UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Medicine

ANTHROPOMETRY, GLUCOSE TOLERANCE AND INSULIN CONCENTRATIONS IN SOUTH INDIAN CHILDREN: RELATIONSHIPS TO MATERNAL GLUCOSE TOLERANCE DURING PREGNANCY

by

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A thesis submitted for the degree of Doctor of Philosophy

December 2005

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE
MEDICAL RESEARCH COUNCIL EPIDEMIOLOGY RESOURCE CENTRE

Doctor of Philosophy

ANTHROPOMETRY, GLUCOSE TOLERANCE AND INSULIN CONCENTRATIONS IN SOUTH INDIAN CHILDREN: RELATIONSHIPS TO MATERNAL GLUCOSE TOLERANCE DURING PREGNANCY

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Earlier studies have shown that individuals whose mothers were diabetic when they were *in utero*, have an increased risk of early obesity, and impaired glucose tolerance (IGT) and type 2 diabetes in adult life. This study was designed to test whether adiposity, glucose tolerance and insulin concentrations are altered in Indian children born to mothers with gestational diabetes (GDM), and are related to maternal glucose and insulin concentrations in pregnancy even in the absence of GDM.

830 pregnant women attending the antenatal clinics of the Holdsworth Memorial Hospital (HMH), Mysore, India underwent an Oral Glucose Tolerance Test (OGTT) at 30+/-2 weeks. 674 of these women delivered at HMH. Detailed anthropometry was performed on the offspring at birth, and annually thereafter. 585 mothers returned with their offspring at 5 years of age for detailed investigations including OGTT for glucose and insulin concentrations, bio-impedance for fat estimation and blood pressure measurement. OGTT was administered to mothers and fasting plasma glucose and insulin concentrations were measured in fathers.

The Mysore babies were small compared to UK neonates, but the deficit varied for different body measurements. While birthweight (-1.1 SD) was considerably lower, crown-heel length (-0.3 SD) and subscapular skinfold thickness (-0.2 SD) were relatively spared. At five years, subscapular skinfold thickness was larger than the UK standards (+0.23 SD, $p<0.001$) despite all other body measurements being significantly smaller. Findings at 5 years were similar in comparison with another standard, based on Dutch children. At 5 years, girls in the cohort had higher insulin concentrations and were more insulin resistant. Body fat was the strongest predictor of glucose and insulin concentrations independent of other body components and parental characteristics.

Newborns of the mothers with gestational diabetes were larger in all body measurements than control neonates (born to non-GDM mothers and non-diabetic fathers). At one year, these differences had diminished and were not statistically significant. At five years, female, but not male offspring of diabetic mothers had larger subscapular and triceps skinfolds ($P=0.01$) and higher 30- and 120-minute insulin concentrations ($P<0.05$) than control females. Even in the control offspring maternal insulin area-under-the-curve was positively associated with 30-minute insulin concentrations, after adjusting for sex and maternal skinfolds ($P<0.001$). Offspring of diabetic fathers ($n=41$) were lighter at birth than controls; they showed no differences in anthropometry at five years.

In conclusion, Maternal GDM is associated with adiposity and higher insulin concentrations in female offspring at 5 years. The absence of similar associations in offspring of diabetic fathers suggests a programming effect of the diabetic intra-uterine environment. With increasing levels of obesity and IGT among Indian mothers, these effects may be contributing to the rise of type 2 diabetes in India. Our continuing follow-up aims to study the long-term effects of higher maternal glucose concentrations in the absence of GDM.

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ABBREVIATIONS

AMA	**Arm-Muscle-Area**
BIA	**Bioimpedance Analyser**
BMI	**Body Mass Index**
BP	**Blood Pressure**
CHL	**Crown-Heel Length**
CRL	**Crown-Rump Length**
DM	**Diabetes Mellitus**
FFM	**Fat-Free Mass**
FM	**Fat Mass**
GAUC	**Glucose Area Under the Curve**
GDM	**Gestational Diabetes Mellitus**
HMH	**Holdsworth Memorial Hospital**
HOMA	**Homeostasis Model Assessment**
IAUC	**Insulin Area Under the Curve**
IFG	**Impaired fasting Glucose**
IGT	**Impaired Glucose Tolerance**
IOV	**Inter/Intra-observer Variation studies**
MUAC	**Mid-Upper Arm Circumference**
NGT	**Normal Glucose Tolerance**
ODM	**Offspring of Diabetic Mothers**
OGTT	**Oral Glucose Tolerance Test**
ONDM	**Offspring of Non-Diabetic Mothers**
PAL	**Physical Activity Level**
SES	**Socio Economic Status**
SGA	**Small for Gestational Age**
SS/TR	**Subscapular-to-Triceps Ratio**
TBW	**Total Body Water**
TEE	**Total Energy Expenditure**
WHR	**Waist-to-Hip Ratio**

ACKNOWLEDGEMENTS

I understand the impossibility of mentioning the names of all the people who helped me throughout the course of this project. I wish to begin by thanking Prof. David Barker, whose inspirational thought set the premises for this study and my mentor Dr. Caroline Fall for the confidence and trust she placed in me by giving this most rewarding opportunity.

I am greatly indebted to Dr. Caroline Fall not only for her constant encouragement and support at every stage, her inspiration and guidance without which this study would not have been possible, but also for warmth and care which made my stay in a 'strange' country endurable. She taught me everything I know about epidemiological research.

I am also thankful to Dr. Jacqui Hill, a former colleague and a friend who was instrumental in my involvement in the project, and helped me develop a taste for epidemiology.

The project would have been a dream but for the greatest help and involvement from my colleagues in Mysore. Dr.Veena's enduring strength, support and friendship steered me through difficult times. I can never forget the support rendered by my present teammates Jayakumar, Geetha, Chachyamma, Saroja, Shobha and Tony Onslow. Great help was also received from my earlier colleagues, especially Tony Gerald, and Gopal Singh.

I am extremely thankful to colleagues at the MRC unit in Southampton for providing me a warm and welcoming environment during my write-up. I thank Sam Leary, Julia Saperia and David Fisher for giving me lessons in statistics and helping me with difficult statistical analysis, and Dr. Clive Osmond for helping to solve tricky problems at crucial times. My sincere thanks to Patsy Coakley for timely help with the data, and Jane Pearce for making my write-up more presentable and attending to my innumerable panic pleas with composed solutions. Hannah Farrant and Inga Mills helped me with my research needs and helped to analyse data on Vitamin D (Hannah) and physical activity (Inga). I am grateful to Vanessa Cox for providing me a 'home' away from home, and Avan Aihie Sayer and her family for their warmth during my stay with them. My heartfelt thanks to Dr. Steve Wootton, Department of Human Nutrition for his valuable guidance with the physical activity data.

Dr. S.C. Karat, the Medical Director of the HMH gave the permission to carry out the study and gave his support throughout. I wish to sincerely thank him and the staff of HMH. I also thank Dr. Yajnik and his staff (KEM Hospital, Pune) for glucose and insulin analysis, and Dr. Mohan and staff (Chennai) for HbA1c assay.

My special thanks to the Parthenon Trust and the Wellcome trust for funding the study, and SNEHA, India for its support.

Most importantly, I would like to remember and thank the study parents for their undying support and kindness which was vital for the success of this project. Association with the affectionate and exuberant children made my field work an enjoyable experience.

Finally, none of these would have been conceivable without the immense support and love from my mother Rukmini, father Vedamurthy, beloved brothers Dashanna, Ravi Kumar, Krishna Kumar, Ramu, Rajeev, dear sisters Pushpa, Komu, Gomu and other family members, whose ceaseless belief in me gave the strength to face all difficulties. I think of my uncle Madanna, who took great pride when I started my study, but could not stay to see me completing this.

AUTHORS CONTRIBUTION

I was instrumental in establishing the cohort in Mysore working in close quarters with Dr. Jacqui Hill during the first part of the study. I managed overall research activities, recruited and trained field workers in anthropometry, blood pressure, bioimpedance measurements, conducted inter- and intra-observer variation studies, trained the research nurse in blood taking techniques in children, and worked with them for clinical data collection with support from my colleague Dr.Veena.

During my stay in Southampton, I analysed the data myself, with lessons and help from Sam Leary, Julia Saperia and David Fisher. I interpreted the results with the guidance from my supervisor Dr. Caroline Fall and wrote the publications with her help.

I have typed this thesis myself.

PUBLICATIONS

Krishnaveni GV, Hill JC, Veena SR, Leary SD, Saperia J, Chachyamma KJ, Karat SC, Fall CHD. Truncal adiposity is present at birth and in early childhood in south Indian children. *Indian Pediatrics* 2005;42:527-538.

Krishnaveni GV, Hill JC, Leary SD, Veena SR, Saperia J, Saroja A, Karat SC, Fall CHD. Anthropometry, glucose tolerance and insulin concentrations in Indian children: relationships to maternal glucose and insulin concentrations during pregnancy. *Diabetes Care* 2005;28:2919-25

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PRESENTATIONS

Anthropometry and Glucose/Insulin Concentrations in Indian Children - Relationships to Maternal Gestational Diabetes. Oral presentation at the Third World Congress on DOHaD, Toronto, November 16-20, 2005.

Anthropometry and Glucose/Insulin concentrations in Indian Children - Relationships to Maternal Gestational Diabetes. Poster presentation at the 5th American Diabetes Association workshop on GDM, Chicago, November 11-13, 2005.

Growth, and body composition at birth and in early childhood - relationships to glucose and insulin metabolism at 5-years - Parthenon Follow up Study. Oral presentation at the Blaire Bell Research Society meeting, Southampton, September 30-October 1, 2004.

Adiposity and risk markers in Indian children: comparison of 2 methods. Poster presentation at the 7th International symposium : In vivo Body Composition Studies, Southampton, September 7-9, 2005.

Also presented my data at the annual workshops of SNEHA, India at the following years:

- Mahabalipuram, India 1999 October.
- Mumbai, India 2000 August.
- Aurangabad, India. 2001 September.
- Kodaikanal, India. 2002 November.
- Jaipur, India. 2004 February.
- Mahabaleshwar, India. 2005 January.

1. INTRODUCTION

The prevalence of type 2 diabetes is rising rapidly in many parts of the world. Recent evidence suggests that the fetal and postnatal environments, as well as the adult lifestyle factors, have a role in the emergence of this trend. This thesis describes a study carried out as part of a programme of research into the maternal and fetal origins of adult type 2 diabetes in India. The objective was to study a large cohort of children born to women whose glucose tolerance was tested in pregnancy in order to examine:

- The relationship between size at birth and pattern of growth up to the age of 5 years on body composition and glucose/insulin metabolism in 5-year old Indian children.
- The effects of maternal diabetes on these outcomes.
- Whether glucose and insulin status of the mother in pregnancy is related to these outcomes 'in the absence of frank diabetes'.

1.1 Type 2 diabetes - background

1.1.1 Pathophysiology: Diabetes mellitus (DM), a disorder associated with a multitude of metabolic abnormalities affecting the utilisation of fuels such as carbohydrates, lipids and amino acids, is a state of chronic hyperglycaemia secondary to absolute or relative deficiency of insulin.

Type 2 DM is the common type affecting more than 90% of all people with diabetes. Two major pathological mechanisms have been attributed for its development. The primary abnormality is thought to be insulin resistance or decreased sensitivity to insulin action at major glucose uptake sites such as liver and skeletal muscle in the presence of normal or increased pancreatic β-cell secretion. Secondarily, there is deficient β-cell activity resulting in impaired secretion of insulin. There is a debate over the primary mechanism as either one of these may lead to the evolution of the second. However, it is unlikely that any one pathology on its own is the cause of type 2 DM[1].

Hyperglycaemia induces a number of micro-vascular complications involving multiple organ systems like the kidneys, cardiovascular and peripheral nervous systems, and retina[1]. Hyperglycaemia itself, and lipid abnormalities associated with diabetes are also associated with macro-vascular complications such as atherosclerosis, a precursor of coronary heart disease (CHD). Diabetes is a major risk factor for CHD, a leading cause of mortality worldwide[1,2].

Type 2 DM is mainly a disease of the middle or older ages. Obesity is common, and contributes to insulin resistance in these individuals[2]. Increased waist circumference and/or larger truncal skinfold thicknesses constituting truncal or central obesity is another common characteristic in some of these individuals[3].

Type 2 DM usually clusters with hypertension. A collection of traits related to diabetes such as insulin resistance, central obesity, impaired glucose tolerance and dyslipidemia, along with elevated blood pressure has been suggested to be present prior to the development of type 2 diabetes[4]. This referred to as the 'metabolic syndrome (insulin resistance syndrome)' has been thought to help the early identification of individuals with high diabetic and cardiovascular disease (CVD) risks[5].

1.1.2 Global picture: The greatest impact of the rising prevalence of type 2 DM is predicted to be on the neo-industrialised countries that are in a phase of rapid 'epidemiologic transition' from mainly rural subsistence economies to urban industrial economies with the attendant lifestyle changes[2]. An *expert group* projected that the number of adults (≥ 20 years) with diabetes in the world will double between the years 2000 and 2030, and the burden of change will be greatest in developing countries[6]. While the majority of people with diabetes in the developed world will be elderly, in developing countries, the burden will be on middle-aged adults (45-64 years). The rise is predicted to be 151% in India and the country is predicted to have 79 million people with diabetes by 2030 (32 million in year 2000)[6].

1.1.3 Economic implications of diabetes: The health care expenditure of individuals with diabetes is estimated to be higher than that of non-diabetic persons[7], a major portion of which is on treating the complications[8]. The economy may also suffer in terms of loss of working hours, decreased life expectancy and premature retirement. Since adequate glycaemic control keeps these complications in check, prompt early diagnosis and treatment of type 2 DM will lessen the tangible as well as hidden costs[9]. Unfortunately, many people in the developing world cannot afford even basic treatment of diabetes. Moreover, awareness about the disease is low due to widespread poverty and illiteracy. Thus, a person from a country like India, who develops diabetes in the middle age, would be at a greater risk of developing complications putting a burden on the economy[10], and would also suffer from loss of own livelihood. Hence, primary prevention of diabetes is most cost effective, particularly in developing countries.

1.1.4 Role of genes: Type 2 diabetes is a disease of heterogeneous aetiology. It is thought to be a familial disease with a polygenic inheritance[11]. The observation of high concordance rates in monozygotic twins in follow-up studies[12], intermediate prevalence rates in populations with genetic admixture[13,14], and a higher incidence of diabetes in the offspring of diabetic fathers than in the general population[15] are consistent with a genetic aetiology. Though susceptibility genes for insulin resistance and type 2 DM (PPAR γ and Calpain-10) [16,17], and genetic determinants of several familial, rare monogenic disorders such as Maturity Onset Diabetes of the Young (MODY)[11] have been identified, they are either extremely rare or have a small effect. The majority of cases of type 2 DM cannot be explained by the genes identified so far.

1.1.5 Role of environmental factors: Despite a strong genetic predisposition of type 2 DM, the following observations suggest a major environmental component in the aetiology of type 2 DM:

- There is a higher prevalence of diabetes in the adult offspring of diabetic mothers than diabetic fathers[15]. Even among siblings, offspring who were born after the mother developed diabetes are at higher risk than those born before[18].
- Higher rates of diabetes are observed in populations with a lower prevalence of disease if they migrate to a higher prevalence area, suggesting a role of the physical environment[19].
- Populations have been genetically stable over many years and the magnitude of the current epidemic cannot be explained solely by genetic involvement.

It is inferred that the genetic factors interact with the environmental factors to bring about a number of intermediate traits such as obesity and insulin resistance. Environmental factors are deemed necessary for the genes to express themselves in bringing the disease to the fore.

Whatever its underlying cause, obesity or excess adiposity is a risk factor for diabetes[20]. Adiposity induces insulin resistance mainly through releasing excess of free fatty acids in to the circulation[21]. Obesity has become a worldwide phenomenon owing largely to rapid urbanisation and altered lifestyle in countries which were traditionally agriculture centred till recently[22]. Neel proposed the presence of a 'thrifty gene', which stored energy in the form of fat during the pre-industrial era and gave a survival advantage

during famine[23]. This gene might have become harmful in these days of plenty, predisposing populations to epidemics of obesity and type 2 DM.

Physical inactivity is associated with obesity[24], and aggravates insulin resistance independently of obesity[25]. Physical exercise may improve insulin sensitivity and glucose homeostasis by altering structure and functions of glucose uptake sites such as skeletal muscles or changes in glucose transport mechanisms[25,26]. It is suggested that physically active people have 30-50% lower risk of developing diabetes compared to sedentary individuals[27]. Studies in adults have shown that a regular physical exercise prevents or postpones the onset of diabetes in high-risk individuals[28,29].

Diets rich in refined carbohydrates, saturated fats, and poor in dietary fibres may increase the risk of obesity and insulin resistance[30,31]. Dietary fatty acids may also related to high insulin resistance independent of obesity, and is thought to be related to excess of free fatty acids in the circulation[31]. Low circulating levels of certain minerals such as zinc, magnesium and chromium are believed to be associated with the development of insulin resistance and type 2 diabetes[32]. Low levels of vitamin D in the body has been shown to be associated with decreased insulin sensitivity and β-cell function[33]. A study among south Asians living in the UK suggested that vitamin D deficiency may contribute to the high prevalence of type 2 diabetes in these populations[34].

1.1.6 Ethnicity and type 2 diabetes: Type 2 DM is particularly prevalent in certain ethnic groups such as Hispanics and south Asian adults[35]. The highest prevalence is seen in the Pima Indian communities of America[2]. What determines the additional risk of diabetes in these populations is still a matter of debate. Though adopting a Western lifestyle (energy-dense diet, reduced physical exertion) is an argument for the causation, it is not clear how this increases the risk in such great proportions. One of the salient features of these individuals is the higher level of insulin resistance than Caucasian adults. While insulin resistance is associated with obesity in native Americans and Hispanics, south Asians are insulin resistant even at lower levels of body mass[36]. Characterisation of their body phenotype suggests that Indians tend to deposit fat in the truncal regions such as the abdomen and subscapular area, and this, termed central obesity, is thought to predispose them to insulin resistance[3].

1.1.7 Childhood obesity, insulin resistance and metabolic syndrome – new global challenge: Recently, there has been a rise in the prevalence of type 2 diabetes in children and adolescents[37,38]. Childhood obesity is becoming a major public health problem in many developed as well as developing populations[39,40]. Many of these children are shown to have insulin resistance, elevated blood pressure and other features of metabolic syndrome[41,42]. In countries like India, central adiposity and insulin resistance are emerging in children even in the absence of increased body mass[43]. A global rise in the incidence of childhood obesity could be playing a role in the increase in adult obesity[44] and of type 2 diabetes.

1.2 Fetal and developmental origins of type 2 diabetes –the thrifty phenotype

A novel concept, linking emergence of chronic diseases like type 2 DM, hypertension and CVD in adults to restricted growth in utero has given new insights into the aetiology of adult diseases. A deficient nutrition supply to the fetus *in utero* resulting in low birth weight, and poor infant growth are said to predispose it to future obesity and insulin resistance by way of altered endocrine function[45].

1.2.1 Initial evidence: The first evidence of the potential hazards of impaired early growth came from the observation of a striking similarity in geographical distribution of deaths due to CHD in adults with that of infant deaths about six decades ago in different parts of England and Wales[46]. Later, by using birth records in Hertfordshire, UK, an association between small size at birth and raised death rates from cardiovascular disease in later life was demonstrated[45].

Subsequently, by studying glucose tolerance in men and women in Hertfordshire it was shown that the prevalence of type 2 DM and impaired glucose tolerance (IGT) was highest in those who had lowest birth weights[45]. The prevalence was three-fold higher in low birth weight men compared to those with higher birth weights.
These findings were replicated in Preston, UK where the prevalence of type 2 DM and IGT fell from 27% in individuals weighing <2.5kg at birth to 6% in those weighing >3.4kg. An association was also observed between thinness at birth and the prevalence of type 2 DM in Sweden, where the prevalence was three-fold higher among men in the lowest compared to the highest fifth of ponderal index ({weight (g)/length $(cm)^3$}*100)[45].

In Hertfordshire and Preston it was observed that current obesity was an important determinant of type 2 DM in low birth weight adults. Men and women with highest risk of IGT, type 2 DM, or insulin resistance syndrome were those who had low birth weights and high current body mass index (BMI) and waist-to-hip ratio[47]. Similarly, in a study involving men and women born in Helsinki, Finland, highest concentrations of 2-hour plasma insulin were found in those with lowest birth weights but high current BMI[48].

1.2.2 Thrifty phenotype hypothesis and fetal programming: The above observations led to the 'thrifty phenotype' hypothesis, which proposed that nutritional insults experienced during critical stages of growth *in utero* permanently programme the fetus[49]. The resulting phenotype offers the maximum survival chances at the available nutrition, by being 'thrifty' or 'economical'. However, it was proposed, the same phenotype predisposes it to insulin resistance, later type 2 DM and the insulin resistance syndrome[49].

The hypothesis is based on the concept of fetal adaptation and later 'misadaptation'. The growing fetus adapts to adverse conditions by prioritising the growth of vital organs like the brain over 'less important' insulin sensitive organs like the pancreas, liver and skeletal muscles resulting in the development of insulin resistance[50]. This prioritisation could occur by redistribution of blood flow to vital organs, reduced secretion of anabolic hormones such as insulin and insulin-like growth factors (IGF) or increased cortisol production encouraging early differentiation and compromising abdominal visceral and musculo-skeletal growth[50]. As long as the individual remains lean in later years, the body maintains glycaemic homeostasis. However, in the face of adult stresses such as obesity, which increases insulin resistance the individual has a lower threshold for developing type 2 DM[45]. This may be due to poor pancreatic reserve because of compromised pancreatic growth, or to increased insulin resistance due to reduced skeletal muscle or altered hepatic development.

Maternal nutritional status is considered as an important determinant of fetal programming[50]. It is known that poor maternal nutrition leads to impaired intra-uterine growth and reduced birth size in the offspring. Animal experiments show that a reduction in either total nutrient or calorie intake during pregnancy alters fetal pancreatic β-cell development, and leads to decreased insulin content of the fetal

pancreas[51]. Feeding pregnant rats on a low-protein normal-energy diet throughout gestation results in reduced pancreatic islet cell proliferation, impaired β-cell granulation, and impaired development of endothelial cells leading to impaired vascularisation of the pancreas in the fetus[52].

Chronic micronutrient deficiencies of the mother also result in impaired fetal growth[53]. Studies among south Asian women have shown that gestational vitamin D deficiency was associated with reduced offspring birth size, and low circulating vitamin D levels in the newborn[54,55]. Vitamin D is known to influence the pancreatic β-cell development and functioning[33], though its role in the development of type 2 DM is not well known. Whether the high prevalence of maternal vitamin D deficiency in certain populations such as south Asians[54,56] contributes to their greater predisposition to type 2 DM is open for further exploration.

Factors related inadequate blood flow such as maternal blood pressure, smoking and placental pathologies can also cause fetal growth retardation by interfering with the delivery of nutrients to the fetus[50].

1.2.3 Confirmatory studies: Following the thrifty phenotype hypothesis, a number of studies from all over the world tested and confirmed the association between small size at birth, and later insulin resistance and type 2 DM[57]. In a systematic review of published literature on the association between birth weight and later glucose and insulin metabolism by Newsome *et al.*, inverse relationships were found in the majority of studies[57]. A study of people born during the Dutch famine of 1944 showed that the individuals whose mothers were exposed to famine during middle and late gestation had lower birth weight, length, and head circumference than those who were not exposed or exposed during early gestation, and had reduced glucose tolerance (higher 120-minute glucose concentrations)[58]. In Finland, after correcting for current BMI, plasma insulin concentrations fell with increasing birth weight or ponderal index in elderly men and women[48]. In China, low birth weight was associated with higher plasma glucose and insulin concentrations after adjusting for current BMI[59]. Some studies demonstrated that small size at birth was related to the development of insulin resistance even in children (Table 1.1)[43, 60-65].

Table 1.1 Main features of studies in children relating glucose/insulin outcomes with birth and current size

Reference	Age	Place	Birth measurements	Childhood measurements	Associations
Yajnik et al[60]	4 years	Pune, India	Birth weight	Weight, height, skinfolds, waist and hip circumference	+ve with current size -ve with birth weight
Bavdekar et al[43]	8 years	Pune, India	Birth weight	Weight, height, skinfolds, waist and hip circumference	+ve with current size -ve with birth weight after adjusting for current size
Whincup et al[61]	11 years	England and Wales	Weight, length, ponderal index	Weight, height, ponderal index	+ve with current size -ve with birth weight after adjusting for current size
Crowther et al[62]	7 years	South Africa	Birth weight	Height, ponderal index Skinfolds at 5 years	+ve with PI and skinfolds, -ve with current height -ve with birth weight after adjusting for current size
Lawlor et al[63]	9 years	Estonia and Denmark	Birth weight	BMI, Height	+ve with current size -ve with birth weight
Wilkin et al[64]	5 years	Plymouth, UK	Birth weight	Weight, BMI, weight change since birth	+ve with current size, no relationship with birth weight
Sayers SM[65]	8-14 years	Australia	Birth weight, ponderal index	Weight, height	+ve with current size, no relationship with birth weight

1.2.4 Fetal insulin hypothesis: Hattersley *et.al.* proposed an alternative explanation for the association between low birth weight and type 2 DM, the 'fetal insulin hypothesis', that low birth weight and insulin resistance as different manifestations of a common genotype[66]. The discovery of mutations in the glucokinase gene which resulted in reduced birth weight, and high insulin resistance is consistent with this hypothesis[67]. This theory is further supported by studies which demonstrated that paternal insulin resistance and diabetes were associated with low birth weight in the offspring[68-70].

Another suggestion was that the strong association between low birth weight and later diabetes is the result of selective survival of low birth weight infants genetically predisposed to insulin resistance[71].

1.2.5 Effects of postnatal size: Some studies linked not only intra-uterine growth, but also growth during infancy and childhood with the development of later type 2 DM. The Hertfordshire study reported an increase in the mean 2-hour plasma glucose in men with the lowest weights at 1-year[45]. In Finland, adults who developed type 2 DM were light and thin at birth, and had a low weight gain during infancy[72]. Risk was increased if there had been rapid gain in BMI during childhood. The risk of diabetes was also increased if there was an early adiposity rebound*; the cumulative incidence of diabetes was 8.6 in adults in whom adiposity rebound occurred before the age of 5 years compared with 1.8% if it occurred after 7 years[72]. Similar findings to Finland were recently demonstrated in a population based study in India[73] (Section 1.4.3).
Conflicting evidence from elsewhere showed that accelerated infant growth favoured insulin resistance in adolescents[74]. It was suggested that policies promoting infant growth might have unfavourable consequences in the future. This issue remains controversial.

* Adiposity rebound: The level of adiposity (BMI) decreases in children after infancy and reaches a minimum before increasing again between 3 and 7 years.

1.2.6 Fetal programming and blood pressure: Blood pressure (BP) is one of the most widely studied outcomes in relation to the 'fetal origins' hypothesis. In Hertfordshire, both systolic and diastolic BP fell between those weighed ≤2.5 kg at birth and those with a birth weight of ≥3.9 kg[45]. Studies in adults have consistently shown an inverse association between BP and birth weight[75,76] suggesting another possible link between fetal growth retardation and CVD risks in later life.

1.3 Fetal and developmental origins of type 2 diabetes -maternal gestational diabetes mellitus (GDM)

1.3.1 Gestational diabetes- background: Gestational diabetes is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy"[77]. Recent evidences indicate that fetal over-nutrition as in the case of maternal GDM, as well as growth retardation, is associated with higher prevalence of adult type 2 DM in the offspring. The higher risk in offspring of diabetic mothers (ODM) underlies the 'U' shaped association between birth weight and type 2 DM observed in some populations[71,78]. Among the Pima Indians, the age adjusted prevalence of the disease was increased in both the lowest (30%) and highest (32%) birth weight groups (Figure 1.1)[71]. The relationship with high birth weight was lost after adjusting for mothers' GDM in this study and in another study from the USA (Figure 1.2)[78]. Thus, maternal GDM could be another important component of the fetal origins of adult disease.

Figure 1.1 Age adjusted prevalence (68% confidence intervals) of diabetes according to birth weight among 1179 Pima Indians aged 20-39 years[71]

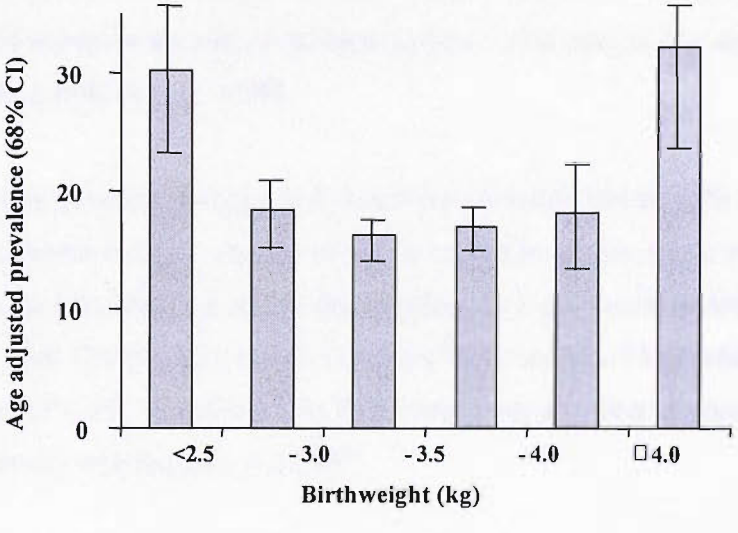
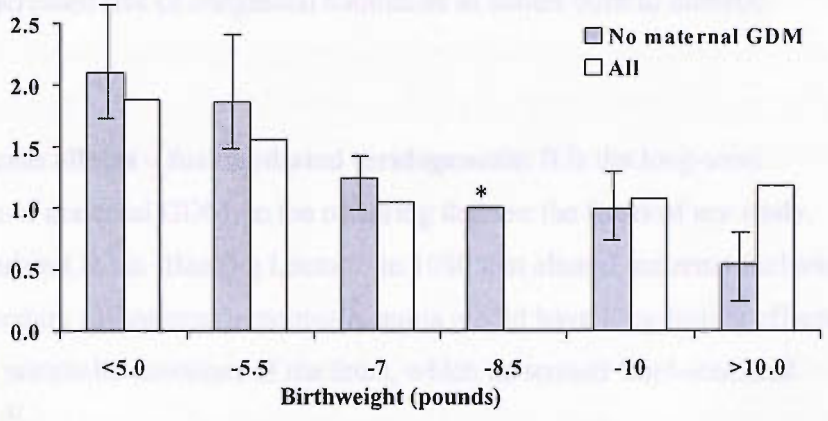


Figure 1.2 Relative risk of NIDDM. US Nurses' Study (n=69,526)[78]



*Reference point

Pregnancy itself is a diabetogenic condition. Gestational steroid hormones facilitate the supply of fuels to the fetus by inducing maternal hyperglycaemia[79]. Peripheral insulin resistance, a common feature of normal pregnancy, also enhances fetal nutrition by diverting glucose from maternal to fetal tissues, and leads to high circulating levels of triglycerides and free fatty acids by way of increased lipolysis during later part of pregnancy. GDM results when the β-cells fail to cope with the increased demand for insulin[79]. GDM increases the risk of developing type 2 DM later in life, and is considered to be a form of type 2 DM.

GDM has a strong genetic aetiology, and shares common risk factors with type 2 DM[77,80]. It is common in older, obese women, increased in certain ethnic minority groups such as the Pima Indians, and is characterised by high insulin resistance. Similar to type 2 DM, adult lifestyle factors like sedentary behaviours and high energy intake increase the risk of GDM. Women's own birth weight has also been shown to be associated inversely with the risk of GDM[81].

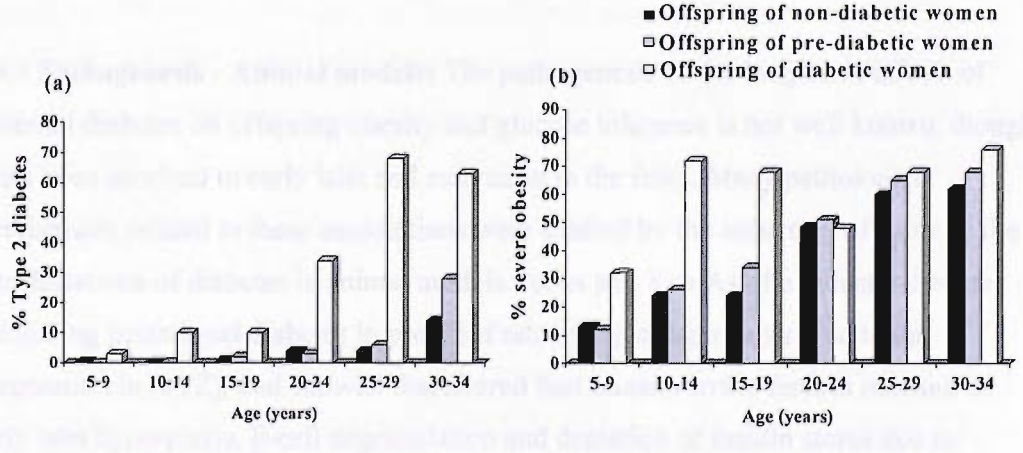
Human studies and animal experiments have shown that maternal hyperglycaemia is associated with higher neonatal morbidity[82,83]. The newborns of diabetic mothers are macrosomic and adipose, due to an excess of 'fuels' (glucose, amino acids and fatty acids) crossing the placenta, and concomitant fetal hyperinsulinaemia[84,85]. Hyperinsulinaemia may lead to increased IGF-I receptors and/or the insulin receptor may itself have growth-mediating properties[85]. Macrosomia itself and shoulder dystocia may hinder vaginal delivery exposing both the mother and the fetus to operative risks[86].

There are other neonatal hazards; fetal hyperinsulinaemia retards lung maturation leading to respiratory distress in the newborn, and causes neonatal hypoglycaemia. There is an increased risk of congenital anomalies in babies born to diabetic mothers[79,82].

1.3.2 Long-term effects – fuel mediated teratogenesis: It is the long-term consequences of maternal GDM on the offspring that are the focus of my study. Freinkel postulated in his 'Banting Lecture' in 1980 that altered maternal fuel transfer to the fetus secondary to maternal hypoinsulinaemia would have long-lasting effects on the structure and metabolic functions of the fetus, which he termed 'fuel-mediated teratogenesis'[87].

Studies in the west have demonstrated that ODM exhibit early obesity and develop IGT/type 2 DM in adult life[15,88-91]. The majority of these were in the Pima Indians, who have high rates of type 2 DM (Figure 1.3). Between 5 and 19 years of age, ODM had higher rates of obesity than offspring of either non-diabetic or pre-diabetic mothers (mothers who developed diabetes after delivery), and had higher glucose concentrations[88-89]. Even among siblings, those born after the diagnosis of the mother's diabetes were at greater risk than their siblings born before[18]. By 20-24 years of age, 45% of ODM had developed diabetes as against 8.6% of offspring of pre-diabetic mothers. Although father's diabetes was associated with higher disease prevalence in the offspring, the association diminished after excluding ODM[15].

Figure 1.3 Prevalence of (a) type 2 diabetes and (b) obesity in offspring of non-diabetic, pre-diabetic and diabetic women (Pima Indians)[88]



There have been few studies of the effects of GDM in childhood. Silverman *et al.* studied anthropometric characteristics and glucose and insulin metabolism in a cohort of the American children born to mothers with GDM or pre-GDM [90,91]. Obesity in ODM was defined by a symmetry index >1.2 (relative weight/relative height*), and the anthropometric data were compared to National Center for Health Statistics (NCHS) standards. At birth the ODM were relatively overweight. This resolved during infancy, and at one year offspring born to mothers with or without diabetes had similar symmetry index[90]. Obesity recurred after that in ODM, becoming obvious by 6-8 years of age. There was no difference in growth pattern between males and females. The authors found a high correlation between childhood obesity and amniotic fluid insulin and proposed that the obesity was an outcome of prematurely activated fetal insulin secretion[91].Th e incidence of IGT was high in the adolescent ODM (19.3% vs. 2.5% in controls)[91].

*Relative weight (or height) = weight(or height)/ NCHS median weight (or height) for age.

In another study from the US, Vohr and McGarvey proposed that size at birth was an important determinant in the development of later obesity in ODM[92,93]. The offspring were categorised as large-for-gestational-age or LGA (birth weight >90th percentile) and appropriate-for-gestational-age or AGA (birth weight 10-90th percentile) in both diabetic and control groups. At one year, LGA offspring of diabetic mothers were larger in BMI, waist circumference and abdominal skinfold measurements compared to other infants[92]. They were heavier, had larger arm, chest, calf and waist circumferences, higher BMI and larger skinfold thicknesses between 4 and 7 years[93]. However, birth size was not a determinant of childhood obesity in other studies of ODM.

1.3.3 Pathogenesis - Animal models: The pathogenesis of the long-term effects of maternal diabetes on offspring obesity and glucose tolerance is not well known, though it has been ascribed to early islet cell activation in the fetus. Many pathological mechanisms related to these associations were studied by the induction of some of the manifestations of diabetes in animal models. Aerts and Van Assche induced diabetes mimicking gestational diabetes in pregnant rats by injecting a pancreatic toxin, streptozotocin (STZ), and showed that altered fuel transfer to the fetuses resulted in early islet hyperplasia, β-cell degranulation and depletion of insulin stores due to constant hyperglycaemia[94]. The β-cells recovered postnatally, and the basal glucose and

insulin levels of the adult offspring were normal. However, when challenged with glucose infusion, the endocrine pancreas failed to cope with the demand, and they became hyperglycaemic. Different mechanisms were related to glucose intolerance in these rat offspring depending on severity of maternal hyperglycaemia. Mild maternal diabetes was associated with fetal hyperinsulinaemia, and increased anabolism resulting in a deficient β-cell response in the adult offspring, probably induced by reduced amino acid turnover. On the other hand, severe diabetes in the mother resulted in extreme hyperglycaemia, disorganised β-cell function and perinatal hypoinsulinaemia in the offspring. These offspring exhibited peripheral insulin resistance as adults[94].

When female offspring of experimentally induced diabetic rats became pregnant, they developed diabetes, and their offspring (third generation rats) exhibited features of offspring of mildly diabetic rats and developed glucose intolerance when stressed during adulthood[95]. The diabetogenic tendency was thus passed on to subsequent generations mimicking genetic inheritance.

The researchers showed that the above outcomes were present in the third generation rat offspring only when their mothers were born to diabetic rats, but not fathers[96]. This suggested that the associations shown in these experiments were mediated through maternal hyperglycaemia, rather than toxic or mutational effects of STZ.

Many of the above findings were replicated in another model, where gestational hyperglycaemia was induced by continuous infusion of glucose in rats during late pregnancy[97]. The newborn offspring were hyperinsulinaemic compared to controls and had altered glucose tolerance postnatally. Glucose intolerance was more pronounced at older ages. There was decreased insulin secretion compared to controls, without a significant decrease in pancreatic insulin content, suggesting that impaired insulin secretion may be related to decreased β-cell sensitivity. This model confirmed that the long-term effects of maternal diabetes were due to maternal hyperglycaemia rather than genes or toxic effects of STZ as might have argued with the other model.

The mechanism by which intra-uterine diabetic environment increases the risk of obesity/adiposity in the offspring is little studied. The inheritance of genes responsible for both obesity and GDM has been implicated by some as a cause[98]. Maternal obesity and sedentary behaviours, usually associated with GDM may influence postnatal

lifestyle behaviours in the offspring and thus lead to obesity. Increased appetite may be a cause of increased adiposity. In rats, hyperinsulinaemia alters neurotransmitter release during critical periods of fetal hypothalamic development and renders the offspring hyperphagic[99,100]. Leptin resistance in ODM may also alter the leptin/insulin feedback system, modifying leptin mediated appetite regulation by the hypothalamus[101].

1.3.4 Offspring of non-diabetic mothers (ONDM): Even in non-diabetic pregnancies, variations within the normal range of maternal glucose have been shown to affect the growth of the fetus[102]. Farmer *et al.* showed a significant, though weak, macrosomic effect of the fasting glucose concentrations of non-diabetic mothers on all the neonatal measurements, particularly skinfolds. It is suggested that a continuous exposure even to a small excess of maternal glucose induces chronic insulin stimulation, and increased growth of insulin-sensitive tissues such as adipose tissue and islet cells in the fetus. In Pima Indians, Pettitt *et al.* showed that maternal 2-hour glucose concentrations, in the non-diabetic range, were significantly associated with the relative weight of the offspring at 5-9 and 10-14 years of age[103]. The relationships were not significant in older ages. They did not look at the associations with other components of body composition.

1.3.5 Effects of good metabolic control: Since hyperglycaemia in the mother is directly linked with the long-term consequences in the offspring, optimum control of gestational diabetes may reduce its effects on macrosomia and later outcomes. In one study, offspring born to GDM mothers with poor metabolic control were heavier, longer and had higher weight to height ratio during the first 4 years of life compared to either offspring of non-GDM mothers or those born to mothers with good metabolic control[104]. There were no significant differences in anthropometry between the latter two groups. However, other studies have demonstrated macrosomia, childhood obesity, and IGT in ODM in spite of achieving good metabolic control (Section 1.3.2). Nonetheless, tight metabolic control of GDM was proposed as a possible means of preventing childhood obesity in the offspring[105].

1.3.6 GDM- issues relating to screening and diagnosis: The cut-offs for the diagnosis of GDM are based on the concentrations of glucose beyond which the fetus is more likely to develop perinatal complications. There are no 'gold-standard' criteria for gestational diabetes. Though several new methods with corrections and refinements

have been introduced, none of the measures is universally accepted. The commonly used criteria are 1) the US National Diabetes Data Group recommendations (3-hour, 100g), which is a modified O'Sullivan and Mahan criteria[106], and 2) the WHO criteria for diabetes and IGT (2-hour, 75g)[107]. There is controversy as to which method is more sensitive in identifying the risks associated with GDM. The studies comparing these two methods have shown that the 75g, 2-hour test is as sensitive as the NDDG method, and may be better tolerated by the women due to relative ease of administration[108]. The Carpenter and Coustan criteria[109], another modification of the O'Sullivan's method is a more sensitive 100g test than the NDDG method[110]. However, clinicians and researchers use different criteria in different parts of the world.

Another contentious issue is the need for universal screening for GDM. It may be important to screen all pregnant women at least in populations that are more prone for diabetes[111].

1.3.7 Maternal obesity: Fetal over-nutrition in the absence of maternal GDM as in the case of maternal obesity/over nutrition also has implications for the development of obesity and type 2 DM in the offspring. In experimental models, dietary induction of obesity in the pregnant rats was associated with increased obesity in the offspring at 16 weeks of age[112]. Normoglycaemic obesity in the mouse mothers was also associated with reduced insulin secretion and impaired glucose tolerance in the female offspring at 50 weeks of age[113]. In humans, gestational obesity is associated with higher offspring birth weight[114], may be due to increased placental transfer of nutrients to the fetus, and may also increase the childhood obesity[115]. In the USA, offspring of obese mothers (BMI $\geq$30 kg/m^2) had more than twice the risk of developing obesity at 4 years of age compared to the offspring of non-obese mothers[115]. However, the information on maternal gestational glycaemic status, which may be a common risk associated with maternal obesity, was not given. Maternal obesity may also influence long-term obesity in the offspring through genes, or by influencing environmental factors such as postnatal diet and activity behaviour[115]. However, there are few studies assessing the association between maternal obesity in the absence of GDM and risk markers of type 2 DM in the offspring. Since gestational obesity and hyperglycaemia are closely related, it is difficult to dissociate the effects of the two.

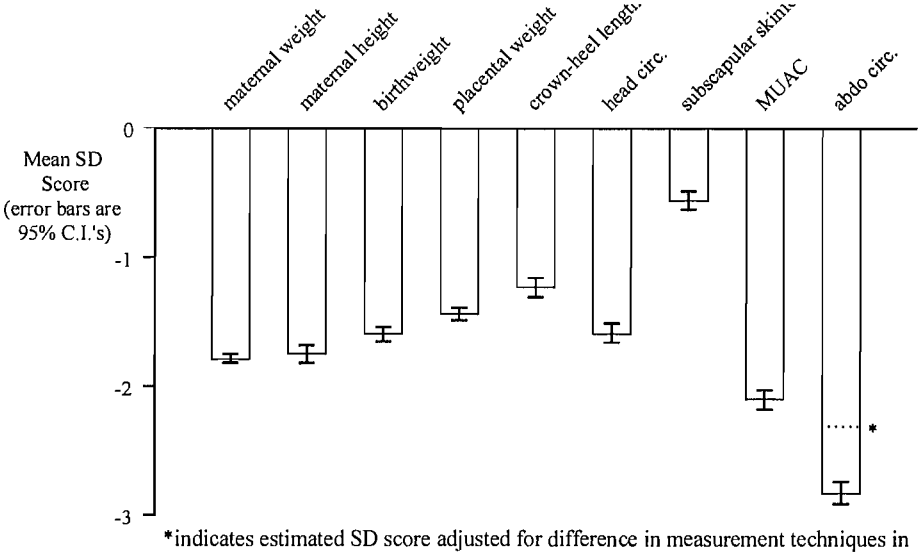
1.4 Fetal origins – the Indian scenario

Epidemiological studies predict that India will become the country with the highest number of individuals with diabetes in the world. Widespread maternal malnutrition and fetal retardation, in association with nutritional transition and a relatively overfed state postnatally might be engineering the rapid emergence of disease in Indian populations.

1.4.1 Low birth weight: About 30% of newborns in India weigh <2.5kg and the average weight of an Indian at birth remains at 2.7 kg, which is much lower than Western standards[116]. The smallness is mostly due to intra uterine growth retardation rather than prematurity[117], probably related to the thinness of Indian mothers with chronic macro and micronutrient deficiencies[53].

It has been shown recently that the body composition of Indian neonates has several potentially adverse features[118] (Figure 1.4). Compared with white Caucasian neonates, they have markedly lower muscle mass (low mid arm circumference) and visceral mass (low abdominal circumference), but are relatively adipose (large subscapular skinfolds).

Fig 1.4 Mean SD scores for maternal pre-pregnant, and neonatal measurements in Pune compared with the Southampton mean (represented by 0)[83]



*indicates estimated SD score adjusted for difference in measurement techniques in Pune and Southampton

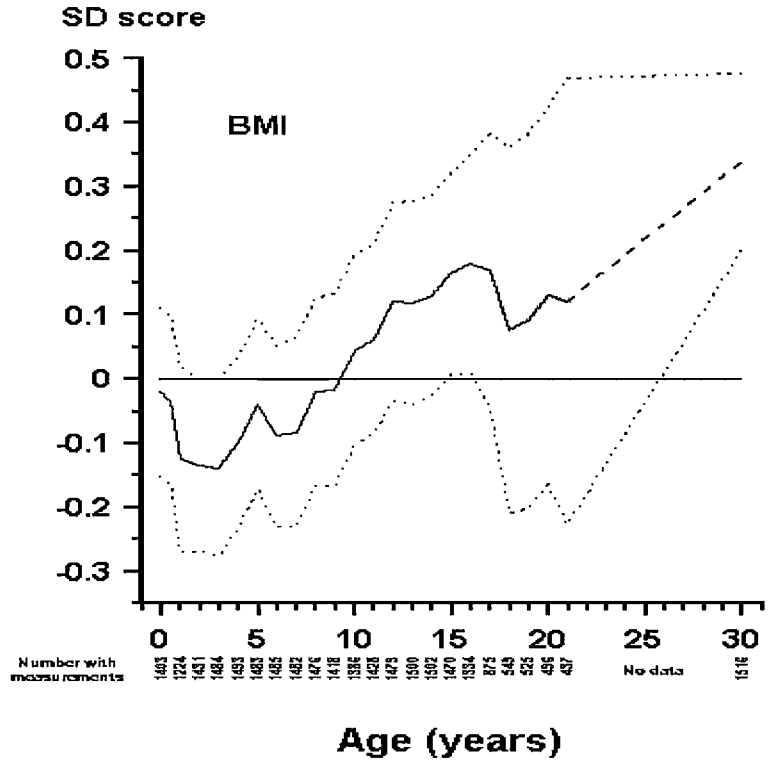
1.4.2 Indian body phenotype: People of Indian origin have a characteristic adult body phenotype: higher body fat for a relatively low BMI and a tendency towards central obesity[3,119,120]. They also have less lean mass (both skeletal muscle and visceral tissue)

and low leg-to-total- muscle ratios than age, sex and BMI matched Caucasian adults[119]. This phenotype is thought to partly explain their higher insulin resistance compared with other populations, and high rates of CVD and type 2 DM. This phenotype, as suggested earlier, could be a manifestation of either a 'thrifty genotype' or a 'thrifty phenotype' resulting from generations of under-nutrition of mothers and consequent fetal programming (Sections 1.1.5 and 1.2.2). With the advent of urbanisation, adults who were genetically vulnerable, or programmed by insufficiency *in utero* develop obesity and disease when exposed to better lifestyles.

1.4.3 'Fetal origins' studies in India: Several studies in India examined the association between poor fetal growth and later glucose/insulin metabolism. The first of these was the Pune children's study, which tested glucose tolerance in a cohort of 4-year old children of known birth weight. This demonstrated higher glucose and insulin concentrations 30 minutes after a standard oral glucose challenge in the low birth weight group[60]. Although the data were difficult to interpret at that time, high 30-minute insulin may have indicated insulin resistance. When the same cohort was studied again at 8 years, it was observed that the children who were small at birth had high insulin resistance (estimated by Homeostasis Model Assessment equation) and other components of insulin resistance syndrome, including higher blood pressure, subscapular/triceps ratio and triglyceride concentrations[43]. The most adverse profile was in children of low birth weight who had the highest weight or fat mass at 8 years.

In a large population-based study in Delhi, North India, glucose tolerance and plasma insulin concentrations were assessed for a group of individuals (26 to 32 years of age) who had been measured at regular intervals from birth up till 12 years of age[73]. The study showed that glucose (120 minutes) and insulin concentrations (fasting and 120 minutes), and insulin resistance were higher in subjects with lower weight and ponderal index at birth. The individuals who went on to develop either type 2 DM or IGT had a low mean BMI at birth and up to the age of 2 years compared to rest of the cohort (Figure 1.5). After 2 years, they had an accelerated increase in BMI, and were in the highest BMI category at 12 years.

Figure 1.5 Mean SD scores for BMI at every age from birth to 21 years, and when studied at 26-32 years, for subjects who developed IGT or diabetes- New Delhi Study[73]. Mean SD scores are indicated by the solid line, and 95% CI's by dotted lines. The mean SD score for the whole cohort is zero.



A study similar to Hertfordshire study was carried out in Mysore, south India, in 1993[121]. The Holdsworth Memorial Hospital (HMH), Mysore has preserved birth records for all babies born there since 1934, recording birth weight, length and head circumference at delivery. These records were used to trace 518 men and women born in the hospital between 1934 and 1954 and still living in Mysore. There was a high prevalence of type 2 DM (15%) in men and women above 40 years, similar to other estimations from India. Although, insulin resistance was associated inversely with birth weight, the adults with impaired insulin secretion and diabetes were short at birth and had a high ponderal index[121]. This was in contrast to the observations made in Pune and among Western populations, where people with low birth weight and low ponderal index at birth were at highest risk of diabetes as adults. There was also a strong association in Mysore between higher maternal weight and pelvic diameters, and type 2 DM. It was suggested that these heavier mothers had gestational glucose intolerance and that this could explain their high risk of diabetes in late middle age[121].

1.4.4 Gestational diabetes in India: Women in India are becoming increasingly adipose owing to widely prevalent fetal growth retardation coupled with rapid urbanisation, resulting in high insulin resistance and glucose intolerance during pregnancy. However, GDM as a cause of increasing prevalence of type 2 DM among Indians has been little studied. A study carried out in Chennai, South India in 1994 estimated a low incidence of GDM (0.87%)[122]. A recent study from Kashmir, published after we established our cohort, reported a rate of 4%[123]. There are no other published incidence studies and no data on follow-up of the offspring of diabetic mothers.
It has been proposed that vitamin D deficiency may contribute to the high prevalence of type 2 diabetes in South Asian populations[34]. There is an increased demand for vitamin D in pregnancy[124], while a deficiency is widely prevalent among south Asian women[56]. This may play a role in the onset of gestational diabetes.

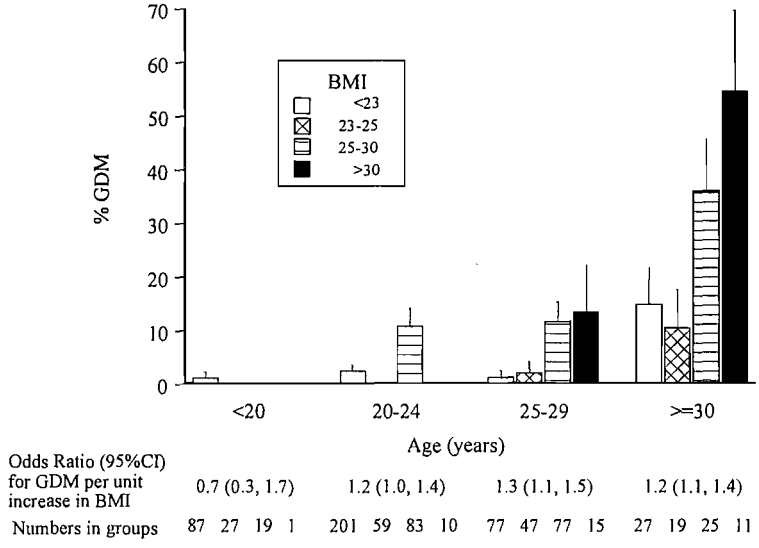
1.5 The Mysore Parthenon study

The Mysore Parthenon Study is a prospective observational study in which glucose tolerance was measured in a cohort of south Indian women during pregnancy[125]. The aim was to observe the incidence of GDM in this population and to study the characteristics of women developing the disease.

1.5.1 Study setting and methods: Between June 1997 and August 1998, we recruited pregnant women from the antenatal clinic of the HMH, Mysore. At 30±2 weeks gestation, a 100g, 3-hour, oral glucose tolerance test (OGTT) was carried out. Of the 830 women who participated in the study, complete OGTT data were available for 785 women.
Six hundred and seventy-four of the women delivered at HMH. Anthropometric measurements of the newborn babies were carried out. Methodology is described in detail in Chapter 2.

1.5.2 Results: The prevalence of GDM was 6.2% (n=49), which was considerably higher than the previous Indian study[122], and marginally higher than a recently published study from Kashmir[123]. Shorter and fatter women had higher concentrations of glucose and measures of insulin resistance and higher prevalence of GDM (Figure 1.6). The prevalence rose with women's age.

Figure 1.6 Prevalence (±SE) of GDM in relation to maternal age and BMI[125]



The babies born to mothers with GDM were bigger in all measurements, especially body fat (skinfold measurements), but also skeletal size (length), and muscle mass (MUAC), compared to neonates born to non-diabetic mothers (Figure 1.7). Even in the offspring of non-diabetic mothers, macrosomic changes were seen across the range of maternal fasting glucose (Figure 1.8). Irrespective of maternal diabetes, the babies were smaller than Western newborns. As in the Pune study, however, they were relatively adipose; while all other body measurements were lower than UK babies by >1 SD, subscapular skinfold thickness were relatively spared.

Figure 1.7: Standard deviation (SD) scores for mothers with GDM and their babies (study population mean = 0)[125]

Values are adjusted for gestation, error bars shown are 95% confidence intervals

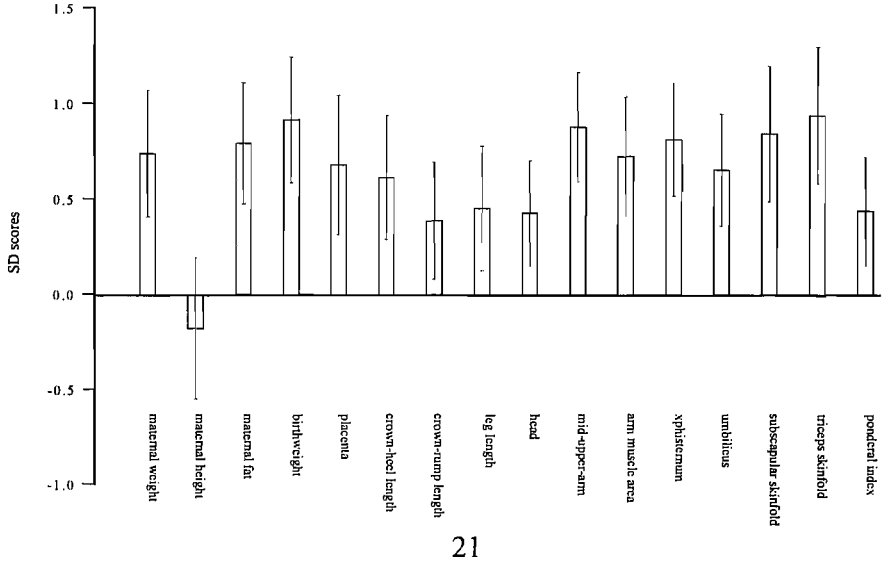
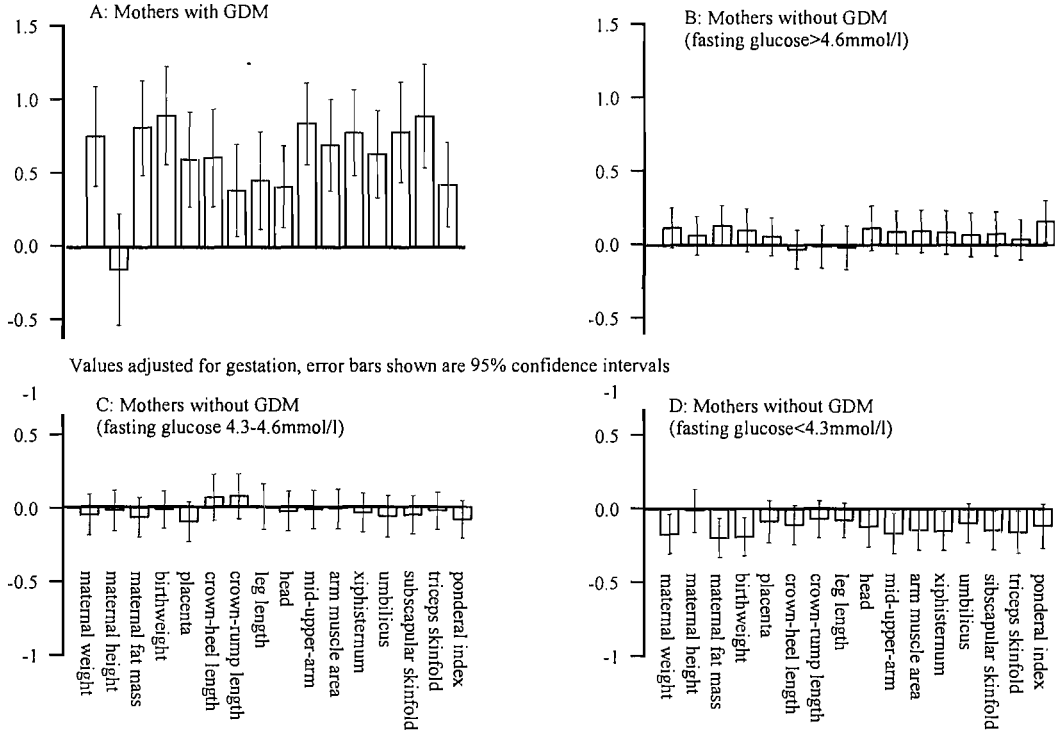


Figure 1.8 Standard deviation (SD) scores for mothers and their babies born in Mysore, South India The mean SD score for the whole cohort is zero.

Graph A shows SD scores for mothers with GDM and their babies. Graphs B, C, D exclude mothers with GDM and show scores for mothers and their babies with normal glucose tolerance in thirds of fasting blood glucose



The Mysore Parthenon Study created a cohort of children who were followed-up annually as part of the present study. The aim was to observe their growth pattern and to study the factors influencing their size and body composition; with a particular interest in maternal diabetes as a risk factor for early obesity and later glucose/insulin metabolism. This formed the basis of my thesis.

The children were measured, and health and developmental monitoring was done during annual visits. At five years, in addition to the routine anthropometry, a 2-hour OGTT was administered to the children to measure plasma and insulin concentrations, and total body fat was measured using bioimpedance. The objective was to test the following hypotheses:

1.5.3 Hypotheses:

- Children born to mothers with diabetes have more central and total body fat at 5-years than children born to non-diabetic mothers, and have elevated plasma insulin concentrations
- Weaker, but significant effects on insulin concentrations and body fat mass and distribution will be found in relation to the mother's gestational glucose and insulin concentrations, even in offspring of mothers without frank diabetes.
- Small size at birth and at one year predicts increased central body fat at 5-years, and higher plasma insulin concentrations.

In the ensuing chapters, I describe the methods adopted and the results obtained in the course of the study. Though not directly related to the above hypotheses, I have also included additional chapters on blood pressure and physical activity in children, and short and long-term associations of maternal vitamin D on offspring size and glucose and insulin concentrations, as these are relevant to the discussions outlined in the current chapter.

2. METHODOLOGY

Figure 2.1 Mysore, South India

2.1 Setting

2.1.1 Mysore – Holdsworth Memorial Hospital (HMH): Mysore, a city with a population of around 370,000, is in the southern Indian state of Karnataka (Figure 2.1). It has a large government hospital catering to the majority of the population of Mysore district, and 6 large and numerous small private hospitals. HMH, a 350-bedded private hospital, is situated in a crowded area at the centre of the city (Figure 2.2). Built in 1905, this mission hospital run by the Church of South India provides medical and maternity care to a large section of the Mysore population. About 25% of the people obtaining its services come from the surrounding villages. The hospital provides quality medical care at an affordable cost.

Figure 2.2 The Holdsworth Memorial Hospital

2.1.2 Birth records and research centre: The hospital has preserved birth records routinely for all deliveries here since 1934. This led to the setting up of a study in 1993 in collaboration with the Medical Research Council Environmental Epidemiology Unit (MRCEEU), Southampton, UK, which had conducted extensive searches all over India before finding the unique collection of records at HMH. The people born here were traced through details from the birth records and were studied for the prevalence of type 2 DM and coronary heart disease. Since then several studies have taken place based on the birth records at HMH as part of this collaboration. The suggestion from one of these studies that maternal hyperglycaemia could increase the incidence of type 2 DM in the offspring led to the current study (Section 1.4.3). A research block was built on the premises exclusively for studies on the Fetal Origins of Adult Disease (FOAD) in the year 2001.

2.2 Study sample

2.2.1 Study on gestational diabetes: Nearly 2500 (10-12% of Mysore deliveries) women come to HMH every year for delivery. Between June 1997 and August 1998, we screened 1539 women booking consecutively into the antenatal clinics of HMH as part of the Ph.D. programme of Dr. Jacqui Hill, a research fellow from Southampton. The women were eligible if they were not diagnosed with diabetes before pregnancy, planned to deliver at HMH, had a singleton pregnancy and were less than 32 weeks gestation at booking (determined by their last menstrual period (LMP) or a first trimester ultrasound scan if the LMP was uncertain). Of the women screened, 1,233 (80%) women were eligible and 830 of them (67%) agreed to participate in the study. The hospital ethical committee approved the study.

2.2.2 Clinical investigations: At 30±2 weeks gestation, detailed anthropometry of the mothers including weight, height, head circumference, external pelvimetry and skinfold thicknesses (biceps, triceps, subscapular and supra iliac) was carried out. Socio-economic status was determined using a questionnaire method, based on education, occupation, and the family's income, the Kuppuswamy Score[126].

An oral glucose tolerance test (OGTT) was administered to all willing women (Figure 2.3). After a fasting blood sample, a 100g oral glucose load was administered and further blood samples taken 30, 60, 120 and 180 minutes after for plasma glucose and insulin assay. This test was used rather than the WHO 75 g OGTT, because it was the established test in clinical use in the hospital. The OGTT was discontinued in 37 due to vomiting, 4 refused full blood sampling and in 5 cases samples were haemolysed. One woman who had already been diagnosed with gestational diabetes was included in the study, but did not have a GTT. Complete OGTT data were therefore available for 785 women.

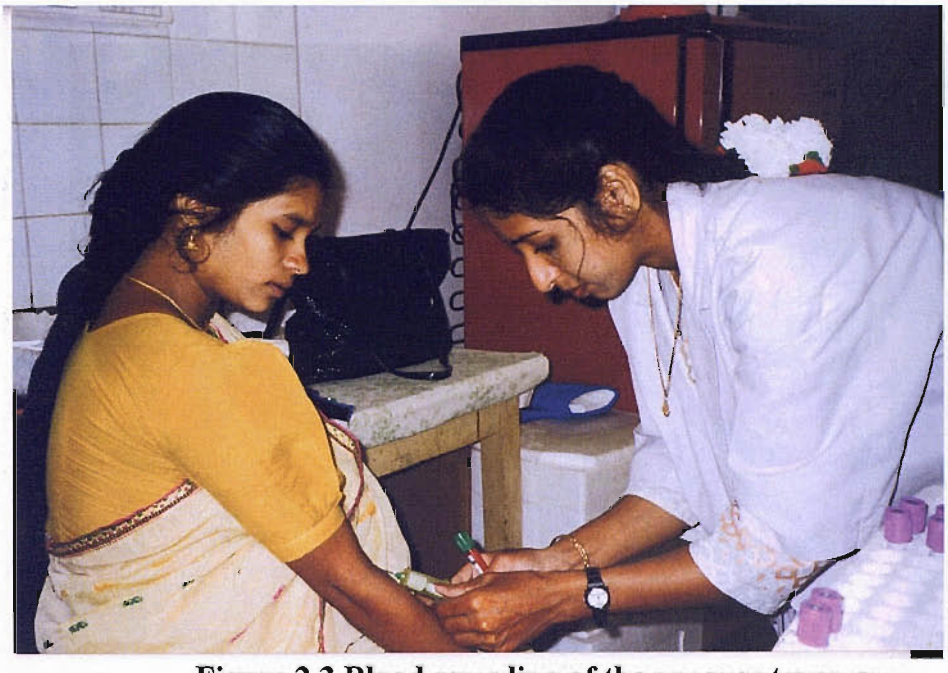


Figure 2.3 Blood sampling of the pregnant woman

Glucose was measured using a standard hexokinase method. Gestational diabetes was defined using the criteria of Carpenter and Coustan[109], by the presence of two or more plasma glucose concentrations greater or equal to 5.3 mmol/l (fasting), 10.0 mmol/l (60 minutes), 8.7 mmol/l (120 minutes) and 7.8 mmol/l (180 minutes). GDM was diagnosed in 49 women, and we informed the woman's consultant obstetrician who managed all further clinical care.

2.2.3 Newborn babies-study cohort: Because of the wide choice of maternity units and the local practice of going to the woman's maternal home for delivery, many women booking into the HMH antenatal clinic ultimately chose another hospital for the birth. Of the 830 women recruited, 674 (81%) delivered at HMH (43 GDM mothers); 7 babies were stillborn (1 born to mother with GDM) and 4 were born with severe congenital anomalies (1 born to mother with GDM). Complete anthropometric measurements including the weight, crown-heel (CHL) and crown-rump lengths (CRL); head, mid-upper-arm (MUAC), abdominal and chest circumferences; and triceps and subscapular skinfold thicknesses were obtained for 663 newborns within 72 hours of their birth (41 born to GDM mothers) Figure 2.5. I took over from Dr. Jacqui Hill as the leader for the subsequent follow-up of these children.

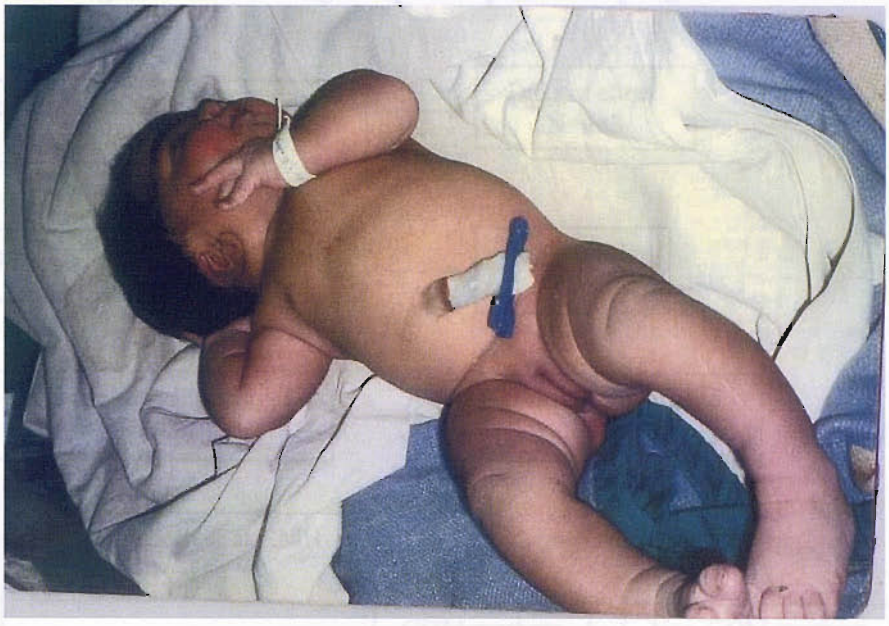


Figure 2.4 New born baby of a study woman

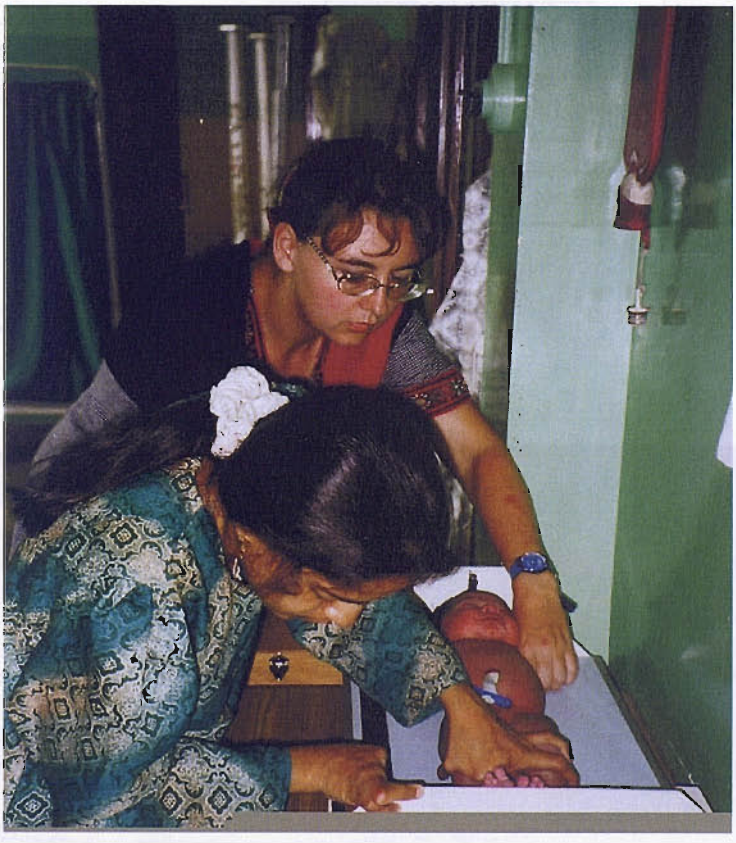
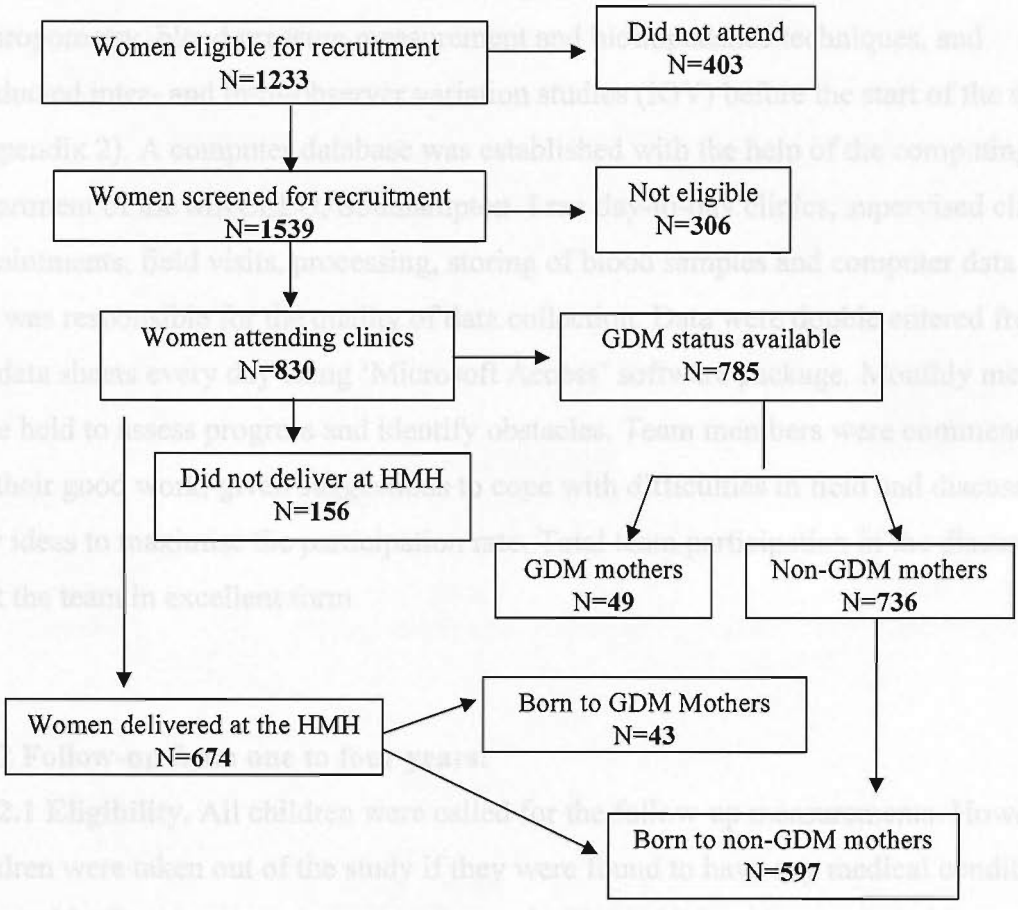


Figure 2.5 Anthropometric measurement of the neonate

Figure 2.6 Flow diagram illustrating the participation of pregnant women



2.3 Follow-up Study

Figure 2.7 Mysore research team

2.3.1 Team (Figure 2.7): I headed a team comprising four social workers, one nurse, one laboratory technician, and two data entry operators. I trained the team members in anthropometry, blood pressure measurement and bioimpedance techniques, and conducted inter- and intra-observer variation studies (IOV) before the start of the study (Appendix 2). A computer database was established with the help of the computing department of the MRCEEU, Southampton. I ran day-to-day clinics, supervised clinical appointments, field visits, processing, storing of blood samples and computer data entry, and was responsible for the quality of data collection. Data were double entered from the data sheets every day using 'Microsoft Access' software package. Monthly meetings were held to assess progress and identify obstacles. Team members were commended for their good work, given suggestions to cope with difficulties in field and discussed new ideas to maximise the participation rate. Total team participation in the discussions kept the team in excellent form.

2.3.2 Follow-up from one to four years:

2.3.2.1 Eligibility. All children were called for the follow-up measurements. However, children were taken out of the study if they were found to have any medical condition that could affect the normal process of growth. Eight children thus excluded from the study after birth. The reasons for the non-inclusion were (Table 2.1):

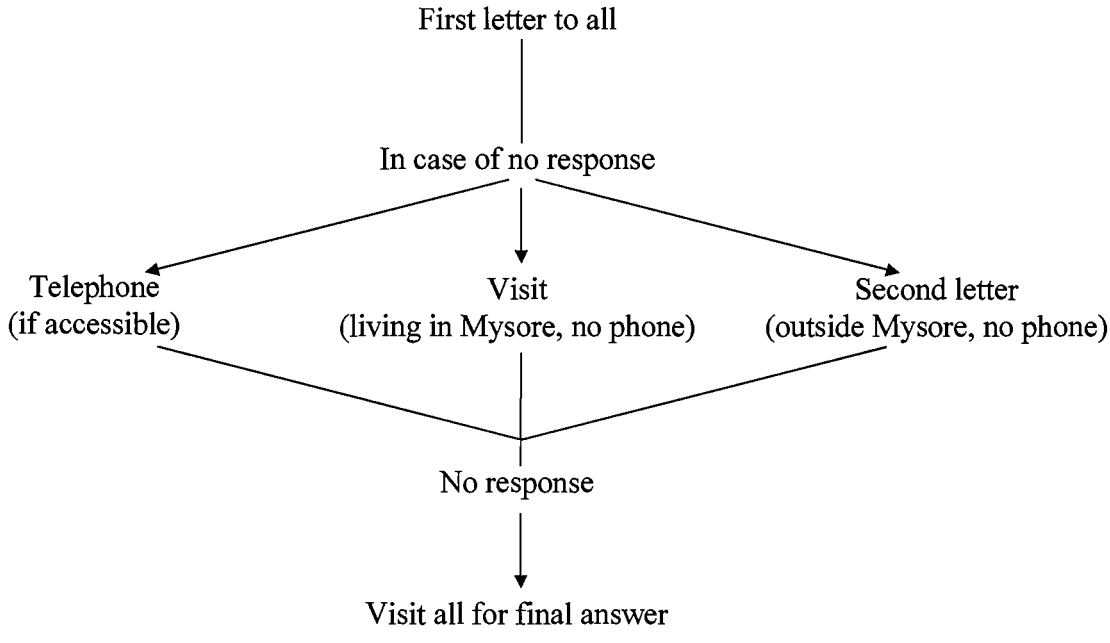
Table 2.1 Reasons for exclusion of children from follow-up study

Medical condition	Total
Congenital heart disease	1
Mental retardation	5
Hydro-cephalus	1
Hereditary spherocytosis (requiring frequent transfusions)	1

2.3.2.2 Appointments. Follow-up at one year was on child's first birthday (± 4 weeks) for term births (gestational age ≥ 37 weeks) and on the anniversary of the expected date of delivery (± 4 weeks) for pre-term births. Follow-ups at subsequent years were on the child's birthday (± 4 weeks) for all children. Letters were sent to the parents 15 days prior to the clinic date, asking them to bring the child to the research clinic. If they did

not attend, they were contacted by phone if possible. If not, another letter was sent requesting them to come at a later date if they were not living in Mysore and home visits were made for Mysore residents. Home visits were made to everyone in the event of non-attendance to get a final answer (Figure 2.8).

Figure 2.8 Mode of booking the children for follow-up



2.3.2.3 Anthropometry and questionnaire. Measurements carried out at all annual visits are shown in Table 2.2 (Figure 2.9, Protocols in Appendix 1). Questionnaires and practical tests were administered at each visit to assess the intellectual development of the children (Figure 2.10). Information on their health and vaccination status over the preceding year was collected. (Appendix 3)

Table 2.2 List of anthropometric measurements carried out at follow-up

Weight (kg)	Measured using an electronic weighing scale (Seca, Germany), naked or in minimal clothing, to the nearest 100g.
Height/length (cm) (crown-heel and crown-buttock/sitting height)	Measured using Harpenden infant stadiometer during first two years and Microtoise wall-mounted stadiometer thereafter, to the nearest 1 mm. An assistant helped to maintain the child's posture. At four years, sitting height was measured using a stool of known height
Head circumference (cm)	Measured using a fibreglass anthropometric tape to nearest 1 mm at the level of maximum occipito-frontal diameter (farthest point of the occipital protuberance in the back and just above the eyebrows in front).
MUAC (cm)	Measured to the nearest 1 mm, at the mid-point between acromion and olecranon processes using a fibreglass anthropometric tape.
Abdominal circumference (cm)	Measured at the level of umbilicus using fibreglass anthropometric tape at the end of expiration.
Chest circumference (cm)	Measured at the level of xiphisternum using fibreglass anthropometric tape at the end of expiration.
Subscapular skinfold (mm)	Measured using Harpenden skinfold callipers just below the inferior angle of the scapula along the natural direction of the skin cleavage. Readings taken at the end of 5 seconds.
Triceps skinfold (mm)	Measured at the intersection of horizontal mid arm line and the vertical line at the most posterior point of the triceps using Harpenden callipers. Readings taken at the end of 5 seconds.

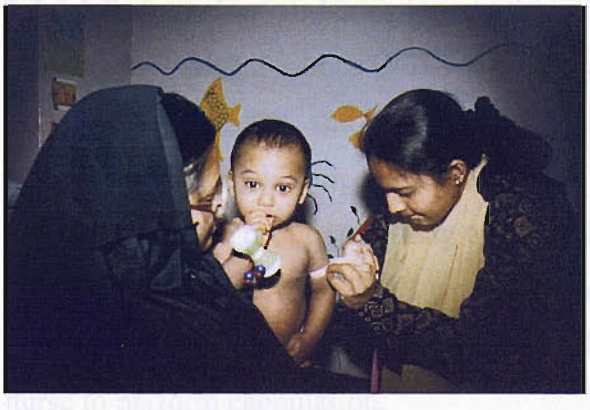
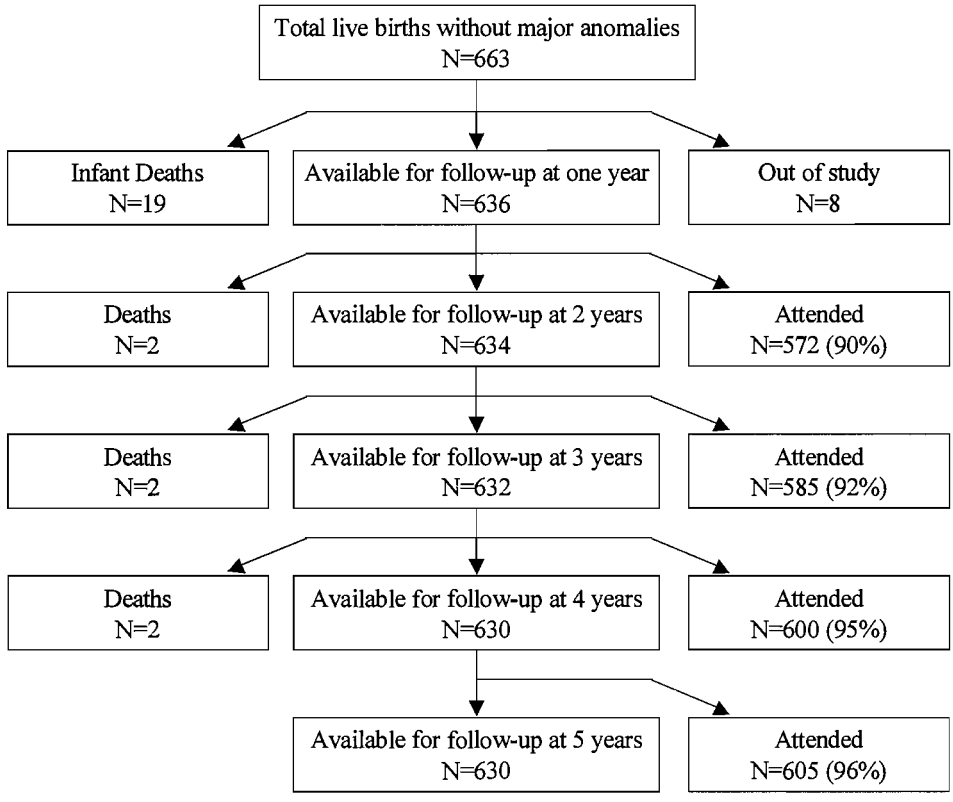


Figure 2.9 Measurement of MUAC

Figure 2.10 Developmental questionnaire

Figure 2.11 Flow diagram showing follow-up numbers at each year



2.3.2.4 Building up rapport. The annual appointments created strong rapport between the families and the research team. These visits gave me an important opportunity to get to know each child and his/her family personally which created a trusting environment.

2.3.3 Five-year follow-up:

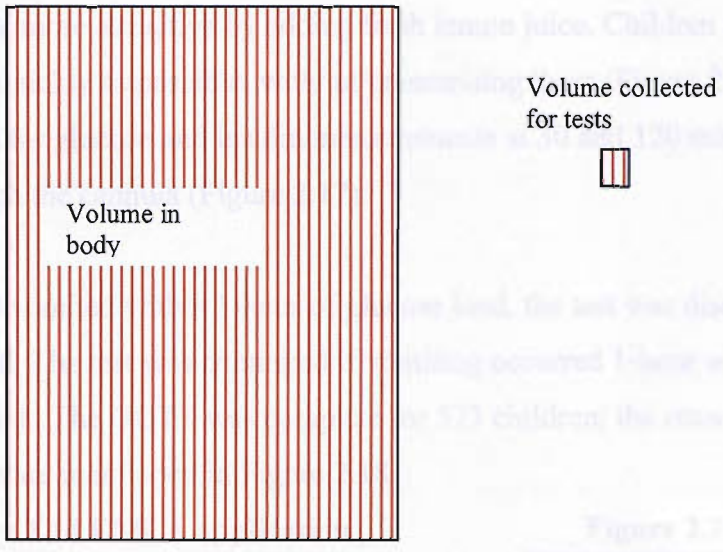
2.3.3.1 Preparation and training. At five years, a more detailed investigation was planned, including a 2-hour OGTT for plasma and insulin concentrations, blood pressure measurement, and in addition to the routine anthropometry, body fat measurement using bioimpedance technique. It was also planned to study the parents at the same time. Since, blood testing of the children required additional skill, I spent time on the paediatric wards at HMH getting trained in intravenous cannulation, and gaining experience in dealing with routine health problems in children. I trained the research nurse to perform cannulation.

Ethical approval for the study was obtained from the Ethical Committee of the Hospital.

2.3.3.2 Interaction through organised meetings. Blood tests are generally feared the world over, and more so if it is for a child. Our previous experiences had shown that blood tests were looked upon suspiciously in this population, as they are believed to cause weakness and long-term illness. Prior to starting the study, we attempted to alleviate these misconceptions, at the same time familiarising parents with our objectives. I organised group meetings with parents in order to inform them of the research background, our past research findings, and to introduce them to the future plans (Figure 2.12). The proportion of blood to be drawn (relative to total blood volume) was shown through illustrations to dispel apprehensions about blood loss (Figure 2.13). Parents were encouraged to ask questions. Children were also invited, and were entertained by recreational activities like colouring, during the discussions. Food was provided for all at the end of the meeting. The team worked optimally to make each one of such gatherings memorable for the families. The interaction was a success, widely approved by parents, and helped to increase the participation rate. Many, who were initially reluctant for blood testing, became enthusiastic to be a part of the study. In total, 6 such meetings were held, with 60-70 families attending each meeting.

Figure 2.12 Interactive meeting with parents

Figure 2.13 Illustration to show the amount of blood taken



2.3.3.3 Booking for the clinic. The appointments for the clinical investigations were given on the child's fifth birthday (± 4 weeks). Social workers visited children's homes a fortnight before the clinic to give parents the details of the tests. During these visits, fasting instructions were given and a written version of the same printed both in the local languages and in English was given (Appendix 3). On the day before the clinic, the same team of field workers visited the respective child's home again to remind parents about tests and to impress upon the need for fasting.

2.4 Clinic – Child

2.4.1 Consent: The parents and the child were questioned to ensure that they had fasted. Investigations started only after the social workers were satisfied that the parents fully understood the nature of the investigations and gave informed verbal consent.

2.4.2 Oral Glucose Tolerance Test: An OGTT based on WHO criteria[107] was administered to all children. This method was used as this is the commonly used test in other studies in children. This is also a convenient method for children as it requires less blood sampling. The skin over the venesection site (preferably ante-cubital fossa) was de-sensitised by applying a local anaesthetic cream for 45 minutes (EMLA™ 5% contains Lidocaine (2.5%) and Prilocaine (2.5%) in a cream base, Figure 2.14). An intra-venous cannula was inserted into a suitable vein to minimise trauma during further samples (Figure 2.15). A fasting blood sample was taken for plasma glucose, insulin,

glycosylated haemoglobin (HbA1c) assays, and a posterity sample for DNA analysis. A load of 1.75g per kg body weight of anhydrous glucose dissolved in water was given to drink, made more attractive by adding fresh lemon juice. Children were encouraged to drink it as quickly as possible, without pressurising them (Figure 2.16). Further samples were taken for glucose and insulin measurements at 30 and 120 minutes after glucose load through the cannula (Figure 2.17).

If the child vomited within 1-hour of glucose load, the test was discontinued and was rescheduled. The test was continued if vomiting occurred 1-hour or more after the glucose drink. The OGTT was complete for 573 children; the reasons for non-completion are mentioned in Figure 2.18.

Figure 2.14 EMLA application

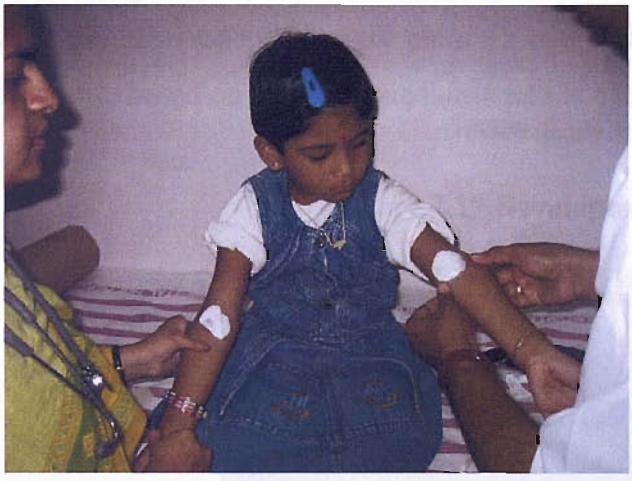
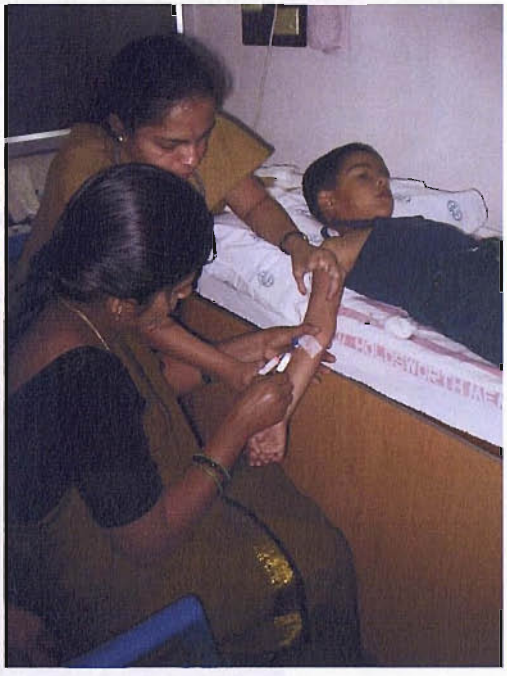


Figure 2.15 Cannulation



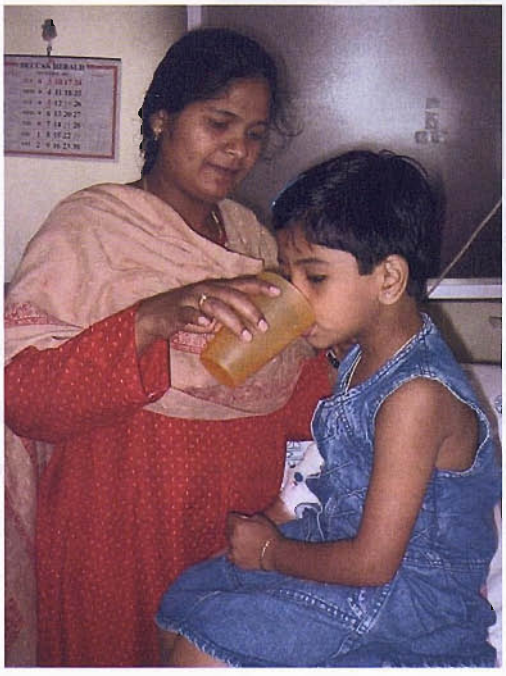


Figure 2.16 Drinking glucose

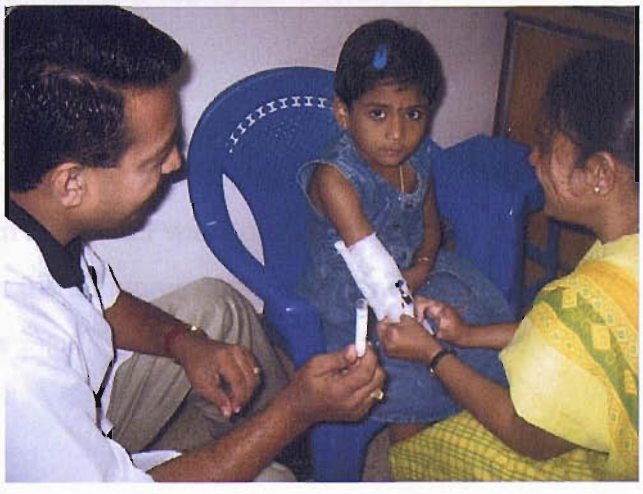
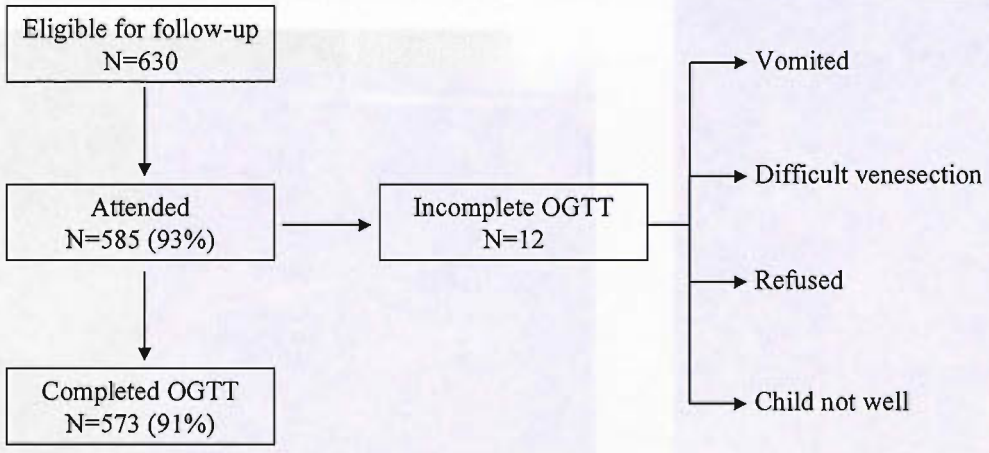


Figure 2.17 Sample through the cannula

Figure 2.18 Participation and reasons for non-completion of OGTT in children



2.4.3 Questionnaire: The questionnaire was designed to screen the children for health and developmental problems as in the previous follow-up years. Simple practical tests were administered to assess the child's motor and reasoning skills (Figure 2.19). All tests were based on standard paediatric text books[127]. (Appendix 3)

Figure 2.19 Developmental testing

2.4.4 Anthropometry (Figure 2.20a and b): The anthropometric measurements were the same as for the previous years with an additional waist measurement in the supine position. Team members were trained for the measurements and the methods were standardised by regular IOVs. (Appendix 2)

Figure 2.20a Head circumference

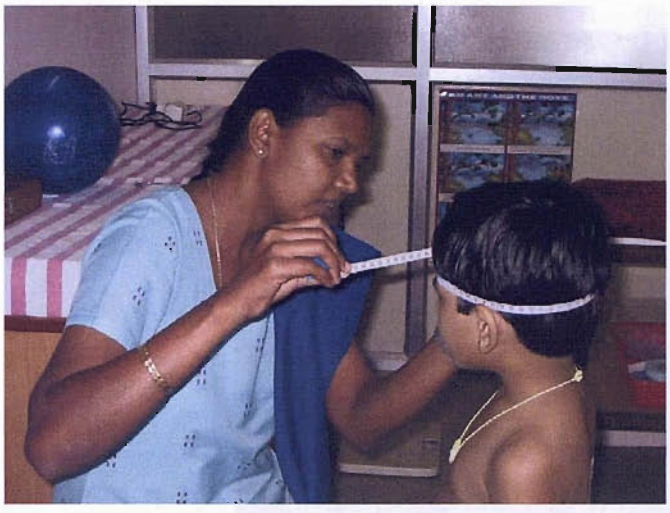
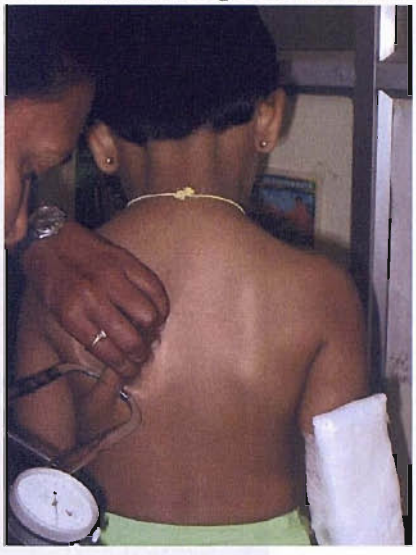


Figure 2.20b subscapular skinfold



2.4.5 Bioimpedance analysis (BIA): The BIA is based on the principal of measuring the resistance/impedance offered by a tissue when a small electric current is passed through it. Fat mass was measured using Bodystat Quadscan 4000 bioimpedance analyser, with the child being rested in supine position for five minutes beforehand (Figure 2.21). Methodology is described in detail in Chapter 5.

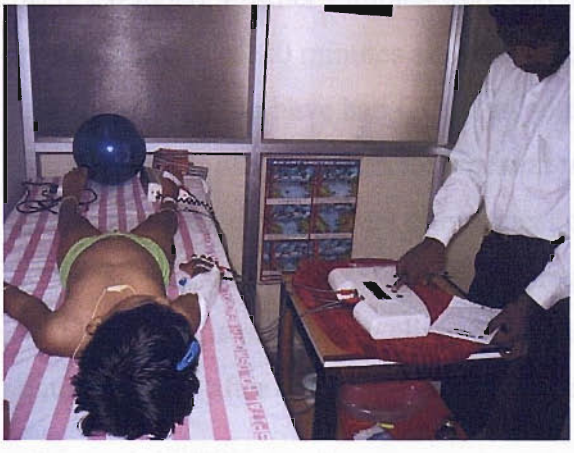


Figure 2.21 Bioimpedance

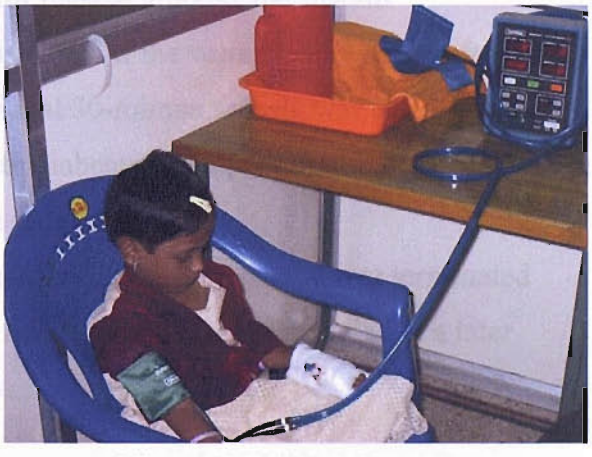


Figure 2.22 Blood pressure

2.4.6 Blood Pressure: Systolic and diastolic blood pressures were measured using a fully automated device (CRITIKON, DINAMAP™ model 8100). The measurements were done following the 30-minute blood sample after allowing the child to relax seated for 5 minutes, on the non-cannulated arm (Figure 2.22, Appendix 1). Team members were trained in blood pressure measurements before the start of the study and methods were standardised.

2.5 Clinic – Parents

2.5.1 Glucose Tolerance Test for Mothers:

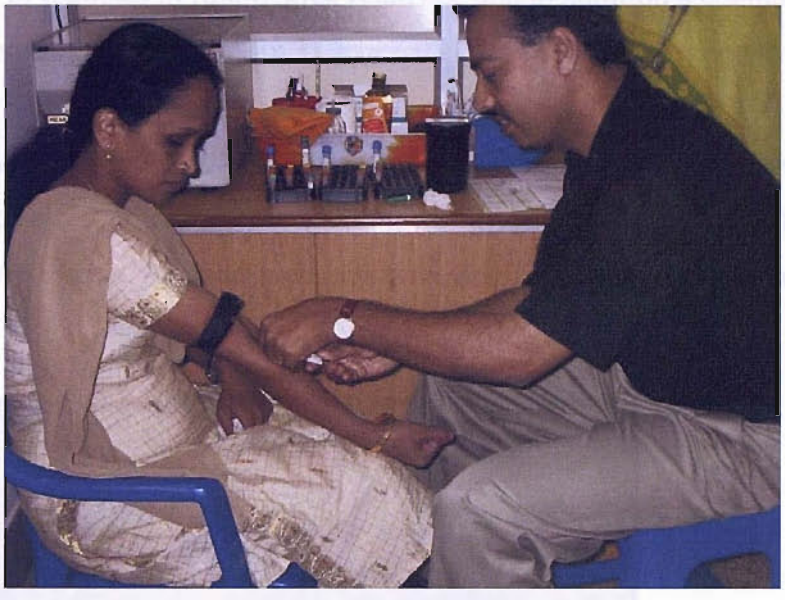


Figure 2.23 Blood sampling of a study mother

After an overnight fast (minimum of 10 hours) blood samples were taken for plasma glucose and insulin assessment and DNA analysis (Figure 2.23). An oral load of 75g anhydrous glucose dissolved in 300ml of water was given. Persons with known diabetes were excluded. Further samples were taken for plasma glucose and insulin measurements at 120 minutes after the glucose load. If the women were known from our earlier study to have had GDM, an additional 30-minute sample was collected. Only the fasting sample was taken for a person with diabetes and a post-prandial glucose assessment was done 2 hours after a meal on request.

Mothers were not eligible if they were pregnant, or if their pregnancy was terminated within 6 months prior to the clinic date. The investigations were scheduled at a later date 6 months after completion of pregnancy.

2.5.2 Fasting sample for fathers: Fasting samples were taken for plasma glucose and insulin measurements from willing fathers after an overnight fast. Further samples were taken on demand. A full OGTT was not administered for fathers, as most men were reluctant to undergo blood tests and agreed to have their fasting sample taken only after much counselling.

If either of the parents was unable to attend on the given date, the tests were rescheduled for a convenient date.

2.5.3 Questionnaire: A questionnaire was administered to obtain information regarding the parents' occupation, alcohol and tobacco consumption, medical and drug history, and family history of diabetes in first-degree relatives, and in addition a detailed obstetric history for mothers. (Appendix 3)

2.5.4 Anthropometry (Figure 2.24): Different teams were selected to measure mothers and fathers respectively. Anthropometric training was given prior to the start of the study, and IOV's were done. All measurements were done according to a set protocol (Appendix 1).

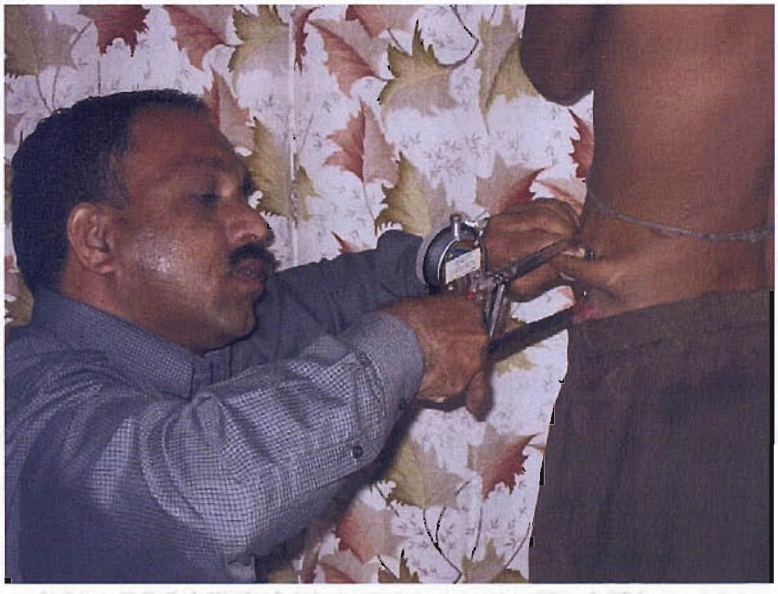


Figure 2.24 Skinfold measurement of a father

The following measurements were carried out on the parents:

Weight

Mid upper arm circumference

Skeletal measurements	Height
	Head circumference
Measurements of adiposity	Waist circumference
	Hip circumference
Skinfold thicknesses	Biceps
	Triceps
	Subscapular
	Suprailiac

Methods used were the same as in children. The hip circumference was measured with a measuring tape, as the maximum measurement around level of the greater trochanter.

2.5.5 Blood Pressure: Blood pressure was measured on the left arm, using similar method as for the children. Measurements were repeated if BP was high (≥150/90), and those with persistent high values were referred to a physician.

2.5.6 Participation Rate: A total of 560 (96%) mothers and 511 (87%) fathers attended the clinic; 551 mothers completed the OGTT. Of the mothers who did not attend, 13 were post partum women, 9 were pregnant, 3 refused to attend, 2 women had died and 1 was out of country. Of the women who did not complete the OGTT, 2 vomited and did not return for a second attempt, 1 refused further samples, 1 was a person with known diabetes, and 5 refused. Reasons for non-attendance of fathers were: 55 refused, 13 were out of country, 6 had died. Of the fathers who attended, 5 refused blood tests and only anthropometric measurements were done for them.

2.6 Blood processing and assaying

Blood samples were centrifuged for 15 minutes at 4000 revolutions/minute and stored at -80°C after separating the plasma into aliquots (Figure 2.25, Appendix 1). A part of the plasma was used to measure glucose in the hospital laboratory and the results were made known immediately to the subjects. If abnormal, the subjects were referred to a hospital consultant and regular follow-ups were done to ensure that the necessary consultation was obtained. The rest of the samples were transported at regular intervals on dry ice to the Diabetic Research Centre at the King Edward Memorial Hospital, Pune for glucose and insulin assay.

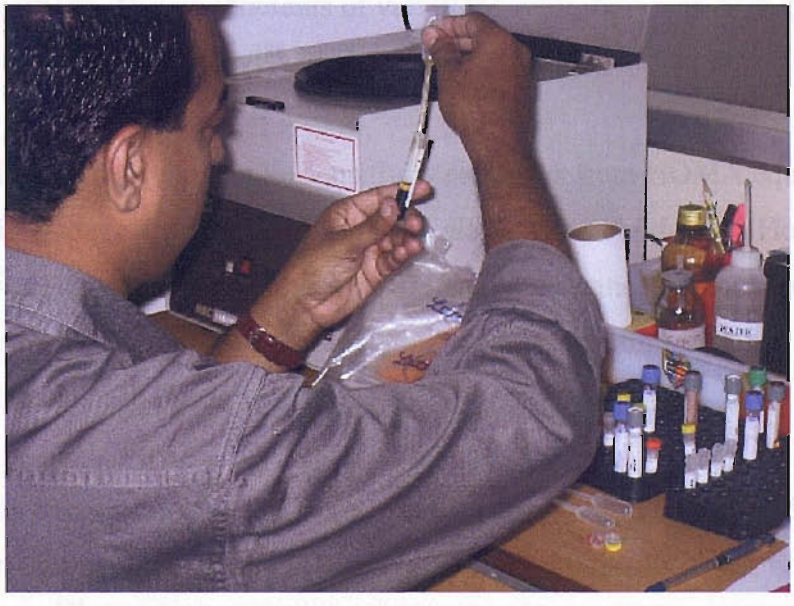


Figure 2.25 Blood processing

The Pune research centre has a well-established set up for specific insulin assay. It has been a part of the UK quality control system for several years (Registered with UK NEQAS quality control scheme). Insulin was assayed a by a time-resolved, flouroimmunoassay (DELFIA) method. Antibody was labelled using Europium labelled neutralite avidine (Southampton, UK). Intra and inter-assay co-efficients of variation using this method were <5% and <10% respectively. Glucose estimations were repeated in Pune, on an Auto analyser (Abbott Laboratories, USA) using a Glucose oxidase-peroxidase method.

Glycosylated haemoglobin (HbA1c) was analysed at the diabetes research centre, Chennai by Variant HbA1c programme (BIORAD Laboratories, USA) using ion-exchange liquid chromatography. This centre, established exclusively for comprehensive diabetic care was recommended by the leading diabetologists. At the time of the study, the centre used the BIORAD internal quality control assessment, but was not a part of any external quality control scheme.

2.7 Derived estimates of insulin resistance and secretion. insulin increment

2.7.1 Homeostasis Model Assessment (HOMA): HOMA, a mathematical model gives an estimation of insulin resistance[128]. Relative insulin resistance was determined using the formula: {fasting insulin (mIU/ml)*fasting glucose (mmol/l)} /22.5. In adults, insulin resistance estimated using HOMA correlated well with that from the euglycaemic clamp (R_s=0.88), and the hyperglycaemic clamp (R=0.69), the 'gold standard' methods. The co-efficients of variations were, however, high (31%) suggesting low reproducibility[128].

High correlations between insulin sensitivity estimates from HOMA equations and euglycaemic clamp estimates (R=0.91) were also seen in children[129]. However, another study showed HOMA as a poor measure of insulin resistance in children[130]. Nevertheless, HOMA provides a simple tool to assess insulin resistance in large field studies and thus is used widely.

2.7.2 Insulin increment: Insulin increment was derived as a measure of first phase insulin secretion in adults from the formula: (30-minute insulin - fasting insulin)/30-minute glucose[131]. Insulin increment estimated by this method was shown to have high

correlations with first phase insulin secretion in an intravenous GTT in adults ($r=0.66$, $P<0.001$). The significance of this measure in children is not known.

Glucose and insulin areas under the curve are calculated from a method proposed for the adults, the trapezoid rule[132].

Table 2.3 Summary Table

Age (Year)	Mothers	Fathers	Children
Birth	Anthropometry, BP OGTT Socio-economic status	Height and weight	Anthropometry Cord blood for plasma glucose and insulin concentrations
First	None	None	Anthropometry Developmental and health data
Second	None	None	Anthropometry Developmental and health data
Third	None	None	Anthropometry Developmental and health data
Fourth	None	None	Anthropometry Developmental and health data
Five	Anthropometry, BP OGTT	Anthropometry, BP Fating samples for glucose and insulin concentrations	Anthropometry, BP Bioimpedance OGTT

3. CHILDHOOD ANTHROPOMETRY FROM BIRTH TO 5 YEARS

In India, under-nutrition in children has been of greater concern than obesity[133]. Recently, however, there has been a rising prevalence of obesity in urban children[134], creating a 'double burden' of disease, and posing new problems for management policies.

At any given BMI, south Asian adults have lower lean mass and higher percentage body fat than adults from the developed world (Section 1.4.2). Accumulation of fat in the truncal region is a feature observed even among Indian neonates[118]. This phenotype, termed, muscle thin but fat or 'thin-fat', has been blamed for their insulin resistance and high risk of type 2 DM in adults. It is not known if this phenotype persists in children.

No previous study in India has looked systematically at the long-term effects of GDM on the offspring body composition, and none have examined the effects of lesser degrees of maternal glucose tolerance. Maternal hyperglycaemia, even in non-diabetic ranges, has been shown to increase the size at birth[102] (Section 1.3.4).

Evidence from a wide range of studies suggests that larger size at birth could be an important factor increasing the risk of adult obesity[135]. In most of these studies, obesity was defined using BMI, which could be a measure of lean body mass, as much as adiposity[135,136]. In contrast, there was a negative association between birth weight and central adiposity (measured by skinfold thickness or waist to hip ratio) in several studies[135]. Other studies have observed 'U' or 'J' shaped associations with higher levels of adiposity seen both among those born with higher and lower weights (Section 1.3.1).

In this chapter, I describe the anthropometric characteristics of the children in our cohort and compare them with reference Caucasian cohorts. I also derive centile curves for different components of body composition from birth to five years, and calculate the prevalence of obesity/ overweight and that of underweight/ stunting based on standard references. I go on to describe their anthropometry in relation to maternal GDM status, while also discussing the major factors influencing size and body composition at five years of age. In the course of the analysis, I test whether:

- Offspring of diabetic mothers, who have experienced fetal overgrowth, have more total and central body fat than the offspring of non-diabetic mothers and offspring of diabetic fathers at five years
- Even in the absence of frank diabetes, weight and body composition of children increase with an increase in maternal gestational glucose and insulin concentrations
- At the other end of the spectrum, children who had small size at birth have increased central body fat at 5-years.

3.1. Data cleaning

Missing values were identified and coded, and range checks were done for each measurement. Outliers were identified by plotting the anthropometric variables against age to spot unlikely values based on previous and subsequent readings. The distribution of weight at each year (Figure 3.1a) shows that the weight of one child was considerably larger than the rest of the group at 5 years of age. Nevertheless, this child's weight had been progressively increasing throughout the preceding five years (Figure.3.1b) and was strongly correlated with the child's height (Figure 3.1c). Hence, the values were deemed true in such cases and were included in the analysis.

Figure 3.1 Scatter plots of weight to check for outliers

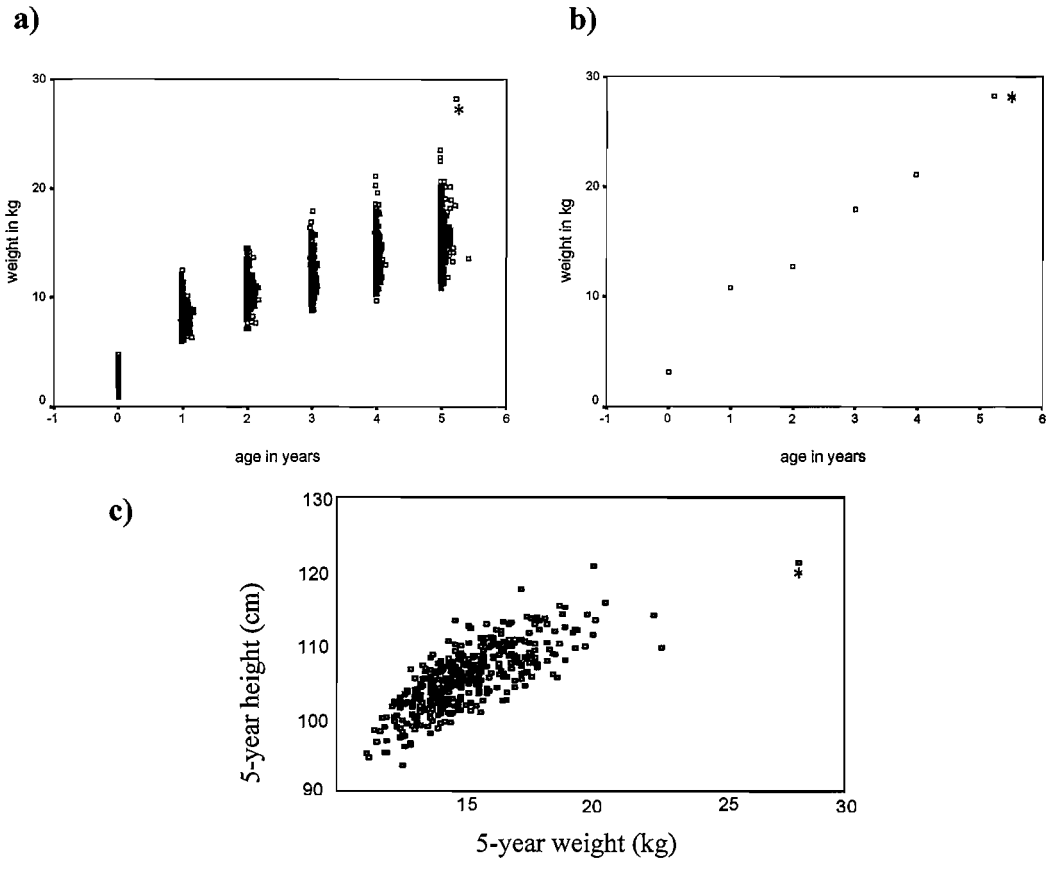


Figure 3.2a shows that the head circumference of one child was unusually small at 2 years of age. The sequence of head circumference measurement over five years revealed considerable shrinkage in size between 1 and 2 years (Figure.3.2b), which was unlikely. The 2-year value was considered spurious and was excluded from the analysis. The total number of values available for anthropometric measurements at each follow-up year, and missing values are given in Table 3.1.

Figure.3.2 Scatter plots of head circumference to test for outliers

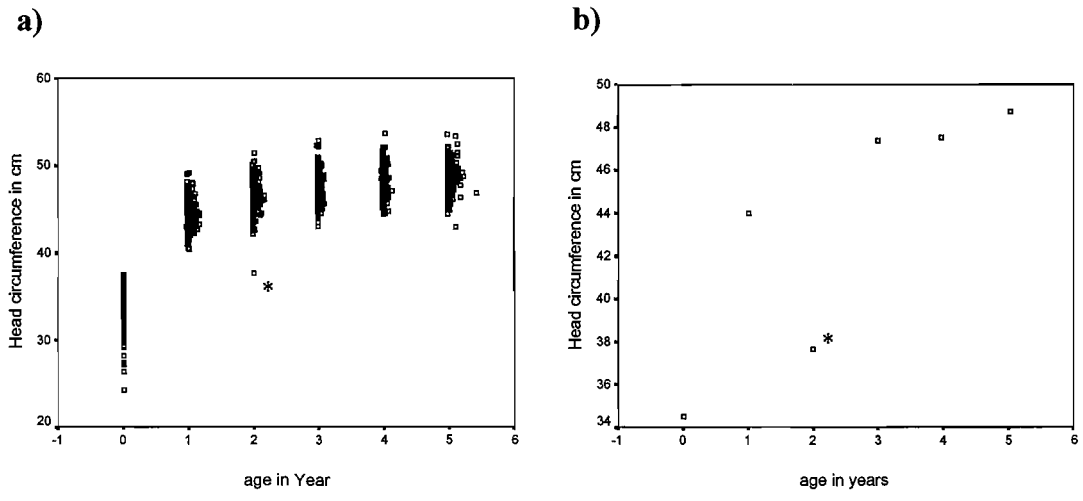


Table 3.1 Total number of anthropometric measurements available for analysis

Anthropometry N=	Birth 663	1-year 572	2-year 585	3-year 600	4-year 605	5-year 585
Weight	663	572	585	600	605	585
Crown-heel length (CHL)/Height	662*	571[¶]	585	600	605	585
Crown-rump length (CRL)/Sitting height	660*	571[¶]	584[¥]	599[¥]	604[¥]	583[¥]
Leg length	660*	571[¶]	584[¥]	599[¥]	604[¥]	583[¥]
Head circumference	662*	572	584[§]	600	605	585
MUAC	660*	572	585	600	605	585
Chest circumference	660*	572	585	600	604	585
Abdomen circumference	660*	572	585	600	605	585
Triceps skinfold	660*	572	585	600	605	585
Subscapular skinfold	660*	572	585	600	605	585

Reasons for missing values: *sick baby [¶]Infant distressed [§]dropped [¥] measurement missed

3.2 Data preparation

3.2.1 Distribution: Histograms were plotted to determine the distributions of the measurements. The skinfold thickness measurements for all years were strongly right skewed and were log transformed to satisfy assumptions of normality.

3.2.2 Adjusting for gestation and age: The mean gestational age of the infants was 39.0 weeks and 52 (8%) children were born before term (<37 weeks). Since gestational age is an important factor influencing size at birth and early postnatal growth, anthropometric measurements at birth, one and two years were adjusted for gestation at 40 weeks for all babies by linear regression: ***gestation adjusted measurement = actual measurement - regression coefficient*(gestation at birth - 280 days)***. Measurements after the age of 2 years were not adjusted as gestation is unlikely to influence the size in later years. All follow-up measurements were adjusted for current age.

Table 3.2 Mean age in years at follow-up from 1 to 5 years.

Follow-up year	Mean age (years)	SD	Minimum	Maximum	Inter-quartile range (IQR)
One	1.00	0.03	0.94	1.17	0.98, 1.00
Two	2.00	0.03	1.98	2.16	1.98, 2.00
Three	2.99	0.02	2.98	3.09	2.98, 3.00
Four	3.99	0.02	3.96	4.13	3.98, 3.99
Five	5.00	0.04	4.97	5.42	4.98, 5.00

3.2.3 Small-for-gestational-age (SGA) or low birth weight babies: The commonly used definition for SGA is a birth weight less than the 10th centile for the population[137]. A cut-off of 2 standard deviations below the mean weight is another frequently used criterion. There are no universally accepted standards for Indian babies and institutions use different criteria to define SGA babies based on different growth references (eg. NCHS standards, locally derived standards). Pediatricians at HMH define low birth weight, SGA babies based on the WHO definition of <2500g in term babies[138]. I categorised babies as SGA if their gestation adjusted birth weight was less than 2500 grams. Seventy-five babies (11%) were SGA by this definition.

3.2.4 Calculations. Arm-muscle-area (AMA) was calculated using the formula $AMA(cm^2)=(MUAC\ (cm)-\pi Triceps\ (cm))^2/4\pi$[139]. This gives a measure of muscle mass. The ratio of subscapular skinfold to the triceps skinfold measurement (SS/TR) was calculated as a measure of central adiposity.

3.2.5 Statistical methods: In the current chapter and the following chapters, the data were analyzed using SPSS (version 10.1) statistical package. Differences between means were assessed using t-tests when outcomes had a continuous distribution, and chi-square tests for categorical variables. Trends in outcome measures with the birth size, parents' anthropometry, glucose and insulin concentrations, and socio-economic status were assessed using multiple linear regressions.

It is suggested that when several dependent or independent variables are tested simultaneously in a study the value for the statistical significance (P value) needs to be lowered, depending on the number of variables to avoid false positive results (the Bonferroni correction)[140]. I did not make any corrections for multiple testing in my study; however, major outcomes and the exposures to be tested had been outlined *a priori.*

3.3 Anthropometry - birth to five years

3.3.1 Descriptions: At birth, male babies were significantly heavier; had longer CHL, CRL and leg lengths; and bigger head ($P<0.001$ for all), MUAC and chest circumferences ($P=0.03$ for both) than female babies (Table 3.3). Other measurements were similar in both sexes. At one year, male infants also had larger abdominal circumference and higher BMI than females ($P<0.001$ for both). By three-years, skinfold thickness measurements in females were significantly larger than those of male children ($P<0.01$), while leg length and abdominal circumference were similar in both sexes. At the age of five years, weight ($P=0.01$), height, head and chest circumferences were larger in boys ($P<0.001$ for all); while girls were significantly more adipose ($P<0.001$) and had bigger abdominal circumference ($P=0.03$, Table 3.3).

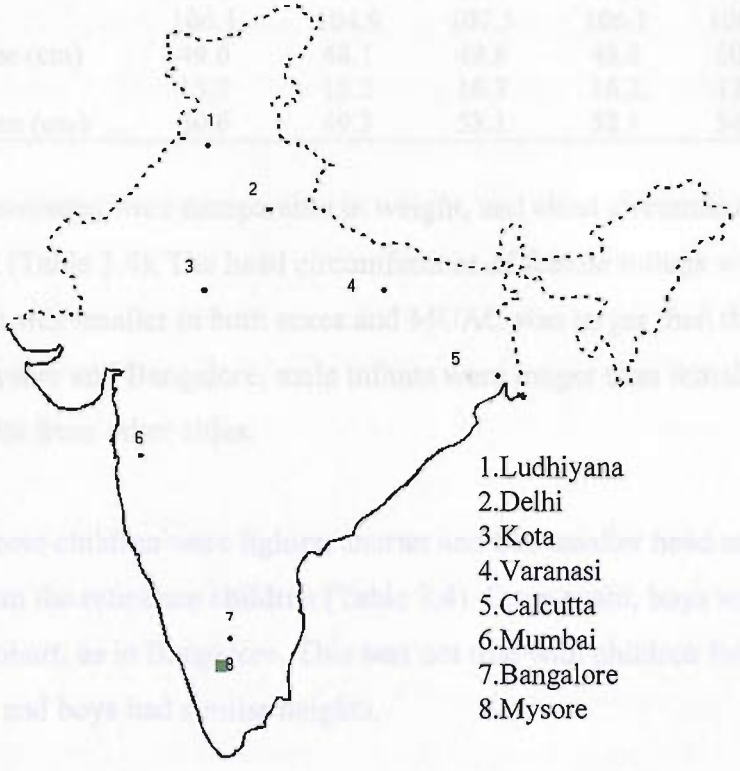
Table 3.3 Anthropometric characteristics of the Mysore children at birth, 1, 2, 3, 4 and 5 years

	Males						Females					
	Birth	**1**	**2**	**3**	**4**	**5**	**Birth**	**1**	**2**	**3**	**4**	**5**
N Max =	**321**	**274**	**282**	**285**	**285**	**280**	**342**	**298**	**303**	**315**	**320**	**305**
Weight (kg)	**3.0** (0.4)	**8.7** (1.1)	**10.8** (1.2)	**12.4** (1.4)	**14.0** (1.6)	**15.4** (1.9)	**2.9** (0.4)	**8.1** (1.1)	**10.3** (1.3)	**11.9** (1.4)	**13.5** (1.7)	**15.0** (2.0)
Height/length (cm)	**49.5** (2.1)	**74.7** (2.7)	**84.7** (3.2)	**92.1** (3.6)	**99.5** (4.0)	**106.3** (4.3)	**48.7** (2.2)	**72.4** (2.7)	**83.0** (3.2)	**90.7** (3.5)	**98.3** (34.0)	**105.0** (4.3)
Crown -rump length (cm)	**32.5** (1.7)	**46.2** (1.9)	**50.8** (2.2)	**54.2** (2.3)	**55.9** (2.4)	**58.5** (2.4)	**32.0** (1.7)	**44.9** (1.8)	**49.4** (2.1)	**53.1** (2.1)	**54.9** (2.7)	**57.7** (2.4)
Leg length (cm)	**17.0** (1.4)	**27.9** (1.5)	**33.9** (1.8)	**38.0** (2.2)	**43.6** (2.3)	**47.7** (2.5)	**16.7** (1.6)	**27.5** (1.6)	**33.6** (1.8)	**37.5** (2.1)	**43.4** (2.6)	**47.3** (2.4)
Ponderal Index (kg/m^3)/BMI (kg/m^2)†	**24.9** (2.7)	**15.9** (1.3)	**15.0** (1.0)	**14.6** (1.0)	**14.1** (1.0)	**13.6** (1.0)	**25.1** (2.8)	**15.4** (1.4)	**14.9** (1.2)	**14.4** (1.1)	**14.0** (1.1)	**13.6** (1.2)
Head circumference (cm)	**34.4** (1.3)	**44.7** (1.3)	**46.9** (1.3)	**48.0** (1.3)	**48.8** (1.3)	**49.0** (1.4)	**33.7** (1.3)	**43.6** (1.3)	**45.9** (1.3)	**46.9** (1.3)	**47.8** (1.2)	**48.0** (1.3)
Mid-arm circumference (cm)	**10.5** (0.9)	**14.4** (1.1)	**14.8** (1.0)	**15.1** (1.0)	**15.4** (1.1)	**15.4** (1.2)	**10.4** (1.0)	**13.8** (1.1)	**14.5** (1.1)	**14.9** (1.1)	**15.3** (1.2)	**15.4** (1.3)
Chest circumference (cm)	**32.4** (1.7)	**44.6** (2.0)	**47.0** (1.9)	**48.4** (1.9)	**49.8** (2.0)	**50.8** (2.2)	**32.1** (1.7)	**43.2** (2.1)	**45.7** (2.1)	**46.9** (2.0)	**48.3** (2.2)	**49.5** (2.5)
Abdominal circumference (cm)	**30.2** (2.0)	**43.4** (2.7)	**44.5** (2.8)	**46.4** (2.6)	**48.1** (2.7)	**48.3** (2.9)	**30.1** (2.0)	**42.4** (3.0)	**44.6** (3.4)	**46.8** (2.9)	**48.4** (3.2)	**48.9** (3.4)
Triceps skinfold (mm)*	**4.1** (3.7,4.8)	**7.8** (6.8,8.9)	**7.6** (6.6,8.5)	**7.5** (6.6,8.7)	**7.5** (6.8,8.5)	**7.2** (6.3,8.2)	**4.2** (3.7,5.0)	**7.7** (6.8,8.9)	**7.9** (6.7,9.0)	**7.9** (6.8,9.2)	**8.2** (7.0,9.6)	**8.3** (6.9,9.8)
Subscapular skinfold (mm)*	**4.4** (3.9,5.0)	**6.4** (5.6,7.4)	**6.9** (5.8,8.2)	**6.3** (5.3,7.2)	**6.0** (5.2,6.7)	**5.5** (4.8,6.2)	**4.5** (4.0,5.2)	**6.4** (5.5,7.4)	**7.1** (6.0,8.3)	**7.0** (5.5,7.9)	**6.7** (5.5,7.9)	**6.4** (5.1,7.7)

Means (SD), or *geometric mean (IQR); †Ponderal index at birth, BMI at subsequent ages

3.3.2. Comparisons with other populations: India: The Mysore anthropometry was compared to a recently published Indian reference data where weight, length/height, and MUAC, head and chest circumferences were available from birth to six years[141]. These were derived using samples from seven cities in India representing different geographic positions; Bangalore, Mumbai, Calcutta, Delhi, Kota, Ludhiyana, and Varanasi (Figure 3.3).

Figure 3.3 Map showing the location of Mysore in relation to reference cities



Children, selected through leading paediatric and obstetric nursing homes, private clinics, nurseries, and private schools, were from affluent backgrounds to provide a reference based on children whose growth was least affected by constraints due to socio-economic deprivation. Affluence was based on parental education, occupation, health awareness, hygiene, sanitation, and child rearing. Children (N=750) were studied between birth and one-year, and between one and six years (N=1885) of age. Not all children were measured at each time point and the numbers available at each year for individual centres were smaller than the Mysore cohort. The anthropometric methods used were similar to those in my study. Geographically Mysore is closer, and socio-cultural characteristics are more similar, to Bangalore than other cities. Table 3.4 shows the anthropometric characteristics of Mysore, Bangalore and Delhi children at birth and five years.

Table 3.4 Measurements of Mysore and reference children[141] (Median)

		Mysore		Bangalore		Delhi	
	N=	663		48		91	
Birth		**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**
Weight (kg)		3.0	2.9	3.1	3.1	3.3	3.2
Length (cm)		49.7	48.9	51.5	50.7	50.4	50.3
Head circumference (cm)		34.7	33.8	34.4	34.5	34.8	34.7
MUAC (cm)		10.6	10.4	10.2	10.3	10.0	10.1
Chest circumference (cm)		32.7	32.2	32.9	32.4	33.2	33.3
Five-year	**N=**	**585**		**180**		**247**	
Weight (kg)		15.1	14.7	17.1	16.4	17.7	17.6
Height (cm)		106.1	104.9	107.5	106.1	106.2	106.0
Head circumference (cm)		49.0	48.1	49.8	48.8	50.7	50.2
MUAC (cm)		15.3	15.2	16.3	16.2	17.1	17.1
Chest circumference (cm)		50.6	49.3	53.1	52.1	54.9	54.3

At birth, Mysore neonates were comparable in weight, and chest circumference to reference children (Table 3.4). The head circumference of female infants was smaller. Crown-heel length was smaller in both sexes and MUAC was larger than the reference values. In both Mysore and Bangalore, male infants were longer than females; this was not true with infants from other cities.

At five years, Mysore children were lighter, shorter and had smaller head and arm circumferences than the reference children (Table 3.4). Once again, boys were taller than girls in our cohort, as in Bangalore. This was not true with children from other cities, where girls and boys had similar heights.

3.3.3 Comparisons with Western standards:

3.3.3.1 SD scores. Neonatal measurements were compared with a cohort of term babies born in Southampton and studied as part of a student research project, between 19th January and 25th April 1987 in the Princess Anne Hospital, Southampton, UK[142]. They were measured and assessed for gestational age using identical methods to those in Mysore. The measurements include birth weight, length, head circumference, circumference at xiphisternum, MUAC and subscapular skinfold. Sex specific standard deviation scores (SD scores/Z scores) were calculated by the formula: ***Mysore SD score = (Mysore observed value - Southampton mean)/Southampton SD.***

Measurements from one to five years were compared to UK and Dutch growth references[143-147]. The SD scores relative to UK children were based on the British 1990 growth reference for weight, height, BMI, head circumference, and triceps and

subscapular skinfolds. The skewness was allowed for by the Box-Cox power transformation (variable L) of the data; data were summarised in three parameters L, M and S. SD scores were derived by the formula: ***Mysore SD score = (Mysore observed value/M)^{L} - 1]/ L*S***, where M is the UK median; S, the coefficient of variation.

The Dutch reference was based on two cross-sectional studies carried out in Netherlands[147]. They provided reference values for MUAC, leg length and CRL, not available in the UK standards. A total of 1518 infants, 407 babies born at 25-40 weeks gestation and 1111 full-term babies, were measured between 0 and 12 months in Maastricht. In addition, 2333 children between 0 and 18 years from Oosterwolde were measured[110]. Two sets of SDs were given to account for the skewness of the data. Measurements at birth were not adjusted for gestation and were likely to have given spurious values, and hence were not used. The SD scores were calculated for measurements from one to five years by the formula: ***Mysore SD score = (Mysore observed value –Dutch median)/Dutch SD***, where SD was different for observations below and above the standard value.

3.3.3.2 Comparison at birth. Mysore babies were significantly smaller than Southampton neonates in all the measurements (Table 3.5, Figure 3.4). However, different body components showed different degrees of deficit. Major deficits were seen for measurements of weight (-1.07 SD) and MUAC (-1.09 SD), while CHL (-0.25 SD) and subscapular skinfolds (-0.19 SD) were relatively larger (Figure 3.4). This pattern was apparent even in SGA babies; the SD score for subscapular skinfold measurement was -1.08 compared with -2.63 for birth weight.

3.3.3.3 One to five years: UK standards. At one year, the smaller body size of Mysore infants compared to UK infants was more pronounced than at birth (Figure 3.4). The greatest deficit was for head circumference (-2.5 SD), and weight (-1.5 SD), BMI (-1.6 SD) and triceps skinfolds (-1.4 SD) were also comparatively smaller than the reference values. As at birth, the deficit was least for CHL (-0.6 SD) and subscapular skinfold thickness (-0.9 SD) (Figure 3.4).

Over the subsequent years the head circumference of Mysore children continued to remain smaller than other measurements. At two years, subscapular skinfold thickness of Mysore children was greater than the UK standards (+0.03 SD) and by three years of

age Mysore children had significantly larger subscapular skinfolds compared to UK standards (+0.08 SD, P=0.04). Height, BMI and triceps skinfolds continued to remain smaller, though there was an increase in the SD scores for triceps from one to five years (Figure 3.4). As at birth, SGA children exhibited a similar phenotype as the whole cohort. At five years, the least deficit was seen for subscapular skinfolds (-0.22 SD), as compared to weight (-2.01 SD) and head circumference (-3.59 SD).

3.3.3.4 One to five years: Dutch standards. Comparisons with the Dutch standards confirmed the above findings (Table 3.5). The SD scores were least for measurements of head circumference at one year and remained small during subsequent follow-up measurements (Figure 3.5). Large negative changes were observed in SD scores from one to five years for MUAC, CRL and weight. Similar to UK comparisons, subscapular skinfold measurements in Mysore were bigger relative to all other measurements and larger than reference values from one to five years (Figure 3.5).

3.3.3.5 Obesity and overweight. The prevalence of obesity and overweight at five-years was determined by BMI values equivalent to 30/kg/m^2 and 25/kg/m^2 respectively in adults recommended by Cole TJ *et al*, where centile curves for BMI from several populations across the world were averaged to give reference values[148]. Only one girl with a BMI of 19.30/kg/m^2 (cut-off = 19.17/kg/m^2) and one boy with a BMI of 19.30/kg/m^2 (cut-off = 19.30/kg/m^2) were obese at five years, and two girls were overweight (BMI >17.15/kg/m^2). All these children also had subscapular skinfold thickness greater than the 95th percentile for their sex (boys= 8.69 mm, girls=10.79 mm).

3.3.3.6 Underweight and stunting. The prevalence of underweight and stunting were determined by weight and height 2 SD scores below the UK median. Based on this 29 (9%) boys and 124 (36%) girls were underweight at five years, and 22 (7%) boys and 39 (11%) girls were stunted.

Table 3.5 Characteristics of the Mysore children at birth, one and five years and the UK and Dutch reference data.

	MYSORE				UK *				DUTCH			
Birth	**BOYS**		**GIRLS**		**BOYS**		**GIRLS**		**BOYS**		**GIRLS**	
Weight (g)	**3.04**	2.8,3.3	**2.9**	2.7,3.2	**3.5**	3.2,3.8	**3.4**	3.1,3.7	**3.1**	2.9,3.3	**3.0**	2.7,3.3
Crown-heel length (cm)	**49.8**	48.6,50.9	**48.9**	47.7,50.2	**49.9**	48.7,51.3	**49.1**	48.2,50.6	**50.4**	49.2,51.8	**49.5**	48.1,50.9
Crown-rump length (cm)	**32.6**	31.7,33.6	**32.0**	31.2,33.0	-		-		**33.8**	32.8,34.8	**33.0**	32.0,34.1
Leg length (cm)	**17.1**	16.2,17.8	**16.9**	16.1,17.6	-		-		**17.1**	16.1,17.7	**16.7**	15.9,17.4
Head circumference (cm)	**34.7**	33.7,35.3	**33.8**	33.0,34.6	**35.5**	34.7,36.3	**34.8**	34.1,35.5	**34.8**	33.8,35.4	**34.0**	33.1,34.8
MUAC (cm)	**10.6**	10.0,11.2	**10.4**	9.8,11.0	**11.5**	10.9,12.1	**11.3**	10.8,12.0	**8.5**	7.4,9.4	**8.5**	7.7,9.1
Abdominal circumference xiphisternum (cm)	**32.7**	31.5,33.5	**32.2**	31.2,33.2	**33.7**	32.5,34.8	**33.2**	32.1,34.4	-		-	
Triceps skinfold (mm)	**4.1**	3.7,4.8	**4.3**	3.7,5.0	-		-		**9.9**	8.2,11.9	**9.4**	7.7,11.16.0
Subscapular skinfold (mm)	**4.5**	3.9,5.0	**4.5**	4.0,5.2	**4.5**	4.0,5.4	**4.7**	4.1,5.6	**6.0**	5.1,7.1	**6.0**	5.1,7.3
ONE YEAR												
Weight (g)	**8.7**	8.0, 9.5	**8.0**	7.4, 8.7	**10.1**	9.4, 10.8	**9.5**	8.8, 10.2	**10.1**	9.51,10.7	**9.4**	8.7,9.8
Crown-heel length (cm)	**73.9**	72.4, 76.0	**72.1**	70.7, 74.3	**75.5**	73.8, 77.2	**73.9**	72.3, 75.6	**77.4**	76.1,78.8	**75.9**	74.0,77.1
Crown-rump length (cm)	**46.2**	44.8, 47.6	**44.7**	43.7,46.0	-		-		**49.2**	48.4, 50.1	**48.0**	46.7, 49.0
Leg length (cm)	**27.9**	26.9, 28.8	**27.5**	26.5,28.4	-		-		**27.8**	26.4, 29.1	**27.7**	26.6, 28.9
Body mass index (kg/m^2)	**15.8**	14.9, 16.8	**15.2**	14.5, 16.2	**17.6**	16.8,18.6	**17.3**	16.4,18.2	**15.9**	15.1,16.7	**15.4**	14.5,16.2
Head circumference (cm)	**44.8**	43.9, 45.5	**43.5**	42.7, 44.4	**47.7**	46.7, 48.7	**46.5**	45.6, 47.4	**48.1**	47.2,48.8	**46.6**	45.8,47.2
MUAC (cm)	**14.3**	13.6, 15.1	**13.8**	13.0, 14.5	-	-	-	-	**15.5**	14.7,16.3	**15.0**	14.1,15.8
Triceps skinfold (mm)	**7.8**	6.8, 8.9	**7.8**	6.8, 8.9	**12.0**	10.2, 13.8	**11.8**	9.9, 13.7	**9.8**	8.1,11.8	**9.4**	7.8,11.1
Subscapular skinfold (mm)	**6.3**	5.6, 7.4	**6.3**	5.5, 7.4	**8.3**	6.8, 10.1	**8.3**	7.1, 9.7	**6.0**	5.0,7.1	**6.0**	5.1,7.3
FIVE YEARS												
Weight (g)	**15.1**	14.2,16.6	**14.7**	13.6,16.1	**18.6**	17.2,20.2	**18.3**	16.8,20.1	**20.2**	18.7,21.6	**18.7**	17.4,20.4
Height (cm)	**106.1**	103.3,109.1	**104.9**	102.1,107.6	**109.6**	106.6,112.6	**108.9**	105.9,111.9	**114.5**	111.2,117.5	**113.1**	110.2,115.7
Sitting height (cm)	**58.5**	56.9,60.0	**57.6**	56.1,59.2	-		-		**65.2**	63.4,66.6	**63.9**	62.4,65.3
Leg length (cm)	**47.6**	45.9,49.3	**47.2**	45.9,48.6	-		-		**49.3**	47.4,51.1	**49.2**	47.4,50.7
Body mass index (kg/m^2)	**13.5**	13.0,14.3	**13.4**	12.7,14.2	**15.5**	14.8,16.4	**15.5**	14.6,16.5	-		-	
Head circumference (cm)	**49.0**	48.0,49.9	**48.1**	47.1,49.0	**52.8**	51.7,53.8	**51.7**	50.9,52.5	**52.8**	51.8,53.7	**51.6**	50.6,52.5
MUAC (cm)	**15.3**	14.6,16.1	**15.2**	14.5,16.1	-		-		**20.3**	19.3,21.1	**20.0**	19.2,20.6
Triceps skinfold (mm)	**7.2**	6.3,8.2	**8.1**	6.9,9.8	**8.7**	7.3,10.4	**10.2**	8.5,12.0	**8.4**	7.1,9.5	**9.6**	8.2,11.1
Subscapular skinfold (mm)	**5.3**	4.8,6.2	**6.0**	5.1,7.7	**5.4**	4.5,6.4	**6.1**	5.1,7.5	**5.1**	4.4,6.4	**5.6**	4.8,6.6

Median (IQR), *Southampton babies for birth measurements

Figure 3.4 Mean SD scores (with 95% confidence intervals) for anthropometry at 1, 2 and 5 years (standardised to the UK population)

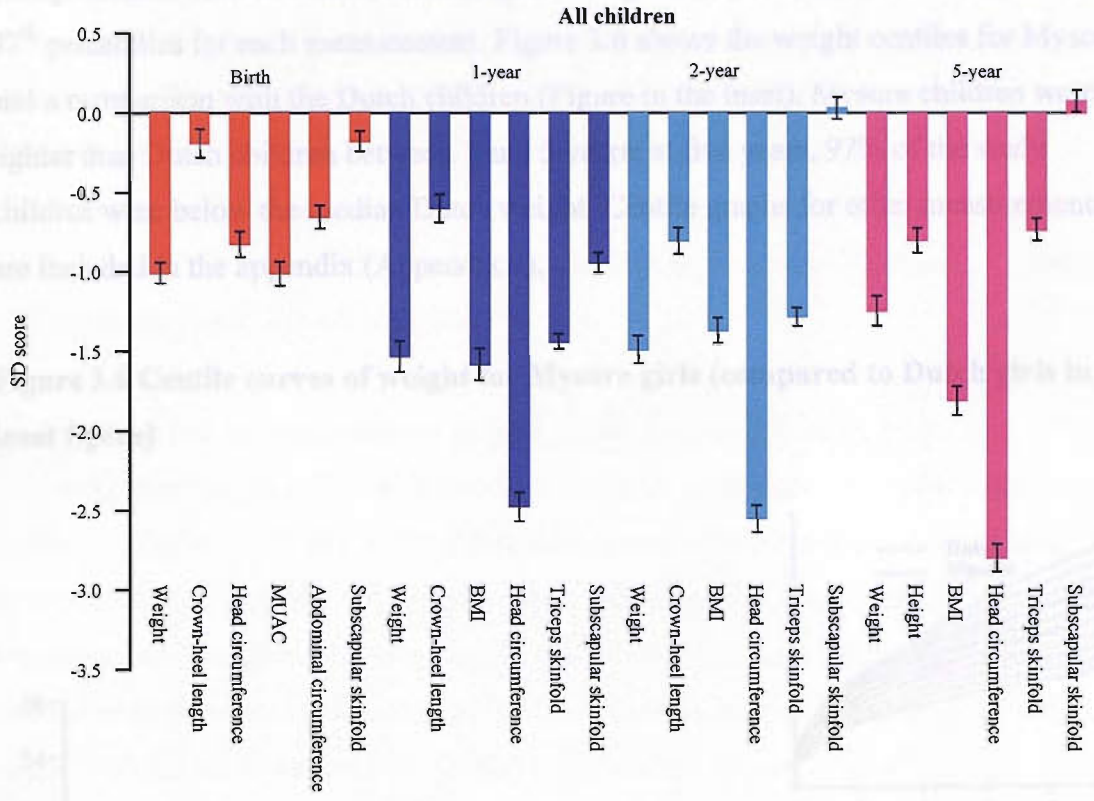
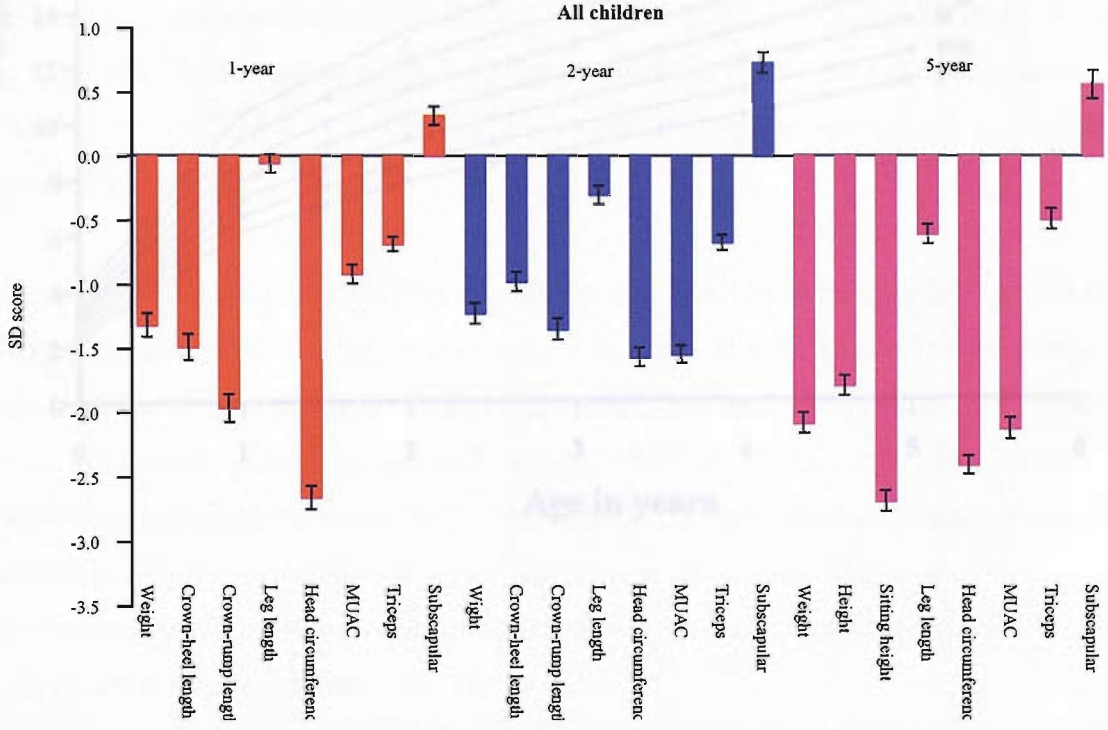
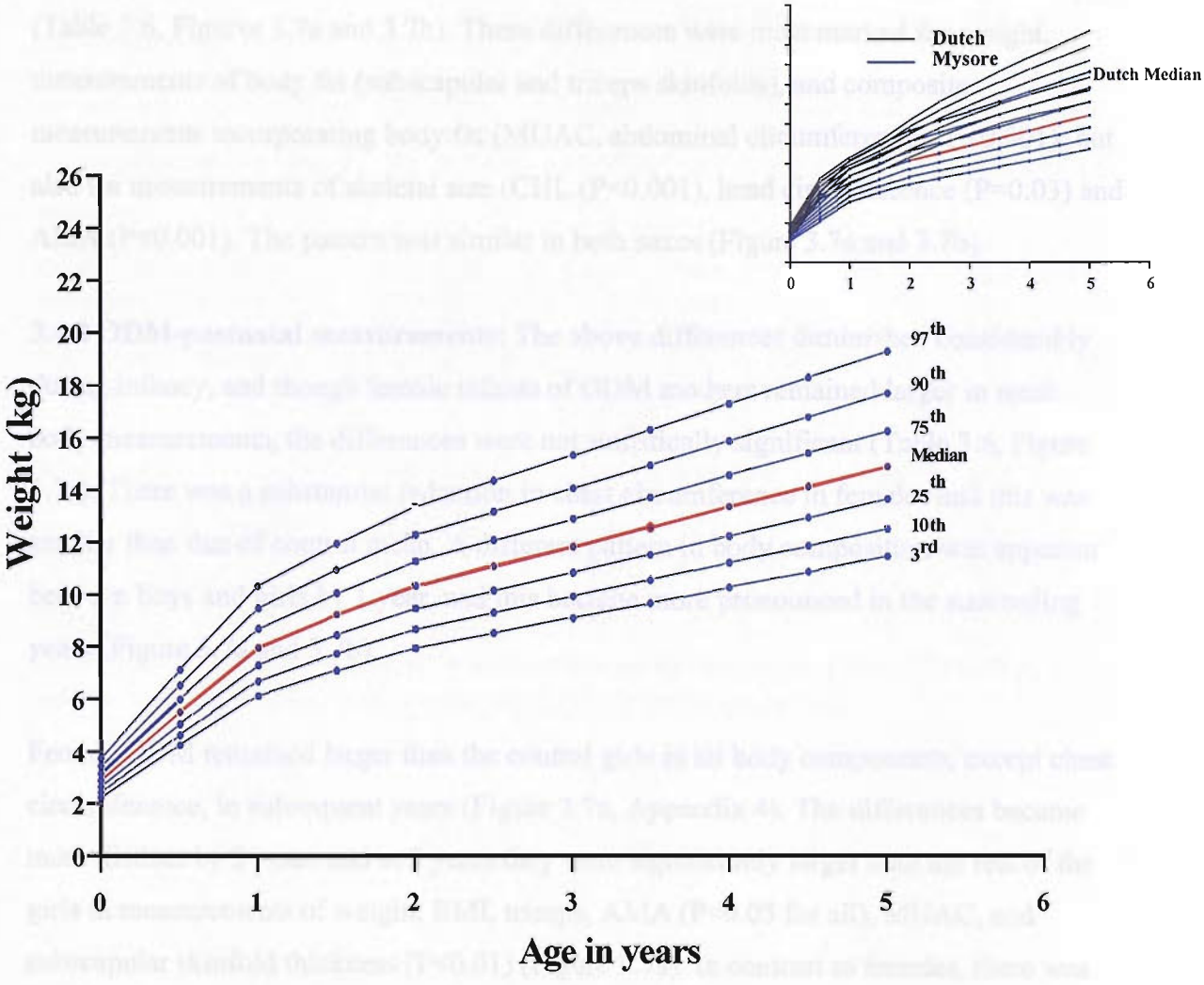


Figure 3.5 Mean SD scores (with 95% confidence intervals) for anthropometry at 1, 2 and 5 years (standardised to the Dutch population)



3.3.4 Centile curves. This study has produced high-quality anthropometric data from birth to five years for children belonging to a wide range of socio-economic backgrounds. I derived centile curves representing the 3rd, 10th, 25th, 50th 75th 90th and 97th percentiles for each measurement. Figure 3.6 shows the weight centiles for Mysore, and a comparison with the Dutch children (Figure in the inset). Mysore children were lighter than Dutch children between 1 and 5 years; at five years, 97% of the study children were below the median Dutch weight. Centile graphs for other measurements are included in the appendix (Appendix 4).

Figure 3.6 Centile curves of weight for Mysore girls (compared to Dutch girls in the inset figure)



3.4 Offspring of Diabetic Mothers (ODM) and fathers

At birth, 630 of 663 babies available for follow-up had their maternal GDM status known. Of these, at five years, fasting plasma glucose and insulin concentrations were measured in 482 willing fathers. Fathers were defined as having diabetes if they were already known to have diabetes or if their fasting glucose concentration was $\geq$ 7.0 mmol/l. In case of overlap between maternal GDM and paternal diabetes, children were categorised as ODM (N=6). The remaining children born to non-GDM mothers and non-diabetic fathers were termed controls.

3.4.1 ODM-birth measurements: At birth ODM were significantly larger than offspring of non-diabetic mothers (ONDM) and control babies in all anthropometric measurements (Table 3.6, Figures 3.7a and 3.7b). These differences were most marked for weight, measurements of body fat (subscapular and triceps skinfolds), and composite measurements incorporating body fat (MUAC, abdominal circumferences, $P<0.001$), but also for measurements of skeletal size (CHL ($P<0.001$), head circumference ($P=0.03$) and AMA ($P<0.001$). The pattern was similar in both sexes (Figure 3.7a and 3.7b).

3.4.2 ODM-postnatal measurements: The above differences diminished considerably during infancy, and though female infants of GDM mothers remained larger in most body measurements, the differences were not statistically significant (Table 3.6, Figure 3.7a). There was a substantial reduction in chest circumference in females and this was smaller than that of control mean. A different pattern in body composition was apparent between boys and girls by 1 year, and this became more pronounced in the succeeding years (Figure 3.7a and 3.7b).

Female ODM remained larger than the control girls in all body components, except chest circumference, in subsequent years (Figure 3.7a, Appendix 4). The differences became more distinct by 2 years and at 5 years they were significantly larger than the rest of the girls in measurements of weight, BMI, triceps, AMA ($P<0.05$ for all), MUAC, and subscapular skinfold thickness ($P<0.01$) (Figure 3.7a). In contrast to females, there was no discernible pattern in size for male ODM during subsequent follow-up. The differences were very small compared to control boys and were not statistically significant at any point after birth (Figure 3.7b).

3.4.3 Offspring of diabetic fathers: Forty-one fathers (8.5%) had diabetes at 5-year follow-up (17 previously diagnosed and 24 diagnosed from fasting blood samples). Their offspring had lower birth weight, and smaller MUAC than control children (Table 3.6, Figure 3.8). Except leg length, which was similar, all neonatal measurements were significantly smaller than those of ODM. There were no differences in anthropometric measurements between offspring of diabetic fathers and controls between 1 and 5 years.

Table 3.6 Characteristics of offspring of diabetic mothers, diabetic fathers and control children

	ODM	P†	Control children	P‡	Offspring of diabetic fathers
N Max =	**41**		**548**		**41**
Birth					
Gestational age (weeks)	**39.1** (1.2)	0.8	**39.0** (1.8)	0.9	**39.1** (1.2)
Weight (g)	**3344** (421)	<0.001	**2973** (408)	0.05	**2869** (305)
Crown-heel length (cm)	**50.5** (2.3)	<0.001	**49.2** (2.1)	0.4	**48.9** (1.9)
Ponderal index (kg/m^3)	**26.0** (2.5)	0.01	**24.9** (2.7)	0.3	**24.5** (1.8)
MUAC (cm)	**11.3** (0.8)	<0.001	**10.5** (0.9)	0.03	**10.2** (0.7)
*Triceps skinfold (mm)	**5.1** (4.6,6.1)	<0.001	**4.2** (3.7, 4.9)	0.06	**4.0** (3.6,4.4)
*Subscapular skinfold (mm)	**5.3** (4.7,6.2)	<0.001	**4.4** (4.0, 5.0)	0.053	**4.2** (3.8,4.6)
One year					
Weight (kg)	**8.5** (1.2)	0.8	**8.4** (1.1)	0.5	**8.3** (1.0)
Crown-heel length (cm)	**73.5** (2.5)	0.6	**73.3** (2.9)	0.3	**72.8** (2.6)
Body mass index (kg/m^2)	**15.6** (1.7)	0.9	**15.7** (1.4)	0.9	**15.6** (1.5)
MUAC (cm)	**14.3** (1.1)	0.2	**14.1** (1.1)	0.8	**14.1** (1.3)
*Triceps skinfold (mm)	**7.8** (6.9,9.5)	0.7	**7.7** (6.8,8.9)	0.995	**7.8** (6.6,8.8)
*Subscapular skinfold (mm)	**6.5** (5.3,7.9)	0.8	**6.4** (5.5,7.3)	0.7	**6.4** (5.6,7.1)
Five years					
Weight (kg)	**15.8** (2.1)	0.09	**15.2** (2.0)	0.96	**15.2** (1.9)
Crown-heel length (cm)	**106.0** (4.5)	0.6	**105.6** (4.4)	0.6	**106.0** (4.3)
Body mass index (kg/m^2)	**14.0** (1.2)	0.03	**13.6** (1.1)	0.7	**13.5** (1.0)
MUAC (cm)	**15.9** (1.3)	0.006	**15.3** (1.2)	0.96	**15.3** (1.2)
*Triceps skinfold (mm)	**8.5** (6.7,10.3)	0.01	**7.7** (6.5,8.9)	0.2	**8.0** (7.1,8.9)
*Subscapular skinfold (mm)	**6.6** (5.2,8.3)	0.01	**5.9** (4.9,6.9)	0.4	**6.1** (5.1,7.1)

Means (SD), or *geometric mean (IQR) †P for the difference between offspring of diabetic mothers and controls ‡P for the difference between offspring of diabetic fathers and controls

Figure 3.7a Mean SD scores for anthropometry relative to control females

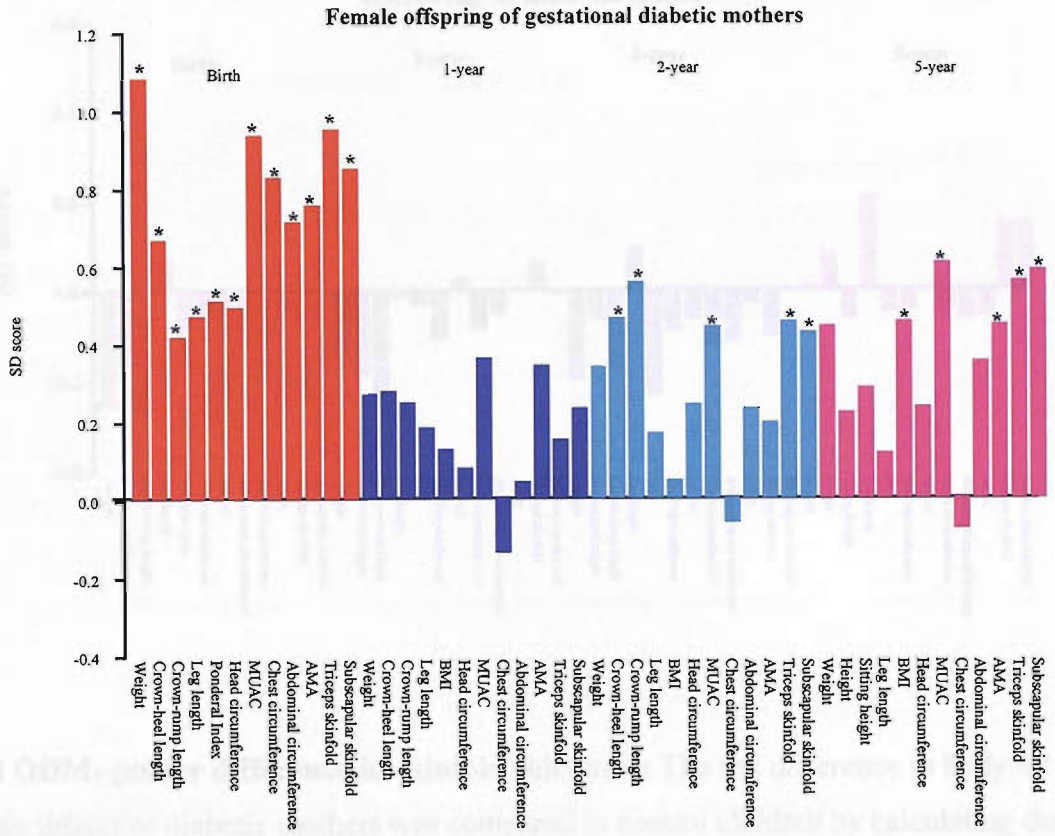


Figure 3.7b Mean SD scores for anthropometry relative to control males

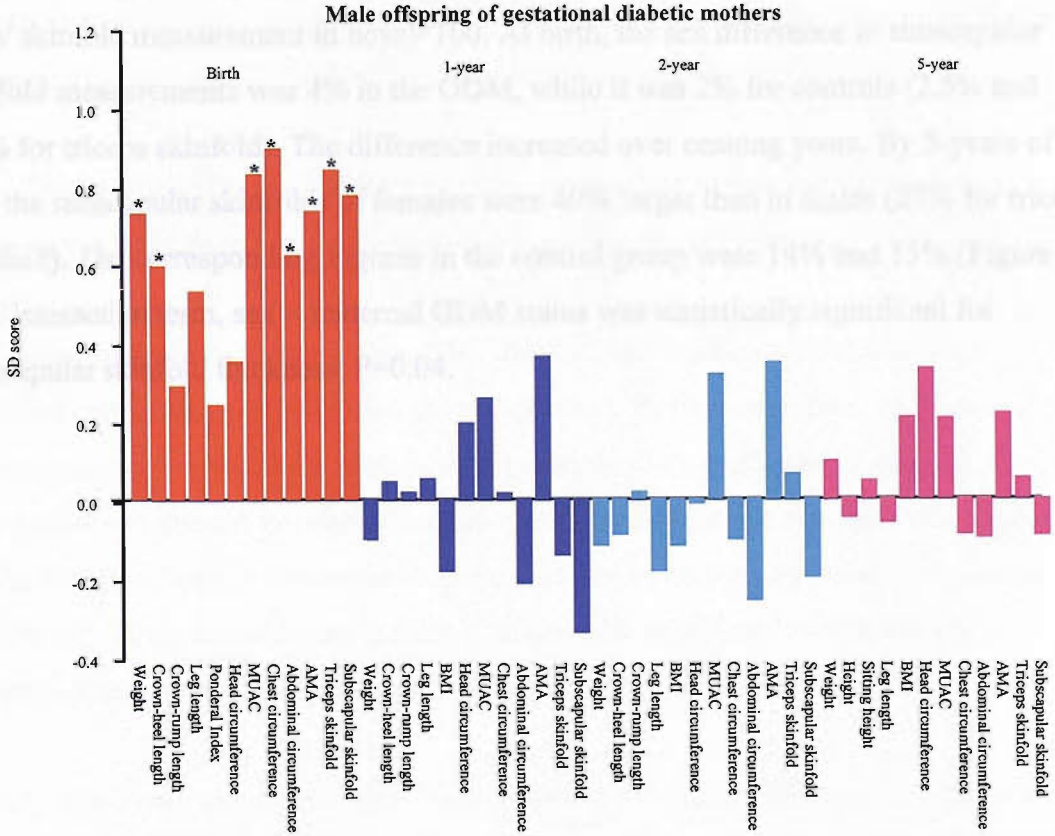
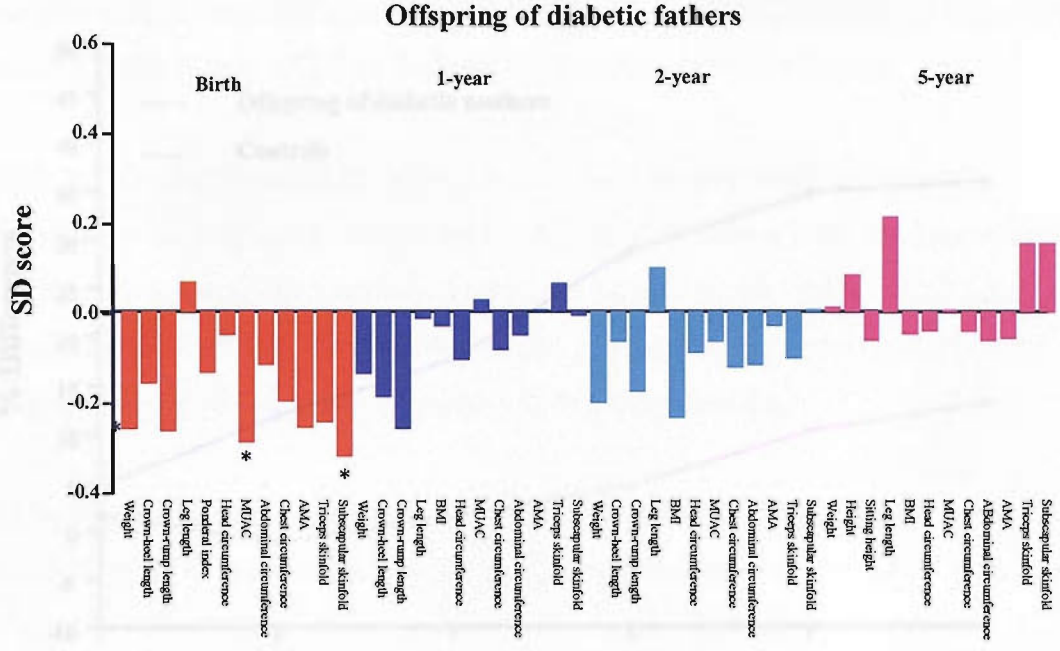
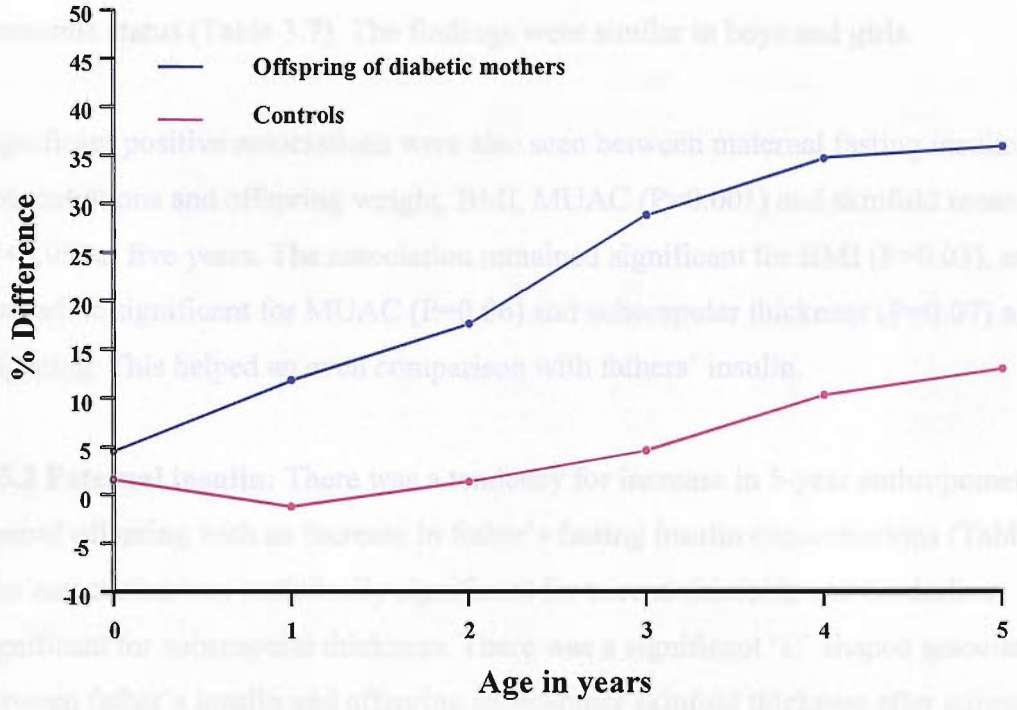


Figure 3.8 Mean SD scores for anthropometry relative to control offspring



3.4.4 ODM- gender difference in skinfold thickness: The sex difference in body fat among infants of diabetic mothers was compared to control children by calculating the percentage difference in skinfold thickness between sexes in both groups: % extra skinfold thickness in females = (skinfold measurement in girls- skinfold measurement in boys/ skinfold measurement in boys)*100. At birth, the sex difference in subscapular skinfold measurements was 4% in the ODM, while it was 2% for controls (2.5% and 1.4% for triceps skinfold). The difference increased over ensuing years. By 5-years of age, the subscapular skinfolds of females were 40% larger than in males (27% for triceps skinfold). The corresponding Figures in the control group were 14% and 13% (Figure 3.9). Interaction term, sex × maternal GDM status was statistically significant for subscapular skinfold thickness, P=0.04.

Figure 3.9: Difference in subscapular skinfold thickness between sexes



3.5 Relationships to parental glucose and insulin in control children

An earlier study has reported a positive association between maternal fasting glucose concentrations during pregnancy in the absence of diabetes and offspring size at birth[102]. I examined for similar associations between maternal glucose and insulin areas under the curve (GAUC and IAUC) and offspring anthropometry in control children.

3.5.1 Maternal GAUC and IAUC: At birth, none of the anthropometric measurements of the offspring born to control mothers was associated with maternal GAUC or IAUC.

At 5 years, offspring weight, height, BMI, MUAC and head circumference were positively associated with maternal GAUC (Table 3.7). However, none of these associations were statistically significant. A statistically significant 'U' shaped association was noticed between maternal GAUC and offspring skinfolds with higher values observed both in lowest and highest quartiles of maternal glucose concentrations (Table 3.7). These associations remained statistically significant after adjusting for maternal skinfolds and socio-economic status of the family (Table 3.7).

Relationships with maternal insulin concentrations were much stronger than those with GAUC. There was a significant positive association between maternal IAUC and all

anthropometric measurements in the children (Table 3.7). The effects diminished, especially with subscapular skinfolds, after adjusting for maternal skinfolds and socio-economic status (Table 3.7). The findings were similar in boys and girls.

Significant positive associations were also seen between maternal fasting insulin concentrations and offspring weight, BMI, MUAC (P=0.001) and skinfold measurements (P<0.05) at five years. The association remained significant for BMI (P=0.03), and borderline significant for MUAC (P=0.06) and subscapular thickness (P=0.07) after adjusting. This helped an even comparison with fathers' insulin.

3.5.2 Paternal insulin: There was a tendency for increase in 5-year anthropometry in the control offspring with an increase in father's fasting insulin concentrations (Table 3.7). The association was statistically significant for triceps skinfolds and borderline significant for subscapular thickness. There was a significant 'U' shaped association between father's insulin and offspring subscapular skinfold thickness after adjusting for paternal skinfolds and socio-economic status (Table 3.7).

Table 3.7 Anthropometry at 5 years in the control children according to fourths of maternal glucose (GAUC), insulin (IAUC) and paternal insulin

Quartiles	N	Weight (kg)	Height (cm)	BMI (kg/m^2)	Head (cm)	MUAC (cm)	Triceps (mm)	Subscapular (mm)
Maternal GAUC in pregnancy								
1 (Lowest)	127	**15.2**	**105.4**	**13.6**	**48.3**	**15.3**	**8.1**	**6.4**
2	141	**15.1**	**105.4**	**13.5**	**48.4**	**15.3**	**7.7**	**6.0**
3	116	**15.2**	**105.8**	**13.6**	**48.7**	**15.4**	**7.6**	**5.8**
4 (Highest)	94	**15.4**	**106.0**	**13.6**	**48.6**	**15.4**	**8.4**	**6.4**
SD	548	2.0	4.4	1.1	1.5	1.2	2.1	1.9
P*		0.3	0.5	0.05§	0.054	0.4	0.001§	<0.001§
P†		0.6	0.2	0.01§	0.6	0.9	0.001§	<0.001§
Maternal IAUC in pregnancy								
1 (Lowest)	123	**15.0**	**105.5**	**13.5**	**48.3**	**15.3**	**7.8**	**6.1**
2	113	**15.1**	**105.5**	**13.5**	**48.4**	**15.2**	**7.7**	**6.0**
3	124	**15.2**	**105.5**	**13.5**	**48.5**	**15.2**	**7.6**	**5.9**
4 (Highest)	101	**15.6**	**106.0**	**13.9**	**48.7**	**15.7**	**8.7**	**6.6**
SD	548	2.0	4.4	1.1	1.4	1.2	2.1	1.9
P*		0.02	0.4	0.02	0.06	0.007	0.002	0.06
P†		0.3	0.5	0.04	0.3	0.1	0.02	0.08
Paternal fasting insulin								
1 (Lowest)	110	**15.0**	**105.4**	**13.5**	**48.5**	**15.2**	**7.6**	**6.1**
2	107	**15.3**	**105.9**	**13.6**	**48.5**	**15.3**	**7.9**	**5.9**
3	97	**15.1**	**105.6**	**13.5**	**48.6**	**15.4**	**8.0**	**6.1**
4 (Highest)	95	**15.3**	**105.9**	**13.6**	**48.5**	**15.4**	**8.1**	**6.2**
SD	409	2.0	4.4	1.1	1.4	1.2	2.2	2.0
P*		0.1	0.1	0.4	0.5	0.07	0.004	0.07
P‖		0.95	0.6	0.6	0.07	0.97	0.08	0.002§

P *adjusted for sex † adjusted for sex, maternal sum of skinfolds and socio-economic status § quadratic association ‖ adjusted for sex, paternal sum of skinfolds and socio-economic status P values were derived by multiple linear regressions, using all variables as continuous.

3.6 Factors influencing anthropometry at five years.

3.6.1 Size at birth: Measurements at 5 years were generally positively correlated with measurements at birth (Table 3.8). The strongest correlations were between skeletal measurements: birth length with 5-year height, head circumference at birth and 5 years. After adjusting for current weight, negative associations were present between birth weight and 5-year triceps skinfold (P=0.02), and birth CHL and both skinfolds at five years (P=0.001 and 0.02 for triceps and subscapular measurements respectively). Higher AMA (muscle bulk, P=0.01) and PI (P=0.04) at birth predicted higher AMA at five years. Birth subscapular skinfold thickness remained a strong positive predictor of adipose measurements (subscapular and SS/TR, $P \leq 0.001$) at five years, even after correcting for current weight and after excluding offspring born to GDM mothers.

3.6.2 Parents' size and socio-economic status (SES): BMI of both parents had significant positive associations with offspring weight, BMI, AMA ($P<0.001$ for all), height ($P<0.05$) and skinfolds ($P<0.05$) at five years, and their heights were associated with skeletal measurements ($P<0.01$) and AMA ($P<0.05$) in children. There was a linear increase in the means of all anthropometric measurements (weight, height, head, AMA: $P<0.001$; BMI and skinfolds: $P<0.05$), except chest and abdominal circumference as the SES of the family increased. The prevalence of underweight (28% vs. 17% in the highest third of SES, $P=0.02$) and stunting (13% vs. 7%, $P=0.03$) was higher in children belonging to the lowest third of SES. All four of the overweight children belonged to the highest SES category.

3.6.3 Multiple regression: The regression model for each 5-year measurement included the corresponding birth measurement, parental height and BMI, SES, and maternal gestational glucose (GAUC) and insulin concentrations (IAUC) (Table 3.9). Except triceps, all birth measurements were positively associated with the equivalent 5-year measurements. Parents' height, BMI, and SES were positively associated with most of the measurements at five years, but had weak associations with subscapular skinfolds. Maternal IAUC was a significant predictor of offspring skinfolds (Table 3.9).

Table 3.8 Correlation coefficients between child's birth size and measurements at five-years (adjusted for sex).

	Weight	Height	Sitting height	Leg length	BMI	Head	MUAC	Chest	Abdomen	Waist	Triceps	Subscapular
Birth weight	0.36	0.28	0.29	0.21	0.29	0.33	0.25	0.27	0.29	0.27	0.11	0.16
Crown-heel length	0.32	0.37	0.36	0.29	0.14	0.27	0.16	0.22	0.23	0.22	0.05	0.07
Crown-rump length	0.33	0.30	0.34	0.20	0.21	0.31	0.21	0.25	0.25	0.23	0.09	0.09
Leg length	0.09	0.18	0.14	0.19	-0.04	0.04	-0.02	0.03	0.04	0.05	-0.03	-0.004
Ponderal index	0.08	-0.09	-0.07	-0.09	0.21	0.11	0.14	0.08	0.09	0.08	0.09	0.12
Head	0.25	0.19	0.19	0.14	0.21	0.44	0.18	0.18	0.19	0.18	0.07	0.08
MUAC	0.28	0.15	0.19	0.08	0.29	0.24	0.25	0.16	0.21	0.17	0.10	0.18
Chest	0.27	0.21	0.19	0.18	0.22	0.25	0.17	0.24	0.25	0.22	0.10	0.15
Abdomen	0.25	0.16	0.16	0.13	0.21	0.24	0.16	0.20	0.23	0.21	0.09	0.16
Triceps	0.19	0.11	0.14	0.05	0.19	0.16	0.15	0.09	0.13	0.11	0.11	0.19
Subscapular	0.18	0.08	0.13	0.03	0.21	0.14	0.15	0.12	0.18	0.16	0.13	0.22

'p' significant at 0.05, <0.001; p not significant

Table 3.9 Multiple regression analysis to show the predictors of anthropometry at five years (adjusted for sex)

		Weight (kg)	Height (cm)	BMI (kg/m^2)	Head circumference (cm)	AMA (cm^2)	Triceps (mm)	Subscapular (mm)
Birth size*	β	1.3	0.62	0.05	0.48	0.36	1.01	1.06
	P	**<0.001**	**<0.001**	**0.01**	**<0.001**	**<0.001**	**0.4**	**<0.001**
SES (score)	β	0.06	0.16	0.02	0.04	0.03	1.004	1.003
	P	**0.001**	**<0.001**	**0.1**	**0.003**	**0.06**	**0.09**	**0.2**
MOTHERS								
Height (cm)	β	0.06	0.18	0.01	0.01	0.01	1.01	1.003
	P	**0.001**	**<0.001**	**0.3**	**0.3**	**0.4**	**0.02**	**0.2**
BMI (kg/m^2)	β	0.04	0.05	0.04	-0.03	0.04	1.004	1.002
	P	**0.04**	**0.2**	**0.004**	**0.02**	**0.04**	**0.2**	**0.4**
Gestational GAUC	β	-0.0002	-0.001	0.00	0.00	-0.0002	1.00	-1.00
(mmol)	**P**	**0.6**	**0.5**	**0.97**	**0.998**	**0.6**	**0.99**	**0.7**
Gestational IAUC	β	0.000	0.00	0.00	0.00	0.00	1.00	1.00
	P	**0.02**	**0.4**	**0.007**	0.02	**0.4**	**0.001**	**0.02**
FATHERS								
Height (cm)	β	0.04	0.17	-0.02	0.02	0.02	-1.003	-1.003
	P	**0.01**	**<0.001**	**0.08**	**0.05**	**0.1**	**0.053**	**0.2**
BMI (kg/m^2)	β	0.04	0.04	0.03	0.03	0.06	1.003	1.001
	P	**0.2**	0.4	**0.09**	**0.1**	**0.02**	**0.4**	**0.9**

* Corresponding birth measurement, exponentiated β for skinfold measurements.

3.7 Summary of main findings

Anthropometric characteristics of the Mysore neonates were similar to those described for babies from other parts of India. Smaller size of the Mysore children at five years may be due to different sampling procedures in the two studies. The diverse genetic makeup of populations in different parts of India and a broad range of socio-economic status in Mysore may be other reasons for these small differences.

Comparison of Mysore neonates with white Caucasian newborns confirmed the 'muscle-thin, but adipose' or 'thin fat' phenotype of Indian neonates. Relatively larger crown heel length and head compared to birth weight may suggest asymmetric restriction of some tissues.

Despite remaining smaller, Mysore children continued to deposit more adipose tissue in later years. The subscapular skinfold thickness of the Mysore children was larger than UK and Dutch standards at 5 years of age. Head circumference was small relative to other body components postnatally.

The prevalence of obesity based on global BMI cut off was negligible in this population. Indeed, there was a high prevalence of underweight. The prevalence of underweight and stunting were highest among lower socio-economic categories.

Offspring of gestational diabetic mothers were larger at birth than the rest of the cohort in all measurements, especially in measurements of truncal adiposity. Truncal adiposity diminished during infancy, but recurred in female ODM. By 5 years, girls born to GDM mothers were significantly larger than control girls in weight, BMI, MUAC and skinfold measurements.

In marked contrast, offspring of diabetic fathers were smaller than control babies at birth. Their anthropometric characteristics were similar to controls during subsequent years.

Maternal insulin concentrations were associated with childhood adiposity even in control children. A significant 'U' shaped relationship was seen between maternal glucose concentrations and offspring's skinfold measurements. Similar, but weaker associations were also seen between father's fasting insulin and offspring anthropometry at 5 years.

Children who were born small and became heavy at 5 years had increased triceps skinfold measurements. Subscapular skinfold thickness at birth was a strong positive predictor of adiposity at 5 years even after adjusting for parental skinfolds and socio-economic status.

3.8 Discussion

3.8.1 Newborn anthropometry – the 'thin-fat' phenotype: The babies born in Mysore were on average 400-500g lighter at birth than babies born in Southampton. The low mean birth weight of Indian babies is well documented and is mainly caused by intra-uterine growth retardation rather than prematurity[117]. The detailed measurements at birth in our study revealed that the smallness of our babies compared with white Caucasian newborns was not proportional. The babies were small and thin with small arm (a measure of muscle bulk), abdominal (an indicator of visceral size) and head circumferences, but were relatively long and adipose. A similar neonatal phenotype was observed in Pune, and it was suggested that muscle and abdominal viscera are the most

'sacrificed' tissues in intra-uterine growth retardation, while fat is relatively preserved[118]. The authors suggest that this fat may have survival advantages for small babies at birth by acting as an energy reserve, helping maintain body temperature, and providing substrates for brain development. Similar results were shown in a study in the UK of immigrant Asian Indians in 1981, but were not remarked upon[149].

3.8.2 Mechanisms for neonatal adiposity: Several mechanisms, acting either independently or collectively, might be responsible for this particular neonatal phenotype. It could be due to the natural selection of a 'thrifty gene' in the past when the food supply was unreliable[23]. Such a gene, which may store surplus calories as fat for future utilization might have persisted though the present nutritional conditions do not require it. Another possible explanation for relative adiposity could be that it is an adaptive mechanism by which the small Indian fetus maximizes its survival chances in the face of inadequate nutrition. An adverse intra uterine environment induces a series of changes in the fetus to help divert the energy supply to vital organs like the brain (Section 1.2.2). Peripheral insulin resistance is one such mechanism, which channels substrate away from the viscera and skeletal muscles[150]. Low muscle mass and truncal adiposity could be mechanisms adopted by the fetus to achieve this. This may be the basis of relatively larger head circumference compared to weight and MUAC in our neonates. A third possible explanation for the phenotype is 'metabolic incompetence'; specific nutritional deficiencies, possibly secondary to maternal under-nutrition could render the fetus unable to lay down lean tissue mass, so energy becomes deposited as fat[151]. Few of our mothers were energy deficient, but Indian women are known to have high prevalence of micronutrient deficiencies[152] and may be able to provide only substrates fit for generating adiposity in the fetus.

3.8.3: Tracking of the neonatal phenotype: The reasons for apparent diminution of adiposity in the first year of life in our children are not clear. I have not come across any studies that could shed light on this phenomenon. However, the adiposity produced by maternal gestational diabetes in the offspring is also masked in infancy[90]. Once released from the constraints of the intra-uterine environment, the infant may change its growth trajectory and utilize the available nutrition for comprehensive growth, thus minimizing the fat development as against other body composition. However, resurfacing of excessive truncal fat later in childhood is a new finding in my cohort. The fat storage

path laid down in intra-uterine life might persist and become manifest as increased adiposity during childhood.

3.8.4: Obesity and under-nutrition. Relevance of global standards: Despite the above findings, the prevalence of underweight and stunting was high in this cohort, while that of obesity and overweight based on global BMI standards was negligible. Obesity is commoner among lower socio economic groups in developed countries[153], but in countries like India under nutrition is a problem in deprived sections whereas obesity tends to increase with higher socio economic conditions. Despite underweight status being more common (28%) among lower socio-economic category in this cohort, the percentage of underweight children was still high (17%)in the higher socio-economic category (Section 3.6.2).

The global standards of obesity and overweight for adults have been shown to be inappropriate for south Asians and new standards were recommended for them[154]. However, no efforts have been made to assess if the same is true in children. The prevailing obesity standards may fail to detect the 'thin-fat' phenotype in these children.

3.8.5 Causes for adiposity in study children-Maternal GDM: One of the obvious causes for adiposity in my study children was maternal gestational diabetes. Even though the power to detect the differences was low due to a small group of diabetic mothers, a recurrence of adiposity was observed in female ODM. The absence of similar associations in the offspring of diabetic fathers support the intra-uterine programming effects of maternal GDM on offspring adiposity. Long-term effects of GDM on offspring obesity have been described by researchers in developed countries (Section 1.3.2). However, most of the data come from studies among Pima Indians, a population predisposed to obesity and type 2 diabetes.

Increased fuel transfer from mother to fetus is known to induce an intra-uterine overfed state, rendering the fetus macrosomic (Section 1.3.1). Maternal hyperglycaemia also induces fetal hyperinsulinaemia, which is an important growth-regulating hormone in the fetus. The mechanism of tracking of adiposity in to later life is not clear. A study of newborn infants in 1960 showed that when fasted, infants of diabetic mothers tend to burn more protein than normal infants, despite having more energy reserves in the form of fat[155]. It was suggested that their hyperinsulinism may slow postnatal mobilisation of

fat. Another explanation could be increased appetite postnatally. Animal models have shown that GDM alters the neurotransmitter release during critical periods of fetal hypothalamic development and renders the offspring hyperphagic subsequently[99,100]. Genes related to obesity in the mother could be another cause for this phenomenon.

There is good evidence that the increased relative weight of ODM is mainly due to increased adiposity[156]. Effects on lean tissue are debated. In our study, female ODM had larger skinfolds than controls. Hunter *et al*, using bioimpedance, observed a higher percentage body fat, but not lean mass, in a small study comparing offspring of pre-gestational type 2 diabetic mothers with ONDM[157]. Durnwald *et al* observed *reduced* lean mass in large-for-gestational-age infants of diabetic mothers[156]. In another study, large-for-gestational-age ODM had larger arm circumferences, as well as skinfolds at seven years than controls[93]. Larger arm circumferences in girl ODM in my study may be due to subcutaneous fat, or may reflect a positive influence of maternal GDM on lean tissue growth.

Since our ODM group was small, apparently greater effects in females may be a chance finding. Alternatively, there may be differences between the sexes. An earlier study suggested that girls contributed most to the differences observed in ODM during early childhood[104]. I can only speculate about the causes of any sex difference. They could be metabolic/endocrine, or behavioral. Girls may be less physically active than boys in this population, though there is no data to support this. Additionally, the closeness of girls to their mothers may have further behavioral effects, as women with GDM may exercise less or have unhealthy diets. If the impact is, indeed, greater in females, this is of concern as they may go on to develop GDM themselves, perpetuating an inter-generational cycle.

This study showed that neonates of diabetic fathers were lighter than control newborns. Several recent studies have described this phenomenon (Section 1.2.4). This is relevant to the well-described association between low birth weight and later insulin resistance and type 2 diabetes (Section 1.2). My study suggests that genes could, at least partly, explain the low birth weight-type 2 diabetes link.

3.8.6 Control offspring: As maternal hyperglycaemia is an important factor inducing fetal overgrowth in GDM, a similar, if smaller, effect could occur when maternal glucose concentrations increase even in the non-diabetic levels. This has been observed for birth measurements earlier in a study in Scotland, and for childhood size in the Pima Indian children (Section 1.3.6). Although not strong at birth, in my study, there was an increase in 5-year anthropometry in control children with an increase in maternal insulin concentrations from lower to higher quartiles. Since the majority of these women were young at the time of pregnancy, strong associations seen with higher insulin concentrations may be due to higher maternal insulin resistance. Adiposity tended to be higher in both higher and lower ranges of maternal glucose concentrations. Lower maternal glucose in these instances may be due to higher insulin secretion maintaining glucose levels in these women. On the other hand, these, indeed, were the children with lowest birth weights, and may be representing the left end of 'U' shaped association as discussed in introduction to the present chapter.

There were similar, albeit weaker, findings in relation to paternal insulin concentrations. Weaker trends for fathers could be because their insulin profile was less completely characterized than for mothers. The data from my study, therefore, are equally compatible with genetic transmission of adiposity and/or insulin resistance from either parent and this issue needs further research. I did not come across any comparable data for fathers.

3.8.7 Size at birth and childhood adiposity: All the measures of adiposity increased significantly with size at birth. Adiposity at birth, as measured by subscapular thickness was the strongest predictor of truncal adiposity at 5 years. Thus any factor giving rise to adiposity at birth might indirectly influence the rise in childhood adiposity. I found an inverse relationship between current triceps skinfold and birth weight only after adjusting for current weight; negative associations were stronger with birth CHL. Small weight at birth has been shown to be associated with measures of central/truncal adiposity in adults[135]; this tended to occur in association with high present size. The findings in my study are in line with the associations noticed among other studies.

Conclusion: I have shown that the children of Mysore accumulate truncal body fat disproportionate to their weight and BMI. The future course of childhood adiposity is still not clear. Nevertheless, a few studies have shown increased incidence of adult obesity in obese children[44]. Persistence of this phenotype in the study children may have adverse implications for later obesity and related disorders in them. I have shown that defining overweight and underweight based on the prevailing obesity standards may under estimate their true adiposity. Although BMI is a widely used index of adiposity, its validity is debatable as it could be a measure of both lean and adipose tissue. I propose the need for better understanding of the childhood phenotype of Indian children to formulate more useful growth indices.

The findings described in this chapter have been published:

Krishnaveni GV, Hill JC, Veena SR, Leary SD, Saperia J, Chachyamma KJ, Karat SC, Fall CHD. Truncal adiposity is present at birth and in early childhood in south Indian children. *Indian Pediatrics* 2005;42:527-538.

4. GLUCOSE AND INSULIN CONCENTRATIONS AT 5-YEARS

Insulin resistance, the precursor of type 2 DM rises with increasing age and adiposity. Evidence from many parts of the world associates small size at birth, especially low birth weight, with increased insulin resistance in both children and adults, and higher rates of type 2 DM in adults (Section 1.2.3). Different theories have been proposed as the cause of this relationship, including the 'fetal programming hypothesis' (Section 1.2.2), and the 'fetal insulin hypothesis' (Section 1.2.4). A few studies also showed that small size at one year and accelerated growth in later childhood years increase the risk of obesity and IGT/type 2 DM (Section 1.2.5).

Some observed a 'U' shaped association, with higher insulin resistance and/or type 2 DM in both low and high birth weight individuals, suggesting maternal GDM as the cause of the latter association (Section 1.3.1). Whether offspring risks of maternal GDM are related to intra-uterine programming or genes is still debated.

More recently, demonstration of association between current size, and elevated risk markers of type 2 DM, such as insulin resistance in children (Section 1.2.3) may provide scope for early intervention to prevent subsequent disease.

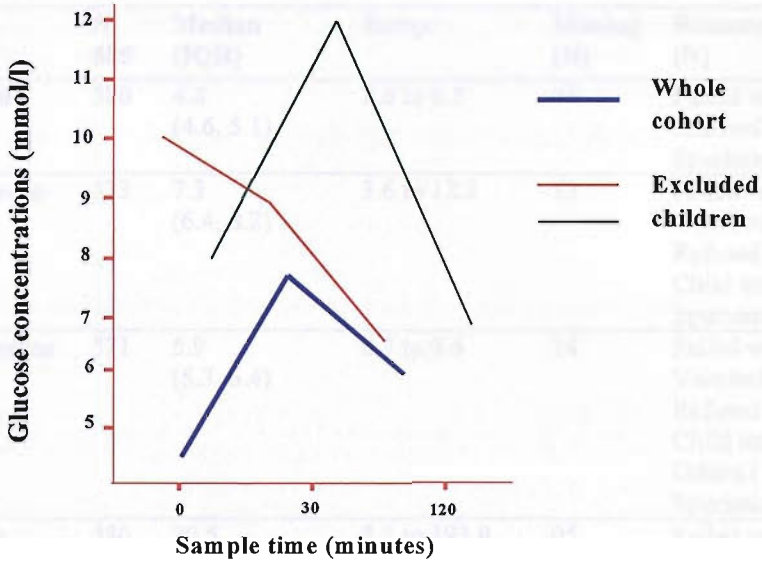
In this chapter I describe the distributions of glucose and insulin concentrations and insulin resistance at 5 years in the Mysore cohort, and examine factors influencing these outcomes. I discuss some of the topics mentioned above while comparing my data to that from similar studies done elsewhere and examine whether:

- Children born to mothers with GDM have elevated plasma glucose and insulin concentrations at 5 years of age
- Weaker, but significant effects on insulin concentrations are seen in relation to the mother's gestational glucose and insulin concentrations, even in the absence of maternal GDM
- Small size at birth and one year predicts higher plasma insulin concentrations at 5-years.

4.1 Data preparation

4.1.1 Data checking and cleaning: Range checks showed that two children had unusually high fasting plasma glucose concentrations. This could have been because the children did not fast or due to errors in sampling procedures or laboratory analysis. These values were deemed spurious and the GTT was considered not valid (Figure 4.1).

Figure 4.1 Mean glucose concentrations of the whole cohort and excluded values



4.1.2 Statistical methods: Glucose concentrations and HbA1c were normally distributed. The distributions of the insulin concentrations at all time points, insulin resistance, and insulin increment at 30-minutes were log-normalised. T-tests were used to compare means between the sexes, and ODM and controls. Linear regression analyses were used to determine the predictors of glucose and insulin concentrations. Determinants of IGT status were tested by logistic regression. For logged variables, exponentiated regression co-efficients (β) were quoted to give the proportion of change rather than the actual effect size.

Diabetes mellitus was defined as a fasting glucose concentration ≥ 7.0, and/or 120-minute glucose ≥ 11.1 mmol/l. Impaired glucose tolerance (IGT) was a fasting glucose concentration < 7.0 mmol/l and 120-minute glucose ≥ 7.8 mmol/l, but < 11.1 mmol/l. A fasting glucose of ≥ 6.1mmol/l was defined as Impaired fasting glucose (IFG).

4.2.Description

Glucose tolerance test results were available for 580 of the 585 children who participated in the study. Of these, complete data were available for 571 children. The number of samples available for each analysis and the reasons for missing values are given in the Table 4.1.

Table 4.1 Total number of samples available, reasons for missing data, and the description of glucose and insulin concentrations

Total	N 585	Median (IQR)	Range	Missing (N)	Reasons for missing (N)
Fasting glucose (mmol/l)	580	4.8 (4.6, 5.1)	2.6 to 6.5	05	Failed venepuncture (1) Refused (2) Spurious (2)
30-minute glucose (mmol/l)	573	7.3 (6.4, 8.2)	3.6 to 12.3	12	Failed venepuncture (3) Vomited (2) Refused (4) Child unwell (1) Spurious (2)
120-minute glucose mmol/l)	571	5.9 (5.3, 6.4)	2.3 to 9.6	14	Failed venepuncture (4) Vomited (2) Refused (4) Child unwell (1) Others (1) Spurious (2)
Fasting Insulin (pmol/l)	580	20.5 (13.0, 30.7)	3.2 to 193.9	05	Failed venepuncture (1) Refused (2) Spurious (2)
30-minute Insulin (pmol/l)	573	149.9 (90.4, 229.7)	4.8 to 1025.0	12	Failed venepuncture (3) Vomited (2) Refused (4) Child unwell (1) Spurious (2)
120-minute Insulin (pmol/l)	569	85.1 (56.5, 127.3)	8.6 to 797.9	16	Failed sample (4) Vomited (2) Refused (4) Sick child (1) Others (1) Spurious (2) Insufficient sample (2)
HOMA (insulin resistance)	579	0.7 (0.5, 1.1)	0.1 to 7.1	08	
Insulin Increment (pmol/mmol)	573	26.7 (14.8, 42.1)	-10.7 to 193.2	14	
HbA1c (%)	390	5.6 (5.3, 5.8)	4.1 to 8.4	195	Facility not available at the start of the study

4.3 Abnormal glucose tolerance

None of the children had diabetes. Four children (all boys) had IFG, and 22 children (12 boys and 10 girls, P=0.5) had IGT. Seventy-five children had above normal (>6.0) HbA1c concentrations.

4.3.1 Impaired Glucose Tolerance: There were no statistically significant differences in anthropometry between children with IGT and normal glucose tolerance (NGT) (Table 4.2). By definition, children with IGT had higher 120-minute glucose concentrations than children with NGT. They also had higher 30-minute glucose and 120-minute insulin concentrations. Their insulin concentrations at 30 minutes were lower than for the NGT children, though this was not statistically significant. HbA1c was similar in both groups.

Table 4.2 Anthropometry, and glucose and insulin concentrations of children with and without IGT

Measurements	IGT (22)		NGT (549)		'P'
Weight (kg)	**15.3**	(2.0)	**15.2**	(1.9)	0.7
CHL (cm)	**105.8**	(3.4)	**105.6**	(4.3)	0.8
BMI (cm)	**13.7**	(1.2)	**13.6**	(1.1)	0.7
Head (cm)	**49.0**	(1.0)	**48.5**	(1.4)	0.08
MUAC (cm)	**15.4**	(1.2)	**15.3**	(1.2)	0.9
Chest (cm)	**49.8**	(1.9)	**50.1**	(2.3)	0.6
Triceps (mm)*	**7.6**	(6.9, 8.2)	**7.7**	(6.6, 8.9)	0.7
Subscapular (mm)*	**6.2**	(5.2, 6.9)	**5.9**	(4.9, 7.0)	0.5
Glucose (mmol/l)					
0-minute	**4.8**	(0.7)	**4.8**	(0.5)	0.9
30-minute	**7.9**	(1.9)	**7.3**	(1.4)	0.04
120-minute	**8.2**	(0.4)	**5.8**	(0.9)	<0.001
HbA1c (%)	**5.5**	0.4	**5.5**	0.5	0.9
Insulin (pmol/l)*					
0-minute	**19.1**	(8.9,36.5)	**19.8**	(13.0,30.4)	0.8
30-minute	**112.8**	(43.9,212.7)	**139.4**	(91.9,230.9)	0.2
120-minute	**189.5**	(124.9,254.5)	**79.5**	(55.2,123.8)	<0.001
Insulin resistance* (HOMA)	**0.7**	(0.3,1.3)	**0.7**	(0.5,1.1)	0.8
Insulin increment* (pmol/mmol)	**28.2**	(9.2,37.2)	**30.4**	(14.8,42.4)	0.7

Mean (SD), or *geometric mean (IQR)

4.4 Age and sex on glucose and insulin concentrations

There were no significant effects of age on glucose and insulin concentrations. Glucose concentrations were similar in boys and girls, while all the insulin values were significantly higher in girls (Table 4.3).

Table 4.3 Glucose and insulin concentrations in male and female children

N Max=	Males 278	Females 302	'P'
Glucose (mmol/l)			
0-minute	**4.8** (0.47)	**4.8** (0.5)	0.2
30-minute	**7.2** (1.45)	**7.4** (1.4)	0.2
120-minute	**5.9** (1.02)	**5.9** (0.9)	0.5
HbA1c (%)	**5.6** 0.5	**5.5** 0.5	0.8
Insulin (pmol/l)*			
0-minute	**18.1** (11.4,28.4)	**22.6** (15.3,52.9)	<0.001
30-minute	**120.2** (5.1,214.7)	**168.4** (108.3,247.0)	<0.001
120-minute	**80.0** (49.9,117.6)	**92.5** (62.7,138.4)	<0.001
Insulin resistance (HOMA)*	**0.7** (0.40,1.01)	**0.8** (0.5,1.2)	<0.001
Insulin increment (pmol/mmol)*	**27.5** (12.4,38.5)	**33.0** (17.8,45.2)	0.006

Mean (SD), or geometric mean (IQR)

4.5 Anthropometry and glucose and insulin concentrations

In the following section, I describe glucose and insulin concentrations in relation to body components indicative of adiposity, skeletal size, and lean mass. Associations were similar in boys and girls; hence the sexes were combined for analyses.

4.5.1 calculations: Fat mass at five years was calculated using a skinfold equation based on pre-school Indian children: ***weight x (5.304 + 0.269 x triceps + 0.50 x subscapular + 0.685 x MUAC - 0.063 x age in months)/100 (for boys), and weight x (7.017 - 0.053 x triceps + 0.201 x subscapular + 0.765 MUAC + 0.052 x age in months)/100 (for girls)***[158]. This equation has been validated using 'deuterated water' method. Lean mass was calculated as the residual of weight after regressing it with height (skeletal measurement) and a measurement of fat (subscapular skinfold thickness). I used this rather than lean mass obtained by subtracting fat mass from weight, as the latter is a combination of skeletal mass also, and analyses using this variable may give an exact opposite effect to that of fat mass. AMA was used as a surrogate of muscle mass.

4.5.2 Measurements of Adiposity (Table 4.4): Larger subscapular and triceps skinfolds, and fat mass were associated with higher glucose and insulin concentrations. Subscapular to triceps ratio was related only to glucose and insulin concentrations at 120 minutes. No significant associations were seen between measurements of adiposity and HbA1c %.

Table 4.4 Mean plasma glucose and insulin concentrations according to subscapular and triceps skinfolds, SS/TR, and fat mass in quartiles

	Glucose (mmol/l)			Insulin (pmol/l)			Others		
	0 mins	30 mins	120 mins	0 mins	30 mins	120 mins	Insulin resistance (HOMA)	Insulin increment	HbA1c
Subscapular Skinfold (mm)									
<4.9	4.8	7.0	5.7	20.7	152.1	79.3	0.74	27.6	5.6
- 5.6	4.8	7.2	5.8	22.5	173.0	98.5	0.80	31.8	5.6
- 7.0	4.9	7.3	6.0	25.8	184.7	103.0	0.94	32.3	5.5
>7.0	4.8	7.6	6.1	30.3	218.4	132.7	1.10	39.5	5.5
β	0.02	0.12	0.09	1.06	1.07	1.07	1.07	1.01	-0.01
P	0.03	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.5
Triceps Skinfold (mm)									
<6.6	4.7	7.1	5.9	21.7	153.8	91.2	0.8	27.7	5.6
- 7.7	4.8	7.2	5.9	23.9	171.2	93.9	0.9	30.6	5.5
- 8.9	4.8	7.6	5.8	24.0	178.6	100.0	0.9	32.3	5.5
>8.9	4.9	7.4	6.0	30.0	225.7	128.6	1.1	40.8	5.6
β	0.02	0.08	0.04	1.05	1.08	1.05	1.05	1.02	-0.01
P	0.02	0.004	0.07	<0.001	<0.001	0.001	<0.001	<0.001	0.7
SS/TR									
<0.67	4.8	7.3	5.8	23.9	183.9	100.9	0.8	33.5	5.6
- 0.76	4.8	7.2	5.8	24.4	179.6	97.3	0.9	32.5	5.5
- 0.87	4.8	7.2	6.0	23.4	183.2	102.6	0.8	33.5	5.6
>0.87	4.8	7.4	6.0	27.6	180.7	111.5	1.0	31.6	5.5
β	0.03	0.53	0.76	1.19	1.01	1.58	1.24	-1.04	-0.06
P	0.8	0.1	0.003	0.3	0.97	0.01	0.3	0.5	0.7
Fat mass (kg)									
<2.5	4.8	7.1	6.0	19.2	130.2	81.7	0.7	23.2	5.6
-3.0	4.8	7.3	5.8	23.6	160.0	88.0	0.8	28.9	5.5
-3.4	4.8	7.3	5.8	24.3	194.9	104.2	0.9	35.4	5.6
>3.4	4.9	7.5	6.0	32.3	242.3	139.0	1.2	43.5	5.5
β	0.10	0.24	0.12	1.30	1.29	1.46	1.34	1.09	-0.04
P	0.003	0.02	0.09	<0.001	<0.001	<0.001	<0.001	<0.001	0.5

In this and the following tables (4.5 to 4.7) sexes were combined, P values were derived using linear regression and all variables as continuous, adjusted for sex. β values were exponentiated for insulin concentrations, HOMA and increment.

4.5.3 Skeletal measurements: Height at 5 years was a strong determinant of insulin concentrations (Table 4.5). This was true for both truncal (sitting height, P<0.001)) and leg lengths (P<0.005). Sitting height was also positively associated with 30-minute glucose concentrations (P=0.05). Larger head circumference predicted higher 30-minute insulin concentrations and insulin increment. No significant associations were seen with glucose concentrations and HbA1c.

Table 4.5 Mean plasma glucose and insulin concentrations according to height and head circumference in quartiles

	Glucose (mmol/l)			Insulin (pmol/l)			Others		
	0 mins	30 mins	120 mins	0 mins	30 mins	120 mins	Insulin resistance (HOMA)	Insulin increment	HbA1c
Height(cm)									
<102.8	4.8	7.3	5.9	22.9	162.7	85.4	0.80	29.3	5.6
- 105.5	4.8	7.1	5.8	22.8	146.7	89.0	0.81	26.3	5.5
- 108.3	4.9	7.3	6.0	25.0	192.7	114.7	0.91	34.65	5.6
>108.3	4.8	7.4	6.0	27.4	225.3	122.4	1.0	41.1	5.5
β	0.01	0.01	0.01	1.03	1.03	1.03	1.03	1.01	-0.003
P	0.2	0.4	0.3	<0.001	<0.001	<0.001	<0.001	<0.001	0.6
Head circumference (cm)									
<47.5	4.8	7.3	5.9	25.5	178.6	95.1	0.90	32.2	5.6
- 48.4	4.8	7.2	5.8	25.0	166.3	104.7	0.89	29.3	5.5
- 49.5	4.9	7.4	5.9	25.7	178.9	97.3	0.93	31.7	5.5
>49.5	4.8	7.4	6.0	23.4	205.8	115.8	0.85	38.1	5.6
β	0.002	0.03	0.03	-1.01	1.05	1.04	-1.01	1.01	-0.002
P	0.9	0.5	0.3	0.7	0.04	0.07	0.8	0.05	0.9

4.5.4 Measurements of lean and muscle mass: Larger AMA predicted higher insulin concentrations at all time points, insulin resistance and insulin increment (Table 4.6). There was a negative association between lean residual and glucose concentration at 120 minutes, and it was positively associated with insulin concentrations (0 and 30 minutes), HOMA and insulin increment. No associations were seen with HbA1c levels.

Table 4.6 Mean plasma glucose and insulin concentrations according to AMA and lean residual in quartiles

	Glucose (mmol/l)			Insulin (pmol/l)			Others		
	0 mins	30 mins	120 mins	0 mins	30 mins	120 mins	Insulin resistance (HOMA)	Insulin increment	HbA1c
AMA (cm^2)									
<11.9	4.8	7.2	5.9	22.8	139.2	84.5	0.8	24.6	5.6
- 13.0	4.8	7.5	5.9	21.9	179.9	104.1	0.8	33.2	5.6
- 14.1	4.9	7.3	5.9	24.9	188.0	105.2	0.9	33.7	5.5
>14.1	4.8	7.2	5.9	29.8	219.6	118.5	1.1	39.3	5.5
β	0.01	0.01	-0.01	1.06	1.10	1.05	1.07	1.02	-0.01
P	0.3	0.8	0.7	<0.001	<0.001	0.001	<0.001	<0.001	0.5
Lean residual									
<-0.68	4.8	7.4	6.0	24.0	162.6	99.3	0.9	29.2	5.6
- -0.03	4.8	7.5	5.9	23.6	181.7	106.6	0.8	32.9	5.6
- 0.62	4.8	7.1	5.9	24.5	167.8	95.7	0.9	30.4	5.5
>0.62	4.9	7.2	5.8	27.2	215.3	111.0	1.0	38.6	5.5
β	0.03	-0.10	-0.09	1.06	1.11	1.00	1.06	1.02	-0.01
P	0.1	0.3	0.03	0.04	0.002	0.99	0.03	0.002	0.6

4.5.5 Composite measurements (Table 4.7): Weight, BMI, MUAC, chest, abdominal and supine waist circumference were positively associated with insulin concentrations, insulin increment and HOMA insulin resistance (P<0.01 for all). Except BMI, others were also associated with higher fasting glucose concentrations.

Table 4.7 Mean plasma glucose and insulin concentrations according to weight in quartiles

	Glucose (mmol/l)			Insulin (pmol/l)					
	0 mins	30 mins	120 mins	0 mins	30 mins	120 mins	Insulin resistance (HOMA)	Insulin increment	HbA1c
Weight (kg)									
<13.8	4.8	7.3	5.9	22.9	143.8	83.8	0.80	25.5	5.6
- 14.9	4.8	7.2	5.9	21.5	167.8	94.6	0.77	30.8	5.6
- 16.3	4.8	7.2	5.9	24.6	173.8	101.7	0.89	30.9	5.5
>16.3	4.9	7.5	6.0	30.3	241.3	132.1	1.10	43.7	5.6
β	0.03	0.05	0.03	1.08	1.12	1.08	1.09	1.03	-0.01
P	0.009	0.1	0.2	<0.001	<0.001	<0.001	<0.001	<0.001	0.4

4.5.6 Multiple regression: Since the measurements at 5-years were highly correlated with one another, the effects observed of any body component on outcome variables may be confounded by other measurements. Hence, I included selected measures of body components (fat, lean and skeletal size) into a multiple regression model along with parental skinfolds and SES of the family. Fat mass was the strongest positive predictor of glucose, and insulin concentrations, insulin increment and insulin resistance even after adjusting for other components of body size, and parental factors (Table 4.8). Smaller head circumference at five years was associated with higher fasting insulin, insulin resistance and insulin increment. Negative associations were seen between lean residual and 30- and 120-minute glucose concentrations. Negative associations were also present between height and 30-minute glucose concentrations (Table 4.8).

4.6 Relationships to parents' size, and socio-economic status (SES)

Paternal skinfolds were positively associated with children's 30-minute glucose concentrations ($P=0.006$), and insulin concentrations at all time points ($P<0.05$), insulin resistance ($P=0.001$) and insulin increment ($P<0.001$). SES ($P<0.01$) and maternal skinfolds ($P<0.05$) were positively associated with 30-minute insulin and insulin increment. Higher SES was associated with an increased risk of IGT ($exp(B)=1.1$, $P=0.04$). The majority of the above associations were not statistically significant after adjusting for the children's body composition. Paternal skinfolds were positively associated with 30-minute insulin, HOMA and insulin increment (Table 4.8).

Table 4.8. Multiple regression analysis of child's glucose and insulin concentrations with child's current anthropometry and parental characteristics (adjusted for sex).

		Glucose (mmol/l)			Insulin (pmol/l) *			Insulin* resistance (HOMA)	Insulin * Increment	HbA1c
		0 mins	30 mins	120 mins	0 mins	30 mins	120 mins			
Child's height (cm)	**β**	-0.001	-0.05	-0.03	1.005	1.002	1.01	1.01	1.003	0.002
	P	**0.8**	**0.03**	**0.07**	**0.7**	**0.9**	**0.6**	**0.6**	**0.3**	**0.8**
Head circumference (cm)	**β**	-0.03	-0.03	0.01	-1.11	-1.05	-1.02	-1.11	-1.01	0.01
	P	**0.2**	**0.6**	**0.8**	**<0.001**	**0.09**	**0.3**	**<0.001**	**0.04**	**0.6**
Fat mass (kg)	**β**	0.14	0.55	0.41	1.35	1.40	1.30	1.38	1.07	-0.02
	P	**0.03**	**0.002**	**0.002**	**<0.001**	**0.001**	**0.004**	**<0.001**	**0.002**	**0.8**
Lean residual	**β**	0.01	-0.20	-0.21	-1.003	1.04	-1.06	1.002	1.01	-0.02
	P	**0.9**	**0.02**	**0.001**	**0.9**	**0.4**	**0.1**	**0.97**	**0.2**	**0.6**
Maternal sum of skinfolds(mm)	**β**	-0.003	0.0004	-0.001	1.0004	1.0001	1.0002	1.0005	1.00	0.0001
	P	**0.6**	**0.8**	**0.4**	**0.6**	**0.9**	**0.8**	**0.5**	**0.9**	**0.8**
Paternal sum of skinfolds(mm)	**β**	0.001	0.005	-0.001	1.002	1.003	1.002	1.002	1.001	0.001
	P	**0.2**	**0.06**	**0.6**	**0.07**	**0.02**	**0.2**	**0.04**	**0.01**	**0.3**
Socio-economic status	**β**	0.002	0.03	0.01	1.005	1.01	1.003	1.01	1.001	-0.004
	P	**0.6**	**0.05**	**0.5**	**0.5**	**0.2**	**0.7**	**0.4**	**0.5**	**0.5**

*Exponentiated co-efficients

4.7 Relationships with maternal gestational glucose tolerance

4.7.1 Offspring of diabetic mothers: Of 22 children who had IGT, 4 (all girls) were born to GDM mothers (11.4% vs. 3.2% in controls, OR=4.0, 95% CI 1.2-13.2, P=0.02 adjusting for sex and BMI).

Generally, insulin concentrations were higher in ODM than in control children (Table 4.9). The differences were more pronounced in female ODM; statistically significant differences were seen for insulin concentrations at 30 and 120 minutes, and insulin increment when compared to control girls (Table 4.9). Differences remained significant for 120-minute insulin concentrations after adjusting for maternal BMI (P=0.04) and borderline significant after adjusting for maternal skinfolds (P=0.08). There were no differences in boys. Glucose concentrations and HbA1c were similar in both groups (Table 4.9).

4.7.2 Offspring of diabetic fathers: Girls born to diabetic fathers had a higher prevalence of IGT (Table 4.9). In contrast to ODM, offspring of diabetic fathers had significantly *lower* 120-minute glucose and insulin concentrations (only boys, Table 4.9). There were no differences between offspring of diabetic fathers and control children in other glucose and insulin variables, or HbA1c.

Table 4.9. Glucose and insulin concentrations according to maternal GDM and paternal diabetes status

	Offspring of Diabetic Mothers			P†			Control children			P‡			Offspring of Diabetic fathers		
	Total	**Girls**	**Boys**	**Total**	**Girls**	**Boys**	**Total**	**Girls**	**Boys**	**Total**	**Girls**	**Boys**	**Total**	**Girls**	**Boys**
N Max=	**35**	**22**	**13**				**474**	**243**	**231**				**41**	**21**	**20**
Plasma glucose (mmol/l)															
Fasting	**4.8** (0.5)	**4.7** (0.6)	**4.9** (0.4)	0.8	0.5	0.5	**4.8** (0.5)	**4.8** (0.5)	**4.8** (0.5)	0.9	0.7	0.8	**4.8** (0.5)	**4.8** (0.4)	**4.8** (0.5)
30-minute	**7.5** (1.4)	**7.7** (1.6)	**7.3** (0.9)	0.3	0.3	0.8	**7.3** (1.4)	**7.3** (1.4)	**7.2** (1.5)	0.5	0.5	0.2	**7.1** (1.3)	**7.5** (1.3)	**6.7** (1.6)
120-minute	**6.1** (1.1)	**6.2** (1.3)	**5.9** (0.7)	0.3	0.2	0.8	**5.9** (1.0)	**5.8** (0.9)	**6.0** (1.0)	0.4	0.1	0.007	**5.7** (1.2)	**6.1** (1.1)	**5.3** (1.1)
HbA1c (%)	**5.6** (0.9)	**5.7** (1.0)	**5.4** (0.4)	0.6	0.5	0.5	**5.5** (0.5)	**5.5** 0.5	**5.6** (0.5)	0.4	0.5	0.6	**5.6** (0.4)	**5.6** (0.3)	**5.6** (0.5)
IGT (N)	**4** (11.4%)	**4** (18.3%)	**-** (0%)	0.01	<0.001	0.4	**15** (3.2%)	**4** (1.7%)	**11** (4.8%)	0.1	0.01	0.97	**3** (7.7%)	**2** (10.5%)	**1** (5%)
Plasma insulin (pmol/l)*															
Fasting	**23** (14,35)	**25** (17,36)	**20** (11,32)	0.1	0.4	0.3	**19** (12,29)	**22** (14,32)	**17** (11,27)	0.2	0.7	0.1	**22** (14,34)	**23** (18,32)	**21** (12,36)
30-minute	**172** (123,275)	**210** (141,291)	**123** (79,241)	0.1	0.05	0.97	**136** (87,225)	**152** (103,242)	**122** (73,213)	0.96	0.5	0.6	**136** (104,252)	**136** (103,242)	**135** (88,254)
120-minute	**105** (73,150)	**122** (74,171)	**82** (52,142)	0.03	0.04	0.7	**82** (57,127)	**90** (62,131)	**76** (52,121)	0.04	0.5	0.008	**63** (29,125)	**81** (62,131)	**49** (25,79)
Other insulin variables*															
Insulin resistance (HOMA)	**0.8** (0.5,1.3)	**0.9** (0.6,1.3)	**0.7** (0.3,1.0)	0.2	0.5	0.3	**0.7** (0.4,1.1)	**0.8** (0.5,1.2)	**0.6** (0.4,1.0)	0.2	0.7	0.2	**0.8** (0.5,1.3)	**0.8** (0.5,1.2)	**0.8** (0.4,1.3)
Insulin increment (pmol/mmol)	**36** (20,47)	**44** (27,54)	**26** (12,38)	0.1	0.04	0.8	**29** (14,40)	**32** (16,44)	**27** (12,38)	0.7	0.8	0.4	**31** (16,46)	**30** (16,44)	**31** (15,48)

Mean (SD) or *geometric mean (IQR), †p for the difference between offspring of diabetic mothers and controls ‡p for the difference between offspring of diabetic father and controls

4.7.3 Control children: In control children, a linear increase in insulin concentrations at 30 minutes and insulin increment was seen with an increase in maternal glucose (GAUC) and insulin (IAUC) concentrations (Table 4.10). The associations with maternal IAUC, but not GAUC, remained statistically significant after adjusting for maternal and offspring skinfolds, and socio-economic status of the family.
When maternal fasting insulin concentrations were used as a predictor, positive associations with 30-minute insulin concentrations (P=0.5), HOMA (P=0.2) and insulin increment (P=0.2) were weak, and remained non-significant after adjusting.

Positive associations were also observed between father's fasting insulin, and offspring 30-minute insulin concentrations and insulin increment at 5 years (Table 4.10). These associations were not significant after adjusting for father's and offspring skinfolds and socio-economic status.

Table 4.10 Insulin concentrations and HOMA at 5 years in the control children according to fourths of maternal GAUC, IAUC and paternal insulin

	Maternal GAUC			Maternal IAUC			Paternal fasting insulin		
	30min insulin	HOMA	Insulin increment	30min insulin	HOMA	Insulin increment	30min insulin	HOMA	Insulin increment
1 (Lowest)	**160**	**0.9**	**28**	**152**	**1.0**	**26**	**151**	**0.8**	**27**
2	**182**	**0.9**	**33**	**161**	**0.9**	**29**	**176**	**0.9**	**32**
3	**183**	**0.8**	**33**	**187**	**0.8**	**34**	**197**	**1.0**	**35**
4 (Highest)	**196**	**0.9**	**36**	**224**	**0.9**	**42**	**201**	**0.9**	**36**
P*	0.04	0.6	0.06	<0.001	0.998	<0.001	0.04	0.02	0.01
P†	0.3	0.2	0.4	<0.001	0.2	<0.001	0.6	0.4	0. 3

P *adjusted for sex † adjusted for sex, maternal/ paternal sum of skinfolds, child's sum of skinfolds at 5 years, socio-economic status. P values were derived by multiple linear regressions, using all variables as continuous.

4.8 Relationships to size at birth

There were no significant linear or 'U' shaped associations between birth weight and glucose and insulin concentrations in the children at 5 years (Table 4.11).

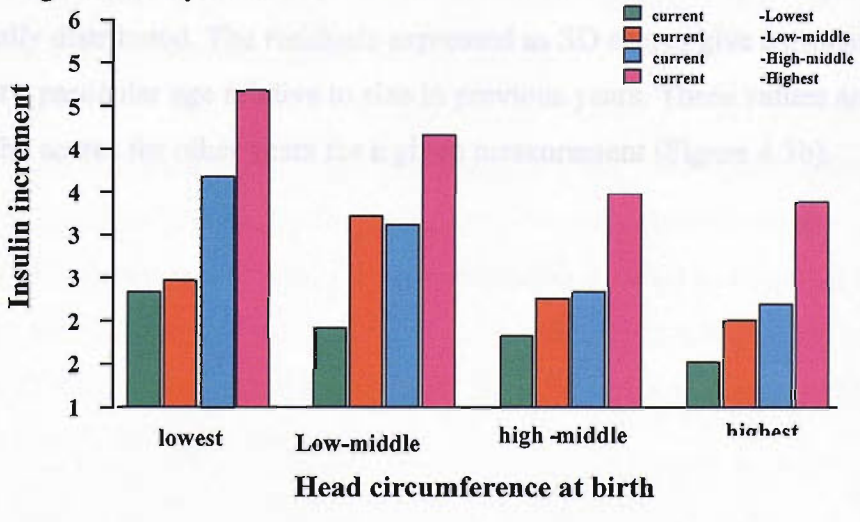
Table 4.11 Mean plasma glucose and insulin concentrations according to birth weight in quartiles

Birth weight quartiles (kg)	Glucose (mmol/l)			Insulin (pmol/l)			Others		
	0 mins	30 mins	120 mins	0 mins	30 mins	120 mins	HOMA	Insulin increment	HbA1c
< 2.72	4.8	7.2	5.8	25.9	187.4	103.4	0.9	33.7	5.5
- 2.97	4.8	7.3	5.9	26.3	181.6	97.2	1.0	32.5	5.6
- 3.24	4.8	7.3	6.0	21.8	176.6	106.9	0.8	32.9	5.5
> 3.24	4.8	7.4	5.9	25.3	182.0	105.2	0.9	32.8	5.5
P*	0.9	0.9	0.7	0.5	0.7	0.4	0.4	0.7	0.95

* Adjusted for sex, birth weight used as a continuous variable.

After excluding ODM from the analysis (the inclusion may mask inverse association with birth size) there were still no associations between any of the birth measurements and glucose/insulin variables at 5 years. After adjusting for current weight, birth weight was inversely related to insulin concentrations at 30 minutes (exp(β)=-1.23, P=0.03) and insulin increment (exp(β)=-1.04, P= 0.05). Head circumference was inversely associated with fasting (exp(β)=-1.06, P=0.03) and 30-minute insulin concentrations (exp(β)= -1.09, P=0.005), insulin increment (exp(β)=-1.06, P=0.03) and also insulin resistance (exp(β)=-1.02, P= 0.02). The highest values for insulin increment (Figure 4.2) and insulin resistance with head circumference were in children who had small heads at birth and were heavy at five years. Birth length was negatively associated with fasting glucose (β=-0.03, P= 0.005) and 30-minute insulin concentrations (exp(β)=1.03, P= 0.02). Except birth CHL with fasting glucose, other associations became statistically non-significant after adjusting current subscapular skinfolds instead of weight.

Figure 4.2 Insulin increment according to quartiles of head circumference at birth and weight at five years



There were no significant differences in glucose/insulin concentrations or in the prevalence of IGT between children who were SGA at birth compared with the rest of the children (Table 4.12). The HbA1c concentrations were higher in children who were appropriate for gestational age at birth compared with SGA children.

Table 4.12. Glucose and insulin concentrations of SGA and AGA children

	SGA	AGA	'P'
N Max=	**57**	**523**	
Fasting glucose (mmol/l)	**4.8** (0.5)	**4.8** (0.5)	0.7
30-minute glucose (mmol/l)	**7.3** (1.8)	**7.3** (1.4)	0.9
120-minute glucose (mmol/l)	**5.7** (1.0)	**5.9** (1.0)	0.4
***Fasting insulin (pmol/l)**	**19.1** (14.2,26.3)	**20.0** (12.9,31.6)	0.6
***30-minute insulin (pmol/l)**	**121.5** (64.3,243.6)	**140.2** (94.8,229.7)	0.2
***120-minute insulin (pmol/l)**	**73.0** (49.9,124.5)	**83.2** (57.8,127.7)	0.2
***Insulin resistance (HOMA)**	**0.7** (0.5,0.9)	**0.7** (0.5,1.1)	0.4
***Insulin increment (pmol/mmol)**	**28.9** (11.2,46.0)	**30.4** (15.5,42.4)	0.6
HbA1c	**5.4** (0.5)	**5.6** (0.5)	0.06

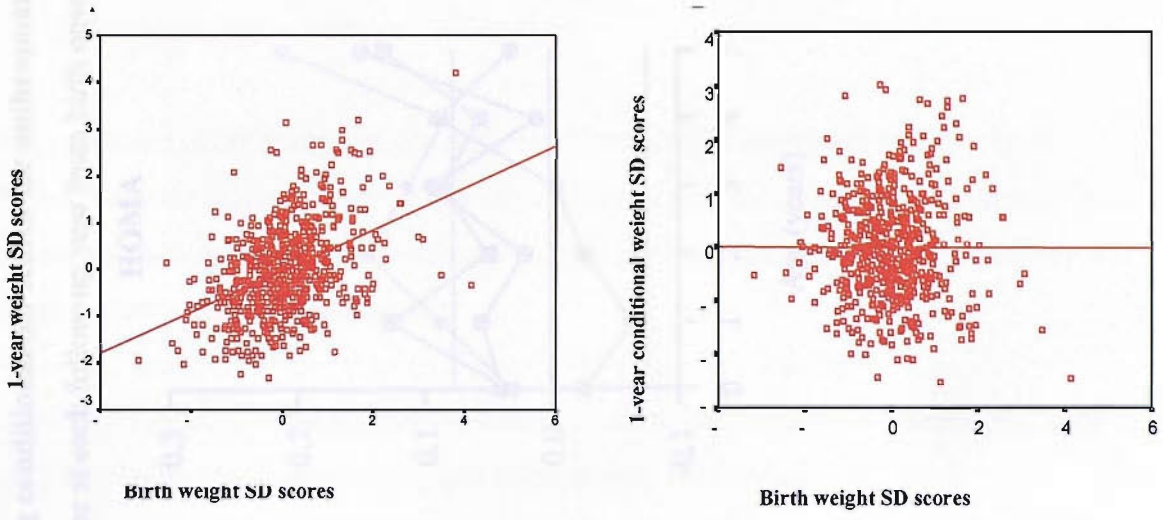
Mean (SD) or *geometric means (IQR)

4.9 Longitudinal growth from 0-5 years

Longitudinal analyses were performed to determine whether growth during particular time periods between birth and five years was related to glucose and insulin concentrations.

4.9.1 Conditional SD scores: High correlations between anthropometric measurements in successive years (Figure 4.3a) make it difficult to ascertain independent associations between change in anthropometry at any given year and glucose and insulin concentrations. I used conditional SD scores to measure these associations. Within cohort SD scores for anthropometric measurements at a given year were regressed on the SD scores for all previous years. The residuals obtained have the property of being normally distributed. The residuals expressed as SD scores give a measure of gain in size at a particular age relative to size in previous years. These values are not correlated with the scores for other years for a given measurement (Figure 4.3b).

Figure. 4.3 Scatter plots showing association between birth weight SD scores and A. one-year weight SD scores B. one-year conditional weight SD scores



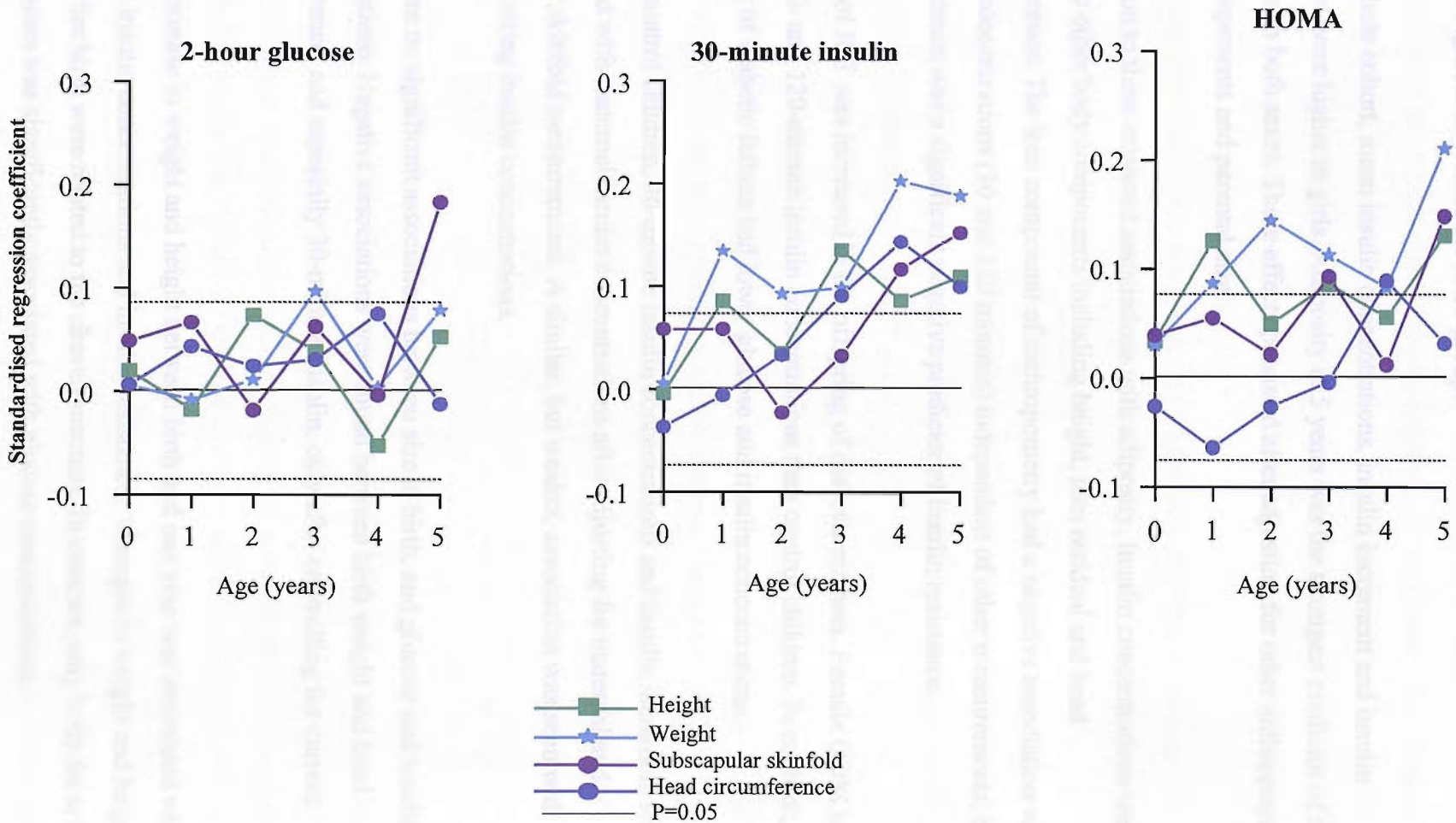
4.9.2 Longitudinal growth, and glucose and insulin concentrations: I included conditional SD scores for individual measurements from 1 to 5 years, along with birth SD score, simultaneously in a regression model together with sex.

There were no significant effects of size at birth or change in size during infancy on glucose concentration at 120 minutes (Figure 4.4). Significant associations were seen only with current body fat measurements (P=0.001). Weak relationships were also noticed with gain in height between 2 and 3 years and 120-minute glucose concentrations (Figure 4.4). Higher weight gain at 5 years was related to higher fasting glucose (P=0.002).

There was no effect of size at birth on insulin concentrations. Greater weight and height gain at all years after birth was related to higher 30-minute insulin concentrations (Figure 4.4). Gain in size for all the body components after 3 years resulted in significantly higher insulin values at 30 minutes.

Size at birth did not have any significant relationships with insulin resistance. Greater changes in weight and height during infancy were significantly related to higher insulin resistance independent of size at 5 years (Figure 4.4). Gain in weight at all years after infancy predicted higher insulin resistance, and the effect was greatest with current weight (P=0.002). Increased gain in body fat at 5 years was significantly associated with higher insulin resistance (P<0.001).

Figure 4.4 Predicting 120-minute glucose, 30-minute insulin and insulin resistance (HOMA) using conditional SD scores for anthropometry from 0 to 5 years. Coloured objects represent SD change in outcome at 5 years per SD gain in size at each follow-up year from birth onwards.



4.10 Summary of main findings

In this cohort of 585 children, 22 had IGT (4%); their anthropometric characteristics did not differ significantly from the NGT group.

In the whole cohort, mean insulin concentrations, insulin increment and insulin resistance were higher in girls. Adiposity at 5 years was the strongest predictor of these outcomes in both sexes. These effects persisted after adjusting for other anthropometric body components and parental size.

In addition to these expected associations with adiposity, insulin concentrations were related to other body components including height, lean residual and head circumference. The lean component of anthropometry had a negative association with glucose concentrations (30 and 120 minutes) independent of other measurements; head circumference was a significant negative predictor of insulin resistance.

The risk of IGT was increased in the offspring of diabetic mothers. Female ODM had higher 30- and 120-minute insulin concentrations than control children. In contrast, offspring of diabetic fathers had lower glucose and insulin concentrations.

Even in control children, 30-minute insulin concentrations and insulin increment were associated with maternal insulin concentrations after adjusting for maternal and offspring skinfold measurements. A similar, but weaker, association was seen with father's fasting insulin concentrations.

There were no significant associations between size at birth, and glucose and insulin concentrations. Negative associations were found between birth weight and head circumference, and especially 30-minute insulin, only after controlling for current weight.

Greater increase in weight and height between birth and one year was associated with increased insulin concentrations and insulin resistance. Changes in weight and height at all ages after birth were related to the above outcomes. In contrast, only body fat at the age of 5 years was significantly associated with glucose concentrations.

4.11 Discussion

4.11.1 Glucose and insulin concentrations-relationships with current Adiposity: Glucose and insulin concentrations increased with measures of fat in both sexes. Higher insulin resistance and insulin concentrations in girls may be related to their higher adiposity (Section 3.3.1) or it may be intrinsic to girls[64]. Current adiposity was the strongest determinant of glucose and insulin concentrations independent of other components of body weight and parental characteristics, the potential confounders.

Associations between current size, particularly adiposity, and glucose and insulin variables have been observed previously in studies in children[43, 60-65]. In a study in India, higher insulin concentrations, increment and insulin resistance (HOMA) were associated with high current weight and subscapular skinfold thickness at 8 years[43]. A subset of the above cohort studied at 4 years in the same setting had shown a positive association between current adiposity, and plasma glucose (post-load) and insulin concentrations (fasting and post-load)[60].

Among British children, ponderal index at 11 years was positively associated with glucose (fasting) and insulin (fasting and post-load) concentrations[61]. In another study of 5-year old British children, positive correlations were seen between weight or BMI, weight gain since birth, and insulin resistance at 5 years[64]. In South Africa, 30 and 120-minute insulin concentrations at 7 years were significantly correlated with current weight and BMI, and 5-year skinfold thickness[62]. Skinfolds were also related to 30-minute glucose.

Current adiposity is well recognised as a risk factor for insulin resistance. Adipose tissue is known to secrete factors like free fatty acids (FFA), leptins, interleukins and tumour necrosis factor-α. (TNF- α)[159]. Induction of lipolysis in adipocytes releases excess of FFA and impairs insulin signalling in glucose uptake sites such as muscle cells. This may be the mechanism for reduced insulin sensitivity associated with adiposity.

4.11.2 Glucose and insulin concentrations-relationships with height: In Mysore, height was a positive predictor of insulin concentrations and insulin resistance. Similar observations were made in Pune, where insulin concentrations and insulin resistance were higher in taller children at 8 years[43]. Similarly in the UK, height was a positive

predictor of fasting insulin in 11-year old children[61], and it was associated with higher HOMA resistance in 9-year old children in another setting[63]. However, a negative association was found with 30-minute glucose concentration in 7-year old South African children[62]. The reasons for these associations are not known. Genes responsible for tallness may stimulate insulin secretion; conversely, genetically or phenotypically induced insulin secretion may stimulate skeletal growth. It may also reflect advanced skeletal maturity of the taller children[60,63]. However, absence of associations after controlling for other body components may be due to confounding effect of other factors, which were highly correlated with one another.

4.11.3 Glucose and insulin concentrations-relationships with lean body mass: Lean tissue was inversely associated with glucose concentrations in my study. None of the studies in children have looked at fat free mass as a predictor of risk factor outcomes. A study comparing Indian migrants with Swedish men found that the Indians had lower lean mass (skeletal muscle and viscera) and this was related to higher glucose concentrations[119]. Larger lean mass in my study children may increase the area available for glucose uptake. However, the lean residual used in my study is not an ideal measure of lean mass as the calculations may not remove completely the effects of intra-fascial or visceral fat, which may have related to positive associations with insulin concentrations (Section 4.5.4).

4.11.4 Glucose and insulin concentrations-relationships with head circumference: In Pune, 4-year old children showed, similar to my findings, an inverse association between current head circumference and fasting and 30-minute insulin concentrations[60]. There was also an inverse relationship with insulin resistance and insulin increment in my study. Since most of the development of head takes place *in utero*, smaller head size may reflect impaired fetal growth. A similar association was seen with small head circumference at birth; however, relationships with size at birth were not strong overall (see below).

The associations were stronger with insulin concentrations in all the above findings, especially with 30-minute insulin, than with glucose variables. Highly active endocrine pancreas may be compensating for insulin resistance by increasing insulin output. The implication of 30-minute concentrations in children is not clear, though in adults, they give a measure of first phase insulin secretion[131]. In 4-year old children in India, low

birth weight was associated with higher 30-minute glucose and insulin concentrations and it was suggested that their higher glucose concentrations were due to insulin resistance[60]. When again studied at 8 years this group of children had high fasting insulin concentrations and insulin resistance (HOMA)[43].

4.11.5 The prevalence of IGT-normal vs. children born to mothers with GDM: The prevalence of IGT was high in the cohort (4%). There are few data available on the prevalence of IGT in normal children. Plagemann quoted <5% in normal children[160], but others suggest a lower prevalence. In Pima Indians, it was less than 3% between 5-9 years[103]. It was 1.5% in Pune 8-year old children[43] and in a group of adolescents born to mothers without diabetes in the USA it was 2.5%[91]. A study of 5 to 10 year old overweight/obese African-American children showed a prevalence of 5.4%[42]. Although children in my study were much younger and generally underweight, the IGT prevalence was comparable to these overweight children.

There was a 3-fold increase in the prevalence of IGT (11.4%) in ODM compared to control children. This was a significant increase despite our ODM group being small. Similar prevalence rates in ODM were reported in two other studies. Pima Indian children had an IGT prevalence of 11% at 5-9 years[103]. In another study in Germany, IGT prevalence among children of GDM mothers was 11.1% at 1-4 years age[160], there was no comparison with offspring of mothers without GDM. In a third study, however, the prevalence of IGT was much lower than in Mysore. The prevalence was low before the age of 5 years (1.2%); but rose to 5.4% between 5 to 9 years and 19.3% after 10 years[91].

The future course of IGT in children is not certain, but high rates of conversion to diabetes are seen in adults with IGT. A survey among urban populations in India has shown an increasing trend of IGT prevalence with age; the percentage of people with IGT rose from around 11% at 20-29 years age group to about 20% at above 69 years age group[161]. The high rates of IGT seen in young children in my study may signal a high future burden of diabetes in the GDM group.

4.11.6 Glucose and insulin concentrations- relationships with maternal GDM: Studies in children comparing glucose and insulin concentrations in ODM with control children are scarce. Among the Pima Indians, offspring born to mothers with diabetes

had higher mean plasma 2-hour glucose and fasting insulin concentrations than children born to mothers with either IGT or normal glucose tolerance at 5-9 years. The differences became more pronounced as the children grew older[103]. In the USA, 120-minute glucose and insulin concentrations were higher in adolescent ODM than in controls[91]. A recent study showed increased acute insulin response and reduced insulin sensitivity (both non-significant associations) in 5-10 year old children of diabetic mothers[157].

In my study, insulin concentrations at 30 and 120 minute were higher in female ODM. The differences were manifest even though children in my study were younger than those in other studies and these may become more distinct as these children get older.

Genes are thought to play a major role in the aetiology of type 2 DM. Among the Pima Indians, offspring of diabetic fathers had more type 2 DM than those of non-diabetic fathers. However, offspring exposed to diabetes *in utero* had a higher prevalence of obesity and type 2 DM than their siblings born before the mother was diagnosed with diabetes, suggesting that this was attributable to the intra-uterine environment[18]. In my study, increased insulin concentrations were seen in ODM, but not in offspring of diabetic fathers, and the association in female ODM was independent of maternal obesity. These findings are consistent with intra-uterine programming effects of GDM.

4.11.7 Maternal glucose and insulin concentrations in the absence of GDM: I have shown that in control children 30-minute insulin concentrations and insulin increment increased with maternal insulin concentrations, independent of maternal and child's adiposity. A linear positive association between maternal 2-hour glucose concentrations in the non-diabetic ranges, and offspring fasting insulin and post-load glucose concentrations at each 4-year age group from 5 to 24 years was also seen in Pima Indians[103]. They did not examine relationships with maternal insulin concentrations. My study gives the first account of an association with maternal insulin concentrations. However, Pima Indians are a group with high prevalence of GDM and type 2 DM, and high degrees of inbreeding may have rendered them genetically different from other populations. Thus, my findings may be paramount in understanding the mechanisms of insulin resistance and type 2 DM in other populations. Persistence of significant effects even after controlling for childhood body composition suggests a parallel increase in the adiposity and body size in these children as an unlikely cause. Possibly, even mild

hyperglycaemia in the mother might have altered pancreatic sensitivity inducing it to produce more insulin for a given glucose challenge.

It may be also due to genes responsible for varying degrees of insulin responsiveness. In my study, an association was also noticed between father's fasting insulin and offspring insulin concentrations in the control group. Weaker trends for fathers could be because their insulin profile was less completely characterized than for mothers. Our data are equally compatible with genetic transmission of adiposity and/or insulin resistance from either parent and this issue needs further research. I did not find similar data reported from other populations.

4.11.8 Size at birth in relation to glucose and insulin concentrations: In my study, glucose and insulin concentrations at 5 years were not directly related to size at birth. A significant inverse association was found between 5-year fasting insulin concentrations and head circumference at birth, and between birth length and 5-year fasting glucose concentrations only after correcting for current body composition. Although, birth weight was inversely associated with 30-minute insulin concentrations and insulin increment after adjusting for current weight, the association was lost when adjusted for body fat instead of weight.

Earlier studies in children have generally demonstrated an inverse relationship between insulin concentrations and size at birth, as in Mysore, only after adjusting for childhood size. In the Pune children, insulin (fasting and 30-minute), and glucose concentrations (30-minute) and insulin resistance were higher in low birth weight children[43]. In another study in the UK, after adjusting for current height and PI, birth weight was inversely related to fasting and post load insulin concentrations and insulin resistance[61]. A similar observation was made in South African children; inverse correlations were seen between birth weight and insulin (30 and 90 minutes) and glucose concentrations (30 minutes)[62]. Increased post natal growth velocity (catch-up) is a feature of low birth weight babies. Intra uterine growth impairment alters endocrine and metabolic functions and lowers the threshold for insulin resistance when the individual gains excess adiposity later in life (1.2.2). In another study in England (EarlyBird study), there was no association seen between birth weight and outcome variables[64]. The researchers proposed that since very few of their babies were growth retarded, birth weight was of no importance as a risk factor for insulin resistance in this population. Thus, for a low

birth weight baby, later gain in size is crucial for development of metabolic abnormalities, while it is not unfavourable if the child remains small subsequently. A high birth weight infant may be at lower risk even if it gains weight later.

In contrast to the above studies in children, a number of studies in adults have shown inverse associations between birth weight and insulin resistance[57]. Intra uterine growth retardation has been thought to induce glucose/insulin abnormalities by variety of mechanisms. Growth of skeletal muscles is compromised in utero due to under nutrition, hence the individuals tend to accumulate adiposity when they gain weight post natally. Pancreatic development may be impaired reducing its capacity to cope with the demands of later adiposity[45]. Abnormal growth hormone secretion[45] and high concentrations of cortisol[162], associated with low birth weight, antagonise insulin actions. The relationships with birth size may become clearer in Mysore, in later years, when the activity of endocrine pancreas starts to diminish.

4.11.9 Longitudinal growth and glucose and insulin concentrations: Very few studies in children have looked at the effects of longitudinal growth in size on glucose and insulin concentrations. A positive correlation has been shown between weight velocity (kg gain in weight per year) from birth to 7 years and insulin resistance and insulin concentrations (30 and 120 minutes) in one study[62]. The correlations were similar for velocities between different age intervals (1-4, 4-5 and 5-7). The associations were, however, not demonstrated for other body components.

I have shown that greater gain in weight and height at all ages from 1 to 5 years was associated with increased insulin concentrations and insulin resistance in the study children. Associations with fat gain were significant only after 3 years for insulin concentrations. Gain in size during early postnatal years did not relate to glucose concentrations, which showed significant associations only with current adiposity. The indications that the relationships between childhood growth and outcome variables are different for different time points may have implications for public health policies. In India, it has been shown that young adults who were small and thin during infancy had accelerated increase in size after 2 years and went on to develop abnormalities of glucose metabolism (section 1.4.3). Thus, intervening at a later stage in postnatal growth may be beneficial. The issue relating to promotion of infant growth remains controversial[74]. However, in countries like India, where infant mortality and morbidity

are major public health concerns, gain in infant size is vital for immediate survival of the child. As the significance of childhood risk markers for future course of the disease are not known, slowing down of infant growth based on few studies may have adverse implications. Thus, I propose the need for more longitudinal studies to understand the importance of infant and childhood growth.

4.11.10 Glycated haemoglobin (HbA1c): About 13% of the children had an HbA1c concentration above the normal level specified by the laboratory (>6.0) where the assay was done. However, this was not associated with a significant difference in IGT prevalence, glucose and insulin concentrations, or anthropometric measurements compared to children with normal levels. HbA1c is not an accepted method of screening/diagnosis of diabetes, and a wide variation in HbA1c levels has been seen before in normoglycaemic adults[163]. Nevertheless, our observation at an young age is a matter of concern. This may be a normal variation or may be an early indicator of an underlying pathology. It may also be related to imprecise assay methods, and thus may be spurious. Our continued follow-up of these children may help us to identify any risks in them in future years.

Conclusion: I have looked at factors influencing glucose and insulin outcome variables at 5 years in a group of normal children.
Glucose and insulin concentrations were strongly related to current size (fat, lean and skeletal components). Associations were stronger with insulin variables than glucose concentrations.
Other factors associated with increased glucose/insulin concentrations at 5 years were:

- Maternal gestational diabetes.
- High maternal and paternal insulin concentrations even in the absence of diabetes.
- Greater gain in weight and height between 1 and 5 years.
- Greater gain in adiposity between 3 and 5 years.

There was little evidence of the effect of small size at birth as its own, although small head circumference at birth and 5 years was associated with higher fasting insulin concentrations. Children of any given 5-year weight, but who were small at birth had higher glucose/insulin concentrations.

In all the above scenarios, the 30-minute insulin had the strongest relationships. The importance of these findings are not known in children. They may reflect insulin resistance. About 4% had IGT, which seems to be high compared to other populations. The prevalence was significantly increased in ODM. Further follow up may reveal the implications of the above factors and clarify the risks of maternal GDM in this population.

The findings described in this chapter have been published:

Krishnaveni GV, Hill JC, Leary SD, Veena SR, Saperia J, Saroja A, Karat SC, Fall CHD. Anthropometry, glucose tolerance and insulin concentrations in Indian children: relationships to maternal glucose and insulin concentrations during pregnancy. *Diabetes Care* 2005;28:2919-25.

5. BIOELECTRICAL IMPEDANCE METHOD OF BODY FAT MEASUREMENT: COMPARISON WITH SKINFOLD METHODS

In the previous chapters, I have discussed the negative implications of excessive adiposity on adult onset disorders such as insulin resistance/type 2 diabetes, cardiovascular diseases and hypertension, and have discussed its pathogenetic mechanisms. I have also shown that the adiposity of the Mysore children was disproportionate to their weight and BMI. The methods used to measure adiposity are varied, ranging from simple and inexpensive anthropometry to sophisticated Dual Energy X-ray Absorptiometry (DEXA). However, there is not a single technique, which is considered ideal in all situations. Given below is a brief description of some of the popular methods.

5.1 Measurement techniques of adiposity

5.1.1 Anthropometry: Anthropometric measurements such as weight, waist circumference and skinfold thickness, and indices derived from them such as BMI (weight/height^2), waist to hip ratio and fat mass are commonly used parameters to define body fat. The advantages of this method are that it is cheap, non-invasive and has a high degree of subject compliance, and can be done by any field workers after thorough training. However, determining obesity/adiposity based on weight or BMI could be misleading, as this does not distinguish between muscle and adipose tissue. Even though skinfolds give a reliable measure of subcutaneous adiposity, this needs repeated training and a high degree of standardisation of methods. Moreover, it does not measure internal adiposity such as visceral fat, and is not ideal for assessing fat-free mass.

5.1.2 Isotope dilution techniques: Total body water (TBW) is calculated by administering isotopes of either hydrogen (^{2}H, ^{3}H) or oxygen (^{18}O) diluted in water. TBW is then determined by estimating the amount of isotope excreted in body fluids (urine, blood or saliva). A fluid sample is collected just before administering the isotope labelled water and another after the ingested isotope reaches equilibrium in the body (usually between 3 and 6 hours after ingestion). The amount of the isotope excreted is used to estimate TBW. This is a reliable 2 compartment model for determining body fat, but is a long procedure and expensive.

5.1.3 Hydro-densitometry/Underwater weighing: This is a 2-compartment model where body mass is divided into fat and fat-free mass. Body volume is estimated by complete submersion of the body under water and residual lung volume (RLV) is measured by oxygen dilution using a closed-circuit spirometer system, after completely exhaling air. Body volume is then corrected for the residual lung volume, and the density is calculated using an equation, which is subsequently used to determine the percentage body fat. The RLV measurement is complex and needs the subjects to be highly compliant and practice the procedure in advance. These difficulties have led to the practice of estimating RLV by a prediction equation, which is bound to introduce errors in the estimated fat%[164]. The equipment is expensive, and requires highly skilled personnel to carry out the measurements. Thus, though considered a gold standard method, this is not suitable for large field studies, especially in children.

5.1.4 DEXA: This is a 3-compartment model, which measures different body components (lean tissue mass, fat mass and bone mineral) by passing a minimal radiation beam (double photon beam) generated by X-ray through the body. The method has been validated against pig carcass analysis, hydro-densitometry and total body potassium. This is a commonly used reference method for fat measurement. However, it is expensive and requires exacting maintenance and skilled operators, and hence is not suitable for routine monitoring of adiposity in small research units.

5.1.5 Magnetic Resonance Imaging: This imaging technique measures different body components based on the signals produced by the hydrogen atoms when a strong magnetic field is applied and removed in a pulse sequence. This gives high-resolution cross-sectional images through selected regions of the body which can be used to estimate volumes of different tissues. This method can be used to differentiate visceral and subcutaneous adipose tissue, and thus is a sophisticated technique. The disadvantages are similar to the DEXA method, and hence is not an ideal field method.

5.1.6 Computed Tomography: This method uses X-ray scanning for imaging of the cross sections of the body components. The images are similar to those from MRI, but have additional information on the densities of the measured area. This characteristic can be used to identify different tissues such as bone, muscle, skin, subcutaneous and visceral adipose tissue, or visceral organs. In addition to disadvantages associated with MRI, it also involves an exposure to radiation.

5.1.7 Bioelectrical Impedance Analyser (BIA):

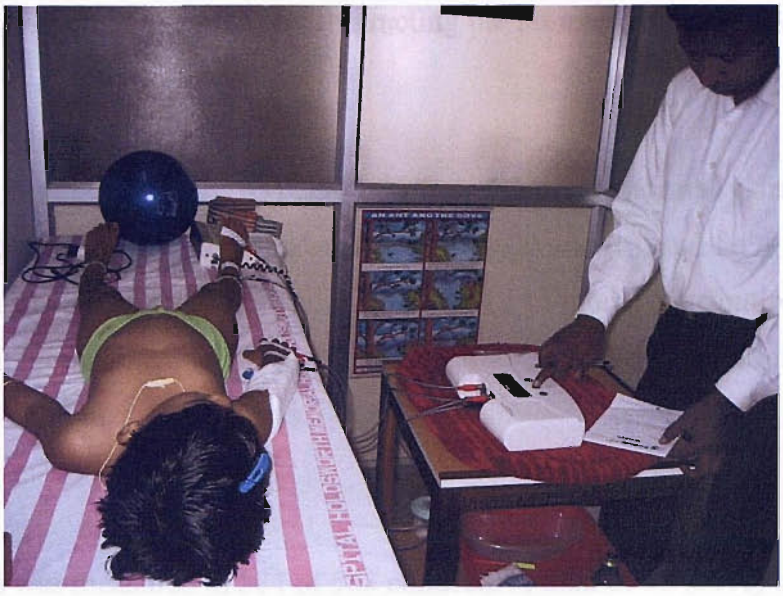


Figure 5.1: Bioimpedance measurement.

Bioimpedance is a 2 compartment model method, which measures TBW, and hence a measure of fat-free mass, by passing a low voltage alternating current through electrodes placed on hand and feet (Figure 5.1). A measure of total body fat is derived by including the impedance value into sex-specific regression equations, along with weight and height. It is a relatively quick method and the equipment is easy to handle, and has been validated using different reference methods for several populations in the world[165-168]. In this chapter I give a description of total fat obtained using bioimpedance technique (Bodystat, Quadscan 4000, Appendix 1) in Mysore children, and compare this with anthropometric indices of fat.

5.1.8 Fat mass (FM) and fat% from skinfolds: Fat% was calculated using a skinfold equation based on (a) pre-school Indian children[158] (section 4.5.1). This equation has been validated using deuterated water method. Fat% was also derived using (b) Slaughter's equation for pre-pubescent Caucasian children: Fat%= K1*(triceps skinfold thickness + subscapular skinfold thickness)-K2*(triceps skinfold thickness + subscapular skinfold thickness)^2-K3, where K1, K2 and K3 are sex specific constants[169]. This method has been validated using multi-compartment models (under-water weighing, deuterated water method, and x-rays of the ulnar and radius for bone mineral density). I chose the above equations since these models use the anthropometric measurements available for my study children. Fat mass was then calculated using the formula: fat% x body weight/ 100.

Fat-free mass (FFM) was obtained directly from BIA estimation. FFM was also calculated from skinfold equations by subtracting the fat mass from body weight.

5.2 Statistical methods

Fat-free mass from all methods, FM and fat% from BIA were normally distributed. Fat mass calculated using skinfolds from both equations (a) and (b), and fat% from (b) were log-normally distributed. Fat% derived from (a) had a bi-modal distribution indicating different distributions for boys and girls. Sex-specific SD scores were used when boys and girls were combined for analyses.

5.3 Results

Bioimpedance was measured in 584 of 585 children. A total of 98 (35%) boys and 221 (72%) girls had greater than 25% of body weight as fat, whereas 23 (8%) boys and 92 (30%) girls had a fat mass of more than 30% of body weight.

Both FM and fat% measured by bioimpedance had higher values compared to those calculated using skinfolds (Table 5.1). The median values and IQR were notably different in all 3 methods. FM and fat% from both BIA and skinfolds were statistically significantly higher in girls and FFM was higher in boys.

Table 5.1. Median (IQR) and range of fat-free mass (FFM), fat mass (FM) and fat% from BIA, and skinfold equations.

	Males (N=280)		Females (N=305)		'P'
		Min-Max		Min-Max	
FFM (BIA) kg	11.7 (10.9-12.7)	8.1,22.2	10.6 (9.9-11.5)	7.1,15.5	<0.001
FM (BIA) kg	3.5 (2.9- 4.2)	1.2,6.7	4.1 (3.5- 4.7)	1.4,10.2	<0.001
Fat% (BIA)	22.9 (20.1- 26.9)	8.6,35.3	27.8 (24.4- 30.9)	11.1,43.4	<0.001
FFM (a) kg	12.6 (11.8-13.6)	9.4,20.4	11.4 (10.6-12.3)	8.5,17.3	<0.001
FM (a) kg*	2.5 (2.3- 2.9)	1.6,7.1	3.3 (3.0- 3.8)	2.2,6.8	<0.001
Fat% (a)*	16.6 (15.7- 17.7)	13.4,25.7	22.6 (22.0- 23.4)	20.1,29.9	<0.001
FFM (b) kg	13.3 (12.5-14.3)	9.8,20.4	12.8 (11.8-13.5)	9.2,17.8	<0.001
FM (b) kg*	1.9 (1.5- 2.3)	1.0,7.1	2.0 (1.7- 2.6)	0.9,7.5	<0.001
Fat% (b)*	12.2 (10.8- 14.0)	7.6,26.5	13.9 (11.9- 16.6)	7.7,32.6	<0.001

(a) derived using equation for Indian children (b) derived using Slaughter's equation * P derived using normalised variables for the difference between the sexes

5.3.1 Offspring of diabetic mothers:

Offspring, especially females, born to mothers with GDM had higher FFM, FM and fat% measured using BIA than ONDM (Table 5.2). The differences were of borderline significance for FM and FFM in females. FM and fat% were smaller in male ODM than in male ONDM. Fat and fat-free mass, and fat% from skinfold equations were significantly greater in female ODM than ONDM. They were similar in boys from both groups.

Table 5.2 Body fat according to maternal GDM status

	Females		Males		P*	
	ODM	**ONDM**	**ODM**	**ONDM**	**F**	**M**
FFM (BIA) kg	**11.3** (1.6)	10.8 (1.4)	**12.5** (1.6)	**11.9** (1.6)	0.09	0.2
Fat mass (BIA) kg	**4.6** (1.3)	4.1 (1.1)	**3.3** (1.0)	**3.6** (0.9)	0.07	0.2
Fat% (BIA)	**28.6** (5.6)	27.5 (5.1)	**20.7** (5.9)	**23.3** (4.8)	0.3	0.06
FFM (a) kg	**12.1** (1.5)	11.5 (11.5)	**12.9** (1.4)	**12.8** (1.4)	0.052	0.8
Fat mass (a) kg¶	**3.7** (3.2,4.2)	3.4 (3.0,3.7)	**2.6** (2.2,3.1)	**2.6** (2.3,2.9)	0.01	0.8
Fat% (a)	**23.5** (1.2)	22.7 (1.2)	**16.9** (1.7)	**16.8** (1.9)	0.002	0.9
FFM (b) kg	**13.2** (1.5)	12.8 (1.4)	**13.6** (1.4)	**13.4** (1.5)	0.1	0.7
Fat mass (b) kg¶	**2.5** (2.0,3.3)	2.1 (1.6,2.5)	**1.9** (1.5,2.4)	**1.9** (1.6,2.3)	0.006	0.8
Fat% (b)¶	**16.0** (13.7,19.4)	13.9 (11.8,16.5)	**12.4** (10.2,14.4)	**12.5** (10.8,14.1)	0.006	0.9

Mean (SD) or ¶geometric mean (IQR); (a) derived using equation for Indian children (b) derived using Slaughter's equation * P for the difference between ODM and ONDM, derived using normalized variables

5.4 Correlations

5.4.1 Bioimpedance and skinfold equations: FFM derived from BIA and that calculated from skinfold equations were strongly, positively correlated (Figure 5.2). Fat mass and fat% measured using BIA were also significantly correlated with those estimated using skinfold equations (a) and (b). The correlations were greater for FM than for fat% (Figure 6.2). Statistically significant correlations were also observed between FM from BIA, and individual skinfolds and BMI (Figure 5.3).

Figure 5.2 Correlations between BIA and skinfold methods.

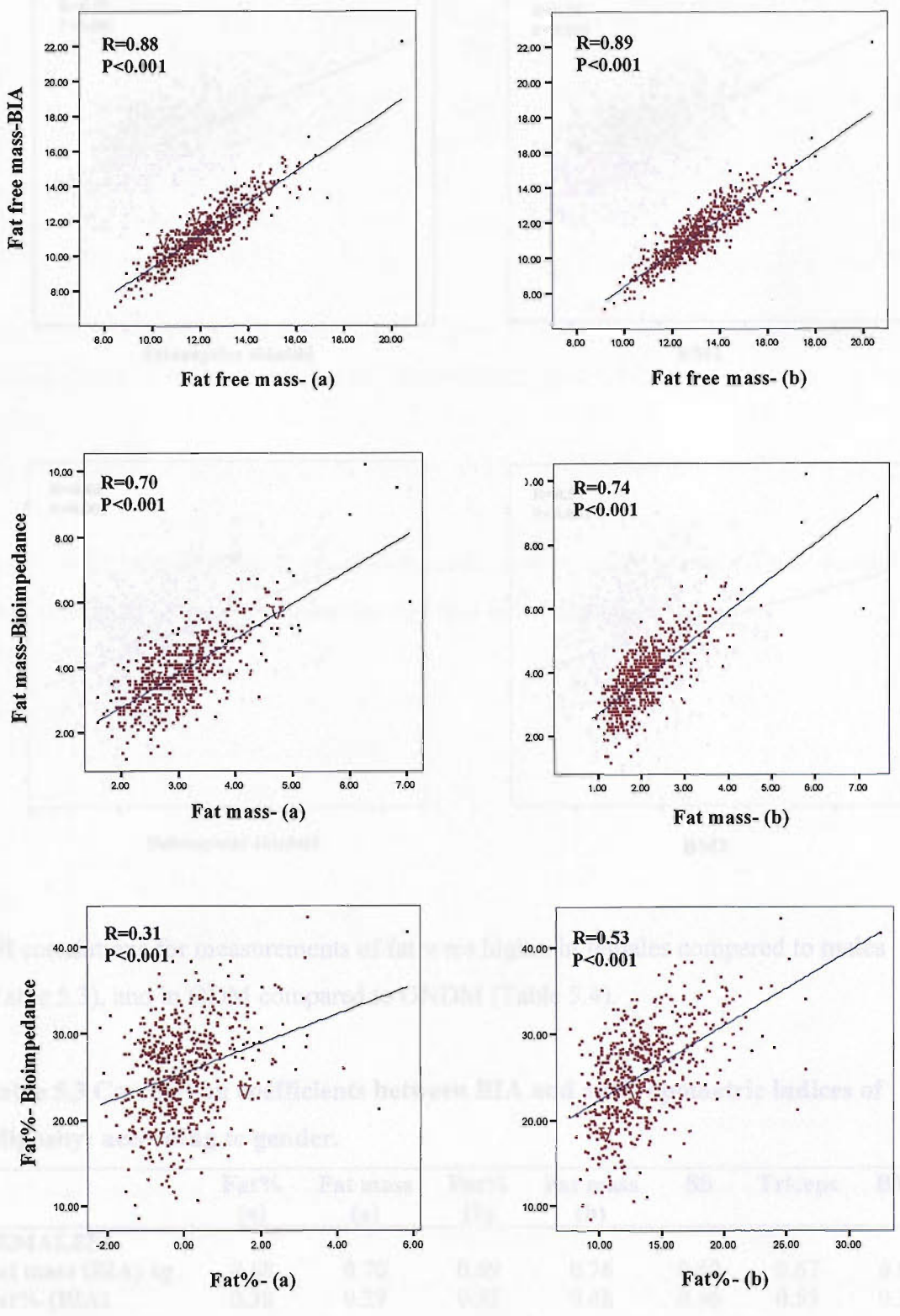
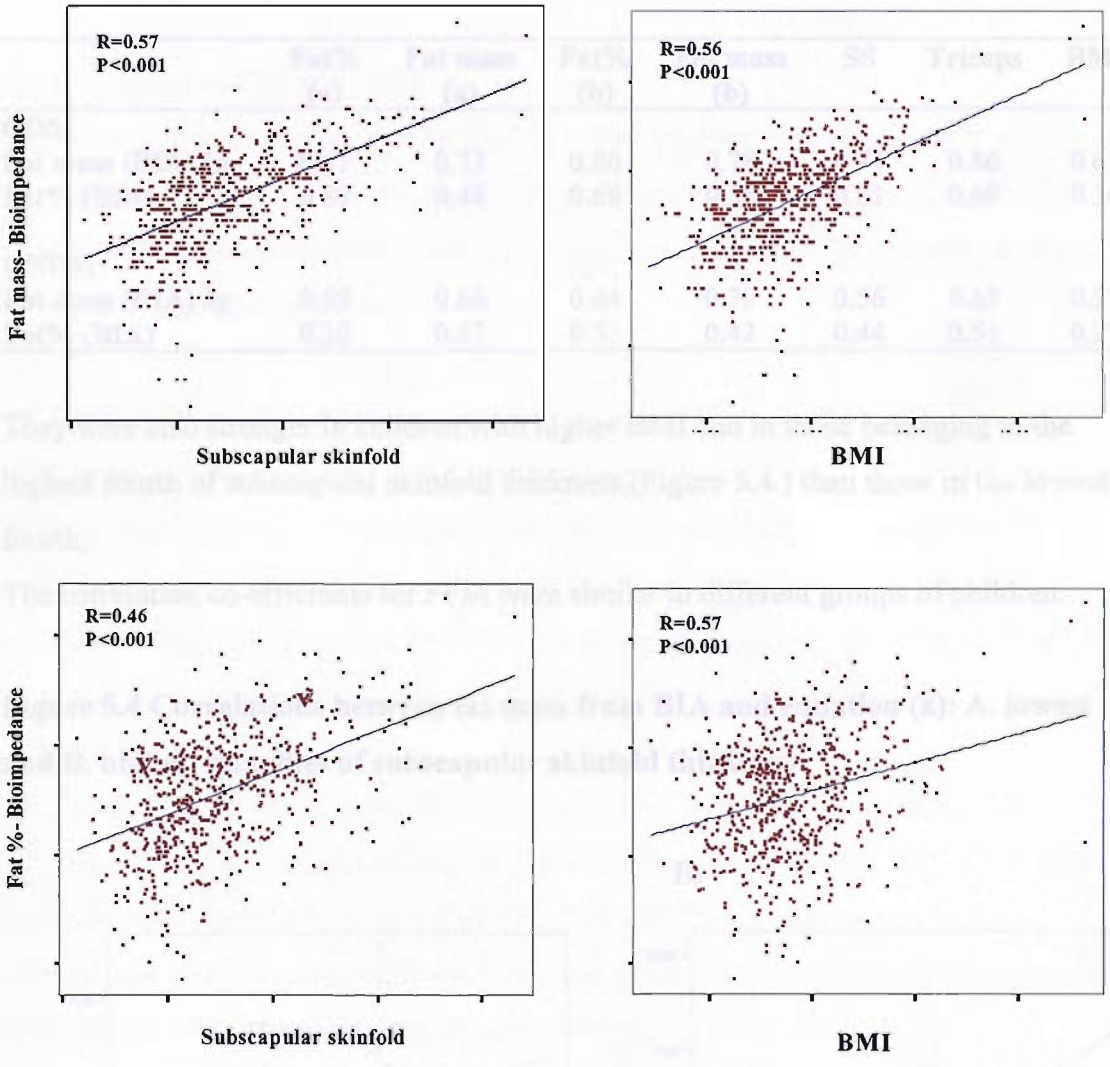


Figure 5.3 Correlations between BIA and anthropometry



All correlations for measurements of fat were higher in females compared to males (Table 5.3), and in ODM compared to ONDM (Table 5.4).

Table 5.3 Correlation coefficients between BIA and anthropometric indices of adiposity: according to gender.

	Fat% (a)	Fat mass (a)	Fat% (b)	Fat mass (b)	SS	Triceps	BMI
FEMALES							
Fat mass (BIA) *kg*	0.68	0.70	0.69	0.76	0.60	0.67	0.67
Fat% (BIA)	0.38	0.29	0.53	0.48	0.46	0.53	0.39
MALES							
Fat mass (BIA) *kg*	0.55	0.60	0.55	0.63	0.46	0.55	0.50
Fat% (BIA)	0.29	0.22	0.35	0.35	0.31	0.40	0.20

Table 5.4 Correlation coefficients between BIA and anthropometric indices of adiposity: maternal GDM status

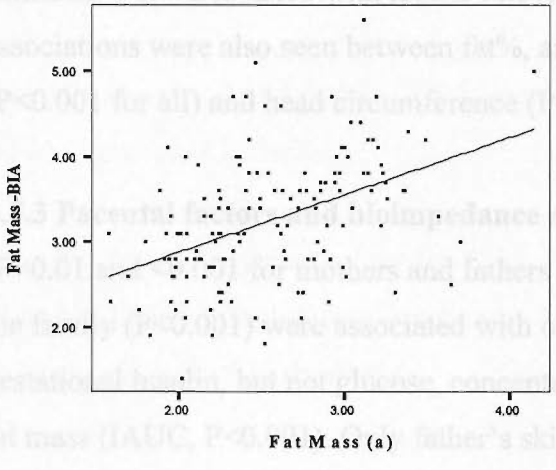
	Fat% (a)	Fat mass (a)	Fat% (b)	Fat mass (b)	SS	Triceps	BMI
ODM							
Fat mass (BIA) *kg*	0.67	0.73	0.80	0.78	0.74	0.80	0.63
Fat% (BIA)	0.39	0.48	0.68	0.55	0.61	0.69	0.36
ONDM							
Fat mass (BIA) *kg*	0.59	0.66	0.64	0.70	0.56	0.63	0.57
Fat% (BIA)	0.30	0.42	0.51	0.42	0.44	0.51	0.27

They were also stronger in children with higher BMI and in those belonging to the highest fourth of subscapular skinfold thickness (Figure 5.4.) than those in the lowest fourth.

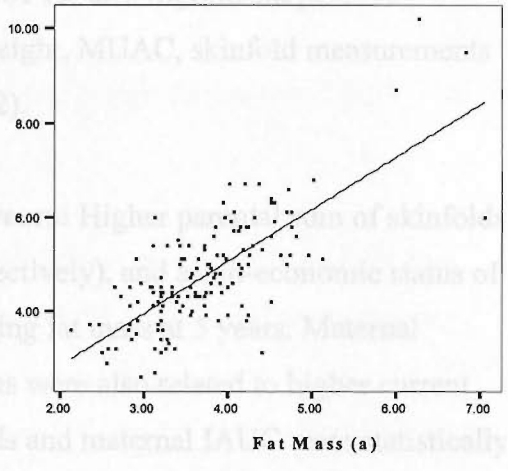
The correlation co-efficients for FFM were similar in different groups of children.

Figure 5.4 Correlations between fat mass from BIA and equation (a): A. lowest and B. highest quartiles of subscapular skinfold thickness

A.



B.



5.4.2 Adiposity and risk markers at 5 years: Fat mass measured by bioimpedance (R=0.27) as well as that derived from skinfold equations (a) (R=0.30) and (b) (R=0.24) showed statistically significant correlations with HOMA resistance. There were also statistically significant correlations between HOMA and fat% (BIA R=0.23, (a) R=0.20, (b) R=0.20). Similarly, statistically significant correlations were seen between FM and systolic BP (BIA R=0.11, (a) R=0.14, (b) R=0.22), while only fat% from skinfold equations (a) and (b) had significant correlations with systolic BP (R=0.22 and 0.17 respectively). The correlations were not strong with the diastolic BP and were

statistically significant only with BIA measurements of FM (R=0.11) and fat% (R=0.09).

5.5 Predictors of FM and fat%

I tested some of the factors associated with current adiposity such as weight at birth and 5 years, parental size, socio economic status of the parents and maternal glucose and insulin status during pregnancy, using regression analyses. All these models included sex as an independent variable.

5.5.1 Size at birth and current adiposity: In a univariate analysis, birthweight (P<0.001), skeletal measurements (length and head circumference, P<0.001), measure of lean tissue (MUAC and AMA, P<0.001) and also skinfold measurements (subscapular, P=0.001 and triceps, P=0.03) were positively associated with FM measured using BIA. Ponderal index at birth was not related to FM (P=0.1). There were no statistically significant associations between birth size and fat%.

5.5.2 Relationships with current anthropometry: Higher current weight and components of body weight such as height, head circumference, MUAC, AMA and skinfolds were associated with higher FM (P<0.001 for all). Significant positive associations were also seen between fat%, and weight, MUAC, skinfold measurements (P<0.001 for all) and head circumference (P=0.02).

5.5.3 Parental factors and bioimpedance at 5 years: Higher parental sum of skinfolds (P=0.01 and <0.001 for mothers and fathers respectively), and socio-economic status of the family (P<0.001) were associated with offspring fat mass at 5 years. Maternal gestational insulin, but not glucose, concentrations were also related to higher current fat mass (IAUC, P<0.001). Only father's skinfolds and maternal IAUC were statistically significant predictors of higher fat%.

5.5.4 Multiple regression analysis: Child's birthweight, maternal IAUC and socio-economic status (P=0.001 for all) were statistically significant predictors of fat mass in a multivariate model. Associations with birthweight and socio-economic status were lost after adjusting additionally for current weight, which was then a strong determinant of fat mass (P<0.001). Maternal IAUC remained a significant predictor even after adjusting for current body weight (P=0.02). Current weight (P<0.001) and maternal

IAUC (P=0.03) were the statistically significant predictors of fat% at 5 years. All the above associations remained little changed even after excluding the ODM from the analyses (Table 5.5)

Table 5.5 Multiple regression model for predictors of fat mass and fat% using BIA in ONDM

	Fat mass		Fat %	
	β	P	β	P
Current weight (kg)	0.33	**<0.001**	0.42	**0.001**
Birthweight (kg)	0.01	**0.9**	0.43	**0.5**
Maternal skinfolds	-0.001	**0.5**	-0.004	**0.6**
Paternal skinfolds	0.002	**0.1**	0.01	**0.1**
SES	-0.00004	**0.997**	-0.001	**0.99**
Maternal GAUC	0.0001	**0.7**	-0.0004	**0.8**
Maternal IAUC	0.000003	**0.02**	0.00002	**0.03**

5.6 Summary of main findings

Body fat, and fat-free mass measured using bioimpedance analyser were well correlated with those measured from skinfold equations. The correlation co-efficients were larger for FFM than for FM. However, the mean values varied widely.

Females in general and female ODM in particular had higher fat mass and fat%. The FFM was greater in males.

Female ODM had higher values for FFM, FM and fat% as measured from BIA than female ONDM. The differences adiposity between ODM and ONDM were greater when measured using skinfolds.

Correlations between fat measurements derived from BIA and those calculated from skinfolds were stronger in children with higher levels of body fat, such as in females, and ODM.

Correlations with risk markers were of similar magnitude for body fat measured using BIA and skinfold method.

In a multiple regression analysis, current body weight and maternal IAUC were the strongest predictors of fat mass and fat% at 5 years.

5.7 Discussion.

The prevalence of overweight/obesity, as defined by BMI cut off is rising among urban Indian children[170]. The amount of body fat, rather than body mass, relates more to disease risk. Bioimpedance, because of its ease of operation, offers a potentially suitable method of quantifying adiposity for wider application in community studies in India.

Bioimpedance does not directly estimate either fat or fat-free mass; it measures the conductivity of a body by passing a low voltage current through it and estimates body fat through statistical correlations[171]. BIA is based on the principal that the volume of a cylindrical conductive body is directly related to its length and inversely to the resistance. Thus, the volume can be estimated by measuring the length (L) and the resistance (R) of the cylinder by the relation $Volume=\rho L^2/R$, where ρ is a constant. In humans the major conductive volume is formed by the TBW and hence FFM, as it offers least resistance to flow of current, while tissues like fat and bone offer relatively more resistance. Thus, by assuming the human body as a cylinder of uniform conductivity, BIA measures the resistance or impedance of the body[171]. TBW is then calculated using the impedance value and the height of the individual by including a parameter h^2/R in to a regression equation along with population specific constants (where h=height (length), and R=impedance (resistance)). Fat mass can then be calculated by a predictive equation, which uses established constants and anthropometric measurements such as weight.

In human body the resistance is based collectively on 5 cylinders (four limbs and the trunk). Since the resistance of the conducting tissue is directly proportional to the length of the conductor and inversely to the cross-sectional area, the limbs that offer greatest resistance determine most of the variability in the estimated fat[172]. Thus, the fat measured using BIA depends on the proportionality of different body parts in a population in comparison with that of the reference population used to model the equation. Thus, it is recommended that the equations be validated for different populations.

In spite of this, BIA has shown to give a reliable estimate of fat and fat-free mass when compared to reference methods. Bioimpedance has been validated for use by several researchers, both in adults and in children, using reference methods such as DEXA, underwater weighing or isotope dilution methods, by developing equations suitable for the populations in question. Several of these studies showed that the agreements between FM or FFM estimated using BIA and that of reference methods were high (r^2 or $r \geq 0.8$)[165-168,173]. Some researchers showed that most of the statistical variability noticed in FM using different equations was explained by the anthropometric variables in their models, while the contribution of the impedance measure itself was minimal[174-176].

My study was not equipped to validate this relatively new method for the Indian context since a suitable reference method was not locally available. Total body fat was measured in my study children at 5 years using the bioimpedance technique, and was compared with the fat measures calculated using existing skinfold equations. There were statistically significant agreement between FFM ($r > 0.8$), FM ($r \geq 0.7$) and fat% ($r \geq 0.3$) from bioimpedance, and those from skinfold methods, but the actual values themselves differed considerably between three methods with bioimpedance measuring consistently higher than skinfolds. The correlations seen in my study are consistent with those observed between BIA measured resistance, and individual skinfolds and sum of skinfolds from an earlier study in children[175]. The maximum correlation seen was less than observed in Mysore ($r=0.45$). The level of agreement seen in the present study is acceptable considering that FM and FFM measured by these 2 methods were based on different principles. While BIA measures TBW primarily, and then FFM, fat mass was derived by deducting the FFM value from body weight. On the contrary, skinfold equations measure body fat, and FFM was derived as a difference from body weight. In deriving their primary outcomes, all the equations use several constants, which may be different for different individuals. Nevertheless, the correlations seen for FFM were consistent with those seen in earlier studies comparing TBW measured using criterion methods and BIA.

A few studies have also reported that BIA gave higher estimation of body fat, similar to Mysore findings, compared to other methods used. One recent study in Pune, India, validated this method for Indian men, using deuterium dilution as the reference method[165]. The researchers observed strong correlations between fat estimated from

bioimpedance by new validated equation, and that obtained from manufacturer's equation on the software. However, they found that the software equation over estimated the fat mass by 1.2 kg in these adult men. Similarly, FM estimated using BIA was higher in children of Sri Lankan origin compared to FM calculated using skinfolds and isotope dilution, and more children were classified as obese based on this method[177]. The values seen with BIA in my study may reflect that this method is measuring internal fat not accessible by skinfold methods, and thus giving a more comprehensive estimation. On the other hand, it may be a spurious finding, especially since the prediction equation was not calibrated for my study population, and estimation of fat mass from BIA is dependent on several assumptions as noted above.

I compared the relationships between body fat measured using BIA and skinfolds, and some of the cardio-vascular and diabetic risk factors in my study children, to assess the validity of higher adiposity as given by BIA. FM and fat% measured from both BIA and skinfold methods were associated with the risk markers in a similar magnitude, and the associations were statistically significant. Thus, it is not possible to deduce any method as superior in my study without undertaking suitable validation of methods using an accepted reference. This is one of the future plans of the ongoing study, where a 'doubly-labelled water method' will be used in a subset of the study children to estimate TBW, which acts as a standard to compare TBW measured by BIA, and a suitable equation can be derived for the local population.

In conclusion, BIA after appropriate calibration could be useful in estimating total body fat, and complementing anthropometric measurements in large field studies, especially since it is easy to operate and is more acceptable by subjects. However, there is a need for a unifying equation to suit all populations rather than having an array of different equations to choose from for each community.

6. BLOOD PRESSURE

Essential hypertension is rare in children, however, increased BP as a component of metabolic syndrome has been demonstrated in a few studies involving children[42,43]. As in adults, low birthweight in combination with high current size was a major risk factor. Blood pressure was also shown to be elevated in the offspring of diabetic mothers, suggesting maternal gestational diabetes as another risk factor for high BP in the offspring[178].

In the present chapter, I analyse the blood pressure at 5 years in my study children in relationship to their current size, birth size and maternal gestational glucose tolerance.

6.1 Methods

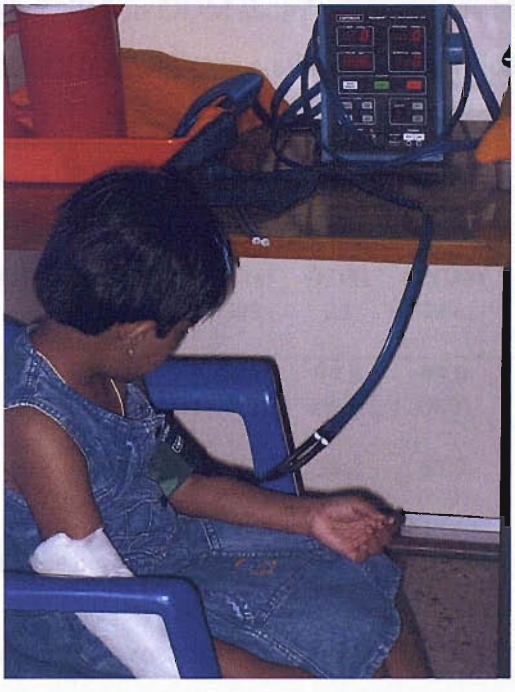


Figure: 6.1. Blood pressure measurement

Systolic (SBP) and diastolic blood pressures (DBP) were measured using a fully automated (CRITIKON, DINAMAP™ model 8100) BP monitor. The device was calibrated manually using a mercury sphygmomanometer. The measurements were done on the non-cannulated arm, following the 30-minute blood sample after allowing the child to relax seated for 5 minutes (Figure 6.1). BP was measured using child cuffs for all children (arm size range: 12 to 19 cm) according to a set protocol (Appendix 1). All

measures were taken in duplicate, with one-minute gap between the two measurements. The average of the two measurements was used for the analysis.

6.1.1 Statistical methods: Blood pressure measurements were adjusted for the room temperature. There were minimal changes in the values of systolic and diastolic BP after adjustments (<0.1 mm Hg). Both systolic and diastolic measurements were normally distributed. All regression and correlation analyses were adjusted for sex.

6.2 Results

Adjusted BP was available for 582 of the 585 children studied at 5 years of age. Of the remaining 3, 2 children refused BP monitoring, and for one, room temperature was not recorded. In the whole cohort boys had higher blood pressure than girls. The differences were of borderline significance only for the systolic BP (Table 6.1). There was a tendency for higher SBP in ODM than in ONDM (Table 6.1). DBP was higher in male ODM. None of the differences was statistically significant.

Table 6.1. Blood pressure values for the study children at 5 years

	Males			Females			All			
	Total	ODM	ONDM	Total	ODM	ONDM	ODM	ONDM	P^1	P^2
N =	279	13	252	303	23	264	36	516		
Systolic BP	**97.3** (8.2)	**100.2** (8.2)	**97.3** (8.2)	**96.0** (8.0)	**98.0** (8.3)	**95.9** (8.0)	**98.8** (8.2)	**96.0** (8.0)	0.053	0.1
Diastolic BP	**58.5** (6.7)	**60.6** (5.0)	**58.5** (6.7)	**57.7** (6.8)	**56.2** (5.6)	**57.9** (6.9)	**57.8** (5.7)	**57.7** (6.8)	0.2	0.8

Mean (SD); P^1 for the difference between sexes, P^2 for the difference between ODM and ONDM.

6.2.1 Determinants of blood pressure: current size. Univariate analysis showed that the current size of the child, especially weight (β=0.98 mm Hg/kg), BMI (β=0.48 mm Hg/kgm^{-2}), MUAC (β=1.49 mm Hg/cm), AMA (β=0.71 mm Hg/cm^2) and skinfolds (β=0.82 mm Hg/mm for both, P<0.001 for all) were the strong predictors of SBP at 5 years. Similarly, weight (β=0.31, P=0.03), height (β=0.13, P=0.05), MUAC (β=0.52, P=0.02), and triceps (β=0.41, P=0.003) were associated with DBP. All the above associations remained statistically significant after excluding the offspring of non-diabetic mothers from the analysis.

6.2.2 Birth size: There was a positive association between birthweight and SBP, and a negative association with DBP, though neither of these was statistically significant. None of the birth measurements had significant associations with blood pressure at 5 years, except MUAC, which was positively related to systolic BP (β=0.8, P=0.04). This association became non-significant (P=0.2) after excluding ODM from the regression.

After allowing for current weight, there were inverse associations between birth measurements and systolic BP (Table 6.2). This was particularly true for birth weight, length and head circumference. The inverse association was statistically significant only with birth length, which became borderline significant after removing ODM from the analysis.

Similarly, non-significant inverse associations were observed between birth measurements and diastolic BP after adjusting for current weight. Relationships were stronger with ponderal index and MUAC.

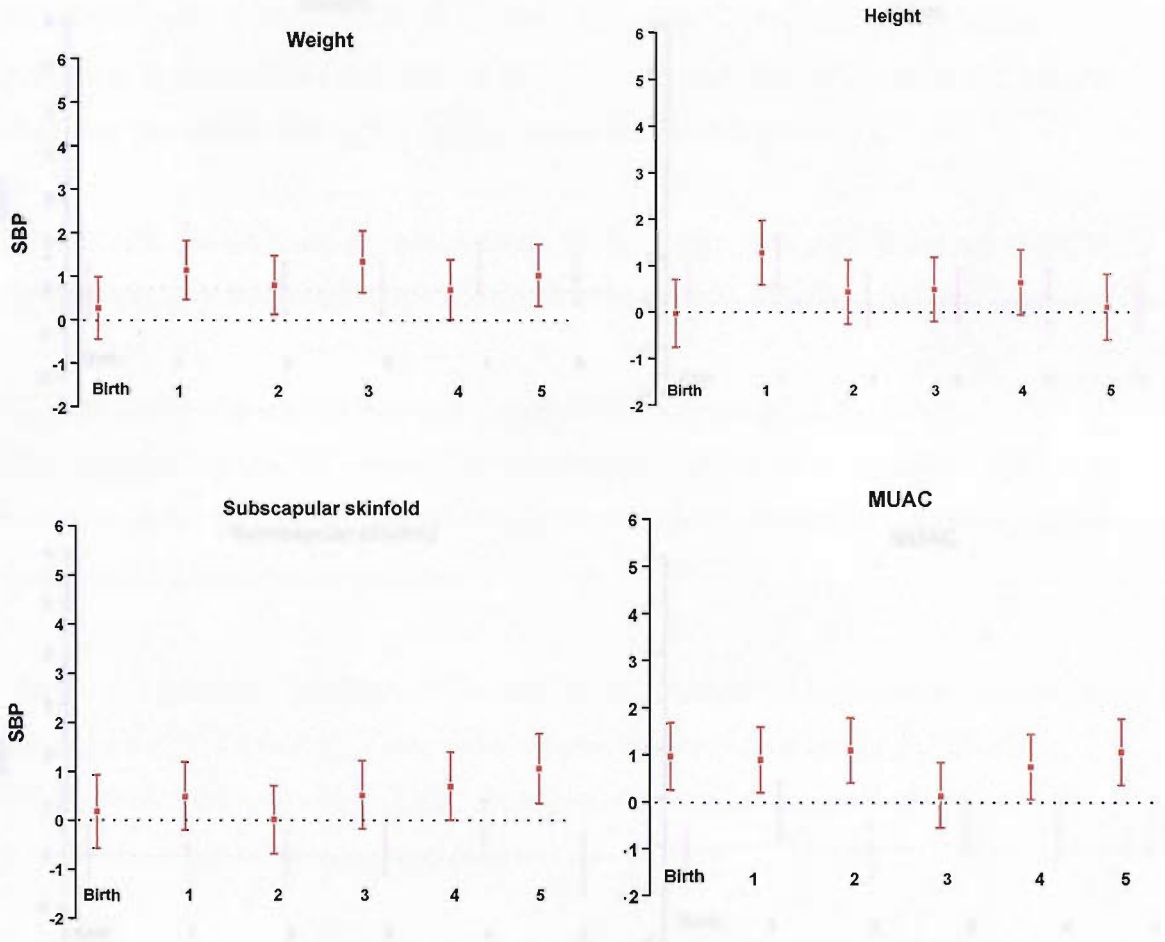
Table 6.2 Regression co-efficients (β) for association between birth size and childhood BP, controlled for childhood weight

	Systolic BP			Diastolic BP		
Birth measurements	**β**	**P**	**P***	**β**	**P**	**P***
Weight (kg)	-1.42	**0.1**	**0.07**	-0.93	**0.2**	**0.2**
Crown-heel length (CM)	-0.36	**0.04**	**0.07**	-0.03	**0.9**	**0.8**
Ponderal Index (kg/m^3)	0.06	**0.6**	**0.998**	-0.16	**0.1**	**0.05**
Head circumference (cm)	-0.37	**0.2**	**0.2**	-0.01	**0.96**	**0.97**
MUAC (cm)	0.21	**0.6**	**0.8**	-0.54	**0.09**	**0.06**

* Offspring of non-diabetic mothers

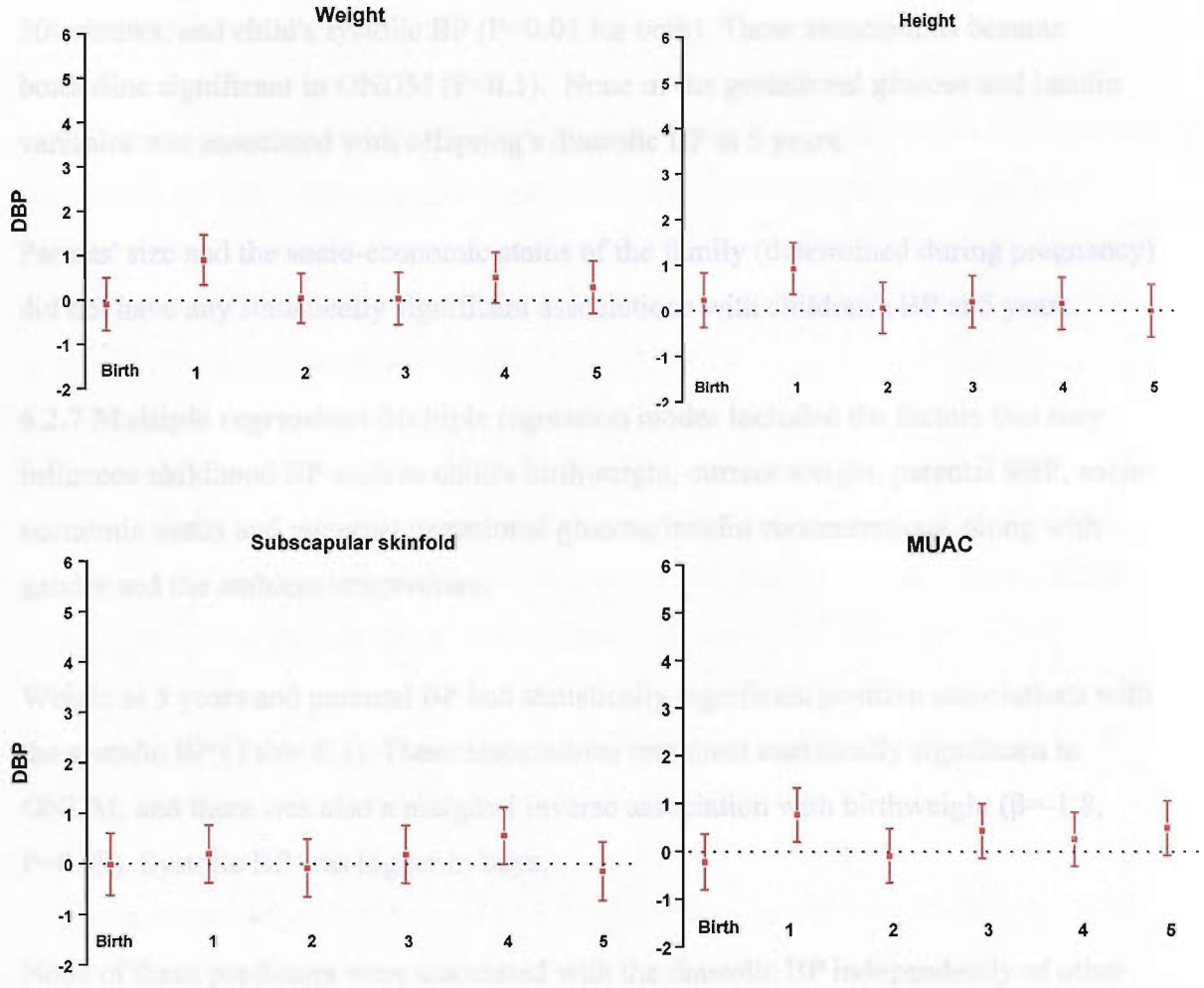
6.2.3 Effects of longitudinal growth from birth to five years: Conditional SD scores: Conditional Z-scores were calculated (Section 4.9.1) to examine the associations between gain in size at each year from birth to 5 years on the BP measurements at five years. The anthropometric measurements used as determinants were weight, height, subscapular skinfold thickness and MUAC.
As noticed earlier, except for a positive association with MUAC, none of the other measurements at birth were associated with SBP (Figure 6.2). However, increased gain in weight and MUAC from birth onwards was associated with higher SBP at 5 years. Gain in adiposity as measured by subscapular skinfold thickness had a positive effect on systolic BP from 3 years of age.

Figure 6.2 Change in the systolic BP (with 95% CI for change) measured at 5 years per SD score increase in anthropometric measurements from birth to five years



In a similar analysis with diastolic BP (Figure 6.3), it was observed that increased gain in weight, height and MUAC during the first year of life was related to higher BP at 5 years. There were no statistically significant associations with change in size at later years.

Figure 6.3 Change in the diastolic BP (with 95% CI for change) measured at 5 years per SD score increase in anthropometric measurements from birth to five years



6.2.4 Correlations with glucose and insulin concentrations at 5 years: There were positive correlations between both the systolic (R=0.18, P<0.001) and the diastolic BP (R=0.11, P=0.007), and GAUC of children. Positive correlations were also observed with HOMA (SBP R=0.1, P=0.01; DBP R=0.09, P=0.03), but not with IAUC or insulin increment. These correlations remained statistically significant even after controlling for maternal GDM status.

6.2.5 Parents' blood pressure: Maternal SBP at follow-up was positively associated with both systolic (B=0.13, P<0.001) and diastolic BP (B=0.06, P=0.02) of the child, and mother's DBP with child's diastolic BP (B=0.06, P=0.04). Similarly, statistically significant positive associations were seen between paternal and child's systolic (B=0.06, P=0.01) and diastolic BP (B=0.06, P=0.04), respectively.

6.2.6 Maternal gestational glucose/insulin concentrations: Maternal GDM did not have any statistically significant associations with the child's BP. There were significant inverse associations between maternal insulin concentrations and insulin increment at 30-minutes, and child's systolic BP (P=0.01 for both). These associations became borderline significant in ONDM (P<0.1). None of the gestational glucose and insulin variables was associated with offspring's diastolic BP at 5 years.

Parents' size and the socio-economic status of the family (determined during pregnancy) did not have any statistically significant associations with children's BP at 5 years.

6.2.7 Multiple regression: Multiple regression modes included the factors that may influence childhood BP such as child's birthweight, current weight, parental SBP, socio-economic status and maternal gestational glucose/insulin concentrations, along with gender and the ambient temperature.

Weight at 5 years and parental BP had statistically significant positive associations with the systolic BP (Table 6.3). These associations remained statistically significant in ONDM, and there was also a marginal inverse association with birthweight (β=-1.8, P=0.09). Systolic BP was higher in boys.

None of these predictors were associated with the diastolic BP independently of other predictors. After excluding ODM from the regression model, there was a significant positive association between maternal SBP and child's DBP (Table 6.3).

Table 6.3. Multiple linear regression co-efficients (β) for association between predictors and childhood BP

	Systolic BP			Diastolic BP		
	β	P	P*	β	P	P*
Current weight (kg)	1.06	<0.001	<0.001	0.29	0.1	0.07
Birthweight (kg)	-1.32	0.2	0.09	-0.88	0.3	0.3
Maternal GAUC (mmol)	-0.001	0.7	0.5	0.001	0.5	0.6
Maternal IAUC (pmol)	-0.00	0.5	0.6	-0.00	0.4	0.4
Maternal systolic BP (mm Hg)	0.13	<0.001	<0.001	0.06	0.06	0.03
Paternal systolic BP (mm Hg)	0.08	0.005	0.007	0.01	0.6	0.6
Socio-economic score	-0.10	0.2	0.1	0.05	0.5	0.7
Gender (M/F)	-1.45	0.052	0.04	-0.85	0.2	0.3
Room temperature (^{0}C)	-0.07	0.7	0.9	0.04	0.8	0.9

* Offspring of non-diabetic mothers

6.3 Summary of main findings

In this cohort, blood pressure tended to be higher in male offspring than in females at 5 years. Measurements were higher in ODM than in ONDM, though the differences were not statistically significant.

Current weight was the strongest predictor of systolic BP at 5 years, even after adjusting for parents' BP and maternal gestational glucose/insulin concentrations. Significant positive associations were also observed between other components of current body size such as height, MUAC and skinfold thickness, and the systolic BP. These associations were less strong with the diastolic BP.

There was a non-significant inverse association between birth weight and blood pressure measurements after adjusting for current weight. Gain in weight and MUAC (surrogate of lean mass) after birth was associated with higher SBP at 5 years. Higher gain in size during infancy, but not other years was associated with higher DBP.

The systolic BP at 5-year follow up in both parents was a strong positive predictor of systolic BP of the offspring, independent of other predictors.

Higher glucose concentrations and insulin resistance in the child were associated with higher BP measurements.

6.4 Discussion

Primary elevation of BP is a major adult disorder associated with CV disease and is one of the most widely studied outcomes in relation to the 'fetal origins' hypothesis. Essential hypertension in children has been emerging as a public health concern in both developed as well as developing populations, and is particularly associated with the growing problem of childhood obesity.

Current size and BP: In my study children current size, including weight as well as components of weight such as height, muscle mass (MUAC, AMA), and adiposity (skinfolds), was the strongest determinant of blood pressure at this age. It is well known that the BP rises directly in relation to current body size, especially body mass. Concomitant adiposity could be an important factor leading to this association. Though, the mechanism for the same is not clear, the endocrine actions of adipocytes are thought

to play a role in such associations[179]. Some of the pathogenetic mechanisms suggested are over stimulation of sympathetic activity (mediated by leptin or free fatty acids), activation of the renin-angiotensin system (mediated by leptin, angiotensin-2 or pro-inflammatory substances such as TNF-α and IL-6), effects of leptin on sodium-potassium ATPase in renal cells, and induction of endothelial dysfunction mediated by inflammatory cytokines such as endothelin-1[179]. Insulin resistance and hyperinsulinaemia associated with obesity may be a contributing factor[180].
Not all associations can be explained by body fat alone. A study from New Delhi, in India, showed significant positive associations between adult lean mass and AMA, and BP measurements in a group of men and women[181]. Similarly, in another study in adolescents, BP measurements were strongly significantly correlated with lean body mass[182]. Though the authors speculated about the possible role of increased muscle tone in elevating BP, this area needs more study. One of the reasons for such associations in my study and the above may be the use of inappropriate lean mass index, which is not completely free from the effects of adipose tissue[181].

It has been suggested that growth and arterial pressure in children are stimulated by a common mechanism, through the actions of insulin and Insulin-like Growth Factors (IGF-1)[183]. Strong associations seen between components of current size and BP in my study may be a reflection of such a mechanism.
On the other hand, the use of a single-sized cuff for all children might have induced greater variations in the BP values at the extremes of MUAC.

Fetal origins of hypertension: While the direct role of current body mass in increasing BP has been universally acknowledged, the 'fetal origins' hypothesis proposed that the above associations were stronger in those individuals who had lower birthweights. It was shown in elderly men and women in the UK that the SBP fell consistently as the birthweight rose from <2.5kg to >3.9 kg[45]. These associations were particularly strong after adjusting for current weight, especially in children, and were mainly for SBP[75,76]. This association was shown to be present in children from developing countries also[43,184]. In my study children, after adjusting for current weight, SBP fell by about 1.5 mm Hg for a kg rise in birthweight. This finding corresponds to the earlier studies both in India and elsewhere. A review by Law *et al.* reported a decrease of 2-3 mm Hg of SBP in children for 1 kg rise in their birthweights[75]. Similarly in another review of later

studies, a 1-2 mm Hg fall of SBP was reported for a kg increase in birthweight, both in adults and in children[76].

Several mechanisms have been proposed relating to fetal origins of BP.

- Intra-uterine growth retardation is thought to activate fetal rennin-angiotensin system leading on to raised BP[185].
- It may be also that IUGR leads to reduction in number of nephrons, which may increase glomerular pressure and lead to essential hypertension, which in turn lead to glomerulosclerosis thus bring about a vicious cycle[185].
- Low birthweight individuals also proposed to have altered HPA axis, which raises cortisol levels in them, which is another potential cause of raised BP[186].
- The endocrine effects may also act through growth harmone-IGF-1 axis[45].
- Reduced elasticity of vascular structure is another characteristic associated with low birthweight. One of the reasons for this may be impaired synthesis of elastin in vessel walls[187].
- Finally, it is proposed that IUGR is associated with sympathetic over activity, which may be another cause of elevated BP in low birthweight individuals[45].

The association with birthweight amplified with age in other populations, but an earlier study from our unit in Mysore did not show any association between low birth size and BP in adults[188]. Our continuing follow-up may help us to understand the future course of this trend.

Maternal hyperglycaemia and offspring BP: A few studies have demonstrated higher BP in children born to mothers with GDM. In my study, SBP values were an average 2 mm Hg higher in ODM compared to ONDM. A difference of similar magnitude was seen in an earlier study in a group of children at 6 and 7 year of age[93]. In another study of children aged 12-13 years, Cho *et al.* showed that the ODM had an average of 8 mm Hg higher SBP than control children, which was statically significant[178]. A study among Pima Indian children showed a significantly higher SBP in children born to mothers with diabetes than in their siblings born before the mothers developed diabetes (118 vs. 107 mm Hg), suggesting intra-uterine programming effects rather than genetic causes[189]. The reasons for this phenomenon are not known. Maternal hyperglycemia may alter renal parenchymal structure and functions[189]. Hyperinsulinaemia could be another reason for the rise in BP in these offspring. Enhanced angiotensin converting

enzyme (ACE) activity in the heart, lungs and kidneys has been observed in an animal model, and it was proposed that alterations in ACE may be associated with altered BP in ODM[190]. However, more studies are needed in this field to understand the mechanism behind the hypertensive effects of maternal hyperglycemia.

Hypertension is known to aggregate in families. It may be either due to genetic link or related to shared lifestyle by the family members. Statistically significant positive association between parents' and children's BP in my study conforms to these findings. Socio-economic status of the family did not predict BP in our cohort. Other factors related to higher BP such as infant feeding and maternal smoking[191] could not be assessed in my study as the breast-feeding was universal and none of the mothers smoked.

Children's BP was positively associated with glucose concentrations and HOMA-resistance at five years. Several studies measuring glucose and insulin concentrations have shown associated BP elevation with higher glucose and insulin concentrations. This may suggest early clustering of factors related to insulin resistance syndrome in our children.

To conclude, my study showed a small, non-significant effect of low birthweight and maternal GDM on the SBP of children at 5 years. Blood pressure is known to rise with age. Even a small rise in pressure during childhood may induce smooth muscle hypertrophy in resistance vessels leading to further rise in BP, and a vicious cycle of vascular hypertrophy and higher BP[192]. Thus, children in the higher BP category, even in non-pathological levels, are more likely to become hypertensive as adults. Therefore, it may be essential to bring about changes in life style factors in those children who are at risk.

7. PHYSICAL ACTIVITY MONITORING

The role of adiposity/obesity in the disease risk in individuals is widely recognised. The prenatal determinants such as genes, fetal nutrition and maternal GDM are clearly important in setting the adipogenic process in motion. Nevertheless, postnatal predictors such as dietary habits and physical activity levels are particularly critical as these can be modified effectively to bring about desirable changes in the risk outcome.

Less physical exercise is associated with obesity even in children. In my study, physical inactivity may be an important contributor to the adiposity in children, especially in certain risk groups such as offspring of diabetic mothers (ODM). Measurement of activity levels may help to determine the comparative role of physical activity in increasing the level of body fat in our children. I briefly describe here some of the methods available for measuring physical activity in field.

7.1 Subjective methods

These are the most commonly used methods in large field studies. The subjects give information about their activity pattern, either by answering a questionnaire, or filling-out detailed activity diaries for a specified period of time (usually a week). Although this is the most cost-effective method, it is crude and has many disadvantages. The questionnaire method requires the subjects to recall their activities, and thus the information is often unreliable, especially in children. The activity diary gives valid information in most instances, but needs a high degree of subject compliance. This method is difficult to use in young children. Reporting can be biased in both the methods.

7.2 Objective methods

These are the more reliable methods as there will be no reporting bias. Some commonly used techniques are:

7.2.1 Direct observation: This method, where the activity is observed by a field worker over a period of time, gives reliable information and can be used as a criterion method to calibrate other objective as well as subjective methods especially in children in free living conditions. However, it requires highly motivated field workers, and cannot be applied for long-term monitoring because the activity pattern may change in front of the observer and the subjects may find it intrusive.

7.2.2 Motion sensors:

7.2.2.1 Pedometers. These are the most commonly used motion sensors in the field. They measure movement of the body by counting steps, and essentially give a measure of body locomotion. This is a relatively cheap method, and easy for self-administration. However, this does not give information about intensity and pattern of activities. In spite of these drawbacks, pedometers are of great value in promoting physical activity in health intervention programmes. Recently, electronic pedometers were found to give highly accurate measure of number of steps accumulated. The steps were highly correlated with walking speed and oxygen consumption. Thus, this could be a useful technique in large field studies that involve mainly locomotor activities.

7.2.2.2 Accelerometers. These are electronic devices that measure more complex body movements, and differentiate different intensities of movements. They can be either uniaxial, measuring acceleration in a single plane, usually vertical, or triaxial measuring omni-directional movements. The activity is measured as counts for a user-specified time interval, usually for each minute. These are small, non-intrusive and have large memory capacity. Hence the movements can be monitored for long periods. However, activities not involving body displacement are not accurately recorded.

7.2.3 Heart rate monitoring: This is based on the assumption that there is a linear relationship between heart rate and the volume of oxygen consumed. Thus, it is a measure of energy expenditure (EE) rather than activity. Heart rate is measured using chest polars and EE is estimated using prediction equations. This method has been validated in laboratory settings using indirect calorimetric method. A major source of error by this method is that the heart rate is altered by factors other than physical exercise, such as emotional stress or ambient temperature.

Some centres have developed new complex devices with multiple functions. Actiheart (Cambridge Neurotechnology Ltd., Papworth, UK) combines heart rate monitoring and movement sensors in one small, unobtrusive unit. This has been validated for adults in a laboratory setting, using ECG electrodes and indirect calorimetry, but has scope for validation in different settings, in free-living conditions, and for children.

Minisun (University of Southampton, UK) has multiple sensors in a bulky unit to measure movements of different body parts to assess both movements and position.

7.3 Criterion methods

In addition to the direct observation method, other criterion methods measure EE, which can be correlated with different parameters of activity measurements obtained from the method to be validated.

7.3.1 Indirect Calorimetry: This calculates volume of oxygen consumption and CO_2 production for an activity, from which EE can be estimated. Previously, this technique required the activities to take place in a laboratory setting, but now there are portable versions to use in free-living conditions. They require the subjects to wear fairly obtrusive equipment, which limits their usefulness in large field studies in children.

7.3.2 Doubly labelled water method (DLW method): This is a gold standard method to measure EE in free-living conditions. Isotopes of hydrogen (^2H) and oxygen (^{18}O), diluted in water are given to drink after a basal fluid (urine, saliva or blood) sample is collected. Another sample is taken after the ingested isotope reaches equilibrium in the body (usually between 3 and 6 hours after ingestion). A further sample is collected at the end of the study period (7-14 days) to measure the amount of excretion of the isotopes. Usually, ^2H is excreted as water and ^{18}O as water and CO_2. The difference between these two rates gives an estimation of oxygen consumption, which can be used to derive EE. Samples may be collected at regular intervals in the course of the study to get a reliable estimation of habitual activity pattern. The high cost of the isotopes limits the suitability of this method in large studies.

In my study, we measured activity levels in a selected group of children using MTI (previously CSA) accelerometers. This was a preliminary attempt to examine the behavioural factors determining adiposity in our cohort, in preparation for developing measures to intervene in the adipogenic pathway. The main aims were:

- To test the feasibility and validity of using accelerometers to measure activity in local children.
- To compare activity levels between offspring born to mothers with and without gestational diabetes to see whether ODM have lower activity levels than controls
- To examine whether reduced activity is associated with higher body fat.
- To examine whether birth weight was a determinant of the level of physical activity in them.

7.4 Methods

7.4.1 Study Sample: Table 1 gives a scheme of planned and the final recruitment figures for the study.

Table 7.1 Number of children monitored in different groups

Groups	Planned	Recruited
Offspring of diabetic mothers	36 (13 boys+23 girls)	31 (9 boys+22 girls)
Sex, BMI matched controls	36 (13 boys+23 girls)	31 (9 boys+22 girls)
High fat category*	20 (10 boys+10 girls)	24 (9 boys+15 girls)
Low fat category*	20 (10 boys+10 girls)	20 (10 boys+10 girls)

* offspring of non-diabetic mothers in highest and lowest quartiles of 5-year subscapular skinfold thickness.

One boy studied as a control was also eligible for the high fat group. Since boys available in high fat group were few, he was considered as being recruited for both groups. Thus, the total study sample was 105.

7.4.2 Actigraph measurements: The uniaxial motion sensor accelerometer, MTI/CSA Actigraph (Model AM7164, MTI Health Services, Florida, USA, Figure 7.1) was used to measure physical activity. This electronic device measuring 2 x 1.6 x 0.6 inches and weighing about 43g is one of the smallest devices for this application available in the market. It measures acceleration in a vertical plane 10 times each second. Total counts are given for each minute epoch (Figure 7.2).

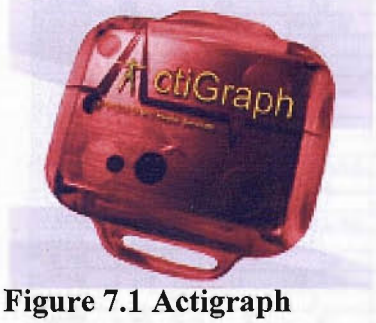
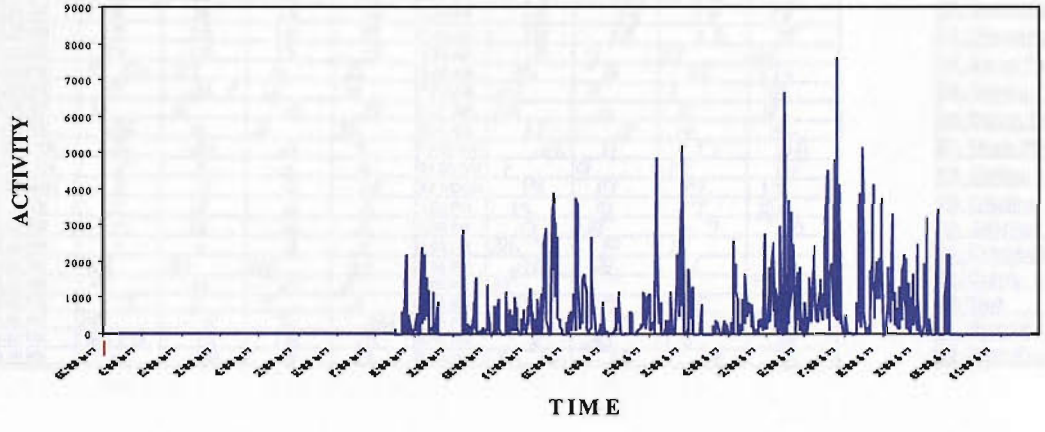


Figure 7.1 Actigraph

Figure 7.2 Activity chart produced by the Actigraph software. Each spike represents the counts accumulated for a minute of activity performed

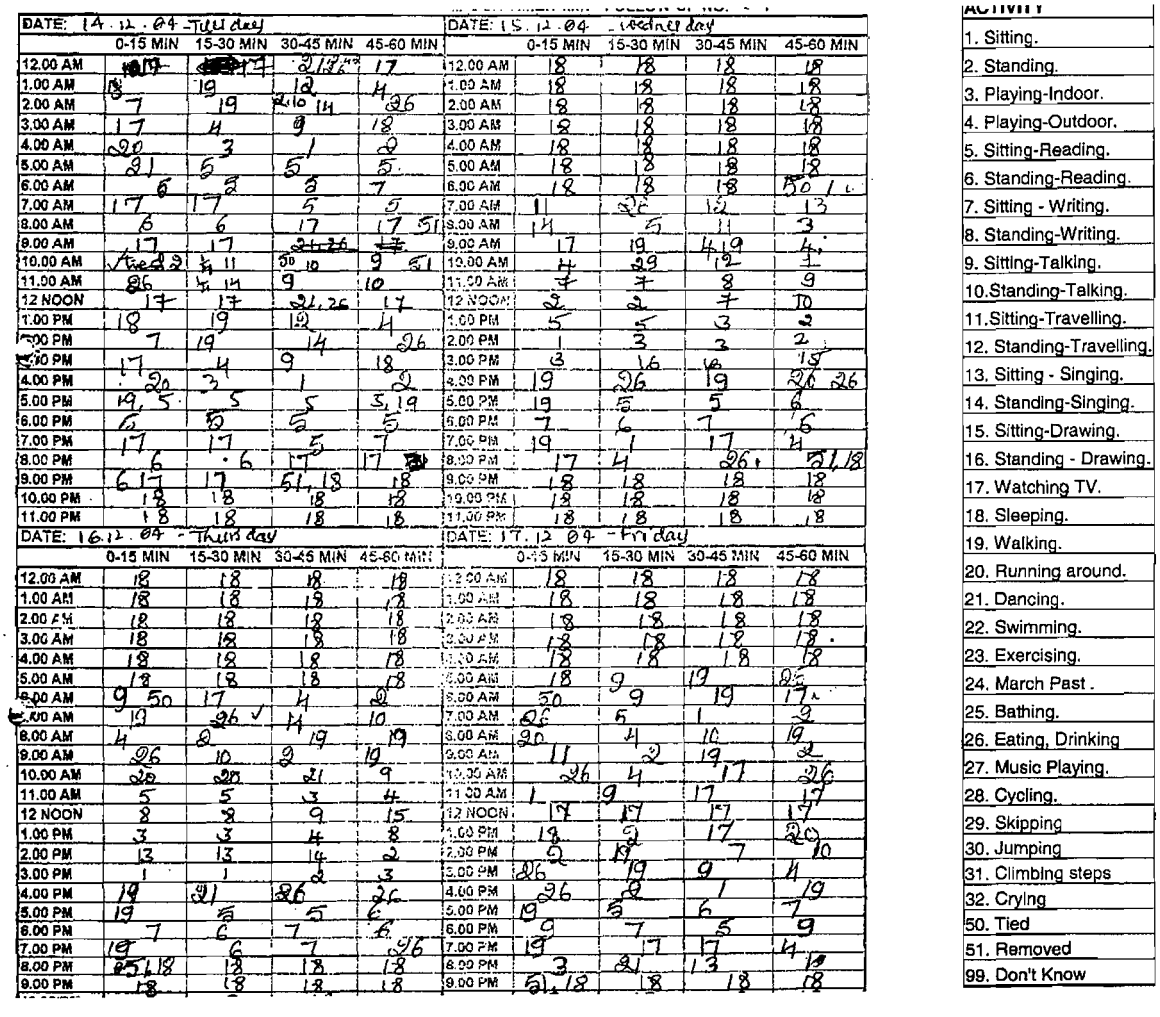


Actigraph, after initialising, was placed in a pouch and tied at the level of the right hip using an elastic waistband. Activity was measured for 7 days during waking hours. Parents were advised to remove the Actigraph during bathing and swimming, as the

machines were not waterproof. The monitors were taken off at night, and replaced when the child got up in the morning. At the end of a week, the data were downloaded into a computer using a reader interface unit supplied by the manufacturer.

Parents and teachers were requested to maintain a record of the child's activities during this period for every 15 minutes interval at home and school. The time of application and removal of the monitor was noted. To make this task simple, they were given log-sheets for all 7 days with empty boxes for each 15-minutes interval (Figure 7.3). Common activities of the local children were listed and coded, and a list of codes was provided to parents/teachers. They were instructed to write the code of the activity in the box against the appropriate time.

Figure 7.3 Sample of a filled-in log-sheet, and list of codes for activities



ACTIVITY
1. Sitting.
2. Standing.
3. Playing-Indoor.
4. Playing-Outdoor.
5. Sitting-Reading.
6. Standing-Reading.
7. Sitting - Writing.
8. Standing-Writing.
9. Sitting-Talking.
10.Standing-Talking.
11.Sitting-Travelling.
12. Standing-Travelling.
13. Sitting - Singing.
14. Standing-Singing.
15. Sitting-Drawing.
16. Standing - Drawing.
17. Watching TV.
18. Sleeping.
19. Walking.
20. Running around.
21. Dancing.
22. Swimming.
23. Exercising.
24. March Past.
25. Bathing.
26. Eating, Drinking
27. Music Playing.
28. Cycling.
29. Skipping
30. Jumping
31. Climbing steps
32. Crying
50. Tied
51. Removed
99. Don't Know

7.5 Validation

The Actigraph monitors were validated using two methods.

7.5.1 Activity diary: All children had activity diaries kept for a period of 7 days by their parents. However, only a few were of good quality. For validation, we planned to use diaries of 50 children (equal number of boys and girls) out of total children monitored, based on the quality of the diaries. We aimed to use legible diaries, which:

- Had activity recorded for most days
- Had activity recorded for a minimum of 10 hours in a day
- Had few empty spaces

Log-sheets for 46 children (25 girls and 21 boys) met the above criteria. Scores (Physical Activity Ratio or PAR) were assigned to each activity based on a published compendium[193]. Total Energy Expenditure (TEE) was calculated by a 'factorial method', which was based on PAR, time spent in activity in each PAR level, and basal metabolic rate (BMR) predicted by sex and age specific equations taking weight and height into account (Appendix 4). Physical activity levels (PAL) were derived by the equation PAL = TEE/BMR. The outcomes of this exercise were compared to Actigraph counts.

Energy expenditure was not calculated using Actigraph counts as the equations used for this (given by the manufacturers) underestimated TEE considerably, and gave a different value to the above method (Table 7.2).

Table 7.2 EE (kJ/minute) calculated using the factorial method from parental records, and using Actigraph counts

		EE/minute (parental log)	EE/minute (Actigraph counts)
Examples:	**1**	5.02	0.63
	2	8.11	1.07
	3	5.66	0.41
	4	4.89	0.65

7.5.2 Direct observation:

7.5.2.1 One-hour logs. For 34 children out of those selected for validation, the team members observed the activities for a period of 1-hour. Activity was recorded for each 5-minute interval; TEE was calculated for 1-hour as above.

7.5.2.2 Structured activity sessions. A separate group of children (not part of the study) of similar age to the study children were requested to take part in a structured activity

session (Figure 7.4). The children performed activities of intensities ranging from sedentary (sitting still) to vigorous (brisk running), within a span of one hour, with Actigraph in place. Two such sessions of 11 and 10 children were carried out. The second session, benefited from the experiences of the first, was more standardised with more specific instructions given for each activity and more uniform speed and distance set for walking and running activities. Counts accumulated for each activity were compared with the intensity of the activity. The second exercise was also used to determine counts for different levels of activity such as sedentary, light, moderate, and hard in the study children.

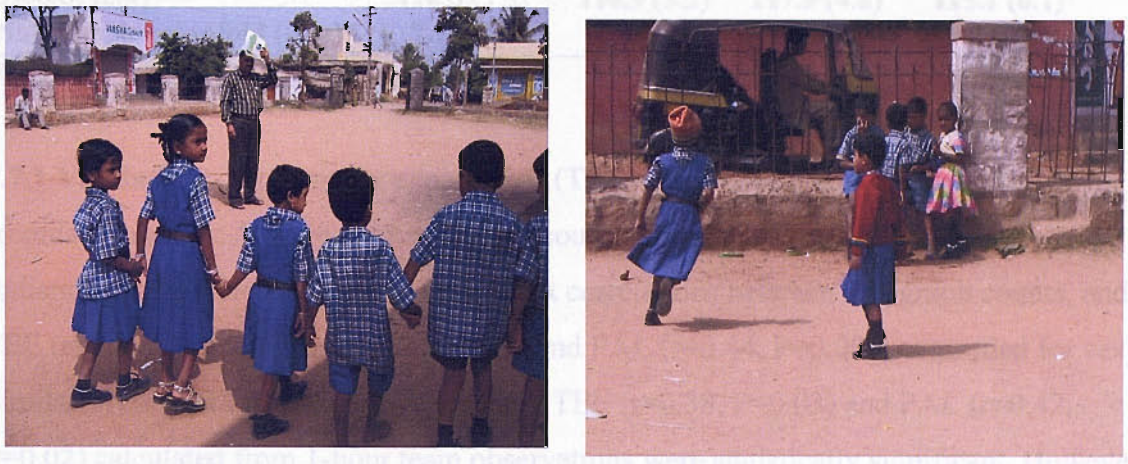


Figure 7.4 Structured activity sessions

7.6 Data reduction

Counts downloaded from Actigraphs were reduced for analysis using a software programme. Only days with a complete record, and a minimum of 500 minutes of recorded activity were used for the analysis to ensure that the full range of the children's activity was recorded. The first and the last days of monitoring were excluded, as counts for these days were incomplete and/or likely to include 'artefacts' related to applying and removing the monitor, and movements recorded after removal, but before downloading the data. Counts and number of minutes spent on different level of activities were summed for each day, and the average of these for all days was used in the analysis. After reduction, the period of monitoring ranged from 1 day (N=1) to 6 days. Only two children had <3 days of monitoring. All available data were used for analysis.

7.6.1 Statistical methods: For validation, correlations were used to compare the Actigraph counts with TEE and PAL from diaries/direct observation. Agreement between the two methods was tested using cross tabulations and *kappa* statistics. T-tests were used

to compare means between different groups. Regression analysis was used to determine the predictors of physical activity.

7.7 Results

Table 7.3 gives the characteristics of the children taking part in the study.

Table 7.3 characteristics of the children monitored for physical activity

	ODM	Control	High fat	Low fat
Age (yr)	**6.6** (0.3)	**6.7** (0.4)	**6.7** (0.4)	**6.4** (0.4)
Weight (kg)	**19.4** (3.2)	**20.0** (2.9)	**21.2** (4.3)	**17.0** (2.5)
Height (cm)	**116.0** (5.5)	**116.9** (5.2)	**117.9** (4.8)	**115.1** (6.1)
Subscapular skinfold (mm)	**7.9** (3.2)	**7.8** (3.0)	**8.8** (3.6)	**4.7** (0.4)

Mean (SD)

7.7.1 Validation: Total energy expenditure (TEE) and PAL calculated from parents' records (Appendix 4) were compared with counts from the Actigraphs using correlation statistics. There were statistically significant correlations between Actigraph counts, and TEE (r=0.40, P=0.007, controlled for sex) and PAL (r=0.44, P=0.002, controlled for sex). Similarly, correlations between counts, and TEE (r=0.38, P=0.03) and PAL (r=0.42, P=0.02) calculated from 1-hour team observations were statistically significant. However, there was a wide scatter of data points on either side of the regression line (Figures 7.5 and 7.6).

Figure 7.5 Comparisons between Actigraph counts and activity diaries

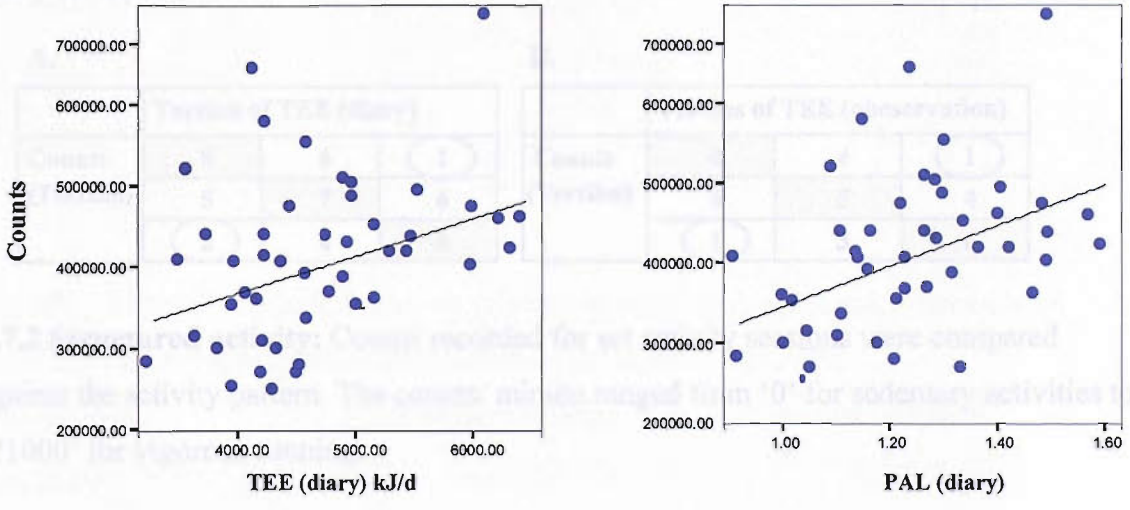
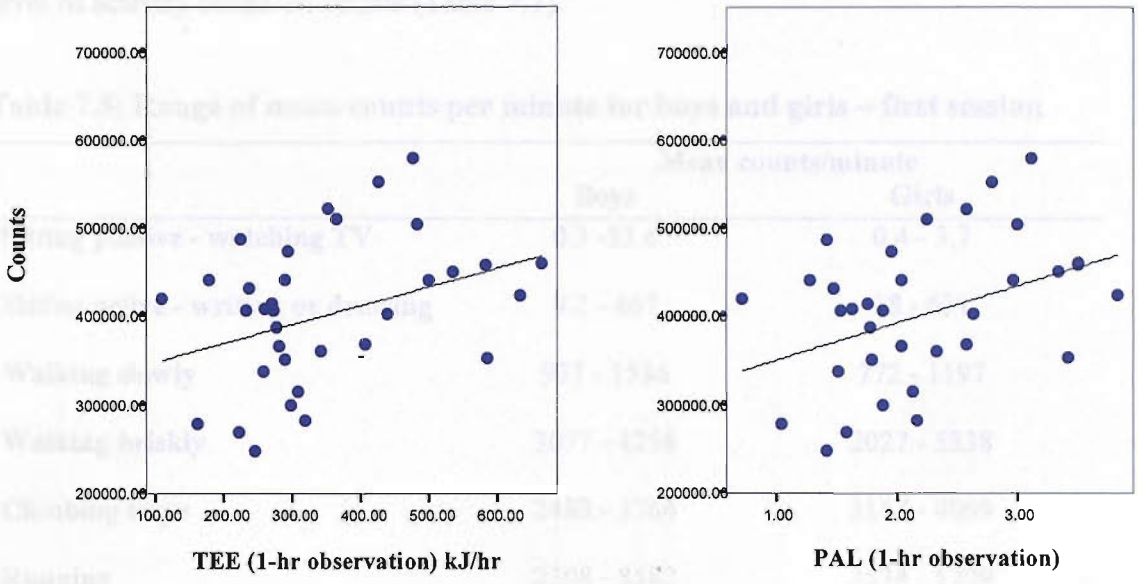


Figure 7.6 Comparisons between Actigraph counts and team observations



Level of agreement measured using *kappa* statistics was statistically significant between counts, and TEE (κ=0.25, P=0.02) and PAL (κ=0.28, P=0.007) from parents' reports. Measure of agreement was lower between counts, and EE (κ=0.16, P=0.2) and PAL (κ=0.21, P=0.08) calculated from direct observations by the team members. However, most of the observations were similarly recorded in both methods, while very few children were categorised differently (Table 7.4). There was no systematic bias in reporting by either of the methods.

Table 7.4 Cross tabulations showing level of agreement between counts, and A. diary B. team observations

A.

	Tertiles of TEE (diary)		
Counts (Tertiles)	8	5	1
	5	7	6
	2	4	8

B.

	Tertiles of TEE (obeservation)		
Counts (Tertiles)	4	4	1
	6	5	4
	1	3	6

7.7.2 Structured activity: Counts recorded for set activity sessions were compared against the activity pattern. The counts/ minute ranged from '0' for sedentary activities to '21000' for vigorous running.

Tables 7.5 and 7.6 show that the mean counts increased corresponding to the intensity of the activity. There was some degree of overlap of counts between activities and a wide

range of counts at any single activity. Nevertheless, it was possible to categorise each level of activity based on counts (Table 7.7).

Table 7.5: Range of mean counts per minute for boys and girls – first session

	Mean counts/minute	
	Boys	**Girls**
Sitting passive - watching TV	0.3 -33.6	0.4 - 3.7
Sitting active - writing or drawing	9.2 - 467	18 - 631
Walking slowly	937 - 1586	772 - 1197
Walking briskly	3077 - 4256	2027 - 5338
Climbing steps	2483 - 3764	3157 - 4099
Running	2308 - 8582	3534 - 5209
Free Play	2340 - 5374	1144 - 5322

Table 7.6: Range of mean counts per minute for boys and girls – second session

	Mean counts/minute	
	Boys	**Girls**
Sitting passive - watching TV	0 -3	0 - 0
Sitting active - writing or drawing	2 - 463	0 - 178
Walking slowly	592 - 2708	1292 - 2832
Walking briskly	2240 - 5226	3135 - 5185
Running	2738 - 8533	3986 - 4758
Free Play	726 - 1462	470 - 1162

Table 7.7 Cut-off counts/minute we selected to represent different intensities of activity compared with those calculated using existing equation

Activity level	**From Mysore sessions**	**Based on equation[194]**
Sedentary	<10	-
Light	10 to 400	<706
Moderate	400 to 3000	- 3137
Hard	>3000	>3137

The above cut-off bands were applied to estimate time spent on light, moderate and hard activities for all children (Figure 7.7).

Figure 7.7 Activity chart with cut-off bands for different levels of activity.

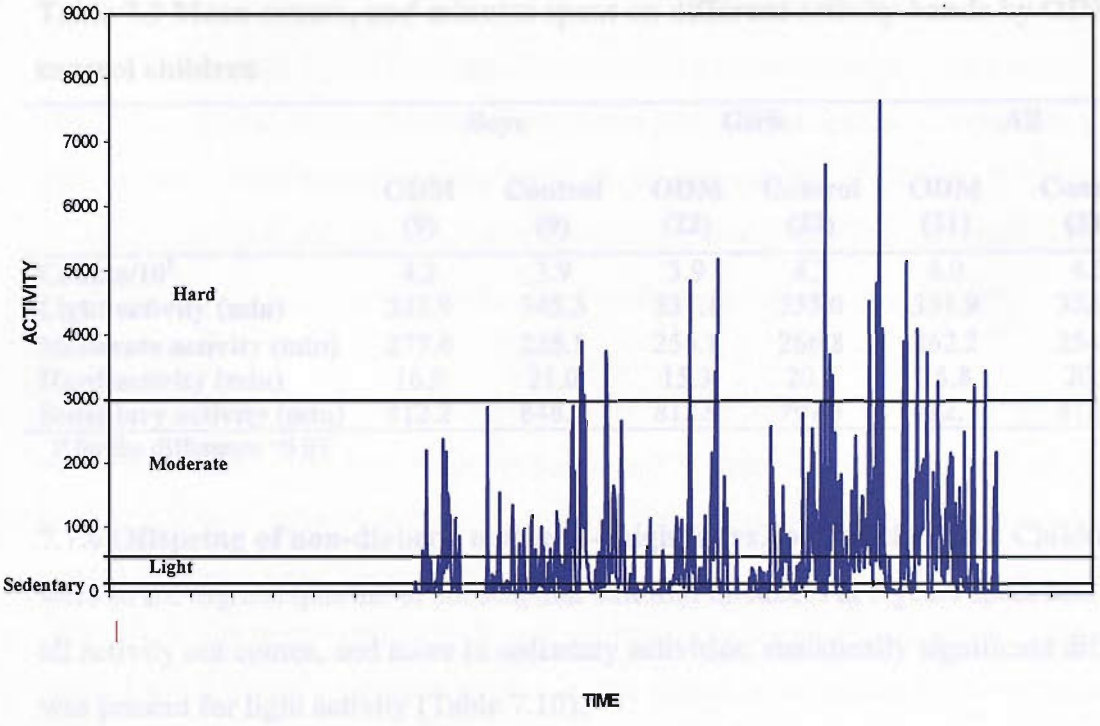


Table 7.8. Mean, range of counts (x 10^5/day), and time spent on different activity levels (minutes/day) by all children.

	Boys (N=35) Mean (SD)	Range	Girls (N=69) Mean (SD)	Range
Counts/10^5 (SD)	**4.1** (1.1)	2.4 to 7.0	**4.0** (1.1)	2.2 to 7.3
Light activity (%)	**346.2** (24.1 %)	236 to 519	**335.7** (23.3 %)	213 to 471
Moderate activity (%)	**252.7** (17.5 %)	144 to 363	**259.1** (18.0 %)	126 to 399
Hard activity (%)	**19.8** (1.4 %)	3 to 52	**17.8** (1.2 %)	2 to 55
Sedentary activity (%)	**821.3** (57.0 %)	683 to 998	**817.5** (56.8 %)	465 to 988

In the whole group, the average counts accumulated ranged from $2.2x10^5$ to $7.3x10^5$ (Table 7.8). Children generally spent very little time (<2%) in a day on hard intensity activities. There were no statistically significant differences between boys and girls for any of the activity parameters.

7.7.3 Offspring of diabetic mothers: Overall, ODM accumulated fewer counts than control children. This difference was clearly due to fewer counts in female ODM, while male ODM accumulated more counts than controls. However, none of these associations were statistically significant (Table 7.9).

Female ODM spent less time in light, moderate and hard activities than control girls, though none of the difference reached statistical significance. In contrast, male ODM spent significantly more time in moderate activities (Table 7.9)

Table 7.9 Mean counts, and minutes spent on different activity bands by ODM and control children

	Boys		Girls		All	
	ODM (9)	Control (9)	ODM (22)	Control (22)	ODM (31)	Control (31)
Counts/10^5	4.2	3.9	3.9	4.3	4.0	4.2
Light activity (min)	333.9	345.3	331.1	355.0	331.9	352.2
Moderate activity (min)	**277.0**	**225.1**	256.1	266.8	262.2	254.7
Hard activity (min)	16.9	21.0	15.3	20.8	15.8	20.9
Sedentary activity (min)	812.2	848.7	812.9	797.3	812.7	812.2

P for the difference <0.05

7.7.4 Offspring of non-diabetic mothers – high fat vs. low fat children: Children who were in the highest quartile of subscapular skinfold thickness at 5 years spent less time in all activity out comes, and more in sedentary activities; statistically significant difference was present for light activity (Table 7.10).

All the differences were more pronounced in females (Table 7.10). Girls in the high fat category also had statistically significantly lower counts than girls in the low fat group, and spent significantly less time in moderate activity. A similar, but weaker trend was present in boys.

Table 7.10 Mean counts and time spent on different activity levels by ONDM (high fat and low fat categories)

	Boys		Girls		All	
	High fat (8)	Low fat (10)	High fat (15)	Low fat (11)	High fat (21)	Low fat (26)
Counts/10^5	4.2	4.1	**3.6**	**4.7**	3.8	4.4
Light activity (min)	**319.1**	**384.2**	**306.4**	**342.4**	**310.8**	**362.3**
Moderate activity (min)	256.8	243.7	**232.9**	**292.1**	241.2	269.0
Hard activity (min)	20.8	19.6	15.3	22.4	17.2	21.0
Sedentary activity (min)	843.3	792.5	**876.0**	**784.2**	**864.6**	**788.4**

P for the difference '**borderline significant, <0.05, <0.01**

7.7.5 Determinants of physical activity: Physical activity levels were similar in both sexes. I examined a number of other possible determinants of physical activity, including birth weight, childhood weight, skinfold thickness, fat and fat-free mass, maternal sum of skinfolds (measured at 5-year follow-up), and socio-economic status of the family.

In the whole group, univariate regressions showed no association between birth weight and the physical activity outcomes. There were borderline negative associations between total fat mass (from bioimpedance), and Actigraph counts ($\beta = -0.16 \times 10^5$, P=0.08) and time spent on moderate intensity activities (β=-7.3, P=0.09). Socio-economic status, and maternal sum of skinfolds were not related to physical activity in children.

In a multiple regression analysis, a statistically significant positive association was seen between fat-free mass (from bioimpedance), and Actigraph counts (P=0.01) and time spent in hard activities (P=0.004, Table 7.11). Borderline significant negative associations were seen between fat mass and total counts (P=0.07) and moderate activity (P=0.07), and positive associations with sedentary activity (P=0.07). There was also a positive trend between mothers' skinfolds, and counts accumulated by the offspring (P=0.07) and time spent in moderate activities (P=0.09, Table 7.11).

Table 7.11 Multiple regression analysis of determinants of physical activity. β(P).

Child	**Counts ($x10^5$)**	***Sedentary activity**	***Light activity**	***Moderate activity**	***Hard activity**
Birth weight (kg)	-0.19 (0.6)	23.0 (0.4)	-0.40 (0.98)	-17.9 (0.3)	-1.10 (0.8)
Fat mass (kg)	-0.20 (0.07)	15.0 (0.07)	-3.88 (0.5)	-9.64 (0.07)	-1.59 (0.2)
Fat-free mass (kg)	0.19 (0.01)	-2.27 (0.7)	-0.69 (0.9)	5.04 (0.2)	2.43 (0.004)
SES	-0.03 (0.3)	0.04 (0.98)	1.80 (0.2)	-1.58 (0.3)	-0.19 (0.5)
Mother					
Sum of skinfolds (mm)	0.01 (0.07)	-0.17 (0.5)	-0.18 (0.3)	0.27 (0.09)	0.06 (0.1)
GAUC (mmol)	0.00 (0.7)	0.01 (0.6)	-0.02 (0.2)	0.01 (0.4)	-0.00 (0.9)
IAUC (pmol)	-0.00 (0.6)	-0.001 (0.1)	0.0001 (0.7)	-0.00 (0.8)	-0.00 (0.40

*minutes/day

I combined different activity levels in a stepwise manner (Appendix 4) and used these new variables in regression models to examine the simultaneous changes in different intensity activities in association with a predictor. The new analysis confirmed the above findings and showed that fat mass of the children was associated with an increase in sedentary activity and a simultaneously reduced moderate activity (P=0.07 for both). No associations were seen with other predictors.

7.8 Summary of main findings

CSA Actigraphs were well tolerated by the Mysore study children.

Counts accumulated by the Actigraphs were significantly correlated with TEE and PAL calculated from diaries.

Structured activity sessions further supported the validity of Actigraphs for use in children by showing clear-cut differences in counts between activities of varying intensities, and a linear increase in counts with increase in intensity of the task.

There was a wide variation between children in average counts accumulated per day. There were no differences in activity measures between boys and girls.

Female offspring of diabetic mothers tended to accumulate fewer counts, and spent less time in moderate and hard activities than control girls. In contrast, male ODM had more counts and spent significantly longer in hard activities than controls.

Children with larger subscapular skinfolds at five years had fewer counts, shorter periods for all activities and more time spent on sedentary tasks.

Physical activity was positively associated with fat-free mass, and negatively with fat mass after controlling for other predictors.

7.9 Discussion

Reduced physical activity is one of the behavioural factors generally blamed for the rise of obesity and associated risks in adults. I speculated in an earlier chapter (Section 3.8.5) that physical activity may be an important determinant of adiposity in this cohort, especially in females and ODM. Evaluation of physical activity using accelerometers in a sub-set of my cohort was the first of its kind to explore the above associations in Indian children.

Actigraphs and validation: The Actigraphs were chosen for our study because of their small size, acceptability in other populations for long-term monitoring in children, and validity of their data in the above studies. In my study, the Actigraph monitor was validated using 2 simple methods, and statistically significant agreement ($r=0.4$) was

found between the methods. The degree of correlation was similar to a recent validation study from the USA comparing the CSA model with parents' proxy records (r=0.3 to 0.4)[195]. One study validating CSA accelerometers in children under controlled settings found stronger correlations (r=0.6 to 0.7) with sophisticated reference methods such as room respiration calorimetry, microwave sensors, and heart rate monitors[196]. This has also been validated in free-living conditions in 9-yr old Swedish children using DLW method, and the correlations between CSA counts, and TEE (r=0.4) and PAL (r=0.6) calculated from the reference method were moderate[197]. Studies comparing counts accumulated using triaxial accelerometers in free-living conditions with either TEE and PAL estimated using doubly-labelled water method[198] or self reported questionnaires[199] have also reported similar correlation co-efficients (r=0.3 to 0.4).

In all the above studies, and in my own study, comparisons were made between two different outcomes: while counts given by accelerometers indicate intensity of movement of the body, reference methods estimate energy expenditure. For a similar intensity of activity, different individuals may expend different amount of energy as it is dependant on the body mass. This may be a reason for moderate degrees of correlation seen in these validation studies. Correlations between physical activity parameters from CSA and parental records in my study was comparable to the above findings, despite the disadvantages such as biased or incomplete reporting, and the use of adult PAR values for children. Direct observation from members of the research team, a superior method to diaries for validation purpose, did not improve the level of agreement. This may be because fewer children were compared by this method than by diaries, the activities of children might have affected by the presence of observers, or the 'factorial method' may not be appropriate to calculate TEE and PAL for 1 hour. Our structured activity sessions in another group of children showed a linear trend between counts and intensity of the activity performed, which confirmed the validity of the method in our children.

Physical activity outcome: Females are generally less active than males during pubertal years[200]. In a study of older children in the USA, girls spent less time in moderate activity (measured by CSA monitor) than boys, and reported that they spent less time in sports[201]. It is not known if it is true in young girls. However, a study in the UK measuring physical activity using CSA accelerometers in pre-pubertal children observed that boys were significantly more active than girls at 5 years (P=0.04)[202]. In Mysore, it is common for

girls to prefer sedentary activities during leisure time. However, my study did not show any effect of gender on physical activity as measured by Actigraphs.

In our study, female ODM exhibited a trend of lower counts and more sedentary activity than control girls, though this did not reach statistical significance. An opposite trend was noticed in male ODM. We had hypothesised that lower levels of physical activity may be contributing to higher adiposity in our ODM, especially in females. Lack of power may be a reason for the absence of statistical significance in girls, or there may not be any effect of maternal GDM on offspring physical activity. Findings in male ODM may be due to chance considering very few boys in our ODM group. I have not come across any comparable data for ODM.

Many studies have examined the association between self-reported physical activity and obesity/over weight in children[203]. Studies using objective methods, especially motion sensors in young children are few. A study in Australia assessing physical activity using doubly labelled water method and a triaxial accelerometer in 5 to 10.5 year old children found a negative correlation between PAL, and fat% and BMI[198]. Children in the highest tertile of time spent in hard activities had lower percentage body fat. In another study of 6-8 year old children in the USA, boys, but not girls who spent more time in light-intensity activities (measured using heart rate monitors) had higher fat mass index (FM/height), while PAL and EE from doubly labelled water method were positively associated with fat-free mass index after adjusting for sex[204]. In my study, children, particularly girls belonging to the higher subscapular thickness category had accumulated fewer counts. They also spent less time in moderate intensity activities and more in sedentary activities.

In my study children, fat-free mass was positively associated with counts accumulated by body movements. Studies have shown that Indian adults are not only centrally adipose, but also have lower muscle mass than Caucasian adults of comparable BMI[119]. Low muscle mass (MUAC) was a feature of Indian neonates in Pune and also in my study[118]. The above findings thus suggest two possibilities: A. children with higher lean/muscle mass may be more fit and thus are inclined to engage in strenuous activities than those with low muscularity, or B. children who are more physically active may develop higher lean mass than those who are more sedentary.

My study showed an association between physical inactivity and body fat. The main objective of our future follow-up is to attempt to achieve sustainable behavioural changes in physical activity in our children. Our calculations using the 'factorial method' show that if a child spends 10 minutes more on hard activities instead of sedentary activities in a day, the TEE increases by 200 kJ. A similar amount of energy will be expended if 30 minutes of sedentary activity is converted into moderate activity. Our current pursuit is to understand the best means of intervening with one activity, without bringing counter-effective changes in the existing activity pattern (eg. Increasing hard activities by 10 minutes may induce a child to decrease moderate activities by 30 minutes to compensate, thus making no changes in the energy expended as whole). Also, some of the major determinants of physical activity are related to psychosocial factors, presently, we are collecting information on the behavioural and social factors associated with physical activity in these children and their parents. A detailed scheme of the proposed future study is included in the Final Discussion chapter.

8. MATERNAL VITAMIN D STATUS DURING PREGNANCY

Vitamin D is an important micronutrient regulating calcium homeostasis in the body. The importance of this in maintaining skeletal growth, especially in children, is well known. Vitamin D is also shown to influence fetal growth, and a deficiency in the mother during pregnancy is associated with lower birthweight[124]. It has been shown in animals that insulin secretion is impaired in vitamin D deficiency, and restored on supplementation [205,206]. In humans, hypovitaminosis D has been shown to be associated with decreased insulin sensitivity and β-cell function[33].

It has been proposed that vitamin D deficiency may contribute to the high prevalence of type 2 diabetes in South Asian populations[124]. Maternal malnutrition is common in India and the intake of many micronutrients is low. There is an increased demand for vitamin D in pregnancy and a deficiency may play a role in the onset of gestational diabetes.

In this chapter I examine whether in my study maternal vitamin D status is related to:

- Maternal GDM and insulin concentrations during pregnancy
- Offspring size at birth
- Postnatal and childhood size
- Glucose/insulin metabolism in the offspring at 5 years

8.1 Clinical investigations

Maternal vitamin D (25-hydroxyvitamin D [25(OH) D]) was measured using stored serum samples, selected from 573 mothers who delivered at HMH, had a term delivery (≥37 weeks gestation) and full OGTT data. Adequate samples were available for 489 of these. Analysis was done at the Departments of Diabetes and Metabolic Medicine and Clinical Chemistry, Royal London Hospital, by radioimmunoassay (IDS Immunodiagnostics Ltd, Boldon, Tyne and Wear, UK. Intra- and inter-assay variations were 8.8% and 10.8% respectively). Mothers were defined as vitamin D insufficient at vitamin D concentrations of 11-20 ng/ml and deficient at concentrations <11 ng/ml[207]. The distribution of vitamin D concentrations was skewed and was log-transformed to normality.

8.2 Calcium and vitamin D supplements

At the time of the study, it was routine practice by general practitioners and

obstetricians to prescribe daily calcium supplements (some of which also included vitamin D) for pregnant women, usually from the beginning of the 2nd trimester. A wide range of products was prescribed, according to each practitioner's preference. Supplements contained between 500mg and 1250mg of calcium carbonate and some contained vitamin D3 (100-250 IU). Medications and supplements being taken by the women were recorded at the time of recruitment, but not subsequently, and were therefore not available at 30 weeks gestation, when blood samples were taken. Of the 489 mothers, 75 were taking calcium, 133 were taking calcium and vitamin D at recruitment and 281 were not being supplemented. Since supplementation was routine practice, many women not on supplements at recruitment would have been prescribed them later in pregnancy.

8.3 Results

Mean maternal serum 25(OH)D was 14.6 ng/ml; 36% of mothers were vitamin D insufficient and 33% were deficient (Table 8.1). Concentrations were not related to the mother's socio-economic status or religious group (Hindus, 57% of population, median 25(OH)D = 15.0 ng/ml, Muslims 34%, 14.3 ng/ml, Christians 8%, 13.6 ng/ml).

Table 8.1. Maternal serum vitamin D concentrations during pregnancy in 3 groups

Vitamin D groups	N Max	Median	(IQR)	Min	Max
Normal (≥20 ng/ml)	159 (31%)	30.3	(23.2,40.0)	20.0	76.8
Insufficient (<20-≥11 ng/ml)	176 (36%)	15.4	(13.1,17.4)	11.0	19.7
Deficient (<11 ng/ml)	154 (33%)	7.5	(5.8,9.2)	1.6	10.8

Of the 489 live born, term babies without major congenital anomalies, 427, 433 and 433 children were followed up at 1,2 and 5 years respectively, and 429 had their glucose and insulin concentrations measured at 5 years.

8.3.1 Vitamin D and gestational diabetes: Maternal 25(OH)D concentrations were similar in the 34 mothers with gestational diabetes (mean 14.7 ng/ml) to those in mothers with normal glucose tolerance (14.7 ng/ml; p=0.98), and the percentages of women with 25(OH)D insufficiency and deficiency were similar in both groups (deficient:32.4%, insufficient:38.2%, normal:29.4% vs. 32.5%, 35.8% and 31.6% in the non-GDM group respectively, P=0.95). Maternal glucose and insulin concentrations were similar in all 3 vitamin D groups (Table 8.2), and this remained true after excluding the women with GDM.

8.3.2 Vitamin D and neonatal size: Neonatal anthropometric measurements were similar in all 3 vitamin D groups (Table 8.2), even after excluding offspring born to mothers with gestational diabetes. In a regression model, maternal vitamin D did not have statistically significant associations with neonatal anthropometry after adjusting for baby's sex, gestational age and maternal parity. There was a statistically significant inverse association between vitamin D and newborn's subscapular skinfold thickness (exp (B)=0.999, P=0.049) after excluding ODM from analysis.

Table 8.2. Maternal and newborn characteristics according to maternal vitamin D

	Maternal Vitamin D groups			P**
	Normal N=154	**Insufficient** N=176	**Deficient** N=159	
Mother				
Weight (kg)	**55.4** (8.5)	**57.3** (8.9)	**56.7** (9.9)	0.2
Height (kg)	**154.0** (5.6)	**155.1** (5.4)	**154.6** (5.5)	0.2
BMI (kg/m^2)	**23.3** (3.5)	**23.9** (3.6)	**23.6** (3.3)	0.4
Sum of skinfolds (mm)	**85.8** (32.2)	**90.8** (33.0)	**91.6** (34.9)	0.1
GDM (N)	**10** (6.5%)	**13** (7.4%)	**11** (6.9%)	0.95
*GAUC (mmol)	**1135.5** (1008,1248)	**1115.9** (980,1215)	**1139.1** (1015,1255)	0.9
*IAUC (pmol)	**50201.5** (35111,79608)	**50307.0** (35167,73432)	**52344.4** (33225,77625)	0.5
Baby				
Birthweight (g)	**2975.4** (405)	**3026.7** (391)	**2988.3** (399)	0.8
CHL (cm)	**49.1** (2.5)	**49.5** (2.0)	**49.3** (1.8)	0.5
CRL (cm)	**32.3** (1.9)	**32.5** (1.4)	**32.4** (1.5)	0.5
Leg length (cm)	**16.8** (1.7)	**17.0** (1.5)	**16.9** (1.3)	0.9
Ponderal Index (kg/m^3)	**25.1** (2.9)	**25.0** (3.0)	**24.9** (2.4)	0.5
Head circumference (cm)	**34.2** (1.3)	**34.3** (1.2)	**34.1** (1.3)	0.9
MUAC (cm)	**10.6** (0.9)	**10.6** (0.9)	**10.5** (0.9)	0.2
Chest circumference (cm)	**32.3** (1.7)	**32.6** (1.5)	**32.3** (1.5)	0.99
Abdominal circumference (cm)	**30.1** (2.1)	**30.6** (1.9)	**30.2** (1.8)	0.9
AMA (cm^2)	**6.8** (1.1)	**6.7** (1.0)	**6.7** (1.1)	0.3
*Triceps skinfold (mm)	**4.3** (3.7,5.1)	**4.4** (3.9,5.0)	**4.2** (3.7,4.6)	0.3
*Subscapular skinfold (mm)	**4.5** (3.9,5.0)	**4.7** (4.1,5.3)	**4.5** (3.9,4.9)	0.9

Means (SD) or *geometric mean (IQR); **ANOVA, 'P' for linearity

8.3.3 Vitamin D and postnatal anthropometry: At one year, anthropometric measurements of the infants were similar in all 3 maternal vitamin D groups (Table 8.3). In a univariate regression analysis there was a statistically significant positive association between maternal vitamin D and offspring AMA (P=0.03) after adjusting for sex. I used conditional z-scores at 1 year for weight, length, subscapular skinfolds, MUAC and AMA to examine the effects of maternal vitamin D on the growth during infancy. There were no statistically significant associations between maternal vitamin D and growth during the first year of life.

At two years, AMA (P<0.001) and MUAC (P=0.003) were larger in the offspring born to mothers with normal vitamin D levels compared to other 2 groups (Table 8.3). Similarly, at 5 years, AMA was bigger in the 'normal' group (P=0.03). Regression analyses showed that the association between maternal 25(OH) D and offspring AMA were stronger at 2 years (P<0.001) and remained statistically significant even at 5 years (P=0.01). These associations remained significant even after adjusting for maternal BMI and socio-economic status of the family and were unchanged after excluding ODM from the analysis.

Table 8.3 Anthropometric characteristics of children according to maternal vitamin D groups

	Maternal Vitamin D groups			P**
	Normal	Insufficient	Deficient	
N Max=	132	151	145	
One year				
Weight (kg)	**8.4** (1.2)	**8.4** (1.1)	**8.4** (1.1)	0.7
CHL (cm)	**73.1** (2.8)	**73.2** (2.9)	**73.3** (2.7)	0.7
BMI (kg/m^2)	**15.7** (1.5)	**15.7** (1.4)	**15.6** (1.4)	0.4
Head circumference (cm)	**44.1** (1.4)	**44.2** (1.5)	**44.1** (1.4)	0.9
MUAC (cm)	**14.2** (1.2)	**14.0** (1.1)	**14.1** (1.1)	0.3
Chest circumference (cm)	**43.9** (2.3)	**43.9** (2.2)	**43.9** (2.2)	0.8
AMA (cm^2)	**11.0** (1.7)	**10.7** (1.6)	**10.7** (1.7)	0.09
*Triceps skinfold (mm)	**7.7** (6.5,9.0)	**7.6** (6.8,8.6)	**7.8** (6.9,9.0)	0.4
*Subscapular skinfold (mm)	**6.4** (5.6,7.5)	**6.5** (5.5,7.6)	**6.4** (5.6,7.4)	0.98
Two years				
Weight (kg)	**10.6** (1.3)	**10.6** (1.3)	**10.5** (1.3)	0.5
CHL (cm)	**83.9** (3.2)	**84.0** (3.4)	**83.8** (3.4)	0.8
BMI (kg/m^2)	**15.0** (1.1)	**14.9** (1.1)	**14.9** (1.1)	0.3
Head circumference (cm)	**46.4** (1.3)	**46.5** (1.5)	**46.3** (1.4)	0.7
MUAC (cm)	**14.9** (1.1)	**14.6** (1.0)	**14.5** (1.0)	0.003
Chest circumference (cm)	**46.6** (2.1)	**46.2** (2.1)	**46.2** (2.1)	0.2
AMA (cm^2)	**12.2** (1.7)	**11.8** (1.7)	**11.4** (1.5)	<0.001
Triceps skinfold (mm)	**7.7** (6.6,8.7)	**7.7** (6.6,8.8)	**7.9** (6.7,9.1)	0.5
Subscapular skinfold (mm)	**6.9** (5.8,8.1)	**6.9** (5.9,8.2)	**7.1** (6.0, 8.4)	0.4
Five years				
Weight (kg)	**15.2** (2.0)	**15.4** (2.2)	**15.1** (2.0)	0.5
CHL (cm)	**105.3** (4.1)	**106.0** (4.5)	**105.7** (4.4)	0.5
BMI (kg/m^2)	**13.7** (1.2)	**13.7** (1.2)	**13.4** (1.0)	0.07
Head circumference (cm)	**48.5** (1.4)	**48.5** (1.5)	**48.5** (1.5)	0.8
MUAC (cm)	**15.5** (1.3)	**15.3** (1.3)	**15.3** (1.2)	0.1
Chest circumference (cm)	**50.0** (2.3)	**50.2** (2.4)	**50.2** (2.4)	0.5
AMA (cm^2)	**13.5** (1.9)	**13.1** (1.9)	**13.0** (1.9)	0.03
*Triceps skinfold (mm)	**7.7** (6.6,8.7)	**7.9** (6.5,9.0)	**7.8** (6.7,8.9)	0.6
*Subscapular skinfold (mm)	**6.0** (4.9,7.2)	**6.2** (5.0,7.1)	**5.9** (5.0,6.5)	0.7

Means (SD) or *geometric mean (IQR); **ANOVA, 'P' for linearity

8.3.4 Glucose and insulin concentrations: The prevalence of IGT was higher in children born to mothers with a vitamin D concentration <20 ng/ml than in those in the normal vitamin D group (5.2% v 2.3%), but the difference was not statistically significant. Glucose and insulin concentrations were similar in offspring in all 3 groups at five years (Table 8.4). HbA1c concentrations were higher in the 'low maternal vitamin D' groups compared to 'normal' group.

Table 8.4 Glucose and insulin concentrations of children according to maternal vitamin D status during pregnancy

	Maternal Vitamin D groups			P**
	Normal N=135	**Insufficient N=153**	**Deficient N=142**	
Glucose (mmol/l)				
Fasting	**4.8** (0.4)	**4.9** (0.4)	**4.8** (0.6)	0.5
30-minute	**7.3** (1.3)	**7.3** (1.3)	**7.4** (1.5)	0.6
120-minute	**5.9** (0.9)	**5.9** (1.0)	**5.9** (1.0)	0.7
HbA1c%	**5.4** (0.5)	**5.6** (0.5)	**5.6** (0.5)	0.04
***Insulin**				
Fasting (pmol/l)	**20.5** (13.3,34.9)	**20.4** (13.6,30.3)	**18.5** (10.9,29.6)	0.2
30-minute (pmol/l)	**133.0** (85.0,239.7)	**142.4** (97.3,224.6)	**136.7** (93.1,229.2)	0.8
120-minute (pmol/l)	**80.2** (52.6,130.8)	**82.4** (53.7,115.2)	**79.2** (55.4,125.5)	0.9
HOMA	**0.72** (0.5,1.2)	**0.73** (0.5,1.1)	**0.65** (0.4,1.1)	0.2
Insulin increment (pmol/mmol)	**29.7** (13.9,44.3)	**30.3** (16.0,39.3)	**30.4** (14.6,41.4)	0.8
IGT (N)	**3** (2.3%)	**8** (5.3%)	**7** (5.0%)	0.3

Means (SD) or *geometric mean (IQR); **ANOVA, 'P' for linearity

8.4 Summary of main findings

More than 2/3rd of the women in the study cohort had lower than normal vitamin D concentrations during pregnancy. The incidence of GDM was similar in both the normal and low vitamin D groups. Vitamin D status was not associated with maternal size or glucose/insulin concentrations during pregnancy.

Maternal vitamin D status was not a predictor of neonatal size or the growth during infancy in the study children. Offspring AMA was positively associated with maternal vitamin D concentrations at 2 years and remained statistically significant at 5 years of age.

At 5 years, the prevalence of IGT tended to be higher in study children in the low maternal vitamin D group (5.2% vs. 2.3% in the normal maternal vitamin D group). There was no statistically significant association between offspring glucose and insulin concentrations and maternal vitamin D concentrations at this age. HbA1c concentrations were higher in offspring born to mothers in the low vitamin D groups.

8.5 Discussion

This chapter describes the neonatal and childhood anthropometric measurements, and glucose and insulin outcomes of the study children in relationship to maternal vitamin D status. A high proportion (69%) of mothers recruited from the antenatal clinic, and giving birth to full term babies were vitamin D insufficient or deficient at 30 weeks gestation according to internationally recognised criteria. This was despite many women being prescribed calcium or calcium and vitamin D supplements from the beginning of the 2nd trimester. There were no significant associations between maternal vitamin D status and maternal body size, glucose and insulin concentrations or risk of developing gestational diabetes. Newborn anthropometry and growth during the first year were unrelated to maternal vitamin D status. At five years, children born to mothers with normal vitamin D status had larger AMA and lower HbA1c concentrations than those in the lower vitamin D groups.

A high prevalence of vitamin D insufficiency/ deficiency has been reported earlier among adult Asians, especially pregnant women, both in their countries of origin[56], and after migration to western countries[34,54,208]. Our findings may appear paradoxical in Indian context because of the abundant supply of sunlight all through the year. However, this has been attributed to skin pigmentation, full clothing, and/or low dietary intake of the precursors on vegetarian diets, which are the likely causes for low vitamin D levels in Mysore mothers. There may also be differences in vitamin D metabolism in south Asians. In vitro studies have shown that they have increased 25-hydroxy-24-hydroxylase activity, leading to increased catabolism of 25(OH)D to 24,25$(OH)_2$D, thought to be a less active metabolite, rather than 1,25$(OH)_2$D[208].

Vitamin D receptors are present in pancreatic β cells and it has been shown using animal models that vitamin D deficiency leads to β cell dysfunction and impaired glucose tolerance[205,206]. In humans hypovitaminosis D was shown to be associated with decreased insulin sensitivity and secretion[33]. In non-diabetic south Asians living in

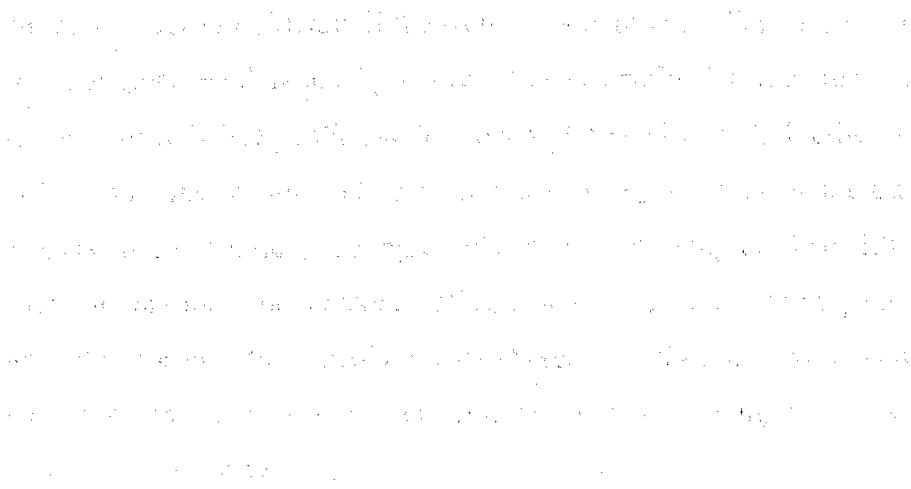
London, glucose tolerance was reduced in vitamin deficient individuals, while 30-minute insulin secretion was improved on supplementing with a single dose of vitamin D[34]. Thus, the high prevalence of vitamin D insufficiency in south Asian populations may contribute to their high incidence of type 2 diabetes[124], and gestational diabetes. Low maternal serum vitamin D concentrations, and an increased risk of neonatal hypocalcaemia have been shown in diabetic pregnancies in Spain[209]. In our study, the mother's 25(OH)D concentrations were not related to her glucose or insulin concentrations, or risk of gestational diabetes. Our data do not, therefore, suggest that vitamin D insufficiency is an important underlying cause of gestational diabetes in this population.

Low levels of vitamin D in pregnant women lead to low vitamin D stores in the fetus[56] and may impair fetal growth[210]. Supplementation trials of vitamin D in Asian women have shown improvements in maternal and neonatal biochemical indices for calcium and vitamin D, and higher birthweights[55]. Our negative findings could be because vitamin D concentrations higher than those found in any of our mothers are required for optimal fetal growth. It is also possible that our mothers lacked other nutrients required for vitamin D function, such as vitamin A, often deficient in pregnancy in India[211]. Activated vitamin D exerts its main effects after binding to its receptor (the VDR) and complexation of the VDR with the retinol-X receptor[212]. It is important to note that the criteria for insufficiency and deficiency were derived in white Caucasian populations and may not be appropriate for Indian women. It could be that low serum 25(OH)D concentrations are well compensated for by the placenta since $1,25(OH)_2D$ is synthesised by the placenta[213].

The absence of associations between maternal vitamin D status and neonatal anthropometry does not rule out other important effects on fetal and postnatal development. A deficiency of neonatal vitamin D stores may retard infant growth in the absence of postnatal supplementation[181]. In a trial involving British Asians, there was no difference in newborn size, but offspring of mothers supplemented with vitamin D during pregnancy were longer and heavier throughout infancy than the control group[214]. Though maternal vitamin D was not related to infant catch-up growth in our cohort, AMA was larger in the higher vitamin D group from 2 years onwards. The significance of AMA is not clear. Though it is used as a proxy for muscle mass, a major part of it comprises bone tissue and hence the association seen with vitamin D in my study may

be reflecting larger bones in these children. This may indicate long-term effects of maternal vitamin D on offspring growth postnatally. Alternatively, offspring of these women may have similar lifestyle habits, and social environment (diet, sun exposure) as their mothers, and thus have normal vitamin D levels in the body.

Maternal vitamin D did not have any significant associations with glucose and insulin concentrations, and insulin resistance of children at 5 years. Since these children are more exposed to sunlight due to less clothing cover and longer periods of outdoor activities, adequate synthesis of vitamin D in them may have compensated for early insufficiency. My data demonstrates higher hbA1c concentrations in children born to vitamin D deficient women. Whether hbA1c levels signal sub-clinical perturbations in glucose/ insulin metabolism in children is not known. Long-term follow-up of these children is essential to understand the effects of maternal micronutrients on growth and metabolism in the offspring.



9. GESTATIONAL DIABETES AND THE PREVALENCE OF DIABETES 5 YEARS AFTER THE INDEX PREGNANCY.

Gestational diabetes mellitus (GDM) in women increases risks both for the mother and the offspring[215]. In the previous chapters, I have discussed the effects of maternal GDM on various offspring outcomes. Since the risk factors for GDM are essentially the same as those for type 2 DM, a woman with GDM is more likely to develop diabetes later in life[80]. Cognisant of the increasing prevalence of GDM as well as type 2 DM in India, I examined the prevalence of IGT, IFG and diabetes in the study mothers 5 years after they were tested for glucose tolerance during pregnancy.

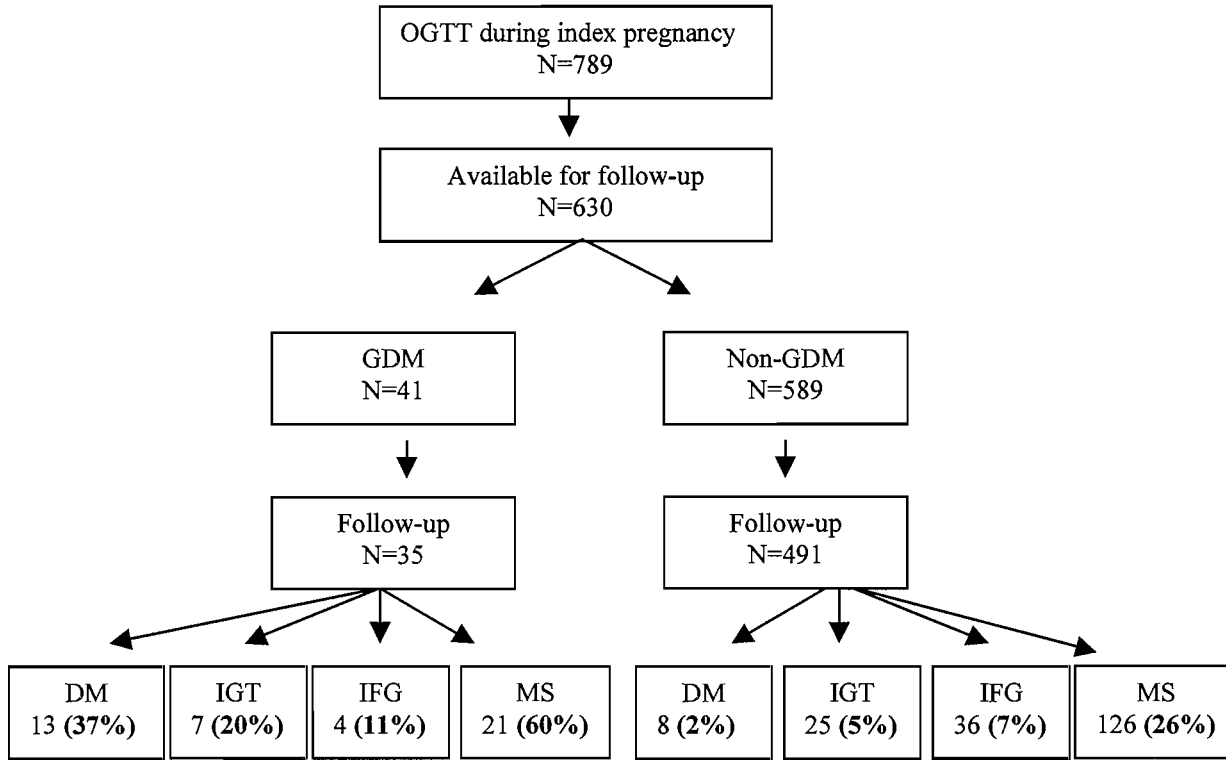
9.1Methods

At the five-year follow-up, a 2-hour, 75 g OGTT was administered to all willing mothers (N=526) whose GDM status was known for the index pregnancy. Blood samples were collected for plasma glucose/ insulin measurements at fasting, and 120 minutes after the glucose load. An additional 30-minute post-glucose load sample was collected in previously GDM women (N=35).

Diabetes was identified by the presence of a fasting glucose concentration ≥7 mmol/l or 120-minute glucose ≥11.1 mmol/l, and if the women were already diagnosed to have diabetes. Abnormal glucose tolerance/ hyperglycemia was defined as the presence of either diabetes, IGT or IFG. Metabolic syndrome was defined by the IDF criteria recommended for south Asian women (waist circumference ≥ 80 cm, and at least two of the following: plasma trigycerides≥150 mg/dl, plasma HDL<50 mg/dl, SBP≥130 or DBP≥85 or on treatment for hypertension, fasting glucose ≥5.6 mmol/l or type 2 DM)[216]. Women diagnosed with clinical diabetes (based on 120-minute values) were referred to a diabetic consultant. The women with IGT and high fasting values were asked to return for a repeat of OGTT.

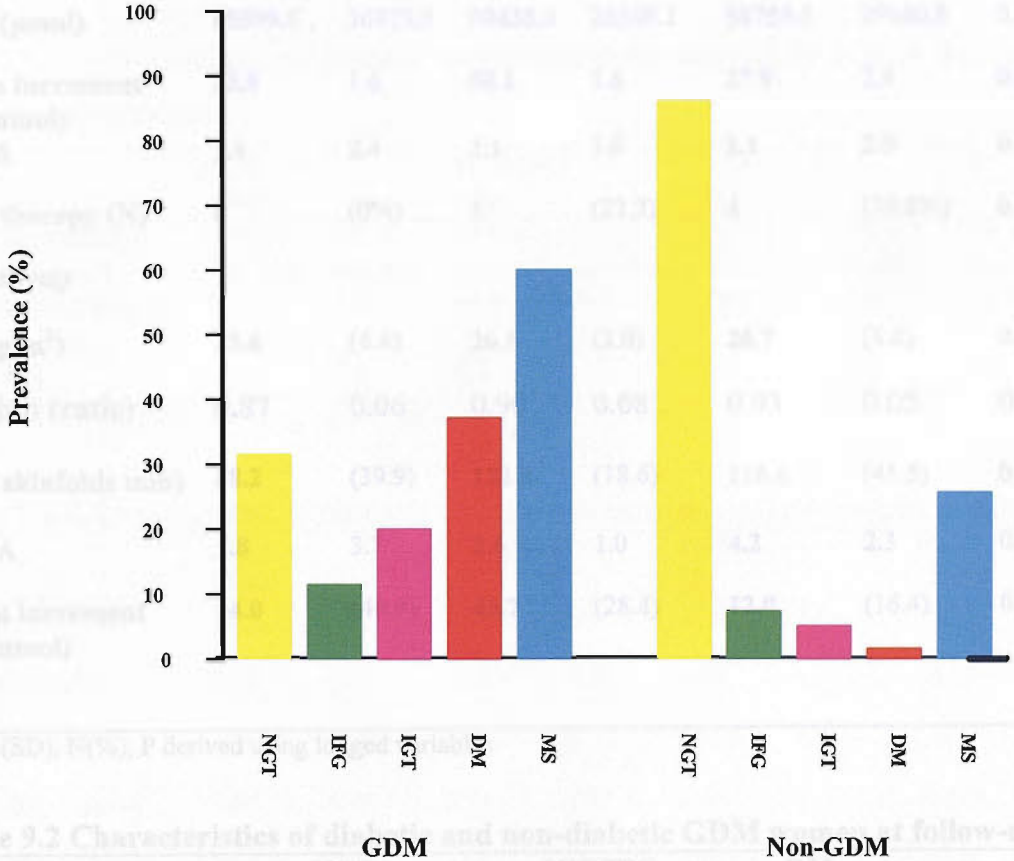
9.2 Results

Figure 9.1: Flow diagram illustrating the participation of women



Type 2 diabetes was diagnosed in 21 women (4%); of these 13 women were previously gestational diabetic (P<0.001 vs. non-GDM group) Figures 9.1 and 9.2. The prevalence of IGT (P<0.001) and metabolic syndrome (P>0.001) was higher in the women with previous GDM. Collectively, 93 (17%) women had abnormal hyperglycaemia 5 years after the index pregnancy, of whom 24 were previously gestational diabetic (69% vs. 14%, P<0.001).

Figure 9.2. Prevalence of abnormal hyperglycaemia and metabolic syndrome in study women according to their GDM status.



9.2.1 GDM and diabetes at follow-up: The GDM women with diabetes at follow-up had higher glucose (border line significant), but lower insulin concentrations during the index pregnancy compared to the women with either NGT or IGT/IFG (Table 9.1). They also had lower insulin increment, though this was not statistically significant). Similar proportion of diabetic and IGT/IFG women were treated with insulin, while none of the NGT women had insulin treatment during pregnancy.

At follow-up, the women who developed diabetes were more centrally obese (Waist-to-hip ratio, WHR), more insulin resistant than both NGT and IGT/IFG women, but had significantly lower insulin increment at 30 minutes (Table 9.2). About 92% of the diabetic women had a first degree relative with diabetes and 85% of the women had metabolic syndrome (insulin resistance syndrome).

Table 9.1 Characteristics of diabetic and non-diabetic GDM women during the index pregnancy.

N=	NGT (11)		IGT/IFG (11)		DM (13)		P
*GAUC (mmol)	1603.4	93.8	1620.7	186.4	2000.8	803.2	0.07
*IAUC (pmol)	85599.5	36973.3	90435.0	26369.1	58759.5	29680.8	0.045
*Insulin increment (pmol/mmol)	33.5	1.6	50.1	1.6	27.9	2.4	0.6
*HOMA	1.9	2.4	2.1	1.6	2.1	2.0	0.7
Insulin therapy (N)	0	(0%)	3	(27.3)	4	(30.8%)	0.05
At Follow-up							
BMI (kg/m^2)	23.6	(4.4)	26.1	(3.0)	26.7	(4.6)	0.08
Waist/hip (ratio)	0.87	0.06	0.90	0.08	0.93	0.05	0.04
Sum of skinfolds mm)	88.2	(39.9)	122.8	(18.6)	116.6	(41.5)	0.08
*HOMA	2.8	3.7	2.6	1.0	4.2	2.3	0.02
*Insulin increment (pmol/mmol)	54.0	(40.9)	43.7	(28.4)	12.0	(16.4)	0.002

Mean (SD), N(%), P derived using logged variables

Table 9.2 Characteristics of diabetic and non-diabetic GDM women at follow-up

N=	NGT (11)		IGT/IFG (11)		DM (13)		P
*Age (yr)	32.6	5.7	34.3	4.3	33.9	4.6	0.4
BMI (kg/m^2)	23.6	(4.4)	26.1	(3.0)	26.7	(4.6)	0.08
Height (cm)	153.9	(7.9)	150.8	(6.5)	152.6	(5.0)	0.6
Waist/hip (ratio)	0.87	0.06	0.90	0.08	0.93	0.05	0.04
Sum of skinfolds mm)	88.2	(39.9)	122.8	(18.6)	116.6	(41.5)	0.08
*HOMA	2.8	3.7	2.6	1.0	4.2	2.3	0.02
*Insulin increment (pmol/mmol)	54.0	(40.9)	43.7	(28.4)	12.0	(16.4)	0.002
Metabolic syndrome (N)	2	(18%)	8	(73%)	11	(85%)	0.002
Family history (N)	5	(46%)	3	(27%)	12	(92%)	0.004

Mean (SD), N(%), P derived using logged variables

9.2.2 Non-GDM women: The non-GDM women who had developed diabetes at follow-up had significantly higher glucose concentration, and lower insulin increment at 30 minutes than NGT women during the index pregnancy (Table 9.3).

At follow-up, they were older, heavier, shorter and more centrally adipose than NGT women, and were more insulin resistant. The prevalence of metabolic syndrome was very high compared to NGT and impaired glucose groups, and they were more likely to have a family history of diabetes (Table 9.4).

Table 9.3 Characteristics of diabetic and non-diabetic non-GDM women during the index pregnancy

N=	NGT (406)		IGT/IFG (75)		DM (8)		P
*GAUC (mmol)	**1092.0**	153.1	**1179.8**	137.2	**1119.1**	175.1	<0.001
*IAUC (pmol)	**591777.4**	37688.6	**56614.9**	28929.1	**46983.8**	20093.9	0.5
*Insulin increment (pmol/mmol)	**70.3**	2.0	**49.0**	2.5	**53.0**	1.4	<0.001
*HOMA	**1.1**	1.8	**1.3**	1.9	**0.95**	1.8	0.5

Mean (SD), N(%), P derived using logged variables

Table 9.4 Characteristics of diabetic and non-diabetic non-GDM women at follow-up

N=	NGT (406)		IGT/IFG (75)		DM (8)		P
*Age (yr)	**28.4**	3.8	**29.6**	4.3	**28.8**	3.2	0.03
BMI (kg/m^2)	**23.2**	(4.4)	**24.8**	(3.0)	**28.9**	4.9	<0.001
Height (cm)	**154.8**	(7.9)	**153.3**	(6.5)	**153.2**	5.1	0.02
Waist/hip (ratio)	**0.88**	0.07	**0.92**	0.07	**0.95**	0.09	<0.001
Sum of skinfolds (mm)	**92.1**	39.9	**108.2**	35.3	**135.7**	38.0	<0.001
HOMA	**1.9**	1.2	**2.8**	1.6	**5.0**	1.88	0.02
Family history of type 2 DM (N)	**101**	(25.0%)	**27**	(36.0%)	**5**	(62.5%)	0.004
Metabolic syndrome (N)	**75**	(18.2%)	**44**	(58.7%)	**6**	(75.0%)	<0.001

Mean (SD), N(%), P derived using logged variables

9.2.3 Predictors of diabetes at follow-up: In all the women, I examined several possible predictors of diabetes using a multiple logistic regression model, including GDM status, GAUC and IAUC at index pregnancy, socio-economic status, age, family history of diabetes and current BMI. The presence of GDM at index pregnancy was a strong risk factor for diabetes (exp (B) =14.3, P=0.03) independent of other predictors (Table 9.5). Similarly, higher prevalence of diabetes in first-degree relatives and higher BMI at follow-up were independently associated with higher rates of diabetes, while gestational IAUC was inversely associated with the prevalence of diabetes. Maternal

age and socio-economic status, and gestational GAUC did not predict diabetes at follow-up (Table 9.5).

Table 9.5 predictors of diabetes at follow-up in study mothers

Predictors	Exp (B)	P
GDM (yes/no)	14.3	0.03
GAUC (mmol)	1.003	0.2
IAUC (pmol)	0.999	0.03
BMI (kg/m^2)	1.3	<0.001
Family history (yes/no)	4.9	0.02
Socio-economic status (score)	0.9	0.09
Age (years)	0.98	0.8

9.3 Summary of main findings

The presence of GDM during the index pregnancy greatly increased the risk of DM in the study women after 5 years (37% vs. 2% in the non-GDM group). Previously GDM women also had higher rates of IGT/IFG and the metabolic syndrome compared to non-GDM women.

Previously gestational diabetic women who were diabetic at follow-up had lower gestational insulin concentrations and insulin increment than those who were normal or impaired glucose tolerant at follow-up for comparable insulin resistance measurements.

None of the GDM women who remained NGT after 5 years had received insulin therapy during pregnancy.

At follow-up, the GDM women with diabetes were more centrally obese, more insulin resistant, and had lower insulin increment at 30-minutes than women with NGT. They were also considerably more insulin resistant, but had lower insulin increment when compared to IGT/IFG group.

A comparison made among the three groups in the non-GDM women showed similar factors associated with diabetes at follow-up.

In the whole cohort, previous diagnosis of GDM, the presence of family history of diabetes, and high current BMI predicted higher rate of diabetes at follow-up independent of other predictors.

9.4 Discussion

As the original part of my study (Chapter 1), the mothers were examined for the incidence of GDM between 28-32 weeks of their gestation. The GDM rate was considerably greater than reported in a study of GDM from India conducted more than 10 years prior (6.1% vs. <1%)[122], and was also greater compared to a recent study in Kashmir in India (vs. 3.8%)[123]. The follow-up of GDM women after 5 years showed the presence of diabetes in more than a third, and a further 20% had IGT. In contrast, only 8 (2%) of the 491 non-GDM mothers followed-up at 5 years were diagnosed with diabetes and 25 (5%) had IGT.

The characterisation of the anthropometric, metabolic and familial factors showed that the GDM women who developed diabetes at follow-up in my study had a strong family history of diabetes (>90%), and had lower levels of insulin concentrations, for a similar degree of insulin resistance, during pregnancy than non-diabetic women. They also had high current body size. My study did not test for the type of diabetes; nevertheless, the characteristics of the women (high current insulin resistance and BMI) were more in accordance with the type 2 diabetes.

Results of this study are consistent with other studies from all over the world that have confirmed an association between GDM and later type 2 diabetes[217-222]. The incidence of diabetes varied from 2% to 70% depending on the length of follow-up (6-8 weeks to 28 years)[217]. Different study groups have reported different rates of conversion. Different diagnostic criteria for GDM, and type 2 diabetes at follow-up make comparisons difficult. Most of the studies used the NDDG criteria for GDM and those of WHO for follow-up rates.

In my study, the pregnant women were tested for glucose tolerance using the criteria of Carpenter and Coustan[109] (ref. Chapter 2), and the WHO criteria at follow-up[107]. Comparisons with NDDG and O'Sullivan's methods suggest that WHO criteria are more sensitive in identifying GDM[108,223]. However, the Kashmir study in India observed no significant difference in the prevalence of GDM by using either the WHO or the Carpenter and Coustan methods[123]. Thus, there is no reason to infer that the methods used in the study gave a markedly different estimation of GDM prevalence than if other methods had been used.

Most of these studies report that the high gestational fasting glucose was an important predictor of diabetes at follow-up[217,218,221]. Higher pre-pregnant weight/BMI and higher gain in weight/BMI post-partum were also independent risk factors commonly associated with later diabetes[217,221]. Family history of diabetes was not an important risk factor in most studies[217].

Hyperglycaemia during 4-16 weeks post-partum has been shown to be an independent risk factor for later diabetes in GDM women[221,224]. Thus, post-partum OGTT may help to identify women at high risks, who need more rigorous follow-up, and may provide scope to modify lifestyle factors. The American Diabetes Association recommends evaluation of glycaemic status for all GDM women 6 weeks after delivery[77]. However, very few of my study women returned for follow-up after delivery, a phenomenon that has been observed in other countries, including developed populations[224,225].

There has been much controversy surrounding screening for GDM, and choice of criteria. Countries like India, which are still struggling with health risks associated with poverty such as infections, are less likely to give priority to detection and management of GDM and risk factors for type 2 diabetes. The absence of consensus on diagnostic and management protocols across the country, lack of specialist diabetic clinics for pregnant women in the majority of the hospitals, and lack of awareness among the public of the harmful effects of GDM are some of the associated problems. Most of these issues are directly related to inadequacy of funds, specialist personnel, and specialist laboratories. The hospitals that can provide facilities for the measurement of indices such as HbA1c are few and are not accessible to the majority of common people. Educating the public about the above risks is as important as impressing upon the policy makers the need for better diagnostic and treatment protocols/facilities for gestational diabetes in public hospitals.

10. FINAL DISCUSSION

10.1 Summary

In this thesis I described the anthropometry from birth to five years, and glucose and insulin concentrations at five years in a cohort of normal south Indian children, whose mothers' glucose and insulin concentrations were measured during pregnancy. Children were small at birth compared to Caucasian children, with smaller arm circumferences (muscle mass), and remained smaller at 5 years, particularly in BMI and head circumference. However, their skinfolds were larger than those of the reference children at 5 years. Twenty-two children had IGT at 5 years. Girls had significantly higher insulin concentrations and insulin resistance (HOMA) than boys. Adiposity, insulin concentrations, and the prevalence of impaired glucose tolerance (IGT) were higher in offspring of diabetic mothers (ODM), especially females, than the rest of the cohort. In contrast, offspring of diabetic fathers were lighter at birth, and had lower glucose and insulin concentrations than ODM and controls. Maternal gestational insulin concentrations even in the absence of gestational diabetes mellitus (GDM) had positive associations with anthropometry and insulin concentrations at five years. Weaker associations were also present with father's fasting insulin concentrations.

10.2 Study sample- selection, follow-up, strengths and weaknesses

This was not a representative sample; all children were born to women booking into one maternity unit and whose glucose tolerance was studied during pregnancy[125]. However, the characteristics of our mothers and babies were similar to those reported for other South Indian urban populations[226,227]. Comprehensive maternal gestational data including glucose tolerance and gestational age was available for all. This was one of the few studies in India with longitudinal data in children, and as far as we know the first to report data on follow-up of the ODM. A high proportion of children in the non-diabetic group were followed-up since birth unlike in other studies where controls were limited and chosen separately. Data on fathers' fasting glucose and insulin concentrations were also available for a majority of them. The children belonged to a wide range of socio-economic status. Quality of anthropometric measurements was high, the measurers were highly trained and methods were standardised regularly. Detailed body composition measurements were taken. Loss to follow-up was minimal; more than 90% of children returned for measurements each year.

One of the main disadvantages was the relatively few ODM in the cohort which limited the statistical power. There were no data available on the diabetic control in GDM mothers which would have added vital information on the significance of tight metabolic control on risk outcomes. The characterization of glucose and insulin concentrations for fathers was different from those for mothers, less detailed, and not available for all. Another limitation was that information on the children's dietary habits, an important determinant of adiposity, was not recorded.

10.3 Conclusions

The study was designed to test three hypotheses (Section 1.5.3) related to cardiovascular risk markers in these young children that may be the forerunner of high cardiovascular disease risk in adults in India.

Hypothesis 1: I proposed that the offspring exposed to diabetes in utero would be more adipose, and have higher insulin concentrations than those born to non-diabetic mothers at five years. This was based on observations from other parts of the world (Section 1.3.2), and from an earlier Mysore study where an association between high ponderal index at birth and adult type 2 diabetes (DM) had prompted the authors to suggest GDM as a potential cause of diabetes in this population[121]. My study supported intra-uterine programming effects of GDM on adiposity and altered glucose/insulin metabolism. These findings were apparent only in females. A sex difference was not observed in other studies. An earlier study, however, observed that female ODM were heavier than other girls up to 4 years of age, while significant differences appeared in males at 4 years[104]. There were very few boys in our ODM group relative to girls. This may explain the absence of a statistical association. On the other hand, these differences may be related to metabolic/endocrine or behavioral differences between the sexes. We speculated that female ODM in this cohort were less physically active and this may have conferred the additional risks noticed in my study. This is of particular concern since these girls may develop GDM themselves as adults and form a vicious cycle of GDM and offspring diabetes. Whether male ODM show greater degrees of altered metabolic milieu when they get older may be answered by future follow-up.

Hypothesis 2: Based on a previous observation from elsewhere[102], and from our own finding that the maternal fasting glucose even in non-diabetic ranges was associated with altered neonatal size, I hypothesised that this would be true for anthropometry and

glucose/insulin concentrations at five years in my study children. Effects of lesser degrees of maternal hyperglycaemia on offspring size and insulin concentrations in childhood have been studied formerly only among Pima Indians[103]. My study supported a positive effect of maternal glycaemia and hyperinsulinaemia on offspring adiposity and insulin concentrations even in non-diabetic pregnancies. A similar association noticed with paternal insulin concentrations is a new finding. Weaker associations noticed here may be because paternal insulin profile was not as detailed as in mothers; weaker relationships were present when only fasting insulin was used for the mothers also (Sections 3.5.1. and 4.7.3). Thus, my study is compatible with either/both intra-uterine programming and genetic aetiologies for the above associations. Given the increasing trends of obesity and insulin resistance in India, hyperglycaemia not reaching to GDM levels may be increasing among pregnant women. This presents new issues relating to screening protocols and glycaemic levels at which the treatment should be started. On the other hand if the above associations have a major genetic role, rigorous metabolic control may be unjustifiable. This is clearly a major area of interest for future research both in India and elsewhere.

Hypothesis 3: My third hypothesis proposed that after excluding ODM, children with lower birth weights would have higher central adiposity and insulin concentrations at 5 years. Though higher birth weight is associated with higher BMI later in life, low birth weight has been shown to be associated with central/ truncal adiposity in adults as well as in children[135]. In view of the high prevalence of low birth weight among Indians, this has been proposed as contributing to their relative adiposity and hyperinsulinaemia[202]. An earlier study in India has shown inverse associations between birth weight, and glucose and insulin profiles in children[43,60]. In my study, the associations between birth weight and risk outcomes were not direct. There were inverse associations between insulin concentrations and insulin resistance at five years and birth measurements only after adjusting for current weight, and the associations were lost after adjusting for current body fat. Inverse associations were observed between current head circumference and insulin resistance. Since most of the growth of the head is completed in the first year of life, smaller heads at 5 years may reflect impaired growth *in utero*[60]. However, my study did not give strong evidence for an association between reduced birth size and altered glucose and insulin metabolism. High current size was the major determinant of these outcomes irrespective of their birth weight.

Through these findings my study attempted to understand the origins of high diabetic and cardiovascular risks among Indians. The body phenotype unique to Indian adults (high truncal adiposity and reduced muscularity relative to Caucasian adults) is thought to predispose them to high insulin resistance and associated risks later in life[119]. A recent study from Pune in India, and my study demonstrated that this phenotype may be apparent very early in life. What determines the emergence of this phenotype is not clear, though it is proposed that it may be related to fetal programming resulting from nutritional deficiencies or may have a genetic role[228]. The adipose neonatal phenotype may be of survival advantage providing substrate for brain development, but may have adverse implications when coupled with unfavourable lifestyle factors.

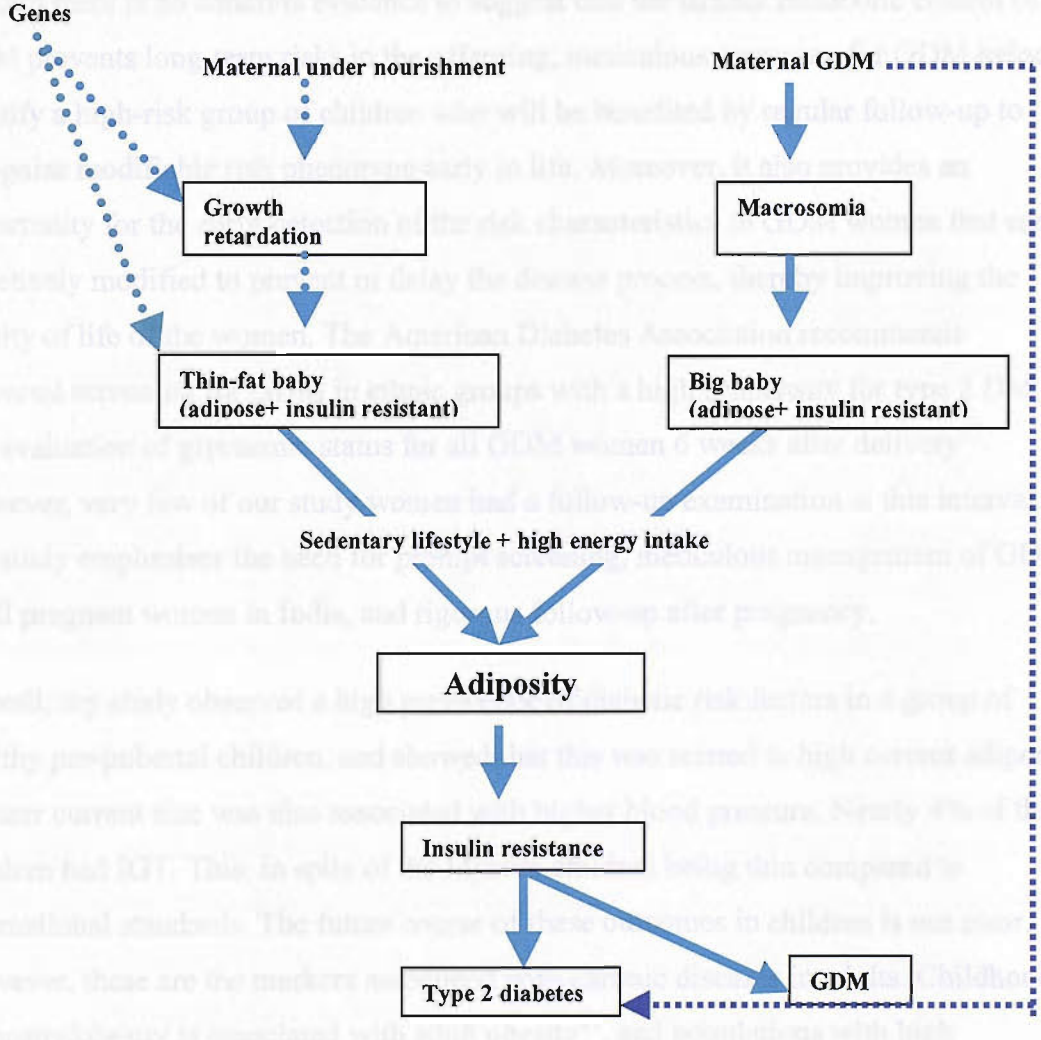
Though little studied, the prevalence of diabetes in pregnant women in India may be rising given the high prevalence of insulin resistance and type 2 DM among adults. GDM increases the risks of future diabetes in the women themselves[217], and is also associated with adiposity and type 2 DM in their offspring (Section 1.3.2). One of the downsides of the study relevant to the above findings was the insufficient information on maternal GDM control. The optimum GDM control is based on the prevention of perinatal complications in the offspring. It is not known if the same degree of metabolic control prevents long-term complications in the offspring. Earlier studies on ODM have observed that risks were present even though GDM control was optimum[90-93]. Long-term prospective studies aimed to address these questions are difficult as it is not clear what should be the length of such follow-ups. Moreover, it may be unethical to carry out controlled trials to determine the role of optimum metabolic control.
Recently, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was established to clarify many of these unanswered questions related to GDM and maternal glycaemia of lesser degrees[229].

Despite limitations in the methodology, my study showed that in contrast to ODM, the offspring of diabetic fathers were smaller at birth than the rest of the cohort. This corresponds to studies reporting low birth weights in the offspring of fathers with type 2 DM, and indicates a possible genetic aetiology, where both reduced birth weight and insulin resistance are determined by common genes that confer risks of future diabetes (The fetal insulin hypothesis, Section 1.2.4).

Most of the above associations were stronger with offspring 30-minute insulin concentrations than other glucose/insulin variables in my study. Though this gives a measure of first phase insulin response in adults, its significance in children is not known. This may be an early indicator of insulin resistance. Since my study children were very young, highly functional β-cells might have masked any insulin resistance present. An earlier study from Pune had observed high 30-minute glucose and insulin concentrations in 4-year old children with lower birth weights[60]. When the same cohort was studied 4 years later, associations with insulin resistance (HOMA) were apparent[43].

Thus, my study findings suggest that several factors may be predisposing this population to type 2 DM. Figure 10.1 illustrates a scheme of associations based on these findings, mechanisms for which need to be explored further.

Figure 10.1 Flow diagram suggesting factors leading to type 2 diabetes in this population



10.4 Population health significance: My study findings that GDM increases adiposity and insulin concentrations in the offspring and the risk of diabetes in the women themselves point towards an important contributing role of GDM in the prevalence of type 2 DM, and are of potential clinical health importance for the primary prevention of type 2 diabetes in India. The diabetic phenotype was more apparent in female offspring which may lead to an additional burden of GDM when they grow-up. Among the Pima Indians, a population with high rates of type 2 DM, it has been estimated that nearly 40% of all type 2 DM in 5-19 year old children could be attributable to the diabetic intrauterine exposure, though such data is not available in other populations[230]. As according to a recent estimation about 10-31% cases of type 2 DM in parous women can also be ascribed to previous GDM[219], the population impact of GDM may be large. Given the enormity of the Indian population, universality of marriage and inclination for childbearing among Indian women, and high rates of type 2 DM among adults, GDM may have a significant impact on the diabetic pool in India.

Though there is no concrete evidence to suggest that the stricter metabolic control of GDM prevents long-term risks in the offspring, meticulous screening for GDM helps to identify a high-risk group of children who will be benefited by regular follow-up to recognise modifiable risk phenotype early in life. Moreover, it also provides an opportunity for the early detection of the risk characteristics in GDM women that can be effectively modified to prevent or delay the disease process, thereby improving the quality of life of the women. The American Diabetes Association recommends universal screening for GDM in ethnic groups with a high propensity for type 2 DM, and evaluation of glycaemic status for all GDM women 6 weeks after delivery[77]. However, very few of our study women had a follow-up examination at this interval. My study emphasises the need for prompt screening, meticulous management of GDM in all pregnant women in India, and rigorous follow-up after pregnancy.

Overall, my study observed a high prevalence of diabetic risk factors in a group of healthy pre-pubertal children, and showed that this was related to high current adiposity. Greater current size was also associated with higher blood pressure. Nearly 4% of the children had IGT. This, in spite of the Mysore children being thin compared to international standards. The future course of these outcomes in children is not clear. However, these are the markers associated with chronic diseases in adults. Childhood adiposity/obesity is associated with adult obesity[44], and populations with high prevalence of IGT also have higher prevalence of type 2 DM[103,161]. In adults, IGT is

associated with a higher risk of developing type 2 DM. It has been shown earlier in obese children and adolescents in the USA that about 33% of subjects with IGT developed type 2 DM on repeating GTT after 18-24 months, and this was associated with significant weight gain[231]. Children who became normal glucose tolerant (33%) had a stable weight during this period.

Identification of risk factors early in life provides immense scope for the primary prevention of type 2 DM in India as the progress can be halted by modification of lifestyle factors. In adults, interventions with low-calorie diets and increased physical activity have been shown to promote weight loss and improved cardiovascular health markers[232]. The evidence for the benefits of dietary interventions alone is not sufficient. In children, there is no evidence to suggest that a reduction in energy intake helps to reduce the risk factors. Moreover, it is not known as to what should be the amount of such reductions or if it is useful in decreasing the regional adiposity in relatively undernourished children as in the Mysore cohort. On the other hand, restricting food intake may impair normal functional development of the children such as cognitive development, and may have several adverse effects which may be unknown currently. Thus, limiting food intake in children as a means of reducing obesity is considered unsuitable, and increasing physical activity may be a better approach.

A few studies in the West have shown an association between lower levels of physical activity and higher current body mass/ adiposity, and features of insulin resistance and metabolic syndrome in children (Section 7.9). Though the beneficial role of intervention measures to increase physical activity in decreasing body fatness is still a matter of controversy, it has been recommended as a means to improve long-term well being of the children. Activity levels are decreasing in urban Indian children consequent to modern lifestyle[134]. This could be a major factor contributing to increased adiposity and diabetes risk factors among our children. Measures to inculcate physical activity behaviour in them may go a long way in reducing these risks in the population as a whole, and this forms the main objective of the future follow-up of the Mysore children.

In my study, we observed a high prevalence of vitamin D insufficiency in a group of healthy, pregnant women belonging to different socio-economic strata. Low circulating vitamin D is thought to contribute to the higher risk of type 2 DM in south Asian adults[34]. A deficiency in the mother may also impair fetal growth[124]. In view of the high prevalence of vitamin D deficiency among pregnant women this may be a risk factor for

GDM in India. Though there were no apparent maternal or newborn risks associated with low vitamin D levels in Mysore, we observed long-term disadvantages in the offspring as suggested by lower arm-muscle-area (a marker of lean tissue) in male children born to women with low vitamin D status. Since deficiency can be modified effectively by supplements and exposure to sunlight, public health policies to improve vitamin D levels in pregnant women may be beneficial.

10.5 Implications for the 'fetal programming' hypothesis: My study examined the relative roles of familial, intra-uterine and postnatal environmental factors in association with increased adiposity and altered glucose/insulin concentrations in the cohort children, and provided strong evidence for a major intrauterine programming effect of maternal diabetes in triggering the risk factors of type 2 DM in the offspring.

In contrast, the evidence for a role of fetal under-nutrition in programming risk (thrifty phenotype hypothesis) was weak. Inverse associations between birth size and insulin concentrations were apparent only after adjusting for current weight, and were lost after adjusting for current adiposity suggesting that in this cohort current adiposity is the major determinant of type 2 DM risks. Even after adjusting, the maximum variation explained by birth weight for any outcome was about 5% (Section 4.8). After adjusting for current size systolic blood pressure (BP) tended to decrease by about 1.5 mm Hg per kg increase in birth weight, but the association was not statistically significant (Section 6.2.2).

Many researchers have argued that the population health impact of birth weight in terms of adult disease is either of small magnitude or absent[64,65,233,234]. Using the data from five studies, Boyko estimated that the population attributable fraction (proportion of cases of diabetes attributable to a given exposure) of type 2 DM for birth weight was small to moderate (1%-25%), while >50% of cases of diabetes in the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study was attributable to a BMI >26kg/m^2.[233] Selective publication of small studies with stronger relationships has been suggested to overestimate the population impact of birth weight, while the true effect based on large studies remained small[234]. However, the above estimations based mainly on large field studies use crude methods of collecting data (self-report) which may in fact underestimate the true association. This, coupled with the complexity of calculating the proportion of cases attributable to low birth weight

considering the continuous association between birth weight and diabetes[45], and the interactions between birth weight and later growth makes the above estimate unreliable. However, my study did not show associations, even though it was methodologically superior with meticulous measurement of birth weights with appropriate adjustments made for the gestational age. This implied that impaired fetal growth may have little or no influence on later risk in this population.

In some studies, an absence of associations between birth weight and outcome measures in children despite using standard methodology[64,65] has led to the argument that the impact of fetal under-nutrition is little in populations where the low birth weight is rare[65], probably because almost all the fetuses are exposed to an optimum intra-uterine nutritional environment leading to little growth retardation. On the other hand, widespread fetal growth restriction among our children may have resulted in a narrow range of birth weights and effect size, and may have masked associations. Moreover, birth weight is a crude indicator of fetal growth retardation, and does not give a complete estimation of the effects of fetal under nutrition on different body components which may be better associated with risk markers, and thus may underestimate the true extent of risks associated with fetal under-nutrition.

Apparent absence of associations may be because of the young age of our children. Systematic reviews have indicated that the strength of association between birth weight and risk outcomes such as glucose and insulin concentrations or blood pressure tend to increase with age[75]. In the UK, the size of association between systolic BP and birth weight increased with increasing age of the participants[235]. In a longitudinal study among British children, Whincup et al. showed that the systolic BP rose from 2.3 mm Hg per kg decrease in birth weight at 5-7 year of age to about 4 mm Hg when the same children were examined at 9-11 years[236]. Thus, even a small effect of birth size on a particular outcome such as adiposity may magnify in the presence of an unfavourable postnatal environment, and accelerate/amplify the onset of adult diseases. The association may become more apparent as the children get older. The adverse influence of low birth size may be apparent only when coupled with accelerated postnatal growth, and the postnatal nutritional status and thus growth may be inadequate in my study children to show an association.

10.6 Future plans

Dietary and physical activity patterns: I have shown earlier that physical activity and body fat are inversely associated in my study children (Chapter 7). Our main objective now is to study the implications of lifestyle factors such as energy intake and physical activity on risk outcomes. We are currently following-up this cohort at 6-monthly intervals. Children's activity patterns are being characterised by administering questionnaires to parents and teachers (Appendix 3). We are also collecting information on the dietary preferences in these children through a 24-hour recall questionnaire administered to parents, and efforts are underway to determine the 'portion size' of the various foods consumed in a selected subgroup.

Validation using Doubly Labelled Water (DLW) method: We plan to use DLW method in a subset of 60 children (30 boys+30 girls of different BMI levels) from the original cohort to estimate energy expenditure, which can be used to validate the activity monitors that will be used in the whole cohort. In addition to validating the Actigraphs, we also plan to assess the validity of Actiheart and Minisun monitors, which combine movement sensors with heart rate monitoring. Currently we are testing the acceptability of these devices by the study children.

Randomised control trial: Our main objective is to develop and test interventions to improve diet, and increase activity levels and energy expenditure preliminary to carrying out a randomised control trial in high risk children. The aim is to see whether there will be a reduction in adiposity, and metabolic risk factors assessed by repeated body composition measurements, and biochemical investigations at the end of the trial.

Presently, we plan to carry out a pilot study for 20 children to test the feasibility and efficacy of the proposed trial. Activity sessions will be organised for them to involve in 'extra activity' for 30 minutes for three days in a week. A review will be done at the end of six months to assess the usefulness of activity sessions on adiposity levels by comparing with matched controls. There is little previous data substantiating the long-term effectiveness of such measures in either changing physical activity behaviours or reducing the risk outcomes in children. Moreover, there is no clear idea as to the amount of increase that would be effective in bringing down the risks, or the long-term risks involved with increasing the activity levels on the growth of the children. The FAO/WHO/UNU joint report recommends a minimum of 60 minutes of moderate

intensity activities per day[237]. However, there is no experimental evidence to corroborate these recommendations. A recent study from the UK observed that the total physical activity of the primary school children did not depend on the amount of timetabled physical education offered in schools[238]. This area needs further exploration before advocating any pubic health measures. The pilot trial may help us to understand/ solve some of these problems, and also to deal with several other issues that require more thought and attention at the preliminary stage, apart from the usual issues related to organisation. One of the major hurdles is to motivate the parents and the children to participate in these sessions. Since these activities need to be undertaken after the school hours, the problems related to timing need sorting out. This should not coincide with the normal daily activities such as schoolwork, and also should not be a substitute for children's routine playtime activities.

Another issue needing attention is the effect of extra activity on existing activity pattern. A child may decide to cut-down on other activities to compensate for energy spent on 'extra' exercise, or may indulge in more energy intake. Therefore, our aim is to choose activities that are attractive to both children and the parents, and are not perceived by the children as 'extra work'.

Genes: Apolipoprotein AV (*ApoAV*): Several genes such as ApoE, ApoC3 and ApoAV control plasma triglyceride concentrations. Variants of *ApoAV* gene (-1131T>C and S19W) have been shown to be associated with increased triglyceride levels in pregnant women, and increased length in the newborns (-1131T>C)[239]. Recently, a raised allele frequency of a variant of ApoAV was demonstrated in Indians living in Singapore[240]. This may be related to high insulin resistance and associated disorders through elevated triglyceride concentrations in Asian Indians.

Triglycerides are known to act as important fuel reserves for the fetus in case of maternal fasting, by releasing non-esterified fatty acids (NEFA, free fatty acids), and contribute to fetal growth. The elevation of NEFA levels may also cause peripheral insulin resistance in pregnancy[241]. Thus, the presence of *ApoAV* variant may predispose women to GDM, and result in higher birth weight in the offspring. During 5-year follow-up, we collected whole blood samples for DNA analysis from both parents and children with their consent. The genetic analysis is underway at the Centre for Cellular and Molecular Biology, Hyderabad, India.

Final comment

My study findings provide an insight into some of the causes for the diabetogenic phenotype of Indians. My study emphasises the need for further work in this cohort to explore the implications of the study findings for future risks in these children, to provide answers to some of the issues raised here such as the offspring effects of meticulous management of GDM and feasibility and long-term benefits of intervention measures to reduce childhood adiposity.

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APPENDIX 1- PROTOCOLS

APPENDIX 2-INTER- AND INTRA-OBSERVER VARIATION STUDIES

APPENDIX 3-QUESTIONNAIRS AND LETTERS

APPENDIX 4-ADDITIONAL ANALYSES, DATA, CENTILE CURVES

APPENDIX 1- Protocols

1 Children's Anthropometry

1.1 Height

Height is measured using the Microtoise wall-mounting stadiometer (CMS Instruments, London). The subject was asked to remove shoes and stand as tall and straight as possible with feet together, arms held loosely by the side and shoulders relaxed with his/her back, including the posterior surface of the head and heels applied to the wall. The head was positioned in the Frankfurt plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal. An assistant was present to check that the child's position was correct. The head plate of the stadiometer, fixed to the wall was pulled down and placed firmly on the top of the head in a horizontal position. The measurer aimed to read the scale from as level a position as possible to minimise the errors due to parallax. The height was read once to the nearest 0.1cm.

1.2 Sitting height

The child was asked to sit erect, with straight back, and head in the Frankfurt plane, on a wooden stool kept under the stadiometer, applied to the wall. The sitting height was measured once to the nearest 0.1cm and the stool height was subtracted from the obtained value.

1.3 Weight

Weight was measured using the electronic digital weighing scale. The base plate of the scale was placed on the most level and stable piece of ground possible. The monitor was placed at a higher level for easy reading. The monitor was checked for 'zero' reading. The child stood bare-feet on the base plate with minimal clothing. One reading to the nearest 100g was taken.

1.4 Head circumference

The child was asked to stand erect and look straight ahead. A measuring tape was passed firmly around the head such that it passes around the most posterior part on the back and just above the eyebrows anteriorly, and the maximum antero-posterior circumference was thus measured three times to the nearest of 0.1cm.

1.5 Abdominal circumference

After ensuring that the abdominal wall is relaxed, the tape was placed horizontally around the abdomen at the level of the umbilicus, taking care not to pull the tape too tight as to indent the skin. Three readings to the nearest 0.1 cm were taken at the end of the expiration.

1.6 Chest circumference

The tape was placed firmly and horizontally at the level of the xiphisternum. Three readings were taken at the end of expiration to the nearest 0.1 cm.

1.7 Mid upper arm circumference (MUAC)

The child was asked to stand with his/her back to the measurer, arm being flexed at 90°. The tip of the acromion (the point of the shoulder) and the olecranon were palpated and a point halfway between them (measured with a tape) marked on the skin. This marks the vertical level at which the skinfold will be made. The child was then asked to relax, with the arm hanging by the side. The tape was placed around the upper arm such that its upper border is at the level of the marking. The tape should be horizontal all round, should be firmly resting on the skin, but should not be pulled too tight. Three readings to the nearest of 0.1cm were taken.

1.8 Supine waist circumference

The child was asked to lie down on the couch in supine position. The iliac crests (highest points of the hipbone) on both sides were palpated and marked at the mid-axillary line. The lower borders of the ribs on both sides were marked at the mid-axillary line. With a measuring tape, mid points of these two levels were marked on either side. Ensuring that the tape is horizontal all round, the circumference was measured at this level to the nearest 0.1 cm in expiration. Three readings were taken.

1.9 Skinfold thickness

It was ensured that the child was as relaxed as possible while doing these measurements. Measurements made with the 'Harpenden' John-Bull calipers (CMS Instruments, London).

1.9.1 Triceps skinfold

The tape was placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line was drawn on the skin posteriorly at the level of the mark. Another vertical line was marked on this line at the most dorsal part of the upper arm. This level was determined by 'eyeballing' the mid-point or by a pen held vertically with one end on the olecranon process and the other end pointing towards the acromion. The point at which the fold is to be measured was now marked by a cross, formed by a horizontal line indicating the vertical level, and a vertical line marking the lateral level.

The skin was picked up over the posterior surface of triceps muscle, above the cross, on a vertical line passing upward from the olecranon to the acromion. The calipers were applied below the fingers such that the marked cross was at the apex of the fold. The readings were taken at exactly 5 seconds after the application of the calipers jaws. Three readings to the nearest 0.1 mm were taken.

1.9.2 Subscapular skinfold

Inferior angle of the scapula was identified and the skin was marked immediately below the angle. The skinfold was picked up above the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The caliper jaws were applied below the fingers, such that the marked cross was at the apex of the fold. Three readings were taken.

2. Bioimpedance

Bioimpedance was measured using a Bodystat, Quadscan 4000 analyser. The measurements were done preferably between 30 and 120-minutes OGTT samples to ensure a uniform hydration status for all. The child was made to lie supine on a couch for 5 minutes before starting the measurements, and any heavy jewellery and metal accessories on the body were removed. The child was asked to lie quietly and as comfortably as possible. After cleaning the area with the surgical spirit, on the hand, one electrode was attached at the level of the ulnar head at the wrist and the other just behind the knuckles. On the foot, the two electrodes were attached at the level of the medial and lateral malleoli and just behind the toes, respectively.

Two sets of the main leads connected to the back panel of the machine were attached to the electrodes. After ensuring that no parts of the body were touching one another, measurements were done by pressing the ENTER button on the machine.

3. Blood pressure

The child was asked to rest quietly for 5 minutes before measuring BP. The measurements were done during 30 and 120 minute samples, on the non-cannulated arm using a fully automated device (CRITIKON, DINAMAP^{TM} model 8100). The arm was exposed and kept comfortably at the level of the heart, making sure that the pushed up sleeve was not tight as to constrict the blood flow. The remaining air in the child cuff was squeezed out completely and the cuff was wrapped around the arm so that the centre mark of the cuff (Artery tubes) was positioned over the brachial pulse and the lower edge of the cuff was 2-3cm above the fossa. Two readings were taken, with one-minute rest between two measurements.

4.Parents' anthropometry

The protocols used for parents' anthropometry were similar to those used for children. Given below are the protocols for extra measurements done in parents or if the protocols were different.

4.1 Weight: Weight was measured in parents using a portable scale (Seca, Germany). The scale was placed on a level ground and the subject was asked to stand on the footplate after removing his/her shoes and heavy items of clothing or jewellery. One reading to the nearest 0.5kg was taken.

4.2 Biceps skinfold: The tape was placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line was drawn on the skin anteriorly at the level of the mark. The subject was asked to face the measurer with his/her arm relaxed and palm facing forward. A vertical line was marked at the level of the most protruding part of the biceps by 'eye-balling'. The callipers were applied below the fingers such that the marked cross was at the apex of the fold.

4.3 Supra-iliac skinfolds: With the subject facing sideways and arms folded, the iliac crest was located and marked at the mid-axillary line. The skinfold was picked up above the mark in the natural cleavage of the skin and the calliper jaws applied at the mark.

The subject was asked to tilt sideways slightly to ease the tension on the skin while picking up the skinfold.

4.4 Hip circumference: The subject was asked to empty the hip pockets and stand sideways with arms folded over chest. The tape was passed around the hip area and adjusted upwards and downwards until the maximum circumference was achieved. Measurements were taken at this level after ensuring the horizontal position of the tape all around.

5. Parents' BP

The measurements were preferably made on the left (non-dominant) arm. An automatic digital instrument (CRITIKON) is used for BP measurements. The left arm (Right, if left side is cannulated) was exposed and kept comfortably at the level of heart. Appropriate sized cuff was selected based on the mid-arm circumference. The measurements were carried out as for children.

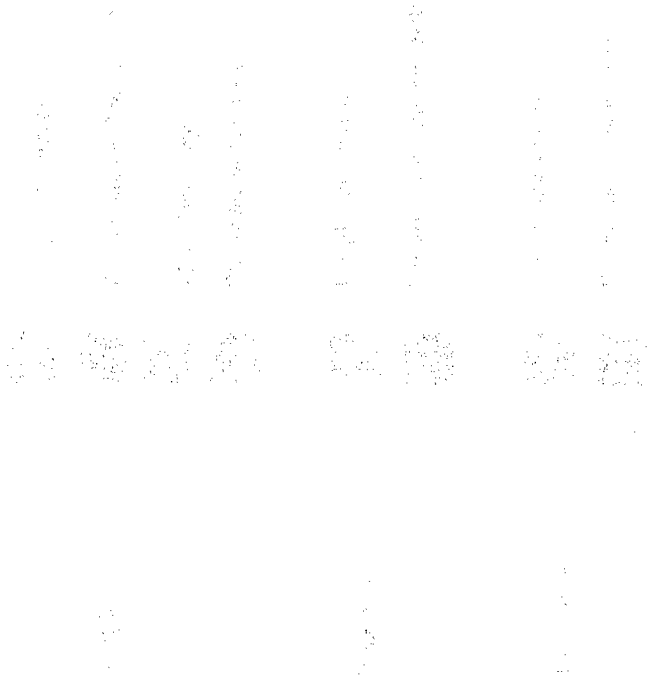
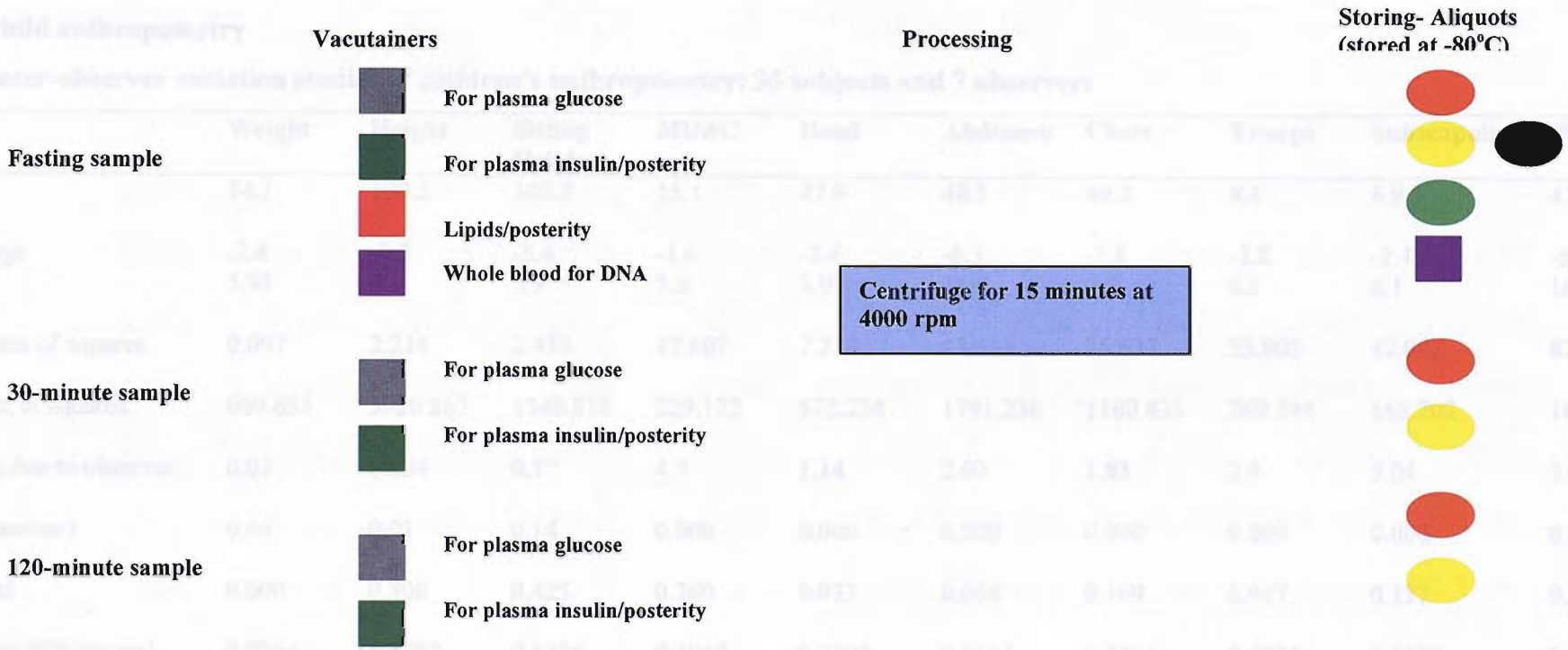


Figure 1 Diagram showing the scheme of blood sampling and processing



APPENDIX 2-Inter- and Intra-observer Variation studies

IOV for child anthropometry

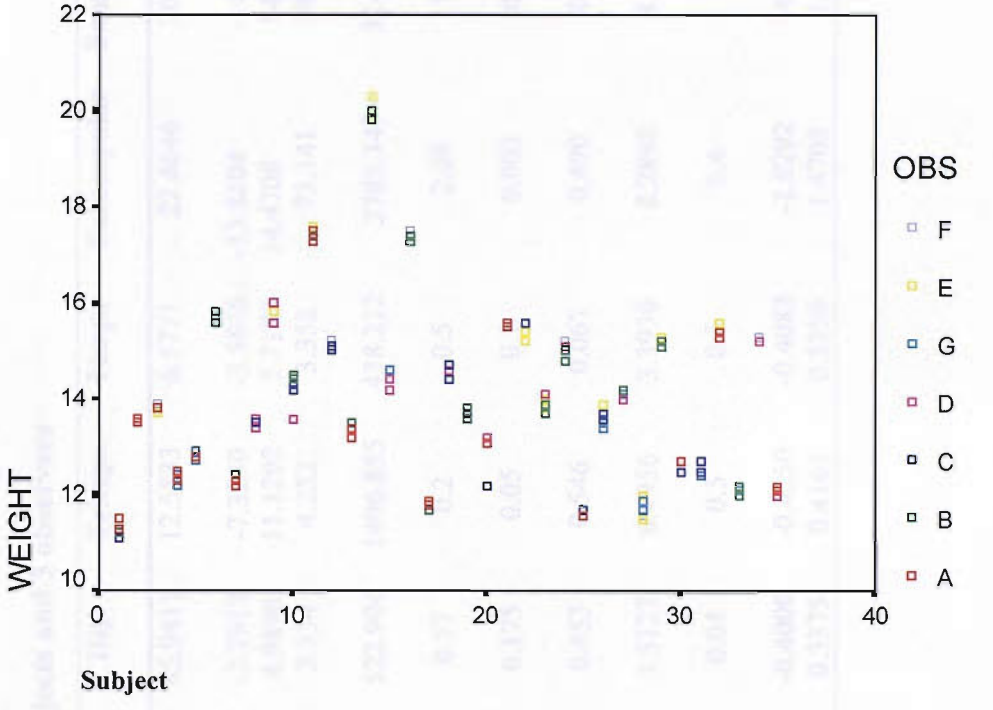
Table I. Inter-observer variation studies of children's anthropometry: 35 subjects and 7 observers

	Weight	Height	Sitting Height	MUAC	Head	Abdomen	Chest	Triceps	Subscapular	Waist
Mean	14.1	100.2	102.3	15.1	47.9	48.5	49.2	8.4	6.9	47.9
Subject range	-2.8 5.93	-9.7 9.1	-5.4 5.9	-1.6 3.9	-2.4 3.9	-6.3 11.6	-3.8 7.2	-2.8 6.1	-2.4 6.1	-5.9 10.9
Observer sum of squares	0.097	2.214	2.453	12.607	7.213	43.531	25.637	25.905	42.042	81.338
Subject sum of squares	669.655	3920.867	1348.818	229.122	572.234	1791.236	1160.835	769.344	665.202	1620.153
% variation due to observer.	0.01	0.054	0.17	4.7	1.14	2.03	1.83	2.9	5.04	3.9
p-value (observer)	0.44	0.01	0.14	0.000	0.000	0.000	0.000	0.000	0.000	0.000
p-value (set)	0.000	0.306	0.425	0.360	0.032	0.064	0.199	0.947	0.137	0.121
SD (observer differences)	0.0264	0.1282	0.1336	0.3040	0.2292	0.5624	0.4301	0.4325	0.5652	0.7751
SD/mean (%)	0.19	0.13	0.13	2.01	0.48	1.16	0.87	5.18	8.19	1.61
Observer range	-0.0255 0.0435	-0.1919 0.1262	-0.1531 0.1956	-0.3972 0.3230	-0.3078 0.3837	-0.7521 0.6070	-0.5377 0.7431	-0.6191 0.4999	-0.5355 0.9417	-0.9241 0.9564

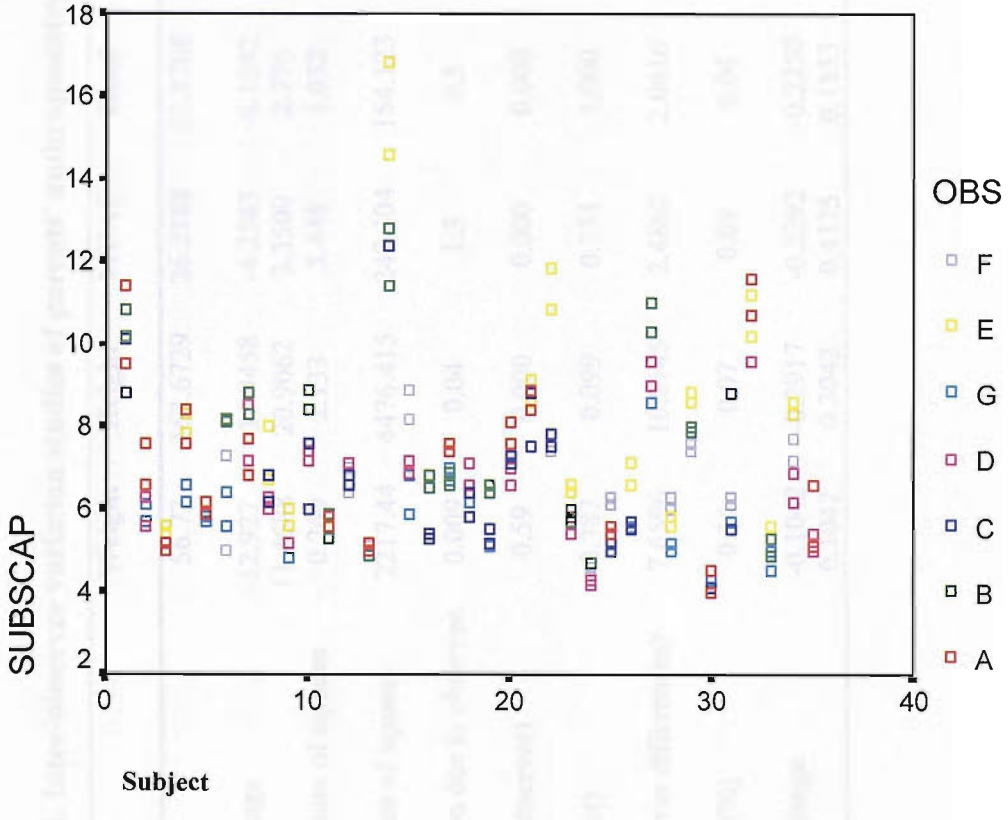
Table II. Intra-observer mean squared differences between sets

Observer	Weight	Height	Sitting Height	MUAC	Head	Abdomen	Chest	Triceps	Subscapular	Waist
A	0.0147	0.3787	0.3773	0.0140	0.0520	0.9633	0.3387	0.2767	0.6773	1.6487
B	0.0307	0.1973	0.7047	0.0513	0.2320	1.5900	0.6733	0.7080	0.2833	1.7273
C	0.0700	0.3233	0.7533	0.0973	0.1313	1.9007	0.3407	0.7300	1.2600	1.6993
D	0.0800	0.1620	0.3447	0.0707	0.2887	0.9100	0.3680	0.4240	0.2720	1.7660
E	0.0447	0.1907	0.4487	0.0800	0.1580	1.7080	0.8520	0.3467	0.6760	1.3193
F	0.0220	0.1807	0.2253	0.0520	0.0840	2.9560	0.5727	0.4540	0.4553	0.8313
G	0.0307	0.2667	0.7887	0.1087	0.2080	0.6107	0.2680	0.2893	0.1800	0.5987
Total	0.0418	0.2428	0.5204	0.0677	0.1649	1.5198	0.4876	0.4612	0.5434	1.3701

Plot of weight with subject by observer



Plot of weight with subject by observer



IOV for parents' anthropometry

Table III. Inter-observer variation studies of parents' anthropometry: 12 subjects and 3 observers

	Weight	Height	MUAC	Head	Waist	Hip	Triceps	biceps	Subscapular	Supra-ileac
Mean	56.72	162.6729	26.2188	52.8708	77.9938	85.9417	12.5833	6.5771	22.8646	20.7333
Subject range	-12.927	-15.9458	-4.2583	-4.1542	-10.2896	-3.7917	-7.3250	-3.5646	-13.8104	-14.4750
	11.4688	20.9062	3.3500	2.775	10.6354	4.9896	11.1292	5.7187	14.4708	14.6292
Observer sum of squares	0.260	2.133	3.448	1.032	127.232	3.334	4.252	3.352	73.141	398.292
Subject sum of squares	2217.44	4476.415	247.304	154.123	2557.829	522.994	1496.855	438.212	2783.347	3240.445
% variation due to observer.	0.009	0.04	1.3	0.5	4.46	0.57	0.2	0.5	2.08	9.15
p-value (observer)	0.59	0.000	0.000	0.000	0.000	0.175	0.05	0.5	0.003	0.000
p-value (set)	0.387	0.099	0.334	1.000	0.993	0.453	0.546	0.067	0.490	0.856
SD (observer differences)	7.6596	10.8145	2.4862	2.0616	7.8200	3.5127	6.4956	3.3930	8.2898	8.8025
SD/mean (%)	0.14	0.07	0.09	0.04	0.1	0.04	0.5	0.5	0.4	0.4
Observer range	-0.1042	-0.2917	-0.2292	-0.2250	-2.6083	-0.4000	-0.4250	-0.4083	-1.9292	-4.5833
	0.1042	0.3042	0.4375	0.1833	1.7500	0.3375	0.4167	0.3250	1.4708	3.2083

Table IV. Intra-observer mean squared differences between sets

Observer	Weight	Height	Head	MUAC	Hip	Waist	Biceps	Triceps	Subscapular	Supra-ileac
A	.3750	.1675	.0788	.0863	.7500	2.2550	.7763	.8062	4.3412	7.6387
B	.6875	.1163	.0688	.4063	1.5750	4.3900	20.1913	2.5888	20.2213	25.9687
C	.7500	.2300	.0375	.0688	1.5950	3.3987	.5737	.5250	1.3037	4.4775
Total	.6042	.1713	.0617	.1871	1.3067	3.3479	7.1804	1.3067	8.6221	12.6950

PARTHENON – WELLCOME – MRC

5 - YEAR FOLLOW-UP STUDY

Parthenon Study Number | | | | |

Follow-up Number | | | | |

Date Of Interview | | | | | | | | |

Child's Name

Mother's Name

Father's Name

Address For Correspondence:

Whether the child attending school ? | | 1. Yes 2. No

CHECK LIST

NO	DESCRIPTION	YES	NO	COMMENTS
1	Consent			
2	Photograph			
3	Child's medical history			
4	Child's medical examination			
5	Mother's questionnaire			
6	Father's questionnaire			
7	Anthropometry –child			
	Anthropometry- mother			
	Anthropometry- father			
8	BP- child			
	BP- mother			
	BP- father			
9	Bio impedance- child			
10	Blood collection – child			
	Blood collection- mother			
	Blood collection- father			
11	Gifts			
12	Reports – child			
	Reports- mother			
	Reports-father			
13	Immunization (On demand)			
14	TA			

CONSENT

We have fully informed the Parents / Father / Mother/ Guardian of the child _____ about the ' Parthenon Study ' conducted by the MRC unit, HMH, Mysore and have covered all the points in the attached check list. They are willing to take part in the study and will undergo the tests specified.

Check list

1. EMLA Cream
2. Glucose tolerance test for child
3. Fasting glucose analysis for the father, GTT for the mother
4. Bio-impedance for child
5. Blood pressure for child and parents
6. Complete anthropometry for child and parents
7. Risks / complications if any

Interviewer's name _____ **Code** ☐

Signature

Date

Developmental check list

1. Does the child define words (5)?

   According to M.R.C. Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

2. Does the child name colours (4)?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

3. Can the child name the opposite for 2 words?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

4. Does the child count up to 20?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

5. Can the child tell his age?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

6. Can the child tell his address / telephone no?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

7. Can the child copy square or a triangle?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

8. Can the child draw a recognizable man?

   According to M.R.C Observer 0. No 1. Yes ☐ Grade ☐

   According to mother (if different) 0. No 1. Yes ☐

9. Does the child walk heel to toe forwards?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

10. Does the child walk heel to toe backwards?

   According to M.R.C Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

11. Can the child jump from 3 steps?

   According to MRC Observer 0. No 1. Yes ☐

   According to mother (if different) 0. No 1. Yes ☐

12. Does the child clean himself after going to toilet?

   According to mother 0. No 1. Yes ☐

13. Does the child take bath on his own?

   According to mother 0. No 1. Yes ☐

14. Can the child climb the ladder?

   According to mother 0. No 1. Yes ☐

15. Does the child obey rules?

   According to mother 0. No 1. Yes ☐

Health And Vaccination Status

Has the child had any major health problems during the past one year? ☐ 1.Yes 2.No

If yes

Code ☐

Does the child have any health problems at present? ☐ 1.Yes 2.No

If yes

Code ☐

Is the child taking any medications currently? ☐ 1.Yes 2.No

If yes

Code ☐

Has the child been vaccinated with DPT booster? ☐ 1.Yes 2.No

Other vaccines given during the past year

Code ☐

General examination: Comments and advice

ANTHROPOMETRY

Child's Name _____ **Study No.** ____

Measurer's Name _____ **Code** ____

Measurements			
Weight (kg)			
Height (cm)			
Sitting Height(cm)			
Circumferences	1	2	3
Head (cm)			
Abdomen (cm)			
Chest (cm)			
MUAC(cm)			
Supine Waist (cm)			
Skinfolds			
Triceps (mm)			
Subscapular (mm)			

Comments: _____

Bioimpedence:

Investigator's Name _____ **Code** ____

Test No ___ **Time** _____ **AM**

Bio impedance Complete: ____ **1. Yes 2. No** **If No, Code** ____

Comments: _____

Blood Pressure

Instructions: 1. Measurements to be made between 30 and 120 minutes samples.
2. Child should be seated quietly for 5 minutes before the measurements.
3. Measured only at non-cannulated side

Time [ ] **AM** **Room Temperature** [ ]

Side [ ] 1. Left 2. Right

	Systolic (mm Hg)	Diastolic (mm Hg)	Pulse (beats/ min)
1			
2			

Glucose Tolerance Test

Amount of glucose given : [ ] gm

Status [ ] 1. Complete 2. Incomplete

Sample Time:

Fasting sample (a.m.)	Glucose finished (a.m.)	30 mins Sample (a.m.)	120 mins Sample (a.m.)

If Incomplete, Reasons [ ] Code [ ]

Blood Results

Routine

Haemoglobin					
Blood group					
HbsAg status					
Total Count					
Differential Count	N:	L:	E:	B:	M:

GTT

Sample	Value (mg/dl)
Fasting	
30 minutes	
120 minutes	

GTT Status [ ] 1. Abnormal 0. Normal

Comments

[ ]

Mother

Date: | | | | | | | | |

Name | | Status | | 1. Alive 2. Dead

Occupation | | Code | |

Is currently pregnant? | | 1. Yes 2. No

Obstetric History: Gravida | | Para | | Living | | Dead | | Abortions | |

Any subsequent pregnancies after the Parthenon baby? | | 1. Yes 2. No

If yes,

Date	GA	BW	L/D /A	M/F	Hospital(name) / Home	MOD	Pregnancy Complications	H/O GDM. If yes Insulin

If any history of Diabetes in the pregnancy? | | 1.Yes 2. No

If yes, age of onset | | | years

Treatment given | | 1. Insulin 2. Tablets 3. Diet

If currently Diabetic? | | 1.Yes 2. No

If yes, age of onset | | | years

Treatment given | | 1. Insulin 2. Tablets 3. Diet

Past and current medical problems:

	Code

Current drug history:

	Code

Family history of diabetes in first degree relative ☐ 1.Yes 2. No

If yes	1. Yes 2. No	Alive / Dead	Age of onset of DM	Insulin
Mother				
Father				
Sibling 1				
2				
3				
4				

Ever used tobacco regularly? ☐ 1. Yes 2. No

If yes 1.Used before, but now stopped ☐

2. Still using ☐

If stopped, age of stopping ☐ Years

Type ☐ Code ☐

Amount / Day ☐

Ever used alcohol regularly? ☐ 1. Yes 2. No

If yes 1.Used before now stopped ☐

2. Still using ☐

If stopped, age of stopping ☐

Type of alcohol ☐ Code ☐

Days / Week ☐

Quantity / time ☐☐ 1. Measures/ spirit 2. Mugs/beer 3. Glasses/wine ☐

Units per week | | | |

Anthropometry

Name |

Measurer's Name | **Code** |

Measurements			
Weight (kg)			
Height (cm)			
Circumferences	1	2	3
MUAC (cm)			
Head circumference (cm)			
Waist circumference (cm)			
Hip circumference (cm)			
Skinfolds			
Biceps (mm)			
Triceps (mm)			
Subscapular (mm)			
Suprailiac (mm)			

Comments:

Blood Pressure

Time [ ] AM **Room Temperature** [ ]

Machine code [ ] 1. Critikon 2.Omron

Cuff size [ ] 1.Small adult 2.Medium 3. Large adult

	Systolic (mm Hg)	Diastolic (mm Hg)	Pulse (beats/ min)
1			
2			

Blood test details

GTT [ ] 1. Yes 2. No

Blood results:

GTT

Sample	**Value (mg/dl)**
Fasting	
30 minutes	
120 minutes	

Comments and advice:

Father

Date: | | | | | | | | |

Name | | Status | | 1. Alive 2. Dead

Occupation | | Code | |

If known Diabetic? | |

If yes age of onset | | | years

Treatment given | | 1. Insulin 2. Tablets 3. Diet

Past and current medical problems:

	Code	

Current drug history:

	Code	

Family history of diabetes in first degree relative | | 1. Yes 2. No

If yes	Yes /No	Alive / Dead	Age of onset of DM	Insulin
Mother				
Father				
Sibling 1				
2				
3				
4				
5				

Ever used tobacco regularly? ☐ 1. Yes 2. No

If yes 1. Used before, but now stopped ☐

2. Still using ☐

If stopped, age of stopping ☐

Type ☐ Code ☐

Amounts / Day ☐

Ever used alcohol regularly? ☐ 1. Yes 2. NO

If yes 1. Used before now stopped ☐

2. Still using ☐

If stopped, age of stopping ☐

Type of alcohol ☐ Code ☐

Days / Week ☐

Quantity / time ☐☐ 1.Measures/ spirit 2. Mugs/beer 3. Glasses/wine ☐

Units per week ☐☐☐

Anthropometry

Name _____

Measurer's Name _____ **Code** _____

Measurements			
Weight (kg)			
Height (cm)			
Circumferences	1	2	3
MUAC (cm)			
Head circumference (cm)			
Waist circumference (cm)			
Hip circumference (cm)			
Skinfolds			
Biceps (mm)			
Triceps (mm)			
Subscapular (mm)			
Suprailiac (mm)			

Comments:

Blood Pressure

Time [ ] **AM** **Room Temperature** [ ]

Machine Code [ ] 1.Critikon 2.Omron

Cuff size [ ] 1. Small adult 2. Medium 3. Large adult

	Systolic (mm Hg)	Diastolic (mm Hg)	Pulse (beats/ min)
1			
2			

Blood test details:

Fasting glucose [ ] mg/dl

Comments and advice:

[ ]

MRC No: _____ Date: _____

_____

Dear Mr. and Mrs.:

You and your child _____ are an integral part of our research project (effects of mother's glucose metabolism on the growth of children). As you are aware, we have examined your child at the time of birth and during 1st, 2nd, 3rd and 4th years. As we have already briefed you, this year's follow-up (5th year) happens to be the most crucial part of the project and we intend to do many relevant tests on the child and also on parents. These include.

1.Blood test for the child - Oral Glucose Tolerance Test, by which we will measure the fasting glucose level in the blood and after 30 and 120 minutes of giving a glucose drink.

- Blood Group, Hemoglobin levels (HB%), HbsAg status (Hepatitis B)
- Blood sampling for DNA and HbA1c assay

2.Measurement of body fat using bio-impedence method and routine body measurements.

3.Blood Pressure (BP) measurements

In addition, we want to see the glucose level in the blood for parents also, measure their BP and will take parents' full body measurements. The total time of all these tests is **approximately 2 and half-hours**.

Therefore, we request you to kindly bring the child on _____ to the MRC building at the Mission Hospital (HMH) at **7.30AM**. Please do follow the instructions given below before coming, to prevent getting the false results.

Kindly co-operate as in the previous years and make this project a success.

The travel expenses will be refunded.

Breakfast will be provided at the end of the tests.

Instructions:

- Make sure that your child has eaten properly for three days prior to the test day.
- Both the child and the parents **should not eat or drink anything except water** after **9 PM** during the previous night.
- Both the child and the parents should not eat or drink **anything (Not even coffee/tea/milk etc) except water** in the morning before coming to the hospital. i,e. Both the child and the parents should come to the clinic after **fasting overnight.**

NOTE: Blood taking procedure will be made PAINLESS for the child by applying a cream (EMLA cream). EMLA cream is not useful for adults.

We seek your kind co-operation and we will assure you that all these tests are very safe and not harmful to your child in anyway.

Date of Clinic: ---------- Time: ----------

PLACE: MRC OFFICE (NEW BUILDING)
CHILDREN'S BLOCK,
MISSION HOSPITAL, MYSORE
Sunday -- Holiday.

Dr. Krishnaveni and the MRC Team
Mission Hospital, Mysore
Phone No.-- 529347

PARTHENON CHILDREN PHYSICAL ACTIVITY QUESTIONNAIRE

Questions for the parents

1. What is the distance from **your house** to the **Child's School**? ☐ **Km**

2. How does your child **mainly travel** to school? ☐

   1.Walk 2.Cycle 3.Two wheeler 4.Auto 5.Bus 6.Car

3. Would it be possible for your child to **Walk or Cycle** to School? 0 No 1 Yes ☐

4. If **Not,** Explain **Reasons** in detail: _____

   _____

   _____

   _____

5. How does your child **mainly travel** to home from school? ☐

   1.Walk 2.Cycle 3.Two wheeler 4.Auto 5.Bus 6.Car

6. Does your Child has formal **Sports or Physical Education** classes at School? 0 No 1 Yes ☐

7. If **Yes, a)** How many minutes / hours per **day**? ☐☐ Minutes ☐☐ Hours

   b) How many minutes / hours per **week**? ☐☐ Minutes ☐☐ Hours

8. What type of activities takes place during these classes?

   _____

   _____

   _____

   _____

9. Does your child **Enjoy** these classes? 0 No 1 Yes ☐

10. Does your Child **Actively Participates** in these classes? 0 No 1 Yes ☐

11. Compared with **Mathematics or Science**, how much do you think **Sports or Physical education** classes are important for your child's future?

    1.More Important 2.Equally Important 3.Less Important ☐

12. What does the child do in the **Evenings** after school has finished?

List the answers eg. Schoolwork, Watching TV, Computer Games, Playing outside, Tuition Class. Then place number 1-5 beside the one which the child spends most time

List Of activities	Time Spent (minutes)	Number
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

13. In a typical evening, how much time does the child spend on **Schoolwork**?

☐☐ Minutes ☐☐ Hours

14.Does the family **Own a TV**? 0 No 1 Yes ☐

15. In a typical day, how much time does the child spend **Watching TV**?

☐☐ Minutes ☐☐ Hours ☐ No TV / Not applicable

16. Does the family own a **Computer at Home**? 0 No 1 Yes ☐

17. Does the child visit any **Cyber Café to use computer**? 0 No 1 Yes ☐

18. In a typical day, how much time does the child spend **Sitting at the computer**?

☐☐ Minutes ☐☐ Hours ☐ Not applicable

19. Does the family **Own a Vehicle (Car / Two wheeler)**? 0 No 1 Yes ☐

20. Does the child have **Space to Play** near the house? 0 No 1 Yes ☐

21. If **Yes,** describe the space:_____

(eg. Private garden, private yard, public park, public playground)

22. On a typical day, how much time does the child spend **In Free Play Out of Doors**?

☐☐ Minutes ☐☐ Hours

23. In a typical **week**, does your child play **formal games / physical education / sports outside of school hours**?

0 No 1 Yes ☐

24. If **Yes,** Which **Activities**? (list in order of preference)

	Activity	**Time (minutes/week)**
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____
7	_____	_____
8	_____	_____

25. How many hours per **week** in total? ☐☐ Hours

26. If the project starts **activities clubs for children** would you be interested? 0 No 1 Yes ☐

27. What **time** would be most **convenient** for you?

a) Weekdays:_____

b) Weekends:_____

28.What activities would you choose for your child at such clubs? (list in order of preference)

1	_____	5	_____
2	_____	6	_____
3	_____	7	_____
4	_____	8	_____

29. Do you **as a family,** do active things together? (eg. walking, sports) 0 No 1 Yes ☐

30. If **Yes,** Which **Activities**? (list in order of preference)

1	_____	5	_____
2	_____	6	_____
3	_____	7	_____

4 _____ 8 _____

31. Does the **Mother** do any formal physical activities/yoga/dance/sports? 0 No 1 Yes ☐

32. If **Yes**, Which **Activities**? (list in order of preference)

	Activity	**Time (minutes/week)**
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____

33. How many hours per **week** in total? ☐☐ Hours

34. Does the **Father** do any formal physical activities/yoga/dance/sports? 0 No 1 Yes ☐

35. If **Yes**, Which **Activities**? (list in order of preference)

	Activity	**Time (minutes/week)**
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____

36. How many hours per **week** in total? ☐☐ Hours

FOOD AND DIET

37. Do you think your child is:
1.Too Thin 2. Too Fat 3. Just Right ☐

38. Does your child **Eat**:
1.Too Much 2. Too Little 3. Just Right ☐

39. Does your child ever **refuse to eat** some things? 0 No 1 Yes ☐

40. If **Yes**, List:

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

41. Do you use **Food / Sweets** as a reward? (eg. For good exam results, achievement in Extracurricular activities)

0 No 1 Yes ☐

42. Which **Items** would your child like as a reward?

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

43. Do You as a family eat in **Cafes / Restaurants** outside the house? ☐

1. Never **2.** Sometimes ≤ 1 per 3 months **3.** > 1 per 3 months **4.** Rare

44. Which **Type of food** do you choose on these occasions? (list in order of preference)

1 _____ 5 _____

2 _____ 6 _____

3 _____ 7 _____

4 _____ 8 _____

45. What **food** does the child **eat at lunchtime at school**?

School Meal (Describe): _____

_____

_____

_____

Lunch box from home (Describe): _____

_____

_____

_____

46. Does the child have **Sweets or Snacks** on the way to or from **School**? 0 No 1 Yes ☐

47. Does the family **eat together at Mealtimes**? 0 No 1 Yes ☐

48. If the child is hungry in between meals, what does he/she get? (list in order of preference)

1 _____ 5 _____

2 _____ 6 _____

3 _____ 7 _____

4 _____ 8 _____

49. What **Sweets / Snacks** are routinely **kept in the house**?

1 _____ 5 _____

2 _____ 6 _____

3 _____ 7 _____

4 _____ 8 _____

50. Does your child get **'Pocket Money'**? 0 No 1 Yes ☐

51. What do they **usually spend it on**? (list in order of preference)

1 _____ 5 _____

2 _____ 6 _____

3 _____ 7 _____

4 _____ 8 _____

Questions for the School Teacher

1.Does the child have **formal classes / lessons devoted to Physical activity / sports / Physical education**?

0 No 1 Yes ☐

2. If **Yes**, how many hours per week? ☐☐ Hours

3. Are **these taught by** specially **trained PE teachers**? 0 No 1 Yes ☐

4. Are these classes **compulsory**? 0 No 1 Yes ☐

5. Do they '**Score**' towards the child's **Overall Marks**? 0 No 1 Yes ☐

6. What **Activities** do they include?

1 _____ 5 _____

2 _____ 6 _____

3 _____ 7 _____

4 _____ 8 _____

7. Do they take place within **School Premises** or do the children get taken **Elsewhere**?

1. School Premises 2. Elsewhere ☐

8. Does the School have a **Playground** for the children to play outside during break times?

0 No 1 Yes ☐

9. Is there any other **curriculum time spent in Physical Activity**?

0 No 1 Yes ☐

10. If **Yes**, Specify: _____

_____

_____

_____

11. Is the **importance of Physical Activity formally taught**? (eg. in Biology or 'health classes')

0 No 1 Yes ☐

12. Is **Diet Education** provided? 0 No 1 Yes ☐

13. Do you think that, **compared with 5 years ago**, formal Physical Education in school has

1. More time 2. Less time 3. About the same 4. Not known ☐

14. Compared with **Mathematics or Science**, how much do you think **Sports or Physical Education classes are important** for your child's future?

1. <u>More</u> Important 2. <u>Equally</u> Important 3. <u>Less</u> Important ☐

15. Do you think there is a problem of **Obesity among School Children** Nowadays?

0 No 1 Yes ☐

16. If **Yes**, what do you suggest to **overcome the problem of obesity among School Children**?

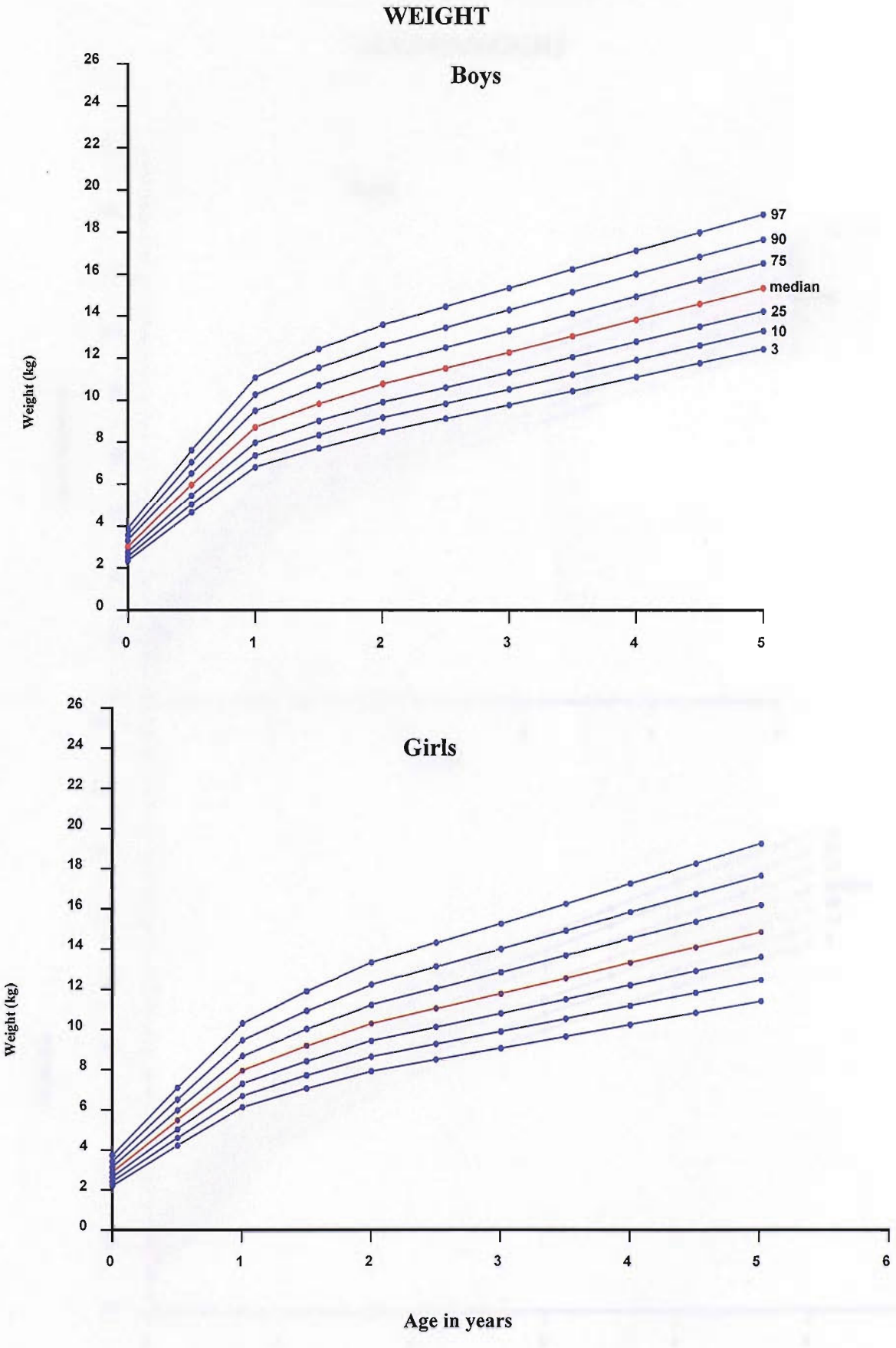
1.Diet education ☐
2.Physical activity/sports/PE
3.Combination of diet and PE / physical activity/ sports
4. Not known

APPENDIX 4 - Additional analyses, data, centile curves

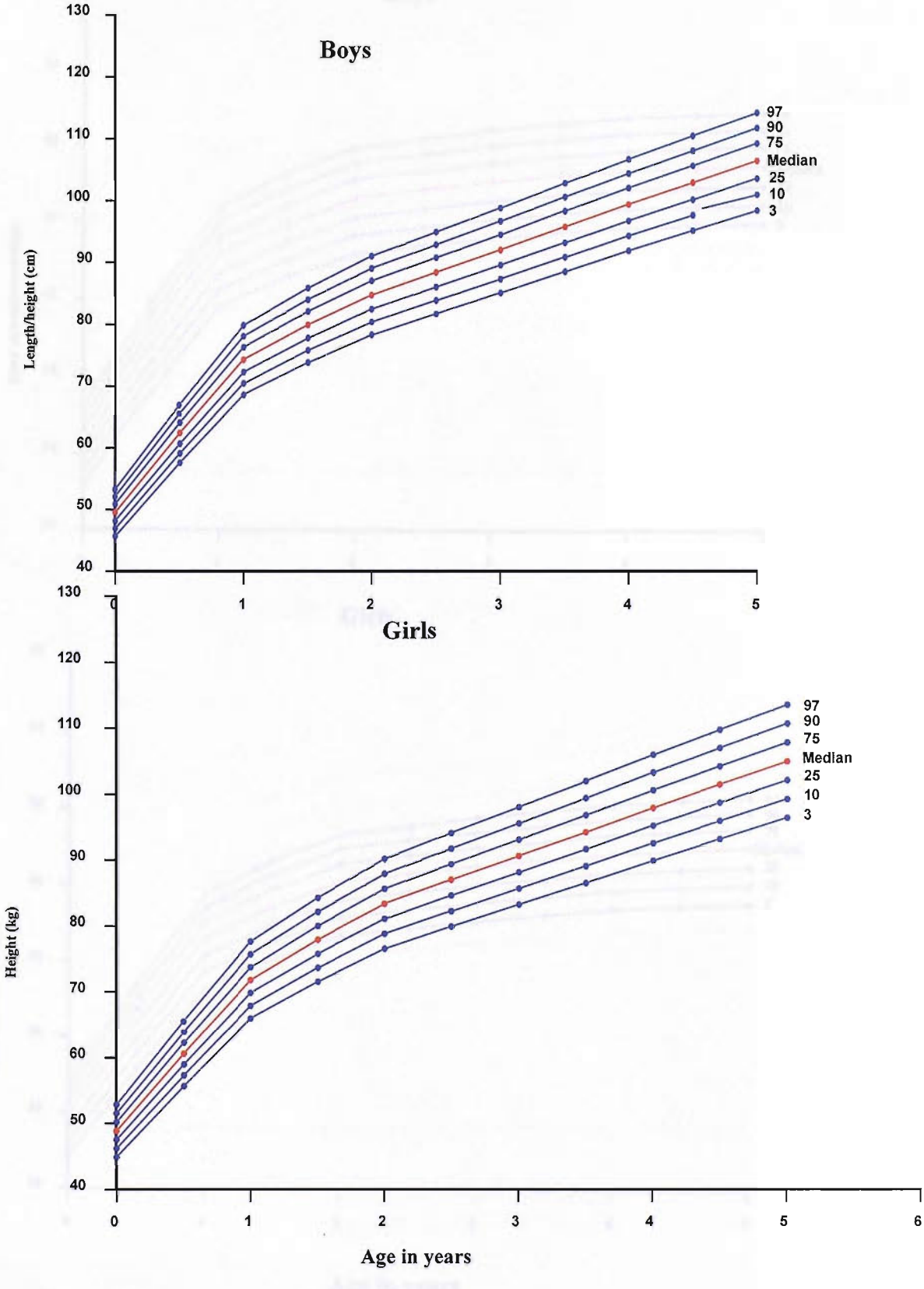
A. Anthropometric characteristics of the offspring of diabetic and non-diabetic mothers at birth, 1, 2, 3, 4 and 5 years (Mean (SD) or *Median (IQR).

	Offspring of diabetic mothers (N Max=41)						Offspring of non-diabetic mothers (N Max=589)						'p' for difference					
	Birth	1	2	3	4	5	Birth	1	2	3	4	5	Birth	1	2	3	4	5
N Max=	**41**	**36**	**39**	**37**	**36**	**36**	**589**	**506**	**516**	**533**	**539**	**519**						
Weight (kg)	3.3 0.4	8.5 1.2	10.7 1.4	12.1 1.0	14.0 2.0	15.8 2.1	3.0 0.4	8.4 1.1	10.5 1.3	12.1 1.4	13.7 1.7	15.2 2.0	<0.001	0.8	0.4	0.8	0.3	0.09
Crown-heel length (cm)	50.5 2.3	73.5 2.5	84.4 3.4	91.4 3.8	98.8 4.6	106.0 4.5	49.2 2.1	73.2 2.8	83.8 3.3	91.2 3.7	98.7 4.0	105.6 4.4	<0.001	0.6	0.3	0.7	0.9	0.6
Crown-rump length (cm)	32.9 1.6	45.7 1.7	50.6 2.0	53.90 2.4	55.3 2.7	58.5 2.6	32.3 1.7	45.6 2.0	50.0 2.3	53.5 2.3	55.3 2.8	58.1 2.4	0.03	0.8	0.1	0.3	0.9	0.4
Leg length (cm)	17.6 1.6	27.8 1.4	33.8 1.9	37.5 2.0	43.5 2.5	47.6 2.4	16.9 1.5	27.7 1.6	33.8 1.9	37.7 2.2	43.4 2.4	47.5 2.5	0.004	0.6	0.9	0.6	0.8	0.9
Ponderal index (kg/m^3)/ BMI (kg/m^2)	26.0 2.5	15.6 1.7	15.0 1.2	14.5 0.9	14.3 1.2	14.0 1.2	24.9 2.7	15.6 1.4	15.0 1.1	14.5 1.1	14.0 1.0	13.6 1.1	0.009	0.95	0.98	0.9	0.1	0.03
Head circumference (cm)	34.6 1.2	44.1 1.4	46.5 1.4	47.3 1.5	48.3 1.5	48.8 1.3	34.1 1.3	44.2 1.4	46.4 1.4	47.5 1.4	48.3 1.4	48.5 1.5	0.03	0.8	0.8	0.4	0.7	0.3
MUAC (cm)	11.3 0.9	14.3 1.1	15.0 1.1	15.0 1.1	15.7 1.3	15.9 1.3	10.5 0.9	14.1 1.1	14.6 1.0	15.0 1.1	15.3 1.1	15.3 1.2	<0.001	0.2	0.03	0.9	0.04	0.005
Chest circumference (cm)	33.7 1.6	43.5 2.4	46.0 2.4	46.7 2.1	48.7 2.5	49.9 2.5	32.3 1.7	43.9 2.2	46.3 2.1	47.7 2.1	49.1 2.2	50.2 2.4	<0.001	0.3	0.4	0.007	0.4	0.5
Abdominal circumference (cm)	31.5 1.8	42.69 3.4	44.9 3.6	46.2 2.6	48.8 3.6	49.4 3.7	30.2 2.0	42.9 2.9	44.5 3.1	46.6 2.8	48.2 2.9	48.6 3.1	<0.001	0.6	0.5	0.4	0.3	0.2
AMA (cm^2)	7.45 1.2	11.12 1.6	12.07 1.5	12.38 1.8	13.38 2.1	13.79 1.9	6.66 1.1	10.76 1.7	11.78 1.7	12.47 1.7	13.02 1.8	13.19 1.9	<0.001	0.2	0.3	0.7	0.3	0.06
Triceps skinfold (mm)*	5.1 4.6,6.1	7.8 6.9,9.5	8.3 7.1,9.7	8.0 6.7,9.4	8.5 6.6,9.9	8.6 6.7,10.3	4.1 3.7,4.8	7.7 6.8,8.8	7.7 6.6,8.8	7.7 6.7,9.0	7.9 6.8,9.0	7.7 6.5,8.8	<0.001	0.8	0.03	0.4	0.1	0.01
Subscapular skinfold (mm)*	5.3 4.7,6.2	6.5 5.9,9.0	7.4 5.9,9.1	7.1 5.7,8.9	7.0 5.2,9.4	6.6 5.2,8.3	4.4 4.0,5.0	6.4 6.0,8.2	7.0 5.9,8.2	6.4 5.4,7.5	6.3 5.3,7.3	5.9 4.9,6.9	<0.001	0.8	0.1	0.03	0.06	0.02

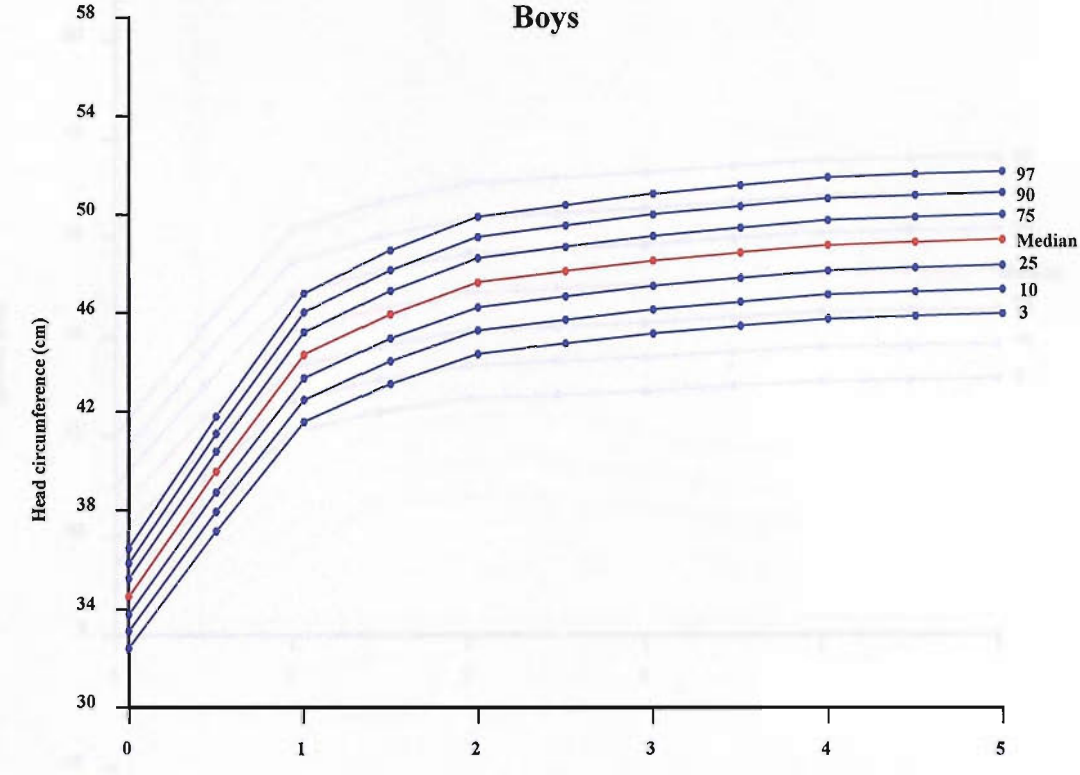
B. Centile curves derived using Mysore anthropometry

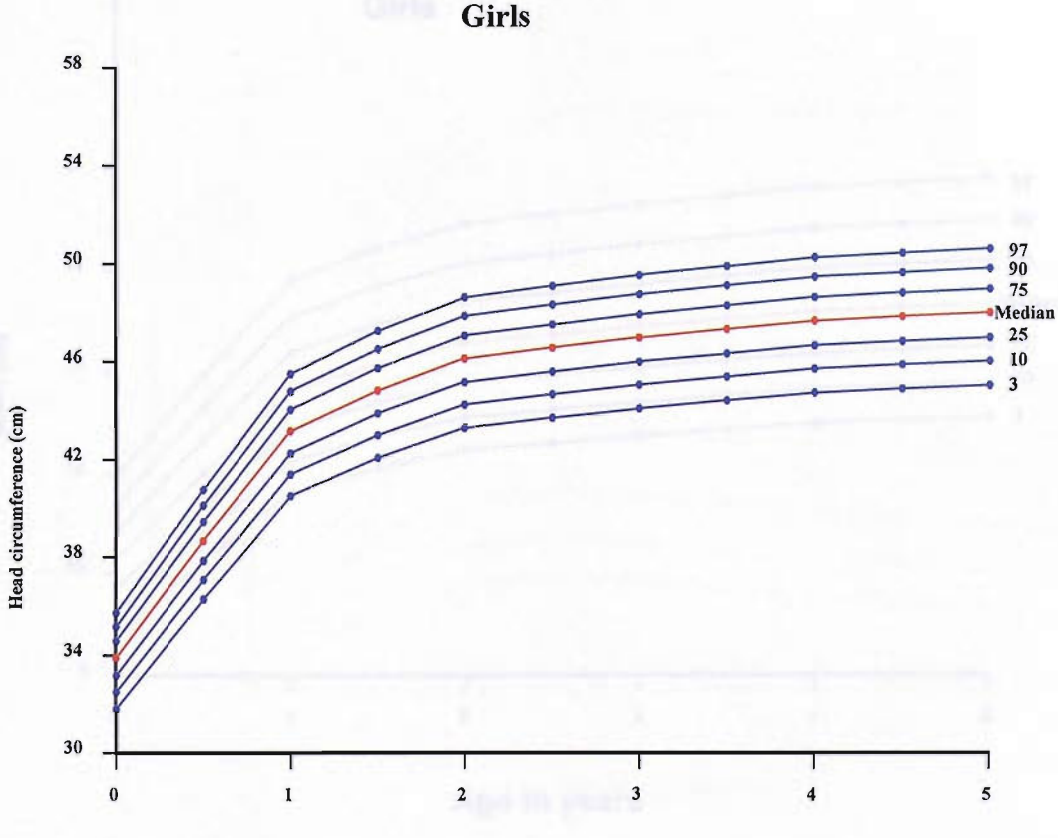


LENGTH/HEIGHT



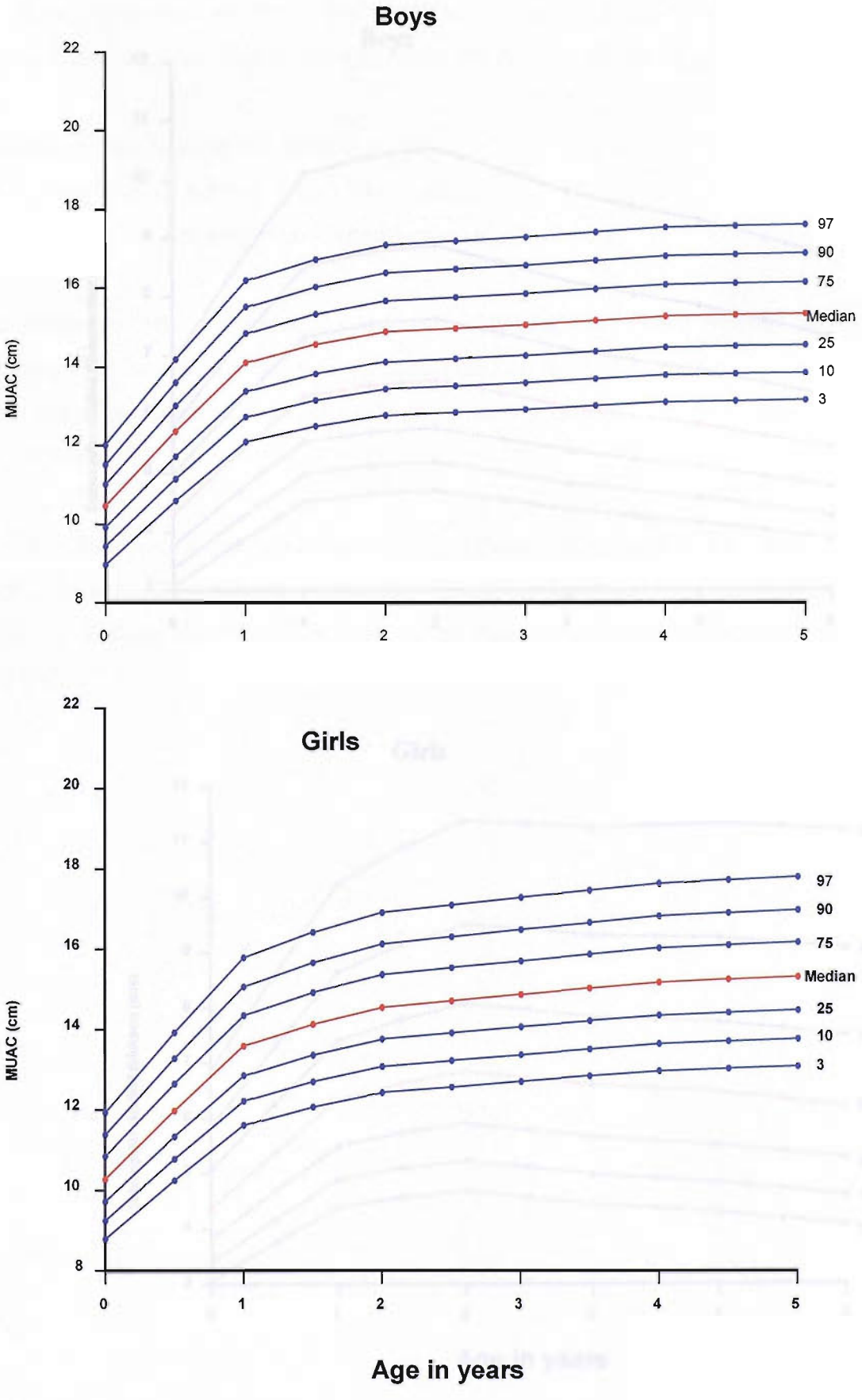
HEAD CIRCUMFERENCE





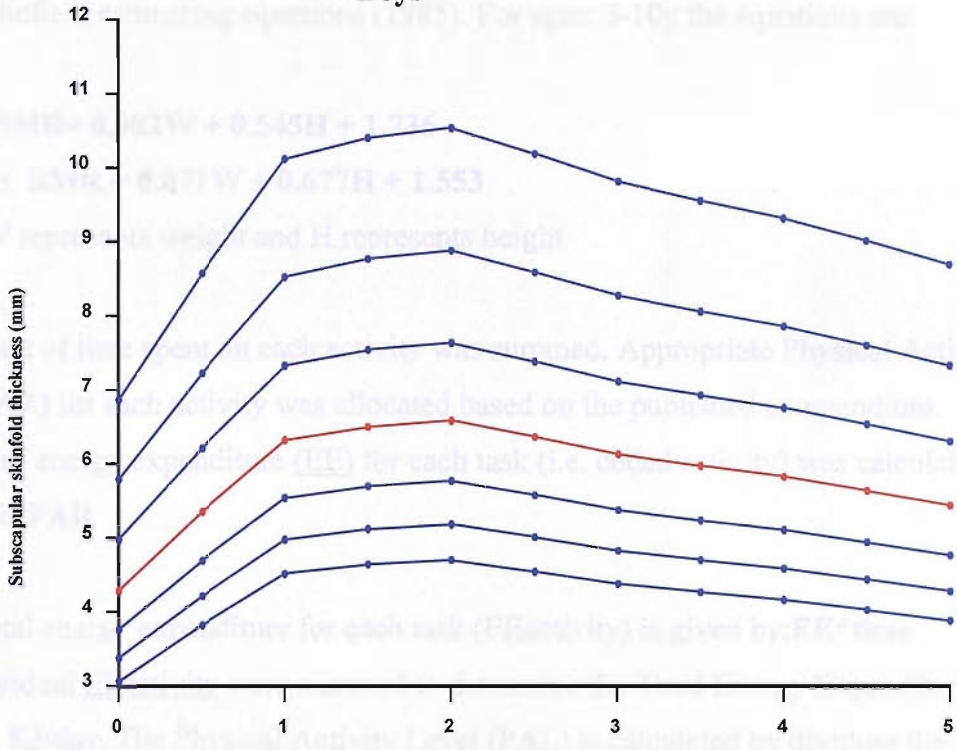
Age in years

ARM CIRCUMFERENCE

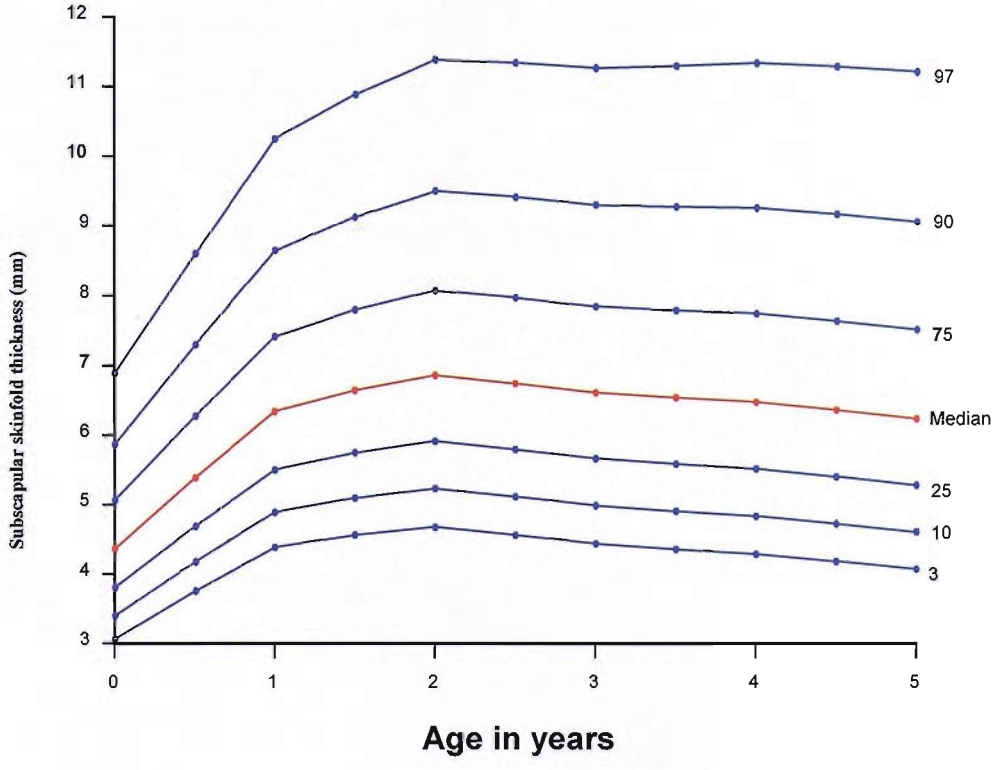


SUBSCAPULAR SKINFOLD

Boys



Girls



C. Factorial method to calculate total energy expenditure (TEE) using parental log

1. Basal Metabolic Rate (BMR) BMR (MJ/day) is estimated from weight and height using Schofield estimating equations (1985). For ages: 3-10y the equations are:

Males: BMR= 0.082W + 0.545H + 1.736
Females: BMR = 0.071W + 0.677H + 1.553
Where W represents weight and H represents height

2. Amount of time spent on each activity was summed. Appropriate Physical Activity Ratio (PAR) for each activity was allocated based on the published compendium. The rate of energy expenditure (EE) for each task (i.e. coded activity) was calculated by: **BMR*PAR**

3. The total energy expenditure for each task (EEactivity) is given by:**EE*time**
The individual EEactivity were summed to determine the Total Energy Expenditure (TEE) in KJ/day. The Physical Activity Level (PAL) is calculated by dividing the TEE by BMR.

D. An example to show calculation of TEE and PAL using logs, and predicted BMR

Child 1	Activity code	Time (min)	PAR (Published compendium)	BMR (kJ/m)	BMR (kJ/d)	EE/m (PAR*BMR)	EEactivity (EE*time)
		1	15	1	2.589861111	3729.4	2.5898611	38.84792
		2	45	1.5	2.589861111		3.8847917	174.8156
		3	60	2.5	2.589861111		6.4746528	388.4792
		5	285	1.3	2.589861111		3.3668194	959.5435
		6	15	1.8	2.589861111		4.66175	69.92625
		7	75	1.8	2.589861111		4.66175	349.6313
		9	30	1.5	2.589861111		3.8847917	116.5438
		10	15	1.8	2.589861111		4.66175	69.92625
		12	15	2.5	2.589861111		6.4746528	97.11979
		13	15	1.6	2.589861111		4.1437778	62.15667
		15	15	1.8	2.589861111		4.66175	69.92625
		17	30	1	2.589861111		2.5898611	77.69583
		19	120	2.5	2.589861111		6.4746528	776.9583
		20	30	6	2.589861111		15.539167	466.175
		26	75	2	2.589861111		5.1797222	388.4792
	50,51		30	0	2.589861111		0	0
		99	15	0	2.589861111		0	0
		18	555	1	2.589861111		2.5898611	1437.373
Total			1440					
			840				**TEE (summed EEactivity)**	4106.225
							PAL {TEE/ BMR(kJ/d)}	1.101042

E. The following steps were followed to examine how the predictors of physical activity are associated concurrently with different intensity activities (sedentary, light, moderate and hard).

Three new variables were created:

- activity1=sedentary/1440(total minutes in a day)
- activity2={sedentary+light}/1440(total minutes in a day)
- activity3={sedentary+light+moderate}/1440(total minutes in a day)

New variables were log-transformed:
Logactivity(1,2 or 3)=log(activity/1-activity).
Hard activity is represented by **1-log(activity3)**.
Logged variables were used in separate regression models along with predictors. The results were interpreted as follows:

1. A statistically significant association between a given predictor and activity1, and no association with activity2 or 3 for the same predictor implies that there was an inverse association with other activities added in the latter models.

2. A statistically significant association with activity3 implies a statistically significant inverse association with hard activity, as it is given by **1-log(activity3).**