athletes) whose FM and FFM proportions might be different from normal; and 2) for clinical purposes. Examples might include: an obesity treatment programme in which the effects of different treatments on health outcomes could depend on whether they mainly affect FM, FFM, or central fat; or patient groups in whom nutritional interventions designed to promote weight gain may change body composition dramatically resulting in a disproportionate gain in FM compared to FFM which could have differential effects on their long-term health.

6.5 Summary

- FM showed a consistent association with most of the cardiovascular risk factors except HDL-C while FFM showed a consistent negative association with HDL-C that was independent of FM and central fat distribution.

- WC as well as trunk and abdominal FM assessed by DXA are good predictors for cardiovascular risk factors.

- Separate measurements of FM, FFM, and central fat distribution can offer more insight into the impact of body composition programming on health outcomes.

Chapter 7

The Influence of Infant Body Composition on Later Outcomes

Chapter 7 The Influence of Infant Body Composition on Later Outcomes

7.1 Introduction

In the previous chapters, I have shown that birthweight and early growth have a significant influence on body composition in childhood and adolescence. In this chapter, I test hypothesis II, that components of *infant* BC are differentially related to later BC, and possibly mediate the relationship between prenatal and postnatal 'growth' and body composition in later childhood or early adult life.

7.2 Methods

7.2.1 Subjects

Only subjects from cohorts A, B, and C (description in **Chapter 3**) had infant BC data available for analysis in this chapter. 54 subjects agreed to come to the study centre and completed the four-component model and cardiovascular risk factor assessment. 5 children had a home visit, with anthropometry and deuterium dilution to assess body composition (see **Chapter 3**).

7.2.2 Infant body composition measurements

The subjects from cohort A had detailed anthropometric measurements at age 2-3 days, 3, 6, 12 weeks, 6 and 12 months as a part of the randomised controlled trial of different energy infant formulas. Doubly-labelled water measurements were performed at 6 weeks and 12 weeks in cohort C and at 12 weeks only in the other cohorts. Infant FM and FFM were adjusted by length squared (giving FMI, FFMI) to adjust for differences in body size. FMI and FFMI SDS were derived using data from the total group of 48 infants from the original cohorts who had BC measured at 6 weeks and 150 infants at 12 weeks.

7.2.3 Body composition outcomes

For the 5 home visit subjects who were measured using deuterium dilution, the two-component model of body composition was calculated from TBW assuming a FFM hydration fraction of 0.751 for boys and 0.746 for girls (obtained from the ongoing body composition reference study, unpublished data). For these 5 subjects, FMI SDS and FFMI SDS were calculated manually by the LMS method (for age and sex); the equations is shown in **Appendix C**. Body composition data from 54 subjects who completed the 4C model were presented as described in **Chapter 5** and **6**.

7.2.4 Statistical methods

Anthropometric data which were not normally distributed were $\log_e$ transformed to reduce skewness in distribution. SF measurements in infancy were adjusted for gender and for the exact age at measurement.

Multiple regression analysis was used to assess the associations between infant body composition (measured both by SF and doubly-labelled water) and later body size and composition adjusted for potential confounders.

7.3 Results

7.3.1 Characteristics of study subjects

7.3.1.1 Infant growth and body composition parameters

Weight SDS at 5 ages during infancy (3, 6, 12 weeks, 6 and 12 months) are shown in **Figure 7-1**. Weight SDS dropped at the age of 3 weeks before stabilising at around zero

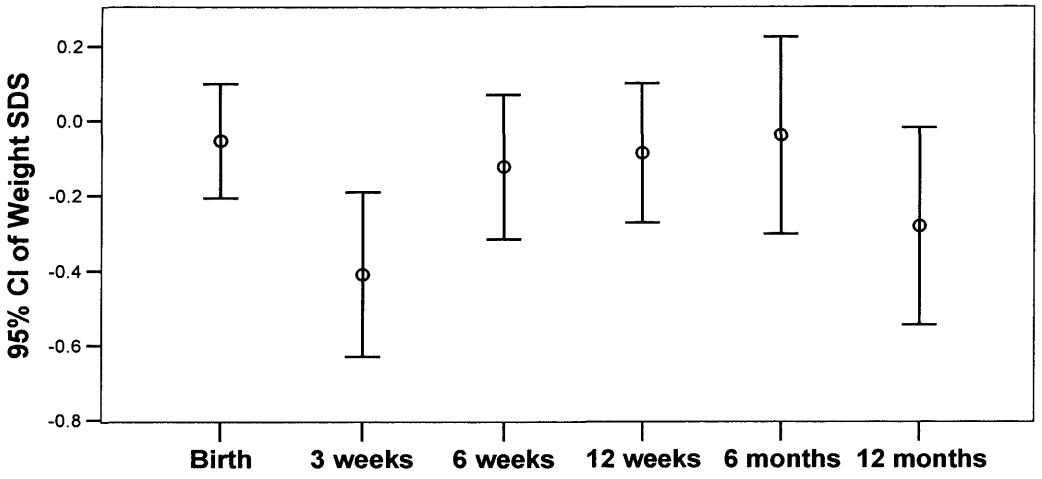
and then dropping again at age 12 months. At these two ages, weight SDS in this dataset was significantly different from the 1990 reference. When boys and girls were analyzed separately, girls showed no significant difference in weight SDS compared to the reference whereas boys had lower weight SDS at most ages except for 12 weeks.

There were no significant differences in triceps and subscapular SF between boys and girls at these 5 ages during infancy. SF increased with age except for at age 12 months when weight SDS also dropped. (**Figure 7-2**)

Infant body composition data are shown in **Table 7-1**. There was a trend towards higher FFM in boys at 12 weeks but the difference was no longer statistically significant after taking length into account using FFMI.

Figure 7-1 Mean and 95% confidence interval of weight SDS at different ages [#]

a) All subjects



b) Separate plots for boys and girls

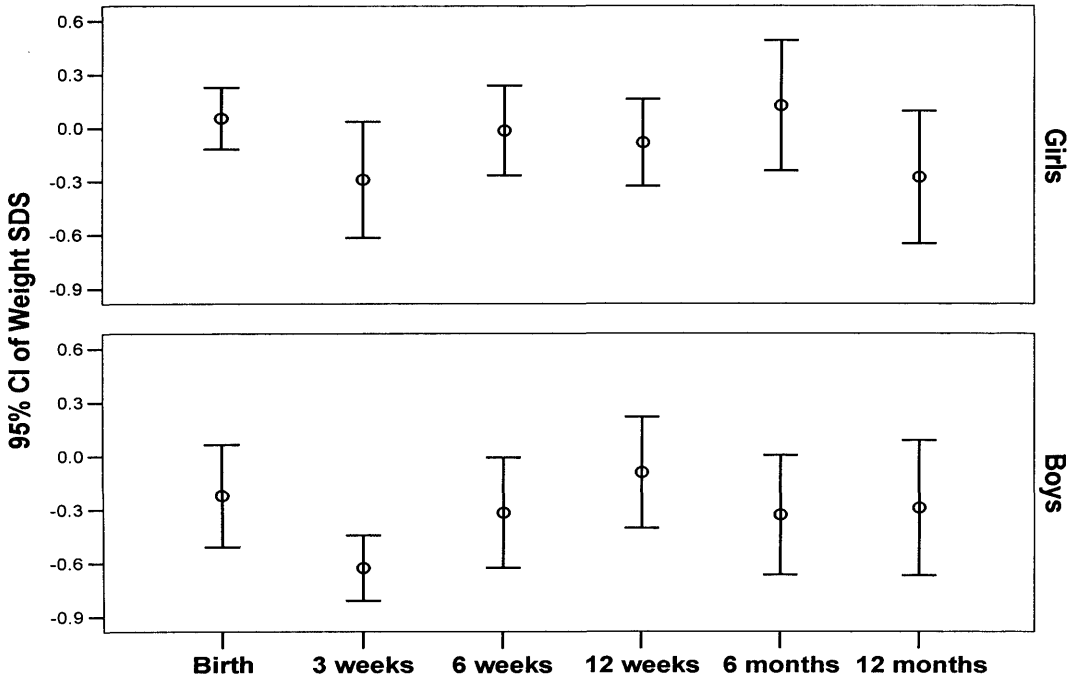
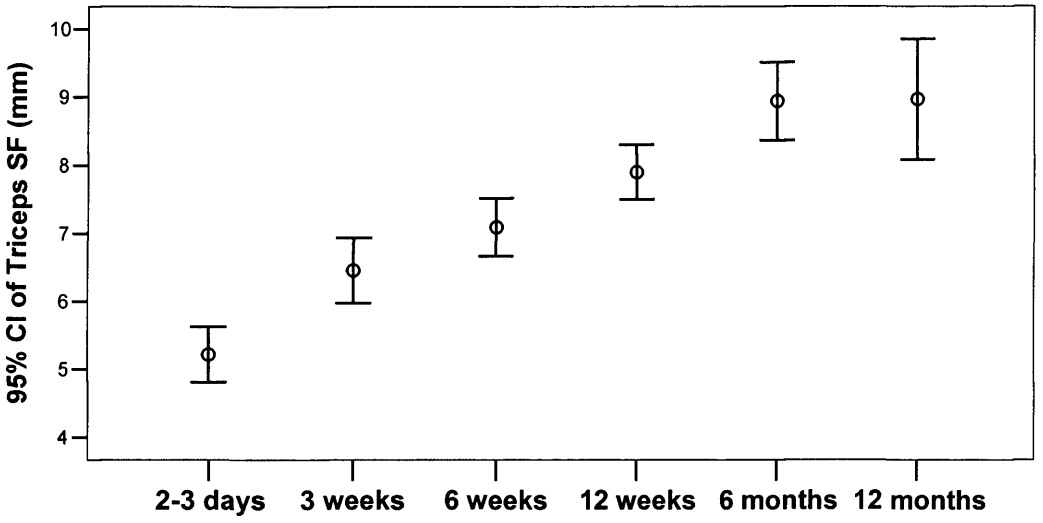
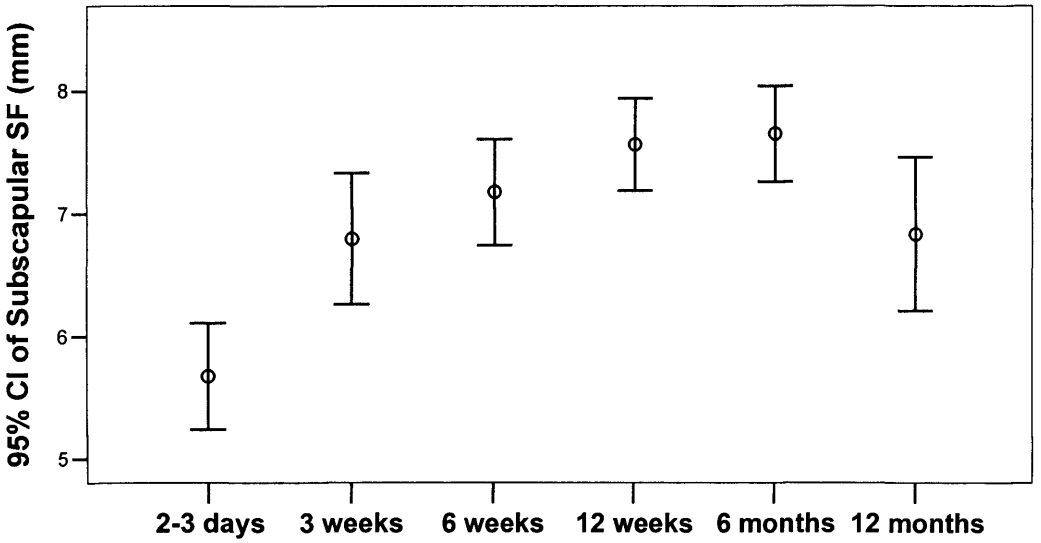


Figure 7-2 Mean and 95% confidence interval of SF at different ages [#]

a) Triceps (mm)



b) Subscapular (mm)



[#] Different samples size at different ages:
3 week measurements only available in cohort A (11 boys, 20 girls)
6 week measurements only available in cohort A and C (15 boys, 27 girls)
12 week measurements available for all cohorts (22 boys, 36 girls)
6 month measurements only available in cohort A and C (14 boys, 25 girls)
12 month measurements only available in cohort A and C (11 boys, 21 girls)

Table 7-1 Characteristics of infant body composition

	All	Boys	Girls	p
FM 6 wk (kg)[#]	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.93
FFM 6 wk (kg)	3.7 ± 0.2	3.8 ± 0.3	3.7 ± 0.2	0.48
FMI 6 wk (kg/m^2)	3.3 ± 0.9	3.4 ± 0.9	3.3 ± 1.0	0.90
FFMI 6 wk (kg/m^2)	12.3 ± 1.2	12.9 ± 0.9	11.9 ± 1.3	0.23
FMI 6 wk SDS[$]	0.63 ± 1.03	0.66 ± 0.94	0.61 ± 1.15	0.94
FFMI 6 wk SDS[$]	-0.16 ± 1.20	0.10 ± 1.06	-0.32 ± 1.33	0.61
FM 12 wk (kg)[##]	1.3 ± 0.5	1.4 ± 0.6	1.3 ± 0.4	0.34
FFM 12 wk (kg)	4.4 ± 0.5	4.6 ± 0.6	4.3 ± 0.4	***0.05***
FMI 12 wk (kg/m^2)	3.6 ± 1.4	3.8 ± 1.5	3.5 ± 1.3	0.59
FFMI 12 wk (kg/m^2)	12.2 ± 1.2	12.4 ± 1.5	12.1 ± 1.0	0.41
FMI 12 wk SDS[$]	-0.26 ± 1.16	-0.01 ± 1.22	-0.41 ± 1.12	0.30
FFMI 12 wk SDS[$]	0.02 ± 1.06	-0.18 ± 1.18	0.14 ± 0.98	0.35

[#] Results only available in cohort C (4 boys, 7 girls)

[##] Results available for 15 boys and 26 girls

[$] SDS was calculated in relation to 48 infants at 6 weeks and 150 infants at 12 weeks.

7.3.1.2 Current body composition outcomes

There were two age bands for BC outcomes measured. Subjects in cohorts A and B, which were set-up in 1991-1993, were 12.0 -14.4 years old (n=48). The other cohort (cohort C) were 18.2-20.4 years old (n=11). Body composition data are shown as SDS where possible. The body composition data presented here are from 54 children who completed the 4C model and 5 children who had the 2C model. All were Caucasian and born in the Cambridge area. For the older cohort, all were Tanner stage 5.

In the younger group (Cohorts A and B—**Table 7-2a**), girls were taller, had higher BMI, waist circumference, triceps and subscapular SF compared to the British 1990 reference. Girls had higher FMI than boys but the difference was no longer statistically significant when SDS were used. However, for some indices (BMI, waist circumference, and subscapular SDS), the differences were beyond normal gender differences.

In the older group, despite a small sample size, gender differences in terms of final adult height, FMI, and FFMI were clearly seen (**Table 7-2b**).

The potential confounders did not differ in boys and girls. The results of all subsequent analyses were similar whether the 5 home visit subjects were included or not.

Table 7-2 Characteristics of study subjects at the time of later BC measurements

a) Cohorts A and B

	All (n=48)	Boys (n=19)	Girls(n=29)	p
Age (y)	13.2 ± 0.7	13.1 ± 0.5	13.3 ± 0.7	0.24
Height (cm)	157.8 ± 7.2	156.7 ± 9.1	158.5 ± 5.6	0.39
Height SDS	0.25 ± 0.88	0.16 ± 0.91	0.31 ± 0.87	0.57
BMI (kg/m^2)	19.9 ± 3.5	18.2 ± 2.6	20.9 ± 3.6	***0.006***
BMI SDS	0.25 ± 1.19	-0.16 ± 1.19	0.53 ± 1.13	***0.05***
FMI (kg/m^2)	5.2 ± 2.5	3.8 ± 1.7	6.1 ± 2.5	***0.001***
FFMI (kg/m^2)	14.7 ± 1.7	14.4 ± 1.8	14.9 ± 1.6	0.35
FMI SDS	0.07 ± 0.86	0.03 ± 0.87	0.09 ± 0.88	0.82
FFMI SDS	-0.02 ± 1.03	-0.40 ± 1.08	0.22 ± 0.94	***0.05***
Trunk FMI SDS[#]	0.05 ± 0.99	-0.06 ± 1.00	0.12 ± 0.99	0.58
WC (cm)	68.6 ± 7.3	70.0 ± 6.7	69.8 ± 7.5	0.20
WC SDS	0.80 ± 1.09	0.29 ± 0.98	1.13 ± 1.04	***0.008***
Triceps SDS	0.48 ± 0.95	0.18 ± 1.08	0.68 ± 0.80	0.07
Subscapular SDS	0.22 ± 1.07	-0.24 ± 1.26	0.52 ± 0.82	***0.02***
Pubertal status (n, %)				
Prepubertal	(3, 6.3%)	(2, 10.5%)	(1, 3.4%)	0.61
Early pubertal	(27, 56.2%)	(10, 52.7%)	(17, 58.6%)	
Late pubertal	(18, 37.5%)	(7, 36.8%)	(11, 37.9%)	
Physical activity (n, %)				
Much less or less	(3, 6.3%)	(1, 5.3%)	(2, 6.9%)	
Same as peers	(23, 47.9%)	(7, 36.8%)	(16, 55.2%)	0.60
More	(20, 41.7%)	(10, 52.6%)	(10, 34.5%)	
Much more	(2, 4.1%)	(1, 5.3%)	(1, 3.4%)	
Social class (n, %)				
Class 1	(5, 10.4%)	(3, 15.8%)	(2, 6.9%)	
Class 2	(17, 35.4%)	(5, 23.6%)	(12, 41.4%)	0.58
Class 3	(11, 22.9%)	(4, 21.1%)	(7, 24.1%)	
Class 4 or more	(15, 31.3%)	(7, 36.8%)	(8, 27.6%)	

[#] n=43 (17 boys, 26 girls) for trunk FMI SDS since DXA data were not available for the 5 home visit subjects

b) Cohort C

	All (n=11)	Boys (n=4)	Girls(n=7)	p
Age (y)	19.6 ± 0.6	19.4 ± 0.9	19.7 ± 0.4	0.60
Height (cm)	173.8 ± 9.0	181.6 ± 6.1	169.3 ± 7.3	***0.02***
Height SDS	0.82 ± 1.07	0.61 ± 0.87	0.94 ± 1.21	0.65
BMI (kg/m^2)	23.1 ± 2.9	22.9 ± 3.0	23.2 ± 3.1	0.88
BMI SDS	0.34 ± 1.08	0.26 ± 1.34	0.39 ± 1.03	0.86
FMI (kg/m^2)	6.7 ± 2.8	4.4 ± 1.9	8.0 ± 2.4	***0.03***
FFMI (kg/m^2)	16.4 ± 2.2	18.4 ± 1.3	15.2 ± 1.6	***0.008***
FMI SDS	0.31 ± 0.81	0.17 ± 0.72	0.39 ± 0.90	0.70
FFMI SDS	-0.30 ± 1.01	-0.28 ± 0.80	-0.31 ± 1.17	0.97
Trunk FMI SDS	0.22 ± 0.78	0.30 ± 0.66	0.17 ± 0.89	0.81
WC (cm)[#]	79.1 ± 6.8	81.3 ± 5.9	77.8 ± 7.4	0.44
Triceps SDS	0.54 ± 0.85	0.28 ± 1.04	0.69 ± 0.78	0.48
Subscapular SDS	0.63 ± 0.96	0.26 ± 1.15	0.84 ± 0.85	0.36
Physical activity (n, %)				
Much less or less	(1, 9.1%)	(0, %)	(1, 14.3%)	
Same as peers	(6, 54.5%)	(1, 25%)	(5, 71.4%)	0.13
More	(4, 36.4%)	(3, 75%)	(1, 14.3%)	
Much more	(0, 0%)	(0, 0%)	(0, 0%)	
Social class (n, %)				
Class 1	(2, 18.2%)	(1, 25%)	(1, 14.3%)	
Class 2	(6, 54.5%)	(2, 50%)	(4, 57.1%)	0.38
Class 3	(1, 9.1%)	(1, 25%)	(0, 0%)	
Class 4 or more	(2, 18.2%)	(0, 0%)	(2, 28.6%)	

[#] No WC SDS calculated for cohort C since the database for waist SDS was available only up to the age of 17

7.3.2 Infant BC and later body size and composition

Unfortunately, the sample size for subjects who had BC measurements at both 6 weeks at later follow-up was too small to give meaningful results (n=11). Only the results from subjects who had BC measurements at both 12 weeks and later follow-up are shown in **Table 7-3**. FFM at 12 weeks was positively associated with later height **(Figure 7-3)**. The association persisted after adjusting for birthweight SDS (data not shown). However, this association depended heavily on one boy with high FFM at 12 weeks (6.08 kg, FFMI SDS = 2.51). There was no significant association between infant BC at 12 weeks and later BC. These results were not substantially changed after adjusting for birthweight SDS, infant diet groups (breast-fed, reduced energy formula, standard term formula), puberty, physical activity, social class, or parental size (one at a time due to the small sample size-data not shown).

Figure 7-3 Scatter plot of FFM at 12 weeks and later height SDS

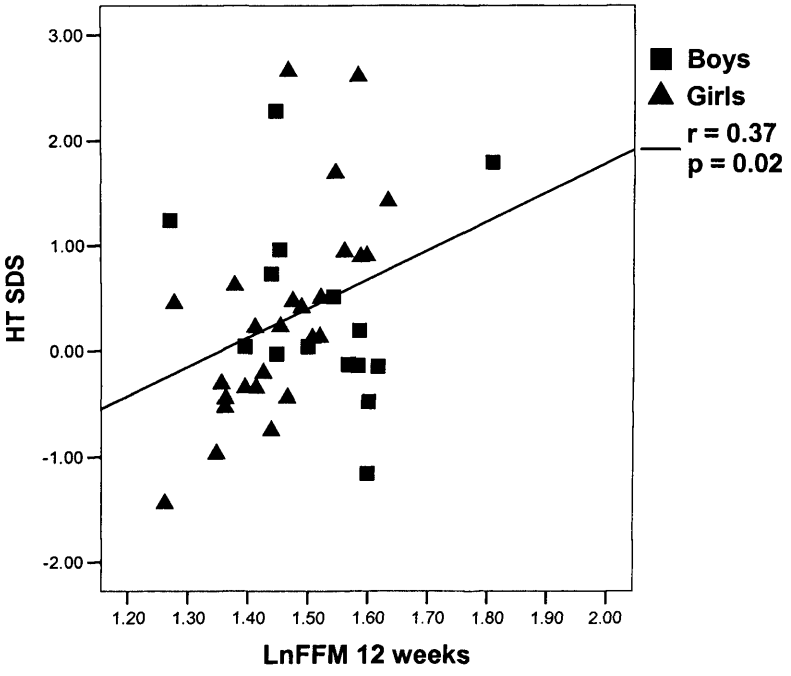


Table 7-3 Association of infant BC at 12 weeks and later body size and composition[++]

	Infant FM				Infant FFM				Infant FMI SDS				Infant FFMI SDS			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.10	0.45	0.82		2.22	1.60	0.17		0.007	0.15	0.96		0.05	0.17	0.75	
Ht SDS	-0.13	0.40	0.76		3.30	1.36	***0.02***	0.14	-0.06	0.13	0.69		0.07	0.15	0.67	
BMI SDS	0.31	0.48	0.52		0.62	1.76	0.73		0.07	0.16	0.66		0.02	0.18	0.92	
FMI SDS	0.18	0.37	0.62		-0.37	1.34	0.78		0.03	0.12	0.84		-0.04	0.14	0.75	
FFMI SDS	0.15	0.43	0.72		0.86	1.55	0.58		0.04	0.14	0.80		0.01	0.16	0.97	
Triceps SDS	0.35	0.38	0.36		0.55	1.39	0.70		0.10	0.13	0.46		0.02	0.14	0.88	
Subscapular SDS	0.55	0.42	0.20		-0.11	1.57	0.94		0.19	0.14	0.20		-0.04	0.16	0.80	
WC SDS[#]	-0.03	0.50	0.96		1.15	1.79	0.53		-0.07	0.17	0.67		0.03	0.19	0.89	
WC[$]	0.01	0.04	0.74		0.02	0.15	0.88		0.002	0.01	0.87		0.002	0.02	0.90	
WC[∞]	-0.01	0.02	0.62		0.06	0.08	0.44		-0.003	0.01	0.71		0.004	0.01	0.64	
TrunkFMI SDS[#]	0.20	0.43	0.64		-0.41	1.57	0.79		0.04	0.14	0.79		-0.06	0.16	0.71	
TrunkFMI SDS[∝]	-0.05	0.17	0.76		-0.49	0.62	0.44		-0.01	0.06	0.83		-0.07	0.06	0.29	

[++] Adjusted for exact age at 12 weeks and gender, (n = 41, 15 boys and 26 girls). Results are from the multiple regression analysis, each row represents a different model with later body size and composition in the left hand column as the dependent variable; infant BC (FM, FFM, FMI SDS, or FFMI SDS) was an independent variable; exact age at 12 weeks and gender were covariates; B = the coefficient of infant BC i.e. the change in later BC per 100% increase in FM and FFM, or per 1SDS increase in FMI and FFMI, SE = standard error, highlighted p value indicate significant at $p < 0.05$, r^2= coefficient of determination (calculated from partial r of infant BC). Infant FM, FFM at 12 weeks were natural log transformed before analysis, WC was natural log transformed and presented in $\log_e$ scale.

[#] n= 31 for WC SDS since the database for waist SDS was available only up to the age of 17, n=37 for trunk FMI SDS since DXA data were not available for the 5 home visit subjects

[$] Adjusted for current height (m)

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

7.3.3 SF and later body size and composition

Due to the small sample size for the isotope results and the possibility that the detailed BC measurements might have been done at a time (12 weeks) that was perhaps 'beyond' the critical period for programming effects, I analyzed the SF at 5 different ages in relation to later body composition. The models with infant triceps SF (which is most sensitive to changes in weight or nutritional status) as an independent variable showed more significant results than those with subscapular SF. Results using the sum of infant triceps and subscapular SF were in the same direction but less strong compared to those with triceps SF. Therefore, results are only shown for infant triceps SF adjusted for age at measurement and gender (**Table 7-4**). The models with adjustment for infant size by length at different ages provided similar results as the models with adjustment for age at infant measurements (data not shown). Triceps SF at 3 weeks was positively associated with adolescent BMI, FFMI, SF, and WC (**Figure 7-4**). Triceps SF at 6 weeks was positively related to BMI, FMI, later SF, WC, and trunk FMI (**Figure 7-5** and **Figure 7-6**). The association persisted after adjusting for birth SDS, infant diet group, puberty, physical activity, social class, or parental size in the model (one at a time due to the small sample size -data not shown). The results were in the same direction if Δ triceps 0-3 weeks (between age 2-3 days and 3 weeks) was used instead of triceps SF at 3 weeks and Δ triceps 0-6 weeks (between age 2-3 days and 6 weeks) was used instead of triceps at 6 weeks (data not shown).

As seen in the scatter plot, there was one boy who was small in infancy (triceps SF 4.8 and 4.4 mm at 3 weeks and 6 weeks old) and in adolescence (current BMI SDS -2.33, FMI SDS -1.57, FFMI SDS -1.34) who appeared to influence the association substantially. Therefore, the same analysis was performed after he was excluded. Triceps SF at 3 weeks was still positively related to later FFMI SDS (n=30, p=0.02, r^2=0.20) and BMI SDS (p=0.03, r^2=0.18). Triceps SF at 6 weeks was still positively related to later WC SDS (n=30, p=0.04, r^2 =0.16) but the relationship with BMI, FMI, and Trunk FMI SDS was no longer statistically significant.

Table 7-4 Association of triceps SF at different periods in infancy and later body size and composition[++]

	Triceps 3wk (n=31)				Triceps 6wk (n=42)				Triceps 12 wk (n=58)				Triceps 6 mo (n=39)				Triceps 12 mo (n=32)			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	1.29	1.05	0.23		1.40	0.83	0.10		0.83	0.85	0.33		0.95	0.88	0.29		-0.05	0.76	0.95	
Ht SDS	-0.86	0.95	0.37		-0.08	0.76	0.92		0.30	0.76	0.69		-.002	0.80	0.99		-0.14	0.67	0.84	
BMI SDS	2.56	1.06	***0.02***	0.18	2.21	0.87	***0.02***	0.14	0.89	0.91	0.33		1.12	0.95	0.25		0.02	0.82	0.98	
FMI SDS	1.24	0.86	0.16		1.36	0.68	***0.05***	0.10	0.42	0.70	0.55		0.90	0.72	0.22		-0.26	0.63	0.68	
FFMI SDS	2.36	0.94	***0.02***	0.19	1.18	0.82	0.16		0.63	0.82	0.45		1.10	0.85	0.20		0.27	0.74	0.72	
Triceps SDS	2.02	0.84	***0.02***	0.18	1.82	0.69	***0.01***	0.16	0.84	0.72	0.24		1.12	0.74	0.14		0.24	0.65	0.71	
Subscapular SDS	1.97	0.94	***0.05***	0.14	2.10	0.76	***0.008***	0.17	0.86	0.81	0.30		0.89	0.81	0.28		0.43	0.70	0.55	
WC SDS[#]	1.81	0.98	0.08		2.47	0.88	***0.009***	0.22	0.86	0.88	0.33		0.23	1.02	0.83		0.002	0.78	0.99	
WC[$]	0.25	0.08	***0.006***	0.26	0.16	0.07	***0.03***	0.12	0.04	0.08	0.58		0.04	0.08	0.63		-0.02	0.07	0.79	
WC[∞]	0.09	0.05	0.09		0.02	0.04	0.64		-0.02	0.04	0.69		-0.01	0.04	0.79		-0.01	0.04	0.71	
TrunkFMI SDS	1.84	0.96	0.07		1.62	0.78	***0.04***	0.11	0.44	0.81	0.59		1.10	0.82	0.19		-0.19	0.72	0.80	
TrunkFMI SDS[∞]	0.02	0.43	0.97		-0.07	0.30	0.81		-0.22	0.31	0.47		0.60	0.31	0.06		-0.11	0.28	0.71	

[++] Adjusted for exact age at 3, 6, 12 weeks, 6, 12 months and gender. Results are from the multiple regression analysis, each row represents a different model with later body size and composition in the left hand column as the dependent variable; triceps SF at different ages was an independent variable; exact age at each time point and gender were covariates; B = the coefficient of triceps SF ($\log_e$ scale) i.e. the change in later BC per 100% increase in triceps SF, SE = standard error, highlighted p value indicate significant at $p < 0.05$, r^2= coefficient of determination (calculated form partial r of triceps SF at each age). Infant triceps SF were natural log transformed before analysis; WC was natural log transformed and presented in $\log_e$ scale

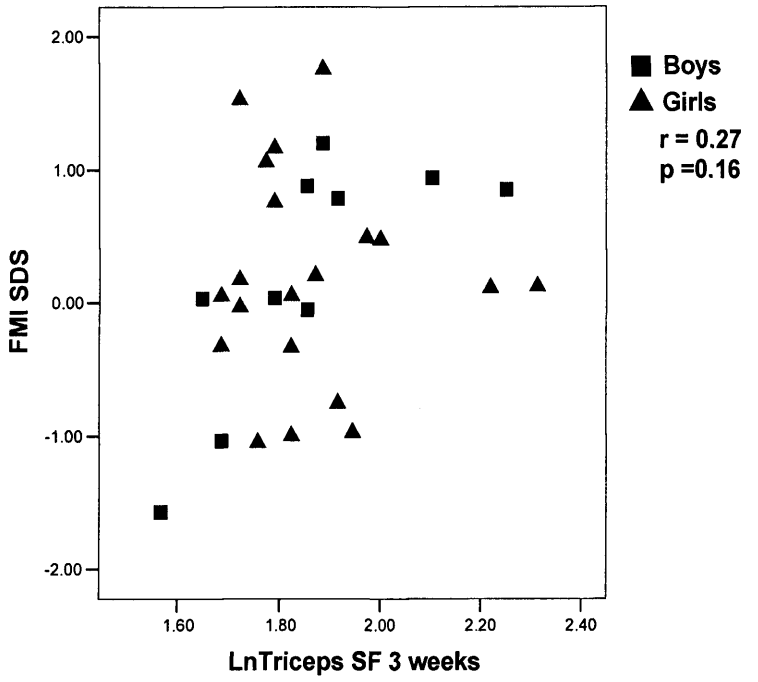
[#] n=31, 31, 48, 29, 29 for the model with WC SDS and triceps SF at 3, 6, 12 weeks, 6, and 12 months, respectively since the database for waist SDS was available only up to the age of 17; n= 28, 39, 53, 36, 29 for the model with trunk FMI SDS and triceps SF at 3, 6, 12 weeks, 6, 12 months, respectively.

[$] Adjusted for current height (m)

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

Figure 7-4 Scatter plots of Triceps SF at 3 weeks and later body composition

a) FMI SDS



b) FFMI SDS

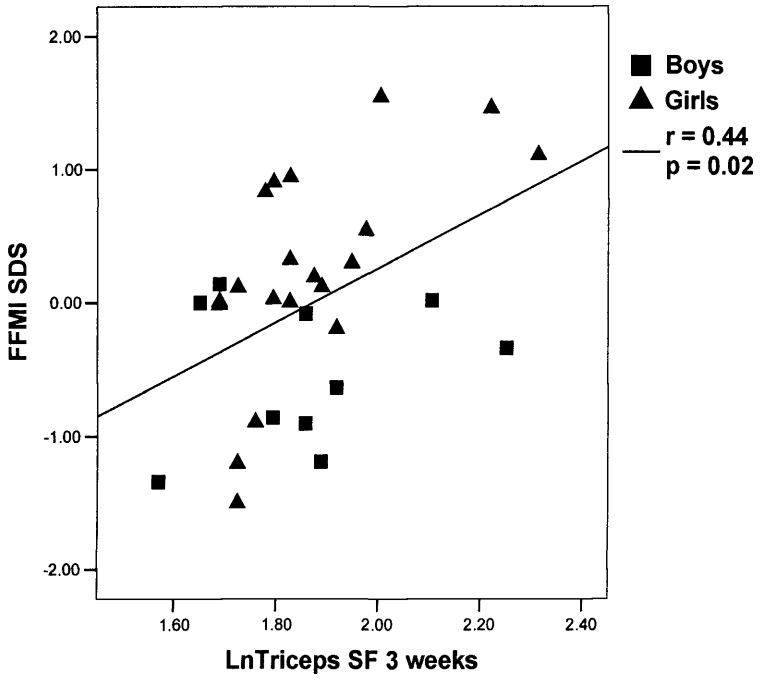
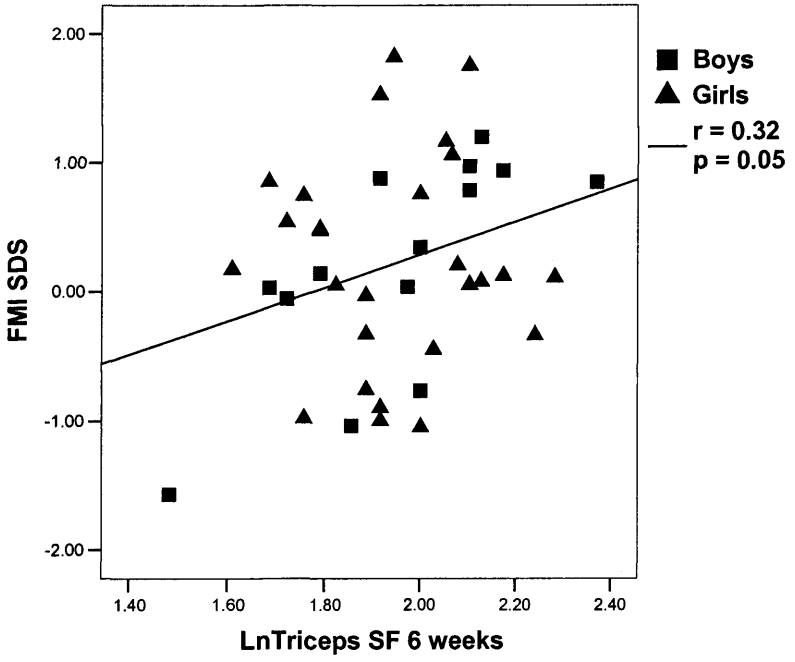


Figure 7-5 Scatter plots of Triceps SF at 6 weeks and later body composition

a) FMI SDS



b) FFMI SDS

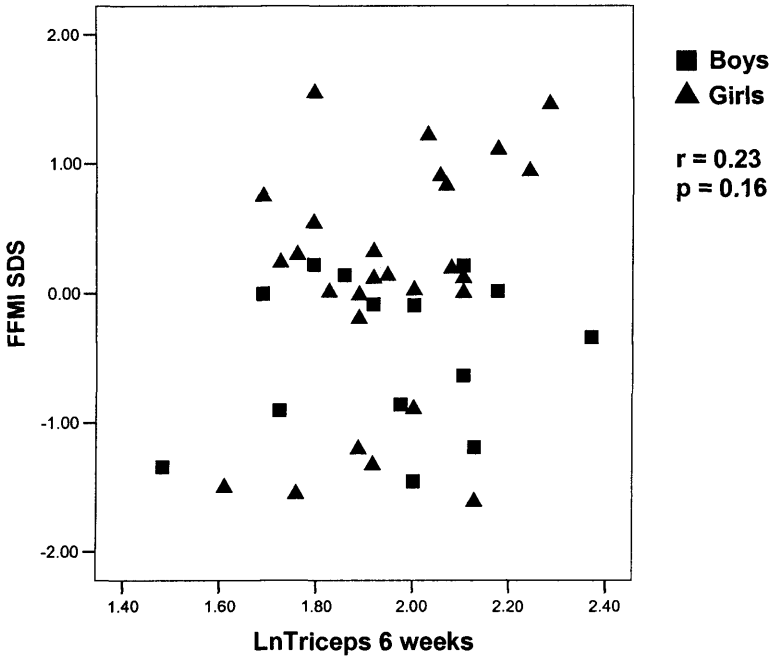
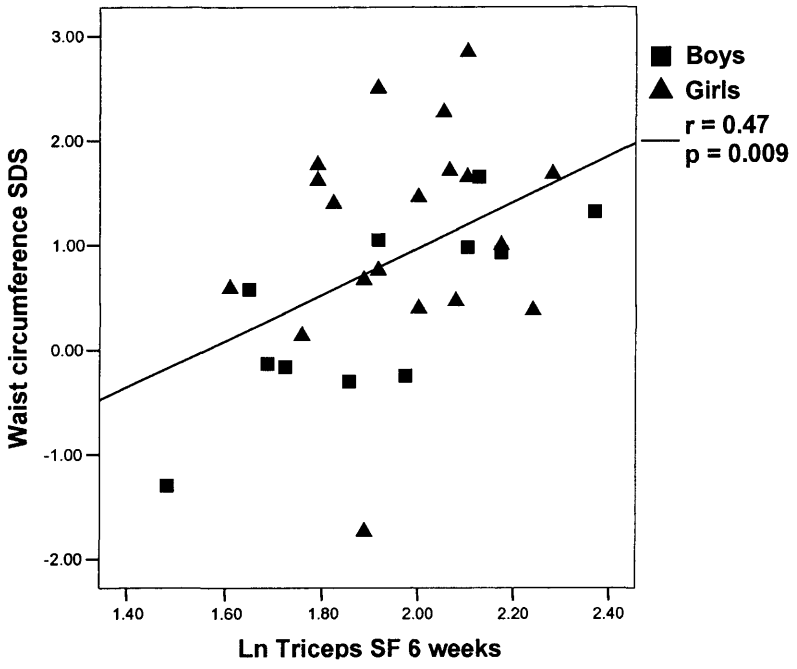
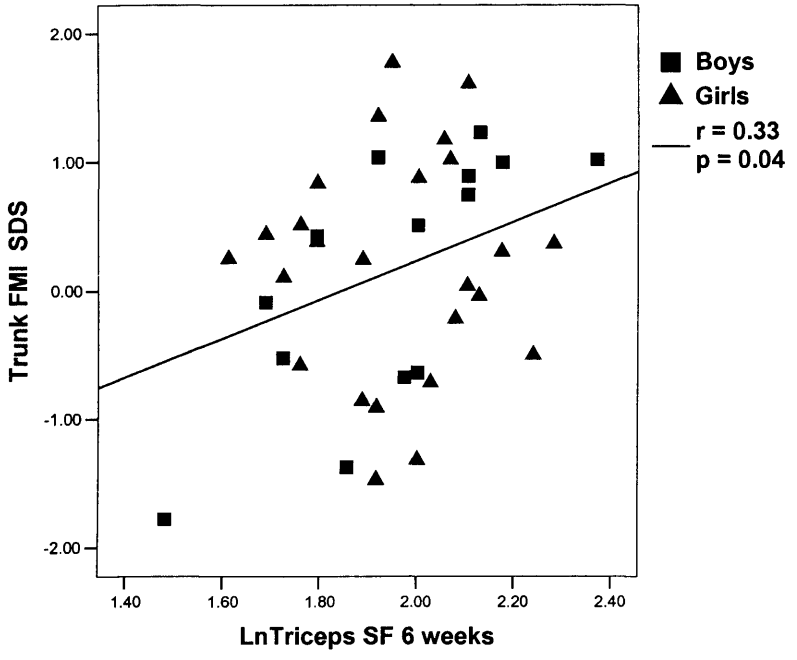


Figure 7-6 Scatter plots of Triceps SF at 6 weeks and later central fatness

a) Waist circumference SDS



b) Trunk FMI SDS



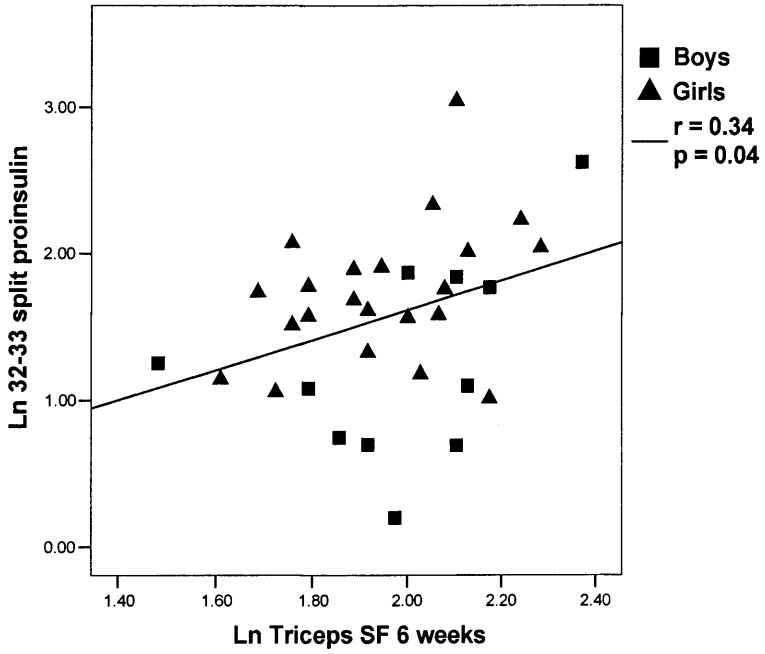
7.3.4 Infant body composition and later cardiovascular risk factors

From the above results, infant triceps SF at a very early age showed correlations with later BC, which was in turn shown in **Chapter 6** to be highly associated with lipid and insulin profiles. Further analysis was therefore performed to examine associations between infant BC and the later cardiovascular risk markers. No statistically significant association between birthweight SDS and later lipid profile, insulin profile or blood pressure found in this dataset, either with or without current body size adjustment (data not shown). There was no significant association between BC at 12 weeks and later cardiovascular risk factors.

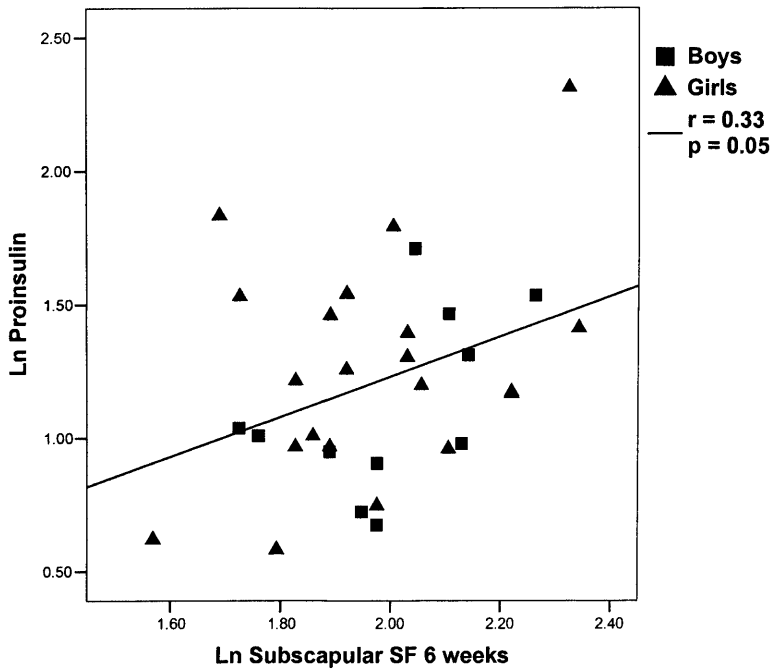
None of the SF (triceps, subscapular, sum of triceps and subscapular) at 3, 12 weeks, 6, 12 months were significantly associated with later cardiovascular risk markers. However, at 6 weeks (n=33) after adjusting for gender and length at 6 weeks, triceps and subscapular SF were positively correlated with 32-33 split proinsulin ($p=0.04$, $r^2= 0.12$ and $p = 0.04$, $r^2= 0.14$ (**Figure 7-7a)** whereas subscapular SF was also positively correlated with proinsulin ($p= 0.05$, $r^2= 0.11$). See **Figure 7-7b**. Due to the small sample size and the fact that one outlier appeared to exert a large effect on the results, I reanalyzed the data without this subject (a girl who had very high 32-33 split proinsulin (21 pmol/L)). Triceps SF at 6 weeks was still positively related to 32-33 split proinsulin ($p = 0.04$, $r^2 =0.12$). However, if the same girl (who also had high proinsulin (10.1 pmol/L)) was excluded, the positive relationship between subscapular SF at 6 weeks and later proinsulin was no longer significant.

Figure 7-7 Scatter plot of SF at 6 weeks and later insulin profiles

a) Triceps SF at 6 weeks and later 32-33 split proinsulin



b) Subscapular SF at 6 weeks and later proinsulin



7.4 Discussion

There have been hardly any studies relating infant body composition to long-term outcomes, largely due to the limitations of infant BC techniques. Sample sizes in existing studies are inevitably small and the results are inconsistent. Butte et al (117) found that differences in body composition (by multicomponent model; deuterium dilution, total body potassium, and DXA) in early infancy (up to 6 months) which were associated with infant feeding mode (40 breast-fed vs. 36 formula-fed infants) did not persist into the second year of life. In contrast, Wells et al. (118) reported that infant fatness (by doubly labelled water study in 30 infants) showed a strong relationship with childhood fatness at the age 2.5-3.5 years.

In my study, I attempted to follow-up a group of infants who had BC measured at 12 weeks into adolescence and early adulthood. The subjects are unique in having detailed measurements in infancy with long-term follow-up. Unfortunately, despite the initial sample size of 199, the total number of subjects who completed both the doubly-labelled water measurement at 12 weeks and the later follow-up was only 41 (out of 59 subjects who had later BC outcomes measured). See **Chapter 3, 3.5.5 Recruitment and follow-up rates for cohorts A, B, and C and 3.6 Key study design features**. Therefore, I recognize that my findings have to be interpreted with extreme caution. In this discussion, I will focus on: 1) the relationship between infant BC and later BC, 2) the possible association between infant BC and later cardiovascular risk factors, and 3) the limitations of this study. The potential mechanism and implication of the findings will be discussed later in **Chapter 9**.

7.4.1 Infant BC and later BC

I did not find any association between infant BC measured by stable isotopes at 12 weeks of age and later BC. There are a number of possible explanations for this lack of relationship. 1) The sample size may be too small (n=41) to detect an association; 2) 12 weeks of age (when the detailed BC measurements were made) could potentially be 'beyond' the critical period for the programming of later BC through

infant BC. The fact that SF at 3 and 6 weeks (which had a comparable sample size, n=31 and 42) were related to later BC whereas SF at 12 weeks (n=58) did not show any association, lends some support to this concept; 3) the long term influence of early growth on later BC might not be mediated through changes in infant BC (see more discussion in **Chapter 9**).

The following discussion is based on infant SF in relation to later outcomes. Although I recognize that the use of SF may not be ideal (it would have been preferable, for example, had detailed BC been measured using stable isotopes at these earlier time-points), but this is one of the first attempts to study the relationship between any measure of infant BC and later outcome. Moreover, the use of SF allows the investigation of infant BC at different ages in infancy, not just 12 weeks which might not be the 'right' window as mentioned previously. Interestingly, triceps SF at 3 weeks was positively correlated with later FFM more strongly than FM. This seems counter-intuitive since triceps SF is most likely to be a proxy for FM rather than FFM. One explanation might be that triceps SF at 3 weeks might simply represent body size i.e. body weight at that time. However, additional analysis showed that after adjusting for weight SDS at 3 week, this positive association was weaken but still statistically significant (p=0.046, data not shown); in other words, higher triceps at 3 weeks was related to higher FFM in later life even in infants with same weight SDS. Another explanation could be that the mechanism of later FFM programming is not as simple as the tracking of FFM from birth and infancy through to adolescence and that early gain in FM predicts later FFM. On the other hand, triceps SF at 6 weeks showed a positive association with later indices of fatness, which supports the idea that higher fatness in infancy might result in higher FM and a more central fat distribution in later life.

In order to test whether infant BC 'explains' the association between early growth and later BC, I performed additional analyses of the early growth data in this small group of subjects (n=59). ΔWeight SDS from birth to 3 weeks was positively associated with FFMI SDS (n=31, p=0.005, r^2=0.26), but not with other BC outcomes, whereas Δweight SDS from birth to 6 weeks was positively related to WC SDS (n=31, p=0.03, r^2=0.17) in adolescence. Furthermore, these positive associations between Δweight SDS and later BC was largely attenuated and became

non-significant if triceps SF at 3 weeks or triceps SF at 6 weeks were added to the models. Therefore, I propose that the relationship between growth in early postnatal life (first 6 weeks) and later BC might be mediated by infant 'fatness' - as measured by SF. Again, this postulation is based on the findings from only 30 to 40 individuals. In order to confirm or refute this hypothesis, a follow-up study (with a bigger sample size) of infants with BC measurements at an earlier age than 12 weeks e.g. 3 or 6 weeks) is required.

In comparison with my findings from **Chapter 5**, there seems to be some discrepancy regarding the 'critical' window for programming of later BC, since in **Chapter 5** I found no association between Δ weight SDS before 6 weeks and later BC. There are a number of potential explanations for this discrepancy. 1) The early weight measurements in the prospective cohorts are likely to be more precise than the measurements obtained from the baby books. This is likely to be most relevant during the first few weeks of life when weight SDS can change dramatically. As a result, the analyses presented in **Chapter 5** might be expected to have more 'noise' during this early period making it more difficult to detect any relationship between very early infant growth and later FFM or FM. 2) Infants from cohort A (only the original RCT had a 3 week measurement) may have had different BC than normal babies due to the trial formula (see **Chapter 8**). 3) The relationship between very early growth and later BC might be apparent only beyond a certain age, as seen in the baboon studies (13;14). However, this last explanation is unlikely, since when I reanalyzed early 'baby book' growth data for subjects in the same age-range as subjects from the prospective cohorts (n=55-60), I still found no association between very early infant growth and later BC.

7.4.2 Infant BC and later cardiovascular risk factors

A number of studies have shown that children with low birthweight have reduced glucose tolerance and that those with low birthweight and high BMI in later childhood are at greatest risk of developing type II diabetes mellitus (34;94;236;237), suggesting that rapid postnatal growth might be associated with later insulin resistance. I did not find any association between birthweight and later insulin profiles; however, my finding that higher SF at age 6 weeks (or change in SF or weight from birth to 6 weeks) was related to higher 32-33 split proinsulin in adolescence seems to support this idea. Nevertheless, these findings were only based on 33 subjects and some of the associations depended substantially on just 1 or 2 individuals. Therefore, my confidence in these findings is limited.

7.4.3 Criticisms of the study

As discussed above, my sample size is really too small to draw firm conclusions. Despite showing 'statistically' significant associations, some of these depended greatly on only 1 or 2 individuals. Moreover, due to the small sample size, I could not adjust simultaneously for potential confounders.

Using the method of Dupont and Plummer (238) I calculated that, with my sample size of 58, I would be able to detect a minimum regression coefficient of 1.87 SDS with 80% power at 5% significance. This has implications for the interpretation of my results, in that I may have missed a 'real' but biologically significant association due to low power. For example, in the case of the association between SF at 12 weeks and later FMI SDS, I only had 49% power to detect the regression coefficient observed in my analysis as statistically significant. It is thus possible that there is a real association - albeit smaller in size than that for SF at 6 weeks.

Despite these limitations, I consider that my analyses have raised some interesting questions which could now be tested in a further, larger, study of infants with body composition measurements. My findings also have implications for the design of

such a study in that they suggest that infant BC measurements should be performed earlier than 12 weeks.

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7.5 Summary

- From this study, there is no evidence that infant BC at 12 weeks is associated with later BC in adolescence but a larger study is required to confirm the findings.

- There is limited evidence from the SF measurements earlier than 12 weeks that infant 'fatness' as measured by SF at 3 weeks seems to be positively related to later FFM whereas higher SF at 6 weeks seems to positively correlate with later FM and waist circumference; this suggests that the period prior to 12 weeks might also be important for BC programming but this hypothesis requires testing in a larger study.

Chapter 8

The Influence of Infant Nutrition on Body Composition

Chapter 8 The Influence of Infant Nutrition on Body Composition

8.1 Introduction

Nutritional intake during infancy has been shown to have a significant influence on growth pattern during infancy (see **Chapter 2**). However, only a limited number of studies have explored its effects on infant BC and none of these studies has included longer term follow-up.

8.2 Methods

8.2.1 Subjects

Infant growth and body composition data from the 2 randomised controlled trial (RCT) groups (cohort A) were compared with data from breast-fed babies from cohorts B and C. Body composition outcomes of the subjects who participated in the follow-up study during adolescence (described in **Chapter 7**) were analysed according to infant diet groups.

8.2.2 Infant diet groups

For the purpose of this analysis, the subjects were divided according to their infant diet into 3 groups. In cohort A (randomised controlled trial of different energy formulas), the reduced energy (60 kcal/100 ml, RE) formula (n=53 during infancy) and standard term (67 kcal/100 ml, TF) formula groups (n=54 during infancy) were fed the assigned study formula as the sole energy source from birth through the initial 12-week study period. Solid food was introduced at a time determined by the parents with advice from their health care professionals (the Department of Health recommendation for term infants at the time was not to introduce solids before 3

months of age). Detailed composition of these 2 formulas and details of the RCT is shown in **Appendix A**. The breast feeding group (n=46 during infancy) were exclusively breast-fed until at least 12 weeks of age by the study design (from cohorts B and C which aimed to study energy metabolism in breast-fed and formula-fed infants at 12 weeks of age).

8.2.3 Statistical methods

Independent sample t-tests were used to test the difference between the 2 RCT diet groups. One-way ANOVA was used to test the difference between the 3 diet groups. Dunnett's post hoc test was used to test pairwise differences between 2 RCT diet groups and breast-fed group.

8.3 Results

Characteristics of the study subjects during infancy and at adolescent follow-up are described in **Chapter 7**. In this chapter, I will focus on the short-term effects of early nutrition on growth and BC in infancy and its long-term effects on BC during the adolescent period.

8.3.1 The effects of early nutrition on infant growth and BC

Between 2 infant formulas with different energy content

Comparisons between the 2 RCT groups are shown in **Table 8-1**. There was no significant difference between the groups in weight or length. Infants fed reduced energy formula (RE) had significantly lower subscapular SF at 6 weeks and 12 weeks than those who received standard term (TF) formula. At 3 months of age, the RE group showed higher milk volume intake than the TF group; consequently, they had a higher protein intake but still a lower fat intake than the TF group. Infant BC results are shown in **Table 8-2**. The RE group had significantly lower FM (mean

difference -0.25 kg; 95%CI -0.49, -0.01), FMI (mean difference -0.62 kg; 95%CI -1.24, 0.01) and higher FFMI (mean difference 0.65 kg/m^2; 95%CI 0.08, 1.23) at 12 weeks of age compared to the TF group.

Between breast-fed and formula-fed groups

Results for the comparison of the 2 RCT groups and breast fed babies are also shown in **Table 8-2**. The number of subjects who were first-born babies was higher in the breast-fed group. Breast-fed babies showed slower weight gain than formula-fed babies from 6-12 weeks (mean difference 0.25 SDS, 95% CI 0.03, 0.49 for the RE group and mean difference 0.21 SDS, 95% CI –0.01, 0.44 for the TF group). They also had lower triceps and subscapular SF than the formula-fed groups (particularly the TF group) at 6 weeks and 12 weeks old. Although there were no statistically significant differences in FM or FFM at 12 weeks between the breast-fed and formula-fed groups, breast-fed infants showed a trend towards lower FM and higher FFM compared to infants fed TF.

Table 8-1 Summary of the results from the RCT of different energy infant formulas [++]

	RE formula[@] (n = 53)	TF formula[@] (n = 54)	p
Weight SDS			
Birth	0.13 ± 0.70	0.10 ± 0.63	0.8
3 weeks	-0.10 ± 0.79	-0.06 ± 0.72	0.8
6 weeks	0.04 ± 0.77	0.19 ± 0.67	0.3
3 months	0.10 ± 0.83	0.21 ± 0.73	0.8
6 months	0.10 ± 0.89	0.09 ± 0.79	1.0
9 months	0.14 ± 0.96	0.07 ± 0.82	0.7
12 months	0.06 ± 0.90	0.02 ± 0.88	0.7
Length SDS			
Enrolment	-0.23 ± 0.94	-0.22 ± 0.85	0.9
3 weeks	0.05 ± 0.90	0.04 ± 0.80	1.0
6 weeks	0.21 ± 0.94	0.33 ± 0.94	0.5
3 months	0.35 ± 1.03	0.36 ± 0.76	0.9
6 months	0.30 ± 1.09	0.42 ± 1.05	0.6
9 months	0.38 ± 1.11	0.39 ± 1.16	1.0
12 months	0.21 ± 1.00	0.47 ± 1.04	0.3
Subscapular SF (mm)			
Enrolment	5.7 ± 0.9	5.9 ± 1.2	0.3
3 weeks	6.8 ± 1.2	7.3 ± 1.3	0.07
6 weeks	7.1 ± 1.2	7.9 ± 1.4	***0.005***
3 months	7.8 ± 1.5	8.5 ± 1.3	***0.014***
6 months	7.6 ± 1.5	7.9 ± 1.6	0.4
9 months	7.7 ± 1.9	8.0 ± 1.8	0.4
12 months	7.2 ± 2.1	7.0 ± 1.7	0.6
Total energy expenditure (kcal/kg/day)[#]	80.6 ± 22.3	78.4 ± 22.6	0.7
Milk intake (ml/kg/day)[#]	172 ± 24.6	158 ± 22.8	***0.03***
Calculated nutrient intake at age 3 months			
Fat (gm/kg)	4.9 ± 0.7)	5.8 ± 0.8	***0.001***
Protein (gm/kg/day)	2.3 ± 0.3	2.1 ± 0.3	***0.04***
Energy (kcal/kg/day)	102 ± 15	106 ± 15	0.4

[++] Data are shown as means ± SD; Independent sample t-test was used to test the difference between the 2 formula groups.

[@] RE= Reduced Energy formula, TF= Standard energy infant formula, detailed composition shown in Appendix A

[#] Measured by doubly-labelled water at the age of 3 months; n=32 and 43 in RE and TF

Table 8-2 Comparison of infant growth and BC according to diet group[&]

	Breast-fed (n=46)	RE formula (n = 53)	TF formula (n = 54)	p
Birthweight (kg)	3.54 ± 0.41	3.54 ± 0.34	3.51 ± 0.32	0.87
Gestational age	40.0 ± 1.3	40.1 ± 1.4	40.0 ± 1.2	0.87
Birthweight SDS	0.14 ± 0.73	0.14 ± 0.71	0.11 ± 0.63	0.96
Boys (n, %)	16, 34.8%	24, 45.3%	26, 48.1%	0.38
First-born (n, %)[§]	23, 50.0%	12, 25.0%	19, 38.0%	***0.04***
Δ weight SDS 0-6 weeks[#]	0.04 ± 0.72	-0.08 ± 0.57	0.06 ± 0.43	0.34
Δ weight SDS 6-12 weeks[#]	-0.19 ± 0.38	*0.07 ± 0.44*	*0.02 ± 0.35*	***0.04***
Δ weight SDS 0-12 weeks	0.03 ± 0.76	-0.02 ± 0.78	0.08 ± 0.56	0.82
Weight SDS 6 weeks	0.31 ± 0.67	0.04 ± 0.77	0.19 ± 0.67	0.31
Length SDS 6 weeks	0.29 ± 0.91	0.24 ± 0.94	0.38 ± 0.84	0.73
Triceps 6 weeks (mm)	6.6 ± 0.8	*7.4 ± 1.5*	*7.6 ± 1.6*	***0.03***
Subscapular 6 weeks (mm)	6.6 ± 0.8	**7.1 ± 1.2**	***7.9 ± 1.4***	***<0.001***
Weight SDS 12 weeks	0.17 ± 0.79	0.10 ± 0.83	0.21 ± 0.73	0.78
Length SDS 12 weeks	0.53 ± 0.90	0.33 ± 0.90	0.45 ± 0.70	0.53
Triceps 12 weeks (mm)	7.6 ± 1.2	8.3 ± 1.7	*8.7 ± 1.8*	***0.004***
Subscapular 12 weeks (mm)	7.2 ± 1.1	**7.8 ± 1.5**	***8.5 ± 1.3***	***<0.001***
FM 12 weeks (kg)[##]	1.4 ± 0.49	**1.35 ± 0.43**	**1.60 ± 0.47**	0.12
FFM 12 weeks (kg)[##]	4.44 ± 0.49	4.49 ± 0.51	4.32 ± 0.51	0.40
FMI 12 weeks (kg/m^2)[##]	3.91 ± 1.27	**3.70 ± 1.14**	**4.31 ± 1.21**	0.15
FFMI 12 weeks (kg/m^2)[##]	12.20 ± 1.20	**12.35 ± 1.06**	**11.69 ± 1.12**	0.07

[&] One-way ANOVA was used to test the mean difference of continuous variables between the 3 diet groups, highlighted p values were significant at <0.05; Chi square test was used to test the difference in categorical variables between groups.

Highlighted and italic figures were significantly different ($p<0.05$) from breast-fed group by Dunnett's post hoc test; bold figures showed the significant difference ($p<0.05$) between the 2 RCT groups by independent sample t-test.

[§] Parity data was missing in 9 subjects

[#] n=21, 47, 48 for 6 weeks measurement and n=46, 46, 48 for 12 weeks measurement in breast-fed, RE formula, TF formula, respectively

[##] n=41, 26, 32 for breast-fed, RE formula, TF formula, respectively

8.3.2 The effects of early nutrition on later BC

The comparison of BC outcomes during adolescence between the 2 RCT diets groups and the breast-fed group is shown in **Table 8-3** and **Figure 8-1**. There was no significant difference between RE and TF groups in later body size or composition. The breast-fed group was significantly older than the two RCT formula groups due to the fact that cohort C was followed-up at 18.2-20.4 years old. Not surprisingly, the breast-fed group also had a significantly higher number of subjects in a higher social class than the 2 formula groups. There was no statistically significant difference in BC between the 3 diet groups despite a trend towards a lower BMI, FMI and waist circumference in the adolescents who had been breast-fed rather than formula-fed.

Table 8-3 Comparison of adolescent body size and composition according to infant diet group[&]

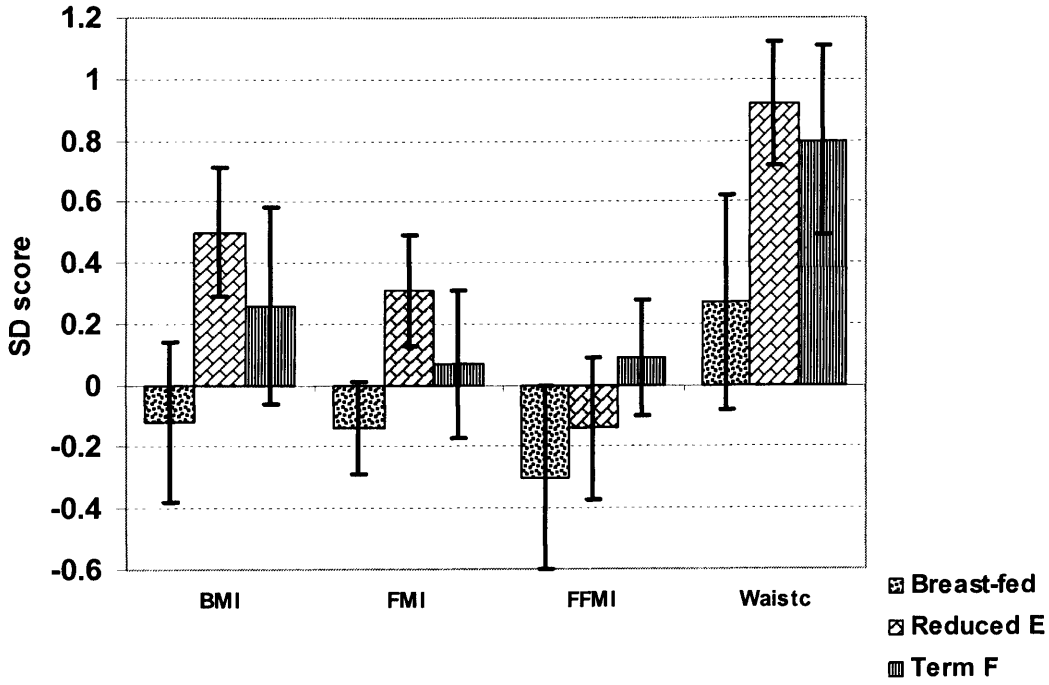
	Breast-fed (n=17)	RE formula (n = 15)	TF formula (n = 16)	p
Current age	16.1 ± 3.3	13.3 ± 0.8	13.0 ± 0.8	***<0.001***
Boys (n, %)	8, 47.1%	5, 33.3%	6, 37.5%	0.72
Early puberty (n, %)	6, 35.3%	10, 66.7%	11, 68.8%	0.10
More/much more active (n,%)	10, 58.8%	5, 33.3%	7, 43.8%	0.20
Social class ≤ 2 (n, %)	14, 82.4%	4, 26.7%	7, 43.8%	***0.005***
Maternal BMI	25.5 ± 5.0	25.5 ± 5.9	26.3 ± 5.1	0.88
Paternal BMI	25.8 ± 3.9	26.0 ± 3.8	26.2 ± 3.6	0.96
Height SDS	0.56 ± 0.99	0.05 ± 1.00	0.42 ± 0.85	0.30
BMI SDS	-0.12 ± 1.09	0.50 ± 0.82	0.26 ± 1.28	0.27
FMI SDS	-0.14 ± 0.63	0.31 ± 0.67	0.07 ± 0.95	0.28
FFMI SDS	-0.30 ± 1.23	-0.14 ± 0.86	0.09 ± 0.76	0.52
Triceps SDS	0.48 ± 0.70	0.64 ± 0.59	0.23 ± 1.24	0.43
Subscapular SDS	0.19 ± 0.83	0.48 ± 0.90	0.08 ± 1.28	0.53
WC SDS[#]	0.27 ± 1.12	0.92 ± 0.76	0.80 ± 1.23	0.30
TrunkFMI SDS[##]	-0.17 ± 0.67	0.23 ± 0.80	0.07 ± 1.09	0.50

[&] One-way ANOVA was used to test the mean difference of continuous variables between the 3 diet groups; Chi square test was used to test the difference between categorical variables between groups.

[#] n=10, 15, 16 for breast-fed, RE formula, TF formula since the database for waist SDS was available only up to the age of 17

[##] n=16, 13, 15 for breast-fed, RE formula, TF formula since DXA data were not available for 5 home visit subjects

Figure 8-1 Comparison of adolescent body composition between the 3 diet groups[#]



[#] n=17, 15, 16 for breast-fed, RE formula, TF formula, respectively. Each bar chart represents means SDS and y-error bar represent SE of mean.

8.4 Discussion

In the following discussion, I will focus on 1) comparison of infant growth and BC between the 2 formula groups and also between the breast-fed and formula-fed infants, and 2) the long-term influence of early nutrition on later BC. The implication of these findings will be discussed later in **Chapter 9**.

8.4.1 The effects of early nutrition on infant growth and BC

Different energy infant formulas

Healthy term infants randomly assigned to receive the RE formula in this trial showed no significant differences in growth in terms of weight, length, or change in weight SDS compared to infants fed the TF formula. Despite the fact that the energy content was reduced to mimic that in breast milk (as measured non-invasively by stable isotopes rather than in samples of expressed breast milk—details are shown in **Appendix A**), infants who received the RE formula did not demonstrate the slower weight gain typically seen in breast compared to formula fed infants (239-243). One explanation could be that infants fed RE formula up-regulated their milk volume intake resulting in similar energy intakes to the TF group by the age of 3 months. This regulation of intake following dietary manipulation has been reported in other studies by Fomon et al (29;30) and Brooke et al (28). It seems not to operate during the first 6-8 weeks of life but to become more obvious after this age. This might also explain why the difference in adiposity indices was greatest at age 6 weeks to 3 months but not significantly different after that.

My analyses confirm that although body *weight* was not different between the 2 diet groups, infant BC can be manipulated by different nutrient intakes in early life. The RE group showed a significantly lower FM and higher FFM at age 12 weeks than the TF group. The results corresponded with the calculated lower fat and higher protein intake in the RE group. Although body composition data earlier than 12 weeks were not available, SF measurements at 6 weeks of age suggested similar findings, with lower values in the RE group. This finding is in agreement with a study by Schulze et

al (105), who studied preterm infants and demonstrated that the relative composition of the new tissue deposited reflected the proportional intakes of protein and energy. In addition, a previous study (103) of protein-energy ratio in term infants showed that infants fed with a lower protein-energy ratio (1.7 gm/100 kcal) gain more weight but not length, suggesting more fat accumulation when compared to a higher protein-energy ratio (1.8 to 2.7 gm/100 kcal). Thus, the fact that RE formula group had a higher protein-energy ratio than TF group (2.25 vs. 2.02 gm/100 kcal) might contribute to the higher FFM and lower FM, even though absolute energy intake was similar between the 2 groups at that time (age 3 months).

Breast-fed and formula-fed groups

There are a large number of studies examining the growth patterns of breast-fed and formula-fed babies. They generally agree that the average weight gain of the former is lower than that of the latter, especially after complementary foods are introduced. Because of this difference, breast-fed infants are generally leaner (by SF) than formula-fed infants by 12 months of age (242;244). In my analyses, breast-fed babies from cohort B and C showed slightly less weight gain than both formula groups from 6-12 weeks of age, a finding supported by Nelson et al, who reported lower weight gain in 419 breast-fed compared to 720 formula-fed infants from 42-112 days of age (245) but slightly earlier than other studies which usually found the difference after 4 months of age (239;240;243). Indeed, this difference can also be seen between the infants who were breast-fed and those who received RE formula, which was designed specifically to be more similar to breast milk; this could probably be explained by the up-regulation of milk volume intake or the higher protein: energy ratio of the diet in the RE group. Moreover, I also demonstrated that breast-fed babies had lower adiposity (measured by SF) than the formula-fed groups (particularly TF group) at 6 weeks and 12 weeks. This SF result was consistent with a trend from the isotope study that showed a lower FM and higher FFM in the breast-fed group compared to the TF group. This finding, again, is supported by other studies comparing breast-fed and formula-fed infants, which generally report lower SF in breast-fed infants; however, in those studies, the difference typically became apparent after the first 4 months of life (242).

To recap, I have demonstrated that infant BC can be influenced by manipulating nutrient intakes during early infancy. Although differences in growth and body composition seem to be transient, data from animal studies show that this could have long-term programming effects which might 'emerge' later in childhood or adolescence.

8.4.2 The effects of early nutrition on later BC

As reported in the previous chapter, I did not find an association between infant BC at 12 weeks of age and later BC, even though there was some evidence (from SF results) that infant 'fatness' at 3 and 6 weeks might have a long-term effect on BC in adolescence. Therefore, it is perhaps not surprising that, although I can demonstrate clear effects of infant diet on infant BC at 12 weeks, there is no evidence that this in turn has any longer term effect on BC. Moreover, the difference in SF at 6 weeks (which is the period during which SF showed a significant association with later BC in the previous chapter) between the 2 diet groups did not seem to have a long-term effect on later BC. In fact, this finding is in agreement with other studies of different infant formulas in that the effect of different diets on infant growth and BC is apparent during infancy (and may be dramatic)(107-110) but does not generally persist at follow-up (111;112). Unfortunately, the sample size in my follow-up study was very small (around 16 in each RCT group). Consequently I only had the power to detect a difference of 1 SD between groups. This limits my ability to draw conclusions about whether BC differences due to dietary manipulation in infancy result in differences during adolescence. Another possibility to be considered is that the effect may not become apparent until later on in life i.e. not until after puberty as in the baboons (13;14).

Despite fairly consistent data from a number of studies, including recent systematic reviews, suggesting a long-term protective effect of breast-feeding against later overweight and obesity (defined by BMI above a percentile cut-off) (246-248) or the reduction of average level of 'adiposity' (i.e. mean values of BMI)(249), the long-term effects of breast feeding on later *body composition* have rarely been reported. Butte et al reported that (117) the differences in infant body composition (by multi-

component model) at 3 and 6 months between breast-fed and formula-fed infants did not persist into the second year of life. However, the DARLING study, which used SF as a measurement of fatness, found that when infants were breast-fed throughout the first year of life, the difference in adiposity between breast and formula-fed infants persisted until at least 18-24 months of age. In my study, despite the fact that there was no statistically significant difference between BC in adolescents previously breast-fed compared to those formula-fed, there was a trend towards lower BMI, FMI, and waist circumference in the breast-fed group, which supports the BMI data above. This effect also corresponds with my findings of slower weight gain and lower SF in breast-fed infants at 6 and 12 weeks, and this slower growth and reduced fatness early in infancy might partly explain any long-term influence of breast-feeding on later BC. Again, due to the very small sample size at this follow-up, a bigger study is needed to verify this assumption.

8.5 Summary

- Early infant growth and infant BC can be manipulated by different nutrient intakes in early life. The reduced energy formula group, who had lower fat and higher protein intakes, showed lower FM and higher FFM compared to the standard term formula group at the age of 12 weeks.

- Breast-fed infants showed a slower weight gain and lesser adiposity by SF measurement at 6 and 12 weeks compared to formula-fed infants.

- Unfortunately, the sample size in this follow-up study was very small. The long-term effects of early nutrition on later BC are not conclusive. There was no evidence for a long-term effect of the different infant formulas on body composition, although subjects who had been breast-fed showed some evidence of reduced fatness. These findings are limited by the small sample size.

Chapter 9

Overall Discussion and Conclusions

Chapter 9 Overall Discussion and Conclusions

9.1 Summary of the findings

In **Chapter 3**, I set out 2 main hypotheses. The first was to test whether early growth can program specific components of body composition later in life, and thereby potentially influence the development of obesity and cardiovascular risk factors. I found that birthweight (regarded as a proxy for prenatal growth) is positively related to later height in both sexes, and positively associated with FFM in boys. In contrast, postnatal growth during the first 6 months of life positively influences later FM and central fat distribution, with a weaker effect on FFM. Hypothesis I was therefore confirmed using the 4C model.

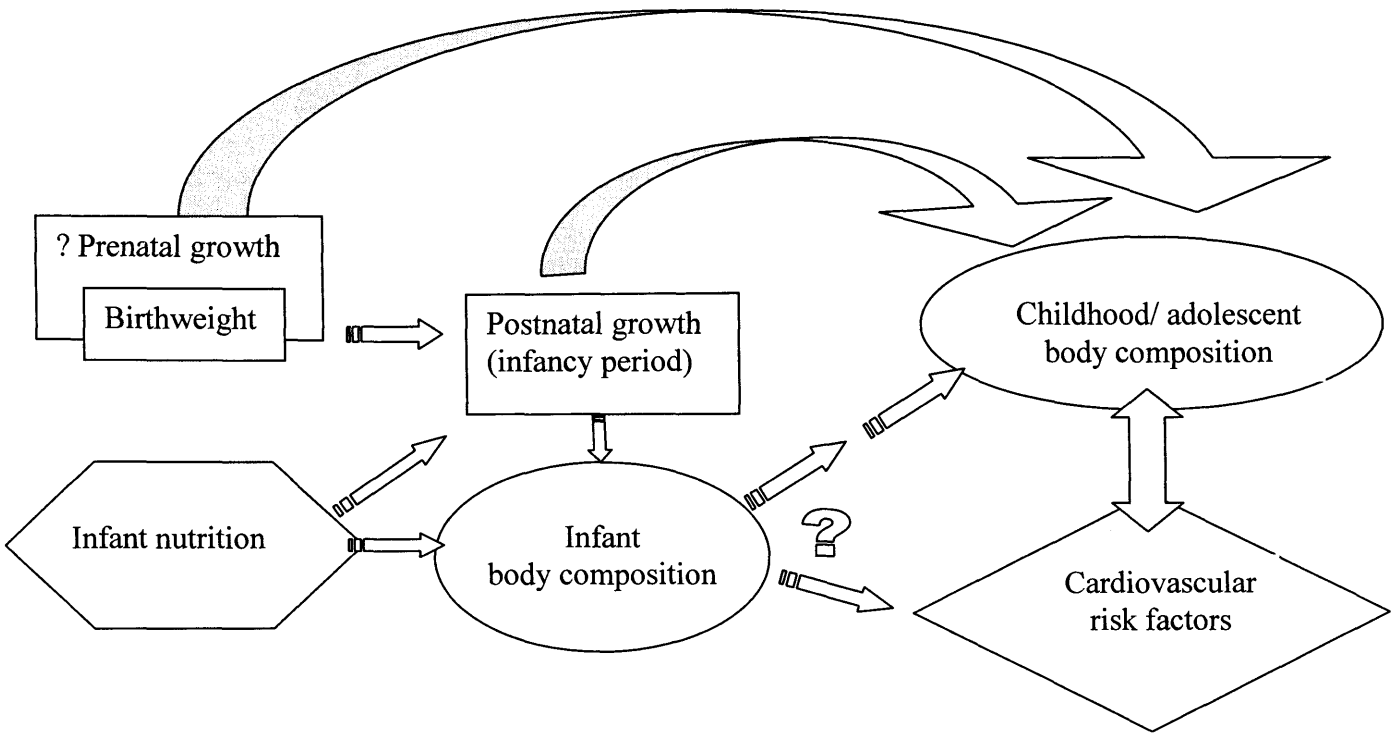
In **Chapter 6**, I demonstrated that whilst FM showed a consistent association with most measured cardiovascular risk factors except HDL-C, FFM showed a strong negative association with HDL-C that was independent of FM and central fat distribution. This supports the concept that BC programming by prenatal or postnatal growth may have a differential impact on health outcomes—at least as assessed using proxy measures in an adolescent population.

I could neither confirm nor refute my second hypothesis that components of infant BC are differentially related to later BC. In **Chapter 7**, I found no significant effect of infant BC at the age of 12 weeks on later BC, but the sample size was rather small. With 41 subjects who had BC measurements at both 12 weeks (by isotope) and later follow-up, I would only be able to detect a significant association if the correlation between infant BC and later BC was higher than 0.42 (183) - i.e. the results would be significant only if infant BC explained around 18% of the variance in later BC (which is around 2.5 times more than the proportion of the variance explained by birthweight or postnatal growth). Therefore, a bigger study is needed to definitively answer this question. On the other hand, based on my findings, it could be argued that 12 weeks of age might be beyond the critical window for programming of BC,

since my SF data support the concept that the critical window could be earlier than this.

Lastly, in **Chapter 8**, I confirmed that postnatal nutrition can affect both infant growth and infant BC. I could not demonstrate any effect of infant nutrition on later BC; however, a bigger study with long-term follow-up is needed to address this issue.

Figure 9-1 Diagram of the potential inter-relationships between early growth, infant BC and later BC



9.2 Potential mechanisms for BC programming

9.2.1 The mechanism by which prenatal growth may program later FFM

There are several plausible explanations for the observed association between birthweight and FFM in childhood and adolescence. For instance, it has been shown that in humans the number of muscle fibres is set before birth (43;250) and hyperplasia does not occur to any significant extent during postnatal life. If this is the case, it would seem unlikely that post-natal growth beyond the 'window' during which new muscle fibres are developing would be associated with FFM later in life. However, there is evidence from rats (251) that protein restriction immediately after birth resulted in reduced muscle mass during adult life, indicating that there is an early postnatal period during which muscle development (e.g. differentiation and hypertrophy) is also highly sensitive to nutritional insults. This could perhaps explain the relationship observed in some studies between early post-natal growth and later FFM (see later).

Fetal growth constraint due to undernutrition could result in lower IGF-1 and thus compromise muscle growth and skeletal length in utero (252). There is indeed some evidence that small for gestational age infants show a larger deficit in lean mass (by DXA) than in FM (253). These 'smaller' babies might then be expected to have lower FFM in later life. It could also be argued that fetal muscle and skeletal growth is determined (at least in part) by an individual's genetic potential (40;254;255) and that this is responsible for the relationship with later FFM. This idea cannot, however, explain the association between the intrapair difference in birthweight of monozygotic twins and later FFM described by Loo et al (78;79). Therefore, I suggest that the mechanism by which FFM is programmed probably involves insults or stimuli (e.g. nutrition) in fetal life, and to a lesser extent, early postnatal life.

Catalano et al (40) showed that male neonates had higher FFM than females, which corresponds with the well-recognized gender differences in body composition during adolescence and adult life. I propose that this might be one of the reasons why I found that the relationship between birthweight and FFM was more obvious in boys.

Using birthweight as a proxy for prenatal growth has some limitations for the study of body composition programming. There is some evidence, for example, that body composition at birth may be different even when birthweight is the same and/or falls within the 'normal' range. Catalano et al (256) reported that the offspring of mothers with gestational diabetes have increased body fat (by total body electrical conductivity) compared with infants of women with normal glucose tolerance. Yajnik et al (257) showed that Indian babies have a more adipose body composition despite being 'small and thin' relative to UK infants. The later effects of having more 'fat' or more 'lean' , albeit with the same birthweight, clearly warrant further investigation and may provide more information about the mechanism by which FFM is programmed.

9.2.2 The mechanism by which infant growth may program later FM and central fat distribution (and, to a lesser extent, FFM)

Prenatal influences on infant growth

Data from the ALSPAC study (Avon Longitudinal Study of Pregnancy and Childhood) confirm that around a quarter of contemporary newborn infants show significant catch-up growth (defined as weight crossing more than one centile band upwards) whereas a further quarter show postnatal 'catch-down' growth (85). Infants who show catch-up growth tend to be longer with a larger head circumference at birth relative to their birthweight (suggesting reduced adiposity). Moreover, maternal factors that might be expected to constrain fetal growth (e.g. nulliparity, poor pregnancy weight gain, and smoking) are closely related to catch-up growth (239). The researchers suggested that the postnatal growth pattern might be driven by the regulation of satiety, based on the

finding that the cord blood level of leptin was positively related to birthweight and subsequent weight gain (258); consequently, they proposed that inherent patterns of increased or decreased appetite and satiety may help to return the infant who experienced growth retardation in late gestation towards its genetic growth trajectory. On the other hand, there is also convincing evidence that infant feeding practices (breast or formula milk, formulas with different composition, age at weaning) have a strong effect on infancy weight gain (24;243;259;260). Moreover, the pattern of growth among small for gestational age infants can also be manipulated by nutritional intake (112), at least initially (28). As a result, I conclude that the pattern of infant growth is determined by both antenatal factors and by the postnatal environment. Important unanswered questions remain: 1) do the factors that determine birthweight (e.g. those related to constraint of fetal growth) influence both the pattern of infant growth and later BC? 2) do the programming effects of infant growth on later BC depend on or interact with birthweight, i.e. does infant growth have a long-term influence on later BC only in low birthweight babies?

With respect to the second question, I showed in **Chapter 5** that early growth in the first half of infancy has a positive association with later FM and central fat distribution regardless of birthweight. In fact, in my dataset only 11 out of 191 subjects had birthweight less than 10^{th} centile for gestation. Moreover, I used separate adjustment for FM and FFM; therefore, the higher FMI associated with infant growth found here is unlikely to be confounded by lower FFMI related to lower birthweight (which might be the case if percentage fat was used as discussed in **Chapter 4)**. Therefore, I propose that rapid infant growth can programme later fatness *regardless* of whether it is driven primarily by preceding slow fetal growth or by postnatal 'overnutrition'.

In **Chapter 5**, I also highlighted the discrepancy in findings from non-western and western populations. In the former, early postnatal growth seemed to have a greater long-term influence on FFM; this was less obvious in the latter. There is evidence that muscle growth during early postnatal life relies largely on protein accretion leading to muscle fibre hypertrophy (261) and is susceptible to protein restriction as discussed

above. One potential explanation for the apparent population differences could be that, in non-western populations where the intrauterine development of muscle fibres might be less optimal, there is a greater potential for growth and nutrition in the early postnatal period to influence later FFM. The findings of Yajnik et al (257) that Indian infants are more 'adipose' despite being smaller at birth than UK infants lends some support to this idea. According to this argument, fetuses in the western world would have (on average) better (or more 'complete') development of muscle mass in utero, hence early postnatal growth might have less influence on their later FFM. In my data, I found a weak association of postnatal growth between 3-6 months and later FFM in boys, which seems to support this concept since males generally develop larger skeletons and higher muscle mass than females. This might make these associations more obvious.

What mediates the association between 'early growth' and later BC?

An important question is whether the association between early 'growth' (generally early weight gain) and later BC is mediated through *structural* changes in infant BC which then track through adolescent and adult life, or through some other mechanism that does not involve infant BC. If the latter is true, weight gain might be regarded as essentially an 'epiphenomenon'. From the results discussed in **Chapter 7**, the evidence implicating infant BC in the programming process was not strong, although my conclusions were limited by the relatively small sample size. Infant BC at 12 weeks of age was not associated with later BC, although a proxy for infant fatness (SF) at 3 and 6 weeks seemed to have some long-term influence on later BC, which might indicate that the critical period for any programming effects of infant body composition are earlier than 12 weeks. The other possibility, not tested in my study, is that early infant growth programs later BC by changing some *functional* component(s) that in turn regulate the subsequent development of fatness. Likely candidates include the set-point of hormones regulating growth or appetite such as insulin, IGF-1, or leptin (26;262;263). Such effects could persist and explain the observed long-term influence on later BC and risk of obesity.

Is early nutrition involved in the mechanism?

From **Chapter 8** and other published work on nutritional manipulation during infancy (see **Chapter 2**), there is good evidence that infant growth and BC can be changed by nutritional intake. There is little evidence for long-term effects of different infant formulas on later body composition, even though they may produce fairly marked differences in infant growth. On the other hand, breast-feeding seems to be associated with long-term effects on later BC (decreased fatness, seen as a trend in my study, and reported in a significant number of studies on breastfeeding and later obesity defined by BMI). This observation might support the idea that the mechanism by which infant growth programs later fatness or obesity risk is more complicated than a simple effect of macronutrient intake on infant tissue accretion. It has been suggested that the apparent benefits of breastfeeding for later obesity risk are due to the slower early growth seen in breast-fed infants. However, if this is the mechanism, it is surprising that the same effects are not seen in infants fed lower energy formulas, and showing reduced fatness in early life. In my opinion, these observations fit with the concept that functional mechanisms link early growth and later BC — for example, learned self-regulation of energy intake or metabolic programming, as proposed by Dewey KG (264).

Studies in animal models support the idea that nutrition in early postnatal life is influential for long-term appetite regulation through the effects of two important hormones—insulin and leptin (26;27;265;266). On the other hand, BC in early life has not been explored in animal models. Available data in humans suggest that plasma insulin, which stimulates greater adipose tissue deposition during childhood (267), can be influenced by the mode of feeding. Formula-fed infants have been shown to have higher plasma insulin levels at 6 days of age compared to breastfed infants (268), although it is not known how long this difference persists. Insulin secretion in formula-fed infants might be stimulated by higher protein intake. An association between high protein intake in early life and overweight in childhood has been reported by some researchers (269;270). Leptin is a key regulator of appetite and body fatness. It has been suggested that breastfeeding might affect leptin metabolism during infancy and later in

life, either via direct exposure to leptin in human milk or indirectly via the rate of early weight gain (264). There is some supporting evidence that the early diet of preterm infants (human milk vs. formula) is associated with plasma leptin concentration at 13-16 years of age (100)—a lower leptin to FM ratio was seen in subjects who were fed with human milk, suggesting they might be less leptin resistant.

To recap, I think that the mechanism by which early growth 'programs' later BC involves both prenatal and postnatal nutrition. Prenatal nutrition influences the development of FFM in utero and also affects postnatal catch-up growth to a certain extent. Infant nutrition might program later FM through effects on hormonal regulation or infant fatness. These potential mechanisms warrant further investigation, since this information could be useful in terms of designing effective early intervention to reduce obesity prevalence in childhood and adolescence.

9.3 Implications for public health

Early intervention

For the purpose of comparison, the relative contributions of birthweight, infancy weight gain, and other factors that might affect later BC are shown in **Table 9-1**. Birthweight can explain 7.8% of the variability in FFMI whereas Δweight SDS 0-6 months can explain a further 3%. Change in weight SDS from birth to 6 months accounts for 7.3% and 9.1% of the variance in FMI and trunk FMI, respectively. The percentages of variance explained by these early growth parameters seem to be similar to the effect of parental BMI, but greater than those of physical activity. Of course, there are other unidentified or unmeasured contributing factors that might have a bigger impact on later BC (e.g. other inheritable factors, current life style, dietary intake or physical activity that cannot be accounted for by the rating assessment used). However, my data show that later BC is determined to a certain extent by early growth, which can be manipulated through nutrition either prenatally or postnatally, and that the effect size is comparable to that of other factors considered important in terms of interventions to

prevent obesity. Hence the findings may have implications for public health; for instance, I propose that interventions aimed at altering early growth might be as effective as those aimed at changing current physical activity levels. In my study the proportions of overweight and obese children (by IOTF cut-off (119)) were around 21.8% and 5.5%, respectively which is similar to the average UK population (4); therefore, my findings should be reasonably generalisable. However, whether the direction and magnitude of the observed associations are similar in normal-weight and obese children is another issue to consider and would require a larger sample size than that was available in my study.

In a recent systematic review, Kramer and Kakuma (271) reported that nutritional interventions during pregnancy aiming to improve birthweight are only moderately successful. They reported that nutritional advice to increase energy and protein intake was successful in achieving those goals, but no consistent benefit was observed on pregnancy outcomes. In 13 trials involving 4665 women (most were from under-nourished populations), balanced energy/protein supplementation was associated with modest increases in maternal weight gain and in mean birthweight, and a substantial reduction in risk of SGA birth. Since SGA infants are more likely to show rapid infant growth, it could be argued that reducing low birthweight might reduce the risk of later fatness/obesity, by removing the stimulus for catch-up during the post-natal period. However, high-protein or balanced protein supplementation alone was not beneficial and was associated with an increased risk of SGA birth. There is a recent study showing that the type of carbohydrate in the maternal diet influences infant birthweight; gravidas with a low dietary glycemic index had reduced infant birthweight and a twofold increased risk of a SGA birth (272) but the effect of increasing the dietary glycemic index on reducing risk of SGA birth still needs more research.

Since my findings suggest that only postnatal growth actually has a long-term influence on later FM, and given the limited success of pregnancy interventions, I consider that, for the purpose of reducing obesity in childhood and adolescence, focusing on infant

growth in the first 6 months of life (which depends greatly on nutrition) may be more successful. This supports the promotion of breast-feeding since from the evidence so far it seems to protect against obesity (defined by BMI) in childhood and in my small study there is also a trend towards lesser adolescent FM in the breast-fed group. Another alternative is to reduce the energy or protein content in infant formula. However, as discussed previously, although infant growth and body composition can be manipulated by differences in energy or protein content of infant formula, so far there is no evidence that the effect lasts beyond infancy period. Clearly, more research is needed in this area.

Table 9-1 Coefficient of determination (r^2) for birthweight SDS, Δ weight SDS 0-6 months, and other confounders for explaining BC outcomes in childhood and adolescent[#]

	Height SDS			BMI SDS			FMI SDS			FFMI SDS			TrunkFMISDS		
Independent variables	**B**	**SE**	**r^2**	**B**	**SE**	**r^2**	**B**	**SE**	**r^2**	**B**	**SE**	**r^2**	**B**	**SE**	**r^2**
Birthweight SDS	0.39	0.09	0.116	0.32	0.11	0.059	0.10	0.09		0.33	0.10	0.078	0.16	0.09	
Δ Weight SDS 0-6 months	0.42	0.09	0.151	0.36	0.10	0.084	0.30	0.08	0.073	0.19	0.09	0.030	0.31	0.08	0.091
Gender	0.07	0.14		0.30	0.17	0.021	0.08	0.14		0.03	0.15		0.09	0.14	
Puberty	0.15	0.09	0.023	-0.11	0.10		-0.11	0.08		0.08	0.09		-0.09	0.09	
Physical activity	0.14	0.09		-0.14	0.11		-0.28	0.09	0.064	0.17	0.10	0.020	-0.21	0.09	0.035
Social class	-0.05	0.07		-0.01	0.09		-0.01	0.07		0.03	0.08		-0.01	0.07	
Ethnicity	0.15	0.24		-0.20	0.29		0.04	0.24		-0.42	0.26		0.04	0.24	
Maternal BMI	0.001	0.02		0.08	0.02	0.121	0.06	0.01	0.104	0.06	0.02	0.074	0.06	0.02	0.104
Paternal BMI	0.02	0.02		0.08	0.03	0.080	0.05	0.02	0.048	0.04	0.02	0.032	0.06	0.02	0.057

[#] From multiple regression analysis, all independent variables were entered simultaneously in the same model for each of the BC outcomes which was a dependent variable; B = the coefficient of each independent variable, SE = standard error, r^2 = coefficient of determination (calculated from partial r) n=150 due to missing data for parental BMI

Puberty was coded as: 1 = prepubertal, 2 = early pubertal, 3 = late pubertal
Physical activity was coded as: 2= much less or less active than peers, 3= same as peers, 4= more active than peers, 5= much more active than peers
Social class ranged from 1 which is the highest to ≥ 4 which is the lower social class
Ethinicity was coded as: 1 = white, 0 = non-white

Impact on later health

In my study, I demonstrated that early growth has a significant influence on BC in childhood and adolescence and that BC in turn had a significant association with cardiovascular risk factors. Thus, I think it is likely that childhood and adolescent BC may provide a 'link' between rapid early growth and cardiovascular risk factors in adolescence, although I could not directly test this concept in my study since cardiovascular risk factors were not measured in the larger cohort. In **Chapter 6**, I showed that FM and FFM in healthy adolescents are differentially related to lipid, insulin, and blood pressure profiles. There is some evidence that these cardiovascular risk factors track from adolescence into adult life (273-275). However, the long-term clinical impact of variation in plasma lipids or insulin during adolescence is not clear, particularly as most values were considered to be within the normal range. It is possible that the 'unfavourable' cardiovascular risk factors found in young people with higher FM might amplify over time. These questions are difficult to answer since the indices measured early on are predictors of the disease, rather than early markers of the pathological process. Assessment of the earliest stages of atherosclerosis might help to clarify the situation. There are recent studies showing that obese children had increased arterial stiffness (276;277) and carotid intima-media thickness (277) which are markers of early atherosclerosis. These effects appear to be mediated, at least in part, by insulin resistance. Therefore, it seems that these changes could begin in childhood and potentially accumulate overtime. However, whether these associations apply over the whole range of lipid and insulin concentration or only beyond a certain cut-off needs further investigation.

We know that BMI tracks from childhood and adolescence through to adult life with a more stable pattern of tracking as individuals grow older (83;278;279), but little is know about the 'tracking' of the FM and FFM components of BMI. Demerath et al (134) reported from 494 children age 8-18 years that whereas FFMI (by hydrodensitometry) had a linear relationship with BMI percentile, FMI tended to increase sharply only at a higher BMI percentiles; therefore, BMI percentile change may not reflect changes in adiposity, especially in male adolescents and children with lower BMI. To date, there is still no direct evidence showing the extent to which

the FM or FFM components of BMI in childhood and adolescence 'track' into adult life.

A further question is whether the fatness that is associated with some markers of cardiovascular *risk* in childhood and adolescence will remained associated with morbidity and mortality from chronic diseases such as coronary heart disease or stroke in later life. Although it is well-recognized that adult BMI is strongly associated with cardiovascular disease morbidity and mortality, there are a limited number studies examining the association between childhood and adolescent BMI and later cardiovascular *disease,* and the results were inconsistent. Some studies have reported that higher BMI in childhood and adolescence is associated with higher coronary heart disease *mortality* among men (123;280) or in both sexes (125). However, more recent studies in 4 UK historical cohorts (124;281) did not support an association between childhood BMI and later risk of coronary heart disease and stroke. In my opinion, since these cohorts were born between the 1920s and 1950s when the prevalence of overweight and obesity was lower than in contemporary children and the environment was less obesogenic (i.e. less energy-dense food and a more active life style), it is difficult to extrapolate the findings to modern children. The findings from Wells et al (52) also support this concept. They found that contemporary children have more FM for the same BMI than Fomon's reference children (36) from the previous 2 decades. In addition, despite a report on the differential effects of FM and FFM on lipid profiles in middle-age men and women (214), the study of the influence of FM and FFM component of BMI in childhood on later health outcomes is hardly reported. Therefore, in order to answer this question more clearly, more longitudinal follow-up study is required.

9.4 Limitations of the study

Despite giving more accurate results, the 4C model requires relatively complex measurements and specialized equipment. In **Chapter 4**, I showed that using the 4C model or DXA to assess body composition in a paediatric population gave a slightly different answer to the research question of whether there was any association

between birthweight and later FM (FM derived from DXA or SF equations showed a weak positive association with birthweight whereas 4C model FM did not). Since it is more difficult, expensive and time-consuming to use the multicomponent models of body composition in a large study, one needs to consider the benefits of a small study with very accurate measurements and a larger study with simpler measurements.

It is important to consider whether BMI is satisfactory as an outcome for research in this area. In my opinion, this depends on the research question. As discussed in **Chapter 6**, for screening purposes at a population level, BMI and WC might be adequate. However, one should keep in mind that BMI is a composite of FMI and FFMI and does not only reflect 'adiposity'. I think that separate measurements of FM and FFM are essential for investigating the long-term health outcomes of BC programming or investigating health outcomes in populations (e.g. athletes) whose FM and FFM proportions might be different from normal. In a large scale study, a 2C model such as isotope dilution would probably be sufficient. However, it is important to appreciate the limitation of each BC technique used before interpreting the results. The validation of 'field' methods against a 'more accurate' method and the use of assumptions suitable for the population of interest are essential.

My ability to draw meaningful conclusions from the analyses in **Chapter 7** and **Chapter 8** was limited by sample size. In such a small study, I could only detect a strong relationship between infant BC or nutrition and later outcomes, and might have missed a smaller effect that could still be important for health. Therefore, I believe that a bigger study with an acceptable method of BC assessment could provide further information on the mechanisms discussed above. However, in order to conduct a long-term follow-up study with detailed measurements, the initial sample size needs to be large enough to allow for a significant loss to follow-up. The alternative would be to use a very simple outcome measure (for example, current weight and height from telephone interview or questionnaire), which would undoubtedly result in a significantly higher follow-up rate, but with less reliable and informative data. Therefore, I think it is important to consider, for each study, the balance between sample size, follow-up rate and quality of outcome measures.

9.5 Future research

Based on my findings I would propose three areas for further research in the area of body composition programming:

1. A study of body composition at birth in relation to later body composition and health outcomes, to investigate the mechanism by which fetal growth influences later outcomes.

2. Cohorts with prospective assessment of both (i) candidate hormones/ appetite regulators such as insulin or leptin, and (ii) infant body composition assessment would be of benefit to clarify the mechanism by which 'infant growth' and 'infant nutrition' influence later health outcomes.

3. Further investigation of the relationship between different components of BMI (i.e. FM vs. FFM) and health outcomes in paediatric populations with follow-up into adulthood.

9.6 Conclusions

The research documented in this thesis has a number of important implications for future research in this area and, potentially, for public health. These are briefly outlined below:

- FFM in later life is influenced by birthweight – a proxy for prenatal growth - as well as other factors such as genetic potential and life style. A study with measurements of body composition at birth in relation to later outcomes might help to clarify the mechanism underlying this observation.

- Whether infant growth influences later FM through structural changes in infant BC which then track through later life, or through functional changes (i.e. the set-point of hormones regulating growth or appetite) is not clear. In my study, the evidence implicating infant BC in the programming process was not strong, although my

conclusions were limited by the relatively small sample size. There is limited evidence that infant 'fatness' as measured by SF earlier than 12 weeks might have a long-term influence on later BC; this suggests that the period prior to 12 weeks might be important for BC programming. However, this hypothesis requires testing in a larger study.

- It is likely that both prenatal and postnatal nutrition are involved in 'programming' later BC. Prenatal nutrition influences the development of FFM in utero and also affects postnatal catch-up growth to a certain extent. Infant nutrition (particularly breast feeding) might program later FM through effects on hormonal regulation or infant fatness. These potential mechanisms warrant further investigation, since this information could be useful in terms of designing effective early intervention to reduce obesity prevalence in childhood and adolescence.

- It is likely that childhood and adolescent BC may provide a 'link' between rapid early growth and cardiovascular risk factors in adolescence, with differential effects of different components on outcome. FM showed a consistent association with most of the cardiovascular risk factors except HDL-C, while FFM showed a consistent negative association with HDL-C that was independent of FM and central fat distribution.

- For screening purposes in a normal population, BMI and WC are valid tools for identifying children who might be at a higher risk for developing cardiovascular disease in later life. On the other hand, more detailed measurements of BC may be useful for investigating the long-term health outcomes of BC programming or investigating health outcomes in populations whose FM and FFM proportions might be different from normal, such as obese or other patient group.

- The method of statistical analysis (especially body size adjustment) and BC techniques (based on different assumptions which vary between populations) chosen to assess these relationships is crucial for the interpretation of the findings.

Acknowledgements

Many people have assisted me with different aspects of this project. I would particularly like to thank my principal supervisor, Dr Mary Fewtrell, whose guidance, knowledge, and patience has been invaluable to me throughout my PhD process. I am also grateful for the help and expertise of my associate supervisors, Dr Nigel Fuller and Dr Jonathan Wells, for their advice and helpful discussions during the data analysis and preparation of this dissertation. I also would like to thank Professor Alan Lucas, Professor Peter Davies, and Dr Jonathan Wells for allowing me to follow-up their cohorts; Professor Tim Cole and Dr Deborah Ridout for their statistical advice; Jane Williams for training me in all practical aspects of body composition measurement; Catherine Wilson for performing and analysing DXA scans; Ian Merryweather for helping with lipid essays and sample collection; Tegan Darch for deuterium analyses; Dr Kirsten Rennie for expert advice about physical activity data; Clare Storry for vascular physiology measurements; Emma Sutton for tracing subjects in Cambridge; and many research nurses as well as Dr Wells who collected the original data on anthropometry and body composition during infancy.

I would like to express my appreciation to all the children and their families who volunteered to take part in this research; and the staff at the MRC Childhood Nutrition Research Centre and Great Ormond Street Hospital for their ongoing help, support and friendship.

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Information about the work in this dissertation

The work presented in this dissertation was carried out at the MRC Childhood Nutrition Research Centre, Institute of Child Health, London. The practical parts of the project were carried out in the Radiology and Vascular Physiology Departments, Great Ormond Street Hospital.

The main project was conceived and developed in collaboration with my supervisors. I analysed infant body composition data and wrote up the data from the original cohort A (**Appendix A**). I developed and implemented the protocols and recruited all the subjects with help in tracing from Emma Sutton in Cambridge. I measured the body composition of all subjects in Cohorts A, B, and C with help from my colleagues, Jane Williams and Catherine Wilson. I collected and entered all the growth records from Cohort D and also helped with body composition data collection in some members of the cohort. I collected and processed the blood samples and saliva samples with help from my colleagues, Ian Merryweather and Tegan Darch. I performed all the data analyses with expert advice and discussion from my supervisors, and statistical input from Professor Tim Cole and Dr Deborah Ridout.

Appendix

Appendix A

Summary of the randomised controlled trial (cohort A)

Randomised trial of low energy versus standard term formula in healthy term infants: methods and results during infancy

Based on the observation that formula-fed infants might effectively be overfed when consuming current infant formulas, a randomised controlled trial (RCT) was set up in 1991 by Professor Alan Lucas at the Dunn Nutrition Unit in Cambridge to test the following hypotheses:

1) The use of a formula with an energy content similar to that in breast milk (metabolisable energy density around 60 kcals/100ml, as determined by doubly labelled water study (144)) would result in the pattern of growth, body composition and energy metabolism seen in breast-fed babies. If the difference in energy metabolism observed between breast-fed and formula-fed infants is solely or largely due to the difference in energy intake, it should be seen in two groups of formula fed infants consuming diets differing only in energy content.

2) Differences in energy intake, energy metabolism and growth in early life would have a "programming" effect on body fatness and metabolism later in childhood. (Specifically, infants fed the lower energy formula would have reduced body fatness. If the lower energy intake and slower early growth and fatness of breast-fed infants is responsible for the lower risk of later obesity, the same effect should be seen between the two groups of formula fed infants consuming diets differing only in energy content.)

Methods

Healthy term infants (appropriate for gestational age, 37-42 weeks) were recruited from the Rosie Maternity Hospital, Cambridge between 1990 and 1992, with 12-month follow up completed in 1993. All had weight at birth within the 10^{th} and 90^{th}

percentiles of the Cambridge Standards and were free from congenital malformations or conditions known to affect growth or development. Infants were eligible for the study if their parents agreed to provide them with the study formula ad libitum as the sole source of calories from birth to at least 3 months and together with supplementary food thereafter for the duration of the study and agreed not to provide any vitamin or mineral supplements to the infants for the duration of the study.

The infants were randomised onto the reduced energy formula (RE) or standard term formula (TF) at enrolment (before planned hospital discharge) and were evaluated at 3 weeks, 6 weeks, and 3, 6, 9, and 12 months. Written informed consent was obtained, and the study protocol was approved by the ethics committee of the MRC Dunn Nutrition Unit, Cambridge. Randomization schedules were prepared at the Dunn Nutrition Unit for the enrolment of 108 subjects (54 males, 54 females), using a permuted block randomization. The sample size represented a compromise between the need for adequate power for detecting differences in outcome measures, and the detailed (and expensive) nature of the stable isotope measurements.

The composition of formulas in comparison with mature human milk is shown in **Table 10-1**. The reduced energy formula differed from the standard formula in its lower fat (and hence energy) content. Both were supplied by Ross Products (Columbus, Ohio). Composition of these formulas complied with the levels of nutrients recommended by the European Economic Community and the Committee on Nutrition of the American Academy of Pediatrics and the Infant Formula Act of 1980. The assigned study formula was fed as the sole energy source from birth through the initial 12-week study period. Solid food was introduced at a time determined by the parents with advice from their health care professionals (the Department of Health recommendation for term infants at the time was not to introduce solids before 3 months of age).

Table 10-1 Composition of study formulas compared with expressed mature human milk

Component per 100 mL	Study formula		Expressed mature human milk		
	RE	TF	DHSS[1]	Macy et al[2]	AAP[3]
Protein (g)	1.34	1.35	1.34	1.45	1.0
Fat (g)	2.86	3.64	4.2	3.8	3.9
Carbohydrate (g)	7.11	7.19	7.0	7.2	7.2
Calcium (mg)	51	51	35	33	28
Phosphorus (mg)	39	39	15	15	14
Sodium (mg)	19	19	15	15	18
Iron (mg)	1.2	1.2	0.08	0.15	0.4
Zinc (mg)	0.51	0.51	0.30	0.53	1.2
Energy (kcal)	59.53	66.92	70	68	68
Protein:Energy ratio (g/100kcal)	2.25	2.02	1.91	2.13	1.47

[1] DHSS Reports on Health and Social Subjects.(282-284)
[2] Macy, Kelly and Sloan (284;285) and Mettler (284;286)
[3] American Academy of Pediatrics(287)

At each follow-up visit, weight, length, OFC (Occipito-frontal head circumference), MUAC (Mid-upper arm circumference) and SF measurements (triceps and subscapular) were performed by the investigators and details of intercurrent illnesses (type, duration, and treatment/management) were recorded on the case report form. Weight was measured using a paediatric scale accurate to ±5 gm, length using a horizontal infant stadiometer (with the infant's head held by one observer, and both legs extended and held by a second observer) and OFC and MUAC using a non-stretchable encircling tape. All SFs were measured using Harpenden callipers.

Subjects were removed from the study if they had poor compliance, vomiting for more than 3 consecutive days, diarrhea necessitating a change in diet for more than 3 consecutive days during the first 3 months, any illness necessitating dietary change for more than 3 days or any condition which would prevent normal growth and development during the study period.

Doubly-labelled water measurements

The doubly-labelled water method (144-147) was used to determine body composition, energy expenditure, energy intake, metabolisable energy intake and milk intake at the 3 month visit. Two samples of urine were obtained from each infant before the isotope study to measure natural concentrations of ^{2}H and ^{18}O in a body fluid. An accurately weighed dose of sterile isotope solution providing roughly 0.28 g $H_2{}^{18}O$ and 0.1 g $^{2}H_2O$ per kg body weight was given orally(146). Subsequent samples of the urine were collected at four and five hours and then daily for seven days (a period that ensured two to three biological half lives of the isotopes) using cotton wool balls left inside the infant's nappy and, after urination, extracting the urine sample by inserting the cotton wool in a syringe (288). The parents were asked to check the nappy frequently for urination (at least once per hour) and the time of voiding was taken as the midpoint between the last two times of checking.

The principle of the doubly labelled water method is that two stable isotopes of water ($H_2{}^{18}O$ and $^{2}H_2O$) are administered simultaneously and their initial enrichment in a body fluid and subsequent rates of disappearance from this fluid are monitored by isotope ratio mass spectrometry. The initial enrichment of either isotope reflects

body water pool size (from the principle of dilution) and permits an estimation of body composition. Subsequently, the rate of disappearance of $^{2}H_2O$ reflects water output (and hence water intake) while that of $H_2{}^{18}O$ reflects water output plus production of carbon dioxide because ^{18}O is free to interchange between water and carbon dioxide through the action of carbonic anhydrase. The difference between the two rates of disappearance is, therefore, a measure of the rate of production of carbon dioxide. With additional knowledge of the subject's respiratory quotient (derived from previous studies on a similar population = 0.85), energy expenditure can be calculated. Details of the method and principles of the calculations used are reported elsewhere(147).

Statistics

The calculated sample size (50 infants per randomised group) permitted detection of 0.6 SD difference in anthropometric measurements at 5% significance level with 80% power. Data for randomised groups were compared using independent sample t-test. Categorical variables were compared using Pearson Chi-Square test. Repeated measures analysis of variance was performed to determine the effect of formula on anthropometric parameter change at each time point. Weight, length and OFC SDS were calculated using British reference data(185). FM and FFM were normalized for infant length by dividing by height^2 to give FM index (FMI) and FFM index (FFMI) (189).

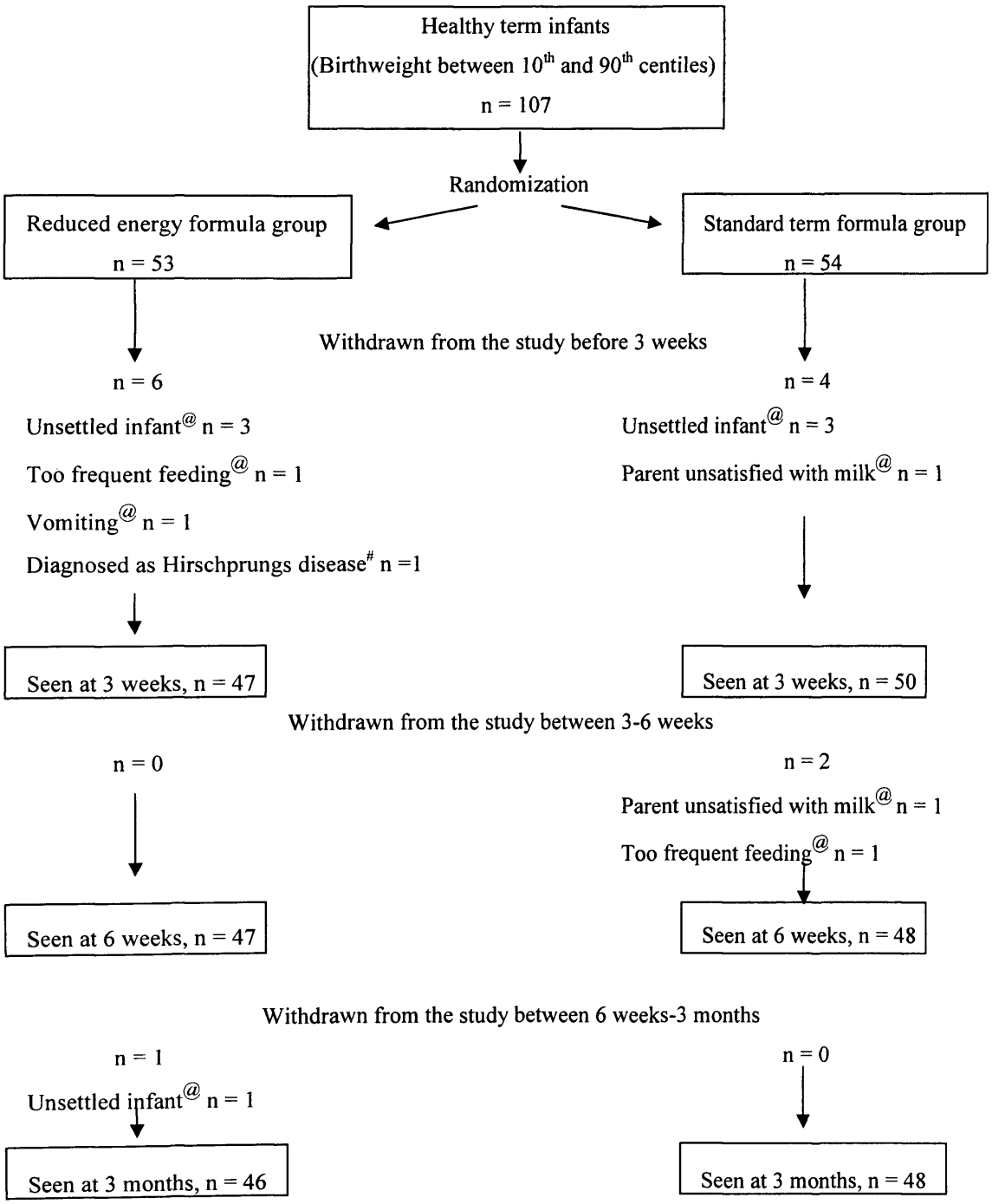
Results

Randomised trial

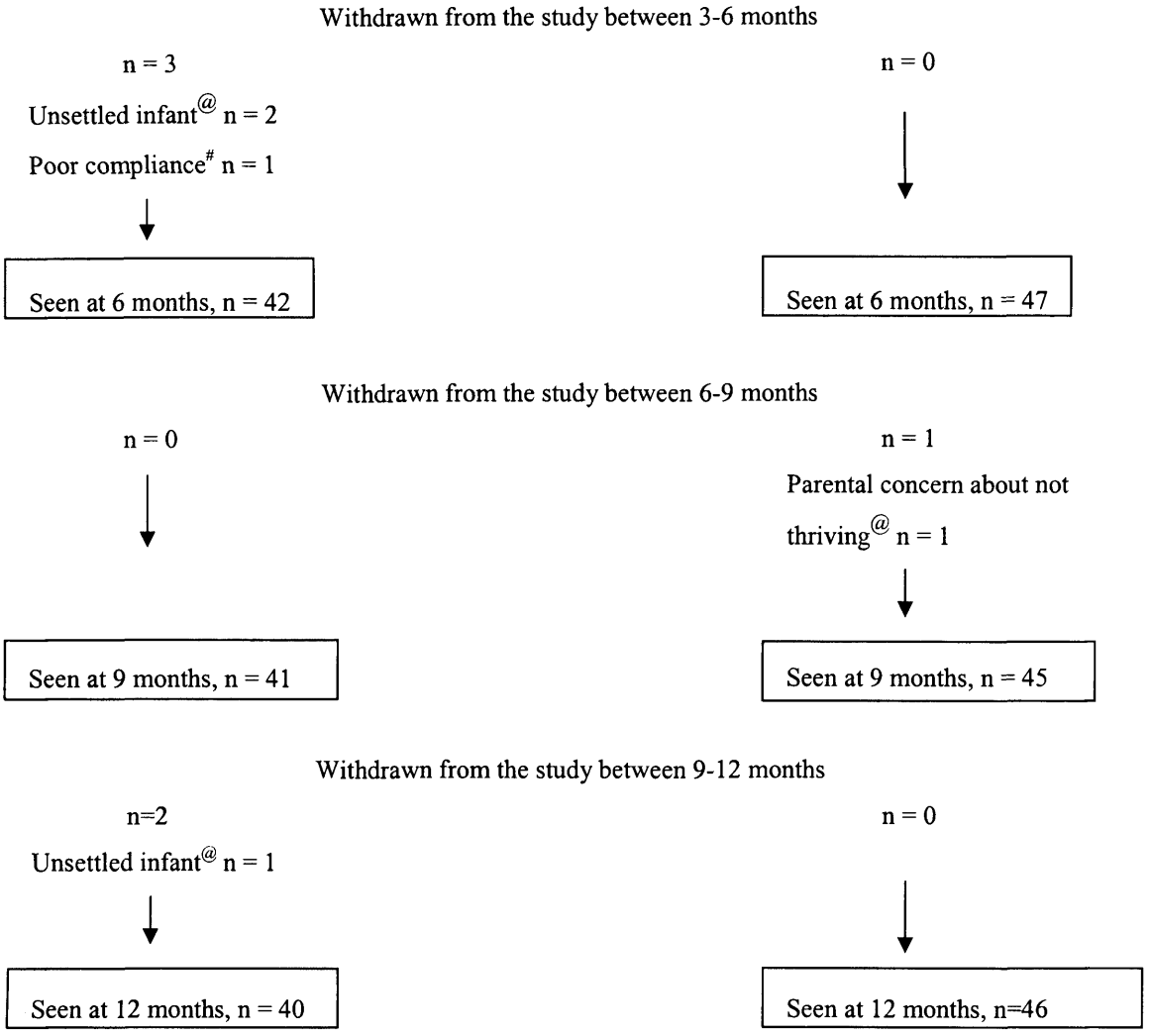
The trial profile is shown in **Figure 10-1**. A total of 107 infants were randomised, 53 to reduced energy formula (RE) and 54 to standard term formula (TF). There were no significant differences in the proportion of infants withdrawn from each formula group or in the number of subjects withdrawn by their parents or investigators; 40, 75.5% of the RE group and 46, 85.2% of the TF group completed the study at age 12 months. However, only 32 and 43 out of 46 and 48 infants (in RE and TF groups

respectively) participated in the doubly labelled water measurements, and only 28 and 33 infants from RE and TF groups had complete results to permit calculation of changes in body composition over the 7-day study period.

Figure 10-1 Trial profile



@ Withdrawn by parents



Withdrawn by investigator
@ Withdrawn by parents

The baseline characteristics of the randomised diet groups are shown in **Table 10-2**. The groups were well matched for birthweight, gestation, gender, rank of birth, parental anthropometry. However, mothers in the TF group had a higher educational achievement (A-level or higher professional qualification 29.6% vs. 8%, p=0.08). No infant was weaned earlier than age 3 months and there were no differences in the percentages weaned at age 3 and 6 months: 37%v.s.35.4%, p=1.0 at 3 months; 95%v.s.100%, p=0.2 at 6 months in RE and TF group respectively.

Table 10-2 Baseline characteristics of infants according to randomised diet group [&]

	RE formula (n = 53)	TF formula (n = 54)	p
Gestation (wk)	40.1 ± 1.4)	39.9 ± 1.2	0.6
Birthweight (g)	3.54 ± 0.34)	3.51 ± 0.32)	0.6
Boys (n, %)	24, 45.3%	26, 48.1%	0.8
Maternal age (yr)	26.9 ± 4.7	27.0 ± 4.2	0.9
Paternal weight (kg) (n = 23, 26)	77.7 ±12.9)	79.4 ± 9.7	0.6
Paternal height (cm) (n = 24, 26)	179.8 ± 6.0)	177.7 ± 5.4	0.2
Maternal weight (kg) (n = 26, 28)	64.6 ± 12.7)	65.1 ± 15.1	0.9
Maternal height (cm) (n = 25, 30)	161.8 ± 6.6)	163.9 ± 6.7	0.2
Mothers with A level/higher professional qualification (%)	8 %	29.6 %	0.08
Rank of Birth (%)			
1st	25 %	38 %	0.6
2nd	48 %	40 %	
3rd	23 %	18 %	
4th	4 %	4 %	

[&] Data are shown as means ± SD; Independent sample t-test was used to test the difference between the continuous variables; Chi-square was used to test the difference between the categorical variable.

Outcome measures

Growth

There were no significant differences between the 2 formula groups in attained weight, length, or OFC, expressed either as absolute values or as SDS (**Table 10-3**). However, the RE group had significantly lower subscapular SF by 0.8 mm (95%CI:-1.31,-0.23; p=0.005) at 6 weeks, by 0.7 mm (95%CI: -1.30, -0.15; p=0.014) at 3 months.

There were no significant differences in age at follow up between the two groups for all six time points although age at 3 week follow up in the RE group was slightly less than for the TF group by 0.6 days (95%CI: -1.22, 0.02; p=0.06).

Table 10-3 Anthropometric data according to randomised group [&]

	RE formula (n = 53)	TF formula (n = 54)	p
Weight SDS			
Birth	0.13 ± 0.70	0.10 ± 0.63	0.8
Enrolment	-0.22 ± 0.78	-0.27 ± 0.66	0.7
3 weeks	-0.10 ± 0.79	-0.06 ± 0.72	0.8
6 weeks	0.04 ± 0.77	0.19 ± 0.67	0.3
3 months	0.10 ± 0.83	0.21 ± 0.73	0.8
6 months	0.10 ± 0.89	0.09 ± 0.79	1.0
9 months	0.14 ± 0.96	0.07 ± 0.82	0.7
12 months	0.06 ± 0.90	-0.02 ± 0.88	0.7
Length SDS			
Enrolment	-0.23 ± 0.94	-0.22 ± 0.85	0.9
3 weeks	0.05 ± 0.90	0.04 ± 0.80	1.0
6 weeks	0.21 ± 0.94	0.33 ± 0.94	0.5
3 months	0.35 ± 1.03	0.36 ± 0.76	0.9
6 months	0.30 ± 1.09	0.42 ± 1.05	0.6
9 months	0.38 ± 1.11	0.39 ± 1.16	1.0
12 months	0.21 ± 1.00	0.47 ± 1.04	0.3

[&] Data are shown as means ± SD; Independent sample t-test was used to test the difference between 2 formula group

	RE formula (n = 53)	TF formula (n = 54)	p
OFC SDS			
Enrolment	0.20 ± 0.85	0.15 ± 0.79	0.8
3 weeks	0.47 ± 0.82	0.44 ± 0.82	0.9
6 weeks	0.48 ± 0.76	0.46 ± 0.77	0.9
3 months	0.29 ± 1.07	0.30 ± 0.84	1.0
6 months	0.21 ± 0.90	0.15 ± 1.01	0.8
9 months	-0.01 ± 0.95	0.02 ± 1.05	0.9
12 months	-0.15 ± 0.94	0.05 ± 1.12	0.4
Triceps SF (mm)			
Enrolment	5.6 ± 1.4	5.5 ± 1.3	0.9
3 weeks	6.6 ± 1.4	7.0 ± 1.5	0.14
6 weeks	7.4 ± 1.5	7.6 ± 1.6	0.5
3 months	8.3 ± 1.7	8.7 ± 1.8	0.3
6 months	9.7 ± 1.8	9.9 ± 2.1	0.7
9 months	9.5 ± 2.0	9.9 ± 2.3	0.4
12 months	9.4 ± 2.5	9.3 ± 2.4	1.0
Subscapular SF (mm)			
Enrolment	5.7 ± 0.9	5.9 ± 1.2	0.3
3 weeks	6.8 ± 1.2	7.3 ± 1.3	0.07
6 weeks	7.1 ± 1.2	7.9 ± 1.4	***0.005***
3 months	7.8 ± 1.5	8.5 ± 1.3	***0.014***
6 months	7.6 ± 1.5	7.9 ± 1.6	0.4
9 months	7.7 ± 1.9	8.0 ± 1.8	0.4
12 months	7.2 ± 2.1	7.0 ± 1.7	0.6
Mid-upper arm circumference (cm)			
Enrolment	10.2 ± 0.6	10.1 ± 0.7	0.5
3 weeks	10.6 ± 0.7	10.8 ± 0.7	0.3
6 weeks	11.2 ± 0.8	11.5 ± 0.8	0.15
3 months	12.3 ± 0.7	12.4 ± 0.9	0.5
6 months	14.0 ± 0.9	14.1 ± 0.9	0.9
9 months	14.6 ± 0.9	14.8 ± 1.2	0.5
12 months	14.9 ± 1.1	15.0 ± 1.4	0.8

Body composition at 12 weeks

Infants in the RE group had a significantly lower FM than the TF group and had a trend towards lower calculated FM accretion: by 2.2 g/day (95%CI: -4.8, 0.3; p=0.08). Furthermore, the RE group also had higher FFM than the TF group although the differences did not reach statistical significant and there were no differences in FFM or protein accretion (**Table 10-4**).

When FM and FFM were normalized for length, FFMI was significantly higher in the RE group: by 0.65 kg/m^2 (95%CI: 0.08, 1.23; p=0.03) while FMI was lower in the RE group: by -0.62 kg/m^2 (95%CI: -1.24, 0.01; p=0.05).

Metabolisable energy intake

From the doubly-labelled water study, there was no difference in total energy expenditure and metabolisable energy intake between the two groups (**Table 10-4**). However, the RE group showed a trend towards lower energy deposited as fat by 20.8 kcal/day (95%CI: -44.2, 2.7; p=0.08) and hence lower total energy deposited in new tissue by 20.9 kcal/day (95%CI: -48.8, 7.1; p=0.14).

Milk and nutrient intake

Infants in the RE group consumed significantly greater volumes of milk than the TF group: by 15 mL/kg/day (95%CI: 3.2, 27.0; p=0.01) at 3 weeks, by 14 mL/kg/day (95%CI: 1.6, 25.9; p=0.03) at 3 months (**Table 10-5**). Consequently, the reduced energy formula group had slightly higher protein intake than the standard infant formula group: by 0.18 gm/kg/day (95%CI: 0.02, 0.34; p=0.03) at 3 weeks, by 0.17 gm/kg/day (95%CI: 0.01, 0.33; p=0.04) at 3 months. However, they still had a significantly lower fat intake than the TF group due to lower fat content in the formula: by 1.1 gm/kg/day (95%CI: -1.50, -0.72; p<0.001) at 3 weeks, by 0.84 gm/kg/day (95% CI: -1.24, -0.44; p=0.001) at 3 months. Thus, the total energy intake was slightly lower in the RE group at 3 weeks but there was no difference between the two groups at 3 months.

Table 10-4 Body composition, energy deposition, energy expended, metabolisable energy intake measured by doubly labelled water method over a 7 day period at age 3 months [&]

	RE formula	TF formula	p
FM (kg)	1.35 ± 0.43	1.60 ± 0.47	***0.04***
FFM (kg)	4.49 ± 0.51	4.32 ± 0.51	0.2
FMI (kg/m^2)	3.70 ± 1.14	4.31 ± 1.21	***0.05***
FFMI (kg/m^2)	12.35 ± 1.06	11.69 ± 1.12	***0.03***
Body composition accretion during 7 days study period[$]			
FFM (g/day)	25.9 ± 13.2	26.1 ± 13.7	1.0
FM (g/day)	7.1 ± 4.7	9.4 ± 5.1	0.08
Protein (g/day)	4.1 ± 2.1	4.1 ± 2.2	1.0
Energy deposited in new tissue[#]			
As fat (kcal/day)	66.0 ± 43.3	86.8 ± 47.3	0.08
As protein (kcal/day)	22.8 ± 11.6	22.9 ± 12.1	1.0
Total (kcal/day)	88.8 ± 51.6	109.7 ± 56.6	0.14
Total energy expenditure			
Kcal/day	478 ± 147	467 ± 134	0.7
Kcal/kg/day	80.6 ± 22.3	78.4 ± 22.6	0.7
Metabolisable energy intake[∞]			
kcal/day	561 ± 156	547 ± 118	0.7
kcal/kg/day	92.8 ± 23.1	90.5 ± 17.3	0.7

[&] Data are shown as means ± SD; Independent sample t-test was used to test the difference between the 2 formula groups. n=28 and 33 for body composition, n=32 and 43 for energy expenditure in RE and TF

[$] FFM accretion (144) (gm/day) = difference in FFM between start and end of period divided by 7; FM accretion = difference in FM between start and end of period divided by 7; Protein accretion = FFM accretion x proportion of protein in FFM (published value=15.7% at age 3 months average for boys and girls)(36).

[#] Conversion factors (9.25 for fat and 5.6 for protein) were used to derive energy stored as fat and protein during the study period (carbohydrate storage was assumed to be negligible). (144)

[∞] Metabolisable energy intake = total energy expended + total energy deposited. (144)

Table 10-5 Milk intakes at age 3 weeks and 3 months and calculated nutrient intake per kilogram body weight [#]

	Reduced energy formula	Standard infant formula	p-value
Milk intake at age 3 weeks *			
mL/day	853 (147)	804 (136)	0.10
mL/kg/day	213 (27)	198 (30)	***0.01***
Milk intake at age 3 months**			
mL/day	1038 (201)	951 (148)	0.06
mL/kg/day	172 (24.6)	158 (22.8)	***0.03***
Calculated nutrient intake at age 3 weeks			
Fat (gm/kg/day)	6.1 (0.8)	7.2 (1.1)	***<0.001***
Protein (gm/kg/day)	2.8 (0.4)	2.7 (0.4)	***0.03***
Energy (kcal/kg/day)	127 (16)	132 (20)	0.15
Calculated nutrient intake at age 3 months			
Fat (gm/kg)	4.9 (0.7)	5.8 (0.8)	***0.001***
Protein (gm/kg/day)	2.3 (0.3)	2.1 (0.3)	***0.04***
Energy (kcal/kg/day)	102 (15)	106 (15)	0.4

[#] Calculate from **Table 10-1 Composition of Trial formulas**
n = 44 and 47 for milk intake at 3 weeks in RE and TF, n = 28 and 33 for milk intake at 12 weeks in RE and TF

* By bottle weighing before and after feeding

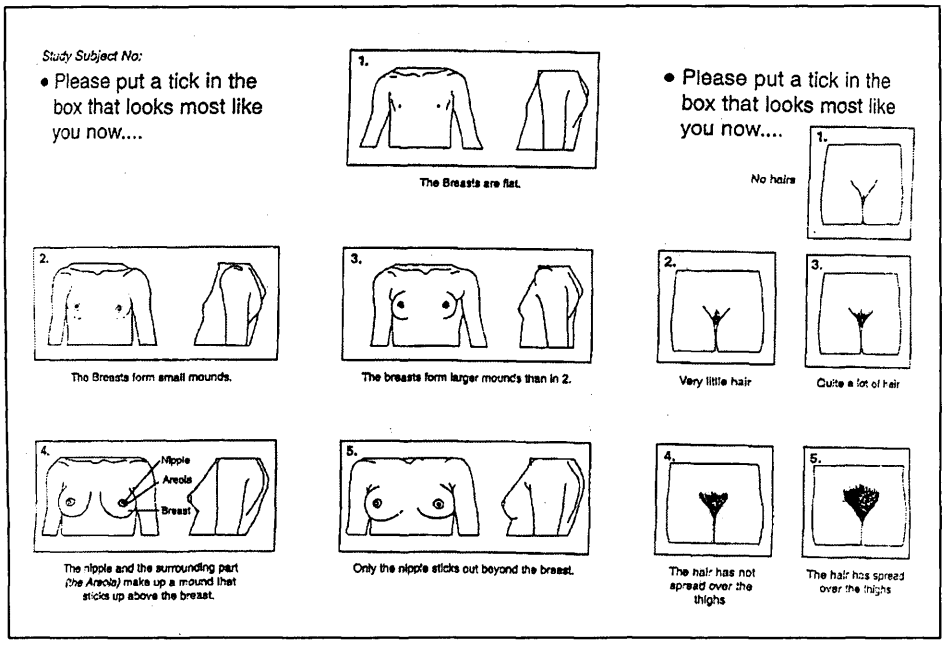
** By doubly labeled water study (147;289)

Appendix B

Examples of the questionnaires used in the study

Figure 10-2 Pubertal status questionnaires

a) For girls



b) For boys

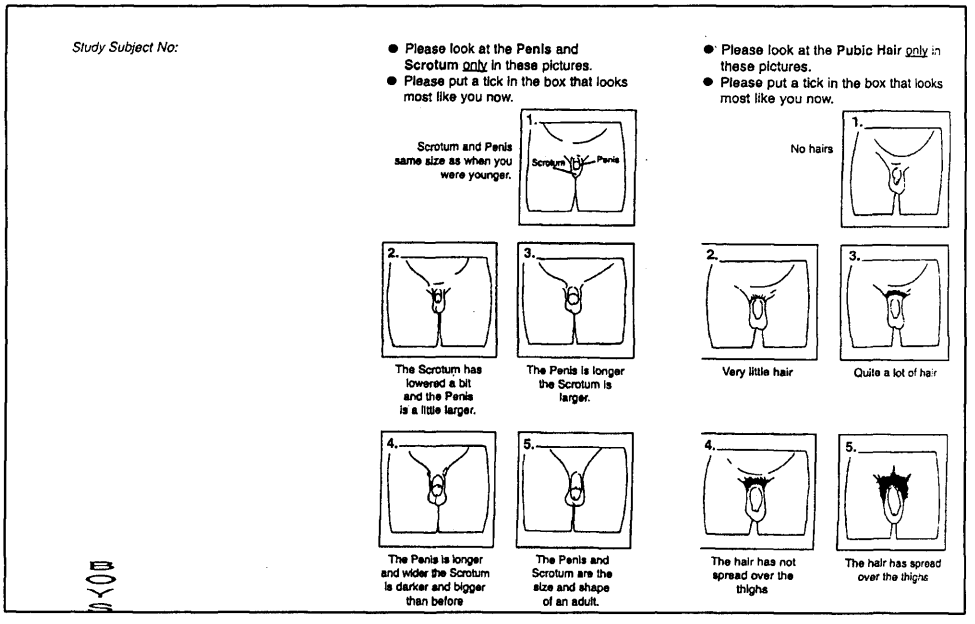
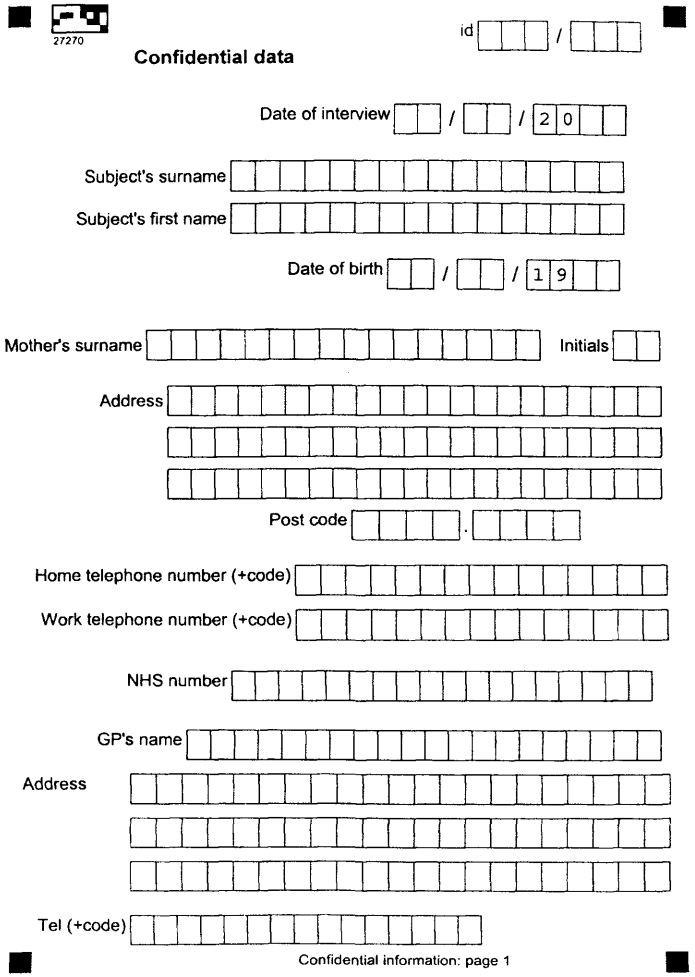


Figure 10-3 Data collection forms



27270

Confidential data

id /

Date of interview / / 20

Subject's surname

Subject's first name

Date of birth / / 19

Mother's surname Initials

Address

Post code .

Home telephone number (+code)

Work telephone number (+code)

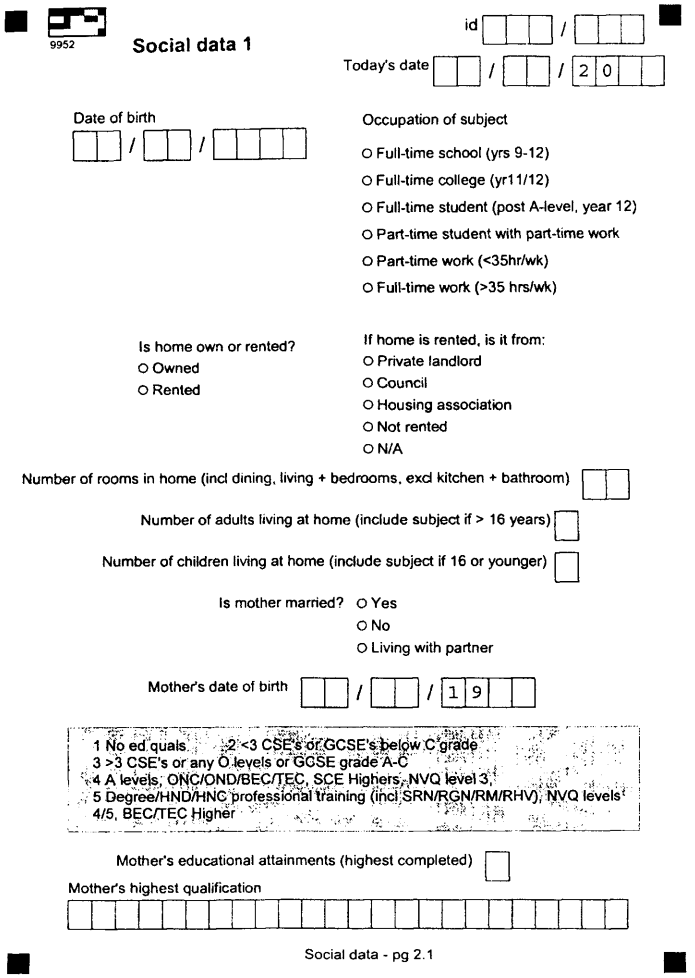
NHS number

GP's name

Address

Tel (+code)

Confidential information: page 1



9952

Social data 1

id /

Today's date / / 20

Date of birth / /

Occupation of subject

- O Full-time school (yrs 9-12)
- O Full-time college (yr11/12)
- O Full-time student (post A-level, year 12)
- O Part-time student with part-time work
- O Part-time work (<35hr/wk)
- O Full-time work (>35 hrs/wk)

Is home own or rented?
- O Owned
- O Rented

If home is rented, is it from:
- O Private landlord
- O Council
- O Housing association
- O Not rented
- O N/A

Number of rooms in home (incl dining, living + bedrooms, excl kitchen + bathroom)

Number of adults living at home (include subject if > 16 years)

Number of children living at home (include subject if 16 or younger)

Is mother married?
- O Yes
- O No
- O Living with partner

Mother's date of birth / / 19

1 No ed quals 2 <3 CSE's or GCSE's below C grade
3 >3 CSE's or any O levels or GCSE grade A-C
4 A levels, ONC/OND/BEC/TEC, SCE Highers, NVQ level 3,
5 Degree/HND/HNC professional training (incl SRN/RGN/RM/RHV), NVQ levels 4/5, BEC/TEC Higher

Mother's educational attainments (highest completed)

Mother's highest qualification

Social data - pg 2.1

9952

id ☐☐☐ / ☐☐☐

Social data 2

Mother's occupation

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

How many months employed in the last year? ☐☐

Father's date of birth ☐☐ / ☐☐ / 1 9 ☐☐

Father's educational attainments (highest completed) ☐

Father's highest qualification

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Father's occupation

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

How many months employed in the last year? ☐☐

Who is the primary earner for the family? O Father
O Mother
O Grandparent
O Both parents
O missing

Social class (use primary earner's occupation) ☐☐

Social code as below ☐

Code as: 1 = 1, 2 = 2, 3N = 3, 3M = 4, 4 = 5, 5 = 6 on primary earner's occupation
Single parent mother unsupported and not working = 7
Parent(s) unemployed and living on benefits = 8
Mother a single parent and a student = 9

3339

Medical History: 1

id ☐☐☐ / ☐☐☐

Fractures

Have you ever had a fracture? ☐ yes=1, no=2 if no, go to medical section

Indicate site/side of fracture

First Fracture

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Second Fracture

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Third Fracture

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Cause of fracture

First Fracture	Second Fracture	Third Fracture
☐☐	☐☐	☐☐

01=fall playground apparatus
02=fall from bike
03= RTA bike
04=RTA in car
05=RTA pedestrian
06=sports injury
07=injury at home
08=other

3339

Medical History: 2

id [ ][ ][ ] / [ ][ ][ ]

For questions below answer: yes = 1, no = 2

Are you clinically well? [ ]

Have you ever suffered with asthma? [ ]

Have you ever suffered with eczema? [ ]

Have you ever suffered with hayfever? [ ]

Is there any family history of asthma, eczema or hayfever? [ ]

Code A - asthma, E - eczema, H - hayfever + relative code below (code 0 for no disease)

1- Mother	2- Father
3- Brother	4- Sister
5- Maternal Grandmother	6- Maternal Grandfather
7- Paternal Grandmother	8- Paternal Grandfather

Asthma	[ ][ ][ ]	[ ][ ][ ]	[ ][ ][ ]
Eczema	[ ][ ][ ]	[ ][ ][ ]	[ ][ ][ ]
Hayfever	[ ][ ][ ]	[ ][ ][ ]	[ ][ ][ ]

3339

Medical History: 3

id [ ][ ][ ] / [ ][ ][ ]

Have you been on long term medication/or currently taking medication? [ ]

(yes=1, no=2 if no, turn to next section)

Current Medication (name, dose, duration and frequency)

Frequency
1= once/day 2= twice/day 3= three/day 4= four/day 5= > four/day 6= alternate days 7= less than alternate days
8= missing, N/A

Past Medication (name, dose, duration and frequency)

Frequency
1= once/day 2= twice/day 3= three/day 4= four/day 5= > four/day 6= alternate days 7= less than alternate days
8= missing, N/A

3339

Medical History: 5

id [ ][ ][ ] / [ ][ ][ ]

Family history of other disease:
With respect to subject (not parent), code 0 for no disease:

1 - mother	**2 - father**
3 - brother	**4 - sister**
5 - maternal grandmother	**6 - maternal grandfather**
7 - paternal grandmother	**8 - paternal grandfather**

High Cholesterol [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Heart disease [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Osteporosis [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Hypertension [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Diabetes - non-insulin dependant [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Diabetes - insulin dependant [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Overweight, requiring medical treatment [ ][ ][ ] [ ][ ][ ] [ ][ ][ ]

Other family history

Have you ever required surgery for any of the following: (1 = yes, 2= no)

lung [ ][ ][ ] heart [ ][ ][ ] GI tract [ ][ ][ ] liver [ ][ ][ ] kidney [ ][ ][ ]

Please give details of any surgery

Have you ever required medical treatment for any of the following :(1 = yes, 2= no)

lung [ ][ ][ ] heart [ ][ ][ ] GI tract [ ][ ][ ] liver [ ][ ][ ] kidney [ ][ ][ ]

Please give details of any medical treatment

3339

Medical History: 4

id [ ][ ][ ] / [ ][ ][ ]

Vitamin and mineral supplements

In the past year have you taken any supplements?

Multivitamins ○ yes
○ no

name of multivitamin

date started [ ][ ] / [ ][ ] / [ ][ ][ ][ ] date stopped [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

no capsules/day [ ][ ][ ]

still taking multivitamins
○ yes
○ no

Calcium supplements ○ yes
○ no

name of calcium supplement

date started [ ][ ] / [ ][ ] / [ ][ ][ ][ ] date stopped [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

no capsules/day [ ][ ][ ]

still taking calcium supplements
○ yes
○ no

Calcium intake (mg)/day [ ][ ][ ][ ] derived from Calquest analysis

18537

Exercise: 1

id R G S / ☐☐☐

Children's Exercise Questionnaire

How many PE lessons do you have per week? ☐☐

How long is each PE lesson? (mins) ☐☐☐

How many hours per week do you spend watching TV and videos, plus playing on the computer and video games (school nights only) ☐☐.☐

How many hours per week do you spend watching TV and videos, plus playing on the computer and video games (weekend) ☐☐.☐

How many hours per week do you spend on each of these activities outside PE classes:

Riding bike	☐☐.☐	Tennis	☐☐.☐
Swimming	☐☐.☐	Netball	☐☐.☐
Running	☐☐.☐	Hockey	☐☐.☐
Football	☐☐.☐	Basketball	☐☐.☐
Aerobics/dancing	☐☐.☐	Rugby	☐☐.☐
Gymnastics	☐☐.☐	Skating	☐☐.☐
Walking	☐☐.☐	Other sport	☐☐.☐

Other, please specify ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Exercise data - 3.1

18537

Exercise data: 2

id R G S / ☐☐☐

Parent's opinion of the child's physical activity levels

Total hours child spents in vigorous activity per week ☐☐

Level of child's activity compared to peers ☐

1= much less than peers
2= less
3= same
4= more
5= much more

Pubertal Development (if 9 year or older)

data to transfer from confidential data sheet

Breast/genital development stage (1-5) ☐

Pubic hair development (1-5) ☐

For girls only:

Have periods started yes o
no o
not applicable o

Age of first period (years:months) ☐☐.☐☐

Date of first day of last menstrual period ☐☐ / ☐☐ / ☐☐☐☐

Number of days in whole menstrual cycle ☐☐☐

For example; 5 days menstruation then 25 days to next menstruation = 30 day cycle.

Oral contraceptive or implant? ☐☐☐ Yes = 1 No = 2

Exercise - pg 3.2

34401

Anthropometry 1

id [ ][ ][ ] / [ ][ ][ ]

Birth Weight (g) [ ][ ][ ][ ]

Birthweight (lb:oz) [ ][ ].[ ][ ]

Sex [ ] 1=male 2=female

Gestation (wks) [ ][ ].[ ]

Mother's reported wt (kg) [ ][ ][ ].[ ]

Mother's weight (st:lb) [ ][ ].[ ][ ]

Mother's reported ht (cm) [ ][ ][ ].[ ]

Mother's height (ft:in) [ ].[ ][ ]

Father's reported wt (kg) [ ][ ][ ].[ ]

Father's weight (st:lb) [ ][ ].[ ][ ]

Father's reported ht (cm) [ ][ ][ ].[ ]

Father's height (ft:in) [ ].[ ][ ]

Subject's Anthropometry

MUAC (cm) [ ][ ][ ].[ ]

Head circum (cm) [ ][ ][ ].[ ]

Waist circum (cm) [ ][ ][ ].[ ]

Hips (cm) [ ][ ][ ].[ ]

Thigh circum (cm) [ ][ ][ ].[ ]

Calf circum (cm) [ ][ ][ ].[ ]

Sitting Height (cm) [ ][ ][ ].[ ]

Skinfold Thickness Measurements

Biceps (mm)	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]
Triceps (mm)	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]
Subscap (mm)	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]
Suprailiac (mm)	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]	[ ][ ][ ].[ ]

34401

Anthropometry 2

id [ ][ ][ ] / [ ][ ][ ]

BODPOD

Date of measurement [ ][ ] / [ ][ ] / [ ][ ]

Time [ ][ ].[ ][ ]

Temp (c) [ ][ ].[ ].

Pressure (mmHg) [ ][ ][ ][ ]

Final Test System

SD [ ][ ]

Mean Volume [ ][ ].[ ][ ][ ]

Test Data

Raw Volume

	Test One	Test Two	Test Three
	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]
	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]
	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]
Best Mean	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]	[ ][ ][ ].[ ][ ][ ]
% Fat	[ ][ ].[ ]	[ ][ ].[ ]	[ ][ ].[ ]
Raw Density (weight/volume)	[ ].[ ][ ][ ][ ][ ]	[ ].[ ][ ][ ][ ][ ]	[ ].[ ][ ][ ][ ][ ]

Weight 1	Weight 2	Weight 3
[ ][ ][ ].[ ][ ]	[ ][ ][ ].[ ][ ]	[ ][ ][ ].[ ][ ]

Height [ ][ ][ ].[ ]

Lung Volume [ ][ ].[ ]

Comments

[ ]

[ ]

34401

Anthropometry 3

id ☐☐☐ / ☐☐☐

DEUTERIUM DILUTION

Full bottle of diluted deuterium (g) ☐☐☐.☐☐

Post dosing bottle weight (g) ☐☐☐.☐☐

Post dose fluid intake (mls) ☐☐☐☐

Time post dose saliva sample taken ☐☐.☐☐

BIOELECTRICAL IMPEDANCE

	Log no.	distance (cm)
Whole arm	☐☐☐	☐☐☐.☐
Lower arm	☐☐☐	☐☐☐.☐
Upper arm	☐☐☐	☐☐☐.☐
Trunk	☐☐☐	☐☐☐.☐
Upper leg	☐☐☐	☐☐☐.☐
Whole leg	☐☐☐	☐☐☐.☐
Lower leg	☐☐☐	☐☐☐.☐
Whole body	☐☐☐	☐☐☐.☐
Whole body	☐☐☐	☐☐☐.☐

584

Step test

id ☐☐☐ / ☐☐☐

Is subject happy to do step-test (Y/N)? ☐

If 'no' state reason ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Does subject currently have asthma (Y/N)? ☐

If Yes to asthma, does subject have inhaler (Y/N)? ☐

If subject has asthma, but no inhaler - do not continue with step-test

Height of subject (cm) ☐☐☐

Estimated step height (0.188 x ht) ☐☐.☐ Step height used (cm) ☐☐.☐

Step height made up of:

1	x Reebok step	15cm	(79.8cm of height)
___	x Reebok inserts	5cm	(26.6cm of height)
___	x 1.2cm platforms	1.2cm	(6.4cm of height)
___	x 0.6cm platforms	0.6cm	(3.2cm of height)

eg. Height of 155cm would need a step of 29.14cm made up of:
1xstep (15cm) + 2xReebok inserts (10cm) + 3x1.2cm (3.6) + 1x0.6cm
= 29.2cm

Set metronome rate:

O Males 96 beats/min O Female 88 beats/min

Resting HR (beats/15 seconds) ☐☐☐

3 minute step test completed (Y/N) ☐

HR immediately post step-test (beats/15 seconds) ☐☐☐

HR 1 minute post step-test (beats/15 seconds) ☐☐☐

21670

id ☐☐☐ / ☐☐☐

Lifestyle data:1

Please fill this questionnaire in as honestly as possible
Please complete all the questions
This information will be treated as CONFIDENTIAL and will not be passed on to anyone outside the study.

Today's date ☐☐ / ☐☐ / 2 0 ☐☐

Smoking

Have you ever smoked a cigarette? O YES
O NO

If yes, do you smoke cigarettes regularly? O YES
O NO

If you smoke, how many per day? ☐☐☐

How long have you been smoking for? (yrs.mths) ☐.☐☐

When did you last smoke a cigarette? ☐☐ / ☐☐ / 2 0 ☐☐

Alcohol

Have you ever drunk alcohol? O YES
O NO

If yes, do you drink alcohol regularly? O YES
O NO

How long have you been drinking alcohol for? (yrs.mths) ☐.☐☐

1 drink means:
1 half pint of beer
1 small glass of wine
1 pub measure of spirits or 1 shot of spirit
0.5 bottle Alcopops for example 'Barcardi Breezer

If you drink regularly, how many alcohol drinks do you have each week? ☐☐

How many days since you last had any alcohol? ☐☐

21670

id ☐☐☐ / ☐☐☐

Lifestyle data:2

Ethnic origin

Please fill the circle that you think best describes your ethnic origin:

O White
O Black - Caribbean
O Black - African
O Black - other
O Indian
O Pakistani
O Bangladeshi
O Chinese
O Asian - other
O Other
O Refused

Thank-you for completing this questionnaire -
please put it in the envelope and give back to the researcher.

Appendix C

Curve fits for internal SDS calculation by LMS methods for body composition variables

The lmsChartMaker program fits smooth centile curves to reference data using the LMS method (290). Reference centile curves show the distribution of a measurement as it changes according to some covariate, e.g. age. The LMS method summarises the changing distribution by 3 curves representing the median (M), coefficient of variation (S) and skewness (L). More details can be found in the User's guide to lmsChartMaker© 1997-2005 Medical Research Council, UK.

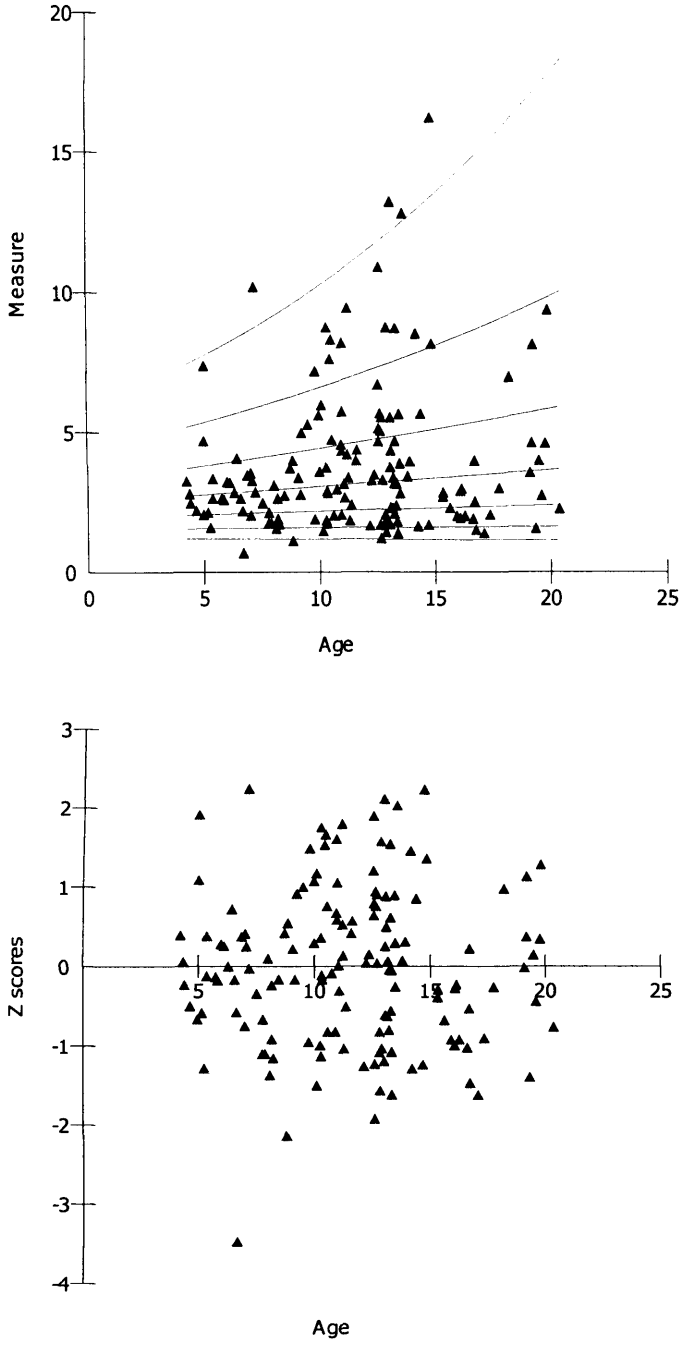
SD score (Z) of a child's measurement (y) is calculated from the L, M and S curves, using values appropriate for the child's age and sex. Two formulae are relevant depending on the value of L;

$$Z = \frac{(y/M)^{L} - 1}{LxS} \qquad \text{if } L \neq 0$$

$$Z = \frac{\log (y/M)}{S} \qquad \text{if } L=0$$

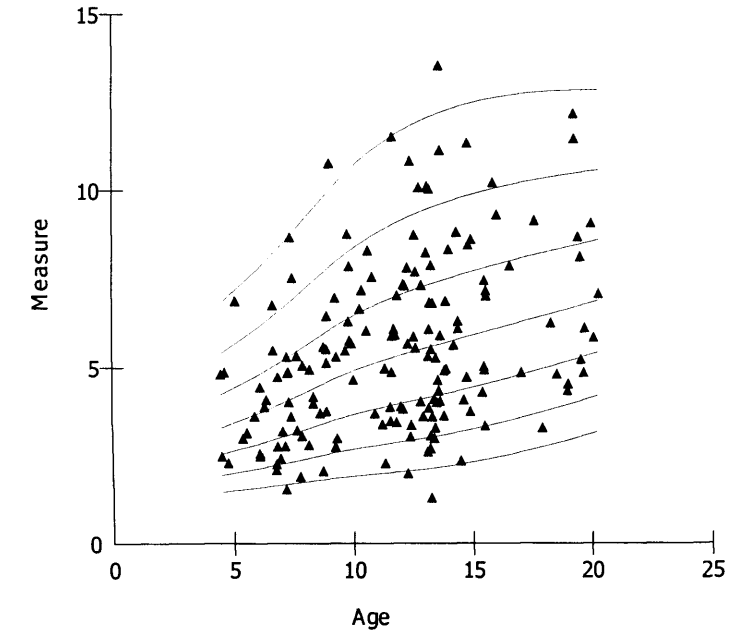
LMS Curve fits used for body composition SDS calculation [#]

Figure 10-4 Boys FMI centile chart (n = 145)



[#] Body composition data were derived from 4-component model and regional composition data were derived from DXA. Subjects used to create the centile charts were part of an ongoing body composition reference study at the MRC Childhood Nutrition Research Centre, Institute of Child Health, London, UK.

Figure 10-5 Girls FMI centile chart (n=163)



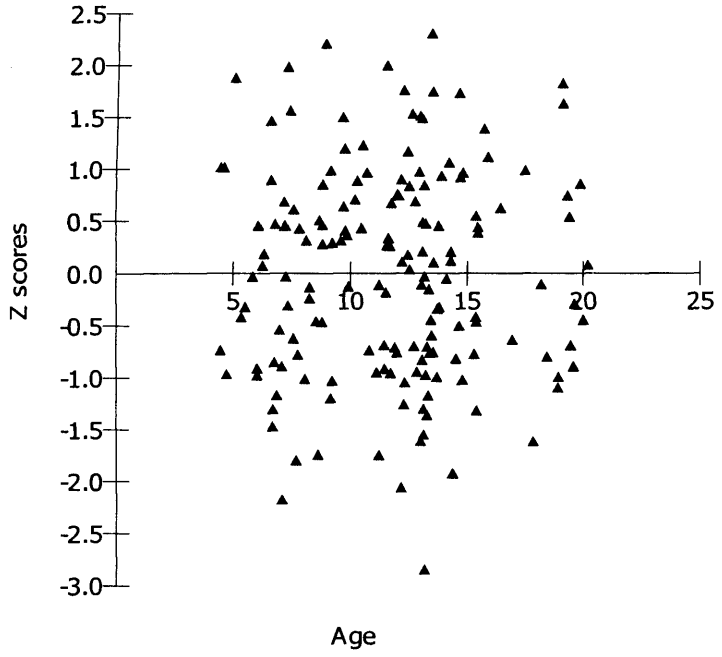
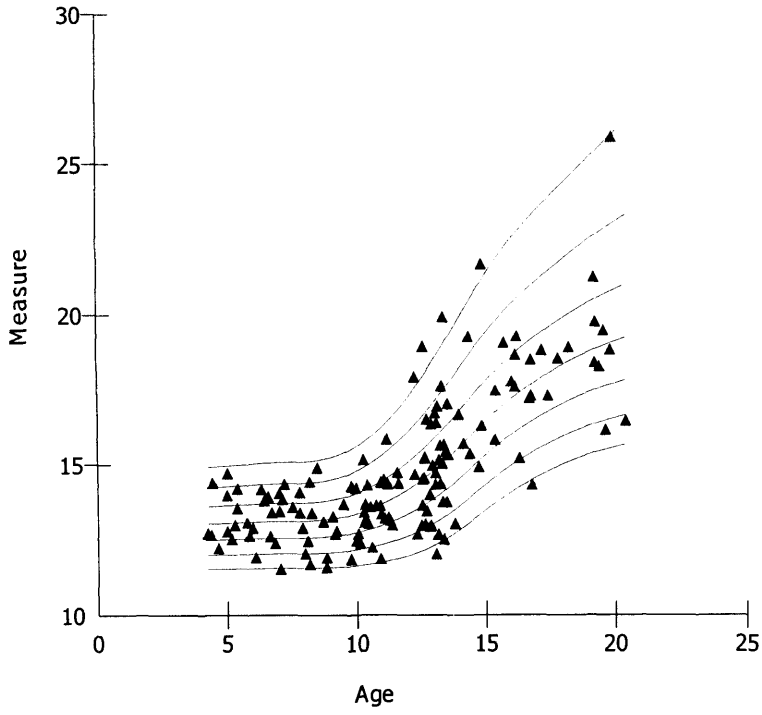


Figure 10-6 Boys FFMI centile chart (n=145)



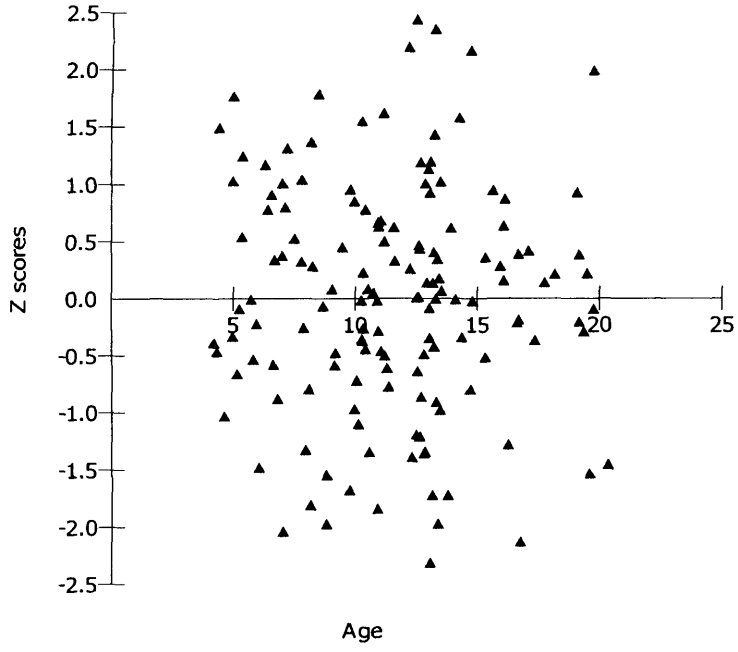


Figure 10-7 Girls FFMI centile chart (n=163)

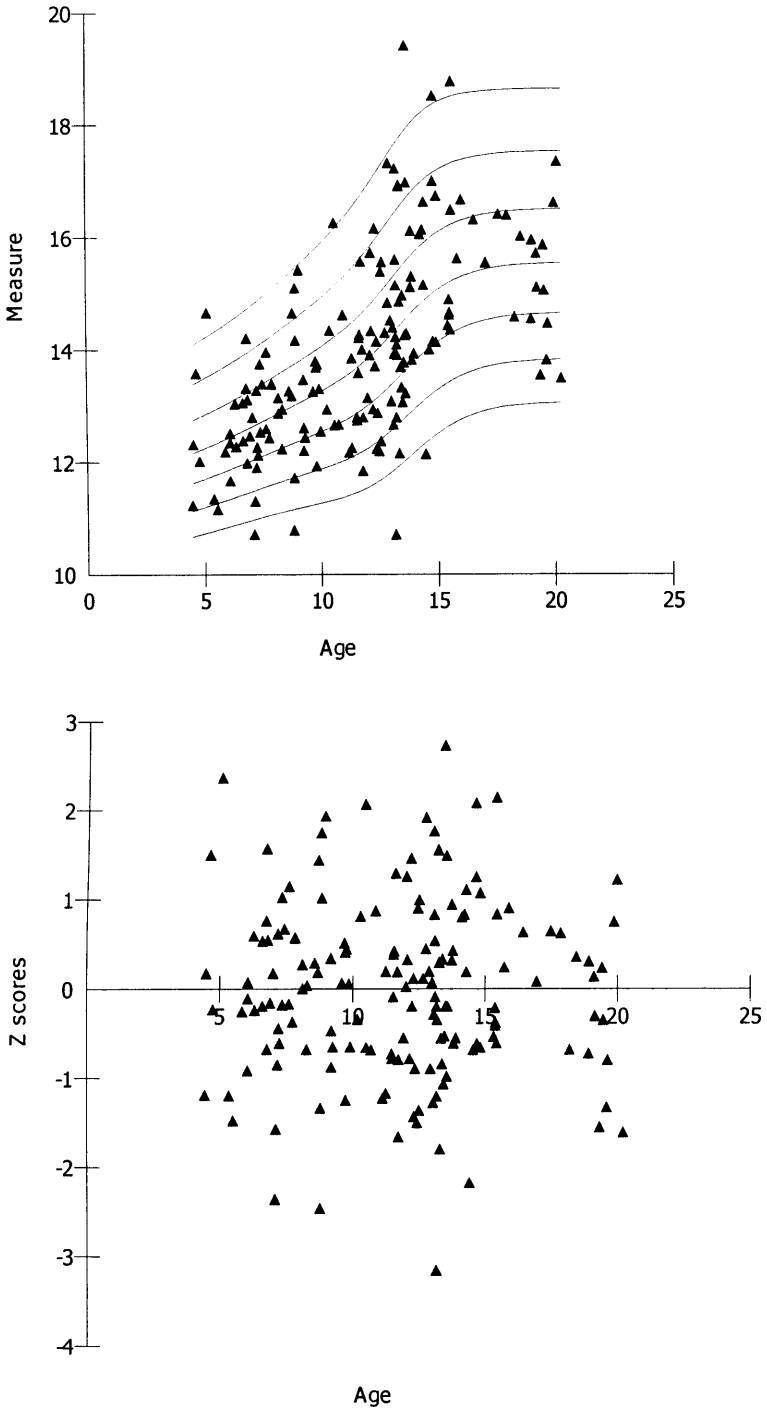
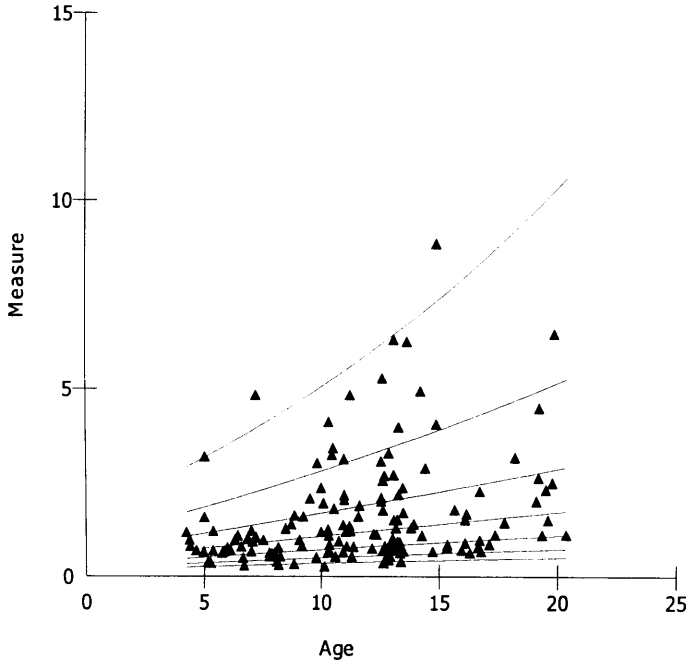


Figure 10-8 Boys Trunk FMI centile chart (n=145)



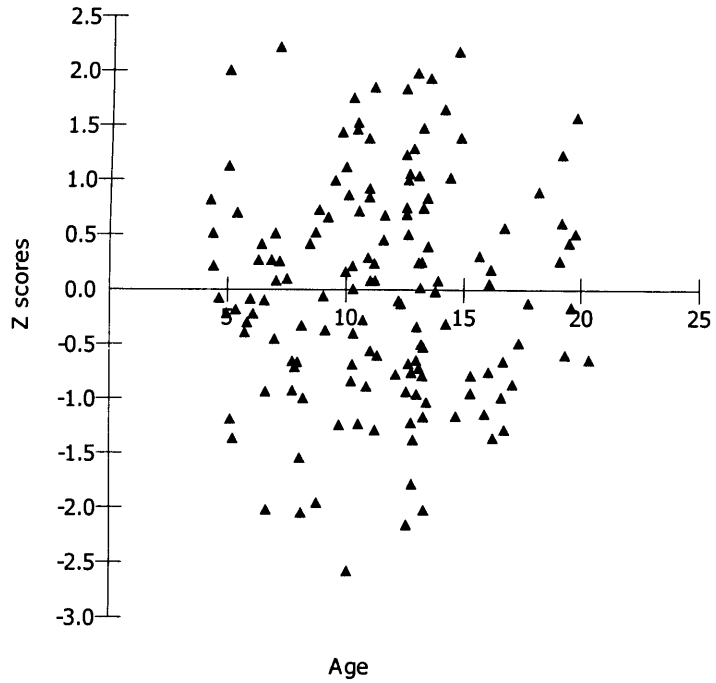


Figure 10-9 Girls Trunk FMI centile chart (n=163)

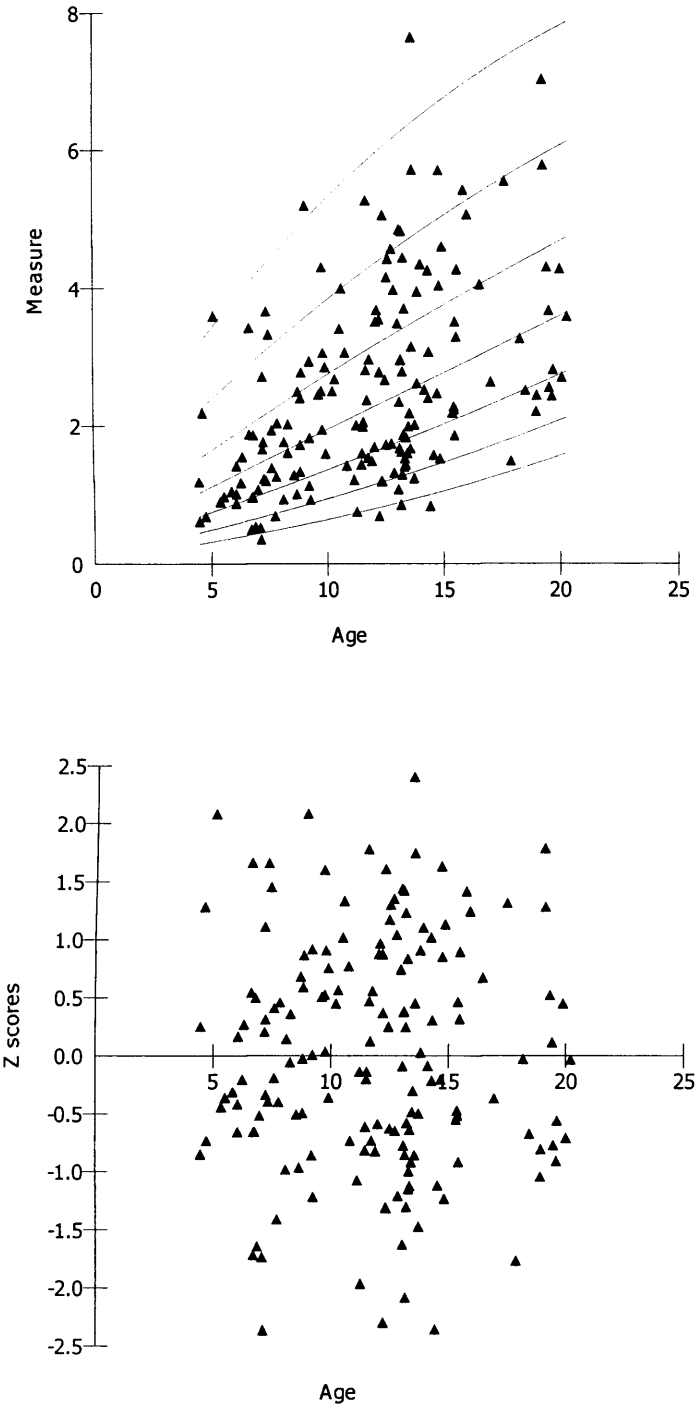


Figure 10-10 Boys Limbs LMI centile chart (n=145)

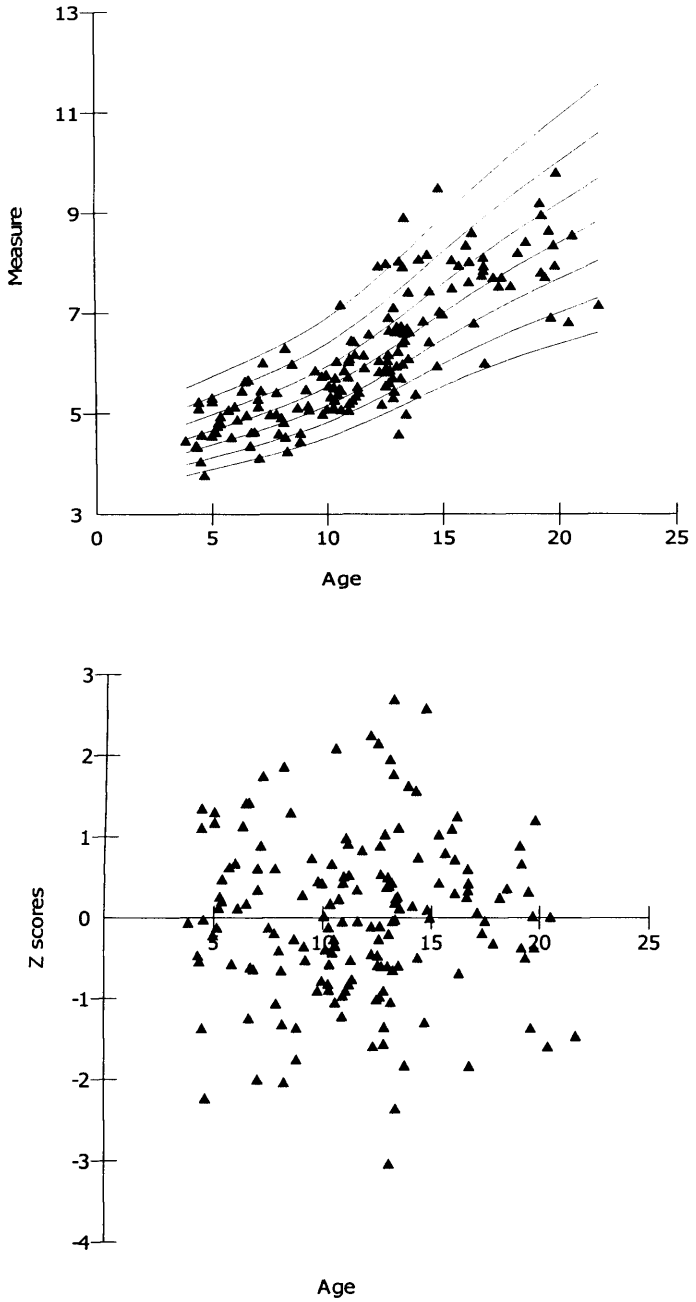
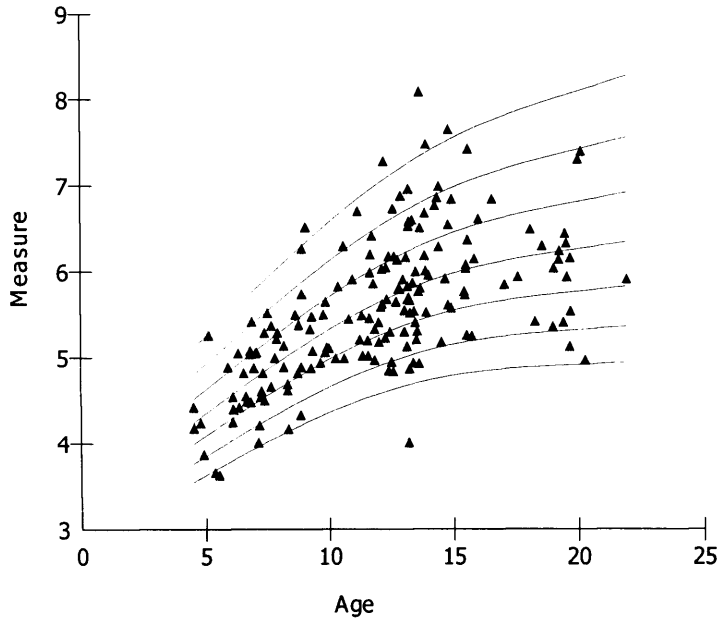


Figure 10-11 Girls Limbs LMI centile chart (n=163)



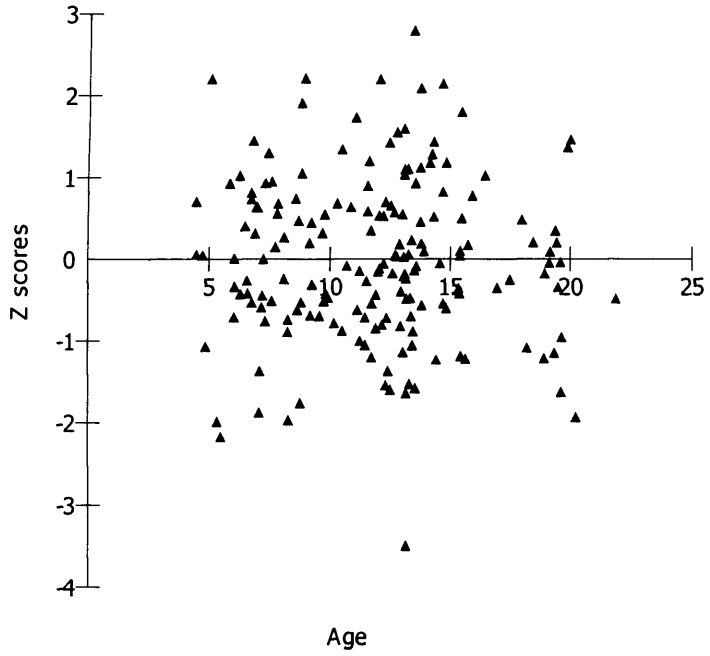
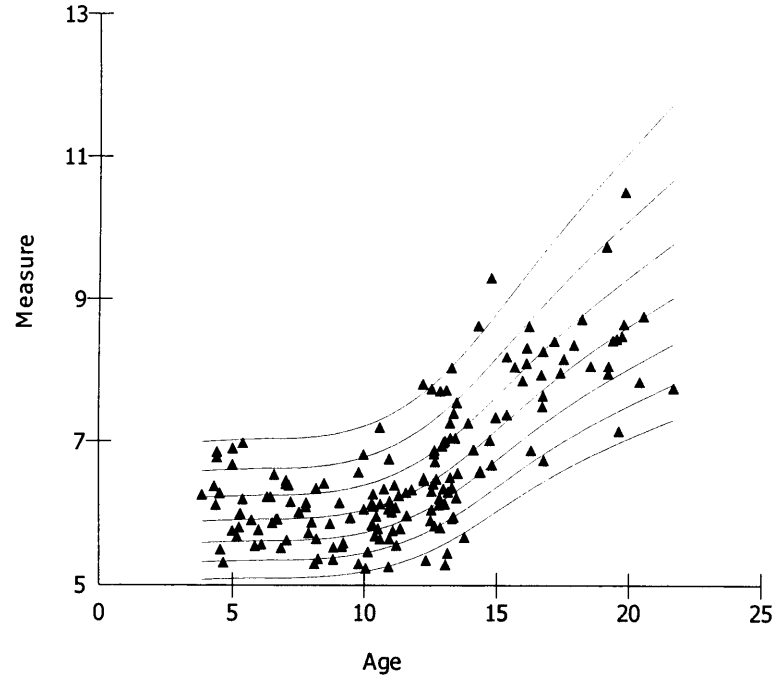


Figure 10-12 Boys Trunk LMI centile chart (n=145)



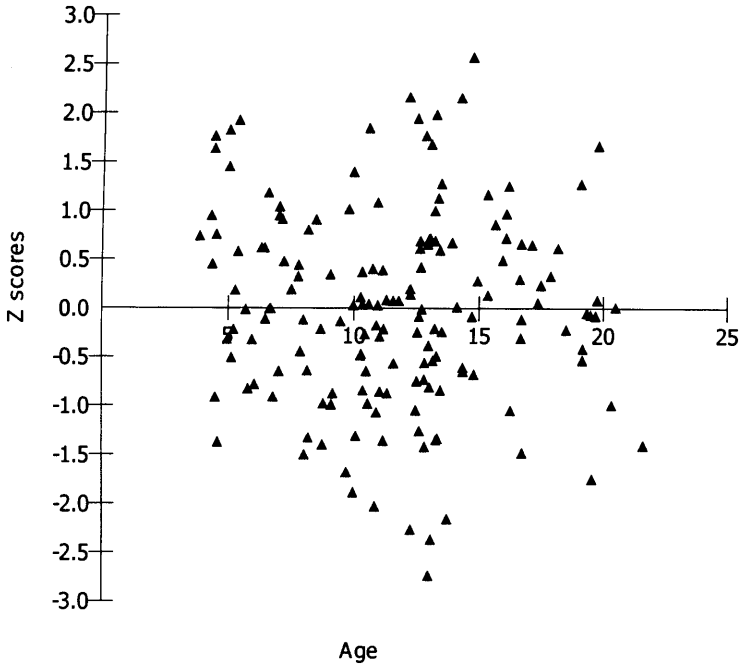
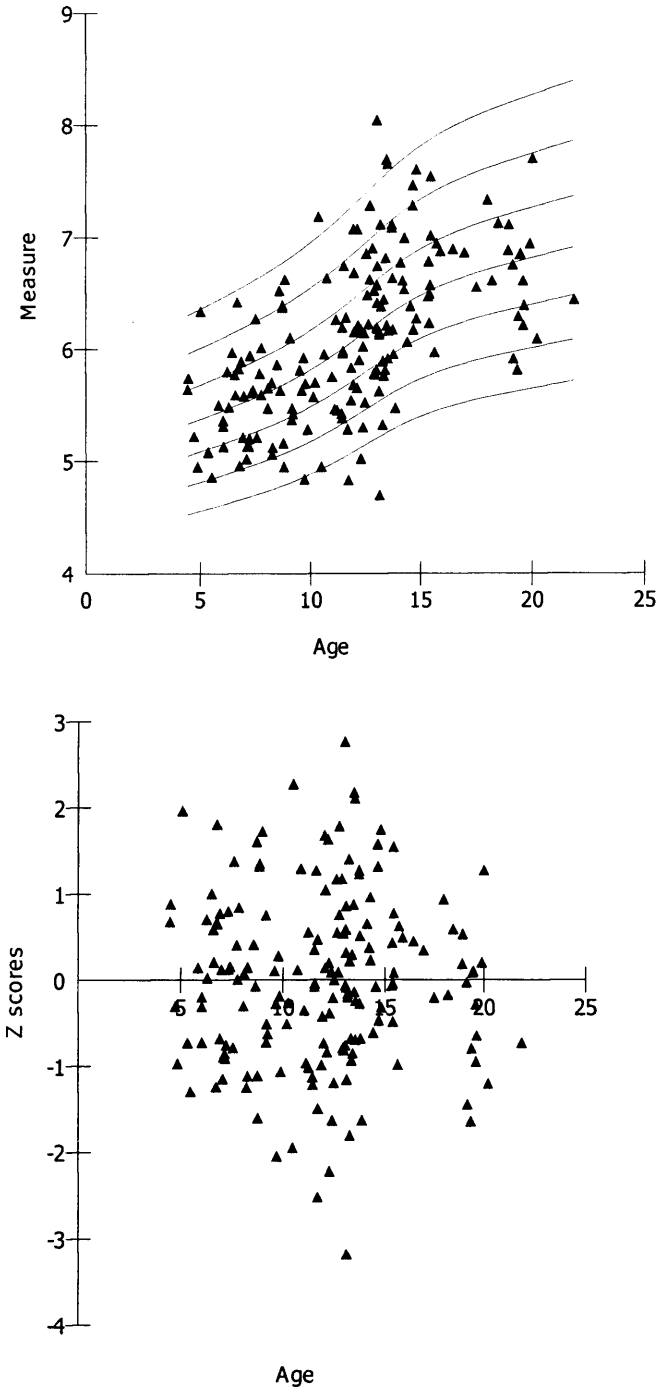


Figure 10-13 Girls Trunk LMI centile chart (n=163)



Appendix D

Example of the information sheets and consent forms

Institute of Child Health – UCL
and Great Ormond Street Hospital for Children NHS Trust

Version 4 5.5.05

YOUNG PERSON'S INFORMATION SHEET

Early Diet and Later Body Composition and Blood Vessel Function

We would like to ask you to take part in this research project. Please read the information sheet carefully and ask us about anything you do not understand. **This study is voluntary which means that it is your choice whether or not to take part in this study and you should only do so if you want to.**

1. The aim of the study.
The aim of this project is to find out whether the type of milk you received as a baby, and your pattern of growth, especially in the first year of life, has any effect on: 1) body composition (how much fat, muscle and bone you have) and 2) how blood vessels work later in life.

2. Why is the study being done?
Many people now think that the type of milk you are fed as a baby, the size you are at birth and how you grow in the first year of your life has an effect on whether or not you get heart and blood vessel disease later on. These factors may also affect your bones and how fat you are later on. We have asked you to take part in this study because we have good records of your early diet and growth. This is because when you were a baby you took part in a baby feeding study and you were weighed and measured during your first year of life.

3. How is the study being done?
If you decide to take part in this **voluntary** study we would ask you to spend a day at our special clinic in London where the study has been arranged. Your parents can come with you. If this is not possible for any reason, we could arrange to come and visit you at home and do a shorter version of the study. We would not be able to do the tests marked * in the list below. Before your clinic visit we will send you a container so that you can provide a sample of the first urine passed on the day of the appointment. If you are willing to have a blood test, we would like you not to have anything to eat or drink (except water) on the morning of your appointment, or if its going to be a long journey, 4 hours before coming to London. Lots of food and drink will be available all day at the centre.

At the clinic we will ask you do the following:

a) After the study has been explained to you, and we have answered all your questions, we would like you to sign a form. Signing this form tells us that you are willing to take part and that we can use the information collected when you took part previously in the baby feeding study to answer our research questions.

To measure how much fat, muscle and bone you have we will ask you to:

b) Lie on a bed with a machine above you that measures your bones using very low dose of X-rays (a DXA machine). This will also take a picture of your skeleton, which you can keep. You should not have a DXA scan if there is any chance you might be pregnant*.
c) Sit inside a chamber (with a window) called a Bodpod for a few minutes. This chamber measures your body volume. You will need t
shorts) (please bring one with you) and a swir
d) To drink a small amount of water that conta
water is entirely safe; it is not radioactive an
sample of your saliva before and after the drir
e) Measure your height and weight, waist, an
fingers and your skinfolds (by pressing the
You will not be asked to undress completely
An electrical signal will be measured to det
body. This measurement is completely painles
times before (even in small babies).
f) Measure the strength of the bones in your wr
The measurements take a few minutes, are co

For the blood vessels part of the study we will ask

g) Rest on a bed for 10 minutes and then meas
occasions. We will then look at the main bloo
(using an ultrasound scanner which is the sam
how fast your pulse travels down the arm (wh
tight cuff will be inflated around your lower
feel some tingling in the fingers before the cu
will be measured. This measurement has bee
as young as 5 years of age and is painless and
main artery in your neck using the same scanr
h) If you agree, some blood for testing will be
numbed the area. The level of a number of
vessel disease in adults and the amount of bo
asked to give us your permission to tell your
this study and if any of the results are abnorm
you do not want to. We will also measure
could be useful for you, because if the level i
lifestyle to a healthier one.
i) Step on and off a low bench for 3-5 minutes to
j) You will be asked to fill in a form to tell us a
function (such as smoking and drinking alcoh
are maturing into adults). A private room w
provided. You will not be asked to undress a
and not known to the people doing the measur
k) We will ask you some questions about your nc
l) We will ask you to wear a small instrument
movement and so will also tell us how physic
this, and the equipment can be posted back to

All of the things we have asked you to do are easy, f
are at the clinic (with lots of breaks!). **You can take**
voluntary and you don't have to do anything that

4. Are there any risks and discomforts?
We do not think that any of the tests we plan to do
scan may be a little uncomfortable but is not painful

5 years of age). Although a cream will be used to numb the skin for blood taking there still may be some discomfort and bruising. The Research Ethics Committee at the Institute of Child Health has given its approval for the study.

5. Who will have access to the case/research records?
Only the people doing the study will be able to see the information collected.

6.What are the potential benefits?
The study will not bring any immediate benefits to you. However it is hoped that the study will help us to understand the things that cause obesity and heart disease. We think that the study may also help you because if your chance of blood vessel disease later on is higher than normal you can be advised on how to reduce the risk for the future.

7. Do I have to take part?
This study is voluntary which means that it is your choice to take part and you should only do so if you want to. If you decide not to take part now or later this will not affect your medical care in any way.

8. Who do I speak to if a problem arises?
If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not sorted out, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, or if, urgent, by telephone on and the committee administration will put you in contact with him.

10. Details of how to contact the Researchers

Please contact either;
Recruitment Nurse,

Sirinuch Chomtho, Study Co-ordinator, Institute of Child Health, London,

Dr Jonathan Wells, Researcher, Institute of Child Health, London,

Dr Mary Fewtrell, Principal Investigator, Institute of Child Health, London,

Thank you for reading this information sheet

Version 4 5.5.05

PARENT'S INFORMATION SHEET

Early Diet and Later Body Composition and Blood Vessel Function

_____ is being invited to take part in a research study. Before you decide whether he/she should take part or not, it is important 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 others if you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

1. The aim of the study.
The aim of this project is to find out whether the type of milk received by a baby, or the pattern of growth, especially in the first year of life, has any effect on: 1) body composition (how much fat, muscle and bone you have) and 2) how blood vessels work later in life.

2. Why is the study being done?
Many scientists now believe that the type of milk you receive as a baby, the size you are at birth and how you grow in the first year have an influence on your body composition later in life and on whether or not you get heart and blood vessel disease. We have asked your child to take part in this study because they previously took part in a study looking at different types of infant feeding and so we have good records of your child's early milk intake, growth and body composition.

3. How is the study being done?
If your child decides to take part, we would like him/her to spend a day at our special clinic in London where the study has been arranged. If this is not possible for any reason, we would arrange to conduct a more limited version of the study at home. In this case, we would not be able to perform the tests marked with an asterisk. Before the study we will send you a container so that your child can provide a sample of the first urine passed on the day of the appointment. If your child agrees to have a blood test, we would like him/her not to have anything to eat or drink (except water) on the morning of the appointment or, if it's going to be a long journey, 4 hours before coming to London. Breakfast, lunch and other refreshments will be available throughout the day and **all travelling expenses will be refunded for both you and your child.**

At the clinic/visit we will ask you do the following:

a) After the study has been explained to you (and we have answered all your questions) we would like you to sign a consent form. This will give us permission to include your child in the study and to use the information collected previously in the infant study to answer our research questions.

To measure your child's body composition (how much fat, muscle and bone they have) we will ask your child to:

b) Lie on a bed with a machine above it (DXA), that measures your child's bones using very low dose X-rays (much less than a day's background radiation). This will also take a picture of his/her skeleton, which you can keep. DXA scans will not be performed if there is any possibility of pregnancy*.
c) Sit inside a chamber (with a window) called a Bodpod for a few minutes. This chamber measures body volume. Your child will need to wear a swimming costume (please bring one with you) and a swimming cap (which we will provide)*.
d) Drink a small amount of water that contains some special water called deuterium. This water is entirely safe; it is not radioactive and occurs naturally. We will ask your child to give a sample of saliva before and after the drink.
e) Measure your child's height and weight, waist, arm, head, hip and calf circumferences, and skinfolds (by pressing the fat gently on the arm, shoulder and abdomen). Children will not be asked to undress completely. Pads will be attached to the hands and feet. An electrical signal will be measured to determine the amount of water and muscle in the body. This measurement is completely painless and harmless and has been done thousands of times before (even in small babies).
f) Measure the strength of the bones in your child's wrist and lower leg using an ultrasound machine. The measurements take a few minutes, are completely painless and do not use x-rays*.

For the blood vessels part of the study we will ask your child to:

g) Rest on a bed for 10 minutes and then measure his/her blood pressure and heart rate. We will then look at the main blood vessel in the right arm and measure its width (using an ultrasound scanner which is the same as the scan used to look at unborn babies) and how fast the pulse travels down the arm (which is not in any way painful or unpleasant). A tight cuff will be inflated around the lower arm for 5 minutes, and then released (there may be some tingling in the fingers before the cuff is released). The change in blood vessel size will be measured. This measurement has been done in over 5000 people, including children as young as 5 years of age and is painless and harmless. We will also measure the size of the main artery in the neck, using the same scanner*.

h) If you and your child agree, we would like to take a blood sample to measure the level of a number of substances thought to affect the chances of blood vessel disease in adults, including cholesterol. We will also measure how fast bone is being made. We will also look for genes that affect how much fat/lean and bone tissue that you have and genes that affect heart disease. The genetic tests are only for research purposes and are unlikely to be have implications for your child's health personally. The sample will be regarded as a gift and may be stored at the Institute of Child Health and measurements may not be done until a later date. **The samples will be identified by a number only and all information collected will be completely confidential.** You will be asked to give us your consent to tell your family doctor (GP) that your child is taking part in this study. If any of the results are abnormal they will be sent to the GP and you will receive a letter advising you to contact the GP. **Children can take part in the study without having a blood test if they want.**
i) To assess physical fitness, your child will be asked to step on and off a low bench for 3-5 minutes and their heart rate measured.
j) Children will be asked to assess their stage of puberty (physical maturity) using pictures as a guide and to fill in a questionnaire about certain factors that could affect blood vessel function (such as smoking and alcohol consumption). A room where they can do this in private will be provided for all children (who will be asked to put their completed questionnaire into an envelope and then seal the envelope). The information collected will be strictly confidential and not known to the people doing the measurements.
k) We will ask you and your child some questions about their diet.
l) We will ask your child to wear a small instrument on his/her belt (for 4 days) that detects movement and so measures physical activity. You can chose when you do this test, and the equipment can be returned to us by post.

All of the things we have asked your child to do will take about 5 hours over the day you are at the centre. There will be lots of breaks, including stops for lunch and refreshments. If we visit you at home to conduct the study, the tests would take about 1 hour.

4. Are there any risks and discomforts?
No risk to your child can be foreseen. The cuff around the arm for the scan procedure may cause minor discomfort but is well tolerated (the scan has been done in children as young as 5 years of age). Although local anaesthetic cream will be used to numb the skin for blood taking there still may be some discomfort or bruising. The Research Ethics Committee of the Institute of Child Health, has given its approval for the study.

5. Who will have access to the case/research records?
Only the researchers and a representative of the Research Ethics Committee will have access to the data collected during this study.

6. What are the arrangements for compensation?
This research has been approved by an independent Research Ethics Committee who believe that it is of minimal risk to your child. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

7. What are the potential benefits?
The study will not bring any immediate benefits to your child. However, it is hoped that this study will further our understanding of the factors that cause obesity and heart disease (and so be of benefit in finding ways to lessen the risk of heart disease). We believe that the information collected could also be beneficial to your child in that if their risk of blood vessel disease in later life is higher than average (eg due to a high cholesterol level or high blood pressure) you can be advised on how to reduce the risk for the future.

8. Does my child have to take part?
If you do decide to take part you will be given this information sheet to keep and asked to sign two consent forms, one for you and one for our records. If you decide, now or at a later

stage that you do not wish to participate in this research project, that is entirely your right and will not in any way prejudice any present or future treatment.

9. Who do I speak to if a problem arises?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, or if, urgent, by telephone on and the committee administration will put you in contact with him.

10. Details of how to contact the Researchers

Please contact either;

Recruitment Nurse,

Sirinuch Chomtho, Study Co-ordinator, Institute of Child Health,

Dr Jonathan Wells, Researcher, Institute of Child Health,

or

Dr Mary Fewtrell, Principal Investigator, Institute of Child Health,

Thank – you for reading this information sheet

Institute of Child Health
and Great Ormond Street Hospital for Children NHS Trust
UNIVERSITY COLLEGE LONDON

Centre Number::
Study Number:
Patient Identification Number for this trial:

The child first and always

ASSENT FORM

Title of Project: Early diet and later Body Composition and Blood Vessel Function

Please initial box

1. I have read and understand the information sheet dated ..... ☐
(version .....) for the above study and have had the chance to ask questions.

2. I understand that taking part is voluntary and that I can withdraw ☐
at any time, without giving any reason, and without my medical care or legal rights being affected.

3. I agree to my GP being told that I am taking part in this study and also being ☐
told of any results that may require further tests

3. I agree to take part in the above study. ☐

Name of young person	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

version 1 14.05.04

The Queen's Anniversary Prizes 2000

UCL

Institute of Child Health
and Great Ormond Street Hospital for Children NHS Trust
UNIVERSITY COLLEGE LONDON

Centre Number::
Study Number:
Patient Identification Number for this trial:

The child first and always

CONSENT FORM

Title of Project: Early diet and later Body Composition and Blood Vessel Function

Please initial box

1. I confirm that I have read and understand the information sheet dated ..... ☐
(version .....) for the above study and have had the opportunity to ask questions.

2. I understand that my child's participation is voluntary and that I am free to withdraw ☐
at any time, without giving any reason, without my child's medical care or legal rights being affected.

3. I agree to my child's GP being notified of his/her participation in this study and also being ☐
notified of any results that may require further investigation

3. I agree to my child .....taking part in the above study. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

version 1 12.03.04

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Institute of Child Health

and Great Ormond Street Hospital for Children NHS Trust

UNIVERSITY COLLEGE LONDON

Centre Number::
Study Number:
Patient Identification Number for this trial:

The child first and always

ASSENT FORM FOR A BLOOD TEST

Title of Project: Early diet and later Body Composition and Blood Vessel Function

Thank you for agreeing to have a blood test. As you know from the information sheet, we would like to take some blood to look at factors that affect bone health and the risk of obesity and heart disease. To do this, we would like to take a small amount of blood using a local anaesthetic cream to numb the skin and stop the test from being painful. Before we can do this we need your permission after you have read the following:

Please initial box

1. I confirm that I have read and understand the information sheet dated ..... (version .....) for the above study, have had the chance to ask questions and been given a copy of the information sheet to keep. ☐
2. I agree to give a sample of blood for research in this project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for the use of this sample at any time without giving a reason, and without my medical or legal rights being affected. I consider the risk of harm in giving the sample is very small. ☐
3. I understand that my doctor (GP) and I will be told if the results of any medical tests done now or as part of the research are important for my health. (Most of these tests are for research purposes only and will not measure anything that directly applies to you) ☐
4. I understand that research using the sample may include genetic research to investigate genetic influences on disease, but the results of these tests are unlikely to have any meaning for my own health ☐
5. I understand that the blood sample will be stored and some tests may be conducted at a later date. I understand that future research using the sample may include genetic research. ☐

Name of young person	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

version 1 14.05..04

The Queen's Anniversary Prizes 2000

UCL

Institute of Child Health

and Great Ormond Street Hospital for Children NHS Trust

UNIVERSITY COLLEGE LONDON

Centre Number::
Study Number:
Patient Identification Number for this trial:

The child first and always

CONSENT FORM FOR A BLOOD TEST

Title of Project: Early diet and later Body Composition and Blood Vessel Function

Thank you for agreeing for your child to have a blood test. As you know from the information sheet, we would like to take some blood to look at factors that affect bone health and the risk of obesity and heart disease. To do this, we would like to take a small amount of blood using a local anaesthetic cream to numb the skin and stop the test from being painful. Before we can do this we need your permission after you have read the following:

Please initial box

1. I confirm that I have read and understand the information sheet dated ..... (version .....) for the above study, have had the opportunity to ask questions and been given a copy of the information sheet to keep. ☐
2. I agree to my child ..... giving a sample of blood for research in this project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for the use of this sample at any time without giving a reason, and without my medical or legal rights being affected. I consider the risk of harm in donating the sample is negligible. ☐
3. I understand that my child's doctor (GP) and I will be informed if the results of any medical tests done now or as part of the research are important for his/her health. (Most of these tests are for research purposes only and will not measure anything that directly applies to your child) ☐
4. I understand that research using the sample may include genetic research aimed at investigating the genetic influences on disease, but the results of these investigations are unlikely to have any implications for my child's own health ☐
5. I understand that the sample will be stored and some tests may be conducted at a later date. I understand that future research using the sample may include genetic research. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

version 1 12.03.04

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Appendix E

Additional data analyses for Chapter 5

Table 10-6 Age range of weight measurements at different period in infancy[#]

	n	Exact age of measurement	range
At 3 weeks	149	3.09 ± 0.49	2.01 – 4.29
At 6 weeks	172	6.06 ± 0.61	4.43 – 7.57
At 12 weeks	173	12.15 ± 0.95	10.00 – 16.29
At 6 months	161	5.94 ± 0.36	5.09 – 6.97
At 12 months	136	11.98 ± 0.77	9.99 – 13.90

[#] Data shown as mean ± SD

Table 10-7 Partial correlation coefficients (r) controlled for sex and current age[&]

a) Relationship between Δ weight SDS at different periods and potential confounders

	ΔSDS0-3wk	ΔSDS3-6wk	ΔSDS6-12wk	ΔSDS 3-6mo	ΔSDS6-12mo
Puberty	-0.14	-0.13	0.11	0.06	0.06
Physical activity	0.07	0.05	-0.03	-0.10	-0.12
Social class	-0.002	-0.09	-0.03	***0.23[b]***	-0.03
Maternal height	0.12	***0.17[a]***	-0.01	0.07	-0.10
Paternal height	0.08	0.11	-0.02	0.09	0.04
Maternal BMI	-0.04	0.004	0.08	0.09	0.17
Paternal BMI	0.07	0.01	0.09	***0.21[a]***	-0.02

b) Relationship between body composition outcomes and potential confounders

	FMI SDS	FFMI SDS	TrunkFMISDS	WCSDS	Triceps SDS	Subsc SDS
Puberty	0.001	***0.27[c]***	0.05	0.06	-0.03	-0.02
Physical activity	***-0.28[c]***	0.04	***-0.22[b]***	-0.12	***-0.26[c]***	***-0.29[c]***
Social class	***0.18[a]***	0.08	***0.16[a]***	0.12	0.09	0.14
Maternal height	***-0.17[a]***	-0.07	-0.13	-0.03	***-0.20[b]***	***-0.18[a]***
Paternal height	***-0.08***	-0.12	-0.07	<0.001	-0.04	-0.05
Maternal BMI	***0.40[c]***	***0.32[c]***	***0.40[c]***	***0.34[c]***	***0.32[c]***	***0.39[c]***
Paternal BMI	***0.33[c]***	***0.28[c]***	***0.35[c]***	***0.31[c]***	***0.25[b]***	***0.25[b]***

[&] All variables which were not normally distributed (Maternal BMI, Paternal BMI) were natural log transformed before analysis; [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$

Puberty coded as: 1 = prepubertal, 2 = pubertal, 3 = postpubertal, Physical activity was coded as: 2= much less or less active than peers, 3= same as peers, 4= more active than peers, 5= much more active than peers, Social class range from 1 which is the highest to ≥ 4 which is the lower social class

Table 10-8 Regression of current body size and composition on weight SDS change at different infancy periods[&]

	Δweight SDS 0-3wk				Δweight SDS 3-6wk				Δweight SDS 6-12wk				Δweight SDS 3-6mo				Δweight SDS 6-12mo			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.21	0.17	0.21		0.62	0.28	***0.03***	0.037	0.53	0.20	***0.009***	0.046	0.66	0.16	***<0.001***	0.103	0.18	0.18	0.31	
Ht SDS	0.20	0.13	0.13		0.29	0.21	0.17		0.23	0.15	0.13		0.39	0.13	***0.003***	0.064	0.20	0.13	0.14	
BMI SDS	0.14	0.19	0.45		0.64	0.30	***0.04***	0.033	0.57	0.22	***0.01***	0.044	0.62	0.18	***0.001***	0.077	0.10	0.19	0.62	
FMI SDS	0.12	0.15	0.41		0.49	0.24	***0.04***	0.031	0.44	0.17	***0.01***	0.042	0.45	0.15	***0.002***	0.065	0.05	0.16	0.73	
FFMI SDS	0.12	0.15	0.45		0.24	0.25	0.35		0.24	0.18	0.20		0.35	0.15	***0.02***	0.037	0.14	0.16	0.37	
Triceps SDS	0.14	0.15	0.34		0.75	0.23	***0.001***	0.076	0.37	0.17	***0.03***	0.031	0.39	0.14	***0.006***	0.053	-.15	0.15	0.32	
Subscapular SDS	0.10	0.16	0.52		0.65	0.26	***0.01***	0.047	0.31	0.19	0.10		0.34	0.16	***0.04***	0.032	0.09	0.17	0.60	
WCSDS[#]	0.28	0.17	0.11		0.63	0.28	***0.03***	0.038	0.23	0.21	0.29		0.50	0.17	***0.004***	0.064	-.05	0.18	0.78	
WC[$]	0.02	0.02	0.33		0.03	0.03	0.27		0.02	0.02	0.22		0.04	0.02	***0.02***	0.039	-.001	0.02	0.95	
WC[∞]	0.01	0.01	0.66		-.01	0.02	0.53		<0.001	0.01	0.99		0.002	0.01	0.83		0.01	0.01	0.64	
TrunkFMISDS	0.12	0.15	0.42		0.65	0.25	***0.01***	0.051	0.47	0.18	***0.009***	0.046	0.50	0.15	***0.001***	0.075	0.07	0.16	0.66	
TrunkFMISDS[∞]	-.04	0.09	0.66		0.18	0.15	0.24		0.27	0.11	***0.01***	0.044	0.11	0.07	0.11		0.15	0.09	0.11	

[&] From multiple regression analysis, each row represents a different model with body size and BC in the left hand column as a dependent variable; ΔweightSDS was an independent variable; birthweight SDS, gender, puberty, physical activity, social class, ethnicity, and parental height were covariates; B = the coefficient of ΔweightSDS i.e. the change in current body size or body composition per early ΔweightSDS (weight SDS change between 2 time points), SE = standard error, highlighted p value indicate significant at $p<0.05$, r^2 = coefficient of determination (calculated from partial r of Δ weight SDS)

[#] n=144, 134, 142, 136, and 116 for ΔweightSDS 0-3wk, ΔweightSDS 3-6wk, Δweight SDS 6-12wk, ΔweightSDS 3-6mo, and ΔweightSDS 6-12mo, respectively since the database for waist SDS was available only up to the age of 17.
WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.
[$] Adjusted for current height (m), WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.
[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

Table 10-9 Regression of current body size and composition on weight SDS change at different infancy periods with interpolation of weight SDS at 6 wk, 12 wk, 6 mo, and 12 mo.[&]

	Δweight SDS 3-6wk (n=147)				Δweight SDS 6-12wk (n=178)				Δweight SDS 3-6mo (n=178)				Δweight SDS 6-12mo (n=178)			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.21	0.17	0.23		0.46	0.18	***0.01***	0.037	0.61	0.15	***<0.001***	0.089	0.36	0.15	***0.02***	0.030
Ht SDS	0.21	0.15	0.17		0.25	0.15	0.11		0.40	0.13	***0.002***	0.052	0.27	0.13	***0.04***	0.024
BMI SDS	0.17	0.20	0.39		0.43	0.20	***0.03***	0.026	0.51	0.17	***0.003***	0.050	0.26	0.17	0.13	
FMI SDS	0.12	0.16	0.47		0.32	0.16	***0.05***	0.023	0.39	0.14	***0.005***	0.045	0.18	0.14	0.20	
FFMI SDS	0.19	0.16	0.24		0.07	0.16	0.69		0.25	0.14	0.08		0.16	0.14	0.27	
Triceps SDS	0.22	0.15	0.15		0.32	0.15	***0.04***	0.025	0.31	0.13	***0.02***	0.030	0.03	0.14	0.83	
Subscapular SDS	0.14	0.17	0.43		0.26	0.17	0.14		0.31	0.15	***0.04***	0.023	0.26	0.15	0.09	
WC SDS[#]	0.23	0.17	0.19		0.22	0.18	0.23		0.47	0.15	***0.003***	0.056	0.13	0.16	0.42	
WC[$]	0.01	0.02	0.56		0.02	0.02	0.31		0.03	0.01	0.82		0.01	0.01	0.37	
WC[∞]	0.003	0.01	0.76		-.011	0.01	0.26		0.01	0.01	0.51		0.004	0.01	0.61	
TrunkFMISDS	0.14	0.16	0.37		0.37	0.16	***0.02***	0.029	0.43	0.14	***0.002***	0.052	0.21	0.14	0.13	
TrunkFMISDS[∞]	0.07	0.07	0.31		0.07	0.07	0.27		0.13	0.06	***0.03***	0.028	0.12	0.06	***0.04***	0.025

[&] From multiple regression analysis, each row represents a different model with body size and BC in the left hand column as a dependent variable; Δweight SDS was an independent variable; birthweight SDS and gender were covariates

[#] n=142, 160, 160, and 160 for Δweight SDS 3-6wk, Δweight SDS 6-12wk, Δweight SDS 3-6mo, and Δweight SDS 6-12mo, respectively since the database for waist SDS was available only up to the age of 17

[$] Adjusted for current height (m), WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

Table 10-10 Regression of current body size and composition on weight SDS change at two different infancy periods in boys and girls[&]

	Boys								Girls							
	Δweight SDS 6-12 wk (n=69)				Δweight SDS 3-6 mo (n=65)				Δweight SDS 6-12 wk (n=88)				Δweight SDS 3-6 mo (n=85)			
Later BC	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**	**B**	**SE**	**p**	**r^2**
Wt SDS	0.58	0.26	***0.03***	0.070	1.09	0.28	***<0.001***	0.196	0.54	0.31	0.09		0.50	0.18	***0.007***	0.086
Ht SDS	0.19	0.22	0.40		0.61	0.25	***0.02***	0.091	0.31	0.29	0.23		0.36	0.17	***0.03***	0.054
BMI SDS	0.68	0.30	***0.03***	0.073	1.03	0.33	***0.003***	0.135	0.49	0.34	0.16		0.41	0.20	***0.04***	0.050
FMI SDS	0.49	0.24	***0.05***	0.059	0.68	0.27	***0.02***	0.089	0.43	0.29	0.14		0.39	0.17	***0.02***	0.065
FFMI SDS	0.26	0.23	0.27		0.61	0.26	***0.02***	0.085	0.07	0.31	0.81		0.15	0.18	0.41	
Triceps SDS	0.52	0.24	***0.03***	0.067	0.60	0.28	***0.03***	0.070	0.41	0.26	0.12		0.31	0.15	***0.05***	0.047
Subscapular SDS	0.43	0.26	0.11		0.56	0.30	0.06		0.34	0.31	0.27		0.29	0.18	0.11	
WCSDS[#]	0.49	0.26	0.07		0.87	0.29	***0.004***	0.136	0.11	0.33	0.74		0.37	0.19	0.06	
WC[$]	0.03	0.02	0.26		0.05	0.03	0.08		0.02	0.03	0.61		0.04	0.02	***0.05***	0.048
WC[∞]	-.01	0.01	0.73		-.003	0.02	0.87		-0.02	0.02	0.23		0.003	0.01	0.79	
TrunkFMISDS	0.57	0.24	***0.02***	0.080	0.83	0.27	***0.003***	0.133	0.39	0.29	0.19		0.37	0.17	***0.03***	0.056
TrunkFMISDS[∞]	0.18	0.09	0.06		0.21	0.11	0.06		0.001	0.11	0.99		0.06	0.07	0.34	

[&] From multiple regression analysis, each row represents a different model with body size and BC in the left hand column as a dependent variable; Δweight SDS was an independent variable; birth SDS was covariates

[#] n=63 and 60 for Δweight SDS 6-12wk, Δweight SDS 3-6mo, respectively for boys; n=79 and 76 for Δweight SDS 6-12wk, Δweight SDS 3-6mo, respectively for girls since the database for waist SDS was available only up to the age of 17.

[$] Adjusted for current height (m), WC was natural log transformed and presented in $\log_e$ scale. For the model with WC, current age was also adjusted for.

[∞] Adjusted for both current height (m) and total FM ($\log_e$ scale)

Bibliography

Bibliography

1. Evans WD, Finkelstein EA, Kamerow DB, Renaud JM. Public perceptions of childhood obesity. Am.J.Prev.Med 2005;28:26-32.

2. Ogden CL, Troiano RP, Briefel RR, Kuczmarski RJ, Flegal KM, Johnson CL. Prevalence of overweight among preschool children in the United States, 1971 through 1994. Pediatrics 1997;99:E1.

3. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA 2002;288:1728-32.

4. Lobstein TJ, James WP, Cole TJ. Increasing levels of excess weight among children in England. Int.J.Obes.Relat Metab Disord. 2003;27:1136-8.

5. Lobstein T, Frelut ML. Prevalence of overweight among children in Europe. Obes.Rev. 2003;4:195-200.

6. Wang Y, Monteiro C, Popkin BM. Trends of obesity and underweight in older children and adolescents in the United States, Brazil, China, and Russia. Am.J.Clin.Nutr. 2002;75:971-7.

7. Hardy LR, Harrell JS, Bell RA. Overweight in children: definitions, measurements, confounding factors, and health consequences. J.Pediatr.Nurs. 2004;19:376-84.

8. Whitaker RC. Understanding the complex journey to obesity in early adulthood. Ann.Intern.Med. 2002;136:923-5.

9. Sowers JR. Obesity as a cardiovascular risk factor. Am.J.Med 2003;115 Suppl 8A:37S-41S.

10. Lucas A. Programming by early nutrition in man. In: Bock GR, Whelan J, eds. The childhood environment and adult disease. Chichester: Wiley 1991:38-55.

11. Lewis DS, Bertrand HA, Masoro EJ, McGill HC, Jr., Carey KD, McMahan CA. Preweaning nutrition and fat development in baboons. J.Nutr. 1983;113:2253-9.

12. Lewis DS, Bertrand HA, Masoro EJ, McGill HC, Jr., Carey KD, McMahan CA. Effect of interaction of gender and energy intake on lean body mass and fat mass gain in infant baboons. J.Nutr. 1984;114:2021-6.

13. Lewis DS, Bertrand HA, McMahan CA, McGill HC, Jr., Carey KD, Masoro EJ. Preweaning food intake influences the adiposity of young adult baboons. J.Clin.Invest 1986;78:899-905.

14. Lewis DS, Bertrand HA, McMahan CA, McGill HC, Jr., Carey KD, Masoro EJ. Influence of preweaning food intake on body composition of young adult baboons. Am.J.Physiol 1989;257:R1128-R1135.

15. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet 1993;341:938-41.

16. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ 1999;319:245-9.

17. Lucas A. Programming by early nutrition: an experimental approach. J.Nutr. 1998;128:401S-6S.

18. Bechard L, Puig M. Body composition and growth. In: Walker WA, Watkins JB, Duggan C, eds. Nutrition in Pediatrics. Hamilton: BC Decker 2003:32-51.

19. Godfrey K, Robinson S. Maternal nutrition, placental growth and fetal programming. Proc.Nutr.Soc. 1998;57:105-11.

20. Cetin I, Alvino G, Radaelli T, Pardi G. Fetal nutrition: a review. Acta Paediatr.Suppl 2005;94:7-13.

21. Hughes IA. Fetal growth. In: Ulijaszek SJ, Johnston FE, Preece MA, eds. The Cambridge Encyclopedia of Human Growth and Development. Cambridge: Cambridge University Press 1998:146-75.

22. Cetin I, Morpurgo PS, Radaelli T et al. Fetal plasma leptin concentrations: relationship with different intrauterine growth patterns from 19 weeks to term. Pediatr.Res. 2000;48:646-51.

23. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull.World Health Organ 1987;65:663-737.

24. Ounsted M, Sleigh G. The infant's self-regulation of food intake and weight gain. Difference in metabolic balance after growth constraint or acceleration in utero. Lancet 1975;1:1393-7.

25. Hill DJ, Hogg J. Growth factors and the regulation of pre- and postnatal growth. Baillieres Clin.Endocrinol.Metab 1989;3:579-625.

26. Cripps RL, Martin-Gronert MS, Ozanne SE. Fetal and perinatal programming of appetite. Clin.Sci.(Lond) 2005;109:1-11.

27. Fernandez-Twinn DS, Ozanne SE. Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Physiol Behav. 2006;88:234-43.

28. Brooke OG, Kinsey JM. High energy feeding in small for gestation infants. Arch.Dis.Child 1985;60:42-6.

29. Fomon SJ, Filer LJ, Jr., Thomas LN, Rogers RR, Proksch AM. Relationship between formula concentration and rate of growth of normal infants. J.Nutr. 1969;98:241-54.

30. Fomon SJ, Filmer LJ, Jr., Thomas LN, Anderson TA, Nelson SE. Influence of formula concentration on caloric intake and growth of normal infants. Acta Paediatr.Scand. 1975;64:172-81.

31. Karlberg J, bertsson-Wikland K. Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatr.Res. 1995;38:733-9.

32. Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ 1999;318:427-31.

33. Hales CN, Barker DJ, Clark PM et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991;303:1019-22.

34. Fewtrell MS, Doherty C, Cole TJ, Stafford M, Hales CN, Lucas A. Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9-12 years. Diabetologia 2000;43:714-7.

35. Ellis KJ. Human body composition: in vivo methods. Physiol Rev. 2000;80:649-80.

36. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am.J.Clin.Nutr. 1982;35:1169-75.

37. Haschke F. Body composition of adolescent males. Part I. Total body water in normal adolescent males. Part II. Body composition of the male reference adolescent. Acta Paediatr.Scand.Suppl 1983;307:1-23.

38. Haschke F. Body composition during adolescence. In: Klish WJ, Kretchmer N, eds. Body composition measurements in infants and children. Report of the 98th Ross Conference on Pediatric Research. Columbus (OH): Ross Laboratories 1989:76-82.

39. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. Pediatr.Res. 2000;47:578-85.

40. Catalano PM, Drago NM, Amini SB. Factors affecting fetal growth and body composition. Am.J.Obstet.Gynecol. 1995;172:1459-63.

41. Baumgartner RN, Roche AF. Tracking of fat pattern indices in childhood: the Melbourne Growth Study. Hum.Biol. 1988;60:549-67.

42. Poissonnet CM, LaVelle M, Burdi AR. Growth and development of adipose tissue. J.Pediatr. 1988;113:1-9.

43. Malina RM. Post-natal growth and maturation. In: Ulijaszek SJ, Johnston FE, Preece MA, eds. The Cambridge Encyclopedia of Human Growth and Development. Cambridge: Cambridge University Press 1998:176-235.

44. White DR, Widdowson EM, Woodard HQ, Dickerson JW. The composition of body tissues (II). Fetus to young adult. Br.J.Radiol. 1991;64:149-59.

45. Lohman TG. Applicability of body composition techniques and constants for children and youths. Exerc.Sport Sci.Rev. 1986;14:325-57.

46. Heymsfield SB, Wang Z, Baumgartner RN, Dilmanian FA, Ma R, Yasumura S. Body composition and aging: a study by in vivo neutron activation analysis. J.Nutr. 1993;123:432-7.

47. Heymsfield SB, Wang J, Lichtman S, Kamen Y, Kehayias J, Pierson RN, Jr. Body composition in elderly subjects: a critical appraisal of clinical methodology. Am.J.Clin.Nutr. 1989;50:1167-75.

48. Wells JC, Fewtrell MS. Measuring body composition. Arch.Dis.Child 2006;91:612-7.

49. Pietrobelli A, Faith MS, Allison DB, Gallagher D, Chiumello G, Heymsfield SB. Body mass index as a measure of adiposity among children and adolescents: a validation study. J.Pediatr. 1998;132:204-10.

50. Wells JC. A Hattori chart analysis of body mass index in infants and children. Int.J.Obes.Relat Metab Disord. 2000;24:325-9.

51. Daniels SR, Khoury PR, Morrison JA. The utility of body mass index as a measure of body fatness in children and adolescents: differences by race and gender. Pediatrics 1997;99:804-7.

52. Wells JC, Coward WA, Cole TJ, Davies PS. The contribution of fat and fat-free tissue to body mass index in contemporary children and the reference child. Int.J.Obes.Relat Metab Disord. 2002;26:1323-8.

53. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. Am.J.Clin.Nutr. 2004;79:379-84.

54. Savva SC, Tornaritis M, Savva ME et al. Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. Int.J.Obes.Relat Metab Disord. 2000;24:1453-8.

55. Maffeis C, Pietrobelli A, Grezzani A, Provera S, Tato L. Waist circumference and cardiovascular risk factors in prepubertal children. Obes.Res. 2001;9:179-87.

56. Tanner JM, Whitehouse RH. Revised standards for triceps and subscapular skinfolds in British children. Arch.Dis.Child 1975;50:142-5.

57. Slaughter MH, Lohman TG, Boileau RA et al. Skinfold equations for estimation of body fatness in children and youth. Hum.Biol. 1988;60:709-23.

58. Wells JC, Fuller NJ, Dewit O, Fewtrell MS, Elia M, Cole TJ. Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models. Am.J.Clin.Nutr. 1999;69:904-12.

59. Williams JE, Wells JC, Wilson CM, Haroun D, Lucas A, Fewtrell MS. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model. Am.J.Clin.Nutr. 2006;83:1047-54.

60. Weststrate JA, Deurenberg P. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. Am.J.Clin.Nutr. 1989;50:1104-15.

61. Dewit O, Fuller NJ, Fewtrell MS, Elia M, Wells JC. Whole body air displacement plethysmography compared with hydrodensitometry for body composition analysis. Arch.Dis.Child 2000;82:159-64.

62. Davies PS, Wells JC. Calculation of total body water in infancy. Eur.J.Clin.Nutr. 1994;48:490-5.

63. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of 2H- and 18O-labeled water in humans. Am.J.Physiol 1994;267:E585-E590.

64. Wells JC, Ritz P, Davies PS, Coward WA. Factors affecting the 2H to 18O dilution space ratio in infants. Pediatr.Res. 1998;43:467-71.

65. Wells JC, Fuller NJ, Wright A, Fewtrell MS, Cole TJ. Evaluation of air-displacement plethysmography in children aged 5-7 years using a three-component model of body composition. Br.J.Nutr. 2003;90:699-707.

66. Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. Clin.Sci.(Lond) 1992;82:687-93.

67. MCCANCE RA. Food, growth, and time. Lancet 1962;2:621-6.

68. Knittle JL, Hirsch J. Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. J.Clin.Invest 1968;47:2091-8.

69. Whitaker RC, Pepe MS, Wright JA, Seidel KD, Dietz WH. Early adiposity rebound and the risk of adult obesity. Pediatrics 1998;101:E5.

70. Parsons TJ, Power C, Logan S, Summerbell CD. Childhood predictors of adult obesity: a systematic review. Int.J.Obes.Relat Metab Disord. 1999;23 Suppl 8:S1-107.

71. Allison DB, Paultre F, Heymsfield SB, Pi-Sunyer FX. Is the intra-uterine period really a critical period for the development of adiposity? Int.J.Obes.Relat Metab Disord. 1995;19:397-402.

72. Phillips DI. Relation of fetal growth to adult muscle mass and glucose tolerance. Diabet.Med. 1995;12:686-90.

73. Stettler N, Tershakovec AM, Zemel BS et al. Early risk factors for increased adiposity: a cohort study of African American subjects followed from birth to young adulthood. Am.J.Clin.Nutr. 2000;72:378-83.

74. Hediger ML, Overpeck MD, McGlynn A, Kuczmarski RJ, Maurer KR, Davis WW. Growth and fatness at three to six years of age of children born small- or large-for-gestational age. Pediatrics 1999;104:e33.

75. Hediger ML, Overpeck MD, Kuczmarski RJ, McGlynn A, Maurer KR, Davis WW. Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics 1998;102:E60.

76. Weyer C, Pratley RE, Lindsay RS, Tataranni PA. Relationship between birth weight and body composition, energy metabolism, and sympathetic nervous system activity later in life. Obes.Res. 2000;8:559-65.

77. Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C. Intrauterine programming of adult body composition. J.Clin.Endocrinol.Metab 2001;86:267-72.

78. Loos RJ, Beunen G, Fagard R, Derom C, Vlietinck R. Birth weight and body composition in young adult men--a prospective twin study. Int.J.Obes.Relat Metab Disord. 2001;25:1537-45.

79. Loos RJ, Beunen G, Fagard R, Derom C, Vlietinck R. Birth weight and body composition in young women: a prospective twin study. Am.J.Clin.Nutr. 2002;75:676-82.

80. Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A. Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am.J.Clin.Nutr. 2003;77:726-30.

81. Yanovski JA. Rapid weight gain during infancy as a predictor of adult obesity. Am.J.Clin.Nutr. 2003;77:1350-1.

82. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109:194-9.

83. Gasser T, Ziegler P, Seifert B, Molinari L, Largo RH, Prader A. Prediction of adult skinfolds and body mass from infancy through adolescence. Ann.Hum.Biol. 1995;22:217-33.

84. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am.J.Clin.Nutr. 2003;77:1374-8.

85. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320:967-71.

86. Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. BMJ 2001;323:1331-5.

87. Stettler N, Bovet P, Shamlaye H, Zemel BS, Stallings VA, Paccaud F. Prevalence and risk factors for overweight and obesity in children from Seychelles, a country in rapid transition: the importance of early growth. Int.J.Obes.Relat Metab Disord. 2002;26:214-9.

88. van Lenthe FJ, van Mechelen W, Kemper HC, Twisk JW. Association of a central pattern of body fat with blood pressure and lipoproteins from adolescence into adulthood. The Amsterdam Growth and Health Study. Am.J.Epidemiol. 1998;147:686-93.

89. Bray GA, Champagne CM. Obesity and the Metabolic Syndrome: implications for dietetics practitioners. J.Am.Diet.Assoc. 2004;104:86-9.

90. Shuldiner AR, Yang R, Gong DW. Resistin, obesity and insulin resistance--the emerging role of the adipocyte as an endocrine organ. N.Engl.J.Med. 2001;345:1345-6.

91. Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J.Epidemiol.Community Health 1992;46:184-6.

92. Malina RM, Katzmarzyk PT, Beunen G. Birth weight and its relationship to size attained and relative fat distribution at 7 to 12 years of age. Obes.Res. 1996;4:385-90.

93. Barker M, Robinson S, Osmond C, Barker DJ. Birth weight and body fat distribution in adolescent girls. Arch.Dis.Child 1997;77:381-3.

94. Bavdekar A, Yajnik CS, Fall CH et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes 1999;48:2422-9.

95. Schroeder DG, Martorell R, Flores R. Infant and child growth and fatness and fat distribution in Guatemalan adults. Am.J.Epidemiol. 1999;149:177-85.

96. Garnett SP, Cowell CT, Baur LA et al. Abdominal fat and birth size in healthy prepubertal children. Int.J.Obes.Relat Metab Disord. 2001;25:1667-73.

97. Choi CS, Kim C, Lee WJ et al. Association between birth weight and insulin sensitivity in healthy young men in Korea: role of visceral adiposity. Diabetes Res.Clin.Pract. 2000;49:53-9.

98. Gillman MW. Breast-feeding and obesity. J.Pediatr. 2002;141:749-50.

99. von Kries R, Koletzko B, Sauerwald T et al. Breast feeding and obesity: cross sectional study. BMJ 1999;319:147-50.

100. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations in later life. Am.J.Clin.Nutr. 2002;75:993-9.

101. Li L, Parsons TJ, Power C. Breast feeding and obesity in childhood: cross sectional study. BMJ 2003;327:904-5.

102. Parsons TJ, Power C, Manor O. Infant feeding and obesity through the lifecourse. Arch.Dis.Child 2003;88:793-4.

103. Fomon SJ, Ziegler EE, Nelson SE, Rogers RR, Frantz JA. Infant formula with protein-energy ratio of 1.7 g/100 kcal is adequate but may not be safe. J.Pediatr.Gastroenterol.Nutr. 1999;28:495-501.

104. Kashyap S, Forsyth M, Zucker C, Ramakrishnan R, Dell RB, Heird WC. Effects of varying protein and energy intakes on growth and metabolic response in low birth weight infants. J.Pediatr. 1986;108:955-63.

105. Schulze KF, Stefanski M, Masterson J et al. Energy expenditure, energy balance, and composition of weight gain in low birth weight infants fed diets of different protein and energy content. J.Pediatr. 1987;110:753-9.

106. van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am.J.Clin.Nutr. 2000;71:816-21.

107. Cooke RJ, McCormick K, Griffin IJ et al. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr.Res. 1999;46:461-4.

108. Pieltain C, De Curtis M, Gerard P, Rigo J. Weight gain composition in preterm infants with dual energy X-ray absorptiometry. Pediatr.Res. 2001;49:120-4.

109. Cooke RJ, Embleton ND, Griffin IJ, Wells JC, McCormick KP. Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr.Res. 2001;49:719-22.

110. Lucas A, Fewtrell MS, Morley R et al. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 2001;108:703-11.

111. Morley R, Lucas A. Randomized diet in the neonatal period and growth performance until 7.5-8 y of age in preterm children. Am.J.Clin.Nutr. 2000;71:822-8.

112. Fewtrell MS, Morley R, Abbott RA et al. Catch-up growth in small-for-gestational-age term infants: a randomized trial. Am.J.Clin.Nutr. 2001;74:516-23.

113. Rolland-Cachera MF, Deheeger M, Guilloud-Bataille M, Avons P, Patois E, Sempe M. Tracking the development of adiposity from one month of age to adulthood. Ann.Hum.Biol. 1987;14:219-29.

114. Schmelzle HR, Fusch C. Body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry. Am.J.Clin.Nutr. 2002;76:1096-100.

115. Butte N, Heinz C, Hopkinson J, Wong W, Shypailo R, Ellis K. Fat mass in infants and toddlers: comparability of total body water, total body potassium, total body electrical conductivity, and dual-energy X-ray absorptiometry. J.Pediatr.Gastroenterol.Nutr. 1999;29:184-9.

116. Ma G, Yao M, Liu Y et al. Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. Am.J.Clin.Nutr. 2004;79:653-60.

117. Butte NF, Wong WW, Hopkinson JM, Smith EO, Ellis KJ. Infant feeding mode affects early growth and body composition. Pediatrics 2000;106:1355-66.

118. Wells JC, Stanley M, Laidlaw AS, Day JM, Davies PS. The relationship between components of infant energy expenditure and childhood body fatness. Int.J.Obes.Relat Metab Disord. 1996;20:848-53.

119. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320:1240-3.

120. McTigue KM, Garrett JM, Popkin BM. The natural history of the development of obesity in a cohort of young U.S. adults between 1981 and 1998. Ann.Intern.Med. 2002;136:857-64.

121. shmukh-Taskar P, Nicklas TA, Morales M, Yang SJ, Zakeri I, Berenson GS. Tracking of overweight status from childhood to young adulthood: the Bogalusa Heart Study. Eur.J.Clin.Nutr. 2006;60:48-57.

122. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. N.Engl.J.Med. 1997;337:869-73.

123. Must A, Jacques PF, Dallal GE, Bajema CJ, Dietz WH. Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935. N.Engl.J.Med. 1992;327:1350-5.

124. Lawlor DA, Leon DA. Association of body mass index and obesity measured in early childhood with risk of coronary heart disease and stroke in middle age: findings from the aberdeen children of the 1950s prospective cohort study. Circulation 2005;111:1891-6.

125. Gunnell DJ, Frankel SJ, Nanchahal K, Peters TJ, Davey SG. Childhood obesity and adult cardiovascular mortality: a 57-y follow-up study based on the Boyd Orr cohort. Am.J.Clin.Nutr. 1998;67:1111-8.

126. Skidmore PM, Yarnell JW. The obesity epidemic: prospects for prevention. QJM. 2004;97:817-25.

127. WHO. Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation on Obesity. 1998. Geneva, World Health Organization.

128. Bosy-Westphal A, Geisler C, Onur S et al. Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors. Int.J.Obes.(Lond) 2006;30:475-83.

129. De LF, Mukherjee M, Kadziola Z, Suleiman S, Kakkar VV. Association of overall adiposity rather than body mass index with lipids and procoagulant factors. Thromb.Haemost. 1998;80:603-6.

130. Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C, Berenson GS. Body mass index, waist circumference, and clustering of cardiovascular disease risk factors in a biracial sample of children and adolescents. Pediatrics 2004;114:e198-e205.

131. Janssen I, Katzmarzyk PT, Srinivasan SR et al. Combined influence of body mass index and waist circumference on coronary artery disease risk factors among children and adolescents. Pediatrics 2005;115:1623-30.

132. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. Pediatrics 1999;103:1175-82.

133. Maynard LM, Wisemandle W, Roche AF, Chumlea WC, Guo SS, Siervogel RM. Childhood body composition in relation to body mass index. Pediatrics 2001;107:344-50.

134. Demerath EW, Schubert CM, Maynard LM et al. Do changes in body mass index percentile reflect changes in body composition in children? Data from the Fels Longitudinal Study. Pediatrics 2006;117:e487-e495.

135. Wells JC, Fewtrell MS, Williams JE, Haroun D, Lawson MS, Cole TJ. Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. Int.J.Obes.(Lond) 2006.

136. Frayn KN. Adipose tissue and the insulin resistance syndrome. Proc.Nutr.Soc. 2001;60:375-80.

137. Kahn BB, Flier JS. Obesity and insulin resistance. J.Clin.Invest 2000;106:473-81.

138. Goran MI, Ball GD, Cruz ML. Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. J.Clin.Endocrinol.Metab 2003;88:1417-27.

139. Maffeis C, Pietrobelli A, Grezzani A, Provera S, Tato L. Waist circumference and cardiovascular risk factors in prepubertal children. Obes.Res. 2001;9:179-87.

140. Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am.J.Clin.Nutr. 2005;82:980-7.

141. Rogers IS, Andy NR, Steer CD et al. Associations of size at birth with body composition and fat distribution at age 9-10 years. Am.J.Clin.Nutr. 2006 (in press).

142. Glickman SG, Marn CS, Supiano MA, Dengel DR. Validity and reliability of dual-energy X-ray absorptiometry for the assessment of abdominal adiposity. J.Appl.Physiol 2004;97:509-14.

143. Lee CC, Glickman SG, Dengel DR, Brown MD, Supiano MA. Abdominal adiposity assessed by dual energy X-ray absorptiometry provides a sex-independent predictor of insulin sensitivity in older adults. J.Gerontol.A Biol.Sci.Med.Sci. 2005;60:872-7.

144. Lucas A, Ewing G, Roberts SB, Coward WA. How much energy does the breast fed infant consume and expend? Br.Med.J.(Clin.Res.Ed) 1987;295:75-7.

145. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. 1949. Nutrition 1990;6:213-21.

146. Schoeller DA. Energy expenditure from doubly labeled water: some fundamental considerations in humans. Am.J.Clin.Nutr. 1983;38:999-1005.

147. Roberts SB, Coward WA, Schlingenseipen KH, Nohria V, Lucas A. Comparison of the doubly labeled water (2H2(18)O) method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants. Am.J.Clin.Nutr. 1986;44:315-22.

148. Wells, JC. Energy metabolism in breast fed and formula fed infants. PhD thesis. 1994. University of Cambridge, United Kingdom.

149. Wells JC, Davies PS. The effect of diet and sex on sleeping metabolic rate in 12-week-old infants. Eur.J.Clin.Nutr. 1995;49:329-35.

150. Wells JC, Davies PS. Sleeping metabolic rate and body size in 12-week-old infants. Eur.J.Clin.Nutr. 1995;49:323-8.

151. Wells JC, Davies PS. Diet and behavioural activity in 12-week-old infants. Ann.Hum.Biol. 1995;22:207-15.

152. Wells JC, Stanley M, Laidlaw AS, Day JM, Davies PS. Energy intake in early infancy and childhood fatness. Int.J.Obes.Relat Metab Disord. 1998;22:387-92.

153. Wells JC, Stanley M, Laidlaw AS, Day JM, Stafford M, Davies PS. Investigation of the relationship between infant temperament and later body composition. Int.J.Obes.Relat Metab Disord. 1997;21:400-6.

154. Davies PS, Ewing G, Lucas A. Energy expenditure in early infancy. Br.J.Nutr. 1989;62:621-9.

155. Davies PS, Cole TJ, Lucas A. Adjusting energy expenditure for body weight in early infancy. Eur.J.Clin.Nutr. 1989;43:641-5.

156. Davies PS, Lucas A. Quetelet's index as a measure of body fatness in young infants. Early Hum.Dev. 1989;20:135-41.

157. Davies PS, Lucas A. The prediction of total body fatness in early infancy. Early Hum.Dev. 1990;21:193-8.

158. Davies PS, Day JM, Lucas A. Energy expenditure in early infancy and later body fatness. Int.J.Obes. 1991;15:727-31.

159. Bray GA, DeLany JP, Volaufova J, Harsha DW, Champagne C. Prediction of body fat in 12-y-old African American and white children: evaluation of methods. Am.J.Clin.Nutr. 2002;76:980-90.

160. Brozek J, Grande F, Anderson JT, Keys A. Densitometric analysis of body composition: revision of some quantitative assumptions. Ann.N.Y.Acad.Sci. 1963;110:113-40.

161. Keys A, Brozek J. Body fat in adult man. Physiol Rev. 1953;33:245-325.

162. Wells JC, Fuller NJ. Precision of measurement and body size in whole-body air-displacement plethysmography. Int.J.Obes.Relat Metab Disord. 2001;25:1161-7.

163. Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. Med Sci.Sports Exerc. 1995;27:1692-7.

164. Nunez C, Kovera AJ, Pietrobelli A et al. Body composition in children and adults by air displacement plethysmography. Eur.J.Clin.Nutr. 1999;53:382-7.

165. Scrimgeour CM, Rollo MM, Mudambo SM, Handley LL, Prosser SJ. A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. Biol.Mass Spectrom. 1993;22:383-7.

166. Wells JC, Strickland SS. Measurement of nutritional status using conventional anthropometry and D2O in Sarawak, Malaysia. Eur.J.Clin.Nutr. 1996;50:668-71.

167. Kiebzak GM, Leamy LJ, Pierson LM, Nord RH, Zhang ZY. Measurement precision of body composition variables using the lunar DPX-L densitometer. J.Clin.Densitom. 2000;3:35-41.

168. Laskey MA. Dual-energy X-ray absorptiometry and body composition. Nutrition 1996;12:45-51.

169. Genton L, Hans D, Kyle UG, Pichard C. Dual-energy X-ray absorptiometry and body composition: differences between devices and comparison with reference methods. Nutrition 2002;18:66-70.

170. McCarthy HD, Jarrett KV, Crawley HF. The development of waist circumference percentiles in British children aged 5.0-16.9 y. Eur.J.Clin.Nutr. 2001;55:902-7.

171. Sobey WJ, Beer SF, Carrington CA et al. Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. Biochem.J. 1989;260:535-41.

172. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin.Chem. 1972;18:499-502.

173. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191-2.

174. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

175. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am.J.Physiol 1979;237:E214-E223.

176. Bonora E, Targher G, Alberiche M et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57-63.

177. Wareham NJ, Byrne CD, Williams R, Day NE, Hales CN. Fasting proinsulin concentrations predict the development of type 2 diabetes. Diabetes Care 1999;22:262-70.

178. Anwar YA, Tendler BE, McCabe EJ, Mansoor GA, White WB. Evaluation of the Datascope Accutorr Plus according to the recommendations of the Association for the Advancement of Medical Instrumentation. Blood Press Monit. 1997;2:105-10.

179. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66:918-20.

180. Standard Occupational Classification: A Publication of the Government Statistical Service. 1991. London, HMSO.

181. Sallis JF, Patterson TL, Buono MJ, Nader PR. Relation of cardiovascular fitness and physical activity to cardiovascular disease risk factors in children and adults. Am.J.Epidemiol. 1988;127:933-41.

182. Euser AM, Finken MJ, Keijzer-Veen MG, Hille ET, Wit JM, Dekker FW. Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am.J.Clin.Nutr. 2005;81:480-7.

183. Cole TJ. Sampling, study size and power. In: Margetts BM, Nelson M, eds. Design concepts in nutritional epidemiology. Oxford: Oxford University Press 1997:64-86.

184. van Belle G. Sample size. In: van Belle G, ed. Statistical rules of thumb. New York: Wiley 2002:29-52.

185. Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. Arch.Dis.Child 1995;73:17-24.

186. Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the UK, 1990. Arch.Dis.Child 1995;73:25-9.

187. Davies PS, Day JM, Cole TJ. Converting Tanner-Whitehouse reference tricep and subscapular skinfold measurements to standard deviation scores. Eur.J.Clin.Nutr. 1993;47:559-66.

188. Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. Stat.Med. 2000;19:3109-25.

189. Wells JC, Cole TJ. Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int.J.Obes.Relat Metab Disord. 2002;26:947-52.

190. VanItallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. Am.J.Clin.Nutr. 1990;52:953-9.

191. Cole TJ. The LMS method for constructing normalized growth standards. Eur.J.Clin.Nutr. 1990;44:45-60.

192. Rodriguez G, Moreno LA, Blay MG et al. Body fat measurement in adolescents: comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. Eur.J.Clin.Nutr. 2005;59:1158-66.

193. Mulligan J, Betts P, Elia M. Programming of body composition: studies in children aged 6.6 to 9.1 years. International Journal of Body Composition Research 2005;3:97 (abstr).

194. Duran-Tauleria E, Rona RJ, Chinn S. Factors associated with weight for height and skinfold thickness in British children. J.Epidemiol.Community Health 1995;49:466-73.

195. Okosun IS, Liao Y, Rotimi CN, Dever GE, Cooper RS. Impact of birth weight on ethnic variations in subcutaneous and central adiposity in American children aged 5-11 years. A study from the Third National Health and Nutrition Examination Survey. Int.J.Obes.Relat Metab Disord. 2000;24:479-84.

196. Wells JC, Hallal PC, Wright A, Singhal A, Victora CG. Fetal, infant and childhood growth: relationships with body composition in Brazilian boys aged 9 years. Int.J.Obes.(Lond) 2005;29:1192-8.

197. Walker SP, Gaskin PS, Powell CA, Bennett FI. The effects of birth weight and postnatal linear growth retardation on body mass index, fatness and fat distribution in mid and late childhood. Public Health Nutr. 2002;5:391-6.

198. Fewtrell MS, Lucas A, Cole TJ, Wells JC. Prematurity and reduced body fatness at 8-12 y of age. Am.J.Clin.Nutr. 2004;80:436-40.

199. Matthes JW, Lewis PA, Davies DP, Bethel JA. Body size and subcutaneous fat patterning in adolescence. Arch.Dis.Child 1996;75:521-3.

200. Labayen I, Moreno LA, Blay MG et al. Early programming of body composition and fat distribution in adolescents. J.Nutr. 2006;136:147-52.

201. Frisancho AR. Prenatal compared with parental origins of adolescent fatness. Am.J.Clin.Nutr. 2000;72:1186-90.

202. Kahn HS, Narayan KM, Williamson DF, Valdez R. Relation of birth weight to lean and fat thigh tissue in young men. Int.J.Obes.Relat Metab Disord. 2000;24:667-72.

203. Li H, Stein AD, Barnhart HX, Ramakrishnan U, Martorell R. Associations between prenatal and postnatal growth and adult body size and composition. Am.J.Clin.Nutr. 2003;77:1498-505.

204. Sachdev HS, Fall CH, Osmond C et al. Anthropometric indicators of body composition in young adults: relation to size at birth and serial measurements of body mass index in childhood in the New Delhi birth cohort. Am.J.Clin.Nutr. 2005;82:456-66.

205. Sayer AA, Syddall HE, Dennison EM et al. Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. Am.J.Clin.Nutr. 2004;80:199-203.

206. Schroeder DG, Martorell R, Flores R. Infant and child growth and fatness and fat distribution in Guatemalan adults. Am.J.Epidemiol. 1999;149:177-85.

207. Cole TJ, Fewtrell M, Lucas A. Early growth and coronary heart disease in later life. Analysis was flawed. BMJ 2001;323:572-3.

208. Pouliot MC, Despres JP, Lemieux S et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am.J.Cardiol. 1994;73:460-8.

209. Hallal PC, Wells JC, Reichert FF, Anselmi L, Victora CG. Early determinants of physical activity in adolescence: prospective birth cohort study. BMJ 2006;332:1002-7.

210. Ekelund U, Ong K, Linne Y et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). Am.J.Clin.Nutr. 2006;83:324-30.

211. Stettler N, Stallings VA, Troxel AB et al. Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. Circulation 2005;111:1897-903.

212. Cole TJ. Modeling postnatal exposures and their interactions with birth size. J.Nutr. 2004;134:201-4.

213. Murphy MJ, Metcalf BS, Voss LD et al. Girls at five are intrinsically more insulin resistant than boys: The Programming Hypotheses Revisited--The EarlyBird Study (EarlyBird 6). Pediatrics 2004;113:82-6.

214. Schubert CM, Rogers NL, Remsberg KE et al. Lipids, lipoproteins, lifestyle, adiposity and fat-free mass during middle age: the Fels Longitudinal Study. Int.J.Obes.(Lond) 2006;30:251-60.

215. Pietrobelli A, Lee RC, Capristo E, Deckelbaum RJ, Heymsfield SB. An independent, inverse association of high-density-lipoprotein-cholesterol concentration with nonadipose body mass. Am.J.Clin.Nutr. 1999;69:614-20.

216. Clarkson PM, Hintermister R, Fillyaw M, Stylos L. High density lipoprotein cholesterol in young adult weight lifters, runners and untrained subjects. Hum.Biol. 1981;53:251-7.

217. Berg A, Keul J, Ringwald G, Deus B, Wybitul K. Physical performance and serum cholesterol fractions in healthy young men. Clin.Chim.Acta 1980;106:325-30.

218. Levak-Frank S, Weinstock PH, Hayek T et al. Induced mutant mice expressing lipoprotein lipase exclusively in muscle have subnormal triglycerides yet reduced high density lipoprotein cholesterol levels in plasma. J.Biol.Chem. 1997;272:17182-90.

219. Murphy MJ, Metcalf BS, Jeffery AN, Voss LD, Wilkin TJ. Does lean rather than fat mass provide the link between birth weight, BMI, and metabolic risk? EarlyBird 23. Pediatr.Diabetes 2006;7:211-4.

220. Toikka JO, Ahotupa M, Viikari JS et al. Constantly low HDL-cholesterol concentration relates to endothelial dysfunction and increased in vivo LDL-oxidation in healthy young men. Atherosclerosis 1999;147:133-8.

221. Lamon-Fava S, Wilson PW, Schaefer EJ. Impact of body mass index on coronary heart disease risk factors in men and women. The Framingham Offspring Study. Arterioscler.Thromb.Vasc.Biol. 1996;16:1509-15.

222. Caprio S, Hyman LD, McCarthy S, Lange R, Bronson M, Tamborlane WV. Fat distribution and cardiovascular risk factors in obese adolescent girls: importance of the intraabdominal fat depot. Am.J.Clin.Nutr. 1996;64:12-7.

223. Richelsen B, Pedersen SB. Associations between different anthropometric measurements of fatness and metabolic risk parameters in non-obese, healthy, middle-aged men. Int.J.Obes.Relat Metab Disord. 1995;19:169-74.

224. Teixeira PJ, Sardinha LB, Going SB, Lohman TG. Total and regional fat and serum cardiovascular disease risk factors in lean and obese children and adolescents. Obes.Res. 2001;9:432-42.

225. Rosenfalck AM, Almdal T, Gotfredsen A, Hilsted J. Body composition in normal subjects: relation to lipid and glucose variables. Int.J.Obes.Relat Metab Disord. 1996;20:1006-13.

226. Landin K, Lonnroth P, Krotkiewski M, Holm G, Smith U. Increased insulin resistance and fat cell lipolysis in obese but not lean women with a high waist/hip ratio. Eur.J.Clin.Invest 1990;20:530-5.

227. Goran MI, Gower BA. Relation between visceral fat and disease risk in children and adolescents. Am.J.Clin.Nutr. 1999;70:149S-56S.

228. Nemet D, Wang P, Funahashi T et al. Adipocytokines, body composition, and fitness in children. Pediatr.Res. 2003;53:148-52.

229. Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc.Nutr.Soc. 2001;60:329-39.

230. Berenson GS, Srinivasan SR, Wattigney WA, Harsha DW. Obesity and cardiovascular risk in children. Ann.N.Y.Acad.Sci. 1993;699:93-103.

231. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. Hypertension 2000;35:1270-7.

232. Brambilla P, Bedogni G, Moreno LA et al. Crossvalidation of anthropometry against magnetic resonance imaging for the assessment of visceral and subcutaneous adipose tissue in children. Int.J.Obes.(Lond) 2006;30:23-30.

233. Lee S, Bacha F, Gungor N, Arslanian SA. Waist circumference is an independent predictor of insulin resistance in black and white youths. J.Pediatr. 2006;148:188-94.

234. Rissanen P, Hamalainen P, Vanninen E, Tenhunen-Eskelinen M, Uusitupa M. Relationship of metabolic variables to abdominal adiposity measured by different anthropometric measurements and dual-energy X-ray absorptiometry in obese middle-aged women. Int.J.Obes.Relat Metab Disord. 1997;21:367-71.

235. Caprio S, Tamborlane WV, Silver D et al. Hyperleptinemia: an early sign of juvenile obesity. Relations to body fat depots and insulin concentrations. Am.J.Physiol 1996;271:E626-E630.

236. Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia 1998;41:1163-7.

237. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 2003;361:1089-97.

238. Dupont WD, Plummer WD, Jr. Power and sample size calculations for studies involving linear regression. Control Clin.Trials 1998;19:589-601.

239. Ong KK, Preece MA, Emmett PM, Ahmed ML, Dunger DB. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: longitudinal birth cohort study and analysis. Pediatr.Res. 2002;52:863-7.

240. Stuff JE, Nichols BL. Nutrient intake and growth performance of older infants fed human milk. J.Pediatr. 1989;115:959-68.

241. Rogers IS, Emmett PM, Golding J. The growth and nutritional status of the breast-fed infant. Early Hum.Dev. 1997;49 Suppl:S157-S174.

242. Dewey KG. Growth characteristics of breast-fed compared to formula-fed infants. Biol.Neonate 1998;74:94-105.

243. Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study. Pediatrics 1992;89:1035-41.

244. Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study. Am.J.Clin.Nutr. 1993;57:140-5.

245. Nelson SE, Rogers RR, Ziegler EE, Fomon SJ. Gain in weight and length during early infancy. Early Hum.Dev. 1989;19:223-39.

246. Arenz S, Ruckerl R, Koletzko B, von KR. Breast-feeding and childhood obesity--a systematic review. Int.J.Obes.Relat Metab Disord. 2004;28:1247-56.

247. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115:1367-77.

248. Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. Am.J.Epidemiol. 2005;162:397-403.

249. Owen CG, Martin RM, Whincup PH, vey-Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. Am.J.Clin.Nutr. 2005;82:1298-307.

250. Rowe RW, Goldspink G. Muscle fibre growth in five different muscles in both sexes of mice. J.Anat. 1969;104:519-30.

251. Desai M, Crowther NJ, Lucas A, Hales CN. Organ-selective growth in the offspring of protein-restricted mothers. Br.J.Nutr. 1996;76:591-603.

252. Gluckman PD, Harding JE. Fetal growth retardation: underlying endocrine mechanisms and postnatal consequences. Acta Paediatr.Suppl 1997;422:69-72.

253. Lapillonne A, Braillon P, Claris O, Chatelain PG, Delmas PD, Salle BL. Body composition in appropriate and in small for gestational age infants. Acta Paediatr. 1997;86:196-200.

254. Godfrey K, Walker-Bone K, Robinson S et al. Neonatal bone mass: influence of parental birthweight, maternal smoking, body composition, and activity during pregnancy. J.Bone Miner.Res. 2001;16:1694-703.

255. Knight B, Shields BM, Turner M, Powell RJ, Yajnik CS, Hattersley AT. Evidence of genetic regulation of fetal longitudinal growth. Early Hum.Dev. 2005;81:823-31.

256. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. Am.J.Obstet.Gynecol. 2003;189:1698-704.

257. Yajnik CS, Fall CH, Coyaji KJ et al. Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. Int.J.Obes.Relat Metab Disord. 2003;27:173-80.

258. Ong KK, Ahmed ML, Sherriff A et al. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J.Clin.Endocrinol.Metab 1999;84:1145-8.

259. Dewey KG. Nutrition, growth, and complementary feeding of the breastfed infant. Pediatr.Clin.North Am. 2001;48:87-104.

260. Kramer MS, Guo T, Platt RW et al. Feeding effects on growth during infancy. J.Pediatr. 2004;145:600-5.

261. Maltin CA, Delday MI, Sinclair KD, Steven J, Sneddon AA. Impact of manipulations of myogenesis in utero on the performance of adult skeletal muscle. Reproduction. 2001;122:359-74.

262. McMillen IC, Muhlhausler BS, Duffield JA, Yuen BS. Prenatal programming of postnatal obesity: fetal nutrition and the regulation of leptin synthesis and secretion before birth. Proc.Nutr.Soc. 2004;63:405-12.

263. Remacle C, Bieswal F, Reusens B. Programming of obesity and cardiovascular disease. Int.J.Obes.Relat Metab Disord. 2004;28 Suppl 3:S46-S53.

264. Dewey KG. Is breastfeeding protective against child obesity? J.Hum.Lact. 2003;19:9-18.

265. Plagemann A. Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav. 2005;86:661-8.

266. Plagemann A, Harder T, Rake A et al. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. J.Neuroendocrinol. 1999;11:541-6.

267. Odeleye OE, de Court, Pettitt DJ, Ravussin E. Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. Diabetes 1997;46:1341-5.

268. Lucas A, Boyes S, Bloom SR, ynsley-Green A. Metabolic and endocrine responses to a milk feed in six-day-old term infants: differences between breast and cow's milk formula feeding. Acta Paediatr.Scand. 1981;70:195-200.

269. Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. Int.J.Obes.Relat Metab Disord. 1995;19:573-8.

270. Scaglioni S, Agostoni C, Notaris RD et al. Early macronutrient intake and overweight at five years of age. Int.J.Obes.Relat Metab Disord. 2000;24:777-81.

271. Kramer MS, Kakuma R. Energy and protein intake in pregnancy. Cochrane.Database.Syst.Rev. 2003;CD000032.

272. Scholl TO, Chen X, Khoo CS, Lenders C. The dietary glycemic index during pregnancy: influence on infant birth weight, fetal growth, and biomarkers of carbohydrate metabolism. Am.J.Epidemiol. 2004;159:467-74.

273. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. Am.J.Epidemiol. 1991;133:884-99.

274. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. The Bogalusa Heart Study. Arch.Intern.Med. 1994;154:1842-7.

275. Porkka KV, Viikari JS, Taimela S, Dahl M, Akerblom HK. Tracking and predictiveness of serum lipid and lipoprotein measurements in childhood: a 12-year follow-up. The Cardiovascular Risk in Young Finns study. Am.J.Epidemiol. 1994;140:1096-110.

276. Tounian P, Aggoun Y, Dubern B et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet 2001;358:1400-4.

277. Iannuzzi A, Licenziati MR, Acampora C et al. Increased carotid intima-media thickness and stiffness in obese children. Diabetes Care 2004;27:2506-8.

278. Trudeau F, Shephard RJ, Arsenault F, Laurencelle L. Changes in adiposity and body mass index from late childhood to adult life in the Trois-Rivieres study. Am.J.Hum.Biol. 2001;13:349-55.

279. Kelly JL, Stanton WR, McGee R, Silva PA. Tracking relative weight in subjects studied longitudinally from ages 3 to 13 years. J.Paediatr.Child Health 1992;28:158-61.

280. Hoffmans MD, Kromhout D, Coulander CD. Body Mass Index at the age of 18 and its effects on 32-year-mortality from coronary heart disease and cancer. A nested case-control study among the entire 1932 Dutch male birth cohort. J.Clin.Epidemiol. 1989;42:513-20.

281. Lawlor DA, Martin RM, Gunnell D et al. Association of body mass index measured in childhood, adolescence, and young adulthood with risk of ischemic heart disease and stroke: findings from 3 historical cohort studies. Am.J.Clin.Nutr. 2006;83:767-73.

282. The composition of mature human milk. DHSS. 12. 1977. London, HMSO. Report on health and social subjects.
Ref Type: Report

283. Artificial feed for the young infant. DHSS. 18. 1980. London, HMSO. Report on health and social subjects.
Ref Type: Report

284. Fewtrell M, Lucas A. Infant feeding. In: Roberton NRC, Rennie JM, eds. Textbook of neonatology. Edinburgh: Churchill Livingstone 1999:305-48.

285. Macy, I. G., Kelly, H. J., and Sloan, R. E. The composition of bulks. 254. 1953. Washington DC, National Academy of Science, National Research Council.

286. Mettler AE. Infant milk powder feeds compared on a common basis. Postgrad.Med J. 1976;52 Suppl 8:3-20.

287. Committee on Nutrition AAP. Appendix A. In: Kleinman RE, ed. Pediatric Nutrition Handbook. Illinois: American Academy of Pediatrics 1999:631-2.

288. Roberts SB, Lucas A. Measurement of urinary constituents and output using disposable napkins. Arch.Dis.Child 1985;60:1021-4.

289. Wells JC, Davies PS. Correction for environmental water influx in measurement of milk volume intake by deuterium turnover in infants. Early Hum.Dev. 1995;41:177-82.

290. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. Stat.Med. 1992;11:1305-19.