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Influence of fetal growth on current body structure and metabolic function

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ABSTRACT

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Fetal growth and development marked by a lower weight at birth is believed to influence the development of both the structure and function of the individual as well as the development of T2DM and CVD in later life. The mechanisms that underlie these effects remain unclear. Previous work has examined the extent to which birth weight is associated with differences in body size and composition in adult life. Most studies have only used simple surrogate measure such as BMI and waist circumference; very few studies have used more detailed reference body composition techniques. It has been consistently reported that adults born with a lower birth weight are shorter and lighter when compared to those born with a higher birth weight. Although, lean mass has been suggested to be positively associated with birth weight, there is little information available on the influence of birth weight on the different components of lean mass such as muscle mass. There have been inconsistent findings on the influence of birth weight on adult adiposity and pattern of fat distribution. Furthermore, although birth weight has been reported to have an effect on some markers of metabolic control such that related to insulin and glucose metabolism, little attention has been directed towards the effect of birth weight on the more fundamental aspects of human metabolism, such as energy and substrate metabolism.

The main purpose of this thesis was to examine the inter-relationship between body structure and metabolic function, and in particular, how fetal growth marked by birth weight may influence this relationship in such a way as to predispose the individual to greater risk of disease in later life. The central hypothesis that underlies the work reported in this thesis is that that the pattern of growth in early life, marked by birth weight, results in structural and functional changes that are evident in adult life, thereby predisposing the individual to an increased risk of obesity, T2DM and CVD.

A group of adult men of differing birth weight were extensively studied using different principle methods to characterise body structure in term of size, composition and fat distribution and metabolic function that is marked by energy, substrate and lipid metabolism both in fasted and fed state.

Adults born with a lower birth weight appear to be shorter, lighter and have lower lean and muscle mass and greater fat mass in particular in the central region of the body when compared to adults with higher birth weight. The observed differences in body composition associated with birth weight remain the same even after accounting for the differences in body size (height + weight). There were differences in body dimensions (both vertically and horizontally) associated with birth weight. The lower birth weight group had shorter leg length and taller trunk and non-limb length when compared to higher birth weight group at the same height. The differences in body composition associated with birth weight could not be simply explained by the observed differences in body dimensions between groups. In addition, adult with a lower birth weight have lower energy metabolism in both the fasted and fed states. These differences in energy metabolism were independent of body size and composition (lean mass). This implies that metabolic function in itself might be programmed an effect greater than that simply explained by differences in size and composition. This less prudent metabolic phenotype associated with differences in birth weight may predispose to more obvious features such as adiposity and central fat which in turn, may increase the risk of developing T2DM and CVD.

List of tables

List of figures

Mean Abbreviation used in the text

AUCi	Area under the curve measured from baseline
AUC	Area under the curve measured from zero
ATPIII	National Cholesterol Education Program
ANCOVA	Analysis of covariance
AFM	Abdominal fat mass
BMI	Body mass index
BMC	Bone mineral content
BIA	Bioelectrical impedance
CHO	Carbohydrate
CVD	Cardiovascular disease
CHD	Coronary heart disease
CETP	Cholesterol ester transfer protein
CT	computed tomography
CV	coefficient of variation
DXA	Dual Energy X-ray absorptiometry
DIT	Dietary induced thermogenesis
FFM	Fat free mass
FFST	Fat free soft tissue
FM	Fat mass
GC	Gas chromatography
HDL	High density lipoprotein
HC	Hip circumferences
HBW	Higher birth weight
IDF	International Diabetes Federation
IRMS	Isotope ratio mass spectrometry
LDL	Low density lipoprotein
LBW	Lower birth weight
MRI	Magnetic resonance imaging
NEFA	Non-esterified fatty acid
NLFM	Non-limb fat mass
REE	Resting energy expenditure
SKF	Skinfold thickness
TUNKFM	Trunk fat mass
T2DM	Type 2 diabetes mellitus

TAG	Triglyceride
TBW	Total body water
UWW	Under water weighing
VLDL	Very low density lipoprotein
VO_2	Whole body oxygen consumption
VCO_2	Whole body breath carbon dioxide excretion
WC	Waist circumference
WHR	waist hip ratio

List of publications

The findings reported in this thesis have been reported, in part, in the following publications:

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Kensara O.A., Elia M., Jackson A.A., Phillips D.I., Hoffman D.J., Wootton S.A. (2005) **Relationship between birth weight and energy expenditure in the fed and fasted state in older Englishmen.** IJBCR 3: P79

Kensara O.A., Elia M., Jackson A.A., Phillips D.I., Hoffman D.J., Wootton S.A. (2005) **Does birth weight predict resting energy expenditure in older adults?** IJBCR 3: P97

Kensara O.A., Elia M., Jackson A.A., Phillips D.I., Patel M., Wootton S.A. (2005) **Birth weight, body composition and muscle mass in adult men after adjustment for body size.** BJOG 112: pp 511-527

Kensara O.A., Wootton S.A., Phillips D.I., Patel M., Elia M (2004) **Does body mass index reflect percentage body fat and body fat distribution in low and high birth weight subjects?** Asia Pac J Clin Nutr: 13 (Suppl):S99

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CHAPTER 1

1.1 Introduction & background to the research

Health or good health is usually and most simply expressed as the absence of disease. This rather negative definition of health states what health is not rather than what health might be. Most broad definitions of health express health within physical, social and mental dimensions and can be seen as the ability to cope with every day activity that is reflected in physical fitness and high quality of life. Despite these different definitions of health, there is no simple way to define health in any easily measurable or quantifiable way.

The most obvious marker which could represent health is body weight. It is a measure of a body structure that is appropriate for the context in which the individual exists. The structure that enables the metabolic and physiological process that are required to cope with the environmental and physiological challenges. In other words, body structure is linked to the functional state. However, there is no optimal body weight which adequately reflects good health. Rather, there is a range of weight over which body structure and function are maintained and where balance is achieved such that the nutrient supply matches the demand. Where there is an imbalance such that supply exceeds demand, changes in body structure occur that are marked by an increase in body weight, fat mass in particular intra-abdominally and an increase in the deposition of fat within organs. This process can be thought of as 'expansive adaptation' – the corollary of those changes observed in the process of reductive adaptation that occurs in malnourishment. These changes in body structure are, in turn, associated with changes in metabolic and physiological function or capacity that reflect the inability of the individual to cope with these changes in body structure and impairment in metabolic control. This loss of metabolic control is associated with marked increases in circulating nutrients in plasma such as glucose, triglyceride (TAG) and cholesterol, elevated levels of hormones such as insulin and perturbations in physiological processes controlling blood pressure and haemostatic control. Such changes in structure (i.e. body mass index and waist circumference) and functions (glucose, TAG, LDL, hypertension, etc) are used to indicate a predisposition towards ill-health. This less prudent phenotype has recently been characterised as the Metabolic Syndrome, a constellation of risk factors that serve as a marker of risk for type 2 diabetes (T2DM) on the one hand and CVD on the other.

Cardiovascular disease (CVD) and diabetes are the major causes of death world wide. In the UK CVD account for just over 216,000 deaths in 2004 and more than one in three people (37%) die from this disease. In addition, around 2 million people have been diagnosed to have T2DM and this number was suggested to rise to 3 million by 2010. In order to understand how it might be possible to reduce the risk of these diseases in later life, it is important to determine the relative contribution of those factors that might influence the development of this less prudent phenotype. Previous studies have indicated that events in early life, as marked by a lower weight at birth, can influence the development of both the structure and function of the individual as well as the development of T2DM and CVD in later life. The mechanisms that underlie these effects remain unclear. One possible explanation is that events in early life alter the processes through which structure and function are acquired in such a way that they have life long effects on adult body structure and metabolic function (this apparently adverse phenotype), which as a consequence, may increase the risk of later life disease. Whilst the relationship between birth weight and disease prevalence has been extensively studied, relatively little attention has been directed towards exploring the effect of fetal development on the acquisition of body structure and composition and how this may in turn, relate to differences in function. Most studies to date have used proxy markers of body composition, such as BMI, best suited for large scale epidemiological studies. Where attempts have been made to provide a more detailed characterisation of body composition, there has been a lack of concordance between observations; a situation that is not helped by the lack of a unifying coherent conceptual framework through which the physical dimensions and structure of the body could be related to differences in body composition or differences in function.

The aim of the work presented in this thesis was to examine the inter-relationship between body structure and metabolic function, and in particular, how fetal growth marked by birth weight may influence this relationship in such a way as to predispose the individual to greater risk of disease in later life. The central hypothesis that underlies the work reported in this thesis is that the pattern of growth in early life, marked by birth weight, results in structural and functional changes that are evident in adult life, thereby predisposing the individual to an increased risk of obesity, T2DM and CVD.

In order to explore this hypothesis, a group of adult men of differing birth weight were extensively studied. Six principle lines of investigation were conducted:

- Firstly, to what extent are differences in birth weight associated with differences in

adult height, weight, body composition and pattern of fat distribution using different principle methods within the same study subjects?

- Secondly, given that height and weight may be associated with differences in body composition and distribution of body fat, to what extent are differences in body composition in adults of differing birth weight attributable to differences in adult height or weight (i.e. body size)?

- Thirdly, given that body shape and proportions may be associated with differences in body composition to what extent are differences in body composition of adults of differing birth weight attributable to differences in adult body shape or body dimensions?

- Fourthly, to what extent are differences in birth weight associated with differences in functional state as most obviously characterised as the rate of energy expenditure and net substrate oxidation at rest in the fasted and fed states?

- Fifthly, given the close association between body size and composition and energy and substrate metabolism, to what extent are any differences in metabolic demand between adults of differing birth weight attributable to differences in body size and composition?

- Finally, to what extent are differences in birth weight associated with differences in functional state characterized by lipid metabolism at rest in the fasted and fed states?

1.2 Thesis outline

The work presented in this thesis is divided into chapters, beginning with an introduction to the field of research (chapter 1). The next section (chapter 2) reviews of the current literature concentrating on the influence of birth weight on adult body structure in terms of size, composition and fat distribution in one hand and metabolic function in term of energy metabolism and metabolic risk factors on the other, highlighting the limitations of existing knowledge. Methods used during the research and validation work to justify the use of the methods are presented in chapter 3. This is followed by chapters describing the results (chapter 4-8) and finally a general discussion (chapter 9).

CHAPTER 2 REVIEW OF THE LITERATURE

2.0 Introduction

The purpose of this chapter is to introduce the area of research presented in the thesis. The review of the literature is presented in four sections. The first section (2.1) provides an introduction to the literature relating body structure and function to health and cardiometabolic disease. The second section (2.2) introduces the concept of fetal programming of adult disease and the evidence that events in early life may influence the risk of CVD and T2DM. In the third section (2.3), attention is directed to consider the evidence that body structure and metabolic function in later life is influenced by fetal growth and development. The fourth section (2.4) provides a summary of the literature review highlighting the key areas requiring further investigation. Thereafter, the central hypothesis and aim of the research are presented in the final section (2.5).

2.1 Health and disease

This section of the review is divided into two sections. The first section (2.1.1) explores how health could be presented within the context of body structure and metabolic function. The second section (2.1.2) shows the evidence that CVD and T2DM are related to body structure and metabolic function and how such adverse changes can be used to identify those at risk of cardio-metabolic risk or metabolic syndrome.

2.1.1 Can body structure and metabolic function reflect health (or good health)?

Health is a broad concept which can embody a range of meanings, from the narrowly technical description of physical state to the all-embracing moral or philosophical. The most commonly used definition of health is a state that is free of disease or the absence of acute and chronic disease. Broader definitions see health as a state of optimal physical, mental and social well-being and not merely the absence of disease and infirmity (1). The World Health Organisation has led much of the debate about definitions of health and currently sees health as:

[Health is] the extent to which an individual or group is able, on the one hand, to realise aspirations and satisfy needs, and on the other hand, to change or cope with the environment. Health is, therefore, seen as a resource for everyday life, not an object of living; it is a positive concept emphasising social and personal resources, as well as physical capabilities (2).

As such, there can be no single measure of health, not least in any direct objective, quantifiable way that embraces all three considerations. Equally, it is beyond the scope of this thesis to consider all aspects of health. Rather, this work focuses on the physical aspects of health and in particular, the inter-relationship between the body structure and functional capabilities of the body and how this might be influenced by events in early life.

The ability to engage in metabolic transformation is the property of the function of cells, acting individually and collectively, within and between organs and tissues. Each of these processes can take place at a defined rate, and the rate within a defined cell or tissue is characterised as its metabolic competence: that is, the specific ability per unit tissue to carry out each specific function. The competence of individual cells will vary from one location to another, between tissues, and at different stages of life. For cells with a similar level of competence, the more cells present or the greater the mass of tissue that is made up by such cells, the greater the overall absolute ability or the greater capacity. It is usual for the metabolic capacity of most functions to exceed the activity required on a day to day basis. There is reserve capacity built into the system such that it is possible to accommodate an increase in demand at short notice. There are however, situations where the capacity just matches the demand and so that an increase in demand cannot be accommodated without a fundamental shift in the setting of the system. In situations where the capacity falls short of the demand, homeostatic status cannot be sustained and changes must take place within the system, resulting in ill-health. The ability to cope effectively to adaptation experiences will be determined by the demands placed upon the system and the capacity of the system to respond appropriately. The nature and pattern of competencies required by a cell, tissue or organism varies at different stages of life and in relation to changes in the physiological state and environmental conditions. An important aspect of growth and development is the progressive acquisition and maturation of competence, and together with increases in the mass of the individual tissues and the body as a whole, resulting greater capacity for important functions with age to maturity and adulthood. Ageing represents a progressive loss of capacity, either through a loss of competency or a decrease in mass, or a combination of both. Health can be seen therefore, as the achievement and maintenance of capacity, and

the ability to ensure sufficient reserve capacity to cope effectively with untoward circumstances.

Measures of mass and structure are often taken to mark the functional wellbeing of the individual and offer a summary statement of health. There is no optimal weight at which an individual could be assumed to be in a good health. Rather there is a range of body weight at which balance can be achieved that is associated with a minimal or lowest observable risk of adverse health consequences. As such, it is reasonable to assume that the functional capacities are appropriate for the environmental stressors and context in which the individual exists – in other words, the structures and function can be seen as 'fit for purpose'. This range is dependent, in part, upon the height of the individual and so expressions of weight with respect to height, such as BMI, are most obviously used to define the range. Whilst it has generally been assumed to reflect body composition and adiposity in particular, there is increasing awareness that for any given BMI, body composition may vary not least with differences in ethnicity and, more recently, body proportions (see section (2.1.2.1)). Above and below this reference range, the greater the risk of ill-health. The implication is that as the individual moves outside this range, the individual is less fit for purpose and less able to cope with the stresses imposed by the environment within which the individuals find themselves.

The net consequence of imbalance between the supply and demand, whereby more energy and nutrients are consumed than needed, requires that the body responds to limit the consequences of this excess. If the magnitude and duration of the excess is modest, then the body may adapt to accommodate the excess with minimal cost and with a modest increase in body weight which could be marked by an increase in the deposition of fat mass. Such partitioning of excess can be seen as a protective response of the body to minimise harm at that point of time. However, should this imbalance be sustained for longer periods in time, further increases in body structure are observed, and are marked by increases in fat mass, particularly centrally or intra-abdominally and within organs such as liver, heart and muscle (3;4). Such abnormal deposition of fat mass, associated with these changes in body structure, may in turn have an effect on metabolic processes exceeding the functional capacity of the individual. Whilst the mechanisms that underlie the influences of such changes in body structure and metabolic function are clearly complex and inter-dependent, such changes in body structure (i.e. increase in BMI and waist circumference) are associated with markers of poor metabolic control which predispose an individual to greater risk for cardiovascular disease and Type 2 Diabetes.

2.1.2 Cardiovascular disease (CVD)

Cardiovascular disease is a collection of diseases affecting both the heart and blood vessels and includes coronary heart disease (CHD) and stroke. CHD causes over 120,000 deaths a year in the UK: approximately one in four deaths in men and one in six deaths in women. This compares to around 34,000 deaths a year from lung cancer, 16,000 deaths from colorectal cancer and 13,000 deaths from breast cancer (5). The underlining basis for the development of CVD is a combination of atherosclerosis and thrombosis. Atherosclerosis occurs when the arterial wall becomes thickened with plaques as results of excessive accumulation of cholesterol-rich lipoprotein (i.e. LDL), resulting in endothelial damage and the narrowing of the lumen, and that in turn, leads to a reduction in blood flow. Although most atherosclerotic plaques are considered stable, a small number are at risk of rupturing, leading to the formation of a blood clot or thrombus. Part of this thrombus is then carried to other site where it can lodge and obstruct the blood flow. The clinical consequence associated with the occurrence of an atherothrombotic event depends on its location. For example, narrowing in the coronary artery may be associated with angina, myocardial infarction, and heart failure.

The factors that predispose an individual to the processes of atherosclerosis and thrombosis have been extensively studied and found to be many and inter-related within a complex web of causality and association. These factors are classified according to whether they are modifiable or non-modifiable risk factors, the former may be changed to alter an individuals CVD risk (i.e. smoking, physical activity and diet). Smoking is clearly important; both in terms of a pro-inflammatory stimulus as well as being associated with differences in other lifestyle considerations such as poor diet and less physical activity (6;7). Food choice and dietary intake is also known to influence the risk of CVD in different ways. For example, total fat intake, specifically saturated fat, has been reported to be associated with increased mortality form CVD (8). In addition, the increase in intake of polyunsaturated fats, such as those found in vegetable oil and fish (i.e. n-3 and n-6 polyunsaturated fatty acid), is associated with a lower risk of CVD. Furthermore, diets high in vegetable and low in sodium are also associated with a reduction in CVD risk. Inadequate physical activity and sedentary life style has also been associated with greater risk of developing CVD in later life. For example, reduction in total and cardiovascular mortality has been reported to be associated with increased physical exercise and cardiorespiratory fitness (9).

Expressing these ideas within the context of characterizing the phenotype in terms of

structure and function enables us to re-examine the literature to identify the extent to which differences in body size, shape and composition on the one hand and markers of poor metabolic control on the other, are associated with increased risk of CVD.

2.1.2.1 How does stature relate to the risk of CVD?

It has been suggested that tall stature of an individual is associated with a reduced risk of CHD, independent of other environmental factors that are known to affect the incidence of CVD. For instance, a previous case control study assessed the risks of myocardial infarction (MI) for tall and short women relative to women of average height after controlling for BMI, socioeconomic status and smoking (10). This study reported a significant and inverse relationship between height and the risk of myocardial infarction. The relative risk for MI estimate for women greater than or equal to 175 cm tall was 0.5 (95% confidence interval (CI) 0.2-0.8); for women less than or equal 150 cm tall, it was 1.5 (95% CI 0.9-2.6). The results were consistent even after adjustment was made for strata of age, BMI and educational status. The author of this study suggested that compared to the women of average height, those of tall stature had a lower risk of myocardial infarction than the shorter group. In addition, another study in a group of women aged 30-50 years demonstrated that the relative risk factor for CHD is greater among shorter women (height < or = 1.55 m), the relative risk of CHD = 0.82 (95% confidence interval (CI) 0.73-0.92), when compared with taller groups (height= >1.70 m), the relative risk of CHD= 0.73 (95% CI 0.65-0.83)(11). Although the mechanisms which underlie the effect of height on risk of CHD remain unclear, several plausible explanations have been suggested. The influence of height on CVD could be the result of the cumulative effect of poor nutritional and environmental factors that occur during childhood growth which in turn may affect the current height. It has also been suggested that the reduction in the risk of CHD associated with taller stature is mediated through the increase in coronary vessel diameter and that might lead to a decrease in risk of luminal occlusion (10). Furthermore, growth hormone and insulin-like growth factors may play important roles in the effect of short stature and risk of CHD. These hormones play an important role during childhood growth and are also thought to have effects on cardiovascular physiology as suggested by the observation that adults with hypopituitarism are at increased risk of CHD and have an adverse cardiovascular risk profile (12;13). In addition, the influence of stature on the risk of CVD could be mediated through differences in body composition, such that the shorter stature individuals may have greater accumulation of body fat, in particular in the visceral region of the body, when compared with those of taller stature. Height also has different components, which include leg length and trunk length.

These components of stature are associated with differences in the proportion of lean and fat mass. Therefore, tall and short individuals with different leg lengths may have different proportions of lean and fat mass over different segments of the body. Such differences in body proportion have been reported to have an effect on BMI as measure of body fatness between different ethnic group (14). Therefore, the greater risk of CVD reported in shorter stature subjects could be attributed to the differences in the body proportion or body segments length (i.e. shorter leg length and the same trunk length) when compared with taller subjects each of which has different tissue composition. There are only a few studies which have assessed the relationship between component of height and CHD. In one, in both men and women, leg length was reported to be responsible for the influence of height on the risk of CVD (15). In another study, leg length measured in childhood (both in boys and girls aged 2-14 years) was reported to be inversely associated with CHD mortality over 52 years follow up (16). In addition, leg length was also found to be inversely associated with other risk factors which may contribute to CVD, such as insulin resistance (17).

2.1.2.2 How do body weight and composition relate to the risk of CVD?

Independent of other risk factors, the increase in body weight and adiposity has been reported to be associated with CVD. This finding was supported by several large scale epidemiological studies conducted in both men and women. For example, the Framingham Heart follow-up Study of 5209 men and women who were free of clinically recognisable CVD at the time of initial evaluation reported that obesity, measured by Metropolitan Relative Weight (MRW), was a significant independent predictor of CVD, especially among women (18). During the follow-up period, 870 men and 688 women developed clinically recognisable CVD. Of these, CHD accounted for 75% of cases in men and 66% of cases in females. Clinically recognisable CVD was defined as angina pectoris, myocardial infarction, coronary insufficiency, and sudden or non-sudden coronary death. The risk of CVD has been reported to increase in both sexes as MRW increases. The increase in risk was most pronounced in those less than 50 years old. After correcting for other risk factors, 8% of males and 18% of females were found to remain in the highest weight class. In this class of body weight the risk of developing CVD increased with elevated MRW and this relationship was more pronounced in males than females. The same finding was also reported in the Nurses Health Study (19). In this study, the Quartelet Index (weight in kilograms divided by the square of the height in meters) which was used as measure of adiposity in large group of women was reported to be associated with risk of nonfatal and fatal myocardial infarction, even after accounting for age and smoking. However, the mechanism that underlies the effect of adiposity on the

development of CVD is not clear, it may in part be related to signalling molecules that originate from or expressed from adipose tissue, particularly that which is found intra-abdominally. It has been proposed that the secretion of these adipokines such as leptin and adiponectin by adipose tissue, combined with the actions of adipose tissue-expressed TNFα in obesity could underlie the association of insulin resistance with endothelial dysfunction, leading to coronary heart disease (20).

2.1.2.3 How do markers of metabolic control relate to the risk of CVD?

The discovery of the role of cholesterol in the formation of fatty plaques resulted in much research based on the effect of dietary cholesterol in the progression of CVD. Cholesterol esters form a fundamental part of the atherosclerotic plaque. Epidemiological studies reported a strong positive relationship between the intake of dietary cholesterol and the incidence of coronary heart disease in humans. Other controlled clinical trails have reported the important of pharmacological lowering of serum cholesterol level in reducing cardiovascular mortality.

Plasma lipoproteins such as LDL and HDL are also classified as important risk factors that are associated with greater CVD risk. Accordingly, the National Cholesterol Education Program of the National Heart, Lung, and Blood Institute has established standards for serum LDL and HDL cholesterol (21) .Although the risk of developing CVD may be very low when the serum LDL cholesterol level is below 100 mg/dl, atherogenesis may occur even at the near-optimal level of a LDL cholesterol of 100 to 129 mg/dl. It is important to note that combination of several risk factors usually provides a more comprehensive picture of the overall risk of CVD. For instance, middle age men may be at an increased risk of developing CVD when their total serum cholesterol level is > 240 mg/dl and/or their LDL cholesterol levels are > 160 mg/dl. In addition, the risk of developing CVD is threefold in a middle-aged man who has LDL cholesterol levels > 160 mg/dl and two additional risk factors as compared with the same individual at the same age who has a lower level of serum LDL cholesterol (22). Lower serum HDL cholesterol has also been demonstrated to be strongly associated with an increased risk of developing CVD (23). Even at optimal plasma cholesterol concentration, lower HDL levels are associated with increased myocardial infarction rates in both men and women (23). It has been suggested that HDL particles may be a marker for both an insulin-resistant state and the presence of other atherogenic lipoproteins, such as VLDL, which have been associated with the development of CVD (24). While low HDL levels are considered a major risk factor for CVD, high levels may be protective. Some studies

demonstrated a significant reduction in major cardiovascular events when HDL levels were raised (25).

Epidemiological evidence now suggested that it is not just the concentration of plasma cholesterol that is important in the development of CVD but also the concentration of TAG in circulation (26). This observation has led to plasma TAG being classed as an independent risk factor for CVD. In addition, the complex process of lipid metabolism after meal ingestion, which involves absorption and partitioning of dietary lipid towards storage or oxidation, and how this process is regulated, should not be overlooked. Several studies have demonstrated that the changes in TAG and non esterified fatty acid (NEFA) concentrations in plasma following the ingestion of a meal (postprandial lipiaemia) are altered in those with CVD when compared to normal subjects (27;28). Alteration in lipid metabolism in those with CVD could be mediated through the increase in adiposity in particular in the abdominal region. Alterations in lipid metabolism in the fasted state, such that marked by an increase in circulating TAG, are associated with an increase in adiposity and visceral fat mass (29). However, evidence now suggests that postprandial lipaemia is also evident in those with greater body fat and visceral fat mass independent of fasting TGA concentration. For example, previous studies reported a greater 24-hours postprandial response (as the area under the curve of the concentration: time plot) for plasma TAG in obese subjects with a higher BMI when compared with lean individuals with a lower BMI (30). Other studies have also reported that postprandial lipiaemia is positively associated with BMI and abdominal fat mass marked by waist: hip circumference ratio and visceral fat mass measured by computed tomography (31;32).

More recently, a study using isotopic tracers where ^{13}C-labelled fatty acids were ingested with a test meal provided a more detailed exploration of the mechanisms that underlie these disturbances in postprandial lipid metabolism (33). This study has shown that there are two related processes that may contribute to the marked hyperlipidaemia with increased cardiometabolic risk, namely a lack of concordance between the processes of TAG-rich lipoprotein delipidation and entrapment of the products into peripheral tissues leading to increased NEFA outflow in the fed state as well as the processes governing the net uptake and release of lipid by the liver during the postprandial period. Whilst differences in lipid metabolism may be attributable in part to differences in body structure, it is important to note that many of these effects persist even after adjustment for differences in weight, BMI and adiposity.

Although the mechanism that underlies the link between postprandial lipiaemia and risk of CVD remains unclear, it has been attributed to the increase in plasma atherogenic lipoprotein particles (34;35). Postprandial lipiaemia may occur as the result of an increase in the production of TAG rich lipoprotein (chylomicrons and/or VLDL) or a reduction in the clearance of these particles from plasma following the ingestion of the meal. This may occur through the rate-regulatory step in TAG hydrolysis and removal following the meal ingestion which is mediated by the action of lipoprotein lipase (36). Any limitation in clearing the dietary lipid from the circulation might be associated with an accumulation of partially hydrolysed chylomicrons and VLDL remnant particles. These remnants are poorly recognised by hepatic receptors and that in turn, may lead to reduced uptake of the cholesterol-enriched particles (i.e. LDL) by the liver (37). The net consequence of this defect is an increased retention of TAG rich lipoprotein (VLDL and chylomicrons) within the circulation; a state that provides greater opportunity for neutral lipid exchange (36). Normally, the neutral lipid in plasma (i.e. cholesterol ester and TAG) is redistributed between particles by the action of cholesterol ester transfer protein (CETP) (38). CETP mediates net transfer of cholesterol ester from HDL to TAG-containing lipoproteins such as VLDL and LDL which may be removed by receptor- mediated uptake in the liver. However, the accumulation of cholesterol ester in VLDL or chylomicron remnants may lead to the formation of atherogenic cholesterol-ester rich remnant particles (39). The net consequence of these transfers is TAG accumulation within HDL and LDL, and cholesterol within chylomicrons, VLDL and their remnants. The TAG-enriched HDL and LDL act as good substrates for hepatic lipase resulting in the formation of small dense HDL and LDL (36). In the normal healthy adult, the ingestion of a fat-containing meal is followed by delipidation of chylomicrons and the resultant remnants are then taken up by the liver for degradation, with little cholesterol-ester transfer occurring. However, in a situation where there is marked postprandial lipaemia, TAG concentrations in plasma are raised for longer, resulting in a greater level of cholesterol-ester transfer and greater production of small dense HDL and LDL particles. These particles have been reported to have a greater atherogenic potential due to their prolonged retention in the circulation (36) as well as their greater ability to induce foam cell formation, a marker for the development of atherosclerosis (40).

Changing the amount and composition of fat in the meal has also been suggested to have an effect on the magnitude and timecourse of the postprandial lipiaemia. This in turn may influence the risk of developing CVD. For example, a previous study which examined the response of serum TAG concentration following meals with increasing fat content in normotriglyceridemic men found that the magnitude of lipiaemia was directly proportional to the fat content of the meal (41). In addition, lower fat content in combination with a high level

of carbohydrate in the diet has also been reported to be associated with an increase in fasting plasma TAG and decrease in HDL in plasma (42;43). Furthermore, long chain n-3 polyunsaturated fatty acid found in fish oils has been reported to reduce both fasting and postprandial TAG concentration (44;45). However, the exact mechanisms that underlie this effect remain unclear.

2.1.3 Type 2 diabetes (T2DM)

The term T2DM embraces a group of chronic metabolic diseases characterised by high blood sugar (glucose) levels, which result from defects in insulin action and secretion, or both. The WHO estimated that 171 million people were affected in 2000 with type 2 diabetes, and this number is expected to be more than doubled by 2030 (46). In the UK, there are currently over 2 million people diagnosed with type 2 diabetes and 750,000 individuals with undiagnosed with this disease (47). People with T2DM have a greater risk of developing both macrovascular and microvascular disease. The risk of clinically demonstrable atherosclerotic disease is increased 2-3 fold in those with diabetes when compared with non-diabetic subjects, including congestive heart failure and coronary heart disease (48;49). Others have reported that the risk of CVD death was higher in men with diabetes than those without even after controlling for age, race, income, systolic blood pressure, cholesterol level and smoking status (50). In addition, the increases in risk of CVD associated with diabetes have been reported to be greater in women than men (51;52). Some studies suggested that such differences in the risk of CVD between diabetic men and women are attributed to the greater adverse differences in the level of several CVD risk factors such as waist to hip ratio, cholesterol and HDL (53). Furthermore, patients with diabetes have a greater risk of developing microvascular disease that presents clinically as retinopathy, neuropathy and renal disease (54;55).

The factors that predispose an individual to the development of T2DM and the association between diabetes and the development of atherosclerosis and thrombosis have been extensively studied and found to be inter-related within a complex web of causality and association. Once again, there is likely to be a genetic component together with a response to environmental stimuli and stressors (56;57). T2DM is most obviously linked to insulin resistance. Insulin stimulates glucose uptake into tissues, and its ability to do so varies greatly among individuals. In insulin resistance, tissues have a diminished ability to respond to the action of insulin. Therefore, compensatory hyperinsulinemia may occur to help maintain normal glucose levels before overt diabetes develops. Eventually the beta cells of

the pancreas are unable to overcome insulin resistance through hypersecretion. As the result, glucose levels in plasma will rise.

2.1.3.1 How does body structure relate to T2DM?

It is well established that obesity, as marked by high BMI, is a risk factor for the development of Type 2 diabetes with an increased amount of intra-abdominal fat producing a particularly high risk. Various measures related to the increase in adiposity and differences in body size in particular height have been found to be associated with the risk of T2DM. In a previous cross sectional study of over 13000 men and women aged 20-59 years Han et al obtained measurements of height, weight, hip and waist circumferences, and information on smoking habits, physical activity, alcohol consumption and education. The definition of Type 2 diabetes was based on subjects either having known diabetes mellitus (not treated with insulin), or having a randomly sampled blood glucose concentration in excess of 11.1 mmol/l. The odds ratio for having Type 2 diabetes was 18.4 times greater in men and 5.3 times greater in women in the largest tertile of waist: hip ratio compared with the smallest. The corresponding figures for largest compared to smallest tertiles of waist circumference were an odds ratio of 4.9 in men and 2.7 in women. Those in the highest tertile of BMI had a greater odds ratio of having Type 2 diabetes than those in the lowest tertile of BMI (men 4.1, women 2.1), whilst those in the shortest tertile for stature had an increased odds ratio compared with those in the tallest tertile (men 4.4, women 1.6). These results confirmed previous demonstrations of an association of T2DM with obesity and particularly with increased central fat depots, and provided important evidence that short stature and smaller hip circumference may represent particularly high risks of T2DM (58).

2.1.3.2 How do markers of metabolic control relate to T2DM?

Diabetes is characterised by several metabolic abnormalities that include insulin resistance, hyperglycaemia and dyslipidaemia (increased plasma TAG, VLDL, cholesterol, LDL-cholesterol concentration, as well as decreased concentration of HDL). Insulin is secreted by the pancreas to maintain blood glucose concentration in the blood in a steady state. It has different actions on different tissues of the body, including liver, muscle and adipose tissue (59). For example, in muscle it stimulates the glucose uptake while in the liver the action of insulin reduces glucose output. In addition, in adipose tissue, it regulates the hydrolysis of TAG and the flux of NEFA in the circulation. Abnormality in insulin secretion or action or both leads to what is called insulin resistance. It represents the situation in which plasma insulin

concentration is higher than the expected for a given plasma glucose concentration i.e. homeostasis assessment model (HOMA) (60). It is estimated that up 90% of individuals with type 2 diabetes to have some degree of insulin resistance that is coupled with hyperglycaemia (61).

Insulin resistance is also associated with an abnormal plasma lipid profile (or dyslipidaemia), that is characterised by increased plasma TAG and reduced HDL-cholesterol concentration (62;63). In addition, insulin resistance has also been documented to be associated with an increase in atherogenic lipoprotein particles such as small dense LDL particles (64). Furthermore, insulin resistance has been reported to be associated with exaggerated postprandial lipaemia (65), and that was also found to be independent of fasting plasma TAG concentration (66). However, the mechanisms underlie the link between insulin resistance and dyslipidaemia remain unclear, in other words it is not clear whether insulin resistance causes dyslipidaemia or is a consequence of dyslipidaemia. It has been suggested that lipids play an important role in the development of insulin resistance. Randle et al initially proposed that an increase in NEFA in plasma is what causes insulin resistance and that it is mediated through an increase in lipid oxidation (67). The increase in lipid oxidation is then associated with elevations in intramuscular acetyl-CoA and citrate content which decrease the activity of the enzymes pyruvate dehydrogenase and phosphofructokinase. This leads to an accumulation of glucose 6-phoshate, which in turn, inhibits hexokinase, glucose uptake and glucose oxidation within the cells. In support of this view, Gomez et al reported that infusion of TAG emulsions is associated with an increase in lipid oxidation and decrease in glucose uptake and also glucose storage (68). In contrast, others (69) have opposed Randle's hypothesis suggesting that insulin resistance and a defect of insulin action, in particular, in adipose tissue, is what leads to the increase in NEFA flux in plasma and this in turn, is associated with an increase in the hepatic production of TAG rich particles.

Although several investigators have suggested that diet may have an effect on the development of insulin resistance, the mechanisms that underlie the effect of diet on insulin resistance are not clear. For instance, diets high in fat, in particular saturated fatty acid, have been reported to induce insulin resistance when compared to those that have relatively low fat (70;71), this result was also evident after adjustment was made for BMI, waist circumference, age , sex and ethnicity (70). In addition, other studies, both in animals and humans, suggest that it is not only the fat content of the diet, which may have an effect on insulin resistance, but also the quality of fat in terms of the balance of saturated and mono-saturated fatty acids that may induce insulin resistance (72).

Both physical inactivity and adiposity, in particular visceral fat, are also reported to have an effect on the development of insulin resistance (73;74). However, the independent effect of these factors is difficult to distinguish given the interaction between these two factors (i.e. an increase in physical activity is inversely related to adiposity). An intervention study by Powell et al demonstrated that an increase in physical activity is associated with an improvement of insulin sensitivity and reduction in risk factors related to CVD (75). On the other hand, the reduction in body weight usually seen in such studies has also been reported to induce insulin sensitivity without increasing activity (76). However, a recent study in overweight subjects suggested that physical activity is inversely associated with insulin resistance, implying the beneficial role of physical activity on insulin resistance that is separate from any influence of physical activity on body compositing or adiposity (77).

T2DM is also associated with hyperglycaemia, it represents the stat in which an increase in fasted plasma glucose concentration of ≥ 7.0 mmol, or impaired glucose tolerance that is characterised by increase in plasma glucose concentration in response to an oral glucose load (2-hours plasma glucose of ≥ 11.1 mmol) (78). Impaired glucose tolerance by itself is now recognized as risk factor for CVD independent of diabetes. For example, individuals with impaired glucose tolerance (IGT) have been reported to have greater relative risks for CVD than those with normal glucose tolerance (79). Whilst the mechanism that underlies the effect of hyperglycaemia on CVD is not fully understood, it has been suggested that this could be mediated through alterations in endothelial cell function of the arterial wall (endothelial dysfunction), which may play a central role in development of atherosclerosis (80).

2.1.4 The imprudent phenotype – differences in structure and function that predispose to increased cardiometabolic risk.

In the previous sections, attention has been directed towards the extent to which structure and function relate to the pathological disease processes underlying CVD and T2DM. Over recent years, it has been increasingly recognised that there is an intermediary phenotypic state that lies between good health and overt disease. This state, in which there are a constellation of small but important changes in both structure and function, has been associated with a high risk of progression to CVD and T2DM. Rather than overt obesity (as a high BMI), the focus is directed towards being overweight (as BMI > 25) together with intra-abdominal fat accumulation (as marked by increasing waist circumference). At the same time, there are indicators that metabolic and physiological control may be compromised with concentrations of blood metabolites such as plasma glucose and triglyceride or blood

pressure being marginally elevated, but not sufficiently raised to exceed the usual clinical criteria that would warrant a diagnosis of T2DM or hypertension. As a consequence in conventional clinical practice, these individual features or symptoms would not trigger a pharmacological intervention. However, each feature individually is indicative of an increased risk of CVD and T2DM and more importantly, when taken together interact to markedly increase cardiometabolic risk and may be thought of as representing an imprudent phenotype. This imprudent phenotype has been repeatedly recognised over the years and in so doing, been ascribed different labels or terms, including Syndrome X or Reavens Syndrome (74), the (Dys) Metabolic Phenotype and more recently, Metabolic Syndrome (81). At the simplest levels, this condition can be thought of as an adaptive response to a situation in which the dietary supply of energy and nutrients exceeds the metabolic demand. This process of adaptation can be thought of as the corollary of the process of reductive adaptation and may be thought of as 'expansive adaptation'.

Metabolic syndrome is a term used to describe a cluster of risk factors that is related to adverse changes in body structure and metabolic function, that when it is present in a person, it increases his/or her risk of developing either type 2 diabetes or cardiovascular disease, or both. The term syndrome X was first introduced by Reaven in 1988 to reflect the impact of insulin resistance, and its related to metabolic abnormalities such as hyperglycemia and dyslipidaemia, on the risk of CVD (64;74). His findings suggested that resistance to insulin is associated to some degree with glucose intolerance that occurs a result of the increases in glucose production from the liver to compensate for the defect on insulin action. Such defects are associated with the development of dyslipidaemia and hypertension. It has also been suggested that resistance to insulin-stimulated glucose uptake does not occur in patients with glucose intolerance of diabetes but also occurs in about 25% of non-obese individuals with normal glucose tolerance. Additional studies by the same author demonstrated that the role of insulin as key risk factors that lead to the development of different risk factors known to be associated with CVD (i.e. hypertension) (74).

The recognition of the inter-relationship between insulin resistance, hyperglycemia and dyslipidaemia, in addition to hypertension and adiposity, has led several investigators to establish diagnostic tools to identify those at most risk of developing CVD or having what is called metabolic syndrome. Three different versions for the definition of metabolic syndrome have been established. The first was that reported by the World Health Organisation (WHO)(82), followed a few years later by that obtained from the National Cholesterol Education Program Adult Treatment Panel (NECP ATPIII) (21). The latest version of the

definition of metabolic syndrome was that reported by the International Diabetes Federation (IDF)(83). Although the criteria that have been used to define metabolic syndrome by these different organisations were not different very much, the values used for some of the criteria were not the same (84). The WHO based their definition of metabolic syndrome on risk factors that are closely related to insulin resistance. This includes at least one abnormality in insulin and insulin related risk factors that include diabetes, impaired glucose tolerance, impaired fasting glucose and insulin resistance. In addition, the subject must have at least two out of four risk factors that are related to visceral adiposity (measured by waist to hip circumference ratio), dyslipidaemia (marked by higher TAG and lower HDL concentration in plasma), hypertension and microalbuminuria.

The NECP ATPIII criteria defined those with metabolic syndrome as having three out of five risk factors that include abnormal fasting plasma glucose, higher visceral fat assessed by waist circumference, higher TAG and lower HDL concentrations in plasma and higher blood pressure. The IDF criteria that are used to define those with metabolic syndrome are the same as that reported by ATPIII but they define different cut-off points for waist circumference in Europeans. The other difference was that the person must have greater visceral adiposity to be defined as having metabolic syndrome. Although the use of these tools could be helpful to identify those at risk of CVD, the results that are obtained must be interpreted very carefully, since the use of three different tools could lead to an individual being categorised as having metabolic syndrome by one definition but not by others. For example, subjects can be defined as having metabolic syndrome based on ATPIII criteria while the same subjects, with all other risk factors except for visceral adiposity, could be considered as not having metabolic syndrome based on IDF criteria (85). It is now suggested that "metabolic syndrome" is not defined and characterised as it is often assumed, and the notion that it is a useful marker of CVD risk above and beyond the risk associated with its individual components is uncertain (86). Kahn et al in a recent review argued that some of the criteria that are used to defined metabolic syndrome are ambiguous or incomplete (86). For instance, he suggested that it is not clear whether the blood pressure definition is systolic pressure ≥130 mmHg and diastolic ≥85 mmHg or whether it is either ≥130 mmHg or ≥ 85 mmHg. In addition, these different definitions did not specify how blood pressure should be measured, e.g., supine, sitting, mean of two measurements. Furthermore, Kahn et al suggested that some of the criteria that are used to define metabolic syndrome such as waist circumference have sex-specific cut points. As a result, this may imply that the relationship between the risk factor level and outcomes differs between the sexes. Since there is no evidence that warrants establishing the sex-specific cut points used in the criteria as they relate to CVD risk, it remains unclear whether the same waist circumference could

carries a different risk in men than in women. An analogous argument has been also suggested to be applicable regarding whether cut points should vary according to differences in race and ethnicity.

2.2 Concept of fetal programming of adult disease

Barker introduced the concept of fetal programming of chronic disease in later life including CVD and type 2 diabetes 15 years ago (87-89). He proposed that alterations in fetal nutrition and endocrine status result in developmental adaptations that permanently change structure, physiology and metabolism of the fetus, thereby predisposing to cardiovascular, metabolic and endocrine disease in adulthood. This hypothesis was based on earlier observational studies in both men and women born in Hertfordshire, UK during 1911 and 1930. Health visitors recorded the birth weight during that time (90). In this study Barker reported that the death rate from coronary artery disease was two times lower in those at the upper end of the distribution of birth weight and weight at one year of age than those at the lower end. In subsequent studies within the same population the prevalence of type 2 diabetes and glucose intolerance and other risk factors were also reported to be reduced with increasing birth weight (91). Although the relationship between birth weight and adult disease in later life has been criticised as being influenced by many environmental factors through childhood and adulthood, many available studies now show that various measures of lower birth weight are associated with chronic disease in later life after correcting for relevant confounding variables, including socioeconomic status, physical activity and alcohol consummation.

The concept of programming of adult disease has been also supported by many experimental studies in animals. These studies show that maternal undernutrition during a critical period of gestation leads to long term effects on offspring that are relevant to that occur in humans, including persisting changes in blood pressure and insulin response to glucose. For example, in rats, maternal protein restriction over a 14 day period before pregnancy has been reported to be associated with high blood pressure in adult offspring (92). Other studies also in the rat, show that the protein-restricted diet given to pregnant rats is associated with impaired glucose tolerance in adult offspring(93). However, the mechanisms that underlie the effect of early growth on developing glucose intolerance and insulin resistance remain unclear.

It appears that the development of chronic disease in later life such as CVD and T2DM reflects the individuals phenotype in terms of structure and function together with the lifestyle

pursued by the individual (i.e. the environmental stressors that the individual faces). The phenotype of the individual is the result of genetic potential and the cumulative exposure to environmental factors that occur in utero and throughout life thereafter. To better predict the risk of chronic disease in later life, one should consider how events in early life might influence the current phenotype in term of structure and function.

2.2.1 Fetal growth and development

Fetal growth is the result of a complex array of genetic and environmental factors that act to provide appropriate conditions to support fetal growth. Although the exact influences of genes on fetal growth remain largely unknown, it is now recognised that the dominant determinant of fetal growth in-utero is the nutritional and hormonal milieu in which the fetus develops, in particular the nutrient and oxygen supply. For instance, observational studies among half-siblings related to only one parent show that those with the same mother have a similar birth weight, while those with the same father have a dissimilar birth weight (94). In addition, a previous epidemiological study suggested that the contribution of environmental factors occurring in utero account for 62% of the variation in birth weight, while maternal and fetal genes account for 20% and 18% of the variation in birth weight, respectively (95).

The prenatal period, from conception until birth, is divided into three parts (96). The germinal period is approximately the first two weeks of development during which the primitive germ layers are formed. The second part of the prenatal period is the embryonic period which lasts from about the second to the end of the eighth week of development, during which the major organ systems come into existence. The last stage of prenatal period is the fetal period, which represents the last 30 weeks of the prenatal period, during which the organ systems grow and become more mature. During the last part of the fetal period, the magnitude of the increase in fetal weight is greater than that associated with length. The fetus grows from about 3 cm in length and 2.5 g in weight at 60 days to 50 cm and 3300 g at term - a 15 fold increase in fetus length and 1300 fold increase in fetal weight. Therefore the variation between infants in weight at birth is greater than that observed in the length of infants at birth. Several hormones, including thyroid hormone (97), glucocorticoids (98;99), insulin like growth factors (100) and leptin (101) regulate the growth of the fetus in utero. However, the exact effect of these hormones on fetal growth and how it relates to infant birth weight or length is not completely understood.

Different anthropometric measurements at birth are used to assess the growth of the fetus in

utero. For instance, birth weight is a crude measure that can be used to assess the growth of the fetus. The measurement techniques are relatively easily standardised and birth weight is often available even in older historical records. Other different anthropometric measurements at birth are also used to assess fetal growth, such as birth length and the circumference of the head or abdomen. However, the length of an infant at birth is more difficult to measure adequately, leading to more variation in this measurement (102). Given the difficulties in measuring birth length in the face of relatively small variations in birth length means that birth length in itself might not be a good proxy measure of fetal growth. Head circumference and Ponderal Index (birth weigh/birth length^3), are also used to assess fetal growth with the PI often interpreted as a statement of lightness or thinness (i.e. low weight for length). Furthermore, placental weight and size (area) are also used as measure of fetal growth. Given that the placenta is the getaway between the mother and the fetus governing the effective nutrient supply (and the impact of the maternal hormonal environment), any changes in placental function will adversely affect the nutritional supply to the fetus (103).

The difficulty arises in the definition of growth restriction. Low birth weight is a term often used to identify those babies who carry the greatest risks in the first year of life and is simply those infants born at less than 2500g irrespective of the normal distribution of birth weight for that given population. More specifically, impaired fetal growth and development can be present in different forms. For example, an infant may be born of a lower birth weight as a result of being delivered prematurely (i.e. less than normal term) or due to impaired growth in utero (intrauterine growth retardation or IUGR). Hence, one approach is to correct birth weight or size for gestational age. Taking this approach, it is possible to identify those infants who are born small for the gestational age (SGA). The WHO criteria for small for gestational age (SGA) include all neonates less than the 10th centile for birth weight. However some babies, though of low birth weight compared to the rest of the population, are not growth restricted, simply constitutionally small. On the other hand, other infants may not have attained their full growth potential, yet may have a weight in the "normal" range (i.e. been subjected to intrauterine growth restriction). Growth restriction in utero may be either symmetrical or asymmetrical (104). This refers to the symmetry of the measurements in terms of centiles, (i.e. if the abdominal circumference is on the 5th centile and the head circumference on the 50th this is asymmetric whereas if both are on the 5th centile then this is symmetric). Symmetrical growth suggests reduced growth due to congenital abnormality, infection/drugs and occurs early in pregnancy. In contrast, asymmetrical growth occurs in the fetus with normal potential as a result of impaired fetal nutrient supply - usually later than 26 weeks (105). The infant may be born with a low weight relative to it's length (i.e. commonly seen as relatively light or thin), short (short length in relation to its head circumference) or

short and heavy (short length in relation to its head circumference as well as being heavy). Asymmetrical growth retardation could be the result of fetal adaptation, which influences blood diversion, and brain-sparing mechanisms in utero that, in turn, may lead to thinness and shortness at birth (106;107).

Being born at a weight which is classified at IUGR, SGA or LBW, that is less than usually seen as the normal range, is known to be associated with risk factors related to CVD in adulthood (108). However, even within the normal range of birth weight, evidence is accumulating that those born in the lower end of what is usually considered the normal range of birth weight are at risk of developing chronic disease in later life.

2.2.2 What determines fetal growth?

The fetal growth in utero reflects the balance between the nutrient supply and the fetal demand. Several factors may influence the balance between this process that includes the trajectory of the fetus that is set at an early stage of development, maternal nutritional status and placental ability to supply nutrients and oxygen to the fetus. Maternal nutritional status can be reflected in the quantity and quality of the diet of the mother and her weight as she enters pregnancy and her weight gain during pregnancy. In addition, her nutritional status reflects maternal body habitus in terms of size (i.e. height and weight), composition and metabolic capacity and function.

2.2.2.1 Fetal growth trajectory

The fetal growth trajectory is set in the early stage of the developmental process. A rapid growth trajectory of the fetus is associated with an increase in the demand of nutrients and oxygen supply. However, if this demand is not met by the supply, especially in later gestation, the rapidly growing fetus may make a series of adaptations in order to survive. Although genetic factors are suggested to determine the growth trajectory of the fetus (109), the identity of these genes, and the influence of other factors on the fetal growth trajectory remain unclear. However, some experimental studies in animals suggest that the action of insulin-like growth factors (i.e. IGF-1) and their receptors may play an important role in determining the growth trajectory of the fetus (110).

2.2.2.2 Maternal nutritional status

Poor maternal nutrition during critical periods of pregnancy has long been considered to impact on fetal growth. The follow-up studies of men and women aged around 50 years and born during the "Dutch hunger winter famine" of 1944 reported that women exposed to the famine during the mid and last gestation delivered babies who were lower in birth weight, length, head circumference and placental area when compared to those exposed to the famine in early gestation (111). However, the role of maternal nutrition within less profound nutritional deprivation is now recognised to have only a minor effect on fetal growth. For example, different studies reported no relationship between the habitual dietary intake of the mother and the weight of infant at birth and placental weight even after adjustment for other factors that are known to influence birth weight such as smoking (112;113). However, it has been reported that the increase in micronutrients intake, in particular vitamin C was positively associated with birth weight (112). However, attempts to increase birth weight by giving mothers protein-dense supplements have been reported to be associated with reduced fetal growth marked by lower birth weight. In an observational study in Southampton, UK, Godfrey et al reported that women who consume a high carbohydrate intake in early pregnancy and low dairy or meat protein intake in later pregnancy had lower placental weights and weight of the infant at birth (114).

Maternal pre-pregnancy weight and height is also a marker of maternal nutritional status. Lower maternal pre-pregnant weight has been reported to be associated with lower infant birth weight (115;116). In addition, a recent study reported that among all other maternal anthropometric measurements (i.e. weight and height), maternal lean mass measured by dual energy X-ray absorptiometry (DXA) has been repeatedly shown to be the major determinant of infant birth weight (117). The relationship between maternal lean mass and fetal growth in utero could be mediated through differences in the metabolic capacity of the mother. Duggleby et al found that mothers with a greater lean mass had a greater protein turnover at the 18 weeks of gestation and that this increased capacity was associated with an increase in infant birth length (118).

2.2.2.3 Placental sufficiency and the delivery of nutrients

The placenta has an important role in providing the nutrients from the mother to the fetus to meet the demand of the fetus to meet the growth of the trajectory of the fetus. The size of placenta provides an indirect measure of its capacity to transfer the nutrients to the fetus.

experimental studies in sheep demonstrated that maternal nutrition in early pregnancy can exert major effect on the size of the placenta and the size of the fetus at birth (119). The size of the placenta is also associated with maternal nutritional intake and that in turn may influence growth of the fetus in utero. An experimental study in animals reported that maternal nutritional intake in early pregnancy is associated with a major effect on the growth of the placenta (120). In addition, animal studies reported that the effect of maternal dietary intake on placental size also depends on the nutritional status of the mother in the preconception period. For instances, a ewe that was poorly nourished prior to conception and then consumes a high intake in early pregnancy would exhibit a smaller placental size. Furthermore, epidemiological study of 538 women who delivered at term showed that those with high dietary intake in early pregnancy, especially of carbohydrate, had smaller placentas, particularly if combined with low intake of dietary protein in late pregnancy (114). The effect of maternal diet on placental size reported in that study was independent of mother's body size, social class and smoking.

The transfer of nutrients from mother to fetus is influenced by several factors such as placenta surface area and availability of specific nutrient transporters on the membrane of the placenta (121). The placenta may also influence fetal growth through its role in the metabolism of certain key nutrients. For example, experimental studies in animals suggested that the placenta may convert glucose to lactate which is then released into the fetal circulation where it provides an important oxidative fuel to the fetal metabolism. In addition, it may plays an important role in determining the fetal amino acid supply, with virtually all fetal glycine requirements synthesised within the placenta rather than taken up from the maternal circulation (122). Furthermore, hormones that are produced from the placenta may also influence fetal growth and development. Both placental lactogen and growth hormone are produced by placenta in large amounts (121). They are believed to contribute to maternal insulin resistance, increasing the availability of glucose and other nutrient in maternal circulation for transfer to the fetus.

2.2.3 Childhood growth and the catch-up process

The growth of infant after birth represents by three discrete stages, infancy, childhood and puberty or pubertal phase. During each of these stages, growth is regulated by a different hormonal system. After birth, the infant period represents the continuation of fetal life and the insulin-like growth factor system is proposed to be the most important factor regulating the growth during this period. Thereafter growth hormone will have a major impact on regulating

growth through to puberty. In puberty, sex steroid hormone also plays an important role on regulating the growth during this period. Thus, perturbations in the normal endocrine control, poor nutrition or adverse environmental factors may have an effect on any of these stages of growth as well as the transition between phases resulting in permanent impairments in stature and body structures.

Infants born with a lower birth weight as the result of impaired fetal growth in utero may achieve similar body size (at least as stature) compared with other children of the same age later in life. This process occurs as the result of the accelerated growth after birth or "catch up growth". For example, observational study in Swedish study reported that 87% of children born small for gestational age fully caught up in terms of height with their peers by the first two years of life (123). Catch up growth has been also reported in twins where the smaller or lighter co-twin at birth, was seen to catch up in weight at the age of one year (124). However, any constraint in the ability of the infant to catch-up for growth after birth may be associated with an increase in risk of having shorter stature in adolescence. For example, it has been reported that infants born small for gestational age have a seven fold increase in the risk of having shorter stature in adolescence (125;126).

Despite the potential normalization of childhood weight and height through the catch-up process, accumulating evidence suggests that this process during childhood can have effects that become evident in later life (127). This could be related to the increases in adiposity that is associated with such accelerated growth. For example experimental study in rats suggested that the combination of pre-natal undernutrition with poor fetal growth, but good post-natal nutrition with accelerated growth, is associated with higher blood pressure, insulin resistance, inactivity and a reduction in life span (128). In the same way, an accelerated weight gain during childhood, in the face of a low weight at birth, is associated with increased risks of developing chronic disease in later life. The weight of infant after birth and during the first year of life has been reported to be associated with CVD and its related risk factors. In Hertfordshire, men with lower weight at the age of one year, and lower weight gain between birth and one year has bee reported to have higher cardiovascular disease mortality (129) and higher prevalence of type 2 diabetes (88) in men but not in women. This finding has been also confirmed in another large cohort study in Finland. For example, in Finland an increase in BMI from birth to 7 years has been reported to be associated with an increased risk of adult CHD only in those who were small or thin at birth (130).

2.2.4 Fetal growth and CVD

The first study reporting an association between birth weight and CVD was that reported by Barker and colleagues 15 years ago. This relationship was obtained from a large follow-up study of more than 10,000 men and women born in Hertfordshire, UK during 1911-1930. The death rates from coronary heart disease expressed as standardised mortality ratios in this cohort fell progressively from 110 to 70 per 100,000 with the increase in birth weight from < 2.5 to >4.3kg in men, and from 70 to 36 in women with the same range of birth weight (129). Another large cohort study in Sheffield UK of 1586 men born during 1907-1925 also reported that infants born with a lower birth weight as the result of growth retardation were associated with greater mortality form CVD in later life (131). The generalisability of this relationship between fetal growth and CVD has been consistently demonstrated in studies throughout Europe (132), USA (133) and India (134) in different settings in both retrospective studies of different ages.

In Finland, in a large epidemiological study conducted in 4630 men born in Helsinki during 1934-1944, the hazard ratio from CHD fell from 1.83 to 1.0 with increasing birth weight from less than 3.5kg to greater than 4.0kg (135). This study also reported an inverse relationship between the growth of the infant at the age of one year marked by BMI, weight and height and mortality from CVD. The hazard ratio fell progressively from 1.83 to 1.0 with increasing body weight at the age of one year from less than 9kg to greater than 12kg. The same result was also reported in relation to BMI and height at the age of one year. The growth of infant at the age of one year was shown in this study to be independent of infant birth weight.

In the United States, the Nurses Health Study reported that the increase in birth weight is associated with a decrease in risk of non-fatal cardiovascular disease (133). The relative risk has been found to be reduced from 1.49 to 0.68 with increasing birth weight from less than 2.27kg to greater than 4.5kg. The inverse relationship between birth weight and non-fatal cardiovascular disease was also apparent for both coronary heart disease and stroke.

In South India, a smaller cohort study of 517 men and women aged more than 47 years and born between 1934 and 1954 reported that infants born with lower birth weight and birth length had a greater prevalence of coronary heart disease when compared with those born with higher birth weight (134). The prevalence of CVD has been reported to be reduced from 11 to 3% in those born with birth weight of 2.5kg or less when compared with those born with birth weight more than 3.1kg. In Sweden a larger follow up study of 14611 men and women

born during 1915-1929 reported an inverse relationship between birth weight and ischemic heart disease in men but not in women (136). This relationship was also apparent even after adjustment was made for socioeconomic circumstances at birth and in adult life. This study reported that the increase in birth weight by one kilogram is associated with reductions in the rate of ischemic heart disease of 0.77.

In a prospective study in Caerphilly, South Wales which included 1258 men, aged 45–59, similar relationship between birth weight and coronary heart disease using a questionnaire (137). During the follow-up period, coronary heart disease occurred in 46 (11.6%) men in the lowest birth weight tertile, 44 (12.0%) of those in the middle tertile, and 38 (9.1%) of those in the highest tertile. Most interestingly, this study reported an interaction between birth weight and adulthood BMI in relation to coronary heart disease. That is the reported inverse relationship between birth weight and coronary heart disease is restricted to men in the top tertile of BMI or who had greater risk of adiposity in adulthood. It was suggested that such interaction between birth weight and adult BMI is attributed to the restriction in growth in early life, which is followed by, accelerated accumulation of fat in adulthood.

Although the influence of birth weight on mortality from CVD is well recognised, the mechanisms that underlie this relationship remain unclear. It could be possible that it is mediated through alterations in adult body structure in term of size, composition and the pattern of fat distribution and metabolic function.

2.2.5 Fetal growth and type 2 diabetes

Many studies have examined the relationship between birth weight, as a crude measure of fetal growth, and the prevalence of clinically established T2DM or metabolic markers related to glucose metabolism such as glucose intolerance, insulin resistance, and insulin secretion. The relationship between birth weight and prevalence of T2DM was first reported by Hales et al in a group of 370 men aged 64 years and born in Hertfordshire during 1920-1930, and whose weight at birth has been recorded by health visitors (91). In this study, the blood glucose concentration 2 hours after a standard glucose load was reported to be inversely associated with birth weight, even after adjustment was made for current BMI. However, fasting plasma glucose in this study was not related to birth weight. Contrary to men born in Hertfordshire, another study of 297 women within the same population group reported an inverse relationship between birth weight and fasting glucose and insulin in addition to glucose and insulin concentration 2 hours after glucose load, and that this inverse

relationship was evident after accounting for current BMI (138).

The Health Professional Follow-up study of 22846 men aged 48-83 years also assessed the relationship between birth weight and diabetic status of the individuals through questionnaire. This study reported that the life time cumulative incidence of diabetes decrease from 7.6% in those with birth weight lower than 2695 g to 4.2% in those born with birth weight greater than 4536 g, and this relationship was evident after adjustment were made for BMI, age and parental history of diabetes (139).

Phillips et al in other study of 81 normoglycaemic subjects, and 22 subjects with impaired glucose tolerance, who were born in Preston, UK, between 1935 and 1943 examined the relationship between fetal growth and insulin resistance (140). In this study, insulin resistance was measured by the insulin tolerance test which uses the rate of fall in blood glucose concentrations after intravenous injection of insulin as an index of insulin resistance. The authors of this study reported that both men and women who were thin at birth, marked by a lower ponderal index, were more insulin resistant. This relationship was reported to be independent of duration of gestation, adult body mass index and waist to hip ratio and of confounding variables, including social class at birth or currently. In another study of 82 normoglycaemic and 23 glucose intolerant subjects from the same authors found no relationship between any measure of fetal growth including birth weight and ponderal index and insulin secretion obtained by intravenous glucose tolerance test (141).

In Sweden, a cross-sectional study of 2237 men born in 1938-1957 in Stockholm examined the influence of birth weight on the prevalence of T2DM and other abnormal glucose metabolism both in those with and without a family history of diabetes (142). This study reported that birth weight was inversely associated with T2DM, impaired glucose tolerance, and impaired fasting glucose, and that this finding were observed after accounting for the differences in body size marked by BMI. This result was most pronounced in those with diabetes in the family, but it was also indicated in those without a family history of diabetes. It has been suggested that men with the combination of low birth weight and family history of diabetes seem to be at particularly high risk of developing T2DM. In a combined cross-sectional study with a prospective arm, 1333 men born in Uppsala, Sweden during 1920-1924 were studied using the intravenous glucose tolerance test at age 50 years and was then followed up for T2DM at the age of 60 years (143). This study reported week but inverse relationship between size at birth and insulin concentration at 60 min at age of 50 years. However, at the age of 60 years' size at birth was found to be inversely associated with the

prevalence of diabetes and glucose tolerance. The prevalence of diabetes was 8% in men whose birth weight was less than 3250 g compared with 5% in men with birth weight 3250 g or more, and that was evident even after accounting for BMI.

The relationship between birth weight and the prevalence of T2DM reported in some studies has not been linear. For example, in the Pima Indians, where the prevalence of T2DM is high, a U-shape relationship has been reported between birth weight and diabetes (144;145). The age-adjusted prevalence for birth weight <2500 g, 2500-4499 g and 4500 g were 30%, 17% and 32% respectively. It has been suggested that the selective survival of low birth weight infants, who are genetically predisposed to insulin resistance and diabetes, provides an explanation for the observed relationship between low birth weight and diabetes. The high incidence of T2DM in infants born with high birth weight could be related to the high incidences of gestational diabetes in the mothers.

2.2.6 Fetal growth and metabolic risk factors related to CVD

The influence of fetal growth on risk of CVD has been suggested to be mediated at least in part through alterations in metabolic control, including that related to lipid and lipoprotein metabolism. Fall and colleagues in a study of 292 women aged 60-71 and born in East Hertfordshire, UK examined the influence of fetal growth marked by lower birth weight on metabolic markers related to lipid and lipoprotein metabolism (138). Birth weight was inversely related to fasting plasma TAG. That is, as birth weight increases from less than 2.5 kg to greater than 4.3 kg, it is associated with a reduction in fasting plasma TAG by approximately 33%. In addition, this study also reported a positive relationship between birth weight and HDL. These differences in TAG and HDL associated with birth weight were found to be independent of adult BMI. However no relationship was reported between birth weight and LDL-cholesterol, total cholesterol and apo-lipoprotein B.

In contrast, Barker et al in a study of 219 men and women aged 50-53 and born in the Jessop Hospital Sheffield, UK during 1939-40 reported that infants born with a lower birth weight and with a smaller abdominal circumference at birth had raised serum concentrations of total and low density lipoprotein cholesterol and apo-lipoprotein B when compared with those who had higher birth weight (146). This relationship was found to be independent of social class, current body weight, cigarette smoking, and alcohol consumption. No relationship was seen in this study between birth weight and plasma TAG and HDL.

Part of the investigation of the risk of insulin resistance syndrome associated with birth weight, Phillips et al measured plasma TAG and non-esterified fatty acid (NEFA) concentrations in the fasted state and NEFA suppression in response to an oral glucose tolerance in a group of 93 men and women aged 50 years born in Preston (147). The purpose of this study was to examine whether any increase in risk of insulin resistance associated with birth weight could be explained by impaired lipid metabolism. However, no significant relationship between birth weight and fasting plasma TAG, NEFA and NEFA suppression during an oral glucose tolerance where observed.

More consistent observations of an inverse relationship between birth weight and fasting plasma TAG have been observed whilst the effect on other markers of cholesterol and lipoprotein metabolism appears more variable. A study of 627 men and women aged around 45 years old in China also reported an inverse relationship between birth weight and plasma TAG. That is, the increase in birth weight from less than 2.5 kg to greater than 3.5 kg was associated with a reduction in plasma TAG by 40%, an effect that remained after adjustment was made for sex and BMI (148). However, this study reported no significant relationship between birth weight and total cholesterol, LDL and HDL. In young Dutch men and women aged between 18 and 32, Clausen et al (1997) reported an inverse relationship between birth weight and plasma TAG only after adjustment was made for age, sex and waist circumference (149). The increase in birth weight by 1-kg was found to be associated with 10% reduction in plasma triglyceride. However, no relationship has been found between birth weight and other lipid profiles that include total cholesterol, LDL and HDL. In another study of 422 adolescent girls and boys in Middleborough, UK also reported an inverse relationship between birth weight and plasma triglyceride level both in boys and girls separately (150). This relationship was found to be independent of BMI and age. No significant relationship has been found between birth weight and other lipid profile which include total cholesterol HDL and LDL. In Sweden, Byberg et al reported that only HDL, amongst all fasting lipid variables is positively associated with birth weight in 70 years old men and that was only evident after adjustment was made for the current BMI and age (151). No significant relationship was found between birth weight and TAG. In Sweden a study of 2478 men and women aged 29-41 and born during 1955-1966, reported an inverse relationship between birth weight and fasting plasma triglyceride in women but not in men (152). However, the increase in birth weight in men was reported to be associated with a decrease in total plasma cholesterol. The differences in TAG and total cholesterol in plasma associated with birth weight in this study was evident after accounting for the differences in BMI and age.

Although the above studies report relationships between birth weight and an unfavorable lipid profile, several others report no relationship between birth weight and any markers related to lipid and lipoprotein metabolism. For example, In a sub-study of 474 men and women aged ≈ 69 obtained from larger cohort study of 7089 subjects born in Helsinki during 1924-1933, Eriksson et al reported no significant association between birth weight and plasma TAG, HDL-cholesterol and LDL-cholesterol even after adjustment was made for age, sex and BMI (153). Similarly, Hulman et al in a study of 137 young African American men and women aged around 28 y also reported no significant association between birth weight and plasma total cholesterol, HDL, LDL and TAG after adjustment was made for anthropometric measures related to body size and fat distribution that include height, weight, and waist and hip circumference (154). Kolack et al also reported the same finding in 192 adult men and women 20 years old (155).

In summary, it appears that whilst fasting TAG appears to be related to birth weight, the effects of fetal growth on cholesterol metabolism and lipoprotein metabolism are modest if any. This may reflect a link between lipids and insulin metabolism mediated through the control and regulation of NEFA and TAG turnover and clearance that is independent of processes that regulate cholesterol metabolism. It might also be related, in part, to the varying power of these studies and differences in study population in terms of age, sex and ethnicity since the relationship between birth weight and these markers were apparent in men but not in women, and in older subjects rather than younger individuals. Another important possibility could be related to the use of BMI as a confounder in most of these studies to account for the differences in adult body size. However, BMI might not reflect the differences in adiposity or central adiposity which in turn are associated with these markers of lipid and lipoprotein metabolism. In other words, differences in body size and composition might be critically important in determining the relationship between birth weight and lipid and lipoprotein metabolism. As such, without a complete characterisation of body composition, attempts to adjust or control for differences in BMI might of itself introduce unwanted variance into the analysis, thereby making the interpretation of the results even more complex.

There is evidence that the prevalence of these risk factors in adulthood, collectively expressed within a diagnosis of metabolic syndrome, may be related to birth weight. Although an inverse relationship between birth weight and the prevalence of metabolic syndrome has been reported (88;156-158), there are important and subtle differences in the interpretation of these results arsing from an interaction between birth weight and postnatal

environmental factors, such as current BMI and physical activity. In some studies, current body size, fatness and lifestyle are seen as obvious confounder for which there is a need to adjust or control for in order to explore the relationship with birth weight. Others, however, recognise that this interaction may be important in determining the ultimate expression of risk. In other words, for those born small, the probability of developing this phenotype is greatest in those with adverse or imprudent lifestyle. For example, some studies have reported that infants born with a lower birth weight and with greater adult adiposity marked by BMI had a greater risk of metabolic syndrome than those born with a lower birth weight and lower adult BMI (158). In the same way, the relationship between size at birth and the prevalence of metabolic syndrome is also related to differences in physical activity assessed by cardorespiratory fitness (as VO_2 max) (156). Those born thin at birth and have lower fitness levels as an adult have a greater risk of metabolic syndrome when compared with those born thin who then had a greater fitness as adults.

2.3 Influence of birth weight on adult body structure and metabolic function

Having considered the evidence relating to events in early life with the risk of developing CVD, T2DM and Metabolic Syndrome, attention is now directed towards a closer examination of the evidence relating to birth weight with differences in structure and function in later life. Before considering the evidence relating to structure, it is necessary to reflect on the methods used to characterise body composition in order to better interpret the work of others and that presented within this thesis.

Many different techniques have been used to determine the different proportions of fat mass (FM) and fat free mass (FFM) in children and adults. Each of these methods is based on differing assumptions relating to the chemical and physical properties of the component tissues, which may be more or less valid in differing contexts and may yield differing results. This section of the review is divided into three parts. Section (2.3.1) discusses what is known about the methods that are used to measure body composition in particular those used to assess the relationship between birth weight and adult body composition which include Hydrodensitometry (or under water weighing; UWW), Air Displacement Plethysmography or BodPod, Bioelectrical impedance (BIA) and Dual energy X-ray absorptiometry and anthropometry (DXA). Section (2.3.2) focuses on what is known about the relationship between measures of adiposity, body size, body composition and the distribution of body fat and fetal growth as marked by birth weight. Finally, section (2.3.3) considers the literature on

energy and substrate metabolism as markers of metabolic function and how that might be influenced by early fetal growth.

2.3.1 Methods used for measuring body composition

Hydrodensitometry and Air Displacement Plethysmography: This method utilises the concept of a two compartmental model which divided the body into two parts: Fat Mass (that tissue which is fat) and Fat Free Mass (FFM - the remaining tissue). This method is based on the principle of determining the density of the body by measuring body mass and volume. Body mass or weight can be obtained easily by regular scales while body volume can be estimated on the basis of Archimedes principle that a body immersed in fluid is acted on by a buoyancy force, which is evidenced by loss of weight equal to the weight of the displaced fluid. Thus, when a subject is submerged in water, body volume is equal to the loss of weight in water, corrected for the density of the water corresponding to the temperature of the water at the time of submersion. The obtained density of the body that is obtained by this method or any other two compartmental methods are then used in well established predictive equations to predict percentage body fat (i.e. Siri's equation (159)) under the assumption that the density of body fat mass and FFM are consistent (0.9 and 1.1g /cm^3 respectively). Once the percentage body fat is obtained the absolute (kg) fat mass are then determine by multiplying total body weight by the obtained percentage body fat, while FFM represent the differences between body weight and absolute fat mass.

Part of the limitation in using this method is related to the assumption underlies the values for the density of fat and lean tissues. For example, although the density of body fat is generally taken as constant, the density of the FFM may vary due to the heterogeneous nature of this compartment. Although principally composed of water (≈ 72.4%), protein (20.5%) and bone mineral (7.1%), the relative proportions of each component may vary. Several studies have demonstrated considerable variation in FFM composition and density attributable to growth and maturation, aging, physical activity, sex and racial differences (160-163). Another limitation that could be associated with this method is related to the technical adjustment for the residual lung volume. Although some investigators measure lung volume during the assessment of body composition (i.e. close circuit or open circuit approaches), others approximate the residual lung volume using predictive equations based on height and weight. Correction for the measured residual lung volume may introduce an absolute error in measuring parentage body fat for around 1% (164). However, if predicted, then the absolute error may be increased to 3-4%. Although underwater weighing provides a non-invasive

method to assess body composition, it is impractical when conducting a larger group of subjects and performed in young children, older subjects and those with disease.

Air displacement plethysmography, as used in commercially with the product known as the BodPod, represents an alternative method for estimating body density especially for those that can not be assess using the under water weighing method (i.e. young children and elderly). The principle of this method is fundamentally the same as underwater weighing, but uses air instead of water. It is based on estimating the volume of air a person's body displaces while sitting inside a comfortable chamber, rather than measuring how much water their body displaces when immersed in a tank in case of the under water weighing method. The volume of the body is determined by measuring the changes in pressure within a closed chamber. Based on body volume and mass the density of the body is obtained, then the percentage body fat is determined by using established predictive equation. There is generally good agreement between estimates of volume, and hence density and fat mass reported in different studies in healthy adults derived using the BodPod and the under water weighing (UWW) method, with the average absolute differences in percentage body fat between BodPod and UWW usually ranging from -4.0 to 1.9% (165;166). For example, study by Collins et al reported significantly lower estimates of the density of the body by UWW than that obtained by BodPod (1.060 v 1.064 g/cc^3 respectively) which corresponds to an estimate of percentage body fat by BodPod that was 13% lower than that derived by UWW (equivalent to a 2% absolute difference in percentage fat) (167). In contrast, Field et al reported that the density of the body in adult women measured by UWW was significantly greater than that measured by BodPod (1.030 v 1.028 g/cm^3 respectively) which corresponds to an overestimation of percentage body fat measured by BodPod by 1% (168). Because the same equation is usually used to convert density obtained from BodPod and UWW (i.e. Siri's equation) to percentage body fat, the differences might be attributed to technical error associated with either method which could influence the estimation of body volume. For instance, differences in subjects clothing, hair, skin surface area and residual lung volume (i.e. predicted or estimated lung volume) may have a compounding effect on measuring the body volume (i.e. excess clothing causes a significant underestimation of body volume and thus overestimation of body density).

Bioelectrical Impedance (BIA): The physical principle behind the BIA technique is that the body's lean (muscle, bone, water) compartment, comprising approximately 60-75% electrolytes and water, conducts electricity far better than the body's fat compartment which is very low in body water content (between 5-10%). These two compartments have,

therefore, very different impedance (or resistance) values to a high-frequency electrical signal. The single impedance measurement reflects the degree of resistance to the flow of current in the body, water being a good conductor but fat a bad conductor. The principle is based on the geometrical relationship between a conductor's shape and its resistance according to Ohm's law. That is, resistance of a length of homogenous conductive material of uniform cross-sectional area is proportional to its length and inversely proportional to its cross-sectional area. The assumptions that underlie the use of this method is that the human body is considered as series of cylindrical or uniform segments with its length proportional to the subject's height and the current that is passed through these segments is uniformly distributed. It is important to note however that the body consists of different segments that include limbs and trunk and each of which has a different characteristic specific resistivity based on their differences in tissue proportion (i.e. muscle v fat). For example, within the limb, resistance is the function of the amount of muscle tissue because of its high water content and the high resistance of bone and fat. In contrast, the resistance in torso could be largely attributed to the amount of fat mass.

In this method, an alternating electrical current (generally at 50 kHz, although in Bioelectrical Spectroscopy systems, this may range from less than <5Hz to >200Hz) is passed between the two electrodes that are placed on one hand and one foot (or in some other cases are placed between the two feet or the hands). The mean resistance of the body as a whole is obtained and is then used to determine total body water (TBW) from predictive equations developed for specific populations. These equations were developed by comparing the values obtained by BIA to the estimates of total body water obtained by a reference method such as deuterated water. Different multiple regression equations have been generated which differ in the variables used to estimate total body water. For example early studies used only height and resistance to predict TBW (169), while more recent equations now include variables such as weight, age, gender and some anthropometric measurements of the trunk (164). Having determined TBW, FFM is then derived on the assumption that the hydration state of the FFM is constant. The principle limitations of this method for predicting FFM lie in part with the specific resistivity constants used for fat and lean compartments and the appropriateness of the hydration constant. Another potential source of bias that could be associated with this method relates to the variation in body shape between individuals in terms of the length and composition of each segment of the body. Such differences in body shape between individuals have been reported to be associated with ethnicity (14), genetic and some postnatal environmental factors (i.e. diet in childhood) (170). For example, those from the Asian population have been reported to have relatively shorter legs when compared with Caucasian at the same BMI. The extent to which such differences in the relative lengths

of body cylinders may affect body composition measurements by bioelectrical impedance has not been adequately tested.

Dual energy X-ray absorptiometry (DXA): This technique was originally developed to determine bone mineral content (BMC) and bone density to aid in the treatment of osteoporosis. However, more recently, this technique has been expanded to include the measurements of body soft tissue that includes fat mass and fat free soft tissue (FFST = FFM-BMC). The DXA machine is composed of a generator emitting two low energy x-rays, the scanning table, a detector, and computer system. The subject lies on the scanning table and scanned rectilinearly from head to toe. The fundamental principle behind the use of DXA is the measurement of the transmission through the body of X-rays with high and low photon energies. When the X-rays of initial photon intensity pass through the body, they are attenuated by photoelectric absorption and Compton scattering, and the intensity transmitted to the detector is reduced. This attenuation is associated with the depth or thickness, density and chemical composition of the subject. The raw scan data, which includes values of the attenuated beams of tissue and bone are then captured and sent to a computer which interprets each pixel through algorithm. This in turn produce two dimensional high quality images that include the quantitative measurement of the bone mineral content, fat free soft tissue and fat mass of the whole body and within each segment of the body. Compared with the methods described above which are based on the two compartmental model, the results obtained by DXA represent three compartments and include BMC in addition to FM and FFST.

There are three commercially manufacturers of DXA: Lunar, Hologic and Norland. Although they are based on the same principle, differences exist in the generation of the low and high energy x-ray beams, the x-ray detectors, the imaging geometry, calibration methodology and the software used for analysing the raw data. The precision of body composition measurements by DXA has been reported to vary according the DXA machine used for assessing body composition. The coefficient of variation for total FM has been estimated to be in the range from 1% to 7% depending on the device and population (171). This in vivo precision has been also reported to be lower in the regional composition measurements when compared with that in whole body (171).

The limitation of DXA for measuring body composition may arise from the assumptions that underlie the use of this method. Given that the DXA can only measure in two dimensions (effectively a flat image), it is assumed that the body thickness within and between individuals

is constant for a given dimension (length or breadth). However, within the same individual, the scans are made over a very wide range of tissue thickness that is from the finger tips to the trunk of the subject's which might range from ≈ 1 to 30 cm. In addition, body thickness of both lean and obese subjects is considered to be the same when using DXA. Several researchers have investigated the effect of variable tissue thickness on the estimates of body composition. For example, whilst consistent and accurate results can be obtained by DXA at moderate depth of soft tissue, increasing the tissue depth to greater than 20-25 cm has been reported to be associated with an overestimation of the amount FM and BMC (172). It has been also reported that for body thicknesses less than 20 cm, fat mass is overestimated by ≈ 4% or less and FFST is overestimated by ≈ 2% or less. The extent to which differences in body thickness that may exist between individuals or between different groups (such as by age, ethnicity or obesity) could contribute to differences in body composition and hence such associations required further research. The other assumption that underlies the use of DXA is related to the hydration status of the subjects which is also assumed to be constant.

Despite these limitations, the DXA is now considered as a reference method for measuring body composition as opposed to the underwater weighing since the former provide three body compartment densities rather than two (171;173). Many studies report good agreement between estimates of body composition derived by DXA and that obtained with two compartment models such as UWW, BIA and skinfold thickness (171;174-176).

One potential application of DXA technology that has not been previously explored is to use the scanned images to provide linear measures of body dimensions. In principle, it should be possible to derive precise and accurate measures of lengths and breadths of the whole body and individual body segments. To date, no studies have been published which present this type of information both in terms of the normal body dimensions of individuals throughout the life cycle, nor of how body proportions might be influenced by events in early life.

2.3.2 Current body size, composition and fat distribution in relation to birth weight

Having considered the principles underlying the methods that may be used to assess body composition, the literature that has examined the relationship between birth weight and body size and composition will be discussed in the subsequent sections.

2.3.2.1 Influence of birth weight on body size - height and weight

Adult body size, as marked by height and weight, is influenced by genotype and different environmental factors throughout the lifecycle. Emerging evidence now suggests that the contribution of pre-natal environmental factors acting in utero that is marked by birth weight have a greater effect on adult height and weight than was previously believed. For example, a longitudinal study of 637 monozygotic twins (which have the same genotype but may differ in birth size) reported that the heavier twin at birth became taller and heavier as an adult when compared to the smaller twin born from the same mother, and these differences has been suggested to be independent of postnatal environmental factors (177). Another study of 2880 identical twin pairs in Minnesota, USA reported that differences in birth weight between these identical twin pairs (intra-pair differences) were directly correlated with adult height and weight but not at all with BMI (178). The differences in adult height and weight associated with intra-pair differences in birth weight remained the same even after adjustment were made for age and of the twin pair.

Loss et al also reported similar results in two separate studies of 113 men and 128 women twin pairs in Belgium. In women, the intra-pair differences in birth weight were associated with greater adult height and weight but not with BMI after adjustment were made for gestational age (179). In men, the same result was reported with adult height and weight but there was a positive association between birth weight and adult BMI (180). In both studies however, no adjustments were made for postnatal factors which might confound the relationship between birth weight and adult body size.

In addition to the above studies, several other epidemiological studies also confirmed the reported positive relationship between birth weight (within the normal range) on the one hand, and adult height and weight on the on the other (181-183). The relationship has been generally reported in these studies to be stronger with adult height than weight, possibly because the variation in adult weight is more marked than that observed with adult height. Although most of these studies also report a positive association between birth weight and adult BMI, others have not been able to support this observation. That may imply that differences in adult body size marked by height and weight might not be observed if BMI is used to assess the differences in adult body size or body composition associated with birth weight.

Stature is the sum of the lengths of the different body segments or body proportion. These

differences in body proportions could contribute to variation in total adult height, such as differences in leg or trunk length or the differences in relative leg length (relative to height). Differences in height between different racial groups (i.e. Asian and Caucasian) have been reported to be attributable to the differences in leg length (14). Even within the same population, variation in stature might be attributed to differences in body proportions or leg length and trunk length. Although both genetic and postnatal factors are likely to contribute to this variation in adult body proportion, the extent to which differences in birth weight may be associated with differences in body proportion later in life, which may contribute to the differences in adult height, has yet to be fully elucidated. There is some indication that body proportion may be programmed. For instance, although Sayer et al found that birth weight was positively associated with both sitting height (a crude measure of trunk length) and leg length as well as height in older men in Hertfordshire, no attempt was made to determine the extent to which differences in height could be attributed to differences in leg or trunk length (184). Equally, no attempts were made to see if such differences were associated with differences in body composition in this study.

It appears that the contribution of fetal growth and development marked by birth weight to the variation on adult body size and proportion is greater than that could be explained by genotype and other postnatal environmental factors. However, the effect of such differences in body size both independently and together on the differences in adiposity or body composition remains to be determined.

2.3.2.2 Influence of birth weight on body size - BMI

Many studies have examined the relationship between birth weight and the risk of development of obesity in later life. Most of these studies use anthropometric measurements related to body mass, such as BMI and body weight, as opposed to more detailed measures of body composition. For example, large cohort study in Sweden in men reported a positive relationship between the weight at birth and BMI at age of 18y (185). In this study, the relationship between birth weight and adult BMI was evident after adjustment was made for living area, mother's age, educational level and parity.

Another cohort study in Denmark in adult men aged around 20y also reported a positive relationship not only between birth weight and BMI but also between the length of the infant at birth and adult BMI (186). In this study, the prevalence of obesity was reported to increase from 3.5% in those with a birth weight <2500 g to 11.4% in those with a birth weight >4501 g.

However, after adjustment was made for mother's age, marital status and occupation, only birth weight but not length was reported to be positively associated with BMI. The positive relationship between birth weight and BMI has also been confirmed in many studies in different populations (187;188) across different age categories (139;187;189), effects that persist even after accounting for the differences in gestational age and postnatal environmental factors. There are other studies however, that have not been able to demonstrate for any relationship between birth weight and BMI both in children (190;191) and adults (158;192;193). This lack of agreement may be attributed, in part, to the limitation of these studies to control for confounding factors, such as socioeconomic status, that effect the risk of obesity.

The positive relationship between birth weight and BMI reported in the above studies may suggests that with increasing birth weight across the normal range, BMI in later life is increased and this may associated with an increased risk of developing CVD and T2DM. This finding runs counter to the fetal programming of adult disease hypothesis, which suggests that a decrease rather than an increase in risk of developing CVD is associated with the increase in the weight of the infant at birth. One explanation for this paradox that is usually offered could be related the influence of other factors (i.e. metabolic risk factors or life style) on the development of CVD in later life which are independent of the differences in adiposity or fatness. Another possible explanation, that has not been adequately explored, could be related to the use of BMI as a crude measure of body fatness or body composition. With this latter explanation, the positive association between birth weight and adult BMI could be mediated through an increase in lean mass rather than fat mass. In addition, using BMI does not take into account any differences in either height or weight which might occur in association with differences in birth weight that might of themselves account for differences in the relative amounts of lean and fat mass either separately or together. The increase in height is well recognised to be associated with lean body mass in particular muscle mass. For example, some studies reported that the increase in height by ≈ 2% in men (from 1.71 to 1,74m) is associated with ≈ 24% increase in muscle mass, while in women the ≈ 2% increase in height was associated with 35% increase in muscle mass (192). In addition, other study have also reported that taller individuals have a greater lean mass measured by under water weighting than the shorter individual does even at the same BMI (194). However, the contribution of height, independent of weight, on the proportion of lean to fat mass has not been adequately examined. As a result, the variation in height and weight (both independently and together) that may be associated with birth weight could have an effect on body composition independent of BMI (i.e. positive association between birth weight and BMI could be related to differences in height or/and weight).

2.3.2.3 Influence of birth weight on body composition

The apparent paradox between birth weight, adult BMI and increased cardiometabolic risk has prompted several groups to study body composition of children and adults in relation to the events in early life. Different methods have been used from the more simple anthropometric measurements, to more detailed assessments using under water weighing and bioelectrical impedance.

Study by Singhal et al use bioelectrical impedance and skinfold thickness and DXA to assess the differences in body composition in two different groups of pre and post pubertal children in relation to birth weight (195). In the post pubertal group of children, FFM but not FM measured by skinfold and BIA has been reported to be significantly associated with birth weight Z score. This relationship remains the same even after adjustment was made for differences in age, sex, physical activity and height. In the pre pubertal group of children FFM but not FM measured by DXA has been also reported in this study to be positively associated with birth weight Z score an effect that remained after adjustment was made for differences in age, sex, physical activity and height. Sayer et al in another study assessed the relationship between birth weight and adult body composition measured by skinfold thickness in a group of adult men born in Hertfordshire, UK between 1931 and 1939 (184). In this study, birth weight has been also reported to be positively associated with FFM but not with fat mass even after accounting for the differences in age, social class at birth and some postnatal environmental factors. Although current height and weight were reported in that study to be positively associated with birth weight, the effect of such differences in body size on body composition was not determined. In other word, it was not clear whether the reported differences in FFM associated with birth weight is related to the differences in body size or the effect of birth weight on current body composition is independent of body size. In a large epidemiological study of 460 adolescent girls and boys age between 13-17y Murtaugh et al used skinfold thickness to assess the differences in body composition associated with birth weight. In this study birth weight has been reported to be significantly and positively associated with lean body mass and positively but not significantly associated with fat mass (196). Birth weight in this study also reported to be positively associated with height and weight. However, lean and fat mass index (kg/h^2) were reported not to be associated with birth weight, implying that the differences in lean and fat mass associated with birth weight is lost when it was accounted for the differences in height.

Loss et al also used bioelectrical impedance to examine the differences in body composition

in relation to birth weight in two separate studies of twins in both men and women. These studies were also aimed to examine whether the relationship between birth weight and body composition are independent of genotype (179;180). Among 388 men and 415 women, birth weight has been reported to be positively associated with FFM but not fat mass, an effect that remained even after adjustment was made for gestational age and body weight. The author of these studies concluded that the differences in birth weight have an effect on current body composition in particular FFM to a greater extent than that could be explained by genotype and postnatal environmental factors. A positive relationship between birth weight and adult height and weight was reported in both these studies. Part of the differences in body composition associated with birth weight in both studies could be attributable to the differences in current weight but not with height.

In addition to the above reported studies which have use simple anthropometry and BIA to assess body composition in relation to birth weight, others have conducted more detailed studies of body composition yielding comparable findings. Previous study assess the relationship between birth weight and body composition in a group of using UWW to assess body composition in a group of 272 Pima Indian men and women aged between 28-50y. In this study birth weight has been reported to be positively associated with FFM, but not with fat mass or percentage body fat, even after adjustment for age and sex (197). Although height and weight but not BMI were positively associated with birth weight, no attempts were made to directly determine the extent to which differences in FFM could be attributable to differences in body size. Another study of 356 young men and women by Li et al also used UWW to characterise body composition in relation to birth weight and found a positive relationship between birth weight and adult height, weight and lean body mass, but no relationship between birth weight and fat mass and percentage body fat (198).

Limited attempts have been made to further differentiate between the components that make up the lean tissue. For example, in a study of 231 young men aged 17-22y, Khan et al assessed the differences in body composition in relation to birth weight using girths and skinfold thickness at mid thigh to estimate thigh muscle + bone area and subcutaneous fat area (199). The author of this study found that birth weight was positively associated with thigh muscle and bone area but not with thigh subcutaneous fat area. Although birth weight was reported to be positively associated with adult BMI, no information on adult height and weight in relation to birth weight were reported in this study. In addition, no information was provided on gestational age or any postnatal environmental factors, which may confound the effect of birth weight on body composition. Using estimates of muscle mass derived from measures of 24 h urinary creatinine excretion in men and women aged between 47-55y,

Philips et al also found that birth weight is positively associated with creatinine excretion and hence, muscle mass in both men and women (192). Although birth weight was also founding this study to be positively associated with both height and weight, the relationship between birth weight and muscle mass could not be attributable to differences in height or weight. However, the percentage of body weight accounted for by muscle mass was found to be greater in the higher than the lower birth weight individuals which suggests that the differences in muscle mass associated with birth weight is independent of adult body weight.

Taken together, it appears that there are consistent reports of a positive relationship between birth weight and height, weight and FFM in both children and adults, whilst any relationship with fat mass is less clear. It is important therefore to determine whether this reflects a true lack of effect on adiposity or whether the inability to demonstrate any relationship may be due, in part, to limitations in methodology and study design. To date, limited attempts have been made to use more detailed imaging techniques to better characterise fat mass and its distribution in relation to birth weight, such as magnetic resonance imaging or computed tomography. Such an approach although desirable is limited due to costs and access considerations. However, valuable information may be derived using DXA to provide total and regional estimates of fat mass, FFM and bone mass at lower costs.

To date, only two studies have used DXA to examine the effect of birth weight on body composition. The first conducted in adults was primarily designed to assess the risk of osteoporosis associated with lower birth weight, although did report information on FFM and fat mass (200). They found, in agreement with previous reports, that birth weight was positively associated with FFM, but only after adjusting for postnatal environmental factors and adult height. This study also briefly commented that less obvious effects were also observed for fat mass, again only after adjusting to body size. The second study used DXA in a group of adolescent girls and boys (201). When both groups were combined, there were no significant relationships between birth weight and FFM or FM. However, when the two groups were examined separately, the weight at birth of the girls but not the boys were inversely associated with FFM but not FM even after adjustment were made for gestational age, physical activity and height square. Summary for all studies that have attempted to examine the influence of birth weight on body composition are illustrated in Table 2.1.

TABLE 2.1 summary results of studies in the literature relating birth weight to subsequent body composition

Reference	Age category	Methods used	Outcome	Adjustment
Singhal et al 2003 - UK	Adolescent and children	SFK and BIA	+ ve with FFM only	Age, sex, Tanner stage and SES
Sayer et al 2004 - UK	Older men aged > 64y	SKF	+ ve with FFM, weakly + ve with FM	Age, social class at birth and at the time of study, smoking and physical activity
Murtaugh et al 2003 - USA	Boy and girls Aged 13-17y	SKF	+ ve with FFM only	No adjustment
Loss et al 2002-Belgium	Adult women 18-34y	SKF	+ ve with FFM, - ve with the sum of SKF thkiness	Body weight
Loss et al 2001-Belgium	Adult men 18-34y	SKF	+ ve with FFM, - ve with the sum of SKF thkiness	Body weight
Weyer et al 2000 - USA	Men and women aged 18-50y	UWW	+ ve with FFM only	Age and sex
Kahn et al 2002 - USA	Adult men Aged 17-22y	Skinfold thickness at midthigh (thigh muscle+ bone area, and subcutaneous fat)	+ ve with Thigh muscle + bone area but not with thigh subcutaneous fat area	Race and height
Phillips et al 1995 - UK	Men and women aged 46-54y	24h Urinary creatinine to estimate muscle mass	+ ve with muscle mass	Age
Glae et al 2001 - UK	Older men and women aged 70-75y	DXA	+ ve with FFM and BMC, weakly and +ve with FM in men but not in women	Life style (cigarette smoking, alcohol consummation and physical activity) and height
Labayen et al 2006 - Spain	Adolescent gilrs and boys aged 13-18y	DXA	+ ve with FFM only in girls but not with boys	Gestational age, physical activity and height square

SKF= skinfold thickness, UWW= under water weighing, BIA= bioelectrical impedance

2.3.2.4 Influence of birth weight on the distribution of body fat

An adverse distribution of body fat, in particular greater central adiposity, is associated with with metabolic risk factors linked to mortality from CVD (i.e. insulin resistance, glucose intolerance and dyslipidemia). The mechanism that underlies this interrelationship remains unclear although appears to be related to an interaction with inflammatory processes, resulting in the release of a host of adipokines and signals from adipocytes that act on peripheral tissues (20). Given that body size and structure may be programmed by events in early life, this raises the question as to whether birth weight is associated with differences in the pattern of fat distribution.

Several studies suggest that differences in the distribution of body fat, in favour of more central fat mass, is programmed in early life. Most of these studies have used simple anthropometric measurement related to the distribution of central and peripheral subcutaneous body fat by measuring the waist and hip circumferences or skinfold thickness at sites on the torso (i.e. subscapular) and limbs (i.e. triceps). For example study by Kuh et al in a group of men and women reported positive relationship between birth weight and waist to hip ratio in men but not in women after adjustment was made for current BMI (202). However, in women, this study reported a weak inverse relationship between birth weight and waist to hip ratio after adjustment was made for BMI. Whilst the positive relationship between birth weight and waist to hip ratio in men may simply reflect a size or scaling effect whereby larger individuals have greater circumferences, more information may be derived of the distribution of body fat by looking at the waist: hip ratio. The finding of this study is in agreement with earlier studies by Barker et al conducted in a group of men born in Hertfordshire (203). However, this study reported a significant interaction between BMI and birth weight. That is the negative association between birth weight and WHR was stronger among men with higher adult BMI. Similar relationships with both waist and hip circumference, but not waist: hip ratio have been reported by others in both cross-sectional (184;193) and longitudinal studies (151).

In the twin study in both men and women, Loss et al found that independent of infant genotype and postnatal environmental factor, the increase in birth weight was inversely associated with waist: hip ratio only after adjustments were made for body weight in both men and women (179;180). Interestingly, the authors also suggested that the higher waist: hip ratio in lower birth weight individuals are likely to be attributable to differences in the hip,

rather than the waist, circumference, implying that such effects are less related to central fat accumulation than previously envisaged. However, given the incomplete data, it was not possible to review whether this observation could be tested in other studies reported above. In much the same way, in discussing the positive association between birth weight and waist circumference, but not waist: hip ratio, in young men applying for military service in the US, Kahn et al proposed that the differences in waist circumference were as likely to be attributable to differences in visceral lean as much as fat mass (199).

Using skinfold thickness measures to characterise fat distribution, Okuson et al found that birth weight was inversely associated with the subscapular: triceps ratio and central to peripheral presented as (subscapular + suprailiac):(triceps + thigh) ratio in either white, black or Hispanic children within the same population in the US after adjusting for the differences in sex, age and BMI (204). In Southampton UK, Barker et al reported that increase in birth weight is inversely associated with subscapular skinfold thickness and by the ratio of subscapular: triceps ratio after adjusting for BMI and social class in study of 348 girls aged 15-16y (205). Byberg et al also found that birth weight was inversely associated with subscapular: triceps ratio (central to peripheral body fat) after adjusting for BMI, an effect largely attributable to differences in triceps skinfold thickness (as a marker of peripheral subcutaneous body fat) but not with subscapular skinfold thickness (central subcutaneous body fat)(151). Although birth weight was reported in that study to be associated with BMI, no adjustment was made for such differences in BMI. In addition, this study also reported no relationship between birth weight and pattern of fat distribution obtained by skinfold thickness presented as triceps, biceps, subscapular and suprailiac.

Taken together, these studies suggest that there is a positive relationship between birth weight and many absolute measures of either waist or hip circumference or individual skinfold thickness which may be largely attributable to a simple size or scaling effect – i.e. bigger babies become bigger adults. At the same time, there is some evidence, albeit not strong, that fat distribution may be related to birth weight when expressed as the ratio between measures at different sites of the body. In essence, low birth weight is associated with higher measures of central fat distribution (i.e. waist: hip ratio or ratio of skinfold at the torso versus limbs). However, it is important to note that each of these measures is crude statements of central adiposity and intra-abdominal fat mass in particular. Waist circumference will be affected by both intra-abdominal and subcutaneous fat mass whilst skinfold exclusively reflect subcutaneous fat mass only. Only one study to-date has used quantitative imaging to assess the relationship between birth weight and the distribution of

body fat. Choi et al measured the distribution of body fat in 22 healthy Korean men using computed tomography (CT) together with waist and hip circumference (206). Although this study reported a weak inverse association between birth weight and visceral fat mass obtained by CT on the one hand and waist: hip ratio on the other, neither of these association were statistically significant. This lack of significance may be largely due to inadequate power associated with the small sample size, but may also may be further confounded by the failure to account for size effects related to height and weight.

A final concern that may influence the interpretation of these results relates to the attempts to control for factors which are seen as confounding, thereby masking important biological effects. There is clearly a lack of consistency in approach across the literature. Some studies attempt to control for confounders by adjusting for size or for BMI, whilst others despite recognising the effect that size might have then make no attempt to control for any size-related effects. At times, others will control for factors which may be unrelated to the outcome variable within the specific study population (i.e. always adjusting for a factor which has not been shown to demonstrably relate to the outcome within the particular dataset out of habit or presumption based on effects seen in other larger or more varied cohorts). In this way, important biological effects may be either overlooked or removed through inappropriate or incomplete statistical adjustments. What is needed however is a clear and coherent framework through which the biological effects may be examined.

2.3.3 Differences in metabolic function in relation to birth weight

Having considered the influence of fetal growth on body structure, there is a need to review the evidence that metabolic function may be programmed in early life. Furthermore, there is a need to consider whether differences in metabolic function are mediated through differences in body structure associated with birth weight. The evidence relating to markers of metabolic control has been discussed in a previous section. In this section, attention is specifically directed towards the summative statement of metabolic function that is the demand for energy and the pattern of substrate oxidation that is used to provide that energy.

It is now well recognised that differences in body structure in term of height, weight, composition and fat distribution are linked to metabolic function. The most fundamental expression of metabolic function can be related to the energy demand and substrate oxidation to meet this demand. Energy demand reflects resting energy expenditure (REE) both in fasted and fed state, Dietary Induced Thermogenesis, and the energy associated with

physical activity. Resting energy expenditure represents a major component (≈ 65-75%) of total energy expended per day (TEE) and it is positively associated with height and weight. Several standard equations have been developed to predict REE in the process of assessing energy requirements in different populations on the basis of weight (207;208) and height + weight (209) taking into account age and gender differences. The equation that is most commonly used in the UK is that referred to as the Schofield equations which were initially derived from a range of published works including cohorts from different ethnic populations (209). However, it is not recognised that there may be important differences between differing ethnic groups that are not adequately reflected in the predictive equations. For instance, Soares *et al* reported that Indian men and women had a lower measured REE (9.3-11.2%) than that predicted from weight using Schofield predicative equation (210). These differences between the measured and predicated REE from weight only or that using height + weight would be expected to be related to differences in body composition associated with ethnicity. Therefore, it appears that although differences in body size (weight and height) can explain much of the variance in REE between different ethnic groups, a substantial proportion of the variance in REE remains that can not be simply accounted for by differences in body size alone.

In addition to the simple size effect on the REE, differences in fat free mass (FFM) between individuals associated with age, gender and ethnicity may influence the relationship between body size and REE since FFM is the major site for energy production and substrate utilisation (211). However, even after accounting for the differences in FFM, a proportion of the variance in REE between individuals remains. For instance, previous attempts to express the differences in REE relative to FFM in both men and women show that shorter and lighter individuals that tend to have less FFM have greater relative REE than those who are taller and heavier who tend to have a greater FFM (194).

This observation has led to the view that the greater relative REE (REE/kg FFM) in lighter individuals with lower FFM is attributed to the differences in the proportion of lean tissue with differing metabolic activities (i.e. muscle to organ mass). It is this biological difference that in turn, may explain the between individual differences in REE associated with FFM. Therefore, it appears that despite the strong relationship between body size and REE, for any given weight and possibly height, the relative proportion of tissue (i.e. fat mass to FFM and muscle to fat free mass) of differing metabolic activity might be associated with differences in REE. Although it is apparent that body size, composition are linked to energy metabolism, the extent to which such inter-relationship between body structure and metabolic function are

influenced by events in early life remain unclear.

There have been only two studies which offer some insight to the link between REE in relation to birth weight. The first, reported no relationship between birth weight and absolute REE, while the relative REE (per unit of fat free mass) was negatively associated with birth weight (212). The authors of this study concluded that the greater relative REE (per unit of FFM) in adults of a low birth weight compared to those with high birth weight could be attributed to the differences in the composition of lean mass which could have different metabolic activity. However, although the relative REE in this study imply that there is a difference in the composition of FFM associated with birth weight, the use of such approach to account for the differences in FFM are now considered to be mathematically biased, since the intercept for the relationship between REE and FFM does not pass through the origin (above zero). This would inherently lead to the inappropriate interpretation that individuals with a lower FFM would have higher relative REE (per unit FFM). A more appropriate approach would be through using the analysis of covariance to adjusted for the differences in FFM or different component of FFM (i.e. muscle, and non-muscle mass). In the second study, Weyer et al reported no relationship between birth weight and 24h energy expenditure while sleeping metabolic rate (SMR) has been reported to be negatively associated with birth weight only after accounting for the differences in FFM and fat mass while there was no reported relationship between 24 hours energy expenditure and birth weight (197). This study suggested an inverse relationship between birth weight and SMR. However such differences in SMR represents smaller proportion of REE that might not affect the total EE associated with birth weight.

Whilst apparent differences in energy metabolism in relation to birth weight may have been stated in the two previous studies, no attempts have been made to determine whether differences in birth weight alter the rate of energy metabolism or pattern of substrate oxidation in the fasted and fed state. Furthermore, the extent to which the reported differences in body composition and fat distribution of themselves could account for any differences in energy metabolism and substrate oxidation need to be determine. In other words do differences in body structure associated with birth weight have an effect on metabolic function in term of energy metabolism and substrate oxidation? Or whether differences in metabolic function associated with birth weight may occur independently of differences in body structure and composition?

2.4 Summary of the literature review

The review of the literature presented in this section initially provided a brief overview on what is known about health or good health in order to set the context for raising questions about the inter-relationship between structure and function and how this relationship may be modulated by events in early life. Although health has been generally defined as the absence of disease, a more positive approach is to express health in terms of complete physical, mental and social wellbeing. What is lacking are objective, quantitative measures of health that can be used to achieve or promote good health. In particular, within the physical dimension, wellbeing can be best described in terms of a body structure and function that is appropriate for the context in which the individual exists. This sense of a 'prudent phenotype' that can be thought of as 'fit for purpose' has not been adequately defined. The most obvious markers, in terms of structure and function, are those associated with body weight and composition and fat distribution on the one hand and indicators of good metabolic control on the other. The latter may be most simply expressed as fasting blood nutrients (glucose, TAG and cholesterol) and hormones (insulin), blood pressure, clotting factors and inflammatory markers within the reference range for those who are free from disease, lacking observable abnormalities or deficiencies. This approach is already recognised within a disease framework as these same markers are used as indicators of risk for cardiometabolic disease, in terms of CVD and T2DM.

It is clear that these elements of the phenotype are inter-related. One direct expression of this inter-relationship is the effect that body size and composition has on the magnitude of the demand for energy and the pattern of substrate utilisation. It is also seen in terms of the relationship between body weight, most obviously expressed as BMI, and the extent to which metabolic control is perturbed or in terms of the probability of developing CVD or T2DM. In addition, it is less clear as to whether changes in metabolic control precede changes in weight, fat mass and distribution or vice versa. However, once acquired, a high central adiposity of itself may serve to further exacerbate poor metabolic control.

This review also considered the evidence relating to the influence of early fetal growth marked by birth weight on structure and function in adulthood. An attempt to summarise the evidence within a casual web is given in Figure 2.1. From the literature, it is generally held that infants born with a lower birth weight are shorter, lighter and have a lower lean mass as both children and adults when compared with those who born heavier. Although, lean mass is consistently reported to be positively associated with birth weight, there is little information

were available on the influence of birth weight on the different components of lean mass such as muscle mass and organ mass. Such changes in the proportion of lean mass may be associated with differences in metabolic function (i.e. REE, glucose metabolism).

Furthermore, there have been inconsistent results on the influence of birth weight on adult adiposity and pattern of fat distribution. This could be related, in part, to the limitation of the methods that have been used to assess body composition or fat distribution in relation to birth weight, since most of studies have used simple anthropometric measurements such as BMI, skinfold thickness and waist circumference. Interpretation of such observations has been further complicated by the failure to recognise the effect that differences in body size and proportions of themselves may alter body composition. For example, that difference in body composition may exist for a given BMI if there are differences in the proportions of trunk to legs given the different structural composition of these body segments. Studies that have examined the relationship between birth weight and body composition did not examine the impact of current body size and proportion on differences on body composition and fat distribution.

The review also considered the evidence examining the relationship between birth weight and metabolic function in term of markers of cardiometabolic risk and disease prevalence in adulthood. There is a large body of epidemiological evidence which consistently demonstrates that those of a lower birth weight are more likely to develop T2DM and CVD. These associations have been supported by experimental studies in animal models where manipulation of the maternal diet has been shown to exert effects on the offspring that alter postnatal growth, survival and some aspects of metabolic function. The putative mechanism through which these changes are brought about are the subject of active investigations and include a potential change in responsiveness to glucocorticoid sensitive systems, enabled by epigenetic modulation of gene structure and altered phenotypic expression. The extent to which being born small predisposes the individual towards this less prudent phenotype in terms of poor metabolic control in humans is less clear. There are consistent reports of an inverse relationship between birth weight and measures of glycaemic control, expressed in terms of glucose and insulin. That is, infants born with a lower birth weight had a greater risk of developing insulin resistance, glucose intolerance, a finding that persists even when attempts are made to control for differences in current body size. In contrast, there have been relatively few studies which have attempted to examine the influence of birth weight on biological markers related to the control of lipid metabolism. Whilst the evidence relating to birth weight and lipaemic control is limited, there are reports of a weak inverse relationship

between birth weight and fasting plasma TAG or plasma cholesterol although the effects of birth weight on more dynamic measures of control such as postprandial lipid metabolism have not been extensively studied. In the same way, there are limited reports of a weak relationship between birth weight and the demands for energy, although there are concerns over the approach used to interpret these observations particularly when attempting to correct energy expenditure for differences in body size and composition. What is lacking is a thorough and systematic examination of the inter-relationship between birth weight and energy and substrate oxidation in both the fasted and fed states within the context of differences in body structure and composition.

In conclusion, our understanding of the relationship between body structure and function serves to limit our ability to examine the way in which events in early life result in a pattern of growth and structure that underlie differences in functional capability, or whether differences in form and structure represent the product of a common underlying metabolic or functional set. There is a need to develop a coherent conceptual framework within which it is possible to explore both the inter-relationship between body structure and metabolic function and the extent to which the predisposition towards a less prudent phenotype may be programmed in early life.

To better understand how the adult phenotype may be influenced by events in early life, the following questions need to be addressed:

- Do differences in body structure in adulthood in term of size, composition and pattern of fat distribution are influenced by events occurs in early life that is marked by birth weight?

- Do different methods that are used to assess in body composition and pattern of fat distribution are consistence in their finding within the same subject groups with differing birth weight?

- If body composition and fat distribution are influenced by birth weight, could this relationship be mediated by differences in body size in terms of height and weight and dimensions both vertically and horizontally, in terms of body proportion (i.e. leg and trunk length) and frame size?

- To what extent is energy and substrate metabolism influenced by birth weight? to

what extent are differences in energy and substrate metabolism attributable to differences in body size and composition?

- To what extent is lipid metabolism both in fasted and fed state influenced by birth weight? If so, is it related to differences in body composition and pattern of fat distribution?

2.5 Hypothesis and aims of the work described in this thesis.

The central hypothesis that underlies the work reported in this thesis is that the pattern of growth in early life, marked by birth weight, results in structural and functional changes that are evident in adult life, thereby predisposing the individual to an increased risk of obesity, T2DM and CVD.

The purpose of the work described in this thesis was to further examine the relationship between current body structure and function (phenotype) and to determine the extent to which this relationship is influenced by events in early life. This was achieved by conducting an extensive series of observations within a single study of a subgroup of subjects recruited from a larger cohort study (The MRC Hertfordshire Cohort) reflecting those adults who were born in the lower and higher centile of birth weight (lower and higher birth weight group).

The study consisted of the following analyses which addressed specific hypotheses:

Hypothesis 1: Differences in body structure in term of size, composition and fat distribution are influenced by birth weight. The magnitude and direction of this effect will be determined in part by the choice of method which may mislead the interpretation of the relationship between fetal growth and current body structure.

This hypothesis was examined by a comprehensive analysis of body size, composition and fat distribution using different principle methods within the same individuals with differing birth weight. Attention was directed to the DXA scan since it provides valuable information on the differences in body composition and fat distribution in relation to birth weight when compared with traditional anthropometry and other two compartmental models such as BIA and BodPod.

Hypothesis 2: Variation in body composition and fat distribution associated with birth weight can be explained by differences in body size in term of height and weight.

Hypothesis 3: At a given BMI, infant born with lower birth weight have greater adiposity in particular at the central region of the body and lower

lean mass when compared to those born with higher birth weight. This may explain the paradox of greater risk of adiposity marked by BMI associated with higher rather than lower birth weight.

These hypotheses were tested by examining the extent to which body size in terms of height and weight, both independently and together, may have an effect on the relationship between birth weight and body composition and fat distribution as measured by DXA. In addition, there was a need to examine the differences in body composition and fat distribution associated with birth weight which might be observed at any given BMI.

Hypothesis 4: Current dimensions of the body both vertically and horizontally are influenced by birth weight and that may explain the differences in body composition associated with birth weight

This hypothesis was tested by examining the extent to which current body dimensions both vertically (segments length), and horizontally (Frame size), are influenced by birth weight, and to examine whether if such a variation in body dimensions, if they persist, may contribute to the differences in body composition associated with birth weight.

Hypothesis 5: current energy-substrate metabolism both in fasted and fed state are influenced by early fetal growth which may be only partly explained by differences in body size and composition

This hypothesis was tested by examining the extent to which current energy-substrate metabolism both in fasted and fed state is influenced by birth weight. Examining the relationship between body size and composition in one hand and energy-substrate metabolism on the other, and whether this relationship is influenced by birth weight. Finally to examine the contribution of body size, composition and birth weight on the variation on current energy metabolism.

Hypothesis 6: Current lipid metabolisms in both the fasted and fed states are influenced by differences in birth weight which may be explained by differences in body composition and fat distribution.

This hypothesis was tested by examining the extent to which lipid metabolism in the fasted state and following the ingestion of test meal that contain the labelled fatty acid (^{13}C-Palmitic acid, to examine the portioning of dietary lipid towards oxidation and storage) is influenced by birth weight, and whether this relationship is mediated through differences in body composition and fat distribution.

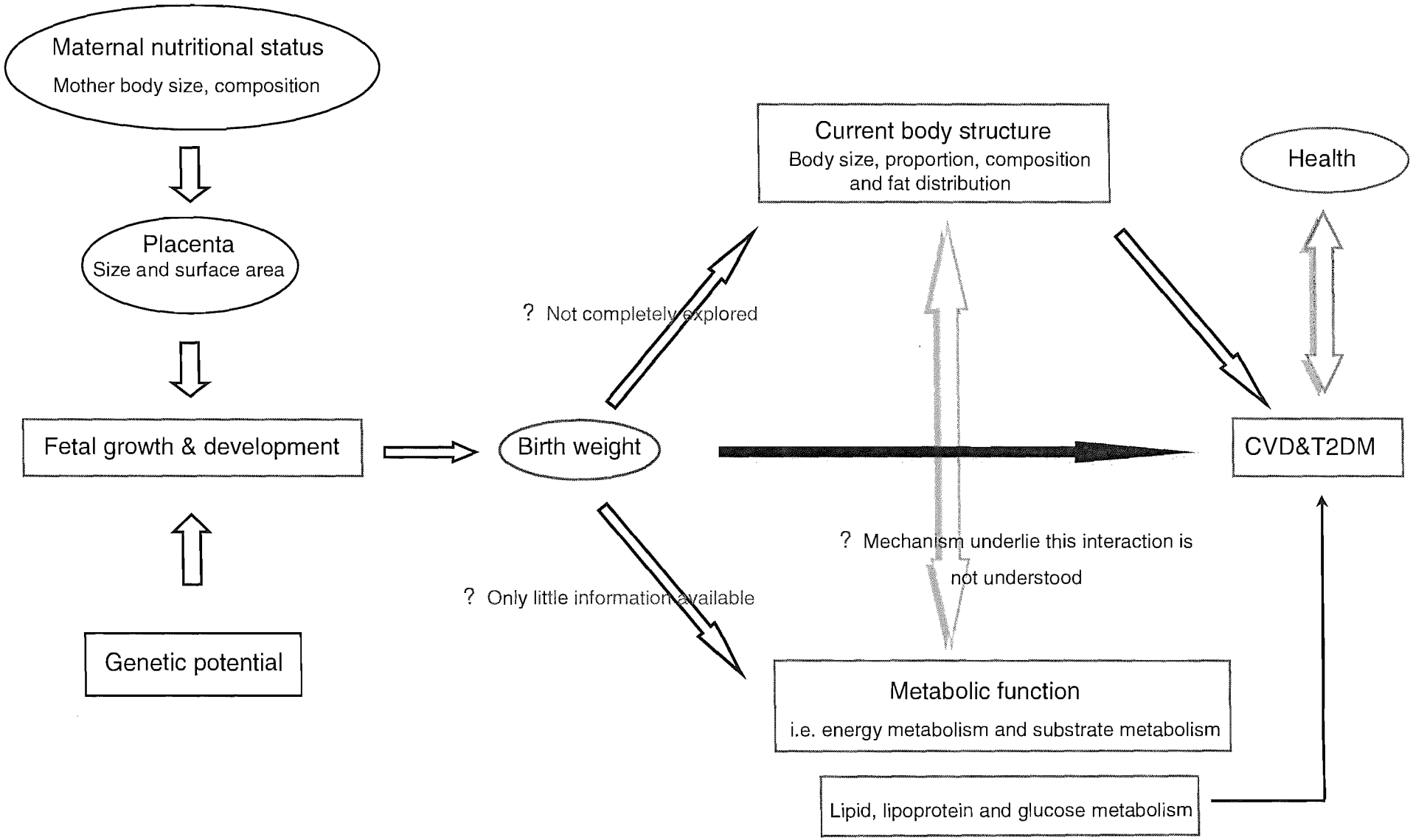


FIGURE 2.1 Summary points for what is not known form the literature based on the conceptual framework of this thesis

CHAPTER 3 METHODOLOGY

3.0 Introduction

The results presented in this thesis reflect a series of investigations conducted within a single activity whereby the same group of subjects were studied on one occasion. Each aspect of the investigation is described and reported separately examining how birth weight might relate to body structure in terms of body size, composition and fat distribution, energy-substrate metabolism and lipid metabolism. The methodology described in this chapter is divided into six sections. Section (3.1) describes the recruitment of the subjects. Section (3.2) highlights the general protocol that is developed for the whole investigation. Section (3.3) describes the assessment of current body structure by different anthropometric and body composition techniques. Section (3.4) describes the analytical methods for the studies and that includes the test meal composition, blood and breathes sample collection and analysis, in addition to the metabolic rate measurements by indirect calorimetry. Section (3.5) describes the data analysis and presentation. Section (3.6) illustrates the power analysis conducted to determine the minimum number of subjects that are required within each study group to observe a significant size effect of the primary outcome variables of the whole study. Any specific details of data processing or statistical analysis that only relates to the information within a given experimental chapter is included in the methods section of that chapter.

3.1 Subject group

The Hertfordshire Cohort Study (MRC-HCS) was initiated by the MRC Environmental Epidemiological Unit in the University of Southampton. It represents a major program of research which has linked the weight at birth of 3000 men and women born between 1920 to 1930 with different functional outcomes related to health and disease in adulthood. Work generated from this Cohort revealed that those born with a lower birth weight had increased death rates from coronary heart disease in later life (90). In addition they had greater cardiovascular risk factors which included impaired glucose tolerance, insulin resistance and raised blood pressure (91;138). From this initial cohort, a group of 1760 older men was identified to still live in East Hertfordshire and registered with a General Practitioner clinic.

Through the GP clinic, a sub-group of 202 men were asked to participate in a study which examined the link between birth weight and carbohydrate metabolism (OGTT after overnight cortisone exposure) under the direction of Prof. David Phillips from the MRC-EEU. During their visit to the GP clinic, a complete description about the study was given to each subject (appendix 2.1). After excluding those with known metabolic disease such as CVD and Type II diabetes, 122 healthy subjects (55%) agreed to participate in this study. Of those, 50 subjects were chosen randomly among those below the 25th centile of birth weight (Lower birth weight group: <3.18 kg (7 lb), n=25) or above the 75th centile (higher birth weight group: >3.86 kg (8.5 lb), n=25) for the entire study population of the HSC. Having recruited these patients to come to Southampton to participate in the study of carbohydrate metabolism, the opportunity arose to conduct a detailed series of metabolic investigation within the Institute of Human Nutrition embracing body habitus in term of size, composition and distribution of body fat, energy metabolism and using stable isotopic tracer to study lipid metabolism in a fed state in relation to birth weight. The original study design was amended to permit these investigations during 3 day inpatient admission to the Welcome Trust Research Facility (WCRF), Southampton General Hospital. Thus the subjects participated in three inter-dependent studies which include: 1) Bone health and calcium absorption, 2) OGTT study following overnight administration of cortisone, and 3) the series of metabolic investigation and body size and composition measurements reported herein. Because the investigations reported in this thesis were nested within the original carbohydrate metabolism trial, the study design of these investigations discussed here was constrained by the design of the original study. As the volunteers were required to be transported from and returned to Hertfordshire and admitted for three days admission to the WCRF, this placed significant financial and time constraints in the overall study design.

The study design required the subjects to be booked to attend the WTCRF in groups of 4, with equal numbers of subjects drawn from the lower or higher birth weight arm on each occasion. Changes in financial support forced the management team to suspend the programme of work, thereby the data reported herein relates to the first 32 of the 50 subjects recruited into the study who had completed the trials (16 subjects in each of the two groups). However, three subjects from the higher birth weight group were removed from the analysis which brings the total subjects presented in this series to 29. One was removed because of a lack of isotopic tracer, and the other two subjects were excluded because of the technical error associated with the indirect calorimetry equipment. Therefore, the results presented in this thesis represent 16 subjects in the lower and 13 subjects in the higher birth weight group. Ethical approval for the study was obtained from the Southampton and South West Hampshire Research Ethics Committee (appendix 2.2).

On admission to the WTCRF, written informed consent was obtained from each subject (appendix 2.3). Information on current socioeconomic status, smoking status, alcohol consumption and physical activity were obtained using questionnaires (appendix 2.4). This questionnaire was originally designed for the concurrent studies other than those reported here. However, it was possible to access this information in describing the study population and determine any differences between the study groups in terms of some postnatal environmental factors. Furthermore, information on socioeconomic status at birth based and the socioeconomic status of the father was obtained from each subjects birth record. An extensive database was established, which included details of physical activity, job history, diseases and medication. Certain aspects of physical activity, socio-economic factors at the time of the investigation and at birth for all subjects and between the study groups are presented in Table 3.1. There were no significant differences between groups in terms of physical activity, socioeconomic status, and smoking status. There was also no significant difference between groups in the gestational age (39 v 40 weeks; $p = 0.459$), prevalence of disease (e.g. an equivalent number of subjects in each groups have chronic bronchitis, phlegm and angina), or medication (i.e. an equivalent number of subject in each group took a non-steroidal anti-inflammatory analgesic, a proton pump inhibitor for indigestion and antacids).

3.2 General protocol

Four subjects (two lower + two higher birth weight subjects) were delivered by taxi to Southampton General Hospital every week over 8 weeks and were admitted to WCRF on the day prior to commencing the study. On admission they were given a brief explanation of the study followed by completion of questionnaires. A series of anthropometric measurements were then undertaken by a single observer (WTCRF nurse) to improve precision and reduce inter-observer variability. The anthropometric measurements included body weight, height, waist and hip circumference. Body composition was then measured by three different methods: air displacement plethysmography unit (BodPod), Dual Energy X-ray Absorptiometry (DXA) and by skinfold thickness (SKF). The measurement of body composition obtained by DXA was carried out by the professional technical staff that operate this machine under the Medical Physics Department of Southampton General Hospital. Both BodPod and SKF measurements were obtained by the WTRCF nurses for all subjects during the study period. Following these measurements, all subjects then ate a standard evening meal (see appendix 2.5). They were permitted to consume non-caffeinated diet drinks and water freely and asked to consume no other foods over the prescribed diet feeding period. In

addition, they were not permitted to undertake any strenuous exercise or consume alcohol. During their stay in WRCF unit, they were directly supervised and observed by the nursing team of the WTCRF. A 24 hour urine collection was started from early morning to measure the total urinary nitrogen execration.

On the morning of the study day (at 08:00 hours) baseline oxygen consumption (VO_2) and carbon dioxide production (VCO_2) (later used to determine the basal metabolic rate and resting energy expenditure over the study period) was measured by indirect calorimetry followed by collection of an end tidal, expired air breath specimen (by OAK). This measurement was obtained by me for all subjects for the whole study period. An indwelling cannula was then inserted (by Dr Patel) into subjects forearm vein and baseline blood sample was also collected.

The subjects then consumed a test meal with a lipid-casein-glucose-sucrose emulsion containing ^{13}C palmitic acid (700mg ^{13}C-palmitic acid, 99 atom percent excess; Cambridge Isotope Laboratories, Mass USA) based on the method of Murphy et al (213). The test meal and the lipid emulsion were of fixed quantity containing 3720 kJ energy (38% of the estimated energy requirement of this particular age group(214)), in which 40%, 45% and 15% represents the relative energy from carbohydrate, lipid and protein respectively. Both the emulsion and test meal composition were typical of present UK diet for adults (215), see appendix (2.5) for the component and macronutrient composition of the test meal.

The emulsion containing ^{13}C-palmitic acid was prepared over a 20 minute period to achieve optimum emulsification and temperature on consumption by the subject (by OAK). The emulsion was consumed at a temperature of 55-65 ^{0}C to keep the label substrate above the melting point to prevent recrystallisation of the tracer fatty acid, with the pre-prepared meal component and beverage. Previous work using balance studies across the gut had demonstrated that in following this protocol, losses of tracer in stool were uniformly minimal thereby reflecting high availability (in excess of 98%) (216).

After ingesting the test meal, indirect calorimetry measurements were repeated at hourly intervals for six hours. On each hour, blood and breath samples were collected over the study period. No additional foods or liquids, except for water were allowed for the subjects during the study period. All subjects were asked to remain seated or in supine position, and they were under constant supervision for the duration of the study period. At the end of the study (after 6 hours) the cannula was removed and an evening meal was given to all

subjects.

3.3 Assessment of current body structure

3.3.1 Height and weight

Body weight was measured by Seca708 electronic weighing scale (Seca Ltd, Medical Scales and Measurement system, Birmingham, UK). Weight was measured after urination and after taking off shoes and heavy clothes and was measured to the nearest 0.1 kilogram. Height was measured by Seca220 electronic stadiometer (Seca Ltd, Medical Scales and Measurement system, Birmingham, UK). The subjects stood as straight as possible with their feet together, arms held loosely by their side and their head was placed in Frankfurt Plane touching the backboard of the stadiometer. The measurements were taken to the nearest 0.1cm. From the body weight and height, body mass index (BMI) was calculated from weight over height squared (kg/m2). The intra-observer variability (% of coefficient of variation) for the measurements of height and weight (three consecutive measurements from a single subject carried out by the same observer in the same day) were 0.56 and 0.72%, respectively.

3.3.2 Waist and hip circumference

Waist and hip circumference were measured by plastic tape to determine the differences in the pattern of fat distribution. The measurements were taken before eating and after empting the bladder. Subjects were asked to be in standing position and wear underwear so that the thickness of clothing did not influence the results. Waist circumferences was obtained by putting the plastic tape in mid-point between the inferior margin of the last chest rib and the crest of the ilium in horizontal plane while in hip circumferences the tape were put in the widest part of the trochanters. Waist and hip circumference measurements were taken by the same investigator for the whole study to reduce the inter-observer differences due to the positioning of the tape. The intra-observer variability for the waist and hip measurements (three consecutive measurements from a single subjects carried out by the same observer in the same day) were 1.1 and 0.9% respectively. The waist and hip measurements for all subjects in this study were made in duplicate and the average was taken for each measurement to improve the accuracy and reproducibility of waist and hip circumference measurements. The obtained results were taken to the nearest 0.1 cm.

3.3.3 Skinfold thickness (SFT)

Skinfold thickness measurements were taken by a Holtain caliper (range 0-40mm x 0.2mm; CMS weighing equipments Ltd. London UK) according to the method of Durnin and Womersley from four different anatomical site and that include Subscapular (Ss) and Suprailliac (Si) Triceps (Tri) and Biceps (Bi) (217). The skinfold measurement during the whole period of the study was taken by the same observer from identical position on each subject when they were in a standing position. Three measurements were taken from each anatomical site for each subjects and the mean value were adopted as value of reference. The caliper was calibrated routinely by machined metal blocks to check the reading. Body percentage fat was estimated using Siri's equation (159) as follows:

$$\%\text{Body Fat} = [(4.95/ \text{Density}) - 4.5] \times 100$$

Where body density in g/cc^3 is determined from the appropriate age and sex regression equation based on the average logged sum of the four skinfold sites (217):

$$\text{Density} = 1.1423 - (0.0700 \times \log \sum 4 \text{ skinfold})$$

The precision of percentage body fat obtained by this procedure has been reported to be ± 3.5% (218). However, the coefficient of variation of three consecutive measurements obtained from single subjects in this study was 4.8%.

Skinfold measurement obtained from each site was taken based on as follows:

Triceps skinfold: the measurement was taken at the mid upper arm which is half way between the tip of the acromion and the olecranon that is measured with a tape when the elbow was bent at 90°. The subjects were then asked to relax and put their hand freely by the their side and plastic tape was placed around the arm at the level of the mark and two horizontal lines were drawn on the skin posteriorly and anteriorly in which the posterior line was used for triceps and the anterior for the biceps skinfold measurement. The side to side mid point for the posterior mid upper arm was determined by eye. The skin was then picked up over the posterior surface of the triceps muscle and the blade of the caliper blade was

then applied to the site.

Biceps skinfold: the anterior mid upper arm line was defined as it described above and the side to side mid point was determined by eye. The subject was facing the investigator with the arm held relaxed and palm facing forward. The skinfold was then picked up over the belly of the biceps and the caliper blade was then applied to the site.

Subscapular skinfold: the subjects were asked to stand with their arm relaxed by their side. The Subscapular site was defined just below and lateral to the bottom tip of the scapula. The scapula was then palpated with the fingertips to find the bottom of the bone and the skinfold was then picked up at the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin and the calliper blade was then applied to the site.

Suprailliac skinfold: the subjects were asked to stand sideway with their arm folded. The anterior superior iliac spine is located at top of the hip bone in the mid-axillary line. The defined point was then marked 1 cm vertically above, and 2 cm medially to the anterior superior iliac spine. The fold was then picked up 1 cm above the mark and then the calliper blade was applied to the site.

3.3.4 BodPod

Body composition was measured using by air displacement plesythmography using the BodPod S/T System (Life Measurement, Inc.; Cranlea, Birmingham, UK). The BodPod utilises the principle of whole body densitometry to estimate the amount of fat and lean tissue in the body. Whole body densitometry is based on the determination of body density by measuring body mass and volume. The body density is calculated as follows:

Density = body mass / volume

The body mass was measured using the electronic scale attached to the BodPod system and body volume measured by the BodPod system. The percentage body fat was calculated using Siri's equation as described in the previous section (skinfold). The BodPod was routinely calibrated with known volume standard (48L) and the weighing scales calibrated against known weight (20kg). Before the measurement, subjects were asked to wear minimal clothing (i.e. swimsuit). Before entering the BodPod an accurate measurement of body

weight was taken for each subject using the electronic scale attached with the BodPod system. The subject then enters the BodPod chamber and asked to sit in a standardised way, with back straight and not touching the back wall of the machine, feet slightly apart and hands placed in a relaxed manner on their lap. Subject's age, height and sex were entered in the computer and four estimates of body volume were obtained after using predicted lung volume. In a previous study, others have shown that the predicted lung volume, on average, was not different from that measured in a group of 50 subjects (36 women, 14 men) (219).

3.3.5 Dual energy X-ray absorptiometry (DXA)

Body composition was also measured by Dual energy X-ray absorptiometry using the Hologic Delphi System (Vertec, Scientific Ltd, Reading, UK) which processed data using software v12.2 to obtain information on the mass of fat (FM), free soft tissue (FFST), bone mineral content (BMC) and percentage of these components in the whole body and in specific body segments.

The DXA machine emits alternating high and low energy X-rays that produce precise, high quality images. The basic principle of DXA data acquisition is based on the differences between bone and soft tissue attenuation at the high and low X-ray levels. As the X-ray beam passes through the subjects, detectors register the varying levels of X-rays that are absorbed by the anatomical structures of the subjects. The raw scan data, which includes values of tissue and bone, are captured and sent to a computer. An algorithm interprets each pixel, and creates an image and quantitative measurement of the bone and body tissues.

Before taking the DXA scan, the subjects were asked to wear loose comfortable cloth with no metal attached to it such as zipper, snaps, or buttons and also to remove all jewellery. During the scan subject was asked to breathe normally when they lie on padded table in which the x-ray generator was set below and a detector (an imaging device) was placed above the table. The DXA Hologic system was calibrated against a spinal phantom and tissue bar. The system and user precision was performed using the ICSD (International Society for Clinical Densitometry) guidelines to estimate individual operator and inter-operator precision (220). Between individuals variability (%CV) for measuring body fat as a percentage of body weight, absolute fat mass and lean mass has been reported previously to be less than 3% when measured by DXA (174;221). The coefficient of variation of regional body composition measurements obtained from single subject also reported to be small (< 5%) but less precise when compared to the whole body measurements (221).

3.3.6 Bioelectrical impedance

Body composition was also measured for all subjects by the WRC nurse by BIA using Bodystat 1500 (Bodystat Ltd;Douglas, UK). The measurements were obtained in the morning after an overnight fasting. The BIA test itself involves the passing of a tiny, undetectable electrical charge between an electrode on the hand and an electrode on the foot. This method allows us to measure the resistance (impedance) of the electric current which pass through the body fluids contained mainly in the lean and fat and from this information one could determine the percent body fat and the percent lean tissue. In practice, a small constant current, typically 400 uA at a fixed frequency, usually 50 kHz, is passed between electrodes spanning the body and the voltage drop between electrodes provides a measure of impedance. This measure, which is related to subject's fat and lean proportion, is registered on the machine LCD display screen. This number, together with the other details of age, height, body weight and gender are used by the regression equations to analyse the data and then this information is translated to a comprehensive body composition statistical analysis. More detail information about this method is illustrated in section (2.3.1).

3.4 Analytical methods

3.4.1 Blood collection and analysis

Blood sample (15 ml) was collected from each subject before the consumption of the test meal and hourly for 6 hours following the ingestion of the test meal. An indwelling cannula was inserted into the forearm vein, permitting repeated sampling of blood with no added discomfort to the subjects. The cannula was inserted following the initial indirect calorimetry measurement, prior to the consumption of the test meal. The blood sample was then collected using a syringe and aliquotted into vacutainer tubes for centrifugation. Plasma was recovered instantly by centrifugation at 2500 g for 15 min at 4^0C and the remaining red cell discarded. The plasma from each time point was separated into 6 x 1ml. Two aliquots which were then used for a) the extraction of the ^{13}C-Palmitic acid in TAG and NEFA fraction and b) to measure glucose, TAG and NEFA both in fasting and fed state by routine enzymatic assay method (see appendix for complete description). Total cholesterol, LDL and HDL was only measured in baseline sample (fasted state) by the same method. Before starting the analysis all plasma samples were kept frozen at -20 ^{0}C.

3.4.2 Plasma Lipid extraction

Total plasma TAG and NEFA was measured to determine the pattern of lipid appearance and clearance from the circulation. However, this method alone did not differentiate between the endogenous lipid (from the adipose tissue or extracted from the liver as VLDL-TAG) and exogenous lipid (from the meal). Using labelled palmitic acid (^{13}C-PA) in conjunction with the meal allows us to trace the pattern of appearance exogenous lipid in plasma. To measure the concentration of ^{13}C-PA, plasma fatty acid in TAG and NEFA pools was extracted and the separated fatty acid was then derivatised and analysed by Gas Chromatograph Isotopic Ratio Mass Spectrometry (GC-IRMS).

3.4.3 Fatty acid extraction and derivatisation

Plasma lipids were extracted according to Folch *et al* (1957). Plasma sample (1ml) was pipetted into a clean glass tube with added surrogate standards for TAG (triheptadecanoin; 100 µg/100µl chloroform: methanol 2:1 v/v) and NEFA (heneicosanoic acid; 30µg / 30 ml chloroform: methanol 2:1 v/v). Chloroform: methanol (2:1 v/v) (5ml) was then added to the plasma and mixed for 15 minutes. After mixing, sodium chloride (1M) solution was added to the tube (1ml) and then spun at 2000rpm for 10 minutes at 4^{0}C. That generated three layers in which the lower (solvent phase) contains the lipid fraction, middle protein rich interphase plug and upper layer (aqueous phase) which contained water and non lipids materials. The aqueous phase was removed and discarded, while the solvent layer was removed to a clean test tube by passing a pasteur pipette through the interphase plug. To ensure high recovery of lipid from the extraction process, the protein interphase plug was homogenized in chloroform: methanol (2:1, v/v) and sodium chloride (1M) and the solvent layer were then removed again to the test tube. The solvent extract was sealed under nitrogen and prepared for the next stage (solid phase extraction).

NEFA and TAG in the extracted lipid phase were then separated by aminopropyl silica column (Varian; 100 mg packed silica per 1 ml cartridge) which has been shown to produce higher lipid recovery compared to the alternative TLC methods. The extracted lipid was dissolved in chloroform (1 ml) and applied to an aminopropyl silica column under gravity. The column was washed with chloroform (2 x 1ml) and the obtained solvent fraction (contains the TAG and cholesterol ester fractions CE) was combined and dried under nitrogen at 40^{0}C and kept for the second separation process in later step. The column was then washed with methanol (1ml) and the NEFA fraction was eluted with chloroform-methanol-acetic acid

(100:2:2 by vol. 2 x 1 ml) under vacuum. In the last step of the separation process, a new fresh aminopropyl silica column (Varian) was preconditioned with hexane (4 x 1 ml). The obtained solvent fraction (which contain TAG and CE) from the previous step was then dissolved with hexane (1 ml) and applied to the column under gravity and washed with hexane under vacuum to elute CE. TAG was then eluted with hexane-chlorform-ethylacetate (100:5:5 by vol. 2 x 1ml) under vacuum.

The eluted fatty acid in NEFA and TAG fraction obtained was methylated to form fatty acid methyl esters (FAME). Toluene (1 ml) and 2% sulphuric acid in methanol (2 ml; v/v) were added to the eluted fatty acid and incubated at 50^0C for 18 h to allow complete methylation of the fatty acid. After that, the reaction mixture was cooled down and the neutralising agent (25 g $KHCO_3$ + 34.55 g K_2CO_3 in 500 ml distilled water; 2 ml) was added to stop the reaction. Hexane (2ml) was added to each sample and the samples were then centrifuged (2500 rpm; 140C; 10 minutes). Two layers was formed after the centrifugation process was ended, the aqueous (upper layer) and solvent (lower layer). The solvent was removed to a round bottomed glass tube and blow down gently under nitrogen. Hexane (2 ml) was added to the remaining aqueous phase, and the process was repeated twice. The solvent layer was then dried under nitrogen until completely dry. Round bottomed tube were washed with dry hexane (200 µl), vortexed and the solvent transfer to GC-mini vials. The process was repeated three times and the solvent dried under nitrogen in the mini vials. When dry, an internal standard was added to each vial for the determination of the recovery of the surrogate standards.

The precision of lipid extraction, separation and derivatisation was established before starting the whole procedure by testing the between samples variability in the enrichment and concentration of ^{13}C-PA by GC-IRMS. The %CV associated with plasma lipid extraction and methylation was <1.29 and <5.36% for the enrichment and concentration of ^{13}C-PA in TAG and NEFA fractions respectively (between sample variability) see appendix (2.6).

3.4.4 Analysis of the ^{13}C- PA enrichment of plasma samples

The ^{13}C-enrichment of the plasma sample was measured using Isotope Ratio Mass Spectrometry (IRMS) (Europa Scientific Ltd, Crewe, UK) interfaced with a gas chromatography system (GC) (5890, Hewlett Packard, Palo, Alto, CA).

Dried fatty acid methyl ester was reconstituted with 20 µl hexane. 1 µl was injected to GC-

IRMS through the capillary gas chromatography column (BPX-70 column, 30 x 0.33 mm 0.d., 0.25 film thickness, 5% phenyl methyl silicone) for the separation of fatty acid. The injector temperature was set at 290^{0}C and the detector temperature at 250 ^{0}C. The injected compound travelled through the GC column by stream of helium gas and combusted to CO_2 and water by platinised copper found in the combustion furnace. The CO_2 and water were then passed to through a water-Nafion membrane where the water was removed and CO_2 is introduced to the reduction chamber, where nitrogen oxide (NO) is converted to nitrogen gas (N_2). Small fraction of CO_2 and N_2 gases were introduced to an IRMS ion source. In the ion source, the CO_2 is bombarded with electrons to generate positively charged CO_2 ions. These ions then pass through a magnetic field and then deflected according to their charge to mass ratio. The detector of the mass spectrometer can detect three different molecule masses i.e. 44, 45, 46 which represented $^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O$ and $^{12}C^{18}O^{16}O$ respectively. The detected ions and enrichment of the sample is processed by software provided by the manufacturer. The enrichment of fatty methyl ester obtained from the plasma samples was calculated with reference with the known enrichment of external standard tricosanoic acid methyl ester [δ ^{13}C – 32.45 $^0/_{00}$ versus Pee Dee Beleminte (PDB)]. The system generates the results via a computer which indicate the carbon mass and the ^{13}C-enrichment of the sample. The ^{13}C-enrichment of the samples was expressed as the isotopic ratio unit delta ($^0/_{00}$). The recovery of ^{13}C-PA in plasma over the study period (expressed as mmol/l) was obtained initially by determining the proportion of total palmitic acid in plasma enriched with ^{13}C. The recovery of ^{13}C-PA in plasma from each time point was then calculated from the proportion of ^{13}C-PA and the total concentration of palmitic acid obtained by routinely established enzymatic assay see appendix (2.7). Finally the concentration of ^{13}C-PA in plasma over the study period was calculated as the area under the time versus the concentration of ^{13}C-PA curve over 6 hours, expressed as mmol/l per 6 hours.

The ^{13}C-PA enrichment and concentration measured by the GC-C-IRMS was validated by testing the within sample variability in the enrichment and concentration of ^{13}C-PA in plasma. The %CV associated with the use of GC-IRMS was 0.73 and 3.46% for the enrichment and concentration of ^{13}C-PA in TAG fraction (within sample variability) (see appendix 2.6).

3.4.5 Breath sample collection and analysis

Breath samples were collected prior to the consumption of the test meal and hourly intervals for 6 hours thereafter. The purpose of this was to measure the excretion of ^{13}C-label, as $^{13}CO_2$ on breath to provide an estimate of the oxidation of exogenous lipid marked by ^{13}C-PA.

The subjects exhaled fully into 750 ml alveolar bag to collect the end tidal breath sample. Three consecutive 10 ml breath samples were transferred from the bag to evacuated gas sample containers for storage and later analysis by using the 10ml syringe. Two samples were used as duplicate for analysis and one was kept for storage. These samples were analysed by continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS; 20/20 IRMS-GSL interface, Europa, Scientific Ltd, Crewe, UK). The samples were loaded into the autosampler rack started with three samples of reference gas (10 ml of 5% CO_2, 95% N_2 gas mix BOC Gases, Manchester, UK). The samples were set in repeated series of 6 samples inter-spaced with three reference samples and finishing with two reference samples. The samples were injected into the combustion chamber and a pulse of pure oxygen was generated and combusted the sample into a mixture of gases containing CO_2, N_2, NO and water. The generated gas was then moved through the system by helium gas through the reduction tube containing copper oxide wires for reducing NO to nitrogen. The water was then removed in the presence of water scrubber which contains magnesium prechlorate. The remaining gases were then introduced to the GC column which is maintained at 125^0C to allow the separation of the gases. Only small amounts of the GC effluent entered into the ionic source of the IRMS while the remainder was passed to the atmosphere. In the ionic source, the sample was bombarded with electron and that generated positively charged CO_2 ions. These ions were then passed to a magnetic filed and were deflected according to their charges to mass ratio. The IRMS detector detects three different molecule masses i.e. 44, 45, 46 which represent $^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O$ and $^{12}C^{18}O^{16}O$ respectively. The detected ions and enrichment of the sample was processed by software provided by the manufacturer.

The proportion of the ^{13}C label on breath $^{13}CO_2$ expressed as percentage of administered ^{13}C-PA was determined using the equation of Watkins *et al* (222) see appendix (2.8). The total of $^{13}CO_2$ over the study period (6 hours) was calculated from the area under the curve of a graph plotted for time versus $^{13}CO_2$ as a percentage of the administrated dose per hour.

3.4.6 Indirect Calorimetry

The rate of energy metabolism and proportion of energy from substrate oxidation was estimated from the oxygen consumption (VO_2) and carbon dioxide production (VCO_2) on breath measured by indirect calorimetry (GEM calorimetry, Europa Scientific Crewe, UK) both in the fasting and fed states. The main assumption in this method is that all oxygen consumed is used to oxidize substrate, and all CO_2 produced is recovered on breath.

Before starting the measurement, subjects were asked to lie in supine position, then the ventilated hood was then placed over the subject's head to provide a constant flow of room air (approx 40 ml/min) which delivers the expired breath to the calorimeter for gas analysis. Baseline VO_2 and VCO_2 measurements were taken 20 min prior to the ingestion of test meal. Following the ingestion of the test meal at hourly intervals measurements were taken over the study period (6 hours). The system was routinely calibrated before the beginning of each study by using reference gas (5% CO_2, 95% O_2). In addition, the system was validated every month to check the flow rate and respiratory exchange ratio by burning a constant amount of pure ethanol.

REE in the fasting and fed state were determined using the equation of Elia *et al* (223) as follows:

$$REE_{fasting}\,(kJ/min) = 15.913\ VO_2\ (l/min) + 5.207\ VCO_2\ (l/min) - (4.646 * U\ nitrogen)_{fasting}$$

$$REE_{fed}\,(kJ/min) = 15.913\ VO_2\ (l/min) + 5.207\ VCO_2\ (l/min) - (4.646 * U\ nitrogen)_{fed}$$

Where U nitrogen (g) is the urinary nitrogen excreted in the fasting and fed state. Although the 24 hours urinary nitrogen (g) was determined from urinary urea and ammonia for all subjects, the exact proportion of the excreted nitrogen in the fasting and fed state was not known. Therefore, the model used in the current analysis to present the oxidative metabolism assumed that total 24 h urine N was distributed between the two 6 hour postprandial periods and the remaining 12 hour period in the ratio of 1:1.4 (protein oxidation in fed state assumed to be 40%) (224). The net oxidative metabolism for was derived using the equation described by Elia and Livesey (223)

The proportion of non-protein REE from carbohydrate and lipid oxidation both in fasting and fed state was estimated from the following equations:

$$\text{Carb Oxi}_{\%REE} = 2112\ (RQ_{mix} - 0.71) / 21.12\ (RQ_{mix} - 0.71) + 19.61\ (1 - RQ_{mix})$$

RQ_{mix} is the non protein respiratory quotient which was determined from the non- protein VO_2 and VCO_2 as follows:

$$_{np}VO_2 = VO_2 - Un \times 116 / 19.48$$

$$_{np}VCO_2 = VCO_2 - Un \times 116 / 23.33$$

The percentage of REE from lipid oxidation was:

$$\text{Lipid Oxi}_{\%REE} = 100 - \%E_{carb}$$

Total exogenous lipid oxidation (TELOX) as a percentage of REE after meal ingestion was determined from the total recovery of the $^{13}CO_2$ in breath over 6 hours (AUC) (as % of the administrated dose / hour) and $\%REE_{Lipid\ Oxi}$ as follow:

$$TELOX = \text{recovery of } ^{13}CO_2 \text{ over 6h (AUC)} \times \text{Lipid Oxi}_{\%REE}$$

3.5 Data analysis and presentation

The date obtained from this study was prepared and analysed using the SPSS statistical package v14.0 (SPSS for windows, SPSS Inc., Chicago, IL, USA). Results were expressed as the mean for the group plus or minus standard error (SEM), except were otherwise stated. Simple t-test was used to compare the mean differences in body composition, fat distribution and metabolic outcome between the lower and higher birth weight groups. Analysis of covariance (ANCOVA) was also used to explore the effect of birth weight (lower and higher birth weight groups as fixed factors) on body composition and fat distribution, and metabolic outcome, whereas height, weight and BMI were used as covariates. Chi squared tests were used to assess the differences in categorical data related to postnatal environmental factors which include physical activity, socioeconomic class between the study groups. Statistical significant was assumed at a level of 5%, above which value were considered to be non-significant (NS). The graphical data illustrated within the results chapters was carried out using Prism 4 for windows (Graph Pad software, Inc. San Diego USA), the results on these graphs were presented as mean differences between groups ± SEM.

3.6 Power analysis

Power analysis can be used to determine the number of subjects that is required to detect the smallest differences in the mean of variables between two populations which may be of biological importance. In addition, from the literature review, the use of power analysis may allow us to estimate the sample size on the basis of likely size of effect or the potential

differences one might expect to see in outcome variables between two populations. For instance, previous studies show that differences in FFM between the lower and higher birth weight groups to be typically between 5-8 kg or 9-16% of the population mean and the within-individual variability (or standard deviation of the mean differences between the two groups) within each group was typically 10-15% (180;200). Using this information, it is possible to predict the minimum effect size (i.e. minimum differences in FFM between groups) at the desirable power of the study (i.e. >80% which mean that there is a 20% chance of missing a difference that is actually there or reflecting the possibility of having type II error) at a given sample size within each groups.

The purpose of this section of the methodology is to determine the anticipated effect size of the major outcome variables (FFM, height, weight and REE) between the lower and higher birth weight groups at the power of 80% when the sample size in each group varies from 10, 15 and 20 in each group. The information obtained from this analysis was used to the design the whole study in terms of the number of subjects that were required within each group to obtain minimum effect size based on previous finding.

The likely variability in the mean and standard deviation of major outcome measures in this study between the lower and higher birth weight groups based on previous literature is presented in Table 3.2. The mean difference in FFM between lower and higher birth weight group was found to range from 9-16% of the population mean. It was also found to be 3, 10 and 12% for height, weight and REE respectively. This finding in addition to the variance observed within each group (SD) was used in the current analysis to predict the effect size at a given number of subjects. The results presented on Table 3.3 show that the increase in the number of subjects within each group from 10 to 20 is associated with a decrease in the anticipated effect size of all major outcome variables at the power of study = 80%. Given the financial constraints of conducting a study with large number of subjects, it has been found that having 15 subjects within each group is associated with an anticipated effect size equal or close to that observed from previous literature. Therefore, in the original design of the study, the 25 subjects planned to be recruited into each of the two birth weight groups would have been in excess of the smallest number of subjects in each arm (15) that would have sufficient power to demonstrate a difference in the key outcome variables of biological significance. Although the trial was stopped after only 32 subjects, and only 29 were included in the analysis, these numbers are comparable to the smallest study group size of 15 identified by the power analysis.

TABLE 3.1 Socio-economic status and physical activity for all subjects and between the two groups

	All subjects (n=29) (%)	Lower BW (n=16) (%)	Higher BW (n=13) (%)	P[1]
Smoker status				
Never	44.83	50.00	38.50	0.481
Ex	51.72	43.80	61.50	
Current	3.45	6.30	0.00	
Alcohol band				
Low (<10 units per week)	55.17	56.25	53.85	0.921
Moderate (11-21 units per week)	17.24	18.75	15.39	
High (>21 units per week)	27.58	25.00	30.77	
Current social class				
I-IIINM [2]	44.44	40.00	50.00	0.464
IIIM-V [3]	55.56	60.00	50.00	
Father social class				
I-IIINM	21.43	20.00	23.08	0.105
IIIM-V	78.57	80.00	76.92	
Physical activity				
1) Degree of walking problem				
No limiting abnormality	93.10	93.75	92.31	1.000
Abnormal gait/walking problem	6.90	6.25	7.69	
2) Walking outdoor				
≤15 min	41.38	56.25	23.08	0.200
1-4 hours	55.17	43.75	69.23	
>4 hours	3.45	0.00	7.69	
3) Walking speed				
Slow	24.14	31.25	15.39	0.699
Normal	48.28	37.50	61.54	
Fairly brisk/fast	27.58	31.25	23.07	
4) House work per week				
<4 hours	86.21	81.25	92.31	0.213
5-8 hours	10.34	12.50	7.69	
>8 hours	3.45	6.25	0.00	
5) Climb stairs				
Occasionally	17.24	18.75	15.38	0.624
Daily	3.45	6.25	0.00	
Several times per day	79.31	75.00	84.62	
6) Carry load				
Occasionally	17.24	18.75	15.39	0.345
Once/several time per week	31.03	31.25	30.76	
Daily	24.14	12.50	38.46	
Several times per day	27.59	37.50	15.39	

† [1] Chi-square test.
[2] Professional, managerial and skilled-nonmanual.
[3] Skilled-manual, partly skilled and unskilled

TABLE 3.2 Differences in outcome variables between lower and higher birth weight group based on previous studies

Variables	Population mean ± SD	Mean differences between groups	SD within each group (as % of mean)
FFM (kg)	50.2 ± 5.2 [a]	8	≈ 10%
	57.7 ± 6.5 [b]	5	≈ 11%
Height (cm)	175 ± 6.3 [b]	5	≈ 4%
Weight (kg)	70.4 ± 9.7 [b]	7	≈ 14%
REE (kJ/min)	5.0 ± 1041 [c]	0.58	≈ 15%

[a] Gale *et al* 2001

[b] Loos *et al* 2001

[c] Weyer *et al* 2000

TABLE 3.3 Anticipated effect sizes of different outcome variables between lower and higher birth weight groups with varying in subjects numbers in each group

Variables	Anticipated effect size at power = 80%. α p<0.05, 2 tailed		
	10 subjects in each group	15 subjects in each group	20 subjects in each group
FFM (kg)	7.2	5.8	5
Height (cm)	8	6.5	5.6
Weight (kg)	13	10.5	9
REE (kJ/min)	0.87	0.66	0.57

CHAPTER 4

Assessment of body habitus in older men with different birth weight by using different methods - First level of analysis

4.0 Introduction

Poor fetal growth in utero, marked by lower birth weight, is associated with increased morbidity and mortality from CVD, type II diabetes and increase in the prevalence of metabolic syndrome (88;89). These chronic diseases are also known to be associated with differences in adult body composition, in particular adiposity, which is marked by an increase in BMI as well as differences in the pattern of fat distribution marked by increased waist circumference (225-227). Because adult body size, composition, and fat distribution (current body habitus) mediate the relationship between birth weight and the occurrence of chronic disease in later life, the impact of poor fetal growth and development on body composition and fat distribution has been studied in both children and adults (180;200;228;229). Two different lines of investigation have evolved. The first, simply attempts to determine the extent to which differences in current body habitus, in both children and adults, might be directly and causally related to fetal growth and development. The second has attempted to control for differences in current body habitus using BMI as a potential confounder in the statistical analysis of the effect of birth weight on markers of disease risk and outcome (i.e. by ANCOVA).

A variety of different approaches have been used to examine the effect of birth weight on body habitus – both in terms of size (stature and mass) as well as in terms of body composition (fat: lean, bone, muscle etc) and fat distribution. These range from simple crude measures of body composition in large scale epidemiological surveys such as using BMI as an index of adiposity (185;187;230) or urinary creatinine excretion as an index of skeletal muscle mass (192) through to more complex and detailed studies using anthropometry (199;228), bioelectrical impedance (180) and few studies using multi compartmental model i.e. DXA (200). Each of these methods is based on differing principles and assumptions, and each with their own errors and limitations.

A summary of the types of studies conducted in this area and their principle findings are shown in Table 2.1 and discussed in detail in chapter 2 Section 2.3. In general, the studies to date can be summarised as follows. Firstly, adults who were born of a lower birth weight are consistently shorter and lighter than those who were heavier at birth – an effect evident in both men and women (198). Such differences in height and weight are also evident in children (196). These differences in body size would be expected to have an impact on the absolute amounts of lean or fat mass or both lean and fat mass (i.e. an increase in height and weight might be associated with proportional increase in lean and fat mass). Secondly, birth weight is consistently reported to be positively associated with absolute lean body mass in children and adults characterised by anthropometry, BIA and in limited number of studies using DXA. Thirdly, there are limited reports that infant born with lower birth weight is associated with lower rates of urinary creatinine excretion and smaller mid-thigh circumference, all of which have been interpreted as indicative of a lower skeletal muscle mass. Finally, attempts to determine fat mass, both in absolute or relative terms and its' distribution have yielded less consistent results. Some studies fail to demonstrate any relationship between birth weight and fat mass whilst others report a positive or negative relationship. As the results, the influence of birth weight on adult fat mass both in absolute and relative to body weight need to be examined further perhaps by using reference methods such as MRI or DXA.

This lack of agreement between studies might be explained, in part, by the limitation and errors of different methods that have been used to measure body composition and fat distribution. The estimation of body composition and fat distribution by different methods are based on different assumption. Therefore these assumptions, together with limited power in some studies, contribute to the lack of consistency observed between studies on the relationship between birth weight and adult body composition or fat distribution. To date, no studies have been conducted to systematically characterise body composition or fat distribution using reference techniques or multiple measures obtained by different methods within the same individuals of differing birth weight.

4.1 Aim

The primary aim of this component of the study was to use different principle methods to characterise current body habitus (body size, composition, and pattern of fat distribution) determined by: a) measuring height and weight b) the amount of lean mass, fat mass and muscle mass in both absolute and relative terms and c) the distribution of body fat in both

central and peripheral region within the same individuals with differing birth weights. This was achieved firstly, by using Dual Energy X-ray Absorptiometry (DXA) and secondly by a series of simple anthropometric and body composition measurements obtained by skinfold thickness, air displacement plethysmography (BodPod) and bioelectrical impedance (BIA). In addition, the pattern of fat distribution was assessed using DXA and by simple anthropometry which included waist and hip circumference as well as skinfold thickness at different anatomical site (triceps, biceps, suprailliac, and subscapular).

The secondary aim of the study reported in this chapter was to evaluate the extent to which the measurements of the relative and absolute amount of lean and fat mass obtained by SKF, BIA and BP for all subjects groups in this study agreed with that obtained by DXA. That was achieved by assessing the strength (or correlation) and agreement of body composition parameter obtained by SKF, BIA and BodPod, relative to that obtained by reference method of DXA.

4.2 Subjects and methods

Twenty-nine healthy adult Caucasian men aged 64-72 y old were studied (detailed information on the recruitment procedure is presented in the general methodology chapter (section 3.1 and 3.2). Body weight, height, waist and hip circumference together with skinfold thickness at the four sites were measured by a single observer. Body fat mass was estimated from skinfold thickness using Siri's equation (detailed information for all anthropometric measurements given in section (3.3). Body composition was also obtained by DXA, BIA and BodPod.

The results from the DXA analysis were taken as the reference method. The different compartments of the FFM computed are illustrated in Figure 4.1 and were ascribed as follows:

1) Bone mineral content (BMC)

2) Fat Free Soft Tissue (FFST) = FFM- BMC

3) Limb FFST = both left and right arms + legs FFM-BMC

4) from limb FFST, muscle mass was estimated based on the equation of Kim *et al* which was

established by relating DXA measurements of limb FFST with whole body measurements of muscle mass obtained using magnetic resonance imaging in healthy adults (231). The equation used for men in this study is as follows:

$$\text{Total body skeletal mass (kg)} = 1.13 * \text{Limb FFST (kg)} - 0.02 * \text{Age (y)} + 1.58$$

5) Non-muscle FFST = FFST - muscle mass.

The pattern of fat distribution or central and peripheral fat mass was also derived from the regional measurements of fat mass obtained by DXA. The central FM which includes intra-abdominal and subcutaneous fat and could be determined form the DXA scans by re-calculating the FM in each sub-region using defined anatomical markers on the scanned image. Central fat mass was defined in three ways: as non-limb fat mass (NLFM), Trunk fat mass (TUKFM) and Abdominal fat (AFM) mass. NLFM was determined from DXA regional computer-generated default lines that include head, chest, abdomen, and pelvis. TUKFM was determined also from DXA regional computer-generated defaulted lines and that include chest, abdomen, and pelvis. AFM determined manually from DXA scan monitor using the DXA software programme v12.2 of Hologic Delphi (see Figure 4.2). The abdominal sub-region was identified as the area between the top borders of the iliac crest to the lower border of the fourth lumbar vertebra. The intra-observer coefficient of variation for the duplicate measurements of abdominal fat measurements was 3.5%. Peripheral fat mass (or Limb fat mass (LBFM)) determined by DXA was that obtained from the sum of fat mass in both the right and left arms and legs defined by the computer generated default lines.

4.3 Statistical analysis

The mean difference in current body size, body composition and pattern of fat distribution measurements obtained by different methods between study groups was compared using independent sample t-test. The comparison of body composition measurements for all subjects obtained by SKF, BIA and BodPod compared with that obtained by DXA was performed using paired samples t-test. Assessment of the agreement of body composition measurement (FM, %FM, FFM, %FFM) obtained by SKF, BIA and BodPod against the reference method of DXA was performed using Bland Altman analysis. The limits of agreement between the methods were defined as the mean differences ± 2SD. Statistical significance was assumed when $P<0.05$. Bivariate correlation coefficient was used to evaluate the strength of the relationship between body composition measurements obtained

by SKF, BIA, and BodPod relative to that obtained by DXA.

4.4 Results

The results presented in this section illustrate the differences in body size, composition and fat distribution obtained by different methods between the lower and higher birth weight groups. These results are presented in five parts. Firstly, the differences in body size and fat distribution obtained by simple anthropometry between the study groups. Secondly, the differences in body composition measurements assessed by DXA between the study groups. Thirdly, the differences in body composition measurements assessed by SKF, BIA and BodPod between the study groups. Fourthly, the differences in the pattern of fat distribution obtained by DXA between the study groups. Finally, the strength of agreement of body composition measurement of all subjects obtained by SKF, BIA, and BodPod when compared to reference method of DXA using Bland Altman analysis.

4.4.1 Anthropometric measurements

The anthropometric measures related to body size and the pattern of fat distribution for all subjects and between the lower and higher birth weight groups in the present study are presented in **Table 4.1**. Based on WHO BMI cut-off point for BMI, most subjects of this study were overweight. The lower birth weight group were significantly shorter (≈ 2.3%; P=0.003) and lighter (≈ 5%; P=0.022) than the higher birth weight group. However, the body mass index (BMI) between the two groups was not significantly different. In addition, there was no significant difference in waist circumference between the two groups, although hip circumference was significantly less in the lower than the higher birth weight group (≈ 3.5%; P=0.033). Despite this difference in hip circumference, there was no significant differences in the waist/ hip ratio (0.95 ± 0.01 v 0.95 ± 0.02; P=0.881). The measurements of subcutaneous body fat in the peripheral (triceps and biceps) and central (subscapular and suprailiac) region assessed by skinfold thickness were also not significantly different between the lower and higher birth weight groups. In addition, the ratio of the sum of subscapular plus suprailiac (as an index of central subcutaneous fat) over triceps plus biceps (as an index of peripheral subcutaneous fat) was also not significantly different between the lower and higher birth weight groups (1.91 ± 0.09 vs 2.00 ± 0.08).

4.4.2 Differences in body composition measurement between groups using DXA

The differences in body composition between the lower and higher birth weight groups both in absolute (kg) and relative (% of body weight) terms obtained by DXA are presented in **Table 4.2**. Compared to the higher birth weight group, the lower birth weight subjects in this study had significantly less absolute amount (kg) of FFM (≈ 16%; P=0.001) and FFST (FFM - BMC)) (≈ 16%; P=0.001). In addition, they had significantly less absolute muscle mass (≈ 19%; P=0.001) estimated from the limb FFST. Furthermore, the non-muscular FFST (muscle mass subtracted from fat free soft tissue) was significantly lower (≈ 13%; P=0.003) in the lower than the higher birth weight group. However, the ratio of non-muscular FFST to muscle mass was not different between the two groups although there was a tendency for this ratio to be greater (≈ 5%) in the lower than the higher birth weight group (0.94 ± 0.07 v 0.98 ± 0.08; p= 0.129). Although BMC was not different between the two groups, it tended to be lower (≈ 12%) in the lower than the higher birth weight group. The relative amount of FFM (% body weight) was not significantly different between the two groups although it tended to be redcued (≈ 12.5%) in the lower compared to higher birth weight groups. The relative fat mass tended to be greater in the lower (≈ 13%) than the higher birth weight groups but this difference was not statistically significant.

4.4.3 Differences in body composition measurement between groups using other methods

The differences in body composition between the lower and higher birth weight groups both in absolute (kg) and relative (% of body weight) terms obtained by SKF, BIA and BodPod are presented in **Table 4.3**. Compared to the higher birth weight groups, the lower birth weight subjects in this study had significantly lower absolute amount of FFM (≈ 12%; P=0.004) estimated by SKF. However, the absolute fat mass was not different between the two groups using this method although it tended to be greater in the higher than the lower birth weight group (≈ 10%). In addition, both FM and FFM as a percentage of body weight were not different between the two groups. Using BIA, the absolute FFM was significantly less (≈ 14%; P=0.003) in the lower than the higher birth weight group. Absolute FM was not different between the two groups, although there was a tendency to be greater (≈ 12%) in the higher than the lower birth weight group. Both FFM and FM as a percentage of body weight were not different between the two groups. Using BodPod, the absolute FFM was significantly less (≈ 19%; P=0.001) in the lower than the higher birth weight group. However, absolute FM was

not different between the two groups. In addition, both FFM and FM were not different between the lower and higher birth weight groups.

The differences in all body composition measurements obtained by DXA, SKF, BIA and BodPod between the lower and higher birth weight groups are also presented in **Figure 4.3**. The mean significant differences in absolute FFM between the lower and higher birth weight groups was greater using BodPod (10.5 kg) when compared with the other methods DXA (8.9 kg), BIA (8.0 kg) and SKF (7.2 kg). Although the absolute FM was not significantly different between the lower and higher birth weight groups the absolute fat mass determined using different methods varied. The greatest difference in absolute fat mass between the lower and higher birth weight groups was observed by using BIA (LBW had 2.6 kg less fat mass than HBW) followed by SKF (2.34 kg) and DXA (0.41 kg). However, the mean difference in absolute fat mass obtained by BodPod between the lower and higher birth weight groups was 1.0 kg, but in this case, the lower birth weight group has a greater FM rather than the higher birth weight groups as reported by the other methods. In relative terms, the mean difference in FFM as percentage of body weight between the lower and higher birth weight groups was reported to be greater by BodPod (LBW have 5.54% less percentage FFM than HBW) followed by DXA (3.19%), BIA (1.7%) and SKF (0.79%). Furthermore, the mean differences in FM as a percentage of body weight between the lower and higher birth weight groups were greater by BodPod (LBW have 5.42% greater percentage body fat than HBW) followed by DXA (3.2%) and BIA (1.75%). The mean differences in percentage body fat between the two groups obtained by SKF was the smallest (0.79%) but in this case, the higher birth weight groups have the greater percentage body fat rather than the lower birth weight groups as reported by the other methods.

4.4.4 Differences in the pattern of fat distribution between groups using DXA

The differences in fat mass in the central and peripheral regions of the body obtained by DXA between the lower and higher birth weight groups are presented in **Table 4.4.** The non-limb (arm + leg fat mass) and trunk fat mass were not different between the lower and higher birth weight groups, although they tended to be greater in the lower than the higher birth weight groups by ≈ 4% and ≈ 7% respectively. Abdominal fat mass and limb fat mass were also not different between the two groups.

The pattern of fat distribution presented as the ratio of non limb: limb, trunk: limb and

abdomen: limb fat mass between the lower and higher birth weight groups estimated by DXA are presented in **Table 4.5**. Compared to higher birth weight, lower birth weight subjects had significantly greater central to peripheral fat mass ratio obtained by DXA which was presented as the ratios of non-limb: limb (≈ 13%; P=0.038) and trunk: limb (≈ 14%; P=0.023). Abdominal: limb fat mass ratio was not different between the two groups. The differences in the pattern of fat distribution between the lower and higher birth weight groups were independent of the absolute and relative body fat mass (see Table 4.5).

4.4.5 Assessment of body composition measurements obtained by different methods

The body composition measurements obtained by different methods in all subjects in are presented in **Table 4.6**. The body composition measurements (FM, %FM, FFM and %FFM) obtained by SKF, BIA and BodPod for all subjects were not significantly different form those obtained by DXA. The correlation coefficient of body composition measurements of all subjects obtained by SKF, BIA, and BodPod relative to DXA are presented in **Table 4.7**. All body composition measurements (FM, %FM, FFM and %FFM) obtained by SKF, BIA and BodPod were highly correlated with those obtained by the reference method of DXA.

The limits of agreements of all body composition measurements (FM, %FM, FFM and %FFM) obtained by SFK, BIA, and BodPod for all subjects in relation to DXA assessed by Bland Altman analysis are presented in **Figures 4.4 to 4.9**. The mean differences in absolute (kg) and relative (%) FM and FFM obtained by DXA and SKF were not different (% differences between the two methods < 2.3 %). The two methods were relatively in good agreement for measuring body composition in all subjects in this study. This analysis also shows that there was a systematic increase in bias between the DXA and SKF in all body composition measurements which were associated with the increases in the mean absolute and relative FM and FFM. The mean differences in absolute and relative FM and FFM between DXA and BIA also were not different between the two methods (% differences between the two methods < 2%). Both DXA and BIA were in a good agreement for measuring body composition in all subjects in this study. The mean differences in absolute and relative FM and FFM between DXA and BodPod were not different in this analysis (% differences between the two methods < 4%). Both DXA and BodPod were in a good agreement for measuring body composition in all subjects in this study. This analysis also shows that there was a systematic decrease in bias between the DXA and BodPod in all body composition measurements which were associated with increases in the mean absolute and relative FM and FFM.

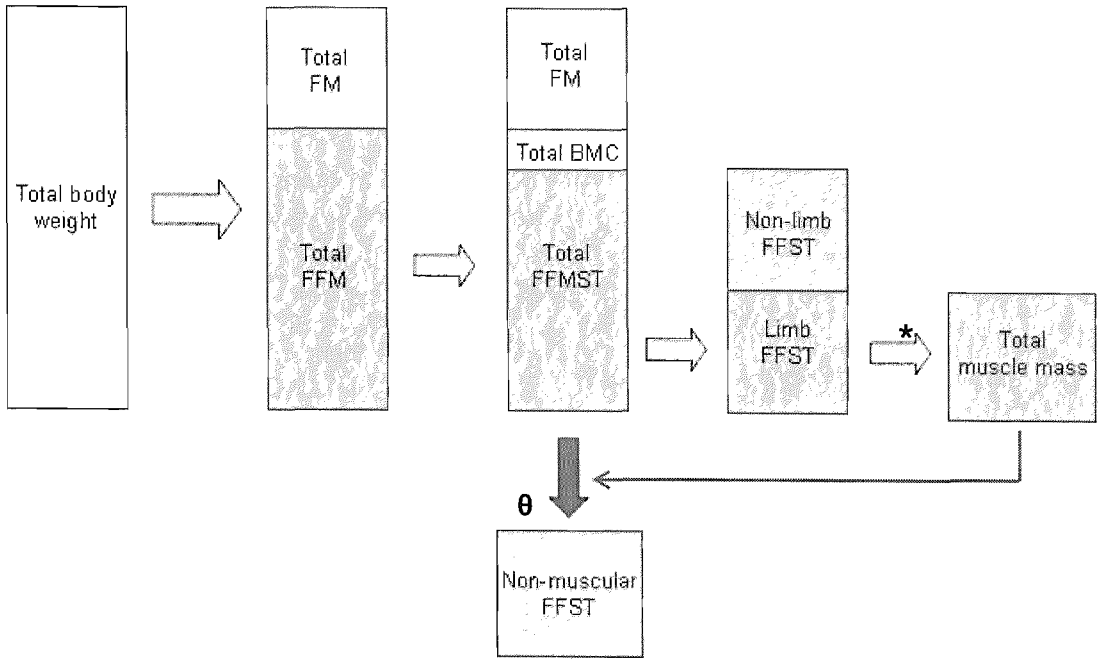


FIGURE 4.1 Body composition measures obtained from the three compartmental model of DXA, and the prediction of muscle mass from this model. FM= fat mass, FFM = fat free mass, BMC= bone mineral content, FFST = fat free soft tissue, * muscle mass predicted from limb FFST from DXA scan according to Kim *et al*, **θ** Non-muscular FFST= total body FFST - muscle mass

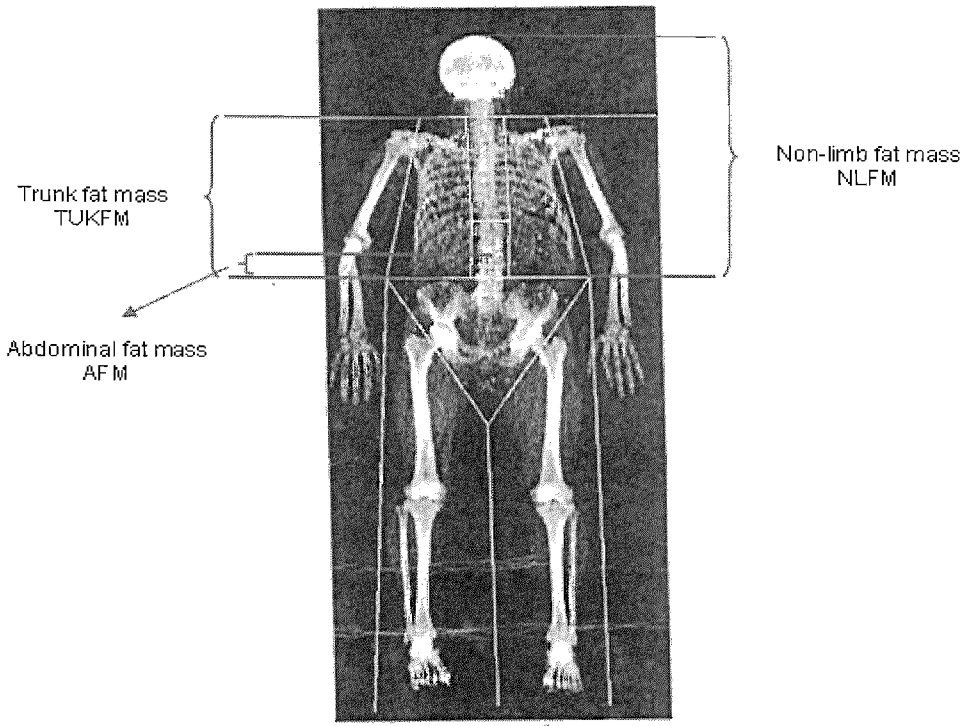


FIGURE 4.2 Different central fat mass sub-region obtained by DXA
NLFM = TUNKFM + head, TUKFM = computer generated default (the top of the shoulder to the top border of iliac crest), AFM = area between the top border of iliac crest to lower border of the fourth lumber vertebra.

TABLE 4.1 Anthropometric measurements for all subjects an between study groups

	All subjects (n=29)	Low BW (n=16)	High BW (n=13)	P value
Height (m)	1.74 ± 0.02	1.70 ± 0.02	1.79 ± 0.02	0.003
Weight (kg)	83.61 ± 1.91	79.45 ± 2.16	88.79 ± 3.36	0.022
BMI (kg/h^2)	27.50 ± 0.54	26.77 ± 0.49	27.97 ± 1.17	0.319
Waist circumference (cm)	102.9 ± 1.85	101.5 ± 1.44	104.7 ± 3.76	0.397
Hip circumference (cm)	108.4 ± 0.89	106.7 ± 0.75	110.5 ± 1.63	0.033
Triceps (mm)	11.81 ± 0.64	12.11 ± 0.71	11.46 ± 1.15	0.623
Biceps (mm)	6.81 ± 0.45	6.46 ± 0.41	7.25 ± 0.88	0.394
Subscapular (mm)	17.98 ± 0.99	18.27 ± 1.09	17.63 ± 1.81	0.753
Suprailiac (mm)	18.07 ± 0.84	18.21 ± 1.06	17.92 ± 1.40	0.870

TABLE 4.2 Differences in body composition obtained by DXA between study groups

	Lower BW (n=16)	Higher BW (n=13)	P value
FFM (kg)	56.55 ± 1.45	65.51 ± 1.61	0.001
FFM (%)	71.28 ± 1.04	74.47 ± 1.86	0.127
FFST (kg)	53.91 ± 1.37	62.55 ± 1.52	0.001
Muscle mass (kg)	26.06 ± 0.71	30.93 ± 0.79	0.001
Non muscular FFST (kg)	27.85 ± 0.78	31.62 ± 0.87	0.003
FM (kg)	22.88 ± 1.06	23.29 ± 2.34	0.867
FM (%)	28.71 ± 1.03	25.53 ± 1.86	0.128
BMC (kg)	2.64 ± 0.16	2.95 ± 0.14	0.110

FFM = fat free mass, FFST = fat free soft tissue (FFM – BMC), BMC = bone mineral content
FM = fat mass

TABLE 4.3 Differences in body composition obtained by SKF, BIA and BodPod

	Lower BW (n=16)	Higher BW (n=13)	P value
Skinfold thickness			
FFM (kg)	58.34 ± 1.57	65.51 ± 1.65	0.004
FFM (%)	72.12 ± 0.74	72.47 ± 1.35	0.596
FM (kg)	22.55 ± 0.86	24.89 ± 1.99	0.258
FM (%)	27.88 ± 0.65	27.53 ± 1.31	0.573
Bioelectrical impedance			
FFM (kg)	58.08 ± 1.75	66.09 ± 1.58	0.003
FFM (%)	72.31 ± 0.88	72.70 ± 2.14	0.426
FM (kg)	22.24 ± 0.95	24.82 ± 2.69	0.325
FM (%)	27.69 ± 0.77	27.30 ± 2.19	0.407
BodPod			
FFM (kg)	55.89 ± 1.97	66.43 ± 1.23	0.001
FFM (%)	69.87 ± 1.40	74.23 ± 2.69	0.065
FM (kg)	24.10 ± 1.26	23.06 ± 3.05	0.738
FM (%)	30.13 ± 1.43	25.77 ± 2.71	0.074

FFM= fat free mass, FM = fat mass

TABLE 4.4 Central and peripheral fat mass obtained by DXA

	Lower BW (n=16)	Higher BW (n=13)	P value
Non Limb FM (kg)	13.74 ± 0.70	13.38 ± 1.42	0.811
Trunk FM (kg)	12.73 ± 0.69	12.31 ± 1.27	0.781
Abdomen FM (kg)	2.67 ± 0.66	2.76 ± 1.03	0.762
Limb FM (kg)	9.14 ± 0.45	9.90 ± 0.95	0.445

TABLE 4.5 Pattern of fat distribution presented as ratios of central to peripheral fat mass obtained by DXA

	Lower BW (n=16)	Higher BW (n=13)	P value
Non limb to limb fat mass			
Unadjusted	1.52 ± 0.06	1.34 ± 0.09	0.038
Adjusted for % body fat	1.49 ± 0.06	1.27 ± 0.07	0.029
Adjusted for kg body fat	1.53 ± 0.06	1.22 ± 0.07	0.002
Trunk to limb fat mass			
Unadjusted	1.40 ± 0.06	1.23 ± 0.04	0.023
Adjusted for % body fat	1.39 ± 0.05	1.24 ± 0.06	0.041
Adjusted for kg body fat	1.41 ± 0.05	1.22 ± 0.06	0.019
Abdomen to limb fat mass			
Unadjusted	0.29 ± 0.01	0.30 ± 0.02	0.912
Adjusted for % body fat	0.30 ± 0.02	0.29 ± 0.02	0.791
Adjusted for kg body fat	0.29 ± 0.02	0.30 ± 0.02	0.882

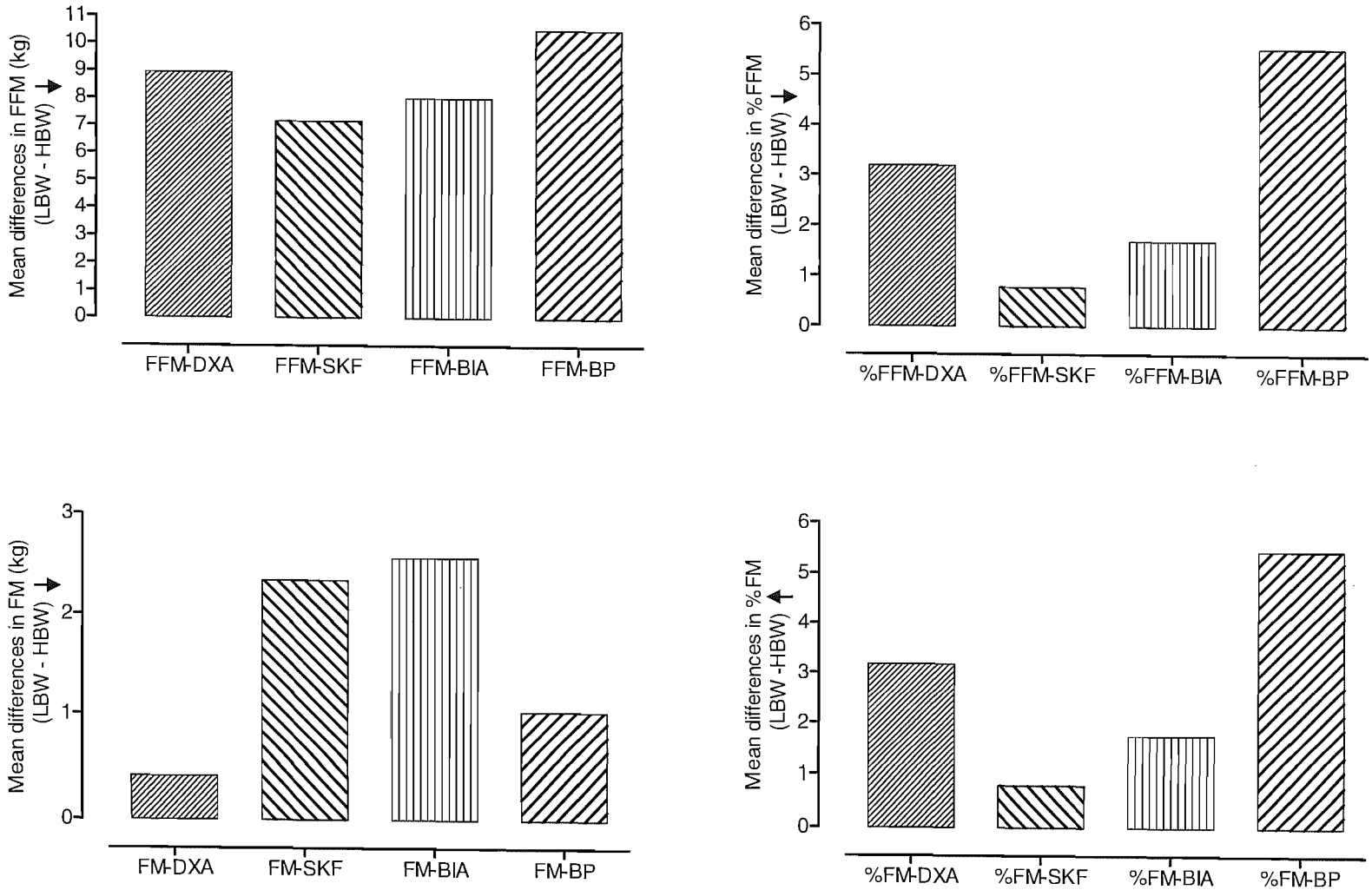


FIGURE 4.3 Mean differences in body composition measurements between the lower and higher birth weight groups obtained by SKF, BIA and BodPod compared with DXA.
(LBW-HBW)↑ = less in lower than higher birth weight group, (LBW-HBW)↓ = greater in the lower than the higher birth weight group.

TABLE 4.6 Body composition measurements by the four different methods for all subjects (n=29)

	FM (kg)	FM (%)	FFM (kg)	FFM (%)
DXA	23.07 ± 1.18	27.28 ± 1.03	60.57 ± 1.35	72.71 ± 1.03
SKF	23.60 ± 1.01	27.48 ± 0.68	61.56 ± 1.31	73.84 ± 0.72
BIA	23.35 ± 1.27	27.52 ± 1.03	61.51 ± 1.41	73.82 ± 1.03
BodPod	23.63 ± 1.51	27.73 ± 1.97	60.61 ± 1.55	72.80 ± 1.49

TABLE 4.7 Bivariate correlation coefficient (r) for body composition measurements (n=29)

	FM (kg)	FM (%)	FFM (kg)	FFM (%)
DXA v SKF	0.874 ($p<0.01$)	0.719 ($p<0.01$)	0.903 ($p<0.01$)	0.709 ($p<0.01$)
DXA v BIA	0.903 ($p<0.01$)	0.885 ($p<0.01$)	0.950 ($p<0.01$)	0.848 ($p<0.01$)
DXA v BodPod	0.932 ($p<0.01$)	0.816 ($p<0.01$)	0.937 ($p<0.01$)	0.919 ($p<0.01$)

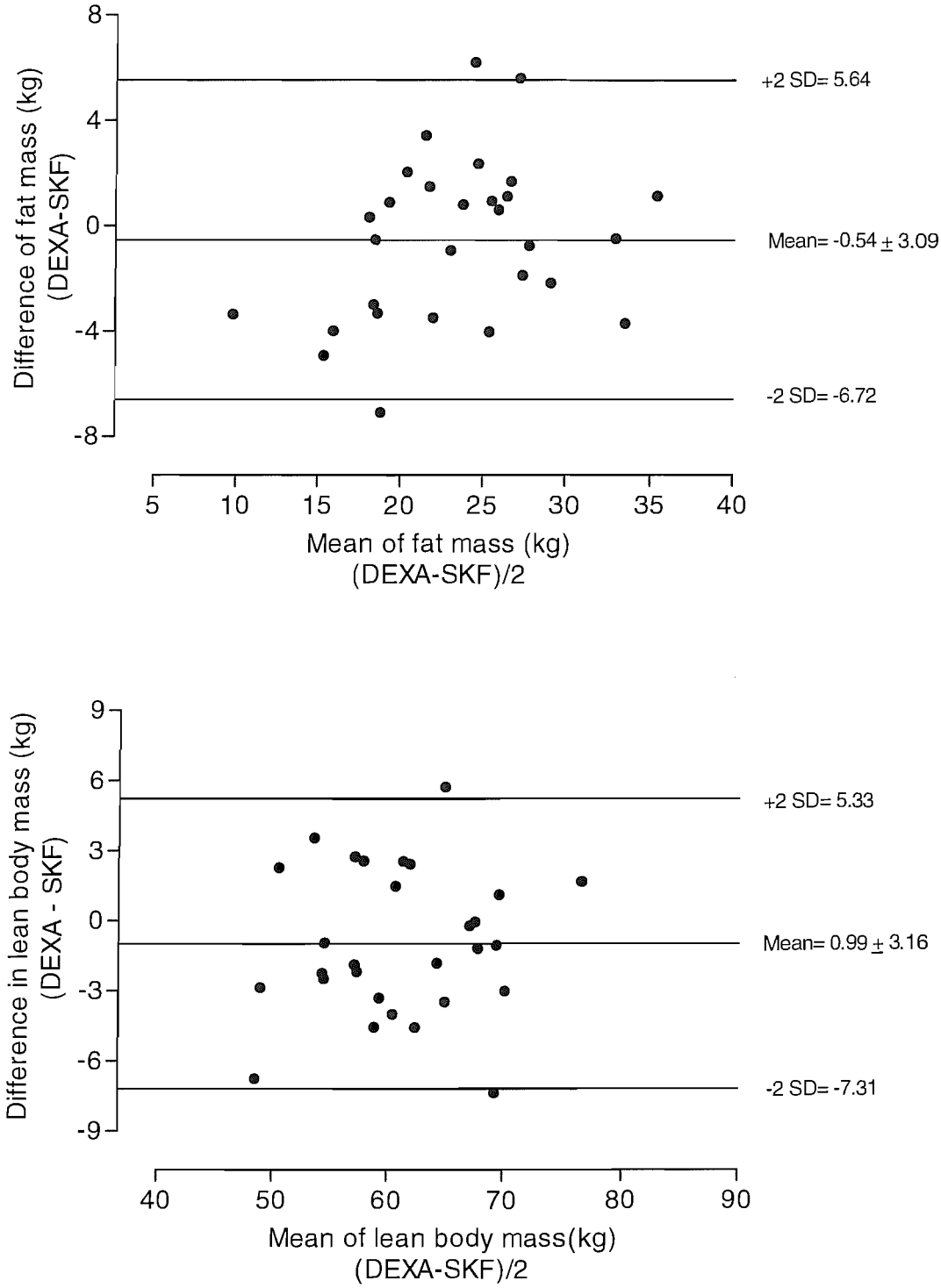


FIGURE 4.4 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and SKF for the Fat mass (upper) and lean body mass (lower)

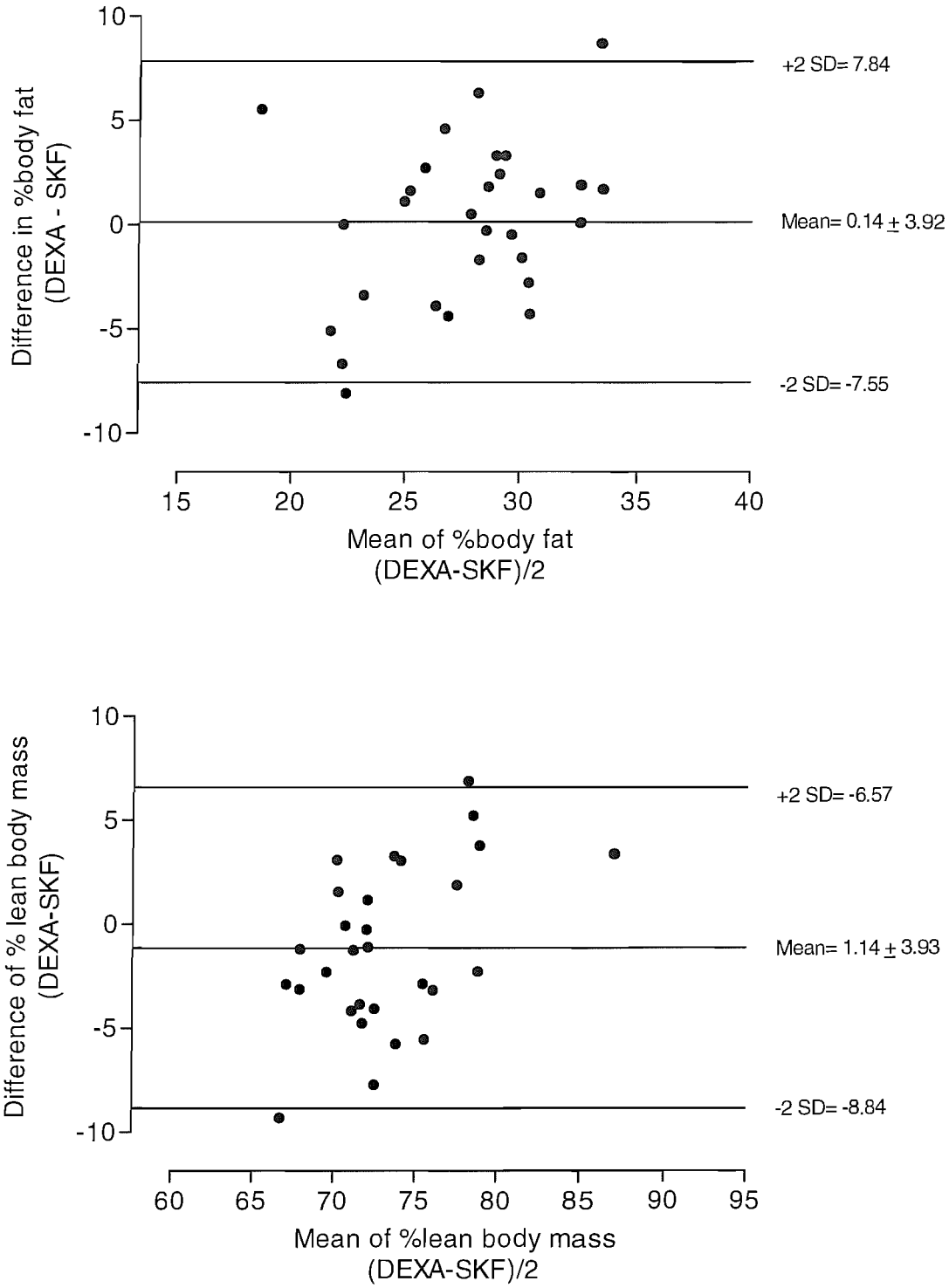


FIGURE 4.5 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and SKF for the %body fat (upper) and % lean body mass (lower)

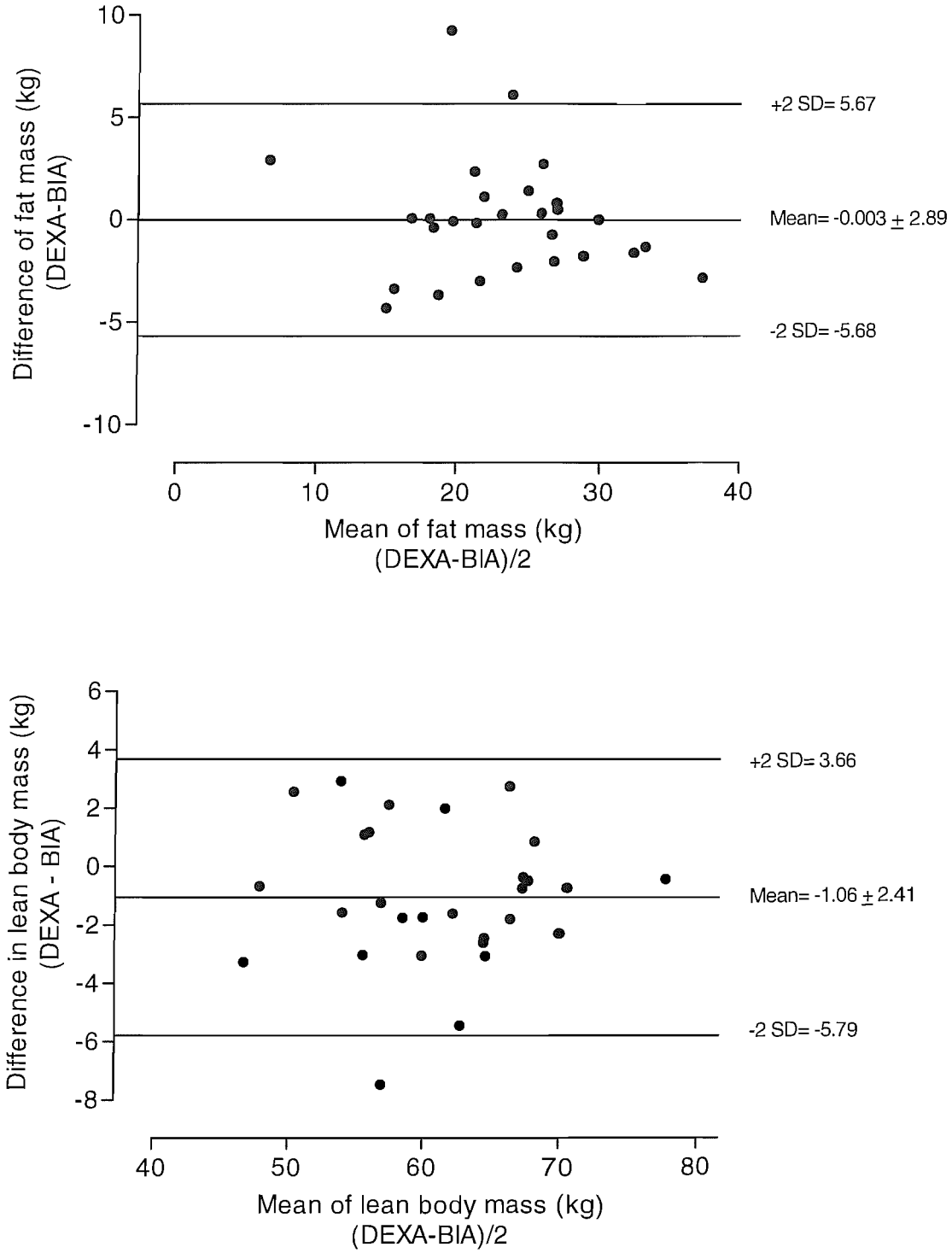


FIGURE 4.6 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and BIA for the Fat mass (upper) and lean body mass (lower)

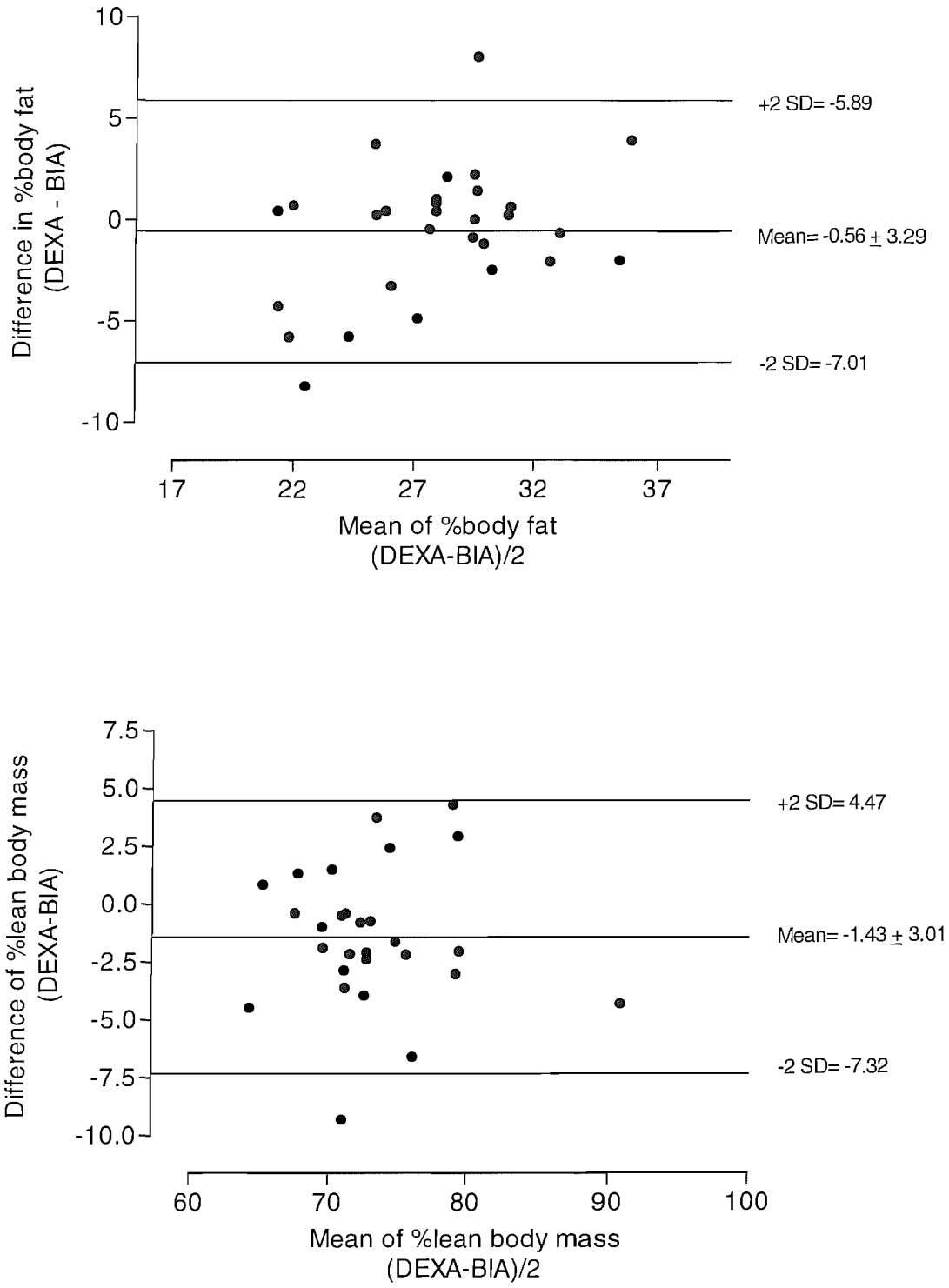


FIGURE 4.7 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and BIA for the %body fat (upper) and % lean body mass (lower)

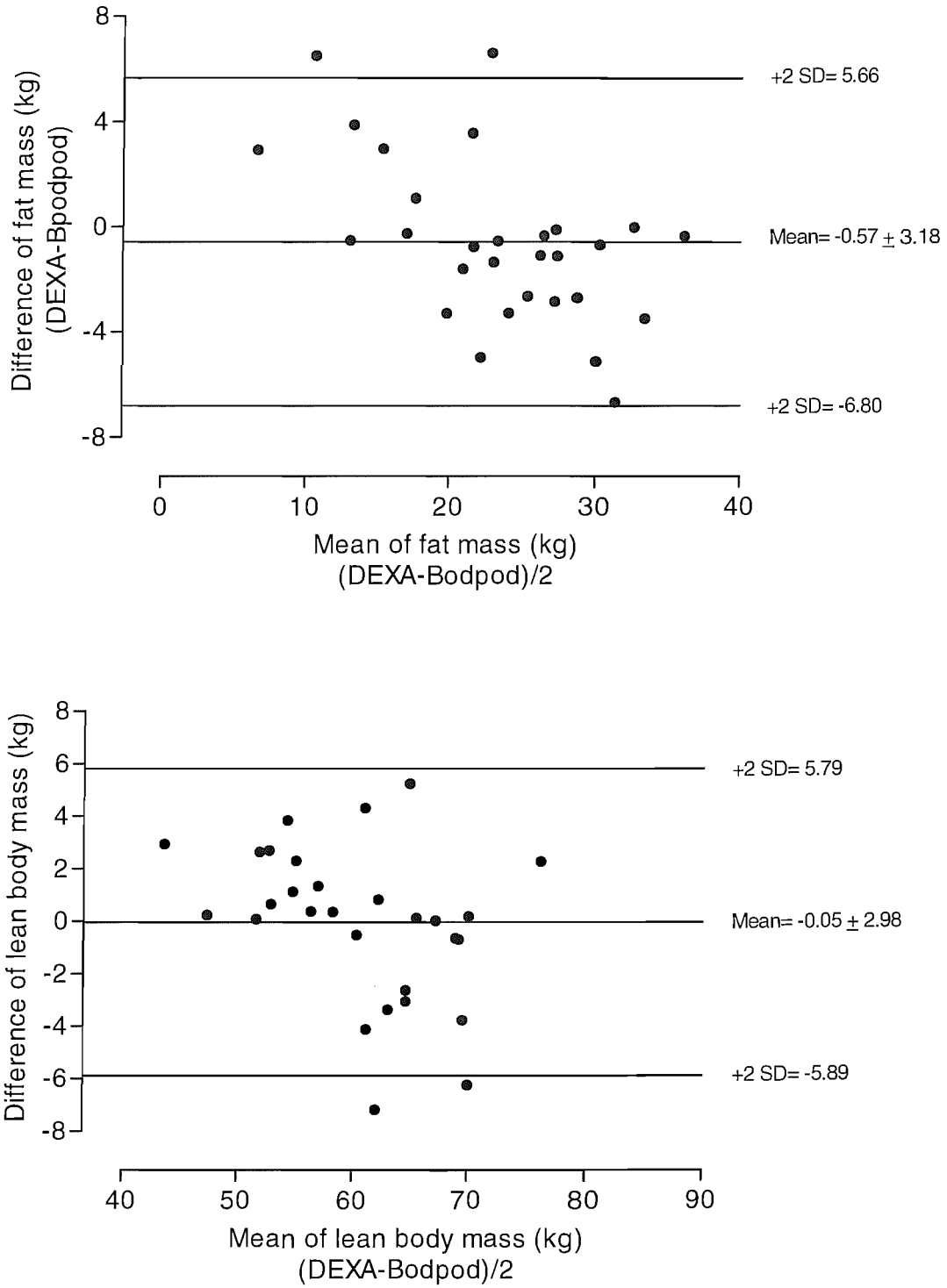


FIGURE 4.8 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and BP for the Fat mass (upper) and lean body mass (lower)

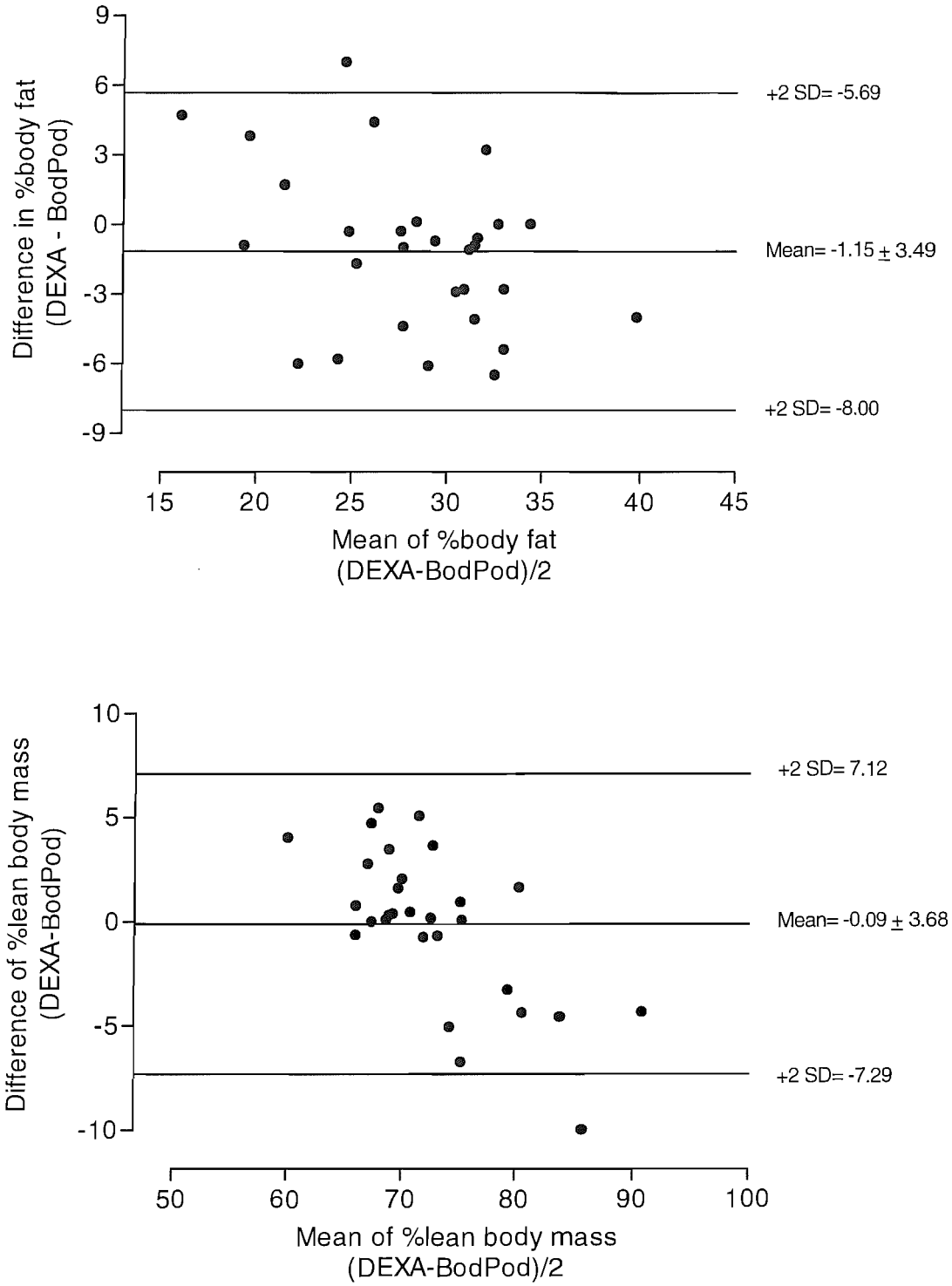


FIGURE 4.9 Bland and Altman plot analysis to evaluate the agreement between the methods of DXA and BodPod for the %body fat (upper) and % lean body mass (lower)

4.5 Discussion

The aim of the analysis presented in this chapter was to examine the influence of birth weight on current body habitus in terms of size, composition, and fat distribution using different principle methods within a group of older men born with different birth weights. This analysis also aimed to compare body composition measurements obtained by SKF, BIA and BodPod against DXA as the reference method by testing the strength and agreement of body composition measurements obtained by SKF, BIA and BodPod relative to that assessed by DXA.

Compared to the higher birth weight group, lower birth weight subjects were shorter, lighter and have lower FFM obtained by four different methods, each of which uses different approaches to estimate body composition. The results obtained by DXA demonstrated that the lower birth weight group have lower absolute amounts of FFST (FFM-BMC), muscle mass and non-muscular FFST (FFST-muscle mass) than the higher birth weight group, a difference that was consistent with the observations derived by using other methods. The absolute FM, by any method, was not different between study groups, neither was the relative FFM. The relative fat mass, measured by all methods, tended to be greater in the lower than the higher birth weight group, although this difference did not attain statistical significance. Although central fat mass (non-limb and trunk fat mass) was not different between the two groups, the pattern of fat distribution assessed by DXA (as the ratios of non-limb: limb and trunk: limb fat mass) show that the lower birth weight group had greater visceral (both subcutaneous and visceral) to peripheral fat mass when compared to the higher birth weight groups. In contrast, the pattern of fat distribution assessed by anthropometric measurements was not different between the study groups. Comparison of the different methods revealed good agreement between the different techniques.

This is the first occasion in which a comprehensive analysis of current body habitus in older men with differing birth weight has been conducted using different principle methods. Using DXA as a reference method, together with other methods not only confirmed previous observations that adults born of lower birth weights are likely to be shorter, lighter and have a lower FFM, but also that there are differences in the components that make up the FFM. Infants born with a lower birth weight would appear to have lower absolute muscle mass (less metabolically active) and non-muscular FFST (more metabolically active since it may represent organ mass) obtained by DXA than those born with higher birth weight. Although the ratio of non-muscular to muscle mass was not significantly different, there was a

tendency for this ratio to be greater in the lower than the higher birth weight group. To avoid making a Type II error due to the risk of being under powered, it was thought prudent to conduct a post-hoc power analysis using the data of this study. In this analysis knowing the magnitude of the mean differences in ratio of non-muscular FFST to muscle mass (obtained from DXA) and the variance within each group, it was possible to determine the appropriate sample size within each group that could lead to reject the null hypothesis. The probability of rejecting the null hypothesis was set at the level of <0.05 and power of study > 80%. The number of subjects determined in this analysis was found to be 30 subjects within each group. This suggests that the inability to observe a significant difference in the ratio of non-muscular to muscle mass between the study groups might be influenced by the relatively small sample size with inadequate power to reject the null hypothesis. Doubling the number of subjects within each group would be needed to derive sufficient power and thereby reject the null hypothesis (at the level of < 0.05 and power of study > 80%)

The greater ratio of non-muscular to muscle mass suggests that fetal programming in early life may not only have an effect on the absolute amount of FFM, but also on the proportion of non-muscular FFST to muscle mass. Such differences in the components that make up the FFM may in turn, effect energy-substrate metabolism. A previous study reported that the increase in birth weight is inversely associated with REE per kilogram of FFM (212). Therefore this difference in REE/FFM could be attributed, at least in part, to the differences in the ratio of non-muscular FFST to muscle mass given their different metabolic activities. Although there are very few studies that have been designed to address the influence of birth weight on energy metabolism, such differences in body composition would be expected to have significant biological effects on energy metabolism measured as REE by varying the amounts of tissues with differing metabolic activities.

The current study also reported a tendency for fat mass relative to body weight obtained by DXA, BIA and BodPod to be greater in the lower than the higher birth weight group but this difference were not statistically significant. Once again, despite the concordance of observations, the possibility that the study was under-powered was considered. In post-hoc power analysis, given the magnitude of the mean differences in relative FM between the study groups and the variance within each group obtained by these methods, it appears that such studies would need 50 (using DXA), 60 (using BodPod) and 120 subjects (using BIA) within each group before the sample size would be sufficient to reject the null hypothesis (at the level of < 0.05 and power of study > 80%). Thus there is a clear risk of making a Type II error and rejecting the hypothesis in the current study.

In the only other study using DXA in adults of differing birth weight, they too found a positive relationship between birth weight and FFM although they made no attempt to further determine the components of the FFM such as muscle mass or regional distribution of FFM (200). They did however; report a weak but significant association between birth weight and whole body bone mineral content, an effect that was not observed in the current study. The observed differences in muscle mass in relation to birth weight in this study are consistent with those reported using indirect measures of muscle mass such as urinary creatinine excretion (192) or measures of thigh circumference (199). In the current study, muscle mass was estimated from DXA using equations based on the established relationship between limb FFST obtained by DXA and the measurements of total body muscle mass using magnetic resonance imaging (MRI). This approach is believed to better predict total body muscle mass as opposed to simple anthropometry and urinary creatinine execration (231;232).

This is the first occasion in which the BodPod has been used to determine whether body density is related to differences in birth weight. Others have used under water weighing to determine body density in similar studies (197;198) and obtained comparable results. Although there was generally good agreement between the different approaches to assess body composition, there were some notable differences compared to DXA, particularly in terms of the results obtained by BodPod. For instance, the mean differences in absolute FFM between the lower and higher birth weight groups were found to be greater by BodPod (≈ 18%) than that obtained by DXA, whilst the absolute FFM was found to be smaller (≈ 25%) than that obtained by DXA. One possible explanation for this apparent anomaly between the two methods may be attributable to the assumptions or technical errors associated with each procedure. For instance, some methods, in particular, those based on two compartmental model assume that hydration of FFM between individuals is constant (i.e. 73% of FFM is water) and unaffected by the composition of the FFM. BIA estimations are based on the assumption that the whole body is reflected as a series of cylinder, each of which represent various body segments. However, there may be important differences between individuals in the proportion of body segments (i.e. leg length and arm length) that may be associated with differences in height.

This is also the first occasion in which differences in the pattern of fat distribution have been reported in relation to birth weight. This finding was only evident using DXA as none of the anthropometric measurements used to assess the pattern of fat distribution were different between the lower and higher birth weight group. The model used in this study to determine visceral and limb fat mass was obtained using DXA to determine composition of the non-

limb, trunk, abdomen and limb compartments. Although, there were no significant differences between the two groups in visceral fat mass (non-limb, trunk and abdomen fat mass) and limb fat mass, the ratios of non-limb to limb and trunk to limb fat mass were significantly greater in the lower than higher birth weight groups. These differences in the pattern of fat distribution are independent of total absolute and relative fat mass. Previous attempts to assess fat distribution on the basis of simple anthropometry, such as waist-to-hip ratio or the ratio of trunk to limb skinfold thicknesses, have produced inconsistent results, with some studies reporting significantly more central fat distribution in individuals with low birth weight (151;191;199;204;205) whilst others found no differences in relation to birth weight (138;190;203;233). One of the reasons for the lack of consistency is that different skinfold-thickness ratios [subscapular to triceps (151;191;204;205), subscapular-suprailiac to triceps-thigh (204) and subscapular-midaxillary to triceps-medial calf (191)] or different circumference ratios [i.e. waist to hip (138;180;190;203;233) or wais to thigh (199)] have been used for this purpose, each of which may better or less well reflect the compartment of interest. Another reason is that some studies have adjusted for weight and not height (138;180;190;203;233), others have adjusted for height and not weight (199), whereas most studies have adjusted for BMI (138;203-205;233), which controls for neither weight nor height, and a few studies make no adjustments at all (234-236).

There are several caveats that need to be considered however. Firstly, in terms of study design, although the differences in lean mass (FFM, muscle mass and non-muscular FFST) between the study groups were seen with less subjects within each group compared to other studies, greater confidence in these observations would be gained by increasing the number of subjects within each group. Furthermore, the study groups included in this analysis were only those born in the lower and higher birth weight category and there would be clear benefit in study birth weight as a continuous variable across the range of birth weight. Secondly, in terms of the observations relating to muscle mass, here limb FFST was derived using a predicative equation which has been established using a different population. Once again, greater confidence in this finding could be gained by using more detailed imaging techniques such as that obtained by CT or NMR. In the same way, improved imaging techniques would better identify compartments of fat mass. Although the pattern of fat distribution obtained by DXA was different between the lower and higher birth weight groups, DXA cannot differentiate between subcutaneous and visceral fat mass within the trunk. It is important to note however, that many studies reported that visceral fat mass obtained from a multiple sub-regions by DXA are comparable to those obtained by more sophisticated and accurate methods of imaging. For example, Gallagher et al compared the correlations between MRI-proxy measurement related to visceral fat mss (the upper edge of the forth lumber vertebra to

the lower edge of the fifth lumber vertebra) and surrogate measures obtained for DXA different abdominal sub-regions (i.e. the upper edge of the second lumber vertebra to the lower edge of the fourth lumber vertebra) in a group of 90 non obese (BMI > 30kg/h^2) healthy men (237). All abdominal fat mass sub-regions determined from DXA and MRI were highly correlated with each other. In addition, both DXA and MRI fat mass sub-regions were highly correlated with total visceral fat mass determined by MRI to the same extent. Others have come to similar conclusions in men and women, in different racial groups and over a wide age range (238;239).

Summary

The main aim of this chapter was to characterise body habitus (size, composition and pattern of fat distribution) in older men with differing birth weight using different methods. In addition, this study aimed to examine the strength and the extent of agreement of body composition measurements obtained by SKF, BIA and BodPod when compared with references method of DXA.

The results of the current study show that:

- Compared to higher birth weight group the lower birth weight subjects were shorter, lighter and have lower absolute FFM, obtained by DXA, BIA, BIA and BodPod. The absolute fat mass was not different between the study groups

- Both FFM and FM relative to body weight were not different between the lower and higher birth weight groups by all methods used in this study. But there was a tendency for relative fat mass to be greater and relative FFM to be lower in the lower than the higher birth weight group.

- Post-hoc power analysis revealed that in order to confidently reject the null hypothesis (and hence avoid a type II error), the variance and magnitude of the differences observed in this study would be considered under powered. The size of the subjects groups would need to be increased in order to avoid type II error. The extent of size increased would differ with each method (i.e. DXA= 50, BodPod= 60 and BIA= 180 subjects).

- The distribution of body fat obtained by simple anthropometric measurements (waist,

hip circumference and skinfold ratio) were not different between the study groups. However, using DXA, the lower birth weight group had greater central to peripheral fat mass.

- Body composition measurements obtained by SKF, BIA and BodPod for all subjects in this study were not different when compared with that obtained by DXA. In addition, all measurements obtained by these methods were highly correlated with that obtained by DXA. Furthermore, these measurements were also in a good agreement when compared with that obtained by DXA.

CHAPTER 5

Assessment of body composition in older men with different birth weight in relation to body size – second level of analysis

5.0 Introduction

The results of the previous chapter confirmed previous observations that adults who were lighter at birth are shorter and lighter when compared to those born of a higher birth weight. Both height and weight are independently related with different components of body composition. For instance, height is known to be positively associated with FFM and muscle mass, whilst body weight is positively associated with body fat both in absolute terms and relative to body weight. Therefore, it would be expected that the lower birth weight group would have lower absolute FFM and FM when compared with higher birth weight subjects simply due to a size or scaling effect such that part or all of the differences in FFM or fat mass might be lost after accounting for differences in body size. The lower birth weight group had lower absolute lean mass (FFM, FFST and muscle mass) when compared to higher birth weight group. Despite the differences in body size, absolute body fat mass was not different between the two groups (LBW=22.9 v HBW=23.3kg). Whilst the differences in the lean compartments might alter when adjusted for size, it is not clear whether fat mass might be similarly affected thereby revealing hidden or masked differences.

Previous attempts examine the link between birth weight and metabolic risk factors associated with CVD have usually tried to control or adjust for differences in adult body habitus marked by BMI by post-hoc statistical analysis (i.e. ANCOVA)(138;240). The assumption that underlies such an approach is that differences in adiposity marked by BMI are a confounder which may be influenced by genetic or postnatal environmental factors. Whilst this may appear reasonable at first glance, there is a need to reflect more carefully on such adjustments. Whilst such an approach may be mathematically desirable in terms of statistical prudency, it may inappropriately weaken associations of biological interest. At the most basic level, a range of biological observations within a population might be observed at the same BMI. For instance, differences in FFM and fat mass would be expected at the same

BMI. This may contribute to the variation in body size (height and weight) associated with birth weight which are not explained simply by the use of BMI alone.

Furthermore, several studies have attempted to assess the risk of obesity in relation to birth weight. These studies use BMI as a simple surrogate measure to reflect differences in adiposity associated with birth weight. Although some reports indicate no relation between birth weight and adult BMI (195;197;228;241) many others indicate a positive relation between the two (133;139;184;199;200;241;242). However, these observations present a conundrum, because a high BMI is generally associated with increased risk of cardiovascular disease, but a high BMI in subjects with a higher birth weight has been shown to be associated with reduced risk (133;137). One explanation for this is that the reduced risk in adults with a higher birth weight is mediated by factors that can override any adverse effects of gross body composition, such as excess adiposity. A second, and very different, explanation is that—compared with individuals with a lower birth weight, persons with a higher birth weight may have relatively less body fat and more lean mass even after adjustment is made for BMI.

Taking these observations together it would be wise to determine the extent to which variance in body size associated with birth weight can explain the differences in body composition and fat distribution between the lower and higher birth weight group. Or whether such differences in body composition and fat distribution are programmed in early life independently of body size?

5.1 Aim

- To examine the contribution of height and weight both independently and together on the reported differences in body composition and fat distribution associated with birth weight.
- To examine the differences in body composition and fat distribution associated with birth weight at any given BMI.

5.2 Subjects and method

The analyses performed in this section are based on the results of body composition and fat distribution between the lower and higher birth weight groups obtained by DXA as reported in

the previous chapter. In this chapter, the reported differences in body habitus between the study groups were examined in relation to height and weight both independently and together, and in addition to BMI, by ANCOVA. Fat mass, different components of FFM which include FFST muscle mass, non-muscular FFST and pattern of fat distribution were used as dependent variables whereas height and weight were used as a covariate in this analysis both separately and together. BMI was also used as a covariate in this analysis. The use of this ANCOVA approach allow to determine what differences in absolute amount of tissue (fat and FFM) and pattern of fat distribution would be expected to observe if we have two groups of people with the same height (or weight) and the same [height + weight] but with different birth weights.

5.3 Results

The results presented in this chapter indicate the extent of the association between measures of body size (height and weight) and all body composition and fat distribution measurements obtained by DXA. Thereafter, the contribution of height on differences in body composition and fat distribution associated with birth weight is described before considering the contribution of weight on differences in body composition and fat distribution associated with birth weight. The contribution of both [height + weight] together on the differences in body composition and fat distribution associated with birth weight is then presented. Finally, the expected differences in body composition and fat distribution between the lower and higher birth weight groups are described when BMI was used to account for the differences in body habitus.

5.3.1 Body size in relation to body composition and fat distribution

The relationships between both measures of body size (height and weight) and composition on one hand and fat distribution on the other, for all study subjects are presented on **Table 5.1**. Height was significantly and positively associated with FFM (P= 0.001), FFST (P=0.001), muscle mass (P= 0.001) and non-muscular FFST (P=0.002). Both absolute fat mass (kg) and relative to body weight (%) were not associated with height although relative fat mass tended to be negatively associated with height but this association did not reach statistical significance. In addition, height was not associated with any measures of fat distribution obtained by DXA although the ratios of non-limb to limb, trunk to limb and abdominal to limb fat mass tended to be negatively associated with height but these associations did not reach statistical significance.

Body weight was significantly and positively associated with FFM (P= 0.001), FFST (P= 0.001), muscle mass (P= 0.001), non-muscular FFST (P= 0.544), absolute fat mass (P= 0.001) and fat mass relative to body weight (P= 0.028). Body weight was also significantly and positively associated with non-limb (P= 0.001), trunk (P= 0.001), abdominal (P= 0.001) and limb fat mass (P= 0.001). However, there were no associations between the ratios of non-limb to limb, trunk to limb, abdominal to limb fat mass and body weight.

5.3.2 Contribution of height on differences in body composition and fat distribution associated with birth weight

The differences in body composition between the lower and higher birth weight groups obtained by DXA adjusted for height are presented in **Table 5.2**. Despite the positive association between height in one hand and FFM, FFST and muscle mass on the other (see Table 5.1), at a given height (1.74m) the lower birth weight group have lower FFM (≈ 9%; P=0.021), FFST (≈ 10%; P=0.017) and muscle mass (≈ 12%; P=0.008) than those in the higher birth weight group. The non-muscular FFST was not different between the two groups although it tended to be lower (≈ 8%) in the lower than the higher birth weight group. Greater proportion of the differences in FFST (5.57kg) between the two groups was attributed to muscle mass (3.17kg) and remaining was related to non-muscular FFST (2.39kg). The ratio of muscle mass to non-muscular FFST was not different between the two groups at the same height. Both the absolute and relative fat mass were not different between the two groups after adjusting for height although relative fat mass tended to be greater (≈ 11%) in the lower than the higher birth weight group.

The differences in the pattern of fat distribution between the lower and higher birth weight groups obtained by DXA adjusted for height are presented in **Table 5.3.** There were no significant differences between the lower and higher birth weight groups in any measure related to central body fat mass (non-limb, trunk and abdominal fat mass) and limb fat mass. Non-limb: limb, trunk: limb and abdominal: limb fat mass ratios were not different between the two groups after adjustments were made for height.

5.3.3 Contribution of weight on the differences in body composition and fat distribution associated with birth weight

The differences in body composition between the lower and higher birth weight groups obtained by DXA adjusted for weight are presented in **Table 5.4**. Despite the positive

association between body weight in one hand and FFM, FFST, muscle mass, absolute and relative fat mass on the other (see Table 5.1), at a given weight (83.6 kg), the lower birth weight group has lower FFM (≈ 8%; P=0.003), FFST (≈ 9%; P=0.002), muscle mass (≈ 11%; P=0.001) and greater absolute and relative fat mass (≈ 22%; P=0.003 and 25%; P=0.002 respectively) than those in the higher birth weight group. The non-muscular FFST was not different between the two groups although it tended to be lower (≈ 5%) in the lower than the higher birth weight group. Greater proportion of the differences in FFST (4.50 kg) between the two groups was attributed to muscle mass (3.04 kg) and remaining was related to non-muscular FFST (1.46 kg). The ratio of muscle mass to non-muscular FFST was also not different between the two groups but it tended to be lower (≈ 7%) in the lower than the higher birth weight group after accounting for the differences in body weight.

The differences in the pattern of fat distribution between the lower and higher birth weight groups obtained by DXA adjusted for body weight are presented in **Table 5.5.** Although body weight was positively associated with non-limb, trunk, abdominal and limb fat mass, at a given weight, the lower birth weight groups had greater non-limb (≈ 29%; P=0.001) and trunk fat mass (≈ 32%; P=0.001) than the higher birth weight subjects. The differences in non-limb fat mass (3.42 kg) or trunk fat mass (3.39 kg) between the two groups represents greater proportion of the differences in absolute fat mass (4.63 kg) after adjusting for body weight. The abdominal and limb fat mass fat mass were not different between the two groups although they tended to be greater (≈ 18% and 14% respectively) in the lower than the higher birth weight groups. Non-limb: limb and trunk: limb fat mass ratios were significantly greater (≈ 14%; P=0.049 and 17%; P=0.015 respectively) in the lower than the higher birth weight groups at a given weight whilst abdominal: limb fat mass was not different between the two groups after adjusting for body weight.

5.3.4 Contribution of height and weight on the differences in body composition and fat distribution associated with birth weight

The differences in body composition between the lower and higher birth weight groups adjusted for height and weight are presented in **Table 5.6.** At a given height (1.74 m) and weight (83.6 kg), the lower birth weight subjects had significantly lower FFM (≈ 6%; P=0.028), FFST (≈ 6%; P=0.017) and muscle mass (≈ 8%; P=0.014). The non-muscular FFST and the ratio of muscle and non-muscular FFST were not different between the two groups although they tended to be reduced (≈ 4% and 5% respectively) in the lower than the higher birth weight group. Greater proportion of the differences in FFST (3.42 kg) between the two

groups after adjustments were made for height + weight was attributed to muscle mass (2.28 kg) and remaining was related to non-muscular FFST (1.14 kg). The absolute and relative fat mass were significantly greater (≈ 16%; P=0.026 and 20%; P=0.018 respectively) in the lower than the higher birth weight group when accounting for height and weight.

The differences in the pattern of fat distribution between the lower and higher birth weight groups obtained by DXA adjusted for height and weight are presented in **Table 5.7**. At a given height and weight, the lower birth weight subjects had significantly greater non-limb (≈ 21%; P=0.010) and trunk fat mass (≈ 18%; P=0.012) than the higher birth weight group. The differences in non-limb fat mass (2.60 kg) or trunk fat mass (2.57 kg) between the two groups represents greater proportion of the differences in absolute fat mass (3.39 kg) after adjustments were made for height and weight. The abdominal fat mass was not different between the two groups although they tended to be greater (≈ 10%) in the lower than the higher birth weight groups. The limb fat mass was also not different between the two groups after accounting for the differences in height and weight. Non-limb: limb, trunk: limb fat mass ratios were not different between the two groups although they tended to be greater (≈ 12 and 15%) in the lower than the higher birth weight group.

5.3.5 Expected differences in body composition and fat distribution associated with birth weight at the same BMI

Differences in body composition between the lower and higher birth weight groups obtained by DXA after adjusting for BMI are presented in **Table 5.8**. At a given BMI (27.3 kg/h^2), the lower birth weight subjects were significantly shorter (1.70 ± 0.02 v 1.79 ± 0.02; P=0.002) and lighter (80.41 ± 1.60 v 87.61 ± 1.78; P=0.006) than those in the higher birth weight group. They also have significantly lower FFM (≈ 14%; P=0.001), FFST (≈ 14%; P=0.001), muscle mass (≈ 17%; P=0.001) and non-muscular FFST (≈ 12%; P=0.001). The ratio of muscle mass to non-muscular FFST was not different between the two groups, although it tended to be less (≈ 5%) in the lower than the higher birth weight group. Although absolute fat mass was not different between the two groups, fat mass relative to body weight was significantly greater (≈ 17%; P=0.014) in the lower than the higher birth weight group.

Differences in the pattern of fat distribution between the lower and higher birth weight groups obtained by DXA after adjusting for BMI are presented in **Table 5.9**. Non-limb, trunk and abdominal fat mass were not different between the lower and higher birth weight groups although the non-limb and trunk fat mass tended to be greater in the lower than the higher

birth weight groups (≈ 9% and 10% respectively). Limb fat mass was not different between the two group. The ratios of non-limb to limb and trunk to limb fat mass were significantly greater in the lower than the higher birth weight group after accounting for the differences in BMI (≈ 13%; P= 0.036 and 16%; P= 0.016 respectively).

TABLE 5.1 Correlation coefficient between body size and composition for all subjects

	Height (r; P-value)	Weight (r; P-value)
FFM	0.644; 0.001	0.845; 0.001
FFST	0.638; 0.001	0.850; 0.001
Muscle mass	0.663; 0.001	0.762; 0.001
Non-muscular FFST	0.544; 0.002	0.849; 0.001
FM	0.094; 0.629	0.792; 0.001
%FM	- 0.193; 0.317	0.408; 0.028
Non-limb FM	0.036; 0.853	0.742; 0.001
Trunk FM	0.028; 0.0.885	0.689; 0.001
Abdominal FM	0.026; 0.893	0.680; 0.001
Limb FM	0.210; 0.273	0.745; 0.001
Non-limb: Limb FM	- 0.234; 0.222	0.115; 0.553
Trunk: Limb FM	- 0.306; 0.106	- 0.022; 0.912
Abdominal: Limb FM	- 0.267; 0.161	0.179; 0.353

TABLE 5.2 Differences in body composition obtained by DXA adjusted for height

	Lower BW (n=16)	Higher BW (n=13)	P-value
FFM (kg)	58.05 ± 1.41	63.67 ± 1.59	0.021
FFST (kg)	55.29 ± 1.34	60.86 ± 1.52	0.017
BMC (kg)	2.76 ± 0.13	2.81 ± 0.14	0.815
Muscle mass (kg)	26.82 ± 0.68	29.99 ± 0.77	0.008
Non-muscular FFST (kg)	28.47 ± 0.80	30.86 ± 0.90	0.077
FM (kg)	23.19 ± 1.78	22.90 ± 2.00	0.920
FM (%)	28.56 ± 1.49	25.71 ± 1.69	0.250
Muscle: non muscular FFST (kg)	0.94 ± 0.02	0.97 ± 0.02	0.384

TABLE 5.3 Differences in the pattern of fat distribution obtained by DXA adjusted for height

	Lower BW (n=16)	Higher BW (n=13)	P-value
Non-limb FM (kg)	13.89 ± 1.10	13.19 ± 1.24	0.696
Trunk FM (kg)	12.86 ± 1.08	12.14 ± 1.23	0.686
Abdominal FM (kg)	2.67 ± 0.23	2.77 ± 0.26	0.789
Limbs FM (kg)	9.22 ± 0.62	10.27 ± 0.70	0.310
Non-limb: Limb FM	1.51 ± 0.06	1.24 ± 0.07	0.151
Trunk: Limb FM	1.39 ± 0.06	1.24 ± 0.06	0.096
Abdominal: Limb FM	0.282 ± 0.02	0.306 ± 0.02	0.524

TABLE 5.4 Differences in body composition obtained by DXA adjusted for weight

	Lower BW (n=16)	Higher BW (n=13)	P-value
FFM (kg)	58.49 ± 0.89	63.13 ± 0.99	0.003
FFST (kg)	55.76 ± 0.82	60.28 ± 0.92	0.002
BMC (kg)	2.73 ± 0.12	2.84 ± 0.14	0.568
Muscle mass (kg)	26.88 ± 0.53	29.92 ± 0.59	0.001
Non-muscular FFST (kg)	28.89 ± 0.48	30.35 ± 0.54	0.065
FM (kg)	25.14 ± 0.88	20.51 ± 0.99	0.003
FM (%)	30.05 ± 1.15	23.88 ± 1.28	0.002
Muscle: non muscular FFST (kg)	0.93 ± 0.02	0.99 ± 0.02	0.084

TABLE 5.5 Differences in the pattern of fat distribution obtained by DXA adjusted for weight

	Lower BW (n=16)	Higher BW (n=13)	P-value
Non-limb FM (kg)	15.11 ± 0.58	11.69 ± 0.65	0.001
Trunk FM (kg)	14.06 ± 0.58	10.67 ± 0.65	0.001
Abdominal FM (kg	2.92 ± 0.16	2.46 ± 0.18	0.073
Limbs FM (kg)	10.03 ± 0.39	8.82 ± 0.44	0.060
Non-limb: Limb FM	1.53 ± 0.06	1.34 ± 0.06	0.049
Trunk: Limb FM	1.42 ± 0.05	1.21 ± 0.06	0.015
Abdominal: Limb FM	0.285 ± 0.02	0.303 ± 0.03	0.617

TABLE 5.6 Differences in the pattern of fat distribution obtained by DXA adjusted for height and weight

	Lower BW (n=16)	Higher BW (n=13)	P-value
FFM (kg)	59.05 ± 0.87	62.44 ± 098	0.026
FFST (kg)	56.25 ± 0.81	59.67 ± 0.92	0.017
BMC (kg)	2.79 ± 0.12	2.76 ± 0.14	0.860
Muscle mass (kg)	27.22 ± 0.53	29.50 ± 0.59	0.014
Non-muscular FFST (kg)	29.03 ± 0.51	30.17 ± 0.57	0.185
FM (kg)	24.58 ± 0.87	21.19 ± 0.98	0.026
FM (%)	29.44 ± 1.16	24.63 ± 1.31	0.018
Muscle: non muscular FFST (kg)	0.94 ± 0.02	0.98 ± 0.24	0.256

TABLE 5.7 Differences in the pattern of fat distribution obtained by DXA adjusted for height and weight

	Lower BW (n=16)	Higher BW (n=13)	P-value
Non-limb FM (kg)	14.75 ± 0.57	12.15 ± 0.64	0.010
Trunk FM (kg)	13.69 ± 0.58	11.12 ± 0.65	0.012
Abdominal FM (kg)	2.82 ± 0.15	2.57 ± 0.1	0.334
Limbs FM (kg)	9.84 ± 0.40	9.05 ± 0.46	0.241
Non-limb: Limb FM	1.52 ± 0.06	1.36 ± 0.07	0.136
Trunk: Limb FM	1.41 ± 0.06	1.23 ± 0.06	0.058
Abdominal: Limb FM	0.280 ± 0.02	0.309 ± 0.03	0.447

TABLE 5.8 Differences in body composition obtained by DXA adjusted for BMI

	Lower BW (n=16)	Higher BW (n=13)	P-value
FFM (kg)	56.90 ± 1.30	65.08 ± 1.42	0.001
FFST (kg)	54.26 ± 1.19	62.13 ± 1.33	0.001
BMC (kg)	2.65 ± 0.13	2.94 ± 0.14	0.145
Muscle mass (kg)	26.19 ± 0.67	30.77 ± 0.75	0.001
Non-muscular FFST (kg)	28.06 ± 0.65	31.36 ± 0.72	0.001
FM (kg)	23.50 ± 0.96	22.53 ± 1.07	0.507
FM (%)	29.13 ± 1.04	25.01 ± 1.16	0.014
Muscle: non muscular FFST (kg)	0.94 ± 0.02	0.99 ± 0.21	0.084

TABLE 5.9 Differences in the pattern of fat distribution obtained by DXA adjusted for BMI

	Lower BW (n=16)	Higher BW (n=13)	P-value
Non-limb FM (kg)	14.12 ± 0.61	12.92 ± 0.68	0.201
Trunk FM (kg)	13.10 ± 0.61	11.86 ± 0.68	0.185
Abdominal FM (kg)	2.75 ± 0.14	2.67 ± 0.15	0.728
Limbs FM (kg)	9.39 ± 0.43	9.61 ± 0.47	0.727
Non-limb: Limb FM	1.53 ± 0.06	1.35 ± 0.06	0.036
Trunk: Limb FM	1.41 ± 0.05	1.22 ± 0.06	0.016
Abdominal: Limb FM	0.291± 0.02	0.296 ± 0.02	0.885

TABLE 5.10 Summary for the differences in body composition and fat distribution between lower and higher birth weight groups (lower compared to higher birth weight group) before and after adjusting for body size

	Not adjusted	Adjusted for height	Adjusted for weight	Adjusted for height + weight	Adjusted for BMI
FFM	LWR (16%)	LWR (9%)	LWR (8%)	LWR (6%)	LWR (14%)
FFST	LWR (16%)	LWR (10%)	LWR (9%)	LWR (6%)	LWR (14%)
Muscle mass	LWR (19%)	LWR (12%)	LWR (11%)	LWR (8%)	LWR (17%)
Non-muscular FFST	LWR (13%)	ND *	ND *	ND	LWR (12%)
FM (kg)	ND	ND	GRT (22%)	GRT (16%)	ND
FM (%)	ND *	ND **	GRT (25%)	GRT (20%)	GRT (17%)
Non-limb FM	ND	ND	GRT (29%)	GRT (21%)	ND **
Trunk FM	ND	ND	GRT (32%)	GRT (18%)	ND **
Abdominal FM	ND	ND	ND **	ND **	ND
Limb FM	Lower	ND	ND **	ND **	ND
Non limb: limb FM	GRT (25%)	ND	GRT (14%)	ND **	GRT (13%)
Trunk: limb FM	GRT (15%)	ND	GRT (17%)	ND **	GRT (16%)
Abdominal: limb FM	GRT (16%)	ND	ND	ND	ND

LWR= Lower, GRT= Greater, ND= not different

* Tendency to be lower

** Tendency to be greater

5.4 Discussion

The aim of the analysis performed in this chapter was to examine the contribution that body size, in terms of height and weight both separately and together, may make to the reported differences in body composition and fat distribution associated with birth weight obtained by DXA. In addition, this analysis aimed to examine the expected differences in body composition and fat distribution associated with birth weight if BMI is used to assess the risk of adiposity associated with birth weight or used as confounder to account for the differences in body habitus when examining the relationship between birth weight and metabolic risk factors associated with CVD in later life.

The results of this section raises important issues about the differences in body composition and pattern of fat distribution associated with birth weight in relation to body size. First, both the variance in height and weight, separately and together, did not explain the reported differences in absolute lean mass (FFM, FFST and muscle mass) between the lower and higher birth weight group. This suggests that even after adjusting for height or weight and both height and weight, there are differences in lean mass remain that cannot be explained simply by differences in body size alone. However, absolute fat mass which was not different between the two groups becomes greater in the lower than the higher birth weight group after considering the differences in weight and height + weight. Secondly, central fat mass (non-limb and trunk fat mass) which was not different between the two groups also becomes greater in the lower than the higher birth weight groups after accounting for the difference in weight and both height and weight. These findings suggested that the differences in absolute lean (FFM, FFST and muscle mass), fat mass and greater central fat mass are programmed in early life independently of any effects on body size. Although differences in lean mass were independent of body size, failing to take account of differences in height and weight may mask differences in absolute fat mass which are considered to be linked with metabolic risk factors associated with CVD.

The increase in lean mass and muscle mass was positively associated with both height and weight. Therefore, it would be expected that accounting for the differences in these measures would explain greater proportion of the variance in lean mass (FFM, FFST and muscle mass) associated with birth weight. Although age, ethnicity and gender also contributes to the variance in lean mass, these factors are not relevant to the subjects of this analysis since all were male,

of similar age and ethnic origin. In this analysis even after accounting for height, weight and height + weight between the lower and higher birth weight groups, there were still variances in FFM, FFST and muscle mass that could not be explained simply by differences in body size. Each of the measures of body size has a different effect on the difference in lean mass seen between the groups. For example, the variance in FFM associated with birth weight was reduced by weight greater than that explained by height alone. However, when both height + weight were considered together, the variance in FFM was further reduced when compared with that observed before or after adjusting for height and weight separately (see Table 4.10). When birth weight was used to predict FFM in post-hoc multiple regression analysis (r=0.622), the residual standard deviation was (5.81 kg). When height + weight were also added to the regression model the residual standard deviation was reduced to 3.18kg. The results presented in this chapter suggested that for each kilogram of body weight and centimetre of height, the lower birth weight group have less absolute FFM (≈ 3.26 kg), FFST (≈ 3.29 kg) and muscle mass (≈ 2.19 kg) than those in the higher birth weight group.

Weight was also reported in this chapter to be positively associated with absolute fat mass. Therefore, it would be expected that the lower birth weight group to have lower absolute fat mass (lighter) than those in higher birth weight group (heavier). However, both groups have comparable absolute fat mass before considering any differences in body size. When the differences in body weight and both height + weight were considered, absolute fat mass was found to be greater in the lower than the higher birth weight group. These results suggested that for each kilogram of body weight and centimetre of height, the lower birth weight group have greater absolute fat mass (3.26 kg) than those in the higher birth weight group. The same finding was also found to be applicable to central fat mass (non-limb and trunk fat mass) before accounting for the differences in body size. When both height + weight were considered, the lower birth weight group have greater non-limb and truck fat mass when compared with those in the higher birth weight group.

Body size marked by height and weight reflects differences in the vertical dimension of the body but does not completely describe body shape (i.e. leg and trunk length) which may contribute to the differences in body composition associated with birth weight. Norgan *et al* suggested that the nature of the relationship between BMI and body composition seen in different ethnic groups may be explained by the differences in body proportions as marked by sitting height relative to stature (243). In addition, differences in frame size (horizontal dimension) are also believed to

contribute to the inter-relationship between BMI and body composition seen in different ethnic groups (244). The extent to which differences in body proportions may explain the differences in body composition associated with birth weight remains to be determined. These issues will be explored in the next chapter.

This analysis also revealed differences in body composition and fat distribution associated with birth weight for any given BMI. At the same BMI, the lower birth weight group was shorter and lighter than higher birth weight group. In addition, they had lower absolute lean mass (FFM, FFST, muscle mass and non-muscular FFST) and surprisingly greater relative body fat (%). Although the central fat mass (non-limb, trunk and abdominal fat mass) was not different between the two groups at the same BMI, the lower birth weight group have greater non-limb: limb and trunk: limb fat mass ratios. These findings suggest that using BMI to assess the risk of adiposity associated with birth weight may of itself, influence the nature of the results. In addition, this may also help to explain (together with differences in fat distribution) the apparent paradox of a lower risk of cardiovascular disease and diabetes in higher birth weight individuals with the same and even greater BMI than in lower birth-weight individuals (133;137). Furthermore, previous studies that have attempted to control for body habitus as a confounder to examine the relationship between birth weight and metabolic risk factors using BMI may have done so inappropriately and inadequately.

An analogous situation has also been reported in relation to ethnicity. For example, at the same adult BMI, Asians population may have 2–6% more of their body weight as fat than do Caucasian (14;245-247), who generally have a lower risk of cardiovascular disease and diabetes compared to Asians. Such differences in relative body fat associated with ethnicity have been attributed to the variation in body size (i.e. stature) and shape (i.e. leg length or sitting height) between individuals within the same or even different ethnic origin.

5.5 Summary

The aim of this chapter were to examine the contribution of height and weight both independently and together on the reported differences in body composition and fat distribution associated with birth weight obtained by DXA. In addition, to examine the expected differences in body composition and fat distribution associated with birth weight at the same BMI. The

results of this analysis suggested that:

- Neither height nor weight independently can explain the reported differences in body lean mass (FFM, FFST and muscle mass) between the lower and higher birth weight group. However, absolute fat mass which was not different between the two groups become greater in the lower than the higher birth weight group after considering the differences in height + weight. The same result was also observed for central fat mass presented as non-limb and trunk fat mass. These finding suggests that being born with lower birth weight is associated with less absolute lean mass in adulthood when compared with adult born with higher birth weight independent of height + weight. Absolute fat mass in whole body and in the central regions of the body were greater in the lower than the higher birth weight group only after considering the differences in height + weight. This suggest that real differences in absolute fat mass in the associated with birth weight could be masked if the difference body size was not considered

- There were differences in both body composition and fat distribution associated with birth weight at the same BMI. Therefore, this finding may explain the paradox associated with the increase rather than decrease in BMI associated with increase in birth weight.

CHAPTER 6

Assessment of body composition in older men with different birth weight in relation to body shape – third level of analysis

6.0 Introduction

The pattern of growth in early life marked by birth weight is recognised to have life long effects on body structure in adulthood. Others have shown that infants born with a lower birth weight are shorter and lighter and have a lower lean mass (FFM and muscle mass) than those born with higher birth weight. The work described in this thesis confirm these findings and also suggest that adult of a lower birth weight have lower lean mass and greater whole body fat mass even at the same body size in terms of height and weight. One possible explanation for such differences in body composition between groups independent of body size (height + weight) might be attributed to the variation in body shape. Body shape can be defined by the dimensions of the body both vertically (i.e. body proportions or leg and trunk length) and horizontally (i.e. frame size or the breadth of sets of bones at shoulder, hips and wrist). Although many studies have found that birth weight is positively associated with height, few have examined the extent to which such increase in height is coupled with variation with both dimensions of the body.

The vertical dimension of the body, largely determined by leg and trunk length reflects two different body segments each of which have different mass and composition (i.e. leg have greater muscle and less fat mass when compared to trunk). If differences in height associated with birth weight are largely due to the length of the leg for example, the mass per unit of length of each segment would be different between groups (length and mass differences between body segments). For instance, the mass per centimetre of the leg would be different from that of the trunk. Thus, it would be reasonable to presume that the reported differences in body composition associated with birth weight may be associated with or due to the variation in segments mass per unit of length. Even if the mass of each segment is assumed to be the same, variation in the length of the segments may contribute to differences in body composition associated with birth weight (i.e. kg/cm leg length would be different from that observe in the trunk). As the lower birth

weight group were shorter than the higher birth weight group, the extent to which such differences in height is associated with differences in segments length needs to be determined.

In large epidemiological studies, BMI has been used as an index to assess the prevalence of cardiometabolic risk within the population. The assumption that underlies the use of BMI is that it marks the major proportion of the variance in fat mass as percentage of body weight (%FM) between individuals. However, it has been suggested that differences in body shape (i.e. leg length) associated with differences in ethnicity may contribute to the observed differences in % body fat at a given BMI. For example, Deurenberg et al have used a slenderness index (height/sum of wrist and knee breadth) and relative sitting height (sitting height as a proportion of stature) as a measure of body shape to correct for differences in % fat between different ethnic groups (244). This study suggested that correction for body shape accounted for about a 4% difference in % fat between a group of Dutch and Chinese from Singapore. This correction was also reducing the variance in the % fat at a given BMI between these two ethnic groups. In addition, Norgan et al regressed BMI (as crude measure of adiposity or body fatness) on relative sitting height (i.e. increase in the ratio of sitting height to stature is associated with shorter leg) to determine the contribution of "long-leggedness" to the lower BMI observed among 26 groups of Australian aborigines (248). The author concluded that the contribution of "long-leggedness" to lower BMI in Australian aborigines was as much as 2 kg/m^2. In other words, the increase in leg length in this group of people would be associated with lower %FM equivalent to that observed with lower BMI by 2 kg/m^2 (assuming BMI is positively associated with %FM). The author suggested that single cut-off values of BMI for assessing adiposity should not be made across populations without allowance for differences in body shape as much as differences in ethnicity. The above studies however, did not take into account the possible interaction between the dimensions of the body and the composition of each segment of the body. This is because these studies only involve anthropometry and densitometry/water dilution techniques to assess body composition, without direct measurements of body dimensions. However, these studies provide some insight into the possible effect of body shape that might contribute to the variation in composition. The extent to which differences in body shape may also explain the reported differences in body composition associated with birth weight within the same population remain to be determined.

6.1 Aim

The current analysis aimed to:

- Examine the extent to which differences in height associated with birth weight was coupled with differences in different body segments length and frame size. That was achieved by measuring the length of the trunk and leg (vertical dimension) and the breadth of the bone at shoulder (horizontal dimension) obtained form enlarged DXA scan.

- Examine the extent to which the reported differences in body composition associated with birth weight at given height + weight can be explained by the mass or composition of different body segments. That was achieved by assessing the differences in the mass and proportion of tissue (lean and fat mass) within each body segment between the lower and higher birth weight group obtained by DXA.

- Examine the extent to which the reported differences in body composition associated with birth weight at given height + weight is attributed by both the dimensions of the body and the mass of each body segments. That was achieved by developing two different statistical ANCOVA models. In these models the length, frame size and mass per unit of each segment length were used as covariate when the differences in body composition between groups were examined.

6.2 Subjects and Methods

The analyses performed in this chapter were based on the information gained from previous chapters which include differences in body composition between the lower and higher birth weight group obtained by DXA. The dimensions of the body both vertically and horizontally were obtained from the DXA scans. The scans were photo-enlarged approximately threefold, and the distances measured accurately to the nearest 0.5 mm with a ruler. These measurements were converted to actual measurements by relating them to the known dimensions of the scanned area (vertical dimension;1 cm from the enlarged scan = 6.745 cm and horizontal dimension; 1

cm from enlarged scan = 5.240 cm). The technique had been previously validated to within 1% by scanning metal rods of known length (0.5-1.5 m). The measurements of segments were made as follows:

1. Head and neck - from the top of the skull to the midpoint of a line joining the top of the two acromial processes

2. Trunk - from the lower end of the neck to the midpoint of a line joining the top of the two greater trochanters

3. Legs - difference between measured height and the length of head + neck + trunk.

Based on examination of healthy men and skeletons, the length of the lower leg was also calculated as 1.14 times the distance between the middle of the knee and ankle joints (the distance to bottom of the heel (calcaneus) was not undertaken because the position of the foot relative to the lower leg was not standardised, with the result that the calcaneus was sometimes obscured by the presence of other bones, and also errors would be introduced by variations in angles in different planes between tibia on the one hand and upper foot bones on the other. Measurements on the legs were made on both the left and right side and an average taken for each subject. The measurement of frame size was that obtained between the two edges of the acromial bone. To be consistent with the segment length measurements obtained from the enlarged DXA scan, the total height of the body was estimated from the sum of its components:

Lower leg - 1.14 x distance from knee joint to ankle joint);

Upper leg - distance from top of trochanter to middle of knee joint);

Trunk;

Head + neck.

The intra-observer coefficient of variation for the measurement of the length of body segments

were as follows: 1.1% (0.4 cm) for the lower leg; 1.35% (0.42 cm) for the bitrochanteric breadth; 1.12% (0.41 cm) for biacromial breadth; and ≤ 1.0% for the other measurements.

The mass and composition of the trunk, legs and arms and remaining parts of the body (head and neck) was that reported by the DXA software. The mass of the trunk included the pelvis, which was defined by a diagonal interface with the leg, which passed though the outer border of the pelvis and through the neck/head of the femur. In calculating mass per cm of legs and trunk, the entire mass of the trunk and legs were divided by corresponding measurements of length, as defined above.

6.3 Results

The results of this section are presented in three parts. The first, illustrate the influence of birth weight on adults body dimensions both vertically and horizontally. The second presents the influence of birth weight on body segments mass and composition. The third illustrate the contribution of the variation of current segments length and mass between groups on the reported differences in body composition associated with birth weight.

6.3.1 Differences in body dimensions associated with birth weight

The differences in body proportion (non-limb, trunk and leg length) and frame size between the lower and higher birth weight group before and after accounting for the differences in height are presented on **Table 6.1** and summarised in **Figure 6.1.** Measured height did not differ significantly from the height calculated for the component segments for all subjects (174.09 ± 1.32 v 174.55 ± 1.32 cm respectively; P 0.185, by paired t-test; r = 0.945, mean ± SE). Similarly, leg length measured by the length of its components (upper leg + (1.14 x lower leg)) did not differ significantly from leg length measured by difference (90.68 ± 0.95 v 89.90 ± 0.82 cm respectively; P = 0.166; r = 0.828).

The lower birth weight individuals were significantly shorter (≈ 9 cm) than those in the higher birth weight group. 80% of the difference in height between the two groups was attributed to the shorter leg length and the remaining differences were related to head & neck (≈ 18%) and trunk length (≈ 2%). Although the differences in leg length was significantly different between the two

groups and was approximately equally distributed between upper and lower legs (3.16 v 3.41 cm), the differences in head & neck and trunk length was not different between the lower and higher birth weight group. Arm length was significantly shorter (≈ 8%; P = 0.001) in the lower than the higher birth weight group and these differences were equally distributed between the upper and lower arm length (≈ 9 v 7%). Biacromial and bitrochanteric breadths (horizontal dimensions) were significantly smaller (≈ 7.6; P = 0.001 and 7.4%; P = 0.003 respectively) in the lower than the higher birth weight group.

Height was positively associated with all measures of body dimension [(Biacromial (r= 0.402; P = 0.045), bitrochanteric (r = 0.715; P = 0.001) breadths and trunk (r = 0.743; P=0.001) and leg (r = 0.667; P = 0.001 length)] for all subjects in this study. However, after accounting for the differences in height between the two groups, the lower birth weight individuals had significantly shorter leg length (≈ 3%; P = 0.003) than the higher birth weigh groups and this difference tended to be equally distributed between the upper (≈ 4.4%) and lower (≈ 5%) leg length. In addition trunk length and non-limb length (head & neck and trunk) were significantly greater (≈ 3.5; P = 0.049 and 3.2%; P = 0.006 respectively) in the lower than the higher birth weight group after accounting for the differences in height between the two groups.

Although, arm length tended to be shorter (≈ 4.5%) in the lower than the higher birth weight group after accounting for the differences in height, this difference was not statistically different between the two groups. The differences in arm length in the lower than the higher birth weight group tended to be related to greater shortness of the upper arm length compared with that in the lower arm length (≈ 6.5 v 2%). Biacromial and bitrochanteric breadths (horizontal dimensions) were significantly smaller (≈ 7.5; P = 0.001 and 3.7%; P = 0.047) in the lower than higher birth weight groups after adjustment was made for the differences in height (see Figure 6.1).

The results obtained in this section for the analysis indicated that at the same height, the lower birth weight group have shorter leg length and greater non-limb and trunk length. In addition, they have a narrower frame size (i.e. Biacromial breadth) when compared to higher birth weight groups (see Figure 6.1). The inclusion of these measurements to the ANCOVA as a covariate did not have an effect on the reported differences in absolute FM and FFM associated with birth weight at the same height and weight. Compared to the higher birth weight group, the lower birth

weight subjects had significantly lower FFM (≈ 10%; P = 0.004) and greater absolute FM (≈ 29%; P = 0.004) when adjustments were made for weight, non-limb length, leg length and frame size (bitrochanteric breadth).

6.3.2 Differences in the mass and composition of body segments associated with birth weight

The differences in the mass and proportion of tissue (lean to fat mass) in each body segments associated with birth weight before and after adjusting for height and weight are presented in **Table 6.2** and **6.3**. Compared to higher birth weight individuals, adults with a lower birth weight had significantly lower limb mass (≈ 15%; P = 0.006). The non-limb mass was not different between the two groups although it tended to be lower (≈ 9.5%) in the lower than the higher birth weight group. The differences in limb mass represented a greater proportion of the differences in total body mass (≈ 54%) between the two groups and remaining was attributed to the differences in non-limb mass (≈ 44%). After accounting for the differences in body size (height + weight) between the two groups, the non-limb and limb mass were not different between the two groups.

Absolute FFM in the non-limb and limb segment were significantly lower in the lower than higher birth weight group (≈ 14.5%; P= 0.001 and 17.5%; P = 0.001 respectively). However, absolute fat mass in both segments of the body were not different between the two groups. Fat mass relative to non-limb and limb mass was also not different between the two groups although relative non-limb fat mass tended to greater in the lower than the higher birth weight group (≈ 15%). The contribution of non-limb and limb FFM to the reported differences in total body FFM (before adjustment was made for height + weight) between the two groups was ≈ 52% and ≈ 48% respectively. After accounting for the differences in height + weight between the two groups, non-limb FFM was significantly reduced (≈ 6%; P = 0.040) in the lower than the higher birth weight group. In contrast, non-limb FM was significantly greater (≈ 22%; P = 0.010) in the lower than higher birth weight group. Limb FFM and FM was not different between the two groups although limb FFM to be lower (≈ 6%) and limb FM tended to greater in (9%) in the lower than the higher birth weight group. Non-limb FFM and FM represents greater proportion of the reported differences in total body FFM and FM between the two groups at the same height + weight (≈ 55% and 76% respectively) and the remaining was related to limb FFM and FM (45% and 24% respectively) (see Figure 6.2).

The non-limb represents the head & neck and trunk segments of the body, the contribution of these segments to the differences in the mass and FFM of the non-limb (before adjusting for height + weight) associated with birth were largely due to the differences in mass and FFM of the trunk (≈ 92 and 93% respectively). However, the mass of the head & neck and trunk were not different between the two groups although trunk mass tended to be reduced (≈ 10%) in the lower than the higher birth weight group. FFM was significantly lower in both segments (head & neck (≈ 8%; P = 0.021) and trunk ≈ 16%; P = 0.001)) in the lower than the higher birth weight group. After the differences in height + weight were considered between the lower and higher birth weight groups, trunk mass was not different between the two groups. However, FFM was significantly reduced in the lower (≈ 7%; P = 0.019) than the higher birth weight group, while trunk FM was greater (≈ 23%; P = 0.012) in the lower than higher birth weight group.

The limb component represents leg and arm segments of the body. The contribution of these segments to the differences in total mass and FFM of the limbs between the two groups were largely due to greater leg mass (≈ 62 v 38%: leg v arm) and FFM (≈ 65 v 35%: leg v arm) between the two groups. Compared to the higher birth weight group, adults with a lower birth weight had significantly lower mass and FFM in the arm (≈ 16; P = 0.002 and 22%; P = 0.001) and in the leg (≈ 13; P = 0.013 and 16%; P = 0.002). Although arm and leg fat mass relative to each segment mass were not different between the two groups, they tended to be greater in the lower than the higher birth weight group (≈ 7 and 9 % respectively). After accounting for the differences in height + weight between the two groups, neither leg FFM and FM were different between the two groups. However, arm mass and FFM were significantly lower (≈ 7 and 13%) in the lower than higher birth weight group.

6.3.3 Variation in segments length and mass in relation to the differences in body composition associated with birth weight

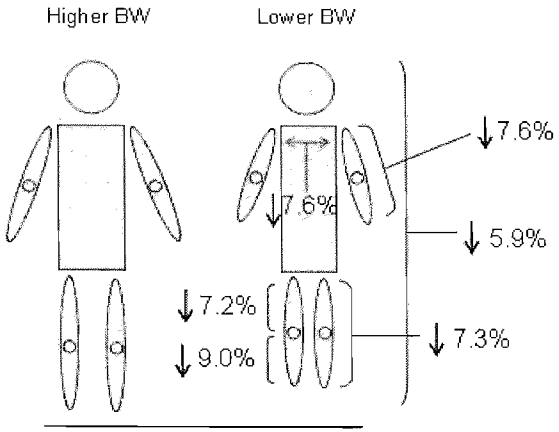
The above results indicted that there were significant differences in segment length and frame size at the same height (i.e. shorter leg length in the lower than higher BW group at the same height), and no differences in segment mass (i.e. non-limb and limb) between the two groups at the same height + weight. Therefore, the mass per unit of length of each of these segments would be expected to be different between the two groups as the result of the differences in length for comparable mass. Because segment length, mass and frame size reflect different

damnations of the body (body shape) which are linked with each other and that in turn, may account in part, for the reported differences in body composition associated with birth weight. To account for such variation in body shape between the two groups, two different ANCOVA models were used. These models included measures of segment length, frame size and mass per unit of length of the non-limb, trunk and leg as a covariate in this analysis. The difference between the two models was only related to the use of non-limb or trunk length and mass. The two models involved segments of the body (segment lengths and mass/cm of each segments) that accounted for a mean of 87.5 $\pm$ 0.15 % of body mass (mass of whole body – mass of arms) and 81.0 $\pm$ 0.17 of total body mass. There was a very high correlation between the mass of segments involved in the model and the mass of the body (r = 0. 998 and 0.997 respectively).

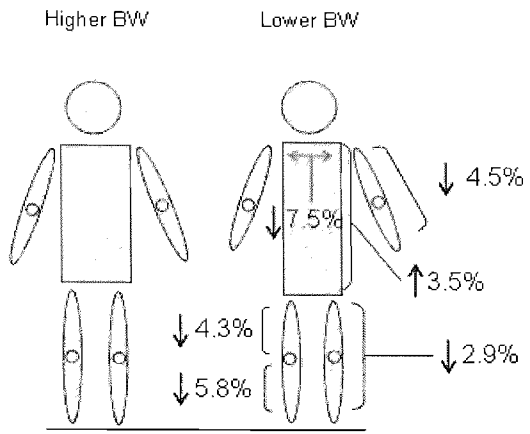
The differences in segments mass per unit of length for each body segments between the lower and higher birth weight group after adjustment was made for height and weight are presented in **Table 6.4**. The mass/cm of the non-limb, trunk and leg were not significantly different between the lower and higher birth weight group before and after adjustment was made for height + weight. However, the mass/cm of the non-limb and trunk tended to be greater (≈ 7 and 8% respectively) in the lower than higher birth weight group and these differences were reduced (2 and 3%) when adjustment was made for height + weight. When these measures as well as segments length and frame size were used in the models as a covariate yet the lower birth weight group had significantly lower FFM (≈ 19%) and greater absolute fat mass (≈ 26%) see Table 6.5.

TABLE 6.1 Differences in body proportion (segments length) associated with birth weight

	Unadjusted			Adjusted for height		
	Lower BW (n=16)	Higher BW (n=13)	P-value	Lower BW (n=16)	Higher BW (n=13)	P-value
Head & neck length (cm)	25.84 ± 0.51	26.39 ± 0.49	0.446	26.38 ± 0.45	25.73 ± 0.51	0.380
Head & neck + trunk length (cm)	83.54 ± 1.03	85.29 ± 1.15	0.265	85.51 ± 0.53	82.88 ± 0.61	0.006
Trunk length (cm)	57.70 ± 0.84	58.90 ± 0.95	0.352	59.12 ± 0.59	57.15 ± 0.66	0.049
Leg length (cm)	86.83 ± 0.94	93.17 ± 0.97	0.001	88.54 ± 0.53	91.12 ± 0.53	0.006
Upper leg length (cm)	44.06 ± 0.53	47.22 ± 0.54	0.001	44.61 ± 0.49	46.55 ± 0.56	0.023
Lower leg (knee to ankle joint) (cm)	37.82 ± 0.49	41.23 ± 0.55	0.001	38.37 ± 0.49	40.61 ± 0.54	0.007
Arm length (to wrist joint) (cm)	56.13 ± 0.69	60.42 ± 0.91	0.001	56.92 ± 0.81	59.47 ± 0.94	0.070
Upper arm length (cm)	29.00 ± 0.59	31.51 ± 0.63	0.008	29.28 ± 0.60	31.17 ± 0.71	0.075
Lower arm (elbow-wrist joint) (cm)	27.13 ± 0.39	28.92 ± 0.67	0.023	27.64 ± 0.48	28.30 ± 0.55	0.406
Biacromial breadth (cm)	35.63 ± 0.37	38.35 ± 0.38	0.001	35.68 ± 0.41	38.36 ± 0.41	0.001
Bitrochanteric breadth (cm)	30.49 ± 0.37	32.75 ± 0.39	0.003	30.99 ± 0.36	32.13 ± 0.34	0.047



Lower birth weight are shorter, have shorter leg length, arm length and frame size than higher birth weight individual



At the same height, Lower birth weight have shorter leg length, arm length and frame size and grater trunk length than higher birth weight individual

FIGURE 6.1 Differences in segments length and frame size (biacromial breadth) between the lower and higher birth weight group before and after accounting for height + weight

TABLE 6.2 Differences in the composition of upper body segments associated with birth weight obtained by DXA

	Unadjusted			Adjusted for height and weight		
	Lowe BW (n=16)	Higher BW (n=13)	P-value	Lowe BW (n=16)	Higher BW (n=13)	P-value
Non-limb						
Mass (kg)	45.70 ± 4.48	50.01 ± 7.54	0.072	47.94 ± 0.43	47.40 ± 0.49	0.853
Fat mass (kg)	13.74 ± 0.70	13.38 ± 1.42	0.811	14.71 ± 0.57	12.15 ± 0.64	0.010
% fat	29.90 ± 1.16	25.95 ± 1.93	0.078	30.77 ± 1.23	24.88 ± 1.39	0.007
FFM (kg)	31.97 ± 0.80	36.63 ± 0.91	0.001	33.23 ± 0.52	35.08 ± 0.59	0.040
Head & neck						
Mass (kg)	5.28 ± 0.53	5.58 ± 0.50	0.134	5.46 ± 0.10	5.51 ± 0.11	0.715
Fat mass (kg)	1.01 ± 0.02	1.07 ± 0.03	0.146	1.05 ± 0.02	1.02 ± 0.02	0.409
% fat	19.21 ± 0.12	19.12 ± 0.20	0.718	19.18 ± 0.15	19.16 ± 0.16	0.958
FFM (kg)	4.28 ± 0.11	4.66 ± 0.11	0.021	4.41 ± 0.09	4.49 ± 0.10	0.589
Trunk						
Mass (kg)	40.42 ± 1.12	44.43 ± 1.98	0.076	42.48 ± 0.35	41.89 ± 0.39	0.315
Fat mass (kg)	12.73 ± 0.69	12.31 ± 1.39	0.781	13.69 ± 0.58	11.12 ± 0.65	0.012
% fat	31.26 ± 1.29	26.74 ± 2.14	0.070	32.26 ± 1.37	25.52 ± 1.55	0.006
FFM (kg)	27.69 ± 0.73	32.12 ± 0.82	0.001	28.79 ± 0.48	30.77 ± 0.54	0.019

TABLE 6.3 Differences in the composition of limbs associated with birth weight obtained by DXA

	Unadjusted			Adjusted for height and weight		
	Lowe BW (n=16)	Higher BW (n=13)	P-value	Lowe BW (n=16)	Higher BW (n=13)	P-value
Limb						
Mass (kg)	33.72 ± 1.03	38.78 ± 1.36	0.006	35.66 ± 0.35	36.39 ± 0.3	0.205
Fat mass (kg)	9.14 ± 0.45	9.90 ± 0.95	0.445	9.84 ± 0.40	9.05 ± 0.45	0.241
% fat	27.11 ± 1.15	24.97 ± 1.80	0.311	27.67 ± 1.27	24.29 ± 1.44	0.117
FFM (kg)	24.58 ± 0.84	28.87 ± 0.66	0.001	25.81 ± 0.54	27.35 ± 0.61	0.094
Arm						
Mass (kg)	9.60 ± 0.29	11.52 ± 0.50	0.002	10.14 ± 0.18	10.86 ± 0.21	0.025
Fat mass (kg)	2.82 ± 0.15	3.25 ± 0.34	0.220	3.09 ± 0.15	2.91 ± 0.16	0.459
% fat	29.31 ± 1.22	27.37 ± 2.05	0.404	30.51 ± 1.41	25.89 ± 1.59	0.058
FFM (kg)	6.78 ± 0.23	8.27 ± 0.24	0.001	7.05 ± 0.19	7.95 ± 0.22	0.009
Leg						
Mass (kg)	24.13 ± 0.78	27.26 ± 0.88	0.013	25.52 ± 0.28	25.54 ± 0.32	0.964
Fat mass (kg)	6.33 ± 0.35	6.66 ± 0.63	0.613	6.75 ± 0.31	6.14 ± 0.35	0.239
% fat	26.20 ± 1.27	23.98 ± 1.78	0.308	26.51 ± 1.35	23.59 ± 1.52	0.199
FFM (kg)	17.80 ± 0.64	20.59 ± 0.49	0.002	18.77 ± 0.41	19.40 ± 0.46	0.353

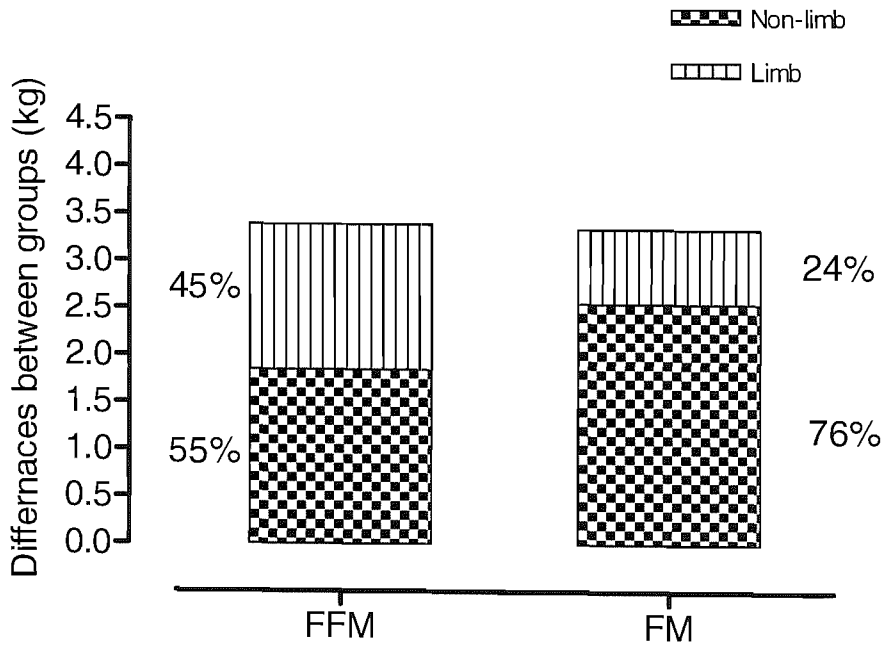


FIGURE 6.2 Contributions of non-limb and limb body composition to the differences in total body composition associated with birth weight after adjusting for height + weight

TABLE 6.4 Differences in mass per cm of body segments between the lower and higher birth weight groups

	Unadjusted			Adjusted for height and weight		
	Lower BW (n=16)	Higher BW (n=13)	P-value	Lower BW (n=16)	Higher BW (n=13)	P-value
Non-limb	0.547 ± 0.012	0.588 ± 0.026	0.140	0.561 ± 0.005	0.570 ± 0.005	0.238
Trunk	0.700 ± 0.015	0.755 ± 0.033	0.120	0.717 ± 0.009	0.735 ± 0.009	0.185
Legs	0.277 ± 0.007	0.293 ± 0.009	0.197	0.288 ± 0.003	0.280 ± 0.003	0.099

TABLE 6.5 Body composition in lower and higher birth weight groups after adjustment for length of body segments and mass/cm of segments

	Adjusted for body proportions[1]			Adjusted for body proportions[2]		
	Lower BW (n=16)	Higher BW (n=13)	P-value	Lower BW (n=16)	Higher BW (n=13)	P-value
Fat mass (kg)	25.49 ± 0.83	20.08 ± 0.96	0.002	25.48 ± 0.90	20.09 ± 1.04	0.003
% body fat	30.53 ± 1.12	23.28 ± 1.29	0.002	30.54 ± 1.18	23.27 ± 1.37	0.003
FFM (kg)	57.91 ± 0.95	63.84 ± 1.09	0.002	57.83 ± 0.94	63.93 ± 1.09	0.002

[1] Adjusted for trunk length, leg length, trunk mass/cm, and leg mass/cm and frame size (biacromial breadth)

[2] Adjusted for head + trunk length, leg length, head + neck mass/cm, leg mass/cm, and frame size (biacromial breadth)

6.4 Discussion

The analyses performed in this chapter aimed to examine the extent to which differences in height between lower and higher birth weight group were associated with differences in the length of body segments (vertical dimension) and frame size (horizontal dimension). In addition, to examine the extent to which differences in birth weight was associated with segments mass and composition. Finally, the current analysis sought to examine the contribution of such variation in body shape to the reported differences in body composition between the lower and higher birth weight group at the same height + weight.

Compared to the higher birth weight group, the lower birth weight group were shorter, predominantly as a result of the shorter leg length, which accounted for 6.3 (≈ 82%) of the 8.0 cm difference in height between the two groups. The length of the head, neck and trunk (non-limb) were preferentially preserved in the lower than the higher birth weight group which might be associated with preferential preservation of the brain and spinal cord. However, the horizontal dimension of the body (biacromial and bitrochanteric), which are sometimes used as measures of frame size, were still different between the two group at the same height. The biacromial and bitrochanteric breadths were two-fold smaller than the vertical (longitudinal) length of the trunk, but the decrements in horizontal breadths were 1.5 to 2.0-fold greater than the decrement in longitudinal length. These differences in body dimensions remained the same after accounting for the differences in height between the two groups.

Although non-limb and limb mass were lower in the lower than higher birth weight group, these differences were not observed after considering the variation in height + weight between the two groups. At the same height + weight, the lower birth weight group have lower non-limb FFM and greater non-limb FM when compared to higher birth weight group. These differences in the non-limb FFM and FM represents greater proportion of the differences in total body FFM and FM at the same height + weight. This finding suggested that the reported differences in total body composition associated with birth weight are not equally affecting all body segments. Although there were no significant differences in the mass per unit length (kg/cm) of the non-limb, trunk and legs segments between the lower and higher birth weight group, the non-limb, trunk and leg mass per unit of these segments length tended to be greater in the lower than the higher birth weight group. These differences remain the same even after adjusting for height and weight

except for leg mass per unit of leg length which tended to be greater in the lower than the higher birth weight group.

Because body dimensions were different between the two groups and segments mass were not, therefore mass per unit of segments length would be expected to be not the same between the two groups. However, these results show that the mass per unit length of the non-limb, trunk and leg segment were not different between the two groups before and after adjustment was made for height + weight. This finding suggests that variation in the vertical dimension of the body (segments length) and segments mass when they were incorporated together did not explain the observed differences in body composition between the lower and higher birth weight groups at the same height + weight. The statistical models used in the current analysis would be expected to largely take into account differences in height and weight between groups because they already incorporate differences in the length and mass per unit of length of major segments, which account for most or all of the total length of the body and most of the weight of the body (the arms, which are not taken into account in any of the models account for <15% of the weight of the body). Indeed, addition of weight and height to the models made very little difference to the results.

It has been repeatedly reported that secular changes in stature are mainly due to greater leg length than upper body length. Environmental factors, such as poor nutrition and disease, operating during childhood have also been repeatedly linked to height in later life, with preferential effects on the length of the leg rather than the trunk (16;249-251). The analysis in the current chapter suggests that the same may happen through environmental factors operating in prenatal life, which appear to affect the length of the limbs to a greater extent than the length of the trunk. Although many studies have reported a link between lower birth weight and lower adult height, there is very little information linking infant birth weight with adult segments length. However, a recent study by Sayer et al conducted within the same population (Hertfordshire cohort study) reported both stature and sitting height of adults according to quintiles of birth weight (lowest quintile <3062 g, highest quintile >3969 g) (184). The difference in adult height between the highest and lowest quintile was only 3.3 cm, of which 1.6 cm was due to differences in sitting height (implying that the rest (1.7 cm) was due to differences in leg length). The reasons for the difference in relative segment lengths with the present study are unclear, but the discrepancy in height between the lower and higher birth weight group was less than half that seen in the study by Sayer et al, the method of measurement of body segments was different,

and sitting height excludes part of the upper leg. In addition, the sample size was much smaller in the current study. No comparisons with horizontal dimensions, such as biacromial or bitrochanteric breadths were possible because these were not reported in the study of Sayer et al. In addition the study of Sayer also did not determine the extent to which the differences in body proportion affect the reported differences in body composition associated with birth weight.

It is also recognised that ethnic differences in height, predominantly due to differences in relative leg length, have changed over time. For example, differences in height between Japanese and Caucasians, have almost disappeared, predominantly as a result of an increase in leg length in the Japanese (251). Furthermore, differences in body proportions between Maya children brought up in better socioeconomic conditions in the USA, than in their home country (where they have relatively shorter legs than upper segment length compared to children in the USA), show a pattern of upper and lower segment length growth that approaches that of other children in the USA (251). Therefore, it seems that at least some of the ethnic differences in stature and segmental lengths, which were previously attributed to genetic factors, have a strong environmental basis. The potential for altering adult body proportions and composition through interventions during gestation remains to be established. In addition, it has been suggested that ethnic differences in body composition (e.g. more % body fat in Asians than Caucasians at the same BMI) are due to differences in body shape, but the extent to which these differences are due to environmental factors operating during pre-natal or post-natal life, also remains to be elucidated.

The present study also provides information about the proportion of fat to lean mass of individual body segments, which is relevant to the risk of cardiovascular disease and diabetes. For instance, after controlling for height and weight, the fat mass in the non-limb was significantly greater in the lower than the higher birth weight group accounting for ≈ 76% of the extra body fat present in the lower birth weight group. In contrast, the difference in the amount of fat in the arms (0.18 kg) and legs (0.61 kg) in combination was smaller (0.79 kg) and was not significantly different between the two groups. Furthermore, the difference in FFM in the non-limb was significantly lower in the lower than the higher birth weight group. That difference accounted for ≈ 55% of the lower whole body FFM present in the lower birth weight group and rest was related to the differences in arm and leg FFM between the two groups. Although socioeconomic factors such as physical activity, smoking, alcohol intake, social class might influence the observed differences in body shape and body composition between groups there was no significant

difference in these variables between the two groups in the current study, and they were not significant when added individually as covariates in the ANCOVA models of body composition.

Previous findings by Deurenberg and Norgan suggest that greater body fat as percentage of body weight seen between different ethnic groups may be attributed to the variation in body proportions between these groups (243;244). However, the results of this chapter did not support this view since such variation in body proportion (i.e. leg length and trunk length) between the lower and higher birth weight groups did not appear to explain the reported differences in body composition between groups. This may be related to smaller sample size used in the present analysis when compared with these studies. Alternatively, the observed differences in body composition associated with birth weight could be programmed in early life independent to the variation in body proportion. However, the mechanism underlies the programming effect on adult body composition remains to be determined.

6.5 Summary

The analyses presented in this chapter aimed to examine the extent to which differences in stature associated with birth weight were coupled with differences in the length of body segments (vertical dimension) and frame size (horizontal dimension). In addition, to examine the extent to which differences in birth weight was associated with variation in mass and composition of each segment. Finally, the current analysis aimed to examine the contribution of such variation in body shape to the reported differences in body composition between the lower and higher birth weight group at the same height + weight.

The results in this chapter show that:

- There were differences in body dimensions (both vertically and horizontally) associated with birth weight. Infant born with a lower birth weight had shorter leg length and taller trunk and non-limb length when compared to higher birth weight individuals at the same height. The variation in these two dimensions of the body between groups did not explain the reported differences in body composition associated with birth weight at the same height + weight.

- Segments mass (non-limb and limb) were not different between the two groups. However, the composition of these segments was not the same between groups. Non-limb FFM was greater in the lower than higher birth weight group, while non-limb and trunk FM were greater in the lower than higher birth weight group at a given height + weight. Limb FFM and FM were not different between the two groups. Non-limb FFM and FM represents ≈ 55 and 75% of the reported differences in total body FFM and FM between the two groups (at the same height + weight).

- The dimensions of the body and the mass of each body segments when they were integrated in two ANCOVA models also did not show an effect on the reported differences in body composition associated with birth weight.

CHAPTER 7

Influence of birth weight on the inter-relationship between body habitus and energy-substrate metabolism

7.0 Introduction

Previous evidence discussed in chapter 2 (section 2.3.3) suggested that factors altering body size and composition or metabolic activity of the tissue may influence the metabolic demand marked by REE. The results of the previous chapter also suggested that adults who were lighter at birth are shorter, lighter, and have lower lean mass (FFM, muscle mass, non-muscular FFST) as an adult. Given that body structure is a primary determinant of the functional capability or competence, such differences in body structure associated with birth weight would be expected to alter the energy demand and substrate oxidation in favour of a lower REE and substrate oxidation in the lower than higher birth weight groups. To date, the extent to which energy and substrate metabolism may be related to differences in birth weight has not been adequately explored.

7.1 Aim

- Examine the extent to which energy & substrate oxidation both in the fasted and fed state are influenced by early fetal growth. This was achieved by assessing the rates of energy expenditure and carbohydrate and lipid oxidation at rest using indirect calorimetry in the fasted state and over following the ingestion of test meal. In addition, by labelling the lipid within the test meal with ^{13}C-palmitic acid and determining the recovery of tracer on breath, it was possible to further differentiate between the extent to which exogenous lipid oxidation (from dietary intake), and by difference endogenous lipid oxidation (from within body stores) contributed net lipid oxidation and whether this was related to birth

weight.

- Examine the relationship between current body structure in term of body size (height and weight), composition in particular lean mass (FFMST, muscle mass and non-muscular FFST) on the one hand and metabolic function in term of energy & substrate on the other independently of birth weight;

- Examine the extent to which fetal growth marked by birth weight may alter the relationship between body size, composition and energy & substrate oxidation. This was achieved by two different approaches; the first was obtained by presenting the results of energy metabolism relative to body size (height and weight) and lean mass. The second was obtained by examining the differences in energy metabolism substrate oxidation associated with birth weight after accounting for body size and lean mass using the ANCOVA.

7.2 Subjects and method

The general methods have been described in the general methods in previous chapters. Resting energy expenditure (REE) was measured using an open circuit indirect calorimetry system that employed a ventilated hood system (GEM calorimetry, Europa Scientific, Crewe, UK). The calorimeter was calibrated using reference gases before each measurement and the validated prior to and on completion of the study using the alcohol burn technique. Gaseous exchange was measured after an over night fast (12-14 hours) for a period of 30 minutes whilst the subjects remained supine on the bed after waking. After this the subjects consumed the test meal (3720 kJ, of which 15% was derived from protein, 40% from carbohydrate, and 45% from fat) together with 700 mg of ^{13}C-palmitic acid (99 atom percent excess; Cambridge Isotopes Massachusetts, USA) mixed with a lipid-casein-glucose-sucrose emulsion) - see chapter 3 (Section 3.2) and Appendix 2.5. Repeat measurements of REE were undertaken at hourly intervals for 6 hours after the meal. All measurements were undertaken in the recumbent position in a quiet room, at an ambient temperature of 22-25^{o} C. Samples of end tidal expired air breath were collected in a bag (Quintron, Milwaukee, USA) before the test meal, and at hourly intervals for the next 6 hours after the test meal.

The enrichment of $^{13}CO_2$ in breath was measured by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS; 20/20 IRMS-GSL interface, Europa, Scientific Ltd, Crewe, UK). A 24 hour urine collection was also obtained for measurement of urine urea and ammonia (Bayer, Berkshire UK, kit number B01-4132-01) and creatinine (Jaffe reaction; Bayer kit number AD286CR), which together was assumed to account for 95% of total urinary nitrogen (N). Energy expenditure was calculated using the equations of Elia and Livesey (223). The model used in the current analysis to present the oxidative metabolism assumed that total 24 h urine N was distributed between the two 6 hour post-prandial periods and the remaining 12 hour period in the ratio of 1:1.4 (protein oxidation in fed state assumed to be 40%) (224). The equation used for net oxidative metabolism was derived, again using the equations and procedures described by Elia and Livesey.

Dietary induced thermogenesis (DIT) expressed as the incremental area under the curve (AUCi) was derived from the difference between the total energy expended over the postprandial period less that which would be attributed to the fasting energy expenditure assuming that it had remained constant over the study period.

Recovery of $^{13}CO_2$ in breath was calculated using the following formula:

% recovery of administrated dose between x and y hours = (mmol excess ^{13}C per mmol CO_2/ mmol ^{13}C administered) x VCO_2 x 100

Where VCO_2 is the volume of CO_2 excreted in breath between x and y hours – (see appendix 2.8)

Exogenous lipid oxidation (proportion of lipid oxidised from meal) was obtained from the measurement of total lipid oxidation (after meal consumption) multiplied by the fraction of the tracer (^{13}C-palmitic acid) recovered as $^{13}CO_2$ in breath which is determined from the above equation. Endogenous lipid oxidation was obtained from the difference between the total lipid oxidation less the exogenous lipid oxidation.

The differences in absolute (kJ/min) and relative energy metabolism and substrate oxidation in the fasted and fed state (per unit of height or weight, and lean mass) between the lower and

higher birth weight groups was analysed by one way analysis of variance (ANOVA). The effect of birth weight on the relationships between height and weight independently and together as [height + weight], lean mass (FFST, muscle mass, non-muscular FFST) on the one hand and energy-substrate metabolism in fasted and fed state on the other was examined by analysis of covariance (ANCOVA), in which birth weight category was used as an independent factor and all energy-substrate metabolism variables were used separately as the dependant factor. Height, weight, and lean mass were used in this model as a covariate. All statistical analysis was carried using SPSS statistical package (v14.0).

7.3 Results

The results of the analyses presented in this chapter divided into three sections. Section (7.4.1) presents the energy metabolism and substrate oxidation both in the fasted and fed state in relation to birth weight. Section (7.4.2) presents the relationship between current body habitus in term of body size and composition on the one hand and energy metabolism and substrate oxidation on the other for all subjects, independently of birth weight. Section (7.4.3) presents the effect of altered body size in term of height, weight and composition associated with birth weight on energy metabolism and substrate oxidation by using two different approaches.

7.3.1 Energy-substrate metabolism and substrate oxidation in relation to birth weight

The differences in REE both in the fasted and fed state (AUC over 6h) in addition to dietary induce thermogenesis between the lower and higher birth weight group are presented in **Figure 7.1**. Compared to the higher birth weight group, adults with a lower birth weight had significantly lower absolute REE (kJ/min) in the fasted (≈ 14%; P = 0.001) and fed state (≈ 15%; P = 0.001). DIT over 6 hours study period was not different between the two groups. DIT relative (%) to energy intake was also not different between the two groups (5.59 ± 0.75 v 6.41 ± 0.85%; P = 0.464).

The differences in the respiratory quotient (RQ) or fuel selection (carbohydrate v lipid) in the fasted and fed state between the lower and higher birth weight groups are presented in **Figure 7.2**. The RQ both in the fasted and fed state were not different between the two groups.

The differences in macronutrient oxidation (carbohydrate, lipid and protein) presented as REE (kJ/min) in the fasted state between the lower and height birth weight group are presented in **Figure 7.3**. Although the rate of carbohydrate oxidation (kJ/min) was not different between the two groups, the rate of lipid and protein oxidation however tended to be lower by ≈ 15 and 29% respectively in the lower than the higher birth weight group but these differences were not statistically significant. In addition, macronutrient oxidation expressed as a percentage of REE in the fasted state was not different between the two groups (carbohydrate; 36.04 ± 3.25 v 32.71 ± 2.63%), (lipid; 42.76 ± 3.20 v 43.67 ± 3.15%), and (protein; 21.19 ± 2.98 v 23.61 ± 2.44%).

The differences in macronutrient oxidation (carbohydrate, lipid and protein) presented as REE in fed state (kJ/min) between the lower and height birth weight group are presented in **Figure 7.4**. Although the rate of carbohydrate oxidation was not different between the two groups, the rate of lipid and protein oxidation however tended to be lower by ≈ 16 and 30% respectively in the lower than the higher birth weight group but these differences were not statistically significant. In addition, there were no differences in macronutrient oxidation expressed as percentage of REE in the fed state between the lower and higher birth weight groups (carbohydrate; 39.57 ± 3.45 v 36.49 ± 2.98%), (lipid; 38.39 ± 2.76 v 38.93 ± 2.78%), and (protein; 22.04 ± 2.83 v 24.57 ± 2.48%).

The total cumulative recovery of $^{13}CO_2$ (derived from the ingested ^{13}C-PA) in breath (AUC over 6h) were not difference between the lower and higher birth weight groups (9.41 ± 0.73 v 9.67 ± 0.63 PDR). Furthermore, there were also no significant differences between the two groups in the absolute endogenous (1.37 ± 0.10 v 1.57 ± 0.09 kJ/min (AUC over 6h)) and exogenous (0.39 ± 0.05 v 0.47 ± 0.06 kJ/min (AUC over 6h)) lipid oxidation. In addition, there were also no differences in the endogenous (78.11 ± 1.59 v 77.79 ± 1.93%) and exogenous (21.89 ± 1.59 v 22.21 ± 1.93%) lipid oxidation relative to the total lipid oxidation after meal ingestion between the two groups. Taken together, this would indicate that there are no differences in lipid oxidation between groups in response to lipid dietary intake within the meal.

7.3.2 Relationship between current body habitus and energy metabolism and substrate oxidation

The relationships between current body size (height or weight) and REE (kJ/min), RQ and

substrate oxidation (kJ/min) in the fasted and fed state for all subjects are presented in **Figures 7.5-7.8**. In the fasted state, height was positively associated with REE (kJ/min; r=0.290), CHO oxidation (kJ/min; r=0.265) and protein oxidation (g/min; r=0.341), but these associations did not reach statistical significance. Height was also positively but weakly associated with RQ and lipid oxidation; height was negatively associated %REE from lipid oxidation (r=-0.241), and positively associated with %REE from protein oxidation (r=0.237) but again, these associations did not reach statistical significance. Height was also positively but weakly associated with %REE from CHO oxidation. In addition, weight was positively and significantly associated with REE (r=0.453; P=0.014), and protein oxidation (r=0.389; P=0.038). Although there were positive associations between weight and RQ, CHO and lipid oxidation, these associations did not reach statistical significance. Weight was negatively associated with %REE from lipid oxidation (r=-0.148) and positively associated with %REE from protein oxidation (r=0.264) but these association did not reach statistical significance. Weight was also positively but weakly associated with %REE from CHO oxidation. Thus, with increasing body size (as height or weight), there was an increase in REE, as well as the absolute rates of CHO and protein oxidation. At the same time, increasing body size was associated with a reduction in the proportion of REE that could be attributed to lipid oxidation and an increase in that derived from protein oxidation.

In the fed state, height was positively associated with the rate of energy expenditure over the postprandial period (AUC over 6h; r=0.346) and protein oxidation (AUC over 6h; r=0.342). However, this association did not reach statistical significance. Height was also positively but weakly associated with RQ, and CHO and lipid oxidation in the fed state. In addition, weight was positively and significantly associated with the rate of energy expenditure over the postprandial period (r=0.472; P=0.010) and protein oxidation (r=0.386; P=0.038). Weight was also positively associated with the rate of lipid oxidation (r=0.307) but did not reach statistical significance. Weight was also positively but weakly associated with RQ and the proportion of energy expenditure derived from CHO oxidation.

The relationships between body composition (FFST and muscle mass) and REE (kJ/min), RQ and substrate oxidation (kJ/min) in the fasted and fed state for all subjects are presented in **Figures 7.9-7.12**. In the fasted state, FFST was significantly and positively associated with REE (r=0.566; P=0.001) and protein oxidation (r=0.491; P=0.007). In addition, it was also positively associated with lipid oxidation, but this association did not reach statistical significance. FFST was also positively but weakly associated with RQ and CHO oxidation. Muscle mass was

positively and significantly associated with REE (r=0.565; P=0.001) and protein oxidation (r=0.491; P=0.007). Muscle mass was also positively associated with lipid oxidation, but this association did not reach statistical significance. Muscle mass was also positively but weakly associated with RQ and CHO oxidation.

In the fed state, FFST was significantly and positively associated with the rate of energy expenditure over the postprandial period (AUC over 6h; r=0.607; P=0.001) as well as the rates of lipid (r=0.381; P=0.042) and protein (r=0.409; P=0.028) oxidation. FFST was also positively but weakly associated with RQ and carbohydrate oxidation. In addition, muscle mass was significantly and positively associated with the rate of energy expenditure over the postprandial period (r=0.653; p=0.001) as well as the rates of lipid (r=0.381; P=0.049) and protein (r=0.491; P=0.007) oxidation. Muscle mass was also positively but weakly associated with RQ and carbohydrate oxidation.

7.3.3 The effect of altered body size (height or weight) and composition associated with birth weight on energy metabolism and substrate oxidation

Given that size and composition have been shown to be associated with differences in energy metabolism and substrate oxidation, attention is now directed towards determining the extent to which differences in body size and composition associated with birth weight could account for the differences in energy metabolism and substrate oxidation. Two different approaches were used. The first, examined the differences in energy metabolism and substrate oxidation in the fasted and fed state relative to body size by expressing the results per kg body weight and centimetre height and composition per kg lean mass. The second was based on ANCOVA to examine the extent to which differences in energy metabolism and substrate oxidation are independent of height, weight and lean mass.

In the fasted state, REE per kg body weight was not different between the lower and higher birth weight groups. In contrast, adults with a lower birth weight have a significantly lower REE per centimetre of height (≈ 9.0%; P=0.042) than those in higher birth weight group. Macronutrient oxidation, in terms of CHO, lipid and protein per kg body weight and centimetre of height, were also not different between groups although lipid and protein oxidation per centimetre of height tended to be lower in the lower than the higher birth weight groups (≈ 10 and 20% respectively).

In the fed state, REE per kg body weight was not different between groups. However, adults with a lower birth weight have significantly lower REE per centimetre of height (≈ 10%; P=0.014) than those in the high birth weight group. Macronutrient oxidation, in terms of CHO, lipid and protein per kg body weight and centimetre of height, were not different between the two groups although lipid and protein oxidation per centimetre of height tended to be lower in the lower than the higher birth weight groups (≈ 9 and 18% respectively).

In the fasted state, REE per kg FFST and muscle mass were not different between the lower and higher birth weight groups. In addition, CHO, lipid and protein oxidation per kg FFST and muscle mass also were not different between the two groups although CHO oxidation per kg FFST and muscle mass tended to be greater (≈ 16 and 18% respectively) in the lower than the higher birth weight groups. In the fed state, REE per kg FFST and muscle mass were also not different between the lower and higher birth weight groups. In addition, CHO, lipid and protein oxidation per kg FFST and muscle mass were not different between the two groups although CHO oxidation per kg FFST and muscle mass tended to be greater (≈ 13 and 15% respectively) in the lower than the higher birth weight group.

Taken together, as the differences in absolute rates of energy expenditure and substrate oxidation in the fasted and fed state between the lower and higher birth weight groups were lost when expressed per unit weight or per unit lean mass, this would support the view that such differences are largely attributable to differences in body size as weight or the mass of metabolically active tissue. However, the differences remain when expressed per unit height suggesting that differences in height could not account for the differences in energy expenditure or substrate oxidation. The above results are also illustrated as Figures presented in Appendix (7.1).

The differences in the rate of energy expenditure and substrate oxidation over the postprandial period between the low and high birth weight groups are presented in **Table 7.1** after adjustments were made for height, weight and height + weight by ANCOVA. In the fasted state, adults with a lower birth weight had significantly lower REE than higher birth weight subjects after adjustments were made for height (≈ 14%; P=0.006), weight (≈ 11%; P=0.014) and height + weight (≈ 12%; P=0.015). In the fed state, REE was significantly lower in the lower than higher birth weight groups after adjustments were made for height (≈ 15%; P=0.002), weight (≈ 12%;

P=0.003) and height + weight (≈ 13%; P=0.004). The dietary induced thermogenesis however was not different between the two groups after adjustments were made for height, weight and height + weight.

When height and weight were used to predict REE in fasted sate in a multiple regression model (r = 0.460) the residual standard deviation was 0.470 kJ/min. When birth weight category was also added to the model the residual standard deviation was reduced to 0.425 kJ/min (and overall r increased to 0.617). In other words, the variance in REE in fasted state between individuals associated with height + weight has been reduced by ≈ 11% when birth weight category was added to the model. Birth weight category alone was found to be a better predictor of REE than height + weight. It was also found to be a better independent predictor than height + weight. In the fed state, the addition of birth weight category to the model reduced the residual standard deviation from 0.485kJ/min to 0.419kJ/min (and over all r increase from 0.491 to 0.675). In other words the variance in REE in fed state associated with height + weight has been reduced by ≈ 16% when birth weight category was added to the model. In addition, the birth weight category was also found to be a better predictor of REE in the fed states than height + weight (see Figure 7.13). These results suggested that although body size in term of height + weight are contributing to the variance in REE in the fasted and fed state, birth weight has an effect on REE greater than that can be explained by differences in body size alone.

The differences in macronutrient oxidation between the lower and higher birth weight group in absolute (kJ/min) and relative (%REE) terms in the fasted state are presented in **Table 7.2-7.3** after adjustments were made for height, weight and height + weight. The rates of CHO, lipid and protein oxidation both in absolute and relative terms were not different between the lower and higher birth weight groups after adjustments were made for in height, weight and height + weight. However, there was a tendency for absolute (kJ/min) lipid oxidation to be lower in the lower than higher birth weight group after adjustments were made for height (≈ 28%), weight (≈ 17%) and height + weight (≈ 28%). In addition, in relative terms, the contribution made by lipid oxidation to energy expenditure over the postprandial period tended to be lower and CHO oxidation tended to be greater (at the same magnitude) in the lower than higher birth weight group after adjustments were made for height (≈ 13%), weight (≈ 7%) and height + weight (≈ 15%)

The differences in macronutrient oxidation between the lower and higher birth weight group in absolute (kJ/min) and relative (%REE) terms in the fed state are presented in **Table 7.4-7.5** after adjustments were made for height, weight and height + weight. The rates of CHO, lipid and protein oxidation were not different between the lower and higher birth weight groups after adjustments were made for height, weight and height + weight. However, there was a tendency for absolute (kJ/min) lipid oxidation to be lower after adjustments were made for height (≈ 28%), weight (≈ 12%) and height + weight (≈ 23%) in the lower than higher birth weight group. In addition, in relative terms, the contribution made by lipid oxidation to energy expenditure over the postprandial period tended to lower and CHO oxidation tended to be higher (effectively balancing each other) after adjustments were made for height (≈ 13%), and height + weight (≈ 11%) in the lower than higher birth weight group.

The differences in the rate of energy expenditure and substrate oxidation over the postprandial period between the low and high birth weight groups are presented in **Table 7.6** after adjustments were made for lean mass height, weight and height + weight by ANCOVA. In the fasted state, the rate of energy expenditure over the postprandial period was not different between the lower and higher birth weight group after adjustment were made for FFST, muscle mass and non-limb FFST. However, energy expenditure tended to be lower in the lower than higher birth weight group after adjustment were made for FFST (≈ 9%), muscle mass (≈ 8%) and non-limb FFST (≈ 10%). In addition, REE was significantly lower in the lower than higher birth weight group after adjustment was made for non-muscular FFST (≈ 10%; P=0.003).

In the fed state, energy expenditure over the postprandial period was significantly lower after adjustments were made for FFST (≈ 10%; P=0.031), non-muscular FFST (≈ 12%; P=0.006) and non-limb FFST (≈ 12%; P=0.011) in the lower than higher birth weight group. Energy expenditure was not different between the two groups after adjustment was made for muscle mass although it tended to be less in the lower than higher birth weight group (≈ 9%). DIT was not different between the two groups after adjustment was made for all components of lean mass.

The differences in macronutrient oxidation (kJ/min) between the lower and higher birth weight group in both the fasted and fed state are presented in **Table 7.7-7.8** after adjustments were made for FFST, muscle mass, non-muscular FFST and non-limb FFST. CHO, lipid and protein

oxidation (kJ/min) were not different between the lower and higher birth weight group after adjustment were made for all components of lean mass.

Taken together, these results suggest that the differences in energy metabolism between the lower and higher birth weight groups in the fasted and fed state cannot be simply attributed to the reported differences in lean mass (i.e. FFST, muscle mass) or differences in visceral lean mass (non-limb FFST).

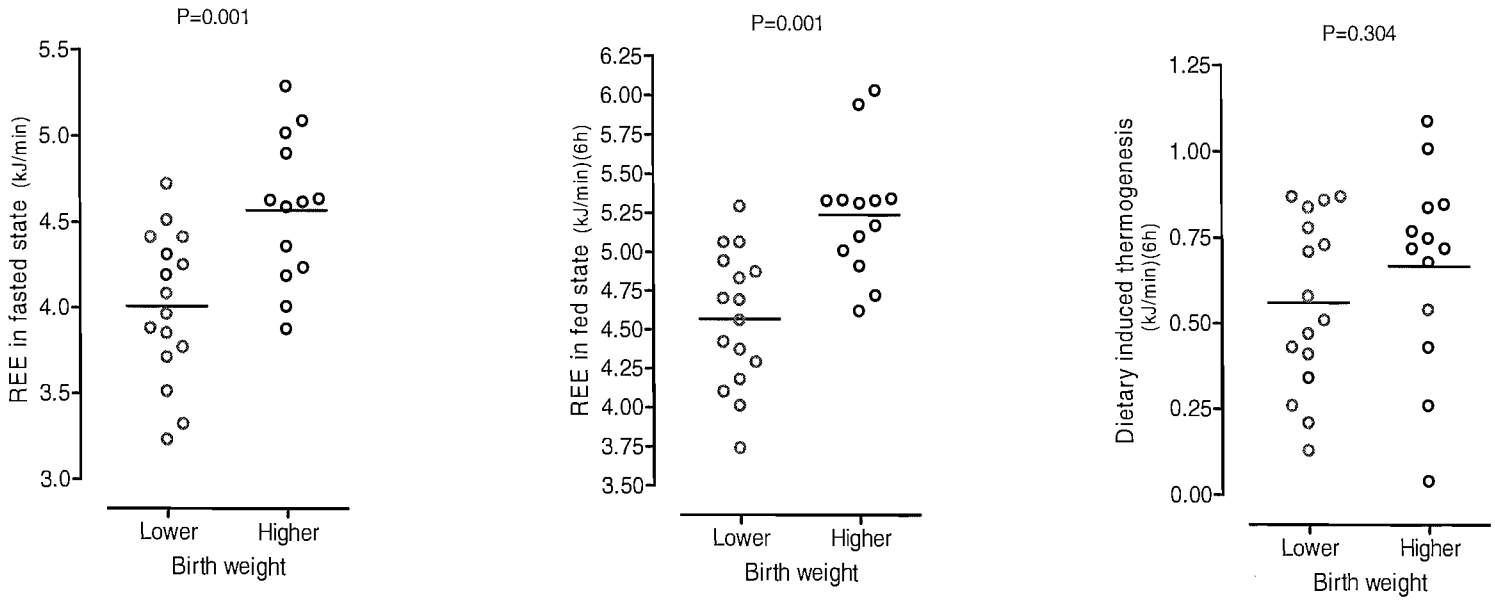


FIGURE 7.1 Differences in REE in the fasted and fed state (AUC over 6 hours) and DIT (AUCi) between the lower and higher birth weight groups

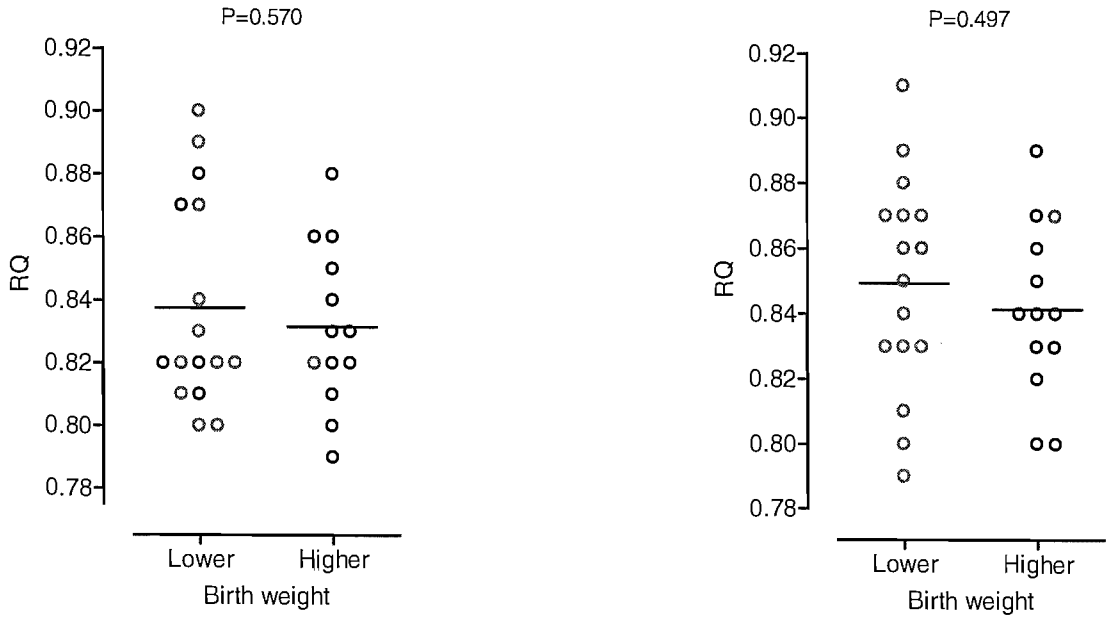


FIGURE 7.2 Difference in RQ in the fasted (lift) and fed state (right) between the lower and higher birth weight groups

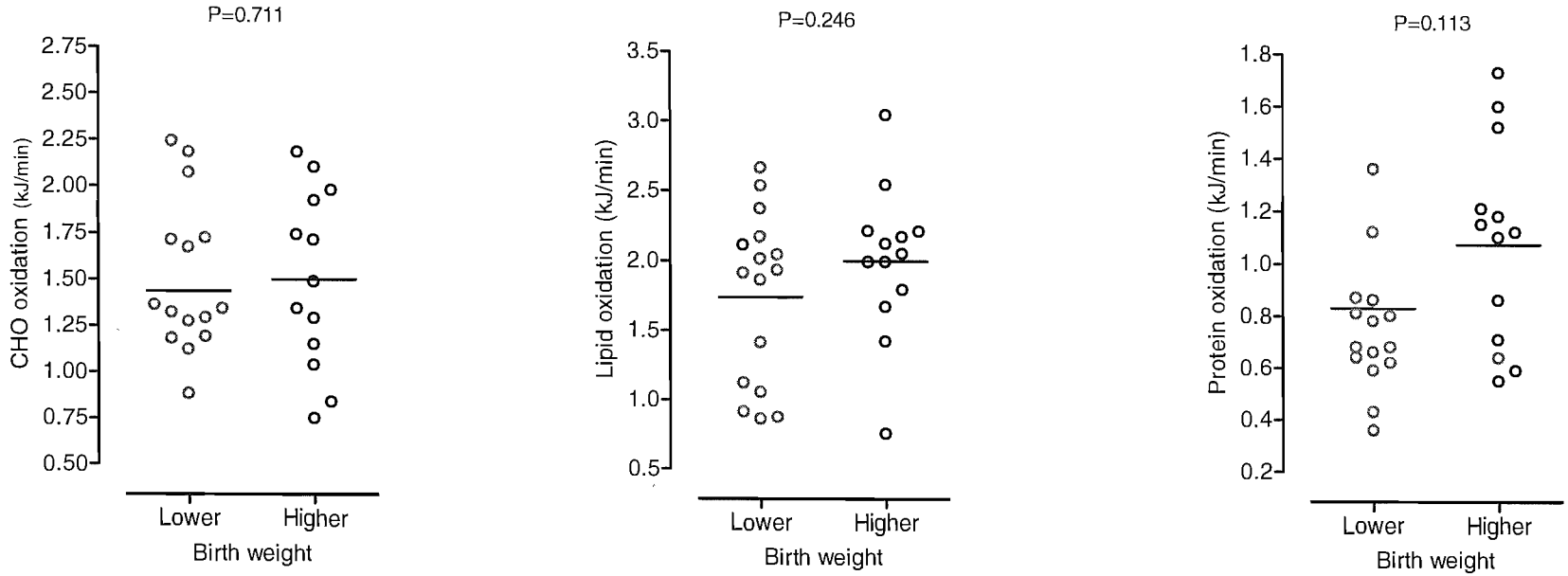


FIGURE 7.3 Differences in macronutrient oxidation between the lower and higher birth weight groups in the fasted state

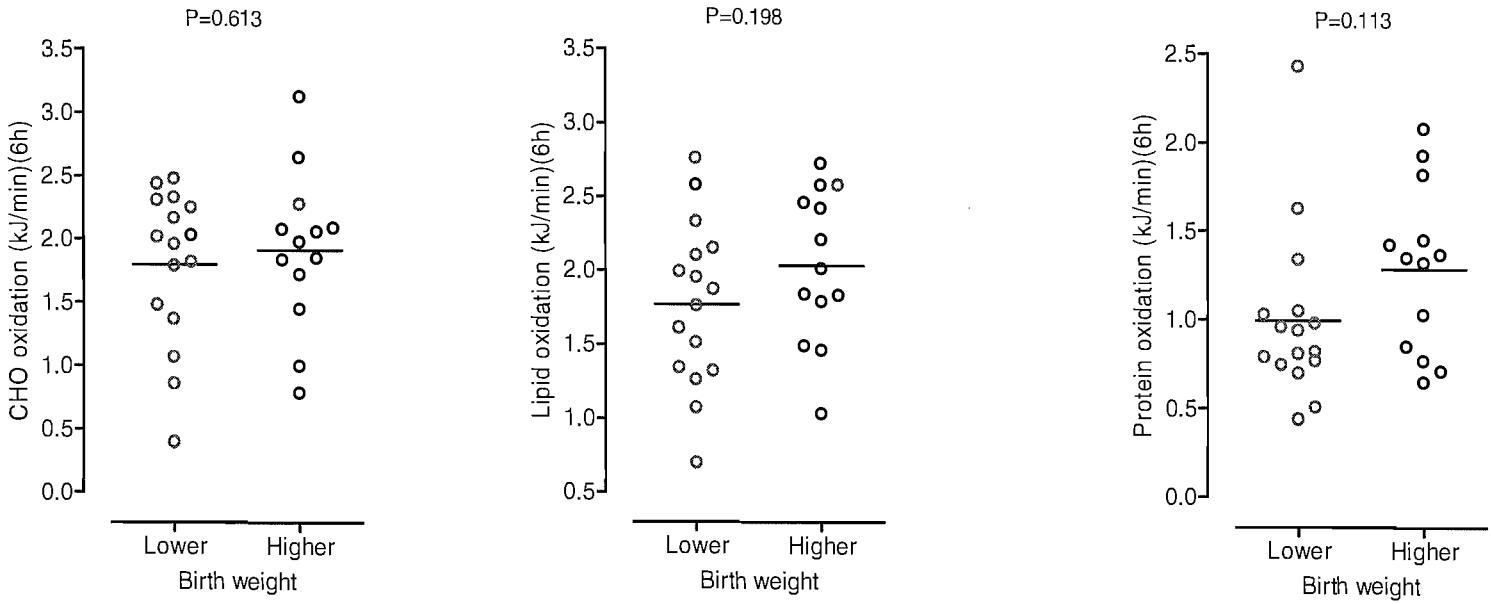


FIGURE 7.4 Differences in macronutrient oxidation between the low and high birth weight groups in fed state

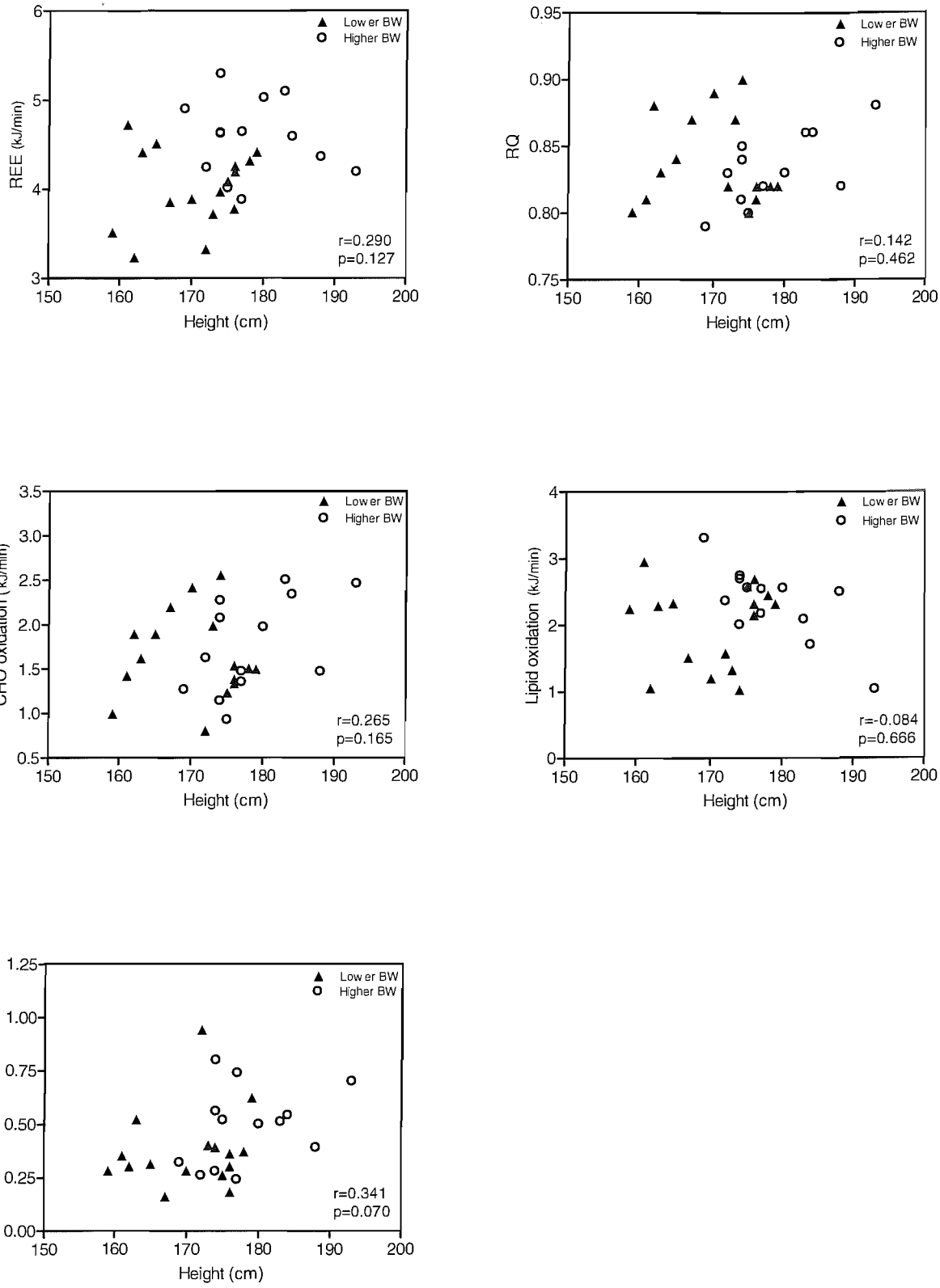
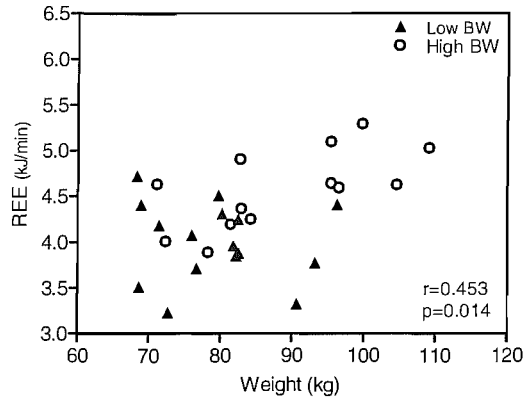
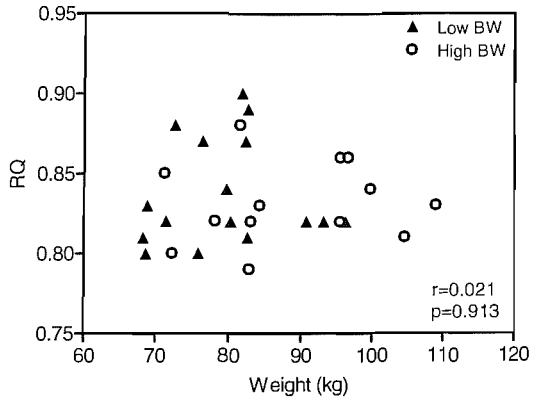
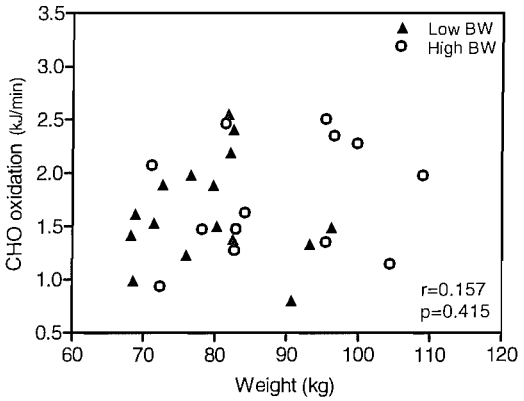
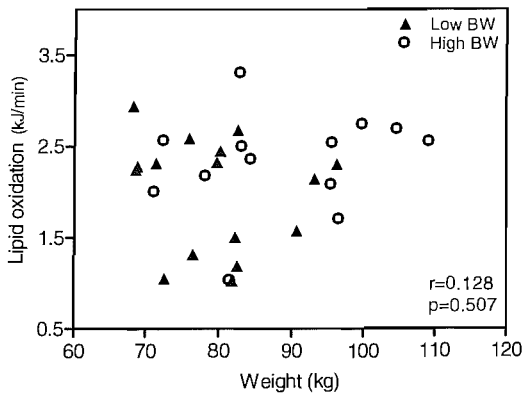


FIGURE 7.5 Relationship between height and energy metabolism & substrate oxidation in fasted state









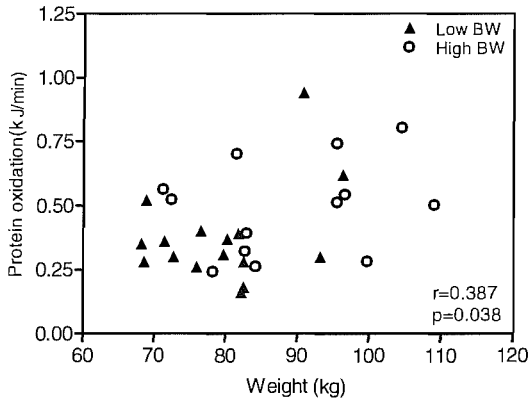
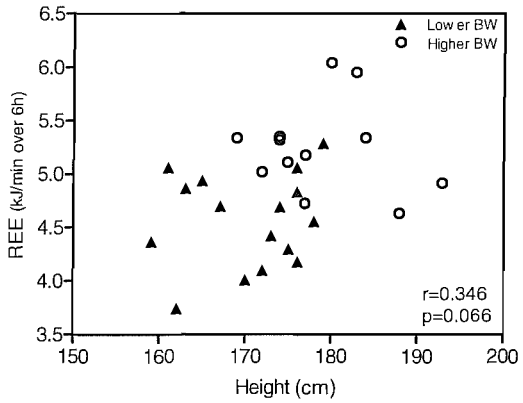
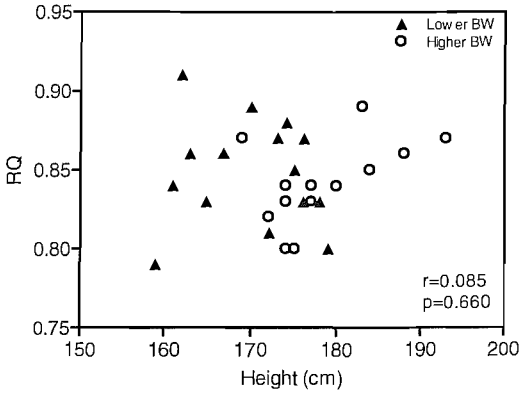
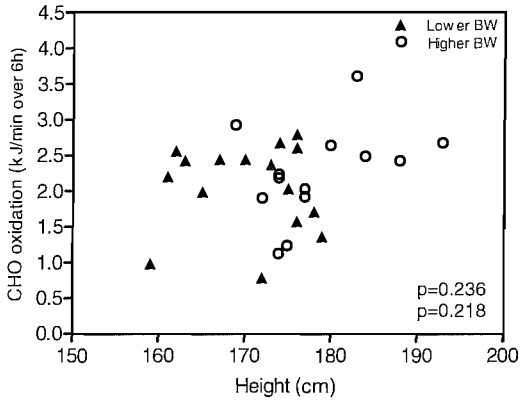
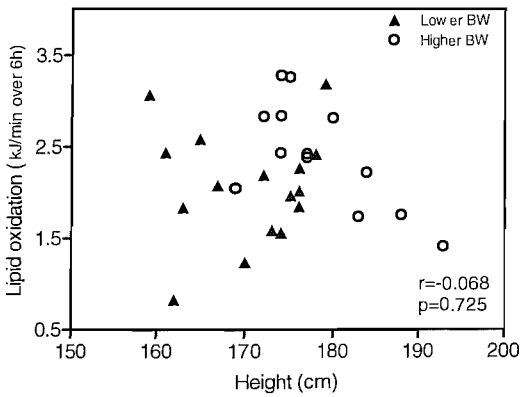


FIGURE 7.6 Relationship between weight and energy metabolism & substrate oxidation in fasted state









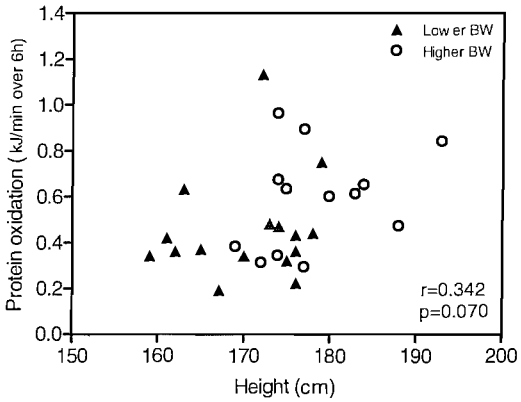


FIGURE 7.7 Relationship between height and energy metabolism & substrate oxidation in fed state

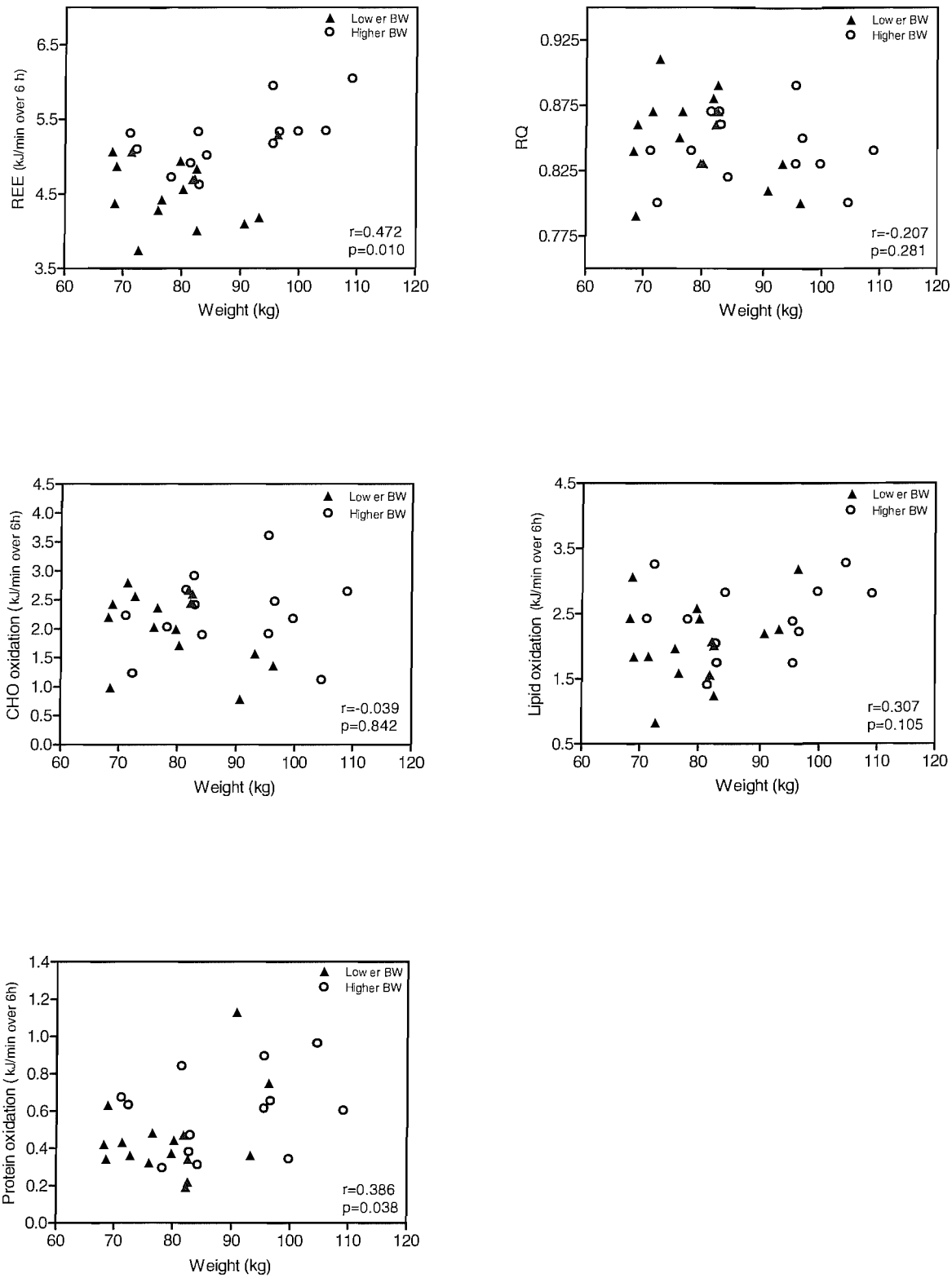
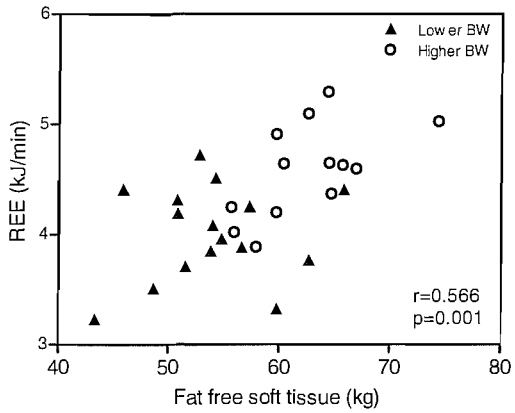
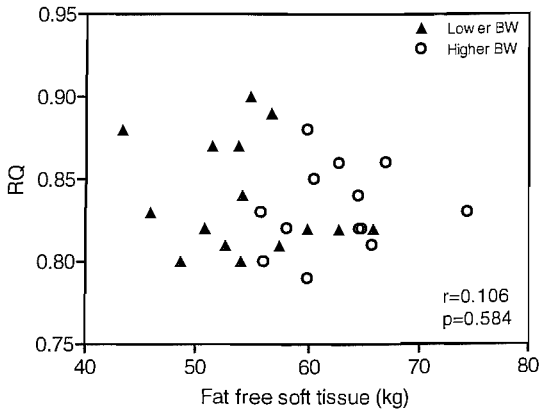
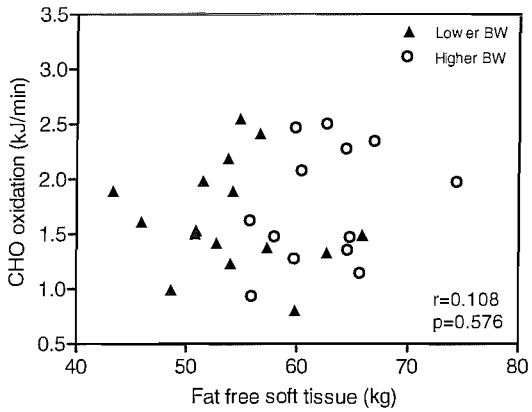
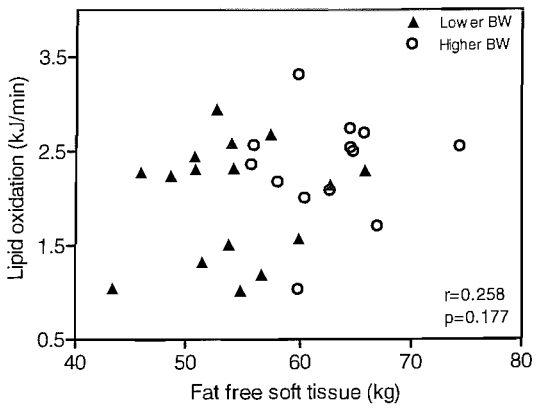


FIGURE 7.8 Relationship between weight and energy metabolism & substrate oxidation in fed state









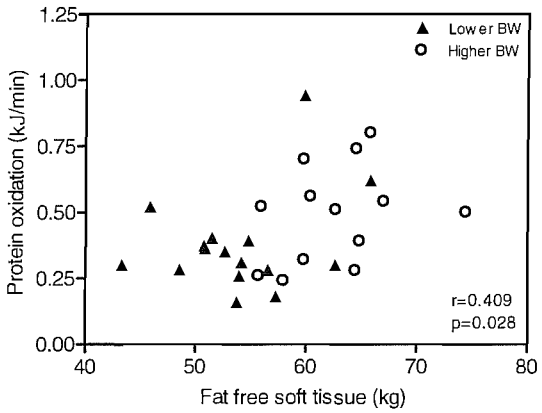
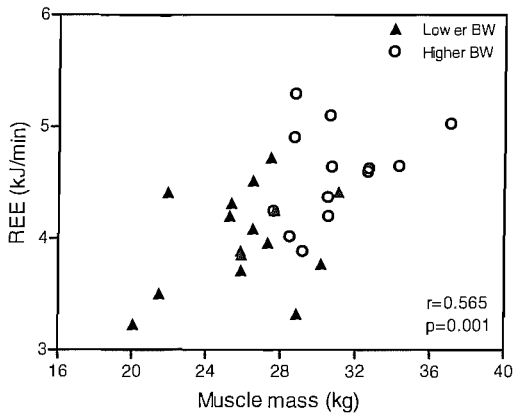
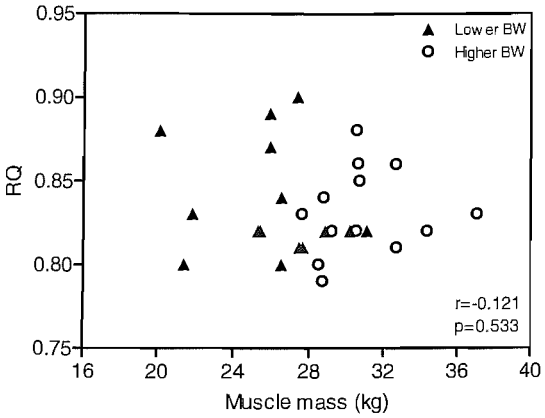
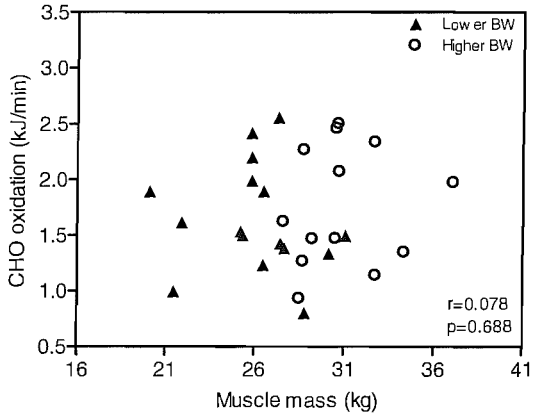
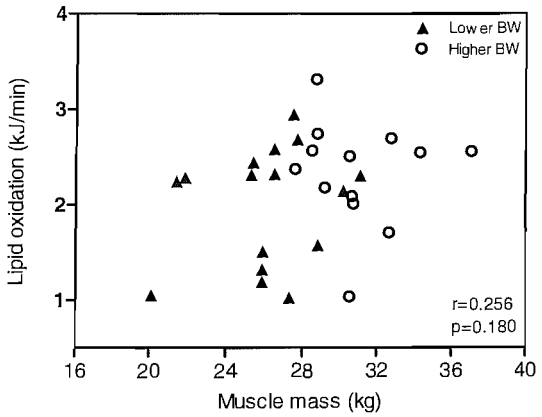


FIGURE 7.9 Relationship between fat free soft tissue and energy metabolism & substrate oxidation in fasted state









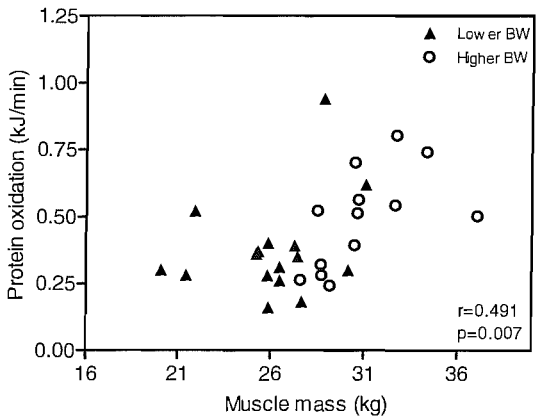
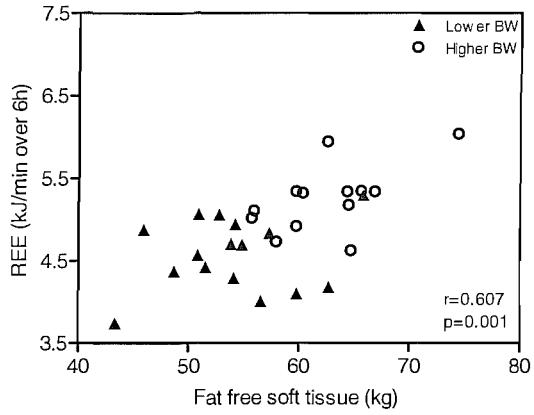
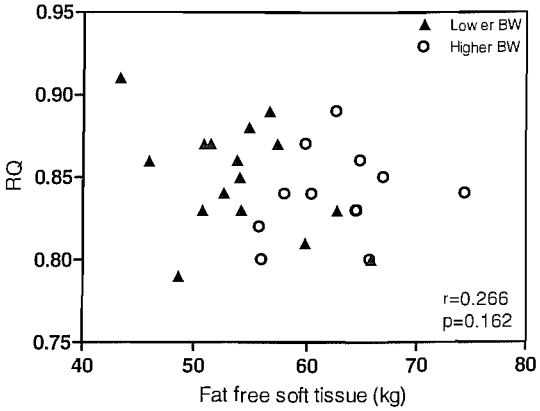
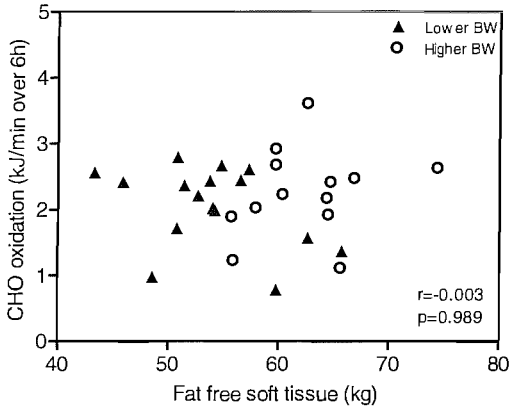
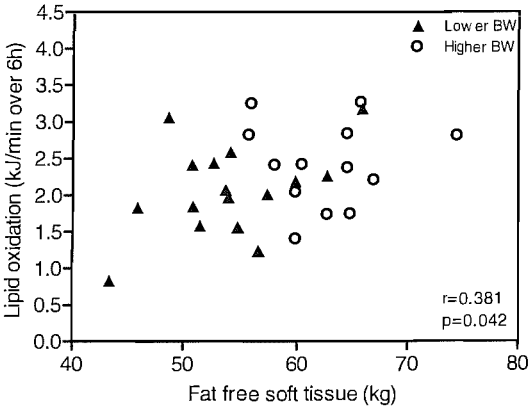


FIGURE 7.10 Relationship between muscle mass and energy metabolism & substrate oxidation in fasted state









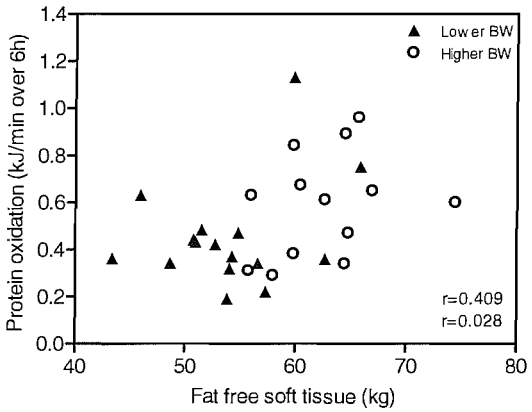
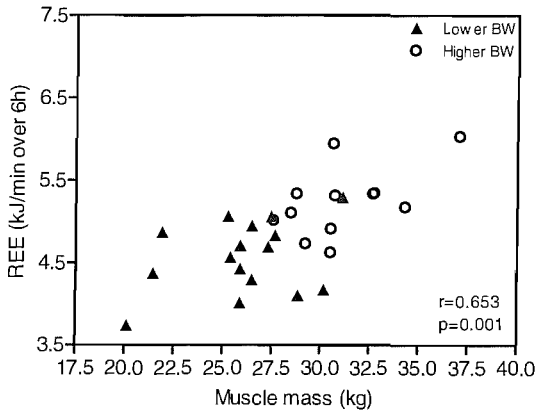
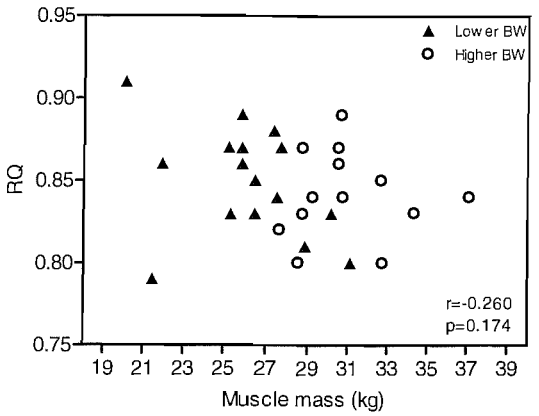
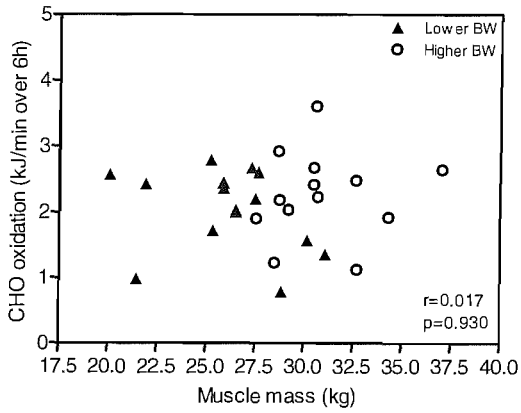
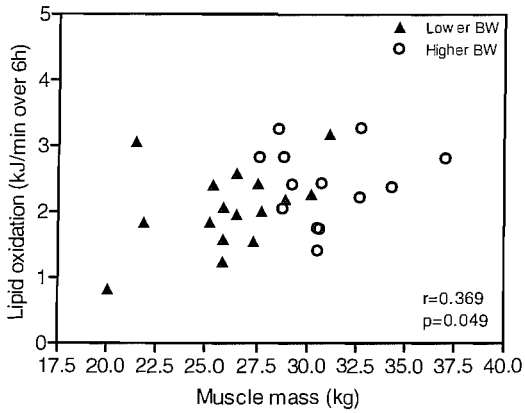


FIGURE 7.11 Relationship between fat free soft tissue and energy metabolism & substrate oxidation in fed state









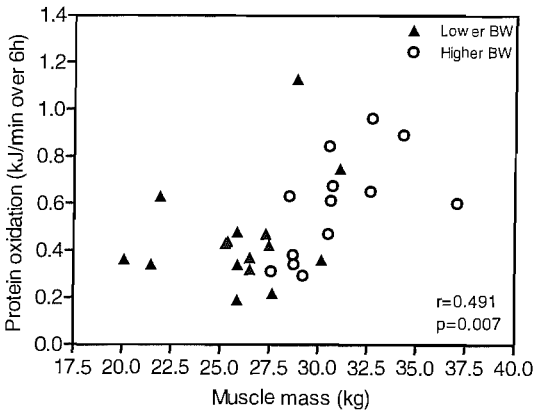


FIGURE 7.12 Relationship between muscle mass and energy metabolism & substrate oxidation in fed state

TABLE 7.1 Differences in energy metabolisms in fasted and fed state between lower and higher birth weight groups in relation to body size

	REE in fasted state (kJ/min)			REE in fed state (kJ/min)(6h)			DIT (kJ/min) (6h)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Height	4.00 ± 0.12	4.58 ± 0.13	0.006	4.57 ± 0.12	5.24 ± 0.13	0.002	0.57 ± 0.08	0.66 ± 0.08	0.454
Weight	4.06 ± 0.11	4.51 ± 0.12	0.014	4.62 ± 0.11	5.18 ± 0.12	0.003	0.56 ± 0.07	0.67 ± 0.08	0.359
Height & weight	4.04 ± 0.12	4.54 ± 0.13	0.015	4.60 ± 0.12	5.20 ± 0.13	0.004	0.57 ± 0.08	0.66 ± 0.09	0.464

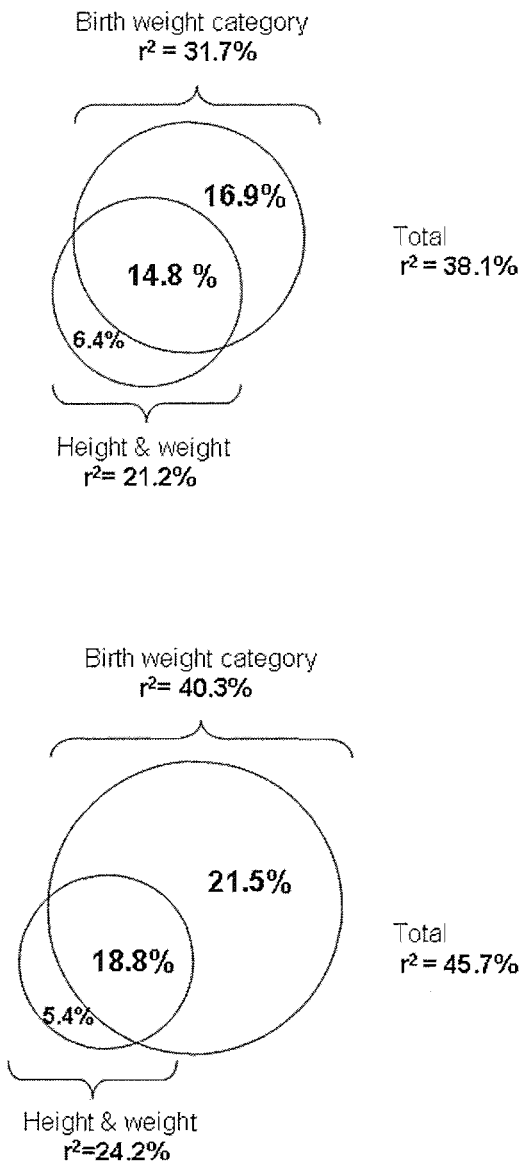


FIGURE 7.13 Independent and combined contributions of birth weight category and height + weight to REE in fasted (upper graph) and fed state (0-6 hours) (lower graph)

TABLE 7.2 Differences in macronutrient oxidation in fasted state between lower and higher birth weight groups in relation to body size

	CHO oxidation (kJ/min)			Lipid oxidation (kJ/min)			Protein oxidation (kJ/min)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Height	1.48 ± 0.13	1.45 ± 0.15	0.913	1.65 ± 0.15	2.11 ± 0.17	0.074	0.88 ± 0.11	1.01 ± 0.12	0.540
Weight	1.45 ± 0.13	1.48 ± 0.14	0.869	1.72 ± 0.15	2.02 ± 0.17	0.244	0.88 ± 0.10	1.01 ± 0.11	0.415
Height & weight	1.48 ± 0.14	1.45 ± 0.15	0.891	1.65 ± 0.16	2.11 ± 0.18	0.095	0.91 ± 0.11	0.98 ± 0.12	0.662

TABLE 7.3 Differences in macronutrient oxidation (%REE) in fasted state between lower and higher birth weight groups in relation to body size

	CHO oxidation (%)			Lipid oxidation (%)			Protein oxidation (%)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Height	36.87 ± 3.16	31.69 ± 3.57	0.323	40.74 ± 3.20	46.17 ± 3.16	0.366	22.39 ± 2.88	22.14 ± 3.24	0.956
Weight	35.91 ± 3.09	32.87 ± 3.46	0.537	41.86 ± 3.20	44.78 ± 3.58	0.566	22.23 ± 2.76	22.34 ± 3.09	0.979
Height & weight	36.65 ± 3.27	31.96 ± 3.69	0.390	40.48 ± 3.29	46.49 ± 3.73	0.279	22.87 ± 2.93	21.55 ± 3.31	0.788

TABLE 7.4 Differences in macronutrient oxidation in fed state between lower and higher birth weight groups in relation to body size

	CHO oxidation (kJ/min) (6h)			Lipid oxidation (kJ/min) (6h)			Protein oxidation (kJ/min) (6h)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Height	1.84 ± 0.17	1.87 ± 0.19	0.906	1.68 ± 0.14	2.15 ± 0.16	0.049	1.06 ± 0.13	1.21 ± 0.14	0.454
Weight	1.77 ± 0.16	1.96 ± 0.18	0.445	1.79 ± 0.14	2.00 ± 0.16	0.357	1.05 ± 0.12	1.21 ± 0.14	0.414
Height & weight	1.81 ± 0.17	1.90 ± 0.19	0.741	1.71 ± 0.14	2.11 ± 0.16	0.093	1.09 ± 0.13	1.18 ± 0.14	0.661

TABLE 7.5 Differences in macronutrient oxidation in fed state (%REE) between lower and higher birth weight groups in relation to body size

	CHO oxidation (%)			Lipid oxidation (%)			Protein oxidation (%)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Height	40.20 ± 3.44	35.71 ± 3.88	0.430	36.54 ± 2.77	41.21 ± 3.13	0.309	23.26 ± 2.78	23.08 ± 3.14	0.969
Weight	38.43 ± 3.26	37.89 ± 3.65	0.919	38.48 ± 2.83	38.83 ± 3.17	0.939	23.09 ± 2.67	23.28 ± 2.99	0.966
Height & weight	39.38 ± 3.43	36.73 ± 3.88	0.643	36.88 ± 2.84	40.79 ± 3.21	0.411	23.74 ± 2.82	22.48 ± 3.19	0.789

TABLE 7.6 Differences in energy metabolism between lower and higher birth weight groups in relation to lean mass

	REE in fasted state (kJ/min)			REE in fed state (kJ/min)(6h)			DIT (kJ/min) (6h)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Fat free soft tissue	4.10 ± 0.12	4.45 ± 0.13	0.094	4.67 ± 0.12	5.12 ± 0.13	0.031	0.57 ± 0.08	0.67 ± 0.09	0.463
Muscle mass	4.11 ± 0.12	4.44 ± 0.14	0.114	4.70 ± 0.12	5.08 ± 0.13	0.063	0.59 ± 0.08	0.63 ± 0.09	0.749
Non-muscular FFST	4.08 ± 0.11	4.49 ± 0.13	0.033	4.62 ± 0.11	5.17 ± 0.12	0.006	0.55 ± 0.07	0.69 ± .084	0.269
Non-limb FFST	4.09 ± 0.12	4.47 ± 0.13	0.060	4.63 ± 0.12	5.17 ± 0.13	0.011	0.54 ± 0.08	0.69 ± 0.87	0.234

TABLE 7.7 Differences in macronutrient oxidation in fasted state between lower and higher birth weight groups in relation to lean mass

	CHO oxidation (kJ/min)			Lipid oxidation (kJ/min)			Protein oxidation (kJ/min)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Fat free soft tissue	1.65 ± 0.15	1.74 ± 0.17	0.715	2.04 ± 0.17	2.26 ± 0.19	0.435	0.41 ± 0.05	0.44 ± 0.06	0.784
Muscle mass	1.63 ± 0.15	1.76 ± 0.17	0.623	2.03 ± 0.17	2.27 ± 0.19	0.449	0.44 ± 0.05	0.42 ± 0.06	0.821
Non-muscular FFST	1.66 ± 0.14	1.74 ± 0.16	0.734	2.02 ± 0.16	2.28 ± 0.18	0.338	0.39 ± 0.05	0.47 ± 0.06	0.357
Non-limb FFST	1.63 ± 0.13	1.78 ± 0.15	0.462	1.99 ± 0.15	2.32 ± 0.17	0.181	0.39 ± 0.04	0.47 ± 0.05	0.268

TABLE 7.8 Differences in macronutrient oxidation in fed state between lower and higher birth weight groups in relation to lean mass

	CHO oxidation (kJ/min)(6h)			Lipid oxidation (kJ/min)(6h)			Protein oxidation (kJ/min)(6h)		
	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value	Lower BW	Higher BW	P-value
Adjusted for									
Fat free soft tissue	1.99 ± 0.18	2.32 ± 0.21	0.301	2.17 ± 0.16	2.27 ± 0.19	0.757	0.50 ± 0.05	0.53 ± 0.06	0.784
Muscle mass	1.99 ± 0.19	2.31 ± 0.21	0.326	2.18 ± 0.17	2.26 ± 0.19	0.776	0.52 ± 0.06	0.49 ± 0.07	0.821
Non-muscular FFST	2.01 ± 0.17	2.30 ± 0.19	0.312	2.14 ± 0.16	2.31 ± 0.18	0.532	0.47 ± 0.06	0.55 ± 0.07	0.358
Non-limb FFST	2.06 ± 0.16	2.24 ± 0.18	0.471	2.06 ± 0.16	2.40 ± 0.17	0.167	0.47 ± 0.06	0.56 ± 0.07	0.268

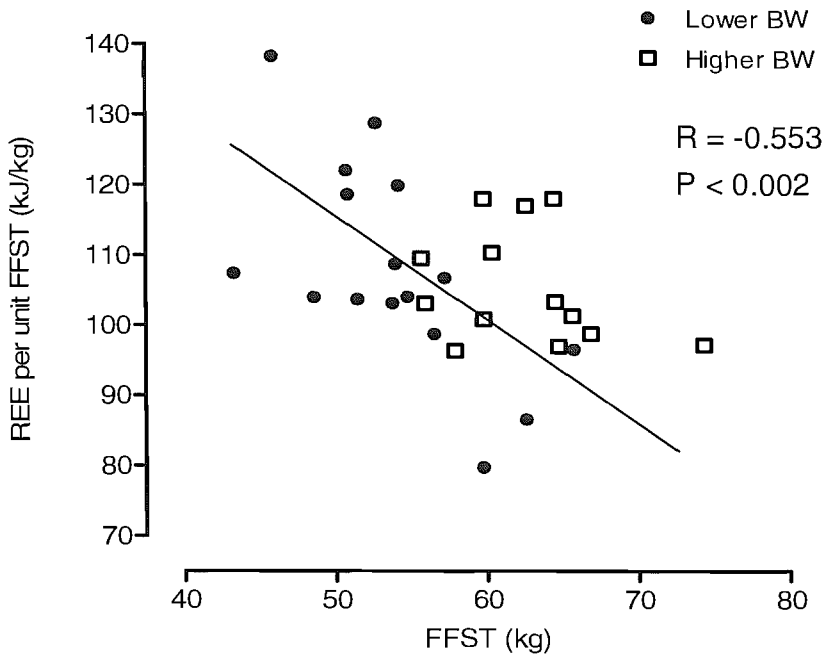


FIGURE 7.14 Correlation between REE relative to kg of FFST and FFST for study subjects

7.4 Discussion

These results raise important issues about the influence of birth weight as a marker of fetal growth on the relationship between body structure and metabolic function. Adults with a lower birth weight had lower absolute REE (kJ/min) than those with higher birth weights in both the fasted and fed states. Although height and weight for all subjects were positively associated with energy expenditure, lower birth weight group had significantly lower REE in fasted and fed states than higher birth weight group even after adjustments were made for height, weight and height + weight. This suggests that neither height nor weight differences associated with birth weight adequately explain the reported differences in REE in the fasted and fed states between the two groups. Indeed, the predictive effect of birth weight category on REE in this group of individuals was substantial and could explain a greater proportion of the variability in REE both in fasted and fed states than height + weight. Furthermore, the independent contribution of birth weight category was found to be greater than that of height + weight (much of the variability due to height + weight could also be accounted by birth weight). REE in the fasted and fed state relative to height but not to weight was found to be significantly less in the lower than higher birth weight groups. This finding suggests that the differences in REE between the two groups are independent of height. In contrast to the results analysed by ANCOVA, REE differences between groups were related to the difference in body weight.

The results of the previous chapter suggested that lower birth weight individuals have lower FFST, muscle mass and non-muscular FFST (non-muscular FFST; more metabolically active than muscle mass) even after adjustments were made for height + weight. In addition, they have lower non-limb FFST (non-limb FFST; more metabolically active than the limb FFST) which is assumed to represent visceral organs mass. Although REE in fasted and fed states were positively associated with all component of lean mass, the lower birth weight group tended to have lower REE in the fasted and fed states after adjustments were made for FFST (9 v 10% for the fasted and fed state respectively), muscle mass (≈ 8 v 12%), non-muscular FFST (≈ 10 v 12%) and visceral FFST (≈ 10 v 9%). This finding suggests that the differences in energy metabolism might be programmed in early life independent to the observed differences in total lean mass (FFST, muscle mass and non-muscular FFST) and visceral lean mass (non-limb FFST), each of which have different metabolic activity.

Although computed tomography (CT) and magnetic resonance imaging (MRI) provide the

most accurate measurement of the mass of different tissue components, useful important information may also be derived from the DXA scan (252;253). We have used DXA to estimate the proportions of muscle (estimated from limbs FFST) or non-muscular FFST (differences between FFST and muscle mass), each of which have different metabolic activity. In addition, it also provides indirect information on the distribution of visceral organ mass (i.e. non-limb FFST) to skeletal muscle mass (i.e. limb FFST), both of which also have different metabolic activity.

An analogous situation to the effect of birth weight on energy metabolism independent of body size and composition has also been reported in relation to race and ethnicity. Soares et al found that Indian men and women had significantly lower REE than their Australian counterparts even after adjustments were made for body weight (254). In addition, measured REE in the Indian population was lower than that predicted from body weight and height using generic equations (255). Furthermore, several studies have reported lower REE in African American women (256), men (257) and children (255;258) than that seen in their white counterparts, even after adjustment were made for lean body mass. Given that comparable differences have been seen within the same population, but of differing birth weights, findings in present study raise the possibility that part of the differences in metabolic rate usually attributed to 'ethnicity' may be equally attributable to differences in birth weight. For example, the average birth weight of the Indian born in South Asia is 2.70 kg, substantially less than children born to white parents in England (3.49kg) (259). The average Indian birth weight would equate to that seen in the lower birth weight group (2.73 kg) in the current study.

The observed differences in metabolic rate even after adjustments for body size and lean mass between the lower and higher birth weight groups might also provide a possible clue to the greater fat mass or adiposity in adults seen in subjects who were born small. Lower rates of energy expenditure, part due to less metabolically active tissue and part due to a lower activity of that tissue, would predispose the individual to be more likely in a positive energy balance for any given energy intake that those who were heavier at birth.

However, the link between resting energy expenditure and predisposition to weight gain is not entirely clear. Although some studies have reported that adults with a lower REE have greater weight gain over a period of years than those with a higher REE (260;261), this has not been corroborated by others (262-264). This may be because REE is only one component of energy expenditure such that the more variable components such that

associated with physical activity are not included in the analysis. Equally important is the effect that differences in energy intake may exist between groups. Unfortunately, it was not possible to examine for such an effect in the current study as no comprehensive assessment of habitual intake was undertaken. However animal studies suggest that alterations in fetal and neonatal growth can lead to alterations in appetite in later life (265;266), whereby they are likely to eat more than those born to dams who were adequately nourished during pregnancy (265). In addition there is the possibility that physical activity and/or fitness are programmed during fetal life, as suggested by a retrospective study of aerobic fitness, which was assessed using endurance and exhaustion tests, in 12 year old boys (267). The inter-relationships between REE, physical activity and appetite would determine the manifestations of obesity and weight gain, but the relative contributions between genetic, environmental factors and programming remain unclear.

Another relevant consideration is that most studies examining the relationship between REE and subsequent weight gain have generally adjusted REE for baseline differences in body composition, whereas in this study, differences in REE between groups are considered to be at least, partly due to differences in body composition (FFM) that persist even after adjustments for height and weight. The reported differences in fat distribution between the lower and higher birth weight groups were not found to be associated with altered REE in this study. This was in keeping with some (268;269) but not all previous reports (270) in which, measurements of REE or sleeping metabolic rate (269) were made in relation to anthropometric measures of fat distribution between trunk and upper legs.

In contrast to the current study, in which REE tended to be reduced in the lower than higher birth weight group after controlling for FFM, a previous study reported a tendency for sleeping metabolic rate to be lower in adults with higher than lower birth weight (197). This study differed from the current study in several ways: it involved obese (mean BMI, 33.5 kg/m^2) rather than non-obese individuals; Pima Indians rather than Caucasians; young adult men and women (mean age 25 years) rather than older men; measurements of energy expenditure made during sleep rather than in woken states; and measurements began 3.5 hours after a snack and 6.5 hours after the start of a meal rather than 12-14 hours after an overnight fast. Another study involving Finnish adults reported higher REE/kg FFM in those with a lower than higher birth weight (212). However, REE/kg FFM typically decreases with increasing FFM (211), probably because of the smaller percentage contribution of organ mass to FFM as FFM increases. This means that the comparison of REE/kg FFM between birth weight categories is likely to be biased towards a higher value in the lower birth weight

group, which has been reported to have a significantly smaller FFM. To examine whether such an effect could bias the interpretation of results in this study, and illustrate how it might account for the observations of others, the REE per kg FFST in the fasted states were plotted against the amount of metabolically active tissue using the results of the present study. As can be seen in see Figure 7.14, there was a significant inverse relationship between relative REE (per hg FFST) and FFST, such that those of a lower FFST would appear to have a greater REE per kg FFST than those who had a greater FFST. Given that the average FFST was less in the lower birth weight group, presenting the results in this way masks the differences in REE seen when adjusting the REE for differences in FFST using ANCOVA.

The greater REE in the fed states observed in the lower than higher birth weight group in the current study could almost entirely be accounted for by the differences in REE in fasted states. No evidence was found of a significant effect of birth weight on dietary induced thermogenesis, macronutrient oxidation or nutrient balance following ingestion of a mixed meal of standard composition. Although there was a tendency for lipid oxidation to be less in the lower than higher birth weight group, this difference did not reach statistical significance. However, since the lower birth weight group had a lower REE, the difference in percentage contribution of fat oxidation to REE was much less marked between the groups.

The calculated nutrient balances in this study were based on estimates rather than direct measurements of net protein oxidation in both fasted and fed states. In this study, the model used to calculate nutrient balance assumed that the contribution of protein to total REE was the same in both groups and fixed at 40%, rather than directly determined for each individual in the study. Furthermore, REE was measured before and after the meal for over part of the day (0-6 hours) and assumed to reflect the whole day. It would be preferable to conduct these measures over the complete 24h period, preferably in the free living state to allow for normal activity behaviour. An alternative approach for measuring REE in free living activity would be the use of doubly-labelled water method (164). This method starts with a baseline urine collection to determine pre-dose values for the hydrogen and oxygen isotopes. The subject is given a single oral bolus dose of heavy water ($^2H_2{}^{18}O$). After the administration of doubly labelled water ($^2H_2{}^{18}O$), the labelled hydrogen ($^2H_2$) is eliminated as water (^2H_2O), corresponding to water output, whereas the oxygen isotope would be eliminated as water ($H_2{}^{18}O$) and as expired carbon dioxide ($C^{18}O_2$). By measuring the differences between the elimination rates of labelled oxygen and hydrogen, the carbon dioxide production rate can be determined. The carbon dioxide production rate is then converted into energy expenditure using the respiratory quotient (RQ) of the food ingested during the observation period. The

method measures integral CO_2 production for several days from the difference in elimination rates of labelled hydrogen (deuterium) and oxygen from labelled body water (164).

7.5 Summary

The analyses performed in this chapter set out to examine the extent to which differences in birth weight could influence energy metabolism and substrate oxidation in both the fasted and fed states and whether any differences, if any, in metabolic behaviour could be attributed to differences in structure.

The results presented in this chapter indicated that:

- Adults with a lower birth weight had lower REE as an adult both in fasted and fed states when compared with those born heavier.

- There was a positive association between height, weight and lean mass (FFST, muscle mass, non-muscular FFST and non-limb FFST) and the absolute rates of energy expenditure and substrate oxidation.

- Expressing the results per unit body size (ie REE/kg weight) would introduce an inappropriate bias in the results when groups of different body size are compared, such that the smaller individuals would appear to have higher values.

- After adjusting for differences in body size between the two groups by ANCOVA, at the same height + weight and lean mass, the lower birth weight group had lower energy expenditures in the fasted and fed states than that seen in the higher birth weight groups. In other words, there were qualitative differences in energy expenditure associated with a lower birth weight. This finding suggests that the differences in energy metabolism and substrate oxidation in the fasted and fed states might be programmed in early life independent of body size and composition.

- The predictive effect of birth weight category on REE in this group of individuals was substantial and could explain a greater proportion of the variability in REE both in the fasted and fed states than height + weight.

CHAPTER 8

Birth weight in relation to lipid and lipoprotein metabolism

8.0 Introduction

The relationships between adiposity in particular in the abdominal regions of the body and abnormality of metabolic control were discussed in chapter 2 (sections 2.1.3 and 2.1.4). For instance, obese individuals, especially those with greater abdominal fat mass have previously been shown to have higher circulating concentrations of plasma triglyceride-rich particles in the fasted and fed states compared with lean individuals (30;32). Such abnormalities in circulating plasma lipid have been found to be associated with insulin resistance and the development of T2DM (271). Whilst fasting lipid and lipoprotein levels in plasma may be used as a marker of metabolic control, disturbances of metabolic control are more obviously apparent when in the fed state as the body is required to accommodate the exogenous lipid. A more precise characterisation of perturbations in lipid metabolism may be obtained by studying the postprandial partitioning of labelled fatty acids consumed within a test meal in order to better differentiate between the processes governing the delipidation of TAG-rich particles and entrapment of the products of this process into peripheral tissues.

There are, however, very few studies that have specifically attempted to examine the effect of fetal growth marked by birth weight on a marker in plasma related to lipid metabolism (see chapter 2, section 2.2.6). Some of these studies reported an inverse relationship between birth weight and fasting TAG in adolescent and adult, although this has not been found by others. The only previous study of postprandial lipid metabolism in relation to birth weight was unable to demonstrate any obvious relationship between the increase in plasma TAG concentration or NEFA suppression in relation to birth weight (147). It should be noted however, that the numbers of subject studied was small and the study had insufficient power.

In previous chapters, adults with a lower birth weight have been reported to have greater fat

mass (kg) and more central fat mass measured by DXA after adjustments were made for height + weight. The extent to which such differences in body composition and fat distribution might alter lipid metabolism in the fasted or fed states or whether lipid metabolism is programmed in early life independent of such differences in body composition and fat distribution remain to be determined.

8.1 Aim

The aims of the analyses presented in this chapter are to:

- Examine the relationship between fat mass and central body fat mass obtained by DXA with biological markers related to lipid metabolism both in the fasted and fed states, independently of birth weight.

- Examine the extent to which lipid, lipoprotein and glucose metabolisms in the fasted state are influenced by fetal growth that is marked by birth weight. This was achieved by measuring the differences in plasma TAG, NEFA, VLDL, LDL, HDL, total cholesterol and glucose concentration in fasted states between the lower and higher birth weight groups.

- Examine the changes in lipid metabolism that might be influenced by differences in birth weight following consumption of test meal containing ^{13}C-labeled palmitic acid. This was achieved by two lines of investigation: firstly by assessing the time course of the plasma TAG and NEFA concentrations over the study period (6 hours), secondly, by assessing the partitioning of dietary lipid by measuring excursion of the ^{13}C-PA concentrations in the TAG and NEFA fractions in plasma.

8.2 Subjects and methods

The subject groups and general protocol were as described earlier. After an overnight fasting, each of the subjects consumed a test meal (containing 3720 kJ, of which 15% was derived from protein, 40% from carbohydrate, and 45% from fat) together with 700 mg of 1-^{13}C-palmitic acid (99 atom percent excess; Cambridge Isotopes Massachusetts, USA) mixed within a lipid-casein-glucose-sucrose emulsion). Blood samples were obtained 30 min before meal ingestion and then at hourly interval for six hours after the meal ingestion. The plasma

was analysed for non-esterified fatty acids (NEFA ACS-ACOD kit; Wako chemicals GmbH, Lewes, UK), triglyceride (TAG), HDL-cholesterol, LDL-cholesterol and total cholesterol (reagent kits from Konelab Labmedics Ltd, Manchester, UK), using the Kone Auto analyzer (Labmedics, Manchester, UK) more information on the principle of these routine analysis is provided in appendix (2.9). Plasma lipids were extracted according to the method of Folch *et al*, fractionated into TAG and NEFA before determining the enrichment of ^{13}C-palmitic acid in each fraction by Isotope Ratio Mass Spectrometry (IRMS) (Europa Scientific Ltd, Crewe, UK) interfaced with a gas chromatography (5890, Hewlett Packard, Palo, Alto, CA) (see chapter 2 for details).

From the graphs of time: plasma analyte concentration following the test meal, it was possible to determine the net excursion as area under the curve (AUC) and the incremental increase over baseline as the AUC less the baseline concentration assumed to be constant over the study period (AUCi). In general, a higher TAG AUC or AUCi reflects poor clearance of TAG from the circulation. A higher AUC of ^{13}C-palmitic acid in the TAG reflects poor delipidation of TAG from circulating lipoproteins (i.e. limited hydrolysis by lipoprotein lipase) such that more of the exogenous lipid remains in the circulation. A higher NEFA AUC, or a lower NEFA suppression as AUCi reflects increased NEFA efflux from adipose tissue. This lack of NEFA suppression may be attributable to either a lesser insulin sensitivity of the adipocyte or impairment in NEFA entrapment following delipidation of TAG from the lipoprotein. The latter will also be reflected as a higher ^{13}C-PA in the NEFA fraction (as AUC). A higher glucose AUC or incremental AUC (AUCi) reflects poor glycaemic control, largely due to decreased insulin sensitivity. Thus, poor metabolic control would be reflected in a higher baseline concentrations of plasma glucose, TAG, NEFA and insulin together with higher AUC and AUCi for plasma glucose, TAG, NEFA and insulin, higher ^{13}C-palmitic acid in the TAG and NEFA fractions as well as a lower AUCi, for plasma NEFA.

The relationship between all measures of lipid metabolism with fat mass and trunk fat were assessed by correlation coefficient (Bivariate correlation). The differences in the mean of all biological markers related to lipid metabolism and metabolic risk factors associated with CVD between lower and higher birth weight were carried by the simple t-test.

8.3 Results

The results presented in this section are divided into two sections. The first examines the relationship between total adiposity and central fat mass measured by DXA on one hand and

biological markers related to lipid metabolism both in fasted and fed states in the other for all subjects. The second part examines the differences in lipid metabolism both in fasted and fed states between the lower and higher birth weight groups.

8.3.1 Adiposity, central fat mass measured by DXA in relation to lipid metabolism in the fasted and fed states

The relationship between fat mass and trunk fat mass measured by DXA and plasma TAG, NEFA, glucose and insulin concentrations in fasted state for all subjects in this study are presented in **Figure 8.1-8.2**. Fat mass (kg) was significantly and positively associated with NEFA concentration in plasma (r=0.795; P=0.006). In addition, fat mass was positively associated with TAG and glucose concentration in plasma but these associations did not reach statistical significance. Furthermore, trunk fat mass was significantly and positively associated with NEFA concentration in plasma (r=0.423; P=0.022). Trunk fat mass was also positively associated with both TAG and glucose concentrations in plasma but this association did not reach statistical significance. These results suggest that with increasing fat mass and central fat mass, there was an increase in plasma TAG, NEFA and insulin concentration in plasma in fasted states for all subjects.

8.3.2 Influence of birth weight on lipid and lipoprotein metabolism

In the fasted states, there were no significant differences in baseline TAG concentration in plasma between the lower and higher birth weight groups although lower BW groups tended to have a greater baseline TAG concentration than those in higher birth weight group (≈ 6%). The time course of plasma TAG concentration over the study period between the lower and higher birth weight groups is illustrated in **Figure 8.3.** Following the ingestion of test meal, TAG concentration rose to peak in excess of 2 mmol/l at 3 hours and steadily decreased to near baseline level by 6 hours. The total TAG concentration over the study period presented as the area under (AUC) was not different between the two groups over the study period (6h). In addition, the incremental area under the curve of TAG concentration (AUCi) was also not different between the two groups although it tended to be less in the lower than higher birth weight group (≈ 30%).

In the fasted states, there were no significant differences in baseline NEFA concentration in plasma between the lower and higher birth weight groups (0.41 ± 0.02 v 0.40 ± 0.04 mmol/l * 6h for lower and higher BW group respectively). The time course of plasma NEFA

concentration over the study period between the low and high birth weight groups is illustrated in **Figure 8.4**. Following the ingestion of test meal, NEFA concentration decrease to near 0.15mmol/l for both groups at 2 hours and steadily rose to baseline level by 6 hours. The AUC for NEFA suppression after the meal ingestion was also not different between the two groups (1.76 ± 0.10 v 1.77 ± 0.11 mmol/l per 6h for lower and higher BW groups respectively) as was the incremental NEFA AUCi (note that the AUCi effectively reflects the extent of NEFA suppression and is a negative value) after meal ingestion (- 0.85 ± 0.20 v - 0.66 ± 0.24 mmol/l * 6h h).

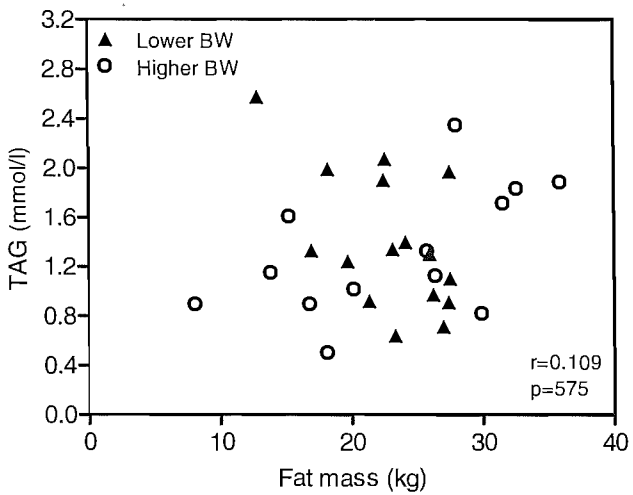
In fasted states, there were also no significant differences in glucose concentration in plasma between the lower and higher birth weight groups (6.16 ± 0.33 v 6.00 ± 0.33 mmol/l respectively). The time course of plasma glucose concentration over the study period between the low and high birth weight groups is illustrated in **Figure 8.5.** After meal ingestion, glucose concentration rose to a peak near 8.0 mmol/l for both the lower and higher birth weight groups at one hour, fell rapidly to near baseline within 3-4 hr. The area under the curve for glucose concentration in plasma over the study period (6h) was not different between the two groups, although it tended to be greater in the lower than the higher birth weight groups (≈ 15%). The total incremental glucose concentration in plasma was also not different between the two groups.

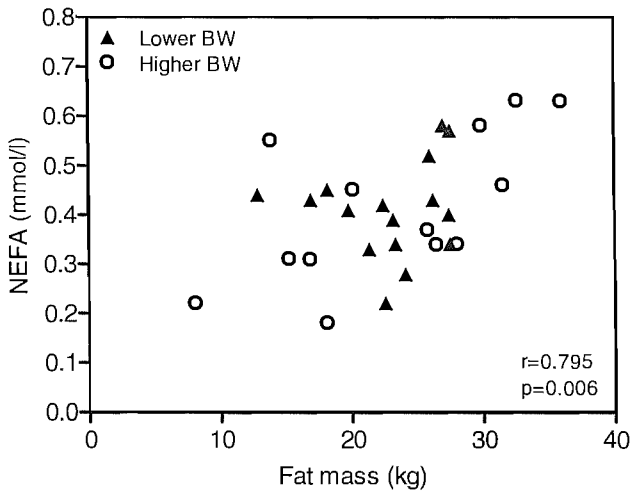
The above results suggested that there were no significant differences between the two groups in fasted TAG, NEFA and glucose. In addition, there were no differences between groups in the pattern of TAG, NEFA and glucose following the ingestion of the test meal.

Following the ingestion of the test meal, the concentration of ^{13}C-Palmitic acid in the TAG fraction increased to peak concentrations at 2 hours, before falling steadily to values half that seen at peak by 6h (see Figure 8.5). ^{13}C-PA in the TAG fraction, either as total AUC or incremental AUCi, was not different between the two groups (AUC; 21.24 ± 3.10 v 23.97 ± 3.40 µg/ml per 6h for the lower and higher birth weight group respectively) and (AUCi; 21.24 ± 3.12 v 22.97 ± 3.46 µg/ml per 6h). The concentration of ^{13}C-NEFA in plasma after meal ingestion followed a similar pattern, peaking at 2-3 hours and remained elevated over the study period (see figure 8.6). There were no significant differences in ^{13}C-PA in NEFA fraction between the two groups either presented as total area under the curve (3.39 ± 0.27 v 3.11 ± 0.23 µg/ml per 6h) or incremental area under the curve (3.08 ± 0.25 v 2.75 ± 0.33 µg/ml per 6h). These results suggested that there were no significant differences between groups in the process of delipidation (or clearance of TAG from the exogenous lipid and

entrapment of NEFA from the exogenous lipids (from meal).

The concentration of cholesterol, LDL-cholesterol, and HDL-cholesterol in plasma for the lower and higher birth weight groups are presented in **Figure 8.8**. The concentrations of cholesterol, LDL-cholesterol, and HDL-cholesterol in plasma were not different between the lower and higher birth weight groups. The prevalence of metabolic syndrome tended to be different between the lower and higher birth weight group. The differences between groups in the criteria of metabolic syndrome that are used in this study were those based on ATPIII-BMI25 (24). The lower birth weight groups tended to have twice the risk of having the metabolic syndrome when compared to those in the higher birth weight group (see Table 8.1).





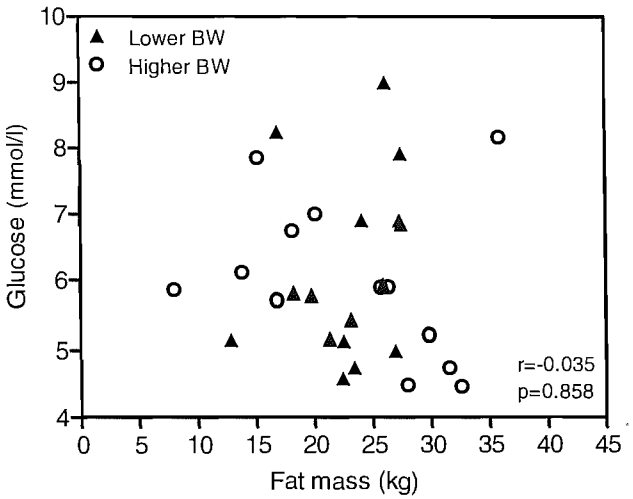
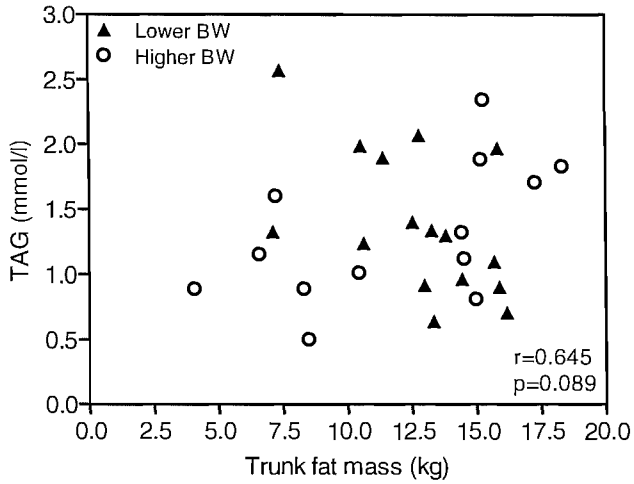
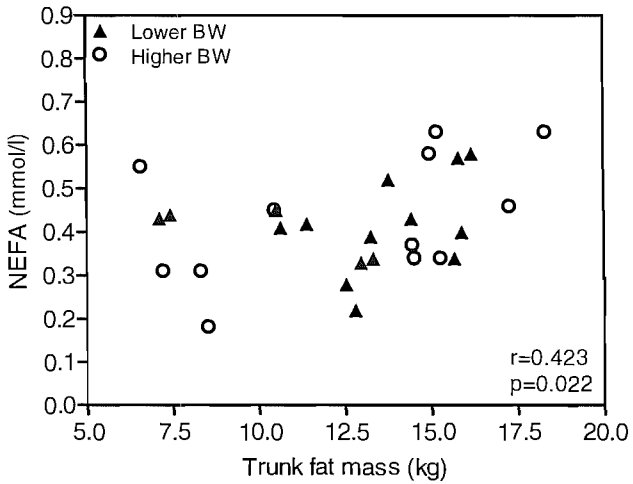


FIGURE 8.1 Relationships between fat mass and plasma TAG, NEFA and glucose concentration in fasted sates





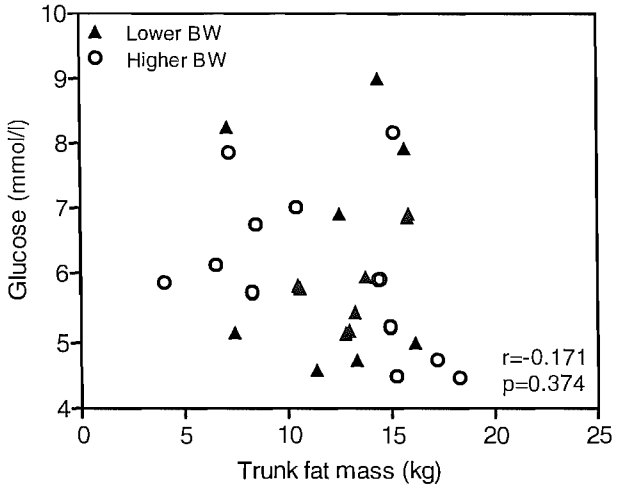


FIGURE 8.2 Relationships between trunk fat mass and plasma TAG, NEFA and glucose concentration in fasted sates

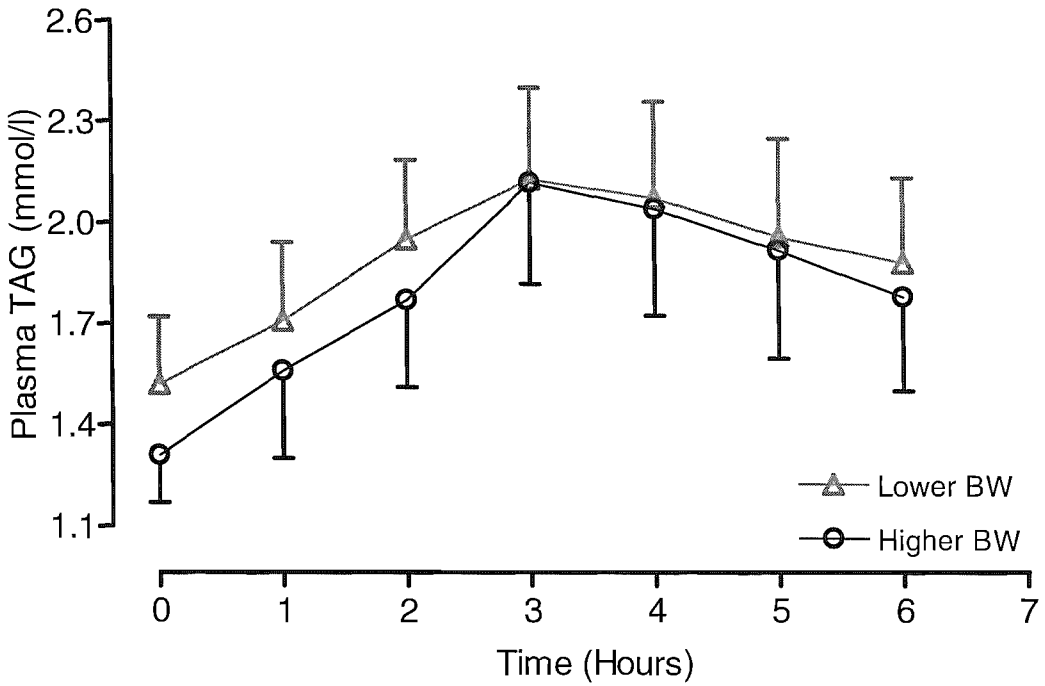


FIGURE 8.3 Pattern of plasma triglyceride concentration (mmol/l) over a six hour study period between the low and high birth weight groups

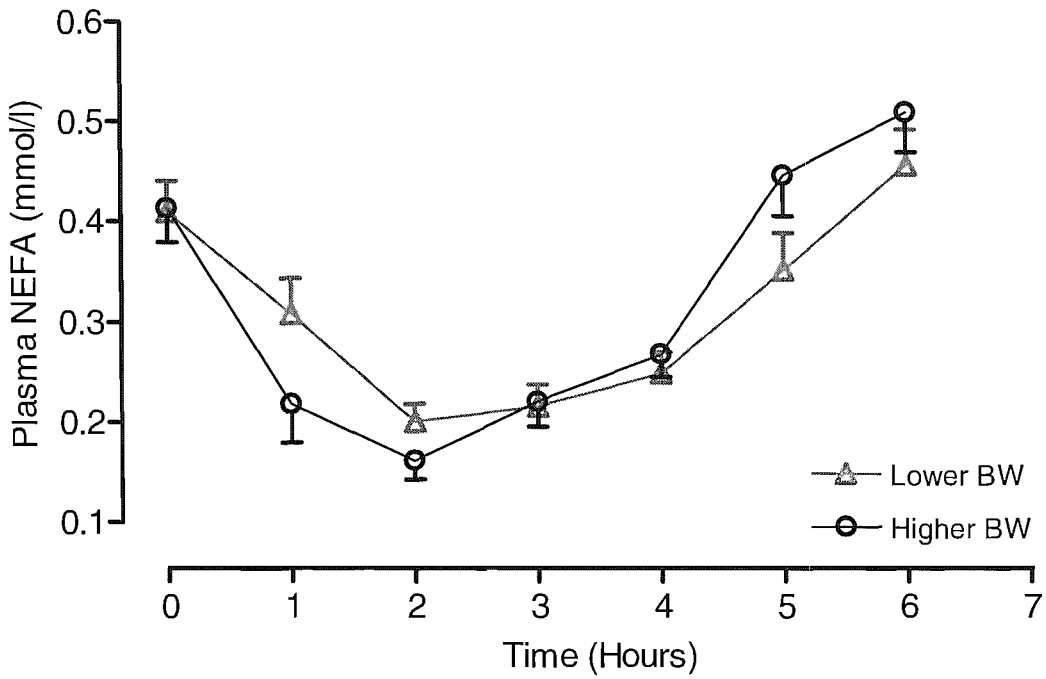


FIGURE 8.4 Pattern of plasma non-esterified fatty acid concentration (mmol/l) over a six hour study period between the low and high birth weight groups

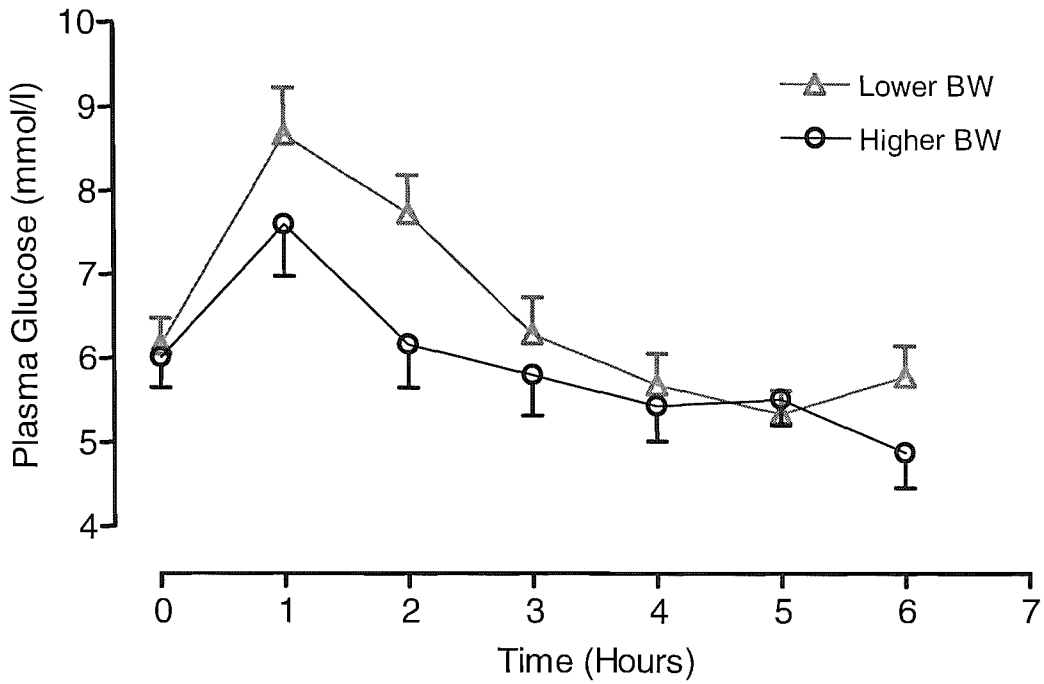


FIGURE 8.5 Pattern of Plasma glucose concentration (mmol/l) over a six hour study period between the low and high birth weight groups

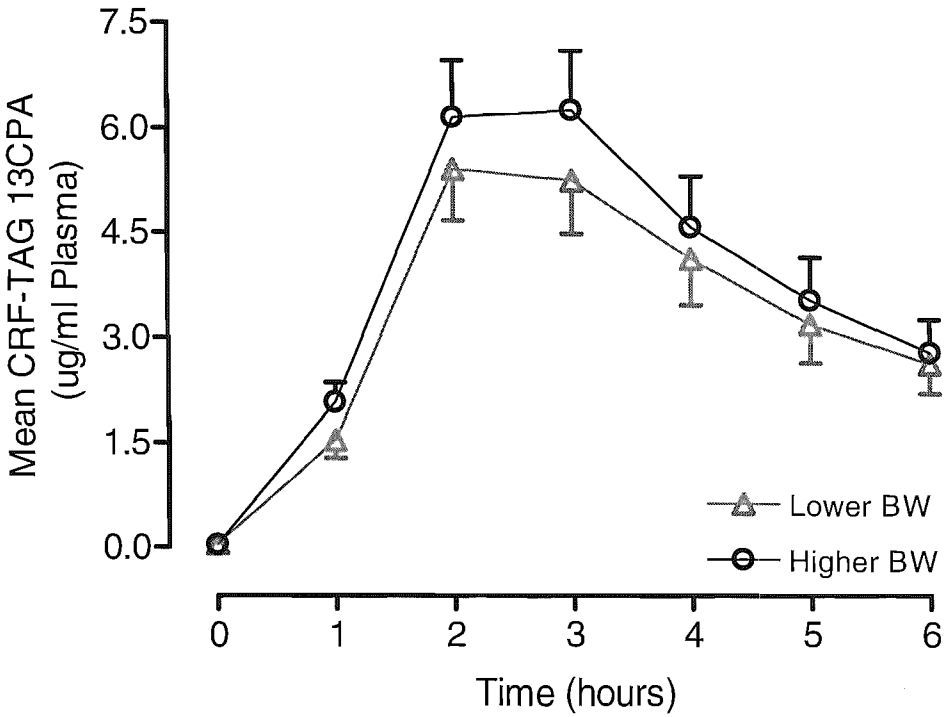


FIGURE 8.6 Pattern of ^{13}C PA in CRF-TAG concentration over a 6 hour study period between the lower and higher birth weight group

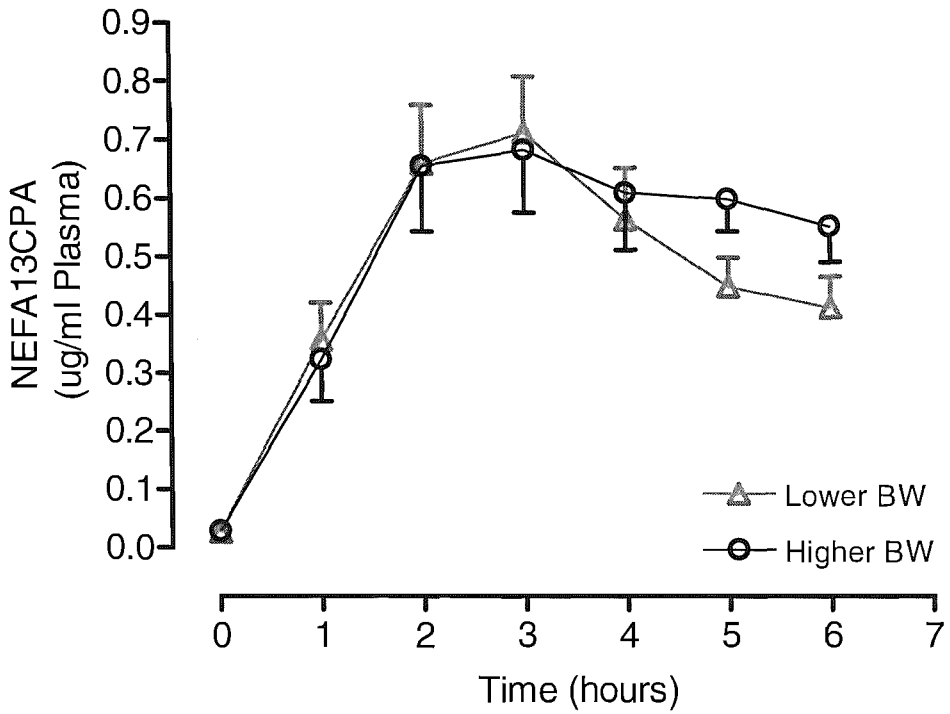


FIGURE 8.7 Pattern of ^{13}CPA in NEFA fraction over a 6 hour study period between the lower and higher birth weight group

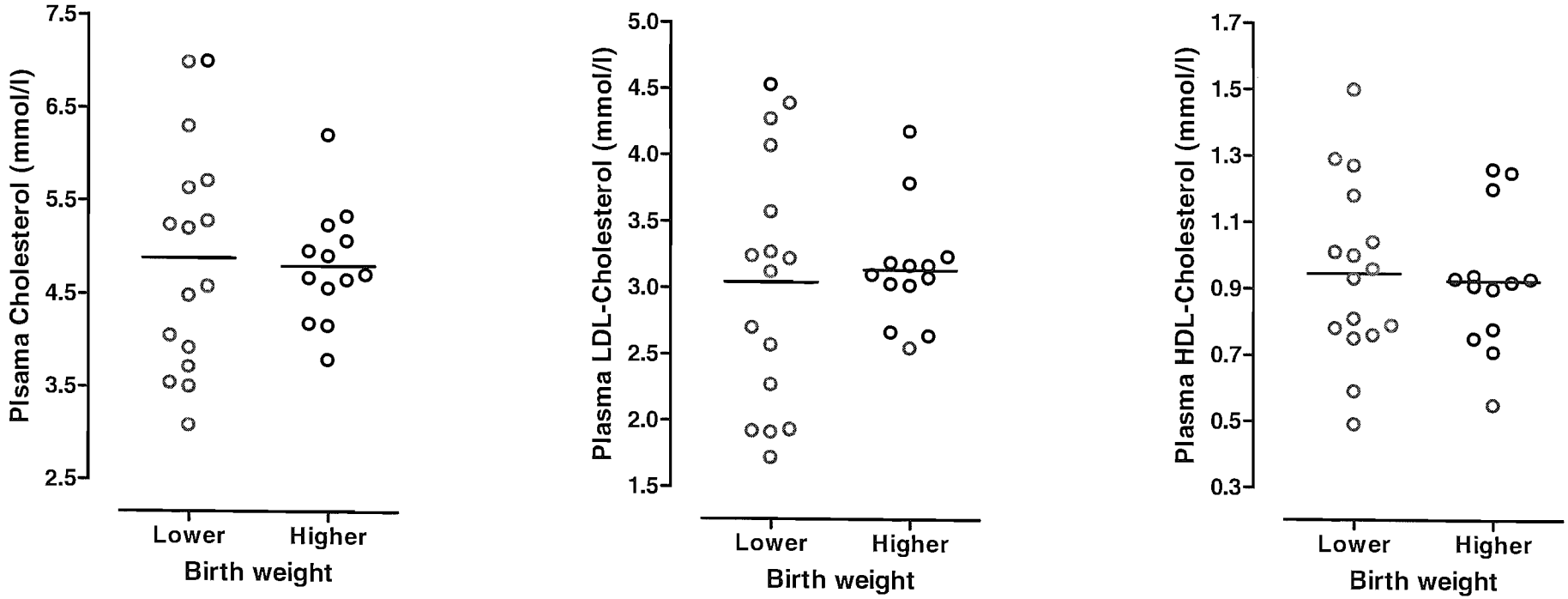


FIGURE 8.8 Differences in baseline plasma cholesterol, LDL-cholesterol, and HDL-cholesterol between the low and high birth weight groups

TABLE 8.1 Components of metabolic syndrome (ATPIII-BMI25 criteria) and its prevalence according to birth weight group

	Low BW (n=16)	High BW (n=13)	P value
BMI (kg/m^2)	26.8 ± 1.49	27.9 ± 1.17	0.319
Blood pressure (mmHg)			
Systolic	148.0 ± 3.44	137.0 ± 5.06	0.089
Diastolic	81.0 ± 1.90	82.0 ± 2.90	0.908
TAG (mmol/l)	134.9 ± 17.9	116.2 ± 12.9	0.426
Cholesterol (mmol/l)	188.9 ±11.90	185.8 ± 6.57	0.826
LDL-cholesterol (mmol/l)	117.8 ± 9.16	121.4 ± 4.76	0.744
HDL-cholesterol (mmol/l)	37.1 ± 2.87	35.8 ± 2.27	0.732
Glucose (mmol/l)	111.0 ± 6.02	108.2 ± 5.9	0.746
Metabolic syndrome (n/N; (%))	11/16 (68.8%)	5/13(38.5%)	0.105

8.4 Discussion

This part of the study aimed to examine the relationship between adult body structures, in particular, fat mass and the distribution of body fat and metabolic markers associated with poor metabolic control marked by lipid and lipoprotein metabolism. In addition, this study aimed to investigate the extent to which birth weight as a marker of fetal growth could have an effect on this relationship that is achieved by first examining the extent to which differences in birth weight could alter lipid metabolism both in fasted and fed states.

The results of this chapter illustrated that fat mass and trunk fat mass obtained by DXA were positively associated with plasma TAG and NEFA at baseline. Similar trend in the fed states has been also observed. In addition, the results presented in this chapter did not demonstrate for any relationship between birth weight and any measures related to lipid and lipoprotein metabolism. Adults born with a lower birth weight has been reported in the previous chapters to have greater fat mass in particular in the central region compared to those born with higher birth weight after adjustments were made for height and weight. Therefore, it would be expected that such differences in body composition and fat distribution to have an impact on lipid metabolism in fasted and fed states or metabolic risk factors associated with CVD. For example, previous study reported that the increase in adiposity and central fat mass has been associated with reductions in clearance of TAG lipoprotein rich particles from plasma after meal ingestion (30). The area under the curve for TAG concentration after meal ingestion and over the study period was reported to be greater in obese subjects (BMI=38.3 kg/h^2) when compared with lean individuals (BMI=21.1 kg/h^2). The area under the curve for TAG concentration after meal ingestion in the present study, however was not different between the lower and higher birth weight groups despite the reported differences in adiposity and central fatness. The area under the curve for ^{13}C-PA in TAG fraction in plasma after meal ingestion which represents the clearance of exogenous lipid from the meal was also not different between the study groups. In addition, after meal ingestion both the area under the curve for NEFA and ^{13}C-PA in NEFA fraction in plasma were not different between the lower and higher birth weight groups. These metabolic markers reflect the differences entrapment of NFEA after meal ingestion. Poor entrapment of NEFA is associated with increase the NEFA flux in plasma and that, in turn, may associated with reduce in tissue insulin sensitivity (272).

The results of this chapter also demonstrated that the prevalence of metabolic syndrome in

the lower birth weight group was almost twice that seen in the higher birth weight group, but due to the small number of subjects, the difference did not reach statistical significance. Previous studies show that the prevalence of metabolic syndrome is inversely associated with birth weight, some of these studies suggested that this inverse relationship is mediated by the interaction of birth weight and current environmental factors such as obesity as marked by BMI and current physical fitness (see chapter 2, section 2.2.6).

Although the results of this chapter did not reported for any significant differences in any lipid profile, it is the only study that has examined the metabolic control marked by differences in lipid metabolism both in fasted and fed states in relation to differences in birth weight.

8.5 Summary

The analysis performed in this chapter set out to examine the extent to which body fatness in particular in central region of the body is associated with biological markers related to lipid metabolism both in the fasted and fed states, independently of birth weight. Furthermore, the analysis also aimed to examine the extent to which lipid, lipoprotein and glucose metabolisms in the fasted state are influenced by event in early life marked by differences in birth weight.

The results of this chapter indicated that:

- Total fat mass and central fatness measured by DXA are associated with fasting plasma TAG, NEFA and glucose. In addition, these measures of body composition were also associated with TAG, NEFA and glucose concentration in plasma after meal consummation over the study period.

- The biological marker related to lipid and lipoprotein metabolism both in fasted and fed state was not influence by differences in birth weight

CHAPTER 9

GENERAL DISCUSSION

9.0 Introduction

The primary aim of the work presented in this thesis set out to examine the extent to which current phenotype in terms of body structure and metabolic function is influenced by fetal growth marked by birth weight. The discussion in this chapter is presented in four sections. The first summarises the principle observations arising from the work present in this thesis. The second considers how the principle observations of this thesis could contribute to our understanding of the way in which events in early life might contribute to the acquisition of an imprudent phenotype in later life and the extent to which being born small might predispose the individual to an increased cardiometabolic risk. The third represents the implication of the mean outcome of the work of this thesis. The limitation of work presented in this thesis and future work are presented in the final section.

9.1 Principle observations from this study

The present analyses show that there are differences in current body structure in terms of size, composition and fat distribution in relation to birth weight confirmed previous studies that adult height, weight and FFM are influenced by fetal growth. That is, an infant born with a lower birth weight is more likely to become shorter, lighter and had shorter leg length with preserve trunk and head length when compared with those born with higher birth weight. These changes in body size and shape may reflect asymmetrical growth associated with poor early fetal growth. In addition, adult with a lower birth weight have a lower FFM as an adult when compared to those with higher birth weight. The relationship between birth weight and FFM was observed using four independent approaches (SKF, BIA, BodPod and DXA) to assess body composition.

The present work also confirmed previous findings that absolute fat mass (kg) was not related to the differences in birth weight, both lower and higher birth weight group having the same amount of FM that is obtained by different methods. Given a similar fat mass between

the two groups in respect to their differences in body size in particular body weight, it was expected that the relative fat mass (%) would be greater in the lower birth weight group. However, although there were no significant differences in relative fat mass between the two groups, there was a tendency towards relatively more fat (%) in the lower birth weight group.

The assessment of body composition by DXA provides additional information on the differences in different components of FFM that could be associated with birth weight. We have shown that muscle mass, FFST and non-muscular FFST are influenced by fetal growth. Infants born with a lower birth weight had lower muscle mass, FFST and non-muscular FFST as an adult when compared to those born with higher birth weight. The proportion of non-muscular FFST to muscle mass tended to be greater in the lower than the higher birth weight group. These differences in the different component of FFM which has different metabolic activity may contribute to the differences in energy-substrate metabolism.

We were able to show, like others, that both height and weight were positively associated with FFM. We were able to go further and show that both measures of body size are also positively associated with muscle mass, non-muscular FFST as obtained by DXA. Although height was not related to total body fat mass, body weight was positively associated with fat mass. Since infants born with a lower birth weight are shorter and lighter as an adult, it would be reasonable to expect that they would also have a lower absolute lean and fat mass through a simple scaling effect. However, we found that at any given height and weight, infants born with a lower birth weight is more likely to have lower FFM and greater absolute FM (kg) than those born with higher birth weight. This suggests that body composition in adulthood is programmed in early life independent of the differences in body size. Differences in FFST, muscle mass and non-muscular FFST associated with birth weight were also evident independent of height and weight. This finding raises an issue about the contribution of different components of FFM, each of which has different metabolic activity and pattern of energy-substrate metabolism, in respect to differences in body size in term of height and weight. In other words, the extent to which differences in the pattern of energy and substrate metabolism could arise from differences in fetal growth and whether such differences could be explained by differences in size and structure, particularly due to differences in the relative proportions of tissues that make up the lean mass.

In agreement with previous studies using simple anthropometry, both waist circumference and waist to hip ratio were not affected by birth weight. However, using the DXA analysis, we found for the first time that the pattern of fat distribution marked by the ratio of non-limb: limb

and trunk: limb fat mass was associated with differences in birth weight. This suggests that adults with a lower birth weight are more likely to have greater visceral fatness than the higher birth weight group. As the pattern of fat distribution marked by the non-limb: limb, trunk: limb and abdominal: limb fat mass ratios were inversely associated with height and positively associated with body weight, it would be expected that such differences in body size might have an impact on the reported differences in the pattern of fat distribution associated with birth weight. For example, adults with a lower birth weight who are shorter and lighter would be expected to have lower visceral fat mass than those with higher birth weight because they are lighter. However, we found that infants born with lower birth weight are more likely to have greater visceral fat mass than the higher birth weight group after adjustment was made for the differences in body size (height + weight). This could be attributable to the interaction between height and weight on the differences in pattern of fat distribution associated with birth weight. The greater amounts of visceral fat mass associated with lower birth weight could mediate the relationship between birth weight and risk of CVD in later life.

The results presented in this thesis also show that at any given BMI, there are differences in body composition and fat distribution associated with birth weight. That is, infants born with a lower birth weight have a lower lean mass (FFST, muscle mass and non-muscular FFST) as an adult when compared with those with higher birth weight at the same BMI. In addition at the same BMI, they had greater fat mass as percentage of body weight and greater visceral fat mass. These findings raise two important issues. The first relates to the validity of using BMI as proxy measures of current body size or adiposity that of itself is related to metabolic risk factors associated with CVD in later life. The second important issue is that the current finding could explain the paradox of greater adiposity marker by higher BMI in the higher rather than lower birth weight individuals since higher birth weight subjects are reported to have lower risk of having CVD and T2DM than those born with a lower birth weight.

The results presented in this thesis also illustrated for the first time that the vertical and horizontal dimensions of the body, as segment length (i.e. trunk and leg length) and the breadth of the bone at shoulder (or frame size), are influenced by birth weight. That is, an infant born with a lower birth weight have a shorter leg length and taller trunk length as an adult at the same height when compared with those born with higher birth weight. Since the composition of each body segment would be expected to be different (i.e. the proportion of fat to FFM in the trunk is different from that observed in the leg), differences in segment length would expected to have an impact on the differences in body composition associated

with birth weight. However, even after adjustment was made for the differences in segment length and frame size, there were differences in body composition associated with birth weight that could not be explained simply by the dimensions of the body. In addition, although the segment mass in particular non-limb mass were not different between the study groups, adults born with a lower birth weight had lower visceral FFM and greater visceral mass at the same height and weight when compared to those born with higher birth weight. In other words, despite the comparable segment mass between the two groups, the composition of these segments was different which by itself could explain the reported differences in total body composition associated with birth weight. The variance in visceral FFM and FM could account for 55% and 76% respectively of the total differences in body composition associated with birth weight.

When the variation in body dimensions and segments mass in relation to birth weight were considered in a statistical model, infants born with a lower birth weight still had greater absolute fat mass and lower FFM as an adult when compared to those born with higher birth weight. These results suggest that although there is variation in the dimensions of the body associated with birth weight, a proportion of the remaining variance in body composition associated with birth weight remains which cannot be explained by body dimensions.

This work has shown that the metabolic demands for energy, as a summative statement of metabolic competence, in both the fasted and fed states are influenced by differences in birth weight. Infants born with a lower birth weight had lower REE both in fasted and fed states when compared to those born with higher birth weight. This finding raise the question about the contribution of body size associated with birth weight on such differences in energy metabolism since height and weight are known as major determinants of REE. However, even after accounting for the differences in height and weight, infants born with a lower birth weight had lower REE as an adult in both the fasted and fed states when compared with those born with higher birth weight. Since body composition is influenced by birth weight, independent of body size, one would expect that the observed differences in energy metabolism are mediated through differences in the lean mass. However, this work has shown for the first time that even after adjusting for the differences in lean mass (FFST, muscle mass and non-muscular FFST) associated with birth weight, adults with a lower birth weight still have lower REE in fasted (8-10%) and fed states (9-11%) when compared with those with higher birth weights. This important finding suggests that the differences in energy metabolism associated with birth weight could be explained qualitatively rather than quantitatively. In other words, the difference in REE associated with birth weight is not solely

related to the differences in the amount of metabolically active tissue (i.e. differences in the proportion muscle to non-muscular), but it could be related to the differences in the demanded of energy that is set for these different tissues. For example, at any given muscle or non-muscular FFST, there could be a variation the in tissue specific metabolic rate because of different metabolic demands of each of these tissue which could be influenced by fetal growth. The mechanism by which differences in fetal growth may program or set metabolic rate of specific tissues to the lead to differences in REE remains unclear. The most obvious candidate process that would need to be discounted before perusing other processes would be to explore the factors that govern the rate of protein turnover in different tissues as this represents the most intense metabolic process consuming energy.

The work presented in this thesis also examined for the first time the influence of birth weight on the pattern of lipid metabolism both in fasted and fed states. Lipid metabolism has been characterised by the concentration TAG and NEFA in the fasted state. In the fed states stable isotope (^{13}C-palmitic acid) has been used to explore the differences in the pattern of exogenous lipid metabolism in terms of both TAG clearance and NEFA suppression. For example, the higher TAG AUC or AUCi reflect poor clearance of TAG from the circulation following the ingestion of test meal. A higher AUC of ^{13}C-palmitic acid in the TAG reflects poor delipidation of TAG from circulating lipoproteins (i.e. limited hydrolysis by lipoprotein lipase) such that more of the exogenous lipid remains in the circulation. A higher NEFA AUC, or a lower NEFA suppression as AUCi reflect increased NEFA efflux from adipose tissue. This lack of NEFA suppression may be attributable to either a lesser insulin sensitivity of the adipocyte or impairment in NEFA entrapment following delipidation of TAG from the lipoprotein. The latter will also be reflected as a higher ^{13}C-palmitic acid in the NEFA fraction (as AUC). The results presented in this thesis however show no differences in lipid metabolism both in fasted and fed states associated with differences in birth weight.

9.2 How this work could contribute to our present understanding

To better understand the influence of fetal growth on the risk of CVD in later life, one should consider how the current phenotype in terms of body structure and metabolic function are linked with each other and how this interaction between body structure and function might be influenced by fetal growth. For instance, could metabolic function be programmed and it is this difference in metabolism that causally determines the pattern of subsequent growth and development that results in the body composition and fat distribution seen in adulthood (i.e. a lower energy expenditure resulting in an increased predisposition to increasing adiposity).

Alternatively, is it the pattern of adiposity that of itself determines the metabolic function? (i.e. higher central fat predisposes the individual to greater insulin resistance). The results of this thesis suggest that as the differences in metabolic function in terms of current energy metabolism associated with being born small are independent body size and composition (lean mass), the possibility that it is metabolic behaviour of itself is programmed and that this may or may not be associated with differences in adiposity. This less prudent metabolic phenotype leads to more obvious features such as adiposity and central fat which in turn, may contribute to the observed increase in adiposity and visceral fat mass which are considered as independent risk factors for T2DM and CVD (see Figure 9.1).

Previous studies examining the role that fetal growth may have on insulin resistance and glucose intolerance conventionally attempted to control for differences in those factors, which in themselves are known to be associated with differences in glucose and insulin metabolism, such as BMI as a marker of body fatness and current lifestyle. Such factors are seen as potential confounders that would mask any effects that could be attributed to differences in events in early life and therefore, their contribution to the observed variance in the outcome variable needs to be excluded. Whilst self-evident at one level, such an approach may be conceptually flawed given that BMI is a poor measure of body composition and that the structure of the body is itself programmed in such a way as to influence height, weight, proportions and composition.

For example, since muscle mass is an important site for glucose uptake and metabolism (273), a failure to acquire muscle mass during growth (either altering metabolic behaviour or patterns of activity behaviour and fitness) may be the critical causal process that links fetal growth and insulin resistance and glucose intolerance, and therefore, T2DM. Hence, before adjusting for size, BMI or specific compartments, particular consideration should be given to the underlying biological processes linking structure and function so as to avoid losing critical information in the statistical analysis of the data.

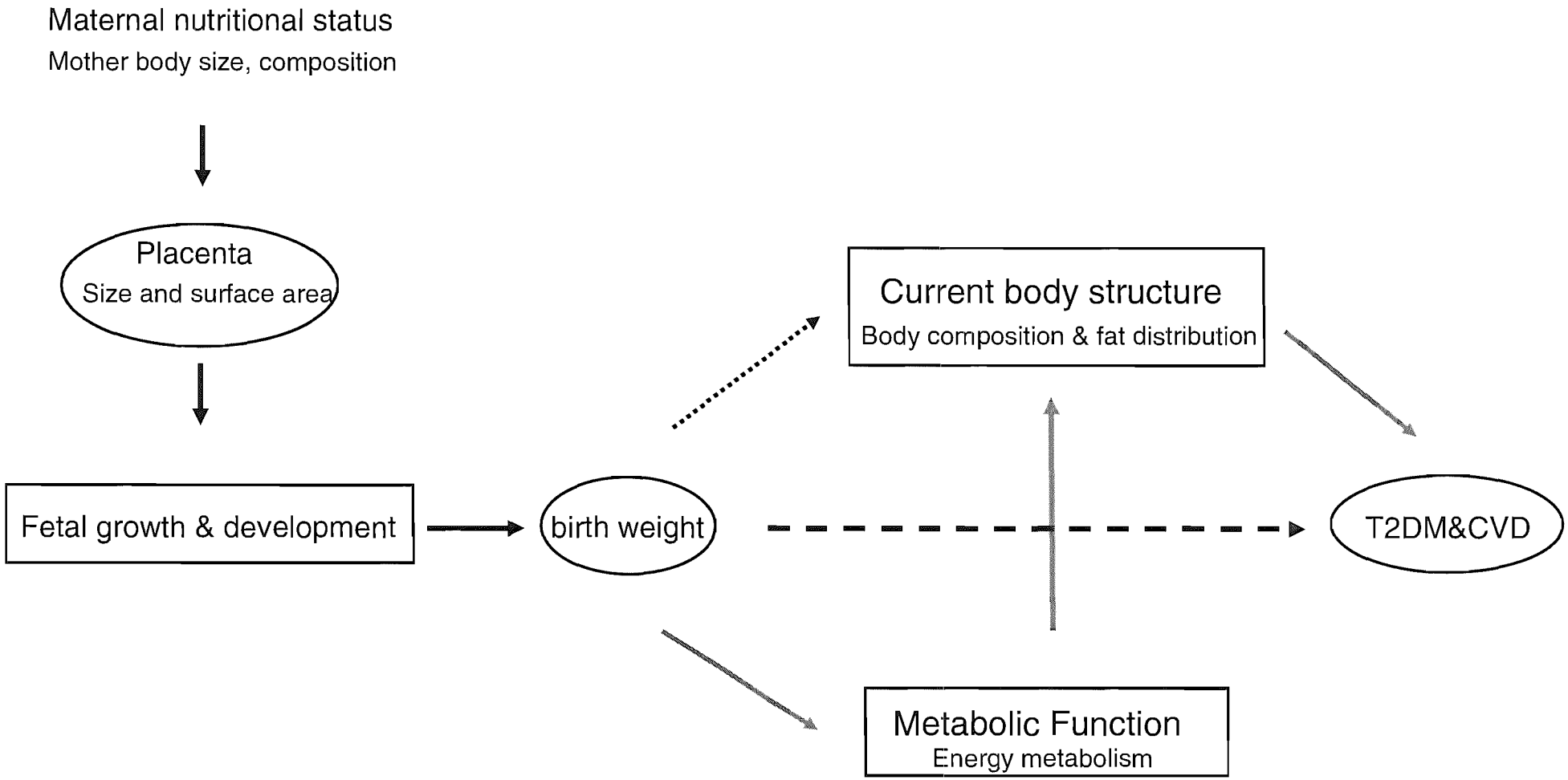


FIGURE 9.1 Influence of birth weight on current metabolic function may describe the differences in body structure

9.2.1 Influence of fetal growth on current body composition and fat distribution: do we need to adjust for body size?

Height is known to be positively associated with lean mass, although the relationship with fat mass is more variable. Weight on the other hand is known to be positively associated with fat mass, although increasing weight or fat mass is also associated with increasing lean mass. Therefore, one would expect that the differences in body composition associated with birth weight is the result of the scaling effect, that is, infants born with a lower birth weight are shorter and lighter and therefore would have lower lean and fat mass when compared with those born with higher birth weight. Therefore, factors that affect height and weight may impact on both lean and fat mass, either directly through simple scaling effects or less obviously through other, more specific processes.

The differences in lean mass associated with birth weight were not influenced by the height or weight differences between groups. Thus, although part of the difference in lean mass could be explained by the difference in height or weight, a substantive proportion of the variance remained that could not be simply accounted for by a simple scaling effect. In other words, the amount and composition of the lean mass of itself appears to be programmed by events in early life by processes other than those that might affect stature such as bone growth.

In contrast, despite weighing less and having a lower lean mass, the fat mass of the two groups was similar. It was only after adjusting for differences in height and weight that the greater fat mass in the lower birth weight group became apparent. In other words, the absolute fat mass of the lower birth weight group was comparable to that seen in the higher birth weight group despite being shorter and lighter. When mathematically adjusted to permit a comparison at the same height and weight, the lower birth weight group would have a greater fat mass and a lower lean mass, hence they would be considered to be relatively fatter. This would suggest that the processes through which fat mass is acquired during growth and development or in adult life, may themselves be programmed.

This appropriateness of adjusting for height and weight in this way needs to considered carefully, to avoid what some have called a 'reversal paradox'. That is, using statistical adjustments to account for a variable which is mediated in the casual pathway (thus not a true confounder) may give rise to an inappropriate interpretation between a measure and outcome (274). Tu et al in recent study which is based on different hypothetical models which

include birth weight, current body weight and blood pressure suggested that the relationship between birth weight and blood pressure as an outcome could be misinterpreted when adjustments were made for current body weight (275). Such that if there is no relationship between birth weight and blood pressure but both are positively associated with body weight, then adjustments for body weight can induce an inverse relationship between birth weight and blood pressure. However, whilst this might be true in some cases, it is proposed that such an approach is biologically sound in current study. For example, the differences in fatness and pattern of fat distribution associated with birth weight were masked with differences in body size. However, when adjustments were made for height and weight, the differences in adiposity and fat distribution are observed. This may explain, in part, the inconsistent results on differences in fatness associated with birth weight. However, the present results show as other, that adults with higher birth weights are taller and heavier than those with lower birth weights and that may imply that they have greater fatness than those with lower birth weights, while the opposite is true when adjustments were made for height and weight.

Although the relationship between birth weight and current FFM is consistent with previous studies, adjustments for body size did not have an effect on the differences in lean mass associated with birth weight. The results were also the same for the pattern of fat distribution, that is, the differences in the pattern of fat distribution associated with birth weight remain the same even after adjustments were made for body size.

In summary, although both height and weight are part of the causal relationship between birth weight and body composition, adjustment for body size could further explain differences in body composition and fat distribution that are independent of body size; this in turn may explain the risk of CVD associated with differences in birth weight.

9.2.2 How early fetal growth could have an effect on adult body composition?

Infants born with a lower birth weight were reported to have lower FFST by ≈ 6% when as an adult when compared to those born with a higher birth weight. The contribution of muscle mass to the differences in FFST is greater than that of non-muscular FFST (2.28 v 1.14 kg). There are several plausible mechanisms which may explain the differences in lean mass, in particular, muscle mass associated with birth weight. Genetically determined insulin resistance may lead to impaired insulin mediate growth of fetal lean mass, and the

continuation of this pattern of body composition during childhood growth could be associated with lower lean mass as an adult. Fetal lean or muscle growth in utero might be sacrificed in favour of brain development as the result of undernutrition. Fetal undernutrition may predispose the fetus to hypoglycaemia. This in turn, may limit insulin secretion, and consequently increase protein breakdown and decrease protein accretion. Furthermore, constrain of nutrients in utero could lower the concentration of insulin like growth factor 1, and that in turn may compromise with a lower fetal lean mass.

The lower muscle mass associated with lower birth weight may also be related to the growth and development of muscle fibre. The number of muscle fibres has been proposed to be determined at or soon after birth (276). In support of this view, animal studies show that the number of muscle fibres are reduced as result of poor intrauterine nutrition (277).

Although the relationship between birth weight and adult adiposity has been reported to be inconsistent, the present work show that infant born with a lower birth weight had greater total fat mass (kg) at the same height and weight when compared with those born with higher birth weight. Yet the mechanism underling the effect of birth weight on adult adiposity remains unclear. This could be possible because the variation in fat mass in adulthood is greater than that of lean mass, since many environmental factors may contribute to this variation. However, the increase in fat mass in the lower than the higher birth weights may be related to the alteration in the number and size of adipocyte (278) which might then respond to subsequate dietary exposure by changes in the size of the adiposity.

Another possibility for the increase in adiposity in relation to birth weight could be attributed to changes in pancreatic islet B cell and impairment of insulin secretion associated with fetal undernutrition. Such changes in insulin secretion, may in turn, associate with grater fat deposition after birth. Studies in animal show that rat exposed to a protein restricted diet during gestation, but who were then nursed by mothers fed a full protein diet, grew fatter than those that had never been protein restricted (279;280). In these studies the relationship between offspring weight at birth and later adiposity has been suggested to be mediated through differences in glucose intolerance.

The increase in adiposity associated with birth weight may be observed as the result of differences in energy metabolism. Although some studies reported that obese subject have greater REE than lean subjects, others suggested that the decrease in REE is what causes an increase in adiposity. Since adults born with a lower birth weight have lower REE as

reported in this thesis, this might explain the greater adiposity associated with lower birth weight. However, the relationship between energy and adiposity is a complex issue. For example, although REE is the major part of total energy expenditure, energy expanded in relation to physical activity is varying between individuals within the same group, that in turn, may confound the influence of lower REE on the risk of adiposity. However, physical activity might be influenced by fetal growth. Birth weight has been reported to be positively associated with aerobic fitness in adolescent boys and girls (267). That may imply that adult physical activity may be influenced by birth weight and that, in turn, might lead to the increase in adiposity associated with lower birth weight. However, the relationship between birth weight and physical activity remains uncertain, since there are limited number of studies were focus in this field.

Variation in dietary intake between individual may also contribute to the differences in total energy expenditure, and without knowing the contribution of such variations to total energy metabolism, it will be difficult to predict the influence of energy metabolism on weight gain and adiposity.

9.2.3 What could contribute to the differences in energy metabolism associated with birth weight?

The results presented in this thesis show that energy metabolism both in fasted and fed states is altered in relation to birth weight and such differences are independent of FFM or different components of FFM which include muscle mass and non muscular FFST. This suggests that there is a qualitative difference in REE associated with birth weight which is attributed to the differences in energy demand of different tissue. The tissue specific energy demand could be related to three different components. The contribution of these components to overall REE could vary from modest or relatively fixed to more highly variable component. These component include the energy cost that is required to set the mechanical work of body organs such as heart, liver and brain. Although the organ size in body varies between individuals, the demand of these organs for energy could be assumed to be constant. Therefore, the contribution of this component of energy demand to overall variation in REE between individuals could be considered constant. The other two major components which involve on the variation in tissue specific energy demand at rest include the process of substrate metabolism, in particular, protein turnover and, the energy involved in cellular membrane ion pump. The energy cost associated with protein turnover represents the energy required during the utilisation of nitrogen and amino acid, in the formation of

polypeptide and their further assembly within or outside the cell and; ultimately in the degradation of proteins and the catabolism of their constituent amino acids. Although the rate of protein turnover is affected by stress and disease, within the normal physiological condition it may influenced by other factors such as thyroid hormone. The thyroid hormone has long been recognised as a major regulator of the oxidative metabolism of energy producing substrates which include protein metabolism. The decrease in thyroid hormone or hypothyroidism is associated with numerous alterations in metabolisms, such as decreased lipolysis (281;282), decreased plasma non-esterified fatty acids and reductions in glucose production (283). Thyroid hormone also has an effect on protein turnover. For instance, in hyperthyroidism, skeletal muscle protein stores suffer depletion which is reflected by an increased urinary nitrogen and methylhistidine excretion (284). On the other hand, hypothyroidism has been reported to be associated with muscular weakness. During hypothyroidism whole body leucine flux and protein synthesis have been reported to be decreased (285). This may suggest that the observed differences in energy metabolism associated with birth weight could be attributed, in part, to the differences in energy demand that is associated with protein turnover. Although protein turnover could be influenced by thyroid hormone, the extent to which early fetal growth may have an effect on the thyroid function and might lead to the difference seen in REE need to be determined.

The other process, which also involves higher energy demand, is related to cellular membrane ion pump, particularly the Na^+, K^+ - ATPase complex. The major function of this pump is to convert the chemical energy from the hydrolysis of ATP into a gradient for Na^+ and K^+; these gradients are used as free energy sources for a number of processes such as formation of the membrane potential, cell volume regulation, and transport of glucose and amino acids into cells against concentration gradients. The demand for energy associated with this process is considerably large, It accounts for 9-45, 16-51 and 17-61% of the oxygen utilisation in the skeletal musculature, liver and gastrointestinal tract, respectively, under a variety of physiological and nutritional conditions (286). The process of cellular membrane pump is also regulated by the thyroid hormone (287). The active form of thyroid hormone (T3) has an important role in regulating the activity and gene expression of the Na^+, K^+ - ATPase protein complex. Therefore, alteration in cellular membrane ion pumps, which might be also influenced by the thyroid hormone, could contribute to the overall energy demand. This in turn, may also explain the differences in energy metabolism associated with birth weight. The question is how could this complex energetic process involved in protein turnover and cellular ion pump are influenced by early fetal growth? Is it mediated to through alteration in the status of thyroid?

Although there is, a difficulty to assess the demand of energy associated with these processes, protein turnover may provide important information on the variation of energy involve in protein synthesis and oxidation. Two important issues could be obtained from assessing protein turnover. It may provide information on the energy demand from protein turnover relative to overall REE, which could be expressed as the ratio of protein to energy. It may also further explain protein oxidation, which may have an effect on the other macronutrients including carbohydrate, and lipid oxidation. The present results of this thesis reported no differences in macronutrients oxidation associated with birth weight obtained by indirect calorometry. However, this method gives crude measure of macronutrients oxidation, which is based on measuring of oxygen consummation and CO_2 production. However, it could possible that, if protein turnover is influenced by birth weight as the results of differences in protein oxidation for example, then this may also have an effect on the differences in CHO and lipid oxidation. To better examine macronutrient oxidation in relation to early fetal growth, one should consider assessing protein turnover. This can be obtained by two different approaches. First, by measuring the rate urinary nitrogen excretion in urine, and secondly, by using stable isotope tracer methodology, which involves measuring the oxidation of amino acid by ^{13}C-leucine or measuring the excretion of nitrogen in urine by ^{15}N-glycine.

Another possibility for the differences seen in REE associated with birth weight might be attributed to the sympathetic nervous system (SNS). SNS play an important role in the regulation of energy expenditure. Previous studies show that the sympathetic ^{ß}adrenergic stimulation evokes an increase in metabolic rate under basal fasted conditions (288;289). Similarly, it has been also reported that the SNS is largely responsible for the facultative component of the thermic effect of acute energy intake in humans (290). However, there is only one study which attempted to examine the influence of birth weight on the SNS activity of the muscle in 272 adult men and women of the Pima Indian cohort. This study reported no relationship between birth weight and SNS activity.

9.2.4 Could birth weight improve the prediction of REE?

The energy demand marked by REE has been predicted from body weight and height since both measures of body size are positively associated with REE. The most commonly used predictive equation is that obtained by Schofield in 1985 and subsequently adopted by FAO/WHO/UNU. This series of equations has been developed covering the differences in body size associated with gender and age from an analysis of 114 studies largely conducted

in individuals of Caucasian origin. It is now recognised that using this equation can both under or overestimate REE between different ethnic groups. For example, using this equation in India has been reported to overestimate REE by 9-11% (210). Such differences might be attributable to differences in body composition, in particular, the proportion of different component of lean mass, that may be captured by using statements of ethnicity. The REE predictions by the original Schofield analysis were largely biased by the greater number of Italian subjects who have higher REE per unit body size than that of the other Caucasian subjects within the combined dataset (291). The analysis of Schofield also did not have enough data points from the tropical region to include in the analysis. Subsequent work by Henry et al within the Oxford Brookes Database of more than 10000 measurements of REE in both men and women offered alternate predictive equations for REE from height and weight which take into account differences in ethnicity (292). In this analysis, fewer Italian subjects were included and replaced with larger number of subjects from the tropic. For any given age, gender and weight, higher values for REE were derived using the Schofield equations than that derived using the Oxford equations. Such differences related to ethnicity are most likely to be explained by differences in the relative proportions of metabolically active tissue per kg weight or cm height, or of differences in the composition or metabolic activity of that tissue. In the same way, the work described in this thesis has indicated that differences in birth weight may also explain part of the variance in REE. However, this effect was independent of current size and composition. Whether differences in birth weight may also be associated with ethnicity (i.e. lower birth weight in South Asia, etc) independently of differences in structure and so act to influence the predictive relationship remains to be determined. Including a statement of birth weight within subsequent attempts to predict REE might account for a greater proportion of the variance in REE or may act as an alternate predictive variable in place of more detailed statements of body composition.

9.3 Implications of these observations for public health initiatives

This work, together with that of others, demonstrates how growth and development in early life may serve to influence the acquisition of a phenotype that may be associated with health from that which might be associated with an increased risk of developing chronic disease in later life. Adverse differences in body structure and metabolic function, mediated by factors that influence intra-uterine growth interact with current lifestyle to influence the processes that contribute to the development of CVD and T2DM. Such observations require some consideration as to what steps might be taken within society to reduce the risk of chronic disease that might be related to birth weight. Given that maternal nutritional status in terms of

size, composition and metabolic capability to nourish her fetus, appear to be important determinants of fetal growth and the weight of infant at birth, there is a need to consider how this knowledge might be used to improve pregnancy outcome in terms of the structure and function of the infant. In the same way, there is a need to consider how those who are born small might be supported to limit their chances of developing an imprudent phenotype into adulthood.

Several differing approaches or interventions could be considered. Firstly, there is a need to consider how it might be possible to improve the general growth and development of young women so that they enter pregnancy in the best health and nutritional state. For example, this might operate at level of groups or populations with a high likelihood of young women moving into childbearing age shorter, lighter and less well nourished with a greater probability to producing smaller babies where a more general public health approach of education and health promotion might be adopted. Alternatively, a more targeted approach would be to identify those young women who are shorter, lighter and had lower lean mass, and work with them to improve their nutritional competency and reserve before they enter pregnancy. This approach has been adopted in programmes such as the Mumbai Woman's Study – Stronger Mothers, Bigger Babies where the investigators are using local produce to promote micronutrient intake within a coherent support structure in an attempt to improve birth weight. A similar approach is likely to develop within the nascent Southampton Initiative for Health, a pragmatic attempt to improve women's health in pregnancy and that of her offspring arising out of the Southampton Women's Study. There is a need to carefully monitor and evaluate the effectiveness of such programmes, specifically, in terms of how they might influence the structure and function of the offspring at birth and during growth.

There is equally a need to consider what could be done to influence growth and development or current lifestyle, particularly in those who were born small. For example, either at the population level or in screened individuals, it would be prudent to follow a diet and lifestyle that would be less likely to be associated with accelerated weight gain in childhood or excess weight gain and central adiposity in adulthood. The increase in physical activity may also help to increase the muscle mass and improve insulin sensitivity and that, in turn, may lower the risk of T2DM and CVD in later life. Whether it is possible to reduce the likelihood of developing an adverse phenotype and lower cardiometabolic risk in those born small through such interventions has yet to be demonstrated and further work is clearly required in this area.

9.4 limitations of study and possible future work

The design of present study included two groups of adults born with lower and higher birth weight. This may not allow us to examine the trend that could be observed between birth weight and current measurement of body structure and metabolic function. In future studies, the addition of one or two intermediary groups will allow better examining the trend of the relationships between birth weight and the outcome instead of comparing the differences in the two extreme groups (lower vs higher). Although the number of subjects within each group was enough to demonstrate the relationship between birth weight and body composition and fat distribution (i.e. birth weight in relation to height, weight and lean mass), power analysis revealed that increasing the number of subjects within each group may help to avoid type II error and increase the chance of demonstrating a relationship between birth weight and some measure related to body composition (i.e. non-muscular FFST: muscle mass ratio). Depending on the outcome measure, this is likely to be of the order of 30 subjects within each weight category. This investigation only considered older men, thereby limiting the observations to his group. Future studies might be extended to include women and younger subjects in different age categories including children, younger and older adults in relation to birth weight. Such approaches could help to better understand how current phenotype is influenced by early fetal growth or influenced by childhood growth and development.

There was no information on the pattern of dietary intake in these groups of subjects. Such information would be helpful in the future study to determine whether the differences in adiposity and visceral fatness associated with birth weight could be mediated by differences in dietary pattern. Although some information of current physical activity was obtained by questionnaire, this was a self-reported current activity and not directly measured. Since adults with a lower birth weight were shorter and lighter and have greater adiposity, in particular, in the visceral region, it would be important to know whether this difference in body composition and fat distribution is attributed to poor fetal growth or it is the subsequence of consumption of more energy and less physical activity.

Energy metabolism in the present study was assessed before consumption of meal (fasted state) and only 6 hours after meal consummation (fed state), this assessment did not reflect the 24 hour energy expenditure which might include the energy related to physical activity and that obtained during the sleep (sleeping metabolic rate). It is impotent to consider that birth weight may have an effect on REE but not with other components of energy expenditure such that associated with physical activity. Since diet and physical activity may have an effect

on the relationship between birth weight and current phenotype, future studies are required to determine whether these two components are part of this relationship between birth weight and differences in current phenotype (not a confounder). In other words, future studies might be implemented to examine the influence of birth weight on current dietary pattern and differences in physical activity.

9.5 Overall conclusion

Previous work has shown that the acquisition of height, weight and lean mass into adulthood may be programmed by events in early life. The work described in this thesis has extended this view by a more detailed, thorough and systematic examination of body structure and composition than has previously been conducted. Moreover, attempts have been made for the first time, to determine the functional aspects of the phenotype and the extent to which differences in energy metabolism may be attributed to differences in body size, shape and composition. This work has shown that the body structure of older men, in terms of composition and pattern of fat distribution, may be programmed in early life, an effect that is only partly explained by differences in size and shape. At the same time, metabolic function as marked by energy metabolism in both the fasted and fed state is influenced by differences in birth weight. Although differences in body size and composition, in particular lean mass, contribute in part to the differences in energy metabolism, a greater proportion of the variance in energy metabolism remains unexplained. These observations suggest that metabolic function, as energy metabolism, in its self might be programmed, suggesting that such effects may be seen as both quantitative and qualitative. This less prudent metabolic phenotype associated with difference in birth weight may leads to more obvious features such as adiposity and central fat which in turn, may contribute to the observed increase in adiposity and visceral fat mass which are considered as independent risk factors for T2DM and CVD. This work has illustrated the need for careful consideration of the statistical approaches used to examine the effect of birth weight on markers of metabolic function. It is important to differentiate between situations where differences in body size and composition serve as potential confounders or whether they act as an integral part of the causal relationship.

It is important to note that these findings are derived from detailed, intensive observational studies conducted in a small group of older men compared across extremes of birth weight. They do serve, however, to inform future work both in terms of experimental design (i.e. effect size and power calculations) and the direction for more mechanistic studies exploring

specific metabolic processes that could underlie both structural and functional effects. Furthermore, there is need for further studies to extend these observations to other groups and also to determine through intervention studies the extent to which such effects may be modulated by diet and lifestyle in both the woman before and during pregnancy and in the offspring throughout childhood into adult life.

APPENDIX 2.1

Patient information sheet

University of Southampton

School of Medicine
Fetal Origins of Adult Disease Division

MRC Environmental Epidemiology Unit
Dr Nigel Arden
Professor Cyrus Cooper
Professor David Phillips

Endocrinology & Metabolism Unit
Dr Richard Holt
Professor Christopher Byrne

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PA: Christine Kyme
Tel 44 (0) 2380 795006

Patient Information Sheet

A study to investigate carbohydrate metabolism in relation to low birthweight

We are inviting you to take part in a research study. Before you decide, it is important that you understand why we are doing the research and what it will involve. Please take time to read the following information carefully. Please discuss it with your friends, relatives and GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

The Purpose of the Study

Previous studies by the MRC unit in Hertfordshire have shown that factors in early life can affect health in later life. However the reason is unclear. There is increasing evidence that how you grow as a baby affects the metabolism of key nutrients such as calcium, glucose and fats.

We therefore wish to see if there is a difference in the way calcium, glucose and fats are absorbed by the body, depending on early life factors, such as birthweight. We also think that these differences are caused by alterations in the production of stress hormones. As part of the study therefore, we will be measuring the levels of activity of these hormones

Why have I been chosen?

We are inviting people from Hertfordshire to participate. We wish to recruit a group of people with different patterns of growth as a baby.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part, we will give you this information sheet to keep. We will ask you to sign a consent form and will give you a copy. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

You will be then invited to attend the Wellcome Trust Clinical Research Facility at Southampton General Hospital. As the tests will be performed over three days, we would like you to stay at the hospital for 2 nights in the research unit itself. Transport to Southampton from Hertfordshire and for the return journey will be arranged by us. All meals and refreshments will be provided by us. Smoking is not permitted within the hospital.

Day 1

You will be collected by car from your home at around 7am on a Monday morning, to arrive in Southampton for 10am. Once settled in, we will measure your height, weight, waist and hip circumference and assess your body fat content. We will conduct questionnaires to gain insight into your general health and degree of physical activity. Some baseline blood tests will be performed. We shall also measure the strength of your bones by means of a DEXA scan. This uses a very small dose of x-rays and is less than that used if you were having an x-ray of your chest.

We will collect a 24 hour urine sample to measure your stress hormone production rate.

In order to measure how well you absorb calcium from your food, we will ask you to perform a strontium absorption test. This entails drinking a glass of orange juice, before your have eaten, which contains a small amount of strontium. This is a mineral, which behaves like calcium. We will take a sample of blood from you just before and one hour after you have drunk the orange juice.

You will be fasted from midnight.

Day 2

You will have some baseline blood tests first thing in the morning, before being given a standard build up drink. A plastic needle will be sited in your arm for 6 hours and then removed. After you have had the drink, you will rest on a bed and be asked to lie still for 6 hours, with regular breaks. Your exhaled air will be analysed at regular intervals. The breath we collect will help us to measure the way in which glucose and fats are metabolised by the body. 10ml of blood will also be taken during each of the 6 hours. Lunch and supper will be arranged by us. Please bring some reading material to pass the time.

Last thing at night, you will be given a small dose of cortisone (a stress hormone), as a tablet. You will then be fasted from midnight again.

Day 3

First thing in the morning, you will be given another cortisone tablet. Over the course of the morning, a plastic needle will be sited in your arm and regular blood tests will be taken, to assess your insulin and steroid response to a glucose drink. You will then be

returned to Hertford, after a late breakfast/early lunch. We shall aim for you to be home by 5 pm on Wednesday afternoon.

What do I have to do?

We ask that you attend the research facility in Southampton for the clinical assessments for 3 days (2 nights). There will be some dietary restrictions in the 2-3 days prior to attending. This is because the components of certain foods may interfere with our investigations, such as maize based products. We shall provide a detailed list of which foods to omit for those 2-3 days. You will of course be allowed to decline having certain tests performed if you so wish during your stay.

What are the possible disadvantages and risks of taking part?

There is a small risk of slight bruising and infection at the site of the plastic needles.

What are the possible benefits of taking part?

This study will not directly affect your medical care. By performing this study we hope to discover more insights into how growth as a baby is associated with health in adult life.

What happens if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If someone's negligence has harmed you, you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of this study, you may contact our department directly and speak to Professor Barker, the Director of the MRC here in Southampton.

Will my taking part in this study be kept confidential?

We will keep all information about you strictly confidential. We will remove your name and address from all information, which leaves the hospital so that you cannot be recognised from it. However, we will inform your GP that you are participating in this study.

What will happen to the results of the research?

The results of the study will be presented at international meetings so we can let other doctors know the results. We will then publish the results in medical journals, although this usually takes about 12-18 months after the completion of the study. You will not be

identified in any publication. We will be very happy to discuss the results with you once these are available. We can also send you a written result summary if you wish.

Who is organising and funding the research?

The MRC Environmental Epidemiology & Endocrinology Units of the University of Southampton are organising the study. The funding is from the National Institutes of Health.

Who has reviewed the study?

The Research Committee of the MRC Environmental Epidemiology Unit and the Southampton and Southwest Hampshire Local Research Ethics Committee have independently reviewed the study.

Contact for further information

If you would like more information at any time, please feel free to contact Professor David Phillips or Dr Mayank Patel on 02380 777624

You will be contacted in due course.

APPENDIX 2.2

Ethical approval letter

Hampshire and Isle of Wight NHS
Strategic Health Authority

Ref: CPW/HH

03 February 2003

Dr M Patel
Research Fellow to Professor D Phillips
MRC Epidemiology Unit
SGH

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

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

General Enquiries: temp1@gp-j82203.nhs.uk
clair.wright@gp-j82203.nhs.uk

Dear Dr Patel,

Submission No. 226/02/t – Studies of carbohydrate metabolism in relation to low birthweight.

In response to your letter dated 27th January 2003, I am pleased to confirm ethical approval for the protocol amendment for the above study.

The following documents were reviewed:

- Letter from Dr M Patel dated 27th January 2003
- Patient Information Sheet, Version 2 dated 27th January 2003

This approval has been granted under Chairman's action by the Vice-Chairman Mr Mervyn Griffiths and will be recorded at the committee meeting in March.

Yours sincerely

Mrs Clair Wright
Research Ethics Manager

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

APPENIDIX 2.3

Study Consent form given to all subjects before the study

University of Southampton

Fetal Origins of Adult Disease Division

MRC Environmental Epidemiology Unit
Dr Nigel Arden
Professor Cyrus Cooper
Professor David Phillips

Endocrinology & Metabolism Unit
Dr Richard Holt
Professor Christopher Byrne

Level D (MP 811)
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Fax: 44 (0) 2380 794154
E-mail: righ@soton.ac.uk
PA: Christine Kyme
Tel 44 (0) 2380 795006

Consent Form

A study of carbohydrate metabolism in relation to low birthweight

Names of Researchers: Dr M Patel, Professor D Phillips, Dr N Arden, Professor C Byrne

Study Number:
Patient Identification Number for this trial:

Please delete as appropriate

1. I confirm that I have read & understand the information sheet dated 28/06/2002 for the above study and have had the opportunity to ask questions

   Yes/no

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

   Yes/no

3. I agree to take part in the above study

   Yes/no

.....	.....	.....
Name of Patient	Date	Signature
.....	.....	.....
Name of Person taking consent (if different from researcher)	Date	Signature
.....	.....	.....
Name of Researcher	Date	Signature

1 for patient, 1 for researcher & 1 to be kept with GP

Transport _____ Serial No [ ][ ][ ]/[ ][ ][ ][ ]

Clinic appt _____

HERTFORDSHIRE HEALTH QUESTIONNAIRE

Name: _____

Address: _____

_____

_____

Telephone: _____

GP _____

Interviewer _____ [ ]

Date of Interview: [ ][ ][ ][ ][ ][ ]
d d m m y y

Date last modified 09/11/00

SECTION 1 GENERAL

Q1 What is your date of birth? [ ][ ][ ][ ][ ][ ]
d d m m y y

Q2 Where were you born? _____

Q3 Are you
1. Single?
2. Married?
3. Divorced or separated?
4. Widowed?
5. Cohabiting?

[ ]

Q4a What is your current or most recent full-time job? ***(Probe if necessary)***

_____

b What industry was that in?

_____

If an ever married woman, continue, otherwise go to Q7

Q5 What was your maiden name?

_____

Q6a What is/was your husband's current or most recent full-time job? ***(Probe if necessary)***

_____

b What industry was that in?

_____

Q7 Please count the number of rooms your household has for its own use.
Do not count: small kitchens (under 2 metres wide), bathrooms or toilets
Do count: Living rooms, kitchens (at least 2 metres wide) bedrooms and all other rooms

The total number of rooms is: [ ][ ]

Q8 Is your accommodation owned/mortgaged or rented by your household?

Owned/Mortgaged ☐

Rented ☐

Other ☐

Q9 How old were you when you finished continuous full-time education?

☐☐ years old

Q10 How many cars and vans would you normally have available for use by you or other members of your household?

None ☐

One ☐

Two ☐

Three or more ☐

SECTION 2 FAMILY HISTORY

Q11 Where was your mother born?

_____

Q12 What was your mother's year of birth?

_____

Q13 Where was your father born?

_____

Q14 What was your father's year of birth?

_____

Q15 How many babies did your mother have? *(including stillbirths and babies that died)*

Order	Name	Date of birth	Sex	Year of death	Cause of death	Live in Herts Y/N

SECTION 3 - CHILDHOOD

Q16 What was your father's job when you were born? (if unemployed, last full-time job)

_____

Q17 Did your father smoke regularly during your childhood?

0. No 1. Yes ☐

Q18 Did your mother smoke regularly during your childhood?

0. No 1. Yes ☐

Q19 Did your family have a cat when you were a child?

0. No 1. Yes ☐

Q20 Did your family keep a dog when you were a child?

0. No 1. Yes ☐

Q21 Did you share a bedroom with your brothers/sisters before you started school?

0. No 1. Yes ☐

Q22 Do you know how much you weighed when you were born?

0. No 1. Yes ☐

If yes, how much did you weigh?

.....lbs .....ozs

Q23 Were you born early, on time, or late?

SECTION 4 PHYSICAL ACTIVITY

Q24 Do you have any problems walking?

0. No limiting abnormality
1. Abnormal gait/walking problems/no aid
2. Uses walking aid
3. Requires help from another person
4. Unable to walk

☐

Q25a Walking out of doors: record all walking yesterday lasting longer than 5 minutes

i	Before 9.00 a.m.	☐☐☐ mins
ii	Between 9.00 a.m. and 12.00 p.m.	☐☐☐ mins
iii	Between 12.00 p.m. and 2.00 p.m.	☐☐☐ mins
iv	Between 2.00 p.m. and 6.00 p.m.	☐☐☐ mins
v	Between 6.00 p.m. and 7.00 p.m.	☐☐☐ mins
vi	After 7.00 p.m.	☐☐☐ mins
vii	Total	☐☐☐ mins

b Was this day unusual?

0. No 1. Yes ☐

If yes, did you walk less or more than usual?

1. Less 2. More ☐

Q26 Which of the following best describes your walking speed?

0. Unable to walk
1. Very slow
2. Stroll at an easy pace
3. Normal speed
4. Fairly brisk
5. Fast

☐

Q27 Which of the following activities do you do at least once a month on average or at least 12 times per year?

Bowls	0. No 1. Yes	☐
Cycling	0. No 1. Yes	☐
Swimming	0. No 1. Yes	☐
Golf	0. No 1. Yes	☐
Fishing	0. No 1. Yes	☐
Dancing	0. No 1. Yes	☐
Other physically active sports or hobbies except gardening *(please specify)*	0. No 1. Yes	☐

Q28 How much time do you spend gardening in a typical week?

0. Less than 1 hour per week
1. 1-4 hours per week
2. 5-8 hours per week
3. More than 8 hours per week

☐

Q29 How much time do you spend doing housework in a typical week?

0. Less than 1 hour per week
1. 1-4 hours per week
2. 5-8 hours per week
3. More than 8 hours per week

☐

Q30 Do you climb stairs?

0. Never
1. Occasionally
2. Once/several times per week
3. Daily
4. Several times per day

Q31 Do you carry loads (equivalent to a full shopping bag or 10 lbs)?

0. Never
1. Occasionally
2. Once/several times per week
3. Daily
4. Several times per day

Q31a Have you had any falls in the last year?

0. No 1. Yes

b ***If yes,*** how many?

SECTION 5 - SOCIAL

Q32a Have you ever smoked regularly?
(i.e. at least once a day for a year or more)

0. No 1. Yes

If yes, continue
If no, Go to Q34

B How old were you when you first smoked regularly?

C If you added up all the years that you smoked, how many would it make in total?

D What was the average amount you smoked over this time?

Cigarettes/day

Roll-ups (ozs)/week

Cigars/week

Pipe tobacco (ozs)/week

e Do you still smoke regularly? 0. No 1. Yes ☐

If yes, Go to Q33
If no, continue

f How old were you when you last smoked regularly? ☐☐

Q33 How much do you smoke now?

Cigarettes/day ☐☐☐

Roll-ups tobacco/week (oz) ☐☐

Cigars/week ☐☐

Pipe tobacco/week (oz) ☐☐

If appropriate, between what ages did you cut down? ☐☐ to ☐☐

Q34a Apart from your own smoking are you regularly exposed to tobacco smoke at home? 0. No 1. Yes ☐

If yes,

b Not counting yourself, how many people in your household smoke regularly? ☐☐

Q35a Do you ever drink alcohol? 0. No 1. Yes ☐

If no, go to 36a

How often do you currently drink shandy/low alcohol beer/lager/cider? (don't include alcohol free lager etc.)

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many pints would you normally have? (if range given code mid-point; 1 average can = 0.8 pints, 1 small can = 0.5 pints) ☐☐ • ☐

35b How often do you currently drink beer/stout/lager/cider? (don't include alcohol free lager etc.)

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many pints would you normally have? (if range given code mid-point; 1 average can = 0.8 pints, 1 small can = 0.5 pints) ☐☐ • ☐

35c How often do you currently drink low alcohol wine?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many glasses would you normally have? (if range given code mid-point) ☐☐ • ☐

35d How often do you currently drink Wine/Sherry/Port /Martini /Cinzano?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many glasses would you normally have? (if range given code mid-point) ☐☐ • ☐

35e How often do you currently drink spirits/liqueurs?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many measures would you normally have? (if range given code mid-point) ☐☐ • ☐

SECTION 6 – CHEST PAIN

Q36a Do you get pain or discomfort in your chest

1. Yes ***go to c***
0. No ***go to b***

b Do you get any pressure or heaviness in your chest?

1. Yes ***go to c***
0. No ***go to l***

c Do you get it when you walk uphill or hurry?

0. No
1. Yes
2. Never hurry or walk uphill

d Do you get it when you walk at an ordinary pace on the level?

0. No 1. Yes

If No to c and d, go to h

e What do you do if you get it while you are walking?

1. Stop or slow down
2. Carry on

(Record stop or slow down if the subject carried on after taking nitro-glycerine)

f If you stand still or slow down what happens to it?

1. Relief
0. No relief

g How long does it take to get relief?

1. 10 minutes or less
2. More than 10 minutes

h Will you show me where it was? Note the number(s) of the site(s) from the chest diagram

i Do you feel it anywhere else?

0. No 1. Yes

If yes, please specify

j Did you see a doctor because of this pain/discomfort

0. No 1. Yes

If yes, what did he/she say that it was?

k How many years ago did this pain or discomfort start?

l Have you ever had severe pain across the front of your chest lasting for half an hour or more?

0. No 1. Yes

If yes, go to m, *if no* go to o

m Did you see a doctor because of this pain?

0. No 1. Yes

If yes, what did he/she say that it was?

n How many of these attacks/episodes have you had?

1. Date 1 (year) _____ Duration of pain _____
2. Date 2 (year) _____ Duration of pain _____
3. Date 3 (year) _____ Duration of pain _____

If subject feels unsure enter 9 here

o Have you ever had an operation to clear the arteries in your heart (coronary artery bypass graft or angioplasty)?

0. No 1. Yes

If yes, go to p, *if no* go to q

p In what year did it occur for the first time?

q Have either of your parents or any of your brothers or sisters suffered from a heart attack?

0. No 1. Yes

If yes, please give details

Relative	Age of first attack

Q37a Do you get pain or discomfort in your legs when you walk?

0. No 1. Yes

If no, go straight to Q39

b Does this pain ever begin when you are standing still or sitting?

0. No 1. Yes

c Do you get it when you walk uphill or hurry?

0. No 1. Yes ☐

d Do you get it when you walk at an ordinary pace on the level?

0. No 1. Yes ☐

e What do you do if you get it when you are walking?

1. Stop ☐ 2. Slow down ☐ 3. Continue at same pace ☐

f Does the pain ever disappear while you are still walking?

0. No 1. Yes ☐

g What happens to it if you stop or slow down?

1. Usually continues for more than 10 minutes
2. Usually disappears in 10 minutes ☐

h Where do you get this pain or discomfort? ***(show card and tick box)***

1. Calf ☐ 2. Thighs ☐ 3. Buttock ☐

4. Groin ☐ 5. Knee ☐ 6. Ankle ☐

Q38 Have you ever had surgery to your aorta or to the arteries in your legs?

0. No 1. Yes 9. Don't know ☐

SECTION 7 – RESPIRATORY

Cough

Q39a Do you **usually** cough first thing in the morning in winter? 0. No 1. Yes ☐

b Do you **usually** cough during the day - or at night in the winter? 0. No 1. Yes ☐

If yes, go to c, ***if no,*** go to d

c Do you cough like this on most days for as much as 3 months of each year? 0. No 1. Yes ☐

Phlegm

d Do you **usually** bring up any phlegm from your chest first thing in the morning in winter? 0. No 1. Yes ☐

e Do you **usually** bring up any phlegm from your chest during the day or at night in the winter 0. No 1. Yes ☐

If yes, go to f, ***if no,*** go to Q40a

f Do you bring up phlegm like this on most days for as much as 3 months each year? 0. No 1. Yes ☐

Q40a Have you had wheezing or whistling in your chest at any time during the last year 0. No 1. Yes ☐

If yes, continue, ***if no,*** go to Q40b

i) Have you had this wheezing when you did not have a cold? 0. No 1. Yes ☐

ii) Have you been at all breathless when the wheezing noise was present? 0. No 1. Yes ☐

b Have you woken with a feeling of chest tightness first thing in the morning at any time in the last year? 0. No 1. Yes ☐

Q41 Have you been woken by an attack of shortness of breath at any time during the last year 0. No 1. Yes ☐

Q42a Are you often troubled by shortness of breath when hurrying on level ground or walking up a slight hill? 0. No 1. Yes ☐

If yes, continue, ***if no,*** go to Q43

b Do you often get short of breath walking with other people of your own age on level ground? 0. No 1. Yes ☐

If yes, continue, ***if no,*** go to Q43

c Do you often have to stop for breath when walking at your own pace on level ground? 0. No 1. Yes ☐

If yes, continue, ***if no,*** go to Q43

d Do you often have to stop for breath after walking about 100 yards (or after a few minutes) on the level? 0. No 1. Yes ☐

If yes, continue, ***if no,*** go to Q43

e Do you get breathless on washing or dressing? 0. No 1. Yes ☐

Q43 Have you had to see your doctor in the last year for your chest 0. No 1. Yes ☐

Have you been admitted to hospital for your chest in the last year? 0. No 1. Yes ☐

Q44 What kind of cooker do you MOSTLY use for cooking? *(Choose one method only)*

1. Gas 2. Electricity 3. Other (specify below) ☐

SECTION 8 – IMMUNITY

Q45 Did you have eczema as a child? 0. No 1. Yes ☐

Q46 Have you ever had hay fever, rhinitis or other nasal allergies? 0. No 1. Yes ☐

Q47 Have you ever had glandular fever? 0. No 1. Yes ☐

If yes, at what age? ☐☐

Q48 Have you ever had your appendix out? 0. No 1. Yes ☐

If yes, at what age? ☐☐

Q49 Have you ever had shingles? 0. No 1. Yes ☐

If yes, at what age? ☐☐

Q50 Have you ever had hepatitis A vaccine e.g. for travel purposes? 0. No 1. Yes ☐

Q51 Have either of your parents, or any of your brothers or sisters ever had asthma, hayfever or childhood eczema? 0. No 1. Yes ☐

If yes, please give details

Relative	Illness

SECTION 9 – BONE

Q52 Have you broken any bones since the age of 45?

If yes, please give details 0. No 1. Yes ☐

Bone	Age when fracture occurred	How did fracture occur?

Q53 Have either of your parents or any of your brothers or sisters fractured a bone when they were more than 45 years old?

If yes, please give details 0. No 1. Yes ☐

Which relative?	Bone	Age when fracture occurred	How did fracture occur?

Q54 Have you ever had back pain in the area shown on the card, which lasted for more than a day? *(do not include pain occurring only during pregnancy, during menstrual periods, or during the course of a feverish illness such as flu)*

0. No 1. Yes

If yes, please answer questions below ☐
If no. go to Q57 ☐

Q55 Has the pain ever spread to your legs?

0. No 1. Yes ☐

If yes, please tell me the furthest point down your leg that the pain reached

Buttock ☐
Thigh ☐
Knee ☐
Calf ☐
Ankle ☐

Q56 When did you last have the pain?

Last week ☐
Last month ☐
Last year ☐
More than a year ago ☐

Occupational History

Q57 Record all jobs/occupations of greater than 1 years duration since the person left full-time education.

Job Title	Age started	Age stopped	Part time/ Full time	Activity		
				Standing	Lifting	Sweating

Record in activity column if the job involved:
1. Standing/walking for 4+ hours per day
2. Lifting 25kg +
3. Physical work enough to make the subject sweat

SECTION 10 OBSTETRIC

MEN ONLY

Q58 How many children have you fathered? ☐☐

WOMEN ONLY. For men go to SECTION 11

Q59 How many times have you been pregnant?

☐☐

Details:

Pregnancy Number	Liveborn (L) Stillborn (S) Miscarriage(M)	If liveborn:		Currently living in Herts	
		Male (M) Female (F)	Birthweight	Name	D.O.B.
1					
2					
3					
4					
5					
6					
7					
8					

Q60a At what age did your periods start? ☐☐

b At what age did your periods stop? ☐☐

c Have you had a hysterectomy (removal of the womb)?

0. No 1. Yes ☐

d ***If yes*** how old were you? ☐☐

e Did the hysterectomy include removal of the ovaries?

0. No 1. Yes 2. Don't know ☐

Q61a Have you ever taken an oral contraceptive pill?

0. No 1. Yes ☐

b ***If yes,*** at what age did you start? ☐☐

c How long in total did you take it for (months)? ☐☐☐

Q62a Have you ever taken hormone replacement therapy?

0. No 1. Yes ☐

b ***If yes,*** at what age did you start? ☐☐

c How long in total did you take it for (months)? ☐☐☐

Q63 have you ever taken tamoxifen (eg for a breast lump)?

0. No 1. Yes 9. Don't know ☐

SECTION 11 – MEDICAL

Q64 Have you ever been told by a doctor or other health professional that you have ever had any of the following:-

a High blood pressure (out of pregnancy only)

0. No 1. Yes 9. Don't know ☐

b Stroke/Transient ischaemic attack

0. No 1. Yes 9. Don't know ☐

c Diabetes (out of pregnancy)

0. No 1. Yes 9. Don't know ☐

If yes, how long have you been diabetic? ☐☐ years

Are you controlled by:

Diet alone ☐

Tablets ☐

Insulin injections ☐

d Have you ever had a head injury severe enough to cause unconsciousness or to require admission to hospital?

0. No 1. Yes 9. Don't know ☐

Q65 Have either of your parents or any of your brothers or sisters had high blood pressure or diabetes?

0. No 1. Yes 9. Don't know ☐

If yes, please give details

Which relative?	Illness	Age when illness occurred	Form of treatment

SECTION 12 - MEDICATION

Q66 What regular medicines/tablets/eye drops/inhalers etc. do you use?
PLEASE USE BLOCK CAPITALS
Please include regular pain killers such as paracetamol

1 _____
2 _____
3 _____
4 _____
5 _____
6 _____
7 _____
8 _____
9 _____
10 _____
11 _____
12 _____

SECTION 13 HEALTH AND DAILY ACTIVITIES

Q67 In general how would you say your health is:

1. Excellent 2. Very good
3. Good 4. Fair
5. Poor

☐

Q68 Compared to one year ago, how would you rate your health in general now?
Please indicate only one

1. Much better than one year ago 2. Somewhat worse than one year ago
3. Somewhat better than one year ago 4. Much worse than one year ago
5. About the same as one year ago

☐

Q69 The following items are about activities you might do during a typical day Does **your health now limit you** in these activities? If so, please indicate how much?

	Yes limited a lot	Yes limited a little	No, not limited at all
a) Vigorous activities, such as running, lifting heavy objects, Participating in strenuous sports	☐	☐	☐
b) Moderate activities, such as moving a table, pushing a vacuum cleaner, Bowling or playing golf	☐	☐	☐
c) Lifting or carrying groceries	☐	☐	☐
d) Climbing several flight of stairs	☐	☐	☐
e) Climbing one flights of stairs	☐	☐	☐
f) Bending, kneeling or stooping	☐	☐	☐
g) Walking more than one mile	☐	☐	☐
h) Walking half a mile	☐	☐	☐
i) Walking one hundred yards	☐	☐	☐
j) Bathing or dressing yourself	☐	☐	☐

Q70 During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of your physical health?** ***Please indicate one answer for each question***

a) Cut down the **amount of time** you spent on work or other activities — 0. No 1. Yes ☐

b) **Accomplished less** than you would like — 0. No 1. Yes ☐

c) Were limited in the **kind** of work or other activities — 0. No 1. Yes ☐

d) Had **difficulty** performing the work or other activities (for example, it took extra effort) — 0. No 1. Yes ☐

Q71 During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems?** ***Please indicate one answer for each question***

a) Cut down the **amount of time** you spent on work or other activities — 0. No 1. Yes ☐

b) **Accomplished less** than you would like — 0. No 1. Yes ☐

c) Didn't do work or other activities as **carefully** as usual. — 0. No 1. Yes ☐

Q72 During the **past 4 weeks,** to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups? ***Please indicate <u>one</u> only***

1. Not at all
2. Slightly
3. Moderately
4. Quite a bit
5. Extremely

☐

Q73 During the **past 4 weeks,** how much bodily pain have you had? ***Please indicate <u>one</u> only***

1. None
2. Very mild
3. Mild
4. Moderate
5. Severe
6. Very severe

☐

Q74 During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)? ***Please indicate <u>one</u> only***

1. Not at all
2. A little bit
3. Moderately
4. Quite a bit
5. Extremely

☐

Q75 During the **past 4 weeks,** how much of the time? ***Please indicate one answer for each question***

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a) Did you feel full of life?	☐	☐	☐	☐	☐	☐
b) Have you been a very Nervous person?	☐	☐	☐	☐	☐	☐
c) Have you felt so down in the dumps that nothing could cheer	☐	☐	☐	☐	☐	☐
d) Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
e) Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
f) Have you felt downhearted and low?	☐	☐	☐	☐	☐	☐
g) Did you feel worn out?	☐	☐	☐	☐	☐	☐
h) Have you been a happy person?	☐	☐	☐	☐	☐	☐
i) Did you feel tired	☐	☐	☐	☐	☐	☐

Q76 During the **past 4 weeks,** how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc)? ***Please indicate <u>one</u> only***

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. None of the time

☐

Q77 Please choose the answer that best describes how TRUE or FALSE each of the following statements is for you. *Please indicate one answer for each question.*

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely false
a) I seem to get sick a little easier than other people	☐	☐	☐	☐	☐
b) I am as healthy as anybody I know	☐	☐	☐	☐	☐
c) I expect my health to get worse	☐	☐	☐	☐	☐
d) My health is excellent	☐	☐	☐	☐	☐

SECTION 14

"The following questions will help you to let us know how you are. Please give the response which comes closest to how you have felt in the last few days. Don't take too long over your replies, your immediate reaction will probably be more accurate than a long thought out response"

Q78 I feel tense or 'wound up':

1. Most of the time
2. A lot of the time
3. From time to time, occasionally
4. Not at all

☐

Q79 I feel as if I am slowed down:

1. Nearly all the time
2. Very often
3. Sometimes
4. Not at all

☐

Q80 I still enjoy the things I used to enjoy:

1. Definitely as much
2. Not quite so much
3. Only a little
4. Hardly at all

☐

Q81 I get a sort of frightened feeling like butterflies in the stomach:

1. Not at all
2. Occasionally
3. Quite often
4. Very often

☐

Q82 I get a sort of frightened feeling as if something awful is about to happen::

1. Very definitely and quite badly
2. Yes, but not too badly
3. A little bit but it doesn't worry me
4. Not at all

☐

Q83 I have lost interest in my appearance:

1. Definitely
2. I don't take so much care as I should
3. I may not take quite as much care
4. I take just as much care as ever

☐

Q84 I can laugh and see the funny side of things:

1. As much as I always could
2. Not quite so much now
3. Definitely not so much now
4. Not at all

☐

Q85 I feel restless as if I have to be on the move:

1. Very much indeed
2. Quite a lot
3. Not very much
4. Not at all

☐

Q86 Worrying thoughts go through my mind:

1. A great deal of time
2. A lot of the time
3. From time to time but not too often
4. Only occasionally

☐

Q87 I look forward with enjoyment to things:

1. As much as I ever did
2. Rather less than I used to
3. Definitely less than I used to
4. Hardly at all

☐

Q88 I feel cheerful:

1. Not at all
2. Not often
3. Sometimes
4. Most of the time

☐

Q89 I get sudden feelings of panic:

1. Very often indeed
2. Quite often
3. Not very often
4. Not at all

☐

Q90 I can sit at ease and feel relaxed:

1. Definitely
2. Usually
3. Not often
4. Not at all

☐

Q91 I can enjoy a good book or radio or TV programme:

1. Often
2. Sometimes
3. Not often
4. Very seldom

☐

APPENDIX 2.5

Component of reference test meal (breakfast meal and labelled emulsion)

Component of test meal

Meal

Rice Krispies (Kelloggs)	40.0 g
Whole milk	200 g
White bread	72.0 g
Flora margarine	14.0 g
Cheddar cheese	50.0 g

Emulsion

Double cream	11.0 g
Extra virgin oil	1.8 g
Sunflower oil	1.5 g
Casein	6.0 g
Glucose	4.5 g
Beet sugar	2.3 g
Chocolate Nesquick	5.0 g
Water	120 ml

Macronutrient composition of test meal (breakfast meal and emulsion)

	Meal	Emulsion	Total
Energy	3115 kJ	605 kJ	3720 kJ
CHO	81.17 g (45.3%)	11.69 g (30.9%)	92.86 g (40%)
Lipid	36.53 g (40.4%)	8.72 g (53.3%)	45.25 g (45%)
Protein	27.42 g (14.3%)	5.64 g (15.8%)	33.06 g (15%)

APPENDIX 2.6

Validation of lipid extraction procedure and measurements by GC--IRMS

This study has been performed before I started to analyse the subject's plasma, to evaluate the precession of the extraction and methylation procedure, and to obtain a considerable confidante on the results obtained by the GC-IRMS.

Subjects and methods

Two blood samples (10ml) was collected from lean health male subjects (Institute of Human Nutrition, Southampton General Hospital) after an overnight fasting and two hours after the ingestion of test meal which containing lipid emulsion (^{13}C-PA (700mg)). The lipid emulsion was given to the subjects with standard break fast meal (3000kJ energy, 37% from fat, 52% from carbohydrate and 11% from protein). Plasma was recovered instantly by centrifugation at 2500 g for 15 min at 4^0C and the remaining red cell was discarded. The plasma was separated into 10 x 1ml independent aliquots and extracted and methylated separately by the methods described in Chapter 2, and analysed by GC-IRMS to evaluate the between sample variability in the enrichment and concentration of ^{13}C-PA in TAG and NEFA fraction (or the reproducibility of the extraction and methylation procedures). In the second step, a single extracted and methylated sample was run 10 times by GC-IRMS to evaluate the within sample variability in the enrichment and concentration of ^{13}C-PA in TAG and NEFA fraction (or the reproducibility of the GC-IRMS).

Results

The between sample variability in the enrichment and concentration of plasma TAG ^{13}C-PA in 10 independent aliquots obtained from single subjects are presented in Table 1. The variability (% COV) in the enrichment and concentration of TAG ^{13}C-PA were 1.28 and 5.36% respectively

The between sample variability in the enrichment and concentration of plasma NEFA ^{13}C-PA in 10 independent aliquots obtained from single subjects are presented in Table 2. The

variability (%COV) in the enrichment and concentration of NEFA ^{13}C-PA were 1.29 and 2.77% respectively.

The within sample variability in the enrichment and concentration of plasma TAG ^{13}C-PA in a repeated measure (10 times) run at the same time by GC-IRMS obtained from a single aliquots are presented in Table 3. The variability (%COV) in the enrichment and concentration of TAG ^{13}C-PA were 0.73 and 3.46% respectively.

TABLE 1 between sample variability in enrichment and concentration of TAG^{13}C- PA in ten different aliquots obtained from single subjects

Sample No.	TAG ^{13}C-PA Enrichment ($^{0}/_{00}$)	TAG ^{13}C-PA Concentration (µg/ml plasma)
1	160.7	1.88
2	162.9	1.94
3	163.6	1.99
4	158.7	1.96
5	160.8	1.96
6	159.8	2.22
7	161.4	2.14
8	165.3	2.18
9	161.6	2.13
10	159.9	2.12
Mean	**160.6**	**2.05**
SDV	**2.06**	**0.11**
%CV	**1.28**	**5.36**

TABLE 2 between sample variability in enrichment and concentration of ^{13}C-PA in NEFA in ten different aliquots obtained from single subjects

Sample No.	NEFA ^{13}C-PA Enrichment ($^{0}/_{00}$)	NEFA ^{13}C-PA Concentration (µg/ml plasma)
1	205.8	4.21
2	203.9	4.38
3	210.3	4.50
4	207.5	4.18
5	203.2	4.36
6	209.0	4.48
7	211.0	4.28
8	210.0	4.33
9	207.0	4.13
10	206.5	4.43
Mean	**207.4**	**4.33**
SDV	**2.67**	**0.12**
%CV	**1.29**	**2.77**

TABLE 3 within sample variability in the enrichment and concentration of ^{13}C-PA in TAG from single sample injected to GC-IRMS ten times

Sample No.	TAG ^{13}C-PA Enrichment ($^{0}/_{00}$)	TAG ^{13}C-PA Concentration (µg/ml plasma)
1	160.6	1.99
2	162.9	1.94
3	161.6	1.99
4	158.7	1.96
5	160.8	1.96
6	159.8	2.02
7	161.4	2.11
8	159.3	2.18
9	161.6	1.99
10	160.1	2.09
Mean	**160.7**	**2.02**
SDV	**1.18**	**0.07**
%CV	**0.73**	**3.46**

Discussion

This study reported a lower between sample variability in measuring the TAG ^{13}C-PA and NEFA ^{13}C-PA enrichment and concentration. This finding confirms the precision of the extraction and methylation procedure of the ^{13}C-PA in both the TAG and NEFA fractions. The results of this validation study show that the between sample variability in the enrichment of TAG ^{13}C-PA was greater than that observed in the within sample variability in enrichment of TAG ^{13}C-PA (1.29 v 0.73% respectively). This could attributed in part to the differences in the enrichment of the 10 independent sample obtained from a single aliquots in oppose to a single extracted and methylated aliquot run by the GC-IRMS ten times in at the same day.

Likewise, the between sample variability in TAG ^{13}C-PA was greater than that observed in the within sample variability (5.36 v 3.46%). This could be also attributed to the variation in the concentration of ^{13}C-PA in ten independent aliquots in oppose to one extracted and methylated aliquots run into the GC-IRMS 10 times. However, despite the smaller variation between sample in the enrichment and concentration of TAG and NEFA ^{13}C-PA observed this study, the extraction and methylation procedures could be considered to be precise. In addition, the lower within sample variability in measuring the TAG ^{13}C-PA enrichment and concentration suggested the ability of the GC-IRMS to obtain the results with considerable confidence.

APPENIDX 2.7

The recovery of ^{13}C-PA from plasma samples expressed as mmol/l

Step 1:

The concentration of palmitic acid in plasma is determined by enzymatic assay as it described in appendix (2.5).

Step 2:

The proportion of the total palmitic acid in the sample enriched with ^{13}C is determined by the following equation:

$$^{*}FA/FA = (^{13}C\ (^{0}/_{00}) + 31.57 / 53.8$$

^{13}C ($^{0}/_{00}$) = enrichment of palmitic acid in delta units

This equation has been derived from a serious of standard curves

Step 3:

The recovery of ^{13}C-PA in plasma (mmol/l) = Concentration of palmitic acid in plasma x Proportion of *FA/FA

Step 4:

The recovery of ^{13}C-PA in plasma over the study period was calculated from the total area under the time verses the concentration of ^{13}C-PA curve over the study 6 hours, expressed as mmol/l per 6 hours.

APPENDIX 2.8

The recovery of $^{13}CO_2$ on breath sample calculated as a percentage of administrated dose of ^{13}C-palmitic acid within a standard meal

Step 1:

Calculating the amount of ^{13}C-PA administrated to the subjects

mmole^{13}C administrated = (amount of ^{13}C-PA in mg / MW ^{13}C-PA) x (P x N / 100)

MW ^{13}C-PA = molecular weight of ^{13}C-palmitic acid

P = purity of the ^{13}C- palmitic acid (99 atom % excess)

N = number of label carbons in the substrate

Step 2:

Conversion of VCO_2 (ml/min) to VCO_2 (mmol/hour)

1 mole of gas = 22.4 litres (Avogadros constant) therefore the VCO_2 (mmol/hour) = VCO_2 (ml/min) / 22.4 litres x 60 (min)

Step 3:

Calculate the mmol excess of ^{13}C per mmol CO_2

mmol excess^{13}C / mmol CO_2 = ($\bar{o}^{13}$Ct – $\bar{o}^{13}$Ct=0) x RPDB x 1/1000

($\bar{o}^{13}$Ct) = ^{13}C-enrichment of breath at time point

($\bar{o}^{13}$Ct=0) = ^{13}C-abundance of baseline sample

RPDB = ^{13}C to ^{12}C ratio of reference standard (0.0112372)

Step 4:

Calculation of the percentage of administrated dose excreted as $^{13}CO_2$ per hour

$$\% \text{ administrated dose / hour} = \frac{\text{*mmol excess } ^{13}\text{C/ mmol } CO_2}{\text{x } VCO_2 \text{ x } 100 \\ \text{**mmol } ^{13}\text{C administrated}}$$

VCO_2 = rate of CO_2 excretion in mmol / hour

* calculated from step 3 ** calculated from step 1

APPENDIX 2.9

Methods used for the analysis of plasma TAG, NEFA, glucose, total cholesterol, LDL and HDL concentration using enzymatic reagent kits.

1. NEFA analysis

NEFA in plasma is treated with acyl-CoA synthetase in the presence of ATP, magnesium cations and CoA, form acyl CoA as well as the byproducts AMP and pyrophosphate. The acyl-CoA is oxidize by added peroxidase allow the oxidative condensation of 3-methyl-N-ethyl-N-aniline with 4-aminoantipyrine to form a purple colored adducted with an absorption maximum at 550nm. The amount of NEFA in the sample is then determined from the optical density measured at 550nm.

Reagent kit:

NEFA ACS-ACOD for the quantitative determination of non-esterified fatty acids by Wako chemicals GmbH

Sample preparation:

Sample and standard can be used without further preparation

2. Triglycerides analysis

Triglycerides are hydrolyzed by lipase to glycerol and fatty acid. The glycerol is phosphorylated to glycerol-3-phosphate, which is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and 4-chlorophenol forming a quinoneimine dye. The absorbance of the formed color is measured at 510nm.

$$TAG \xrightarrow{Lipase} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{GK} Glycerol\text{-}3\text{-}phosphate + ADP$$

$$Glycerol\text{-}3\text{-}3phosphate + O2 \xrightarrow{GPD} Dihydroxyacetone\ phosphate + H_2O_2$$

$$2H_2O_2 + Aminoantipyrine + 4\text{-}Chlorophenol \xrightarrow{POD} Quinoneimine + HCL + H_2O$$

Reagent kit:

Konelab Triglyceride kit

Sample preparation:

Sample and standard can be used without further preparation

3. HDL-Cholesterol analysis

The cholesterol assay is homogeneous enzymatic colorimetric test, where in the presence of magnesium sulphate, dextran sulphate selectively from water–soluble complex with LDL, VLDL and chylomicrons, which are resistance to PEG modified enzyme. The cholesterol concentration of HD-cholesterol is determined enzymatically by cholesterol oxidase coupled with PEG to the amino groups.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{PEG cholesterol esterase}} \text{cholesterol} + \text{RCOOH}$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{PEG cholesterol oxidase}} \Delta^4\text{- cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ + 5H_2O \xrightarrow{\text{Peroxidase}} \text{purple blue pigment} + H_2O$$

Reagent kit:

Konelab HDL-cholesterol kit

Sample preparation:

Sample and standard can be used without further preparation

4. LDL-cholesterol

The LDL cholesterol test is a homogeneous enzymatic colorimetric test, where in the presence of magnesium ions, a sugar compound markedly reduces the enzymatic reaction for the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in plasma.

$$\text{LDL-cholesterol} + H_2O \xrightarrow{\text{Detergent}} \text{cholesterol} + \text{RCOOH}$$

$$\text{LDL-cholesterol} + O2 \xrightarrow{\text{Cholesterol oxidase}} \Delta^4\text{- cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ + H_2O \xrightarrow{\text{Peroxidase}} \text{purple blue pigment} + 5H_2O$$

Reagent kit:

Konelab LDL-cholesterol kit

Sample preparation:

Sample and standard can be used without further preparation

5. Glucose analysis

Glucose is phosphorylated by ATP, in a reaction catalyzed by hexokinase (HK). The glucose-6-phosphate (G-6-P) formed is oxidated to 6-phosphogluconate (6-PG) by gucose-6-phosphate dehydrogenase (G-6-PDH). In the same reaction an equimolar amount of NAD is reduced to NADH, with a resulting increase in the absorbance at 340nm.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NAD}^+ \xrightarrow{\text{G-6-P-DH}} \text{6-PG} + \text{NADH} + \text{H}^+$$

Reagent kit:

Konelab Glucose (HK) kit

Sample preparation:

Sample and standard can be used without further preparation

6. Cholesterol analysis

Cholesterol esters in the plasma are hydrolyzed by an esterase to cholesterol and fatty acid. The obtained cholesterol is then oxidized by cholesterol oxidase with the side production of H_2O_2. Peroxydase catalyzes the formation of quinoneimine dye with aid of H_2O_2. The formation of the coloured compound is then measured at 510nm.

Cholesterol esters ----Esterase----> Cholesterol + fatty acid

Cholesterol + O_2 ----Cholesterol oxidase-> 4-cholestenone + H_2O_2

$2H_2O_2$ + phenol + amino-4-antipyrine ---Peroxydase-> Quinoneimine dye + $4H_2O$

Reagent kit:

Konelab Cholesterol kit

Sample preparation:

Sample and standard can be used without further preparation

APPENDEX 2.10

Energy metabolism in fasted and fed stated relative to body size and composition between the lower and higher birth weight group

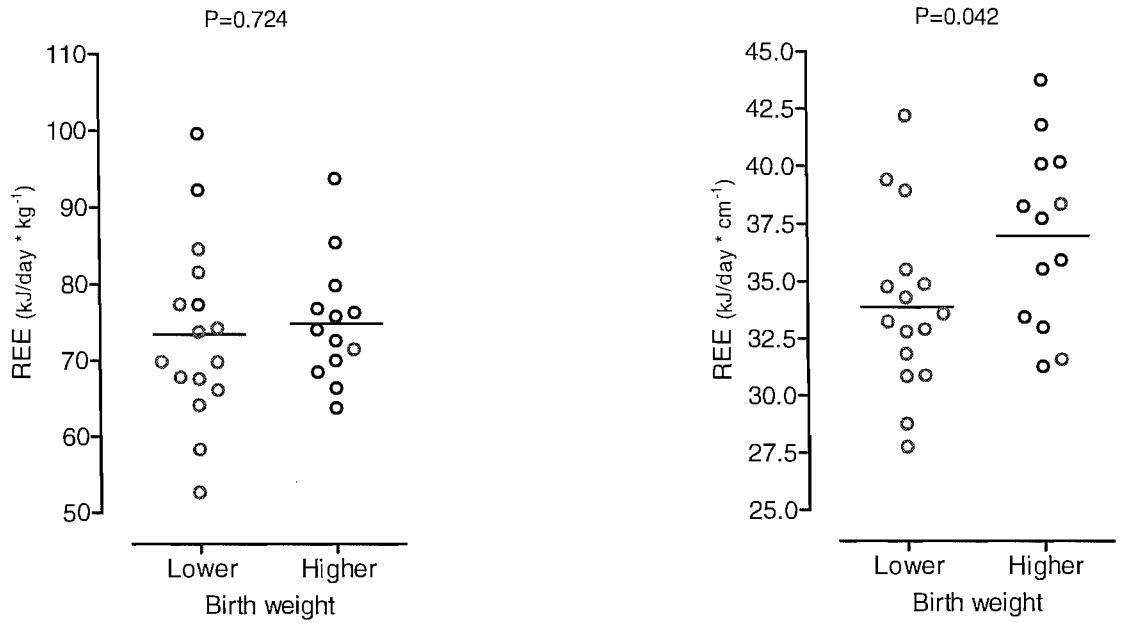

FIGURE 1 Differences in REE per kg body weight (left) and centimetre of height (right) between the lower and higher birth weight groups in fasted state

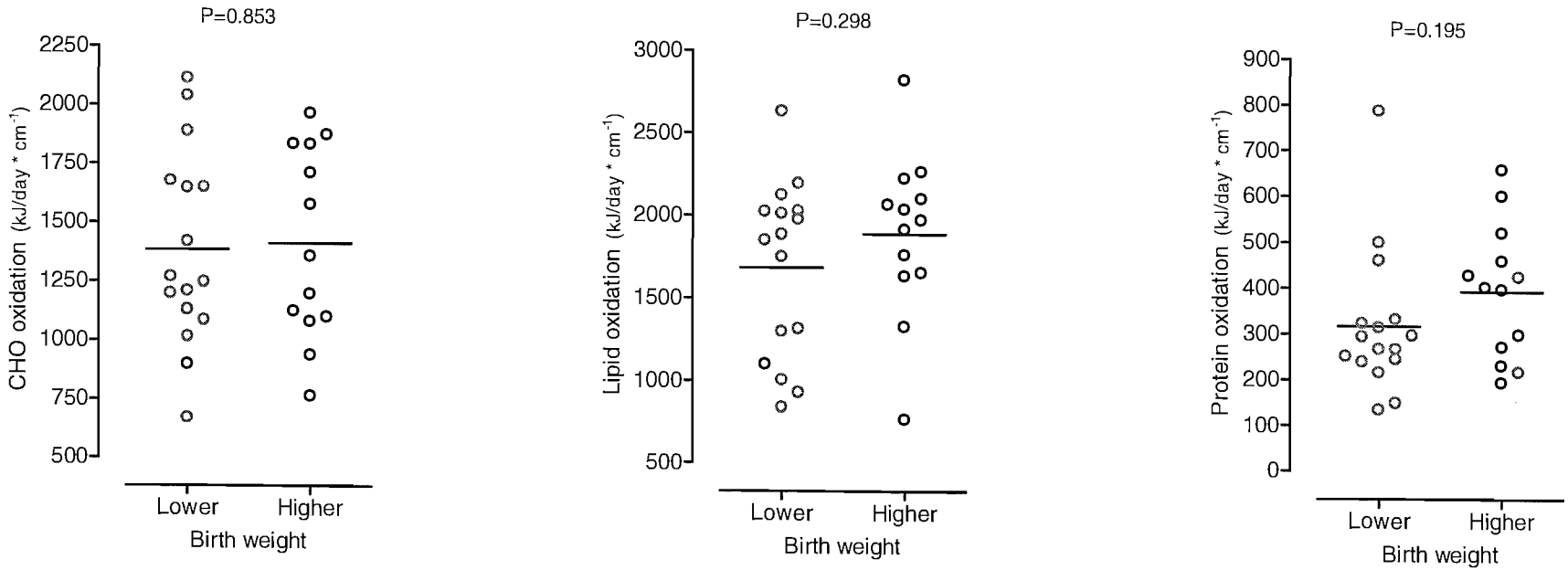

FIGURE 2 Differences in macronutrient oxidation per centimetre of height between the lower and higher birth weight groups in fasted state

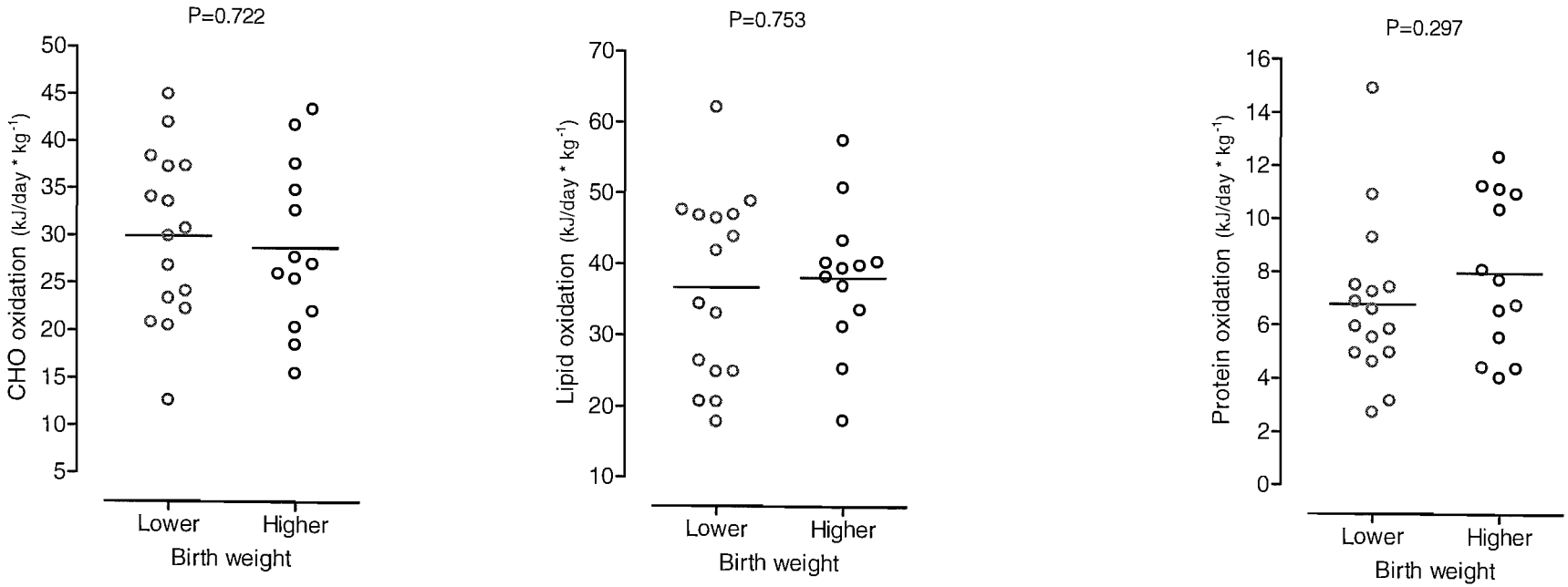

FIGURE 3 Differences macronutrient oxidation per kg body weight between the lower and higher birth weight groups in fasted state

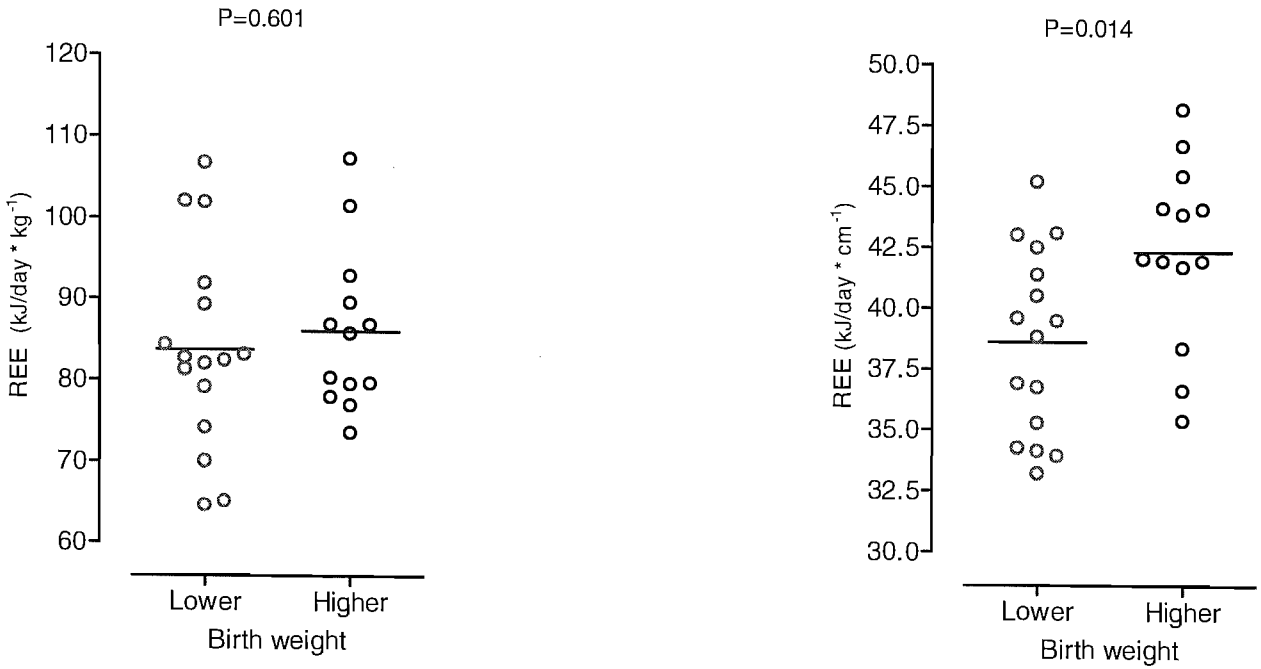

FIGURE 4 Differences REE per kg body weight (left) and centimetre of height (right) between the lower and higher birth weight groups in fed state

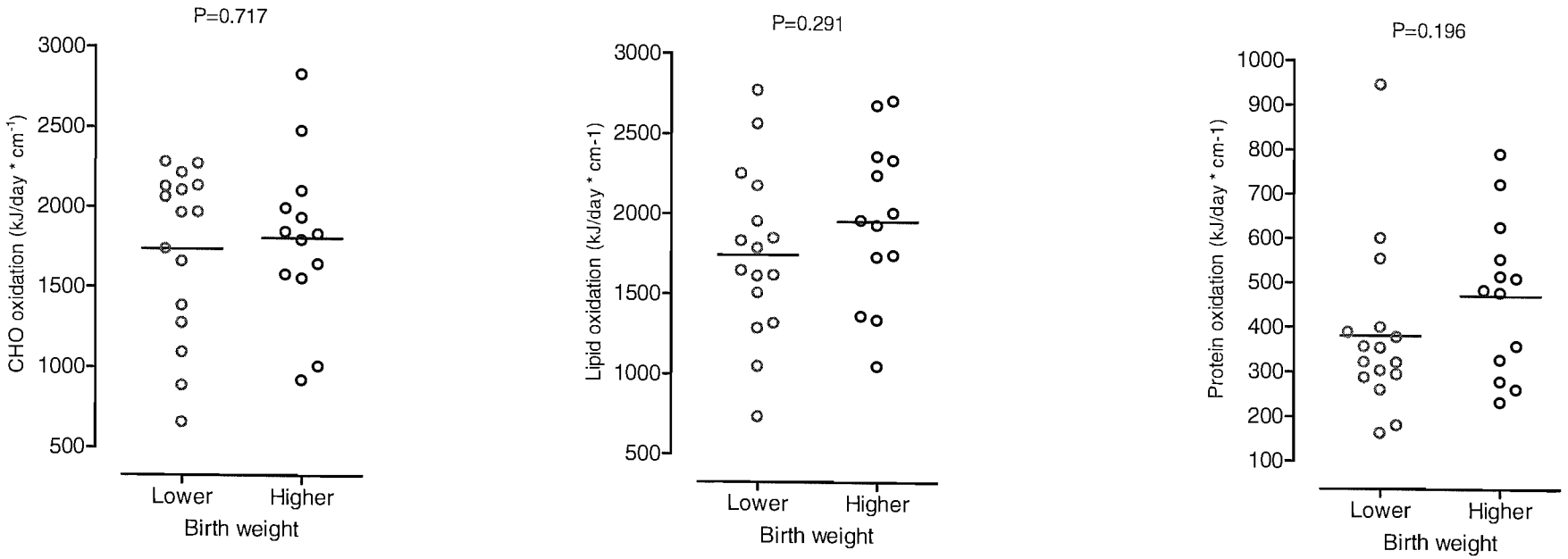

FIGURE 5 Differences in macronutrients oxidation per centimetre of height between the lower and higher birth weight groups in the fed state

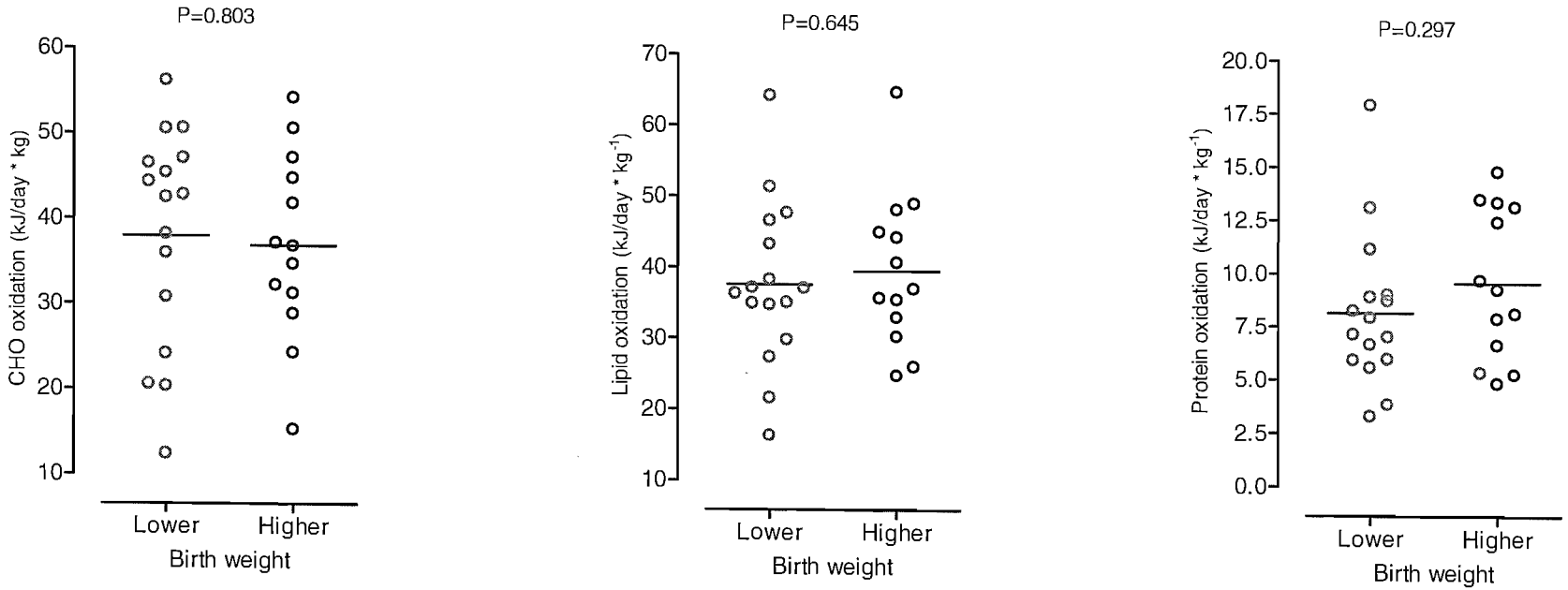

FIGURE 6 Differences in macronutrient oxidation per kg body weight between the lower and higher birth weight groups in the fed state

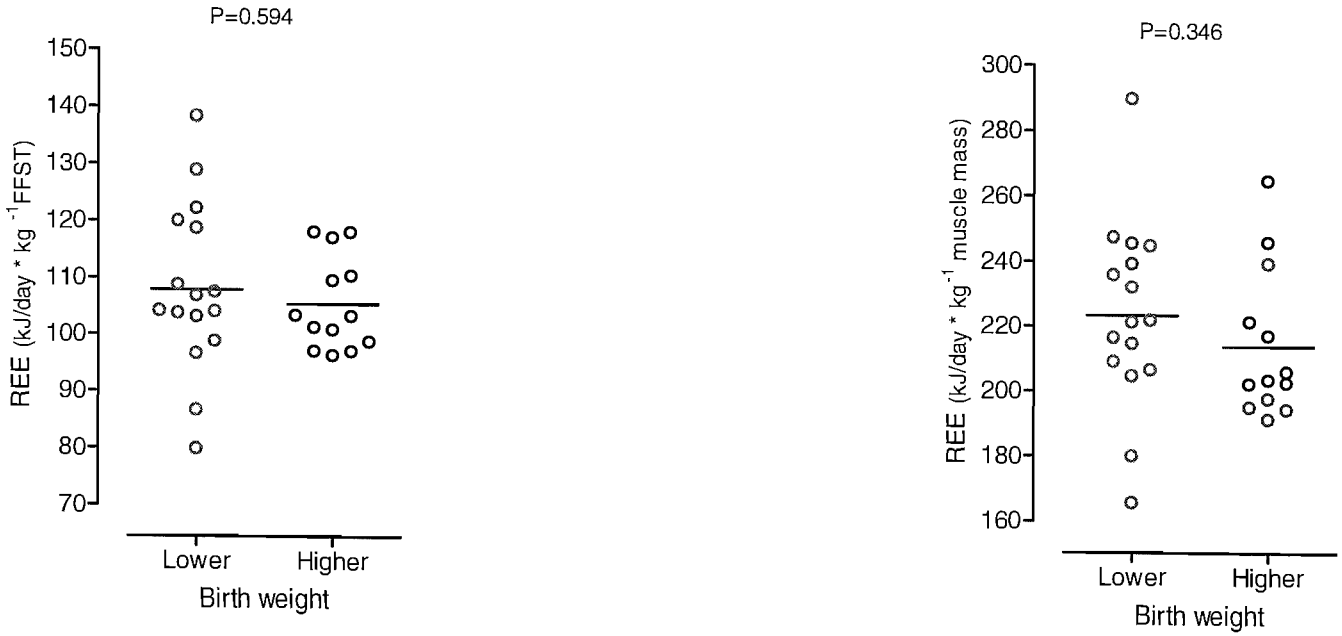

FIGURE 7 Differences REE per kg FFST and muscle mass between the lower and higher birth weight groups in the fasted state

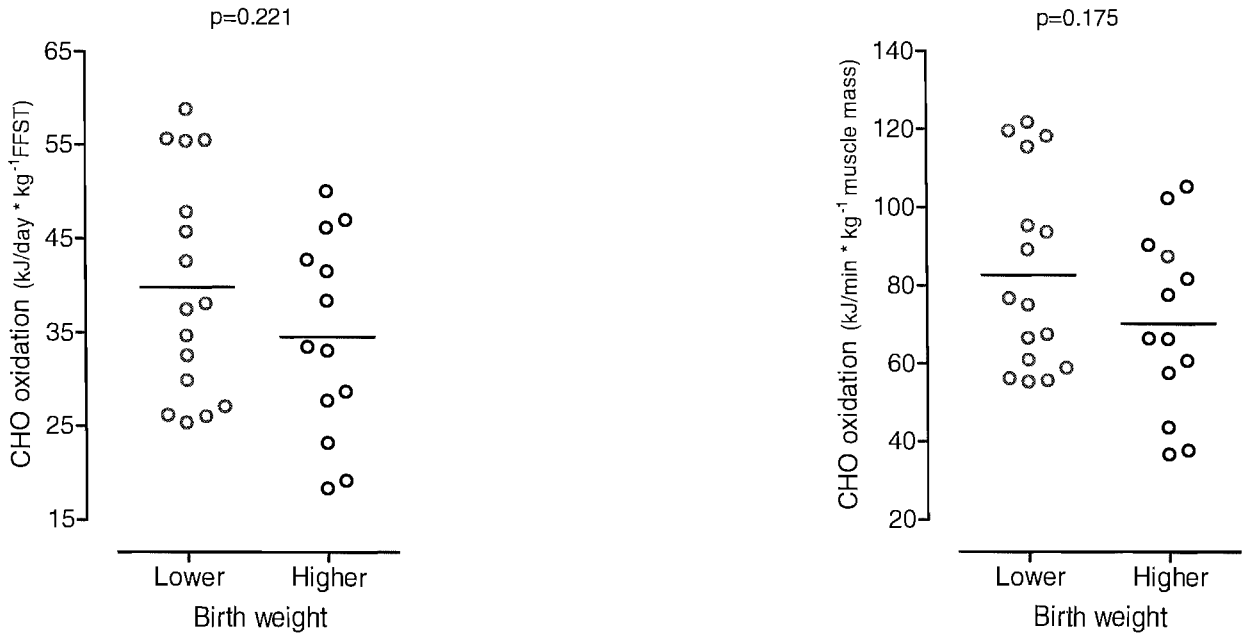

FIGURE 8 Differences in CHO oxidation per kg FFST, and muscle mass between the lower and higher birth weight groups in the fasted state

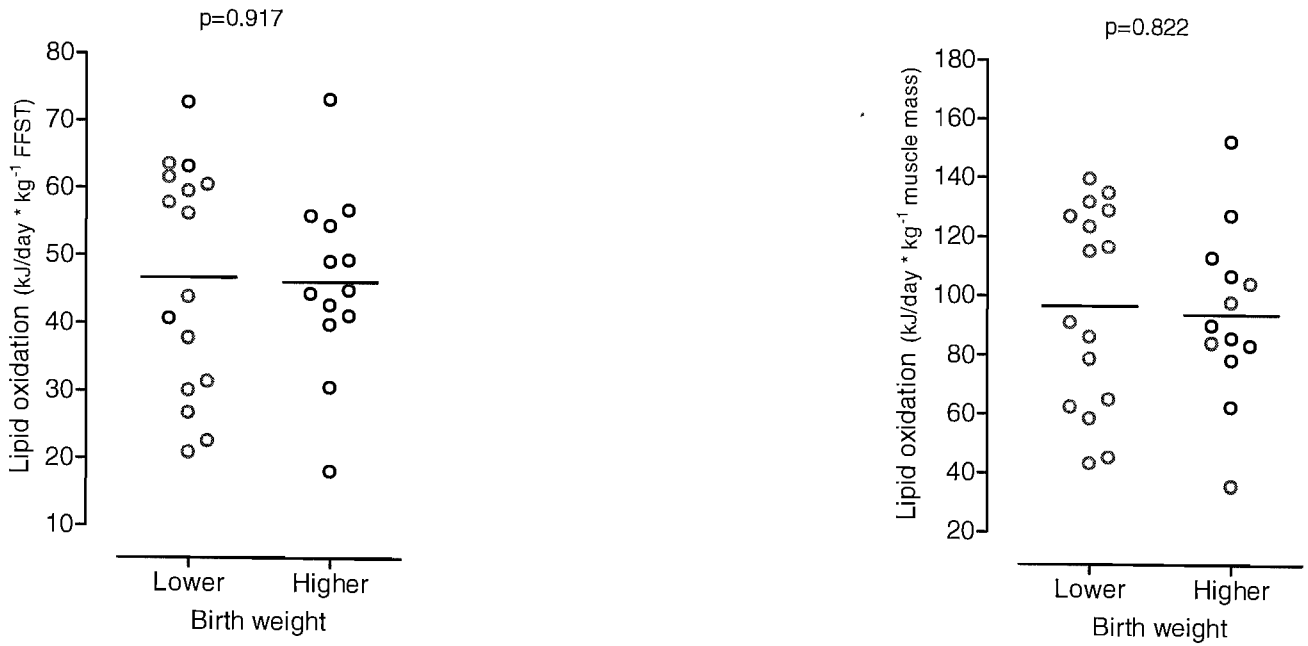

FIGURE 9 Differences lipid oxidation per kg FFST and muscle mass between the lower and higher birth weight groups in fasted state

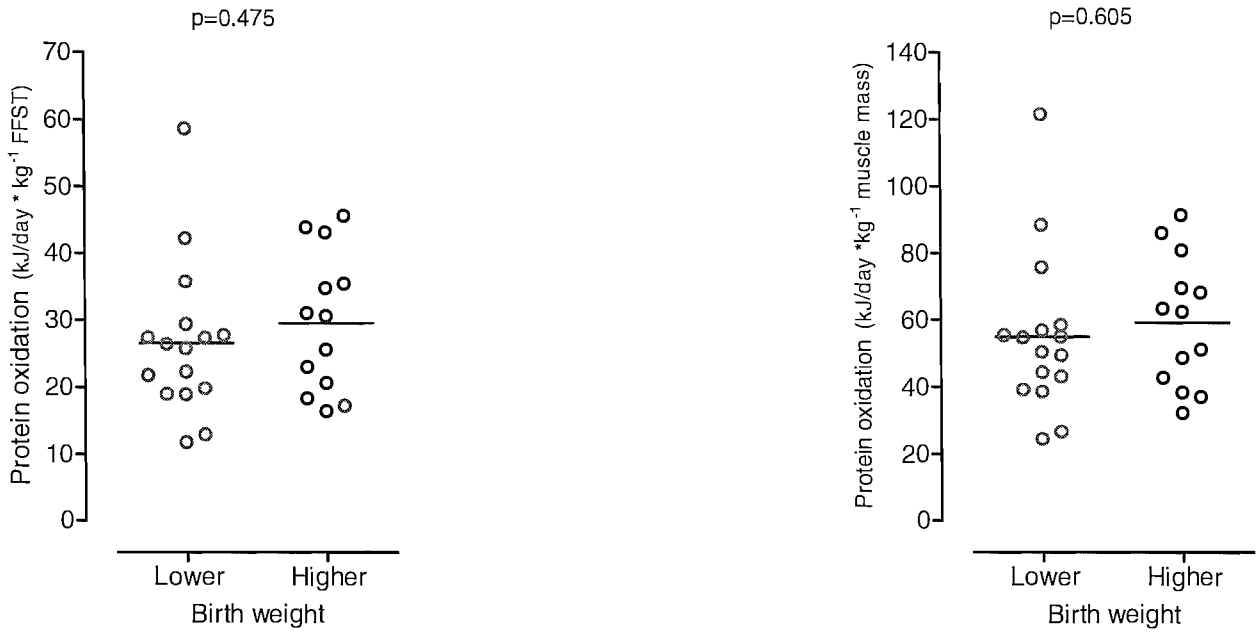

FIGURE 10 Differences protein oxidation per kg FFST and muscle mass between the lower and higher birth weight groups in fasted state

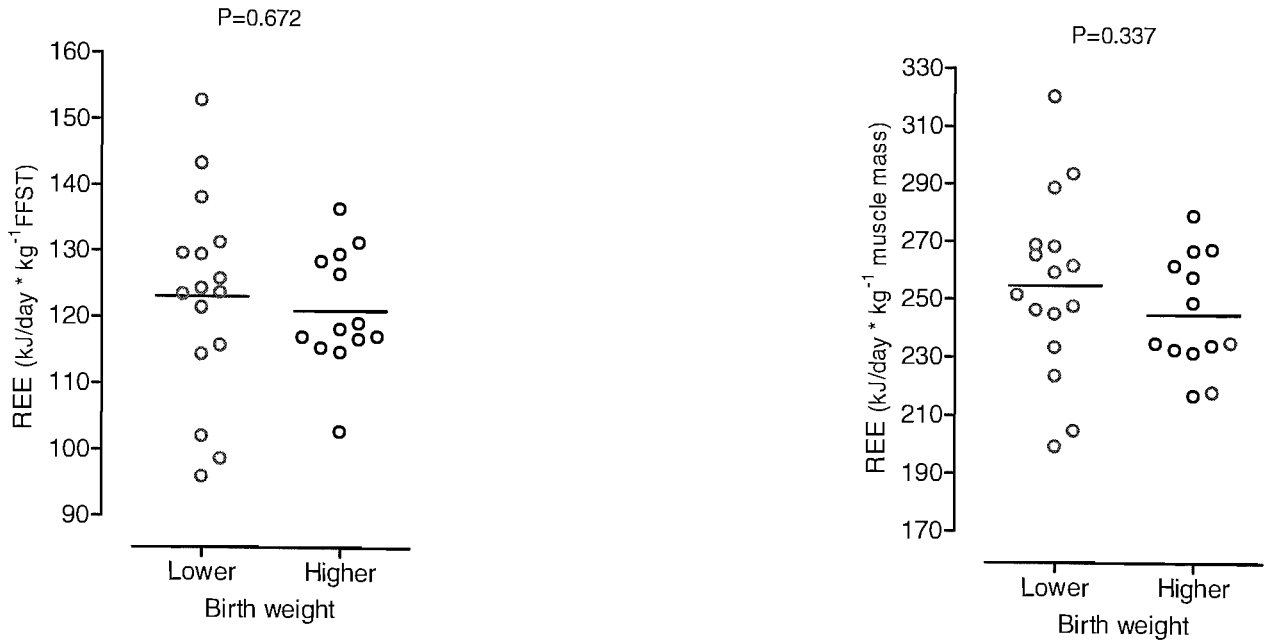

FIGURE 11 Differences REE per kg FFST and muscle mass between the lower and higher birth weight groups in fed state

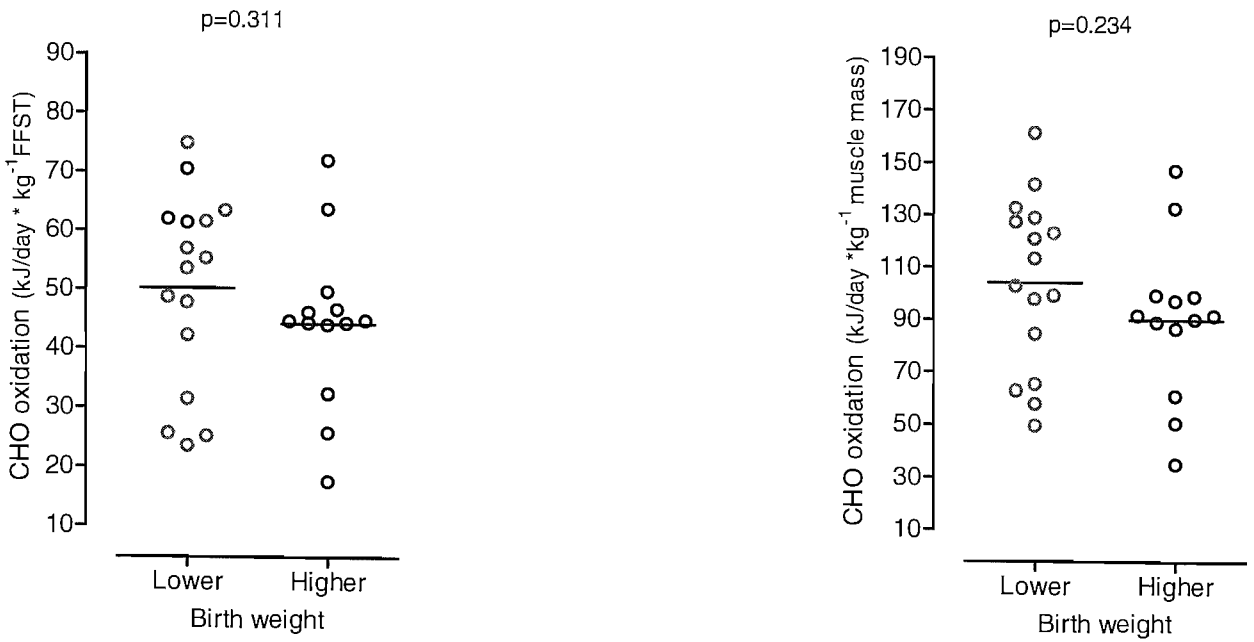

FIGURE 12 Differences in CHO oxidation per kg FFST and muscle mass between lower and higher birth weight groups in the fed state

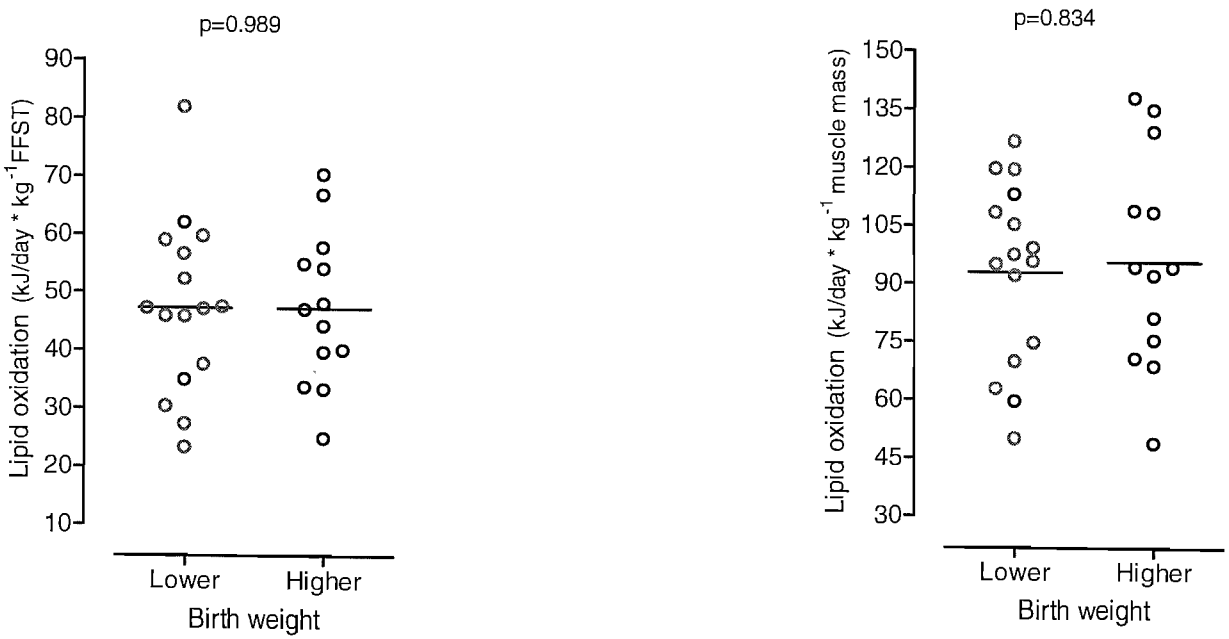

FIGURE 13 Differences in lipid oxidation per kg FFST and muscle mass between the lower and higher birth weight groups in fed state

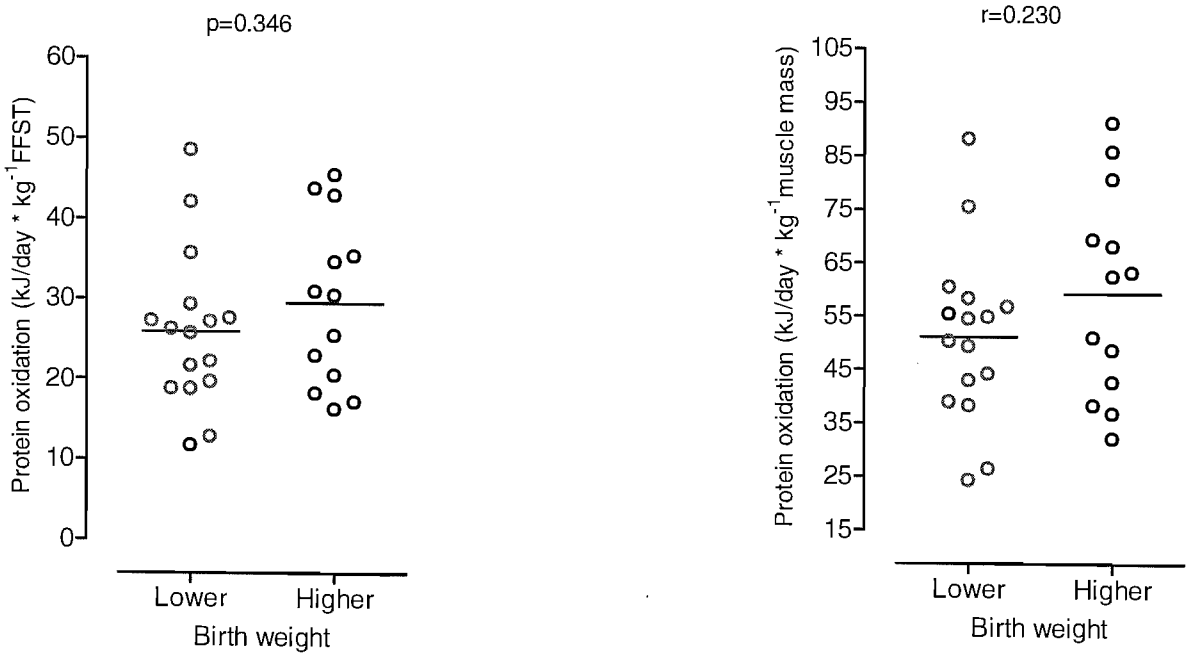

FIGURE 14 Differences in protein oxidation per kg FFST and muscle mass between the lower and higher birth weight groups in fed state

CHAPTER 11

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