UNIVERSITY OF SOUTHAMPTON

GLUCOSE TOLERANCE AND INSULIN STATUS DURING PREGNANCY IN SOUTH INDIA: RELATIONSHIPS TO MATERNAL AND NEONATAL BODY COMPOSITION

An observational study

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Abstract

Faculty of Medicine - Doctor of Philosophy

Glucose tolerance and insulin status during pregnancy in South India: Relationships to maternal and neonatal body composition.

by Jacqueline Charlotte Hill

This study was designed to test the hypothesis, that women, whose growth is impaired in early life (as evidenced by short stature, small head circumference and/or low birthweight) and who become 'fat' as adults, are insulin resistant, become hyperglycaemic in pregnancy and give birth to fat, hyperinsulinaemic babies who are at increased risk of diabetes in adult life.

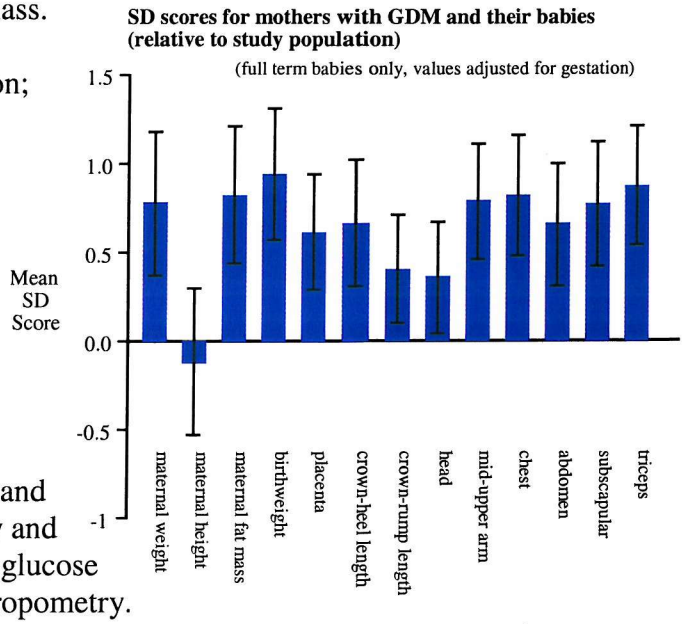
832 women recruited from ante-natal clinics at the Holdsworth Memorial Hospital (HMH), Mysore, South India underwent an oral glucose tolerance test (OGTT) and anthropometry at 30+/-2 weeks gestation. 676 went on to deliver their babies at HMH. Mean maternal weight was 55.2kg, height was 154.6cm and BMI was 23.1kg/m^2. The prevalence of gestational diabetes (GDM) (Carpenter and Coustan) was 6.1%. It was higher in older women ($p<0.001$) and fatter women ($p<0.001$) and had a U-shaped distribution with height ($p=0.05$) and head circumference ($p=0.05$). The highest blood glucose concentrations and measures of insulin resistance were in short, relatively fat women and in women with small head size who were also relatively fat.

Among 82 women with birth records available, those with GDM had been lighter and shorter with smaller head circumference at birth, although these findings were not statistically significant ($p=0.3$).

Overall, full-term babies in Mysore (urban India) were heavier (mean birthweight, 2956g) than those born in Pune (rural India) (2665g), but lighter than Southampton babies (3441g). Neonatal body composition was similar to that in Pune, with relative fat-sparing and decreased muscle mass.

Neonatal body composition was related to maternal body composition; fatter mothers had fatter babies, taller mothers had longer babies and mothers with smaller head size had babies with smaller heads.

Babies of mothers with GDM were significantly larger in all measurements, especially body fat (Figure). These 'macrosomic' changes were present across the range of 'normal' maternal glucose concentrations. Cord blood glucose and insulin concentrations were strongly and positively related to maternal blood glucose concentrations and to neonatal anthropometry.



In conclusion, GDM prevalence was high in this population. The highest glucose concentrations and insulin resistance indices were found in mothers with evidence of impaired growth in early life and who had become relatively fat as adults. 'Macrosomic' changes were seen across the range of 'normal' maternal glucose concentrations. These babies are being followed up annually to study the effects of maternal glycaemia on the child's growth and glucose/insulin metabolism.

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Abbreviations

AMA	Arm-muscle-area
AUGC	Area-under-the-glucose-curve
BMI	Body mass index
CHL	Crown-heel length
CRL	Crown-rump length
EDD	Estimated date of delivery
GDM	Gestational diabetes mellitus
HMH	Holdsworth Memorial Hospital
IDDM	Insulin dependent diabetes mellitus
IGT	Impaired glucose tolerance
IGTT	Intravenous glucose tolerance test
IOVS	Inter-/ Intra- observer variation studies
IUGR	Intra-uterine growth retardation
LBW	Low birthweight
LMP	Last menstrual period
MUAC	Mid-upper-arm circumference
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
PI	Ponderal Index
RIR-HOMA	Relative Insulin Resistance – Homeostatic Model Assessment
SS/TR	Ratio of subscapular skin-fold to triceps skin-fold thickness

Acknowledgements

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Tinkerbell, who supported and helped me in more ways than she knows. There are so many others whom it would be impossible to name but whom I wish to thank also. Professor CN Hales and his staff of the Department of Clinical Biochemistry, Addenbrookes Hospital, Cambridge analysed all our insulin and glucose samples and I am grateful to them for their help and advice. Dr Judith Darmady was the first to recognise the worth of this project and supported its funding from the Parthenon Trust, Switzerland.

I also wish to thank Eric for reminding me of what is important in life and for his support and encouragement during the writing up of this thesis. Lastly, I thank my parents whose faith and love have inspired mine. They have always been there for me, have encouraged and supported me in whatever I have done. I love them very much and could not have written this without them.

Authors Contribution

During my 18 months in India, I organised premises, employed and trained the fieldworkers and worked with them to recruit subjects. I carried out the clinical data collection supported by my colleague Dr. Krishnaveni.

On return to the UK, I carried out the analysis of the data myself with help and tuition from Ms Sam Kelligray. Dr Caroline Fall helped me to interpret the results and with her I wrote the publications listed overleaf.

Publications

GV Krishnaveni, JC Hill, CHD Fall, SD Kellingray. Maternal diabetes during pregnancy and infant growth. Published in the proceedings of the sixth annual international workshop on the fetal origins of adult disease, Mahabalipuram, India, 19th October 1999.

JC Hill, GV Krishnaveni, CHD Fall, SD Kellingray. Glucose tolerance and insulin status in pregnancy in South India: Relationships to maternal and neonatal body composition. Journal of Endocrinology March 2000 Volume 164 Suppl P 252.

1. Introduction

This thesis describes a study carried out as part of a programme of research into the maternal and fetal origins of adult type 2 (non-insulin dependent) diabetes (NIDDM) in India. My aim was to test the hypothesis, based on earlier studies in India, that women whose growth is impaired in early life are more likely to develop gestational diabetes (GDM), and that GDM increases the risk of type 2 diabetes in their offspring. I measured glucose tolerance during pregnancy in women living in the South Indian City of Mysore in order to:

- Define the relationship of the mother's size at birth, height growth in childhood and adult anthropometry to her glucose metabolism in pregnancy.
- Define the relationship of the mother's glucose and insulin concentrations in pregnancy to the size and body composition of her baby at birth.
- To create a cohort of babies who could be followed up through childhood, to study the effects of maternal glucose intolerance in pregnancy on the child's risk of developing abnormal glucose/insulin metabolism and ultimately type 2 diabetes.

1.1 Background

Type 2 diabetes mellitus is a disease generally thought to be of a genetic aetiology, in which resistance to the peripheral actions of insulin, in addition to defects in insulin secretion, leads to loss of glycaemic control. Hyperglycaemia can be present for many years before type 2 diabetes develops. The disease is often asymptomatic at the time of diagnosis but is associated with major complications such as blindness, renal insufficiency and cardiovascular disease including hypertension and myocardial infarction. It is widespread throughout the world, affecting male and female alike, with a prevalence of 3-5% in white Caucasians but up to 50% in specific populations like the Nauruans or the Pima Indians.[1]

Two processes are involved in the pathology of type 2 diabetes. The first, insulin resistance or decreased insulin sensitivity can be demonstrated by a reduced uptake of glucose in response to infused insulin.[2] This occurs in both major sites of insulin action, the liver, where insulin inhibits glucose production, and skeletal muscle, where insulin increases blood flow and stimulates glucose uptake.[3] Insulin resistance leads to persistently elevated insulin concentrations, even when fasted, due to compensatory pancreatic hypersecretion.

The second process, insulin deficiency or decreased insulin secretion can be shown by a reduced first phase insulin response in an intravenous glucose tolerance test (GTT),[4,5] a low 30-minute insulin response in an oral GTT, or a high plasma concentration of inactive insulin precursors, such as intact and 32,33-split proinsulin.[6,7] Whether insulin resistance or deficiency is the primary defect in type 2 diabetes is controversial. The general view however, is that insulin resistance comes first, and that diabetes occurs when the chronically over-stimulated β-cells become 'exhausted' and insulin secretion fails to match demand.[8]

The causes and exact nature of the defects responsible for producing insulin resistance and poor insulin secretion remain largely unknown.[3] Environmental factors, such as obesity and physical inactivity exacerbate insulin resistance, probably explaining the link between these factors and type 2 diabetes itself.[3, 9] Clinical studies have shown that both acutely and with regular training, exercise increases insulin sensitivity and glucose tolerance.[10, 11] Many studies, mainly in developed countries, have shown that the prevalence of type 2 diabetes is higher in more sedentary people and people with the disease are less active than those without.[12-15] A randomised control study of men and women with IGT in Da Qing [16] found that after six years of follow-up, the cumulative incidence of type 2 diabetes was 68% among controls and 40-50% among men and women who had been exercising regularly. The reduction in incidence of type 2 diabetes seen with exercise was similar to that seen in those who modified their diet and there did not seem to be any added benefit of combining both diet and exercise.

Himsworth [17] showed reduced mortality and hospital admissions for type 2 diabetes in the UK during periods of wartime food rationing when calorie intakes decreased.

Differences in fat but not carbohydrate intakes, have been shown to correspond well with population differences in the prevalence of type 2 diabetes.[18] However, prospective studies looking for dietary determinants of diabetes have been disappointing and several prospective dietary studies have failed to show a link between carbohydrate or fat intakes and incidence of diabetes.[19-22] These studies have been confined to developed countries. Recenty, Boucher [23] highlighted an association between vitamin D deficiency, impaired insulin secretion and diabetes but more data is required on dietary micronutrient quality and risk of diabetes.

Neel proposed a genetic predisposition to abnormal insulin secretion: the 'thrifty genotype hypothesis'. He suggested that genes associated with the abnormal insulin responses seen in diabetes may have conferred a survival advantage in conditions of limited or erratic food supply in the past, but lead to decompensation and disease in modern conditions of plentiful food and reduced physical activity.[24, 25] Evidence for a genetic aetiology comes from; twin studies, which show a higher concordance rate among monozygous than dizygous twins,[26] familial studies, where the risk of developing type 2 diabetes is increased in first-degree relatives of diabetic patients [27,28] and from ethnic groups with exceptionally high rates of type 2 diabetes, such as the Micronesian islanders, in whom, the excess of type 2 diabetes is reduced in families where there has been admixture with people from populations with lower rates of disease.[29]

Type 2 diabetes also occurs in association with monogenetic defects in β-cell function. These defects were formally grouped together and termed maturity-onset diabetes of the young (MODY). Although there were various types of MODY, they were all characterised by impaired insulin secretion with minimal or no defects in insulin action.[30] Specific genetic defects in insulin action are more unusual and include mutations of the insulin receptor sometimes seen in association with polycystic ovarian syndrome.[31] However, despite these associations, genetics alone cannot account for the majority of cases of type 2 diabetes.[32-34]

People with the greatest risk of developing type 2 diabetes come from developing countries, minority groups and disadvantaged communities in industrialised countries.[1] The increased risk is thought to be partly due to alterations in traditional modes of life and behavioural patterns, mainly in response to urbanisation, industrialisation and changes in socio-economic profile, leading to a move away from poverty to relative affluence with its associated decrease in physical activity, increased dietary intake and resulting obesity.

In India, the effects of rapid urbanisation have resulted in a rising prevalence of type 2 diabetes.[35, 36] The high rates of type 2 diabetes seen in people from the Indian subcontinent were first demonstrated in those who had migrated from India and Pakistan to other countries.[37] In Britain, their diabetes prevalence is up to five times that of the white indigenous population.[38, 39] In a large multi-centre study in 1975, a low prevalence of type 2 diabetes was reported in India [40] and low rates of the disease persist in rural areas.[41] However, recent studies have shown high rates, comparable to those found in the studies of migrants, in Indian cities [41-43] and in a recent survey in Chennai, 12% of adults had diabetes,[44] a 40% rise in prevalence in 6 years. Most of the studies which have assess the prevalence of type 2 diabetes in India have used oral glucose tolerance tests and World Health Organisation (WHO) criteria.[45] Those studies which have used questionnaire methods alone, have found lower prevalence rates (Table 1.1), illustrating the fact that type 2 diabetes is often under-reported and can remain asymptomatic for many years.

Indians are characteristically insulin resistant and have been found to be centrally obese.[39] McKeigue [50] suggested a variation on Neel's hypothesis i.e. that there is a gene for central obesity which may have been selected for under conditions of unreliable food supply and high physical activity levels, but which leads to insulin resistance and type 2 diabetes in the modern, urban situation of plentiful food and low levels of physical activity.

Table 1.1: Prevalence of type 2 diabetes in India

WHO = World Health Organisation

* reported as age-adjusted prevalence rates

Reference	Place	Rural/ Urban	N	Sex	Age	Criteria for diagnosis	% NIDDM
Ramachandran 1992 [41]	Chennai						
		Urban	900	M&F	>20	WHO	8.2*
		Rural	1038	M&F	>20	WHO	2.4*
1999 [36]		Peri-urban	1637	M&F	>20	WHO	5.9*
1997 [44]		Urban	2183	M&F	>20	WHO	11.6*
Zargar A.H. 2000 [46]	Kashmir						
		Urban	1038	M&F	>40	WHO	5.2
		Rural	4045	M&F	>40	WHO	4.0
		Urban	1098	M&F	>40	Questionnaire	2.2
		Rural	4993	M&F	>40	Questionnaire	1.8
Singh R.B. 1999 [47]	Moradabad Trivandrum Calcutta Nagpur Bombay	Urban	3257	F	25-64	Questionnaire	2.6
Singh R.B. 1998 [48]	Moradabad						
		Urban	1806	M&F	25-64	WHO	6.0
		Rural	1769	M&F	25-64	WHO	2.9
Fall C.H.D. 1998 [49]	Mysore						
		Urban	506	M&F	39-60	WHO	15.0

1.2 The fetal origins of type 2 diabetes

Recent evidence suggests two environmental factors, which by acting during intra-uterine life, predispose the individual to type 2 diabetes:

1. Fetal growth retardation
2. Maternal gestational diabetes

1.2.1 Fetal growth retardation

Early clues to the possible importance of pre-natal growth in determining adult disease came from geographical studies, undertaken in an attempt to gain a better understanding of the aetiology of coronary heart disease. These studies showed that differences in death rates for cardiovascular disease in different areas of England and Wales were closely related to differences in neonatal mortality.[51, 52] As the majority of neonatal deaths are associated with low birthweight (LBW), the findings suggested that cardiovascular disease in adulthood could be linked to poor fetal growth. Systematic searches of archives and hospital record departments were then carried out to find birth records of subjects in middle and old age and a series of longitudinal studies undertaken in which birth measurements were related to cardiovascular disease, type 2 diabetes and insulin resistance.

The first set of records identified was from Hertfordshire, UK. Every baby born in that county from 1911 onwards had been weighed at birth and again at one year of age. Among 370 men born in Hertfordshire during 1920-30, the prevalence of impaired glucose tolerance (IGT) (plasma glucose 7.8-11.0 mmol/l at 2 hours) and type 2 diabetes (plasma glucose $\geq$ 11.1 mmol/l at 2 hours) fell progressively with increasing birthweight and weight at one year of age.[53] The relative risk of IGT or type 2 diabetes was six times higher in those who weighed 5.5 lb (2.5 kg) or less at birth compared with those who weighed more than 9.5 lb (4.3 kg) (Table 1.2).

Table 1.2: Percentages of men aged 64 years with impaired glucose tolerance (IGT: 2 h glucose 7.8 - 11.0 mmol/l) or diabetes (NIDDM: 2 h glucose ≥11.1mmol/l) according to birthweight.

*1 lb = 454g

Birthweight (lb)	N	% Men with 2 h glucose of			Odds ratio for IGT/NIDDM adjusted for body mass index (95% confidence interval)
		7.8-11.0	≥11.1	≥7.8	
< 5.5	20	30	10	40	6.6 (1.5 – 28)
5.5 - 6.5	47	21	13	34	4.8 (1.3 –17)
6.6 - 7.5	104	25	6	31	4.6 (1.4 – 16)
7.6 - 8.5	117	15	7	22	2.6 (0.8 - 8.9)
8.6 - 9.5	54	4	9	13	1.4 (0.3 - 5.6)
> 9.5	28	14	0	14	1.0 -
All	370	18	7	25	**p value for trend < 0.001**

The prevalence of IGT and diabetes in men with birthweights ≤2.5 kg was 40% compared to 14% in those with birthweights >4.3 kg. The associations between weight at birth and glucose tolerance 60 years later were independent of the subjects' current body mass index (BMI), and were seen in each social class. Of men whose birthweights were below the median and whose body mass indices were above the median, 41% had IGT or diabetes while only 6% of men who were above the median for birthweight and below the median for BMI were affected. These findings suggest that good fetal growth may protect against the effect of higher BMI in adult life, while low BMI may protect against the deleterious effects of reduced early growth.

The same findings were seen in Preston, Lancashire, UK, where 140 men and 126 women aged between 46 and 54 years were studied.[54] The importance of this study was that gestational age and more detailed birth measurements; length, head circumference and placental weight were available. The prevalence of IGT and type 2

diabetes fell from 27% in those who weighed ≤2.5 kg at birth, to 6% in those of ≥3.41 kg. This trend was statistically significant and remained so after allowing for current BMI. In addition, low ponderal index (birthweight/length^3, a measure of fatness) at birth and a high head circumference-to-length ratio were independently associated with raised 2-hour plasma glucose and insulin concentrations, and individuals with IGT and type 2 diabetes had a high ratio of placental weight to birthweight. This study not only confirmed the findings of the Hertfordshire study, it demonstrated that the association between fetal growth and IGT existed in both men and women and was independent of gestational age and therefore not due to prematurity. The association of particular patterns of fetal growth, thinness and shortness at birth, with increased risk of IGT in adult life suggested that it was not simply retarded growth that was important but disproportionate growth.

These findings from Hertfordshire and Preston led to the fetal origins ('thrifty phenotype') hypothesis,[55] which proposed that adaptations made by the fetus in response to undernutrition, lead to persisting changes in metabolism and organ structure (including blood vessels, pancreas, liver and lungs), which lead to disease in adult life. It is thought that type 2 diabetes is 'programmed' in-utero; the principle being that a stimulus or insult at a critical period of development has lasting or lifelong significance.[56] 'Programmed' changes which occur in-utero, may then be magnified by environmental factors in postnatal life, which amplify the expression of adult disease.

An early criticism of the work in adults was that the observed association could be due to unknown confounding factors occurring at some point during childhood or adult life. This led to studies of glucose tolerance and insulin concentrations in children.[57-60] Results from these studies show that the link between events in-utero, and insulin resistance and type 2 diabetes in adult life can be observed from an early age, and therefore the associations between fetal growth and adult disease do not simply reflect the confounding influence of adverse environmental factors during childhood and adult life.

Another alternative explanation for these findings is the 'fetal insulin hypothesis' as proposed by Hattersley.[61] This suggests that insulin-regulated fetal growth and hence birthweight, is determined by fetal insulin secretion and insulin action, which are in turn regulated by fetal genotype. Genetic factors, which alter both fetal and adult insulin secretion and/or insulin action could explain the observed association between low birth weight and adult glucose intolerance. However, the genetic factors, which have been identified, can account for only a very small proportion of cases of type 2 diabetes.

A number of other studies in Europe[62] and in the USA[63] have confirmed the associations between reduced fetal growth, thinness at birth and type 2 diabetes. However, the mechanisms linking reduced fetal growth with diabetes in adult life are not fully understood. Insulin resistance is an early metabolic defect, which predicts the disease, but reduced pancreatic β-cell function is also characteristic of diabetes. It is currently uncertain whether the diabetogenic effect of reduced fetal growth is a result of poor development of the pancreas – especially the β-cells, which in later life are not able to compensate adequately for insulin resistance – or whether reduced early growth could predispose to insulin resistance per se.

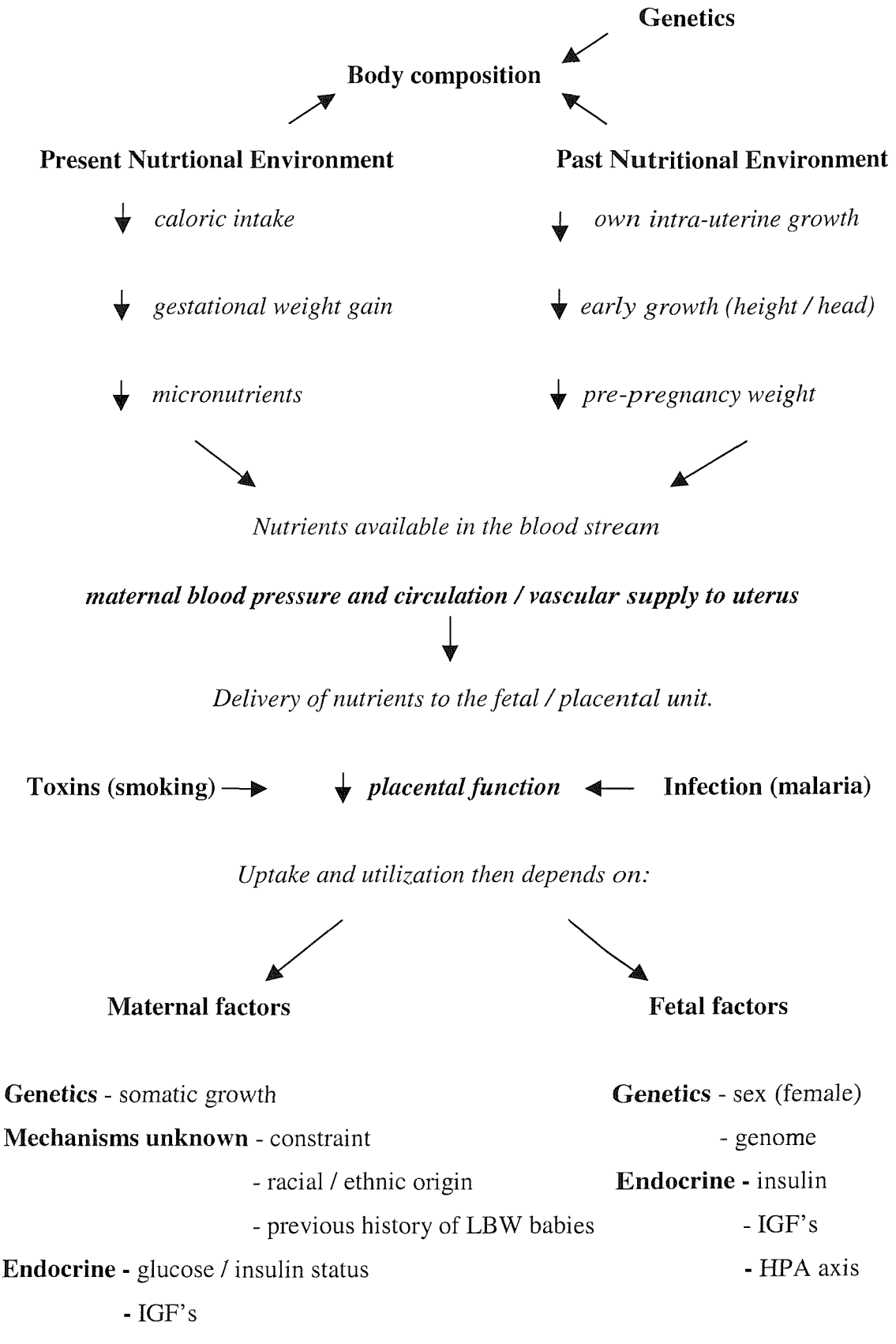
That insulin resistance plays an important role in the link between LBW and diabetes is demonstrated by the fact that LBW is associated with a higher prevalence of the metabolic syndrome (the coexistence of raised blood pressure, glucose intolerance and dyslipidaemia), which is known to be associated with insulin resistance. In the Hertfordshire study, the percentage of men with the metabolic syndrome fell progressively from 30% in those whose birthweight was ≤5.5lb to 6% in those who weighed ≥9.5lb.[64] A similar relationship was demonstrated in Preston and in a study of 30-year-old Mexican-Americans and non-Hispanic whites in San Antonio, Texas.[63] Studies were carried out to determine whether there was an association between size at birth and insulin resistance in the adult. In the Preston study, men and women who were thin at birth (low ponderal index) were insulin resistant in adult life and subjects who were thin at birth but obese as adults were the most resistant to insulin.[65] These findings were confirmed in four other populations in Europe,[66, 67] the USA[63] and in India,[49] implying that insulin resistance originates through impaired development in fetal life.

Undernourishment of the fetus may also be responsible for abnormalities of insulin secretion, as reduced early growth is also related to a raised 32,33-split proinsulin, which can be interpreted as a sign of pancreatic β-cell dysfunction [53] but may simply reflect insulin resistance. No relationships with reduced early growth have been shown with the first phase insulin response or with proxies for it, such as the insulin increment.[68]

Many factors are thought to be responsible for LBW. These include a poor maternal diet, poor nutritional reserves in the mother, inadequate uterine blood flow, or defects in the passage of nutrients across the placenta. It is difficult to assess which of these exert independent causal effects and the magnitude of the effect. Kramer [69] undertook a critical assessment and meta-analysis of the determinants of LBW, excluding those due to chronic maternal illness or pregnancy complications (Figure 1.1).

It is known that maternal nutrition plays an important role in fetal development, and that malnutrition during pregnancy leads to intrauterine growth retardation and microsomia in humans as well as in experimental animals. This is thought to be due to decreased availability of nutrients for placental transport and decreased placental blood flow, resulting in a decreased nutrient supply to the fetus.[70] Since adequate uterine blood flow depends, to some extent, on maternal haemodynamics, systolic and diastolic blood pressure or maternal plasma volume might be expected to have an association with birthweight. Until recently and with the exception of women who develop pre-eclampsia or severe hypertension, no association had been found between maternal blood pressure during pregnancy and the birthweight of the baby.[71, 72] Churchill et al.[73] used ambulatory blood pressure measurements and showed a continuous inverse association between birthweight and maternal blood pressure, throughout the range seen in normal pregnancy. Kramer however, felt that demonstration of the effects of maternal blood pressure on fetal growth should be based on measurements made prior to pregnancy to avoid confusing a determinant of body weight with an intermediate outcome of pregnancy, such as pregnancy induced hypertension. No studies in his meta-analysis met these criteria.

Fig.1.1: **Determinants of low birthweight**

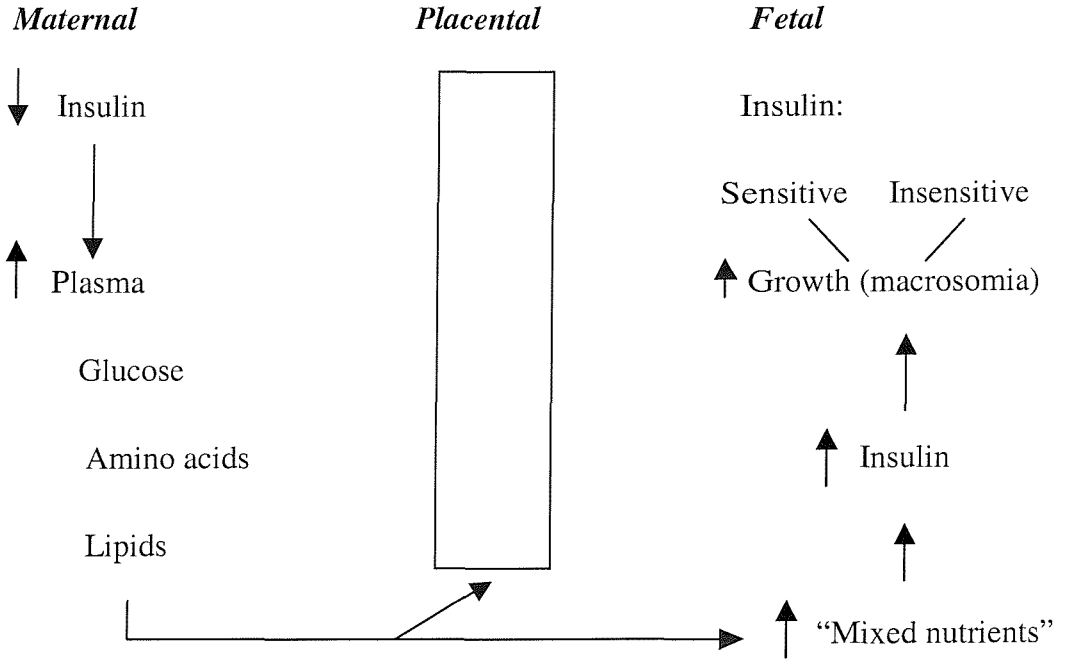


The long-term effects of fetal undernutrition have been demonstrated in experimental animals where maternal malnutrition during pregnancy, whether qualitative (protein deficiency) or quantitative (50% restriction) leads to offspring with impaired glucose tolerance,[74, 75] suggesting that fetal undernutrition can cause insulin resistance. There is also evidence, from both humans and animals, that developing fetal pancreatic β-cells are vulnerable to poor nutrition in-utero. Growth retarded neonates have been shown to have reduced numbers of β-cells and reduced insulin secretion.[76] This reduction in islet cell function is thought to be due not only to changes in the β-cells but also to abnormal development of the more complex aspects of islet cell structure and function, such as vasculature and innervation.[77] The implication being that a low-protein diet during gestation, transmits signals to the intra-uterine milieu which impair the normal maturation of two major cell types, the β-cell with its growth-promoting hormone (insulin) and the endothelial cell.

1.2.2 Maternal gestational diabetes

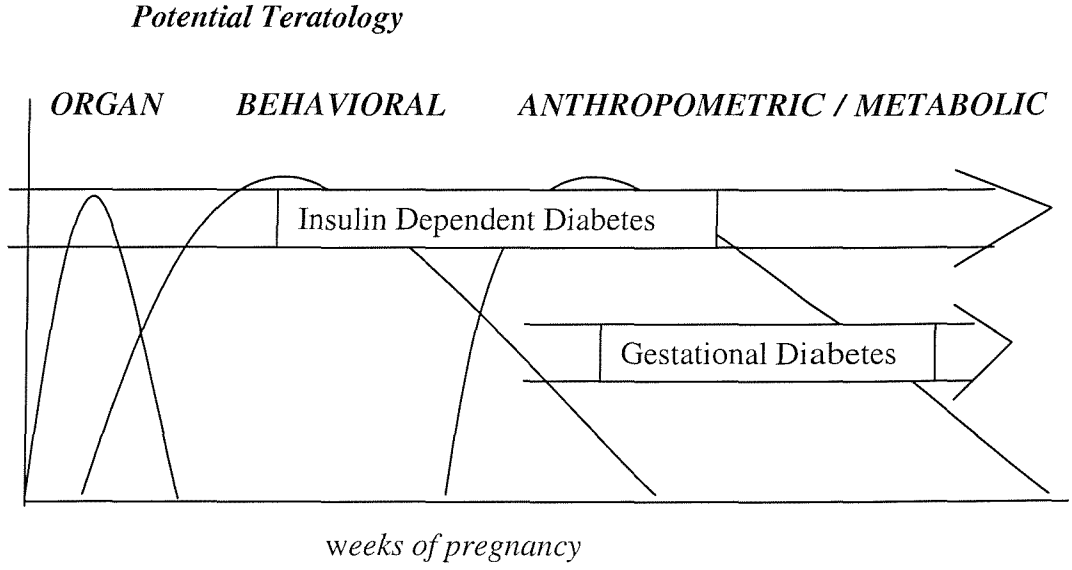
Freinkel, in his 1980 Banting Lecture [78] modified Pederson's classical "hyperglycaemia-hyperinsulinism" hypothesis. According to the original hypothesis, diminished maternal insulin causes diminished glucose utilization in the mother. The resultant rise in maternal glucose effects a rise in fetal glucose. The latter stimulates fetal insulin and greater growth. Freinkel suggested that in diabetic pregnancies, maternal fuels (lipids and aminoacids) along with elevated glucose concentrations, reach the fetus and stimulate β-cell development and secretion. As a consequence, fetal fuels are consumed more intensively, resulting in near normal glycaemia and low aminoacid levels, increased anabolism of fetal tissues and finally macrosomia (Fig.1.2).

Fig.1.2: Fetal development according to the modified Pedersen hypothesis



In the human fetus and newborn, islet hypertrophy and β-cell hyperplasia have long been recognised as features of a diabetic pregnancy and can be observed as early as 19 weeks gestation.[79] From 32 weeks onwards, a positive correlation is present between the degree of islet and β-cell hyperplasia, maternal glycaemia and body weight.[80] It is well known that babies born to diabetic mothers are macrosomic and hyperinsulinaemic [81] but the long-term effects of these adaptations on the fetus have not been well studied. Freinkel, in the same lecture, postulated that long-term anatomical and functional changes may occur in the fetus exposed to altered maternal fuels during pregnancy. This he termed 'Fuel-mediated teratogenesis' (Fig.1.3).

Fig.1.3: Freinkel's hypothesis of fuel-mediated teratogenesis



In studies using experimental animals, hyperglycaemia in the mother in late pregnancy produces offspring which appear as normal healthy animals in adult life with normal body weight, normal basal glucose and insulin levels and morphologically normal endocrine pancreas,[82, 83] but when stressed, are glucose intolerant. The effect on the offspring differs depending on whether the maternal hyperglycaemia experienced by the fetus was mild or severe. Mild maternal hyperglycaemia resulting in fetal

hyperinsulinaemia induces an increase in amino acid turnover which is maintained in adulthood, and the β-cell response to glucose stimulation is deficient, while the insulin receptor system appears to be unaffected.[83-85] However, when the maternal hyperglycaemia is severe and results in fetal hypoinsulinaemia, the adult offspring not only displays a deregulation of the stimulated insulin output, but also insulin resistance in the liver and the peripheral tissues.[84, 86]

Pregnancy itself is an insulin resistant state and is responsible for considerable stress on the glucose and insulin metabolism of the mother, implying a need for adaptation of the maternal endocrine pancreas at both the structural and functional level. In rats, the mass of islet tissue doubles in pregnancy and the activity of the β-cells are enhanced.[87] When offspring of diabetic or malnourished dams with IGT become pregnant, they develop gestational diabetes (GDM),[83, 88, 89] and their offspring (also developing in an abnormal intra-uterine milieu) display the typical features of offspring from mildly diabetic mothers. When they become adult they develop IGT and GDM.[83, 88] A diabetogenic tendency is thereby transmitted from one generation to another, without any genetic involvement, but as a result of the fact that the fetus developed in an abnormal intra-uterine environment.

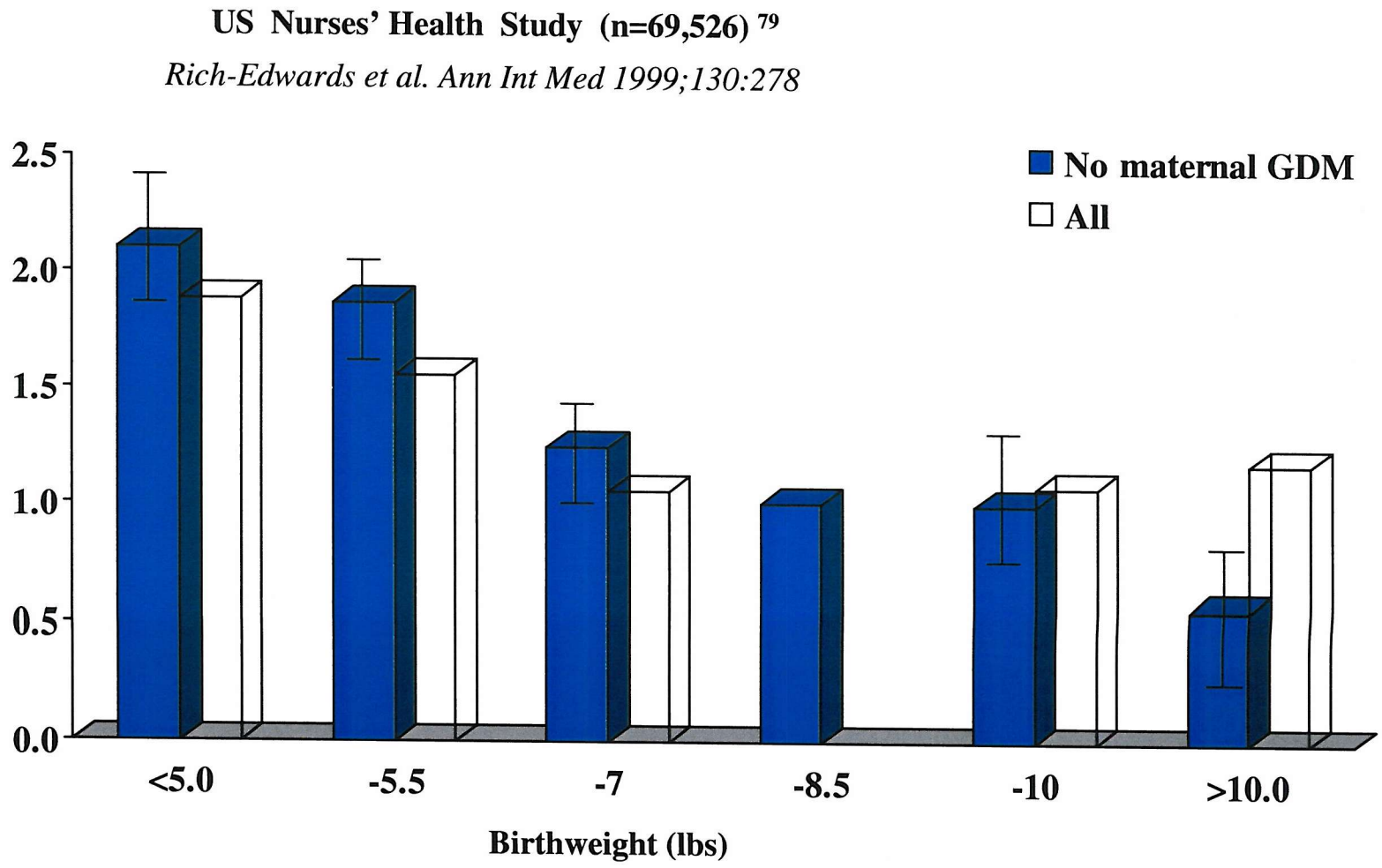
In the human, there have been a number of epidemiological studies which, have pointed to the importance of maternal GDM for the development of type 2 diabetes in the offspring. A higher incidence of type 2 diabetes and of GDM is reported in children from diabetic mothers than from diabetic fathers,[90] and a higher incidence of diabetes is present in offspring from diabetic great-grandmothers via the maternal than via the paternal line.[91] Systematic treatment of diabetic mothers during pregnancy may result in a decreased incidence of diabetes in their children.[92] However, the most convincing data on the intra-uterine transmission of the diabetogenic tendency in the human derive from studies of Pettitt and co-workers on the Pima Indians of North America. In this population, diabetes is very prevalent and is commonly seen during pregnancy. The studies show that the diabetic intra-uterine environment can induce a diabetogenic tendency in the offspring. IGT is more frequent in young adults whose mothers were diabetic during pregnancy compared to young adults whose mothers developed diabetes after pregnancy: 33% versus 1.4% at ages of 15-19 years.[93]

These data indicate that the genetic predisposition, a factor for offspring of both diabetic and pre-diabetic women, is a less important determinant of type 2 diabetes in this population than is the intra-uterine environment. This finding is reinforced by the much smaller effect of paternal than of maternal diabetes on the prevalence of diabetes in the offspring.[93] A study by Silverman [94] confirmed that these findings were not unique to the Pima Indians. Silverman studied a racially mixed group of children of diabetic mothers. He followed them from birth and compared them with 10-16 year-old controls. He showed that almost 20% of the offspring of diabetic women had IGT by age 10-16 years i.e. eight times the rate in 10-16 year old children whose mothers did not have diabetes during pregnancy.

In the Pima Indian population, the association between birthweight and the future development of type 2 diabetes is 'U'-shaped,[95] i.e. a high prevalence of IGT and type 2 diabetes was associated with both the lowest and highest birthweights. Age-adjusted prevalence of diabetes at birthweights under 2.5 kg was 30%, while at birthweights of 3.5 - 4.49 kg it dropped to 17%, increasing to 32% at birthweights $\geq$4.5 kg. The association between birthweights >4.5 kg and a raised prevalence of diabetes was not surprising in that previous work by Pettitt et al. had shown that maternal diabetes in pregnancy, which is known to be associated with macrosomia, can lead to increased prevalence of diabetes in successive generations.[93] By excluding from the analysis subjects whose mothers may have had GDM, the authors were able to demonstrate a significant reduction in the prevalence of diabetes in the high birthweight group.

A 'U'-shaped association, consistent with that seen in the Pima Indians, was demonstrated by Rich-Edwards in 69,526 women from the USA, Nurses Health Study.[96] In this study, LBW was associated with increased risk for type 2 diabetes. After adjusting for age, the relative risks suggested a 'U'-shaped (or reverse J-shape) association which was lost again after adjusting for maternal history of diabetes and BMI (Fig.1.4). The fact that a 'U'-shaped relationship has not been noted in the UK studies, is probably due to the fact that GDM is less prevalent and that survival of infants of diabetic pregnancy born more than 60 years ago is likely to have been poor. In such populations the effect can only be seen when the study sample is very large as in the USA, Nurses Health Study.

Fig.1.4: Relative risk of NIDDM



1.3 The fetal origins of type 2 diabetes in India - the Mysore study

India has a high prevalence of low birthweight babies. Approximately 30% are less than 2.5 kg and the mean full-term birthweight is 2.7 kg, almost 1 kg lower than in Western Europe.[97-99] With its high prevalence of type 2 diabetes, India became the obvious place to continue research into the fetal and maternal mechanisms involved in the development of type 2 diabetes.

In 1993, the MRC, Environmental Epidemiology Unit, conducted their first research project in the South Indian city of Mysore. An extensive search had discovered a Mission Hospital, the Holdsworth Memorial Hospital (HMH), where uniquely detailed records, similar to those found in Preston, UK, had been kept on each birth since 1934. The study set out to test the 'fetal origins hypothesis' in urban India by determining the prevalence of coronary heart disease and type 2 diabetes in 506 adults born at the hospital during 1938-53.[49]

Consistent with other studies in India,[42, 43] the prevalence of diabetes was high. In Mysore, rates of diabetes and IGT were twice those (15% and 19%) of a British population of similar age (6% and 12%),[100] despite the fact that the Mysore population was thinner. 44% of those over 50 years of age had abnormal glucose tolerance and as expected, diabetes was commoner in obese individuals and in those with greater central fat distribution. The insulin profile showed that Mysore men and women were extremely insulin resistant, even if they had normal glucose tolerance. Those with type 2 diabetes had low 30- and 120-minute insulin concentrations, suggesting that in addition to being insulin resistant, they were also insulin deficient.

Consistent with studies elsewhere,[54, 63, 65-67] insulin resistance was highest in those of LBW, especially if they became fat as adults, with high fasting insulin and 32,33-split proinsulin concentrations. Unlike these studies however, these individuals did not have the highest rates of diabetes. Rates of type 2 diabetes were highest in men and women who were short and fat at birth and whose mothers were heavy with large intercristal pelvic diameters (Table 1.3). In contrast to the insulin resistance of the LBW group, the insulin profile of these men and women showed that they were insulin deficient, with a low 30-minute response to the oral glucose load.

Table 1.3: Percentage of subjects with type 2 diabetes (NIDDM) according to their ponderal index and their mother's size.

PI , ponderal index

PI at birth (kg/m^3)	(n)	% NIDDM
≤ 23	(182)	11.0
- 27	(149)	15.4
> 27	(170)	18.9
ALL	(501)	15.0
p for trend ,		0.03

wt., weight

Mother's wt. (kg)	(n)	% NIDDM
≤ 43	(68)	10.3
- 49	(70)	12.9
> 49	(66)	24.2
ALL	(204)	15.7
p for trend,		0.008

ICD, intercristal diameter

Mother's ICD (cm)	(n)	% NIDDM
≤ 24	(75)	9.3
- 25	(84)	16.7
> 25	(121)	23.1
ALL	(280)	17.5
p for trend,		0.009

Maternal weight (a summary of height, fatness and lean body mass) predicted diabetes more strongly than the subjects' own size at birth or current BMI. The Mysore records did not contain direct measurements of height, fatness or lean body mass, but the pelvic diameters gave some indirect insight. In Mysore, they increased with maternal parity and with age, beyond the age of skeletal maturity. It was concluded that they were a measure of maternal subcutaneous fat as well as bony diameters and that the association between type 2 diabetes and maternal weight reflected a link with increased maternal adiposity. A possible link between maternal fatness, a high ponderal index at birth and subsequent insulin deficiency associated with type 2 diabetes is gestational diabetes. The following hypothesis was proposed by the investigators to explain their findings [49] and is illustrated further in Figure 1.5.

1.4 Hypothesis

A large proportion of women in India experience undernutrition during their own fetal life and in both childhood and adulthood. This is reflected in their low weight, short stature and small head circumference. As mothers, they remain undernourished during their pregnancy and subsequently give birth to small, undernourished babies who have a 'thrifty phenotype', and who will become insulin resistant if they become even mildly obese in adult life. With increasing urbanisation and industrialisation, many low birthweight female babies are becoming relatively obese women. This increase in fatness compounds their insulin resistance. When they become pregnant, their insulin resistance increases still further, requiring increasing insulin secretion from the pancreatic β-cells to counteract this resistance. When the β-cells are no longer able to match the demand, maternal hyperglycaemia results and ultimately gestational diabetes.

Maternal hyperglycaemia is responsible for fetal hyperglycaemia and hyperinsulinism which in turn causes increased fetal growth, especially of the soft tissues, "relative macrosomia", measured by a high ponderal index at birth. This abnormal intra-uterine milieu also alters the development of the fetal pancreas and predisposes the offspring to reduced insulin secretion and type 2 diabetes in adult life.

Studies that have looked at the prevalence of gestational diabetes (GDM) throughout the world have found a markedly increased risk in women of Asian origin.[101-105] Compared with white European women, Dornhorst [106] reported a higher prevalence of GDM in London of approximately 11-fold in women from the Indian subcontinent, eight-fold in Southeast Asian women and six- and three-fold in Arab/Mediterranean and Black/Afro-Caribbean women respectively. There is however, very little data from India itself on the prevalence of GDM.[107] Interestingly, Ramachandran has twice studied a South Indian urban population and found low rates of GDM.[108] Unlike type 2 diabetes where WHO criteria have been universally accepted for its diagnosis, GDM has no universally accepted criteria and its diagnosis remains a hugely controversial issue. Studies on GDM prevalence are difficult to compare because of these differences in diagnostic criteria (Table 1.4).[109]

Fig.1.5 **Hypothesis**

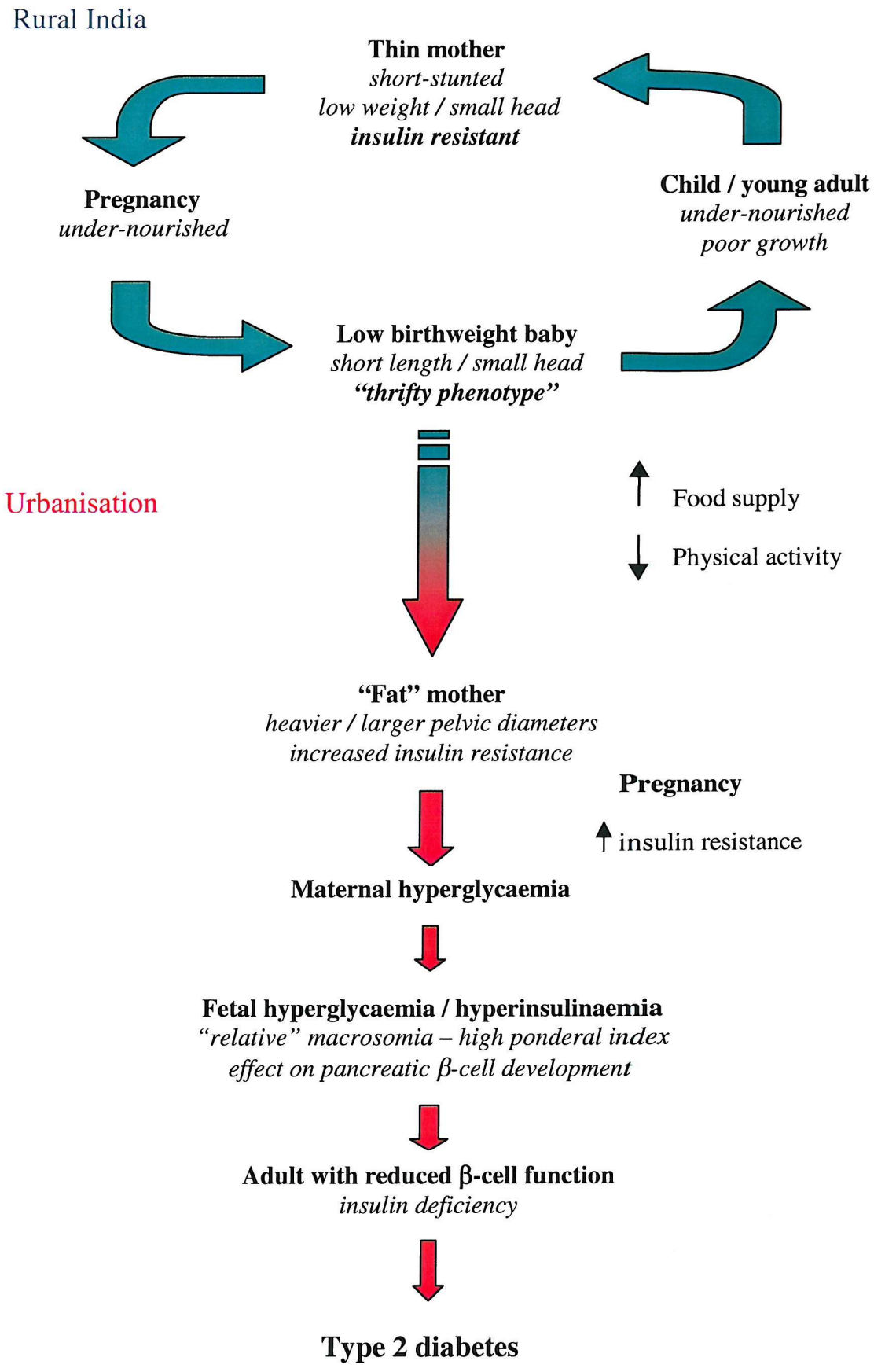


Table 1.4: Prevalence of GDM among Asians in India and Abroad

Reference	Country	N	Criteria for diagnosis	% GDM
Ramachandran A. 1998 [109]	India	1036	National Diabetes Data Group	0.9
Solomon C.G. 1997 [101]	USA	248	Questionnaire	10.5
Yue D.K. 1996 [105]	Australia	114	Australian Diabetes in Pregnancy Society	16.7
Koukkou E. 1995 [103]	UK	49	Diabetic Pregnancy Study Group (Lind)	5.8
Dornhorst A. 1992 [102]	UK	1218	Area-under-the-glucose-curve (Gilmer)	4.4
Samanta A. 1989 [104]	UK	314	World Health Organisation	0.2

As pregnancy itself may be viewed as diabetogenic, in that many pregnancy hormones, particularly human placental lactogen, antagonise the effects of insulin, any predisposition to the development of glucose intolerance in later life may be unmasked during pregnancy. It has been suggested that GDM and type 2 diabetes are the same disease, manifesting at different time points.[110] If this is true, factors responsible for an increased prevalence of type 2 diabetes in certain populations may also be responsible for an increased prevalence of GDM. The two most important risk factors for the development of GDM are also associated with insulin resistance, namely obesity and ethnicity. Both the incidence of GDM and the progression to diabetes post-partum is highest in those ethnic groups characterised by high insulin resistance, women from the Indian subcontinent, native American women and women of Hispanic origin being examples of this phenomenon.

It was proposed that the rise in type 2 diabetes in urban Indian populations was due to hyperglycaemia occurring during pregnancy in insulin-resistant mothers who had impaired nutrition and growth in early life and had become relatively obese and inactive in adult life. According to this model, women who are fatter will be more insulin resistant and hyperglycaemic during pregnancy, especially if they themselves

were undernourished in early life, indicated by their own low birthweight, short stature or small head circumference, and will deliver heavier, fatter, hyperinsulinaemic babies.

Maternal plasma glucose concentrations are known to correlate with maternal BMI and even mildly elevated glucose concentrations within the normal range are associated with increased birthweight, ponderal index and neonatal skinfold thickness.[111-117] These relationships have been shown to be continuous with no evidence of a threshold and seem to be stronger in Asian than in white Caucasian mothers.[115] The shortness and higher ponderal index at birth of men and women with type 2 diabetes in Mysore could be explained as an effect of maternal hyperglycaemia if it could be shown that:

1. Higher maternal weight and wider pelvic diameters indicate an increase in fat, rather than height or lean mass.

2. Maternal glucose concentrations are related to this increase in fat, so that the fatter the mother, the higher her blood glucose concentrations and the more likely she is to develop GDM.

3. Maternal hyperglycaemia is more likely if the mother's early growth was impaired leading to insulin resistance in addition to adult obesity.

4. Maternal glucose concentrations are related to the body composition of the baby, such that the higher the glucose levels, the bigger the baby, and that this increase in neonatal size follows a specific pattern whereby the soft tissues increase to a greater extent than the skeleton.

5. Maternal glucose concentrations are related to neonatal cord blood glucose and insulin concentrations, which in turn relate to neonatal size.

These five assumptions form the basis of this thesis. Showing them to be true would strengthen the original hypothesis: that women whose growth is impaired in early life (evidenced by short stature, small head circumference and/or low birthweight) have higher levels of insulin resistance if they become fat as adults. They then become hyperglycaemic in pregnancy and give birth to short, fat, hyperinsulinaemic babies.

In addition to the points above, the babies born to these mothers now form a cohort who are being followed annually throughout their childhood in order to look for evidence of abnormal glucose/insulin metabolism and ultimately the development of type 2 diabetes in adult life.

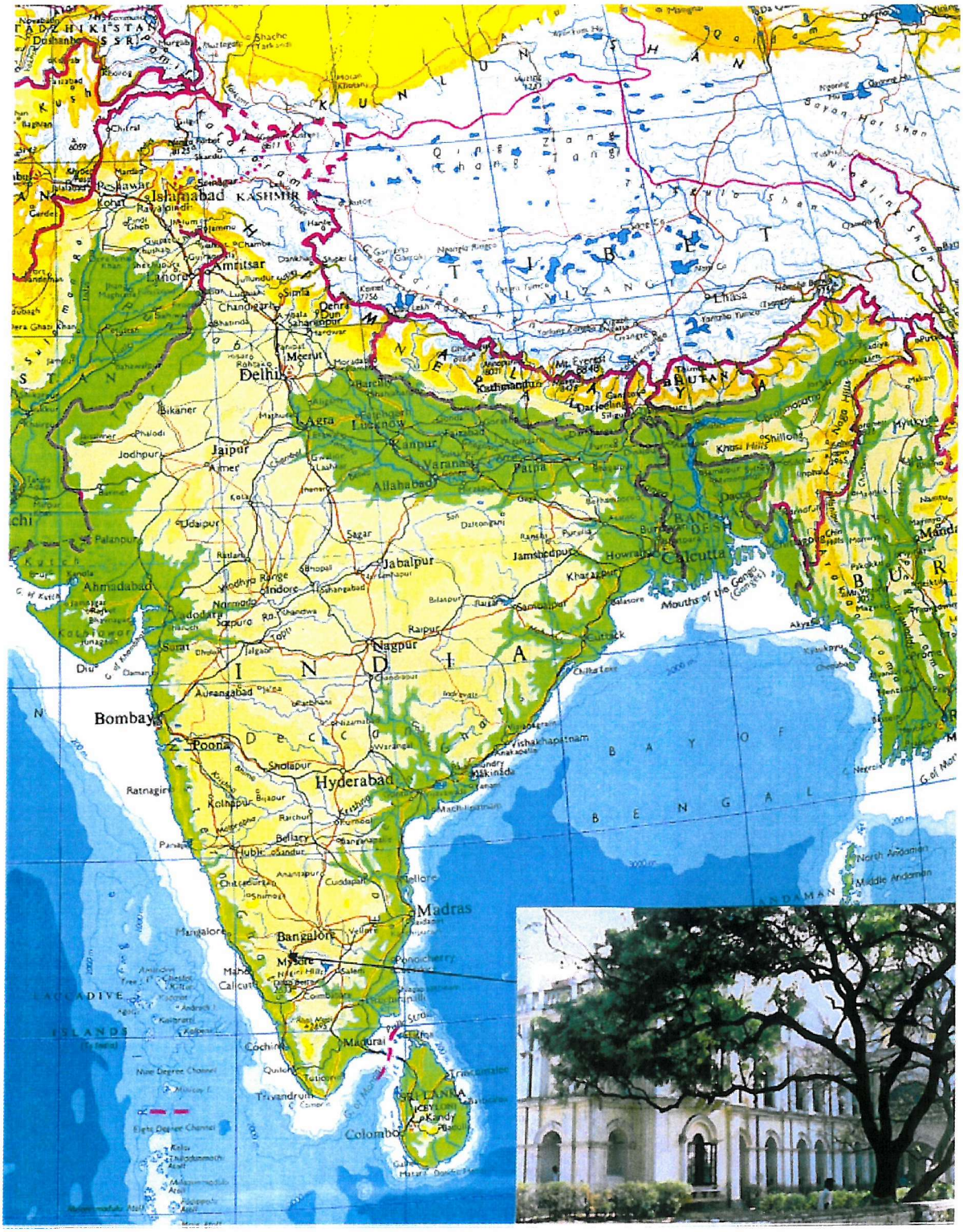


Fig. 2.1: Holdsworth Memorial Hospital (HMH), Mysore City, South India.

2. Methods

2.1 Setting

The Holdsworth Memorial Hospital (HMH) in Mysore, South India (Fig.2.1), is a mission hospital governed by the Church of South India. It was built as a maternity hospital in 1905 in a poor crowded area of the city and for the first half of the century was one of three main hospitals offering obstetric care to people from all socio-economic groups in Mysore. In 1993 it was chosen as a research base by the MRC for the study of the fetal origins of adult disease. More than three hundred other long-established hospitals throughout India were contacted and HMH was chosen over and above the others because detailed obstetric records had been kept routinely for all babies born in the hospital from 1934 onwards. These records contained the babies' birthweight, crown-heel length, head circumference and placental weight. In addition to the babies' measurements, they also contained the parents' names, occupations, address, religion or caste and the mother's age, parity and obstetric history along with her weight and external pelvic measurements i.e. the intercristal, external conjugate and interspinous diameters. Today there are close to eighty hospitals of varying sizes in Mysore, a city with a population of almost one million.

2.2 Study Population

HMH caters for approximately 20% of the hospital deliveries that take place in Mysore i.e. approximately 2,500 deliveries per year. 50% of mothers who deliver in the hospital will not have booked in the ante-natal clinics beforehand and will be seen at the hospital for the first time in labour. Many of these mothers live in villages on the outskirts of the city and travel long distances to the hospital.

It is traditional for women to deliver their first baby in their mother's village and in subsequent pregnancies to spend their seventh or eighth month there, thus creating an extremely mobile pregnant population. Despite these factors, our study population was mainly urban and mainly from Mysore.

HMH provides a wide range of medical services at a reasonable cost and operates a policy of subsidising the very poor. Other private hospitals in the city are more expensive and the government hospitals, although free of charge, provide a lower quality of service. Those attending HMH are accordingly from all socio-economic groups with the majority being from the middle and lower brackets. This distribution of social classes was reflected in the study population.

2.3 Data collection

The project team consisted of seven people, myself and six others who originated from Mysore: a local doctor, an experienced social worker (who had worked on the previous MRC research projects based at HMH), two nurses, a laboratory technician, and a data entry operator. Data was double entered from the questionnaires and data sheets on a daily basis using the software package 'FoxPro'. Monthly progress and feedback meetings helped the team to work together effectively.

2.4 Recruitment

2.4.1 Method

Women were recruited consecutively from the ante-natal clinics held at HMH. Recruitment began in June 1997 and ended in October 1998. Every woman of less than 32 weeks gestation, as determined by her last menstrual period (LMP) or first trimester ultrasound scan if the LMP was unknown, was approached and invited to take part in the study. A written explanation of the study, in Urdu, Kannada or English was also given to each woman and a 'screening' form completed, which included details such as name, address, age, religion, eligibility and willingness to participate in the study. This form was completed for 1,541 women.

2.4.2 Eligibility

Women were eligible for the study if they planned to deliver at HMH, had a singleton pregnancy and were less than 32 weeks gestation at the time of interview (Fig.2.2). Gestation was calculated from the last menstrual period (LMP), using an obstetric calculator, or from an early ultrasound scan, performed before 14 weeks gestation when the LMP was unknown or uncertain. Women whose LMP was unknown and who were clinically greater than 14 weeks gestation were excluded, as were women whose LMP was initially thought to be certain but for whom a later ultrasound scan adjusted the expected date of delivery (EDD) by more than three weeks.

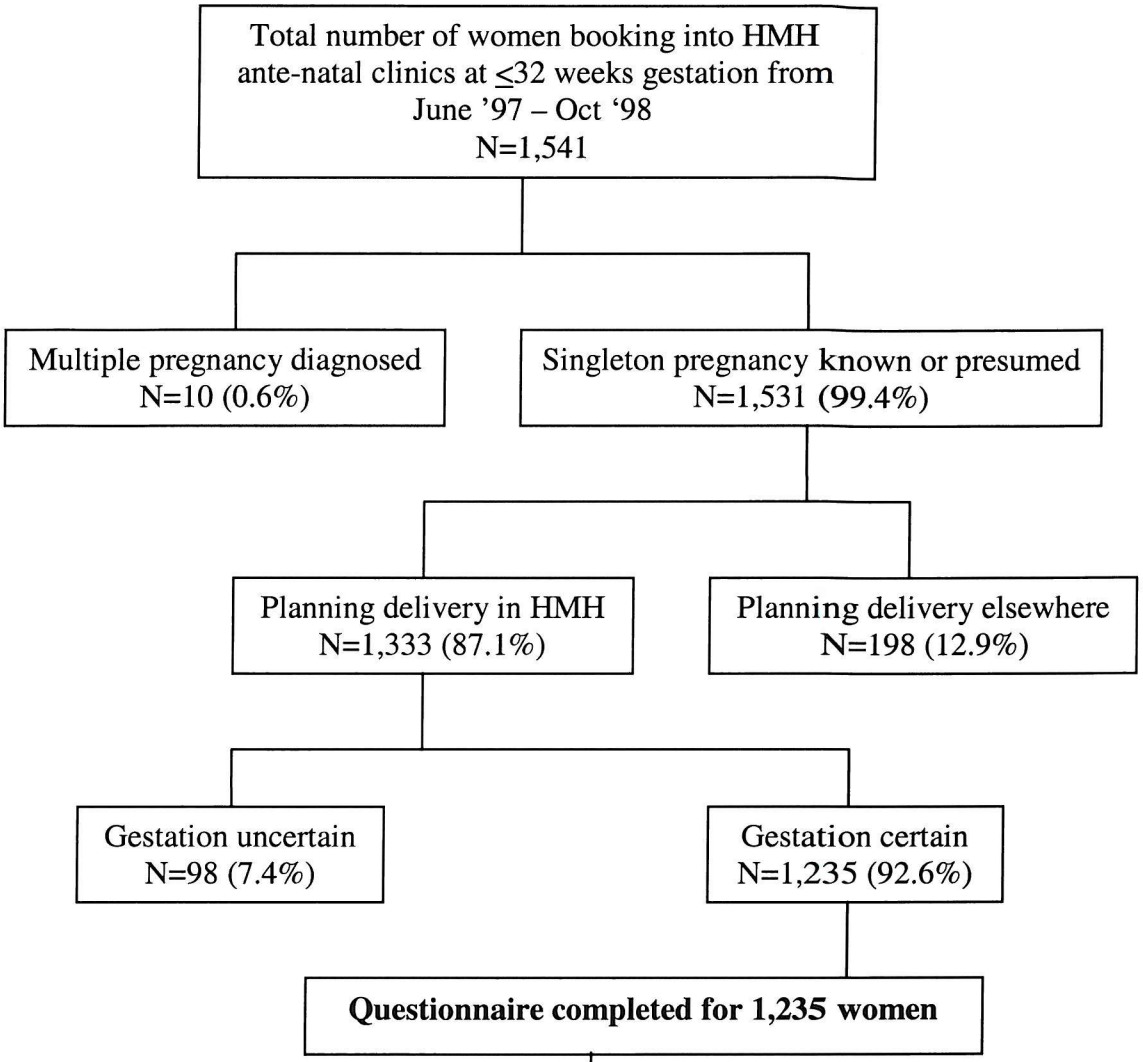
2.4.3 Questionnaire

1,235 women were eligible and completed a questionnaire (Appendix 1) designed to elicit factors that could affect fetal growth. Details included age, religion, marital status and consanguinity. A menstrual history was taken and it was noted whether or not contraception or lactation could have affected the estimation of gestation. Ultrasound scan details, if available, were recorded. A full obstetric, medical and drug history was obtained and a family history of diabetes in first-degree relatives was recorded. The social history included questions pertaining to occupation and tobacco and alcohol consumption.

2.4.4 Kuppuswamy score

Socio-economic status was ascertained using the Kuppuswamy score,[118] a standardised questionnaire method for urban Indian populations which uses information on family size, type and location of housing, availability of water and sanitation, education, occupation and income (Appendix 1). Before this project began, the classification of incomes was revised so that it was in line with contemporary earnings in Mysore. The scores grouped subjects into five social classes with group 5 being the least advantaged.

Fig.2.2: Flow chart showing recruitment and eligibility (June '97 – Oct '98)



2.4.5 Birth record tracing

184 women reported that they had been born in HMH (15% of those recruited). They completed a 'tracing' form (Appendix 1) designed to collate the information required in order to match the women with their birth records. This information included parents' names, occupations, religions and address at the time of birth, as well as a record of siblings in the order in which they were born and including those who had died. If the birth record was found and it matched the information given, details of the woman's birthweight, length, head circumference and placental weight were entered on a separate form.

2.5 Clinic

2.5.1 Method

Following recruitment, women were given a date on which to attend the research clinic for an oral glucose tolerance test (OGTT) and anthropometric measurements. The date given was as close to 28 weeks gestation as possible and no later that 32 weeks. The pattern of clinic attendance is shown in Figure 2.3. Women were instructed to fast for at least ten hours overnight prior to their clinic visit, and to ensure they ate their usual diet during the previous three days. Expenses incurred in getting to the clinic were reimbursed and on completion of the OGTT, breakfast was provided.

832 women (67.4% of those recruited) attended the clinic. Those who missed their appointment were either visited at home or sent a letter, depending on where they lived, and invited to make a new appointment. Almost one third of non-attendees were women who lived on the outskirts of the city, for whom attending the clinic would have been time-consuming and difficult and viewed by the family as largely unnecessary. Another third were women within the city who were visited and who agreed to a further appointment but who again did not attend. In this population all blood tests are viewed with suspicion and this was a major reason for women not participating in the study. Reasons for non-attendance are shown in Table 2.1.

Fig.2.3: Distribution of gestation at clinic attendance

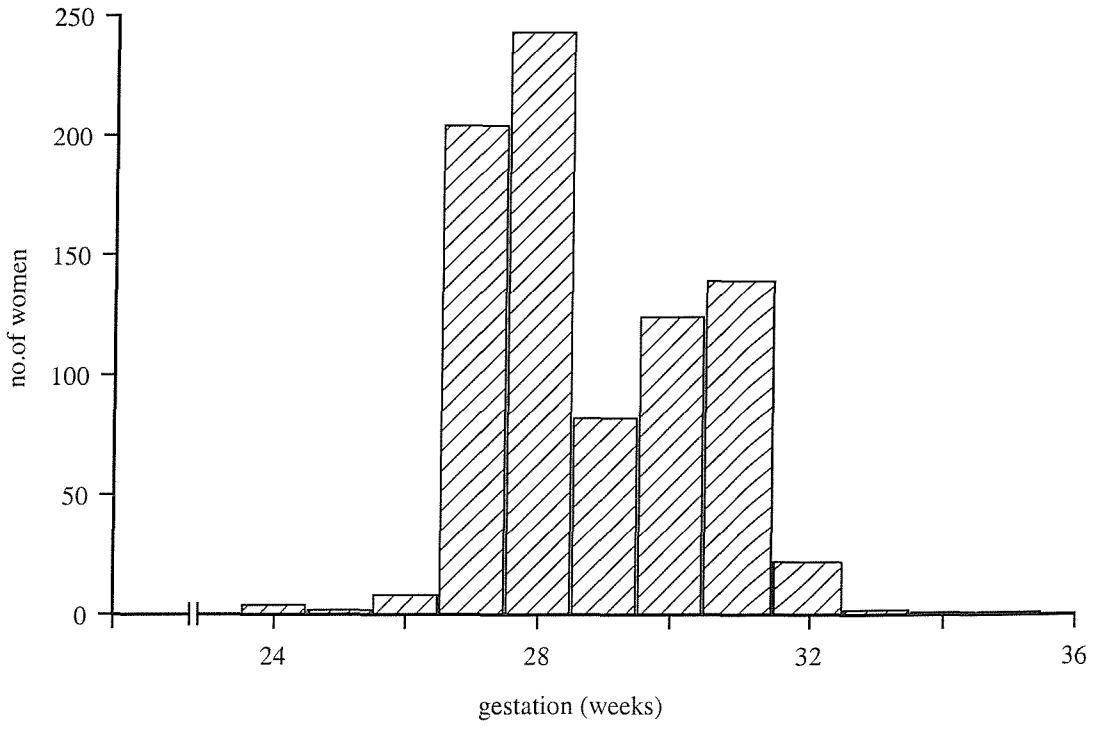


Table 2.1 Reasons for non-attendance at the clinic

Reasons	No. of women	% of non-attendees
Letter sent – no response	124	30.8
Visited – new appointment - did not attend	128	31.8
Attempted visit – home not found	65	16.1
Staying with mother – not in Mysore	23	5.7
Refused blood testing	29	7.2
Aborted	22	5.5
Premature home delivery	2	0.5
Delivering elsewhere	9	2.2
> 32 weeks gestation	1	0.2
Total	**403**	100

2.5.2 Blood sampling, processing and assaying

A fasting blood sample was taken by the laboratory technician for measurement of plasma glucose, insulin, proinsulin and 32,33-split proinsulin concentrations (Fig.2.4). Excluding two women already known to be diabetic, a 100g oral glucose load dissolved in 400ml water was given to each woman to drink over a period of five minutes. Further blood samples for plasma glucose and insulin concentrations were taken at 30-, 60-, 120- and 180-minute intervals.

Many women complained of severe nausea, which was helped by the addition of fresh lemon juice to the glucose drink. If vomiting occurred prior to the 60-minute sample being taken, the test was discontinued and rescheduled for another day. If after the 60-minute sample, the test was allowed to continue. 41 women did not complete the OGTT: 37 vomited and 4 refused further blood sampling. In a further 5 women, the samples were haemolysed. OGTT data was therefore complete in 784 women. Reasons given for missing values are shown in Table 2.2.

Fig.2.4: Blood sampling during an oral glucose tolerance test

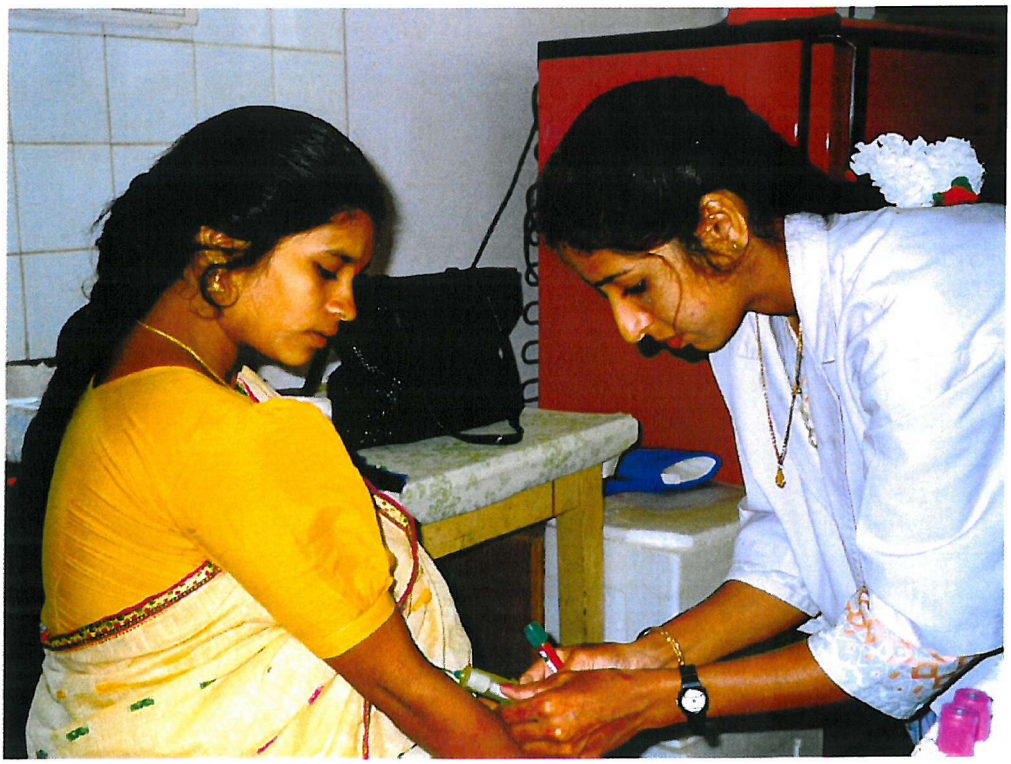


Table 2.2: Maternal blood samples. Total number of samples available for analysis and reasons for missing data.

	Total no. of samples	No. of missing values	Reasons for missing values (n)
Fasting	**832**	**0**	-
Glucose (mmol/l)	832	0	-
Insulin (pmol/l)	815	17	Haemolysed (17)
Proinsulin (pmol/l)	814	18	Haemolysed (17)
32,33-split proinsulin (pmol/l)	814	18	Insufficient (1)
RIR-HOMA	813	18	
HOMA-β	798	33	Haemolysed (17) Insufficient (1) Uncalculable (15)
30 minutes	**825**	**7**-no further sampling	Vomited (3) Diabetic (2) Refused (2)
Glucose (mmol/l)	825	0	-
Insulin (pmol/l)	821	4	Insufficient
30-minute insulin increment (pmol/mmol)	799	26	Insufficient (4) Uncalculable (22)
60 minutes	**792**	**33**-no further sampling	Vomited
Glucose (mmol/l)	790	2	Failed sample
Insulin (pmol/l)	785	7	Failed sample (3) Haemolysed (3) Insufficient (1)
120 minutes	**790**	**2**-no further sampling	Vomited
Glucose (mmol/l)	786	4	Failed sample (3) Haemolysed (1)
Insulin (pmol/l)	777	13	Failed sample (4) Haemolysed (6) Insufficient (3)
180 minutes	**788**	**2**	Refused
Glucose (mmol/l)	784	4	Failed sample (3) Time expired (1)
Insulin (pmol/l)	774	14	Failed sample (4) Haemolysed (6) Insufficient (3) Time expired (1)

All blood samples were centrifuged for twenty minutes at 4,000 rpm and separated into aliquots before being stored at -80°C and transported to the UK on dry ice at a later date. Plasma taken for measurement of glucose was divided in two: half was used to measure glucose in the HMH laboratory in order that results could be made available immediately, and half was stored and transported to the UK along with the rest of the samples. Plasma glucose, insulin, proinsulin and 32,33-split proinsulin concentrations were measured in the department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK.

Insulin samples were assayed individually on the Access Immunoassay System (Sanofi Pasteur Diagnostics) using a one step chemiluminescent immunoenzymatic assay. Cross-reactivity with intact proinsulin was <0.2% at 400 pmol/l and with 32,33 -split proinsulin was <1% at 400 pmol/l. Between run coefficients of variation were 6.6% at 28.6 pmol/l (n=99), 4.8% at 153.1 pmol/l (n=102) and 6.0% at 436.7 pmol/l (n=99) respectively. The limit of detection has been shown to be 0.2 pmol/l. Assay range 2100 pmol/l.

Intact proinsulin and 32,33-split proinsulin samples were assayed in duplicate using a time-resolved, fluometric (Delfia) assay. The solid phase antibody, bound to a microtitre plate was the same in each case. This and the labelled antibody for intact proinsulin were those previously described.[119] Tracer antibody was labelled using the Delfia Europium labelling kit 1244-302 (Wallac [UK] Ltd. Milton Keynes). Intact proinsulin cross-reactivity was <1% with insulin and 32-33 split proinsulin at concentrations of 2500 pmol/l and 400 pmol/l respectively. Between batch coefficients of variation were 10.5% at 4.5 pmol/l, 8.5% at 20 pmol/l and 8.1% at 92.9 pmol/l (n=50) respectively. The 32,33-split proinsulin assay showed 87% cross-reaction with intact proinsulin and it was therefore necessary to take account of the proinsulin in the specimen in order to obtain a specific measure of 32,33-split proinsulin. Cross-reaction with insulin was <1% at 2500 pmol/l. Between batch coefficients of variation were 8.6% at 6.6 pmol/l, 6.4% at 41 pmol/l and 5.3% at 101.2 pmol/l respectively (n=50). The limit of detection of the intact proinsulin and the 32,33-split pro-insulin assay was 1.25 pmol/l. Assay range 400 pmol/l.

Glucose samples were assayed individually on the Dimension XL clinical chemistry system (Dade International, Gamidor Ltd., Oxfordshire), using an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method.[120] Assay range was 0-27.8mmol/l and between batch coefficients of variation were 1.4% at 3.7 mmol/l (n=54) and 1.1% at 26 mmol/l (n=99) respectively.

2.5.3 Measurement of glucose tolerance, insulin resistance and insulin secretion

In non-pregnant populations diabetes is diagnosed universally according to WHO criteria.[45] However, there is great controversy over the use of these criteria in pregnancy.[121, 122] In 1964, O'Sullivan and Mahan described the current standard procedure for the diagnosis of gestational diabetes (GDM) in the USA using a 100g 3-hour OGTT.[123] Their criteria became the basis for the generally accepted North American thresholds for the diagnosis of GDM.

The National Diabetes Data Group (NDDG) in 1979 responded to the general change in laboratory standards from whole blood to plasma or serum with the formulation of a diagnostic criterion that is approximately 14% higher than that of simultaneously measured whole blood values.[124] Carpenter and Coustan[125] further refined the threshold value by accounting for the effect of glucose oxidase and hexokinase methods, which measure only glucose. Most recently, Sacks et.al.[126] simultaneously used the original methodology of O'Sullivan on whole blood and the more current, plasma glucose oxidase method on the same 995 venous blood samples from pregnant patients. They demonstrated the true translation for the laboratory differences between the techniques should be even lower than previously proposed (Table 2.3). None of the above criteria have been universally accepted and indeed there are many other sets of diagnostic criteria in use.

At HMH, the criteria used to diagnose GDM were those of Carpenter and Coustan. The obstetric consultants felt strongly that these criteria should be maintained and hence they were adopted in this study. Diabetes was diagnosed if two of the four measured values were raised.

Table 2.3: Comparison of cut-off values used in the 100g OGTT.

	O'Sullivan		NDDG		Carpenter		Sacks	
	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l
Fasting	90	5.0	105	5.9	95	5.3	96	5.3
1-hour	165	9.2	190	10.6	180	10.0	172	9.6
2-hour	145	8.1	165	9.2	155	8.7	152	8.4
3-hour	125	7.0	145	8.1	140	7.8	131	7.3

The area-under-the-glucose-curve (AUGC) in the oral glucose tolerance test was calculated from the trapezoidal rule [127] and was used as a continuous measure of glucose tolerance in analyses.

The 'gold-standard' methods for measurement of insulin resistance and β-cell function are the euglycaemic/hyperglycaemic clamp studies and the intravenous glucose tolerance test respectively. In epidemiological studies, insulin concentrations measured during an oral glucose tolerance test can be used as proxy measures.[128-130] Fasting insulin, 120-minute insulin,[129] and relative insulin resistance estimated by homeostasis model assessment (RIR-HOMA), calculated from the fasting glucose and insulin concentrations,[128] correlate with insulin resistance measured in clamp studies. The insulin increment ((insulin concentration at 30-minutes – fasting insulin concentration) /30-minute glucose concentration) and HOMA estimates of β-cell function (HOMA-β) correlate with first phase insulin secretion in an intravenous glucose tolerance test.[130] The 30-minute insulin increment and RIR-HOMA are useful as separate markers of insulin deficiency and resistance. HOMA-β appears to correlate with measures of both insulin secretion and resistance.

A proportion of 'insulin' measured in a standard radio-immuno assay is inactive proinsulin and other insulin precursors. The development of radio-immunometric

assays have allowed insulin, proinsulin and the hydrolysed proinsulin, 32,33-split proinsulin to be measured specifically.[131] Proinsulin and 32,33-split proinsulin were included in this study as measures of β-cell pathology. They were thought to be a useful measure of β-cell failure, the release of incompletely processed insulin by a stressed cell. A recent study has shown that high circulating concentrations in fasting blood correlate strongly with insulin resistance and not with first-phase insulin secretion.[129] It remains unclear whether they measure insulin resistance alone (the stress) or an abnormal β-cell response to it (stress damage).

In order to assess whether or not an individual's level of insulin secretion was appropriate for their degree of insulin resistance, a new variable was created from a simple linear regression of RIR-HOMA with HOMA-β as the dependent variable (β=0.5, $p<0.001$). Each woman's own value was subtracted from the predicted value and termed the 'residual'. A negative residual implied that the level of insulin secretion (as calculated by HOMA-β) was less than that predicted for the level of insulin resistance (calculated by RIR-HOMA).

2.5.4 Clinical management of GDM

Following the OGTT, results of the test were made available to the woman and her consultant obstetrician, usually on the same day, allowing GDM cases to be managed according to the hospital protocol. This involved initial dietary advice and a further appointment to re-check fasting and post-prandial plasma glucose concentrations. If these values were high (>5.3mmol/l fasting or >7.8mmol/l post-prandial), the woman was admitted for a blood sugar series taken over 24 hours and on the basis of these results, the decision to start insulin therapy was made.

Interestingly, but inexplicably, the laboratory estimates of blood glucose concentrations in Mysore, although strongly correlated, were lower than the corresponding values measured in Cambridge, UK (Table 2.4) and 24 out of the 48 cases of GDM diagnosed in Cambridge went undiagnosed in Mysore.

Table 2.4: Mean blood glucose concentrations in mmol/l

Sampling time (minutes)	Mysore	Cambridge	Correlation coefficient
0	4.22	4.57	0.69
30	6.69	7.33	0.73
60	6.51	7.16	0.86
120	5.57	6.06	0.85
180	5.07	5.55	0.69

2.5.5 Blood Pressure

Blood pressure was measured in all 832 women at the time of their OGTT, usually following the fasting sample and after being seated quietly for at least five minutes. Blood pressure was taken by one of four observers using a standard mercury sphygmomanometer. Two readings were taken with a standard cuff according to a set protocol (Appendix 2) and room temperature was recorded. Prior to the start of the project, team members were trained in the measurement technique, and intra- and inter-observer variation studies (IOV's) were carried out in order to standardise these techniques and minimise measurement error.

If the blood pressure recorded was greater than or equal to 140/90, it was measured again after one hour and if still raised, the obstetrician was informed and the appropriate action taken.

2.5.6 Anthropometric measurements

Protocols for the anthropometric measurements made are set out in Appendix 2. Team members were trained in the measurement techniques prior to the start of the project, and IOV's were carried out, as for blood presssure measurments in order to standardise these techniques and minimise measurement error (Appendix 3).

The anthropometric measurements made on each woman were as follows (Fig.2.5):

a) Weight (kg)	
b) Height (cm)	
c) Circumferences (cm)	Head
	Mid-upper arm (MUAC)
	Mid-thigh
d) Skin-fold thicknesses (mm)	Biceps
	Triceps
	Subscapular
	Suprailiac
e) External pelvic diameters (cm)	Intercristal
	Interspinous
	External conjugate

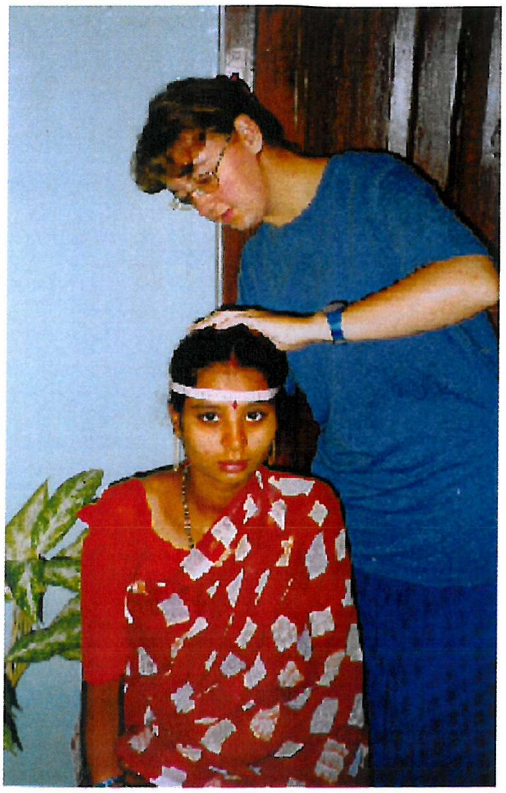
Body mass index (BMI kg/m^2) was calculated from weight (kg) / height (m)^2 and used as a measure of fatness.

The ratio of subscapular skin-fold to triceps skin-fold (SS/TR) was calculated as a measure of central fatness. A tendency to store fat centrally (abdominally and truncally) rather than in the limbs, described as a male (android) pattern of fat distribution, is associated with an increased risk of type 2 diabetes.[132] The most commonly used index of central obesity in epidemiological studies is the ratio of waist to hip circumferences. This has been shown to be a strong risk factor for diabetes and has been shown to predict gestational diabetes when measured early in pregnancy.[133] Other measures of central obesity such as the subscapular skin-fold thickness and SS/TR ratio show similar associations.[134] This ratio is perhaps more difficult to interpret in pregnant populations due to the fact that central skin-folds tend to increase to a greater extent than peripheral skin-folds during pregnancy.[135, 136]

Fig.2.5: Anthropometric measurements made in the clinic

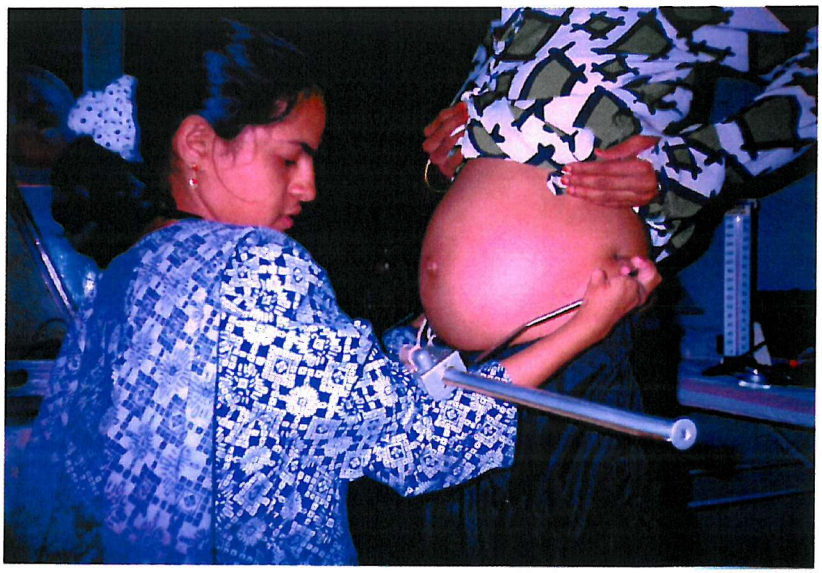
a) Head circumference

Measured with anthropometric tape



b) Intercristal diameter

Measured using a Harpenden anthropometer



2.5.7 Fat mass and muscle mass calculations

A major limitation in all research on body composition is that there is no direct measure of body fat other than carcass analysis, which is obviously not suitable for most purposes. Body composition models rely on assumptions about the relations among these compartments of the body that can be measured and those that cannot. For this study, body density and hence percentage body fat was calculated from equations (1) and (2) devised by Durnin and Womersely[137] using the average skin-fold measurements from all four sites. This method has been validated before in a South Indian population.[138]

(1) Density = c – [m – log total skin-folds]

(where c and m are found according to a nomogram using the subject's age c=1.1599 and m=0.0717 when age group = 20-29).

(2) Body Fat % = [(4.95/density) – 4.50]* 100

Fat mass was calculated from equation (3), devised by Joop MA van Raaij[139] and designed to take into account the changes in body water and therefore fat-free mass that occur during pregnancy.

(3) At 30 weeks gestation:

Fat mass = body weight/100* ([510.8/body density] – 467.5) kg

Muscle mass was calculated using equations (4) and (5) devised by Heymsfield et al.,[140] where arm muscle area (AMA, cm^2) is calculated from triceps skin-fold thickness (TSF, cm) and mid-upper arm circumference (MUAC, cm) and corrected for gender difference.

(4) Corrected AMA = $[(MUAC - \pi * TSF)^2 / 4\pi]$

(5) Muscle mass (kg) = height (cm^2)*(0.0264 + 0.0029 * corrected AMA)

2.5.8 Husbands

Women were asked to bring their husbands with them to the clinic in order that their height and weight could be measured. If a woman's husband was not able to attend the clinic at that time, an alternative arrangement was made which often involved a home visit or a visit to the work place if necessary. A total of 676 out of 832 husbands were measured.

2.6 Delivery

2.6.1 Method

Having attended the research clinic, an identifying label was attached to the woman's obstetric record by which the midwives were able to recognise women participating in the study and inform the team of their admission to the delivery suite. The team member who attended the delivery recorded on the delivery data forms (Appendix 1) any complications that had arisen during the pregnancy and had been recorded in the obstetric notes. Labour and delivery details were documented along with the newborn's sex, gestation, apgar scores and the presence or absence of birth injury or congenital anomaly. Any admission to the neonatal unit was recorded along with the reason for admission.

2.6.2 Cord blood

Following delivery of the baby, the cord was wiped with a piece of cotton and 10ml of venous blood taken, preferably before delivery of the placenta. The blood was then transferred into the relevant vacutainers and centrifuged as soon as possible at 4-5000 rpm for 20 minutes. It was used for measurement of plasma glucose, insulin, proinsulin and 32,33-split proinsulin concentrations and was processed in the same manner as the maternal blood samples.

Samples were obtained from 576 of the 597 term births (96.5%). Cord blood was not available in 11 cases where the team was not informed of the delivery and in 10 where an insufficient sample was obtained. Reasons for missing values are shown in Table 2.5.

Table 2.5: Cord blood samples. Number of values available for analysis and reasons for missing data.

Cord blood variables	Total no. of samples available for analysis	Reasons for missing values (n)
Glucose (mmol/l)	571	Haemolysed (4) Insufficient (1)
Insulin (pmol/l)	554	Haemolysed (22)
Proinsulin (pmol/l)	547	Haemolysed (22) Insufficient (4) Reading failed (3)
32,33-split proinsulin (pmol/l)	547	Haemolysed (22) Insufficient (4) Reading failed (3)

2.6.3 Placenta

Placentae were trimmed and weighed according to a specific protocol (Appendix 2). The cord clamp was released allowing the blood to drain from the placenta, which was checked for completeness. The amnion was stripped off and the chorion trimmed close to the placental edge. The cord was cut off flush with the placenta. Obvious clots were removed. The electronic weighing machine was zeroed and the placenta weighed.

2.6.4 Anthropometry of the neonate

Whenever possible, anthropometric measurements were carried out on the new born baby at the time of delivery i.e. while mother and baby were still on the delivery suite. If the baby had been admitted to the neonatal unit, measurements were delayed until the baby was in a stable condition, as judged by the paediatricians. This delay was never more than 72 hours. In eleven cases where the team had not been informed of the delivery, the baby was measured on the post-natal ward within 24 hours of the delivery (Fig.2.6).

Measurements were made according to a set protocol (Appendix 2) and included:

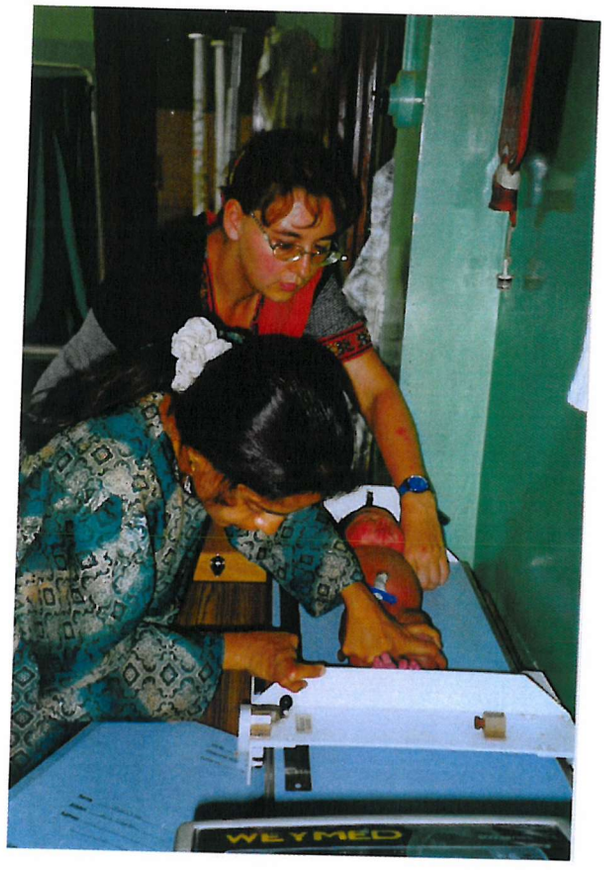
a)	Weight (g)	
b)	Lengths (cm)	Crown-heel (CHL)
		Crown-rump (CRL)
c)	Circumferences (cm)	Head
		Chest (xiphisternum)
		Abdomen (umbilicus)
		Mid-upper arm (MUAC)
d)	Skinfold thicknesses (mm)	Triceps
		Subscapular

Ponderal index (PI) was calculated from birthweight / crown-heel length^3 (kg/m^3) and used as a measure of neonatal fatness.

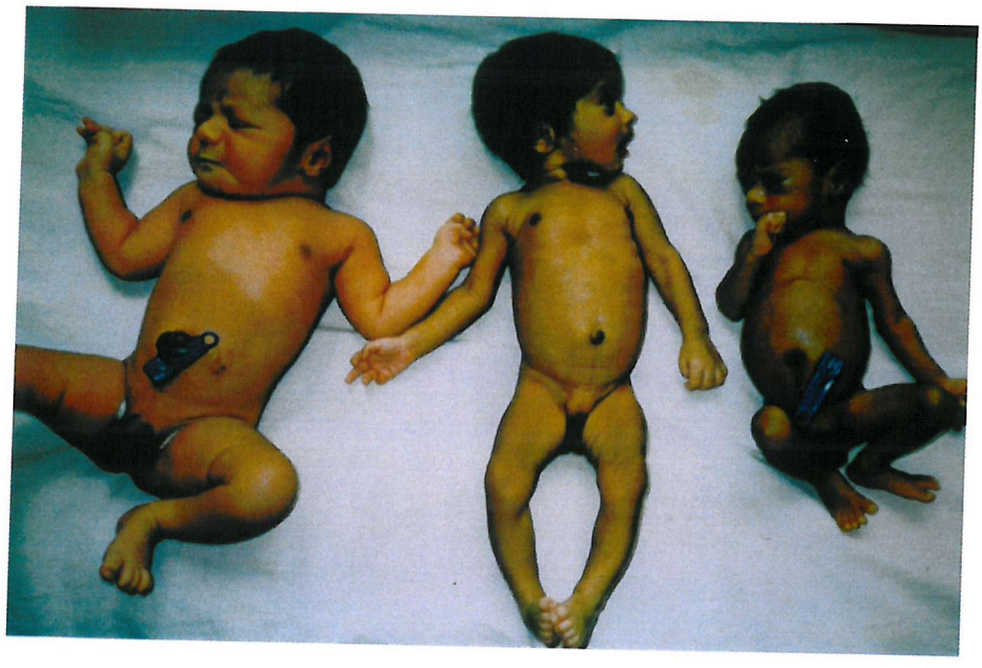
Leg length was calculated from (crown-heel length) – (crown rump length) (cm).

The head to abdomen ratio was calculated from head circumference / circumference at xiphisternum and was used as an indicator of asymmetrical growth, 'brain-sparing'.

Fig.2.6: Measuring crown-heel length using a Harpenden infant stadiometer



Mysore babies



2.7 Statistical Methods

Anthropometric variables that were not normally distributed were log transformed (i.e. weight, BMI, SS/TR, triceps, biceps, subscapular skin-fold thicknesses and calculated fat mass) except for the suprailiac skin-fold thickness, which was square-rooted (Table 3.5). Maternal blood glucose concentrations were normally distributed but the insulin concentrations, including 32,33-split proinsulin, the 30-minute insulin increment, RIR-HOMA and HOMA-β were skewed and were log transformed. The distribution of maternal proinsulin concentrations was extremely skewed due to 38.8% of values falling below the lower limit of assay detection. Proinsulin was therefore analysed as a categorical variable (Table 4.1). Neonatal anthropometric variables were normally distributed apart from the skin-fold thicknesses and placental weight, which were log transformed to satisfy assumptions of normality (Table 3.15). Neonatal cord blood variables were similarly skewed and were logged to achieve a normal distribution.

Analyses have been carried out with all available data using multiple linear and logistic regression with the SPSS/PC 7.5 statistical computer package. Continuous variables were used where appropriate and adjustment made as necessary for sex, gestation, maternal age, parity, social class and fat mass. In Figure 4.1, standard deviation (SD) scores were calculated for each subject's anthropometric measurements relative to the study population as (subject's value – population mean value) / standard deviation of population.

Differences in means were tested using the Student's t-test. The Mann-Whitney U-test was used for non-parametric data and the Chi-Squared test for differences in proportions.

Literature searches were carried out in Southampton using 'MEDLINE'.

Ethical approval for this study was given by the Ethical Committee of the Holdsworth Memorial Hospital and informed consent was obtained from all women prior to their attendance at the research clinic.

3. Results – Maternal and Neonatal Anthropometry

3.1 Introduction

Many factors, both genetic and environmental are known to be important determinants of adult size and body composition. The extent to which maternal size and body composition, rather than genome, is directly responsible for the size and body composition of the newborn is not well known. Animal studies, such as Walton and Hammond's Shire-horse-Shetland–pony cross experiments,[141] show that growth of a fetus genetically destined to be large, is down regulated if the mother is small. The mechanisms responsible for this adaptation are not well understood. Studies in humans suggest that size at birth is largely determined by environmental influences in-utero generated by the mother and that there is relatively little contribution from the fetal genome.[142,143]

In this chapter I have described the women who participated in the study, the influence of age, parity and social class on their size and body composition and the correlations that exist between their different anthropometric measurements. I have gone on to describe their babies, attempting to define the relationships that exist between the mother's body composition and the size of her newborn. In so doing, I have compared my study population (urban India) with a rural Indian population and with mothers and babies from Southampton. Finally, I have attempted to define the genetic contribution made by the father to the size of the baby.

3.2 Women who participated in the study

3.2.1 Age and parity

832 women participated in the study by attending the research clinic at 30+/-2 weeks gestation. Their ages ranged from 16 to 40 years (median 23) (Fig.3.1), and their parity from 0 to 4 (Fig.3.2). Women who did not participate in the study were younger (median 22 years, $p<0.001$) but with no difference in parity.

3.2.2 Social Status

Using the modified Kuppuswamy score as described previously, women were classified as belonging to one of five social groups, group 5 being the least advantaged (Table 3.1). The distribution of social class in the study population may reflect the fact that although HMH attempted to cater for all social classes, it remained too expensive for the very poor. Women in social classes 4 & 5 were more likely to deliver at home or in government hospitals. Conversely, women in social class 1 were more likely to deliver in the more expensive, private, nursing homes.

Table 3.1: Social status according to Kuppuswamy scoring

Social Class	Kuppuswamy Score	No. of women	% of women
1	26-29	8	1.0
2	16-25	311	37.4
3	11-15	330	39.7
4	5-10	181	21.8
5	≤ 4	2	0.2
	Total	**832**	**100**

Fig.3.1: Age distribution of women who attended the research clinic

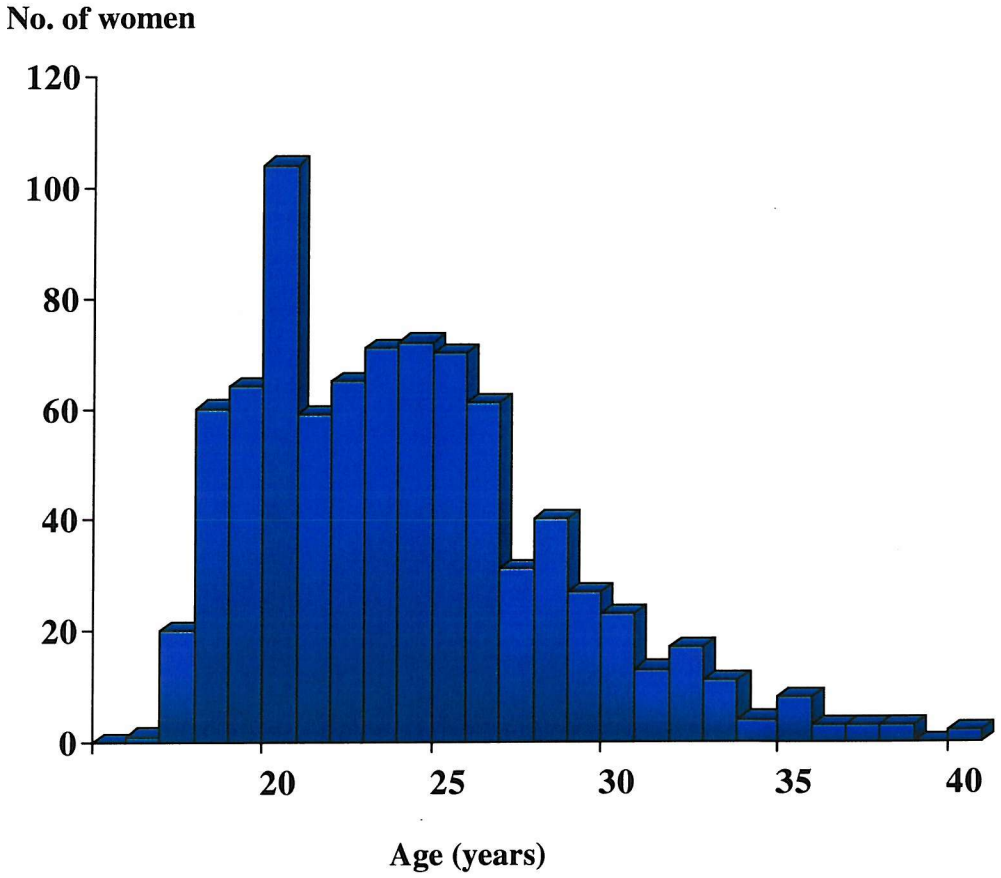
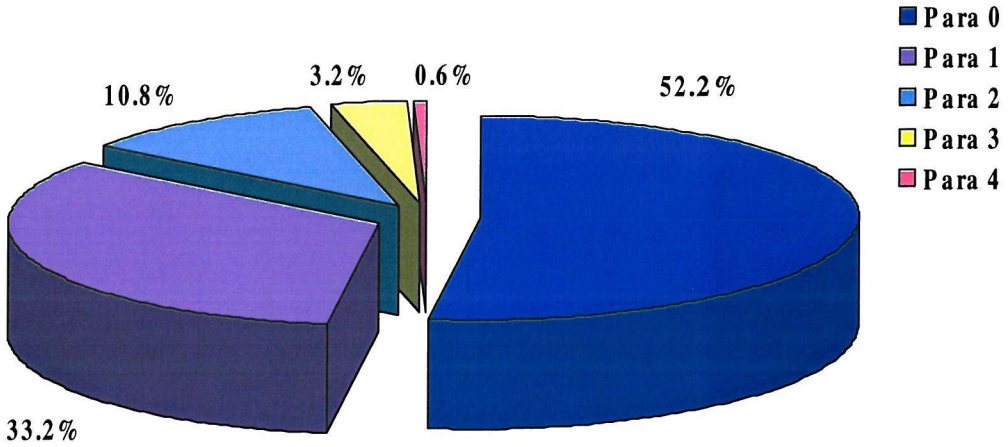


Fig.3.2: Parity of women who attended the research clinic



Women in social classes 4 & 5 had more children and were younger than women in classes 1–3 (Table 3.2).

Table 3.2: Mean age (years) with number (N) and percentage (%) of women in each social class according to their parity.

Social Class	Para 0		Para 1		Para 2 +		Total
	Age	N (%)	Age	N (%)	Age	N (%)	N
1 & 2	23	*176* (55.2)	26	*107* (33.5)	27	*36* (11.3)	*319*
3	21	*182* (55.2)	24	*110* (33.3)	27	*38* (11.5)	*330*
4 & 5	22	*76* (41.5)	23	*59* (32.2)	25	*48* (26.2)	*183*

3.2.3 Medical and surgical history

31 women gave a positive history of a significant medical problem and 27 (3.2%) required medical treatment during the study period. 7 women (0.8%) had undergone a surgical procedure prior to the study and one woman underwent an appendicectomy in the first trimester of the study pregnancy. No one was excluded from the study on the basis of a medical or surgical problem. Tables 3.3a and 3.3b list the medical and surgical conditions and the management of them.

No woman in this study used tobacco or drank alcohol on a regular basis.

Table 3.3a: Medical conditions and treatment given during the study period.

Medical condition	No. of women	Medication used
Asthma	9	Inhalers: salbutamol, terbutaline, betamethasone / bromhexine Oral: salbutamol,theophylline
Tuberculosis	1	Oral: ethambutol
Pneumonia	1	Oral: ampicillin, cephalexin
Epilepsy	2	Oral: carbamazepine, Phenytoin, phenobarbitone
Rheumatic fever	4	IM: benzothinepenicillin
Rheumatoid arthritis	1	IM: penicillin
Rheumatic heart disease (mitral stenosis)	1	IM: benzothinepenicillin Oral: digoxin
Congenital heart disease (atrial septal defect)	1	IM: benzothinepenicillin
Ischaemic heart disease	1	Oral: isosorbide-5-mononitrate
Diabetes	2	Subcutaneous: insulin
Hypothyroidism	2	Oral: thyroxine
Cholera	1	IV: rehydration
Depression	1	Oral: diazepam, amitryptilline
Total	**27**	

Table 3.3b: Surgical procedures undergone prior to and during the study period.

Surgical procedure	No. of women undergoing surgery	
	Prior to study (n=7)	During study (n=1)
Myomectomy	3	
Tubectomy	1	
Appendicectomy	1	1
Partial thyroidectomy	1	
Lithotripsy	1	

3.2.4 Family History of Diabetes

The reported frequency of diabetes among first degree relatives of women who participated in the study was high (18.6%, n=155), and probably underestimated the true frequency, as diabetes often remains undiagnosed. Of these 155 women, 66.5% had a father with diabetes, 41.3% a mother and 7.1% a sibling. 16 women had both father and mother affected. 4, their father plus a sibling and 3 their mother plus a sibling. The fact that more fathers than mothers were reported as having diabetes may simply reflect an increasing prevalence of diabetes with age, as husbands were invariably older than their wives.

3.2.5 Marriage

All the women were married, although one was recently widowed. Consanguinity is common in this community and was present in 19.5% of these marriages. 89.5% of the consanguineous marriages were of first degree consanguinity i.e. the woman was married to her mother's or father's brother, or to a first cousin. Second degree consanguinity (marriage to a second cousin) was present in 10.5%. 70.8% of consanguineous marriages involved the mother's side of the family and in only 29.2%, the father's side.

3.2.6 Occupation

90.7% of women described themselves as housewives (Table 3.4).

Table 3.4: Occupations of women attending the research clinic.

Occupation	N	%
Housewife	755	90.7
Teacher / social worker / police woman	38	4.6
Laboratory technician / nurse / optician	11	1.3
Secretary / typist / stenographer / receptionist / clerk	10	1.2
Lecturer	6	0.7
Worker on farm / factory / beedi rolling	4	0.5
Accounts officer / supervisor / manager	2	0.2
Research assistant / trainee / PhD student	2	0.2
Tailor / screen printer	2	0.2
Own business	1	0.1
Doctor	1	0.1
Total	**832**	100

3.2.7 Religion

In India, according to the 1991 census, 82% of the population were Hindu, 12.1% Muslim and 2.3% Christian. In Mysore, according to the same census, 72% were Hindu, 16.3% Muslim and 3% Christian. Within the study population, 59.3% were Hindu, 33.3% were Muslim and 7.1% were Christian (Fig.3.3).

The differences in the distribution of religions in the study population compared to that of Mysore, probably reflects the fact that HMH is a Christian mission hospital and is situated in an area of Mysore with a high Muslim population.

3.2.8 Maternal Anthropometric Measurements

There were no significant correlations between any maternal anthropometric measurements and the gestational age at measurement, probably due to the narrow gestational age range in which the women were studied. The values used in analyses were therefore unadjusted for gestational age and are shown in Table 3.5.

Fig. 3.3: Distribution of religions in A. India, B. Mysore, and C. Women who attended the research clinic

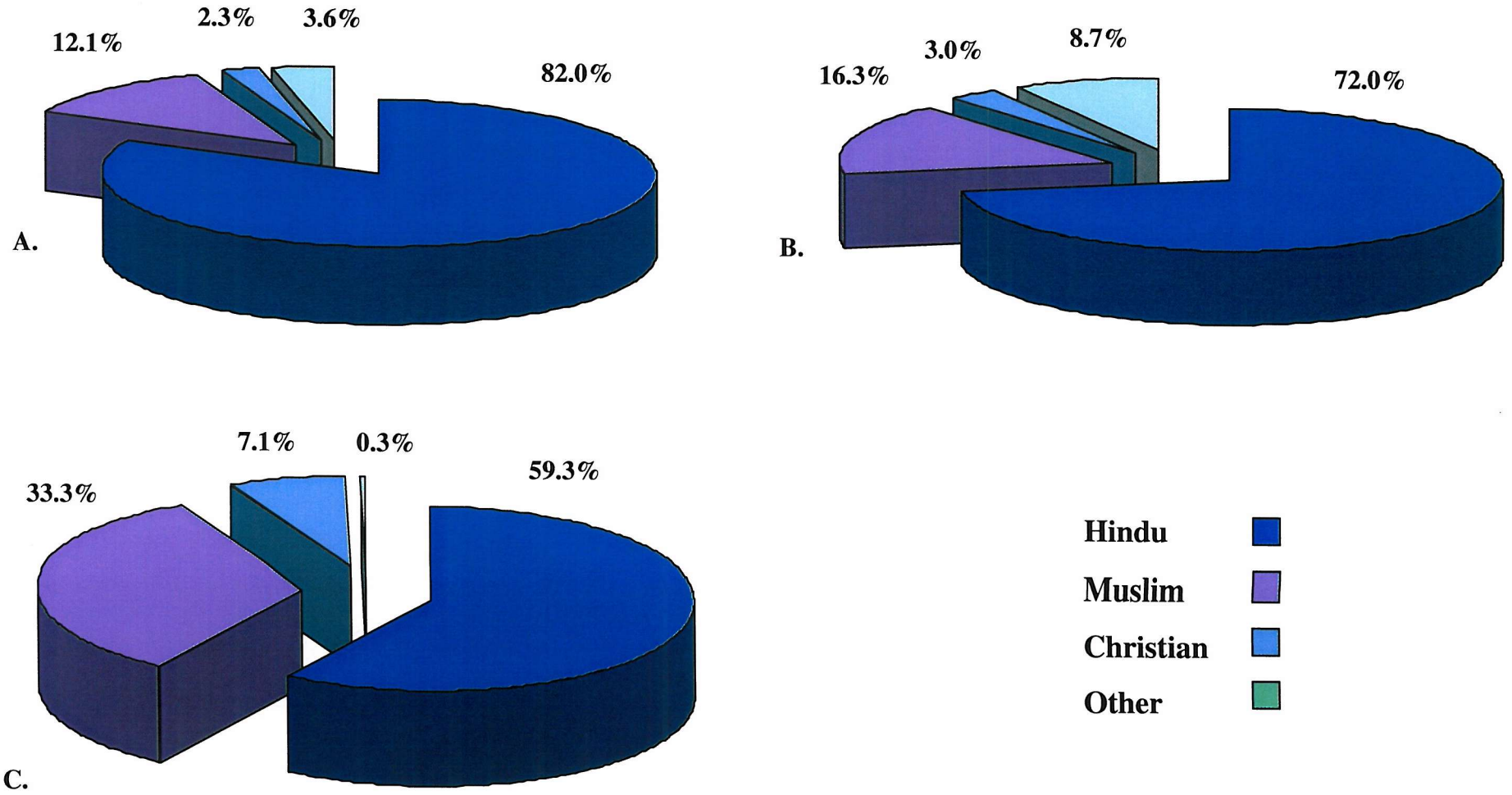


Table 3.5: **Anthropometric description of women who participated in the study. Means and SD's given** (geometric means and interquartile ranges (IQR) for logged variables) * denotes logged variables.

Anthropometric Variables	No. of women measured	Mean *geometric mean	SD *IQR
Height (cm)	832	154.6	5.5
Weight (kg)*	832	55.2	49.1, 61.5
BMI (kg/m^2)*	832	23.1	20.7, 25.7
Circumferences			
Head (cm)	749	53.4	1.5
MUAC (cm)	832	24.5	2.9
Mid-thigh (cm)	831	46.3	5.2
Skin-fold thicknesses			
Triceps (mm)*	832	16.7	12.1, 23.8
Subscapular (mm)*	832	23.7	17.5, 32.4
SS/TR*	832	1.4	1.2, 1.7
Biceps (mm)*	832	8.8	6.2, 12.5
Suprailiac (mm)*	832	31.7	23.2, 41.7
External pelvic diameters			
Intercristal (cm)	832	25.7	2.5
Interspinous (cm)	832	23.5	2.2
External conjugate (cm)	831	21.0	2.3
Fat mass (kg)*	832	17.2	13.6, 22.0
Muscle mass (kg)	832	13.8	2.3

BMI=body mass index
MUAC=mid-upper-arm circumference
SS/TR=ratio of subscapular to triceps skin-fold

3.2.8.1 Anthropometry and Social Class

Maternal anthropometry varied with social class (Table 3.6). Women in the lower social classes (i.e. 4 & 5) were markedly shorter and thinner with smaller head, mid-upper-arm and mid-thigh circumferences. All four skin-fold measurements were reduced. Interestingly, the ratio of subscapular to triceps was increased, suggesting a tendency to central fatness amongst women of lower social classes. The external pelvic diameters were also reduced in these women and the measured fat mass decreased from a mean of 18.8 kg in women of social class 1 & 2 to 15.3 kg in women of social class 4 & 5. There was no significant difference in muscle mass.

3.2.8.2 Anthropometry and Age

Older women were significantly heavier than younger women but with no difference in height (Table 3.7). Mid-upper arm and mid-thigh circumferences increased with age as did all the skin-fold thickness measurements and the external pelvic diameters. There was a large increase in the calculated fat mass (12.7 kg in the lowest third of age and 18.7 kg in the highest) and although muscle mass and head circumference were also shown to increase with age, they did not do so to the same extent. In summary, older women were heavier mainly due to increased fat mass.

After adjustment for social class, there was a strong inverse relationship between age and height (β=-0.1 cm/year, p=0.008) (Table 3.7). This reflected a relationship between age and social class, with the younger women coming from the lower social classes (Table 3.8a). The relationship between age and head circumference lost its significance (p=0.3) (Tables 3.7 and 3.8b).

Table 3.6: Relationship of social class to maternal anthropometry.
Mean values shown and p for trend

Social Class				Circumferences			Skinfold thicknesses					External pelvic diameters				
	height (cm)	**weight** (kg)	**BMI** (kg/m^2)	**head** (cm)	**mid-arm** (cm)	**mid-thigh** (cm)	**triceps** (mm)	**biceps** (mm)	**sub-scap.** (mm)	**supra-iliac** (mm)	**SS/ TR**	**inter – cristal** (cm)	**inter-spin.** (cm)	**ext. conj.** (cm)	**fat mass** (kg)	**musc. mass** (kg)
1 & 2 (n=319)	156.1	57.8	23.7	53.8	25.0	47.4	18.2	9.5	24.8	34.6	1.4	26.1	23.8	21.3	18.8	13.9
3 (n=330)	154.6	54.5	22.8	53.2	24.4	46.0	16.5	8.8	23.7	31.3	1.4	25.6	23.4	20.9	17.0	13.8
4 & 5 (n=183)	151.9	52.1	22.6	52.9	24.1	45.1	14.9	7.8	21.9	27.5	1.5	25.1	23.0	20.5	15.3	13.7
p for trend	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.3

Table 3.7: Relationship of age to maternal anthropometry.
Mean values shown and p for trend before and after adjustment for social class

Age (years)	height (cm)	weight (kg)	BMI (kg/m^2)	Circumferences: head (cm)	mid-arm (cm)	mid-thigh (cm)	Skinfold thicknesses: triceps (mm)	biceps (mm)	sub-scap. (mm)	supra-iliac (mm)	SS/TR	External pelvic diameters: inter-cristal (cm)	inter-spinous (cm)	ext. conj. (cm)	fat mass (kg)	musc. mass (kg)
16 – 20 (n=249)	154.4	52.2	21.9	53.2	23.5	44.5	14.8	7.7	21.2	30.5	1.4	25.0	23.0	20.4	12.7	13.5
21 – 25 (n=337)	154.8	55.6	23.2	53.3	24.6	46.7	16.8	9.0	24.5	31.6	1.5	25.7	23.5	20.9	17.5	13.9
26 – 40 (n=246)	154.5	57.8	24.3	53.5	25.5	47.7	18.8	9.9	25.4	33.0	1.4	26.4	24.0	21.5	18.7	14.1
p for trend	0.9	< 0.001	< 0.001	0.02	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.03	0.02	< 0.001	< 0.001	< 0.001	< 0.001	0.002
p adj. for social class	0.008	< 0.001	< 0.001	0.3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.1	0.04	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.8a: Mean height (cm) of women according to their social class and age (in thirds). *(Number of women shown in parenthesis)*

	Age (years)			
Social Class	**16 – 20**	**21 – 25**	**26 – 40**	**All**
1 & 2	156.4 (68)	157.0 (127)	155.1 (124)	156.1 (319)
3	154.6 (116)	154.5 (131)	154.6 (83)	154.6 (330)
4 & 5	152.0 (65)	151.8 (79)	151.9 (39)	151.9 (183)
All	154.4 (249)	154.8 (337)	154.5 (246)	154.6 (832)

Table 3.8b. Mean head circumference (cm) of women according to their social class and age (in thirds). *(Number of women shown in parenthesis)*

	Age (years)			
Social Class	**16 – 20**	**21 – 25**	**26 – 40**	**All**
1 & 2	53.7 (68)	53.9 (127)	53.7 (124)	53.8 (319)
3	53.1 (116)	53.1 (131)	53.5 (83)	53.2 (330)
4 & 5	52.8 (65)	52.9 (79)	53.0 (39)	52.9 (183)
All	53.2 (249)	53.3 (337)	53.5 (246)	53.4 (832)

3.2.8.3 Anthropometry and Parity

Several anthropometric measurements were related to parity, including height, weight, BMI, muscle mass, mid-upper-arm circumference and the suprailiac skin-fold (Table 3.9). After adjusting for age and social class effects, these relationships remained significant only for the suprailiac skin-fold and muscle mass. Although the suprailiac skin-fold was lower in women of higher parity, the triceps skin-fold was higher. Women of higher parity tended to be from the least advantaged social classes and were both thinner (Table 3.10a) and shorter (Table 3.10b).

Table 3.10a: Mean BMI (kg/m^2) of women according to their social class and parity. *(Number of women shown in parenthesis)*

Social Class	Para 0	Para 1	Para 2 +	All
1 & 2	23.1 (176)	24.3 (107)	25.0 (36)	23.7 (319)
3	22.5 (182)	23.0 (110)	23.9 (38)	22.8 (330)
4 & 5	22.0 (76)	23.0 (59)	23.2 (48)	22.6 (183)
All	22.7 (434)	22.6 (276)	23.9 (122)	23.1 (832)

Table 3.10b: Mean height (cm) of women according to their social class and parity. *(Number of women shown in parenthesis)*

Social Class	Para 0	Para 1	Para 2 +	All
1 & 2	156.4 (176)	156.0 (107)	155.2 (36)	156.1 (319)
3	154.9 (182)	154.4 (110)	153.7 (38)	154.6 (330)
4 & 5	152.5 (76)	151.9 (59)	151.0 (48)	151.9 (183)
All	155.1 (434)	154.5 (276)	153.1 (122)	154.6 (832)

Table 3.9: Relationship of parity to maternal anthropometry.

Mean values shown and p for trend before and after adjustment for age and social class

Parity				Circumferences			Skinfold thicknesses					External pelvic diameters				
	height (cm)	**weight** (kg)	**BMI** (kg/m^2)	**head** (cm)	**mid-arm** (cm)	**mid-thigh** (cm)	**triceps** (mm)	**biceps** (mm)	**sub-scap.** (mm)	**supra-iliac** (mm)	**SS/ TR**	**inter – cristal** (cm)	**inter-spinous** (cm)	**ext. conj.** (cm)	**fat mass** (kg)	**muscle mass** (kg)
0 (n=434)	155.1	54.5	22.7	53.3	24.1	46.0	16.2	8.6	23.1	33.1	1.4	25.7	23.5	20.9	17.1	13.6
1 (n=276)	154.5	55.9	23.5	53.4	24.9	46.9	17.2	9.1	24.3	30.6	1.4	25.7	23.5	20.9	17.5	14.1
2 + (n=122)	153.1	56.0	23.9	53.5	25.2	46.3	17.4	9.0	24.5	29.2	1.4	25.8	23.4	21.2	17.4	14.2
p for trend	< 0.001	0.003	< 0.001	0.4	< 0.001	0.2	0.06	0.2	0.1	0.001	0.6	0.7	0.7	0.4	0.5	< 0.001
p adj. age & social class	0.06	0.4	0.08	0.1	0.04	0.8	0.7	0.99	0.7	< 0.001	0.96	0.3	0.1	0.7	0.7	0.007

3.2.8.4 Intercorrelations between maternal anthropometric variables

There are various reasons why anthropometric variables may correlate with each other:

- They may have common biological determinants e.g. effects of social class on height, fat mass and head circumference. This is the type of correlation that we are most interested in.
- They may be correlated if one variable forms part of the other e.g. triceps and biceps skin-fold thicknesses are included in the measurement of mid-upper-arm circumference and, the measurement of weight inevitably contains height.
- One variable may correlate with another if it is used in the calculation of the other e.g. weight is used to calculate fat mass and mid-upper arm circumference and height to calculate muscle mass.

Scatter plots were used to look at relationships between anthropometric variables; no non-linear relationships were identified. Table 3.11 shows the Pearson correlation coefficients between the variables. Taking each variable in turn and looking at its relationship to the others, the following statements can be made:

1) Weight correlated strongly and positively with all the maternal anthropometric variables apart from the ratio SS/TR, with which it correlated negatively. Its measurement includes; height, head circumference, direct measures of fat as well as composite measures of fat, muscle mass (mid-upper arm and mid-thigh circumference) bone and pelvis.

2) Taller women were significantly heavier: They were fatter (seen mainly with the suprailiac skin-fold), had bigger pelvises, increased muscle mass and head circumference.

3) BMI is used as a measure of fatness. It did reflect direct measures of fat but was also negatively correlated with height and was therefore less specific than direct fat measurements.

Table 3.11: Correlation coefficients between maternal anthropometric variables, n=832, (*p < 0.05 **p < 0.01)

	Weight	Height	BMI	Circumferences			Skinfold thicknesses					External pelvic diameters			Fat Mass	% Body fat	Muscle mass	Arm-muscle area
				Head	Mid-Arm	Mid-Thigh	Triceps	Biceps	Sub-scapular	Supra-iliac	SS/TR	Inter-cristal	Inter-spinous	External conjugate				
Weight	**1.00**																	
Height	0.36**	**1.00**																
BMI	0.90**	-0.09**	**1.00**															
Head	0.49**	0.31**	0.38**	**1.00**														
Mid-arm	0.86**	0.08*	0.88**	0.38**	**1.00**													
Mid-thigh	0.88**	0.10**	0.89**	0.37**	0.83**	**1.00**												
Triceps	0.78**	0.05	0.80**	0.32**	0.83**	0.76**	**1.00**											
Biceps	0.70**	0.05	0.72**	0.28**	0.76**	0.69**	0.81**	**1.00**										
Subscapular	0.73**	0.03	0.76**	0.28**	0.76**	0.70**	0.82**	0.75**	**1.00**									
Suprailiac	0.72**	0.16**	0.69**	0.33**	0.68**	0.66**	0.76**	0.68**	0.76**	**1.00**								
SS/TR	-0.12**	-0.04	-0.11**	-0.08*	-0.17**	-0.15**	-0.37**	-0.15**	0.24**	-0.06	**1.00**							
Intercristal	0.65**	0.27**	0.57**	0.25**	0.58**	0.54**	0.48**	0.44**	0.48**	0.49**	-0.04	**1.00**						
Interspinous	0.48**	0.26**	0.38**	0.18**	0.40**	0.36**	0.31**	0.30**	0.32**	0.33**	-0.01	0.88**	**1.00**					
Ext. conjugate	0.56**	0.22**	0.50**	0.22**	0.49**	0.48**	0.45**	0.41**	0.43**	0.44**	-0.05	0.83**	0.82**	**1.00**				
Fat mass	0.93**	0.22**	0.89**	0.43**	0.87**	0.85**	0.89**	0.82**	0.88**	0.87**	-0.08*	0.60**	0.42**	0.54**	**1.00**			
% Body fat	0.80**	0.09**	0.81**	0.34**	0.82**	0.76**	0.91**	0.85**	0.92**	0.92**	-0.05	0.52**	0.42**	0.48**	0.96**	**1.00**		
Muscle mass	0.42**	0.27**	0.32**	0.25**	0.49**	0.32**	-0.02	0.12**	0.13**	0.07	0.23**	0.33**	0.28**	0.24**	0.26**	0.09**	**1.00**	
Arm muscle area	0.35**	0.05	0.35**	0.19**	0.50**	0.31**	-0.03	0.12**	0.12**	0.03	0.25**	0.28**	0.23**	0.20**	0.22**	0.07*	0.98**	**1.00**

4) Head circumference showed strong, positive correlations with the other anthropometric variables apart from the ratio SS/TR with which it was negatively correlated. Interestingly, head circumference had a far stronger correlation than height with fat mass (r=0.43 cf. r=0.22).

5) Mid-upper-arm and mid-thigh circumferences correlated with all the other variables, probably reflecting the different components (fat, muscle and bone) included in their measurements. Mid-upper-arm circumference is also used in the calculation of muscle mass and would therefore be expected to correlate with it.

6) The skin-fold measurements correlated well with each other. Only the subscapular and triceps skin-folds were related to muscle mass (triceps is subtracted in the calculation of muscle mass). The suprailiac skin-fold was related positively to height. All four skin-fold measurements were strongly and positively correlated to the external pelvic diameters (weakest correlations were with the interspinous diameter).

7) The ratio SS/TR is a measure of central fatness in the non-pregnant population. During pregnancy central skin-folds increase to a greater extent than peripheral skin-folds and the ratio of SS/TR may be describing the amount of fat gained. It correlated positively with muscle mass and arm muscle area but rather weakly and negatively with other variables apart from those directly included in its calculation. Women who were more centrally fat had perhaps gained more fat during the pregnancy. They tended to be lighter, with less total fat mass and smaller head circumferences.

8) The external pelvic diameters correlated strongly and positively with all the other variables (apart from SS/TR) and with each other. They were more strongly correlated with body fat than with height or muscle. For measures of muscle and skeleton, the external conjugate was the weakest. For measures of fat and head, the interspinous diameter was the weakest.

9) Maternal fat mass was most strongly correlated with weight, which is used in its calculation. It was strongly and positively correlated with BMI, mid-arm and mid-thigh circumferences and all four skin-fold thicknesses ($r>0.8$ for all). It showed strong correlations with the external pelvic diameters and head circumference ($r>0.4$ for all) and was less strongly correlated with height and muscle mass ($r>0.2$ for both). There was a weak negative correlation with the ratio SS/TR discussed previously. Percentage body fat showed similar though weaker relationships than those seen with fat mass, apart from those with the four skin-folds, which were stronger.

10) Muscle mass correlated strongly and positively with all the maternal measurements apart from the triceps and suprailiac skin-folds. It was strongly correlated with weight and also with height, which is involved in its calculation. Arm-muscle-area showed similar correlations as for muscle mass except that there was no correlation with height.

3.2.8.5 The External Pelvic Diameters

One aim of this study was to determine whether measurement of the external pelvic diameters could be used as an indirect measure of maternal body fat.

All three external pelvic diameters correlated positively and significantly with height, muscle and body fat but the strongest correlations were with fat (Table 3.12). This remained true whether absolute fat mass was used, skin-fold thickness measurements or percentage body fat.

Table 3.12: Pearson correlation coefficients are shown for relationships of the external pelvic diameters with height, muscle mass, arm-muscle area (AMA), and measures of fat (n=832).

External Pelvic Diameters	Height	Muscle mass	AMA	Fat mass	% Body fat	Suprailiac skin-fold
Intercristal	0.27	0.33	0.28	0.60	0.52	0.49
Interspinous	0.26	0.28	0.23	0.42	0.35	0.33
External conjugate	0.22	0.24	0.20	0.54	0.48	0.44

all correlations are significant at p<0.01

The stronger relationships of the pelvic diameters with fat rather than with height are shown again in Table 3.13 using the intercristal diameter and the maternal fat mass.

Table 3.13: Mean intercristal diameter (cm) according to maternal height (cm) and fat mass (kg) in quarters. P value for trend calculated within each group. *(Number of women in parenthesis)*

	Height (cm)				
Fat mass (kg)	**<151**	**-154**	**-158**	**>158**	**p for trend**
<13.6	23.4 (67)	24.0 (64)	24.0 (40)	24.4 (37)	**0.01**
-17.7	24.6 (57)	24.8 (54)	25.0 (50)	25.5 (47)	**0.02**
-22.0	26.0 (52)	25.7 (47)	26.3 (55)	26.8 (54)	**0.02**
>22.0	27.4 (30)	27.2 (44)	27.6 (66)	28.4 (68)	**0.009**
p for trend	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	

3.3 Women who delivered at HMH

Of the 832 women who participated in the study, 676 (81.2%) went on to deliver their babies at HMH. Those who did not, were younger (median age 21 cf. 23, p<0.001), tended to be from the lower social classes (30.8% were from social classes 4 and 5 cf. 20%, p<0.001) and from a Hindu background (68.6% cf. 57.1%, p=0.007). There was no significant difference in parity between the groups (p=0.08).

These differences may reflect the fact that the women least likely to deliver in the hospital are those living on the outskirts of the city, furthest from the hospital. These districts are predominantly Hindu, relatively rural and less wealthy and the women from them are more likely to marry at a younger age.

3.3.1 Babies born at HMH

Of the 676 babies born at HMH, 330 (48.8%) were male and 346 (51.2%) female. The range of gestation at delivery was 29 to 44 weeks. There were eight, macerated still-births, one of which was anencephalic, the other seven unexplained, born at gestations between 31 and 40 weeks. There was one intrapartum death due to birth asphyxia, which occurred during a breech delivery in a 31-week infant. Thirteen babies (2.0%) were born alive with major congenital anomalies (Table 3.14), five of whom were excluded from further analysis. A further 65 babies were born prematurely (<37 weeks) i.e. 9.7% of live births, and for the purpose of anthropometric analysis we excluded them also. The anthropometric characteristics of the remaining 597 babies are shown in Table 3.15. Variables were adjusted for gestational age at birth using 40 weeks gestation as the reference point.

Table 3.14: Congenital malformations and gestation at birth (weeks) in 676 singleton births. (n = number of babies with the condition)

Type of congenital malformation	n	Gestation (wks)
1. cleft lip and palate alone	1	40
2. *cleft lip and palate as part of a syndrome	1	38
3. bilateral talipes alone	1	38
4. *bilateral talipes as part of a syndrome	2	40 / 40
5. *retrognathia/extended neck ? syndrome	1	40
6. *rocker bottom feet/absent talus/deformed upper limbs	1	39
7. bilateral hydroceles	1	40
8. scrotal hypospadias	1	34
9. cystic hygroma	1	37
10. haemangioma	1	39
11. congenital heart disease (VSD)	2	34 / 39
Total	**13**	

* *denotes babies whose anthropometric details were not included in later analysis.*

Male babies were significantly larger than females in terms of birthweight, head circumference and length (both crown-heel and crown-rump). Chest and mid-upper arm circumferences appeared to be greater in males but this did not reach statistical significance. The abdominal circumference was the same for both sexes. Female babies had bigger skin-folds, ponderal indices and placentae than the males, although these differences were not statistically significant. The difference in the subscapular skin-fold was of borderline significance.

Table 3.15: Description of babies born alive at term excluding those with major congenital anomalies (n=597).

*Means (*geometric means for logged variables) and SD (* interquartile range for logged variables) shown in brackets. P values for differences in the means were calculated from the Student's t-test. Values shown are unadjusted for gestation.*

Neonatal Anthropometric Variables	**Male** (n=291)	**Female** (n=306)	**p** for Difference in means	**All** (n=597)
Birthweight (kg)	2.956	2.866	0.009	2.910 (0.42)
Head circumference (cm)	34.2	33.6	<0.001	33.9 (1.32)
Abdomen (umbilicus) (cm)	30.0	30.0	0.8	30.0 (1.97)
Chest (xiphisternum) (cm)	32.1	32.0	0.4	32.0 (1.71)
MUAC (cm)	10.4	10.3	0.2	10.4 (0.93)
CHL (cm)	49.2	48.6	0.001	48.9 (2.16)
CRL (cm)	32.2	31.9	0.006	32.0 (1.71)
Triceps SFT (mm)*	4.2	4.3	0.1	4.1 (3.6, 4.8)
Subscapular SFT (mm)*	4.4	4.6	0.07	4.4 (3.9, 4.9)
P I (kg/m^3)	24.8	25.0	0.6	24.9 (2.76)
Placenta (g)*	415.9	416.4	0.9	407.4 (355.0, 465.0)

MUAC=mid-upper-arm circumference
CHL=crown-heel length
CRL=crown-rump length
PI=ponderal index
SFT=skin-fold thickness

3.3.2 Relationships between maternal and neonatal anthropometry

Scatter plots were used to visualise relationships between maternal and neonatal anthropometric variables and to look for non-linear associations. None were found. Pearson correlation coefficients between variables are shown in Table 3.16, and the following observations made:

1. Maternal weight correlated more strongly with birthweight than any other maternal measurement. Weight was significantly positively correlated with all the neonatal measurements except leg length. Lighter mothers had babies with an increased head to abdomen ratio, which may indicate a degree of brain sparing.

2. Maternal height was significantly, positively correlated with the crown-heel length of the baby, less so with the crown-rump length and not at all with leg length. There was some correlation with neonatal chest and abdominal circumference but none with head circumference, ponderal index, skin-fold thickness or placenta.

3. BMI was strongly correlated with birthweight and showed similar, though weaker correlations as for weight.

4. Maternal head circumference correlated strongest with neonatal head circumference although it was significantly positively related to all the neonatal measurements apart from leg length.

5. Maternal mid-upper-arm and mid-thigh circumferences had their strongest relationships with birthweight, although both continued to have strong, positive relationships with neonatal mid-upper-arm circumference, chest circumference, skin-fold thicknesses, ponderal index and placenta. There was also a relationship with length, which was stronger than the relationship of maternal height to length. There was a significant, negative correlation with the head to abdomen ratio.

Table 3.16: Correlation coefficients between maternal and neonatal anthropometric variables (adjusted for gestation), n=597. (*p < 0.05, **p < 0.01)

Maternal variables

Neonatal variables	weight	height	BMI	Circumferences			Skin-fold thicknesses					External pelvic diameters			fat mass	% body fat	muscle mass	arm-muscle area
				head	mid-arm	mid-thigh	triceps	biceps	sub-scapular	supra-iliac	SS/TR	inter-cristal	inter-spinous	external conjugate				
birthweight	0.36**	0.09*	0.35**	0.19**	0.26**	0.27**	0.22**	0.21**	0.17**	0.19**	-0.09*	0.23**	0.18**	0.15**	0.30**	0.21**	0.14**	0.12**
head circ.	0.28**	0.07	0.26**	0.22**	0.19**	0.18**	0.15**	0.11**	0.10**	0.14**	-0.08	0.17**	0.13**	0.12**	0.22**	0.14**	0.14**	0.14**
chest circ.	0.33**	0.11**	0.30**	0.17**	0.25**	0.23**	0.19**	0.18**	0.17**	0.18**	-0.05	0.23**	0.20**	0.15**	0.27**	0.20**	0.15**	0.13**
abdo. circ.	0.28**	0.10*	0.25**	0.18**	0.19**	0.20**	0.16**	0.16**	0.12**	0.15**	-0.08	0.18**	0.15**	0.14**	0.22**	0.16**	0.09*	0.07
mid-arm circ.	0.28**	0.03	0.28**	0.14**	0.23**	0.22**	0.19**	0.17**	0.16**	0.16**	-0.06	0.16**	0.12**	0.11**	0.24**	0.18**	0.10*	0.10*
crown-heel	0.23**	0.12**	0.18**	0.18**	0.12**	0.15**	0.10*	0.09*	0.07	0.12**	-0.05	0.14**	0.12**	0.10*	0.17**	0.10**	0.08	0.05
crown-rump	0.26**	0.10*	0.23**	0.12**	0.14**	0.19**	0.09*	0.08	0.07	0.13**	-0.05	0.17**	0.12**	0.10*	0.19**	0.11**	0.14**	0.12**
leg length	0.02	0.05	0.00	0.02	0.01	-0.00	0.03	0.04	0.03	0.01	-0.02	-0.00	0.04	0.03	0.02	0.02	-0.05	-0.06
triceps	0.29**	0.03	0.29**	0.15**	0.25**	0.23**	0.24**	0.21**	0.19**	0.19**	-0.10*	0.18**	0.13**	0.16**	0.26**	0.22**	0.08	0.07
subscapular	0.27**	0.02	0.27**	0.06	0.22**	0.24**	0.21**	0.19**	0.19**	0.16**	-0.05	0.16**	0.11**	0.15**	0.24**	0.19**	0.07	0.07
P.I.	0.20**	-0.03	0.22**	0.10*	0.18**	0.16**	0.14**	0.15**	0.12**	0.11**	-0.05	0.12**	0.07	0.08	0.18**	0.14**	0.10*	0.11**
placenta	0.29**	0.07	0.27**	0.17**	0.20**	0.22**	0.18**	0.15**	0.12**	0.13**	-0.10*	0.17**	0.14**	0.15**	0.23**	0.15**	0.13**	0.12**
head/abdo.	-0.14**	-0.08	-0.11**	-0.06	-0.09*	-0.11**	-0.09*	-0.11**	-0.07	-0.08*	0.04	-0.09*	-0.08*	-0.07	-0.11**	-0.09*	-0.01	0.01

6. Maternal skin-folds had their strongest correlations with birthweight and measures of neonatal fat, i.e. the skin-fold thicknesses and the ponderal index. Maternal triceps skin-fold was the strongest correlator. Interestingly, the suprailiac skin-fold correlated more strongly than any other skin-fold with length. Again, there was a negative correlation with the head to abdomen ratio, suggesting that thinner mothers have babies who undergo a degree of brain-sparing during their intra-uterine growth.

7. Correlations with the maternal ratio SS/TR were all weak and negative except for that with the head/abdomen ratio, which was positive. Relationships that reached significance were with birthweight (r=-0.09), neonatal triceps and placental weight (r=-0.1 for both), suggesting that mothers who were more centrally fat gave birth to lighter, less fat babies with smaller placentas.

8. The external pelvic diameters showed similar relationships with all the neonatal anthropometric variables but the intercristal diameter had the strongest relationships throughout. It correlated with all the neonatal variables but was strongest with birthweight and chest circumference. There was a negative relationship with the head to abdomen ratio although this was small.

9. Maternal fat mass had similar relationships to the individual skin-fold measurements except that it was also positively related to length. Percentage body fat had similar but weaker relationships to all the neonatal measurements as those seen with fat mass.

10. Muscle mass was related to birthweight, head circumference, chest circumference more than abdominal circumference and crown-rump length but not crown-heel. It was weakly correlated with the ponderal index but not with the other measures of neonatal fat. Arm-muscle area showed similar but weaker relationships throughout.

In an attempt to look at individual components of maternal weight, which are intercorrelated, independently of each other, I divided maternal weight into four measurable components: height, head circumference, body fat and muscle. For this model I have used percent body fat rather than the calculated fat mass because unlike fat mass, percent body fat is not dependent on weight for its calculation. Similarly, I have used arm-muscle-area rather than muscle mass because unlike muscle mass, arm-muscle-area is not dependent on height for its calculation. Multiple regression analyses were performed in order to examine the independent effects of each component of maternal weight on neonatal anthropometry (Table 3.17). Adjustment was made for neonatal gestation and sex and for maternal parity. Maternal age did not independently predict neonatal size and adjusting for social class did not alter the relationships shown.

From these analyses, maternal height (skeleton) predicted the length of the baby, crown-heel more than crown-rump, although both were very similar. Maternal head circumference predicted birthweight, neonatal head circumference ($p<0.001$), abdominal circumference and placental weight. Maternal body fat was strongly predictive of all the neonatal variables apart from leg length and maternal arm-muscle area predicted head circumference, chest circumference, crown-rump but not crown-heel length, ponderal index or placental weight.

Table 3.17: Multiple linear regression analysis of mothers' height, head circumference, % body fat and arm muscle area (simultaneously) on their babies' anthropometric measurements at birth, using live, term, normal babies (n=597).
Gestational age, sex and maternal parity were included in all analyses.

Neonatal Measurements	Maternal Measurements							
	Height (cm)		Head circumference (cm)		Percent body fat (%)		Arm muscle area (mm^2)	
	β	p	β	p	β	p	β	p
Birthweight (g)	*3.4*	**0.3**	*29.4*	**0.02**	*13.5*	**< 0.001**	*6.7*	**0.06**
Head circ. (cm)	*0.002*	**0.8**	*0.1*	**< 0.001**	*0.02*	**0.006**	*0.02*	**0.02**
Abdominal circ. (cm)	*0.02*	**0.1**	*0.1*	**0.02**	*0.04*	**0.002**	*0.01*	**0.4**
Chest circ. (cm)	*0.02*	**0.1**	*0.08*	**0.1**	*0.05*	**< 0.001**	*0.03*	**0.03**
Mid-upper arm circ.(cm)	*- 0.0004*	**0.96**	*0.05*	**0.09**	*0.03*	**< 0.001**	*0.01*	**0.1**
Crown-heel length (cm)	*0.04*	**0.03**	*0.07*	**0.2**	*0.04*	**0.02**	*0.01*	**0.6**
Crown-rump length (cm)	*0.03*	**0.06**	*0.06*	**0.2**	*0.03*	**0.03**	*0.04*	**0.02**
Leg length (cm)	*0.02*	**0.3**	*0.01*	**0.8**	*0.01*	**0.4**	*- 0.02*	**0.07**
Triceps SFT* (mm)	*- 0.00008*	**0.96**	*0.01*	**0.08**	*0.008*	**< 0.001**	*0.001*	**0.5**
Subscapular SFT* (mm)	*0.0009*	**0.6**	*- 0.003*	**0.6**	*0.007*	**< 0.001**	*0.002*	**0.3**
Ponderal Index (kg/m^3)	*- 0.03*	**0.2**	*0.1*	**0.1**	*0.06*	**0.007**	*0.05*	**0.05**
Placental weight* (g)	*0.001*	**0.5**	*0.01*	**0.03**	*0.005*	**0.001**	*0.004*	**0.03**

**denotes logged variables*
circ.= circumference
SFT = skin-fold thickness

3.4 Population Comparisons

I compared the mothers and babies from this study in Mysore (an urban Indian population), with those studied in the Pune Maternal Nutrition Study [144] (a rural Indian population) and in the Princess Anne Study, Southampton, UK [145] (Table 3.18). Mothers from Pune were much lighter and less fat (smaller skin-folds) than mothers from Mysore, although the ratio SS/TR was the same. The Pune mothers were shorter by approximately three centimetres. The Southampton mothers were far heavier and were taller than the Mysore mothers. Their triceps skin-fold was increased in comparison but their subscapular skin-fold reduced, producing a ratio of SS/TR less than that found in both Indian populations, suggesting that Indians, both urban and rural, are more centrally fat, or gain more fat centrally during their pregnancies.

Pune babies were lighter, shorter, had smaller head circumferences, mid-upper arm circumferences, chest circumferences and placentas than Mysore babies who were similarly smaller in all these measurements to Southampton babies. Skin-fold measurements were not made in the Southampton babies in this study. Pune babies had smaller skin-folds and were less fat than Mysore babies.

Using the same Southampton values along with a subscapular skin-fold value from another Southampton study (T. Wheeler, personal communication) as a reference, SD scores were calculated on Pune and Mysore mother and baby measurements and shown graphically in Figure 3.4. All the scores were below the Southampton mean, with Pune scores further from the Southampton values than Mysore scores. Interestingly, neonatal subscapular scores were closer to the mean than any other neonatal measurement suggesting a degree of fat-sparing in Indian babies. In Mysore babies, the mid-upper-arm circumference was furthest from the Southampton mean, suggesting comparatively less muscle in these babies.

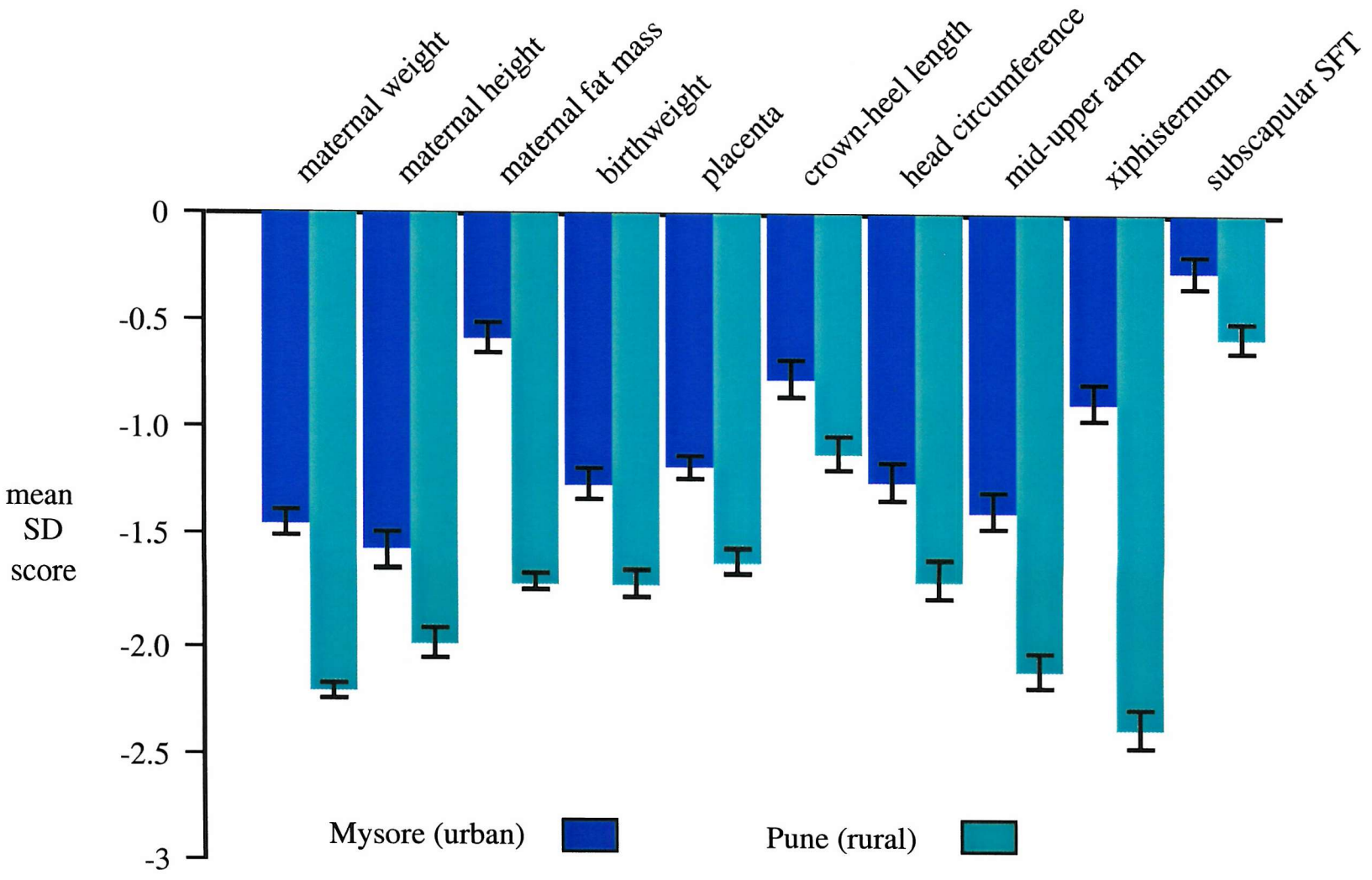
Table 3.18: Comparison of Mysore urban (HMH) mothers and babies with Pune rural (PMNS)[144] and Southampton, UK (Wellbeing)[145] mothers and babies using term babies only.

Maternal measurements made at 30+/-2 weeks gestation. Medians and inter-quartile ranges given. P values for differences in medians calculated using Mann-Whitney 'U' Test: p(MP)=differences between Mysore and Pune, p(MS)=differences between Mysore and Southampton.

	p (MP) Values	Pune (PMNS) n=633	Mysore (HMH) n=597	Southampton (Wellbeing) n=519	p (MS) values
MOTHERS					
Weight (kg)	<0.001	**47.0** (43.7, 50.4)	**55.0** (49.5, 62.0)	**71.8** (64.6, 80.4)	<0.001
Height (cm)	<0.001	**152.0** (148.5,155.5)	**154.5** (151.0,158.1)	**163.0** (160.0,167.8)	<0.001
BMI (kg/m^2)	<0.001	**20.3** (19.2, 21.6)	**23.2** (21.0,25.9)	**26.5** (24.3, 30.0)	<0.001
Triceps (mm)	<0.001	**9.1** (7.1, 11.4)	**17.0** (12.3, 24.6)	**19.5** (15.4, 24.7)	<0.001
Subscapular (mm)	<0.001	**13.0** (10.1, 15.9)	**24.8** (17.9, 33.7)	**17.7** (13.3, 25.1)	<0.001
SS/TR	0.5	**1.4** (1.2, 1.6)	**1.4** (1.2, 1.7)	**1.0** (0.8, 1.1)	<0.001
BABIES					
Birthweight (kg)	<0.001	**2.7** (2.5, 2.9)	**2.9** (2.6, 3.2)	**3.5** (3.2, 3.7)	<0.001
CHL (cm)	<0.001	**47.7** (46.5, 49.2)	**48.8** (47.7, 50.1)	**49.9** (48.5, 51.3)	<0.001
PI (kg/m^3)	0.006	**24.4** (23.0, 25.8)	**24.8** (23.2, 26.3)	**27.7** (26.3, 29.1)	<0.001
Head circ.(cm)	<0.001	**33.1** (32.2, 34.0)	**34.0** (33.1, 34.8)	**35.0** (34.1, 35.9)	<0.001
TSFT (mm)	0.9	**4.2** (3.6, 4.6)	**4.1** (3.6, 4.8)	Not measured	
SSFT (mm)	<0.001	**4.2** (3.6, 4.6)	**4.3** (3.9, 4.9)	Not measured	
Chest circ. (cm) (xiphisternum)	<0.001	**29.7** (28.5,30.8)	**32.1** (31.0, 33.1)	**33.4** (32.4, 34.6)	<0.001
Abdominal circ. (cm) (umbilicus)	<0.001	**28.7** (27.5, 29.9)	**30.0** (28.8, 31.2)	Not measured	
MUAC (cm)	<0.001	**9.7** (9.1, 10.3)	**10.3** (9.8, 11.0)	**11.6** (11.0, 12.2)	<0.001
Placenta (kg)	<0.001	**0.36** (0.31, 0.41)	**0.41** (0.36, 0.47)	**0.56** (0.48, 0.64)	<0.001

Note: Circ.=circumference, CHL=crown-heel length, PI=ponderal index, MUAC=mid-upper-arm circumference, TSFT=triceps skin-fold thickness, SSFT=subscapular skin-fold thickness.

Fig.3.4: Maternal and Neonatal SD scores (relative to mothers and babies in Southampton)
Full term babies only



3.5 Paternal effects on neonatal size

Of the 597 term deliveries, 496 (83.1%) fathers were available to be weighed, have their height measured and their age recorded. Mean weight was 64.4 kg (SD=10.5), height was 167.2 cm (SD=6.2), BMI was 23.0 kg/m^2 (SD=3.4) and age was 31.5 years (SD=4.7). As expected, age, height and weight of husbands significantly correlated with that of their wives, evidence of assortive mating (r=0.6 for age, r=0.3 for height and weight: $p<0.001$ for all).

In order to determine the independent effects of paternal height and BMI on neonatal size, multiple linear regression was used (Table 3.19). Effects of maternal and paternal height and BMI were looked at individually and then paternal height and BMI after adjusting for maternal height and BMI. From these analyses, the following observations were made:

1. Father's height was a significant predictor of birthweight, crown-heel, crown-rump and leg length. The regression coefficients (β) and p values indicated that in general, paternal effects on birth size were weaker than maternal effects. Striking exceptions were with crown-heel and leg length where the relationship with paternal height was greater than that with maternal height. Interestingly, the reverse was true for crown-rump length, suggesting that the paternal effect on length was due to increased limb length. Probably because of paternal effects on length, ponderal index was inversely related to parental height, more strongly with paternal.

2. Father's BMI was a significant predictor of all the birth measurements except crown-heel and leg length. The regression coefficients and p values indicated that paternal effects were considerably weaker than maternal effects (β=19.2g/kgm^{-2} cf. 40.8g/kgm^{-2} , for birthweight).

3. Adjusting for maternal height and BMI reduced the paternal effects considerably, suggesting that they were partly mediated by 'assortive mating'. The effect of paternal height on crown-heel and leg length remained strongly

Table 3.19: Multiple linear regression analysis of 1). Maternal height and BMI 2). Paternal height and BMI and 3). Paternal height and BMI adjusted for maternal height and BMI with 496 full-term, neonates' anthropometric measurements.
Gestational age, sex and maternal parity were included in all analyses.

Babies Measurements	1. Mother				2. Father				3. Father adjusted for mother			
	Height (cm)		BMI (kg/m^2)		Height (cm)		BMI (kg/m^2)		Height (cm)		BMI (kg/m^2)	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Birthweight (g)	7.8	***0.01***	40.8	***<0.001***	6.9	***0.02***	19.2	***<0.001***	5.7	***0.06***	10.7	***0.04***
Head circ. (cm)	0.02	***0.05***	0.1	***<0.001***	0.01	***0.1***	0.06	***<0.001***	0.01	***0.2***	0.04	***0.008***
Abdo. circ. (cm)	0.04	***0.006***	0.1	***<0.001***	0.001	***0.9***	0.08	***0.001***	- 0.009	***0.5***	0.06	***0.02***
Chest circ. (cm)	0.04	***0.002***	0.1	***<0.001***	0.01	***0.2***	0.07	***0.001***	0.006	***0.6***	0.04	***0.04***
MUAC (cm)	0.007	***0.3***	0.7	***<0.001***	0.008	***0.2***	0.04	***0.001***	0.006	***0.4***	0.04	***0.03***
CHL (cm)	0.05	***0.002***	0.1	***<0.001***	0.06	***<0.001***	0.04	***0.1***	0.005	***0.002***	0.02	***0.5***
CRL (cm)	0.04	***0.005***	0.1	***<0.001***	0.02	***0.04***	0.06	***0.005***	0.02	***0.2***	0.04	***0.07***
Leg length (cm)	0.02	***0.2***	0.001	***0.9***	0.03	***0.007***	- 0.02	***0.3***	0.03	***0.01***	- 0.02	***0.2***
TSFT (mm)	0.002	***0.3***	0.02	***<0.001***	0.001	***0.5***	0.009	***0.001***	0.0006	***0.7***	0.006	***0.04***
SSFT (mm)	0.001	***0.4***	0.01	***<0.001***	0.0007	***0.6***	0.007	***0.01***	0.0004	***0.8***	0.003	***0.2***
PI (kg/m^3)	- 0.01	***0.6***	0.2	***<0.001***	- 0.02	***0.4***	0.1	***0.006***	- 0.02	***0.5***	0.07	***0.07***
Placenta (g)	0.003	***0.04***	0.02	***<0.001***	0.003	***0.07***	0.008	***0.004***	0.002	***0.2***	0.004	***0.1***

CHL = crown-heel length, CRL = crown-rump length, TSFT = triceps skin-fold thickness, SSFT = subscapular skin-fold thickness, PI = ponderal index

statistically significant. The relationship of paternal BMI remained strongly significant for head circumference and significant for birthweight, abdominal, chest and mid-arm circumferences and the triceps skin-fold.

Adjusting for social class did not significantly alter the relationships shown.

3.6 Summary of main findings

1. Social class, age and parity all influenced maternal body composition. Women from lower social classes were both shorter and thinner (due to less body fat) than women from higher social classes. They also tended to be younger and of higher parity. Older women were heavier than younger women due mainly to increased body fat, and this remained true following adjustment for social class. Multiparous women were heavier than primiparous women due to an increase in muscle, and this remained true after adjusting for social class and age.

2. Measurements of the external pelvic diameters correlated significantly and positively with maternal height, muscle and body fat but were strongest with measures of fat.

3. Maternal body composition was related to neonatal body composition. Maternal weight was the strongest correlator with birthweight and this appeared mainly due to maternal body fat. Direct and indirect measures of maternal fat correlated well with measures of neonatal fat and had a negative relationship with the head/abdomen ratio, suggesting that thinner (less fat) mothers have babies who undergo a degree of brain-sparing during their intra-uterine growth. Height correlated most strongly with crown-heel length and maternal head circumference with neonatal head circumference.

4. Comparison of mothers and babies of three different populations (Pune, Mysore and Southampton) showed that mothers and babies born in rural India are shorter and lighter (less fat) than those born in urban India, who are in turn, shorter and lighter than mothers and babies in Southampton. Indian mothers are more centrally fat than Southampton mothers, and their babies show evidence of fat-sparing, possibly at the expense of their muscle mass.

5. In order to determine the independent effect of the father on neonatal size, maternal height and BMI were controlled for. The results of these analyses show that paternal height and BMI have significant positive effects on fetal growth, the strongest of these being with paternal height and neonatal crown-heel and leg length. The effects on birthweight and other measures of neonatal size are however much smaller than the effects seen with maternal height and BMI.

3.7 Discussion

In this chapter I have shown that social class, age and parity all influence maternal size and body composition. By comparing mothers from different populations it is evident that rural Indian mothers are shorter and thinner than mothers in urban India who are in turn shorter and thinner than mothers in the UK. Despite having lower body mass indices, Indian mothers (both rural and urban) were more centrally fat than UK mothers. Central obesity has been linked to insulin resistance and has been described before in Indian populations.[39] However, its significance in pregnancy is unclear due to the fact that mothers tend to gain fat centrally rather than peripherally during pregnancy[135, 136] and therefore a larger SS/TR ratio may indicate a higher fat gain.

The mean birthweight in India is 2.7 kg,[97, 146] similar to that found in Pune (rural India),[144] and slightly lower than in Mysore (2.9 kg). Compared with Southampton babies, Indian babies were significantly smaller. There was however a pattern to their smallness; Indian babies were relatively fat sparing, suggesting that subcutaneous fat

accumulated possibly at the expense of muscle during intra-uterine development. The observation that low birthweight and specific neonatal phenotypes are associated with increased risk of developing adult disease[147] has given new purpose to understanding the determinants of fetal growth. India's poor fetal growth is at least partly caused by maternal chronic energy deficiency and stunting,[99] implying that poor maternal nutrition may well underlie these associations with disease in later life.

Although it is well known that maternal nutrition, as assessed by pre-pregnancy weight, height and gestational weight gain predict birthweight,[69, 98] few investigators have examined the relationships between individual components of maternal body composition and the detailed phenotype of the baby at birth. Maternal weight in this study and in a similar study [148] correlated not only with birthweight, but also with other measures of neonatal size such as length, head circumference, skin-folds and mid-upper-arm circumference. Maternal weight can be thought of as a composite of the mother's own intra-uterine, infant, childhood and pubertal growth as well as her energy and protein balance in adult life. Her nutritional experiences at these different times are reflected in her head circumference (intra-uterine and infancy), height (childhood and puberty) and fat and muscle mass (adult energy and protein balance). For this reason, I used a 'four compartment' model, similar to that used in the Pune Maternal Nutrition Study [144] in order to look at the independent effects of these components of maternal body composition on neonatal size.

From this model, maternal body fat predicted neonatal size to a greater extent than maternal height, head circumference or muscle. The strongest relationships were with measures of neonatal fat such as the skin-folds and the ponderal index and the weakest relationships were with length. These findings agree with those of other studies, which have used either skin-fold measurements or total-body electrical conductivity (TOBEC) to show that fatter mothers give birth to fatter babies.[149-151] A study by Udall et.al.[152] indicated that maternal weight gain during pregnancy was associated with both increased fatness and length in the newborn, while high pre-pregnancy weight for height was associated with fatness independent of length. A large study by Neggers et.al.[148] also found that maternal fat predicted neonatal fat to a greater extent than length.

Maternal height in my study population was an important predictor of neonatal crown-heel length and maternal head circumference of neonatal head circumference but also of birthweight and placental weight. Similar although stronger relationships of maternal head circumference to neonatal size were found in the Pune study.[144] Maternal muscle predicted head and chest circumferences, crown-rump length and placental weight. In a Peruvian urban population, maternal muscle had a greater influence on linear growth of the neonate than maternal fat.[151]

Any contribution by the father to neonatal size must be genetic. Evidence supporting the fact that genes are important in determining size at birth comes partly from studies of gene knockout mice where mutations of genes encoding insulin and insulin-like growth factors (IGF-1 and IGF-11) result in impaired fetal growth,[153] whereas, mutation of the gene encoding the IGF-11 receptor results in fetal overgrowth.[154] Fetal growth retardation in humans can be found associated with mutations at the gene encoding IGF-1,[155] and fetal overgrowth sydromes with over-expression of IGF-2.[156] In addition, fetal growth is affected by mutations at genes which influence insulin action, such as the insulin receptor[157] and glucokinase.[158] Although these mutations are rare, they illustrate the possibility of genetic effects on fetal growth. Recently, the insulin gene (*INS*) VNTR (variable number of tandem repeats) locus, a functional candidate polymorphism, was found to be associated with size at birth in 758 children,[159] although this relationship was not confirmed in a smaller study where it was thought that maternal-uterine restraint and nutritional factors may have had a greater effect.[160]

In my study, effects of the fathers' height and BMI on neonatal size were considerably weaker than those of the mothers, apart from the relationship with length, for which the fathers' height was a stronger predictor than the mothers'. Other studies which have looked at effects of parental size on fetal growth have found positive correlations between paternal height and weight (adjusted for height) and birthweight which are significantly smaller than those of maternal height and weight.[69 161-163] These findings suggest that the mother's size and nutritional status exert strong environmental effects on fetal growth.

Finally, we included measurement of the external pelvic diameters in this study in order to define what they measured. External pelvic measurements were abandoned by obstetricians in the middle of this century when x-ray pelvimetry demonstrated that these measurements bore little relation to the actual size of the bony pelvis and did not sufficiently predict birth outcome.[164] Data relating external diameters are few, but they are known to increase during pregnancy [165] and have been shown to increase with parity and with age, beyond that of skeletal maturity.[49] In this study they were found to have a significant and positive relationship with age but not with parity, possibly because the range of parity in this study was small (0-4).

All three external pelvic diameters correlated positively and significantly with height, muscle mass and body fat (whether measured directly by individual skin-folds or calculated indirectly as fat mass) but the strongest correlations were with fat mass. The correlation with height is contrary to that found in a previous study.[166] The diameter which correlated most strongly with each component of maternal body composition was the intercristal diameter. The fact that the external pelvic diameters show stronger correlations with fat than with height or muscle make it possible to conclude that the external pelvic diameters can be used as an indirect measure of maternal body fat.

4. Results – Glucose and Insulin Data: Relationship to the Mother

4.1 Introduction

Pregnancy itself is an insulin resistant state,[167] and carbohydrate metabolism in the pregnant women is profoundly different to that out-with pregnancy. The increased insulin resistance encountered in pregnancy helps optimise metabolic efficiency and therefore fetal growth. As insulin resistance increases, insulin secretion also increases in order to maintain maternal glucose tolerance.[167, 168] Although most women have the necessary β-cell reserves to meet the extra demands of pregnancy, a minority do not and develop glucose intolerance that is detected for the first time when pregnant.

Gestational diabetes mellitus (GDM) is thought by some to be a pre-diabetic condition, and certainly the original O'Sullivan criteria [123] were formed based on the fact that up to one third of women with GDM would go on to develop type 2 diabetes following their pregnancy. Risk factors associated with GDM are similar to those associated with type 2 diabetes, namely; increasing age, obesity, ethnicity and a family history of diabetes. Higher social class has also been shown to be a risk factor for type 2 diabetes among urban Indian women [47] and may similarly be a risk factor for GDM. More recently associations have been found with short stature [169-172] and low maternal birthweight [173-175] and risk of GDM, suggesting that early life events are important determinants of GDM. One aim of this study was to see if India, with its high proportion of low birthweight babies and childhood stunting and wasting, had evidence that early life undernutrition (short stature, small head circumference) was related to adult β-cell function and GDM prevalence.

In this chapter I have described the glucose and insulin concentrations found in mothers who participated in the study in relation to their age, parity, social class, anthropometry, and family history of diabetes in order to determine the prevalence of GDM in this urban Indian population and associated risk factors.

4.2 Relationship of maternal age, parity and social class to GDM prevalence and glucose and insulin concentrations.

784 women completed the OGTT and 48 (6.1%) were found to have GDM as diagnosed by the Carpenter & Coustan criteria described previously. Women who did not complete the OGTT (n=48) were no different in terms of age, parity and social class. They were however thinner (mean BMI=21.9 kg/m^2 cf. 23.2 kg/m^2: p=0.01), with less muscle (mean muscle mass=13.0 kg cf.13.9 kg: p=0.008) but no difference in fat mass or in height. There were no differences in mean fasting glucose or insulin concentrations in women who completed the OGTT compared with those who did not.

Women with GDM had higher mean glucose and insulin concentrations at all time points except for at 30-minutes when the insulin concentration was lower than in women with normal glucose tolerance, suggesting a reduced insulin response (Table 4.1). Other measures of insulin secretion i.e. HOMA-β and insulin increment were also lower in women with GDM. Measures of insulin resistance: fasting insulin, proinsulin, 32,33-split proinsulin and RIR-HOMA were higher.

4.2.1 Age and Parity

Older mothers were more likely to have GDM (Table 4.2). They had higher plasma glucose concentrations at all time points and higher insulin concentrations at 0, 60, 120 and 180-minutes. RIR-HOMA rose with increasing age but there was no trend with 32,33-split proinsulin. In contrast, HOMA-β tended to fall but there was no corresponding trend with insulin increment (Table 4.3). Older women had reduced insulin secretion (HOMA-β) in relation to their degree of insulin resistance (RIR-HOMA) as calculated by the residual (p<0.001). These patterns remained when women with GDM were excluded from the analysis, although the relationships between age and fasting glucose and RIR-HOMA lost their significance.

Table 4.1: Maternal blood results showing number of samples analysed (N). Comparisons between mothers with normal glucose tolerance and those with GDM were made using the student's t-test.

(Means and SD's are shown for normally distributed variables, geometric means and interquartile ranges (IQR) for logged variables and median and range for categorised variables) * denotes logged variables ** denotes categorised variables

Maternal blood variables	N	Mean / SD (*geometric mean/IQR ** median/range)		Normal OGTT (n=736)	GDM (n=48)	p for difference in means
Fasting	**832**					
Glucose (mmol/l)	832	4.6	(0.6)	4.5	5.6	<0.001
Insulin* (pmol/l)	815	32.1	(22.0, 45.0)	31.3	43.7	<0.001
Proinsulin**(pmol/l)	814	1.4	(<1.25–9.8)	1.4	1.8	0.003
32,33 split-proinsulin* (pmol/l)	814	3.8	(2.4, 5.8)	3.8	5.4	<0.001
RIR-HOMA*	813	1.2	(0.8, 1.6)	1.1	1.8	<0.001
HOMA-β*	798	118.5	(83.4,156.4)	121.1	84.7	<0.001
30 minutes	**825**					
Glucose (mmol/l)	825	7.3	(1.3)	7.2	9.8	<0.001
Insulin* (pmol/l)	821	327.7	(213.0,546.5)	332.4	216.1	<0.001
Insulin increment* (pmol/mmol)	799	63.0	(41.2,111.5)	64.4	34.8	<0.001
60 minutes	**792**					
Glucose (mmol/l)	790	7.2	(2.0)	6.9	11.7	<0.001
Insulin* (pmol/l)	785	333.0	(221.0,554.5)	330.1	389.4	0.1
120 minutes	**790**					
Glucose (mmol/l)	786	6.1	(1.6)	5.8	9.9	<0.001
Insulin* (pmol/l)	777	237.2	(157.0,431.0)	224.7	545.1	<0.001
180 minutes	**788**					
Glucose (mmol/l)	784	5.6	(1.2)	5.4	7.6	<0.001
Insulin* (pmol/l)	774	174.8	(114.0,303.0)	168.0	311.7	<0.001

Table 4.3: Mean maternal plasma glucose and insulin concentrations according to age (in quarters).

Age (years)	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures			
Quarters	**0 mins** n=832	**30 mins** n=825	**60 mins** n=790	**120 mins** n=788	**180 mins** n=784	**0 mins** n=815	**30 mins** n=821	**60 mins** n=785	**120 mins** n=777	**180 mins** n=774	**32,33 split proinsulin** (pmol/l) n=814	**RIR-HOMA** n=813	**HOMA β** n=798	**Insulin increment** (pmol/mmol) n=799
16-20	4.4	7.0	6.4	5.5	5.3	29.4	320.9	284.9	171.1	140.0	3.7	1.03	123.5	63.7
21-23	4.5	7.2	7.0	5.9	5.5	33.3	346.4	349.4	237.2	167.8	4.1	1.19	129.6	65.1
24-26	4.6	7.4	7.4	6.1	5.6	33.7	318.6	344.5	267.0	190.1	3.7	1.20	119.0	61.8
27-40	4.8	7.9	8.1	7.0	6.0	32.8	327.7	375.8	322.0	223.5	4.0	1.24	101.2	61.4
p for trend	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.04	0.98	<0.001	<0.001	<0.001	0.6	0.001	<0.001	0.5
p adjusted for fat mass	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.5	0.1	0.06	<0.001	<0.001	0.1	0.5	<0.001	0.09

Table 4.2: Percentage of women with GDM according to age in quarters

(Number of women in parenthesis)

Age (years) in quarters	% Women with GDM
16 – 20 *(232)*	**0.9** *(2)*
21 – 23 *(186)*	**4.3** *(8)*
24 – 26 *(195)*	**5.6** *(11)*
27 – 40 *(171)*	**15.8** *(27)*
All *(784)*	**6.1** *(48)*
p for trend	**<0.001**

There was no relationship between maternal parity and GDM prevalence, nor did parity relate to any of the individual maternal glucose or insulin concentrations independently of age.

4.2.2 Social Class

GDM prevalence was not significantly related to social class (Table 4.4), although women of higher social class did have a higher prevalence and had higher blood glucose and insulin concentrations at all time points ($p<0.001$ for all) apart from the fasting glucose concentration ($p=0.3$) (Table 4.5). Higher social class was related to increased insulin resistance (32,33-split proinsulin, RIR-HOMA) and secretion (HOMA-β, insulin increment). There was no relationship with the calculated residual. Adjusting for age alone did not significantly alter these relationships. However, following adjustment for maternal fat mass, relationships between social class and maternal glucose concentrations lost their significance, apart from that at 180-minutes, suggesting that women of higher social class may be more glucose intolerant. Relationships with measures of insulin resistance also lost their significance, but those with insulin secretion remained strongly significant, suggesting that women of lower social class have poorer insulin secretion.

Table 4.5: Mean maternal glucose and insulin concentrations according to social class grouping on Kuppuswamy scoring.

Social Class	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures			
	0 mins n=832	**30 mins** n=825	**60 mins** n=790	**120 mins** n=788	**180 mins** n=784	**0 mins** n=815	**30 mins** n=821	**60 mins** n=785	**120 mins** n=777	**180 mins** n=774	**32,33 split proinsulin** (pmol/l) n=814	**RIR-HOMA** n=813	**HOMA β** n=798	**Insulin increment** (pmol/mmol) n=799
1 & 2 (n=319)	4.6	7.6	7.5	6.3	5.7	33.9	348.7	376.7	285.3	212.5	4.1	1.2	121.3	66.9
3 (n=330)	4.6	7.2	6.9	5.8	5.4	32.9	340.4	315.2	210.7	158.7	4.0	1.2	120.9	64.5
4 & 5 (n=183)	4.5	7.2	7.0	6.0	5.4	27.6	273.8	296.0	212.7	147.6	3.2	1.0	109.3	53.9
p for trend	0.3	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.05	<0.001	0.001
p adjusted for age	0.9	0.02	0.03	0.2	0.005	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.006	<0.001	<0.001
p adjusted for age & fat mass	0.3	0.3	0.2	0.7	0.04	0.1	0.001	0.001	0.1	0.001	0.09	0.1	0.1	0.002

Table 4.4: Prevalence of GDM according to social class. The p value for differences in prevalence between social class groups was calculated from a linear-by-linear Chi-Squared test.
(Number of women in parenthesis)

Social Class	% Women with GDM
1 & 2 *(301)*	**7.3** *(22)*
3 *(308)*	**6.2** *(19)*
4 & 5 *(175)*	**4.0** *(7)*
All *(784)*	**6.1** *(48)*
p for difference in prevalence	**0.3**

4.3 Relationship of maternal anthropometric variables to GDM prevalence and glucose and insulin concentrations

4.3.1 Body Fat

Fatter mothers were more likely to have GDM (Table 4.6). They had higher blood glucose and insulin concentrations at all time points, independently of age (Table 4.7). Excluding women with GDM from the analysis did not alter these relationships. When age and fat mass were examined together, GDM prevalence rose with both age and increasing body fat, so that the highest prevalence of the disease was found in the oldest, fattest women. 20% of women in the oldest age group (26-40 years) and highest third of fat mass (>20.2 kg) had GDM (Table 4.8, Fig.4.1A). There was no evidence of interaction between age and fat mass.

There were also strongly significant relationships between fat mass and the calculated values for insulin resistance and secretion. (Table 4.7), suggesting that fatter women were more insulin resistant and had a stronger 30-minute insulin response. Similar trends were seen if fat mass in Table 4.6 was replaced with weight ($p<0.001$), BMI

Table 4.7: Mean maternal plasma glucose and insulin concentrations according to fat mass (in quarters).

Fat mass (kg)	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures			
Quarters	0 mins n=832	30 mins n=825	60 mins n=790	120 mins n=788	180 mins n=784	0 mins n=815	30 mins n=821	60 mins n=785	120 mins n=777	180 mins n=774	32,33 split proinsulin (pmol/l) n=814	RIR-HOMA n=813	HOMA β n=798	Insulin increment (pmol/mmol) n=799
< 13.6	4.4	7.1	6.8	5.8	5.3	23.9	269.3	255.3	173.1	122.1	2.9	0.8	100.2	53.7
-17.7	4.4	7.1	6.8	5.8	5.4	28.9	328.2	313.3	207.8	154.5	3.4	1.0	116.8	66.2
-22.0	4.6	7.4	7.0	6.0	5.6	34.2	367.3	362.9	257.2	200.7	4.4	1.2	119.9	69.0
> 22.0	4.8	7.9	8.0	6.7	5.9	45.0	353.6	417.9	337.7	243.5	5.1	1.7	139.8	64.2
p for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02
p adjusted for age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02

(p<0.001), mid-upper-arm circumference (p<0.001), mid-thigh circumference (p<0.001), any of the pelvic diameters (p<0.001) or any of the individual skin-fold measurements (p<0.001). These trends are likely to reflect maternal body fat, as they become largely non-significant after adjustment for maternal fat mass.

Table 4.6: Percentage of women with GDM according to fat mass (in quarters)
(Number of women in parenthesis)

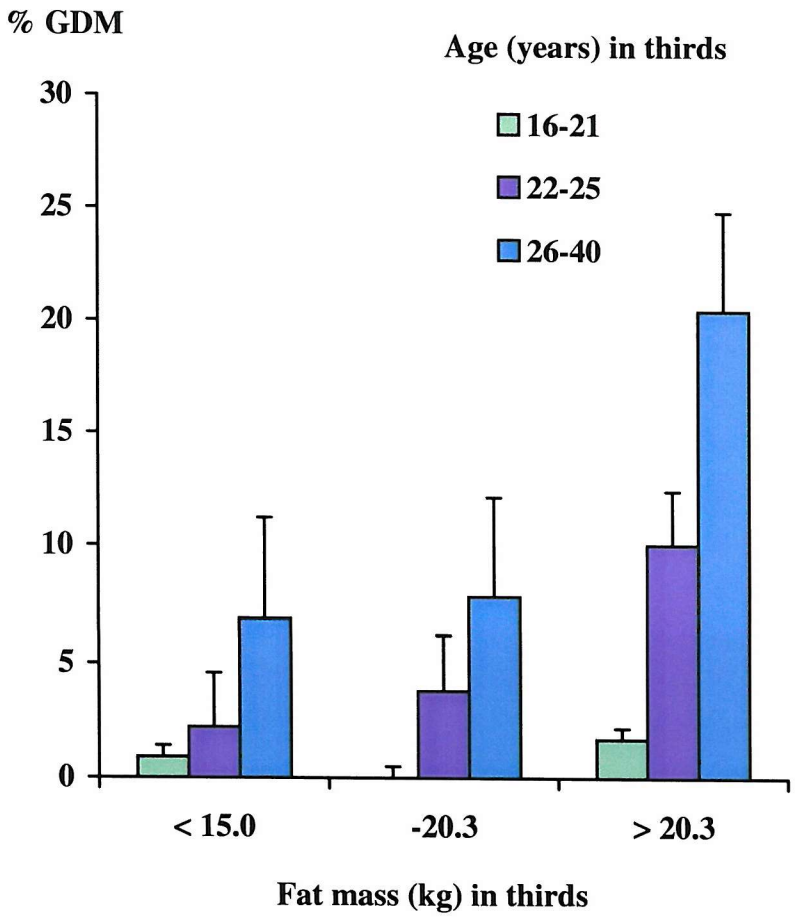
Fat mass (kg) in quarters	% Women with GDM
< 13.6 *(191)*	**2.1** *(4)*
- 17.7 *(195)*	**3.1** *(6)*
- 22.0 *(199)*	**5.5** *(11)*
> 22.0 *(199)*	**13.6** *(27)*
All *(784)*	**6.1** *(48)*
p for trend	**<0.001**
p *adjusted for age*	**<0.001**

Table 4.8: Prevalence of GDM (%) in thirds of age (years) and fat mass
(Number of women in parenthesis)

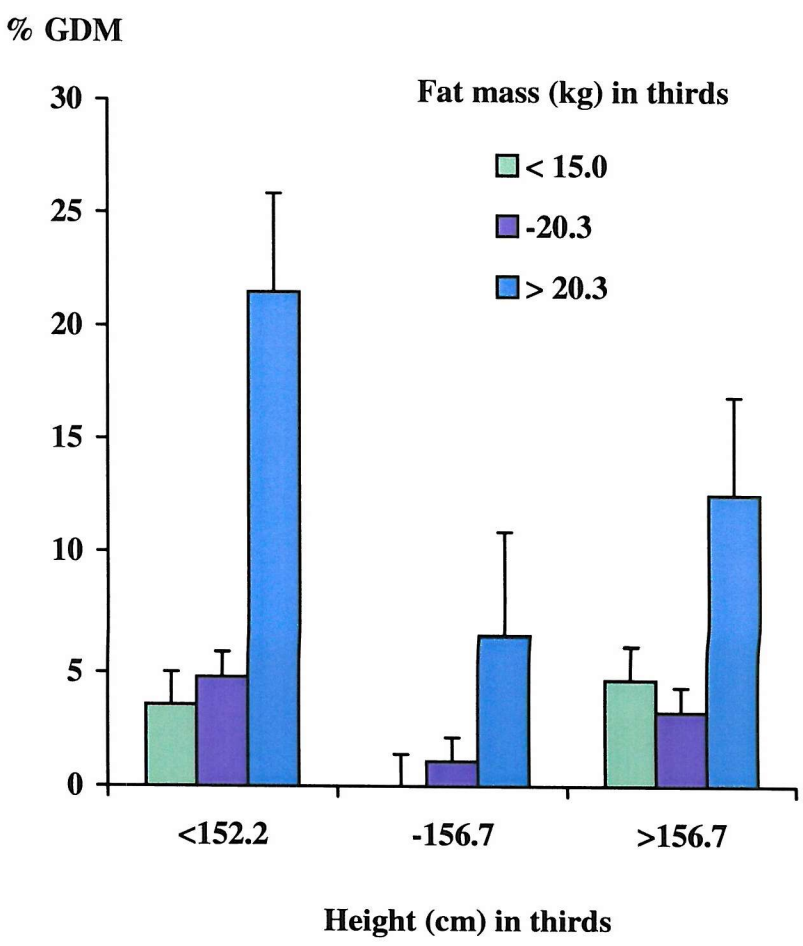
Fat mass / **Age**	**< 14.8**	**- 20.2**	**> 20.2**	**All**
16 – 20	1.1% *(90)*	0% *(99)*	2.3% *(43)*	0.9% *(232)*
21 – 25	1.9% *(106)*	2.9% *(102)*	9.4% *(117)*	4.9% *(325)*
26 – 40	6.9% *(58)*	7.7% *(65)*	**20.2%** *(104)*	13.2% *(227)*
All	2.8% *(254)*	3.0% *(266)*	12.5% *(264)*	**6.1%** ***(784)***

Fig.4.1: **Prevalence of GDM in women who completed the OGTT (n=784) in relation to A) age and fat mass and B) height and fat mass (Standard Error bars shown).**

(A)



(B)



The trends of increasing plasma glucose concentrations with age were independent of maternal fat mass. The trends with age for measures of insulin resistance were, however, no longer statistically significant after adjusting for maternal fat mass (Table 4.3), suggesting that women become more insulin resistant with age only because they become fatter as they get older. This is illustrated in Table 4.9, where insulin resistance increased with increasing fat mass and was highest in the oldest, fattest women, but did not increase with age within the thirds of fat mass. Interestingly, women in the highest third of fat mass did appear to show a relationship between insulin resistance and age although a significant interaction could not be demonstrated.

Table 4.9: Mean RIR-HOMA in thirds of age (years) and fat mass (kg)
(Number of women in parenthesis)

Fat mass / **Age**	**< 14.8**	**- 20.2**	**> 20.2**	**All**
16 – 20	0.84 *(101)*	1.10 *(102)*	1.39 *(46)*	1.03 *(249)*
21 – 25	0.95 *(112)*	1.14 *(104)*	1.56 *(121)*	1.20 *(337)*
26 – 40	0.87 *(64)*	1.04 *(72)*	**1.65** *(110)*	1.22 *(246)*
All	0.89 *(277)*	1.10 *(278)*	1.56 *(277)*	1.15 *(832)*

Multiple linear regression analysis of RIR-HOMA with age (years) and fat mass (kg):
(y variable = RIR-HOMA, R^2 = 0.18)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.003*	*1.00*	*0.004*	*0.5*
Fat mass (kg):	*0.04*	*1.04*	*0.003*	*<0.001*
Constant:	*-0.69*			

It is possible that GDM was associated with age because, as women grow older and therefore fatter and increasingly insulin resistant, their pancreatic β-cells, which have been compensating for the increased resistance by increased secretion, become 'exhausted' and are no longer able to match the increasing demand for insulin. The fact that insulin secretion was reduced relative to higher insulin resistance in older women is shown in Tables 4.10a and 4.10b. This was more clearly demonstrated using HOMA-β values than insulin increment.

Table 4.10a:

Mean HOMA-β in thirds of age (years) and fat mass (kg)

Table 4.10b:

Mean insulin increment (pmol/mmol) in thirds of age (years) and fat mass (kg)

(Numbers of women per cell are the same as shown in Table 4.7)

Fat / Age	< 14.8	- 20.2	> 20.2	All	< 14.8	- 20.2	> 20.2	All
16 – 20	107.6	131.1	145.5	**123.5**	52.2	81.4	57.1	**63.7**
21 – 25	115.1	118.6	144.5	**126.2**	56.6	66.3	64.8	**62.4**
26 – 40	84.7	102.4	118.1	**104.1**	57.5	61.6	67.8	**63.2**
All	**104.7**	**118.3**	**113.8**	**118.5**	**55.2**	**70.2**	**64.5**	**63.0**

a. Multiple linear regression analysis of HOMA-β with age (years) and fat mass (kg): (y variable = HOMA-β, $R^2 = 0.07$)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*- 0.02*	*0.98*	*0.005*	*<0.001*
Fat mass (kg):	*0.02*	*1.02*	*0.003*	*<0.001*
Constant:	*4.94*			

b. Multiple linear regression analysis of insulin increment (pmol/mmol) with age (years) and fat mass (kg): (y variable = insulin increment (pmol/mmol), $R^2 = 0.009$)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*-0.01*	*0.99*	*0.007*	*0.09*
Fat mass (kg):	*0.01*	*1.01*	*0.005*	*0.02*
Constant:	*4.19*			

4.3.2 Height

There was a quadratic (U-shaped) relationship of borderline significance between height and GDM (Table 4.11), with an increased prevalence of GDM in the shortest and in the tallest women. There were no significant relationships between height and individual plasma glucose or insulin concentrations (Table 4.12), nor was height related to insulin resistance as measured by RIR-HOMA, although there was a significant positive trend with 32,33-split proinsulin (p=0.02) which was lost after adjusting for fat mass. There were no relationships with measures of insulin secretion.

When height and fat were looked at together, the highest plasma glucose concentrations at any given time point and the highest rates of GDM were found in the shortest, fattest women (Table 4.13, Fig.4.1B). The same pattern was shown for insulin resistance, as measured by RIR-HOMA (Table 4.14), although in the regression model, the *p* value for height was not significant.

Table 4.11: Percentages of women with GDM according to height (in quarters) *(Number of women in parenthesis)*

Height (cm) in quarters	**% Women with GDM**
< 151.0 *(195)*	**8.2** *(16)*
- 154.5 *(194)*	**4.1** *(8)*
- 158.1 *(200)*	**5.0** *(10)*
> 158.1 (195)	**7.2** *(14)*
All *(784)*	**6.1** *(48)*
p for linear trend	**0.3**
p *adjusted for age and fat mass*	**0.06**
p for quadratic effect	**0.06**
p *adjusted for age and fat mass*	**0.05**

Table 4.12: Mean maternal plasma glucose and insulin concentrations according to height (in quarters).

Height (cm)	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures (pmol/l)			
Quarters	0 mins n=832	30 mins n=825	60 mins n=790	120 mins n=788	180 mins n=784	0 mins n=815	30 mins n=821	60 mins n=785	120 mins n=777	180 mins n=774	32,33 split proinsulin (pmol/l) n=814	RIR-HOMA n=813	HOMA β n=798	Insulin increment (pmol/mmol) n=799
< 151.0	4.5	7.3	7.3	6.0	5.6	30.3	310.1	321:5	229.7	170.1	3.6	1.1	115.0	60.0
- 154.4	4.6	7.3	7.0	6.0	5.6	32.5	314.2	306.8	207.1	166.9	3.8	1.2	123.9	62.4
- 158.0	4.6	7.4	7.2	6.1	5.5	33.3	347.2	357.7	258.4	187.5	4.0	1.2	117.0	65.6
> 158.0	4.6	7.4	7.2	6.2	5.5	32.2	340.2	347.1	256.3	175.0	4.1	1.2	118.3	64.1
p for trend	0.4	0.6	0.98	0.3	0.6	0.2	0.08	0.09	0.06	0.5	0.02	0.08	0.9	0.3
p adjusted for age	0.8	0.99	0.6	0.5	0.3	0.2	0.03	0.02	0.04	0.6	0.02	0.1	0.5	0.1
p adjusted for age & fat mass	0.3	0.2	0.05	0.7	0.05	0.1	0.3	0.4	0.7	0.2	0.8	0.3	0.2	0.3

Table 4.13: Prevalence of age-adjusted GDM (%) in thirds of fat mass (kg) and height (cm) *(Number of women in parenthesis).*

Height \ Fat mass	< 14.8	- 20.2	> 20.2	All
< 152.3	3.8% *(112)*	5.3% *(87)*	**14.9%** *(66)*	8.0% *(265)*
- 156.7	0.0% *(85)*	1.4% *(85)*	5.0% *(92)*	2.2% *(262)*
> 156.7	4.5% *(64)*	3.5% *(89)*	11.3% *(104)*	6.6% *(257)*
All	2.6% *(261)*	3.4% *(261)*	10.4% *(262)*	5.6% *(784)*

Logistic regression analysis of GDM with age (years), fat mass (kg) and height (cm): (y variable = GDM [no=0, yes=1]).

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.2*	*1.21*	*0.03*	*<0.001*
Fat mass (kg):	*0.1*	*1.13*	*0.03*	*<0.001*
Height (cm):	*-0.06*	*0.94*	*0.03*	*0.06*
Constant:	*-1.04*			

Table 4.14: Mean RIR-HOMA in thirds of fat mass (kg) and height (cm) *(number of women in parenthesis).*

Height \ Fat mass	< 14.8	- 20.2	> 20.2	All
< 152.3	0.89 *(119)*	1.12 *(90)*	**1.58** *(67)*	1.10 *(276)*
- 156.7	0.93 *(93)*	1.09 *(90)*	1.56 *(98)*	1.17 *(281)*
> 156.7	0.83 *(65)*	1.08 *(98)*	1.55 *(112)*	1.18 *(275)*
All	0.89 *(277)*	1.10 *(278)*	1.56 *(277)*	1.15 *(832)*

Multiple linear regression analysis of RIR-HOMA with age (years), fat mass (kg) and height (cm): (y variable = RIR-HOMA, R^2 = 0.18).

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.003*	*1.00*	*0.004*	*0.6*
Fat mass (kg):	*0.04*	*1.04*	*0.003*	*<0.001*
Height (cm):	*- 0.005*	*0.10*	*0.004*	*0.2*
Constant:	*0.09*			

Relationships with measures of insulin secretion were inconsistent. The values for insulin increment (Table 4.15b) suggested that short, fat women tended to have lower insulin secretion, implying that these women were not only insulin resistant but became insulin deficient as well. However, HOMA-β values did not confirm this impression and if anything suggested the opposite effect (Table 4.15a). In both regression models, the *p* values for height were not significant.

Table 4.15

a: Mean HOMA-β in thirds of fat mass (kg) and height (cm)

b: Mean insulin increment (pmol/mmol) in thirds of fat mass (kg) and height (cm)

(Numbers of women in each cell are as shown in Table 4.14)

Fat / Height	< 14.8	- 20.2	> 20.2	All	< 14.8	- 20.2	> 20.2	All
< 152.3	110.2	124.1	134.8	120.3	54.2	72.2	58.1	60.4
- 156.7	99.5	113.5	140.8	117.5	60.8	64.6	68.5	64.7
> 156.7	102.6	117.8	127.4	117.7	50.0	73.7	65.1	64.0
All	104.7	118.3	133.8	118.5	55.2	70.2	64.5	63.0

a) Multiple linear regression analysis of HOMA-β with age (years), fat mass (kg) and height (cm): (y variable=HOMA-β, R^2=0.07)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*-0.02*	*0.98*	*0.005*	*<0.001*
Fat mass (kg):	*0.02*	*1.02*	*0.004*	*<0.001*
Height (cm):	*-0.004*	*0.10*	*0.004*	*0.3*
Constant:	*5.49*			

b) Multiple linear regression analysis of insulin increment with age (years), fat mass (kg) and height (cm): (y variable=insulin increment, R^2=0.01)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*-0.01*	*0.99*	*0.007*	*0.1*
Fat mass (kg):	*0.01*	*1.01*	*0.005*	*0.04*
Height (cm):	*0.006*	*1.01*	*0.005*	*0.3*
Constant:	*3.31*			

When the analysis was re-run excluding women with GDM, the shortest, fattest women still had the highest glucose concentrations at 60-minutes (Table 4.16), suggesting a continuum of effect, not confined only to women with GDM. The regression coefficients remained negative for relationships with the 180-minute glucose concentrations and RIR-HOMA although the *p* values for height were not significant.

Table 4.16: Mean glucose concentration at 60-minutes (mmol/l) in thirds of fat mass (kg) and height (cm), excluding women with GDM
(Number of women in parenthesis).

Height \ Fat mass	< 14.8	- 19.8	> 19.8	All
< 152.3	6.49 *(105)*	6.65 *(81)*	**7.48** *(57)*	6.77 *(243)*
- 156.6	6.89 *(83)*	6.92 *(76)*	7.07 *(84)*	6.96 *(243)*
> 156.6	6.53 *(57)*	6.62 *(89)*	7.30 *(104)*	6.88 *(250)*
All	6.63 *(245)*	6.72 *(246)*	7.26 *(245)*	6.87 *(736)*

Multiple linear regression analysis of 60-minute plasma glucose concentration (mmol/l) with age (years), fat mass (kg) and height (cm) and excluding women with GDM: (y variable=60-minute glucose concentration, R^2=0.08)

	Regression coefficient	*Standard Error*	*P value*
Age (years):	*0.08*	*0.01*	*<0.001*
Fat mass (kg):	*0.04*	*0.01*	*<0.001*
Height (cm):	*- 0.007*	*0.01*	*0.5*
Constant:	*5.43*		

4.3.3 Head

Head circumference was similar to height in its relationship to GDM i.e. women with the smallest and largest heads were more likely to be glucose intolerant in pregnancy (Table 4.17). Head circumference was positively related to the 60-minute plasma glucose concentration and to plasma insulin concentrations at all time points apart from at 30-minutes (Table 4.18). Significance was lost after adjusting for age and fat mass. A larger head circumference was associated with increased measures of insulin resistance and HOMA-β, a measure of insulin secretion, but not with the insulin increment. These relationships remained significant after adjusting for age but lost their significance following adjustment for fat mass.

Table 4.17: Percentage of women with GDM according to head circumference (in quarters) *(Number of women in parenthesis)*

Head circumference (cm) in quarters	**% Women with GDM**
< 52.4 *(171)*	**7.0** *(12)*
- 53.4 *(184)*	**3.8** *(7)*
- 54.5 *(173)*	**5.2** *(9)*
> 54.5 *(177)*	**8.5** *(15)*
All *(705)*	**6.1** *(43)*
p for trend	**0.3**
p *adjusted for age and fat mass*	**0.2**
p for quadratic relationship	**0.07**
p *adjusted for age and fat mass*	**0.06**

Table 4.18: Mean maternal plasma glucose and insulin concentrations according to head circumference (in quarters).

Head (cm)	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures			
Quarters	0 mins n=832	30 mins n=825	60 mins n=790	120 mins n=788	180 mins n=784	0 mins n=815	30 mins n=821	60 mins n=785	120 mins n=777	180 mins n=774	32,33 split proinsulin (pmol/l) n=814	RIR-HOMA n=813	HOMA β n=798	Insulin increment (pmol/mmol) n=799
< 52.3	4.5	7.3	7.0	6.0	5.5	29.0	329.4	308.4	209.9	163.3	3.5	1.0	114.0	64.4
- 53.3	4.6	7.3	7.2	6.0	5.5	28.9	295.8	336.3	231.5	157.4	3.8	1.1	107.2	55.8
- 54.4	4.7	7.4	7.2	6.2	5.7	33.0	330.1	321.8	232.2	178.9	3.8	1.2	116.4	64.7
> 54.4	4.6	7.4	7.5	6.2	5.6	36.7	343.7	368.0	277.1	199.9	4.4	1.3	127.0	63.3
p for trend	0.2	0.1	0.04	0.2	0.3	< 0.001	0.3	0.05	0.008	0.02	0.002	< 0.001	0.02	0.7
p adjusted for age	0.3	0.5	0.2	0.6	0.9	< 0.001	0.2	0.03	0.01	0.02	< 0.001	< 0.001	0.01	0.7
p adjusted for age & fat mass	0.2	0.07	0.2	0.09	0.1	0.9	0.6	0.6	0.6	0.4	0.6	0.7	0.99	0.6

When head circumference and fat mass were examined together, the fattest women with the smallest heads had the highest prevalence of GDM (Table 4.19). They also had the highest insulin concentrations at all time points and the highest values for RIR-HOMA (Table 4.20). Although the *p* values for head circumference were non-significant, the slopes were negative. There were no apparent relationships with measures of insulin secretion.

Table 4.19: Prevalence of GDM (%) in thirds of fat mass (kg) and head circumference (cm) *(Number of women in parenthesis).*

Fat mass / Head	< 14.8	- 20.2	> 20.2	All
< 52.7	2.4% *(126)*	3.0% *(66)*	**18.4%** *(38)*	5.2% *(230)*
- 54.0	1.6% *(64)*	1.0% *(103)*	12.0% *(83)*	4.8% *(250)*
> 54.0	5.6% *(36)*	4.2% *(71)*	11.1% *(117)*	8.0% *(224)*
All	2.6% *(226)*	2.5% *(240)*	12.6% *(238)*	6.0% *(704)*

Logistic regression analysis of GDM with age (years), fat mass (kg) and head circumference (cm): (y variable = GDM [no=0, yes=1])

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.2*	*1.2*	*0.04*	*< 0.001*
Fat mass (kg):	*0.1*	*1.1*	*0.03*	*< 0.001*
Head circ. (cm):	*- 0.2*	*0.9*	*0.1*	*0.2*
Constant:	*- 2.81*			

Even when women with GDM were excluded from the analysis, the fattest women with the smallest heads had the highest plasma glucose and insulin concentrations at all time points as well as the highest values for RIR-HOMA: suggesting again a continuum of effect, not confined only to women with GDM.

Table 4.20: Mean RIR-HOMA in thirds of fat mass (kg) and head circumference (cm) *(number of women in parenthesis)*

Head \ Fat mass	< 14.8	- 20.2	> 20.2	All
< 52.7	0.81 *(137)*	1.16 *(69)*	**1.82** *(41)*	1.03 *(247)*
- 54.0	0.97 *(70)*	1.02 *(108)*	1.44 *(88)*	1.13 *(266)*
> 54.0	0.95 *(39)*	1.17 *(74)*	1.55 *(123)*	1.31 *(236)*
All	0.88 *(246)*	1.10 *(251)*	1.55 *(252)*	1.15 *(749)*

Multiple linear regression analysis of RIR-HOMA with age (years), fat mass (kg) and head circumference (cm): (y variable = RIR-HOMA $R^2 = 0.19$)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.002*	*1.00*	*0.005*	*0.6*
Fat mass (kg):	*0.04*	*1.04*	*0.004*	*<0.001*
Head circ. (cm):	*- 0.005*	*0.10*	*0.01*	*0.7*
Constant:	*- 0.46*			

4.3.4 Muscle Mass

Muscle mass was not related to the prevalence of GDM (Table 4.21) although there were significant positive trends with fasting glucose and insulin as well as with the 60-minute insulin concentration, 32,33-split proinsulin and RIR-HOMA (Table 4.22). These relationships were lost after adjusting for age and fat mass. As muscle mass and fat mass were significantly correlated (r=0.26), more muscular women also being fatter women, the relationships seen may simply be due to fat.

Interestingly, there was a strong negative relationship between muscle mass and the 180-minute glucose concentration after adjustment for fat mass and age (p=0.006, β=-0.05 mmol/kg), suggesting that more muscular women may in fact have better glucose tolerance, even though this is not reflected in rates of GDM.

Table 4.22: Mean maternal plasma glucose and insulin concentrations according to muscle mass (in quarters).

Muscle mass (kg)	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures (pmol/l)			
Quarters	0 mins n=832	30 mins n=825	60 mins n=790	120 mins n=788	180 mins n=784	0 mins n=815	30 mins n=821	60 mins n=785	120 mins n=777	180 mins n=774	32,33 split proinsulin (pmol/l) n=814	RIR-HOMA n=813	HOMA β n=798	Insulin increment (pmol/mmol) n=799
< 12.3	4.5	7.3	7.0	6.0	5.6	30.1	359.9	324.8	240.4	183.9	3.7	1.1	115.8	72.5
- 13.6	4.6	7.4	7.2	6.0	5.6	31.5	297.4	309.6	210.1	158.5	3.7	1.1	115.7	56.0
- 15.2	4.6	7.3	7.1	6.1	5.6	30.7	312.1	315.8	231.4	162.9	3.8	1.1	113.2	61.4
> 15.2	4.6	7.4	7.3	6.1	5.5	36.4	345.5	385.8	270.0	196.7	4.2	1.3	129.8	63.4
p for trend	0.04	0.6	0.1	0.4	0.6	0.002	0.7	0.02	0.1	0.4	0.05	0.001	0.08	0.2
p adjusted for age	0.5	0.6	0.5	0.8	0.05	0.04	0.4	0.05	0.3	0.95	0.04	0.02	0.03	0.1
p adjusted for age & fat mass	0.6	0.06	0.6	0.2	0.006	0.7	0.06	0.5	0.6	0.1	0.9	0.4	0.8	0.03

Table 4.21: Percentage of women with GDM according to muscle mass (kg) (in quarters) *(Number of women in parenthesis)*

Muscle mass (kg) in quarters	% Women with GDM
< 12.3 *(189)*	**5.8** *(11)*
- 13.6 *(196)*	**8.9** *(15)*
- 15.2 *(199)*	**4.5** *(9)*
> 15.2 *(200)*	**6.5** *(13)*
All *(784)*	**6.1** *(47)*
p for trend	**0.7**
p *adjusted for age and fat mass*	**0.2**

4.3.5 Subscapular skin-fold / Triceps skin-fold (SS/TR)

The ratio, SS/TR showed a significant negative trend with the diagnosis of GDM (Table 4.23), suggesting that women who were *less* centrally fat were more likely to become glucose intolerant during pregnancy. After adjusting for age and BMI, this trend was of borderline significance only. A similar negative trend was seen with plasma glucose concentrations at all time points apart from at 180-minutes, although significance was lost after adjusting for maternal BMI and age (Table 4.24).

There were no significant trends with individual insulin concentrations, or with measures of insulin resistance or secretion until after adjustment for age and BMI, when a significant positive trend with HOMA-β was seen, implying that women with less central fat may have lower insulin secretion.

Table 4.23: Percentage of women with GDM according to their ratio of SS/TR (in quarters) *(Number of women in parenthesis)*

SS/TR in quarters	% Women with GDM
<1.2 *(192)*	**7.8** *(15)*
- 1.4 *(195)*	**10.3** *(20)*
- 1.6 *(196)*	**5.6** *(11)*
>1.6 *(201)*	**1.0** *(2)*
All *(784)*	**6.1** *(47)*
p for trend	**0.003**
p *adjusted for age and BMI*	**0.06**

Logistic regression analysis of GDM with age (years), BMI (kg/m^2), triceps and subscapular skin-folds: (y variable = GDM (yes/no))

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Age (years):	*0.2*	*1.21*	*0.03*	*<0.001*
BMI (kg/m^2):	*0.08*	*1.09*	*0.07*	*0.2*
Triceps (mm):	*0.09*	*1.09*	*0.04*	*0.02*
Subscapular (mm):	*-0.02*	*0.98*	*0.03*	*0.4*
Constant:	*-10.93*			

The results of the logistic regression analysis above suggest that the inverse relationship of SS/TR with GDM prevalence may be due to the effect of larger triceps skin-fold thickness rather than smaller subscapular skin-fold thickness.

Table 4.24: Mean maternal plasma glucose and insulin concentrations according to the ratio of subscapular/triceps skin-fold thickness (SS/TR) (in quarters).

SS / TR	Mean Plasma Glucose (mmol/l)					Mean Plasma Insulin (pmol/l)					Other Insulin Measures (pmol/l)			
Quarters	0 mins n=832	30 mins n=825	60 mins n=790	120 mins n=788	180 mins n=784	0 mins n=815	30 mins n=821	60 mins n=785	120 mins n=777	180 mins n=774	32,33 split proinsulin (pmol/l) n=814	RIR-HOMA n=813	HOMA β n=798	Insulin increment (pmol/mmol) n=799
< 1.2	4.6	7.5	7.4	6.0	5.5	31.4	323.1	340.2	222.9	156.0	3.7	1.1	108.1	59.9
- 1.4	4.6	7.5	7.4	6.4	5.7	34.5	337.9	346.0	282.3	206.5	4.1	1.2	126.5	67.4
- 1.7	4.6	7.3	7.0	5.9	5.6	32.2	326.6	329.3	238.0	173.2	4.0	1.2	118.6	62.3
> 1.7	4.5	7.1	6.9	5.8	5.4	30.3	323.5	317.3	211.2	166.6	3.7	1.1	121.9	62.9
p for trend	0.01	0.001	0.006	0.02	0.1	0.3	0.9	0.3	0.2	0.97	0.9	0.2	0.1	0.8
p adjusted for age	0.2	0.1	0.6	0.7	0.4	0.6	0.7	0.8	0.4	0.3	0.5	0.8	0.02	0.7
p adjusted for age & BMI	0.3	0.2	0.96	0.99	0.5	0.1	0.5	0.4	0.2	0.09	0.1	0.2	0.004	0.7

4.4 Relationship of family history of diabetes to GDM prevalence

Of the 48 women diagnosed with GDM, 21 (43.8%) gave a history of diabetes in a first degree relative. 16 (76.2%) had fathers with diabetes, 7 (33.3%) had mothers, 4 (19.0%) had both father and mother affected, 3 (14.3%) had a sibling affected and in one (4.8%), both father and sibling had diabetes. Using logistic regression to calculate odds ratios (Table 4.25), having any first degree relative with diabetes increased the risk of developing GDM almost four-fold (OR=3.8).

Table 4.25: Relative risk of GDM with a positive family history of diabetes. Number of women shown. Logistic regression used to calculate odds ratios and 95% confidence intervals (CI).

	Family history of diabetes	Women with GDM		Risk for GDM Odds Ratio	95% CI
		No	Yes		
Any relative	No	612	27		
	Yes	124	21	3.8	2.1 – 7.0
Father	No	654	32		
	Yes	82	16	4.0	2.1 – 7.6
Mother	No	684	41		
	Yes	52	7	2.2	1.0 – 5.2
Father + Mother	No	724	44		
	Yes	12	4	5.5	1.7 – 17.7
Sibling	No	729	45		
	Yes	7	3	6.9	1.7 – 27.8
Father + Sibling	No	734	47		
	Yes	2	1	7.8	0.7 – 87.7

In logistic regression analysis, women with a family history of diabetes tended to be older ($p=0.005$) and fatter ($p<0.001$) with no significant difference in height or head circumference. Using the Student's t-test to compare groups, women who had fathers with diabetes were fatter ($p<0.001$) but with no difference in age. Women with mothers who were diabetic were older ($p=0.03$) and only slightly fatter ($p=0.05$). The age at first diagnosis in mothers with diabetes ranged from 25-62 years and did not predict the development of GDM in their daughters.

The significant predictors of GDM in a logistic regression model (Table 4.26) were age, fat mass, a positive family history of diabetes and shorter stature.

Table 4.26: Predictors of GDM
Logistic regression model where the dependent variable is GDM (yes=1, no=0)

	Regression coefficient	SE	P	Exponential β
Age (years)	0.19	0.04	<0.00001	1.2
Fat mass (kg)	0.11	0.03	0.0002	1.1
Height (cm)	-0.06	0.03	0.05	0.9
Social class (Kuppuswamy)	0.01	0.03	0.8	1.0
Parity (0-4)	0.03	0.19	0.9	1.0
Family history of diabetes (0=no, 1=yes)	1.03	0.35	0.003	2.8

4.5 Summary of Main Findings

1. Mothers with GDM tended to be older, heavier (fatter), shorter and with smaller head circumferences. There was some evidence of a relationship with higher social class although the prevalence rates were not significantly different. Increasing maternal age was shown to be associated with increasing insulin resistance and decreased insulin secretion. The data suggest that GDM was associated with age because, as women grow older and therefore fatter and increasingly insulin resistant, their pancreatic β-cells, which have been compensating for the increased resistance with increased secretion become 'exhausted' and are no longer able to match the increasing demand for insulin.

2. There was evidence that early growth, measured by height and head circumference influenced maternal glucose and insulin status. The highest rates of GDM and of insulin resistance were found in the fattest, shortest women and in the fattest women with the smallest heads. There did not appear to be any consistent relationships between measures of insulin secretion and fat, height or head circumference.

3. A positive family history of diabetes was significantly predictive of GDM. A history of diabetes in any first degree relative increased the risk of GDM almost four-fold (OR=3.8). Interestingly, the risk was higher with a father who was diabetic than with a mother and was higher still if both mother and father were affected.

4.6 Discussion

Although India is known to have a high prevalence of type 2 diabetes,[35] there are few data on the prevalence of GDM. Ramachandran *et al* studied a South Indian urban population of comparable age and BMI to my study population and found a low prevalence (<1%),[108] perhaps due to the use of different diagnostic criteria. Ramachandran used a 75g OGTT and WHO criteria to define women with impaired

glucose tolerance (4.9%) who then underwent a 100 g OGTT, and diagnosed GDM using the National Diabetes Data Group (NDDG) criteria. My study appears to be the only recent study from India in which GDM has been assessed using a single standard test.

It is possible that GDM prevalence has been slightly overestimated because women who were eligible for the study but did not participate were younger (median age 22 years cf. 23 years). However, those who underwent the OGTT but did not deliver in the hospital were also younger (median age 21 years cf. 23 years) and their prevalence of GDM was 4.1%. Based on this figure, the overall prevalence may be closer to 5.5%, still significantly higher than that found by Ramachandran et.al. Consistently higher prevalence rates have been found in South Asian Indians compared to white Caucasians in the UK (4.4% v 0.4%),[102] Australia (16.7% v 3.0%),[105] and USA (10.5% v 4.8%).[101]

As shown in other populations, the prevalence of GDM in Mysore rose with maternal age, probably due to a combination of increased insulin resistance and decreased insulin secretion. Although the insulin increment did not decrease with age, it was low in older mothers relative to their insulin resistance. Although these women were relatively non-obese (mean BMI=23.1 kg/m^2), GDM was also strongly related to body fat. After adjusting for age and body fat, the prevalence of GDM was increased in women with evidence of reduced growth in early life as shown by height and head circumference. The association with reduced height agrees with data from other populations but are shown for the first time in India. Studies from Greece, Korea and the USA showed higher rates of GDM in shorter women.[169-172] The association with smaller head circumference has not been shown before.

There was a small increase in GDM among tall women and in those with larger head circumferences. This finding was unexpected and not explained by increased insulin resistance or low secretion in these women. U-shaped relationships between height and GDM and between head circumference and GDM were not part of my *a priori* hypothesis and were of borderline significance. The association with tall mothers and mothers with larger head size may therefore be a spurious finding. There was no

evidence of a secular trend in height or head circumference among the study women and although taller mothers and those with larger head size who had GDM tended to be younger, adjusting for age did not alter the quadratic relationship. Assuming the U-shaped relationships are real, one explanation would be that taller women with larger heads were themselves products of GDM pregnancies and therefore macrosomic at birth, but with no information on their mother's glucose tolerance during pregnancy we are unable to test this.

A family history of type 2 diabetes is a known risk factor for diabetes in the offspring and in this study, a positive family history was associated with an almost four-fold increase in risk of GDM. The mechanisms involved in the 'inheritance' of the disease remain largely unknown. Most family studies have found a higher risk of developing both type 2 diabetes and GDM in individuals with diabetic mothers as opposed to diabetic fathers,[90, 176-178] and the investigators have suggested that the intra-uterine environment may play an important role in the transmission of diabetes to the offspring. The finding in this study that more women with GDM reported fathers with diabetes than mothers is difficult to explain, but may simply reflect a higher prevalence of the disease in fathers, perhaps because they were older or fatter or because they sought medical advice and were diagnosed earlier.

The possible association of poorer glucose tolerance with higher social class in this study is also interesting. This association has been reported before among urban Indian women [47] in relation to type 2 diabetes. It fits with the hypothesis that it is the transition from poverty and early-life undernutrition towards greater affluence and relative obesity in adult life that is responsible for the increasing prevalence of type 2 diabetes in India,[35] and may also lead to high rates of GDM in Indian mothers.

5. Results – Glucose and Insulin Data: Relationships to the Baby

5.1 Introduction

It is well known that maternal diabetes during pregnancy is responsible for increased fetal growth (macrosomia).[179] It has also been shown that increasing maternal glucose concentrations, even in the 'normal' range are responsible for increased growth in the new-born.[111, 180] The main substrate for fetal development is glucose, which is completely derived from the maternal circulation, since it cannot be synthesised by the fetus itself. Glucose is supplied from mother to fetus across the placenta by facilitated diffusion, mainly determined by maternal plasma glucose levels. The maternal metabolic condition is therefore an important determinant of fetal growth.

What is less well known is the mechanism by which glucose is utilized by the fetus in order to effect growth. Rising glucose concentrations stimulate the fetal pancreas to produce insulin. Insulin is the major factor responsible for fetal growth throughout the last trimester of pregnancy and for normal insulin-related growth, two related effects are needed. Insulin must be produced by the fetal pancreatic β-cells in appropriate quantity and quality and on the other hand, insulin must effect appropriately the uptake of glucose by the fetal tissues, which must be equipped to do so.

Most studies that have examined relationships between maternal glucose and insulin concentrations and neonatal size have concentrated on birthweight as the outcome variable. In this study, with detailed neonatal anthropometry, I was able to define more clearly the effects of increasing maternal glycaemia on neonatal phenotype and on cord blood glucose and insulin concentrations. These relationships are examined in this chapter.

5.2 Relationship of neonatal anthropometric variables to maternal glucose and insulin concentrations.

5.2.1 Birthweight

Mothers with GDM had heavier babies, independently of neonatal sex, gestation at delivery, maternal age, parity and fat mass (Table 5.1).

Table 5.1: Birthweight (kg) in quarters and percentage of mothers with GDM
(Number of women shown in parenthesis)

Birthweight (kg) in quarters	% Mothers with GDM
< 2.7 *(141)*	**0.7** *(1)*
- 2.9 *(142)*	**3.5** *(5)*
- 3.2 *(141)*	**9.2** *(13)*
> 3.2 *(141)*	**12.8** *(18)*
All *(565)*	**6.6** *(37)*
p for trend	**< 0.001**
p adjusted for sex and gestation	**< 0.001**
p adj. for sex, gestation, maternal age, parity, fat mass	**< 0.001**

There were strong positive trends with birthweight and maternal plasma glucose concentrations at all time points ($p<0.03$) and with the area-under-the-glucose-curve (AUGC: $p<0.001$). Following adjustment for sex, gestation, maternal age, parity and fat mass, significant trends remained with fasting glucose ($p=0.02$), the glucose concentration at 60-minutes ($p=0.01$) and the AUGC ($p=0.02$) (Table 5.2).

Table 5.2: **Relationship of maternal area-under-the-glucose-curve (AUGC: $mmol.l^{-1} h^{-1}$) in quarters to the anthropometric measurements of normal, term neonates** (all measurements have been adjusted for gestation).

(SD's and interquartile ranges in brackets).

AUGC ($mmol.l^{-1}h^{-1}$) Quarters	Neonatal Anthropometric Measurements											
	Birth weight (g) n=565	Circumferences (cm)				Skinfolds (mm)		Lengths (cm)			Ponderal Index (kg/m^3) n=565	Placental weight (g) n=556
		head n=565	chest n=563	abdomen n=563	mid-arm n=563	triceps n=563	subscap. n=563	CHL n=565	CRL n=563	Leg n=563		
< 16.8	2885.8	33.9	32.1	30.1	10.3	4.1	4.4	48.9	32.0	16.9	24.7	402.3
- 18.7	2930.1	34.0	32.0	30.0	10.4	4.2	4.5	49.1	32.3	16.8	24.9	402.1
- 20.7	2972.8	34.2	32.3	30.1	10.5	4.2	4.4	49.2	32.2	17.0	25.0	413.1
> 20.7	3062.9	34.2	32.6	30.5	10.6	4.4	4.6	49.4	32.5	16.9	25.3	430.5
All	2963.0 (412.4)	34.1 (1.3)	32.2 (1.6)	30.2 (2.0)	10.4 (0.9)	4.2 (3.7,4.9)	4.5 (3.9,5.0)	49.1 (2.1)	32.2 (1.7)	16.9 (1.5)	25.0 (2.8)	411.9 (360.1,470.4)
p for trend	< 0.001	0.1	< 0.001	0.01	< 0.001	< 0.001	< 0.001	0.03	0.04	0.4	0.007	0.001
p adj. for sex	< 0.001	0.09	< 0.001	0.01	< 0.001	< 0.001	< 0.001	0.02	0.04	0.4	0.007	0.001
p adj. for sex maternal age, parity, fat mass.	0.02	0.96	0.08	0.3	0.09	0.03	0.03	0.3	0.7	0.3	0.1	0.03

Birthweight was positively related to maternal insulin concentrations at 60- and 120-minutes ($p=0.03$, $p=0.05$ respectively) and to measures of insulin resistance: 32,33-split proinsulin and RIR-HOMA ($p=0.001$ for both) (Table 5.3). Significance remained following adjustment for sex and gestation but was lost after adjustment for maternal age, parity and fat mass. There were no significant relationships with measures of insulin secretion.

5.2.2 Fat

Mothers with GDM had significantly fatter babies, shown by larger skin-fold thicknesses (subscapular and triceps) and a higher ponderal index (PI) (Table 5.4). Following adjustment for maternal fat mass, age and parity, the relationship with PI lost its significance although strong significant trends with the skin-folds remained.

Measures of neonatal fatness (skin-fold thicknesses and PI) were positively related to maternal glucose concentrations at all time points ($p<0.05$) and to the AUGC ($p<0.01$) (Table 5.2). These relationships were considerably weakened following adjustment for maternal age, parity and fat mass but remained significant with the fasting glucose concentration ($p<0.05$) and the AUGC ($p=0.03$ for both skin-fold thicknesses).

Larger skin-fold thicknesses and higher PI were related to higher maternal insulin resistance: RIR-HOMA ($p<0.01$ for all) (Table 5.3) and 32,33-split proinsulin ($p<0.001$ for all). Following adjustment for maternal age, parity and fat mass, significant relationships remained between the skin-folds and the 32,33-split proinsulin concentration. There was a negative trend of borderline significance between PI and insulin increment which was strengthened after adjusting for maternal age, parity and fat mass ($p=0.05$), suggesting that mothers with poorer insulin secretion were more likely to have fatter babies.

Table 5.3: **Relationship of maternal RIR-HOMA in quarters to the anthropometric measurements of normal, term neonates** (all measurements have been adjusted for gestation).

(SD's and interquartile ranges in brackets).

RIR-HOMA Quarters	Neonatal Anthropometric Measurements											
	Birth weight (g)	Circumferences (cm)				Skinfolds (mm)		Lengths (cm)			Ponderal index (kg/m^3)	Placental weight (g)
		head	chest	abdomen	mid-arm	triceps	subscap.	CHL	CRL	Leg		
	n=584	n=584	n=582	n=582	n=582	n=582	n=582	n=584	n=582	n=582	n=584	n=575
< 0.8	2898.5	34.0	31.9	29.9	10.3	4.0	4.3	49.1	32.2	16.9	24.5	398.8
- 1.2	2940.9	33.9	32.2	30.1	10.4	4.1	4.5	49.2	32.3	16.9	24.7	408.2
- 1.7	2911.2	34.0	32.0	30.0	10.4	4.1	4.4	48.9	32.1	16.8	24.9	404.0
> 1.7	3082.5	34.4	32.7	30.7	10.7	4.5	4.7	49.3	32.4	16.9	25.6	438.5
All	2958.3 (414.5)	34.1 (1.3)	32.2 (1.7)	30.2 (2.0)	10.4 (0.9)	4.2 (3.7,4.8)	4.5 (3.9,5.0)	49.1 (2.1)	32.2 (1.7)	16.9 (1.5)	24.9 (2.8)	412.2 (360.3,469.8)
p for trend	0.001	0.004	<0.001	0.001	< 0.001	< 0.001	< 0.001	0.3	0.5	0.5	0.002	< 0.001
p adj. for sex	< 0.001	0.001	<0.001	0.001	< 0.001	< 0.001	< 0.001	0.2	0.4	0.4	0.003	< 0.001
p adj. for sex, maternal age, parity, fat mass.	0.5	0.2	0.1	0.2	0.1	0.2	0.05	0.6	0.2	0.6	0.1	0.04

Table 5.4: Comparison of babies born to mothers with and without gestational diabetes (GDM). All babies were full-term and measurements were adjusted for gestation. Means and geometric means for logged variables (denoted*) shown. P values obtained from T-tests.
(Number of babies in parenthesis)

Neonatal anthropometric variables	**GDM** (n) Yes or No	**Means**	**p** for difference in means	**p** adjusted for sex	**p** adjusted for sex + maternal age, parity, fat
Birthweight (g)	**No** (528)	2938.5			
	Yes (37)	3311.4	< 0.001	< 0.001	< 0.001
Lengths (cm)					
a)Crown-heel	**No** (528)	49.0			
	Yes (37)	50.4	< 0.001	< 0.001	0.001
b)Crown-rump	**No** (526)	32.2			
	Yes (37)	32.8	0.05	0.03	0.4
c)Leg	**No** (526)	16.8			
	Yes (37)	17.6	0.006	0.004	0.001
Circumferences (cm)					
a)Head	**No** (528)	34.0			
	Yes (37)	34.4	0.09	0.03	0.3
b)Chest	**No** (526)	32.2			
	Yes (37)	33.5	< 0.001	< 0.001	< 0.001
c)Abdominal	**No** (526)	30.1			
	Yes (37)	31.4	< 0.001	< 0.001	0.002
d)Mid-upper arm	**No** (526)	10.4			
	Yes (37)	11.1	< 0.001	< 0.001	< 0.001
Skinfolds (mm)					
a)Subscapular*	**No** (526)	4.4			
	Yes (37)	5.2	< 0.001	< 0.001	0.001
b)Triceps*	**No** (526)	4.1			
	Yes (37)	5.0	< 0.001	< 0.001	< 0.001
Ponderal Index (kg/m^3)	**No** (528)	24.9			
	Yes (37)	26.0	0.02	0.02	0.2
Placental weight* (g)	**No** (521)	408.3			
	Yes (35)	466.4	< 0.001	< 0.001	0.002

5.2.3 Length

Crown-heel length (CHL), crown-rump length (CRL) and leg length (LL) were all significantly increased in babies born to mothers with GDM (Table 5.4). Significant relationships remained for CHL and LL after adjusting for maternal age, parity and fat mass, but were lost for CRL. Significant positive trends were seen with CHL and CRL and maternal plasma glucose concentrations at 60- and 120-minutes and with the AUGC ($p<0.04$ for all) (Table 5.2).

Longer length (CHL and CRL) was related to higher measures of maternal insulin resistance: insulin concentrations at 60- and 120-minutes and 32,33-split proinsulin ($p<0.05$ for all). Significance was largely lost after adjusting for maternal age, parity and fat mass, but remained for the relationship between CRL and the 120-minute insulin concentration ($p=0.03$). There were no trends seen with measures of maternal insulin secretion.

5.2.4 Head

Mothers with GDM had babies with larger head circumferences (Table 5.4). This relationship became significant only after allowing for neonatal sex ($p=0.03$). If neonatal fat (triceps skin-fold) was added to the regression equation, the relationship between head circumference and GDM in the mother was lost, suggesting that the increase in head circumference in these babies may actually be due to an increase in neonatal fat deposition.

There were weak, positive trends with head circumference and maternal glucose concentrations at fasting, 30- and 60-minutes ($p<0.05$ for all), which were strengthened after adjusting for neonatal sex. Following adjustment for maternal age, parity and fat mass these relationships lost their significance and a strong negative relationship appeared between head circumference and the maternal insulin concentration at 180-minutes ($p=0.002$).

Neonatal head size was related positively to measures of maternal insulin resistance: 32,33-split proinsulin (p=0.03) and RIR-HOMA (p=0.004)(Table 5.3). Again, these relationships were strengthened after adjusting for sex but lost after adjusting for maternal age, parity and fat mass. There were no significant relationships with measures of insulin secretion.

5.2.4 Chest, abdominal and mid-upper arm circumferences

Chest, abdominal and mid-upper-arm (MUAC) circumferences were significantly larger in babies born to mothers with GDM (p<0.001 for all)(Table 5.3). This appeared to be due mainly to increased fat in these babies: when neonatal fat (triceps skin-fold) was added to the regression model, abdominal circumference and MUAC were no longer significantly related to GDM and the relationship with chest circumference was weakened (p=0.03).

There were significant, positive trends with all three circumferences and maternal glucose concentrations at fasting, 60- and 120-minutes (p<0.05 for all) and with the AUGC (p<0.01) (Table 5.2). Following adjustment for maternal fat mass, age and parity, significance remained only for the relationships between chest circumference and the fasting (p=0.01) and 60-minute (p=0.03) glucose concentrations.

Larger chest, abdominal and mid-upper-arm circumferences in the neonate were related to higher measures of insulin resistance in the mother: fasting insulin concentration (MUAC: p=0.01), 32,33-split proinsulin and RIR-HOMA (chest, abdomen and MUAC: p<0.01 for all) (Table 5.3). After adjusting for maternal age, parity and fat mass, significant relationships remained between chest and abdominal circumferences and the 32,33-split proinsulin concentration (p<0.03).

There were no significant relationships with measures of insulin secretion.

5.2.5 Placenta

Babies born to mothers with GDM had larger placentas ($p<0.001$) (Table 5.4). Placental weight was positively related to maternal glucose concentrations at all time points ($p<0.04$) and to the AUGC ($p=0.001$) (Table 5.2). After adjusting for maternal age, parity and fat mass, significance remained for relationships with the 60- and 180-minute glucose concentrations and the AUGC ($p=0.03$).

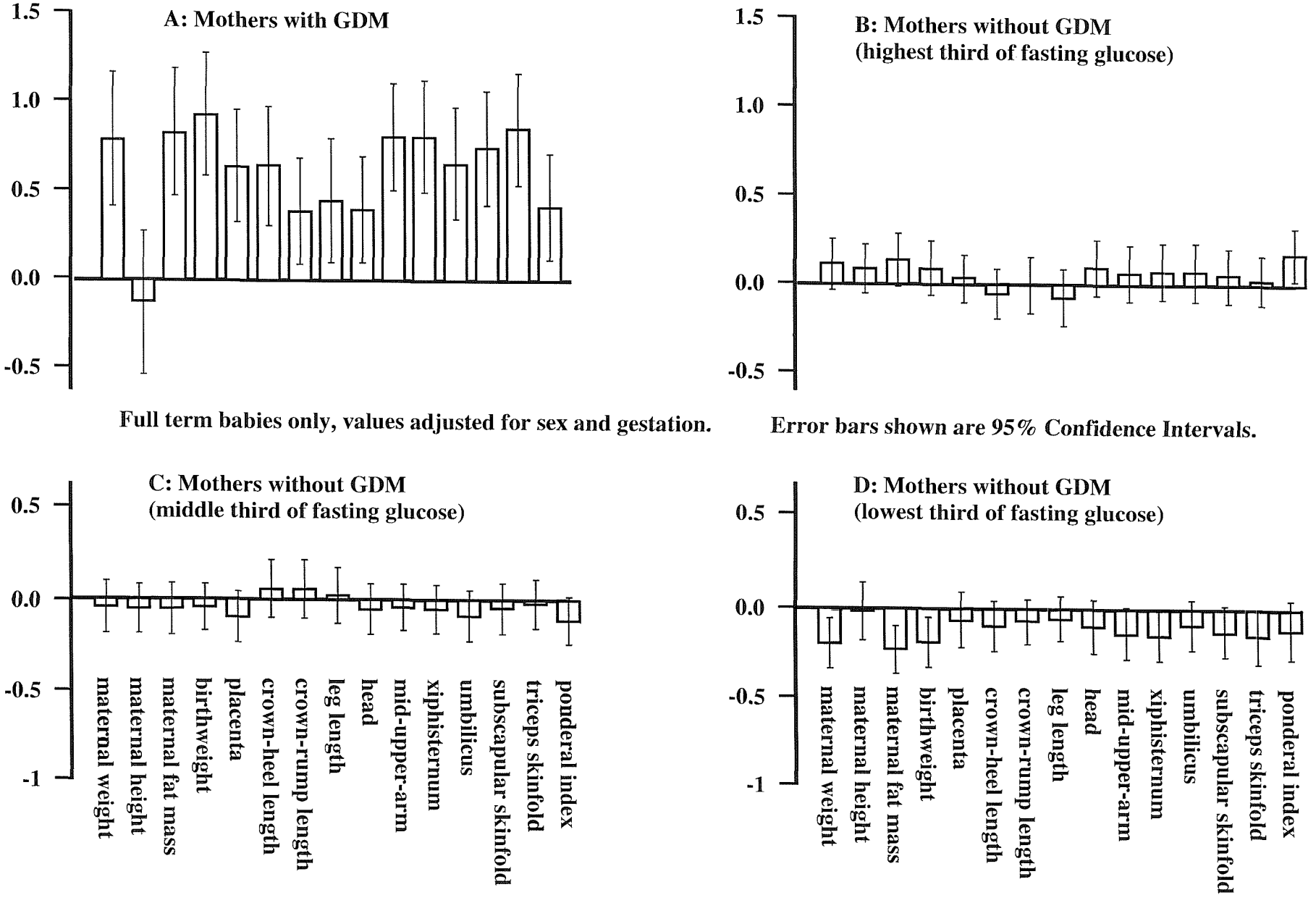
Larger placentae were also related to higher measures of maternal insulin resistance: fasting insulin ($p=0.05$), 32,33-split proinsulin ($p=0.005$) and RIR-HOMA ($p<0.001$). After adjusting for maternal age, parity and fat mass, significance remained only for the relationship with RIR-HOMA ($p=0.04$) (Table 5.3). There were no significant relationships with measures of insulin secretion.

In summary, babies born to mothers with GDM were bigger in all their anthropometric measurements. However, relatively bigger differences were seen for measures of neonatal fat than for skeleton, either head circumference or length. The size of the effect of maternal glucose intolerance on neonatal anthropometric measurements is illustrated graphically in Fig.5.1A, using standard deviation (SD) scores calculated for mothers with GDM and their babies relative to the study population as a whole.

Excluding from these analyses babies born to mothers with GDM, similar but weaker relationships were seen between neonatal anthropometric variables and the blood glucose and insulin concentrations of the mother. This suggests a continuum of effect across the range of glucose and insulin concentrations in mothers with normal glucose tolerance. The size of this effect on neonatal anthropometry is illustrated in Fig.5.1 B,C,D. In these three graphs, mothers with GDM and their babies have been excluded from the study population and the SD scores shown are for mothers with normal glucose tolerance and their babies in three levels of fasting glucose (low, middle and high). Evidence of a continuum of effect is best illustrated by neonatal ponderal index (PI). Mothers with 'high' fasting glucose concentrations have babies with a higher PI than mothers with 'middle' fasting glucose, who in turn have babies with a higher PI

Fig 5.1: Standard deviation (SD) scores for mothers and their babies (standardised to whole study population).

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



Full term babies only, values adjusted for sex and gestation.

Error bars shown are 95% Confidence Intervals.

than mothers with 'low' fasting glucose. Fig.5.1 as a whole, emphasises the enhanced effect on fetal growth, which occurs in mothers with GDM.

5.3 Relationship of maternal glucose and insulin concentrations to neonatal cord blood glucose and insulin concentrations.

Geometric means and interquartile ranges for cord blood glucose, insulin proinsulin and 32,33-split proinsulin concentrations are given in Table 5.5.

Table 5.5: Cord blood samples: numbers (N), geometric means and interquartile ranges (IQR) shown.

	N	Mean (geometric)	IQR
Glucose (mmol/l)	571	6.1	5.1, 7.5
Insulin (pmol/l)	554	22.8	13.0, 40.0
Proinsulin (pmol/l)	547	7.8	5.9, 11.0
32,33-split proinsulin (pmol/l)	547	9.4	5.9, 14.0

Mothers with GDM had babies with significantly higher cord blood glucose and insulin concentrations (Table 5.6). Higher cord blood glucose and insulin concentrations were strongly related to higher maternal plasma glucose concentrations at any time point during the OGTT and independently of maternal age, parity and fat mass ($p<0.01$ for all). Thus implying that maternal hyperglycaemia at 30+/-2 weeks gestation was associated with neonatal hyperglycaemia and hyperinsulinaemia at delivery.

Table 5.6: Cord blood glucose and insulin concentrations in babies born to mothers with and without GDM. Number of samples and geometric means are shown. P value for difference in means was calculated by the Student's t-test.

Cord blood concentrations	GDM	N	Mean	P value for difference in means
Glucose (mmol/l)	No	*506*	6.0	
	Yes	*35*	7.2	**0.001**
Insulin (pmol/l)	No	*490*	21.5	
	Yes	*35*	41.1	**< 0.001**
Proinsulin (pmol/l)	No	*483*	7.5	
	Yes	*35*	11.4	**< 0.001**
32,33-split proinsulin (pmol/l)	No	*483*	8.7	
	Yes	*35*	21.9	**< 0.001**

Cord insulin but not cord glucose concentrations were related to maternal insulin concentrations at all time points apart from 30-minutes and to measures of maternal insulin resistance: RIR-HOMA ($p<0.001$), 32,33-split proinsulin ($p<0.05$). There were no relationships with measures of maternal insulin secretion.

When mothers with GDM were excluded from the analysis, relationships were weakened but remained significant for cord glucose and insulin concentrations with maternal fasting glucose ($p<0.05$ for all) and for cord glucose, proinsulin and split proinsulin with the 30- and 180-minute glucose concentrations. Cord glucose and insulin concentrations also remained significantly related to maternal fasting insulin and RIR-HOMA ($p<0.05$ for all). Cord insulins but not cord glucose were related positively to HOMA-β ($p<0.05$) but not to the insulin increment.

5.4 Relationship of maternal anthropometric measurements to neonatal cord blood glucose and insulin concentrations

Mothers' anthropometric measurements were not related to the cord glucose concentration of their babies. However, fatter mothers had babies with higher cord insulins (Table 5.7). The strongest trends were with measures of maternal body fat (skin-fold thicknesses, % body fat, fat mass) and cord 32,33-split proinsulin ($p<0.001$ for all). These relationships were weakened following adjustment for maternal glycaemia (AUGC) and significance was lost if insulin resistance (RIR-HOMA) was also adjusted for. However, excluding mothers with GDM did not significantly alter these relationships.

Table 5.7: Mean cord blood insulin (Ins) (pmol/l), proinsulin (Pro) (pmol/l) and split proinsulin (Split) (pmol/l) concentrations in three equal groups of maternal fat mass (kg) and insulin resistance (RIR-HOMA) *(number of women in parenthesis).*

Fat mass (kg)	**< 14.8**			**- 20.2**			**> 20.2**			**All**		
RIR-HOMA	***Ins.***	***Pro.***	***Split.***	***Ins.***	***Pro.***	***Split.***	***Ins.***	***Pro.***	***Split.***	***Ins.***	***Pro.***	***Split.***
< 1.0	30.1	7.8 *(103)*	9.3	20.4	6.8 *(65)*	8.5	32.7	9.1 *(26)*	12.4	27.3	7.7 *(194)*	9.5
- 1.5	27.0	8.5 *(63)*	10.6	29.8	8.1 *(70)*	10.6	44.1	8.9 *(62)*	13.0	33.5	8.5 *(195)*	11.4
> 1.5	37.0	9.3 *(28)*	12.0	42.2	8.4 *(60)*	12.4	**53.7**	**10.5** *(107)*	**18.2**	47.9	9.7 *(195)*	15.6
All	30.0	8.2 *(194)*	10.1	30.6	7.8 *(195)*	10.5	47.7	9.8 *(195)*	15.7	36.2	8.6 *(584)*	12.2

Multiple linear regression analysis of cord blood insulin with maternal fat mass (kg), area-under-the-glucose-curve (mmol.l^{-1}.min^{-1}), RIR-HOMA, neonatal sex and gestation: (y variable=logged cord blood insulin, R^2=0.07)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Fat mass (kg):	*0.01*	*1.01*	*0.007*	*0.07*
AUGC (mmol.l^{-1}.min^{-1}):	*0.0005*	*1.00*	*0.000*	*0.009*
RIR-HOMA:	*0.2*	*1.2*	*0.07*	*0.01*
Sex (1=M,2=F):	*-0.05*	*0.9*	*0.08*	*0.5*
Gestation (weeks):	*-0.08*	*0.9*	*0.04*	*0.03*
Constant:	*5.33*			

5.5 Relationship of cord blood glucose and insulin concentrations to neonatal anthropometric measurements

Controlling for neonatal sex and gestation, cord insulin concentrations were positively related to all neonatal anthropometric measurements, but more strongly to measures of neonatal fat (ponderal index, triceps and subscapular skin-fold thickness; $p<0.001$ for all), than to measures of skeleton (CHL: p=0.004. CRL: p=0.1. Leg length: p=0.02. Head circumference: p=0.06. Table 5.8). Controlling for maternal fat mass did not significantly alter these relationships. In contrast, cord glucose concentrations were positively related to measures of skeleton: CHL (p=0.02), leg length (p=0.04) and head circumference (p=0.03) but not to neonatal fat (Table 5.9). Following adjustment for maternal fat mass, cord glucose remained significantly related to CHL (p=0.05).

The significant trends shown were not altered by excluding mothers with GDM from these analyses, suggesting that the associations between cord blood insulin and glucose and neonatal size were not exclusive to babies born to mothers with GDM.

Table 5.8: Relationship of neonatal cord blood insulin concentrations (in thirds) to the anthropometric measurements of normal, term neonates (all measurements have been adjusted for gestation).

(SD's and interquartile ranges in brackets).

Cord blood insulin (pmol/l) (thirds)	Neonatal Anthropometric Measurements											
	Birth weight (g) n=554	Circumferences (cm)				Skinfolds (mm)		Lengths (cm)			Ponderal Index (kg/m^3) n=554	Placental weight (g) n=553
		head n=554	chest n=552	abdomen n=552	mid-arm n=552	triceps n=552	subscap. n=552	CHL n=554	CRL n=552	leg n=552		
< 14.0	2885.7	34.0	32.0	29.8	10.2	4.1	4.4	48.9	32.1	16.7	24.7	402.3
– 31.0	2913.3	33.9	32.1	30.1	10.4	4.2	4.5	49.1	32.1	17.0	24.5	402.1
> 31.0	3076.1	34.2	32.7	30.6	10.7	4.2	4.4	49.3	32.4	16.9	25.7	413.1
All	2958.0 (417.0)	34.0 (1.3)	32.2 (1.7)	30.2 (2.0)	10.4 (0.9)	4.2 (3.7,4.9)	4.5 (3.9,5.0)	49.1 (2.1)	32.2 (1.7)	16.9 (1.5)	25.0 (2.8)	411.9 (360.1,470.4)
p for trend	< 0.001	0.06	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004	0.1	0.02	< 0.001	< 0.001
p adj. for sex	< 0.001	0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.1	0.02	< 0.001	< 0.001
p adj. for sex and maternal fat mass	< 0.001	0.3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.03	0.4	0.03	0.02	< 0.001
p adj. for sex and cord glucose	< 0.001	0.1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.01	0.2	0.06	< 0.001	< 0.001

Table 5.9: **Relationship of neonatal cord blood glucose concentrations (in thirds) to the anthropometric measurements of normal, term neonates** (all measurements have been adjusted for gestation).

(SD's and interquartile ranges in brackets).

Cord blood glucose (mmol/l) (thirds)	Neonatal Anthropometric Measurements											
	Birth weight (g) n=571	Circumferences (cm)				Skinfolds (mm)		Lengths (cm)			Ponderal Index (kg/m^3) n=571	Placental weight (g) n=570
		head n=571	chest n=569	abdomen n=569	mid-arm n=569	triceps n=569	subscap. n=569	CHL n=571	CRL n=569	leg n=569		
< 5.3	2933.1	33.9	32.1	30.1	10.4	4.2	4.4	48.9	32.2	16.7	25.0	403.4
– 6.8	2947.9	34.0	32.2	30.1	10.4	4.2	4.4	49.1	32.1	16.9	24.9	415.6
> 6.8	2986.2	34.2	32.3	30.3	10.5	4.3	4.5	49.3	32.4	16.9	25.0	421.6
All	2955.6 (417.0)	34.0 (1.3)	32.2 (1.7)	30.1 (2.0)	10.4 (0.9)	4.2 (3.7,4.9)	4.4 (3.9,5.0)	49.1 (2.1)	32.2 (1.7)	16.9 (1.5)	25.0 (2.8)	413.3 (360.1,470.4)
p for trend	0.2	0.03	0.2	0.5	0.8	0.7	0.8	0.02	0.2	0.04	0.7	0.06
p adj. for sex	0.2	0.06	0.2	0.5	0.8	0.6	0.7	0.03	0.3	0.05	0.7	0.06
p adj. for sex and maternal fat mass	0.4	0.1	0.4	0.7	0.9	0.9	0.98	0.05	0.5	0.06	0.6	0.1
p adj. for sex and cord insulin	0.8	0.2	0.7	0.95	0.4	0.6	0.6	0.07	0.3	0.1	0.3	0.3

5.6 Summary of Main Findings

1 Babies born to mothers with GDM were bigger in all dimensions. This was most marked for measures of neonatal fat, but these babies were also significantly longer, indicating effects on skeletal growth as well as fat. This increase in growth was independent of gestational age, sex, maternal age, parity and fat mass and was evident across the range of maternal blood glucose concentrations, as measured by the maternal AUGC. It was not therefore a specific effect seen only in mothers diagnosed with GDM, although in those mothers, the effect was far more pronounced.

2 Babies born to mothers with GDM had higher cord blood glucose and insulin concentrations. Cord insulin concentrations were positively correlated with all the neonatal anthropometric measurements but were strongest with measures of neonatal fat and weakest with length and head, suggesting that the increased soft tissue growth seen in these babies was due to fetal hyperinsulinaemia. Cord glucose concentrations were related to neonatal crown-heel length, leg length and head circumference but not to measures of fat.

5.7 Discussion

Detailed neonatal anthropometry in this study has allowed better than usual characterisation of the features of macrosomia. It is well known that babies born to diabetic mothers are fatter.[179] Increased birth length, although well documented,[179] has not been highlighted, and an increase in head circumference has not, to my knowledge been described before. Increased neonatal size was more closely related to maternal fasting glucose concentrations than to post-load values. It is not surprising that insulin-sensitive tissues like fat should increase, but interesting that skeletal growth (traditionally insulin-insensitive) is also increased. Mechanisms of fetal overgrowth in GDM are not well understood. It has been suggested that chronic

insulin excess leads to an increase in IGF-1 receptors, or that the insulin receptor does after all have growth-mediating properties.[181]

Cord glucose and insulin concentrations were significantly related to maternal glucose concentrations and neonatal anthropometry. For cord insulin concentrations, stronger associations were seen with measures of neonatal fat, while for cord glucose concentrations stronger associations were seen with skeletal measurements.

In addition to these changes in babies of diabetic mothers, we demonstrated an increase in fetal size and cord glucose/insulin concentrations across the range of glucose concentrations in mothers with normal glucose tolerance, suggesting that lesser degrees of maternal hyperglycaemia may stimulate the fetal pancreas and influence fetal growth. This has been shown in other studies, mainly in relation to birthweight,[180] but also in relation to length, head circumference and fat as measured by the thigh skin-fold thickness.[111]

Freinkel postulated that long-term functional changes may occur in the fetus exposed to altered fuels during pregnancy.[78] A number of epidemiological studies have shown that GDM is a risk factor for type 2 diabetes and obesity in later life in the offspring.[93, 94] In experimental animals, offspring of hyperglycaemic mothers are glucose intolerant in adult life.[88, 182] Convincing evidence in humans that a diabetic tendency is transmitted from one generation to the next by the intra-uterine environment in GDM comes from studies of the Pima Indians of North America.[93] In this population there are high rates of diabetes in people of low birthweight, but also in those of high birthweight born to mothers with GDM, creating a U-shaped relationship between type 2 diabetes and birthweight.[95] As India has both a high prevalence of low birthweight and childhood stunting, and a high prevalence of GDM, these may be important factors leading to a vicious cycle of inter-generational transmission of diabetes. As the prevalence of GDM and type 2 diabetes increases, birthweight will also increase and a U-shaped relationship of diabetes with birthweight may emerge in the future.

6. Results – Maternal Birth Size

6.1 Introduction

With the discovery over recent years that size at birth is a risk factor for disease in later life, much interest has been generated in the determinants of fetal growth. In the UK and in Europe, studies have shown that babies of low birth weight and low ponderal index are at greater risk of developing insulin resistance and diabetes in adult life, especially if they become fat as adults.[51-53, 49] In a recent study in India, low birthweight was related to higher levels of insulin resistance in children.[183] In the Pima Indians, a population with an extremely high prevalence of type 2 diabetes, a U-shaped relationship was found between birthweight and prevalence of diabetes,[95] the heavier babies being those born to mothers with GDM during the pregnancy. From the first Mysore study, men and women with the highest prevalence of diabetes had been heavier babies, fatter and shorter, and it was suggested that they may have been born to mothers with GDM also.[49]

One aim of this study was to determine whether early-life undernutrition was related to GDM prevalence. These relationships were assessed using height and head circumferences as indicators of early growth. However, a small proportion of women who participated in this study were born in the hospital and had birth records available. From this group I was able to analyse relationships between maternal birthweight and GDM prevalence and between maternal birthweight and the offsprings birthweight.

6.2 Relationship of mother's size at birth to her adult anthropometry

118 (14.2%) women had been born in the hospital and birth records were found for 84 (71.2%). Data were excluded for one woman who was a twin and another who was born prematurely at 31 weeks gestation. Birth data for the remaining 82 women are shown in Table 6.1. Placental weight was not used in further analyses, as the numbers were too small to be meaningful.

Table 6.1: Maternal size at birth. Means and standard deviations (SD) shown. (All births took place at HMH between 1958 & 1981)

Maternal birth data	N	Mean	SD
Birthweight (g)	82	2821.8	446.4
Head circumference (cm)	70	33.6	1.8
Crown-heel length (cm)	69	48.6	3.0
Placental weight (g)	26	437.2	104.2

Women with higher birthweight were taller adults (p=0.02, β=0.003 cm/g), and women who were longer at birth were heavier (p=0.01, β=0.02 kg/cm) with higher BMI's (p=0.04, β=0.01). This appeared to be due to an increase in fat, rather than skeleton or muscle as there were no significant relationships between length as a baby and height, head or muscle mass. Whereas, there were strong, positive relationships between length and all measures of body fat i.e. mid-upper arm and mid-thigh circumferences (p=0.03 for both), biceps, triceps and suprailiac skin-folds (p=0.02 for all), all three external pelvic diameters (p=0.02 for all) and the calculated fat mass (p=0.009). All these relationships remained significant after adjustment for adult age. Head circumference at birth was not related to adult head circumference or to any other adult anthropometric variable.

6.3 Relationship of mother's size at birth to her adult glucose / insulin status

77 mothers with birth records available had completed the OGTT and 9 (11.7%) had GDM. Table 6.2 shows differences in mean birthweight, length and head circumference at birth for mothers with and without GDM.

Table 6.2: Birth size of mothers with and without GDM. Mean and SD shown. P value for difference in means calculated by the student's t-test.

Birth measurements	GDM Yes / No	N	Mean	SD	p for difference in means
Birthweight (g)	No	68	2830.4	439.8	
	Yes	9	2680.6	462.4	**0.3**
Crown-heel length (cm)	No	59	48.5	3.2	
	Yes	5	48.0	1.9	**0.7**
Head circumference (cm)	No	59	33.7	1.9	
	Yes	6	32.8	0.4	**0.008**

Although the relationships of GDM to birthweight and length did not reach statistical significance, the data suggest that mothers with GDM may have been smaller at birth: lighter and shorter, with smaller head circumferences.

Birthweight and length were not significantly related to individual maternal plasma glucose or insulin concentrations or to measures of insulin resistance or secretion. Head circumference at birth was negatively related to maternal plasma glucose concentrations at 30- and 120-minutes ($p=0.02$, $p=0.01$ respectively) and to plasma insulin at 120-minutes ($p=0.04$). However, these relationships were lost after adjusting for maternal age, suggesting that the trend with neonatal head circumference is present simply because mothers with smaller heads at birth were older.

There was a significant negative trend in neonatal head circumference over time ($p=0.02$, $\beta=-0.1$ cm/year). Babies born in the 1980's had larger heads than those born in the 1970's, who had larger heads than those born in the 1960's (Table 6.3). This may reflect an improvement in fetal growth over time, which has affected head size to a greater extent than either length or birthweight. Interestingly, a similar trend was

found in data from the first Mysore study, for babies born between 1934 and 1957 (p=0.02, β=-0.04 cm/year) (C.Stein, personal communication). In data from the Mysore Intergenerational Study (C.Fall, personal communication), the head circumference at birth of women born between 1955 and 1975, exceeded that of their mothers born between 1934 and 1955 (33.9 cm cf. 33.2 cm).

Table 6.3: Mean head circumference at birth according to adult age
(number of women shown in parenthesis)

Age (years)	Mean head circumference (cm)	
16 – 20	34.3	*(24)*
21 – 25	33.5	*(26)*
26 – 40	32.7	*(21)*
p for trend	**0.02**	

6.4 Relationship of mothers' birthweight and adult fat mass to GDM prevalence

After correcting for maternal age, mothers with birthweights below the mean, had a higher prevalence of GDM (12.9% v 4.8%) (Table 6.4). There were no cases of GDM in mothers whose birthweight was above the mean and adult fat mass below the mean. Although numbers were small and the *p* value for the association with birthweight non-significant (p=0.4), these differences in prevalence were very striking.

Table 6.4: Prevalence of age-adjusted GDM (%) according to mother's birthweight and current fat mass (kg)

(number of women shown in parenthesis)

Birthweight \ Fat mass	< 18.0	> 18.0	All
< 2.81	**16.5** *(25)*	**9.4** *(15)*	**12.9** *(41)*
> 2.81	**0.0** *(14)*	**7.8** *(23)*	**4.8** *(37)*
All	**9.8** *(39)*	**8.2** *(39)*	**8.9** *(77)*

Logistic regression model contained the predictor variables; age (years), fat mass(kg) and birthweight (g) for the dependent variable: GDM (no=0, yes=1)

	Regression coefficient	*Exp(β)*	*Standard Error*	*P value*
Birthweight (g):	*- 0.001*	*0.99*	*0.001*	*0.4*
Age (years):	*0.5*	*1.62*	*0.2*	*0.006*
Fat mass (kg):	*0.03*	*1.03*	*0.1*	*0.8*
Constant:	*-12.9*			

6.5 Relationship of mother's size at birth to that of her baby

71 mothers with birth records delivered at HMH; 7 babies were born prematurely (at <37 weeks) and one was stillborn at term. Analyses were performed using data for the remaining 63.

Mother's birthweight was positively related to all the baby's anthropometric measurements. There were strong relationships with baby's birthweight ($p<0.001$, $\beta=0.4$ g/g), head, chest, abdominal and mid-upper-arm circumferences ($p\leq0.005$), crown-heel and crown-rump lengths ($p\leq0.002$) and triceps skin-fold ($p=0.01$). There were non-significant but positive relationships with subscapular skin-fold ($p=0.09$), ponderal index ($p=0.07$) and placental weight ($p=0.2$). There was no evidence of a trend with leg length ($p=0.9$).

Following adjustment for maternal glycaemia (AUGC) and adult body composition; height, fat mass, muscle mass and head circumference, all these relationships were strengthened (apart from that with leg length) and mother's birthweight was shown to be a significant factor in predicting her baby's birthweight (Table 6.5).

Table 6.5: Predictors of neonatal birthweight (g) (n=63). Multiple linear regression model (r^2 value = 0.6). Regression coefficients (β) and p values shown.

	β	P
Mother's birthweight (g)	0.5	< 0.001
Mother's current size: height (cm)	- 27.6	0.007
head (cm)	65.2	0.02
fat mass (kg)	10.1	0.7
muscle mass(kg)	24.5	0.3
Mother's parity (0-4)	- 101.2	0.1
Mother's AUGC ($mmol.l^{-1}$ min^{-1})	0.4	0.02
Baby's sex (male=1, female=2)	- 60	0.5
Baby's gestation (weeks)	137.0	0.003

Mother's head circumference at birth was not significantly related to any of her baby's anthropometric measurements until after allowing for maternal glycaemia and adult body composition. At which time, significant trends with neonatal birthweight (p=0.05, β=27.1 g/cm), chest circumference (p=0.01, β=0.2 cm/cm) and subscapular skin-fold (p=0.02, β=0.009 mm/cm) were seen.

Mother's crown-heel length was positively related to baby's birthweight ($p=0.02$, $\beta=40.1$ g/cm), head circumference ($p=0.01$, $\beta=0.1$ cm/cm), chest, abdominal and mid-upper-arm circumferences ($p<0.01$ for all) and ponderal index ($p=0.02$, $\beta=0.2$). There were no relationships with neonatal length (crown-heel, crown-rump or leg length), triceps or subscapular skin-folds or placental weight. Adjusting for maternal glycaemia and adult body composition did not significantly alter these relationships.

6.6 Relationship of mother's size at birth to baby's cord blood glucose and insulin concentrations.

There were no significant relationships between mother's size at birth and her baby's cord blood glucose, insulin, proinsulin or 32,33-split proinsulin concentrations.

6.7 Summary of Main Findings

1. Longer babies became heavier, fatter adults and heavier babies became taller adults. Head circumference at birth did not predict adult head circumference or any other adult anthropometric variable.

2. Head circumference at birth was associated with maternal age, younger women having bigger heads, and may reflect an improvement in fetal growth over time.

3. Women who developed diabetes during pregnancy were more likely to have been smaller babies, lighter and shorter with smaller heads. This is consistent with the starting hypothesis, that impaired growth in early life is a risk factor for diabetes in the adult, but may be an artefact caused by changes in birth size with time.

4. Mother's own birthweight was strongly and positively related to her baby's birthweight. After adjusting for maternal glycaemia and adult body composition, maternal birthweight was related to all the anthropometric measurements in the

baby (apart from leg length) and was a significant predictor of the baby's birthweight.

5. Mother's head circumference at birth related was positively related to the birthweight of her baby but not to head circumference. Mother's length at birth was similarly related to the birthweight of her baby but not to length.

6. There did not appear to be any significant relationships between the mother's size at birth and the glucose, insulin, proinsulin, or 32,33-split proinsulin concentrations measured from the cord blood of her baby.

6.8 Discussion

The association between low birthweight and future development of diabetes has already been discussed in the introduction to this thesis. By tracing the birth records of women participating in this study, who had been born in the hospital, I was hoping to show a similar association between low birthweight and the development of GDM. However, fewer women than originally supposed, had been born in the hospital and had birth records available. The data shown in this chapter suggest a link between GDM and low maternal birthweight although numbers were small and this relationship was not statistically significant. I calculate that I would need 177 each, of women with and without GDM, of known birth size, to show a statistically significant effect. Three studies from the UK and USA with larger sample sizes have shown an association between GDM and low birthweight.[173-175] The one study which did not show this association had a smaller sample size.[184] Despite small numbers, the findings from this study are consistent with the hypothesis that poor growth in fetal life and / or in early childhood, are risk factors for the later development of GDM.

7. Results – Maternal Blood Pressure

7.1 Introduction

Until recently and with the exception of women who develop pre-eclampsia or severe hypertension, no association had been found between maternal blood pressure during pregnancy and the birthweight of the baby.[71, 72] However, studies that had looked for an association relied on routine blood pressure measurements made in the ante-natal clinic and there is much evidence that blood pressure is not measured reliably in that setting.[185] Churchill et al.[73] used ambulatory blood pressure measurements and has shown a continuous inverse association between birthweight and maternal blood pressure, throughout the range seen in normal pregnancy.

Changes in the maternal cardiovascular system during pregnancy have an important role in optimising utero-placental blood flow and thus the supply of oxygen and nutrients to the growing fetus. In 'normal' women, these changes include an increase in cardiac output, a decrease in total peripheral resistance and an associated decrease in diastolic blood pressure with little change in systolic blood pressure. The fall in blood pressure is maximum by mid-pregnancy and thereafter blood pressure rises to pre-pregnant levels by term.[186] Size at birth has been shown to be an important determinant of adult hypertension,[187] the hypothesis being that poor maternal nutrition or supply to the fetus at critical periods of development, programmes the subsequent development of hypertension and other cardiovascular risk factors.

In this study blood pressure was measured in 832 women at 30+/-2 weeks gestation in order to define relationships between blood pressure and maternal anthropometry, maternal glucose and insulin concentrations and neonatal size. In a small proportion of women with birth records available, relationships between maternal size at birth and blood pressure during pregnancy were examined. Blood pressure measurements were made by one of four observers using a standard mercury sphygmomanometer and Korotkoff sound V to represent the diastolic pressure. Prior to starting this study, blood pressure measurements were standardised and inter-observer variation studies

performed in order to minimise error. Two readings were taken with a standard cuff according to a set protocol (Appendix 2) and room temperature was recorded. If the blood pressure was greater than or equal to 140/90 mmHg, it was measured again one hour later and if still raised, the woman was immediately referred to the consultant obstetrician caring for her and managed according to the hospital protocol.

7.2 Relationship of blood pressure to maternal age, parity, social class and anthropometry

Two women with hypertension were excluded from the analysis; one was known to be hypertensive and was receiving treatment, the other was newly diagnosed and treatment was initiated the same day. Mean systolic blood pressure (SBP) in the remaining 830 women was 103.1 mmHg (SD 9.6) and mean diastolic blood pressure (DBP) was 63.4 mmHg (SD 0.04). Blood pressure was significantly related to room temperature; the higher the room temperature, the lower the blood pressure (SBP; $p=0.001$, $\beta=-0.8$ mmHg/ ^{o}C and DBP; $p=<0.001$, $\beta=-0.9$ mmHg/ ^{o}C). Room temperature ranged from 25^{o}C to 31^{o}C and was adjusted for in all further analyses. There was no relationship between maternal blood pressure and gestation at the time of the clinic visit.

Higher blood pressures were associated with older age and lower parity. SBP was more strongly related to parity than to age (parity: $p=0.003$, $\beta=-1.2$, age: $p=0.2$, $\beta=0.1$), while DBP showed a stronger relationship with age than with parity (age: $p=0.004$, $\beta=0.2$, parity: $p=0.06$, $\beta=-0.7$). Neither SBP nor DBP were related independently to social class. Both SBP and DBP were strongly and positively related to maternal weight, BMI, fat mass, muscle mass ($p<0.001$ for all) and head circumference ($p=0.002$). These relationships remained significant following adjustment for parity and age. The relationships with head circumference lost their significance after adjusting for fat mass. There were borderline positive relationships with height ($p=0.05$ for both), which were lost when age and parity were adjusted for.

7.3 Relationship of blood pressure to maternal blood glucose and insulin concentrations

Two women known to have pre-gestational diabetes and receiving insulin therapy at the time of the clinic visit were excluded from this analysis.

SBP was positively related to GDM ($p=0.004$). Higher glucose concentrations at all time points and higher insulin resistance indices: 120-minute insulin concentration, 32,33-split proinsulin and RIR-HOMA, were associated with higher SBP ($p<0.001$ for all). Following adjustment for maternal age, parity and fat mass, relationships with individual glucose concentrations were weakened ($p<0.05$ for all) and significant relationships with insulin resistance and GDM were lost ($p=0.08$ for the relationship with GDM). DBP was not related to GDM, although it showed significant positive trends with fasting and 120-minute glucose concentrations ($p<0.05$) and with insulin resistance indices: 32,33-split proinsulin ($p=0.002$) and RIR-HOMA ($p=0.003$). Following adjustment for maternal age, parity and fat mass, significance was lost for these relationships.

7.4 Relationship of maternal blood pressure to neonatal size at birth

This analysis was restricted to full-term neonates. Maternal blood pressure was not significantly related to any neonatal anthropometric measurement in univariate analysis. However, after adjustment for gestational age, sex, maternal parity, age and adult body composition (fat mass, height, head circumference and muscle mass), women with higher blood pressure gave birth to smaller babies and DBP was a stronger predictor of neonatal size than SBP.

Both SBP and DBP were inversely related to neonatal birthweight (SBP; $p=0.04$, β=-3.7 g/mmHg and DBP; $p=0.006$, β=-5.7 g/mmHg), chest circumference (SBP; $p=0.05$, β=-0.01 cm/mmHg and DBP; $p=0.02$, β=-0.02 cm/mmHg), abdominal circumference (SBP; $p=0.07$, β=-0.02 cm/mmHg and DBP; $p=0.001$, β=-0.03 cm/mmHg), mid-upper-arm circumference (SBP; $p=0.03$, β=-0.009 cm/mmHg and DBP; $p=0.006$, β=-

0.01 cm/mmHg) and crown-rump length (SBP; p=0.04, β=-0.02 cm/mmHg and DBP; p=0.003, β= -0.03 cm/mmHg). In addition, DBP was strongly related to crown-heel length (p<0.001,β=-0.04 cm/mmHg) and had a borderline relationship with leg length (p=0.06,β=-0.02 cm/mmHg). There were borderline inverse relationships between blood pressure and neonatal fat as measured by the subscapular skin-fold (SBP; p=0.07, β=-0.002 mm/mmHg and DBP; p=0.08, β=-0.002 mm/mmHg) and between DBP and the triceps skin-fold (p=0.06, β=-0.002 mm/mmHg). There were no significant relationships with ponderal index, head circumference or placental weight.

Table 7.1a: Relationship of mothers' DBP (mmHg) and BMI (kg/m^2) measured at 30+/-2 weeks gestation to the birthweight (g) of her baby (DBP adjusted for room temperature and birthweight adjusted for sex and gestation). *(Number of babies shown in parenthesis).*

	Maternal DBP (mmHg) in thirds			
BMI (kg/m^2) in thirds	**< 59**	**- 68**	**> 68**	**All**
< 21.6	2844.7 (98)	2787.7 (55)	2817.6 (46)	2822.7 (199)
- 24.6	2964.2 (64)	2986.0 (73)	2844.2 (62)	2934.8 (199)
> 24.6	3170.2 (50)	3110.5 (60)	3079.8 (89)	3111.8 (199)
All	2957.6 (212)	2967.7 (188)	2944.5 (197)	2956.4 (597)

Table 7.1b: Relationship of mothers' SBP (mmHg) and BMI (kg/m^2) measured at 30+/-2 weeks gestation to the birthweight (g) of her baby (SBP adjusted for room temperature and birthweight adjusted for sex and gestation). *(Number of babies shown in parenthesis).*

	Maternal SBP (mmHg) in thirds			
BMI (kg/m^2) in thirds	**< 99**	**- 106**	**> 106**	**All**
< 21.6	2873.6 (80)	2814.8 (73)	2746.8 (46)	2822.7 (199)
- 24.6	2925.6 (51)	2985.3 (86)	2872.4 (62)	2934.8 (199)
> 24.6	3088.0 (41)	3097.6 (68)	3133.4 (90)	3111.8 (199)
All	2940.1 (172)	2964.1 (227)	2961.8 (198)	2956.4 (597)

7.5 Predictors of birthweight

In order to assess the magnitude of the effect that maternal blood pressure has on the birthweight of the baby, a multiple linear regression model was constructed to include known predictors of birthweight:

1. Multiple linear regression analysis of birthweight with predictors as listed below: (y variable = birthweight (g), $R^2 = 0.20$)

	Regression coefficient	Standard Error	P value
DBP (mmHg):	-6.0	2.1	0.004
Room temp.(°C):	16.8	13.0	0.2
Sex (M=1, F=2):	-128.7	33.2	<0.001
Gestation (weeks):	91.1	14.6	<0.001
Parity:	22.8	21.6	0.3
Age (years):	2.9	4.1	0.5
Fat mass (kg):	18.7	3.2	<0.001
Height (cm):	0.3	3.3	0.9
Head circ. (cm):	21.7	12.2	0.08
Muscle mass (kg):	10.1	8.0	0.2
Fasting glucose (mmol/l):	94.5	34.2	0.006
Constant:	-2800.2		

2. Multiple linear regression analysis of birthweight with predictors as listed below: (y variable = birthweight (g), $R^2 = 0.19$)

	Regression coefficient	Standard Error	P value
SBP (mmHg):	- 4.1	1.8	0.02
Room temp.(°C):	19.4	12.9	0.1
Sex (M=1, F=2):	-135.1	33.4	<0.001
Gestation (weeks):	93.0	14.7	<0.001
Parity:	20.4	21.8	0.4
Age (years):	2.0	4.2	0.6
Fat mass (kg):	18.7	3.3	<0.001
Height (cm):	0.3	3.3	0.9
Head circ. (cm):	21.0	12.2	0.09
Muscle mass (kg):	9.8	8.0	0.2
Fasting glucose (mmol/l):	98.0	34.4	0.004
Constant:	-2824.1		

From the above analyses, approximately 20% of neonatal birthweight can be accounted for by sex and gestation at birth, maternal fat mass, fasting blood glucose and blood pressure, systolic or diastolic. Maternal age, parity, height, head circumference and muscle mass were not significant predictors.

7.6 Relationship of mother's birth size to her blood pressure

In women with birth records available, maternal birth size was not related to DBP in pregnancy. However, mothers who had been longer at birth, had higher SBP in pregnancy (p=0.006, β=0.95 mmHg/cm), independently of parity, age and adult BMI (Table 7.2). There were no significant relationships between mother's birthweight or head circumference at birth and her SBP in pregnancy.

Table 7.2: Relationship of mother's crown-heel length (cm) at birth (in thirds) and her adult BMI (kg/m^2) (in thirds) to her SBP (mmHg) in pregnancy. *(Number of women in shown in parenthesis).*

		Crown-heel Length (cm)			
		< 48.0	**- 50.0**	**> 50.0**	**All**
BMI (kg/m^2)	**< 21.4**	94.5 (9)	101.0 (13)	105.6 (7)	100.1 (29)
	- 24.6	98.1 (6)	100.6 (5)	99.7 (9)	99.4 (20)
	> 24.6	100.0 (6)	106.2 (7)	110.2 (8)	106.0 (21)
	All	97.1 (21)	102.4 (25)	104.9 (24)	101.7 (70)

7.7 Summary of Main Findings

1. Both systolic and diastolic blood pressure were inversely related to parity and increased with maternal age, fat mass and muscle mass.

2. Higher maternal blood pressure was associated with higher maternal glucose concentrations and with increasing insulin resistance although, these effects were probably mediated by older age and greater body fat. The relationships were strongest and most consistent with fasting glucose and RIR-HOMA.

3. After adjustment for maternal size, mothers with higher blood pressures gave birth to smaller babies. DBP was a stronger predictor of neonatal size than SBP, and was strongly related to shorter length.

4. In the small number of mothers with birth records available, longer length at birth was associated with a higher SBP in pregnancy.

7.8 Discussion

Pregnant women are usually young and fit and the range of blood pressures therefore tend to be narrower than in the general population. Nevertheless, as has been found in the non-pregnant population, higher blood pressure is associated with increasing age and weight. Its association with decreasing parity perhaps reflects the increased risk of pregnancy induced hypertension in primiparous women. From this study there was some evidence that higher blood pressure was associated with higher maternal glucose concentrations and insulin resistance, although these associations were probably mediated via age and body fat.

The recent finding by Churchill et.al.,[73] that maternal blood pressure has a continuous, inverse relationship with fetal size suggested to these investigators that maternal blood pressure could be an important confounding factor in the relationship of small size at

birth to adult hypertension and that this association could be genetically mediated. However, the data can also be interpreted as showing an intergenerational effect of the intrauterine environment on fetal growth,[188] suggesting that raised maternal blood pressure may reflect an increase in total peripheral resistance associated with a decrease in placental blood flow.

In this study we found similar inverse relationships between maternal blood pressure and size at birth. These were significant only after adjusting for maternal size. Relationships were stronger with diastolic blood pressure than with systolic. Detailed anthropometry of the neonates in our study allowed us to examine more closely relationships of maternal blood pressure to neonatal body composition. There were strong relationships between maternal DBP and birthweight, chest, abdominal and mid-arm circumferences, crown-rump and crown-heel length. There were borderline relationships with neonatal fat (triceps and subscapular skin-fold thicknesses) and no relationships with head circumference, ponderal index or placental weight. One interpretation of the association between maternal blood pressure and poor fetal growth is that the placenta can modify its own perfusion by influencing maternal blood pressure. Thus, high blood pressure during pregnancy may be a sign or consequence of suboptimal placental perfusion, rather than a cause of retarded fetal growth. Mechanisms by which maternal blood pressure may influence fetal growth are as yet largely unknown.

In women with birth records available, we found a significant relationship between longer length at birth and higher SBP in pregnancy. The association of size at birth and blood pressure in later life has been examined in two large systematic reviews,[189 190] which have demonstrated that in children, adolescents and adults there is an inverse relationship between birthweight and SBP after adjustment for current weight. However, in a previous study in Mysore, India,[191] small size at birth was not shown to be associated with increased adult blood pressure and in fact the association was between longer length at birth and higher SBP in adult life, similar to the relationship found in my study. One possible interpretation of these findings is that a combination of growth retardation in-utero and catch-up growth postnatally is required to raise adult blood pressure,[192] and in South Indian populations, poor post natal growth has

prevented a rise in blood pressure in people of lower birthweight. In a recent review, [190] skeletal and non-skeletal catch up growth were positively associated with blood pressure, with the highest blood pressures occurring in individuals of low birth weight but high rates of growth subsequently.

The findings from this study suggest that higher maternal blood pressure, within the normal range for pregnancy, affects fetal growth and is associated with lighter, shorter babies. Interestingly, mothers with higher systolic pressures in pregnancy had been longer at birth and although this finding is similar to that of the previous study in Mysore [191] it remains difficult to explain. Further research is required to elucidate mechanisms whereby maternal blood pressure affects fetal growth and whereby fetal growth effects adult blood pressure.

Chapter 8 (Pregnancy Outcomes) contains data not immediately pertinent to the hypothesis on which the study is based. The data are however interesting and I decided to include them for that reason.

8. Results – Pregnancy Outcomes

8.1 Introduction

Studies, which have looked at adverse outcomes in pregnancies associated with gestational diabetes (GDM), have not shown consistent results. Indeed the validity of the diagnosis itself has been questioned.[193] In an effort to resolve some of the underlying issues, an international study, Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) has begun, involving 25 field centres and 25,000 pregnant women (Boyd Metzger, personal communication). The aims are to define the maternal glucose concentrations that place the mother, fetus and neonate at increased risk and to arrive at an internationally accepted set of criteria for the diagnosis and classification of hyperglycaemia in pregnancy based on the identification of specific adverse outcomes.

Of 676 women who delivered at HMH, two had pre-gestational insulin-dependent diabetes; one delivered a stillborn infant at term, the other a 34-week premature infant following induction of labour for fulminating pre-eclampsia. 639 women had completed the OGTT at 30+/-2 weeks gestation and 42 (6.6%) were found to have GDM. However, due to differences in Cambridge and Mysore measurements of blood glucose (page 53), only 21 women with GDM were recognised and therefore treated in Mysore; 14 with diet alone and 7 with insulin.

Pregnancy complications and outcomes were recorded for all women and comparisons made between women with normal glucose tolerance, women with recognised/treated GDM and women with unrecognised / untreated GDM.

8.2 Pregnancy Complications

Pregnancy complication rates among women who delivered in this study were lower than expected although there were no data from the obstetric population as a whole with which to compare. It is possible that complications or problems arising during the

pregnancy have been underestimated due to poor recording in the obstetric notes during the pregnancy. From the data available there did not appear to be an increase in complications during pregnancy in women with GDM (Table 8.1) apart from a possible association with pre-eclampsia (PET). This diagnosis was made by the obstetricians on the basis of two blood pressure readings of greater than or equal to 140/90 mmHg (taken one hour apart) and the presence of proteinuria (one plus or more) on urine dipstix. PET occurred in two women with GDM (only one of whom was diagnosed in Mysore).

Table 8.1: Maternal Pregnancy Complications Recorded

Maternal Pregnancy Complications	All women (n=676)	Women with normal OGTT (n=597)	Women with GDM (n=42)
1. Pre-eclampsia	13 (1.8%)	10 (1.7%)	2 (4.8%)
2. Urinary tract infection	5 (0.7%)	5 (0.8%)	-
3. Ante-partum haemorrhage	1 (0.1%)	1 (0.2%)	-
4. Cervical incompetence – circlage	7 (1.0%)	7 (1.2%)	-

8.3 Induction of Labour

Induction of labour was performed routinely at HMH on or around the expected date of delivery. A prostaglandin pessary was inserted if the cervix was thought unfavourable. This was followed by rupturing the membranes and establishing an oxytocin infusion once the cervix was deemed favourable. With this policy of routine induction at term, the induction rate was high, occurring in 33.7% of women in this study, 80% of whom were induced for no other reason than that their pregnancies had reached term (Table 8.2). Overall, the induction rate was similar in women with GDM (28.6%), however, in women with GDM, a higher proportion were induced for the reason that their fetus was large-for-dates (LFD). Of the 12 women with GDM who

were induced, 9 had been recognised and were being treated and the 3 who had not were induced only for term pregnancies.

Table 8.2: Reasons given for induction of labour

Reasons given for induction	All women induced n=228 (%)		Women with normal OGTT n=201 (%)	Women with GDM n=12 (%)
1. Post-dates	132	(57.9)	115 (57.2)	5 (41.7)
2. Term	51	(22.4)	48 (23.9)	2 (16.7)
3. Reduced fetal movements	3	(1.3)	3 (1.5)	-
4. Suspicious CTG	3	(1.3)	3 (1.5)	-
5. Small for dates ?IUGR	6	(2.6)	6 (3.0)	-
6. Oligohydramnios / low BPS	9	(3.9)	9 (4.5)	-
7. Prolonged ROM's	5	(2.2)	5 (2.5)	-
8. PIH	5	(2.2)	3 (1.5)	1 (8.3)
9. Large for dates	4	(1.8)	1 (0.5)	3 (25.0)
10. Previous IUD at term	6	(2.6)	5 (2.5)	-
11. Current IUD	3	(1.3)	2 (1.0)	1 (8.3)
12. Maternal request	1	(0.4)	1 (0.5)	-

8.4 Mode of Delivery

75.3% of women achieved a spontaneous vaginal delivery, 1.6% of whom delivered breech. Assistance with forceps or ventouse was required in 5.8% of women and caesarean sections were performed in 18.9%, 16.4% of which were elective (Table 8.3). When comparing mode of delivery in women with and without a diagnosis of GDM, women with GDM were twice as likely to be delivered by caesarean section

(Table 8.4) although the significance of this finding was borderline (95% confidence intervals: 1.0–4.2). Whether or not the diagnosis of GDM was known in Mysore did not significantly effect the mode of delivery (p=0.5 from Chi-Square testing).

Table 8.3: Mode of delivery and reasons for operative deliveries.
Number of women shown (percentages in parenthesis).

Mode of delivery and reasons for operative deliveries	All women N=676 (%)	Women with normal OGTT n=597 (%)	Women with GDM n=42 (%)
Vaginal cephalic	**498 (73.7)**	**447 (74.9)**	**26 (61.9)**
Vaginal breech	**11 (1.6)**	**9 (1.5)**	**-**
Forceps delivery	**35 (5.2)**	**30 (5.0)**	**2 (4.8)**
1. delay in second stage	19	16	1
2. fetal distress	9	8	1
3. maternal exhaustion	4	4	-
4. maternal cardiac disease	2	2	-
5. PIH	1	-	-
Ventouse delivery	**4 (0.6)**	**3 (0.5)**	**1 (2.4)**
1. delay in second stage	4	3	1
Emergency LSCS	**107 (15.8)**	**92 (15.4)**	**9 (21.4)**
1. fetal distress	48	40	3
2. failure to progress	32	26	5
3. failed induction	4	4	-
4. breech (undiagnosed)	11	11	-
5. unstable lie	3	3	-
6. malpresentation	3	3	-
7. fulminating PET	3	2	1
8. APH	1	1	-
9. failed forceps	1	1	-
10. cord prolapse	1	1	-
Elective LSCS	**21 (3.1)**	**16 (2.7)**	**4 (9.5)**
1. previous LSCS	11	7	4
2. two previous LSCS	5	5	-
3. breech	4	4	-
4. maternal request	1	-	-

8.5 Neonatal Outcomes

20.3% of babies born in this study were low birthweight (LBW) (defined as weight less than 2.5kg). 31.4% of them were born at less than 37 weeks gestation, implying that the majority (68.6%) of LBW babies were small for dates (SFD) rather than premature. The prevalence of LBW among mothers with GDM was 9.5% and none of these babies were premature. Rates of premature delivery were high in this study population (10.5%) and this may have been due in part to the high induction rates around term. Two premature births were to mothers with GDM (4.8%).

A total of 92 (13.6%) babies were admitted to the special care nursery for the reasons given in Table 8.5, 21.4% of them were born to mothers with GDM. One third of nursery admissions were for respiratory distress, only one of these babies was born to a mother with GDM. Birth asphyxia and a birthweight of less than 2.3 kg accounted for another third of admissions, none of these babies were born to mothers with GDM. Seven babies weighing over 3.5 kg were admitted for observation, two of these were born to mothers with GDM. Babies of diabetic mothers on insulin were routinely admitted for observation and exclusion of hypoglycaemia. Hypoglycaemia was tested for in all babies admitted to the nursery and was diagnosed in 20 babies (21.7% of admissions), two of whom were born to mothers with GDM (22.2%). The complications of prematurity, suspected meconium aspiration and congenital anomaly accounted for the rest of the nursery admissions and apart from one baby born prematurely, did not involve babies born to mothers with GDM.

The incidence of birth injury, associated with or without shoulder dystocia, is increased in macrosomic infants. In this study however, there were no significant birth injuries and only two cases of mild / moderate shoulder dystocia. In neither case was the mother diabetic. The birthweight of the baby in each case of shoulder dystocia was 3150g and 3470g.

Unexplained stillbirth is another known complication of maternal diabetes. In this study population there were 9 stillbirths (1.3%) one occurred as described at the

beginning of this chapter in a woman with pre-gestational diabetes and the other occurred in a woman with gestational diabetes, treated only with diet.

Table 8.5: Reasons for neonatal admission to the nursery

Reasons for admission	All babies N=92 (%)		Babies born to mothers with GDM n=9 (%)
1. respiratory distress (RDS or infective)	29	(31.5)	1 (11.1)
2. birth asphyxia (requiring resuscitation)	16	(17.4)	-
3. birth weight < 2.3 kg	14	(15.2)	-
4. prematurity (< 34 weeks)	12	(13.0)	1 (11.1)
5. suspected meconium aspiration	5	(5.4)	-
6. maternal diabetes (insulin usage)	7	(7.6)	5 (55.6)
7. birth weight > 3.5 kg	7	(7.6)	2 (22.2)
8. major congenital anomaly	2	(2.2)	-

8.6 Summary of Main Findings

1. The relative risks of various obstetric outcomes occurring in women with GDM were examined using logistic regression and summarised in Table 8.4. From this analysis, the only significant increased risks for women with gestational diabetes were those of having a large-for-dates baby (in this population, birthweight greater than 3.5 kg at term) and an increased likelihood of delivery by caesarean section.

2. As only 50% of woman with GDM were actually recognised and treated in Mysore, I was able to compare caesarean section rates and rates of LFD's

babies in women in whom the diagnosis was known and in those in whom it wasn't. Using Chi-Squared tests, I concluded that there were no significant differences in either the caesarean section rate or in the rate of LFD's babies between these two groups, suggesting that knowledge of the diagnosis did not significantly influence the obstetrician's management of the case.

Table 8.4: Obstetric outcomes in women with GDM (n=42) compared to those in women with a normal OGTT at 30+/-2 weeks (n=597).

Obstetric Outcomes		GDM		Odds Ratio	95% CI
		No	Yes		
Pre-eclampsia	No	587	40		
	Yes	10	2	2.9	0.6 – 13.9
Labour induced	No	380	26		
	Yes	201	12	0.9	0.4 – 1.8
Mode of delivery	SVD	447	26		
	LSCS	108	13	2.1	1.0 – 4.2
Nursery admission	No	519	33		
	Yes	78	9	1.8	0.8 – 3.9
Stillbirth	No	591	41		
	Yes	6	1	2.4	0.3 – 20.4
Congenital anomaly	No	584	41		
	Yes	13	1	1.1	0.1 – 8.6
Pre-term delivery	No	533	40		
	Yes	64	2	0.4	0.1 – 1.8
LFD (> 3.5 kg)	No	563	32		
	Yes	34	10	5.2	2.3 – 11.4

8.7 Discussion

The assessment of poor outcomes associated with a diagnosis of GDM has been difficult due to different criteria used to define the disease, different screening programmes in place to discover it and different treatment strategies following the diagnosis. Not only that, many studies have failed to account for other risk factors such as maternal age and obesity. Initial studies showed an increase in perinatal mortality in women with GDM although the difference was not statistically significant,[194] and the published analyses did not sufficiently examine the potential confounding variables, of which age and obesity were the most obvious. Subsequent data from Belfast suggest that 'any putative risk associated with GDM must be small',[195] and it would require very large numbers of observations to provide statistical significance and to adjust for confounding variables. Arguments used in favour of screening for GDM cite increased morbidity (maternal and neonatal) rather than mortality.

Maternal risks in the short term appear to be those associated with an increased likelihood of caesarean section and it has been argued that knowledge of the diagnosis itself is associated with an increased caesarean section rate.[193] However, in this study, although women with GDM were twice as likely to be delivered by caesarean section, there was no difference in rate between those in whom the diagnosis was known and in those in whom it wasn't. Some studies have shown an association between hypertension in pregnancy and GDM. This study suggests a possible association with pre-eclampsia although this was not statistically significant. Pre-eclampsia is associated with failure of trophoblast invasion of the spiral arterioles of the placenta, but the complete pathophysiological process in poorly understood. As pregnancy is itself an insulin resistant state, and insulin resistance is a feature of essential hypertension in non-pregnant individuals, it has been suggested that pregnancy-induced hypertension is related to insulin resistance. This may still be the case in non-proteinuric gestational hypertension but has so far not been verified in cases of pre-eclampsia.

In the long term, the risk to the mother with GDM is that she goes on to develop type 2 diabetes. The rate of progression to type 2 diabetes depends predominantly on ethnicity and the degree of glucose intolerance in pregnancy and immediately afterwards.[196] Other contributing factors are weight during pregnancy and subsequent weight gain, age, parity, and family history.[197] Although there is no data from India itself, in other high risk populations, such as Hispanic American women, about 40% of women with GDM develop diabetes within 6 years after the birth.[198]

It is well known that birthweight is increased in babies born to mothers with GDM and that increased size at birth puts the neonate at risk of a traumatic delivery. However, Spellacy et al.[199] found that the risk of macrosomia was higher with maternal obesity and post-term pregnancy than with maternal glucose intolerance. In my study population where the average birthweight among term deliveries was 2.9 kg, mothers with GDM were five times more likely to deliver babies over 3.5 kg, but there were no cases of birth injury in these babies. Although minor morbidity such as neonatal hypoglycaemia and polycythaemia almost certainly occurs in association with GDM and may require admission to the neonatal nursery, there is no evidence of significant prolonged neonatal morbidity.[200]

There continues to be a great deal of controversy over the significance of adverse outcomes in women with GDM, benefits of screening and levels of glycaemia at which to start treatment. Until the HAPO study is completed, these issues are unlikely to be resolved.

9. Final Discussion

This thesis has described a large study of glucose tolerance during pregnancy in a South Indian population, which has included detailed anthropometry of mothers and their babies. The prevalence of GDM in the study population was 6.1%. Maternal glucose intolerance and insulin resistance indices were strongly related to older age and higher body fat mass. GDM prevalence was increased in women who were relatively fat but had evidence of impaired growth in early life (short adult stature, small head circumference and /or low birthweight). Babies born to mothers with GDM were larger in all measurements. This was most marked for measures of body fat, but these babies were also significantly longer with larger heads. Small effects on neonatal anthropometry were also seen at maternal glucose concentrations within the normal range.

The study was designed to test the hypothesis, based on earlier studies in India, that women whose growth was impaired in early life (as evidenced by short adult stature, small head circumference and /or low birthweight) are more likely to become insulin resistant if they become fat as adults. Insulin resistance increases in pregnancy and these mothers become hyperglycaemic, develop GDM and give birth to fat, hyperinsulinaemic babies who are at increased risk of developing type 2 diabetes in adult life.

When designing the study, it was calculated that a sample size of 1,000 women would be required to show a statistically significant effect of maternal anthropometry on maternal blood glucose concentrations. 832 women participated (67% of those eligible). Women who did not participate (n=403), were younger (median age 22 years cf. 23 years: $p<0.001$). It is possible therefore that I have overestimated the prevalence of GDM in the study population because younger women, with a presumably lower prevalence of GDM, failed to take part. However, women who attended the clinic but did not deliver in the hospital (n=156) were also younger (median age 21 years cf. 23 years: $p<0.001$), as well as being thinner (mean BMI 22.3 kg/m^2 cf. 23.1 kg/m^2: $p<0.001$), and their prevalence of GDM was 4.1%. Based on this figure, the overall prevalence of GDM may be closer to 5.5%.

Although the study sample was not strictly a 'population' sample; the women all delivered in one hospital in Mysore, nevertheless, characteristics of mothers and babies were similar to those reported for other South Indian urban populations.[109, 201] Fewer than hoped for women had been born in the hospital and had birth records available. With such small numbers for this part of the study, it was not possible to demonstrate a statistically significant relationship between maternal birthweight and GDM prevalence although the data suggest that lower birthweight may be associated with a higher prevalence of GDM. It was calculated that 177 each of women with and without GDM, and of known birthweight, would be required to show a statistically significant effect.

Five assumptions were made when forming the starting hypothesis (listed in the Introduction to this thesis: page 38) and these will now be discussed:

Assumption 1.

Higher maternal weight and wider pelvic diameters indicated an increase in fat, rather than height or lean mass.

The starting hypothesis was proposed following a previous study by the MRC in Mysore, where the prevalence of type 2 diabetes was determined in 506 adults who had been born in HMH between1938-53 and whose birth records were available.[49] Maternal weight but not height was recorded on the birth records and was found to predict diabetes in the offspring. The only other maternal anthropometric measurements recorded were the external pelvic diameters. These had been measured routinely during labour and were similarly found to relate to the prevalence of diabetes in the offspring. Subsequent analysis showed that these external diameters increased with parity and with age beyond the age of skeletal maturity. It was concluded that they were a measure of maternal subcutaneous fat as well as bony diameters and that the association between type 2 diabetes and maternal weight reflected a link with increased maternal adiposity. My study provided the opportunity to test this assumption.

I found a significant, positive relationship between maternal age and the external pelvic diameters, similar to that found in the previous Mysore study, but unlike the previous study, there was no relationship with parity. Possibly because the range of parity in my study was small (0-4) compared to that of the previous study (0-10). I also found that the external pelvic diameters correlated positively and significantly with maternal height, muscle mass and body fat (whether measured directly by individual skin-fold thicknesses or calculated indirectly as fat mass), but that the strongest correlations were with measures of fat. For measures of muscle and skeleton, the external conjugate was the weakest correlate. For measures of body fat, the interspinous diameter was the weakest and the diameter with the strongest relationships to each component of maternal body composition was the intercristal diameter.

External pelvic measurements were abandoned by obstetricians in the middle of this century when x-ray pelvimetry demonstrated that external measurements bore little relation to the actual size of the bony pelvis and did not sufficiently predict birth outcome.[164] Data relating external pelvic diameters are few, but they are known to increase during pregnancy[165] and have been shown to increase with parity[49] and in both this study and the previous Mysore study, with age, beyond that of skeletal maturity. Post-natal x-ray pelvimetry has shown relationships of the pelvic diameters to height.[166] In my study, although the external diameters correlated with height and with muscle mass, the strongest correlations were with maternal body fat. It is therefore possible to conclude that the external pelvic diameters are an indirect measure of maternal fatness, thus proving this first assumption true.

Assumption 2.

Maternal glucose concentrations were related to this increase in fat, so that the fatter the mother, the higher the blood glucose concentrations and the more likely the development of GDM.

As shown in other populations, the prevalence of GDM in Mysore rose with both age and increasing measures of body fat; both direct measures e.g. skin-fold thicknesses

and indirect measures e.g. BMI, fat mass or the external pelvic diameters. Although the majority of women in my study were not obese (mean BMI=23.1 kg/m^2) according to WHO criteria, fatter mothers still had higher blood glucose and insulin concentrations at all time points of the OGTT and a higher prevalence of GDM. They were also shown to have higher insulin resistance and secretion. The highest prevalence rates of GDM were seen in the oldest fattest women. This suggests that as women become older and therefore fatter and more insulin resistant, their pancreatic β-cells, which have been compensating for the increased resistance by increased secretion become 'exhausted' and are no longer able to match the increasing demand for insulin. These relationships between maternal 'fatness' and maternal glucose concentrations prove this assumption true.

Assumption 3.

Maternal hyperglycaemia was even more likely if the mother had impaired early growth leading to insulin resistance in addition to adult obesity.

Short adult stature, small adult head circumference and/or low birthweight were the three measures used in this study as proxies of impaired growth in early life. The determinants of adult height and indeed head circumference reflect a complex interplay of genetic and environmental factors. It is known that length at birth correlates with both maternal and paternal height, suggesting a genetic component to fetal growth.[188] Animal studies, such as Walton and Hammond's Shire-horse-Shetland–pony cross experiments,[141] show that skeletal growth of a fetus genetically designed to be large, is down regulated if the mother is small. The mechanisms by which this occurs are not understood, but it may be an adaptation to prevent obstructed delivery in small mothers.

Apart from the effects of small maternal size, intra-uterine growth retardation from any cause may reduce fetal length growth,[202, 203] and this is a strong risk factor for short adult height.[204] This suggests that there are critical periods for skeletal growth pre-natally, and/or that genetic and environmental factors which reduce fetal growth, also influence the mechanisms controlling post-natal growth, such as the growth

hormone/IGF-1 axis.[205, 206] Post-natal growth depends on genetic potential, and on the post-natal environment, especially adequacy of nutrition and frequency of infections.[207] The important point is that short adult height may reflect reduced growth in fetal life and infancy.

After adjusting for age and body fat, maternal hyperglycaemia and the prevalence of GDM was increased in women with evidence of reduced growth in early life as shown by their short stature and small head circumference. The association of short stature with GDM has been shown in other populations but not in India. Studies from Greece, Korea and the USA showed higher rates of GDM in shorter women.[169-172] My data also suggest a link between GDM and low maternal birthweight although numbers were small and this relationship was not statistically significant. Three studies from the UK and USA that have shown an association between GDM and low birthweight,[173-175] had a larger study sample size. Findings from my study are consistent with the assumption that poor growth in childhood or before birth are risk factors for the later development of GDM and that this effect is mediated via increased insulin resistance rather than reduced insulin secretion.

Assumption 4.

Maternal blood glucose concentrations were related to the body composition of her baby, such that the higher the maternal glucose levels, the bigger the baby and that this increase in neonatal size followed a specific pattern whereby the soft tissues increased to a greater extent than the skeleton 'relative macrosomia'.

Detailed neonatal anthropometry in this study allowed better than usual characterisation of the features of macrosomia. In neonates born to mothers with GDM, measures of neonatal body fat (skin-fold thicknesses, abdominal circumference) increased to a greater extent than measures of skeleton (crown-heel length, leg length, head circumference). It is well known that babies born to diabetic mothers are fatter.[179] Increased birth length, although well documented[179] has not been highlighted, and an increase in head circumference has not to my knowledge been described before. Increased neonatal size was more closely related to maternal fasting glucose

concentrations than to post-load values. It is perhaps not surprising that insulin-sensitive tissues like fat should increase, but interesting that skeletal growth (traditionally insulin-insensitive) is also increased. Mechanisms of fetal overgrowth in GDM are not well understood. It has been suggested that chronic insulin excess leads to an increase in IGF-1 receptors, or that the insulin receptor does after all have growth-mediating properties.[181]

In addition to these changes in babies of diabetic mothers, I have demonstrated a small increase in fetal size across the range of blood glucose concentrations in mothers with normal glucose tolerance, suggesting that lesser degrees of maternal hyperglycaemia may stimulate the fetal pancreas and influence fetal growth. This has been shown in other studies, mainly in relation to birthweight,[180] but also in relation to length, head circumference and fat as measured by the thigh skin-fold thickness.[111] In my study, significant relationships were found with birthweight, ponderal index and head circumference. These findings are consistent with the assumption that maternal blood glucose concentrations, even in the normal range have an effect on fetal growth and that this effect is seen to a greater extent with fetal soft tissues than with skeleton.

Assumption 5.

Maternal glucose concentrations were related to neonatal cord blood glucose and insulin concentrations, which in turn related to neonatal size.

Cord blood glucose and insulin concentrations were significantly related to maternal glucose concentrations and to neonatal anthropometry. Even in mothers of normal glucose tolerance there was an increase in neonatal cord glucose/insulin concentrations across the range of maternal glucose concentrations. For cord insulin concentrations, stronger associations were seen with measures of neonatal fat, while for cord glucose concentrations stronger associations were seen with skeletal measurements. Little is known about the mechanisms responsible for fetal overgrowth in neonates born to mothers with GDM. The findings from this study suggest that different mechanism may be involved for skeletal growth than those involved in soft tissue growth. It may

be that fetal hyperglycaemia leads to a rise in fetal IGF-1 concentrations and that it is this, rather than insulin that gives rise to increased skeletal growth.

Having shown the assumptions made at the beginning of this study to be true, the findings are consistent with the starting hypothesis: that women whose growth is impaired in early life (as evidenced by their short stature, small head circumference and/or low birthweight), are more insulin resistant if they became 'fat' as adults and are more likely to become hyperglycaemic in pregnancy. Gestational diabetes is responsible for fat, hyperinsulinaemic babies who may themselves be at risk of GDM in adult life.

A number of epidemiological studies have shown that GDM is a risk factor for type 2 diabetes and obesity in later life in the offspring.[93, 94] In experimental animals, offspring of hyperglycaemic mothers are glucose intolerant in adult life.[88, 182] Convincing evidence in humans that a diabetic tendency is transmitted from one generation to the next by the intra-uterine environment in GDM comes from studies of the Pima Indians of North America.[93] In this population there are high rates of diabetes in people of low birthweight, but also in those of high birthweight born to mothers with GDM, creating a U-shaped relationship between type 2 diabetes and birthweight.[95] As India has both a high prevalence of low birthweight and childhood stunting, and of GDM, these may be important factors leading to a vicious cycle of inter-generational transmission of diabetes. As the prevalence of GDM and type 2 diabetes increases, birthweight will also increase and a U-shaped relationship of diabetes with birthweight may emerge in the future.

Although India is known to have a high prevalence of type 2 diabetes, there are few data on the prevalence of GDM. Ramachandran *et al* studied a South Indian urban population of comparable age and BMI to mine and found a low prevalence (<1%).[108] This may be due to different diagnostic criteria. Ramachandran used a 75g OGTT and WHO criteria to define women with impaired glucose tolerance (4.9%) who then underwent a 100 g OGTT, and diagnosed GDM using the National Diabetes Data Group (NDDG) criteria. This study appears to be the only recent study from India in

which GDM has been assessed using a single standard test. Further studies are required to assess GDM prevalence in India and to ensure that the high rates found in this study were not atypical.

The fact that GDM prevalence was highest in women who were short with small head circumferences (implying poor growth in early life) and who became fat as adults, has implications for programmes to improve fetal and childhood growth in developing countries. It suggests that improving the nutrition and growth of girls during infancy and childhood should be given high priority. However, it would seem that increasing fat mass alone may be disadvantageous, especially in women who were deprived in early life as this is likely to be responsible for an increasing prevalence of GDM which increases the risk of diabetes in the offspring.

The finding of an increase in GDM prevalence among tall women in this study was unexpected and not explained by increased insulin resistance or low secretion in these women. A U-shaped relationship between height and GDM was not part of my *a priori* hypothesis and was of borderline significance. This association with tall mothers may therefore be a spurious finding. There was no evidence of a secular trend in height among the study women and although taller mothers with GDM tended to be younger, adjusting for age did not alter the quadratic relationship. Assuming the U-shaped relationship is real, one explanation would be that taller women were themselves products of GDM pregnancies and therefore macrosomic at birth. Diabetic mothers of above mean height (154.6cm) had a mean birthweight of 3193.9g (n=3) compared with 2423.9g (n=6) in those of below mean height. However, with such small numbers and no data on glucose tolerance in the subjects' mothers during pregnancy, I was unable to test this. Further studies are required, where a greater number of mothers have birth records available and the presence or absence of GDM in their mothers has been documented, to allow the relationship of maternal height to GDM to be further defined.

Future research is also needed to identify the mechanisms by which fetal overgrowth occurs in babies born to mothers with GDM, how the fetus adapts to an increased nutrient supply and how these adaptations program the structure and physiology of the

body. The fact that fetal overgrowth does occur in babies born to mothers with GDM is well known and has been demonstrated again in this study. Detailed anthropometry has shown that babies born to mothers with GDM are not only fatter, but are also longer, with bigger head circumferences. The findings from this study also suggest that fetal growth increases even within the normal range of maternal glucose concentrations, but that the increase in growth that occurs is much smaller than that seen in babies born to mothers with GDM. Measurement of IGF-1 concentrations in stored maternal serum and neonatal cord samples would be one way to begin investigating the mechanisms involved in fetal overgrowth.

Debate continues as to whether or not glucose intolerance in pregnancy should be screened for and treated. Macrosomic babies, born to mothers with GDM, have been shown in some populations to have an increased risk of type 2 diabetes in adult life. Screening for GDM and subsequent treatment of the disease may, by avoiding hyperglycaemia in the mother and consequent hyperinsulinaemia in the baby, prevent fetal overgrowth and ultimately prevent the increased risk of diabetes in the adult offspring. Prospective studies need to be carried out in Indian populations in order to determine firstly, whether maternal gestational diabetes is a risk factor for diabetes in the adult offspring and secondly, if it is, whether treatment of maternal hyperglycaemia is able to reduce this risk. Annual follow-up of the babies born in my study is currently underway in order to define the long-term effects that the mothers' body composition and glucose/insulin metabolism during pregnancy have on the growth and development of her child and his/her glucose and insulin metabolism.

Overall, the findings from this study imply that a woman's own fetal growth, body composition and glucose and insulin status during pregnancy play a major role in the growth and development of her baby and consequently in programming the future health of her children.

Appendix 1

Questionnaire

Recruitment Form 1

Study Number: | | | | |

Date of Interview: | | | | | | |

Out-patient Number: | | | | | | |

Name of subject: _____

Address of subject: _____

_____

_____

_____

Age: | | years **DOB:** | | | | | | |

Religion: | | **1.** Hindu **2.** Muslim **3.** Christian **4.** Other

Marital Status: | | **1.** Married **2.** Widowed **3.** Divorced **4.** Single

Consanguinity: | | **1.** Yes **2.** No **9.** Don't know

If Yes, describe type: _____

Recruitment Form 2

Study Number: | | | | |

Menstrual cycle: | | | | | If range given, code midpoint

If < 3 cycles / yr , length = 99

| | **1.** Regular — Irregular if periods out by >

2. Irregular — or = 7 days in last 3 months

Date of LMP: | | | | | | |

| | **1.** Certain **2.** Approximately **3.**Uncertain

EDD by Dates: | | | | | | |

Gestation (in weeks) | | + | | days

Contraception: is this likely to have affected estimation of gestational age?

| | **1.** Yes

2. No

If Yes, why ?

Lactation: is this likely to have affected estimation of gestational age?

| | **1.** Yes

2. No

If Yes, why ?

Ultrasound Scan Details:

Date of scan | | | | | | |

Femur Length (mm) | | |

Biparietal Diameter (mm) | | |

Crown-Rump Length (mm) | | |

Recruitment Form 3

Study Number: | | | | |

EDD by Ultrasound | | | | | | |

Gestation (in weeks) | | | + | | Days

Other ulltrasound findings:

Obstetric History:

Gravida: |

Parity: | + |

Date	GA	BW	L/D or A	M\F	Hosp\Home (name of hospital)	MOD	Pregnancy Complications, inc. gestational DM

Recruitment Form 4

Study Number: | | | | |

Past and Current Medical Problems:

Known Diabetic: ☐ **1.** Yes **2.** No

If Yes:

Age of onset ☐☐ yrs

Insulin ☐ **1.** Yes **2.** No

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

If Yes:

Mother ☐ **1.**Yes **2.**No **Age of onset** ☐☐ **Insulin** ☐ **1.**Yes **2.**No

Father ☐ **1.**Yes **2.**No **Age of onset** ☐☐ **Insulin** ☐ **1.**Yes **2.**No

Sibling ☐ **1.**Yes **2.**No **Age of onset** (of youngest) ☐☐ **Insulin** ☐ **1.**Yes **2.**No

Drug History: (names and quantities of drugs currently taken)

Recruitment Form 5

Study Number: | | | | |

Occupation: _____

Have you ever used tobacco regularly? [ ] Yes **1.** No **2.**

If Yes: [ ]
- Before this pregnancy **1.**
- During this pregnancy **2.**
- Still using **3.**

Type: [ ]
- Cigarettes **1.**
- Tobacco (chewed) **2.**
- Beedis (smoked) **3.**

Amounts per day (describe): _____

Have you ever taken alcohol regularly? [ ] Yes **1.** No **2.**

If Yes: [ ]
- Before this pregnancy **1.**
- During this pregnancy **2.**
- Still using **3.**

If Yes: Type of alcohol: _____

How many days/week [ ]

Quantity per time [ ][ ] Measures/spirits

[ ][ ] mugs of beer

[ ][ ] glasses of wine

Units per week [ ][ ][ ]

Born in HMH [ ] **1.** Yes **2.** No **9.** Don't know

If Yes, fill in a Tracing form.

Kuppaswamy

Study Number:

a) Locality in the town ☐

1	Slum
2	Low class
3	Middle class
4	High class

b) People per room ☐

1	4 or more
2	3 to 3 . 9
3	2 to 2 . 9
4	1 to 1 . 9

	Separate	Common	Not available	**Score**
c) Water	**3**	**2**	**1**	☐
d) Bathroom	**3**	**2**	**1**	☐
e) Toilet	**3**	**2**	**1**	☐

f) Education level of subject ☐

g)Education level of husband ☐

7. Professional degree, MA, MSc, MCom, MTech, MBBS, BE, MSW, Postgrad Diploma
6. B.A., B.SC., B.Com., DME, DHMS, BPNA,
5. HSC, ITI, Intermediate D.Ed. Post- high school Diploma
4. High school certificate, S.SIC.
3. Middle school completion
2. Primary school / literate
1. Illiterate

Kuppaswamy (cont.) **Study Number:**

h) Occupation of main breadwinner

10. Professional, University teacher class 1, Gazetted officer.
6. Semi-professional officer, Inspector, Teacher, Chemist, Diploma in Engineering, Maintenance (in charge), Personnel Manager, Advocate, Businessman.
5. Shopowner, Clerical, Assistant in government or private service, Farm owner, Dairy, Telephone exchange business, Medical rep, Police inspector.
4. Skilled worker, fitter, lower clerical, carpenter, goldsmith, army jawan, Police constable, telephone operator.
3. Semi-skilled worker, rickshaw driver, plumber, salesman.
2. Unskilled worker, labourer, coolie, builder, vegetable seller.
1. Unemployed.

i) Income of the head of the family

		Rs / month

12. Rs 7000 or more
10. Rs 5000 to 6999
6. Rs 3000 to 4999
4. Rs 2500 to 2999
3. Rs 2000 to 2499
2. Rs 1000 to 1999
1. Less than Rs 1000

j) Per Capita Income Total income from all the members of the household divided by the total number in the household per month.

Household member 1: Income:-_____
Household member 2: Income:-_____
Household member 3: Income:-_____
Household member 4: Income:-_____
Household member 5: Income:-_____

Total income into the household per month _____

Number of members of household _____

Rs / month / head.....to be calculated by computer

Husband's Form

Study number: | | | | |

Date of visit: | | | | | | |

Name of interviewer _____

Name of husband _____

Age | | yrs **Date of birth** | | | | | | |

Born in HMH: | | **1** Yes **2** No **9** Don't know

Address _____

_____

_____

_____

Occupation _____

Measurements:

Height | | | | • | | cm

Weight | | | | • | | kg

Tracing Form – to be filled in for women born in HMH

Study Number:

Full name of subject : _____

Religion: _____

Present Address: _____

Address when born: _____

Age | | | yrs **Date of birth** | | | | | | |

Mother : Alive / Dead
Occupation _____

Full name: _____

Mother's age when married | | yrs **Mother's age at birth** | | yrs

Father : Alive / Dead
Occupation _____

Full name: _____

No.	Brothers / Sisters Names	Age	Sex	Birth Place	Alive / Dead	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Delivery Form 1

Study number: | | | | |

Date of Birth of newborn: | | | | | | |

Gender: | | Male **1.** Female **2.**

EDD (accepted) | | | | | | |

Gestation (at birth) | | + | |

Labour | | Spontaneous **1** Induced **2**

If Induced, give reason: _____

Mode of Delivery | |

S.V.D.	**1**
Vaginal Breech	**2**
Forceps	**3**
Ventouse	**4**
Emergency LSCS	**5**
Elective LSCS	**6**

If Forceps, give reason: _____

If Ventouse, give reason: _____

If Emergency LSCS: _____

If Elective LSCS: _____

Complications:

Shoulder dystocia | | Yes **1** No **2**

If Yes | |

mild	**1**	(hyperflexion of hips only)
moderate	**2**	(rotational manouveres requ.)
severe	**3**	(extreme measures requ.)

Delivery Form 2

Study Number: ☐☐☐☐

Other birth injury ☐ Yes **1** No **2**

If Yes **describe type:** _____

Hypoglycaemia (< or = 40mg/dl) ☐ **1.** Yes **2.** No **3.** Not measured

R.D.S. (paediatric diagnosis) ☐ **1.** Yes **2.** No

Admission to S.C.B.U. ☐ Yes **1.** No **2.**

If Yes: length of stay (in days) ☐☐☐

reason for admission _____

Congenital malformation ☐ Yes **1** No **2**

If Yes: type: _____

Mother's Blood Pressure in Labour (on admission)

Room temp. ☐☐ °C

Blood pressure *systolic* **1** ☐☐☐ **2** ☐☐☐

diastolic **1** ☐☐☐ **2** ☐☐☐

Complications during this pregnancy: (medical and obstetric)

_____

_____

_____

Last gestation urinalysis recorded ☐☐ Weeks

Proteinuria ☐ **1.** Yes **2.** No

Glycosuria ☐ **1.** Yes **2.** No

Appendix 2

Protocols

1 Blood Pressure

The subject was asked to expose her left upper arm and rest it comfortably, palm up, on a table at heart level ensuring that the arm was not constricted by rolled up clothing. A standard mercury sphygmomanometer was used. Any remaining air was squeezed out of the cuff and it was then wrapped around the arm, positioned so that the centre mark on the cuff was lying over the brachial pulse, the lower edge 2-3 finger-breadths above the ante-cubital fossa. The diaphragm of the stethoscope was positioned in the ante-cubital fossa where the brachial pulse could be felt. The subject was asked to sit quietly throughout the measurement with legs uncrossed. Systolic blood pressure was recorded as the Korotkov sound was first heard and diastolic as the sound disappeared. The cuff was completely deflated before being pumped up again and the second set of measurements taken. Two measurements of blood pressure were taken and the average used in analysis.

2 Maternal anthropometric measurements

2.1 Height

A portable stadiometer (Microtoise, CMS Instruments, London) was used to measure height. The base-plate was placed on the floor, on as firm and level a surface as possible, and preferably near a perpendicular, such as a door architrave, which helped the eye to ensure that the tape was vertical. The subject was asked to remove her shoes and stand on the base-plate with her back to the tape. She was told to stand as tall and straight as possible with feet together and arms held loosely at her side and shoulders relaxed (to avoid lordosis). She stood far enough forward on the base-plate so that the tape was not distorted when pulled to vertical. The tape was checked for correct insertion into the base plate. It was then raised vertically and the head plate placed on the top of the subject's head, using the spirit level to check horizontality. 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 was horizontal. The scale was then read from as level a position as possible. If there was a lot of height disparity, the measurer used a chair to get level with the scale. Height was read once to the nearest 0.1 cm.

2.2 Weight

A portable scale (Seca, Germany) was placed on the most level and stable piece of ground possible and zeroed, ensuring that the dial was read vertically from above to avoid errors due to parallax. The subject was asked to remove his/her shoes and heavy items of clothing or jewellery. One reading of weight to the nearest 0.5 kg was recorded.

2.3 Head circumference

The subject was asked to sit up straight and to look straight ahead. A standardised measuring tape was placed just above the eyebrows at the front and across the occipital prominence at the back to obtain the maximum diameter. The tape was pulled round firmly and one measurement made to the nearest 0.1 cm.

2.4 Mid-upper arm circumference

The subject was seated with her back to the measurer and her left arm flexed at 90^{o}. The tips of the acromion and the olecranon were palpated and a point halfway between (measured with a tape) was marked on the skin. The subject was then asked to relax and with her arm hanging by her side the tape was placed around the upper arm at the level of the mark, resting on the skin but not indenting it. Three readings of mid-upper arm circumference were made to the nearest 0.1 cm and the average used for analysis.

2.5 Mid-thigh circumference

With the subject standing straight, the proximal border of the left patella was marked, and the subject then asked to place her left foot on a chair to allow hip flexion to 90^{0}. The inguinal crease was located and a reference point taken on the inguinal crease at

the mid-point of the long axis of the thigh. A tape measure was then used to measure and mark the mid-point between these two marks with the hip still flexed.
With the subject standing, her feet 10cm apart and weight evenly distributed, the circumference of the thigh was measured at the mid-point ensuring that the tape was horizontal. Three readings were taken to the nearest 0.1 cm and the average used for analysis.

2.6 Skinfold thicknesses

Harpenden ('John-Bull' model) skinfold callipers (CMS Instruments, London, UK) were used throughout and all measurements made on the left side of the body. The technique used was as described by Cameron.[208] The dial was read to the nearest 0.2 mm at a count of six seconds even if it was still moving. Where the needle did not fall on a marked division, the measurement was taken to the nearest lower value. Three measurements were taken at each site, releasing the skinfold and starting afresh each time. The average value was used for analysis.

2.6.1 Triceps and Biceps

The subject sat with her back to the measurer, her arm flexed at 90°. The tips of the acromion and the olecranon were palpated and a point halfway between (measured with a tape) marked on the skin. This marked the vertical level at which the skinfold was made. With her arm relaxed and hanging by her side, the tape was placed around the upper arm at the level of the mark, as if to measure mid-upper arm circumference. A horizontal line was drawn on the skin posteriorly and anteriorly at the level of the first mark. The posterior line was used for the triceps skinfold and the anterior line for the biceps skinfold. To determine the side-to-side position at which to take the skinfold, it was simply 'eye-balled', as the mid-point and most dorsal (i.e. the part which sticks out furthest posteriorly) part of the upper arm at the level of the horizontal mark. A pen, held vertically with one end on the olecranon process and the other end pointing towards the acromion, was also used to get the correct line. The point at which the skinfold was to be measured was marked by a cross.

The skin was picked up over the posterior surface of the triceps muscle, at least 1cm above the cross, on a vertical line passing upward from the olecranon to the acromion. The woman was asked to bend her arm and then straighten it before the calliper was applied. In this way, any muscle that may have been picked up was pulled out from the skinfold by the contracting action of the triceps. The callipers were applied below the fingers, such that the marked cross was at the apex of the fold.

The subject was then asked to face the measurer with her arm relaxed and palm facing forwards. The anterior horizontal line already made marked the vertical level at which the skinfold was measured. The side-to-side mid-point was determined as for the triceps skinfold. The calliper were applied below the fingers, such that the marked cross was at the apex of the fold.

2.6.2 Subscapular

The subject was positioned as for the triceps skinfold measurement with the shoulders and arms relaxed. The inferior angle of the scapula was identified and the skin marked immediately below this point. The skinfold was picked up above the mark and measured on the mark. The skin was picked up with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The callipers were applied below the fingers, such that the marked cross was at the apex of the fold.

2.6.3 Suprailiac

With the subject standing sideways and arms folded, the iliac crest was located and marked in the mid-axillary line. The skinfold was picked up above the mark and the calliper jaws applied at the mark itself. The subject was asked to tilt sideways slightly to ease the tension on the skin while picking up the skin-fold. As with the subscapular fold, the suprailiac fold was picked up to follow the natural cleavage of the skin.

2.7 External Pelvimetry

A Harpenden anthropometer (CMS instruments, London, UK) was used for these diameters. The subject was asked to stand up straight with feet slightly apart and with the lower abdomen completely exposed so that the bony landmarks could be identified and marked. Each calliper blade was held between forefinger and thumb and reasonably firm pressure applied so that the tip of the blade was felt to be resting on bone. Measurements were taken in triplicate to the nearest 0.1cm and the anthropometer removed from the skin site completely each time. The average was used for analysis.

2.7.1 Intercristal diameter

With the subject standing face on, this diameter was measured by placing the tips of the callipers on the outer margins of the iliac crests and taking the widest transverse measurement. These points were marked with pen and the measurement repeated.

2.7.2 Interspinous diameter

With the subject standing face on, the tips of the callipers were placed on the outer edges of the anterior superior iliac spines. Again these points were marked before the measurement was repeated.

2.7.3 External conjugate (Baudeloque's diameter)

This was measured with the subject standing side on. One calliper tip was placed on the anterior, upper margin of the pubic symphysis and the other on the spine of the last lumbar vertebra. The space below the last lumbar vertebra was found by taking a line joining the posterior superior iliac spines. A point 3cm above its centre denotes the position of the last lumbar spine. For most women, the position of the posterior superior iliac spine was indicated by a dimple in the skin.

3. Neonatal anthropometric measurements

3.1 Weight

The baby was placed naked on the digital weighing scales and one reading taken.

3.2 Lengths

An infant stadiometer (Harpendon) was used for these measurements and three readings taken to the nearest 0.1cm. The average was used in analysis.

3.2.1 Crown-heel

The baby's head was held against the end of the head plate and the legs extended until they were flat. The foot plate was brought up to the heels ensuring that the feet and knees were flat and the length was read off the dial.

3.2.2 Crown-rump

The baby's head was held against the end of the head plate, both hips and knees were flexed, making sure that the back was still flat on the mat. The foot plate was brought up to the buttocks and the length read off the dial.

3.3 Circumferences

Circumferences were measured by firstly marking on a blank tape and then measuring the tape against a fixed ruler. Measurements were taken in triplicate to the nearest 0.1cm and the average used for analysis.

3.3.1 Head

This measurement was taken with the baby's head on one side, so that the maximum occipito-frontal circumference could be found. The tape was placed on the forehead, on

the most anterior point (just above the eyebrows) and passed around the head to the most posterior part of the head ensuring that the maximum circumference was found.

3.3.2 Chest

The tape was placed around the chest at the level of the xiphisternum ensuring that it was horizontal and that the measurement was made at the end of expiration.

3.3.3 Abdomen

The tape was placed around the baby immediately above the umbilicus ensuring that it was horizontal and that the measurement was made at the end of expiration.

3.3.4 Mid-upper arm

This measurement was made with the arm bent, allowing the measurement to be taken with the baby in its natural position rather than having to straighten out and hold the arm. Preferably, the arm should be relaxed rather than held in position. The mid-point should be eyeballed and marked accordingly. The blank tape used as already described.

3.4 Skinfold thicknesses

Harpenden callipers were used as for the maternal skinfold measurements. Three measurements were taken unless this caused too much distress, in which case, one measurement was taken.

3.4.1 Triceps

The vertical level at which the skinfold was made was that marked for the measurement of the mid-arm circumference. The tape was placed around the upper arm at the level of the mark and a horizontal line was drawn on the skin posteriorly. To determine the side-

to-side position at which the skinfold would be taken, the mid-point and most dorsal (i.e. the part which sticks out furthest posteriorly) part of the upper arm was eyeballed at the level of the horizontal mark. The point at which the skinfold was to be measured was marked by a cross. The skin was picked up over the posterior surface of the triceps muscle, above the cross, on a vertical line passing upward from the olecranon to the acromion. The callipers were applied below the fingers, such that the marked cross was at the apex of the fold.

3.4.2 Subscapular

The inferior angle of the scapula was identified by following the medial border of the scapula downwards. The skin was marked immediately below the inferior angle and the skinfold picked up above the mark and measured on the mark. The skin was picked up with the fold inclined downward and laterally, in the natural cleavage of the skin. The calliper jaws were applied below the fingers, such that the marked cross was at the apex of the fold.

Appendix 3

Inter- and Intra-Observer Variation (IOV) Studies

Prior to leaving the UK for India, I was made familiar with the measurement protocols (Appendix 2) and was trained in all the measurement techniques by staff at the MRC, Environmental Epidemiology Unit. In order to be sure that I was measuring correctly and consistently I undertook IOV studies using female staff in the unit as volunteers and neonates at the Princess Anne hospital, Southampton. I was then in a position to train my research team in India in the same techniques.

In Mysore, two observers performed all the maternal measurements (myself and Dr.Krishnaveni). Four observers (myself, Dr. Krishnaveni and two research nurses) performed all the neonatal measurements. Before our research clinics got underway, we undertook IOV studies in order to standardise our measurement techniques and therefore minimise sources of measurement error. During the 18 months of data collection, IOV studies were performed on a 6 monthly basis, thus enabling us to maintain good quality data throughout the study.

Inter-observer variation

Data were initially looked at graphically (Fig.i & ii) using a plot of the difference of the observers' measurements against their mean. This allowed us to investigate any possible relationship between measurement error and the true value.

1. Where two observers were involved, IOV was assessed using the standard Bland-Altman method for assessing agreement whereby within each study, comparisons are made between the two observers. Order of measurement had a non-significant effect on all the maternal anthropometric variables. Data for maternal anthropometric measurements are shown (Table ia and ib).

Fig.i: Inter-observer plot - crown-heel length

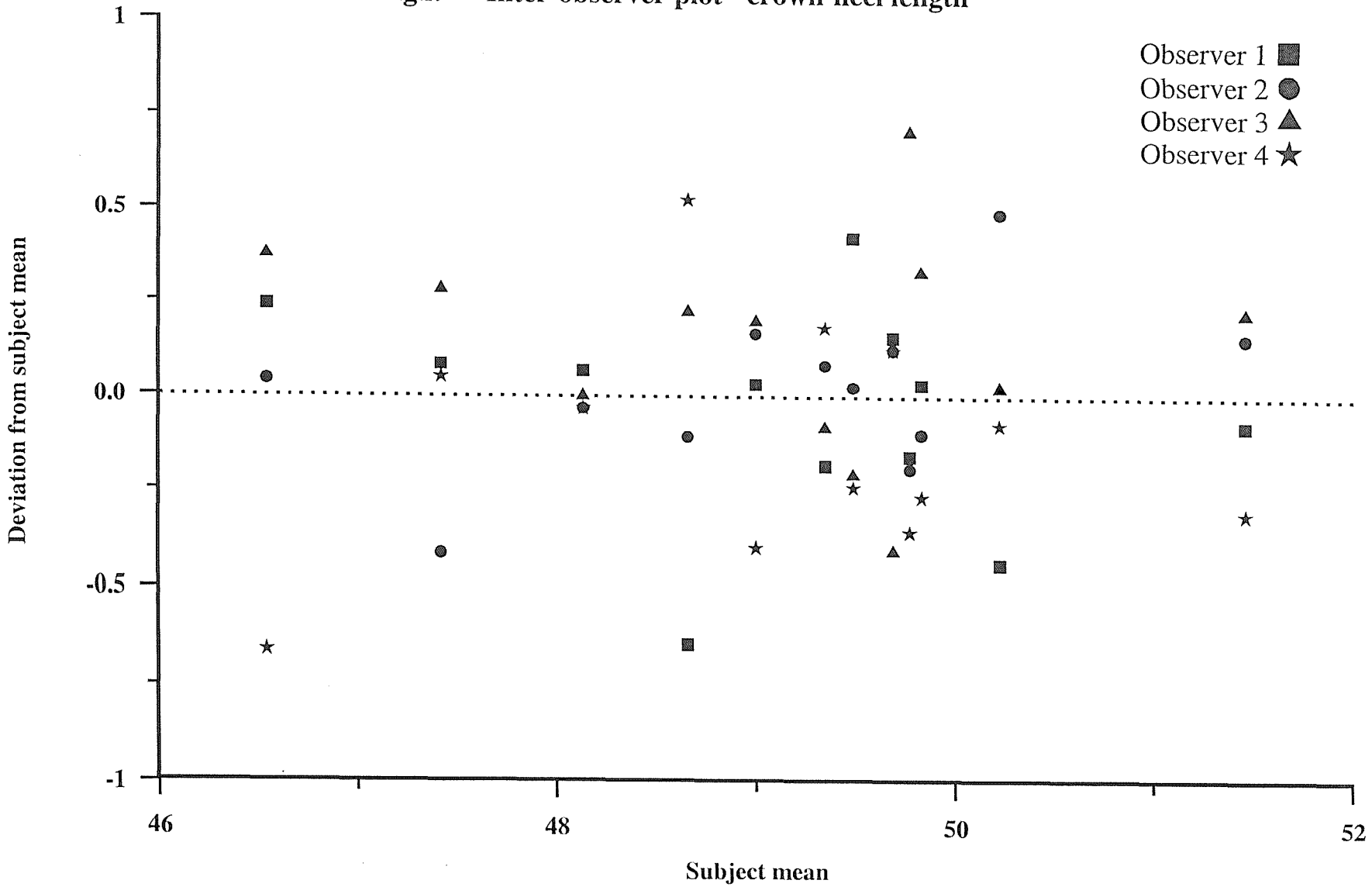


Fig.ii: Inter-observer plot - external conjugate

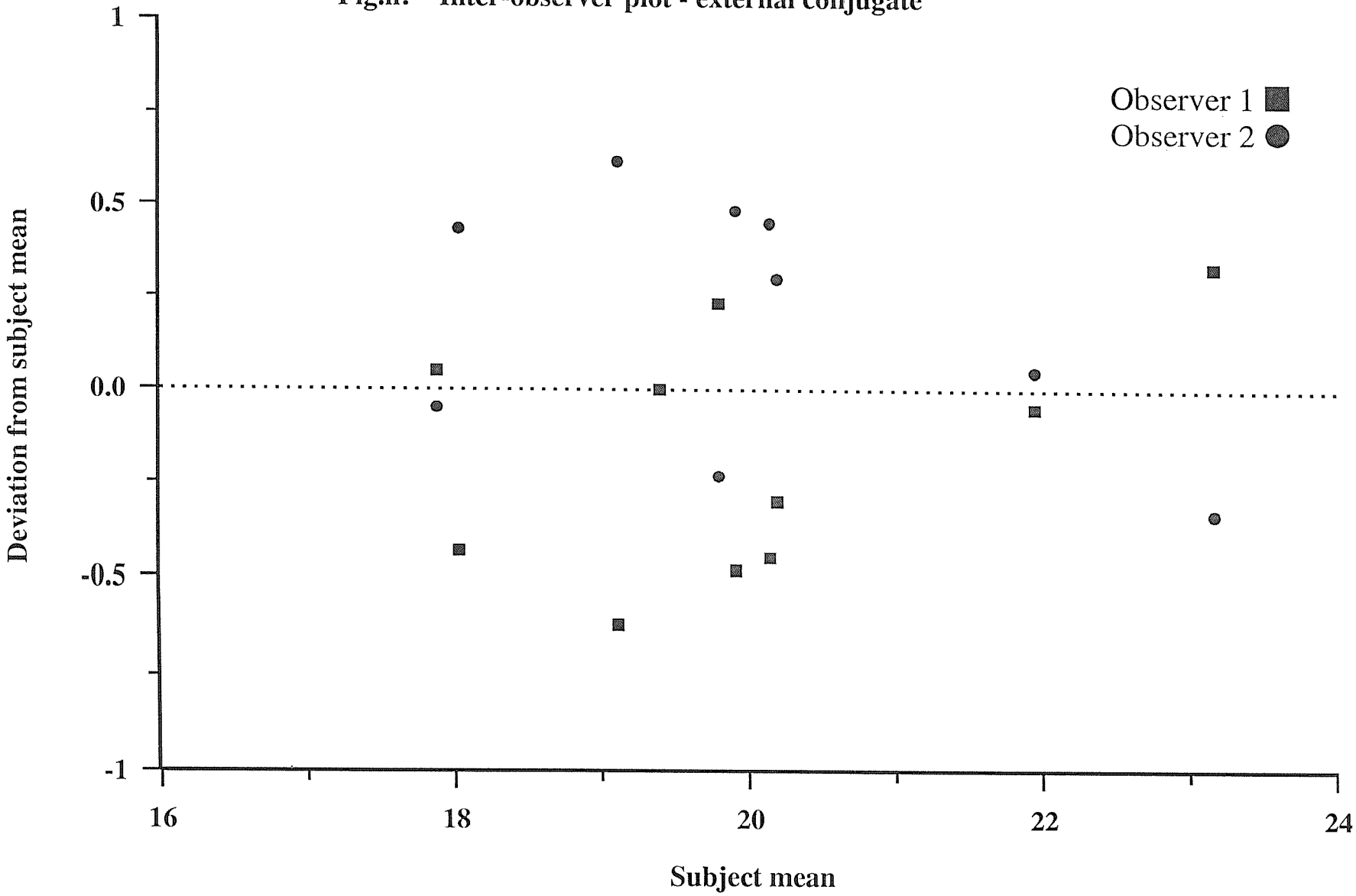


Table i: Inter-observer variation data on maternal anthropometric measurements shown for a) July 1997 and b) Feb 1998 – Twelve subjects, Two observers

d = mean difference (observer 1 – observer 2).
p value is for test of d = 0.
limits = 95% limits of agreement of observer 2 with observer 1 (d-2s, d+2s), where s = standard deviation of the differences between the observer and the gold standard (as each measurement was made by each observer three times, 's' values have been corrected to take account of this).

a) July 1997

Anthropometric Variables	d	95% CI	p	limits
Height (cm)	- 0.8	- 1.4, - 0.1	0.03	- 3.1, 1.6
Weight (kg)	0.0	- 0.5, 0.6	0.9	- 2.0, 2.1
Mid-thigh circumference (cm)	- 0.6	- 1.1, 0.0	0.05	- 2.5, 1.4
Mid-upper-arm circumference (cm)	- 0.3	- 0.6, 0.1	0.1	- 1.5, 1.0
Intercristal diameter (cm)	- 0.5	- 1.2, 0.3	0.2	- 3.1, 2.2
Interspinous diameter (cm)	0.0	- 0.4, 0.5	0.9	- 1.6, 1.6
External conjugate diameter (cm)	0.4	- 0.3, 1.1	0.3	- 2.1, 2.8
Triceps skinfold (mm)	1.9	0.4, 3.5	0.02	- 3.5, 7.4
Biceps skinfold (mm)	- 0.7	- 1.8, 0.5	0.2	- 4.6, 3.3
Subscapular skinfold (mm)	- 0.4	- 2.2, 1.4	0.6	- 6.7, 5.9
Suprailiac skinfold (mm)	5.3	1.5, 9.1	0.01	- 7.9, 18.4

b) February 1998

Anthropometric Variables	d	95% CI	p	Limits
Mid-thigh circumference (cm)	0.5	- 0.2, 1.1	0.1	- 1.6, 2.5
Mid-upper-arm circumference (cm)	0.2	- 0.0, 0.5	0.05	- 0.5, 1.0
Intercristal diameter (cm)	0.6	0.0, 1.2	0.04	- 1.2, 2.4
Interspinous diameter (cm)	0.3	- 0.4, 1.1	0.3	- 2.1, 2.7
External conjugate diameter (cm)	0.3	- 0.1, 0.8	0.1	- 1.1, 1.8
Triceps skin-fold (mm)	- 0.5	- 1.3, 0.3	0.2	- 3.0, 2.0
Biceps skin-fold (mm)	- 0.7	- 1.5, 0.1	0.1	- 3.3, 1.9
Subscapular skin-fold (mm)	- 0.5	- 2.0, 0.9	0.4	- 4.9, 3.9
Suprailiac skin-fold (mm)	0.4	- 1.4, 2.2	0.6	- 5.2, 6.0

2. Where four observers were involved, IOV was assessed using an adaptation of Bland-Altman for assessing agreement [209], so that within each study, individual observers compared with a gold standard based on all the observers. Data for neonatal measurements on two occasions are shown (Table iia and iib).

Intra-observer variation studies

Initially data were plotted (Fig.iii) to demonstrate the difference between the observers measurement.

IOV data was assessed using the standard Bland-Altman method for assessing repeatability. Data shown is that for the maternal skin-fold measurements.

Table iii: Intra-observer variation data showing maternal skin-fold measurements taken on ten subjects by two observers measuring each subject twice – July 1997.

d = mean difference (set 1 – set 2).
p value = test for d = 0.
r = repeatability coefficient, i.e. 95% of the time the observer will measure to within r of the true value.

	Observer 1				Observer 2			
Skin-folds (mm)	**d**	**95% CI**	**p**	**r**	**d**	**95% CI**	**p**	**r**
Triceps	- 0.0	- 1.3, 1.2	0.9	3.4	0.2	- 0.9, 1.2	0.7	2.8
Biceps	- 0.3	- 1.3, 0.7	0.5	2.8	0.4	- 0.6, 1.4	0.4	2.9
Subscapular	- 0.2	- 1.3, 0.9	0.7	2.9	- 0.3	- 1.9, 1.3	0.7	4.2
Suprailiac	0.3	- 0.7, 1.3	0.5	2.6	- 1.5	- 2.7, - 0.2	0.02	4.4

Notes:
1) Mean of 3 values within a set used in calculations for each observer.
The mean difference between suprailiac measurements for observer 2 is significantly less than zero, so 'r' should be interpreted with caution.

Fig.iii: Intra-observer plot - maternal triceps

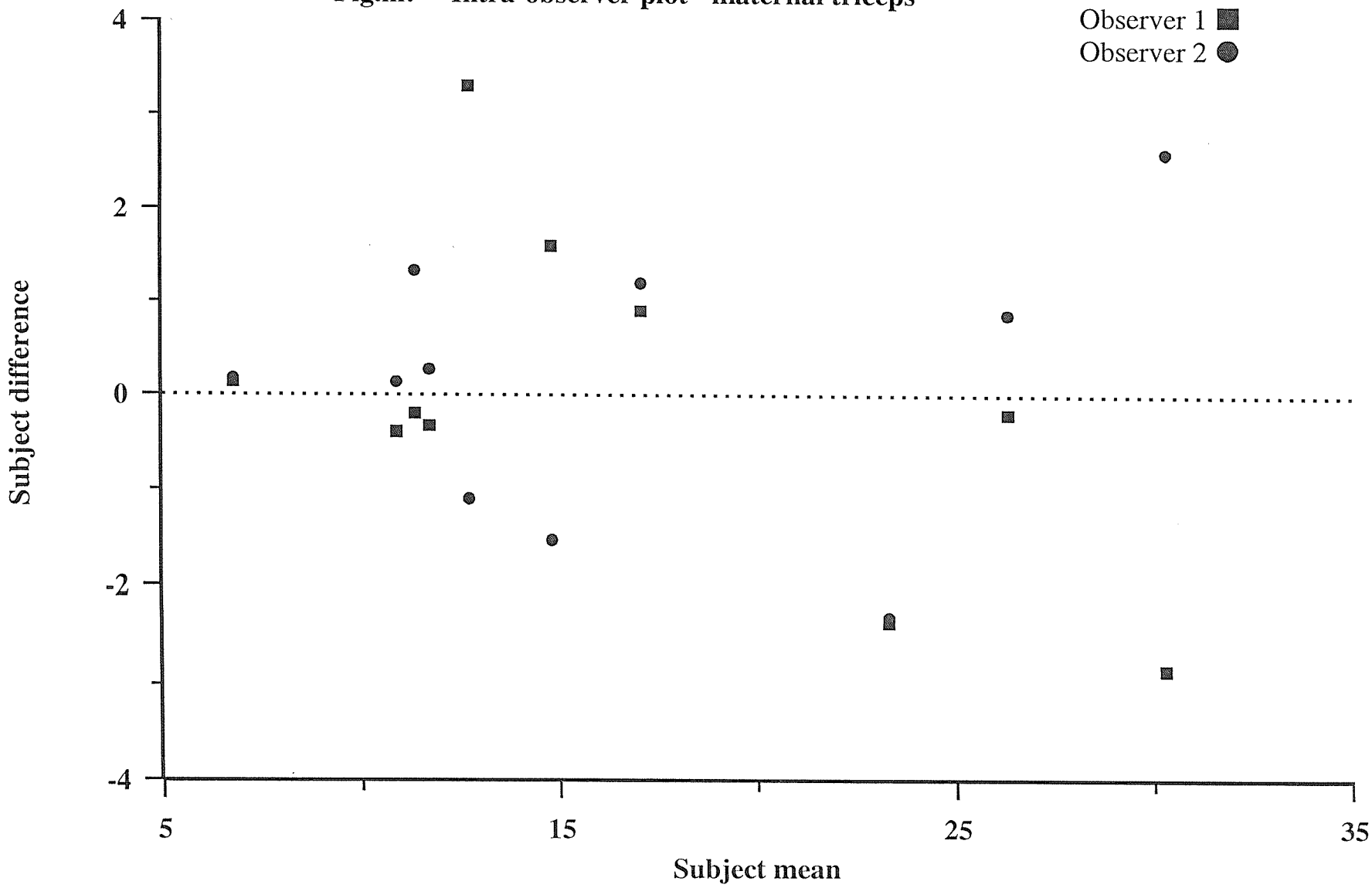


Table ii: Inter-observer variation studies on neonatal anthropometric measurements in a) July 1997 and b) January 1998. 4 observers measured 12 subjects (each measurement was performed three times and the average taken in analysis).

d = mean difference between observer and gold standard.
p value is for test d = 0.
limits = 95% limits of agreement with the gold standard (d-2s, d+2s), where s = standard deviation of the differences between the observer and the gold standard and is corrected for repeated measurements by observers on the same occasion.

a) July 1997

Neonate	Observer 1				Observer 2				Observer 3				Observer 4			
	d	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**
Weight	-0.0	-0.0,0.0	0.2	-0.0,0.0	-0.0	-0.0,0.0	0.1	-0.0,0.0	-0.0	-0.0,0.0	0.7	-0.0,0.0	-0.0	-0.0,0.0	0.8	-0.0,0.0
CHL	-0.0	-0.2,0.2	0.9	-0.7,0.7	0.2	0.1,0.3	0.002	-0.3,0.7	0.0	-0.2,0.3	0.8	-0.9,1.0	-0.2	-0.4,-0.0	0.04	-0.9,0.5
CRL	-0.1	-0.3,0.1	0.3	-0.7,0.6	0.3	0.1,0.5	0.003	-0.4,1.0	-0.2	-0.3,-0.1	0.007	-0.7,0.4	-0.0	-0.2,0.1	0.5	-0.7,0.6
Head	0.0	-0.1,0.2	0.4	-0.4,0.5	-0.0	-0.1,0.1	0.9	-0.6,0.6	0.0	-0.1,0.1	0.6	-0.4,0.4	-0.1	-0.2,0.1	0.3	-0.5,0.4
Chest	0.0	-0.1,0.1	0.97	-0.5,0.5	-0.0	-0.1,0.1	0.7	-0.5,0.4	-0.0	-0.1,0.1	0.99	-0.6,0.6	0.0	-0.1,0.1	0.7	-0.4,0.5
Abdo.	-0.2	-0.2,-0.1	0.002	-0.7,0.4	0.3	0.1,0.5	0.008	-0.5,1.1	-0.0	-0.2,0.2	0.99	-0.8,0.8	-0.1	-0.3,0.0	0.1	-0.8,0.6
MUAC	-0.1	-0.2,0.0	0.1	-0.7,0.5	0.0	-0.1,0.2	0.7	-0.7,0.5	0.2	0.0,0.3	0.04	-0.4,0.7	-0.1	-0.2,0.0	0.1	-0.6,0.4
Triceps	0.0	-0.1,0.1	0.9	-0.3,0.3	-0.0	-0.1,0.1	0.9	-0.3,0.3	-0.0	-0.1,0.1	0.7	-0.3,0.3	0.0	-0.1,0.1	0.7	-0.3,0.3
Subscap.	-0.0	-0.1,0.1	0.9	-0.5,0.4	-0.1	-0.2,0.0	0.2	-0.5,0.4	0.1	-0.1,0.2	0.3	-0.5,0.6	0.0	-0.2,0.2	0.9	-0.7,0.7

CHL = crown-heel length, CRL = crown-rump length, Abdo.= abdominal circumference, MUAC = mid-upper arm circumference, Subscap = subscapular skinfold

b) January 1998

Neonate	Observer 1				Observer 2				Observer 3				Observer 4			
	d	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**	**d**	**95% CI**	**p**	**limits**
Weight	0.0	0.0,0.0	0.01	-0.0,0.0	-0.0	-0.0,0.0	0.4	-0.0,0.0	0.0	-0.0,0.0	0.6	-0.0,0.0	-0.0	-0.0,0.0	0.2	-0.0,0.0
CHL	0.0	-0.1,0.2	0.7	-0.7,0.7	-0.0	-0.2,0.1	0.8	-0.6,0.5	-0.1	-0.3,0.0	0.1	-0.8,0.6	0.1	-0.1,0.3	0.2	-0.7,0.9
CRL	0.1	-0.1,0.3	0.3	-1.1,1.3	0.1	-0.1,0.3	0.1	-0.6,0.9	-0.1	-0.2,0.0	0.2	-0.7,0.5	-0.2	-0.3,-0.0	0.05	-0.8,0.5
Head	0.1	-0.0,0.2	0.2	-0.4,0.6	0.0	-0.1,0.2	0.7	-0.6,0.6	0.1	-0.1,0.2	0.3	-0.4,0.5	-0.0	-0.3,0.0	0.1	-0.7,0.5
Chest	0.2	0.1,0.3	0.003	-0.3,0.8	0.0	-0.2,0.2	0.97	-0.8,0.8	-0.1	-0.3,0.0	0.1	-0.9,0.6	-0.1	-0.2,0.1	0.2	-0.7,0.5
Abdo.	0.2	-0.1,0.4	0.2	-0.9,1.2	-0.0	-0.3,0.3	0.9	-1.1,1.1	-0.1	-0.5,0.2	0.4	-1.3,1.1	-0.1	-0.2,0.2	0.9	-0.9,0.9
MUAC	0.0	-0.1,0.1	0.7	-0.3,0.3	0.1	-0.1,0.2	0.4	-0.5,0.6	0.0	-0.1,0.2	0.8	-0.6,0.6	-0.0	-0.2,0.0	0.1	-0.5,0.3
Triceps	-0.1	-0.2,0.1	0.3	-0.7,0.6	0.0	-0.1,0.1	0.8	-0.5,0.5	0.1	-0.1,0.3	0.4	-0.8,1.0	-0.0	-0.2,0.1	0.7	-0.8,0.7
Subscap.	0.1	0.0,0.2	0.02	-0.4,0.6	0.1	0.0,0.2	0.04	-0.3,0.6	-0.1	-0.3,0.0	0.1	-0.7,0.5	-0.1	-0.2,-0.0	0.04	-0.6,0.4

CHL = crown-heel length, CRL = crown-rump length, Abdo.= abdominal circumference, MUAC = mid-upper arm circumference, Subscap = subscapular skinfold

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