University of Sheffield

LONGITUDINAL CHANGES IN BODY COMPOSITION WITH ADVANCING PREGNANCY AND THE RELATIONSHIP OF MATERNAL FAT DEPOSITION TO FETAL GROWTH

A thesis submitted to the Faculty of Medicine for the Degree of Doctor of Philosophy

by

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Abstract

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Obesity has long been recognised as a risk factor for the development of variety of life threatening diseases; examples include diabetes mellitus and cardio vascular disease. In particular, in young women, it is widely thought to predispose towards impaired glucose tolerance and gestational diabetes. Obesity is not only common but it is increasing in prevalence despite the efforts made by individuals and their health advisors to avoid it.

Identification of factors contributing to obesity could be useful in planning preventative policies.

Many women relate the onset of their obesity to one or more of their pregnancies. We now know that there is more to obesity than the actual amount of excess weight. The adverse metabolic effects of obesity are proved to be greater when excess fat deposition is central rather than peripheral. The idea that fat deposition may be directed to different body sites with different effects on glucose tolerance and insulin levels, is a relatively new concept.

The majority of investigators have focused only on the magnitude of weight changes during pregnancy and postpartum. There is not much information with regard to the

compartmental changes in body composition during and after delivery. Most studies in the developed world show maternal net fat gain accompanying pregnancy. The extent to which such fat gain is physiological, and what is its beneficial effects is unknown. It is therefore important to study the impact of other factors with possible effects on fetal growth and maternal body composition. The present work examines further the question of fat storage by means of a longitudinal study to establish changes in body fat content during normal pregnancy and postpartum and also its relationship to maternal glucose tolerance, fetal metabolism, new-born anthropometry and infant growth.

For this purpose, 123 women were recruited at their first visit to Antenatal Clinics of the Northern General Hospital, from which 73 completed 3 visits during pregnancy and 46 participated in the whole course of the study. Maternal anthropometry and body composition were measured at 13 (5 - 15), 24 (23 - 32) and 36 (34 - 41) weeks gestation during pregnancy and also 6 weeks and 6 months postpartum. Skinfold thickness measurements and Bio-electrical Impedance were used to assess the changes in body composition during pregnancy and postpartum.

Glucose tolerance tests were performed at 28-31 weeks gestation. Insulin and/or C-peptide levels were also collected at birth and assessed later. Infant anthropometry (weight, length and head circumference) were measured at birth, 6 weeks and 6 months postpartum.

The subjects were divided into four groups of under-weight, normal weight, over-weight and obese, based on their early pregnancy BMI. The statistical analyses were performed on the whole group and also the groups were then compared for the above mentioned variables.

The two methods (Skinfold thickness measurements and Bio-electrical Impedance) showed a reasonable agreement in predicting the fat changes during pregnancy. A considerable variation was observed, however, between the two methods in the results of changes from early pregnancy to postpartum. Therefore, direct interpretation of skinfold thickness (which was consistent with the converted version of it to fat mass) was alone chosen to derive the conclusions.

During pregnancy, a formula which is corrected for hydration changes during gestation, was used to convert skinfold thicknesses to fat mass. The results confirmed that a substantial net gain of fat was made during pregnancy of which a significant amount was still retained at 6 month postpartum. Maternal weight increased (10.87 kg ± 4.67), but it reverted to the early pregnancy values by 6 months postpartum. The rate of fat and weight changes was significantly higher in the first half of pregnancy compared to later. The observed changes in maternal weight and fat mass, during pregnancy were not significantly different between the BMI groups (normal weight, over-weight and obese group). At the postpartum period, the obese group retained more of the net fat gain than the other two groups. A state of redistribution of fat tissue was observed in the subjects in particular in the obese group who had a tendency towards central fat retention.

Gestational fat gain was not directly related to infant birth weight. In this well nourished population, maternal early pregnancy lean body mass (LBM) was the most significant predictor of infant birth weight.

There was a positive correlation between maternal BMI and cord insulin ($r=0.44$, $p=0.002$) and/or C-peptide ($r=0.33$, $p=0.008$). In addition higher levels of insulin were observed in large for gestational age (LGA) babies in comparison with the average for gestational age (AGA) babies. These observations might have an important impact on the care of overweight women. It may be that reduction of maternal BMI in the prepregnancy period in the obese women would reduce the risk of gestational diabetes and also the rate of fetal hyperinsulinemia and macrosomia. Further investigation on this hypothesis is needed.

In summary; obese women had significantly heavier babies than the normal weight women. Maternal fasting glucose was significantly correlated to infant birth weight. On the other hand, maternal fasting glucose and the level of fetal insulinisation was significantly associated with maternal early pregnancy BMI. This is likely to be a metabolic effect operating throughout gestation. What we can suggest is that , in the studies of maternal glucose metabolism and fetal growth, the confounding effects of maternal BMI should be seriously considered.

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Abbreviations

BMI	Body mass index
BIA	Bio-electrical Impedance Analysis
BI	Bio-electrical Impedance
TSF	Total skinfold thickness
STM	Skinfold thickness measurements
LBM	Lean body mass
FM	Fat mass
TBW	Total body water
UWW	Under water weighing
WHR	Waist hip ratio
LGA	Large for gestational age
AGA	Average for gestational age
SGA	Small for gestational age
GDM	Gestational diabetes mellitus
SD	Standard deviation
$\overline{dif}$	mean difference
ΔAUC	Delta Area Under the Curve

Units

g	gram
Kg	Kilogram
L	Litre
wks	weeks
mo	months
µmol	micromole
nmol	nanomole
hr	hour
Kcal	kilocalorie
cm	centimetre
mmol/l	millimole/litre
µU/l	microunit/litre

Chapter 1

Introduction and background to the studies

During pregnancy, the maternal body undergoes tremendous changes. Some of these changes are visible and easily detectable, whereas some others are more complicated and require more sophisticated methods to be appreciated. The morphologic, physiologic and metabolic changes occurring during pregnancy are presumed to facilitate fetal growth and development.

As it has been suggested by Knopp et al. (1981), fetus does not act solely as a parasite to drain the fuels from the mother. He believed that as well as the "fetal pull" in respect to nutrient flow, across the placenta, there is a "maternal push" through some physiologic

and metabolic adaptation. Some of these adaptations can be understood by longitudinal studies of weight changes during pregnancy. Body composition measurements, however, would give a more detailed insight to this matter.

Studies on fat deposition during pregnancy, have produced various results. The majority of the studies suggest that a considerable portion of weight gain is body fat mass (Taggart et al. 1967, Van Raaij et al. 1987, Forsum et al. 1988). Eighty five percent of energy cost of pregnancy was accounted for fat gain (Hytten 1991). Hytten et al. (1966) found that maternal fat stores reach a maximum in the mid second trimester. Fat deposition thereafter remained constant or even diminished in the third trimester, in average weight women. This is concurrent with a physiologic state of reduced insulin sensitivity in late pregnancy which would leave glucose available for the use of the fetus. This sequence of events seem to benefit fetal growth, since its maximum growth is in the third trimester.

It has already been shown that maternal weight gain is positively correlated with infant birth weight (Eastman and Jackson 1968) but which component of weight gain has the closest association with intrauterine growth is still to be investigated. The data from Gambian studies indicates that poorly nourished women store less fat than do well-nourished women, they also deliver smaller babies (Lawrence et al. 1987). The literature is not consistent in the matter of the relationship between fetal growth and fat retention during pregnancy in well nourished Western women.

In the postpartum period, knowledge of the magnitude of fat mobilisation could have an important practical impact on maternal well-being. It may help to understand the relation of maternal gestational fat gain/retention to consequent development of obesity. The

present study was designed to examine further the question of fat storage by means of a longitudinal study to establish the pattern of anthropometry and body composition changes during gestation. It was also aimed to investigate the relationship of the above mentioned factors with maternal glucose tolerance, fetal metabolism, newborn anthropometry and infant growth. This section commences with illustrations of terminology, methodology of body composition measurements, and their application to gestational period.

Human body composition

A complete chemical analysis of the human is a formidable task which has been carried out on a number of occasions, but not sufficiently often to give the range of variations in people of different age and sex and in different stages of life cycle. Chemical analyses on small animals have been made, but the results do not necessarily apply to the human. However, for more than a century, it has been known that the human body contains many of the elements of the earth, twenty five of which appear to be essential for normal physiological functioning. About 4-6 percent of the human body is composed of minerals, primarily calcium and phosphorus in the bones, but also including others such as iron, potassium, sodium, chloride, and magnesium. The vast majority of the human body consists of four elements; carbon, hydrogen, oxygen and nitrogen. These elements

are the structural basis for body proteins, carbohydrate, fat and water. For practical purposes, the human body is divided into two, three, four, or five compartments. The terminology and the background of each model of body composition will be described.

Terminology and current knowledge on human body composition

Fat and fat free mass are two terms used in scientific literature relating to body composition. The total amount of fat in the body consists of both essential and storage fat. Essential fat is necessary in certain body structures such as the brain, nerve tissue, bone marrow, heart, and cell membranes. Essential fat in adult males represents about 3 percent of the body weight. Adult females also have additional essential fat associated with their reproductive process. This additional 9-12% of sex specific fat gives them a total of 12-15% essential fat. Storage fat is simply a depot for excess energy and the quantity of body fat in this form may vary considerably. Some storage fat is found around body organs for protection, but over 50% of total body fat is found just under the skin and is known as subcutaneous fat.

Fat free mass primarily consists of protein and water, with smaller amounts of minerals and glycogen. The tissue of the skeletal muscles is the main component of fat free mass, but the heart, liver, kidneys, and other organs are included also. Another term that is often used interchangeably with fat free mass, is lean body mass (LBM). However, LBM is slightly different from fat free mass because the fat in the cell membranes is excluded

from FFM, but is retained in LBM due to the anatomical proximity of this small amount of fat to lean tissues (Roubenoff and Kenaylas 1991). Total body water (TBW) overlaps with LBM, but these two compartments differ in that the TBW does not include extracellular connective tissues or intracellular non water structures. Total body water forms a fairly constant proportion of LBM of about 73% (Behnke 1961, Pace and Rathbun 1945). The common terms to describe body composition are summarised in a review by Jensen (1992) of research techniques for body composition measurements. These are presented in table 1.1.

Table 1.1. Body composition terms (Jensen 1992)

Term	Explanation
Body fat mass	Quantity of triglyceride fat in the body
Adipose tissue mass	Fat (~83%) plus its supporting cellular and extracellular structures (consisting of ~2% protein and ~15% water)
Lean body mass	Nonadipose tissue body mass
Fat free mass	Lean body mass plus non-fat components of adipose tissue
Body cell mass	Cellular components of the body
Extracellular Solids (ECS)	Total body bone mineral (skeleton, ~85% of ECS) + fascia, cartilage, etc. (~15% of ECS)

Background to the physiological studies of human body composition

Although an interest in human body composition can be traced to the late 1800s, research to establish indirect measurements of body composition began during the 1940's. In fact attempts to assess body fat content from density arose as a result of a practical problem. Some men on a draft for recruitment into the US navy were marked unfit for the service by a medical board because their weight exceeded the maximum permissible for their heights. As these men were professional footballers in their civilian life, it seemed unlikely that they were unfit for their naval service because of obesity. A US naval surgeon, Behnke et al. (1942) examined the men. They were weighed in air and under water. Their density was calculated and found in some cases to be as high as 1.090 to 1.097, indicating a fat content of 4 percent or less. From this it was concluded that the excess weight was mainly due to extra muscle and not fat. Since that time a variety of methods based on different assumptions of body composition have been introduced, which are discussed below.

Two-component model of body composition

It was through the pioneering efforts of Behnke and his co-workers that the first truly noninvasive *in vivo* technique of measuring body composition was developed. They actually established the first two-compartment model of body composition:

"Wt = Fat + Non-fat (or lean mass)"

where the densities of the fat and lean compartments are assumed constant. Behnke et al. (1942) reasoned that if whole body volume or density could be measured and if the body could be considered as only two compartments, each with constant but different densities, then the proportional masses of each component could be calculated. This model is still used by many investigators, mainly because of the advantage of simplicity. The non-invasive technique developed by Behnke et al. has often been referred to as the "reference method". Many of the methods developed since then have been evaluated or calibrated against the original densitometric technique, thus elevating it to a "standard" by some investigators. Virtually all of the equations based on anthropometric measurements, for example, have relied directly or indirectly on estimates of body fat that use underwater densitometry as their absolute measure or "standard," independent of possible density changes in the lean tissue related to sex, race, age or special circumstances like pregnancy and disease. Over the last few decades, however, investigators have developed newer procedures, both direct and indirect, and have recognised differences in density of the various subcomponents of the non-fat or lean tissues in the body. These observations have led to multicompartmental models of body composition.

Multicompartmental models of body composition

The chemical analysis of human cadavers reported by Forbes and Lewis (1956), Widdowson and co-workers (1951, 1964) indicated that lean body mass (LBM) had a

relatively constant composition, at least for its water and potassium content. This observation stimulated the development of water and potassium measurements *in vivo* as indices of fat free mass. The conceptualisation of a metabolically active component of lean tissue mass, called the body cell mass (BCM), was then introduced by Moore et al. (1963). He believed that the human body can be divided to three compartments:

"Man = CM + EST + Fat"

CM is the cell mass which is the active tissue, carrying out all the work of the body. EST is the extracellular supporting tissue which supports the cell mass. This again can be subdivided into two parts: the extracellular fluid, and minerals and protein fibres in the skeleton and other supporting tissue. The extracellular fluid comprises the blood plasma and lymph and fluid which bathes the cells. The advantage of this model was that BCM could be calculated directly from total body potassium (TBK) measurements and extracellular fluid volume could be calculated by bromide dilution. Alternatively, many investigations continue to assume, on the basis of the initial cadaver studies of Forbes et al. (1961) that the TBK/LBM ratio is a constant for adults.

During the derivation of the two compartment model, Keys and Brozek (1953) divided the mammalian body into four chemical groups; water, protein, ash or bone mineral, and fat. Anderson (1963) later used measurements of body potassium and water to estimate these components. Only recently has technology been available that allows for the *in vivo* determination of these four compositional variables.

The current model of body composition, and the most informative, consists of five major compartments (Halliday and Miller 1977, Lukaski and Johnson 1985):

"Weight = protein + Water + Minerals + Glycogen + Fat"

Although this five-compartment model is traceable to the chemical data obtained from human cadaver analyses, the recent development of *in vivo* elemental analyses has made it more practical (Schoeller et al. 1980). No single measurement technique, however, has the capacity to provide such a detailed resolution of body composition at this time. Body glycogen stores, for example, are usually assumed to be approximately 0.6% by total weight of fat free mass (FFM) of the body.

The two and multicompartmental models served as the basis upon which all body composition methods were developed. The two compartment model, however, continues to have the widest acceptance and application, mainly because of the simplicity of its interpretation.

Methodology of body composition measurements

In this section, some of the theoretical and practical aspects of the methods of body composition measurements will be discussed. These methods are summarised as a practical guide in a review by Jeb and Elia (1993). They described the techniques under two groups of reference body composition methods (densitometry, total body water, total body potassium, in vivo neutron activation analysis, dual energy x-ray absorptiometry, computed tomography and magnetic resonance scanning) and bedside or field techniques (anthropometry, bioelectrical impedance analysis, infrared interactance, and urinary metabolic excretion). Field techniques are derived from a statistical comparison with measurements from the reference methods. Thus they are less direct and produce less accurate results than the reference methods, but practically they are more accessible and subject friendly than the reference methods.

Reference methods:

Densitometry

The assessment of human body composition by measurement of whole body density is a common method used for normal healthy people. This method is based on the two compartmental model, in which the density of the fat and fat-free mass is known and constant.

Density is measured as the mass of the body in air divided by the volume of body tissues, and in body composition analysis is usually expressed as grams per millilitre (g/ml) or grams per cubic centimetre (g/cc^3). The standard for the comparison is water, which has a density of 1 g/ml. Corresponding densities for the fat and fat free tissue are considered to be 0.9 and 1.1 subsequently (Brozek et al.1963, Mendez et al. 1960). The density of the human body as a whole may have a wide range, approximately 1.02 to 1.10.

The most widely used technique of measuring whole body density is the determination of the body volume according to Archimedes' principle, which states that the volume of an object submerged in water equals the volume of water the object displaced. If one measures mass in air and mass in water, the difference, corrected for the density of the water corresponding to the water temperature at the time of underwater weighing, is the apparent body volume. With this technique it is mandatory to determine the lung volume during submersion (residual lung volume) which makes a sizeable (1-2 L) contribution in the estimate of total body volume. A second volume, gastrointestinal gas, is considerably smaller in magnitude and is never measured (Buskirk 1961). It is however assumed to be ~100 ml, based on previous radiological studies. Because the intra-individual variability in gastrointestinal gas volume can be quite large (50-300mL), this variable can compromise the precision of the densitometric method (Bedell et al. 1956). An alternative way of measuring volume, is to use a form of body volumeter in which the volume of the water displaced is measured directly using a tilted burette attached to the side of the tank of water that the subject is submerged in. As weight is generally more accurately recorded than volume, the under water weighing is more acceptable.

A common equation for the calculation of percent body fat (%F) from body density (D_b) is that proposed by the Brozek et al. (1963): "%F = (4.570 / D_b) - 4.142". Another equation was developed by Siri (1956): "%F = 4.950 / D_b) - 4.50". Within densities of 1.10-1.03, the two equations give results within 1% body fat. For subjects with more than 30% fat, the Siri equation yields higher values than the equation of Brozek (Lohman 1981). Very useful body compositional data can be gained from the hydrodensitometric technique. However, its use in populations unaccustomed to swimming or apprehensive about submersion in water may result in longer periods of time required to densitize the subjects to obtain valid and reproducible body composition estimates. This technique requires a great deal of cooperation from the study subject and is relatively expensive.

Total body water

The findings that water is not present in stored triglyceride and that water occupies a relatively fixed fraction (73.2%) of the fat free mass (Pace and Rathburn 1945) have stimulated the determination of total body water (TBW) as an index of human body composition:

"FFM (kg) = TBW (kg)/ 0.732"

The isotope dilution technique has been used to estimate TBW. Some general assumptions of the isotope dilution technique are that the isotope has the same

distribution volume as water, it is exchanged by the body in a manner similar to water, and it is nontoxic in the amounts used (Pinson 1952).

In this technique the known tracers which nowadays are stable, non-radioactive isotopes of water containing deuterium or 18-oxygen have been used to estimate TBW. Tritium is commonly used since the analysis is rapid, cheap and simple to perform, although since it is radioactive, it is not the method of choice, particularly for children and women of childbearing age.

In practice the subject must receive a precisely known amount of the tracer, orally or intravenously. After equilibration, the tracer is measured in a sample of a body fluid; plasma, urine or saliva.

Implementation of this technique is difficult outside of a specialised research laboratory and it is also time consuming and rather expensive.

Total body Potassium

Potassium is the major intracellular cation in lean tissue but it is virtually absent in adipose tissue, therefore, knowledge of total body potassium permits estimation of lean body mass (Jensen 1992). A small percentage of all potassium is the naturally occurring radioactive isotope ^{40}K. Sufficiently sensitive detectors surrounded by adequate shielding can detect the decay of body ^{40}K. Comparing this radioactivity to known amounts of potassium permits calculation of total body potassium and thus lean body mass (Garrow

1982). Alternatively, ^{42}K can be administered to a subject and the radioactivity can be measured by methods that do not require extensive shielding and sensitive detectors (Pierson et al. 1984).

Once total body potassium (TBK) is determined , LBM could be estimated with a factor dependant upon the potassium content of the LBM.

LBM = TBK/concentration of K per kg of LBM

The potassium content of the lean tissue is often taken as 60 mmol/kg for females and 66 mmol/kg for males (Forbes et al. 1961, Pierson et al. 1974).

The accuracy of measuring body potassium is good and its precision depends on the efficiency and the duration of counting. One of the major drawbacks to TBK measurements is the influence of body size and geometry on the measured counts. Adipose tissue exerts a shielding effect and fewer counts can be recorded in a obese subject compared to lean individual for a similar amount of potassium.

Although absolute measurements of TBK can be obtained with the use of highly sophisticated equipment, the cost of instrumentation and technical support, may be prohibitive for some research applications.

Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DEXA) was originally used to assess bone density. It works based on scanning the body with photons at two different energy levels. The

differential absorption of the photons is then measured and from this, it calculates both bone mineral and soft tissue. It subsequently divides the latter into fat and fat free soft tissue. The data may be analysed to yield information about the composition of the whole body or individual segments, e.g. limbs or abdominal regions.

The method requires patients to lie on a table while they are scanned. The time taken varies depending on the machine employed, but it does not exceed 20 minutes for a single whole body composition analysis. The coefficient of variation of fat free tissue measurements is approximately 2%, which is acceptably precise. The accuracy of DEXA appears to be excellent in comparison with other techniques (Mazess et al. 1990). One of the limiting factors for the use of this machine, is the size of the scanning area which is too small for very obese or tall people. The radiation exposure, makes this technique unsuitable for children or pregnant women.

In vivo neutron activation analysis

In vivo neutron activation analysis (IVNAA) involves irradiating an individual with neutrons to change some of the body's nitrogen, chloride, and calcium to radioactive isotopes. The isotopes revert to a stable condition by the emission of energy which can be measured. It is possible then to calculate total body nitrogen (a major indicator of lean body mass), body chloride, and body calcium (Cohn et al. 1985).

IVNAA is probably the ultimate multi-compartment model, allowing elemental analysis of the body. However, of the techniques mentioned, this is the most restricted since it is available in only a few centres world-wide. Because of the high radiation, it is also unsuitable for pregnant women and children.

Imaging techniques

These techniques provide a visual image of adipose tissue and non-fat tissue with whole body scans, but the level of precision for the whole body measurements is generally worse than those can be obtained by the other means (e.g. UWW and DEXA).

However, such techniques offer a uniquely direct approach to the estimation of a particular body compartment. They have the advantage over other body composition methods in being able to assess the size of the individual organs or tissues, as well as providing information about adipose tissue or muscle and its distribution at particular site of the body. The disadvantages are that they are expensive, time consuming and in the case of X-ray or computerised tomography (CT) scans, they involve a radiation dose to the subject. Therefore, they are not recommended for pregnant women or children. They are also complex and need professional assistance to perform. Access to such equipment is often limited.

CT scanning. Computerised tomography is based on irradiation of the body with X-rays. Depending on the density of the tissues, a signal would be reflected to the detector. The maximum signal is received when there is no tissue between the X-ray source and the

detector. The image is constructed from differences in the physical density of tissues. High image contrasts can be observed between different body compartments (bone, fat free tissue, adipose tissue and air which appear as grey shading), ranging from black to white for high to low densities, respectively (Foster et al. 1988).

Magnetic resonance. This approach is based on the fact that atomic nuclei, made up principally of neutrons and protons, can behave like magnets. When a radiofrequency wave is applied to the body, some nuclei absorb energy and align themselves in the direction of magnetic field. When the radio wave is turned off, the nuclei return back to their original position, releasing the energy that they had earlier absorbed. This emission of energy can be detected and from its frequency and intensity images of tissues can be constructed.

MRI is safe and needs minimal cooperation from the subjects. The procedure is however, slow and expensive. There is limited experience of its use in pregnancy.

Field techniques:

Anthropometry

Simple measurements of body weight, height and subsequently body mass index (BMI), are of great use in nutritional assessment, but they hardly give any information about body composition. BMI which is weight divided by a power of height, usually $(height)^2$, has found to be useful in evaluating groups of people. Individuals with high indices are

classified as overweight, even obese, and those with subnormal indices as undernourished. BMI is not however, an accurate index of body composition. This is evident from the fact that despite the well demonstrated difference in body fat mass the average BMI is about the same for both sexes during the adolescent and young adult years.

Measurements of skinfold thicknesses and body circumferences have been used for decades as rapid, inexpensive methods of estimating body fat content and its distribution (Deurenberg 1992).

Skinfold thicknesses. This method is probably the most widely-used technique for estimating fat mass. The assumption behind this approach is that the majority of the body fat resides in the subcutaneous regions and that there is a fairly consistent relationship between subcutaneous and visceral fat. The measurement is made by grasping the skin and adjacent subcutaneous tissue between the thumb and forefinger, shaking it gently to exclude underlying muscle, and pulling it away from the body just far enough to allow the jaws of the calliper to impinge on the skin. The callipers exert a standardised pressure on the measurement site. Duplicate readings are made at each site to improve the accuracy and the reproducibility of the measurements. Subcutaneous fat fold measured at one or more sites can then be interpreted using previously validated equations into an estimate of fat mass. In the UK, the equations of Durnin and Womersley (1974) are probably the most widely used for adults, based on measurements made at four sites - triceps, biceps, subscapular and suprailiac. A logarithmic transformation of the sum of four skinfold

thicknesses produces a linear relationship with body density which is age and sex specific. Fat mass is then calculated using the equation of Siri (1956) or others.

The suprailiac, subscapular, triceps, and thigh skinfolds appear to be particularly important in predicting total body fat in women. The selection of the site and the technique of the measurement can lead to substantial inter observer variation, but performed by a single observer the method can yield extremely reproducible results (Fuller et al. 1991). For longitudinal studies the measurements should ideally be made by a single observer. The absolute accuracy is difficult to establish and will vary with the prediction equation.

Body circumferences. The measurements of body circumferences do not give information on body composition, but they are useful indices in the assessment of the changes over a period of time or inter-individual differences. Some measures such as waist and hip circumferences are valuable in fat distribution determination, and have been used as risk indices for disease. Arm circumferences, particularly in association with triceps skinfold thickness, have been used to estimate mid-arm muscle circumference as an indicator of protein energy malnutrition.

A tape which does not stretch and is sufficiently long to cover the entire circumference, is required to measure any circumference. What should be carefully defined is the site of measurement which is usually chosen with respect to anatomical landmarks. The position of the subject, whether supine or standing, can have major effect on the measurement at some sites and should be controlled. The WHO (1988) standards for waist and hip circumferences are based on measurements made in the standing position.

Infrared interactance

Infrared interactance, a relatively recent technique, is based upon the principle of light absorption and reflection using near infra-red spectroscopy. When electromagnetic radiation strikes a material, the energy is reflected, absorbed or transmitted depending on the scattering and absorption properties of the sample. Energy transmitted into the sample is scattered and reflected back out of the sample. The pattern of reflection gives information about the chemical composition of the sample. For estimation of human body composition, a computerised spectrophotometer is used with a single, rapid scanning monochromator and fibre optic probe. The probe emits electromagnetic radiation from the monochromator to a selected site on the body, collects interactive energy, which is the combination of reflected and scattered energy, and conducts it to the detector. The signal penetrates the underlying tissue to a depth of 1 cm and the composition is assessed only at the examined site. It would seem unlikely that one could validly extrapolate compositional data from a limited (1 cm depth) subcutaneous depot to the entire body and expect consistent and valid results.

Urinary metabolite excretion (Creatinine)

As a result of nitrogen metabolism, mainly in skeletal muscle, creatinine would be formed from two precursors; creatinine and creatine phosphate. Therefore, 24 hour

measurements of excreted creatinine by an individual could be used in order to assess the muscle mass (Heymsfield et al. 1983). Although the correlation of the excretion rate with lean body mass is reasonable, the need for restricted diets, the high variability, and the practical difficulties of urine collection severly limits the ability of this technique to be used as a body composition measurement or as measure of changes in lean body mass.

Bioelectrical impedance Analysis (BIA)

This method is based upon the nature of the conduction of an applied electrical current in an organism. In biological structures application of a constant, low-level alternating current produces an impedance to the spread of a current that is frequency dependent. The living organism contains intra- and extracellular fluids that behave as electrical conductors and cell membranes that act as imperfect reactive elements. At low frequencies (~1 KHz) the current mainly passes through the extracellular fluids while at higher frequencies (500-800 KHz) it penetrates the intra- and extracellular fluids (Nyboer 1970, 1972).

Electrical conduction is related to the water and electrolyte distribution in the biological conductor. The hypothetical relationship between impedance and electrical volume was proposed by Nyboer et al. (1943), who demonstrated that electrically determined biological volumes were related inversely to impedance (Z). Assuming that body has the simple geometric shape of a cylinder, the theoretical relation between body water and

impedance is of the form of: $TBW=\rho \times H^2/Z$, where TBW is total body water, ρ is the resistance of the body fluid, H is the body height and Z is the measured impedance value (Lukaski et al. 1985). Although there are difficulties in applying this general principle in a system with as complex geometry and bioelectrical characteristics as the human body, this relationship has been used to derive models for the prediction of human body composition (Thomasett 1962, Hoffer et al. 1969, Lukaski et al. 1985) by assuming that the body is a series of connected cylinders. Using densitometric data but lacking accurate residual volume measurements, Nyboer et al. (1983) developed preliminary statistical relationships between conductance and body composition variables in college students. Segal et al. (1985) attempted to verify these regression equations and found unsatisfactory predictors of fat free mass in men and women aged 18-50. Lukaski et al. (1986a) conducted a study on 47 men and 67 women to validate the relationship between conductance and fat free mass. Comparison of densitometrically determined and impedance predicted fat free mass yielded an error of prediction of 2-2.5 kg and a calculated error of relative body fatness of 2.7%. Relative to densitometry the observed error of calculated body fatness was larger by anthropometry (3.9%) than by impedance (2.7%). In the studies by Lukaski (1985, 1986b), Kushner and Schoeller (1986) the conductance method was proved to be the best single predictor of total body water. Because fat free mass, including the protein matrix of adipose tissue, contains virtually all the water and conducting electrolytes in the body, conductivity is far greater in the fat free mass than in the fat mass of the body (Pethig 1979).

In practice, a small current, usually 800 μA, is passed between electrodes on the hand and foot and the voltage drop is measured to give an estimate of the body resistance or impedance. Prediction equations are required to translate the measured impedance into an estimate of water volume. When total body water assumed to be a fixed proportion of the lean tissue, LBM could then be calculated. Fat mass is also calculated by difference from body weight and the calculated lean tissue.

The measurement itself takes only a matter of seconds. Minimal undressing is required to yield the appropriate sites. Also, minimal preparation of the site is required, except in particularly hairy subjects. Since the technique is measuring body water, it is important to ensure as far as possible that subjects are normally hydrated. To assist in this, usually measurements are made ~2 hours after eating and within 30 minutes of voiding. There is less observer error associated with BIA than skinfold thicknesses (Fuller et al. 1991) especially in the obese. Although the position of the electrodes are important, the sites are easier to define than for skinfold thicknesses. In terms of electrode positioning, that of the wrist is the single most important site, since the arm makes such a large contribution to the total measurement. In recent years, some influential surveys with multiple observers, notably The National Health And Nutrition Examination Survey (NHANES), have turned to impedance as a measure of fatness (Woteki et al. 1988).

In summary, BIA offers the advantage of rapid, safe, relatively cheap and noninvasive measurements that can be done at the bedside. Its disadvantages include the possible need for prior knowledge of the degree of fatness in subjects who are obese and the possibility that changes in electrolyte milieu of the body will affect measurements (Cohn

1985). Deurenberg (1996) suggested that that the predicted body fat mass from BIA in obese subjects must be interpreted with care. He believed that changes more than 1-2 kg TBW or 2-3 kg FFM can be more reliably detected in the obese group.

Application and limitation of body composition measuring methods to pregnancy and lactation

The additional calorie increment estimated as necessary during pregnancy (about 250-300 kcal/day) was determined to a large extent by a calculation of net fat gain in healthy women (WHO 1986, Hytten 1980). Weight gain recommendations during pregnancy therefore assume certain fat changes occur during normal pregnancy.

Fetal growth may be influenced more by specific maternal tissue changes, for example, by accretion of lean tissue, fat, or body water.

Gestational diabetes mellitus (GDM) could endanger maternal and fetal life. Twenty five percent of infants born to diabetic mothers are hypoglycaemic and hyperinsulinemic Andersen et al. 1982). The association of excess fat and particular patterns of fat distribution with metabolic disturbances has been well established (Kissebah et al. 1982). To what extent and how obesity during pregnancy could be related to GDM is to be investigated. All these reasons make the information on maternal body composition, a valuable addition to the clinical care and evaluation of the nutritional needs of pregnant women. However, due to practical limitations, data on body composition and its changes during pregnancy have been limited.

In identifying appropriate methods to study longitudinal changes in maternal body composition, the type and magnitude of the expected changes must be taken into consideration. Basically, determination of fat storage is more complicated in pregnant women than non-pregnant women, because within a wide range of maternal weight gains

there are considerable changes in many components of body composition during pregnancy. The question of the methodology for the assessment of body composition of pregnant women therefore has remained unanswered over the years. Some of the methods are unsafe during pregnancy, others are not potentially valid in this period. Most techniques currently used to estimate body composition are based on the measurements of LBM. Of the commonly used methods, only densitometry is dependent on both fat and lean tissue, but the fat estimate is still highly influenced by the variability of the lean tissue density. In the two compartment model, as explained above, the weight of fat is the difference between the two large masses; body weight and lean body mass. Therefore, a small relative error in the lean body mass estimation will produce a much larger error in calculated body fat. In the light of expected changes in the maternal body composition , the theoretical assumptions behind the three standard methods (UWW, TBW, TBK) as well as the other most commonly used methods of measuring body composition will be discussed.

Under water weighing: Under water weighing (densitometry) provides a reliable estimation of density and has been used as the criterion measure for the estimation of fat for more than 50 years. Although, under water weighing provides an accurate estimation of density, the conversion from body density to percentage of body fat is open to controversy. Basically, the assumptions behind the criterion methods would be violated by the changes which are known to occur during pregnancy. The fact that FFM of the developing fetus is less than the average density of 1.10 g/ml suggest that an underestimation of maternal FFM could result. Furthermore, the assumption that 73.2%

of the FFM is water can be challenged in pregnancy due to the large and significant increase in fluid retention which occurs in mid to late gestation. In a study by Van Raaij et al. (1988), new equations were developed specifically to take into account the alterations in density and composition of the maternal FFM throughout pregnancy, but validation of these equations have not been reported.

The problem of validity of hydrostatic weighing is of particular concern when attempting to use other methodologies, such as bioelectrical impedance in measuring FFW, in that such methods have been developed using regression from the hydrostatic weighing technique.

Total body Potassium: The measurement of TBK is again based on the assumption of a standard value for the concentration of the potassium in the lean tissue. The vast majority of the body's potassium is intracellular (approximately 98%), therefore, total body potassium is actually a reflection primarily of the intracellular compartment. Substantial changes in the extracellular compartment can go undetected which is obviously of importance during pregnancy. To have a reasonable estimation of the weight of lean tissue, the ratio of the intracellular to the extracellular tissue must be either close to the norm or assessed independently.

Total body water: To calculate the lean tissue from TBW, the water content of the lean tissue must be known. The average percentage of water in the lean tissue of adult women is known with fair accuracy but the addition of nonfat tissues during pregnancy would make a considerable difference in the hydration of lean tissue from approximately 72.5% at 10 weeks gestation to about 75% at 40 weeks in women with generalised oedema (Van

Raaij et al. 1988). This difference could lead to underestimation of fat deposition by about 50% or more in women gaining 3 to 4 kg of fat.

The interpretation of body water changes might be improved with a measure of extracellular water. Variation in the extracellular water could be substantial and it can be determined either with an extracellular tracer such as bromide or by subtraction of intracellular water measured by TBK from total body water.

Despite their limitations, TBW, UWW, and TBK are considered to be the three best methods for studying body composition in pregnancy and lactation. However, the measurement of TBK and UWW require special, large equipment and considerable patient cooperation, and TBW estimation requires special isotopes that are relatively expensive.

In the case of relatively new techniques such as DEXA, IVNAA, and CT scanning, radiation exposure makes these techniques unsuitable for pregnant women. Magnetic resonance imaging is safe and needs minimal cooperation from the subjects, but being slow and expensive would exclude this method from the list of options for many researchers. A consideration of the cost, availability, safety and acceptability of the methods has encouraged the use of simpler methods, such as Skinfold thickness measurements (STM) with callipers and Bio electrical impedance (BIA).

Skinfold thickness measurement: Skinfold thickness changes have been widely used to estimate variation in the fat content of pregnant women. Skinfold can be measured quickly with relatively inexpensive equipment. However, the method is open to

inaccuracy. A high degree of standardisation and extensive training is required to achieve reliable and reproducible measurements (Taggart et al. 1967).

To convert skinfold thickness measurements to estimate of body fat, standard regression equations are used. Generally, these are based on studies correlating skinfold measurements to body fat measured by total body water, body density or total body potassium. Because the most widely used regression equation during pregnancy is developed in studies of nonpregnant subjects (Durnin and Womersley 1974), the results should be interpreted with caution.

On the other hand, longitudinal studies on STM in pregnancy (Taggart et al. 1967) suggest that skinfold thicknesses may be increased by water retention. Therefore, the variation in the skinfold may not necessarily indicate a change in body fat content. The magnitude of this hydration effect may also vary from one site to the another (Adair et al. 1984, Taggart et al. 1967, Forsum et al. 1989). Some specific regression equations for pregnant women have been developed which were derived from small number of subjects (Forsum et al. 1989). The usefulness of the equations is partly dependent on the comparability of the measurement techniques used in the study population and the reference population. It may also be affected by differences in age, ethnic background, and exercise patterns of the reference and the study population. Bearing in mind the limitation of STM, in several studies skinfold thickness values have been used without calculating body fat content. In this approach, either individual (Frisancho et al. 1977, Maso et al. 1988, Viegas et al. 1987) or sum of the several measurements (Taggart et al.

1967, Lawrence et al. 1984, Arroyo et al. 1978) have been used, but they did not assume that all the measured changes directly reflect changes in body fat.

Bio electrical impedance Analysis (BIA): As explained above, this technique is thought to be an accurate means of assessing TBW in non-pregnant human and that the lean weight is assumed to be 72.3% H_2O. The applicability of this method in pregnancy was studied by Lukaski (1994), who indicated that the significant increase in the TBW can be accurately assessed by tetrapolar BIA, but the conversion of TBW to FFM will be in error. The higher water content will indicate a much greater FFM that actually exists. The overestimation of FFM may or may not cause an error in the evaluation of fat. Although more research is required to test the validity of this method, there are no suitable measuring techniques to provide the criterion measure at this time. In fact, due to lack of validation, there is no way of verifying which method is the best nor can it actually be suggested that any method would provide acceptable results.

In brief, there are many different methods that can be utilised to measure body composition in the living organism, some are highly practical and some of which are highly limited, due to economics, to a very few research or clinical institutes. As all of these methods are relatively indirect or predictive, there are errors (or variations) associated with each. These variations are caused by the errors of measurement (in relation to a specific technique) and biological variation of the subject. Conditions such as pregnancy make the matter more complicated, due to water retention and alterations in hydration constant of LBM. Hence, the vicious circle of alterations in maternal body composition and inaccuracy of the methods of measuring body composition, severely

limits the selection of an appropriate technique. Attempts has been made to develop pregnancy specific formula to predict maternal fat mass from densitometric (Van Raaij et al. 1988) and skinfold measurement (Forsum et al. 1989) techniques. However, depending on the type of study, what should be considered besides the accuracy and reliability of the method, is the availability and the convenience of the subjects.

The state of current knowledge on body composition during pregnancy and lactation

Body composition changes during pregnancy:

During pregnancy weight is gained and fat is stored in some areas more than the others. It is hypothesised that the excess weight and stored fat which could partly be an energy reserve for lactation, is mobilised during the postpartum period. The changes in the relative contribution of the components of weight during the reproductive cycle, are not well defined. The basic changes which occur during pregnancy are the addition of products of conception (fetus, placenta and amniotic fluid), there are also changes in uterine and breast tissue, body water (intracellular and extracellular water), and maternal fat. They change in variable amounts among women during pregnancy, thereby distinctly affecting the interpretation of individual weight gain.

The average weight gain after a typical 40 week pregnancy amounts to approximately 12.5 kg, about a 20% increase in body weight for most of women (average weight = 60

kg) (Forbes 1962). Most of this weight gain (68%) occurs during the last 20 weeks of pregnancy. It is known that about 40% of the total gain is represented by the products of conception. The remaining 60%, therefore, is the mothers' body weight change.

These figures could be different in developing countries, as the average weight gain in developing countries like Gambia, Thailand and Philippines, are estimated about 7-8 kg (Durnin 1987), of which 50% is accounted for by the products of conception.

To have a comprehensive understanding of body composition during pregnancy, it is imperative to know about the composition of the products of conception. Obviously, the proportion of fetal weight varies throughout the course of gestation. At term, the fat content of the fetus has been estimated at approximately 14% of body weight (Widdowson and Dickerson 1964). In another attempt to assess total maternal fat by Forsum et al. (1989), the fat content of the fetus was reported to be about 500 g in late pregnancy.

In addition to the fat percentage, the total water weight of the fetus, placenta, and amniotic fluid amounts to 3.8 kg of a total 4.7 kg (81%). In fact, it has been estimated that 60% of the total weight gain of the fetomaternal unit is water (Forbes 1987).

There is evidence that some of the weight gain of the pregnant female is due to increases in mammary, uterine, kidney and heart tissues. There is also, an increase in plasma volume and other fluid volumes (mostly extracellular fluid) (Hytten 1980). An increase of ~9 litres of water during pregnancy has been reported. In fact, TBW changes during pregnancy have been studied by many investigators. The least water change was an

increase ~5.7 kg in the study by Forsum et al. (1988) and the greatest was ~8.5 kg in the study by Campbell (1983).

Comparison of the incremental gestational changes in total body potassium also reveals substantial differences among the studies. The incremental potassium values in the study of Forsum et al. (1988) from early to late pregnancy is approximately one-third that of the other two studies by Pipe et al. (1979) and Emerson et al. (1975). In Forsum study a loss of lean tissue was observed. Clapp et al. (1988) however, showed an increase of both fat and lean tissue during the first 7 weeks of gestation. In fact, in the study by Forsum, the increase from prepregnancy to term is so low that it is insufficient to account for the amount of the potassium others have estimated to be required for the contribution of the conceptus alone. Nevertheless, all three above mentioned studies (Forsum, Pipe, Emerson) show postpartum figures that are very close to their early pregnancy values.

Using the potassium values, a maternal gain of 1.2 to 2.1 kg of lean tissue between early and late pregnancy, is estimated, excluding the conceptus (Pipe et al. 1979). Bone, however, is likely to increase relatively little. Putting all these findings together, there are only slight increases in the dry weight of lean tissue but substantial changes in the composition of the lean mass during gestation, because of increased water content.

Fat stores, also, increase during pregnancy but there has been a difficulty in quantifying this change. Deposition of fat is mainly on the hips, thighs (the classical sites for female fat distribution), back and abdomen. Several researchers have attempted to monitor maternal fat changes during the gestational period. Hytten (1980) estimated that well-nourished women retain ~4 kg body fat whereas studies in Scotland (Durnin 1987) and

the Netherlands (Van Raaij et al. 1987) suggest that this figure could be too high. On the other hand, Forsum et al. (1988), reported that Swedish women retain an average of 5-6 kg body fat during pregnancy and that 60% of this fat is gained before weeks 16-18. Also, during lactation these Swedish women were slow to mobilise the fat retained during pregnancy.

The variation in the results of the studies mentioned above could partly be explained by the different population and designs used in those studies. However, in a recent study by Lederman et al. (1993), using several methods of body composition measurements in the same subjects concurrently, considerable discrepancies were found between the results of each method. Three standard methods (TBW, TBK and UWW), which are based on the two compartmental model were used in this study. They also used a multicompartment model (discussed by Selinger 1977) based on direct measurement of the compartments (water and bone) that contribute most to variation in body density, particularly during pregnancy. In their study, body fat mass was measured in 65 pregnant women at 14 and 37 weeks gestation. The widely different results of fat gain from 14 to 37 weeks, obtained with each method, were as follows; TBW: 0.3 kg, TBK: 8.1 kg, UWW: 5.6 kg and MCM: 2.6 kg. This study confirmed the potential influence of methodological error on the results.

Body composition changes during postpartum period:

Assessment of body composition during the postpartum period would suffer less from the lack of certainty and precision. In the post partum period falls in the body weight and

fatness, particularly at the hips and thighs, are expected. Up to now, the composition of the tissue lost is not well established nor whether the compartments vary according to the degree of weight gained. In weight reduction of the obese the composition of the weight loss is on average, 75% adipose tissue and 25% lean body mass (Garrow et al. 1979). The composition of tissue lost also depends on the initial level of fatness, being higher in the fatter subjects (Forbes 1987). The maternal changes are not simply decreases in the adipose tissue mass, however. There is also a reduction in the volume of maternal organs, e.g. the breast and uterus.

As with pregnancy, the interpretation and the comparisons of reported studies on post partum changes are complicated by differing methodologies and different study designs. Ohlin & Rossner (1990), presented the results of the body changes of 1423 Swedish women during and after pregnancy. There was a fall in full term body weight of 12.5 kg over 12 months, with half the postpartum weight loss occurring in the first three months and a residual weight difference at 12 months of $+1.5 \pm 3.6$ kg (mean±SD) in comparison with prepregnancy weight. Post partum weight losses were higher in women with high scores for duration and intensity of breast feeding at 2.5 months but not at 12 months. Butte et al. (1984) observed a weight loss of 2 kg, on average, in 45 American women between 1 and 4 months postpartum.

Measurements of skinfold thicknesses showed falls at the subscapular and supra-iliac sites and paradoxical small but not significant increases at the triceps site. Adair et al. (1984) reported a marked increase in both triceps and subscapular skinfold thicknesses in the mother after parturition. Studies in less affluent countries (mainly countries with

marked seasonality variation) give a different view. The average weight gain in these countries is estimated about 7-8 kg. In Gambia (Lawrence et al. 1987), a successful outcome of pregnancy was observed with less fat storage (comparing with developed countries). In this population, lactating women gained weight in the dry season but lost weight in the wet season, when less food was available. This illustrates the importance of climatic factors above all the previously mentioned factors in causing the variation in this study area. However, Schutz et al. (1980) found that the energy cost of lactation was met mainly by fat losses in the rural Guatemalan women. The discrepancy between the results of different studies during postpartum period may also be explained by the lack of consistent definition of true breastfeeding.

In brief, a substantial weight loss occur mainly during early postpartum. Nevertheless weight losses during lactation can not always counter-balance the weight gained during pregnancy in well nourished women. This could be due to food availability and reduced physical activity in the developed countries. The possibility of retention of extra weight and fat may lead to development of obesity with each pregnancy.

The controversy surrounding the field would urge the need for repeated experiments, particularly on local populations, which would add valuable information on the physiology of maternal-fetal energy requirements.

Intermediary metabolism with an emphasis on glucose metabolism, insulin action and adipose tissue

Intermediary metabolism has been extensively reviewed by Lönnroth and Smith (1992) and also by Jefferson and Neely (1982). Some aspects of these reviews which are thought to be important in the consideration of diabetes mellitus and obesity are discussed below. A detailed description of biochemical pathways in fuel metabolism are beyond the scope of this thesis, so it is focused on the issues of metabolism that are most relevant to the subsequent research protocol.

Glucose metabolism:

Of the major nutrients or metabolic fuels that the body relies on, for its energy requirements (e.g. glucose, fatty acids, ketone bodies, to a lesser extent lactate, pyruvate and amino acids), glucose is the most important. The concentration of glucose is tightly controlled, because it is essential for the central nervous system. If the rate of glucose entry into the extracellular fluid compartment were to become less than the rate of glucose exit out of the compartment, hypoglycaemia would develop. Conversely, hyperglycaemia would result if the rate of glucose exit from the extracellular fluid were to become less than the rate of glucose entry. Entry of the glucose to extracellular spaces is from either exogenous or endogenous sources and it should be maintained within a limited range (3 to 7 mmol/l in different nutritional states). In the fed state, when exogenous glucose is abundant (e.g. after a high

carbohydrate meal), the body stores the excess glucose as glycogen in liver and to a lesser extent in muscle, and also as triglycerides in adipose tissue. During the postabsorptive state (after overnight fasting), the liver is the source of endogenous glucose which is derived from glycogenolysis (breakdown of glycogen to glucose) and from conversion of amino acids, lactate, pyruvate, and glycerol to glucose by the process of gluconeogenesis. Whatever the source of glucose to the plasma, it is taken up from the circulation by body tissues to be metabolised. Organs which are involved in glucose metabolism are divided to four groups by Bonadonna and Defronzo (1992). These four groups are summarised as follows:

1. Glucose dependent organs: This group, including brain and red blood cells (RBC), utilises glucose in a fixed rate. The brain and the RBCs are strictly dependent on glucose for their normal metabolism. Glucose levels below a certain level (3.6 mmol/l) lead to a life threatening situation due to damage to neurones. A complex homeostasic mechanism is then readily activated to improve the situation.

Although brain and RBC's glucose utilisation is constant over time (about 100 mg/min), feeding and consequent exogenous glucose availability are phasic. At times in the day, glucose availability greatly exceeds the needs of glucose dependant organs, and because glucose is not allowed to accumulate in the extracellular space, the excess glucose must be disposed of by the other organs. The next group of organs are responsible for this function.

2. Insulin dependent organs: This class of organs disposes of the carbohydrate that exceeds the need of brain and RBCs. These organs which include skeletal muscle and adipose tissue, should adjust their glucose uptake to effective glucose availability. Their glucose uptake is also dependent on the changes in the circulating insulin.

Skeletal muscle and adipose tissue are both sensitive to insulin, the former has a greater impact on glucose homeostasis, the latter, however, is not regarded as important for glucose metabolism (Bjorntorp and Sjostrom 1978). This is because the fat-free cytoplasm (i.e. the glucose metabolising mass), in the adipose tissue is only 0.7 to 0.8 kg, much less than the 28 kg of muscle mass in a 70 kg person.

3. Glucose sensor organs: Liver and pancreatic beta cells belong to this group. Plasma glucose concentrations would regulate pancreatic insulin secretion. Hepatic glucose production is also influenced by the plasma glucose levels. Hormonal regulation, however, has a much greater influence on the hepatic glucose production than glucose levels per se.

4. Glucovorous organs: The fourth group of organs which have essential roles in carbohydrate metabolism, are the gut and the kidneys. The gut is primarily responsible for absorption and entry of new glucose and the kidneys are able to re-absorb and re-enter the glucose to the circulation. Above a certain plasma glucose (~10 mmol/l), however, the kidneys, are not able to absorb all of the filtered glucose and this is when glucosuria appears.

In general, all of these four types of organs described earlier, share a common function; transferring glucose from one physio-anatomical space to another. In the most relevent organs to our subject of study: adipose tissue, muscle and also liver, metabolism is regulated by insulin and its counterregulatory hormones: glucagon, catecholamines, glucocorticoids and growth hormone. When insulin is ascendant, secretion of antagonists is suppressed and, when it is in short supply, the antagonists tend to be abundant. Insulin dominance favours anabolic processes, i.e., glycogen synthesis, fatty acid and triglyceride synthesis, protein synthesis, whereas dominance

of the antagonists promote catabolic processes, i.e., triglyceride hydrolysis, fatty acid oxidation, ketogenesis, proteolysis, glycogenolysis.

The Insulin Action:

Insulin is produced in the pancreatic beta-cells as the primary product "preproinsulin". This peptide is rapidly converted to proinsulin which is in turn, converted to insulin plus C-peptide by specific proteolytic steps within the beta-cell secretory granule.

The normal products are therefore, insulin, an equimolar amount of C-peptide and a small amount of unconverted proinsulin. Upon their release from the B cell to the blood stream, a large amount of the insulin is cleared from the portal vein during the first passage through the liver; the most important insulin target for glucose homeostasis. This clearance results in a relatively constant peripheral level of circulating insulin . Proinsulin which has about 5% of the biological activity of insulin is cleared less efficiently and may form 20% of the insulin in the peripheral circulation. C-peptide is excreted into the urine and can be assayed there or in plasma. This is a useful procedure since the C-peptide is produced in amounts equimolar to insulin and its radioimmunoassay can be used to evaluate a patient's endogenous insulin synthetic capacity (Horowitz et al. 1978).

Insulin is primarily responsible for glucose homeostasis but it is involved in the prevailing anabolic states and in suppressing catabolic processes. It also functions as an essential factor for the maintenance or growth of virtually all types of cells.

Insulin encourages protein synthesis and inhibits protein breakdown in skeletal and cardiac muscle and in other tissues, thus reducing the amino acid supply of the liver

from those sources. Similarly the anti-lipolytic effect of insulin in fat cells inhibits the release of FFA from adipose tissue and thus decreases the FFA concentration of liver output. By acting directly on the liver cell, insulin promotes glycogen deposition and inhibits its glucose release.

Adipose tissue:

This tissue is the major energy store in the body, capable of satisfying the basic energy requirements for considerable length of time. Its major metabolic functions are removal from plasma of fatty acids and other substrates used to synthesise fatty acids, storage of these fuels as triglycerides in lipid droplets during periods of nutrient excess, and breakdown (hydrolysis) of triglycerides with release of fatty acids and glycerol into the blood during the periods of increased energy demands or deficiency of exogenous nutrients. Glucose seems to be of a great physiological importance for lipogenesis, since it provides the triglyceride backbone, the glycerol part, through glycolysis (Jefferson and Neely, 1978).

Fat cells in the adipose tissue are uniquely adapted for their main function of storage and release of energy. The fat within the adipocyte is triglyceride of a composition giving a semifluid central fat droplet at body temperature. The adipocyte is morphologically a rather conventional cell as far as its size is concerned. When accumulating triglyceride, the cell size increases enormously with perhaps a 20-fold increase in diameter and consequently their capacity by thousandfolds (Björntorp 1982). The flexibility in size not only means a vast ability to vary storage capacity but also may explain the metabolic aberrations in some obese individuals. Björntorp

(1982), suggested that excessive storage of fat in adipocyte stretches the cytoplasm which is a metabolically active part of the cell, in a thin layer over the central globule. The cell surface, containing receptors for hormonal binding, would also be expanded. Another possible result of increased size of fat cell that was pointed out by the same author, was the decreased extracellular space, allowing less abundant flow of various components in this area. He then concluded that the morphology of adipose tissue because of geometrical reasons can vary the metabolic capacity. This could partly explain the metabolic abnormalities in obesity where an increased amount of fat is stored in enlarged adipocytes or in an increased number of fat cells or combination of both (see below).

Pathogenesis of obesity in association with diabetes

Non-insulin dependant diabetes (NIDDM) is usually, associated with obesity. A number of studies have shown that obesity is a strong risk factor for the later development of this disorder. The evidence for that could be summarised as follows:

(1) adult subjects with recent onset of NIDDM are more obese than those without the disease (Knowler et al. 1981),

(2) a correlation has been seen between the degree of obesity in population and the prevalence of diabetes (West 1978). Also, in a study by Hanson et al. (1995), weight gain was strongly correlated to diabetes incidence (in this study, in the case of women, it was only true for the ones who were not initially overweight. This finding of Hanson et al., shows the importance of weight gain in women in relation to the incidence of NIDDM but it does not rule out the relationship between long term obesity and diabetes),

(3) weight reduction in NIDDM, especially when the disease is of recent onset, improves glucose tolerance (Henry et al. 1986).

(4) the incidence of diabetes in obese subjects is greater than in the non-obese (Haffner et al. 1990).

Furthermore, from the studies on the endocrine and biochemical abnormalities of overweight, it has been known that a state of hyperinsulinemia exists in obesity (Elahi et al. 1982, Polonsky et al. 1988). This could, in turn, reflect a resistance to the effect of insulin on glucose clearance in target cells.

It has been suggested that obesity shares the characteristic of insulin resistance with diabetes. Although both of these conditions are believed to be determined at least in part genetically, literature surveys suggest that obese individuals and the majority of non-insulin dependent diabetic patients have altered body composition and patterns of insulin resistance (Bogardus et al. 1984, Evans et al. 1984, Campbell et al. 1988, Defronzo 1988, Groop et al. 1989).

From these observations stems the long held hypothesis that obesity may be considered a pre-diabetic state. In the next part, considering the basic concepts of normal glucose metabolism and its regulation in relation to adipose tissue, the link between these two developing disorders; "obesity and diabetes" will be discussed. Before that it is necessary to be familiar with the basic definition and common characteristics of each of these relatively well known diseases.

Human obesity

Obesity may simply be defined as a condition in which there is an "excessive storage of calories as body fat". Apparently it is not a single condition but is a syndrome of varied aetiology with regard to its natural history, associated disease and complications.

Obesity runs in families but this does not necessarily mean that it is inherited genetically. It could rather be as a result of metabolic, social and environmental influences. Interestingly, many women relate their onset of obesity to their pregnancy.

No matter what the underlying aetiology, the common denominator of all forms of obesity is an imbalance of energy intake and energy expenditure, and it is now clear that there is a great variation among individuals, both in the ease with which obesity develops and in the factors regulating energy balance.

A high calorie intake has long been considered to be the major cause of obesity in man. In fact, the term of "obesity" is derived from a Greek word, meaning "overeating" and implies that the primary defect is on the intake side of the energy balance equation.

It is however, worth noticing that many individuals developing obesity, do so primarily because of a decreased energy expenditure. A significant example could be the increasing incidence of obesity in the 'modernised' societies in which physical activities are reduced, therefore, minimum energy for daily activities is required. The prevalence of obesity as measured by BMI has increased gradually in the UK during the last 50 years (National Dairy Council 1993). Based on a comparison between "The dietary and nutritional survey of British adults" in 1986/7 with a "Health survey for England" in 1991, the rate of overweight and obesity has increased clearly in spite of little difference in dietary intake (White et al. 1991).

It is the case that most of obese individuals, after reaching a certain point of body weight, stay in equilibrium for many years.

Despite all of these social and environmental changes, the question that still remains unanswered is why some people seem to be more prone to put weight on than the others, and to what extent it could be related to life style or conditions like pregnancy.

Classification of obesity:

Obese individuals can be classified in several ways: according to the anatomical characteristics of the adipose tissue, fat distribution, and based on the age and onset of obesity or etiological factors. Obesity could also be classified based on its prevalence in association with mortality and morbidity indexes. Each of these set of classifications are described below.

Fat cell size and number: Anatomically, obesity has been considered as an increased triglyceride content per adipocyte, and from this it could be subcategorised to two forms. In a hypothesis by Hirsch et al. (1979), these two forms are described as hypertrophic and hyperplastic obesity. Based upon their theory the stored fat could either be distributed in the increased number of normal weight adipocyte (hyperplastic) or in the enlarged fat cells (hypertrophic obesity). It could also be in the combination of both which seems to occur in most cases.

When obesity develops in adulthood or during pregnancy, mature fat cells are less able to replicate in human (Hirsch et al. 1979, Bjorntorp and Sjostrom 1971). Therefore, it is more likely that adulthood obesity is of the hypertrophic form and this type of obesity is associated with metabolic abnormalities (hyperglycaemia, hyperlipidemia and hyperinsulinism). It has also been seen that under these conditions, the increased fat deposition is predominantly truncal (see below). In contrast, childhood obesity appears to be more from the hyperplastic form and clinical diabetes is observed to be much less common in obese children (Bray 1992). A protective factor in this regard could well be the ability of the adipocytes to replicate

in response to overfeeding. Adults having predominantly hyperplastic (hypercellular) obesity but without enlargement of fat cells as well are relatively uncommon. An increased total number of fat cells is usually present in individuals who are more than 75% above their desirable weight (Hirsch et al. 1989).

Overweightness: Apart from the theory of adipose tissue cellularity which points to the obesity as a serious medical condition, there is a milder condition of excess fat categorised as "overweight". This situation is defined as an excess of weight in relation to height. Overweightness is also seen to be associated with increased prevalence of heart diseases and diabetes mellitus (Van Italli 1985, Stamler 1979).

To distinguish between different degrees of obesity, although there are sophisticated techniques for measuring body fat, they have not been used in larger population studies. Therefore, the degree of obesity and overweightness is expressed with simpler indices such as weight for height, body mass index and waist/hip ratio. They have been constructed to standardise weight for height. The most widely used of these indices is BMI, which is addressed above.

Body mass index has been recognised as a surrogate measure of body fatness, especially for epidemiological purposes. Nevertheless, persons with similar BMI might show a quite wide variation in body fat. Therefore, it must be borne in mind that the BMI characterises groups rather than individuals.

Various statistical cut-off points have been used to distinguish between obese, overweight, and also normal weight people and these are designed based upon the data on mortality and morbidity. In large samples ($N>10^5$) the relation between mortality

and BMI is J or U shaped, and average mortality rates appear to be at approximately BMI>28 kg/m^2 (Waaler 1984). However, an international classification of obesity has been proposed by Garrow (1981) and endorsed by a panel of the World Health Organisation (1988). The categories in this thesis accord with those adopted by the WHO classification which are as follows:

Level of BMI index	Description
20 or less	Underweight
over 20 to 25	Desirable (Normal-weight)
over 25 to 30	Moderate overweight
30 to 40	Severe overweight or Obese
over 40	Morbidly obese

It is interesting to know the rate of obesity in United Kingdom, based on this classification is 13% for men and 16% for women and the mean BMI for women and men were 25.4 and 25.6, respectively (White 1991). In my own study of early pregnant women attending Antenatal Clinics of Northern General Hospital in Sheffield, the mean BMI was also 25.4 and the rate of obesity (BMI>29) was 17.5% (Soltani 1993, M Med Sci dissertation).

Regional fat distribution

In this regard, obesity could be categorised based on accumulation of fat in a particular location. According to this criterion obesity consists of two main

subgroups; upper body segment (truncal, central or android obesity) and lower body segment (peripheral or gynoid obesity). The first type is characterised by an excess amount of fat accumulation in the shoulders, abdomen and nape of the neck. In contrast, the latter type is associated with a predominant accumulation of buttock and thigh fat. These two subgroups can simply be evaluated by measuring skinfold thickness on the trunk and extremities or by measuring circumferences. Waist to hip girth ratio (WHR) is the most common index that is been used for this purpose. WHR is obtained by the measurement of the waist girth dividing by the maximal hip girth. WHR>0.85 is considered as a characteristic of upper body segment obesity in women (Kissebah et al. 1982).

In summary, BMI represents a simple index of obesity in population studies, with no detailed information on body composition. Although different weight for height indices may be optimal risk predictors in different populations, adipose tissue distribution is optimally predicted by indices such as waist-hip ratio. In a recent study (Han et al. 1995) waist circumference in women was shown to be an even better predictor of intra-abdominal fat mass (IFM) than WHR. The correlation between BMI and IFM was almost as good as waist circumference.

Diabetes mellitus

Diabetes is a state of abnormal carbohydrate metabolism and its common symptoms involve; thirst, polyuria, weight loss, fatigue, pruritus vulvae (Vaginal candidiasis), hunger, blurred vision and other diabetic complications.

Current knowledge of the aetiology and pathogenesis of diabetes, although still incomplete, has indicated that this disease is heterogeneous in nature. Thus diabetes is considered as a syndrome.

The syndrome of diabetes has been classified into diabetes mellitus type I, type II, impaired glucose tolerance (IGT) as well as gestational diabetes, previous abnormal glucose tolerance and potential abnormality of glucose tolerance (Harris et al. 1979).

Diabetes Type I (Juvenile onset diabetes), has been relatively well characterised as having a positive association with genetic HLA (histocompatibility antigens) on the Chromosome six. It could also be due to some viral infection destroying beta cells. Islet cell antibodies are present at the onset, and there is a defect in insulin secretion of such severity that exogenous insulin is mandatory for sustaining life. The term insulin dependent diabetes mellitus (IDDM), for type I, comes from the last feature.

In contrast, type II diabetes mellitus (non insulin dependent diabetes mellitus (NIDDM) or maturity onset diabetes), has been less well characterised. In this type, although having a genetic background, there is a strong association with insulin resistance and abnormality in insulin secretion.

Both type I and type II diabetes mellitus are defined either by fasting hyperglycemia (>8 mmol/L) or levels of plasma glucose during a glucose tolerance test above certain limits (2 hr value > 12 mmol/L after 75 g glucose).

The state of impaired glucose tolerance (IGT) is characterised by plasma glucose levels during a glucose tolerance test which lie above normal but below the frankly diabetic levels. What should be remembered is that the limits of plasma glucose have

been defined differently by various investigators for each type of diabetes mentioned above.

Gestational diabetes (GDM): This is defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy (Metzger 1991). Women with GDM are normally older and tend to be more obese than women who have normal glucose tolerance (NGT) in pregnancy (Gabbe 1986). GDM during pregnancy is associated with an increased perinatal morbidity and mortality and an increased loss of viable fetuses. There are certain important risk factors associated with gestational diabetes (Sacks et al. 1987):

- Previous history of gestational diabetes;
- Family history of diabetes (first degree relatives);
- Obesity;
- Maternal age over 25;
- Previous infant with congenital anatomical malformation;
- Previous macrosomic infants;
- Previous poor obstetric history.

Since gestational diabetes is associated with the same complications as the other types of diabetes, including congenital malformations (Hod et al. 1995), it is important that this disease be diagnosed early and controlled carefully. In the past, urine examination for glucose has been used as a screening test for GDM. This approach is no longer used in our Maternity Unit in Northern General Hospital (Sheffield) because it resulted in too many false positive screens. Although it is still being practised in

some other ante natal clinics in UK. Fifty percent of healthy pregnant women have glucosuria at some stages while their blood glucose levels remain well within the normal limits (Davison and Hytten 1975). More recently, the oral glucose tolerance test has become the most common screening test, generally carried out between 28 and 32 weeks gestation.

Once the diagnosis is made, the principles for the treatment of GDM are similar to those women with pre-existing diabetes. The frequent feeding diet, composed of high carbohydrate (55 to 60% of total energy), low fat (20 to 30%) and low protein (15 to 20%) should be advised. Although some authors report good results using diet therapy alone or in combination with oral hypoglycaemic agents in certain patients with gestational diabetes, most authorities believe that better control can be achieved using diet therapy and small doses of regular and intermediate-acting insulin (Berne et al. 1985). Because rigid control of blood sugar level is so important, as with the other types of diabetes, a team management, including a physician and a dietician, is necessary.

There is no doubt that GDM must be considered a disease entity that presents significant perinatal risks and entails a considerable enhanced risk for later development of diabetes in the mother (Gabbe 1986). There is growing evidence that the perinatal risks can be reduced by a systematic approach to identification and managment of this disorder (Gabbe 1985). There are however, arbitrary views regarding the diagnostic cut off points between normal and abnormal glucose tolerance in pregnancy (Naylor 1989).

Aetiologically, despite the differences between all types of diabetes mellitus, a state of reduced insulin sensitivity (insulin resistance) plays an important role, with the

exception of IDDM which is due to a loss of beta cell function (insulin deficiency). This common characteristic of different type of diabetes is briefly discussed below.

Insulin resistance: definition and mechanism

Insulin resistance exists when a given, known quantity of insulin produces less than the normal expected biological effect. The causes of insulin resistance according to its known aetiologic mechanisms are classified by Olefsky (1982). These are summarised as follows:

- An abnormal beta-cell secretory product;

a. abnormal insulin molecule

b. incomplete conversion of proinsulin to insulin

- circulating insulin antagonists;

a. elevated levels of counterregulatory hormones, e.g., growth hormone cortisol, glucagon, catecholamines, progestrone, or human placental lactogen (HPL).

b. antiinsulin antibodies

c. antiinsulin receptor antibodies

- A target tissue defect in insulin action;

a) insulin receptor defects

b) post receptor defects.

In nonpregnanct states, a target tissue defect in insulin action may contribute a rather more significant role in the causes of insulin resistance.

Regarding insulin action, the first step is binding to its cell surface receptors. The insulin receptor complex is then formed and one or more signals are generated. This signal or "second messenger" of insulin action could be responsible for the variety of insulin actions. In many instances, the effect is through a multistep sequentially linked enzymatic system (i.e., activation of glycogen phosphorylase) or a series of enzymes involved in the degradation of a particular substrate (i.e., glucose). Clearly, abnormality in any stage of insulin action cascade, can lead to insulin resistance. Insulin resistance is comprised of two states; decreased sensitivity and/or decreased responsiveness. In the former state, an increase in hormone concentration is required to produce a normal biological response. In the latter state, the dose response is unchanged but the maximal possible response is reduced. Usually a combination of these states is responsible for insulin resistance.

For convenience, these abnormalities are defined under subheadings of insulin receptor defects and postreceptor defects.

Insulin receptor defects: Decreased insulin receptors which reflect reduced insulin sensivity, have been described in a variety of pathophysiologic studies. The most common of these are obesity. It has also been described following glucocorticoid or oral contraceptive therapy.

What should be noticed is the fact that the function of insulin receptors is not as simple and it has been suggested that cells possess 'spare receptors' to maximise the effect of insulin (Olefsky 1976). This concept is based on the observation that a maximal insulin effect is achieved at an insulin concentration which occupies less than the total number of cellular receptors.

Postreceptor defects: At this level within the cells of insulin sensitive tissues a change in the function of hormone-receptor complexes and/or enzyme deficiency could result in tissue resistance to insulin action. This, of course, could be related to insulin responsiveness.

Diagnosis of insulin resistance: Many studies of insulin activity are focused on the blood glucose regulatory effects of insulin. The sensitivity and responsiveness to insulin in controlling blood glucose levels is measured. This is partly due to the technical ease of measuring blood glucose levels and partly because of the interrelationship between blood glucose levels and insulin secretion. The methodology of diagnosis of insulin resistance is described recently by Martinez et al. (1993), a summary of which is outlined below.

The *in vivo* methods in use can be classified as 'closed loop' techniques in which insulin and glucose concentrations are allowed to interact freely (e.g. Glucose tolerance tests with or without concomitant intravenous boluses of insulin), 'open loop' techniques in which insulin and/or glucose levels are fixed (e.g. Euglycemic glucose clamp) and 'model methods' in which a mathematical model would be used to analyse the interactions between insulin secretion patterns and glucose disposal.

The euglycemic hyperinsulinemic glucose clamp, has become the gold standard for measurement of insulin action. This method uses a variable glucose infusion to maintain a constant plasma glucose level, whilst a high dose of insulin is infused systematically. The insulin infusion tends to increase peripheral glucose uptake and to suppress endogenous glucose output. The rate of glucose infusion is calculated to match the total reduction in the plasma glucose level induced by the insulin infusion.

It is thus a marker of insulin sensitivity. This method requires expensive equipment and considerable technical skill and is also labour intensive, therefore, it does not seem to be appropriate for population studies (Stanley et al. 1993).

In the case of pregnant women, *in vitro* methods if accurate, would be more acceptable. Using the C2C12 mouse myoblast cell line, Bruce et al. (1994), have demonstrated that changes in insulin sensitivity with advancing pregnancy can be provoked by human pregnancy serum. This technique will be described later. What is basically examined in this technique is the possible effect of counter-regulatory hormones in the pregnant women's serum on insulin resistance. From a comparison of the results gained from this model system to those studies using the Euglycemic clamp technique at various gestational stages, it was concluded that the model was relatively accurate (Bruce et al. 1994).

The relationship of obesity (insulin resistance) and type II diabetes mellitus

Obesity as a state of chronic hyperinsulinemia is believed to be associated with insulin resistance. The fact that hyperglycaemia is a characteristic of obesity has been known since the early 1960s but the mechanism behind this remains mysterious.

From a review of the available data, the hyperinsulinemia is initially due to hyper secretion by beta-cells to compensate the insulin resistance in obesity (Bonora et al. 1984, Polonsky et al. 1988). Decreased hepatic removal of the hormone, although not as evident, is one of the other factors that has been suggested to have a role in hyperinsulinemia (Davidson et al. 1987). With duration or severity of obesity, there

may be a further deterioration in insulin sensitivity. Nonetheless, glucose tolerance is only mildly impaired because the beta-cells are able to further augment their secretion of insulin, although it has been shown, that this increase is not fully compensatory. Beta-cells in obese persons are unable to secrete enough insulin to maintain the same glucose disposal rate as in lean controls (Bonadonna et al. 1990). Consequently, any event leading to a deterioration in glucose homeostasis, causes more sustained hyperglycaemia in obese persons than in normal weight individuals. If, moreover, for either genetic or environmental reasons, a modest loss of beta-cell mass occurs, the remnant pancreas, which under normal circumstances should still be able to guarantee glucose homeostasis, in obese persons fails to do so .

It is also hypothesised that even mild hyperglycaemia could impair insulin secretion and aggravate peripheral insulin resistance. Based on the glucose toxicity concept which was proposed first by Unger and Grundy (1985), higher abnormal blood glucose levels per se could impair the ability of beta-cells to respond to a hyperglycaemic stimulus. The mechanism by which hyperglycaemia per se causes insulin resistance seems to be related to down-regulation of glucose transport in the insulin dependent tissues (Sasson et al. 1987). Another factor that is supposed to aggravate glucose metabolism and insulin resistance is the presence of elevated free fatty acids (FFA). By a substrate competition mechanism, FFA can potentially increase hepatic glucose output (gluconeogenesis) and impair glucose removal by the peripheral tissues (Struck et al. 1966). One study suggests that elevated FFA concentrations can also impair the interaction of insulin with its own receptor and some of the postbinding events involved in the transduction of the signal from the receptor to the effectors (Svedberg et al. 1990). These two last mentioned factors are

well documented in animal models of diabetes and *in vitro* systems. Slight hyperglycaemia and increased FFA supply/oxidation may account for the presence of the metabolic/hormonal abnormality that differentiate obese NIDD patients from non-diabetic obese individuals. In fact, once this pathogenic cascade has started, it has the potential to progress and become a vicious cycle that eventually leads to overt hyperglycaemia. However, prospective studies are still needed to confirm or disprove the concepts of this hypothesis.

Relation of body fat distribution to metabolic complications of obesity

Recent interest in the importance of metabolism in relation to fat distribution stems from the work of Vague (1956) which has gone on to demonstrate that women with an "android" type of fat distribution, predominantly in central, truncal sites have a greater frequency of diabetes than those with a more peripheral "gynoid" fat distribution. Indeed, epidemiological studies have documented that a central pattern of fat distribution, as characterised by an increased ratio of the waist to hip circumference, is a strong, independent predictor for the later development of NIDDM (Haffner et al. 1990).

In a study by Kissebah et al. (1982), plasma glucose and insulin levels during oral glucose loading were significantly higher in women with predominantly upper body segment obesity than in women with lower body segment obesity. Furthermore, significant correlations were observed between the abdominal fat cell size and glucose or insulin areas.

From this and other similar studies, the insulin resistance associated with obesity is clearly more pronounced in subjects with centrally localised obesity. On the other hand, some studies have defined visceral (intra-abdominal) fat mass to show the strongest correlation with insulin resistance (Björntorp 1989). Because WHR and visceral fat mass are highly correlated and both closely associated with insulin resistance, it seems likely that the risk subcompartment of the WHR is in fact, the visceral fat mass. Intra-abdominal fat storage is supposed to be from the hypertrophic type of adipocytes.

Apparently, visceral fat has a higher turnover than other adipose tissue regions. This is probably because the lipolytic potential and FFA release, are amplified by the low effectiveness of insulin to inhibit lipolysis in these fat tissues (perhaps due to predominant hypertrophic adipocytes). The effect of FFA in abnormal metabolism, apart from increasing gluconeogenesis and impairing glucose storage, is suggested to involve diminishing insulin clearance as well (Strömblad and Bjöntorp 1986).

In addition, increased levels of testosterone and cortisol (Jung 1984), as insulin antagonistic hormones have been observed in obese subjects with a central pattern of obesity.

In summary, a specific pattern of metabolic, morphological, and hormonal features distinguish obese persons with a central fat distribution from obese individuals with peripheral fat localisation. Still, there are more studies needed to reveal whether this association of central fat distribution to abnormal metabolism is a cause-effect relationship or just a coincidence.

Carbohydrate Metabolism in Pregnancy

During pregnancy fetal demand for fuel increases and this demand is met by increased energy intake, decreased energy expenditure and changes in fuel metabolism. The primary adaptation in energy utilisation during pregnancy includes a shift in the fuel sources. Fat becomes the major maternal fuel whereas glucose is the major fetal fuel. Approximately 50%-70% of the kilocalories required daily by the fetus in the third trimester are derived from glucose (Battaglia 1986).

The changes in fuel metabolism has been described by Knopp et al. (1981) as two different metabolic phases in early and late pregnancy (figure 1.1). They called early pregnancy an "anabolic state" when maternal body is able to accelerate fat storage and enhance glucose removal from the circulation. At this stage the fetus is small in size and requires relatively few nutrients. In the latter part of gestation when fetal growth is much more rapid, they believed that there is a competition for glucose supply between the mother and the fetus. Consequently, the mother uses fat as an alternative fuel supply to glucose. This is based on the concept of "accelerated starvation" during pregnancy which was originally suggested by Freinkel (1965). According to his assumption, there is a tendency for an accelerated rate of fat mobilisation in this stage of pregnancy. This stage was called "catabolic phase" which comprises the last trimester of pregnancy.

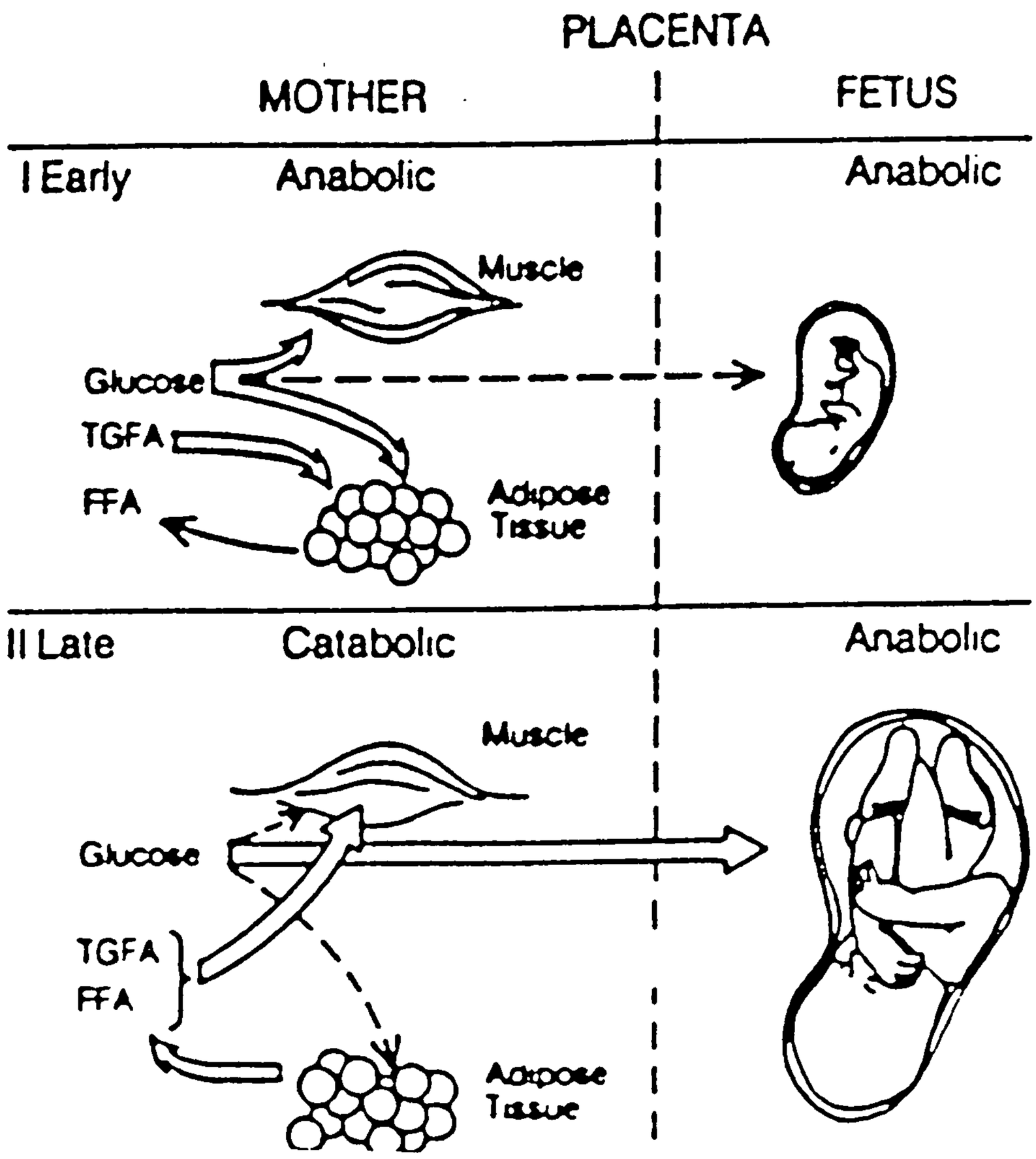


Figure 1.1. Fuel disposition in pregnancy. The upper panel (I) represents early gestation in which glucose is the primary maternal metabolic fuel and triglyceride fatty acids (TGFA) are stored. The lower panel (II) represents late gestation in which free fatty acids (FFA) are seen as the primary maternal metabolic fuels and glucose is diverted from maternal tissues for fetal use. (Knopp et al. 1981, with permission).

It has been reported that basal fasting glucose is slightly but significantly lower in pregnancy (Kitzmiller 1980). Also, a number of studies have shown a fall in the level of fasting plasma glucose as pregnancy advances to the third trimester (Lind et al. 1973, Fisher et al 1974). This is in line with the above mentioned assumption of Knopp et al. The fall in the maternal plasma glucose may well be due to the enhanced consumption of glucose by the fetus, placenta and also by some of the expanding maternal tissues. The mother's body adapts to the situation to maintain glucose homeostasis, in part, by using other fuel substrates like fatty acids and sparing glucose for the tissues the most in need, fetus and placenta (Fraser 1991). Another way of adapting maternal body to the increased glucose requirements, is shown to be by increasing the capacity of gluconeogenesis (Kalham et al 1979). Perhaps the most effective way of adaption of maternal body to increased nutrient requirements in late pregnancy, is 'the state of increased insulin resistance'. A loss of insulin action will result in greater substrate availability for feto-placental growth and energy needs (Catalano et al. 1991).

Serial measurements of individuals and groups have shown a progressive elevation of insulin concentrations into the third trimester of pregnancy (Jovanovic et al. 1981). Nevertheless, despite the considerable increase in the insulin levels, there is a gradual decline in oral glucose tolerance during normal pregnancy (Kühl et al. 1975). These observations led the investigators towards the assumption of insulin resistance during pregnancy. Many investigators using different techniques, focused on verification of this assumption and the mechanism behind it. Using the euglycemic clamp technique, Catalano et al. (1991) showed that insulin sensitivity decreases significantly (56%) through to 36 weeks gestation from preconception level.

Further evidence that pregnancy induces a state of insulin resistance is the fact that gestational diabetes usually appears in late pregnancy and that such patients often require large amounts of insulin to attain glycemic control (Roversi et al. 1980).

The reason for the insulin resistance in pregnancy is still unknown. Theoretically, it may be induced by either changes in insulin -receptor binding, postreceptor changes or a combination of both (Pederson 1984). As has been discussed previously, decreased insulin-receptor binding has been observed in human obesity and non-insulin dependent diabetes mellitus, which are like pregnancy, characterised by decreased glucose tolerance and increased insulin resistance (Pederson 1984).

Most of the available data point to insulin-receptor binding, not being significantly decreased in the target tissues investigated, despite the hyperinsulinemia in late pregnancy. Furthermore, significant differences in insulin receptor binding between pregnant women with normal glucose tolerance and normal weight women with untreated GDM have not been found. This implies that the pregnancy-induced insulin resistance must be due to postreceptor changes and that the difference in glucose tolerance between normal pregnant women and women with GDM can not be attributed to a different insulin receptor binding (Kühl 1991).

The other possible mechanism explained for insulin resistance in pregnancy is the high rate of anti-insulin hormones such as oestrogen, prolactin, human placental lactogen (HPL), cortisol and progesterone (Jovanovic and Peterson 1991). The timing of hormonal presentation in pregnancy is observed to be in inverse order to their relative gluconeogenic properties. For example, human chorionic gonadotropin (HCG), is in higher values in early pregnancy which has no gluconeogenic effect and

cortisol, a relatively late appearing hormone, has the most potent gluconeogenic effect.

In contrast to the report of Kühl (1991), insulin resistance during pregnancy, has been suggested to be due to the alterations of the number or affinity of insulin binding sites on receptor cells (Pagano et al. 1980). The changes in insulin binding are believed, however, to be mediated at least in part by hormonal changes that occur during pregnancy (Jovanovic and Peterson 1991).

Despite all the above mentioned changes, in normal situations, the pancreas can increase insulin production to compensate the effect of resistance to insulin. However, 1% to 5% of women fail to do so and develop gestational diabetes, or carbohydrate intolerance (Fraser 1994). Having said that, there is still controversy concerning the true defect in gestational diabetes and this argument may arise from the fact that gestational diabetic women constitute a heterogeneous group with varying patterns of insulin secretion (Hadden 1985, Hollingsworth 1983).

Based on our previous discussion, maternal obesity could represent a significant risk factor for the development of gestational diabetes mellitus and subsequent adult onset diabetes. However many overweight women do not manifest carbohydrate intolerance when they are carefully screened during pregnancy.

It has also been mentioned that upper body obesity is an apparent marker for both impaired glucose tolerance and hyperinsulinemia. In addition obese women who formerly had gestational diabetes mellitus have elevated waist to hip ratios compared with control women matched for body weight (Ward et al. 1985). Hence, maternal

body shape (body fat distribution) may explain more the heterogeneity of overweightness and carbohydrate metabolism during pregnancy.

Currently, however, there is little data available concerning the relationship between maternal fat distribution and carbohydrate metabolism during pregnancy. We therefore sought to investigate whether maternal obesity per se, or more specifically body fat distribution is associated with alterations in carbohydrate tolerance observed during normal pregnancy.

The effect of maternal anthropometry and metabolic changes on fetal growth

As it has been pointed out earlier, certain changes occur during pregnancy in maternal anthropometry and physiology. In terms of lipid metabolism, fatty acid oxidation and production increase (Knopp et al. 1973). The consequent changes; increased glucose production, hyperinsulinemia, increased insulin resistance and elevation of plasma lipids are consistent with early diabetes in the nonpregnant person. Therefore, pregnancy has been described as a "diabetogenic" state. As long as these physiological changes are within a normal range, not only are they beneficial to the fetus but also essential for its normal growth. If these alterations exceed significantly those levels in normal pregnancy, because of other causes of pancreatic deficiency or conditions such as obesity, gestational diabetes will occur. In this state one is concerned about fetal growth.

In understanding both the maternal adaptations to pregnancy and their effects upon the infants, the flow of the substrates across the placenta could be important. It is known that glucose freely crosses the placenta by facilitated diffusion (Jovanovic and Peterson 1991). Observations in many species, including man, indicate that fetal blood glucose concentration is always lower than that of the mother and this could be because the main process for glucose transfer is dependant on the capacity for passive diffusion of glucose.

Nevertheless the transport of insulin through the placenta in either direction is shown to be impaired (Spellacy et al. 1973c). Normally the fetus produces its own insulin in low amounts and this is an important hormone regulating its growth. The blood glucose of the fetus is normally controlled on the maternal side of the placenta by the maternal insulin. The fetal beta cells are relatively unresponsive to a glucose stimulus (Spellacy et al. 1973d). In the case of infants of diabetic mothers however, hyperinsulinaemia occurs which frequently results in neonatal hypoglycaemia. Hyperglycaemia and hyperinsulinaemia *in utero* produces infants which are excessively large and obese (macrosomic) and this typifies the infants of diabetic mothers.

The metabolic fuels that are transferred in excess from mother to fetus are potent insulin secretagogues. Chronic exposure of the fetal pancreas to these substrates could result in beta cell hyperplasia and augmented insulin secretion. Insulin is believed to promote and control the utilisation of metabolic fuels by the fetal tissues cultured in-utero and hence, plays a pivotal role in the control of fetal development.

Although, studies on offspring of twins in a Scandinavian population have provided evidence that the major part of the variation in birth weight is genetic (Magnus et al. 1984), the wide variation of birth weight in normal pregnancy could be due to the physiological variation in maternal glucose metabolism. Maternal fat mass has been found to correlate with maternal insulin levels in late pregnancy (Karam et al. 1963). Moreover, various parental morphometric factors, in particular, maternal pregravid weight and weight gain during gestation, have been suggested to correlate with fetal growth and birth weight. Including other factors such as gestational age and sex can improve the correlation but the overall correlation remains low (Thomson et al. 1968).

This triggers the assumption that in addition to genetic factors, other maternal environmental factors must have an important effect on fetal growth.

Despite the wide investigations on fetal growth, much remains to be understood. The regulation of fetal growth involves a complex mechanism; including the mother and the placenta as well as the fetus. The majority of the studies on fetal growth, considered only one or few factors (mainly maternal pregravid weight and gestational weight gain) in relation to infant birth weight. Therefore, in addition to assessing the magnitude of the changes in maternal anthropometry during and after pregnancy, the purpose of the following study is to evaluate the relationship of maternal carbohydrate metabolism with placental weight and neonatal anthropometric measurements. It also seemed to be of interest to study the interrelationship between maternal BMI, maternal fat mass, fetal insulinisation and fetal growth.

Aims and Objectives:

Our intention was to examine further the question of fat storage by means of a longitudinal prospective study to establish changes in body fat during normal pregnancy and the postpartum period, and also its relationship to fetal metabolism, new-born anthropometry and early infant growth. More precisely the aims were to:

- Study maternal anthropometry and body composition longitudinally during pregnancy in healthy volunteers.
- Investigate the effect of initial maternal BMI on the magnitude of the anthropometric changes during pregnancy.
- Conduct postpartum follow up studies of maternal fat distribution in relation to changes seen in pregnancy and also in relation to maternal body type.
- Establish any relationship of obesity/type of fat distribution to glucose tolerance test results in late pregnancy.
- Investigate the metabolic status of the new-born in relation to maternal body composition and degree of obesity, by measurements of neonatal anthropometry and insulin levels of cord blood.

Chapter 2

Material and Methods

Subjects:

A verbal approach was made to 150 pregnant women to see if they were interested in taking part in the study. A total of 127 agreed to participate and were actually measured for the first time. They all gave written consent after the aims and methods of the study had been explained.

Women were classified to four groups of under-weight (UW: BMI<19.8), normal-weight (NW: 19.8-26), over-weight (OW: >26-29) and obese (OB: >29) based on their BMI in early pregnancy (Institute of Medicine 1990). Attempts were made to have 30 subjects in each BMI group but due to restriction of timetable, 9 UW, 32 OW, 36 OB and 50 NW women were recruited. It was observed that there was hesitation

on the part of overweight group to discuss body composition , surprisingly however, the obese group were amongst the most co-operative subjects.

The subjects had singleton pregnancies and were not on any chronic drug therapy. At the time of recruitment, none of the subjects was known to be diabetic.

Racial characteristics:

The majority of the subjects were Caucasians, there were only one Indian (NW), two Afro-Caribbean (NW), one Pakistani (UW), and one Arab subject (OW). From the last two mentioned subjects; one withdrew due to lack of time and the other one however was excluded due to premature labour (24 wks).

Study Design

Women were recruited from the Antenatal Clinics of Northern General Hospital in Sheffield. The recruitment was started on March 1st 1994 and it was completed on 11.10.94. The measurements on the last mother and baby was performed on 11.11.95.

Total body fat (TBF) was estimated in a longitudinal study by using Bioelectrical Impedance (BI) and Skinfold Thickness Measurement (STM).

Serial measurements of anthropometric and body fat composition were made on three occasions during pregnancy and in two occasions after delivery on mothers. The gestational age was calculated from the date of the last menstrual period, confirmed by

the ultrasound scanning at booking visit. The time and the number of subjects measured in each occasion are as follows:

Table 2-1. The time in which measurements were made:

Visits during pregnancy	gestational age (weeks) Median (range)	Number of subjects
1	13 (9 -15)*	127
2	25 (23-32)†	78
3	36 (33-40)	80
	postpartum	
4	6 weeks	51
5	6 months	48

*: Only one subject was studied at 5 weeks.

†: Four subjects failed to attend when first were requested and were studied at 32 weeks.

Anthropometric data (e.g. weight, length, head circumference) was also collected on 50 infants within 48 hours of birth. Birth weights and placental weights were obtained later from the hospital notes. Fifty one women and their babies remained in the study at six weeks and 48 at six month postpartum (of three women who left the study, one had a subsequent pregnancy at 8 weeks postpartum, the other two could not afford the time).

Cord blood samples were collected from 62 new-borns (of women who were studied during pregnancy) at birth in order to assess the insulin and C-peptide levels.

Glucose tolerance tests were performed on 38 volunteer pregnant women who were already taking part in the longitudinal study (including 2 UW, 6 NW, 11 OW and 19 OB) at 28-31 weeks gestation.

In vitro assessments of insulin sensitivity were performed on plasma from 12 pregnant women. For the details of collected data refer to appendix 2.

Ethics Committee Approval:

The protocol was submitted on February 14th 1994 and approved by the Ethics Committee of Northern General Hospital . An information sheet and a consent form was made available for the subjects (these were attached to the protocol, Appendix 3).

Based on the guideline 4 of the Data Protection Act (1984), the content of the study was described for women (verbally and in writing). The subjects were given the opportunity to ask any question in relation to the clarity of the study. There was no pressure on women to take part or continue taking part in the study. Volunteers were assured about the confidentiality of the collected data. The subjects were also offered feedback following the completion of the study. A summary of the findings was sent to the volunteers who were interested in the results.

Methods

Skinfold thickness measurements

All skinfold measurements were made by one observer (the author), with a Holtain skinfold calliper, exerting a constant pressure of 10 g/mm^2. Its dial is marked in divisions of 0.2 mm but readings of 0.1 can be easily estimated (Holtain LTD, Crymych, UK). The procedure was carefully standardised, and each measurement was made in triplicate on the left side of the body; the results were averaged. The skinfold measurements quoted include double layers of skin and of subcutaneous tissue. During the measurements, the subjects were standing comfortably erect with the upper extremities relaxed at the sides of the body. The calliper jaws were applied 1 cm distal to the thumb and finger raising the fold, and the thickness was recorded to the nearest 0.2 mm. The sites and the relative techniques used, are as follows:

Triceps: The triceps skinfold was measured in the mid-line of the posterior aspect of the arm, midway between deltoid process and olecranon, over the triceps muscle.

Biceps: This was measured as the thickness of a vertical fold raised on the anterior aspect of the arm (at similar place to the triceps), over the belly of the biceps muscle.

Suprailiac: This was measured in the mid-axillary line immediately superior to the iliac crest. An oblique skinfold was grasped just posterior to the mid-axillary line following the natural cleavage lines of the skin (which is aligned inferomedially 45° to the horizontal).

Subscapular: Just below the inferior angle of the scapula was picked up and measured approximately 45° to the horizontal plane in the natural cleavage lines of the skin.

Mid thigh: The thigh skinfold was measured in the mid-line of the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella. This was performed while the body weight was shifted to the other foot, to leave the measuring leg relaxed.

Hip Circumference:

An inelastic tape was placed around the buttocks at the point yielding the maximum circumference in a horizontal plane without compressing the skin, while the subject was standing erect with her arms at the sides and the feet together. The subjects wore only non-restrictive briefs. The measurement was recorded to the nearest 0.1 cm.

Waist Circumference:

The subjects were undressed and the measurements were performed directly on the skin. The measurements were made at the level of umbilicus with the subject standing, her arms by her sides and her feet together. The measurements were recorded to the nearest 0.1 cm.

Maternal height

Maternal height was measured in the subject (without shoes) with the heels together and with the so-called Frankfurt plane of the head in a horizontal position (WHO 1987). The subject was asked to breathe in deeply with the legs stretched but the feet flat on the ground.

Presence of generalized oedema

Generalized oedema was assessed by physical examination of the mothers, and asking questions about tightening of their rings. It was also checked in the maternal records following the routine antenatal check up.

Maternal body weight and fat mass

Maternal body weight (MBW) was measured in a light clothing (without shoes) with a Seca scale (760 Cranlea, Birmingham). MBW was measured to the nearest 0.1 kg. The same balance was used throughout the study. Body fat mass was assessed by two methods: the Skinfold method and Bioelectrical Impedance method.

By the Skinfold method body fat mass was derived from the sum of the four skinfold measurements (triceps, biceps, suprailiac, and subscapular) and body weight using equations presented by Durnin and Womersley (1974):

$$\text{Density} = 1.1599 - 0.0717 \times \log(\text{triceps} + \text{biceps} + \text{subscapular} + \text{supra-iliac})$$

Percentage body fat was then calculated from the body density values obtained, using Siri's equation (1956):

$$\text{Percentage body fat} = [(495/\text{body density}) - 450] \times 100 \qquad (1)$$

For measurements made throughout pregnancy, the specific pregnancy equations (Van Raaij et al. 1988) that account for the altering composition of the maternal fat-free body throughout pregnancy, were also used to convert body density to percentage body fat:

for 12 wk gestation $\quad FM = W/100 \times (497/D - 452.3) \qquad (2)$

for 24 wk gestation $\quad FM = W/100 \times (504.3/D - 460.4) \qquad (3)$

and for 36 wk gestation $FM = W/100 \times (516.3/D - 473.7)$ (4)

These were compared with the results using the equation of Siri (1956) for conversion of body density (from STM) to percent body fat.

In addition to four above mentioned skinfolds, midthigh skinfold was also measured as an indicator of peripheral pattern of fat distribution. From direct comparison of these skinfolds, changes in fat distribution during pregnancy and postpartum were studied.

BMI: Body mass index was measured as: weight/height^2 (kg/m^2).

WHR: Waist hip ratio was measured as: Waist circumference/Hip circumference (cm).

In order to determine the reliability of the measurements, 6 non-pregnant women were measured on four occasions within the same day. Table 2.2 shows the mean and coefficients of variation between each measurement on the same subject.

Table 2.2. Mean and coefficients of variation between skinfold measurements on the same subjects (n=6)

	Mean	Coefficients of Variation
Triceps	23.51	3.1%
Biceps	11.39	8.2%
Subscapular	18.01	7.0%
Suprailiac	15.59	7.3%
Midthigh	39.65	2.4%
Total skinfolds	108.15	2.1%

The results of our experimental study was consistent with the similar one (which was performed with a single experienced observer) in the study by Taggart et al. (1967). As in their study, biceps and suprailiac involved a higher degree of inaccuracy than the others. However, the agreement between replicates were shown to be reasonably good (in fact the variability of our study was slightly less than the study by Taggart). In this part, the subjects were divided to two groups of BMI≥25 and BMI<25. As we expected, the values of coefficients of variation were higher in the group with the greater BMI, indicating less reliability of skinfold measurements in the overweight group (table 2.3).

Table 2.3. A comparison of coefficients of variation on skinfold measurements in two groups of under and over BMI: 25.

	BMI ≥ 25		**BMI < 25**	
Measurement sites	Mean	Coefficients of Variation	Mean	Coefficients of Variation
Triceps	28.36	3.3%	18.66	2.9%
Biceps	15.37	11.9%	7.41	4.6%
Subscapular	17.68	6.4%	18.34	7.5%
Suprailiac	18.33	8.3%	12.82	5.8%
Mid-thigh	43.36	2.1%	35.94	2.8%
Total skinfolds	123.09	2.3%	93.16	1.6%

The coefficients of variations between the 4 consecutive measurements of waist, hip, mid-thigh and up-thigh circumference on the same subjects (n=6) were 1.2%, 0.7%, 0.5%, 0.6% respectively.

Bioelectrical Impedance

The four electrode Bioelectrical Impedance Analyser (BIA EZ Comp 1500 Cranlea UK) was used to measure the body composition of the women.

In our experimental study (6 non-pregnant women), we actually noticed a great deal of variability after having a big meal (less than 2 hours), coffee and tea did not show any effect on the obtained data. The measurements therefore, were made on average, at about 3 hours after a meal.

During the measurements the subjects wore clothes but no shoes or socks, and were supine on a couch. Measurements were taken on the left side of the body. After cleaning with alcohol and drying all skin contact areas, arginine foil spot electrodes were placed on the dorsal surfaces of the hands and feet at the distal metacarpals and metatarsals respectively, and also between the medial and lateral malleoli at the ankle (Figure 2-1).

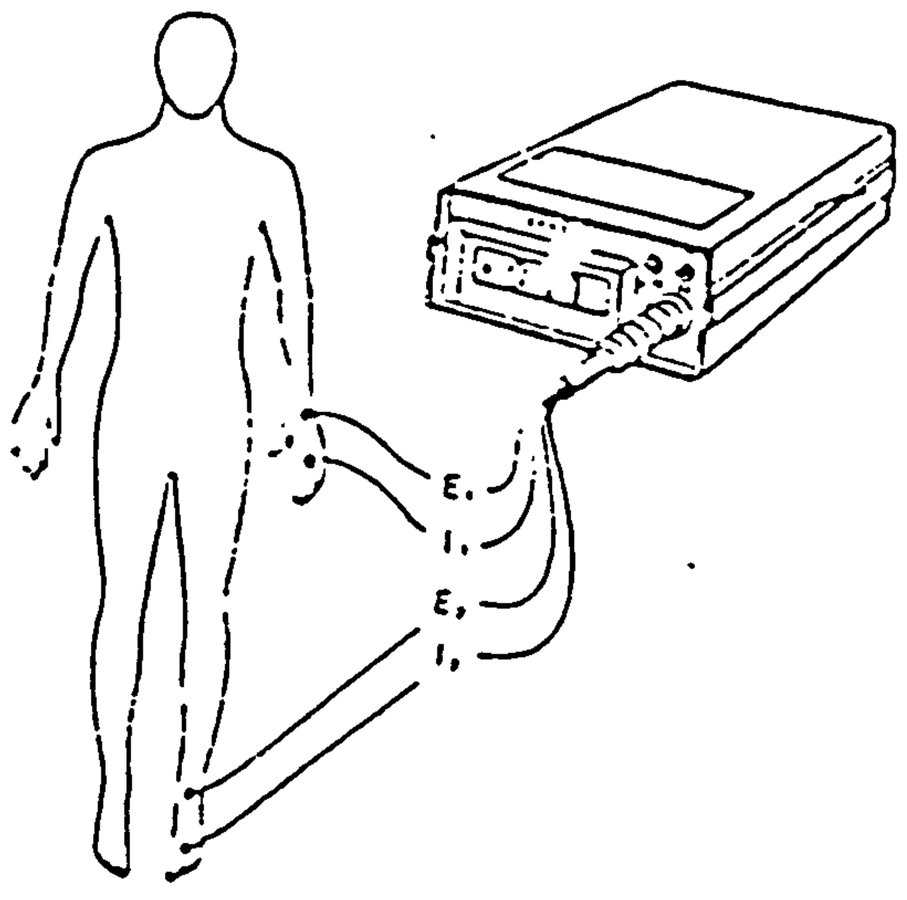


Figure 2.1. Diagrammatic representation of impedance using a four electrode arrangement.

The more detailed placement of the sensors are described below:

On the back of one hand the sensor pad was placed on the third knuckle of the middle finger. The second pad was placed on the arm just behind the wrist at the crease observed when the hand is bent back. The edge of the sensor pad was placed to just touch the crease. The pad on the foot was applied just below the place where the big toe and the first toe join. The position for the sensor pad at the ankle was found by placing the thumb and forefinger on the two protruding bones on either side of the ankle and then bringing the fingers forward to the midway point between the two bones. The sensor pad was then placed at the bend of the ankle, in line with the tibia.

Infant weight measurements

An Electronic Digital Baby Scale (727 digital scale, Seca, Cranlea, Birmingham) was used. A light weight towel was left on the scale and then the scale was calibrated to zero each time. The infant was weighed without any clothes and nappy (nude). The baby was placed on the scale so that the weight was distributed equally on each side of the center of the pan. Weight was recorded, to the nearest 5 g, with the infant lying quietly (which sometimes required a great deal of patience!).

Infant length measurements

Recumbent length of the infant was measured using the rollometer (511A 100 cm, Raven, Castlemead publication, UK). This equipment is made of material that is warm to the touch and is especially made for infants. The infant's head was held in the Frankfurt plane position aligned perpendicular to the measuring table. The legs were extended at the hips and knees so they would lie flat against the tabletop. Giving

a brief instruction to the mother, she was asked to position the infant's head against the headboard. In the meanwhile, putting one hand on the knees of the baby to ensure the legs remain flat on the table, the movable board was firmly pushed against the heels. The measurement was read direct from a cursor line. The length was recorded to the nearest 0.1 cm.

The pressure applied to the foot-board was tried to be sufficient to compress the soft tissues of the foot but not enough to alter the length of the vertebral column. The accuracy of the instrument is claimed to be zero to minus 3 mm.

Infant head circumference

The head circumference of the baby was measured when lying on the bed. An adjustable round and non-stretching tape about 0.6 cm wide (Lasso, Child growth, London) was used. Anteriorly, the tape was placed just superior to the eyebrows and posteriorly it was placed over the bony occiput so that the maximum circumference was measured. The tape was pulled tightly enough to compress the hair. The measurement was recorded to the nearest 0.1 cm.

Glucose Tolerance Test

Glucose tolerance tests were performed on 38 pregnant women at 28-31 weeks gestation (mean 29 weeks). The subjects had been instructed:

-Not to eat anything after 10.00 p.m. on the night before the test and not to have breakfast on the day of the test.

-Not to drink or eat or smoke during the test.

The test was started at 9.00 am when a capillary blood sample of 0.2 ml by finger prick was obtained. A standard glucose drink (75 g glucose dissolved in 200 ml water) was given to the women. They remained at rest until another capillary blood sample was taken two hours after the drink.

Blood samples were collected in the mini-tubes containing fluoride heparin (anticoagulant agent) and they were assayed within 2 hours of collection.

The blood samples were then centrifuged and serum glucose was measured by the glucose oxidase method with a Glucose Analyser (Yellow Springs Instrument [YSI], Model 23 AM, Clandon Distributor, UK). The principles of the Glucose Analyser method is described in appendix 1.

Criteria from the Report of the Second Meeting of the World Health Organisation Expert Committee on Diabetes Mellitus (WHO 1985) were applied to classify the impaired glucose tolerance and gestational diabetes. The diagnostic criteria for capillary plasma, which was used in our study are as follows:

Table 2.4. Diagnostic criteria for gestational diabetes from capillary plasma (WHO 1985).

	Glucose concentration, mmol/litre (mg/dl)	
	Impaired Glucose Tolerance	Gestational diabetes
Fasting values	< 7.8	≥ 7.8
2 hrs value after 75 g glucose load	8.9 - 12.2	≥ 12.2

Measurement of infant insulin and C-peptide levels:

Cord blood collection:

Cord blood samples were collected in test tubes containing fluoride heparin. Thereafter, they were centrifuged at 2000 r.p.m. for 15 minutes to separate the plasma from the blood cells. The golden coloured plasma was pipetted off and placed in a 7 ml sample tube, labelled with the mother's name and were stored at -20° C before assay.

Serum insulin and C-peptide levels were measured by a double antibody radioimmunoassay technique in our laboratory (Clinical Sciences Centre). The limit of sensitivity for each assay is insulin 2.5 μU/ml, C-peptide 0.47 ng/ml. The interassay and intraassay coefficients of variation were 7.4% and 6.8% for insulin, 8.5% and 6.4% for C peptide.

C-peptide measurements

Guildhays's C-peptide RIA kit which is especially designed for the determination of C-peptide in human serum was used (Appendix 1).

Standards, non specific binding (NSB) controls and subjects samples were incubated with C-peptide antiserum reagent for 24 hours. The C-peptide tracer reagent (labelled with Iodine-125 isotope) was then added and allowed to react for a further 24 hours at +4°. Separation was achieved by incubation with the separation reagent for 30 minutes, followed by centrifugation and decantation of supernatants. C-peptide

concentrations were calculated from the measurement of the bound fractions (separation reagent pellets) in a gamma counter.

The sample and standard counts were expressed as a percentage bound of total counts, minus the "NSB Control" counts.

A standard curve was drawn by plotting the percentage bound of each standard against the respective concentration. Each sample concentration (C-peptide level in mmol/L) was obtained by interpolating the sample percentage bound on the standard curve.

Insulin measurements

The technique for the measurements of plasma insulin levels was very similar to that of C-peptide measurements. The antisera was guinea-pig antisera, again from Guildhay. Known aliquots of samples (50 μL) were incubated with the antisera for approximately 16 hours. The insulin was then labelled with Iodine-125 (this is from life screen LTD) during a 24 hour incubation. The assay was terminated by a 2 hour phase separation using a second antiserum (anti guinea-pig precipitating rabbit serum). The difference with the C-peptide measurement was that a separate polyethyleneglycol (PEG) accelerator solution and pH buffer was added with the second antibody. The sample tubes were again centrifuged and the supernatant removed. The tubes were then counted for gamma radiation.

The plotting of the standard curve and calculation of the insulin levels were attained in a similar manner to that of C-peptide.

Haemolysis to any degree could greatly affect on the accuracy of the insulin assay but not the C-peptide (O'Rahilly et al. 1987). Therefore, in samples that appeared to be haemolysed only C-peptide was measured.

Insulin was measured in mU/l. This value can be converted to mmol/ml for a direct comparison to C-peptide which is measured in mmol/ml (Lin et al. 1981).

In vitro measurements of insulin sensitivity:

To study longitudinal changes in insulin sensitivity throughout pregnancy blood samples were taken from 32 women at early (10-15 weeks gestation), and late gestation (33-36 weeks). Seventeen samples were also available from the middle of pregnancy (24-28 weeks gestation). From the total (32 women) 3 were excluded, due to having GDM (two cases) and IGT (one case). Efforts were made to have the blood samples at the same time as routine blood sampling at the ante natal visits. The data on blood samples are enclosed in appendix 2.

The blood samples were collected in test tubes containing lithium heparin and then they were centrifuged within 2-3 hours (they were kept in the fridge before the centrifugation). The plasma was pipetted and stored in -20° C. Because of the time consuming nature and expense of these experiments, the blood samples of 12 (randomly selected) subjects (see appendix 2, marked subjects) were assessed. The samples from the rest remain frozen for the possible future studies.

An *in vitro* method of tissue cell culture; the myoblast cell line (developed by Bruce et al. 1994) has been used to evaluate the effect of pregnant sera on the insulin stimulated glucose uptake by the C2C12 mouse myofibres.

C2C12 myoblasts and myofibres :

Cells were seeded into 12 well plates at 10^4 cells per well in high glucose (25 mM) DMEM (Dulbeccos Modified Eagles Medium) containing 10% fetal calf serum (FCS) and grown until confluent when the medium was changed to high glucose DMEM containing 2% horse sera. Differentiation was usually complete within 4 days and cells were used between 4-7 days.

Incubation with sera from pregnant subjects:

Fully differentiated myofibres were incubated with media supplemented with 10% sera from pregnant subjects for 24 hours with and without 10^{-7} M insulin. Media was removed and cells assayed for glucose uptake.

Glucose uptake measurements:

The growth media was removed from the wells and replaced by 0.5 ml of glucose free RPMI[1] medium containing 2% horse serum and the appropriate concentration of insulin. Cells were then incubated at 37° C for 15 minutes when 50µl of 2.22 mM H^3 2-deoxyglucose were added (final concentration 0.2 mM) and the cells incubated for a

[1] RPMI: Roswell Park Memorial Institute (liquid media for cell culturing)

further 10 min. The medium was then removed, the cells washed twice with cold phosphate buffered saline (PBS) and then dissolved in 1.0 ml of 1.0 M NaOH. Aliquots were then taken for counting and protein determination.

(Insulin and C-peptide measurements as well as the actual experiment of *in vitro* insulin sensitivity was conducted by Mrs Bruce; Senior technician in the laboratory of Obstetrics and Gynaecology, Clinical Sciences Centre).

The full details of each experiment can be seen in appendix 1.

Statistical Analysis

The data are presented as mean± SD for quantitative variables. Statistical significance was considered to be present at the 0.05 level. All the data were analysed using statistical package for social sciences (SPSS).

In order to assess the relationship between the two methods of measuring body fat mass (Bioelectrical Impedance and Skinfold thicknesses Measurements), the analysis of measurement method comparison data (Bland and Altman, 1986) were performed. Mean differences ($\overline{dif}$) and the level of agreements ($\overline{dif} \pm 2$ SD) were calculated to know by how much these two methods are different. Pearson correlation coefficient (r) were determined to investigate the strength of the relationship between the two methods. The two values of fat mass by STM, using two different formulae were also compared in the same way.

Maternal body weight (MBW), total skinfold thickness (TSF), fat mass (FM), lean mass and water content of the body were studied during pregnancy and postpartum. Serial changes of each variable were reported for the all subjects, and the results were compared between the groups using summary measures.

Summary measures (Mathews et al. 1990) were obtained for each study, allowing comparison of the selected features of each woman over time. This method is believed to be the most useful approach to the analysis of serial measurements. It uses the individual as the basic unit and has the ability to cope with missing observations and variable timing of the observations. The summary measures calculated, were rate

of changes, the absolute differences between certain points and also delta area under the curve (ΔAUC). The summary measures were then compared using one way ANOVA (Tukey's honestly significant difference test).

Rate of changes in maternal body weight, total skinfold thickness and fat mass were compared for the whole study group from 13 weeks to 24 weeks (rate 1-2) with 24 to 36 weeks (rate 2-3), using student paired t-test.

Pearson and Spearman correlation coefficient were used as appropriate to correlate infant birth weight to various maternal and fetal variables.

Multiple linear regression analyses (forward method) were performed to assess the interrelationships between each of the new-born anthropometric indicators (weight, length and head circumference) with maternal anthropometry and lifestyle habitus as well as fetal insulinisation, sex and length of gestation. The Coefficient of multiple determination (R^2) is given to express the extent of variation in a given birth parameter explained by the subset of variables included in the model. Caution should be used in interpreting the coefficients because they reflect the influence of other predictor variables in the equation.

Chapter 3

Results

Of 127 subjects who agreed to take part in the study, four cases were later excluded from the study. One of them developed pregnancy induced hypertension (PIH), leading to premature delivery. There was also one case of idiopathic premature labour (24 weeks), one subject moved out of the country and the baby of one subject died on the first day postnatal, secondary to unexplained Antepartum Hemorrhage (APH). This left 123 pregnant women of whom 68 completed 3 visits during pregnancy (semi-longitudinal); 48 of whom completed the whole longitudinal study. The remainder made less frequent visits, the number of subjects in each visit can be seen in table 2-1. The main reason for not being able to participate in all stages of pregnancy was stated to be the lack of time due to pressure of motherhood. Because of the variability of the numbers of participants in each stage of the study, the numbers of subjects will be given in the relevant section.

A compilation of anthropometric, obstetrics and basic characteristics of the whole group and also of each BMI group at the first visit can be seen in table 3.1-5.

Table 3.1. Characteristics of the whole study group (n=123)

	mean	SD	range
Early pregnancy weight (kg)	71.41	16.24	(39.00 - 117.50)
prepregnancy body weight (kg)*	67.52	15.80	(36.00 - 110.00)
Height (m)	1.62	0.05	(1.50 - 1.77)
Body mass index (kg/m^2)	26.95	5.82	(16.44 - 45.89)
Waist-hip ratio	0.90	0.11	(0.75 - 1.74)
Age at first visit (y)	25.89	4.78	(15.00 - 43.00)
Gravida	2.21	1.20	(1.00 - 7.00)
Parity	0.82	0.85	(0.00 - 4.00)
Gestational age at 1st visit (wks)	13.17	1.73	(5.00 -15.00)
TSF (mm)†	111.97	40.64	(41.95 - 193.70)
fat mass (kg) (STM)‡	23.57	9.85	(6.56 - 50.23)
fat mass (kg) (BI)♣	25.66	11.34	(7.00 - 58.00)
lean mass (kg) ♣	46.06	6.19	(32.00 - 67.20)
water content (L) ♣	30.46	3.93	(22.00 - 40.00)
Length of gestation (wks)	39.20	1.40	(35.00 - 42.00)
Infant birth weight (g)	3456.20	492.96	(2510.00 - 4480.00)
Placental weight (g)	620.04	126.56	(335.00 - 945.00)

*: n = 85, self reported values.

†: Total skinfolds (sum of skinfolds: triceps, biceps, subscapular, supra-illiac and midthigh).

‡: As measured by Skinfold thickness measurements at first visit.

♣: As measured by Bioelectrical Impedance at first visit.

Table 3.2. Characteristics of Under-weight group (n = 9)

	mean	SD	range
Early pregnancy weight (kg)	51.77	6.75	(39.00 - 59.00)
prepregnancy body weight (kg)*	51.26	9.46	(36.00 - 58.00)
Height (m)	1.65	0.07	(1.54 - 1.74)
Body mass index (kg/m^2)	18.77	1.01	(16.44 - 19.48)
Waist-hip ratio	0.85	0.05	(0.78 - 0.95)
Age at first visit (y)	22.33	4.41	(15.00 - 29.00)
Gravida	2.33	1.11	(1.00 - 4.00)
Parity	1.00	0.70	(0.00 - 2.00)
Gestational age at 1st visit (wks)	12.55	2.35	(9.00 -15.00)
TSF (mm)†	56.50	10.66	(41.95 - 68.41)
fat mass (STM) (kg) ‡	10.49	2.13	(6.56 - 13.18)
fat mass (BI) (kg) ♣	11.44	2.06	(7.00 - 14.00)
lean mass (kg) ♣	40.33	4.94	(32.00 - 45.00)
water content (L) ♣	26.77	3.89	(22.00 - 32.00)
Length of gestation (wks)	38.66	0.86	(37.00 - 40.00)
Infant birth weight (g)	3151.11	323.70	(2740.00 - 3820.00)
Placental weight (g)	601.11	142.69	(410.00- 830.00)

*: n = 5, self reported values.

†: Total skinfolds (sum of skinfolds: triceps, biceps, subscapular, supra-illiac and midthigh).

‡: As measured by Skinfold thickness measurements at first visit.

♣: As measured by Bioelectrical Impedance at first visit.

Table 3.3. Characteristics of Normal-weight group (n = 48)

	mean	SD	range
Early pregnancy weight (kg)	60.17	6.18	(46.00 - 71.00)
prepregnancy body weight(kg)*	56.97	4.98	(45.21 - 66.04)
Height (m)	1.62	0.06	(1.50 - 1.76)
Body mass index (kg/m^2)	22.68	1.30	(20.02 - 24.67)
Waist-hip ratio	0.87	0.06	(0.75 - 0.99)
Age at first visit (y)	25.35	5.16	(16.00 - 43.00)
Gravida	1.93	1.15	(1.00 - 6.00)
Parity	0.62	0.81	(0.00 - 4.00)
Gestational age at 1st visit (wks)	13.16	1.50	(9.00 -15.00)
TSF (mm)†	82.46	23.22	(44.15 - 161.33)
fat mass (STM) (kg) ‡	16.44	3.69	(9.51 - 24.85)
fat mass (BI) (kg) ♣	16.89	3.45	(10.00 - 25.00)
lean mass (kg) ♣	43.85	5.66	(34.00 - 67.20)
water content (L) ♣	28.97	3.13	(23.00 - 37.00)
Length of gestation (wks)	39.43	1.44	(35.00 - 42.00)
Infant birth weight (g)	3341.97	481.07	(2565.00 - 4450.00)
Placental weight (g)	589.70	131.73	(335.00 - 945.00)

* : n = 35, self reported values.

†: Total skinfolds (sum of skinfolds: triceps, biceps, subscapular, supra-illiac and midthigh).

‡: As measured by Skinfold thickness measurements at first visit.

♣: As measured by Bioelectrical Impedance at first visit.

Table 3.4. Characteristics of Over-weight group (n = 32)

	mean	SD	range
Early pregnancy weight (kg)	71.32	6.24	(59.00 - 83.00)
prepregnancy body weight (kg)*	67.29	9.26	(57.15- 86.99)
Height (m)	1.60	0.05	(1.51 - 1.70)
Body mass index (kg/m^2)	27.53	1.34	(25.39 - 29.93)
Waist-hip ratio	0.91	0.07	(0.78 - 1.06)
Age at first visit (y)	26.70	4.92	(18.00 - 36.00)
Gravida	2.29	1.34	(1.00 - 7.00)
Parity	0.93	0.78	(0.00 - 2.00)
Gestational age at 1st visit (wks)	12.96	1.64	(9.00 -15.00)
TSF (mm)†	124.90	21.72	(66.86 - 166.46)
fat mass (STM) (kg) ‡	24.60	3.88	(14.86 - 32.59)
fat mass (BI) (kg) ♣	26.79	3.48	(20.00 - 33.50)
lean mass (kg) ♣	44.53	4.04	(37.00 - 55.00)
water content (L) ♣	28.97	3.13	(23.00 - 36.00)
Length of gestation (wks)	39.15	1.52	(35.00 - 42.00)
Infant birth weight (g)	3363.12	499.95	(2510.00 - 4460.00)
Placental weight (g)	610.96	111.32	(345.00 - 890.00)

*: n = 21, self reported values.

†: Total skinfolds (sum of skinfolds: triceps, biceps, subscapular, supra-illiac and midthigh).

‡: As measured by Skinfold thickness measurements at first visit.

♣: As measured by Bioelectrical Impedance at first visit.

Table 3.5. Characteristics of Obese group (n = 34)

	mean	SD	range
Early pregnancy weight (kg)	92.57	11.11	(76.00 - 117.50)
prepregnancy body weight (kg)*	86.52	13.49	(61.60 - 110.00)
Height (m)	1.63	0.05	(1.53 - 1.77)
Body mass index (kg/m^2)	34.60	3.98	(30.09 - 45.90)
Waist-hip ratio	0.98	0.16	(0.82 - 1.74)
Age at first visit (y)	26.85	3.72	(20.00 - 35.00)
Gravida	2.50	1.11	(1.00 - 5.00)
Parity	0.97	0.96	(0.00 - 4.00)
Gestational age at 1st visit (wks)	13.53	1.94	(5.00 -15.00)
TSF (mm)†	156.56	23.24	(116.10 - 193.70)
fat mass (STM) (kg) ‡	36.14	6.31	(25.36 - 50.23)
fat mass (BI) (kg) ♣	40.50	7.05	(31.00 - 58.00)
lean mass (kg) ♣	52.07	4.38	(45.00 - 61.00)
water content (L) ♣	34.15	2.73	(30.00 - 40.00)
Length of gestation	39.12	1.34	(36.00 - 41.00)
Infant birth weight (g)	3681.91	463.23	(2590.00 - 4480.00)
Placental weight (g)	676.44	114.59	(480.00 - 875.00)

*: n = 24, self reported values.

†: Total skinfolds (sum of skinfolds: triceps, biceps, subscapular, supra-illiac and midthigh).

‡: As measured by Skinfold thickness measurements at first visit.

♣: As measured by Bioelectrical Impedance at first visit.

The data on prepregnancy weight was reported by the mothers and the correlation coefficient between the self reported weight and measured weight at early pregnancy was highly correlated ($r=0.93$, $p<0.05$). Therefore the self reported prepregnancy weights seem to be a useful guide.

Lifestyle habitus:

The Northern General Hospital (where the subjects were recruited), serves women from a wide range of social classes (above average, average, below average and the most deprived) in Sheffield (Sheffield Health Authority and Family Health Authority, 1994). However, the most prevalent one is the last mentioned. By the above mentioned Authority, deprivation has been defined as "a state of observable and demonstrable disadvantage relative to the local community or the wider society or nation to which an individual, family or group belongs". Nevertheless, we classified the subjects in social classes, based on their occupation using the criteria established by the Great Britain Office of Population Censuses and Surveys (1991). Figure 3.1 shows this classification of the subjects in the whole group as well as in each BMI group.

Mothers were also asked about alcohol consumption and smoking. Most of them were not smokers and took no alcohol. Some of them however were smokers and had occasional drinks (1-2 units per week). The data on cigarette smoking, alcohol consumption and parity can be seen in Figure 3.2-4.

Social class based on maternal occupation

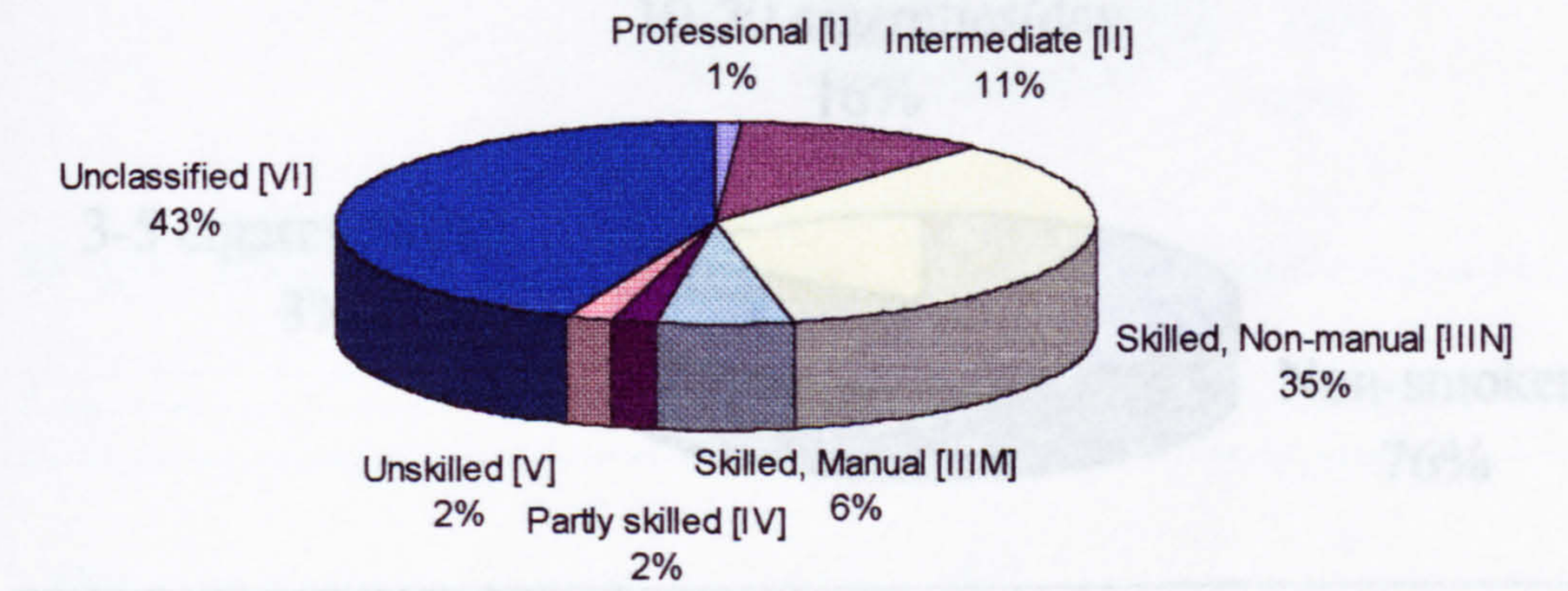


Figure 3.1. Maternal social class based on occupation a) whole group b) under-weight group c) normal-weight group d) over-weight group e) obese group.

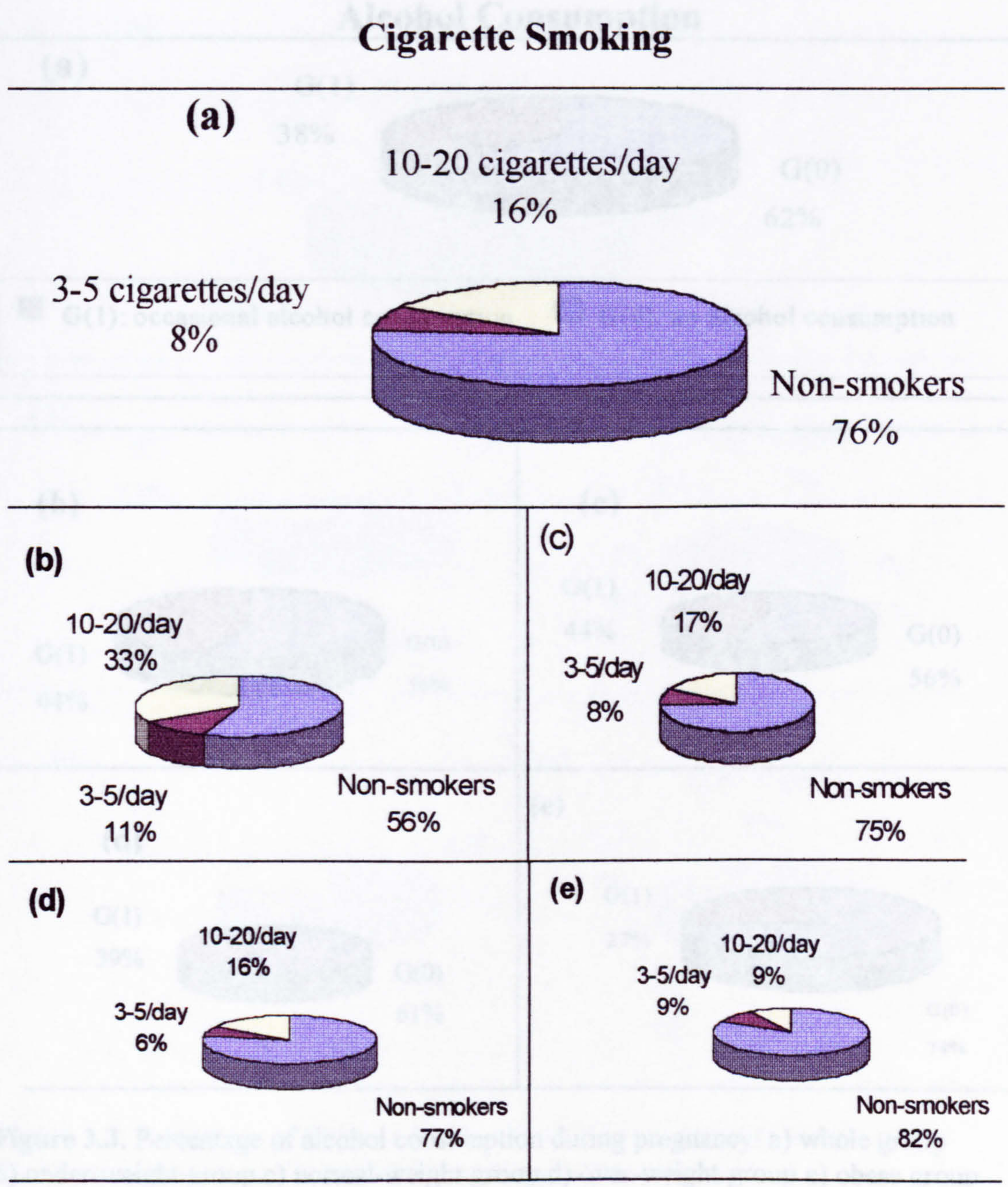


Figure 3.2. Percentage of cigarette smoking in the study subjects: a) whole group b) under-weight group c) normal-weight group d) over-weight group e) obese group

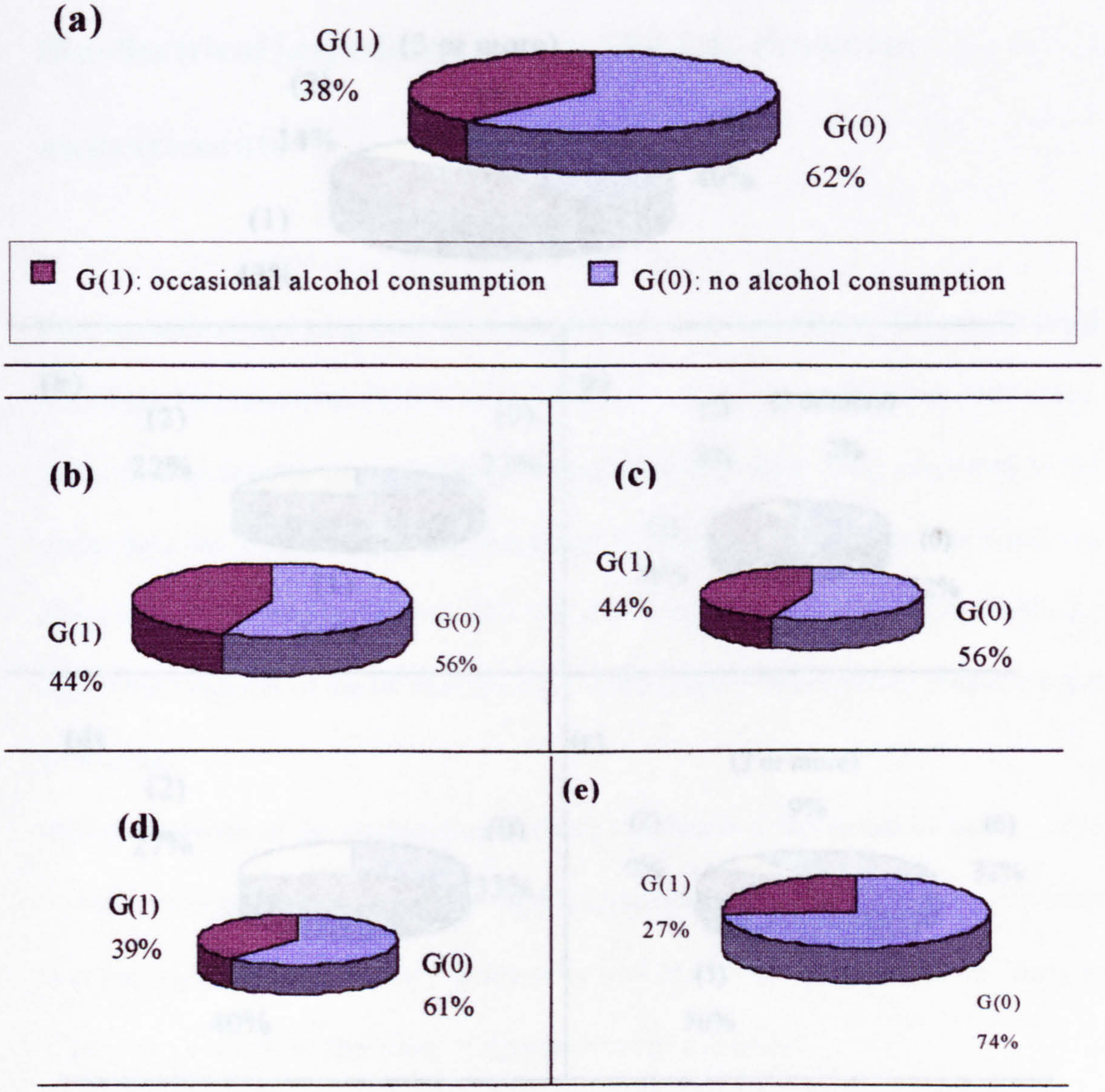


Figure 3.3. Percentage of alcohol consumption during pregnancy: a) whole group b) under-weight group c) normal-weight group d) over-weight group e) obese group

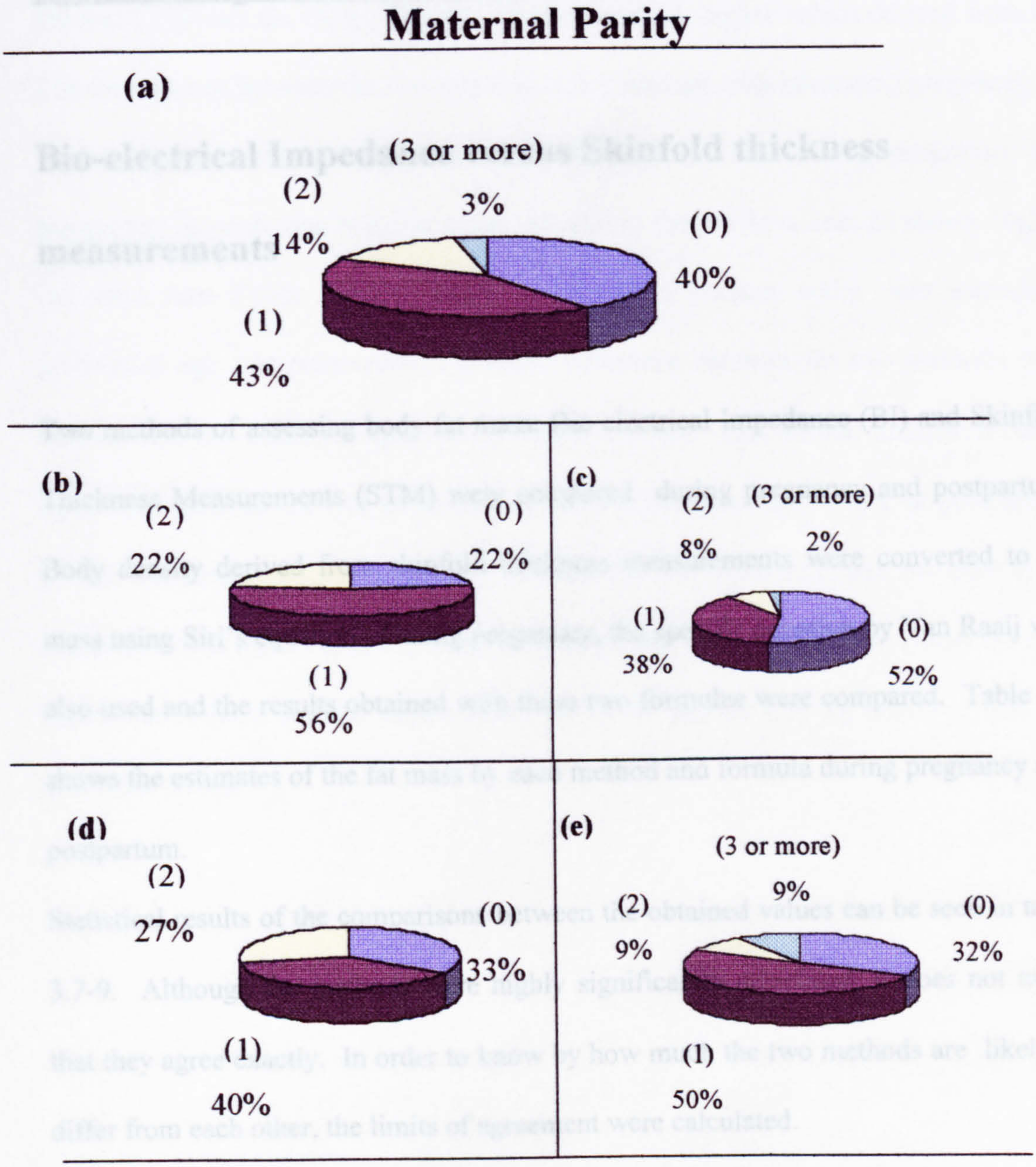


Figure 3.4. Percentage of maternal parity: a) whole group b) under-weight group c) normal-weight group d) over-weight group e) obese group

A) Comparison of the methods:

Bio-electrical Impedance versus Skinfold thickness measurements

Two methods of assessing body fat mass; Bio electrical Impedance (BI) and Skinfold Thickness Measurements (STM) were compared during pregnancy and postpartum. Body density derived from skinfold thickness measurements were converted to fat mass using Siri's equation. During pregnancy, the specific equation by Van Raaij was also used and the results obtained with these two formulae were compared. Table 3.6 shows the estimates of the fat mass by each method and formula during pregnancy and postpartum.

Statistical results of the comparisons between the obtained values can be seen in table 3.7-9. Although the methods were highly significantly correlated, it does not mean that they agree exactly. In order to know by how much the two methods are likely to differ from each other, the limits of agreement were calculated.

Table 3.7. shows a comparison of fat mass obtained by skinfolds using two different formulae of Van Raaij (Fmv) and of Siri's equation (Fms). The mean difference was 0.25 kg between the two obtained values at the first visit (13 weeks gestation) and it increased with advancing pregnancy. As we expected, limits of agreement were small in early pregnancy, they became wider, however, by late pregnancy.

Table 3.8. shows the comparison of BI with STMv. A considerable difference was observed between the values obtained by each method, higher values derived from BI. The discrepancy between the two methods was increased with advancing pregnancy.

Table 3.9. represents the comparison of the BI and STMs during pregnancy and postpartum in predicting body fat mass. Similar to the previous one, BI shows higher estimates than STMs and the limits of agreement became wider with increasing gestational age. At postpartum, the mean difference between the two methods was, reduced and the limits of agreement became smaller. This should indicate a better agreement between the two methods and less variability of the results, at postpartum period. Nevertheless the discrepancy between the two methods is still too high to be acceptable. For instance, at 6 months postpartum, on average BI shows values 0.79 kg higher than STMs, and the values estimated by BI may be 4.75 kg below or 6.33 kg above the STMs.

Table 3.6. Estimates of body fat mass* at different stages of reproduction and postpartum, using Bio-electrical Impedance and skinfold thickness measurements

Weeks gestation♦	FM_{BI} (kg)	FM_V (kg)	FM_S (kg)
13	25.52 ± 11.40	23.32 ± 9.82	23.57 ± 9.85
25	31.17 ± 11.51	27.63 ± 9.75	28.63 ± 9.88
36	34.14 ± 11.11	29.29 ± 9.93	31.60 ± 10.17
Post partum			
6 weeks	30.65 ± 11.03	-	29.62 ± 9.74
6 months	28.80 ± 12.13	-	28.01 ± 10.62

*: ($\overline{X}$ ± SD)

♦: median of gestational weeks

FM_{BI} : Fat mass derived from Bio-electrical Impedance

FM_V: Fat mass derived from Skinfold Thickness measurements (using Van Raaij formula)

FM_S: Fat mass derived from Skinfold Thickness measurements (using Siri formula)

Table 3.7. Comparison of fat mass obtained by Siri's formula (FM_S) with fat mass obtained by Van Raaij formula (FM_V)

	correlation* coefficients	mean differences (kg)	limits of agreement (kg)
Visit 1	1.00	0.25	(0.17 to 0.33)
Visit 2	1.00	1.00	(0.70 to 1.30)
Visit 3	1.00	2.30	(1.68 to 2.92)

*: All the correlation coefficients are statistically significant.

Visits: Gestational weeks and number of subjects for each visit were explained in table 2.1.

Table3.8. Comparison of fat mass obtained from Bio-electrical Impedance (FM_{BI}) with FM_V

	correlation* coefficients	mean differences (kg)	limits of agreement (kg)
Visit 1	0.97	2.31	(-3.23 t0 7.85)
Visit 2	0.96	3.36	(-3.24 to 9.96)
Visit 3	0.96	4.48	(-1.21 to 11.03)

*: All the correlation coefficients are statistically significant

Visits: Gestational weeks and number of subjects for each visit were explained in table 2.1.

Table 3.9. Comparison of FM_{BI} with FM_S

	correlation* coefficients	mean differences (kg)	limits of agreement (kg)
Visit 1	0.97	2.06	(-3.44 to 7.56)
Visit 2	0.96	2.35	(-4.07 to 8.77)
Visit 3	0.96	2.54	(-3.26 to 8.34)
Visit 4	0.98	1.04	(-3.78 to 5.86)
Visit 5	0.96	0.79	(-4.75 to 6.33)

*: All the correlation coefficients are statistically significant

Visits: Gestational weeks and number of subjects for each visit were explained in table 2.1.

B) Changes in body composition and anthropometric indices during pregnancy and postpartum

Maternal body weight (MBW), total skinfold thickness (TSF), fat mass (FM), lean mass (LM) and water content of the body were studied during pregnancy and postpartum.

The subjects were divided to four groups of under-weight, normal-weight, over-weight and obese based on their early pregnancy BMI. The distribution of each BMI group in the population under the study is shown in figure 3.5.

The absolute changes of each variable during pregnancy and postpartum were reported and also certain summary measures were compared between the BMI groups.

The ratio of subjects who continued participating in the study up to 6 month postpartum for each group of underweight, normal weight, over-weight and obese are 1/9, 18/48, 12/32 and 17/34 respectively. The rate of withdrawal from the study was not significantly different between the groups, except for the obese and under-weight group. The latter group was not considered separately in the postpartum period, since there was only one UW person left.

The results of one way analysis of variance suggest that there is no significant differences between the groups in respect to "maternal age, parity, gestational age , breast feeding and in having generalised oedema during pregnancy. Therefore, the groups are considered to be matched.

The rate of breast feeding at 6 weeks post partum were 35/51, including: UW: 1/1, NW: 13/18, OW: 9/13 and OB: 12/19. At 6 month postpartum, however, they were only 4/48 who were breast feeding; UW: 0/1, NW: 3/18, OW: 1/12 and OB: 0/17.

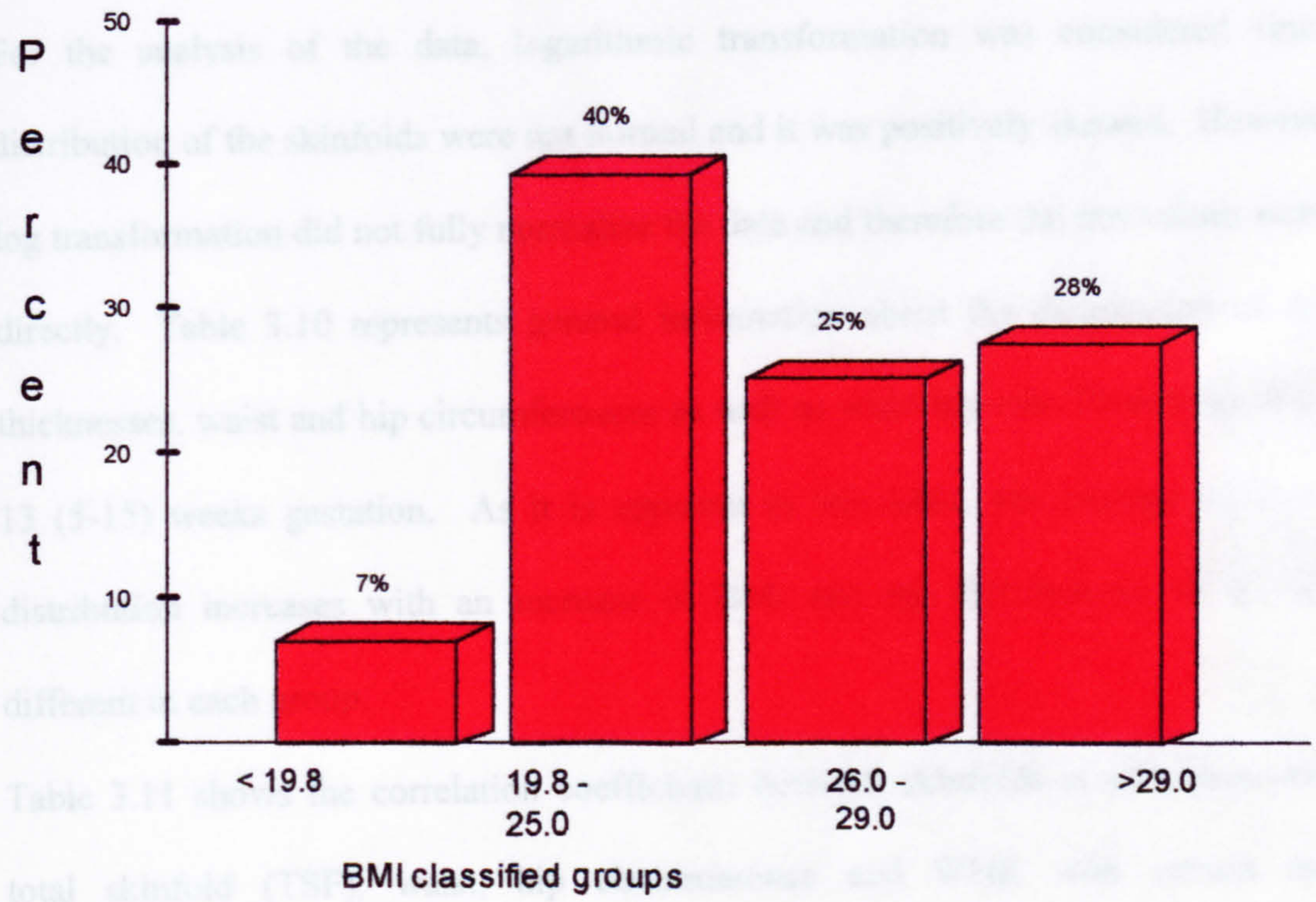


Figure 3.5. Bar charts presenting distribution of each BMI group in total population under the study (classification based on maternal early pregnancy BMI: Under-weight, Normal weight, Over-weight and Obese respectively).

Skinfolds and fat distribution during pregnancy and postpartum

Skinfolds and fat distribution in early pregnancy

For the analysis of the data, logarithmic transformation was considered since the distribution of the skinfolds were not normal and it was positively skewed. However, the log transformation did not fully normalize the data and therefore the raw values were used directly. Table 3.10 represents general information about the distribution of skinfold thicknesses, waist and hip circumferences as well as waist hip ratio (waist/hip: WHR) at 13 (5-15) weeks gestation. As it is apparent in this table, the average indices of fat distribution increases with an increase in BMI and the distributions of fat are also different in each group.

Table 3.11 shows the correlation coefficients between skinfolds at each measured site, total skinfold (TSF), waist, hip circumference and WHR with certain maternal characteristics (i.e. weight, height, fat mass (from BI and STM), BMI and WHR.

Each site of subscapular and suprailiac had the highest correlation with TSF. Weight had the highest correlation with subscapular skinfold and the least with mid-thigh. There was little, if any correlation between the measured values for skinfolds, waist, hip and WHR with maternal height. Interestingly, fat mass values from Bio-electrical Impedance did

not show a great difference with BMI and also weight in correlating with skinfolds and circumference measurements. Expectedly, all skinfolds show a high correlation with fat mass (from STM), and the highest was with TSF. The correlation of WHR with skinfolds were not impressive. Waist circumference per se was found to have a better correlation with the above mentioned maternal characteristics than WHR. It should be also noticed that by 13 weeks gestation, there is a slight increase in waist circumference due to enlarged uterus.

Table 3.10. Descriptive statistics of skinfolds (mm) and waist-hip circumferences at 13 (5-15) weeks pregnancy in the whole subjects and each BMI group

	Whole group	Under-weight	Normal weight	Over-weight	Obese
Numbers	127	9	50	32	35
Triceps	22.47 ± 7.76	11.07 ± 2.54	17.43 ± 5.27	25.21 ± 4.47	30.11 ± 4.76
Biceps	12.47 ± 6.88	4.88 ± 1.18	8.34 ± 3.22	13.45 ± 3.98	19.44 ± 7.28
Subscapular	21.50 ± 10.96	8.67 ± 2.43	13.72 ± 5.13	23.20 ± 7.39	34.36 ± 6.85
Suprailiac	20.26 ± 10.25	7.30 ± 2.14	12.97 ± 5.81	23.85 ± 7.02	30.71 ± 6.65
Mid-thigh	35.26 ± 9.90	24.57 ± 5.19	30.00 ± 9.49	39.19 ± 4.41	41.95 ± 8.65
TSF	111.97 ± 40.64	56.50 ± 10.66	82.46 ± 23.22	124.90 ± 1.72	156.56 ± 23.24
Waist (cm)	91.86 ± 15.98	74.50 ± 7.32	80.99 ± 6.06	93.44 ± 8.07	110.42 ± 14.26
Hips (cm)	100.56 ± 11.31	87.63 ± 4.33	92.85 ± 5.69	102.32 ± 3.98	113.28 ± 10.19
WHR	0.91 ± 0.11	0.85 ± 0.05	0.87 ± 0.06	0.91 ± 0.07	0.98 ± 0.16

WHR: waist/hip ratio

TSF: sum of skinfolds at all sites.

Table 3.11. Correlation coefficients between skinfolds, waist-hip ratio and certain maternal characteristics (n=126)

(Comparison of the results at 13 weeks gestation)

	TSF	Weight	Height	BMI	Fat mass (BI)	Fat mass (STM)	WHR
Triceps	0.91	0.74	0.05	0.76	0.78	0.84	0.34
Biceps	0.87	0.80	0.02	0.84	0.83	0.87	0.54
Subscapular	0.93	0.85	0.04	0.88	0.88	0.93	0.53
Suprailiac	0.93	0.80	0.07	0.81	0.83	0.89	0.45
Mid-thigh	0.80	0.59	0.06	0.60	0.60	0.67	0.28
TSF	1.00	0.85	0.05*	0.88	0.88	0.94	0.48
Waist	0.83	0.90	0.11*	0.92	0.91	0.91	0.70
Hip	0.79	0.86	0.26	0.83	0.86	0.85	0.13*
WHR	0.48	0.50	-0.07*	0.56	0.52	0.54	1.00

*: non-significant

Pattern of changes in skinfolds and fat distribution during gestation

Changes in skinfold thicknesses and circumferences between different measurements are shown in table 3.12. Of the women who completed the study, 12 (3 NW, 3 OW and 6 OB) had generalised oedema. Presence of generalized oedema did not have a significant effect on the pattern of the changes in skinfolds during pregnancy and postpartum.

The suprailiac site showed the highest and biceps the least contribution to the total increase of skinfolds during pregnancy. The increase in subscapular and triceps are not significantly different. The increase in mid-thigh skinfold was much less than the suprailiac but slightly higher than the other measured sites. The table also shows that the increment in total skinfolds is higher between the first and second trimester measurements during pregnancy.

The suprailiac skinfold decreased considerably at 6 weeks postpartum from 36 weeks pregnancy, the others showed only small changes. From 6 weeks to 6 months postpartum, triceps and biceps (unlike the others) seem to increase. Although there were some apparent losses in most of the measured sites from 36 weeks pregnancy to 6 months postpartum, at 6 month postpartum all the skinfolds were higher than 13 weeks gestation values. This suggest that a considerable amount of subcutaneous fat is retained at 6 months postpartum in the whole population under the study. WHR seems to not vary

all, however, each of the waist and hip circumferences increased from 13 weeks gestation to 6 month postpartum.

Figure 3.6. shows the schematic presentation of the changes of five measured skinfolds at each stage of gestation from the first visit value at 13 weeks gestation in all subjects.

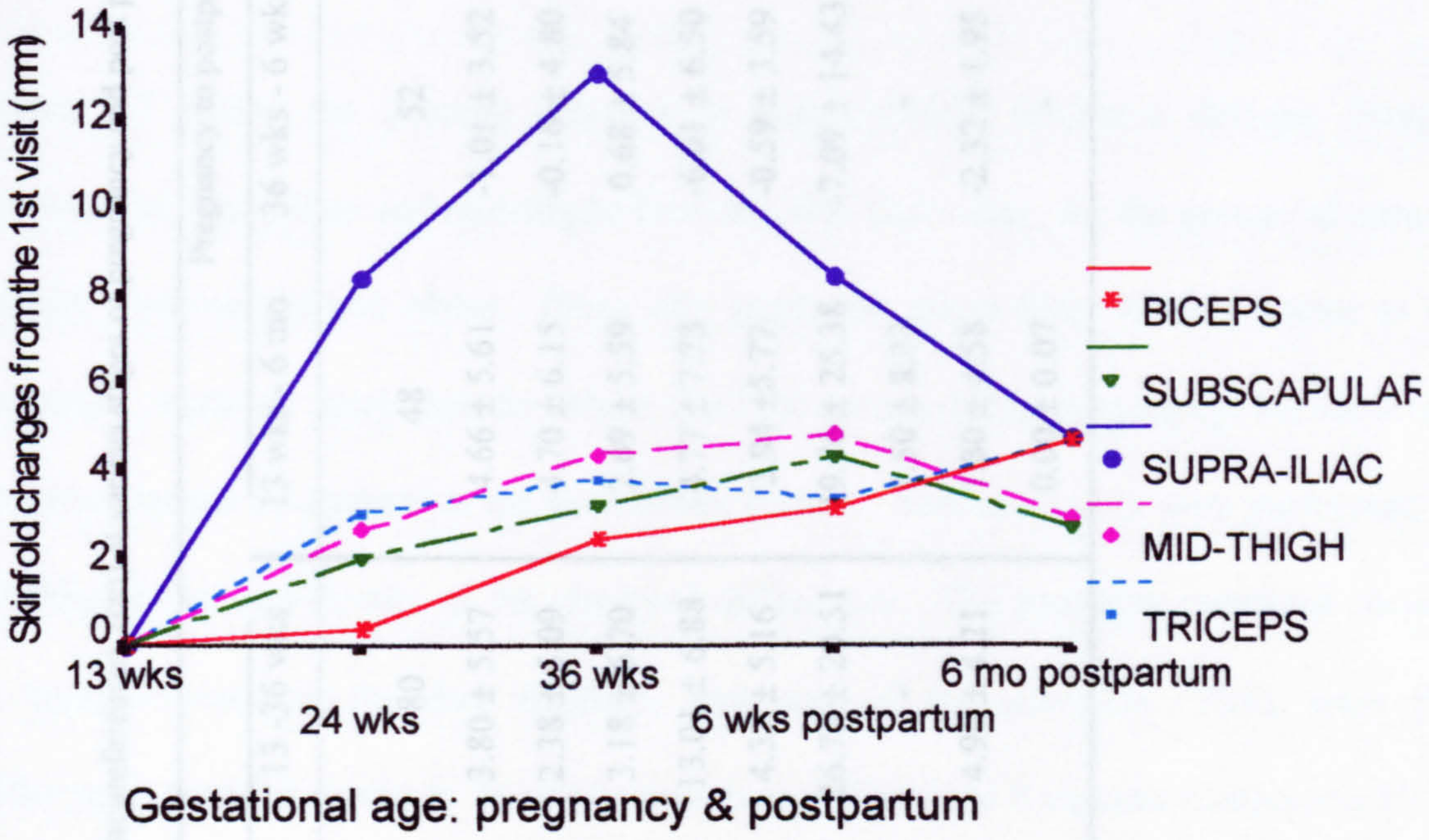


Figure 3.6. Changes in five skinfolds during pregnancy and postpartum in all the subjects. The changes are expressed as the difference of the mean values at each stage from the first visit value. Number of subjects during pregnancy 77 and at postpartum was 48.

Table 3.12. Average changes in skinfolds (mm) and body circumferences (cm) at various stages of pregnancy and post partum

	Pregnancy			Pregnancy to postpartum			Postpartum
	13 -25 wks	25 -36 wks	13 -36 wks	13 wks - 6 mo	36 wks - 6 wks	36 wks - 6 mo.	6 wks - 6 mo.
Subjects (n)	77	68	80	48	52	48	48
Triceps (mm)	2.95 ± 4.14	0.98 ± 3.96	3.80 ± 5.57	4.66 ± 5.61	-1.01 ± 3.52	0.61 ± 5.23	1.59 ± 4.71
Biceps (mm)	0.49 ± 3.13	2.23 ± 4.80	2.38 ± 5.09	4.70 ± 6.15	-0.16 ± 4.80	1.50 ± 6.40	1.67 ± 5.95
Subscapular (mm)	1.98 ± 4.11	1.24 ± 6.02	3.18 ± 6.70	2.69 ± 5.59	0.68 ± 5.84	-0.78 ± 7.66	-1.56 ± 4.43
Suprailiac (mm)	8.40 ± 5.89	5.38 ± 5.12	13.01 ± 6.88	4.77 ± 7.73	-6.01 ± 6.50	-9.63 ± 9.76	-3.30 ± 6.96
Mid-thigh (mm)	2.60 ± 4.65	1.91 ± 4.22	4.37 ± 5.16	2.94 ±5.77	-0.59 ± 3.59	-3.50 ± 3.52	-1.43 ± 3.74
TSF (mm)	16.25 ± 14.16	11.88 ± 15.07	26.75 ± 20.51	19.76 ± 25.38	-7.09 ± 14.43	-10.35 ± 4.11	-3.03 ± 18.73
Waist (cm)	-	-	-	1.50 ± 8.33	-	-	-0.77 ± 5.11
Hips (cm)	3.53 ± 2.81	1.56 ± 2.32	4.96 ± 4.21	1.80 ± 4.58	-2.32 ± 1.95	-3.50 ± 3.52	-1.01 ± 3.14
WHR	-	-	-	0.00 ± 0.07	-	-	0.00 ± 0.04

Comparison of the pattern of skinfold changes between the groups of normal weight, over-weight and obese women

Figure 3.7 shows the average changes of each skinfold thickness (triceps, biceps, subscapular, supra-iliac and mid-thigh) from the first visit value, for the groups of normal weight, over-weight and obese. From this graph, the supra-iliac skinfold seems to be increased relatively more than the others in all the groups during pregnancy, but there is a different pattern of changes in the postpartum period. Statistical tests were performed to investigate the significance of the observed differences. The summary measures chosen to compare each of the five skinfolds and sum of the skinfolds (TSF), were the differences from 13 weeks to 36 weeks gestation (1-3) and to 6 months postpartum (1-5) as well as the differences from 36 weeks pregnancy and 6 weeks postpartum to 6 months postpartum (3-5 and 4-5 respectively). The results of one way ANOVA for the summary measures suggest that TSF changes are significantly different between the groups of normal and obese in the postpartum period (36 weeks gestation to 6 month postpartum). The changes in triceps and subscapular are not significantly different between the groups. The increase in biceps is significantly higher in the obese and over-weight groups than in the normal weight group from 13 weeks pregnancy to 6 months postpartum. The increment in mid-thigh skinfold was also higher in the obese group than the normal

weight group. At the supra-iliac site, again, the obese group showed the highest increase through the whole course of the study (13 wks gestation to 6 months postpartum) and the least decrease during the postpartum period. The changes in the latter site were not significantly different between the groups, only over the pregnancy period (13 wks to 36 wks gestation). The significant differences in skinfold changes between the BMI groups, can be seen in table 3.13.

The individual plots of total skinfold (TSF) changes from 13 weeks gestation, for each BMI group are presented in figure 3.8. The normal weight group show a homogeneous pattern of TSF changes over the whole course of the study. Whereas, the over-weight and obese groups seem to be more scattered and mostly tend to increase or stay higher in the postpartum period. From the comparison of the selected summary measures of TSF, the obese group were significantly different from the normal group in respect to changes from 36 weeks gestation to 6 months postpartum. While the normal weight group tend to have a reduction of TSF, the obese group seem to increase total skinfold thicknesses at postpartum (table 3.13).

The changes in waist circumference (WC) and WHR from 6 weeks to 6 months postpartum were also compared between the three BMI groups. The results for each BMI group are as follow: (WC: -3.25, 0.04, 1.54 and WHR: -0.18, 0.01, 0.01). The changes in the obese group were significantly different from the normal group in respect to both indices of fat distribution.

Table 3.13. Statistically significant mean differences in skinfolds (mm) between the groups of normal weight, over-weight and obese subjects.

	Pregnancy	Pregnancy to postpartum				Postpartum
Gestation	13 - 36 wks	13 wks - 6 mo		36 wks -6 mo		6 wks - 6 mo
Skinfolds	Mid-thigh	Biceps	Supra-iliac	TSF	Supra-iliac	Supra-iliac
Normal weight	2.43*	1.53†*	0.64†*	-23.96*	-16.69†*	-6.61*
Over-weight	4.60	6.67†	5.76†	-9.48	-9.62†#	-2.47
Obese	6.29*	7.01*	10.08*	3.93*	-2.01*#	-0.26*

TSF: Total skinfolds; sum of five measured skinfolds; triceps, biceps, subscapular, supra-iliac and mid-thigh.

*: Expressing the significant difference between the normal weight and the obese group.

†: Expressing the significant difference between the normal weight and the over-weight group.

#: Expressing the significant difference between the over-weight and the obese group.

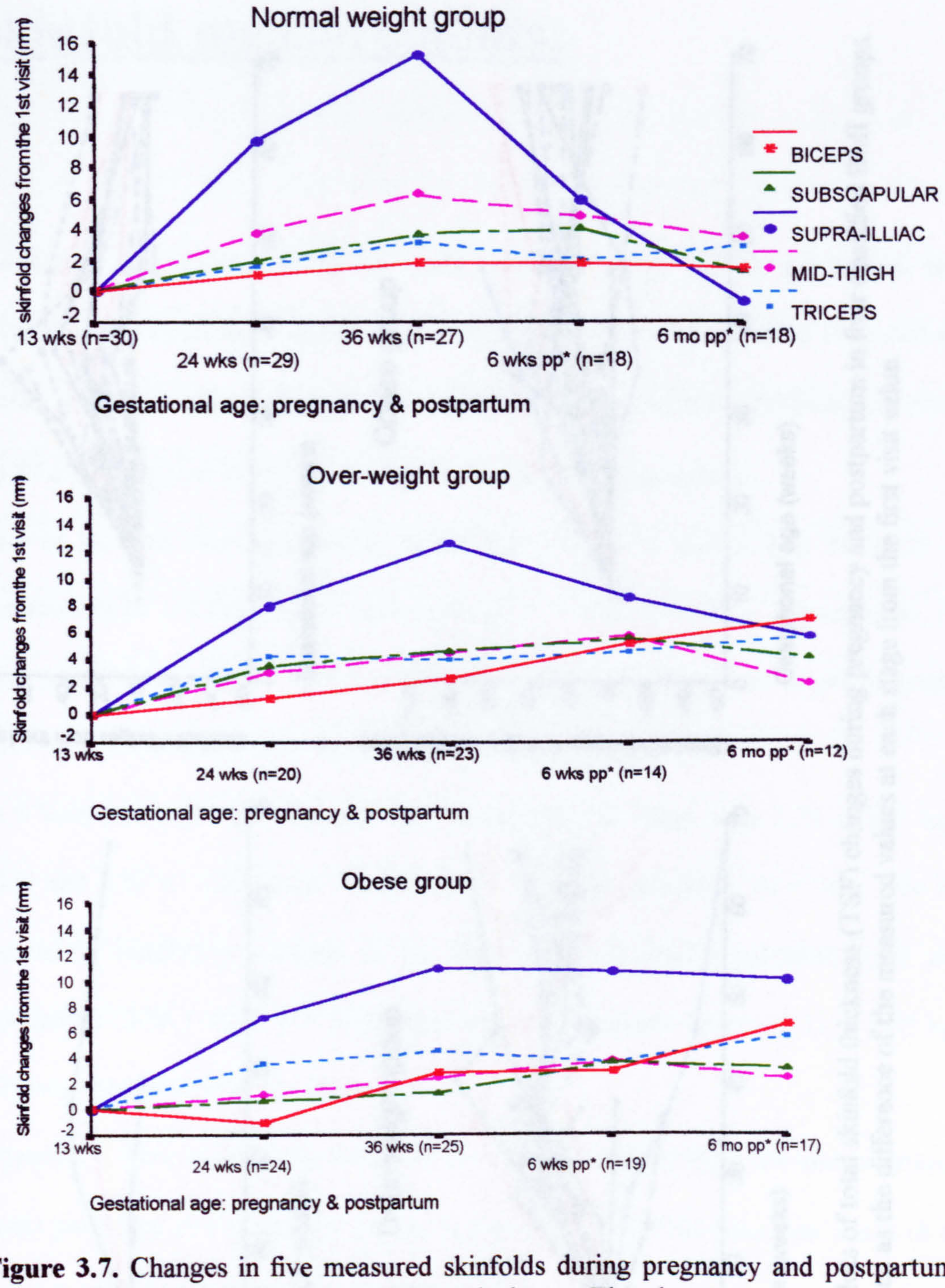
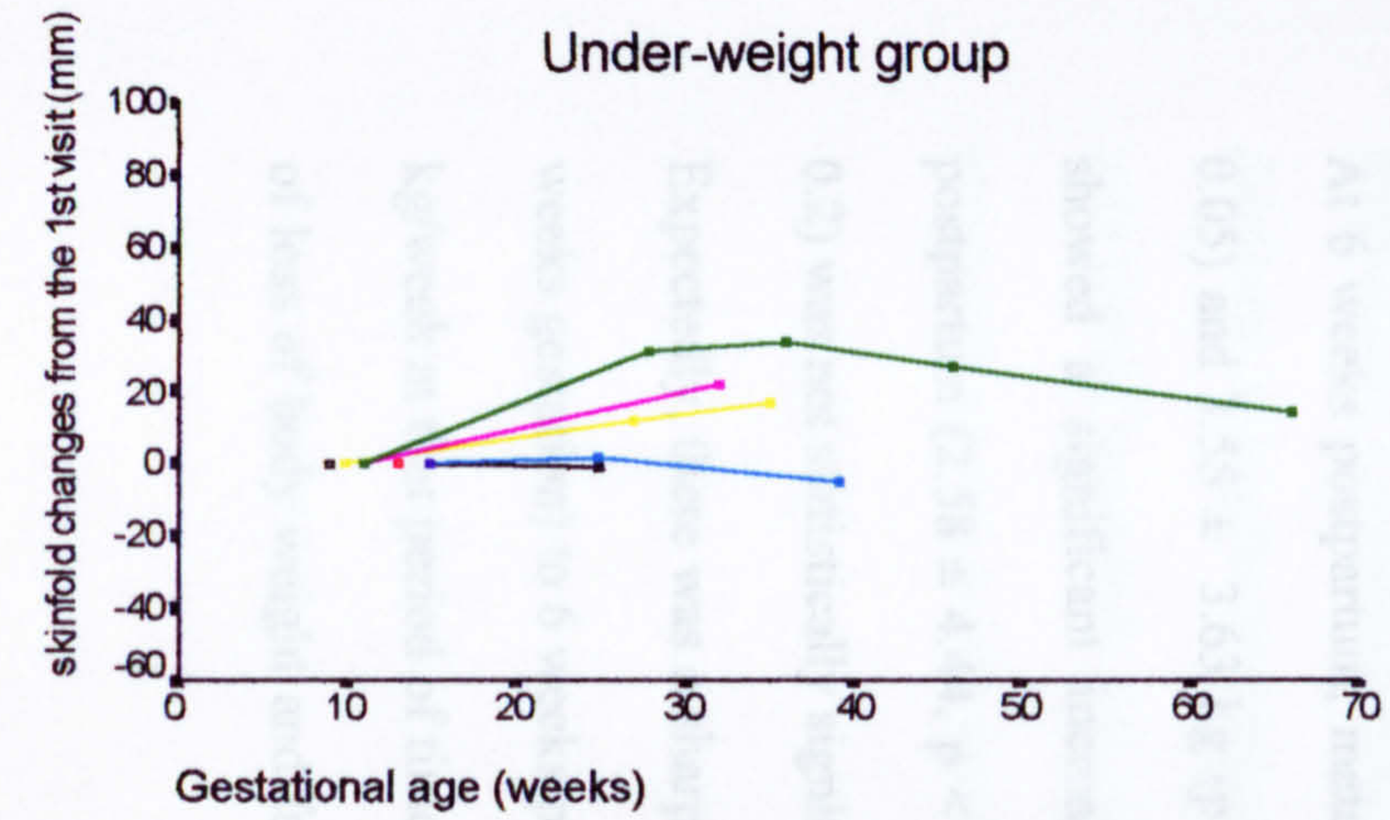
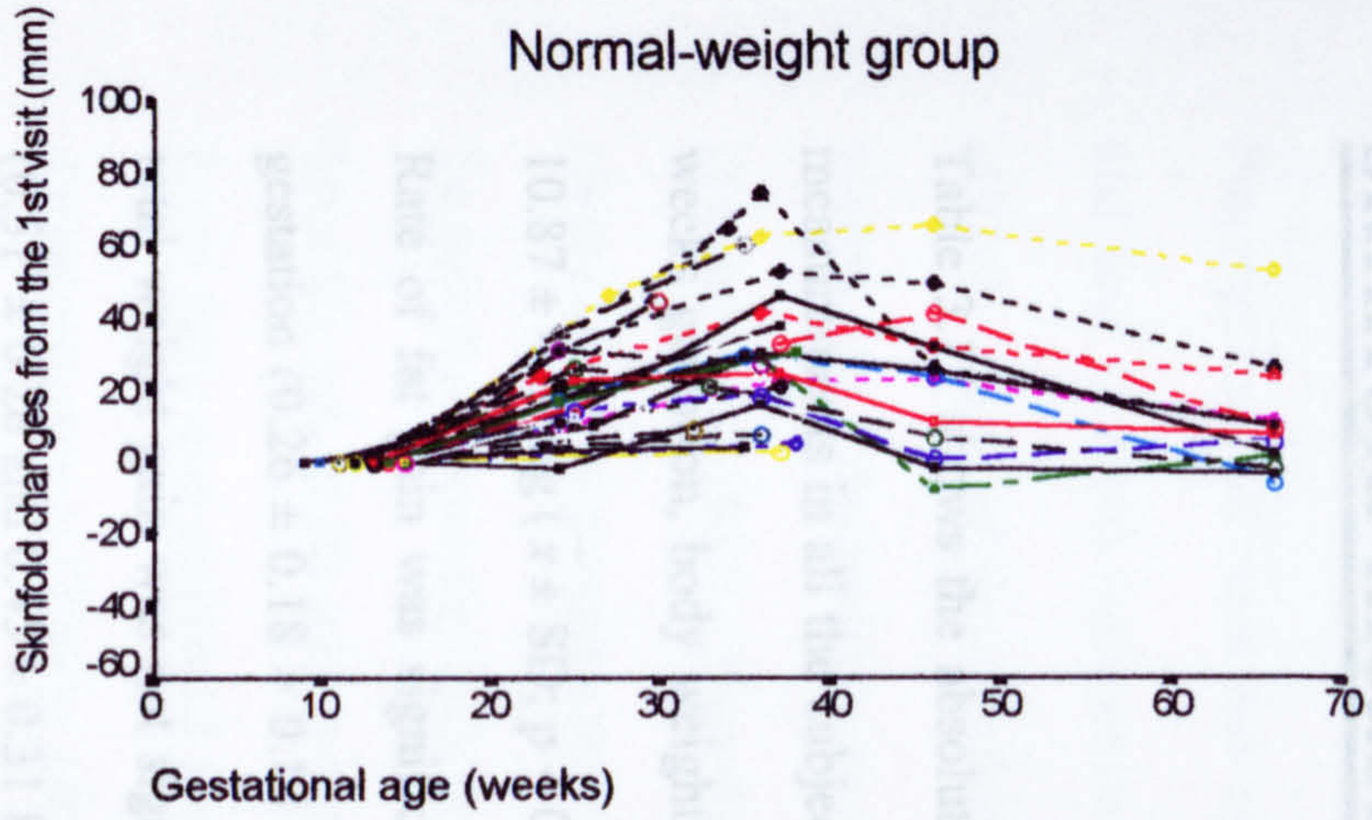
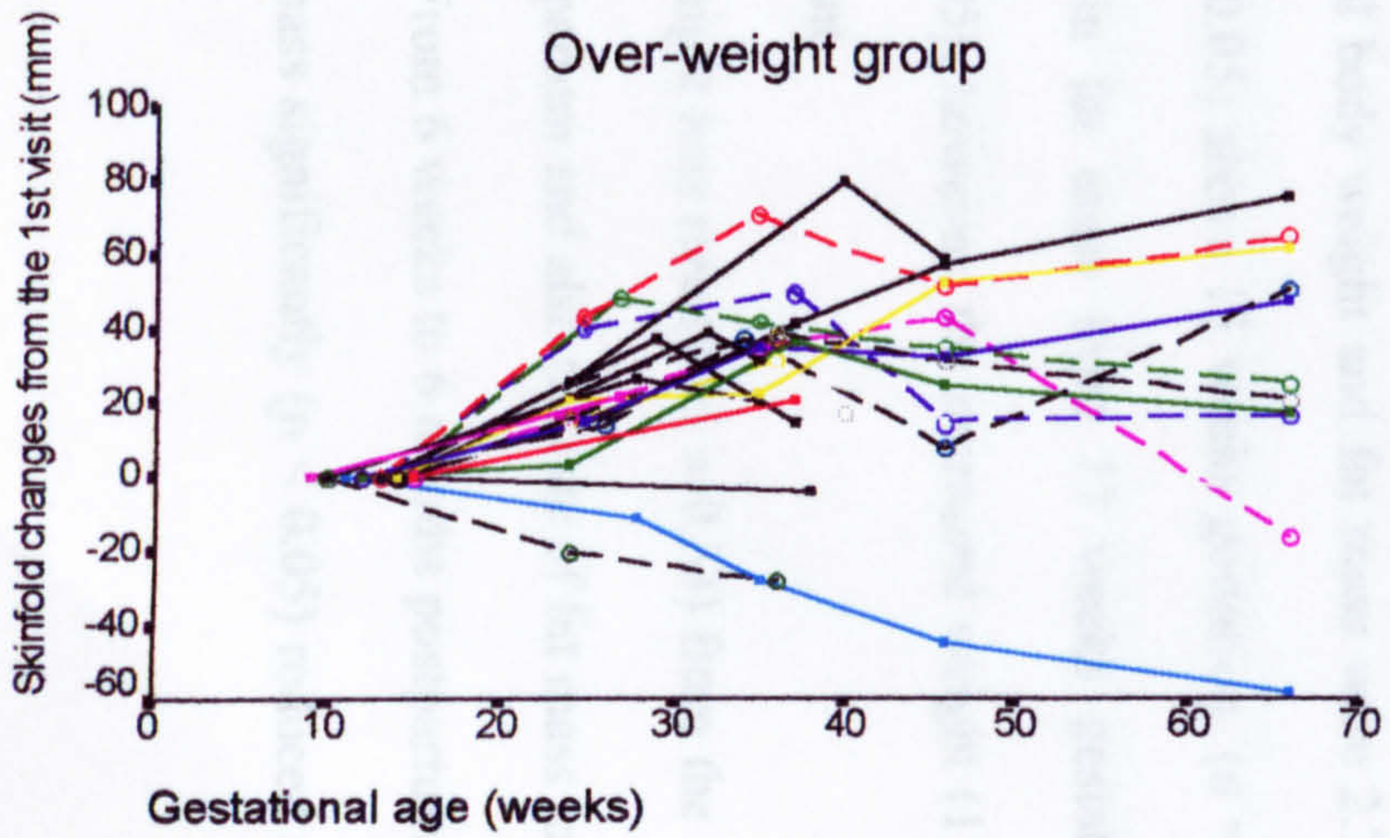


Figure 3.7. Changes in five measured skinfolds during pregnancy and postpartum in groups of normal weight, over-weight and obese. The changes are expressed as the difference of the mean values at each stage from the first visit value.







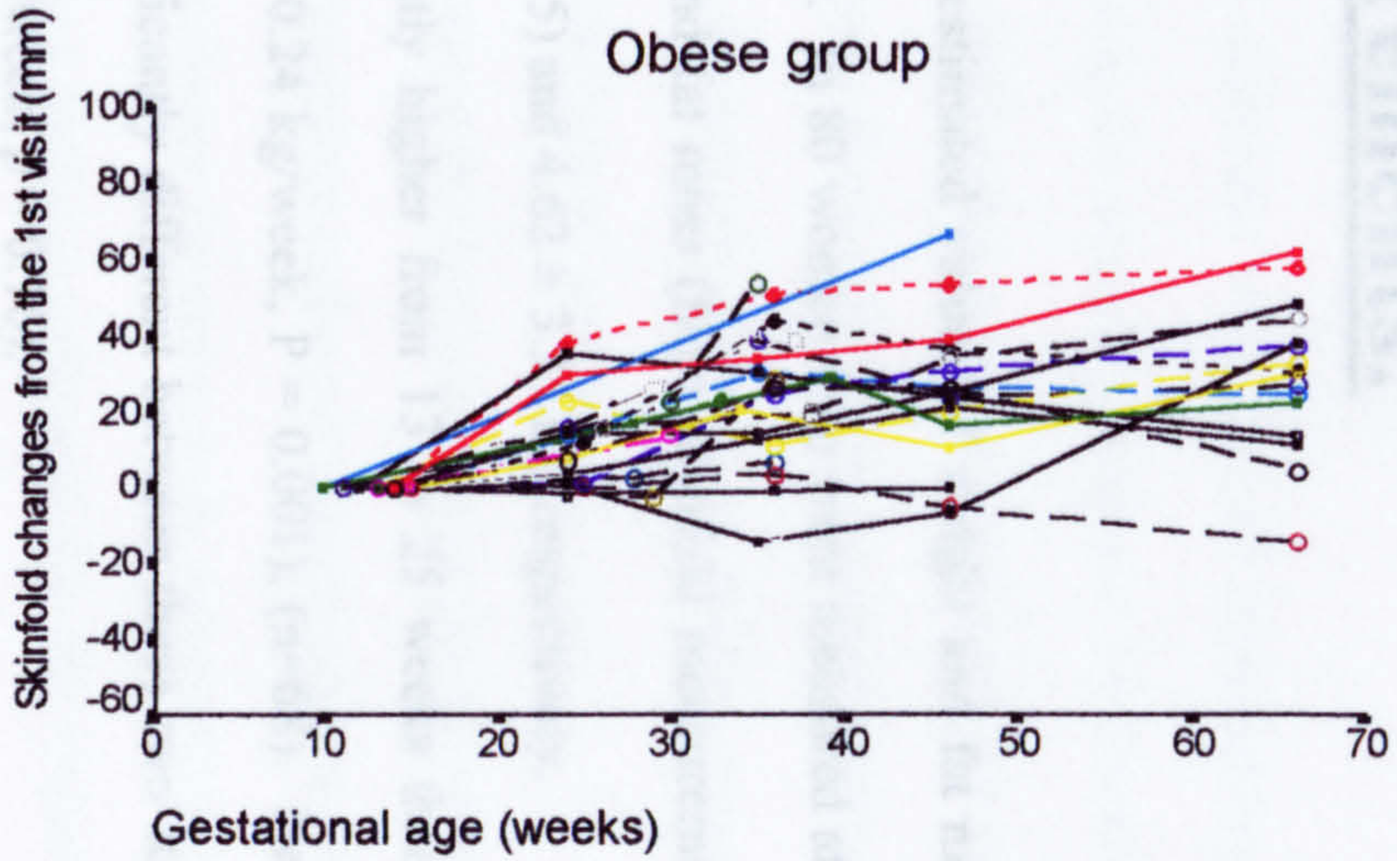


Figure 3.8. Individual plots of total skinfold thickness (TSF) changes during pregnancy and postpartum in four classified BMI groups. The changes are expressed as the difference of the measured values at each stage from the first visit value.

Body weight and body fat mass using skinfold measurements:

Table 3.15 shows the absolute estimated values of weight and fat mass from skinfold measurements in all the subjects. In 80 women who were measured at 13 weeks and 36 weeks gestation, body weight and fat mass (from skinfold measurements) increased by 10.87 ± 4.67 kg ($\bar{x} \pm$ SD; $p < 0.05$) and 4.62 ± 3.35 kg, respectively.

Rate of fat gain was significantly higher from 13 to 25 weeks than 25 to 36 weeks gestation ($0.26 \pm 0.18 > 0.14 \pm 0.24$ kg/week, $P = 0.001$), (n=68). Nevertheless, rate of total weight gain was not significantly different between these two stages of pregnancy (0.51 ± 0.26 and 0.45 ± 0.31 kg/week, $p = 0.16$).

At 6 weeks postpartum, maternal body weight and fat mass were 2.74 ± 5.01 kg ($p < 0.05$) and 3.55 ± 3.63 kg ($p < 0.05$) above 13 weeks gestation, ($n = 48$). The results showed a significant increase in fat mass from 13 weeks gestation to 6 months postpartum (2.58 ± 4.44, $p < 0.05$), however, the increased weight (1.11 ± 5.97 kg, $p = 0.2$) was not statistically significant.

Expectedly, there was a sharp weight loss rate (0.93 ± 0.33) from the late pregnancy (36 weeks gestation) to 6 weeks postpartum and also the rate of fat mass loss was 0.18 ± 0.20 kg/week at that period of time. From 6 weeks to 6 months postpartum, however, the rate of loss of body weight and fat mass significantly ($p < 0.05$) reduced to 0.02 ± 0.07 and

0.01 ± 0.6 kg/week, respectively. In fact the weight and fat mass losses from 6 weeks to 6 months postpartum (1.12 ± 4.31, p = 0.08 and 0.07 ± 3.35, p = 0.15 respectively) were not statistically significant.

Prepregnancy fat mass was also calculated from the self reported prepregnancy weight and four skinfold thicknesses using the equation proposed by Durnin and Womersley (1974). Three of the skinfolds which were measured and used for calculation (biceps, triceps and subscapular) are among those which change least during early pregnancy (Taggart et al 1967). Therefore, the extrapolation of skinfold thicknesses from week 13 (5-15) to the prepregnant state is likely to be justified.

To gain the values for body fat mass at full term pregnancy, linear extrapolation was used from the rate of fat gain in late pregnancy and measured fat mass at 36 weeks gestation. From these and estimated prepregnancy fat mass, were calculated the gain in fat mass during pregnancy (7.03 ± 3.91, $p < 0.05$), (n =62) and also the retained fat mass due to pregnancy at 6 months postpartum (4.37 ± 4.61, $p < 0.05$), (n = 45).

Similarly, using the prepregnancy weights and extrapolated weight at term, total weight gain during pregnancy was estimated to be 17.58 ± 7.56 kg and retained fat gain due to pregnancy up to 6 month was 6.24 ± 7.93 kg.

Body composition using Bio-electrical Impedance:

Body composition of the subjects were also measured by Bio-electrical Impedance technique. The values for each body compartment (fat mass, lean mass and body water) obtained using BI, are shown in table 3.14.

Using this method, body fat mass, body lean mass and water content of body increased significantly ($p < 0.05$) from 13 weeks to 36 weeks gestation (7.04 ± 3.65 kg, 3.76 ± 2.34 kg and 3.32 ± 2.79 respectively, n=80). At 6 weeks postpartum (n=51), body fat mass were on average 2.37 ± 4.18 above 13 weeks gestation values whereas the small gain in body lean (0.48 ± 2.27, $p = 0.14$) and body water (0.43 ± 2.36) were not significant.

Comparing the values at 13 weeks pregnancy to those at 6 months postpartum (n=48), none of the changes including fat mass (1.16 ± 5, $p = 0.12$), lean mass (-0.01 ± 3.09, $p = 0.99$) and body water (-0.05 ± 1.57) were significant.

Table 3.14. Maternal weight and body composition in the whole group as assessed by Skinfold measurements and Bio-electrical Impedance during pregnancy and postpartum.

Weeks gestation (mean)	Number of subjects	Weight (kg)	*Fat $mass_{STM}$ (kg)	¤Fat $mass_{BI}$ (kg)	Lean $mass_{BI}$ (kg)	$Water_{BI}$ (L)
Prepregnancy	86	67.56 ± 15.72	22.39 ± 9.30#	–	–	–
Pregnancy						
13	123	71.18 ± 16.33	23.32 ± 9.82	25.52 ± 11.40	45.94 ± 6.23	30.37 ± 3.96
25	76	79.22 ± 16.82	27.63 ± 9.75	31.17 ± 11.51	48.42 ± 6.31	32.32 ± 5.07
36	80	84.50 ± 16.32	29.29 ± 9.93	34.14 ± 11.11	50.28 ± 6.21	34.23 ± 5.15
Postpartum						
6 weeks	51	78.03 ± 16.23	29.62 ± 9.74	30.65 ± 11.03	47.46 ± 6.05	31.43 ± 4.62
6 months	48	75.44 ± 16.83	28.01 ± 10.62	28.80 ± 12.13	46.65 ± 5.86	30.84 ± 3.81

#: Fat mass calculated from self reported prepregnancy weight and sum of four skinfold measurements at week 13 by means of equations proposed by Durnin and Womersley and Siri's equation.

*: Fat mass values derived from sum of four skinfold thickness using Van Raaij formula during pregnancy and Siri formula for postpartum measurements.

¤: BI stands for Bio-elctrical Impedance.

Comparison of Normal weight, over-weight and obese group in respect to weight and body composition:

Table 3.15 shows the absolute values of estimated maternal weight and body composition during pregnancy and postpartum in groups of under and normal weight as well as over-weight and obese groups.

Total weight gain and fat gain in kg (STMv) for the course of measurements during pregnancy (13 to 36 weeks gestation) for each group are as follows:

	13 to 36 weeks gestation	
Groups	fat mass (STMv)	Weight
Normal weight	4.92 ± 2.71	11.01 ± 3.22
Over-weight	5.29 ± 4.50	11.91 ± 6.38
Obese	3.75 ± 2.76	9.68 ± 4.30

Table 3.15. Maternal weight and body composition (mean ± SD) as assessed by Skinfold measurements and Bio-electrical Impedance during pregnancy and postpartum in different BMI groups.

Weeks gestation	Subjects (n)	Weight (kg)	*Fat $mass_{STM}$ (kg)	¤Fat $mass_{BI}$ (kg)	Lean $mass_{BI}$ (kg)	$Water_{BI}$ (L)
	Normal weight & Under-weight group					
Pregnancy						
13	59	58.53 ± 7.04	15.28 ± 4.09	15.84 ± 3.90	43.07 ± 5.69	28.46 ± 3.37
25	33	64.86 ± 8.09	18.97 ± 4.83	20.98 ± 5.53	44.15 ± 4.27	29.70 ± 3.23
36	32	70.74 ± 8.63	20.66 ± 5.54	23.84 ± 5.38	46.92 ± 5.29	32.02 ± 4.04
Postpartum						
6 weeks	19	62.54 ± 8.76	20.12 ± 5.31	19.61 ± 5.73	42.95 ± 4.52	29.11 ± 4.19
6 months	19	60.16 ± 8.51	18.15 ± 4.98	17.42 ± 5.22	42.74 ± 4.17	28.42 ± 2.43
	Over-weight group					
Pregnancy						
13	32	71.33 ± 6.24	24.35 ± 3.87	26.80 ± 3.48	44.53 ± 4.05	29.69 ± 3.32
25	20	79.63 ± 8.42	28.82 ± 4.51	29.86 ± 6.41	47.94 ± 5.47	32.90 ± 7.10
36	23	83.89 ± 10.35	29.86 ± 6.41	34.93 ± 5.94	48.96 ± 5.24	33.63 ± 5.39
Postpartum						
6 weeks	14	78.18 ± 9.99	30.04 ± 6.00	31.11 ± 6.49	47.07 ± 4.70	31.14 ± 3.96
6 months	12	75.46 ± 11.56	28.53 ± 7.69	28.75 ± 8.48	46.71 ± 4.31	30.92 ± 3.80
	Obese group					
Pregnancy						
13	35	92.36 ± 11.02	35.84 ± 6.29	40.37 ± 6.99	51.98 ± 4.35	34.03 ± 2.78
25	24	98.63 ± 9.80	38.55 ± 5.63	44.38 ± 6.06	54.25 ± 4.19	35.33 ± 3.00
36	25	102.68 ± 9.24	39.83 ± 5.64	46.58 ± 5.75	55.78 ± 4.16	37.60 ± 4.57
Postpartum						
6 weeks	19	93.42 ± 9.73	38.80 ± 5.52	41.37 ± 5.60	52.26 ± 4.64	33.97 ± 4.36
6 months	17	92.50 ± 8.52	38.67 ± 5.11	41.56 ± 5.03	50.97 ± 5.52	33.50 ± 3.41

*: Fat $mass_{STM}$ values derived from sum of four skinfold thickness using Van Raaij formula during pregnancy and Siri formula for postpartum measurements.

¤: BI stands for Bio-elctrical Impedance.

The individual plots of weight and body compartment changes (fat mass from STM and fat mass, lean mass, water from BI), during pregnancy and up to 6 months postpartum in each BMI group can be seen in figures 3.9 to 3.13. As shown in the graphs, there are some differences in the rate of fat gain during pregnancy and in particular at postpartum the trend of changes seem different between the groups.

Figure 3.9. shows that the pattern of weight changes follow a homogenous trend in normal weight individuals. They all seem to increase their weight during pregnancy whereas a considerable loss of weight is observed at 6 weeks postpartum. From then to 6 months postpartum they tend to either reduce slightly or stay at the same level. Over-weight women show a divergent pattern. The maximum weight gain and also the maximum weight loss are seen in this group. The majority of obese women seem to be heavier at 6 month postpartum in comparison to 13 weeks gestation.

Figure 3.10 and 3.11 represent the individual changes of fat mass obtained by the two methods of BI and STM. By the two methods, normal weight women show similar patterns of changes in fat mass, over-weight show a very scattered figure and the obese women have mostly higher values of fat mass at 6 months postpartum than in early pregnancy.

Figure 3.12 reveals the changes in the lean mass of the individuals. In this set of graphs, conversely, over-weight women show a closer pattern of curves. The changes in the lean mass seem to be relatively similar in all the groups.

The least variation is observed in the changes of body water within and also between the groups (figure 3.13). Body water is increased considerably from 24 weeks gestation to 36

weeks, followed by a sharp fall after delivery at 6 weeks postpartum. Thereafter, it remains relatively constant at 6 months postpartum.

There were only a few people in the under-weight group who completed the course of study. They, however, showed a similar pattern to the normal weight subjects.

In our study a multivariate analysis of variance (MANOVA) on fat mass and weight changes during and after pregnancy suggests that BMI and parity of the subjects have significant effects on the rate and extent of the changes. Since the groups are matched in respect to parity, a one way ANOVA was performed to elaborate the magnitude of the effect of BMI on the above mentioned variables. The summary measures calculated, were rate of changes, the absolute differences between certain points and also delta area under the curve (ΔAUC) for all the variables. The calculated rates included the rate of changes from 13 weeks gestation to 25 weeks (rate 1-2) and from 25 weeks to 36 weeks gestation (rate 2-3).

The absolute mean changes during pregnancy from 13 to 36 weeks gestation (1-3), the changes from 36 weeks gestation to 6 month postpartum (3 - 5) and also the whole course of study from 13 weeks gestation to 6 months postpartum were obtained and compared between the groups. ΔAUC was estimated from 13 weeks gestation to 36 weeks gestation, to 6 months postpartum and also from 36 weeks gestation to 6 months postpartum. (ΔAUC3, ΔAUC5, ΔAUC3-5 respectively).

The results showed that there are no significant differences between the rates of fat gain and weight gain between the groups. From the comparison of the other summary measures, the obese group proved to be significantly different from the normal group in

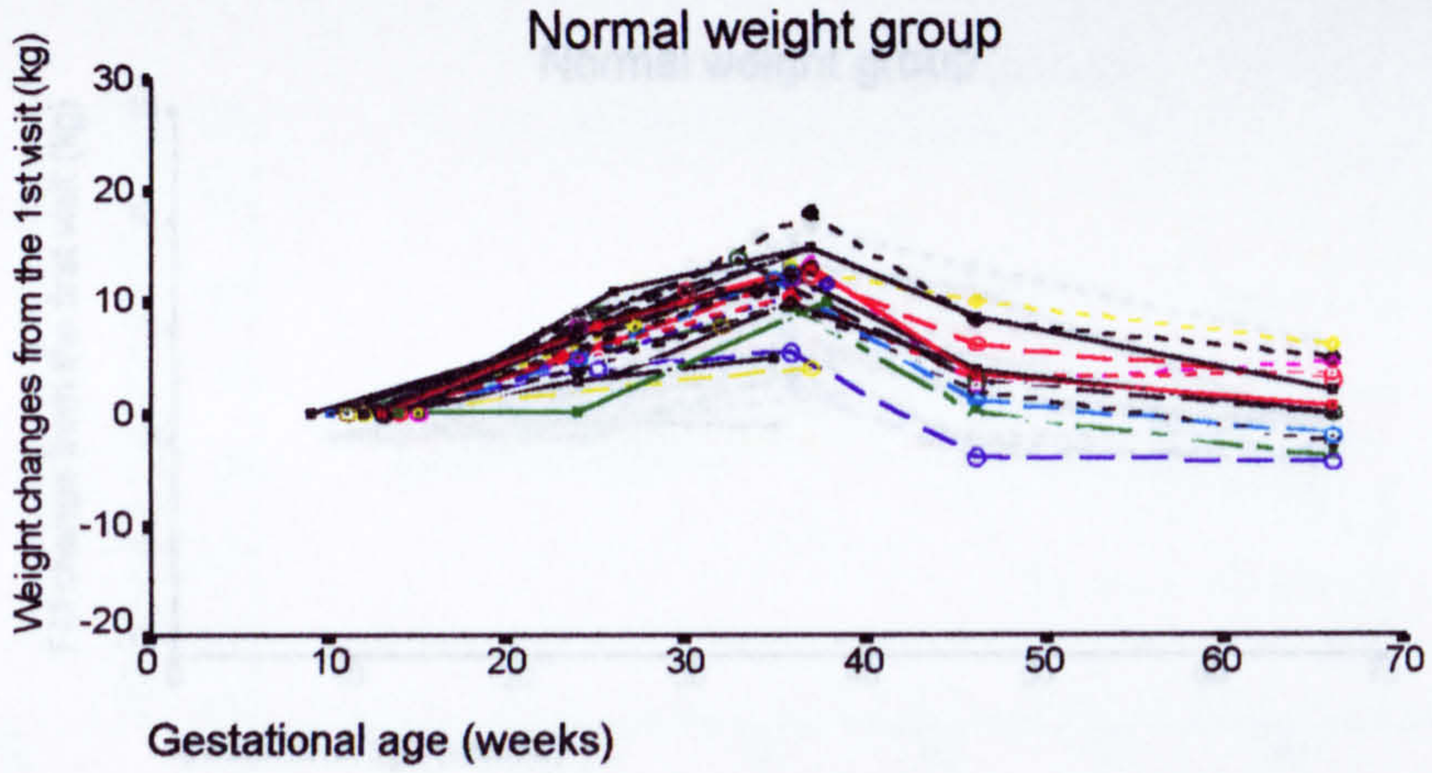
three aspects: Δfat mass (STM, 3-5), ΔAUC for fat mass (STM, 3-5) and ΔAUC for lean mass (BI, 1-3). The obese group lost less fat mass (by STM) at postpartum (as measured from 36 weeks gestation to 6 month postpartum) than the normal weight group. Delta area under the curve (ΔAUC) was also significantly different between these two groups at postpartum period. Using BI, the only significant difference was observed between the normal and the obese group in ΔAUC of lean mass during pregnancy. Nevertheless the net changes of lean mass was not significantly different between the groups. This would indicate a different pattern of lean mass changes between these two groups. These differences are presented in the following table:

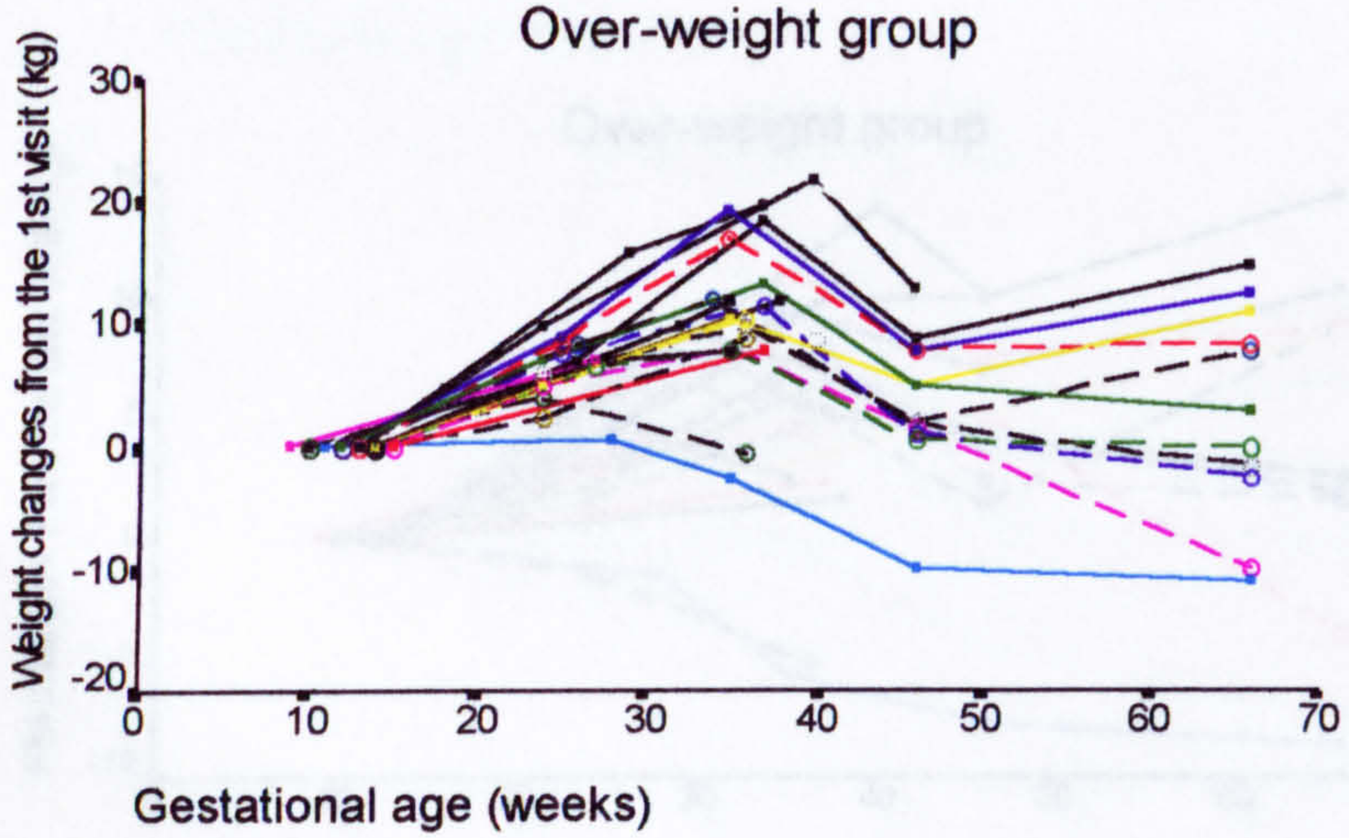
Table 3.16. The significantly different variables observed between the groups of normal weight, over-weight and obese women.

	36 wks pregnancy to 6 mo postpartum		13 to 36 wks pregnancy
	ΔFM (STM)	ΔAUC (FM: STM)	ΔAUC (LM: BI)
Normal weight	-4.11*	-198.94*	25.20*
Over-weight	-1.97	-129.61	40.30
Obese	-0.91*	-65.83*	57.33*

*: indicates significant difference.

Comparing direct measurements of skinfolds with fat mass derived from those measurements (STM), show identical trends between 36 weeks gestation and 6 months postpartum. The obese group are less likely to lose fat over this time period than either the normal weight group or the over-weight group (see figures 3.8 and 3.10).





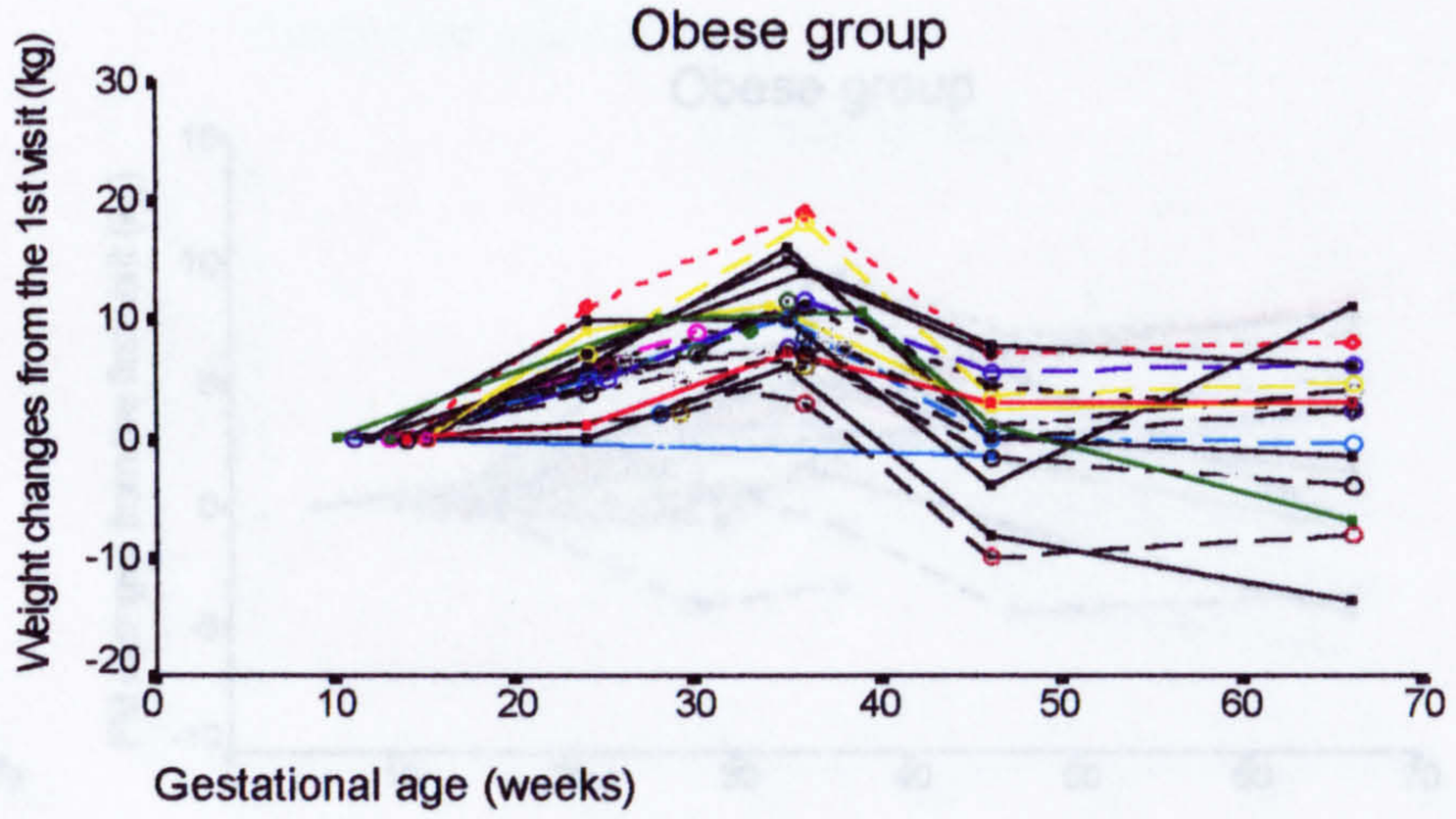
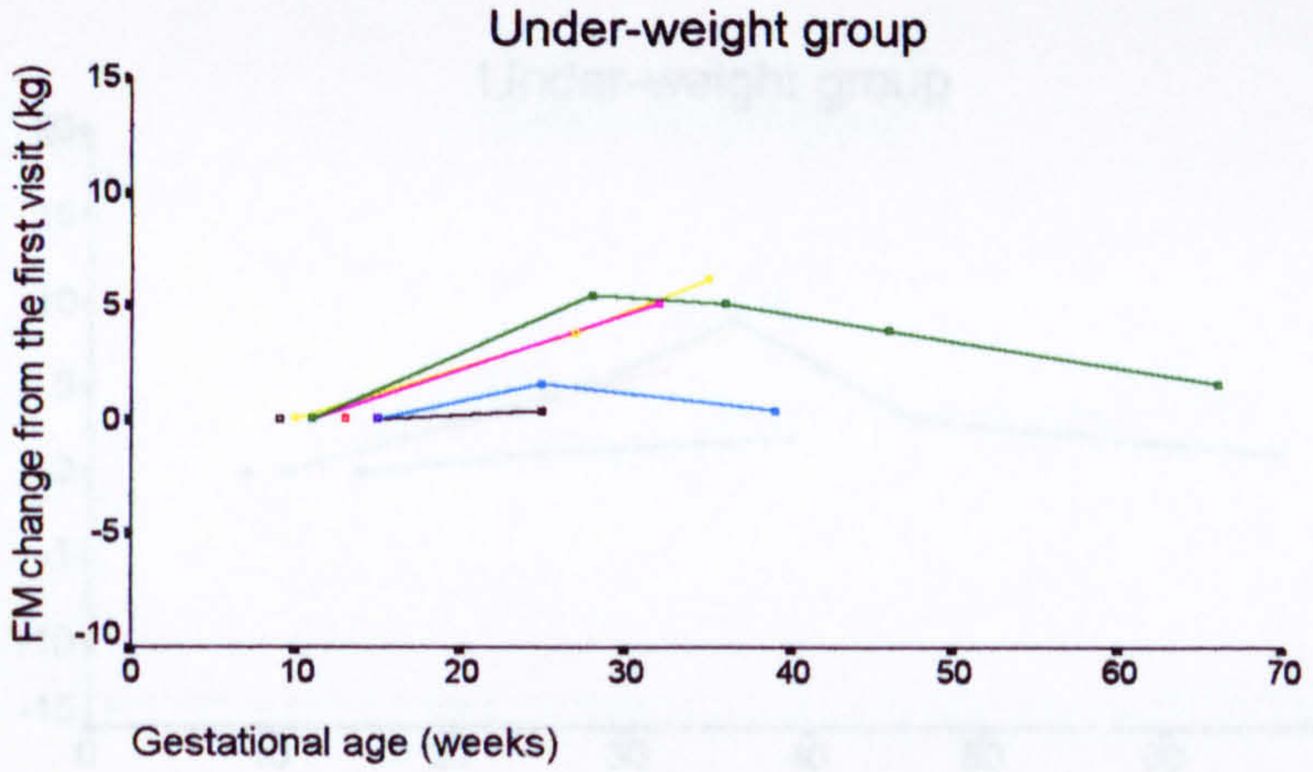
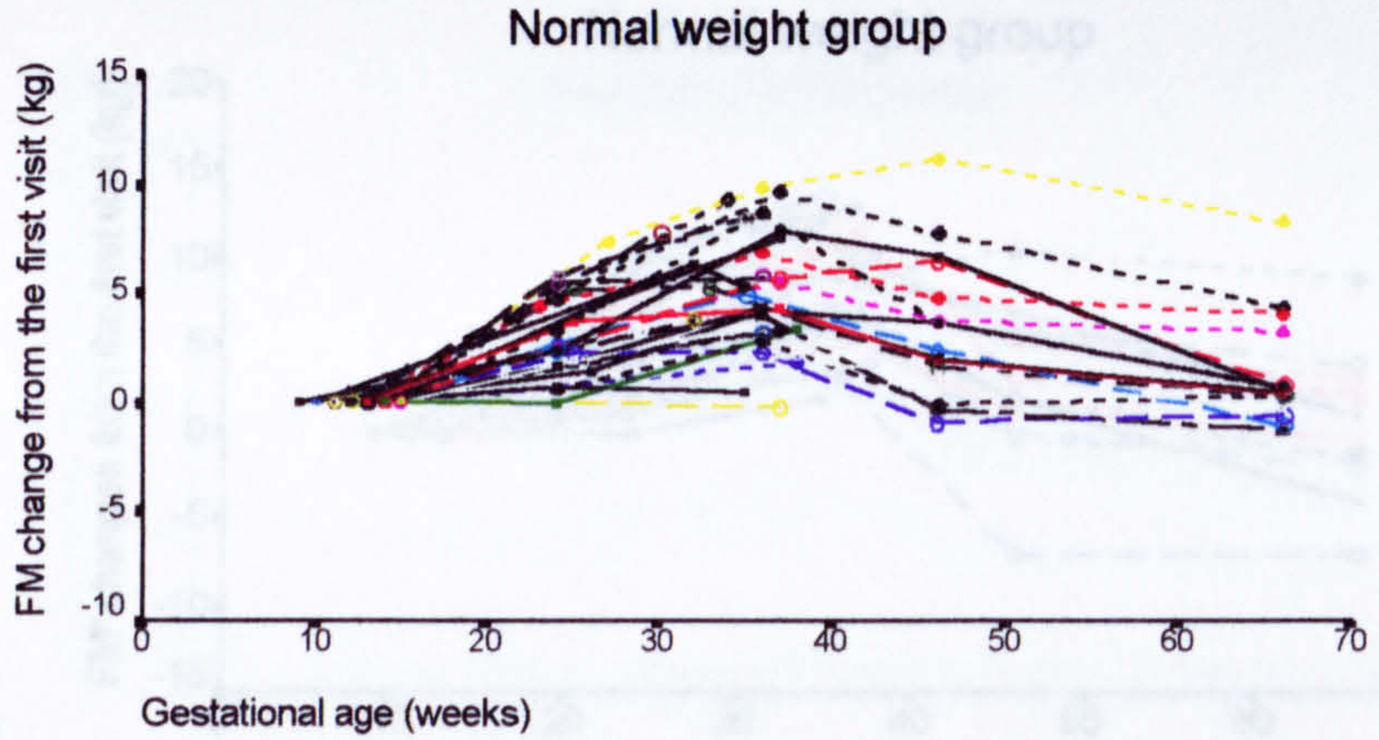
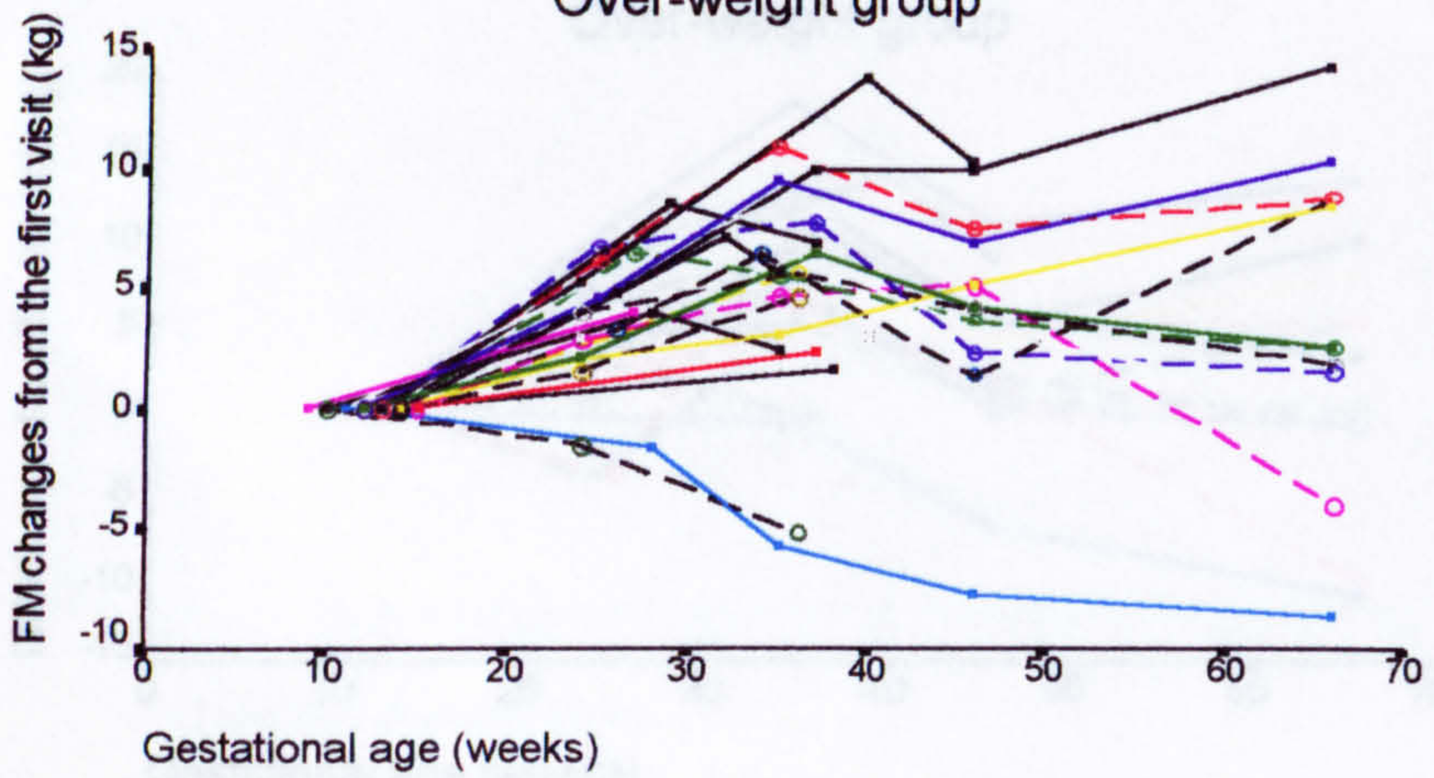


Figure 3.9. Individual plots of weight changes during pregnancy and postpartum in four classified BMI groups. The changes are expressed as the difference of the measured values at each stage from the first visit value.







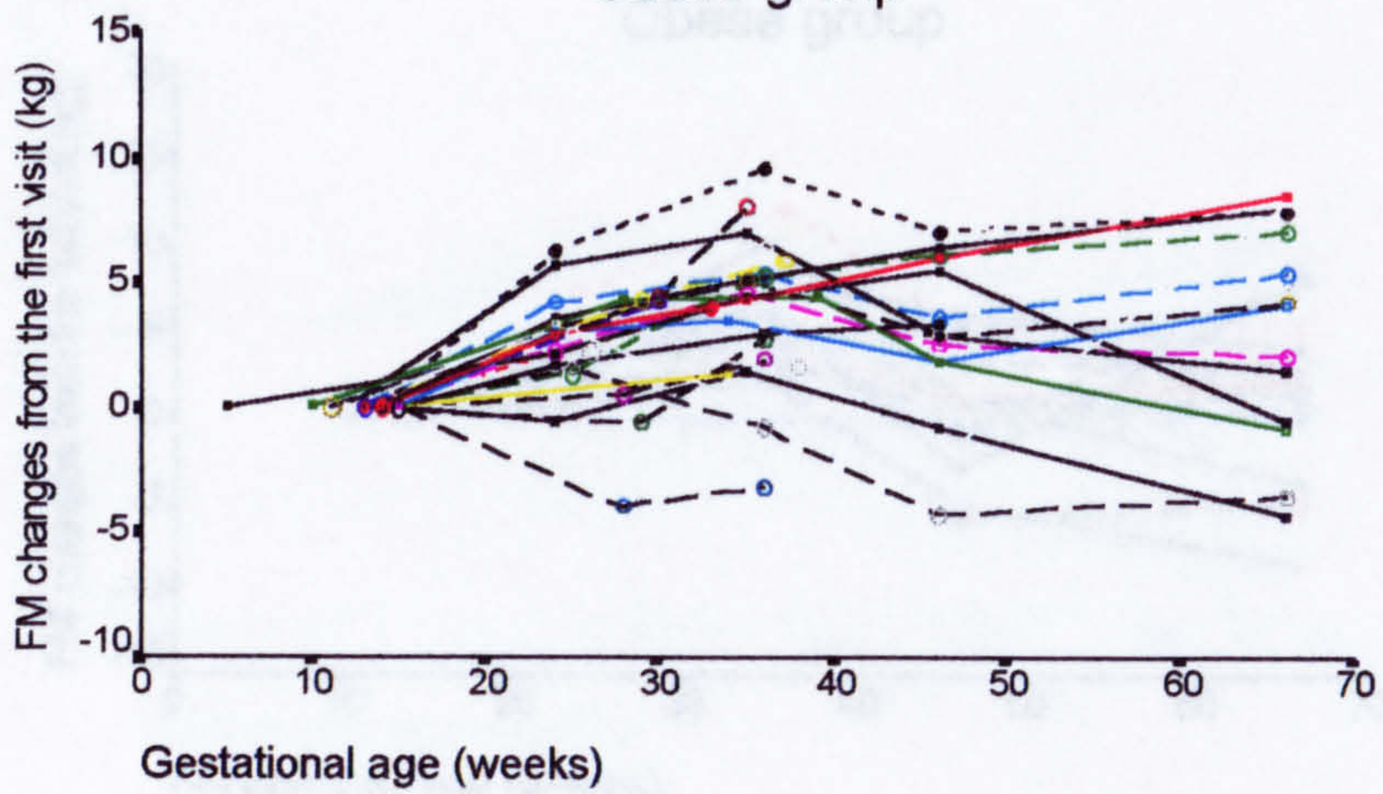
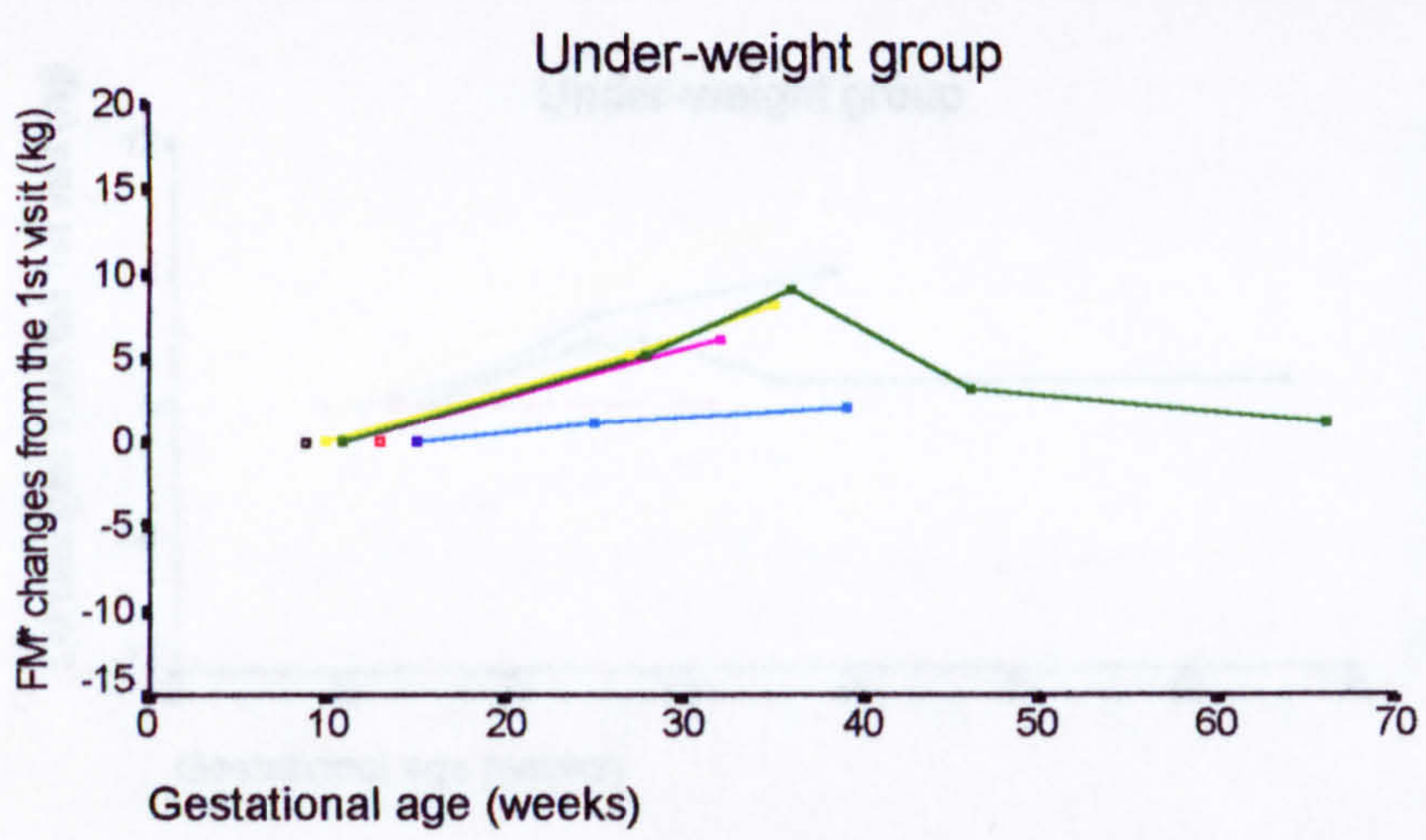
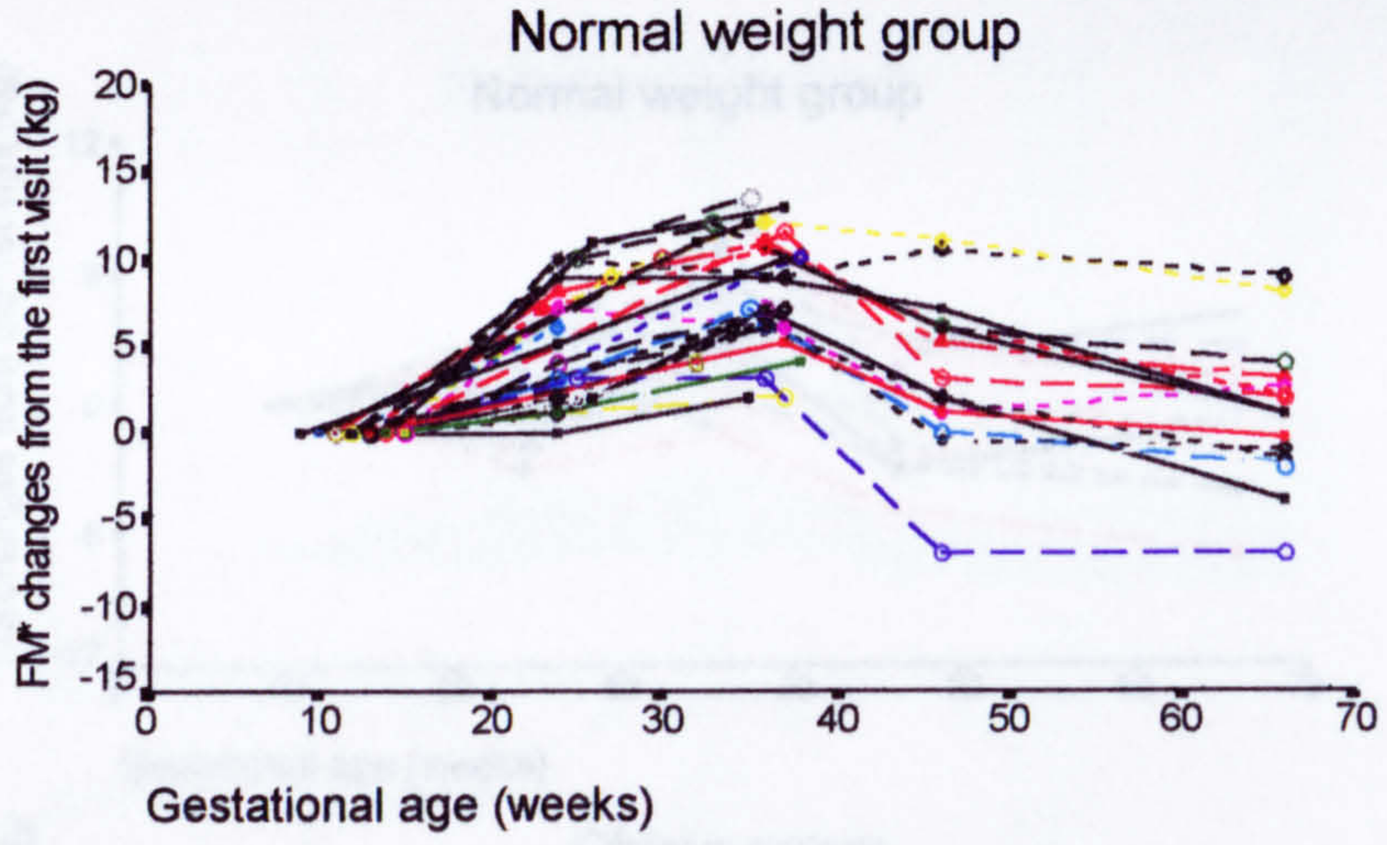
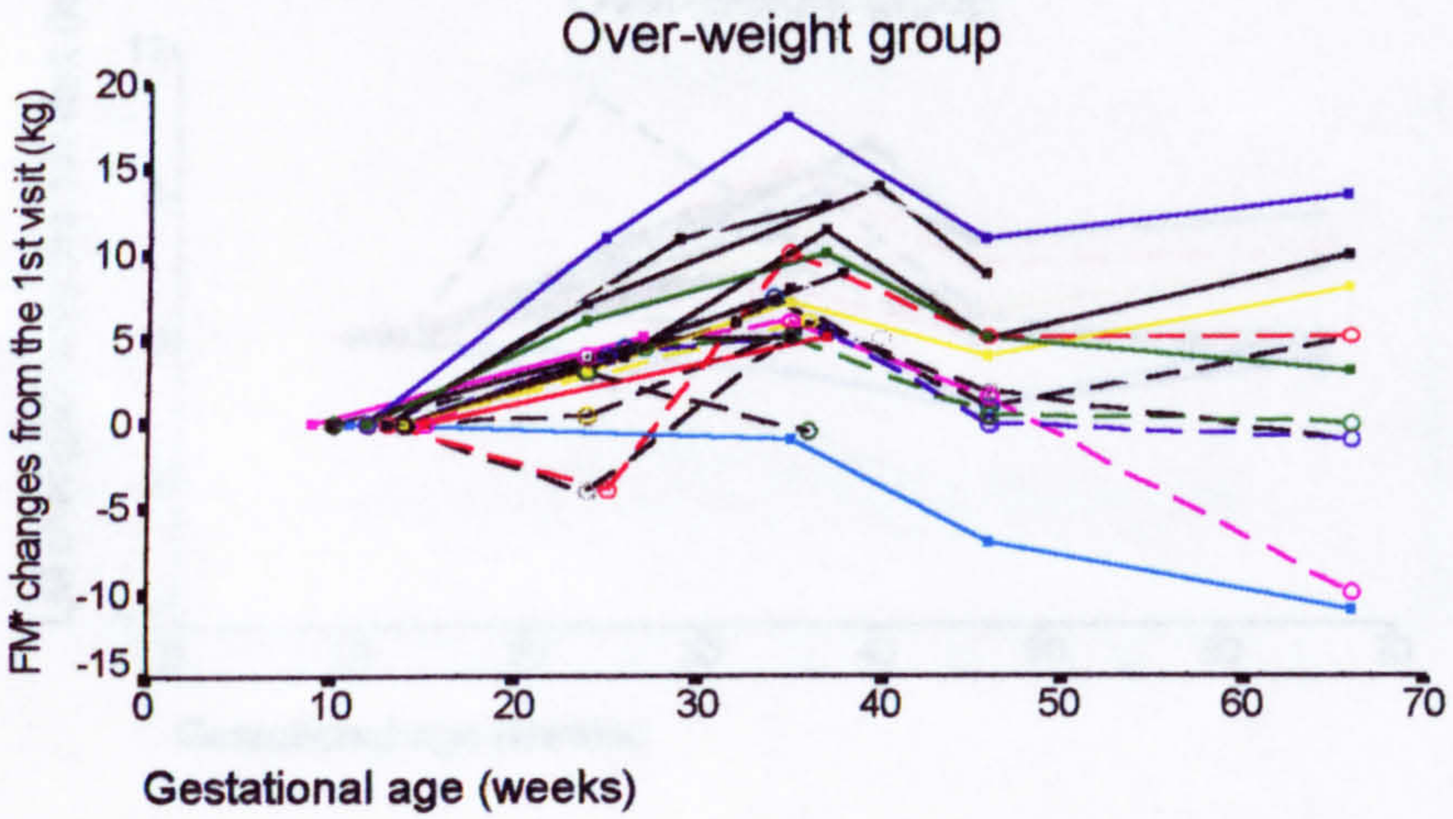


Figure 3.10. Individual plots of fat mass changes during pregnancy and postpartum, in four classified BMI groups. Fat mass changes are expressed as the difference of the values at each stage from the first visit values.
Fat mass values are derived from the skinfold thickness using Durnin and Womersley (1974) equation and Van Raaij formula (1988).







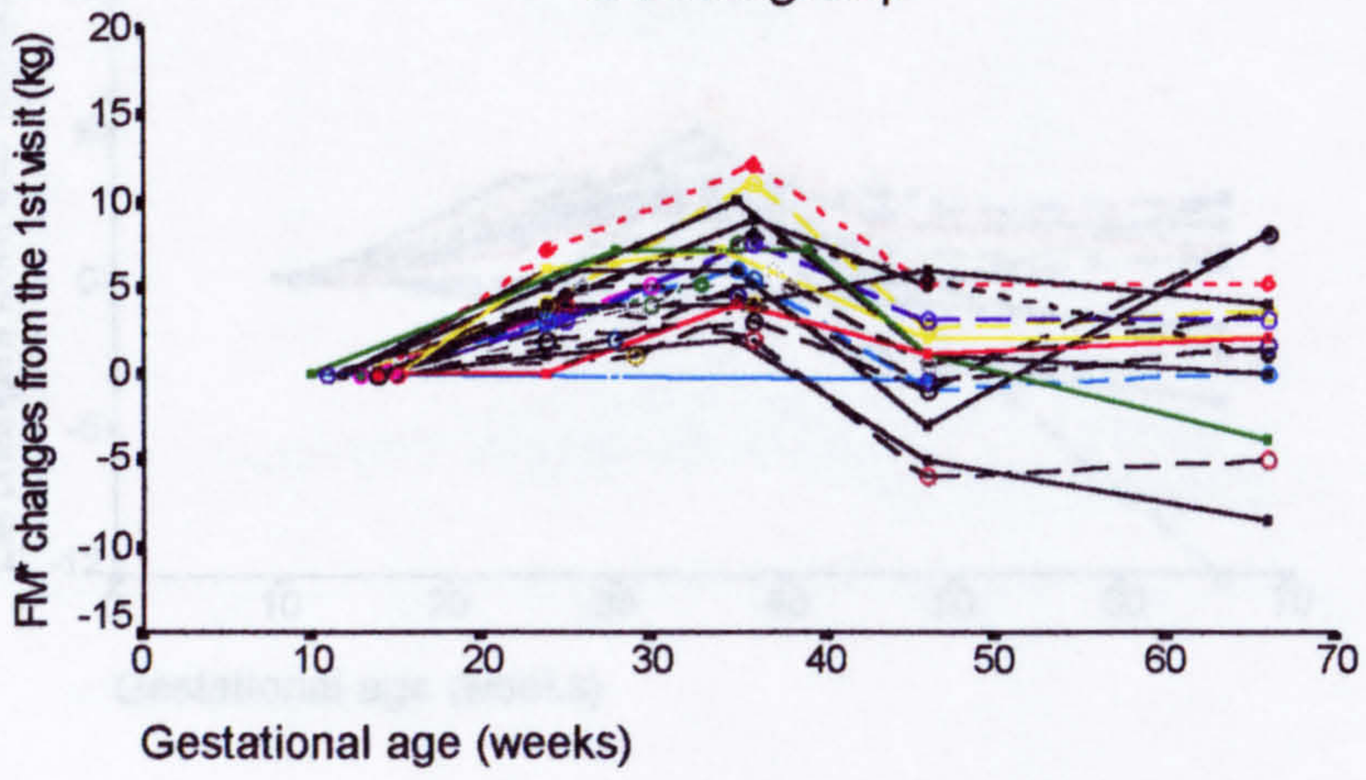
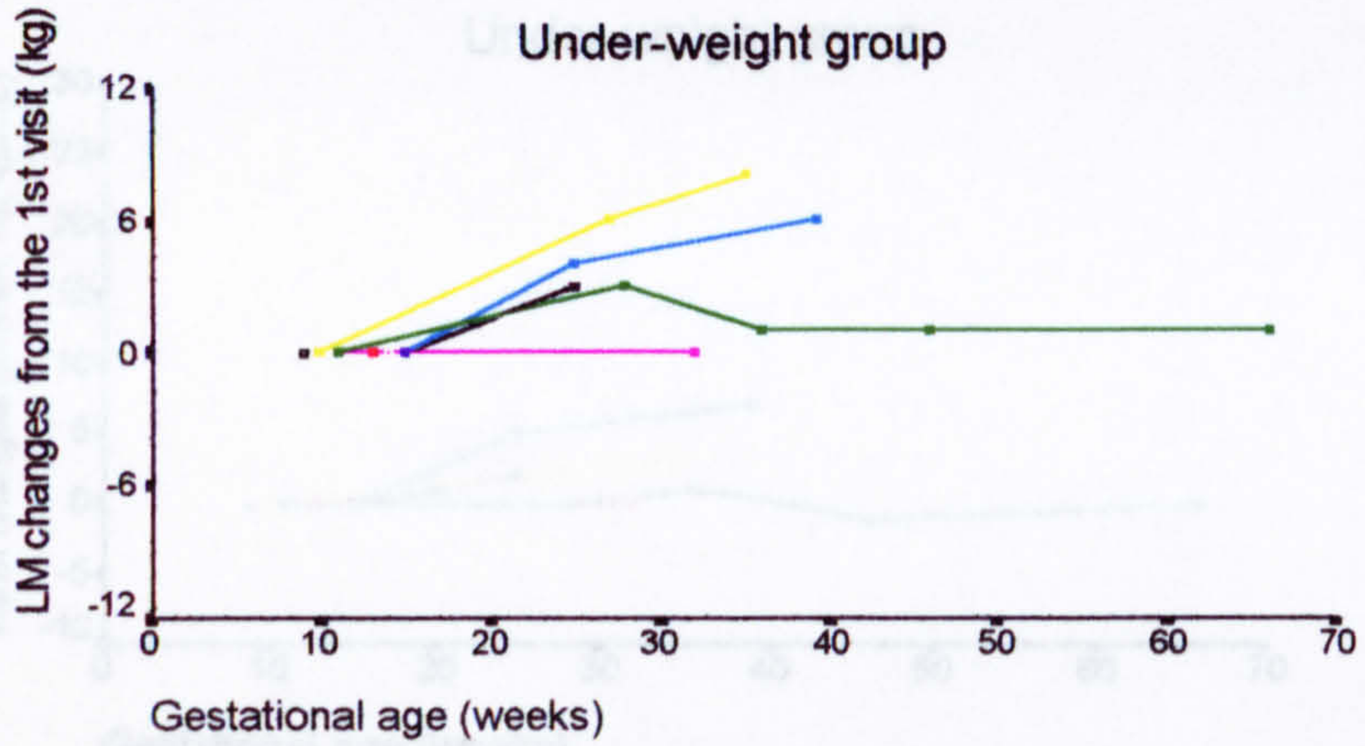
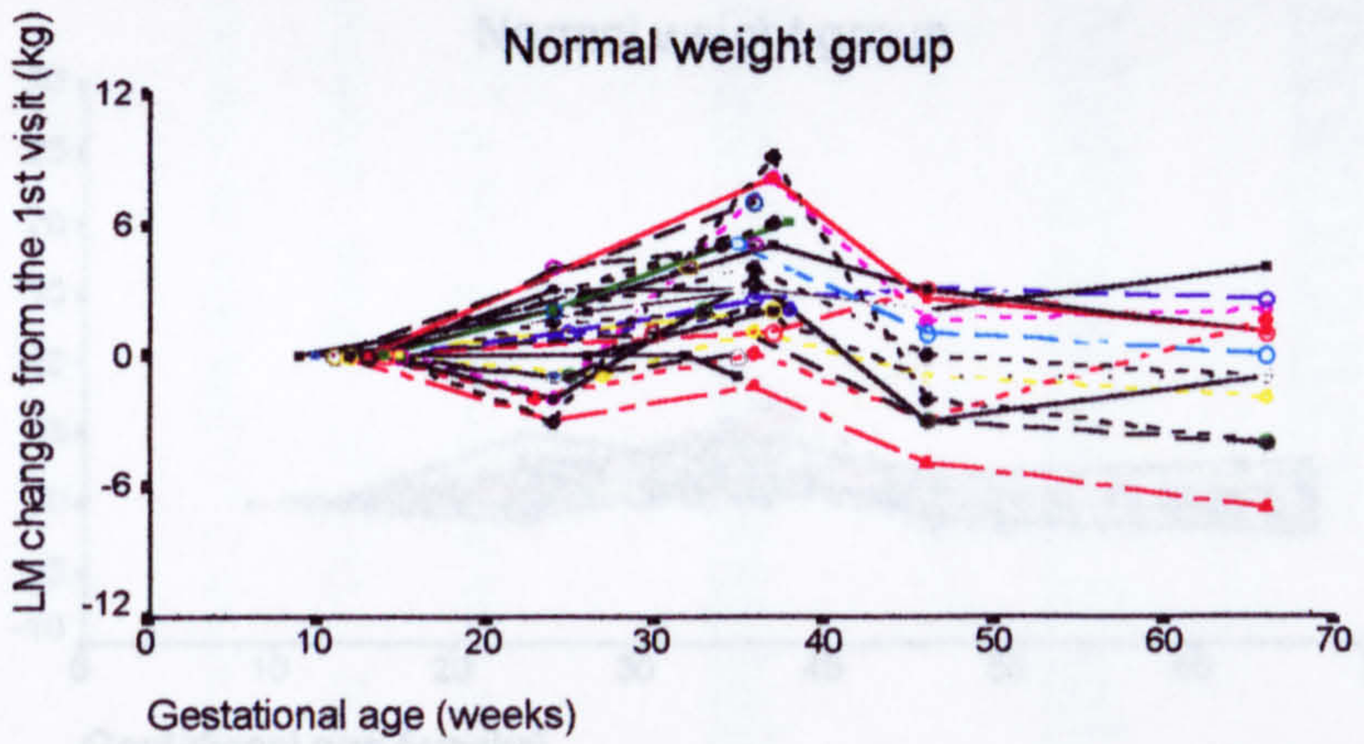
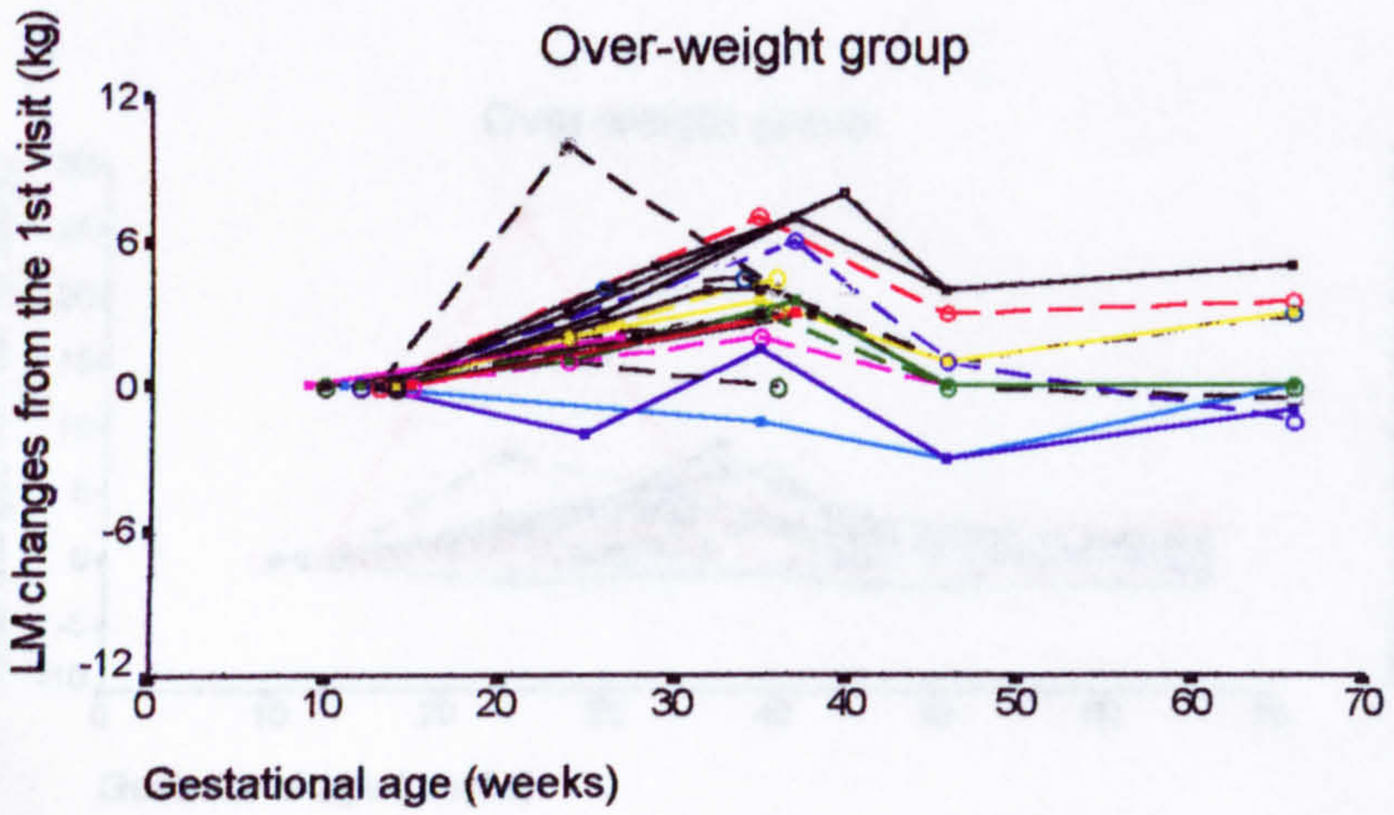


Figure 3.11. Individual plots of fat mass* changes during pregnancy and postpartum in four BMI classified groups. Fat mass changes are expressed as the difference of the values measured at each stage from the first visit value.
*: Fat mass estimated by Bio-electrical Impedance







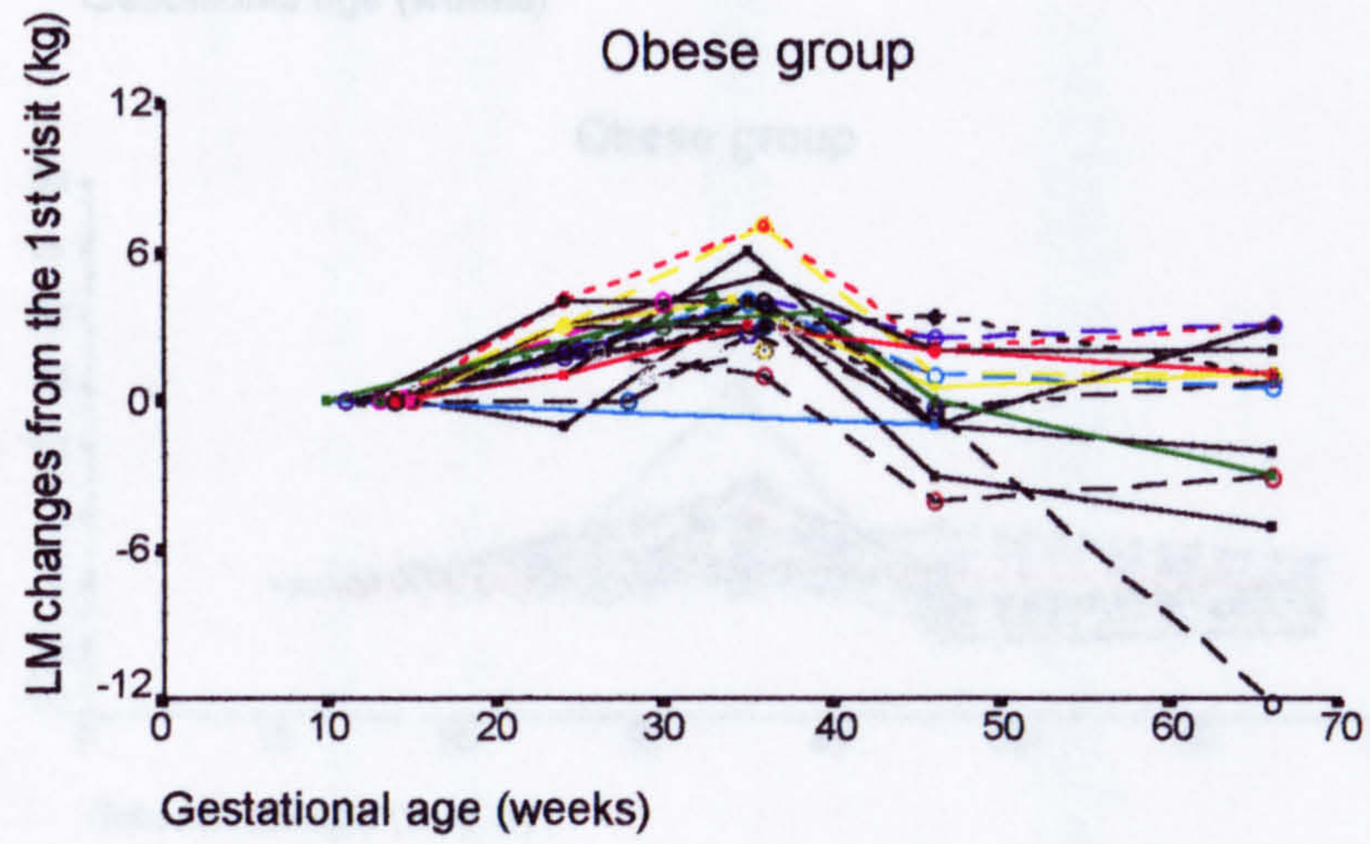


Figure 3.12. Individual plots of lean mass changes during pregnancy and postpartum in four BMI classified groups. Lean mass changes are expressed as the difference of the values measured at each stage from the first visit value.
Lean mass estimated by Bio-electrical Impedance.

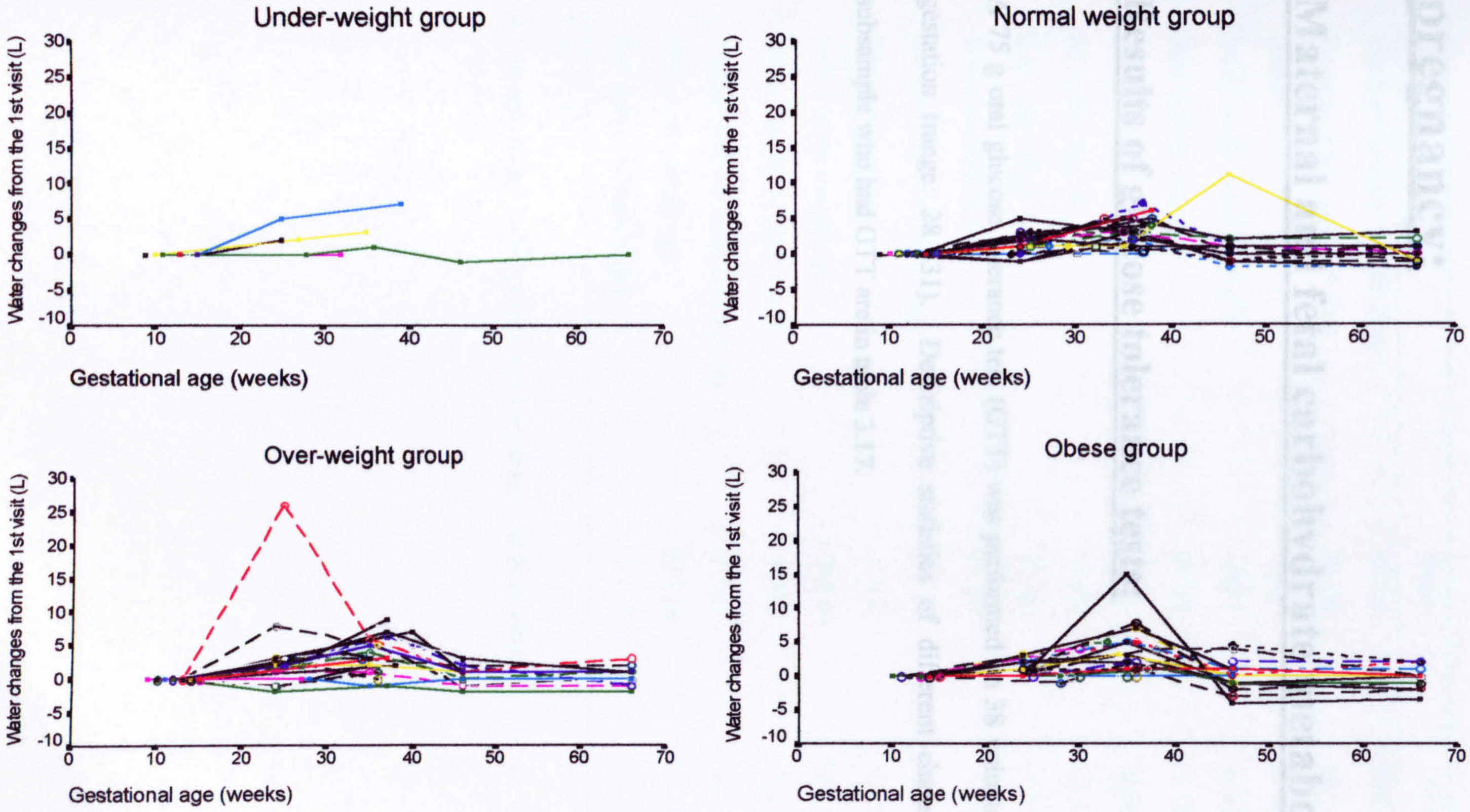


Figure 3.13. Individual plots of body water changes during pregnancy and postpartum in four BMI classified groups. Body water changes are expressed as the difference of the values measured at each stage from the first visit value.
Body water calculated by Bio-electrical Impedance.

C) Study of fetal growth in normal pregnancy:

Maternal and fetal carbohydrate metabolism

Results of glucose tolerance tests:

A 75 g oral glucose tolerance test (GTT) was performed on 38 volunteers at 29 weeks gestation (range: 28 -31). Descriptive statistics of different characteristics for the subsample who had GTT are in table 3.17.

Table 3.17. Characteristics of the subgroup volunteers who had glucose tolerance test (n=38)

	mean	SD
Early pregnancy weight (kg)	80.21	19.07
Prepregnancy weight* (kg)	75.78	18.16
Height (m)	1.63	0.06
Body mass index (kg/m^2)	30.07	6.42
Age at first visit (y)	27.13	4.29
Parity	0.95	0.66
fat mass (kg) ♣	32.29	12.42
lean mass (kg) ♣	47.90	7.05
water content (L) ♣	31.55	4.46
Length of gestation (wks)	39.16	1.42
Waist-hip ratio	0.94	0.10
Infant birth weight (g)	3508.68	548.38
Infant length at birth (cm)	51.06	2.25
Infant head circumference (cm)	37.32	1.34
Placental weight (g)	620.26	130.94
Sex ratio (female/male)	21/17	

*: n = 31

♣: As measured by Bio-electrical Impedance at first visit.

Based on WHO diagnostic criteria (WHO 1985), there were two cases of impaired glucose tolerance in this study group (5%). There was also one case of abnormal glucose tolerance (fasting glucose: 5.5 mmol/l and 2 hrs value: 12.7 mmol/l) who was referred to the antenatal ward in Northern General Hospital for glucose profiles. The results were normal, no treatment was prescribed. She remained in the study.

The other two subjects who had impaired GTT, were referred to the diabetic team in our hospital (including diabetic specialist, dietitian and obstetricians) for special care. They were prescribed a diabetic diet (high fibre, low fat and high complex carbohydrate). These two subjects were also excluded from the analysis in this part of the study.

Table 3.18. shows the correlation between fasting glucose and maternal obesity and fat distribution indices. Fasting glucose level is significantly correlated to each of the indices. However, multiple linear regression (stepwise method) with fasting glucose as the dependent variable and maternal BMI, WHR, fat mass and lean mass in early pregnancy (as independent factors), revealed that only fat mass is independently related to fasting glucose ($r = 0.54$, $p = 0.0004$).

Table 3.18. Correlation between maternal fasting glucose level with maternal anthropometry (n=38)

	BMI	Waist/hip	Fat mass	Lean mass
r	0.51	0.36	0.54	0.52
p	0.001	0.02	0.0001	0.001

Comparison of the groups:

The subjects were divided to three groups of under & normal weight, over-weight, and obese groups, based on their early pregnancy BMI. In this part of the study, there were no significant differences between the three groups in respect to age, parity, gestational age, smoking, WHR (index of fat distribution). Table 3.19 shows the values for fasting level, and 2 hrs level, after 75g oral glucose load in all subjects and in each BMI group.

Table 3.19. Fasting and 2 hrs glucose concentration after 75 g glucose tolerance test in groups of under & normal weight, over-weight and obese pregnant women.

Groups (n)	fasting values (mmol/l)	2 hrs values (mmol/l)
Normal & under weight (7)	4.13 ± 0.16*	6.03 ± 0.59
Over-weight (9)	4.63 ±0.59	6.79 ± 0.55
Obese (19)	4.96 ± 0.61*	6.26 ± 1.30
Total (35)	4.71 ± 0.62	6.36 ± 1.06

*: indicates a significant difference.

As is apparent in the above table the fasting blood glucose concentrations rise with increasing BMI. Fasting blood glucose, 2 hrs values and the difference between the two values (fasting and 2 hrs postprandial) were compared between the groups using one way ANOVA. The results show that fasting glucose level is significantly higher in the obese group than in the normal & under weight group.

To investigate the effect of fat distribution on the glucose tolerance test, the subgroup (n=38) were then divided to two groups based on WHR at first visit (13 weeks gestation); lower body: WHR < 0.9 and upper body: WHR ≥ 0.9. Using independent sample t-test, fasting blood glucose , 2 hrs postprandial blood glucose levels, the difference between the two values were compared between these two groups. Fasting blood glucose and AUC (0-2) were significantly higher in the subjects with upper body fat distribution than in the other group (table 3.20).

Table 3.20. The effect of fat distribution (Waist/hip ratio) on glucose tolerance in early pregnancy; (WHR<0.9: lower body, WHR≥0.9: Upper body fat distribution) (n=38).

fat distribution	fasting level*	2 hrs values	diff (0 - 2hrs)
upper body (24)	4.86 ± 0.68	6.53 ± 1.22	-1.65 ± 1.32
lower body (14)	4.43 ± 0.38	6.03 ± 0.62	-1.60 ± 0.75

*: significantly different ($p<0.05$)
diff (0- 2hrs): difference of fasting glucose and 2 hrs postprandial values.

Glucose metabolism in relation to fetal growth:

Multiple regression between each of the measured characteristics of the newborn at birth (weight, length and head circumference) as independent factors with each glucose indicators (dependent), revealed that only fasting glucose is related significantly to the birth weight and length. The Spearman correlation coefficients between maternal fasting

glucose and birth weight, length, head circumference, placental weight appear in table 3.21. Fasting glucose was highly significantly correlated to birth weight and placental weight. Its significant correlation with baby's length was borderline.

Table 3.21. Correlation between maternal fasting glucose level with newborn anthropometry (numbers in brackets).

	birth weight (38)	length (34)	head cir. (34)	placental wt (38)
r	0.42	0.34	0.24	0.38
p	0.009	0.05	0.18	0.02

To find out which compartment of body composition (in early pregnancy) might predict the fasting glucose level, a multiple regression (Backward) was performed between fasting level as the dependent factor and each compartment of the body (measured by BI: fat mass, lean mass and body water) as independent factors. Fat mass proved to be the only significant predictive factor of the three (table 3.22).

Table 3.22. Backward multiple regression model to predict fasting blood glucose. Body water, lean mass and fat mass were considered as independent factors.

Variable	Coefficient b	se (b)	Beta	p
fat mass	0 .029645	0.007360	0.574128	0.0003
(Constant)	3.724664	0.260197	14.315	

Fetal insulinisation: measurements of cord insulin and C-peptide

Cord blood samples were collected from 62 newborns whose mothers were studied longitudinally during pregnancy (two subjects who had impaired GTT were excluded from the analyses). C-peptide levels were measured in 60 samples but insulin levels were only assessed in those which were not haemolysed (n=48). Physical characteristics of these subjects are in table 3.23.

Table 3.23. Characteristics of the volunteers whose cord blood samples were studied (n=60, otherwise indicated in brackets)

	mean	SD
Early pregnancy weight (kg)	72.87	18.02
Prepregnancy weight (kg) (n=48)	68.78	17.21
Height (m)	1.63	0.06
Body mass index (kg/m^2)	27.24	6.38
Age at first visit (y)	26.34	4.58
Parity	0.79	0.75
fat mass (kg) ♣	26.48	12.59
lean mass (kg) ♣	46.31	6.34
water content (L) ♣	30.67	3.81
Length of gestation (wks)	39.27	1.33
Waist-hip ratio	0.91	0.09
Infant birth weight (g)	3518.31	480.96
Infant length at birth (cm) (n=46)	51.07	2.29
Infant head circumference (cm) (n=46)	37.19	1.43
Placental weight (g)	622.26	118.64
Sex ratio (female/male)	29/31	

♣: As measured by Bio-electrical Impedance at first visit.

Table 3.24. C-peptide and insulin concentration in groups of under & normal weight, over-weight and obese pregnant women.

Groups	C-peptide (n) (μmol/l)	Range	Insulin (n) (μU/l)	Range
Normal & under weight	0.07 ± 0.05 (n=28)	(0.01- 0.19)	1.61± 2.75 (n=23)	(0.20 -12.00)
Over-weight	0.10 ± 0.05 (n=13)	(0.03 - 0.21)	0.84 ± 0.96 (n=10)	(0.20 - 2.90)
Obese	0.10 ± 0.06 (n=19)	(0.03 - 0.23)	5.28 ± 5.14* (n=13)	(0.20 -15.00)

*: significantly higher than the other two groups ($p<0.05$)

As table 3.24 shows, C-peptide levels are not significantly different between our three BMI groups. However, insulin level in the obese group is significantly higher than in the other two groups (over-weight group and the group including normal & under weight).

As we expected, a significant correlation was observed between the two measured hormones in the cord blood; Insulin and C-peptide levels ($r=0.41$, $p=0.002$). Neither of them, however, were significantly correlated to birth weight, length, head circumference or placental weight. On the other hand cord c-peptide and insulin were highly significantly associated with maternal BMI, fat and lean mass at early pregnancy. The results of Spearman correlation between C-peptide and insulin levels with each of the new born and maternal anthropometric indices are shown in the following table:

Table 3.25. Correlation coefficientsφ of Cord blood C-peptide and Insulin level with individual infant and maternal anthropometric characteristics.

	New-born			
	Birth weight	Length	Head cir.	Placental weight
C-peptide (n=48*)	0.13 (0.32)	0.08 (0.61)	0.02 (0.87)	0.14 (0.26)
Insulin (n=34)	0.08 (0.57)	-0.20 (0.26)	-0.18 (0.32)	0.09 (0.55)
	Maternal early pregnancy			
	BMI	Waist/hip	Fat mass	Lean mass
C-peptide (n=60)	0.33 (0.008)	0.21 (0.09)	0.31 (0.01)	0.24 (0.06)
Insulin (n=48)	0.44 (0.002)	0.20 (0.18)	0.42 (0.003)	0.39 (0.006)

φ: correlation coefficients (p values in brackets)
*: Number of compared cases of birth weight was 60.

Multiple regression analysis (forward method) on each of cord insulin and C-peptide (individually) as the dependent factor and maternal early pregnancy LM and FM mass as explanatory variables; revealed that maternal fat mass explains the variation in cord insulin (p=0.002) and C-peptide (p=0.02) rather than the lean mass.

Birth weights were corrected for gestational age using birth centiles (Gairdner and Pearson 1985). Using birth weight centiles did not improve the correlation of the variable with either cord insulin or C-peptide.

In this part, infants were grouped according to their birth weight centiles into three groups: small for gestational age (SGA); defined as birth weight below 10th percentile for gestational age, average for gestational age (AGA); between the tenth and ninetieth percentiles and large for gestational age (LGA); above the ninetieth percentile. Although in the whole group, there was no significant correlation between birth weight (birth centiles) and either of cord insulin or C-peptide level, comparing the three birth centile

groups (using one way ANOVA) revealed that LGA babies have significantly higher levels of cord insulin compared to AGA babies. C-peptide levels were not significantly different between these three groups of infants. The values for mean, median and ranges for each classified centile are reported in table 3.26.

Table 3.26. Descriptive of cord insulin and C-peptide for different birth weight centiles.

Birth weight centile	Cord insulin (μU/l) Mean (range)	Cord insulin (μU/l) Median (n)	C-peptide (μmol/l) Mean (range)	C-peptide (μmol/l) Median (n)
≤10	2.00 (0.20 -3.80)	2.90 (5)	0.10 (0.05 - 0.21)	0.09 (6)
11-89	1.31 (0.20- 7.60)*	0.20 (27)	0.07 (0.01- 0.19)	0.07 (31)
≥90	4.46 (0.20 -15)*	1.10 (16)	0.10 (0.01- 0.23)	0.08 (23)

*: indicates significant difference ($p<0.05$)

The Box and Whisker plot distribution for birth weight centiles against C-peptide are shown in figure 3.14 and against insulin in figure 3.15. As it is apparent in the case of insulin; for babies with birth weight under 10th centile the distribution was skewed towards the left indicating a high number of low values. For babies with birth weight above the 90th centile the distribution was skewed toward the higher values. In case of C-peptide, there is a similar trend only for babies above 90th centiles. There was no clear lack of overlap between these two cord hormone levels for various centile groups that we used.

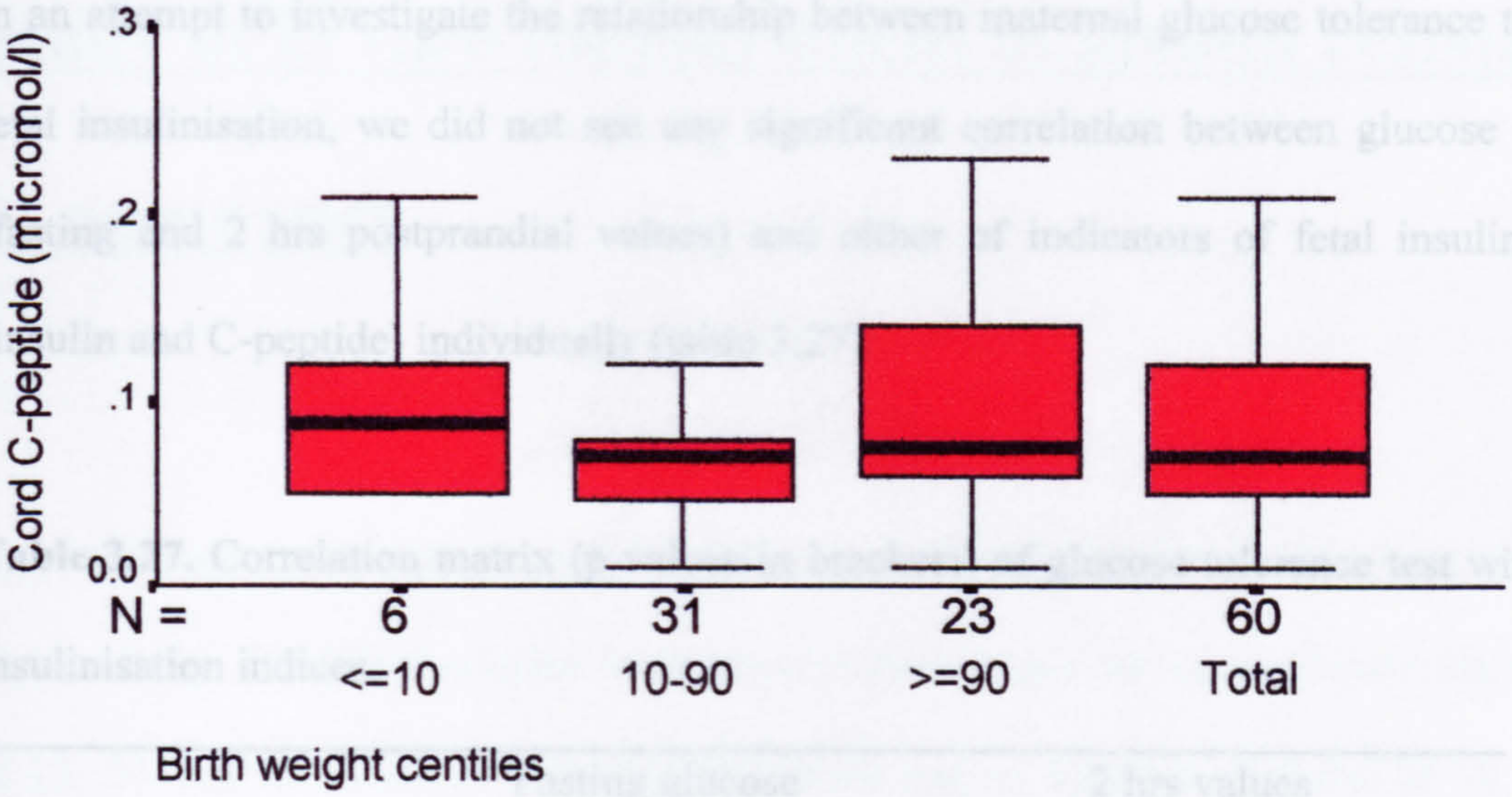


Figure 3.14. The relation between cord C-peptide (μmol/l) and birth weight centile for gestational age. Boxes contain 50% of values falling between 25th and 75th percentiles. Whiskers (I): lines that extend from the boxes presenting 10th-90th centile. Horizontal lines (-) indicate median.

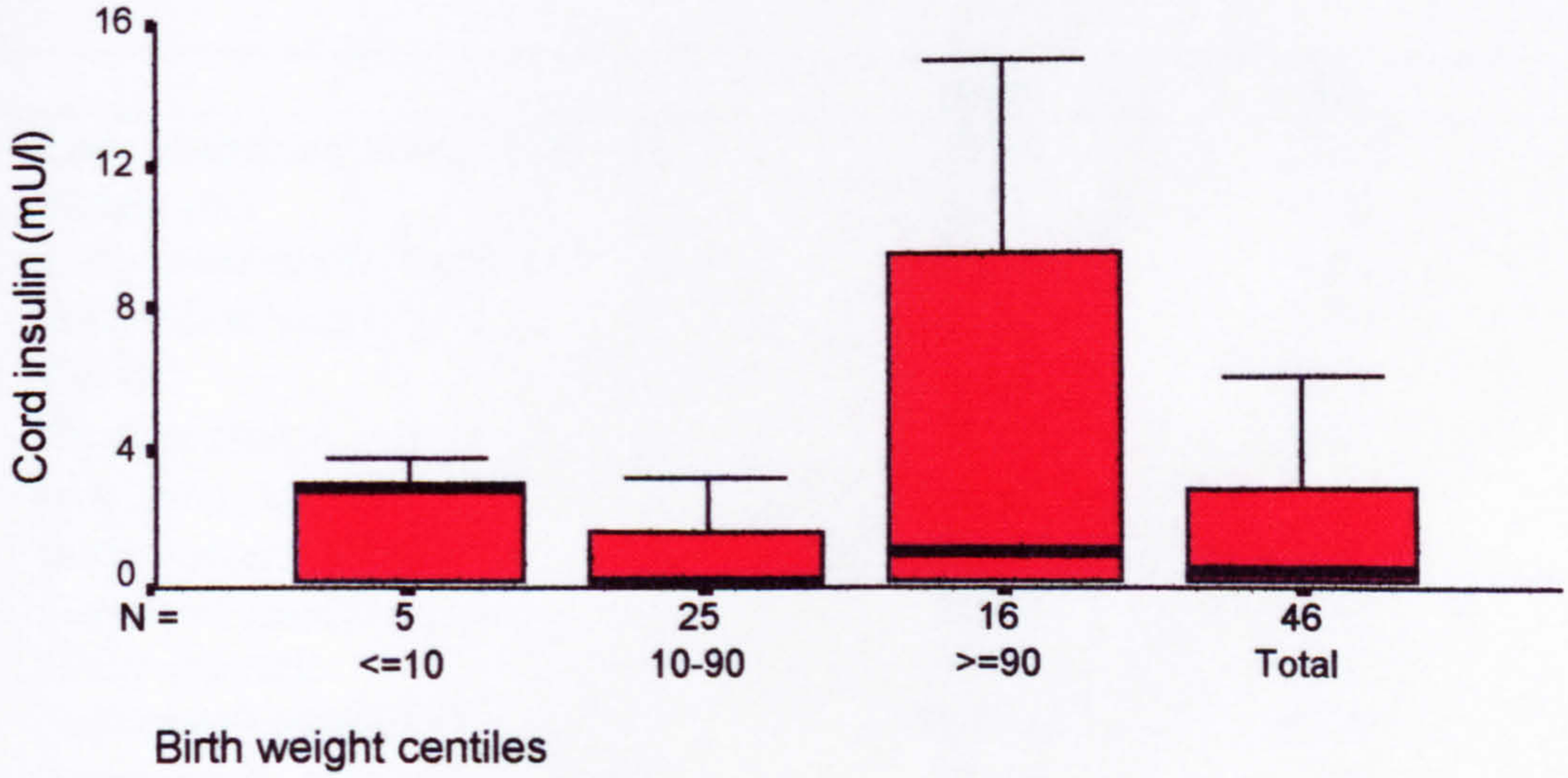


Figure 3.15. The relation between cord Insulin (μU/l) and birth weight centile for gestational age.

In an attempt to investigate the relationship between maternal glucose tolerance test and fetal insulinisation, we did not see any significant correlation between glucose indices (fasting and 2 hrs postprandial values) and either of indicators of fetal insulinisation (insulin and C-peptide) individually (table 3.27).

Table 3.27. Correlation matrix (p values in brackets) of glucose tolerance test with fetal insulinisation indices.

	Fasting glucose	2 hrs values
C-peptide (25)	-0.42 (0.84)	-0.005 (0.99)
Insulin (19)	0.02 (0.92)	-0.31 (0.21)

Study of *in vitro* measurement of insulin resistance in pregnancy

In vitro insulin resistance was studied using serum blood samples of 12 pregnant women in our study. The blood samples were taken at three stages during pregnancy: on average at 13, 24 and 35 weeks gestation. There were only 6 samples available at 24 weeks, mainly because of haemolysis. Summary characteristics of these subjects are presented in table 3.28.

Table 3.28. Characteristics of the volunteers who were studied for insulin resistance (n=12, otherwise indicated in brackets).

	mean	SD
Early pregnancy weight (kg)	73.14	13.12
Height (m)	1.61	0.06
Body mass index (kg/m^2)	28.05	4.61
Age at first visit (y)	28.09	4.50
Parity	1.60	1.58
fat mass (kg) ♣	27.27	8.87
lean mass (kg) ♣	45.82	5.95
water content (L) ♣	30.55	4.06
Length of gestation (wks)	39.00	1.61
Waist-hip ratio	0.96	0.06
Infant birth weight (g)	3504.55	596.95
Infant length at birth (cm) (n=9)	51.19	1.76
Infant head circumference (cm) (n=9)	36.96	1.12
Placental weight (g)	642.09	169.36
Sex ratio (female/male)	7/5	

♣: As measured by Bio-electrical Impedance at first visit.

The basal mean glucose uptake (nmols mg^{-1} protein 10 min^{-1}) in the presence of maternal sera with and without insulin stimulation at each stage of pregnancy are as follows:

Mean glucose uptake	13 weeks (n=11)	24 weeks (n=6)	35 weeks (n=12)
Basal	21.30	24.61	23.47
Insulin stimulated	27.03	31.39	29.53

Figure 3.16 represent the changes in insulin responsiveness of myofibres in the presence of pregnant sera. Although a proportionate reduction of insulin sensitivity is apparent in this diagram, the differences are not statistically significant. Only the difference in percentage increase of insulin stimulated glucose uptake from 13 weeks to 24 weeks was borderline with p value of 0.07, the others were not significant.

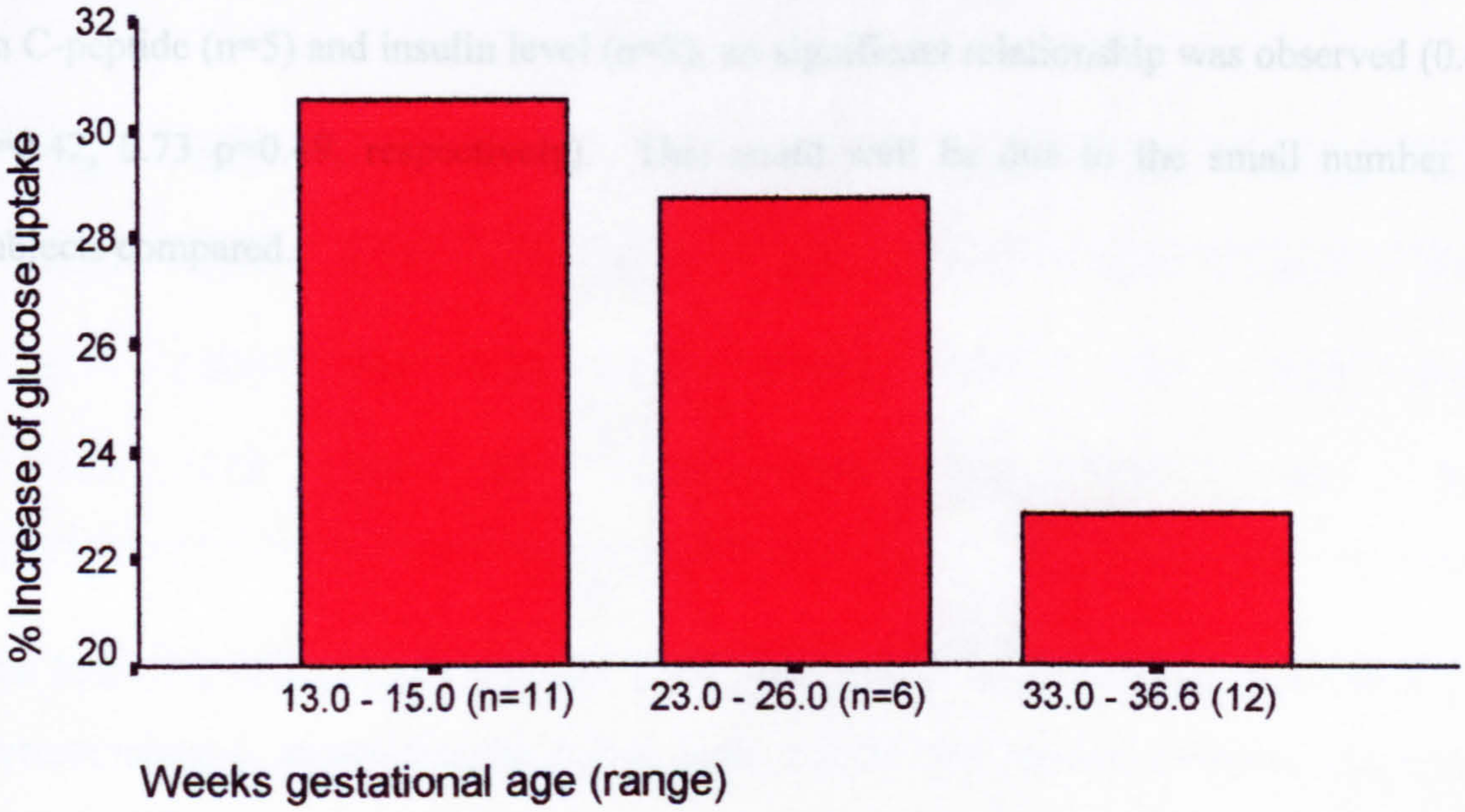


Figure 3.16. Diagrammatic presentation of insulin resistance changes throughout gestation; presented as % increase of glucose uptake above the basal level, with insulin stimulation in mouse myofibres in presence of pregnant sera. (% increased G uptake above the basal level = ((stimulated G uptake-basal G uptake)/basal G uptake) × 100).

The difference between percentage increase of stimulated glucose uptake at early and late pregnancy (the insulin resistance index) was compared with certain maternal and infant anthropometric characteristics, borderline significant difference is seen between the index and infant length only. The results are in table 3.29.

Table 3.29. The correlation coefficient of in vitro index of insulin resistance with maternal and infant anthropometry.

	Maternal (early pregnancy)			Infant (at birth)		
	BMI	Waist/hip	Fat mass	Birth wt	Length	Head cir.
r	-0.16	-0.34	-0.03	0.32	0.65	0.29
p	0.64	0.30	0.93	0.33	0.05	0.44

The relationship of the insulin resistance index was also assessed with the available data on C-peptide (n=5) and insulin level (n=3), no significant relationship was observed (0.47 p=0.42, 0.73 p=0.49, respectively). This could well be due to the small number of subjects compared.

The effect of maternal anthropometry and metabolism on fetal growth

Table 3.30 shows the relationship between infant birth weight and various maternal factors including BMI.

Table 3.30. Matrix correlation coefficients of infant birth weight (IBW) with maternal early pregnancy age, body mass index (BMI), height (MH), maternal fasting blood glucose (FBG), fat mass and lean mass by the two methods of skinfold (FMv) and Bio-electrical Impedance (FM_{BI} and LM_{BI}), gain in fat and lean mass by the two methods (FMGv, FMG_{BI}), gestational weight gain (WG), and prepregnancy weight (PPW).

	Age	BMI	MH	FBG	FMv	FM_{BI}	LM_{BI}	FMGv	FMG_{BI}	WG	PPW
r	0.13	0.34	0.23	0.42	0.37	0.35	0.42	0.17	0.17	0.23	0.26
p	0.16	0.0001	0.01	0.01	0.0001	0.0001	0.0001	0.13	0.12	0.04	0.02
n	123	123	123	38	123	123	123	80	80	80	85

The following tables (table 3.31 and 3.32) also present the correlation coefficients and corresponding p values for the infant birth weight and various maternal and infant characteristics.

Table 3.31. Spearman correlation coefficient between birth weight and maternal parity, infant gestational age and sex.

	Maternal parity	Gestational age	Infant sex (1:male, 2:female)
Infant birth weight (r)	0.22	0.36	-0.17
p	0.01	0.0001	0.07
n	123	123	123

Table 3.32. Correlation coefficient between maternal weight gain and infant birth weight in different BMI groups.

	Normal weight	Over-weight	Obese
r	0.33	0.44	0.17
p	0.07	0.04	0.43
n	29	23	25

Finally the interrelations between new-born anthropometry (weight, length and head circumference) with indices of fetal insulinisation as well as maternal anthropometry and lifestyle habitus were studied. Multiple linear regression analysis (forward method) were performed on birth weight as the dependent variable and other independent factors, including: maternal fat mass, lean mass in early pregnancy and weight gain during pregnancy, maternal lifestyle (smoking and parity) and fetal insulinisation indices (cord insulin and C-peptide). Gestational age and sex of the baby were also taken into account (since previous studies suggested these factors are individually important in relation to fetal growth).

As a result, lean mass in early pregnancy turned out to be the most significant predictor of Birth weight (table 3.33 a.). Looking at the coefficient of determination (R^2), lean mass explained 13% of variation in birth weight.

Table 3.33 a. Multiple linear regression analysis of birth weight (n=36).

	Regression coefficient (B)	SE B	R^2	Adjusted R^2	Sig
Lean mass (kg)	26.35	11.65	0.13	0.10	0.03
Constant	2250.20	546.20			0.0002
Excluded variables at the level of 0.05 significance					
	Partial correlation				Sig
Weight gain (13-36 wks)	0.33				0.05
Smoking	-0.05				0.78
Infant's sex	-0.28				0.10
Gestational age	0.30				0.08
Parity	-0.04				0.80
Cord C-peptide	-0.15				0.40
Cord Insulin	-0.10				0.56

Table 3.33 b. Multiple linear regression analysis of birth weight, maternal fasting glucose level added to the previous independent variables (n=19).

	Regression coefficient (B)	SE B	R^2	Adjusted R^2	Sig
Lean mass (kg)	31.66	14.18	0.23	0.18	0.04
Constant	1976.50	676.34			0.01
Excluded variables at the level of 0.05 significance					
	Partial correlation				Sig
Weight gain (13-36 wks)	0.16				0.51
Gestational age	0.16				0.52
Cord C-peptide	-0.04				0.88
Maternal fasting glucose	-0.16				0.53
Cord Insulin	-0.07				0.77
Parity	-0.05				0.84
Smoking	-0. 04				0.87
Infant's sex	-0.46				0.06

In table 3.33 b. (n=19) maternal fasting glucose was added to the independent variables presented in the previous analysis. Lean mass was still the most significant predictor of birth weight with 10% increase in the coefficient of determination (R^2: 23%>13%).

The same process was performed for the length of the baby at birth as the dependent variable and the above mentioned variables as independent factors (in table 3.33 a). Lean mass again came out to be the most significant denominator (table 3.34) (n=30). However in the case of head circumference (as the dependent variable) with same factors, fat mass in early pregnancy and also baby's sex and gestational age were shown to have the highest contribution to the outcome variable (table 3.35).

Table 3.34. Multiple linear regression analysis of new-born length (n=30)

	Regression coefficient (B)	SE B	R^2	Adjusted R^2	Sig
Lean mass (kg)	0.15	0.06	0.21	0.18	0.01
Constant	43.88	2.65			0.0001
Excluded variables at the level of 0.05 significance					
	Partial correlation				Sig
Weight gain (13-36 wks)	0.30				0.12
Gestational age	0.33				0.09
Cord C-peptide	-0.01				0.97
Cord Insulin	-0.17				0.40
Parity	-0.28				0.14
Infant's sex	-0.37				0.06
Smoking	0.001				0.10

Table 3.35. Multiple linear regression analysis of new-born head circumference (n=30).

	Regression coefficient (B)	SE B	R^2	Adjusted R^2	Sig
Baby's sex*	-1.16	0.45	0.15	0.12	0.02
Gestational age (wks)	0.52	0.18	0.29	0.24	0.008
Lean mass (kg)	0.08	0.03	0.41	0.34	0.03
Constant	14.80	7.56			0.06
Excluded variables at the level of 0.05 significance					
	Partial correlation				Sig
Weight gain (13-36 wks)	0.09				0.64
Cord C-peptide	-0.29				0.15
Cord Insulin	-0.22				0.29
Parity	0.17				0.42
Smoking	0.18				0.40

*: Baby's sex: Female: 2, Male:1.

Adding fasting glucose to the list of independent variables, in the case of infant's length, maternal early pregnancy lean mass was still the most sinificant predictor (B: 0.19, Constant: 47.78, R^2=0.39 and Sig=0.007). In the case of head circumference, however, none of the variables remained significant.

It should be noticed that in all of these regression models, fat mass has been deleted from the computer analyses and that was stated to be because of its correlation with lean mass.

Follow up of infant growth up to 6 months post partum:

Weight, head circumference and length of the babies who were followed by 6 month postpartum are presented in the following table.

Table 3.36. Anthropometric characteristics of infants during the postpartum period (Birth weight: 3532.02 ± 540.75 g) (n=48).

	1 days pp	6 wks pp	6 mo pp
Weight (gr)	3361.51 ± 516.38	4686.83 ± 691.90	7610.30 ± 1431.40
Length (cm)	50.94 ±1.97	56.45 ± 2.62	67.73 ± 2.52
Head cir. (cm)	37.12 ±1.42	40.42 ± 1.18	45.58 ±1.35

The ratio of breast to bottle feeding in this population was 31/17 at birth, 26/21 at 6 weeks and 4/43 at 6 month postpartum. The pattern of breast feeding was not significantly different between the BMI groups at each stage.

Splitting this group based on maternal BMI, there were no significant differences in changes in weight, head circumference and length of the babies of normal, over-weight and obese group, up to 6 weeks or 6 month postpartum.

To have an insight into early infant growth (from the available data), a multiple regression analysis was performed using infant weight gain as outcome variable and maternal factors (body composition and lifestyle habitus) as independent factors. Sex of

the baby, gestational age and type of feeding were also included in the list of independent factors.

No parameters were picked up for the infant weight gain up to 6 wks. Weight gain up to 6 months was however only significantly correlated with sex of the baby as the male infants gained more weight (table 3.37)

Table 3.37. Multiple linear regression analysis of weight gain up to 6 mo postpartum (female: 2 and male:1) (n=48).

	Regression coefficient (B)	SE B	R^2	Adjusted R^2	Sig
Baby's sex	-889.30	354.63	0.12	0.10	0.02
Constant	5429.30	589.26			0.0001
Excluded variables at the level of 0.05 significance					
	Partial correlation				Sig
Gestational age (wks)	0.09				0.54
Lean mass (kg)	-0.04				0.77
Parity	0.01				0.94
Smoking	-0.09				0.57
Breastfeeding at 6 mo pp	-0.01				0.95

Chapter 4

Discussion

A) Comparison of the methods:

(Skinfold thickness measurements Vs Bio-electrical impedance)

Because of the limited knowledge on the actual changes occurring in pregnancy and the limitations of the methodology of this subject, it is difficult to judge the validity of the methods. Skinfold thicknesses have been previously used in a number of studies to estimate maternal body fat changes during pregnancy and postpartum. The most

distinctive one is the study by Forsum et al. (1989) in which gestational fat changes were studied longitudinally during the reproductive cycle in 29 Swedish women, using TBW, TBK and STM. They also developed new equations for the estimation of percent body fat for different stages of pregnancy from STM. It is unwise to extrapolate too freely from a study of 29 women but interestingly, they found similar results by each method. They concluded that due to the basic alterations occurring in fat free mass during pregnancy, each of the current methods of body composition measurements is open to question. Bearing in mind that these method related limitations, it seems to be appropriate for population studies to use the simpler methods which are practically more acceptable.

Bio-electrical impedance is also a convenient method of measuring body composition which is associated with less observer error than STM. BIA is known as an accurate method of measuring body water. It has not been validated against other relatively standard methods during pregnancy. Since both STM and BIA are convenient methods of body composition measurement, it is of interest to compare these methods, to see how pregnancy would affect on their results.

The fact that the two methods were highly correlated, is not enough to ensure the comparability of the results obtained by them. The lowest limits of agreement were seen in early pregnancy compared to the later stages. The least difference in the mean and the limits of agreement are at 6 months postpartum. This would indicate that in the non-pregnant state these two methods are more similar in estimating body fat. Nevertheless, the limits of agreement are still too wide to claim that these two methods can be alternatives to each other.

The results of these comparisons show the widely different findings for body fat changes during pregnancy, when different methods are used. This is consistent with the previous findings of Lederman et al. (1993) who found a variety of figures for fat changes during pregnancy using different methods. In their study of 65 pregnant women from 14 weeks to 37 weeks gestation, they reported a net fat gain of 0.3 kg by TBW and 8.1 kg using TBK. These differences may explain discrepancies between studies that use various methods. They also indicate that estimated dietary needs for pregnancy will be influenced to a large extent by the method selected to determine body composition changes.

B) Body composition and anthropometric measurements during pregnancy and postpartum

In our previous study on the distribution of obesity in the women attending Northern General Hospital in Sheffield (aged 15 - 43), the distribution of BMI was as shown in figure 4.1 (Soltani 1993). This compares to figures from the UK National Survey (White et al 1993), in which the mean BMI was estimated to be 25.4 kg/m^2, and the BMI distribution was as follows: 12% UW, 55% NW, 22% OW and 11% OB. The only small difference was in the higher proportion of OB women and lower proportion of UW. This may reflect the local social class and nutritional status differences. A significant correlation between BMI and social class was reported by White et al. (1993).

The mean height of our current study population was 1.62 m and the mean prepregnancy weight (n = 85) was 67.5 kg. The mean height and weight of women in health survey for England was reported to be 1.61 m and 65.9 kg respectively.

The mean BMI in our study, in which some women were selected as obese, was 26.95 including 7% UW, 40% NW, 25% OW and 28% OB.

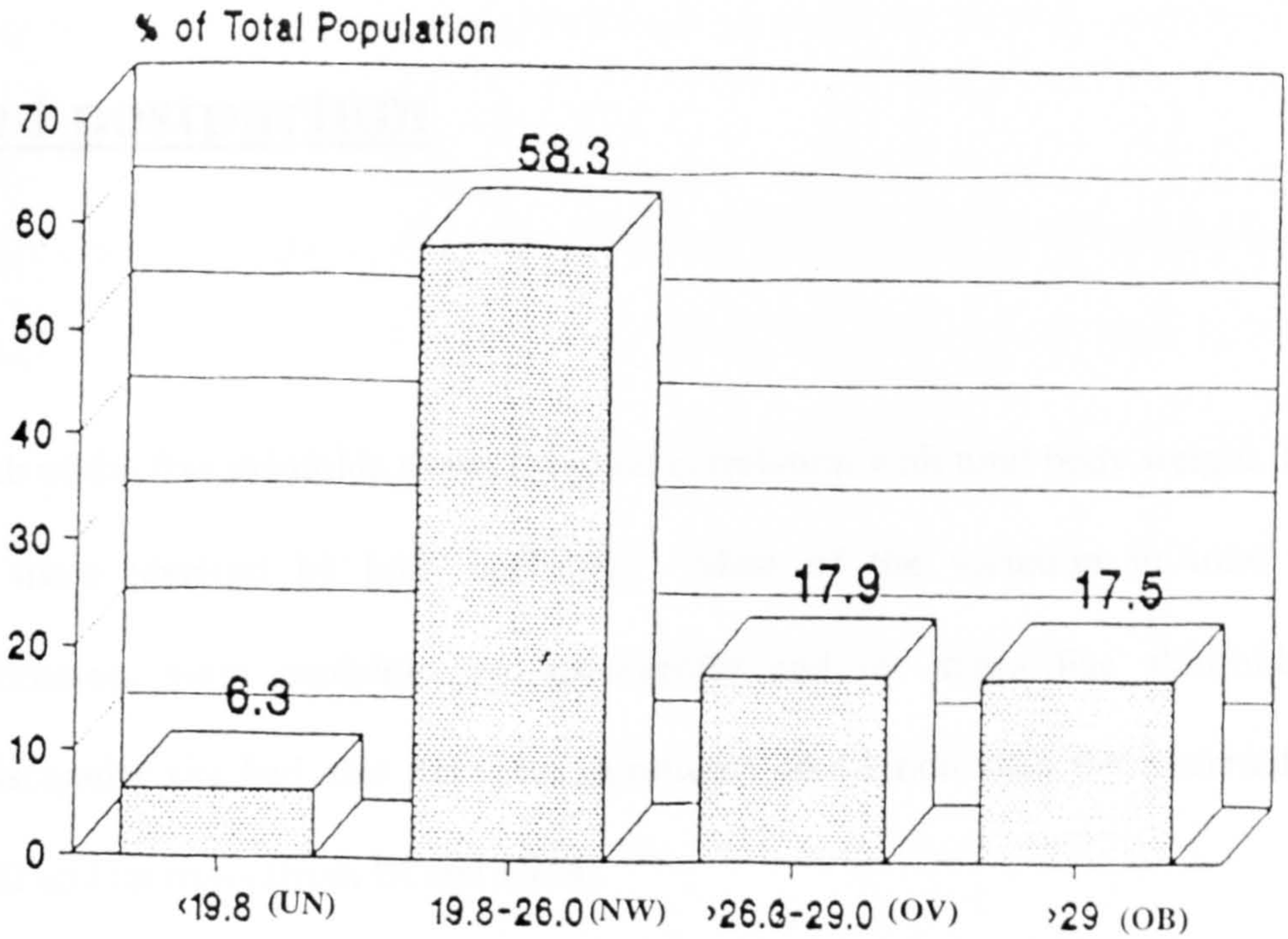


Figure 4.1. Bar charts presenting distribution of BMI groups in women attending Northern General Hospital in Sheffield (n=85) (The results of the previous study, Soltani 1993).

Skinfold thickness changes during pregnancy and postpartum

Each of the five skinfolds showed a good correlation with total body weight, BMI and fat mass (derived by both methods). Most of the variation in total skinfold thicknesses were explained by subscapular and /or supra-iliac skinfolds. The subscapular site had also the most significant correlation with the maternal weight, BMI and fat mass (from BI and STM).

Skinfold thicknesses at all sites (except for biceps) increased relatively rapidly from 15 weeks to 24 weeks gestation and thereafter the rate of increment reduced to a lesser extent up to 36 weeks gestation. Like Taggart et al. (1967) and Forsum et al. (1989), the results of our study also show that the skinfolds during pregnancy have a tendency to increase at central site rather than peripheral sites. Mid-thigh was an exception. It increased considerably from 13 to 36 weeks gestation, but of course may be influenced by the presence of dependent oedema.

Triceps and supra-iliac skinfolds decreased by 6 weeks postpartum, the former one started to increase afterwards while the latter decreased even further. This trend of change in triceps was also observed by Forsum et al. (1989).

The biceps skinfold changed in a different magnitude from the other sites through pregnancy and postpartum. The rate of increase in the biceps was higher in later stage of pregnancy (24 - 36 wks) and at the postpartum period it still carried on increasing

up to 6 weeks and 6 month postpartum. While triceps and biceps both increased from 6 weeks to 6 month postpartum, the other three skinfolds showed a clear reduction in this period.

The sudden reduction in the supra-iliac and triceps from 36 weeks gestation to 6 weeks postpartum might partially be explained by the loss of extra fluid retained subcutaneously. It is however difficult to explain these changes. If it was a simple relationship to clearance of interstitial fluid, a similar pattern should have been observed at mid-thigh skinfold. We could not see this reduction in the other sites by 6 weeks postpartum. There is a possibility that either the changes in subcutaneous fat was that much to cover the changes in the extracellular water retained cutaneously or the loss of subcutaneous fluid happened in an earlier stage of postpartum before our measurements. Dynamic changes in the early puerperium in fat deposition may have resulted in these contradictory observations.

In total, at 6 weeks or 6 months postpartum, none of the measured skinfolds returned to the values measured at 13 weeks of pregnancy. This differs from the findings of Taggart in which most of skinfolds had reverted close to their size at early pregnancy (at 10 weeks).

In summary:

If 80% of fat tissue is stored subcutaneously (Sohlström et al. 1993), the measurements of skinfolds in our study does confirm what is believed that pregnancy is accompanied by a significant storage of fat.

If the skinfold measurements can be said to reflect relative changes in adipose tissue deposition there is a definite regional rearrangement in pregnancy. This is

demonstrated by the relatively great increases in the supra-iliac skinfold in particular. In our total population, there is a tendency to develop adipose tissue centrally, during pregnancy and sustain the pattern at postpartum.

Comparison of the pattern of skinfold changes between the BMI groups

There have been studies looking at the pattern of skinfold changes throughout pregnancy and postpartum but little information exists on the possible variation between different body types.

Our study strongly suggests that there are essential differences in the magnitude of subcutaneous fat storage and mobilisation between BMI groups. Total skinfold thickness decreases are significantly divergent between each BMI group. The least reduction was seen in the obese group and the highest was seen in the normal weight group from 36 weeks gestation to 6 months postpartum.

The individual diagrams of TSF, compared between the groups, would also show that most of the women follow a similar pattern during pregnancy and return close to original values by 6 months postpartum. Over-weight women however, show more individual variation. In both the over-weight and obese groups there was a tendency

to loose less skinfold thickness or even to expand these measurements during the postpartum period.

Despite the fact that all the BMI groups increased fairly similarly at the supra-iliac site during pregnancy, the reduction at this site at postpartum period was significantly less in the obese group than the normal weight group.

Indicators of central fat distribution (WHR and waist circumference), were reduced significantly in normal weight group from 6 weeks to 6 month postpartum while the obese group showed a significant increase in that period.

All these observations imply that pregnancy affects the pattern of fat distribution, differently, in women with higher BMI, than in normal weight women. Obese women are prone to develop their obesity further. Central deposition of the new fat depots is typical in this group.

Maternal body weight and body fat mass changes at antepartum and postpartum period

There have been many previous studies investigating maternal gestational weight gain and postpartum weight loss/or retention. Some of them also looked at the contribution of body composition to the weight changes. The extent of variation between the results of these studies is remarkable. This variability is mainly related to the differences in methodology, design of the study but biological variation may be contributing as well. Repeated studies on maternal anthropometry and body composition in different populations would give a more detailed insight to the energy requirements during pregnancy. It may also give some information on the impact of pregnancy on subsequent development of obesity and fat retention.

In our study, from 13 to 36 weeks gestation , mean maternal weight gain was 10.9 kg which is very similar to the other studies on Western women of Hytten et al. (1966), Emerson et al. (1975), Taggart et al. (1967), Pipe et al. (1979), Campbell (1983), Forsum et al. (1988). To achieve a better comparison, a graph of maternal weight gain (Hytten and Leitch 1971) was used to estimate the weight that would have been gained before and after the measurements made. On the basis of this standard, expected additional weight gains are 0.5 kg before 5 weeks, 1 kg before 10 weeks, 4 kg before 20 weeks, 1.3 kg after 37 weeks, and 0.8 kg after 38 weeks of gestation. Applying these corrections, total weight gain in our study would have been a mean of 13.2 kg.

Alternatively assuming a constant rate of weight gain to term and extrapolating backwards (from our own data), we obtained an estimation of 17.58 kg net weight gain in term pregnancy. This is in line with the study of Swedish women who gained about 19 kg over the whole course of pregnancy (Sohlström 1993). It should however be noticed that the rate of weight gain might be different after 36 weeks gestation and extrapolation based on rate of weight gain in late pregnancy (from 25 to 36 weeks gestation) may not be appropriate.

Using STM technique, fat mass gain from 13 - 36 weeks gestation, was 7 kg. This is when, we used Siri equations to convert estimated fat% to fat mass. This way the values were considerably higher than original reports by Pipe et al. (1979), Dibblee and Graham (1983), Langhoff-Roos et al. (1987) from developed countries, and also from study of 5 countries (including: Scotland, Netherlands, Gambia, Thailand, and Philippines) by Durnin (1987) (the amount of fat gains were 2.8, 4.4, 4 and also 2.3, 2, 0.6, 1.4, 1.3 kg respectively). The same method and equations were computed in those studies.

Importantly, the Van Raaij equation (another formula to convert fat% to fat mass), which allows some correction for alteration of hydration constant in maternal tissue, resulted in about 4.6 kg gestational fat gain during the course of our study.

Fat gains from industrialised countries using different techniques, are in the same range as the latter estimation of ours. Forsum et al. (1988) using TBW method, reported a fat gain of 5.6 kg through pregnancy and Sohlström et al. (1993) using MRI also reported a total of 5.5 ± 3.2 kg during a full term pregnancy.

In 1991 the panel on Dietary References Values (DRVs) of the Committee of Medical Aspects of Food Policy (Department of Health 1991) estimated the average cost of pregnancy to be approximately 70,000 kcal for a woman with a prepregnant weight of 60 kg, depositing 2 to 2.4 kg of fat during pregnancy. The estimation of fat gain during pregnancy was considerably higher (with either of the methods) in our study. This could be explained by either methodological differences or reduced energy expenditure and basal metabolic rate, reduced activity or more possibly it could be due to increased energy intake. A survey on energy intake of pregnant women and also the information of midwives to advise the patients about the dietary intake would be useful to improve the standard of health care provided for pregnant women.

From a total weight gain of 13.2 kg through whole pregnancy in our study, 34.8% was accounted for the products of conception (fetus and placenta). This was consistent with the estimation of 35% for affluent countries, compared with the 50% in less developed countries (Norgan 1992).

At 6 weeks postpartum, there was still 2.74 ± 5.01 kg weight retained above the mean at 13 weeks gestation. The fat retention in the same period was however, somewhat higher than the rate of weight; 3.55 ± 3.63. This finding is similar to the study of Forsum et al. (1988), in which 22 healthy Swedish women with mean BMI of 22.3 ± 3.50 and mean age of 28.7 ± 4.00 were longitudinally studied through pregnancy and postpartum. The methods were used to assess maternal body fat in their study were TBW and TBK. The amount of fat retention (3.59 ±3.14 kg) from prepregnancy to 6 month postpartum was higher than the retained weight (1.34 ± 3.62). This might suggest that there is an internal interchange of body compartments; fat increase and

concurrent reduction of lean body mass (or water). Nevertheless, it could well be as a result of methodological limitations associated with the conversion of absolute skinfolds to fat mass.

Another important aspect of this similarity in our findings and those of Forsum is that all the women in Forsum's study, started breastfeeding at hospital and half of them were still breastfeeding at 6 months postpartum. Conversely, in our study, there were only few women (4/48) breastfeeding at 6 months postpartum.

At 6 month postpartum, there was no significant weight retention, however, fat mass was still 2.58 ± 4.4 kg higher than 13 weeks gestation.

Although weight gain neutralised by 6 month postpartum, using skinfold thickness measurements, a substantial amount of fat retention was observed in our study. This was also confirmed by direct comparison of skinfolds (previously illustrated).

Body composition using Bio-electrical Impedence

The values of fat mass obtained by BI (7.04 ± 3.65 kg), was very close to the values from STMs with the uncorrected formula (Siri equation; 6.67 ± 3.38 kg). However, it was significantly higher than the results by STMv (4.62 ± 3.35, using Van Raaij formula to convert fat% to fat mass). Furthermore, BI overestimated fat gain in comparison with previous studies mentioned earlier, using various methods.

The Impedance method is believed to be an accurate means of assessing total body water (Lukaski et al. 1994).

The present study, showed a significant increase of 3.32 ± 2.79 L water from 13 to 36 weeks gestation. Considering the timing of the study, this is similar to the finding of Forsum et al. (1988), who reported the change from prepregnancy to delivery. She used Oxygen-18 labelled water and sampled saliva (rather than blood) to monitor the dilution of the isotopically labelled water.

Other studies (Emerson et al. 1975, Campbell 1983), reported a weight gain of 8.2 to 12.8 kg from 10 to 38 wks gestation. The estimated gestational changes in total body water in those studies ranged from 6.3 to 8.5 L. Lukaski (1994), using Bio-electrical Impedance observed an average increase of 7.2 L water, with 14.3 kg weight gain from prepregnancy to 38 weeks gestation. His finding was consistent with those

reported in cross sectional studies of human pregnancy but notably higher than our results or those in the longitudinal study of Forsum et al. (1988).

Beside the methodological variations, the inconsistency among the results of these studies may reflect the differences in experimental design.

In our study, at 6 weeks postpartum, a retention of 2.74 ± 5.01 fat mass was obvious but lean mass was reverted to the original measured values at 13 weeks gestation.

Like weight changes, at 6 months postpartum, none of these measured body compartments were different from the values measured at the first visit at 13 weeks pregnancy. This reflects an important difference for the values derived from STM.

Both methods of STM and BI are supposed to be more reliable during postpartum (Norgan 1992). Unfortunately a lack of consistency between the overall results of the two methods was observed. One (BI) indicated the complete restitution of body composition to the early pregnancy values, the other one however, showed a retention of considerable amount of fat at 6 month postpartum.

This disagreement would of course, complicate the interpretation and conclusion. Due to the nature of validation, it is difficult to exclude either of the methods. Nevertheless, we prefer the results of STM with Van Raaij formula, since some corrections are made and the results are compatible with the previous studies that used TBW, densitometry which is believed to be the Gold Standard method in the field. More research is required to resolve these methodological problems. There are no valid measuring tools to provide the criterion measure at the present time (Girandola et al. 1991).

Comparison of maternal anthropometry and body composition in different BMI groups

Individual plots of changes in total weight and body composition show that the normal weight group show a more homogenous pattern of changes while the other two groups are more variable. A similar variation was observed among the groups, in respect to total skinfold thickness. Total body water, however, seemed to change in a similar pattern in all the groups.

Although the net changes in the lean mass did not differ significantly between the groups, ΔAUC was significantly higher in the obese group than the normal weight group during pregnancy. The obese group gained more lean mass but reverted to mean values in the way that the overall net LBM changes during pregnancy were not significantly different on the same time scale from the other two groups.

In the postpartum period (at 6 months), the obese group were again different from the normal weight ones, in net fat mass changes as well as in the ΔAUC of fat mass (STM). Obese women lost almost no fat mass from 36 weeks gestation to 6 months postpartum, whereas, the normal weight group tended to revert to the values similar to early pregnancy.

The pattern of postpartum weight loss could be variable. The general observation is that in association with delivery an average of 4.5-5.8 kg weight is lost. This weight loss is attributed to the delivery of the infant and placenta, amniotic fluid and blood

loss. During the first three days, some weight gain is typical. This is mainly related to the hormonal changes (adrenocortical hormone and arginine vasopressin associated with the stress of the labour) which lead to the retention of water and sodium (Blackburn and Loper 1992). By the end of the first week, 2 -3 kg weight loss is noted, which is mainly secondery to diuresis (Cunningham et al. 1993).

In many midwifery and obstetrical textbooks, it is assumed that if women gain 11.3 - 13.5 kg, they will return to their prepregnancy weight by 6 to 8 weeks postpartum. In our study however, women weighed about 2 - 3 kg heavier than in early pregnancy at this stage. Whatever the pattern of changes in the early puerperium, the greatest weight loss occurs in the first 3 month of postpartum. After 3 months a steady loss of weight may be observed (Sadurskis et al. 1988). After 9 months, no changes in BMI have been found other than normal weight gain associated with ageing (Tooke Crowell 1995).

Factors affecting postpartum weight loss are reported to be maternal age, parity, prepregnancy weight, weight gain during pregnancy, marital status, length of lactation, exercise and the mother's desired weight (Tooke Crowell 1995). In our study, the original BMI did not have any effect on the postpartum weight changes. However, in the postpartum period, obese women were less likely to lose the extra fat gained during pregnancy. Therefore, although things look settled on the surface (reversal of weight gained), there is always a possibility, in particular for obese mothers to retain extra fat with each pregnancy. Excess fat leading to further obesity can have serious hazards including; hypertension, cardiovascular disease, diabetes mellitus, elevated blood lipids, gallbladder disease, arthritis, and an increase in the incidence of endometrial, cervical, ovarian and breast cancer (Pi- Sunyer 1991). Considering these

risks and also other psychological dissatisfaction of women at postpartum with their body size, would warn the professionals to take this matter more seriously.

The Institute of Medicine, in 1990, published its recommendation for weight gain during pregnancy based on pregravid BMI (Under-weight: 12.5 kg, normal weight: 11.5 - 16 kg, over-weight: 7 - 11.5 kg and obese: 6 kg). To what extent these recommendations influence practice is not known. Health care providers as well as the women in their care (mothers) need to have a deep and detailed understanding of the magnitude and the implications of weight changes during pregnancy and postpartum to ensure a better standard of care. A period of at least 6 month postpartum is necessary to facilitate the weight loss.

C) Study of fetal growth:

Maternal and fetal carbohydrate metabolism

Glucose tolerance tests:

Carbohydrate intolerance is frequently observed in women who exhibit obesity in both pregnant and non-pregnant individuals. In non-pregnant state, regional fat distribution is also regarded as a risk factor for the development of diabetes mellitus.

A recent study by Landon et al. (1994) shows the relevance of body fat distribution as a factor contributing to the metabolic heterogeneity found during pregnancy in obese women. In their study, although a small number of pregnant women were studied (9 lean and 14 obese), a maximal deterioration of carbohydrate tolerance was observed in early pregnancy in upper body obese women compared with women of other body subtypes. Previously, Kissebah et al. (1982) in a study of 25 obese and 9 non-obese women, reported that plasma glucose and insulin levels during oral glucose loading were significantly higher in women with upper body segment obesity than in women with lower body obesity. They also showed that the large adipocytes from the abdominal region of the former group exhibit a higher rate of lipolysis compared to cells obtained from similar sites of the latter group. The increased flux of free fatty acids associated with fat cell hyperplasia, might explain the metabolic alterations in the upper body segment obese group. Similarly, Evans et al. (1984) discovered a

positive correlation between WHR and fasting and postprandial insulin as well as glucose levels in 80 premenopausal women.

Basically, fasting glucose concentration is dependent upon endogenous (primarily hepatic) glucose production. In fact, fasting plasma glucose levels are the results of the balance between hepatic glucose production and glucose uptake by the tissues (De Fronzo 1988). Basal hepatic production primarily represents glycogenolysis (the breakdown of the stored glycogen) and to a lesser extent, gluconeogenesis (the synthesis of glucose from three carbon precursors and amino acids). In contrast , postprandial glucose concentrations are the results of increased glucose availability and the interaction of increased insulin (and decreased glucagon) concentrations on insulin sensitive tissues. These alterations result in decreased hepatic glucose production and increased glucose uptake by insulin sensitive tissues.

Of course, a single glucose tolerance test would not be sufficient to judge insulin insensitivity and there is a need for more sophisticated and more invasive methods for this purpose (i.e. insulin clamp). However, the observed positive correlation between excess fat mass (/central fat distribution) and plasma fasting glucose in our study, would indicate that obese people would either produce more glucose endogenously or have a lesser glucose uptake by insulin sensitive tissues (or a combination of both).

In our study, although in a normal range of healthy women, the positive correlation observed between excessive fat content/central fat distribution and plasma fasting glucose, would confirm the tendency of obese pregnant women (centrally fat distributed) towards carbohydrate intolerance.

Putting the results of our study together with the related previous findings would indicate that including a simple fat distribution index (WHR) to the list of the criteria for gestational diabetes, may be a valuable step.

We did not find any significant association between 2 hour postprandial glucose concentration with indices of obesity. Concurrent measurement of insulin level may be more informative in this regard.

Cord insulin and C-peptide levels

Glucose is the major substrate for placental and fetal energy metabolism. All glucose supplied to the placenta and the fetus is provided by maternal glucogenesis or ingested in the pregnant woman's diet. Although maternal arterial glucose concentration is the driving force for glucose supply to the placenta, its control is far more complex than a simple direct relationship. Glucose crosses the placenta via carrier mediated facilitated diffusion and this is believed to be independent of insulin concentration.

There is no evidence that insulin can cross the placenta from the mother to the fetus or vice versa in any significant amount in the human (Kalhan et al. 1975). Although the molecular weight of C-peptide is about half of Insulin, insignificant placental transfer of this peptide has been reported (Lin et al. 1981). In the model of diabetic pregnancy, it is presumed that excessive maternal glucose crosses the placenta and stimulates insulin production in the fetus.

Some animal studies, have demonstrated a significant increase in fetal weight (in monkey) with infusion of insulin (Susa et al. 1979, Susa and Schwartz 1985). Since body length and head circumference was not measurably increased and excessive adipose tissue was seen, it was concluded that the increase in weight could simply be explained by fat and glycogen increment.

This was not confirmed in a more rigorous study by Stangenberg et al. (1981) (in rats) who showed no effect of insulin on fetal dry weight, body weight, body lipid or nitrogen. Although species differences may be coming into play here.

Many studies in humans demonstrate a positive correlation of cord C-peptide and/or insulin levels with infant birth weight (Stanley et al. 1992). Lin et al. (1981), suggested a persistently low production of insulin in SGA and a high production of insulin in LGA infants may lead to the different intrauterine growth rates observed. Moreover, the high incidence of macrosomia in the infants of diabetic mothers, are considered as an insulin stimulation experiment on intact fetuses.

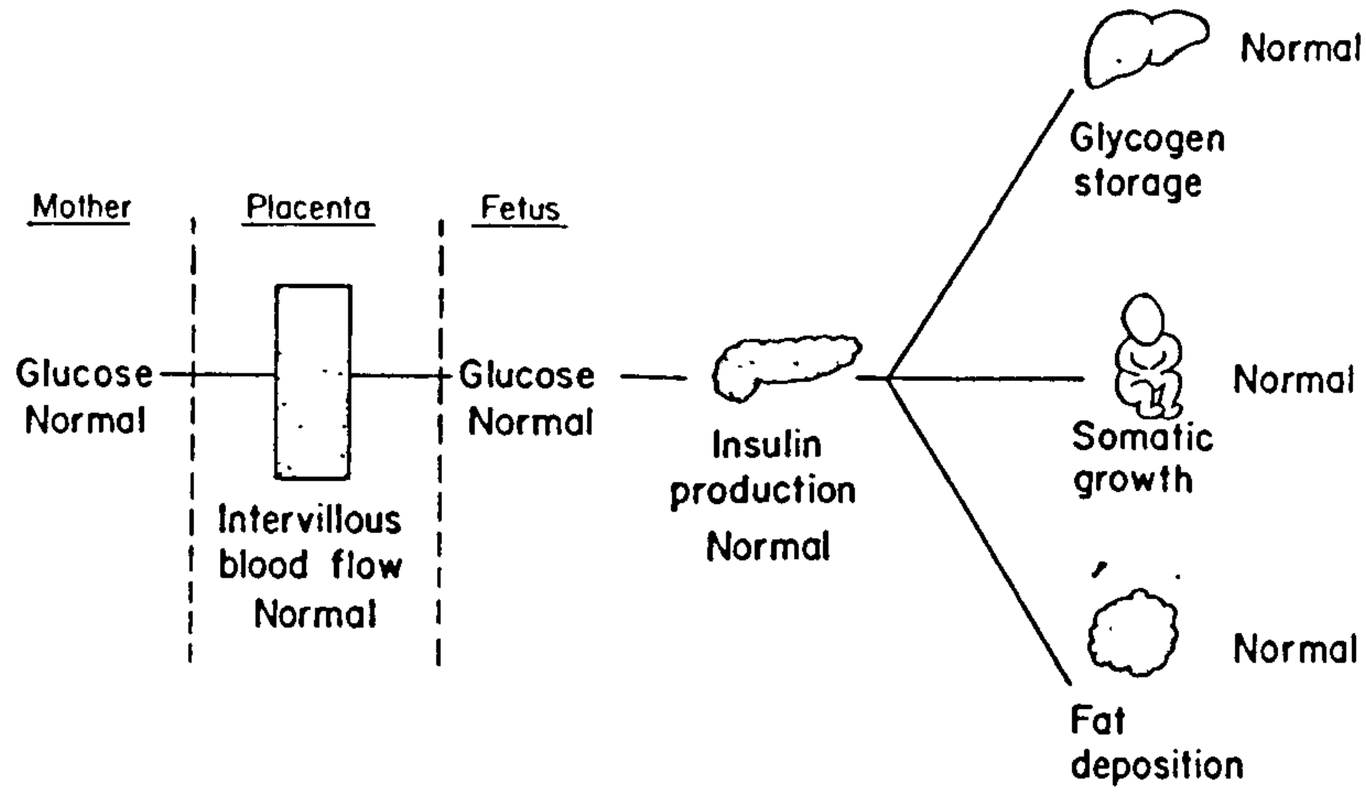
In our study, which reports observations within the normal range, a significantly higher value of insulin was observed in cord plasma of LGA babies in comparison with the AGA babies. This is in line with previously mentioned studies, however, we did not observe any significant correlation between birth weight, length or head circumference of the babies and cord insulin/C-peptide levels.

Cord insulin and C-peptide levels were significantly correlated to each other, this is understandable since they are secreted in equimolar amounts. Stanely et al. (1992) was reported similarly in this respect, nevertheless, Lin et al. (1981) reported no close correlation between these two variables. They explained this discrepancy could be due to the differences in hepatic clearance of insulin (40% - 60%) vs. C-peptide (<5%)

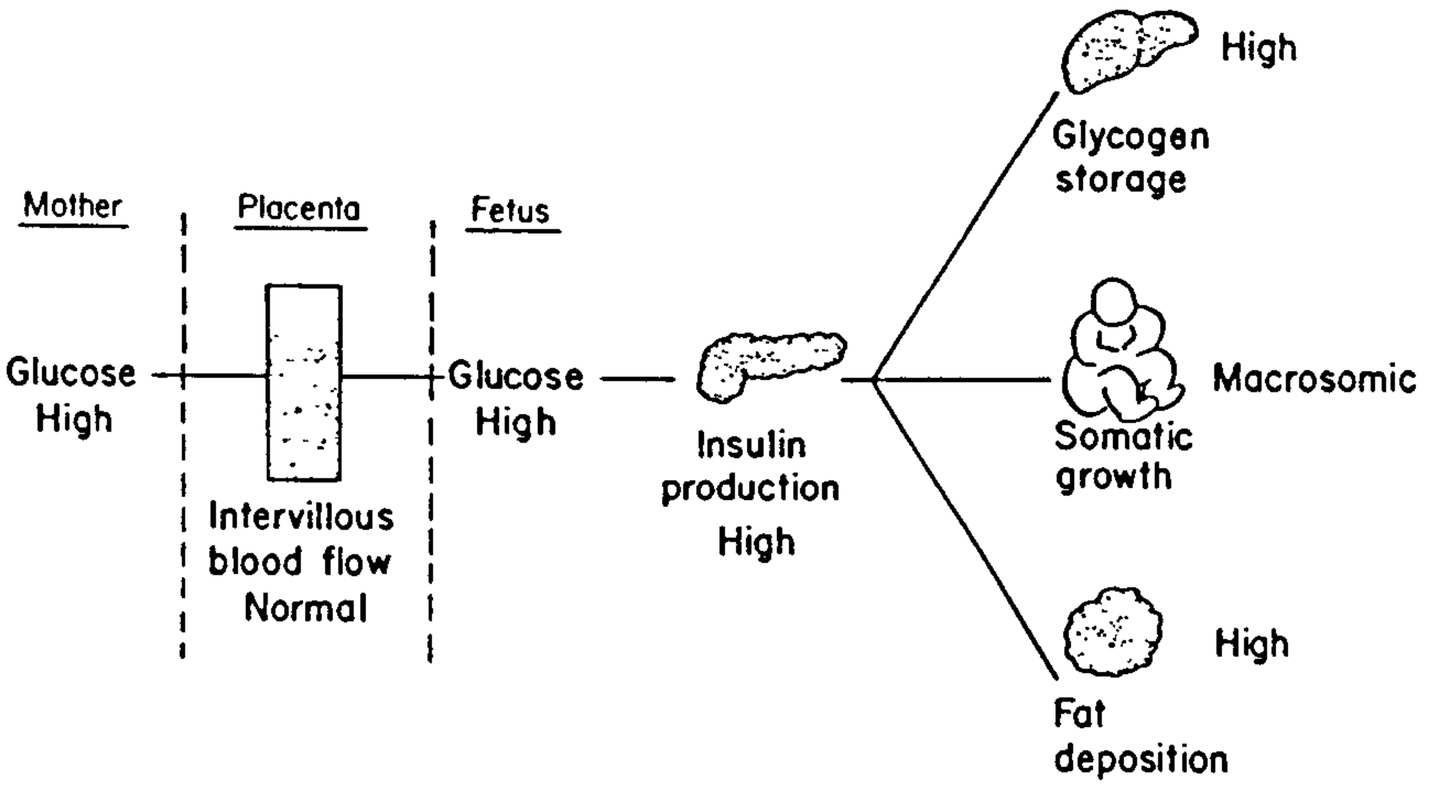
and also the slower peripheral clearance of C-peptide than insulin. The first issue could be argued by the fact that the clearance of C-peptide will be mainly handled by kidneys instead of liver (Steiner 1978).

What makes our study unique is the finding of a positive significant correlation with both cord insulin/C-peptide levels and maternal early pregnancy BMI. This relation was in particular explained by maternal fat mass in early pregnancy. Previously, maternal fat mass has been found to correlate with insulin level in late pregnancy (Karam et al. 1963) but we are not aware of any report of this association of maternal fat mass (or BMI) and fetal insulinemia.

Three basic factors can be speculated from our study: 1. a positive correlation of maternal BMI and maternal fasting glucose level 2. a positive correlation of maternal early pregnancy BMI (fat mass) with fetal insulinisation 3. significantly higher level of insulin in LGA infants. Although within a normal range, our findings are in favour of the proposed assumption that excess maternal glucose would cross the placenta and may stimulate fetal insulin secretion leading to excessive fetal growth (following figure). Above all, maternal BMI has shown a significant role in the production of higher glucose in the mother, higher insulin secretion in the baby and eventually producing a bigger infant.



The fetus of a normally pregnant woman receives a normal supply of glucose



The fetus of the diabetic woman receives an increased supply of glucose

Figure 4.2. Insulin regulation of intrauterine growth in normal and diabetic women. (Lin et al. 1981, with permission)

We were not able to see a statistically significant relation between maternal fasting glucose and cord insulin or C-peptide directly. This could well be due to the small number of samples available for this purpose (19/60 cord blood samples).

The difference in our study in comparison with Stanley et al. (1992) and Lin et al. (1981) was in that we did not find a significantly lower level of cord insulin in SGA babies. This could also be, because of smaller number of SGA infants in our study or more possibly due to the special characteristics (selected BMI) of the mothers involved in our study. There was no (or little) information about the maternal physical characteristics in last two mentioned studies or other related studies.

Silliman and Kretchmer (1995) who studied the relationship of maternal obesity and fetal adiposity in 37 pregnant women at 35-36 weeks gestation, observed a higher insulin concentration in the obese group than the lean women. Applying multiple regression analysis, however, they found that maternal adiposity, fasting glucose and gestational age were independently associated with infant adiposity.

The significant correlation of maternal BMI, in particular fat mass, with fetal insulinisation observed in our study of women in a normal range, is of interest but the mechanism behind it should be further investigated.

In conclusion, reducing maternal BMI or more precisely maternal fat mass might have an impact on the care of diabetic or potential diabetic women, in the sense that it would reduce the risk of fetal hyperinsulinemia and consequent macrosomia.

In vitro measurement of insulin responsiveness

This method which was presented by Bruce et al. (1994), is an *in vitro* method for investigating the changes in insulin responsiveness caused by sera from pregnant women. Stanley et al. (1993) demonstrated that this technique would give similar results to that of the insulin clamp *in vivo*.

There was a significant increase in basal glucose uptake with advancing pregnancy. We could see a reduction in insulin stimulated glucose uptake, but these changes were not statistically significant. The reduction in insulin sensitivity was borderline only in the middle of pregnancy (23 - 26 weeks, $p = 0.07$).

The index of insulin resistance (decrease in insulin stimulated glucose uptake from first to third measurements), was correlated with infant length at birth at borderline statistical level ($r=0.65$, $p=0.05$). It did not show any significant correlation with infant birth weight or head circumference.

It is basically difficult to draw any conclusion, having such a small number of subjects. However, the results of our study confirm the previous reports showing a significant decrease in insulin sensitivity during pregnancy. The studies of Fisher et al (1980); using a high dose glucose infusion test, showed that normal pregnant women <85th percentile standard of body weight at 38 to 40 weeks gestation had a decrease of about 80% in the insulin sensitivity index of that observed in a non-pregnant group. Buchanan et al. (1990) with the minimal model technique, found that insulin sensitivity in normal pregnant women at 29 to 36 weeks gestation was only one third of non pregnant values. Both these studies were cross sectional. Catalano et al.

(1991), in a longitudinal study of non obese pregnant women on a special diet, using hyperinsulinemic euglycemic clamp, showed a significant decrease in insulin sensitivity through pregnancy. They were however, unable to show any significant change in insulin sensitivity during gestation by other estimates that they used (the insulin/glucose ratio with OGTT or glucose disappearance index with the intravenous GTT). They blamed this discrepancy between the results of these two latter methods and the insulin clamping technique, down to the relatively small number of subjects used in their study.

Our study differs from the previous reports by the nature of longitudinal study design and the method used. Considering the fact that the present method is less invasive and needs the least co-operation from the volunteers, it seems worthwhile to develop it further. Perhaps using maternal tissue (biopsy of fat or lean tissue) would add to the value of the technique.

Fetal growth and subsequent neonatal growth

Birth weight is a convenient measure of fetal growth which has both clinical and biological significance. The genetics of birth weight has been the subject of many studies over the past half century.

Our study confirms the previous reports of greater neonatal birth weight in males compared with females (Catalano et al. 1995). Length and head circumference were also reported to be higher in male than female neonates. This pattern of difference between boy and girl infants was also observed in our study. Nevertheless, we did not observe a difference in the levels of cord blood C-peptide and insulin. Gestational age was not significantly different between the two sexes.

Earlier investigators (Thomson et al. 1968, Naeye 1979, Dougherty and Jones 1982) have described a positive correlation between maternal prepregnancy weight, weight gain during pregnancy, parity, infant sex and neonatal birth weight. Maternal weight and height were also reported to correlate positively with new-born birth weight.

We also observed a statistically significant correlation between birth weight and early pregnancy BMI. When we considered each individual BMI group, there was still a highly significant correlation of birth weight and maternal BMI in the over-weight group and a border line significant correlation in the normal weight group. A significant correlation was not present in the obese group.

Abrams and Laros (1986), also reported similar findings. They observed a strong positive relation between maternal weight gain and new-born birth weight in under-weight, normal weight and over-weight group, but not in very over-weight ones. The

mean birthweight for each group in the above mentioned study was 3593 ± 514 g, after adjusting for maternal age, socio-economic status, cigarette consumption and gestational age. In our study, when the groups were statistically matched in the above mentioned variables, the mean birth weight for obese group (3681.9 g) was significantly higher than the normal weight group (3296.7 g). The over-weight women delivered babies with mean birth weight of 3463.1 g, which lies between the two other groups. Head circumference and length were not significantly different between the BMI groups. The overall mean birth weight was 3433.60 ± 536 g (n = 123). Above all, maternal age and parity were shown to have a positive correlation with infant birth weight. When maternal age was adjusted for parity, there was no correlation between maternal age and birth weight (Catalano et al. 1995).

Spearman correlation showed that infant birth weight has a significant correlation with each of maternal early pregnancy fat mass and lean mass, but not with the fat gain. The changes in the lean mass had a border line significant relation to the birth weight. These results possibly demonstrate a relationship between prepregnancy maternal energy stores and fetal growth rather than the energy stores during the time of pregnancy. Even if there was going to be a positive correlation between maternal fat gain and birth weight that went undetected in the present study, it would appear that a quite substantial increase in maternal fat gain would be needed before there was any marked effect on the birth weight.

Lawrence et al. (1991), in a study of 115 women with average body weight and fatness, also concluded that there may not be a direct causal link between maternal fat gain, energy intake during pregnancy and birth weight. Thus, women who gain more fat during pregnancy, may not have bigger babies.

Langhoff-Roos et al. (1987), using the skinfold thickness method (and two other convenient methods; retained weight gain at 3 days postpartum and factorial method), could not find any significant correlation between maternal fat accretion and infant birth weight in a sample of 56 healthy Swedish women.

Although the methods that were used to estimate the fat accretion were not without their inherent problems, the results of our study are in accordance with the previous studies. No increase in fetal growth is obtained from the increasing maternal fat deposits in a well nourished population.

Unfortunately, we did not have enough under-weight subjects to make any comments but it is possible that in women who begin pregnancy with a reduced body weight or depleted maternal fat stores, an increase in fat content during pregnancy may be of a greater physiological importance.

Apparently, it is not advisable to encourage a strict diet program and weight control for all women (as practised by US physicians in the 1960s). Nonetheless, a modest reduction in energy intake for some women is most unlikely to have any adverse effect on birth weight, but it may prevent the problem of excessive fat gain by the women.

Our study is in agreement with the hypothesis that various maternal morphometric factors, in particular, prepregnancy weight and gestational weight gain correlated with fetal growth. In addition, to genetic and environmental factors, metabolic status may have an important effect on the determination of infant size.

In a sample of 38 women, on whom glucose tolerance tests were performed, maternal fasting glucose was found to be related significantly to the neonatal bithweight. This was also observed by Langhoff-Roos et al. (1989) who studied 52 healthy women. However, the pattern of this relation in conditions like gestational diabetes is a matter

of controversy (Tallarigo et al. 1986, Letters 1987). Only twenty percent of infants of diabetic mothers are macrosomic or LGA (Miller et al. 1988). Nonetheless, macrosomia is considered a hallmark of the pregnancy complicated by gestational or pregestational diabetes.

The positive correlation of fasting glucose with infant birth weight to some extent supports the hypothesis that maternal hyperglycaemia, results in greater amounts of glucose being available for placental glucose transport to the fetus and subsequent over-growth of the fetus. The excess transferred glucose, is supposed to stimulate fetal insulin secretion which finally would increase the deposition of lean body mass and adipose tissue in the fetus.

We could not see a statistically significant correlation between C-peptide/insulin level and infant birth weight. This could be because of the small number of subjects or the composition of the study population, including considerable number of subjects with higher BMI. Maternal BMI could be a confounding factor for the effect of glucose metabolism on fetal growth, since 39% of the mothers of macrosomic babies were found to be obese (Modanlou et al. 1980).

However interestingly, a direct significant correlation between maternal early pregnancy BMI and fetal insulinisation was observed in our study. Fasting glucose was also higher in women with greater BMI. Reduced insulin sensitivity during late pregnancy, would also facilitate fetal growth by allowing more glucose transfer to the fetus.

Briefly speaking, the present study shows that heavier mothers have higher level of fasting glucose and also bigger babies. Furthermore, infants of mothers with higher BMI, showed a significantly higher level of insulin and c-peptide in their cord blood.

But why we could not see a positive significant relation between fetal insulinisation and the birth weight throughout its range, remains uncertain.

Further investigations on the interrelationship of maternal carbohydrate metabolism and infant size in each BMI group may provide more information to eliminate the confounding effects of maternal body habitus on this matter.

Admittedly, the factors affecting fetal growth are highly complicated and involve a large number of processes at maternal, fetal and placental levels. Placental hormones such as human placental lactogen (HPL), oestradiol, and progestrone are suggested to be correlated with placental function and fetal growth (Langhoff-Roos et al. 1989).

Finally, all possible factors that we found in our study to have a relation to birth weight were analysed in a multiple regression model. Maternal early pregnancy lean mass turned out to be the most significant predictor of infant birth weight and length. The results of multiple regression should be interpreted with caution, since adding or excluding a factor may change the final outcome. However, our findings are consistent with previous study by Langhoff-Roos et al. (1987) who only considered 3 factors (fat mass, lean mass and gestational weight gain). Since, lean body mass is believed to be hereditarily determined (Pollock 1973, Langhoff-Roos et al. 1987), in this population of healthy, well-nourished women, heredity has a major influence on fetal growth.

Vohr et al. (1980), who studied the effect of maternal diabetes on the growth of offspring, suggested that macrosomia in the infants of diabetic mothers may be a predisposing factor for later obesity. As a follow up study we tried to discover if there is any relation between infant growth and various factors investigated in the present study (maternal BMI, body composition, maternal lifestyle habitus, cord insulin and

C-peptide level). Cord level of C-peptide and insulin did not show a significant correlation with subsequent infant growth up to 6 months. There were only few of mothers who were breast feeding up to 6 month postpartum therefore it was not possible to see a significant difference between breast and bottle fed babies. Maternal body status (BMI) did not show any effect on the pattern of infant growth at postpartum period. Male infants appeared to have a greater rate of growth up to 6 month than the female infants.

Chapter 5

Conclusion and practical implications

Conclusions extracted from the present study are summarised as follows:

- Direct measurement of skinfold thicknesses demonstrates a definite redistribution of adipose tissue during pregnancy and postpartum. The magnitude of these changes are towards central accumulation of fat tissue.

- Various methods in this study confirm the storage of significant amounts of fat during pregnancy.

- The fat gained during pregnancy does not appear to be beneficial for fetal growth, in a well nourished population. The mothers (particularly the obese ones), however, find it difficult to lose the extra fat subsequently. This would promote the risk of developing obesity after delivery.

- The lack of association between maternal energy intake reflected in fat storage, and birth weight was noticeable in the obese group. The predisposition to fat retention at postpartum in the obese group, may have an impact on the implications for recommended weight gain during pregnancy. Carefully controlled trials of diet during pregnancy and exercise programs at postpartum are necessary to reduce the risk of further development of obesity.

- The positive correlation between maternal obesity and central type of fat distribution with fasting glucose, would implicate that simple measurement of waist/hip ratio at prepregnancy or early pregnancy would be valuable addition to the present screening criteria for gestational diabetes.

- The association of maternal body type (BMI) with level of fetal insulinisation, would again, emphasise the importance of weight reduction at prepregnancy in the obese women, to reduce the risk of fetal hyperinsulinemia and macrosomia. Further investigations are required in particular in the obese diabetic women.

- The close relationship between maternal fasting glucose and maternal BMI, and also the significant correlation of each of these variables with infant birth weight could simply lead to the conclusion that perhaps the effect of BMI is mediated through higher values of glucose produced in the heavier mothers. However, the control of fetal growth is more complex than this simple relation. Therefore, we only suggest that in the studies of glucose tolerance and fetal growth, physical characteristics of the mother should be considered as important confounders.

- Finally, lean body mass is believed to be predominantly controlled by genetics and it could also be modified by exercise. If lean mass is the most significant predictor of fetal growth, as we could observe in this population of well nourished healthy women, manipulation of lean mass (by exercise, etc..) in the prepregnancy period might be considered. These findings in the other hand, highlight the complexity of fetal growth.

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Appendix 1

Principles of the Glucose Analyser technique:

This model is highly specific for glucose and it uses an oxidase enzyme hydrogen peroxide sensor. The conversion of glucose and oxygen to gluconic acid and hydrogen peroxide, Reaction I, forms the basis for this method.

Reaction I:

glucose oxidase

$$\text{D-glucose} + \text{oxygen} \longrightarrow \text{Gluconic acid} + H_2O_2$$

The probe, then oxidises a constant portion of the hydrogen peroxide at the platinum anode, Reaction II. The current thus created is directly proportional to the glucose level in the diluted sample.

Reaction II:

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

The circuit is completed by a silver cathode at which oxygen is reduced to water, Reaction III.

Reaction III:

$$4H^+ + O_2 \rightarrow 2H_2O - 4e^-$$

The tip of the probe is covered by a compound porous membrane which serves to protect the electrodes and to define a diffusion pass to them. It is a two layer membrane of differing materials, premounted on an "O" ring and prefilled between the layers with an enzyme. The outer membrane is a polycarbonate material with a pore size of .03 to .05 micrometers. This is large enough to readily pass glucose, oxygen, hydrogen peroxide, water and salt, but small enough to exclude cells and to restrict the diffusion of enzymes.

The glucose oxidase is immobilised between the polycarbonate membrane and the inner membrane in a thin layer of resinous material. The inner membrane is a cellulose acetate material with much smaller pore size. These pores are large enough to pass H_2O_2 , oxygen, water, salts, etc., yet small enough to exclude glucose, ascorbic acid and most other potential interfering substances.

Glucose in the vicinity of the probe will diffuse through the outer membrane and come in contact with glucose oxidase. At this point Reaction I takes place, converting the glucose to gluconic acid and hydrogen peroxide. The hydrogen peroxide in turn diffuses through the inner membrane, coming in contact with the platinum anode. Reaction II and III then take place, yielding a current proportional to the quantity of hydrogen peroxide diffused.

The probe is mounted in a measurement chamber controlled at 37° C and filled with pre-warmed buffer solution. Standards, controls and samples are injected to the chamber with YSI Syringepet (Combination syringe and pipette). The chamber contents are stirred by an air-driven silicone diaphragm which assures adequate stirring of the buffer and sample, plus an abundant supply of oxygen to support the reaction.

The current created is proportional to the glucose level in the sample. Integrated electronics arrange a display readout of the glucose level. The sample volume is 25 mm^3 and the sensitivity of the instrument 0.2 mmol/l.

DIABETES SCREEN™

C-PEPTIDE RIA

(Product Code: G029)

CLINICAL APPLICATIONS

The measurement of serum C-Peptide levels is of great value in the investigation and diagnosis of a number of clinical conditions. Serum C-Peptide levels correlate well with those of insulin as they are secreted in equimolar amounts. However, due to different metabolic clearance rates C-Peptide levels are higher than insulin in the peripheral circulation.

In Insulin dependant diabetics C-Peptide measurement canbe used to monitor beta cell function as it is an indicator of endogenous insulin in the presence of exogenous insulin.

C-Peptide measurement is useful in the clinical investigation of hypoglycaemic states,for example factitious hypoglycaemia due to surreptitious injections of insulin and in the detection of insulinomas in both diabetics and non-diabetics.

In hyperglycaemic states C-Peptide measurement can beused to monitor pancreatic status after pancreatectomy and pancreatic tumour removal due to nesidioblastosis and insulinomas. C-Peptide levels can be indicative of residual pancreatic tissue, therecurrence of the tumour or functional metastasis.

ASSAY DESCRIPTION

Guildhay's C-Peptide RIA kit is for the precise determination of C-Peptide in unextracted human serum or plasma. Standards and samples are incubated with C-Peptide Antiserum Reagent for 2 hours at +4°C. C-Peptide Tracer reagent is then added and allowed to react for a further 24 hours at +4C. Separation is achieved by incubation with the Separation Reagent for 30 minutes, followed by centrifugation and decantation of supernatants. C-Peptide concentrations are calculated from the measurement of the bound fractions (Separation Reagent pellets) in a gamma counter.

The sensitivity of the assay may be increased by extending the first incubation from 2 hours to 24 hours

KIT CONTENTS

1. C-Peptide Antiserum Reagent — 1 vial
   Contains C-Peptide sheep antiserum in a phosphate buffer with human serum albumin and sodium azide 0.1% w/v

2. C-Peptide Tracer Reagent — 1 vial
   Contains Iodine-125 labelled C-Peptide, < 1u Ci per vial, in phosphate buffer with inert red dye, human serum albumin and sodium azide 0.1% w/v. (11.0ml per vial)

3. C-Peptide Standards — 7 vials
   Standard concentrations range between 0 - 8.0 ng/mL. See Standard vials for actual concentrations. Contains human serum with thiomersal 0.1% w/v. 0.5ml per vial.

4. C-Peptide Separation Reagent — 1 vial
   Contains donkey anti-sheep serum and sheep serum in polyethylene glycol solution with sodium azide 0.1% w/v. (44ml per vial).

5. Non Specific Binding (NSB) Control — 1 vial
   Contains lyophilised human serum and sodium azide 0.1% w/v. Reconstitute with 0.5ml distilled/de-ionised water. (0.5ml per vial).

6. Non Specific Binding (NSB) Reagent — 1 vial
   Contains phosphate buffer with human serum albumin and sodium azide 0.1% w/v. 6.0ml per vial.

MATERIALS AND EQUIPMENT REQUIRED

1. Plastic tubes, 12 x 75mm.
2. Precision pipettors and disposable tips.
3. Vortex Mixer.
4. Gamma counter calibrated to detect 125-Iodine.
5. Centrifuge (capable of 3000g).
6. Distilled or De-ionised water.

WARNINGS AND PRECAUTIONS

1. This kit is for *IN VITRO* use only.

2. The standards in this kit have been prepared from sterile filtered human serum which has tested negative for Hepatitis B and HIV. However, because no test method can offer complete assurance that Hepatitis B, HIV or any other infectious agents are absent, these standards should be considered a potential biohazard and handled with the same precautions applied to any other blood product.

3. Wash solution and waste should be properly decontaminated with bleach or other strong oxidising agents before disposal.

4. Caution should be exercised in the handling of alkali or other hazardous chemicals in accordance with Good Laboratory Practice.

5. Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing are necessary to achieve good assay performance.

6. Never pipette by mouth

STORAGE AND STABILITY

1. All reagents should be stored at 2° - 8°C on delivery.

2. Standards and NSB control should be stored at -20°C after reconstitution.

3. Do not freeze other reagents.

4. Do not use after the stated expiry date.

5. Avoid exposure to sunlight

6. Do not mix reagents from different lots.

REAGENT PREPARATION

1. Reconstitute standards and NSB control with 500µl distilled/de-ionised water and allow to stand for 15 minutes with occasional mixing prior to use.

2 Allow all reagents to come to room temperature and mix gently before use.

ASSAY PROCEDURE

1. Label tubes as follows: 2 tubes for Total counts, 2 tubes for NSB Control, 2 tubes for each standard, 2 tubes for each sample,and 2 tubes for Sample NSB (if required).

2. Pipette 100µL of standards, controls and patient samples into the appropriate tubes. All samples should be run in duplicate. Samples should be retained at 2-8°C until successful results are confirmed.

3 Dispense 100µL of C-Peptide Antiserum Reagent into all standard and sample tubes.

4. Dispense 100µl NSB Reagent into "NSB Control" and "Sample NSB" tubes

5. Mix gently and incubate at +4°C for 2 hours.

6. Dispense 100µL C-peptide Tracer Reagent into each tube, including "Total Counts" tubes.

7. Mix gently and incubate at +4°C for 24 hours.

8 Mix C-Peptide Separation Reagent gently. Dispense 400µL to all tubes except the "Total Counts" tubes Mix thoroughly and incubate for 30 minutes at room temperature

9 Centrifuge all tubes at 3000g for 30 minutes

10 Decant the supernatent from all the tubes except the "Total Counts" tubes. Allow to drain. Blot on absorbent paper to remove remaining drops.

11 Count each tube for 100 seconds in a gamma counter calibrated to detect 125-Iodine.

NOTES ON ASSAY PROCEDURE

Increased sensitivity may be obtained by increasing the first incubation to 24 hours.

CALCULATION OF RESULTS

1. Express the standard counts as a percentage bound of total counts, minus the "NSB Control" counts.

2. Express the sample counts as a percentage bound of total counts, minus the appropriate "Sample NSB" counts.

3. Draw a standard curve by plotting the percentage bound of each standard against the respective concentration. Obtain each sample concentration by interpolating the sample percentage bound on the standard curve.

4. As an alternative to (1) - (3) above, a suitable data reduction computer package may be used.

PERFORMANCE CHARACTERISTICS

Sensitivity

The lowest concentration of C-Peptide that may be detected in this assay is 0.04ng/ml. This may be increased by extending the first incubation to 24 hours.

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Insulin assay

Reagents

1 Buffer (0.4M Phosphate pH 7 4 , containing 0.01M EDTA)
Dissolve 4.6g Na_2HPO_4 anhydrous, 1 2g $NAH_2PO_4.2H_2O$ and 1.25g Thiomersalate in approx 800 cm3 water Check pH and add 3.64g Disodium EDTA. Make volume up to 1L

2 Working buffer. Add BSA to 0 5% to the required volume of buffer

3 Antisera Guildhay HP/GP/16/5A .Reconstitue in working buffer to dilution of 1/50,000 (Store aliquoted at dilution of 1/500)

4. Label LIFE SCREEN LTD L538. Iodinated insulin is stored in solution and diluted for use 1/80 such that 50pg is added to each assay tube

5. Standards (NIBSC66/304) .Dissolve the ampoule contents (3 units) in 1cm3 0 07M Barbitone buffer pH 8.6 and make up to 30cm3 with 0.04m phosphate buffer containing 0 5%BSA 1cm3 aliquots (100mU/cm3) of stock solution are stored frozen for use. Dilute 1cm3 stock solution to 500cm3 with BSA /buffer (200mU/l)

6. Second antiserum IDS anti guinea pig precipitating serum rabbit. Dilute 1:15 for use with working buffer

7. PEG Solution 15g PEG 6000 in 100cm3 distilled water.

8 Normal guinea pig carrier serum .Use at 1/500 dilution.

Method

The method involves a 16 hr preincubation with sample,standards and primary antibody The label is added followed by a further 24 hr incubation. The assay is terminated by a 2 hr phase seperation with PEG accelerated second antibody.

Protocol

Day 1
: Prepare insulin antiserum All standards, controls, test samples etc, are set up in duplicate

Antisera mixture

No of tubes	100
Working buffer cm3	19.8
Insulin A/S (1/500)	200µl
Guinea pig sera	40µl

Tube	Antiserum	Stripped serum	Standard	Control serum	Test serum
Total cts	-	-	-	-	-
NSB	200 µl WB	50µl	-	-	-
Zero	200µl	50µl	-	-	-
Standard	..	-	50µl	-	-
Control	..	-	-	50µl	-
Test	..	-	-	-	50µl

Day 2
Make up sufficient working label solution for the number of assay tubes

Working label mix

No of tubes	120
Working buffer cm3	24.0
Stock label (µl)	300

Add to all tubes 200 µl working label
Mix and incubate at room temperature for 24hr.
Day 3
Make sufficient working second antiserum solution for the number of assay tubes

Second antibody mix

No of tubes	80	160
Working buffer cm3	9.0	18.0
Second A/S cm3	0.6	1.2
PEG cm3	8.0	16.0

Add to all tubes (except total) 200µl working 2nd antiserum. Vortex and incubate at room temperature for 2hr Centrifuge all tubes except blanks and totals at 2,500rpm for 30min at 4 Decant the supernatent and count the pellets for 180 secs or 10,000 counts

2.1 MATERIALS USED

The C2C12 myoblast cell line was obtained from The Centre for Applied Microbiology and Research, European Collection of Animal Cell Cultures, Porton Down Salisbury, Wiltshire, UK. (P7 was used in all experimentation).

The culture media and foetal calf and horse serum were obtained from Gibco BRL. Insulin was purchased from Novo and the H^3-2-deoxyglucose label from Amersham International Plc.

The Tissue culture disposable plastics i.e. flasks, pipettes and plates were purchased from Sterlin or Costar.

2.2 GENERAL TISSUE CULTURE METHODS

2.2.1 Standard practices for Cell Culture work.

The following practices were performed each time Cell Culture work was conducted; emphasis was placed on aseptic technique:-

i. Before entering the Tissue Cell Culture (TCC) laboratory, laboratory coats were donned and were removed on leaving.

ii. Hands were washed with antiseptic prior and following cell handling

iii. Protective gloves were worn at all times when working in the TCC laboratory.

iv. Class II safety cabinets, Heraeus HRR 2448 (Laminar Flow), were used for all cell culture manipulations.

v. The bench surfaces and all inside surfaces of the Laminar Flow hood were wiped down with 70% alcohol before conducting any work.

vi. Media etc. were brought from cold store and the bottles were swabbed with 70% alcohol before being placed in the hood.

vii. All plastics used were sterile (their packaging was removed within the cabinet), ensuring that all utensils coming into contact with the cells were uncontaminated.

viii. All cultures and supernatants were handled as infectious material.

ix. Mechanical pipetting devices (Gilson:pipette boy) were used for all procedures.

x. All contaminated liquid or solid wastes were decontaminated with neat chloros before disposal.

xi. All plastics were placed in water and chloros after use before waste disposal.

2.2.2 Storage of the cell line upon receipt

The cell lines were stored in $1.0cm^3$ plastic ampoules in a liquid nitrogen storage vessel at -70°C, until required for experimental purposes.

2.2.3 Handling of the cell line - Resuscitation of cells from liquid nitrogen

After storage in liquid nitrogen the ampoules were only handled wearing full protective clothing - full face shield, insulated gloves and laboratory coat.

The following procedure was conducted:-

- The ampoule cap was unscrewed ¼ turn on removing from the liquid nitrogen to release the pressure. It was then left at room temperature for approximately one minute within the Class II cabinet and the cap re-tightened.
- The ampoule was then transferred to a 37°C water bath for 1-2min until fully thawed. It was important that the ampoule was not immersed below the screw cap to ensure water did not enter.
- The ampoule was wiped with 70% alcohol and placed in the Class II cabinet. $0.5cm^3$ of the cells were slowly pipetted from the ampoule using a $1.0cm^3$ pipette and washed in $10.0cm^3$ phosphorus B saline (PBS) in a $25.0cm^3$ universal container to remove the

DMSO they were stored in.

- The cells were centrifuged at 1000-1200rpm, for 3min at room temperature. A pellet of cells became clearly visible at the end of this procedure.
- Within the Class II cabinet the supernatant was carefully removed using a $10.0cm^3$ pipette. The pellet was then re suspended in $2.0cm^3$ of the remaining supernatant; the cells were mixed gently to leave a cloudy suspension.
- The cells were then added to $10.0cm^3$ growth medium (high glucose (25mM) Dulbeccos Modified Eagles Medium (DMEM) containing 10% foetal calf serum and PS to prevent bacterial growth) in a T25 culture flask using a $1.0cm^3$ pipette.
- The flask lid was then tightly screwed within the class II cabinet and placed in the incubator (Heraeus 6000) with a humidified atmosphere of air/CO_2 (95:5 v/v) at 37°C. This atmosphere buffered the medium at an optimal pH range of 6.9-7.4 (Butler, 1992).
- Within the incubator the flask was positioned with its neck away from the incubator door to minimise exposure when the incubator door was opened and shut.
- The cells remained in this state until enough cells had been obtained to seed 12 well plates at 10^4 cells $well^{-1}$ - this was usually a time span of approximately 2-3days.

2.2.4 Cell maintenance

The growth and environment of the cells was viewed and therefore checked daily using a lightphase microscope, objective 10 (x 100 magnification).

The growth medium was removed approximately every 2days and replaced with fresh media using a $10.0cm^3$ pipette.

2.3 SEEDING THE WELL PLATES

2.3.1 Obtaining a cell suspension

- The T25 culture flask was removed from the incubator and placed in the class II cabinet. The growth medium was then removed from the cells using a $10.0cm^3$ pipette and discarded. A rinse of $10.0cm^3$ PBS was then added to the cells.
- $1.0cm^3$ of trypsin (10%) was added to the cells ensuring that an even coverage had occurred across the cells by gently swirling the culture flask.
- The cells were then viewed under the lightphase microscope to ensure that the trypsinization was complete. The flask was tapped gently to help loosen the cells from the plastic surface they were adhered to.
- $10.0cm^3$ of PBS was then added to the culture flask in order once again to wash the cells.
- Following this the suspension of cells plus PBS was transferred to a universal container and was spun down in a centrifuge for 3-4mins at 1000rpm.
- The majority supernatant was then carefully removed leaving the cell pellet residue in approximately $1.5cm^3$ of the remaining PBS.
- By flicking the universal container the cell pellet was re suspended and $5.0cm^3$ of the 10% growth medium added.
- To prevent clumping the cells were passed through a 23 gauge needle and syringe.

2.3.2 Cell counting

The coulter counter Z1 was used for the counting of all cells.

Prior to using the coulter counter the aperture range had to be found for the C2C12 cell line. This was conducted as follows:-

- $0.5cm^3$ of the resuspended cells plus $19.5cm^3$ of isoton diluent were placed in a $20.0cm^3$ measurement vile. This gave a 1:40 dilution of cells.

The aperture range was then altered and the cells counted in order to plot a graph and thus find the correct range for the cell count.

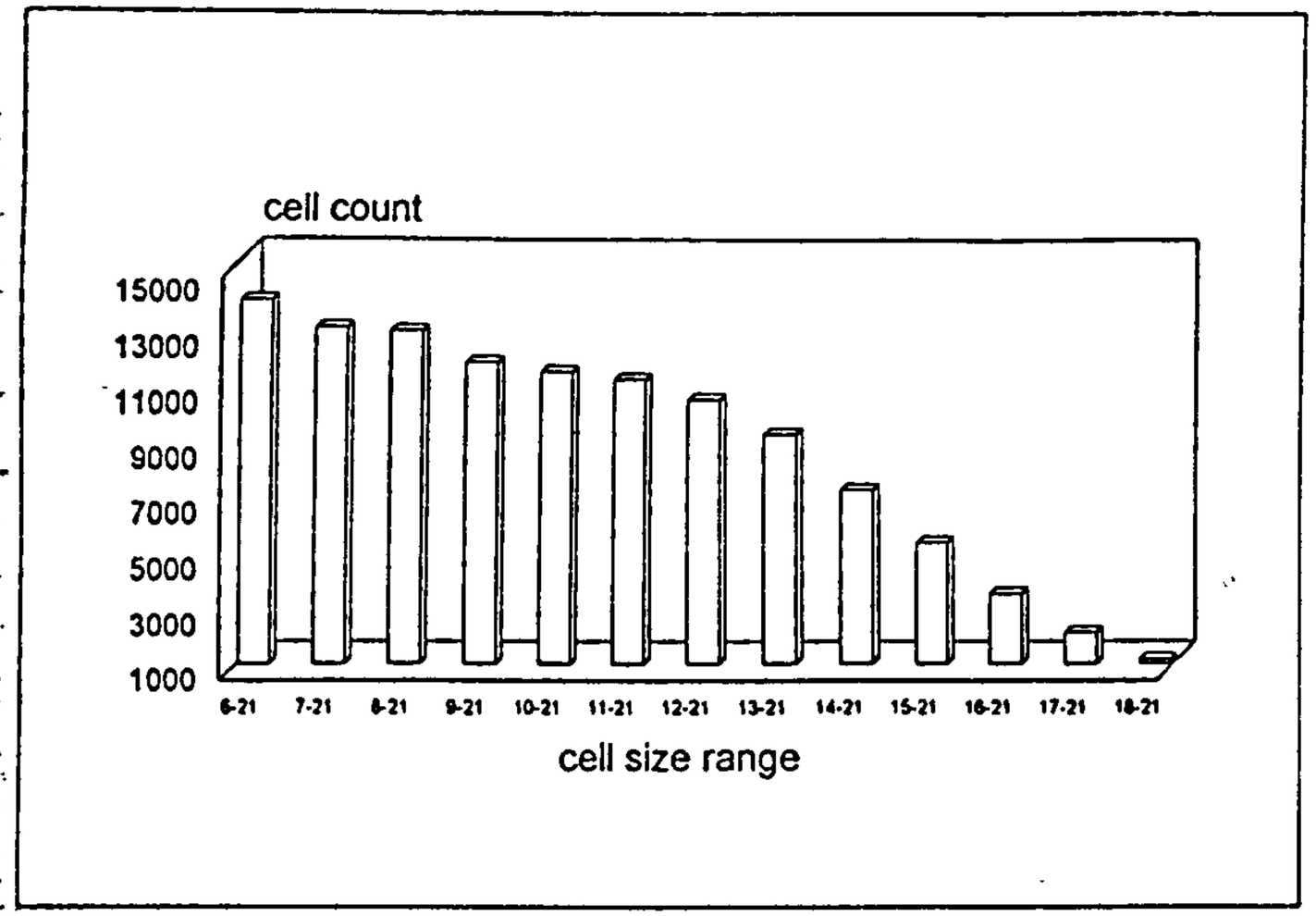

Figure 2.1. A calibration gate for the correct aperture range.

- From this graph it was calculated that an aperture range of 12-21μ would be most accurate in counting the C2C12 cells.
- Each cell count was conducted 5x and the mean calculated hence reducing the chance of random error.

2.2.3 Calculation of the cell dilution factor

The dilution factor of the cells needed to be calculated in order to seed the plates out at 10^4 cells per well, where:-

cell number in $1.0cm^3$ medium = total no. cells ×dilution ×volume

(dilution factor) x × 20 × 2

- Once the dilution factor had been calculated the growth medium could be added at an appropriate volume. The cells were then seeded in 12 well plates at $1.0cm^3$ $well^{-1}$.
- The cells were incubated for approximately 3-4days until confluent (as observed under the microscope).

2.4 THE INDUCTION OF CELL FUSION AND DIFFERENTIATION

Once the cells had become confluent the cell medium was altered as follows:- The growth medium was sucked off the cells using a 25 gauge needle and $10.0cm^3$ syringe.

- $2.0cm^3$ of high glucose DMEM with 2% horse serum was then added using a $1.0cm^3$ pipette. The medium was added slowly (drip wise) in order to ensure that the cells were not damaged.
- Following this the plates were placed back into the incubator.

Fusion normally appeared complete after 3-4days and the cultures were therefore generally used after 4-7days

2.5 INCUBATION WITH HUMAN SERUM

2.5.1 Choice of human serum samples

In order to obtain a cross section of longitudinal samples throughout full term pregnancy, samples were gained for early (10-15weeks gestation), middle (20-25weeks gestation) and late (32-36weeks gestation) pregnancy. The women who gave blood samples also varied in BMI and were categorised accordingly. Some cord bloods were also obtained and

analysed for effect on insulin resistance.

2.5.2 Human serum samples

The human blood serum for both pregnant and non-pregnant subjects was run at 10% for each sample. (The blood samples had been obtained previously as approved by the Ethical Committee and were in freezer storage.)

- After defrosting the human blood serum samples by placing them in a water bath at 37°C they could be added in an appropriate manner to the growth medium.
- For each 10% human blood serum sample; 4.5cm^3 high glucose DMEM with 1% PS
  0.5cm^3 human blood serum
- This was then filtered through a 0.22 µ Acrodisc filter and 1.0cm^3 added to each of the wells.
- Insulin was then added to the appropriate wells at a factor of 10^{-7} via the following dilution process:-

(1) 2.75cm^3 + 0.5cm^3 = 10^{-4}
HG DMEM + Novo
2% Horse serum insulin
(to prevent sticking)

(2) 4.5cm^3 + 0.5cm^3 = 10^{-5}
HG DMEM (1)

- 10 µl was then added to each of the samples requiring the insulin using a Gilson pipette hence reaching a concentration of 10^{-7}.

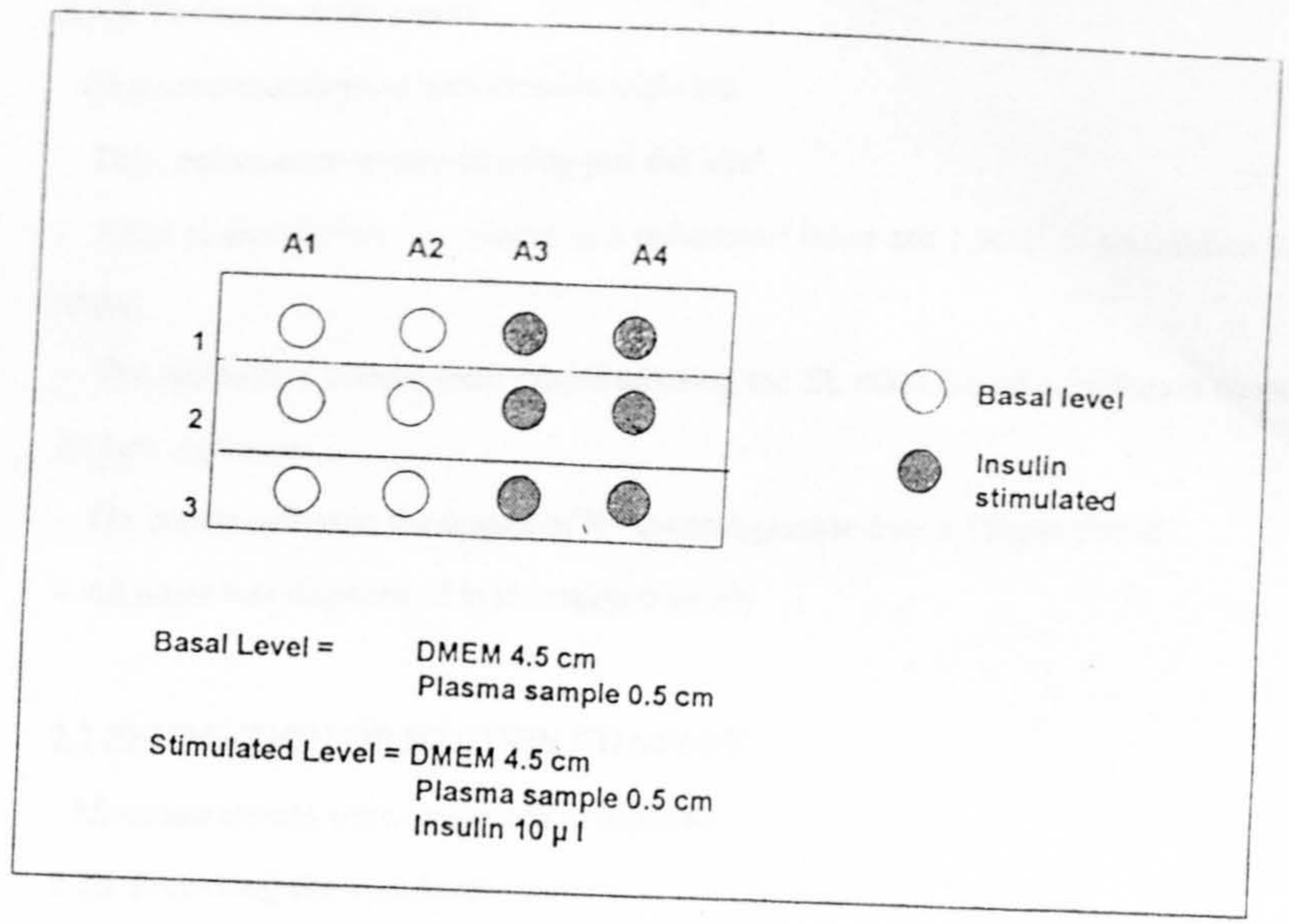

Figure 2.2. A schematic representation of the 12 well plate following the addition of the plasma sample and insulin.

2.5.3 Plating the wells

In order to plate the wells the following procedure was carried out:-

The plates were removed from the incubator and the medium removed from the wells using a 10.0cm^3 syringe and 25 green gauge needle.

- The cells were then washed with 1.0cm^3 of RPMI. This was removed once again with a syringe and needle and 1.0cm^3 of high glucose DMEM containing 10% human blood sera added as described above. The insulin was then added as described to the appropriate wells.
- The plates were then incubated for 24hours before the glucose uptake was measured.

2.6 GLUCOSE UPTAKE

The glucose uptake was measured using H^3-2-deoxyglucose, following the method of Walker *et al*, (1989).

2.6.1 The H^3-2-deoxyglucose mixture

For two plates i.e. 24 wells the mix was as follows:-

1.4cm^3 2-deoxyglucose stock

130µl RPMI/HS(2%)

10µl label

2.6.2 Glucose uptake procedure

The procedure was conducted as follows:-

- Following the experimental incubations of 24hours, the growth medium was removed from the wells using a 25 green gauge needle and 10cm^3 syringe.
- The cells were washed twice with 1.0cm^3 of RPMI and the medium replaced by 0.5cm^3 (in each well) of glucose-free RPMI medium containing 2% horse serum.
- The cells were then incubated at 37°C for 15mins.
- On removal from the incubator 50µl of 2.2mM H^3-2-deoxyglucose were added (final concentration 0.2mM), following the standard procedures set by the University for handling radioactive materials The cells were then incubated at 37°C for a further 10mins.
- Following the 10mins time span the medium on the cells was removed using a 25 green gauge needle and 10cm^3 syringe. The cells were washed twice with 1.0cm^3 cold PBS and then dissolved in 1.0cm^3 of 1.0M NaOH.
- Aliquots could then be taken after 1hr of incubator storage, for counting and measurement of protein content.

2.6.3 The radioactive count

All measurements were performed in triplicate

- Total counts were measured using just the label.
- 100µl of each sample was placed in a radioactive insert and 1.0cm^3 of scintillation fluid added.
- The radioactive counts were measured using the SL 4000-Liquid scintillation counter, IN Intertechnique.
- The counts indicated the uptake of H^3-2-deoxyglucose over a 10mins period.
- All waste was disposed of in the radioactive bin.

2.7 ESTIMATION OF PROTEIN CONTENT

All measurements were performed in triplicate.

2.7.1 Preparing the standard

The standard for the assay was prepared as follows:-

- 1mg/cm^3 albumin was added to 1.0M NaOH; A range of 0-50 µl was then used for measurement. The standards were made up with 2M NaOH and 50µl of HCl to reach a volume of 100µl.

2.7.2 Preparing the samples

The samples were prepared in 2.5cm^3 cuvettes as follows:-

25.0µl sample

25.0µl 1.0M HCl

50.0µl distilled water

2.8.4 Calculation of sample protein content

- A standard calibration curve was plotted.

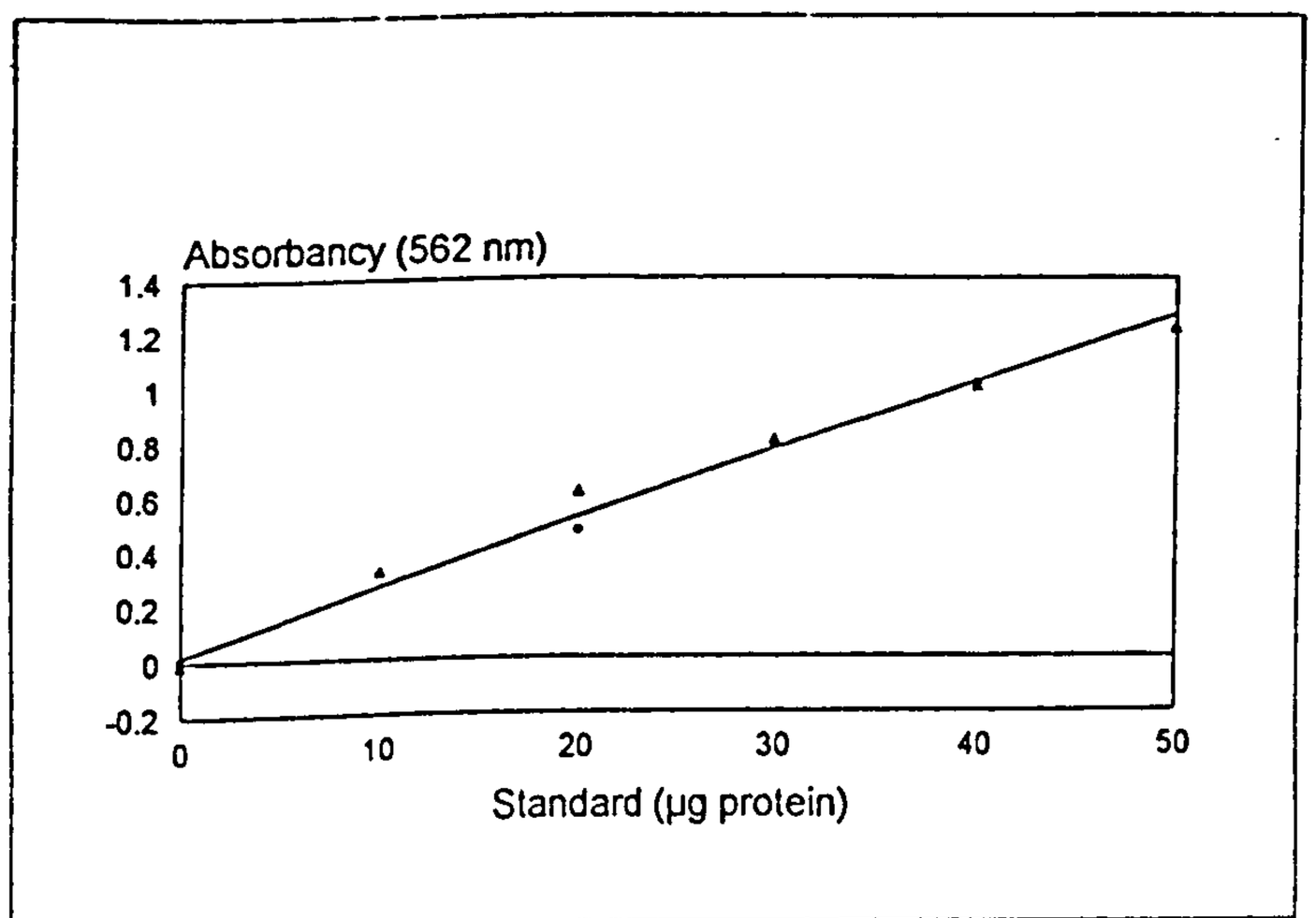

Figure 2.3 A standard protein calibration curve.

- From the calibration curve the protein content in µg could be related to the absorbancy, by finding the gradient of the curve.
- For each sample the protein content in µg could be calculated.

gradient × absorbancy

- In order to calculate µg cm^3 for each sample the following calculation was conducted:-

µg protein ×40 [dilution factor]

2.7.3 Measuring absorbancy

The BCA protein assay reagent (manufactured by Pierce) was used for all experimentation. The procedure was as follows:-

- 0.5cm^3 of BCA reagent (made up according to the instructions) was added to each cuvette and mixed.
- The samples were then incubated at 37°C for 1hr.
- On cooling 0.5cm^3 of distilled water was added to each cuvette and mixed.
- The absorbency was then measured at 562nm for each tube Vs water reference using the Pye Unicam SP6-350 visible spectrophotometer.

2.8 THE CALCULATION OF GLUCOSE UPTAKE RATES

The uptake rates of H^3-2-deoxyglucose were expressed as nmol/10 min/mg protein.

- The mean total count was calculated and divided by 22.2, to obtain counts per minute (cpm)/nmol.
- The following calculation was then used:-

$$\frac{\text{cpm per 100 µ l (sample)}}{\text{cpm per nmol (mean total count)}} \times \frac{10 \times 1000}{\text{µg protein / cm}^3}$$

Appendix 2

Anthropometric data collected at first visit								
Under weight	data1	weight1	height	BMI	waist1	hips1	thigh.cir1	upth.cir1
1	45	39	1.54	16.44459	63	81	40	
2	77*	57	1.73	19.04507	71	89	47	50.5
3	129	46	1.55	19.14672	73	84	43	46
4	65	51	1.63	19.1953	78	92	45.5	50.5
5	57	56	1.7	19.37716	77	87	46.8	48.5
Normal weight	data1	weight1	height	BMI	waist1	hips1	thigh.cir1	upth.cir1
6	132	51	1.6	19.92188	84	88	44	48.4
7	17	50	1.58	20.02884	78	82	43	
8	31	62	1.73	20.71569	81	95	50	
9	69	56	1.64	20.82094	75.3	88.4	46.5	50.5
10	20	60	1.69	21.00767	83	93	53	58
11	116	49	1.52	21.20845	74.5	83.4	43	49.5
12	42	57	1.63	21.45357	79	91	48	36
13	12	58.5	1.65	21.4876	82.5	89.5	44.5	
14	1	53	1.57	21.50189	75	83	46	
15	23	52.5	1.56	21.57298	82	89	36	48
16	48	54	1.58	21.63115	80	92	47	
17	78	56.5	1.61	21.797	73.5	90	48.3	52.4
18	53	58	1.63	21.82995	73.5	89.5	48.5	54
19	68	65	1.72	21.97134	78.5	93.5	49.3	54.4
20	82	64	1.7	22.14533	84	97	53.2	57.3
21	86	66	1.72	22.30936	83	96	50.5	55.5
22	85	70	1.76	22.59814	87	101	48.2	56
23	133*	63	1.66	22.86253	85.5	96.2	49	56
24	71	68	1.72	22.9854	87.7	95	50.1	56.5
25	70	56	1.56	23.01118	72	92.5	49	54.2
26	21	65	1.68	23.03005	89	91.5	51	
27	3	57	1.57	23.12467	84.8	91.9	50.8	
28	74	63	1.64	23.42356	80.3	95	49.4	54.6
29	80	63	1.64	23.42356	80.5	96.5	51	57
30	14	61	1.61	23.53304	89	97	53	
31	13	62	1.62	23.62445	91	95	50	
32	123	62	1.62	23.62445	82	100	48.5	56
33	104	67	1.68	23.73866	83	95.5	51.4	56.5
34	37	66	1.66	23.95123	77	103	55	
35	52	67	1.67	24.02381	80.5	96	52	57
36	95	69	1.69	24.15882	78	101	53.7	60
37	41	62	1.6	24.21875	83	98	53	
38	44	66	1.65	24.24242	88	97	54	
39	15	57	1.52	24.67105	86	87	48	
40	120	65	1.6	25.39063	86.5	99.2	53.3	59.2
41	99	69	1.64	25.65437	85	100.5	52	61.5
42	64	63	1.56	25.88757	81.8	93.8	49.8	54.8
	To be Continued in page 233							

tri1.1	tri1.2	tri1.3	m tri1	bi1.1	bi1.2	bi1.3	m bi1	sub1.1	sub1.2
8.1	8.2	8.2	8.166667	3.3	3.3	3.2	3.266667	8	8
8.4	8.4	8.4	8.4	5.1	5	5.1	5.066667	5.4	5.3
12	12.2	11.8	12	4	3.6	3.6	3.733333	7	7.2
15.3	15.2		15.25	5.1	5.1		5.1	14	14
8.2	8.3		8.25	4.3	4.3		4.3	7.2	7.2
tri1.1	tri1.2	tri1.3	m tri1	bi1.1	bi1.2	bi1.3	m bi1	sub1.1	sub1.2
22	21	22	21.66667	11.4	11.2		11.3	14.2	14.2
12	11	11	11.33333	6	7	7	6.666667	14	15
17	17	17	17	6	5	5	5.333333	13	13
9	9	9	9	4.1	4.1	4.1	4.1	5.3	5.3
21	20	20	20.33333	6	6	6	6	12	13
10	9.6	9.6	9.733333	4.8	4.8	4.6	4.733333	8.4	8.6
9	8	8	8.333333	4	5	5	4.666667	8	8
16	16	16	16	6	6	6	6	15	14
23			23	8			8	9	
14	13	13	13.33333	6			6	13	
16	16.2	16	16.06667	10	10	11	10.33333	15	14.5
21.2	21.2	21.2	21.2	7.1	7.2	7.1	7.133333	9.1	9.1
10.3	10.3		10.3	4.2	4.2	4.2	4.2	12.2	12.2
12.2	12.3		12.25	6.4	6.4	7	6.6	8.1	8.1
20.3	20.2	20.2	20.23333	8.3	8.4	8.3	8.333333	10.3	10.2
16.3	16.2	16.2	16.23333	6.2	6.2	6.2	6.2	9.2	9.2
14.4	14.4	15	14.6	5.1	5.1		5.1	14.2	14.2
33	34	33.2	33.4	19	18	18.6	18.53333	35	34.6
14.1	13.3	14.1	13.83333	7	7.4	7.4	7.266667	8.1	8.3
21.1	21.1	22	21.4	11.2	11.2	12	11.46667	17.4	18.2
14	14	14	14	7	6	6	6.333333	15	14
20	19	18	19	6.4	6.3	6.1	6.266667	13	15
20.1	20	20	20.03333	8.2	8.4	8.4	8.333333	15.2	14.9
20	20.1	20.4	20.16667	15.3	15.2	15.3	15.26667	12.1	12.1
14	15	15	14.66667	10	10	10	10	22	23
22	22	21	21.66667	10	10	9	9.666667	14	15
14.8	14.4	13.8	14.33333	6.2	6.4	6.2	6.266667	9.8	10.4
23	23.8	2.2	16.33333	13	12.2	13	12.73333	13	13.2
20	21	20	20.33333	10	9	9	9.333333	16	17
24.3	25	24.3	24.53333	10.2	10.2	12	10.8	18.1	19.1
30	29.4	29.6	29.66667	14.4	14.8	14.6	14.6	14	13.6
24			24	15	16	16	15.66667	18	19
21	21	21	21	6	7	6	6.333333	15	15
21	21	19	20.33333	9	9	9	9	17	16
26	25.8	25.8	25.86667	12.6	14	12.8	13.13333	23.2	22.2
21	20	21	20.66667	8.8	8	10.4	9.066667	15	15.2
15	14.4	14.4	14.6	6.2	6		6.1	8.3	8.3

sub1.3	m sub1	sup1.1	sup1.2	sup1.3	m sup1	th1.1	th1.2	th1.3
8	8	5	5	5	5	23	22	23
5.3	5.333333	4.2	4.3		4.25	19.1	19.2	20
7	7.066667	11	11.6	10	10.86667	29	28	26
14	14	7	7.1	7.1	7.066667	27	27	27
	7.2	6.2	6.2		6.2	16	16	
sub1.3	m sub1	sup1.1	sup1.2	sup1.3	m sup1	th1.1	th1.2	th1.3
14	14.13333	24.8	25.8	26	25.53333	38.2	39.2	39.4
15	14.66667	9	9	10	9.333333	17	18	17
13	13	11	11	12	11.33333	32	33	32
5.3	5.3	6.3	6.4		6.35	19.4	19.4	19.4
12	12.33333	14	14	15	14.33333	38	37	36
9	8.666667	8.2	7.8	7.6	7.866667	16	15	15.8
7	7.666667	6	7	7	6.666667	17	17	17
14	14.33333	9	8	7	8	21	21	21
	9	9.3	9.1	9	9.133333	35	33	33
	13	15	16	14	15	15	16	15
15.2	14.9	15	16	16	15.66667	29	30	29
9.1	9.1	8	8.2	8	8.066667	36.2	35.4	35.3
12	12.13333	6	6	6.1	6.033333	19	19	19
8	8.066667	9.2	9.4		9.3	23.4	23.4	
10.2	10.23333	14.2	14.2		14.2	31.2	31.2	31.1
9.2	9.2	7.3	7.3	7	7.2	24.2	24.4	24.1
14.3	14.23333	13.2	13.2	13.3	13.23333	31	32	28.3
35	34.86667	30	31.2	32.6	31.26667	43	42.4	44.4
8.3	8.233333	11.1	11.1	10.3	10.83333	20.1	20	20
18	17.86667	18	17.4	17.4	17.6	39.3	40.1	39.3
13	14	9	10	9	9.333333	32	33	34
15	14.33333	12.2	12.2	12	12.13333	24	18	18
15	15.03333	13.2	13.1	12.4	12.9	37.2	38	40
	12.1	10.1	10	10.1	10.06667	39.3	39	39
22	22.33333	19	20	19	19.33333	32	33	36
16	15	15	15	14	14.66667	37	37	37
10.6	10.26667	9.8	10	10	9.933333	33.4	32.8	32.2
1.6	9.266667	13.6	13.4	13	13.33333	41	41.2	41.6
15	16	16	15	15	15.33333	41	40	41
19.1	18.76667	19	19	18	18.66667	41	39	40
14	13.86667	19.4	19.4		19.4	43	42.4	43.2
19	18.66667	12	12	12	12	36	35	35
15	15	11	12	11	11.33333	31	30	31
17	16.66667	12	14	14	13.33333	31	35	36
23.2	22.86667	18.4	19.8	20.2	19.46667	38.4	38.2	36.2
15	15.06667	16.4	16.2	14	15.53333	35.2	34.4	34
9	8.533333	5.1	5.2	5.1	5.133333	32	33.2	32.3

data1	m th1	(c) tbsi	(m) tbsi	den tbsi1	%fat tbsi1	fat%1	lean%1	fat(kg)1
45	22.66667	1.1599	0.0717	1.060382	16.81306	17.9	82.1	7
77*	19.43333	1.1599	0.0717	1.062196	16.01547	21.1	78.9	12
129	27.66667	1.1599	0.0717	1.0504	21.24916	23.9	76.1	11
65	27	1.1599	0.0717	1.043949	24.1613	23.5	76.5	12
57	16	1.1599	0.0717	1.058506	17.64008	23.2	76.8	13
data1	m th1	(c) tbsi	(m) tbsi	den tbsi1	%fat tbsi1	fat%1	lean%1	fat(kg)1
132	38.93333	1.1423	0.0632	1.024676	33.07943	25.5	74.5	13
17	17.33333	1.1599	0.0717	1.043513	24.3592	24	76	12
31	32.33333	1.1599	0.0717	1.040232	25.85529	24.2	75.8	15
69	19.4	1.1599	0.0717	1.059981	16.98965	23.2	76.8	13
20	37	1.1599	0.0717	1.036269	27.675	31.7	68.3	19
116	15.6	1.1599	0.0717	1.052969	20.09915	22.4	77.6	11
42	17	1.1423	0.0632	1.051501	20.7556	24.6	75.4	14
12	21	1.1423	0.0632	1.038227	26.77451	24.1	75.9	14
1	33.66667	1.1599	0.0717	1.038628	26.59012	24.5	75.5	13
23	15.33333	1.1599	0.0717	1.039791	26.05743	32.7	67.3	17
48	29.33333	1.1599	0.0717	1.034022	28.71322	24.1	75.9	13
78	35.63333	1.1599	0.0717	1.041021	25.49492	26.8	73.2	15
53	19	1.1549	0.0678	1.052244	20.42335	24.1	75.9	14
68	23.4	1.1599	0.0717	1.048126	22.27135	24.6	75.4	16
82	31.16667	1.1599	0.0717	1.036269	27.675	26.6	73.4	17
86	24.23333	1.1599	0.0717	1.045954	23.25216	25.8	74.2	17
85	30.43333	1.1599	0.0717	1.0399	26.00714	27.1	72.9	19
133*	43.26667	1.1423	0.0632	1.011342	39.44889	27	73	17
71	20.03333	1.1599	0.0717	1.044903	23.72826	25	75	17
70	39.56667	1.1599	0.0717	1.028357	31.35042	26.8	73.2	15
21	33	1.1599	0.0717	1.042301	24.91069	26.2	73.8	17
3	20	1.1549	0.0678	1.038706	26.55432	35.1	64.9	20
74	38.4	1.1599	0.0717	1.034389	28.54358	27	73	17
80	39.1	1.1599	0.0717	1.033678	28.87267	27	73	17
14	33.66667	1.1599	0.0717	1.029282	30.91785	26.2	73.8	16
13	37	1.1599	0.0717	1.031892	29.70143	27.4	72.6	17
123	32.8	1.1423	0.0632	1.040506	25.72995	27.4	72.6	17
104	41.26667	1.1549	0.0678	1.038744	26.5369	34.4	65.7	23
37	40.66667	1.1599	0.0717	1.031892	29.70143	34.8	65.2	23
52	40	1.1599	0.0717	1.026399	32.26838	28.4	71.6	19
95	42.86667	1.1423	0.0632	1.022884	33.92569	27.5	72.5	19
41	35.33333	1.1333	0.0612	1.020254	35.17343	37.1	62.9	23
44	30.66667	1.1423	0.0632	1.032983	29.19487	27.3	72.7	18
15	34	1.1599	0.0717	1.032754	29.30075	35.1	64.9	20
120	37.6	1.1599	0.0717	1.022934	33.90231	36.9	63.1	24
99	34.53333	1.1599	0.0717	1.032234	29.54241	36.2	63.8	25
64	32.5	1.1423	0.0632	1.045216	23.58627	36.9	63.1	22

data1	lean(kg)1	water1	age	job	gravida	para	abortion	smok/day
45	32	22	28	travel cons	2	1	0	1-2/week
77*	45	32	21	bar worker	4	2	1	10
129	35	23	29	h/w	2	1	0	10/d
65	39	25	22	H/W	2	1	0	0
57	43	28	21	h/w	4	2	1	5-10/day
data1	lean(kg)1	water1	age	job	gravida	para	abortion	smok/day
132	38	24	35	h/w	3	1	1	0
17	38	25	22	food opera	1	0	0	12
31	47	31	22	h/w	3	1	1	0
69	43	32	25	sell assis	2	1	0	5
20	41	27	27	tel operat	4	1	2	0
116	38	26	23	h/w	3	2	0	0
42	43	29	30	h/w	4	1	2	15/d
12	44	30	36	shopkeep	2	1	0	0
1	40		27	typist	1	0	0	0
23	35	23	23		1	0	0	0
48	41	29	20	hmade	1	0	0	00-Jan
78	41	26	23	sell assis	1	0	0	10
53	44	30	16	hair dress	1	0	0	0
68	49	33	23	h/w	1	0	0	0
82	47	31	28	admin offic	2	0	1	0
86	49	34	23	h/w	2	1	0	15/day
85	51	33	24	secretory	1	0	0	0
133*	46	31	33	Dr	1	0	0	0
71	51	37	27	clarck	2	1	0	0
70	41	26	25	hair dress	1	0	0	0
21	48	31	27	h/w	3	1	1	0
3	37	24	17	st. yts	1	0	0	5
74	46	31	26	secretory	1	0	0	0
80	46	31	27	h/w	3	1	1	0
14	45	30	22	h/w	1	0	0	0
13	45	29	28	h/w	6	4	1	0
123	45	29	30	market ass	2	1	0	0
104	44	28	18	h dresser	1	0	0	0
37	43	28	26	nurse	1	0	0	0
52	48	31	28	pension as	1	0	0	0
95	50		33	sell assiss	1	0	0	0
41	39	27	43	teacher	1	0	0	0
44	48	31	30	h/w	3	1	1	0
15	37	25	22	care assis	1	0	0	0
120	41	27	26	teacher	2	1	0	0
99	44	29	27	h/w	2	1	0	0
64	41	30	34	nurse	3	2	0	10

						Glucose Tolerance test		
data1	alcohol	meal time	ges.age.s	ges.age.d		fast. level	GTT (wks)	120 min
45	1	3.0	11	10		4.2		9.3
77*	0	4.0	10					
129	0	f	15.0	15		3.8	28w	6.1
65	1	4.0	15	16				
57	0	3.5	11					
data1	alcohol	meal time	ges.age.s	ges.age.d		fast. level		120 min
132	1	3.5	14	14				
17	1	1.5	13	14		4.2		
31	0	2.0	14	15				
69	0	3.0	11	15				
20	0	4.0	9	9		4.2		6.6
116	0	4.0	14	14		4.3	29w	5.9
42	1	f	12	13				
12	1	3.5	15	16		4.1		6
1	0	3.5	13	14				
23	0		13	14				
48	0	1.5	12	16				
78	1	2.5	15	15				
53	0		11					
68	0	1.0	15	17				
82	1	2.0	12	12				
86	1	3.0	11	12		4.2	29	5
85	0	1.0	14	15				
133*	0	15.0	11	11				
71	1	2.0	13	14				
70	1	3.5	13	14				
21	1	4.0	14	15				
3	1		14					
74	1	0.5	13	14				
80	0	2.0	15	15				
14	1	4.0	14	14				
13	0	4.0	10	10				
123	1	2.0	15	15				
104	0	5.0	13	14				
37	1	3.0	14	15		4.1		6.6
52	0	4.0	12	15				
95	1	3.0	14	14				
41	0	3.0	15	17				
44	1	1.0	14	13				
15	1		12	15				
120	0	2.0	14	14				
99	0	3.0	15	16		5.5		12.7
64	1	3.0	14	16				

	Continued from page227(Data collected at 1st visit							
Over-weight	data1	weight1	height	BMI	waist1	hips1	thigh.cir1	upth.cir1
43	54	62	1.54	26.14269	101	98	53.2	56.2
44	108	68	1.61	26.23356	85.5	101.2	52.4	59
45	98	71	1.64	26.39798	85.5	97.5	54.4	58.5
46	92	69	1.61	26.61934	87.4	100.5	52.7	58.5
47	93	67	1.58	26.83865	85.7	100	55.1	59.6
48	62	77	1.69	26.95984	92	102	55	60
49	46	64	1.54	26.986	90	98	55	
50	58	74	1.64	27.51338	88	101.8	54.5	60
51	49	69	1.58	27.6398	102	98	49	
52	25	65	1.53	27.7671	96	103	55	
53	50	80	1.69	28.01022	109	105.5	53.2	
54	47	70	1.58	28.04038	95	105	54	
55	73	73	1.61	28.16249	81.5	103.9	56.4	62.5
56	29	80	1.68	28.34467	102	108		60
57	109	72	1.59	28.47989	96.5	102	57.3	61.4
58	19	70	1.56	28.76397	100.5	104	56	
59	66	77	1.63	28.98114	106.6	101	53	61.2
60	56	77	1.62	29.34004	110.6	108.3	50	
61	*117	73	1.57	29.61581	105.3	102.7	55.2	62
62	110	82	1.66	29.75758	99.5	107.6	60	67.5
63	128	82.5	1.66	29.93903	95	112.2	60	69.2
Obese	data1	weight1	height	BMI	waist1	hips1	thigh.cir1	upth.cir1
64	39	78	1.61	30.09143	99	106	59	
65	125	85	1.68	30.11621	101.8	109	57	64.7
66	83	90	1.72	30.42185	102	113.5	61	73.5
67	90	83	1.65	30.48669	106	107.7	58.7	64.7
68	112	77	1.58	30.84442	97.5	101.5	53.5	58
69	24	92	1.72	31.09789	106.5	117	63	
70	87	88	1.68	31.17914	108.5	107.5	57	62.5
71	122	78	1.58	31.24499	86.5	103.5	60.5	65.5
72	118	91	1.68	32.24206	105	112	58	65
73	96	96.5	1.7	33.391	121	114.5	58.7	65
74	105	91	1.645	33.62866	113	109.7	56	64.5
75	7	84	1.58	33.64845	112.5	117.5	61	
76	30	106	1.77	33.83447	106	125	67	
77	27	89	1.62	33.91251	118	120	58	
78	103	96	1.67	34.42217	108.5	117	64.2	71.5
79	34	82	1.53	35.02926	114	110	59	
80	4	99	1.68	35.07653	101.9	125	69.3	
81	114	98	1.67	35.1393	106.5	114.2	63.5	68
82	63	85	1.55	35.37981	93	114	66	72
83	26	93	1.62	35.43667	117	122	65	
84	100	100	1.66	36.28974	129	121.5	61.3	71.5
85	107	93.5	1.59	36.9843	105.2	115.3	65	72
86	89	101	1.61	38.96455	123.7	115.5	65.2	71
87	94	116	1.69	40.61482	131.5	125	65.4	72
88	35	113	1.64	42.01368	131	124	75	
89	60	108	1.6	42.1875	129	115	65.8	71

tri1.1	tri1.2	tri1.3	m tri1	bi1.1	bi1.2	bi1.3	m bi1	sub1.1	sub1.2
18.2	18.5	18.1	18.26667	8.2	8.2	8.2	8.2	21	22
24.4	24	23.6	24	12	12.4		12.2	18	18
23	21.2	21.2	21.8	8.2	8.2		8.2	12.4	12.4
29	29	29.8	29.26667	17	17.2	18	17.4	30	30
22	21.6	21.6	21.73333	15.2	15.4	14.8	15.13333	20	21.4
20	20.4	21	20.46667	15.3	15.2	15.1	15.2	16.1	16
22	21	22	21.66667	11	11	11	11	17	18
26.1	26	26.1	26.06667	14	13.9		13.95	16.1	16.3
25	25	25	25	14	15	15	14.66667	30	31
30	29	29	29.33333	20	21	22	21	27	28
30	31	31	30.66667	15.2	15	15.2	15.13333	42	42
33	32	32	32.33333	15	15	16	15.33333	29	30
27.1	27.2	27.4	27.23333	14.3	14.2		14.25	24.2	24.2
27	26	27	26.66667	9	8	8	8.333333	30	32
30	31.4	32	31.13333	21	20.4	19.6	20.33333	27	27.2
22	23	23	22.66667	12	11	10	11	18	18
18.3	18.3	19	18.53333	15	15.1		15.05	28.1	28.3
28.2	28		28.1	15.2	15.8	16	15.66667	38.1	38
30	31.2	32	31.06667	16	17	18	17	23	23.6
28	28	28.4	28.13333	12	11	10.6	11.2	20	20
25.4	26	25.4	25.6	9.2	9.6	9.2	9.333333	20	19.4
tri1.1	tri1.2	tri1.3	m tri1	bi1.1	bi1.2	bi1.3	m bi1	sub1.1	sub1.2
25	26	25	25.33333	12	13	14	13	28	27
30	30	30.6	30.2	18	17	17.4	17.46667	35	32.6
20	20.1	20.2	20.1	11.1	11.3	10.3	10.9	20	20.1
30.2	31.2	31	30.8	12.1	12.1	12.2	12.13333	34	33.4
22	23	21.8	22.26667	11.8	12	13	12.26667	31.6	29.8
31	30	31	30.66667	14	15	14	14.33333	28	26
28	29	28.3	28.43333	12	12.3	12.1	12.13333	39	39.3
31.2	32	32.4	31.86667	19	17.2	16	17.4	30	28
32	31	30	31	17	16.4	16.4	16.6	36.4	36.4
33	34	33.2	33.4	21	19	20.4	20.13333	36	37.2
24	24.6	26	24.86667	10.4	10.2	10	10.2	34	35
33	32	33	32.66667	22	21	22	21.66667	28	26
35	35	35	35	28	27	27	27.33333	39	38
37	37	36	36.66667	26	25	27	26	42	40
31.4	32	32.2	31.86667	17.8	19.2	18.2	18.4	30.8	30.1
22	21	23	22	12	13	12	12.33333	31	29
26	24	25	25	15	18	17	16.66667	34	35
33	34	33	33.33333	20	21	19.8	20.26667	38	37.4
35	35.1	35	35.03333	25.1	25.2	25	25.1	35	34.4
33	34	32	33	23	24	23	23.33333	40	39
31.4	31.2	31	31.2	16.4	15.6	16.2	16.06667	43	42
33	31	33.4	32.46667	18.6	19.2	17.8	18.53333	41	39
38	39		38.5	24	25	26	25	45	45
20.4	20.4	20.8	20.53333	34.6	36	35	35.2	40.4	40.6
36	36	36	36	26	27	26	26.33333	44	47
31.3	31.3	31.1	31.23333	26	26		26	38	37.1

Subject NO.								
Over-weight	sub1.3	m sub1	sup1.1	sup1.2	sup1.3	m sup1	th1.1	th1.2
54	23	22	19	20		19.5	38	39
108	18	18	24	23.2	22	23.06667	35	37.6
98	12.6	12.46667	16	15		15.5	37	37.8
92	31	30.33333	20.2	20.6	21.2	20.66667	40	40.2
93	21	20.8	18	18.4	18	18.13333	41.6	42
62	15.9	16	24.1	24		24.05	41	41
46	18	17.66667	14	15	15	14.66667	35	35
58	16.3	16.23333	23	23	21	22.33333	37	38
49	30	30.33333	22	23	22	22.33333		42
25	28	27.66667	23	22	23	22.66667	42	42
50	43	42.33333	34	35	37	35.33333	43	
47	30	29.66667	26.2	26	26	26.06667	44	44
73		24.2	23.4	23.3	24	23.56667	42	41.1
29	31	31	19	19	18	18.66667	42	42
109	27	27.06667	30	32	30	30.66667	43	44
19	18	18	28	27	27	27.33333	35	36
66		28.2	27	27.1		27.05	32.4	32.4
56	39	38.36667	33.3	33.2		33.25	42	42.5
*117	23.4	23.33333	35	36	37	36	45	45.2
110	20	20	26	25.8	26	25.93333	43.6	44
128	18.6	19.33333	29	31	32	30.66667	42	41
Obese	sub1.3	m sub1	sup1.1	sup1.2	sup1.3	m sup1	th1.1	th1.2
39	27	27.33333	16	15	17	16	43	43
125	32.4	33.33333	38	39	39	38.66667	46	47
83	20.4	20.16667	25.3	25.3	25	25.2	44	44.2
90	33.2	33.53333	23.4	24	24	23.8	44	43.4
112	30.2	30.53333	26.4	26	27	26.46667	27	24.4
24	28	27.33333	31	30	31	30.66667	41	40
87	38	38.76667	33	32		32.5	46	45
122	28	28.66667	30	31		30.5	34	34.8
118	38	36.93333	38	37	39	38	47	47
96	38.4	37.2	34	32	33	33	43.8	43.8
105	34.8	34.6	33.8	33	32.4	33.06667	43	41.2
7	28	27.33333	26	27	26	26.33333	43	42
30	39	38.66667	36	35	34	35	47	47
27	42	41.33333	28	27	29	28	47	
103	31.6	30.83333	31.6	32	31.2	31.6	42	41.2
34	30	30	23	24	26	24.33333	47	44
4	32	33.66667	24	25	22	23.66667	44	42
114		37.7	39	41		40	47	
63	34.1	34.5	27	24	24	25	44	47
26	42	40.33333	28	29	30	29	47	
100		42.5	34	37.2	38	36.4	47	47
107	39	39.66667	38	36	35.4	36.46667	48	
89		45	37	38		37.5	47	44
94	40.8	40.6	32.4	32.6	33	32.66667	44.2	44
35		45.5	36	33	36	35	47	47
60		37.55	34	37		35.5	44	47

Subject NO.								
data1	th1.3	m th1	(c) tbsi	(m) tbsi	den tbsi1	%fat tbsi1	fat%1	lean%1
54	38	38.33333	1.1423	0.0632	1.026499	32.22166	38.7	61.3
108	37.4	36.66667	1.1549	0.0678	1.026894	32.03606	36.8	63.2
98	37.4	37.4	1.1599	0.0717	1.03348	28.96422	28.2	71.8
92	39.8	40	1.1599	0.0717	1.017235	36.61313	37.7	62.3
93	42	41.86667	1.1599	0.0717	1.025128	32.86666	37.3	62.7
62	41	41	1.1599	0.0717	1.025162	32.85053	37.7	62.3
46	35	35	1.1599	0.0717	1.029914	30.6226	37.5	62.5
58	39	38	1.1549	0.0678	1.026397	32.26972	37.8	62.2
49	42	28	1.1423	0.0632	1.018089	36.20487	39.1	60.9
25	42	42	1.1599	0.0717	1.016293	37.06422	38.5	71.5
50		43	1.1599	0.0717	1.009936	40.13012	40	60
47	44	44	1.1599	0.0717	1.015459	37.46435	38.6	61.4
73	42	41.7	1.1599	0.0717	1.020041	35.27443	38.4	61.6
29	47	43.66667	1.1599	0.0717	1.021683	34.49469	31.3	68.7
109	43.6	43.53333	1.1599	0.0717	1.013759	38.28152	38.9	61.1
19	34	35	1.1423	0.0632	1.02237	34.16915	40	60
66	33.1	32.63333	1.1599	0.0717	1.020187	35.20511	39	61
56		42.25	1.1423	0.0632	1.011973	39.1437	39	61
*117	46	45.4	1.1599	0.0717	1.014277	38.03237	41.1	58.9
110	45	44.2	1.1423	0.0632	1.020275	35.16343	40.2	59.8
128	41	41.33333	1.1599	0.0717	1.021585	34.54113	40.6	59.8_59
data1	th1.3	m th1	(c) tbsi	(m) tbsi	den tbsi1	%fat tbsi1	fat%1	lean%1
39	43	43	1.1599	0.0717	1.022806	33.96256	39.7	60.3
125	47	46.66667	1.1599	0.0717	1.010909	39.65816	41.2	58.8
83		44.1	1.1423	0.0632	1.0233	33.72889	41.1	58.9
90	43	43.46667	1.1599	0.0717	1.016417	37.00481	41	59
112	24	25.13333	1.1599	0.0717	1.019255	35.64894	40.3	59.7
24	41	40.66667	1.1423	0.0632	1.015089	37.64212	41.3	58.7
87	47	46	1.1599	0.0717	1.013017	38.63917	40.9	59.1
122	35	34.6	1.1599	0.0717	1.013979	38.17587	41	59
118	46	46.66667	1.1423	0.0632	1.010322	39.94265	41.8	58.2
96	22.6	36.73333	1.1423	0.0632	1.010055	40.0724	42.7	57.3
105	40.8	41.66667	1.1423	0.0632	1.01516	37.60794	44	56
7	42	42.33333	1.1423	0.0632	1.013788	38.26795	44	56
30	47	47	1.1599	0.0717	1.006925	41.59557	43.4	57.6
27		47	1.1423	0.0632	1.00828	40.93519	43.8	56.2
103	39.2	40.8	1.1599	0.0717	1.012777	38.75515	44.8	55.2
34	46	45.66667	1.1599	0.0717	1.020246	35.1773	43.9	56.1
4	40	42	1.1599	0.0717	1.016813	36.8152	45.5	54.5
114		47	1.1599	0.0717	1.00802	41.06148	42.9	57.1
63		45.5	1.1599	0.0717	1.010918	39.65396	44.7	55.3
26		47	1.1599	0.0717	1.009386	40.39717	44.1	55.9
100	47	47	1.1423	0.0632	1.00952	40.33189	46	54
107		48	1.1599	0.0717	1.009025	40.57278	45.45	54.8_54.3
89		45.5	1.1423	0.0632	1.005513	42.28608	46.5	53.5
94	44.2	44.13333	1.1599	0.0717	1.008571	40.79355	47.4	52.6
35	47	47	1.1599	0.0717	1.005399	42.34198	47.8	52.2
60		45.5	1.1599	0.0717	1.008262	40.94359	47.2	52.8

Subject NO.								
Over-weigt	fat(kg)1	lean(kg)1	water1	age	job	gravida	para	abortion
54	24	38	24	34	h/w	2	1	0
108	25	43	29					
98	20	51	36	22	h/w	2	1	0
92	26	43	28	24	dental	1	0	0
93	25	42	28	25	clarical as	1	0	0
62	29	48	31	29	educ.& dev	2	0	1
46	24	40	28	27	care assis	2	1	0
58	28	46	30	19	hair dress	1	0	0
49	27	42	28	33	shop keep	3	2	0
25	25	40	26	24	care assis	1	0	0
50	32	48	29	29	pharmaci	1	0	0
47	27	43	27	20	unemploy	1	0	0
73	28	45	31	26	secretory	1	0	0
29	25	55	36	24	h/w	3		
109	28	44	30	27	cook	7	2	4
19	28	42	29	30	officer	3	2	0
66	30	47	31	25	clark	2	1	0
56	30	47	34	31	civil serant	2	1	0
*117	30	43	26	24	bakery	2	1	0
110	33	49	35	35	nurse	4	1	2
128	33_34	49_49	32	24	h/w	2	1	0
Obese	fat(kg)1	lean(kg)1	water1	age	job	gravida	para	abortion
39	31	47	32	26	secretory	2	1	0
125	35	50	31	20	un employ	4	0	3
83	37	53	37	31	sells ass	2	1	0
90	34	49	32	26	receptionis	1	0	0
112	31	46	33	28	h/w	3	2	0
24	38	54	35	30	h/w	2	1	0
87	36	52	34	21	h/w	3	1	1
122	32	46	31	28	cashier	1	0	0
118	38	53	37	32	blood trans	3	1	1
96	41	55	37	32	h/w	3	2	0
105	40	51	34	33	h/w	5	3	1
7	37	47	30	32	nurse	3	1	0
30	46	60	38	27	nurse	1	0	0
27	39	50	33	31	h/w	5	4	
103	43	53	33	23	videograph	2	1	0
34	36	46	31	24	h/w	3	1	1
4	45	54	68	28	ex.teacher	2	1	0
114	42	56	38	22	credit cont	3	1	0
63	38	47	30	23	h/w	2	1	0
26	41	52	34	29	cook	1	0	0
100	46	54	33	35	h/w	2	1	0
107	42_43	51.5_51	32	24	glass artist	1	0	0
89	47	54	36	30	h/w	3	1	1
94	55	61	37	27	care taker	3	1	1
35	54	59	37	25	nurse	1	0	0
60	51	57	38	26	h/w	3	1	0

					Subject NO.	Continuation from page 6		
smok/day	alcohol	meal time	ges.age.s	ges.age.d	Over-weight	fast. level	GTT (wks)	120 min
0	0		10	13	54	5.2		7.2
					108			
5	1	3.0	13	13	98			
0	0	3.0	13	14	92			
0	0	1.0	14	14	93			
0	1	0.5	14	15	62			
10	1	1.0	9	9	46			
15	1	2.0	14		58	4.2		6.4
0	1	4.0	14	14	49	4.1		7
0	0	2.0	12	16	25	4.1		6.8
0	0	2.0	11	10	50			
2-3/day	0	2.0	11		47			
0	0	2.5	13	14	73	4.4	29w	5.6
0	0		12	14	29			
0	1	4.0	15	15+	109			
20	0	3.5	15	14	19			
0	1	2.5	13	14	66	4.5		7
0	0	4.0	13	15	56	4.4		9.4
0	0		10		*117	4.6	29w	7.3
0	1	3.5	13	14	110	5.9	28w	6.5
0	0	10.0	13	14	128	4.7	31w	7.3
smok/day	alcohol	meal time	ges.age.s	ges.age.d	Obese	fast. level		120 min
0	0	3.0	12	15	39	4		5.3
0	0	2.0	13	14	125			
0	1	3.0	15	15	83	4.7		5.6
0	0	1.5	13	14	90			
0	0	f	14	14	112	5.2	28	6.4
0	0	2.0	15	15	24	4.9		8.2
15/day	0	3.0	13	15	87	5.1	30	5.1
0	1	1	14.0	15	122			
0	1	2.0	15	15	118			
0	0	2.0	13		96	6.3	29	7.4
0	0	4.0	13	15	105	4.1	29w	6.4
0	0	4.5	10		7			
0	0	0.5	15	16	30	5		5.6
0	1	3.0	12	14	27			
0	0	4.0	13	15	103	5.1	30w	5.1
3	1	4.0	14	15	34	4.4		7.3
0	0	3.0	14	14	4	5.1		6.1
10	0	4.0	15	15	114	5	28	3.9
0	0	3.5	14	15	63	4.5		5.6
0	0	4.0	15	16	26		gest. diab.	
10	1	1.5	15	15	100	5.5	28 w	5.3
0	1		11	11	107	4.3	29w	7
0	0	3.0	14	15	89	6.1		8.8
0	1	4.0	13		94	4.5	29	6
0	1	2.0	15	15	35	5		8.5
1	0	0.0	5		60	5.3	29 wks	5.5

Cord blood samples collected at birth

NO	UNIT NO	HAEMOLYSIS	INSULIN mU/l	C-PEPTIDE ng/ml
1. (17)			2	0.19
2. (63)	543457		<0.2	0.080
3. (64)			<0.2	0.060
4. (73)	581266		1.6	0.13
5. (21)	569439		3.2	0.07
6. (30)	449763		2.9	0.075
7. (7)			1.7	0.030
8. (36)			<0.2	0.040
9. (104)	771023		<0.2	<0.02
10. (6)			<0.2	0.025
11. (39)	574048	++	NA 6.0	0.070
12. (20)	516357		0.5	0.050
13. (54)	539152		<0.2	0.060
14. (60)	454654		10.2	0.17
15. (90)	473979	+	NA 0.5	0.040
16. (106)	522812		<0.2	0.065
17. (115)	351491		0.5	0.050
18. (125)	762227		7.6	0.075
19. (55)	719813		<0.2	<0.02
20. (71)	288438		2.3	0.065
21. (19)	426481		2.9	0.12
22. (113)	536631		6.0	0.17
23. (50)			1.9	0.08
24. (94)	472905		8.8	0.14
25. (100)	392084		1.6	0.075
26. (52)	569559		12	0.060
27. (107)	579905		12.8	o.17
28. (78)	361802		<0.2	0.05
29. (26)	569641		15	0.14
30. (68)	578815		<0.2	0.025
31. (1)	730812	++	NA 2.9	0.075
32. (77)	526533	++	NA <0.2	0.070
33. (82)	545138			0.070
34. (84)	516944		3.0	0.080
35. (124)	513960		<0.2	0.12
36. (10)	316100		<0.2	0.05
37. (43)	425149		<0.2	0.04
38. (128)	505104		<0.2	0.14
39. (49)	530166		<0.2	0.21
40. (80)	714410	++	NA	0.19
41. (105)		++	NA	0.06
42. (62)	468906		<0.2	0.065
43. (58)	714629		0.5	0.075
44. (118)	479299			0.23
45. (96)	410145	++	NA 16.5	0.12
46. (99)	522036		<0.2	0.13
47. (32)	518081		1.12	0.18
48. (23)	522911		1.5	0.070
49. (42)	498442	++	NA	0.050
50. (75)	481850	++	NA<0.2	0.080
51. (89) **GDM**	316936	++	NA	0.035
52. (37)	467459	+++	NA	0.045

NO	UNIT NO	HAEMOLYSIS	INSULIN mU/l	C-PEPTIDE ng/ml
53. (109)	513539	++	NA	0.030
54. (116)	235078		3.8	0.10
55. (4)	429048	++	NA	0.030
56. (35)	741932		<0.2	0.085
57. (45)	539149		<0.2	0.020
58. (48)	265069			0.050
59. (101)	581298	++	NA	0.10
60. (121)	271522	++	NA	0.12
61. (24)	265636	++	NA	0.11
62. (12)	546118		<0.2	0.070

Blood samples taken for the purpose of insulin sensitivity measurements.

*: samples that were used in our study.

No.	Subject No.	BMI (kg/m^2)	1st blood sample (wks)	2nd sample (wks)	3rd sample (wks)	GTT	Cord blood sample
1	35) *	42.01	-	25	35	+	+
2 (GDM)	89)	38.96	15	24	35	+	+
3	100)	36.28	-	25	36	+	+
4	114)	35.13	-	28	36	+	-
5	34) *	35.02	14	24	36	+	-
6	27) *	33.91	12	28	36	-	+
7	105) *	33.62	13	-	36	+	+
8	96)	33.39	13	-	38	+	+
9	118)	32.24	15	25	36	-	+
10	39)	30.09	12	-	35	+	-
11	128)	29.93	14	26	34	-	+
12	110) *	29.75	13	24	36	+	+
13 (GDM)	56)	29.34	14	26	34	-	+
14	29) *	28.34	12	25	35	-	-
15	73)	28.16	13	-	36	-	+
16	49) *	27.63	14	25	35	+	+
17	58)	27.51	-	26	37	+	+
18	62) *	26.95	14	-	35	-	+
19	92)	26.61	13	25	35	-	-
20	120)	25.39	14	-	36	-	-
21	15) *	24.67	12	-	35	-	-
22	95)	24.15	14	24	-	+	-
23	37)	23.95	14	-	36	+	+
24	13) *	23.62	10	-	35	-	+
25	14)	23.53	14	-	36	-	-
26	80) *	23.42	15	-	37	-	+
27	23) *	21.57	13	-	35	-	+
28	1)	21.50	13	-	37	-	+
29	12)	21.48	-	-	37	+	+
30	116)	21.20	-	24	36	+	+
31	17)	20.02	13	-	35	+	+
32 (IGT)	45)	16.44	11	29	36	+	+

Appendix 3

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF OBSTETRIC AND GYNAECOLOGY

HORA SOLTANI
MIDWIFE, M MED SCI IN HUMAN NUTRITION & PhD STUDENT

ROBERT B. FRASER
CONSULTANT OBSTETRICIAN AND GYNAECOLOGIST

TITLE

LONGITUDINAL CHANGES IN BODY COMPOSITION WITH ADVANCING PREGNANCY AND THE RELATIONSHIP OF MATERNAL FAT DEPOSITION TO FETAL GROWTH:

BACKGROUND

Obesity has long been recognised as a risk factor for the development of diabetes mellitus, particularly in later life and in young women it widely thought to predispose towards impaired glucose tolerance and gestational diabetes. Obesity is not only common but it is increasing in prevalence despite efforts to avoid it made by many people. We now know that, there is more to obesity than the actual amount of excess weight. The distribution of subcutaneous fat and type of obesity might have significant effects on glucose tolerance and insulin levels.

In my M Med Sci dissertation (1), the prevalence of obesity among pregnant women in Sheffield was compared with a city in north of Iran (Tabriz). The results showed that more than 35% of the population under study in Sheffield were overweight or obese. This was contrasted with an obesity rate of 14.6% in Tabriz.

Studies on body fat during pregnancy, have produced variable results. Hytten and Leitch (2), suggested that pregnant women store body fat at least up to 30 weeks gestation to provide an energy bank for the demands of late gestation and lactation.

On the other hand, a study on a randomised series of pregnant, non-pregnant and postpartum women (3) did not show a consistent trend for fat deposition.

A study by Lanchoff-roos et al (4), in which maternal fat stores and fat accretion during pregnancy were estimated, in a small group of women, showed a wide range of maternal fat accretion during pregnancy, and the amount of fat accumulated was correlated to the total weight gain. Maternal fat accretion during pregnancy was found to have little relationship with the infant birthweight at term.

A recent investigation on 22 Swedish healthy pregnant women (5), suggests that 60% of net pregnancy fat gain was already present at 16 gestational weeks. In that study fat gain was not correlated with infant birth weight.

There are several studies on maternal body composition and outcome of pregnancy (6, 7, 8, 9) but few data exist on the effect of maternal fatness on fetal metabolism.

The present work will examine further the question of fat storage by means of a longitudinal study to establish changes in body fat during normal pregnancy and postpartum, and also its relationship to fetal metabolism, new-born anthropometry and early infant growth.

AIMS

The aims are to:

1. Study Body Composition (BC) longitudinally in pregnancy and its relation to BC after delivery in healthy volunteers selected on the basis of initial body weight.

2. Establish any relationship of obesity/type of obesity to Diabetes and/or Impaired Glucose Tolerance in late pregnancy.

3. Investigate the metabolic status of new-born in relation to maternal body composition and degree of obesity, by measurements of new-born anthropometry and insulin levels of cord blood.

4. Short term follow up of maternal fat distribution (6 months postpartum) in relation to changes seen in pregnancy and infant feeding behaviour.

SUBJECTS

Cohorts of normal weight, underweight, overweight and obese women will be identified amongst the attenders to N.G.H antenatal clinics.

30 volunteers subjects with Body Mass Index less than 19.8 as underweight, 30 volunteers subjects with BMI: >26-29 as overweight, and 30 volunteers subjects with BMI more than 29 as obese subjects will be recruited at first visit to NGH Antenatal Clinics at 10-12 weeks gestation. In addition, 30 volunteers subjects with BMI: 19.8-26 will be recruited as normal weight controls.

DESIGN

Women will be recruited from the antenatal clinics of all the consultants at NGH (with permission).
During pregnancy, serial measurements will be made on three occasions on each patients, at 10-14, 24-28 weeks and at 34-38 weeks gestational age, calculated from the date of the last menstrual period, confirmed by Ultrasound scanning.
Two more measurements will be made at 6/52 and 6/12 after delivery.

Waist and hip circumferences will be measured at first visit to identify body type of pregnant women.
Body composition will be analysed by electrical impedance using EZ COMP 1500 (Body Composition Analyser) and skin fold thicknesses, using Harpenden Callipers, will be measured at 5 sites of body (triceps, biceps, sub scapular, suprailliac and mid thigh).

A 75g oral glucose tolerance test will be performed at 28-34 weeks.
Ten ml blood samples will be collected at the booking visit and late pregnancy (34-36) to measure insulin sensitivity, by an in vitro technique using cultured rat myoblasts.

Birth weight, placental weight, new-born anthropometry (length, head circumference,) and cord blood for fetal insulin levels will be assessed for each women's baby.
Maternal BC, skin fold thickness and infant anthropometry will be performed at 6/52 and 6/12.

The measurements and tests will be performed on the maternity unit at NGH. Each test will require 20 minutes to perform.
An explanatory consent form will be signed.
Travelling and child care expenses will be paid.

DATA ANALYSIS

Using SPSS, statistical package an Analysis of variance (ANOVA) will be made to find the significance of differences between the four study groups, and within the overweight & obese groups by type of obesity.
The Δ–weight and Δ–fat distribution in each group will be compared in relation to variables such as age, parity and etc.
The type of obesity will be characterised.
To relate outcome of pregnancy to the different variables measured during study, we will use the multiple regression technique the whole group.

ETHICAL CONSIDERATIONS

The measurement of the body fat by Harpenden callipers and bioelectrical impedance is quite safe, and was the subject of an earlier successful application to this committee.

Bioelectrical Impedance makes use of the fact that body fluids are excellent conductors of electrical current. This technique, uses a very small current and has been extensively evaluated in pregnancy and is known to be safe.

The blood samples will be taken at the same time as routine blood check (booking visit and late pregnancy for Hb checking).

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Information Sheet

STUDY

Project to study fat changes during pregnancy, after delivery and effects of maternal fat on baby growth:

This study is important as it will help us to understand the pattern in which fat might be laid down in the body in pregnancy. It should help to get more information about whether there is a relationship with body weight and risk of diabetes when you are pregnant.
At the first visit which is your booking visit in the Antenatal Clinics at Northern General Hospital, we would measure your body weight and height. Also your body composition will be estimated by measuring your skin thickness and using a small electrical instrument which will measure your body composition. This method is totally safe and will not harm you or your baby.
The measurements will be repeated at 24-28 weeks and 34-38 weeks of your pregnancy and also 6 weeks and 6 months after the birth of your baby.
Glucose Tolerance Test (GTT) will be performed at 28-34 weeks (Information sheet about GTT is also available).
Blood samples will be taken as the same time as routine blood check.
After birth blood will be taken to measure the insulin levels, this blood is not taken from the baby but from the after birth once it has been separated.
Baby's weight, length, head circumferences will be measured at birth and 6 weeks and 6 months later.
All visits will be in Antenatal Clinics of NGH.
Travelling and child care expenses will be paid.
You can change your mind and withdraw from the study at any stage.

Hora Soltani, Midwife, M Med Sci in Human Nutrition & PhD Student

CONSENT FORM

STUDY: Project to study fat changes during pregnancy, after delivery and effects of maternal fat on baby growth:

I have read the above information sheet and any other queries have been answered. I understand that I may withdraw from the study at any time.

NAME:
ADDRESS:

SIGNED:

TEAM:
NO.
HOSP.NO.

WEIGHT
HEIGHT
BMI

W/H ratio

WAIST:

HIP:

THIGH:

SKINFOLDS

TRICEPS:

BICEPS:

SUBSCAPULAR:

SUPRAILLIAC:

THIGH:

BODY COMPOSITION

FAT%:

LEAN%:

FAT(Kg):

LEAN(Kg):

WATER:

DATE:
AGE
JOB
G P

SMOKING
ALCOHOL
TIME OF LAST MEAL
PREPREGNANCY Wt

EDD

GEST.AGE (BY SCAN):
(BY DATE):
SECOND Appt.
THIRD Appt.

MOTHER'S NAME
NO.

DATE.OF DELIVERY
DAY OF DELIVERY
TYPE OF DELIVERY

TYPE OF FEEDING

BABY'S SEX
BIRTH WEIGHT
PLACENTAL WEIGHT
BABY'S GESTATIONAL AGE

BABY'S WEIGHT

LENGTH

HEAD CIRCUMFERENCE

DATE