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Metabolic, methodological and developmental aspects of body composition

Studies in women and children with special reference to early life mechanisms behind childhood obesity

Britt Eriksson

Department of Clinical and Experimental medicine
Faculty of Health Science
Linköping University Sweden
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Klokast är den som vet vad han inte vet

Wisest is he who knows what he does not know

Sokrates

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1. ABSTRACT

In recent decades the number of children with overweight has increased worldwide. To understand the mechanisms behind this development, knowledge regarding metabolism and physiology in relation to the nutritional situation in early life is of importance. In particular, information about body composition development during early childhood is relevant. This thesis presents three studies in this area. In the **pregnancy study** serum samples, collected from 23 women before, during and after pregnancy, were analysed for serum levels of leptin, adiponectin and resistin and used to assess insulin resistance (HOMA-IR) in relation to the total body fat (TBF) content of the women. TBF (%) and leptin were significantly correlated with HOMA-IR before and during pregnancy. When HOMA-IR was regressed on TBF (%) the slope of the regression line was 0.111 in gestational week 32 and significantly ($p<0.05$) higher than the value before pregnancy, 0.046, indicating that healthy pregnancy enhances the relationship between body fatness and insulin resistance. In the **HF-study** hydration of fat-free mass (hydration factor, HF) was assessed in 12 newborns using the doubly labelled water (DLW) method and air displacement plethysmography (PeaPod). HF was 80.9% with a low biological variability (0.81% of average HF). In the **longitudinal study** the body density of 108 healthy fullterm infants (53 girls, 55 boys) was measured at one and 12 weeks of age using PeaPod. Body composition was calculated using two models (Fomon's and Butte's). BMI values for the mothers of the infants were assessed before pregnancy. Body composition and total energy expenditure using the DLW-method were assessed in 20 of these children at the age of 1.5 years, when their sleeping metabolic rate was measured using indirect calorimetry and their resting energy metabolism was calculated using prediction equations. Butte´s model gave significantly ($p<0.05$) lower values for TBF than Fomon´s model, and invalid results for five newborns. Using Fomon´s model, at one week of age girls contained 13.4 ± 3.7 % and boys contained 12.5 ± 4.0 % TBF. The corresponding figures at 12 weeks were 26.3 ± 4.2 % and 26.4 ± 5.1 %. The mothers' BMI values before pregnancy were correlated with the body weight but not with the TBF (g,%) or fat-free mass (g) of their infants at one week of age. At 1.5 years of age girls (n=9) contained 28.0±2.8 % and boys (n=11) 28.3±3.7 % TBF. Between one and 12 weeks of age all infants increased their TBF content, while 13 children increased and seven children decreased their TBF content between the ages of 12 weeks and 1.5 years. The results demonstrated that predicting rather than measuring resting energy metabolism involves a risk for spurious correlations between TBF and physical activity level. The level of physical activity (x), was negatively correlated with [TBF (%) at 1.5 years minus TBF (%) at 12 weeks] (y), $r=-0.52$, $p=0.02$. In conclusion, the results suggest that the body fat content of a woman has a stimulating effect on the growth, rather than on the fat retention, of her foetus. They also show that the Fomon model is the best available model when calculating the body composition of infants from body density. Finally, the results indicate that physical activity at the age of 1.5 years is important regarding the rate at which the high level of body fat, typical of infancy, decreases in early childhood.

2. LIST OF PUBLICATIONS

I. B Eriksson, M Löf, H Olausson, E Forsum. Body fat, insulin resistance, energy expenditure and serum concentrations of leptin, adiponectin and resistin before, during and after pregnancy in healthy Swedish women. Br J Nutr 2010; 103: 50-7.

II. B Eriksson, M Löf, U Hannestad, E Forsum. Hydration of fat-free mass in human newborns: assessment and implications when calculating body composition from body density. 2010. (Manuscript)

III. B Eriksson, M Löf, E Forsum. Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. Acta Paediatrica 2010; 99:563-8.

IV. B Eriksson, H Olsson, M Löf, U Hannestad, E Forsum. Body composition and energy expenditure in response to physical activity in 1.5 year old children studied by means of the doubly labeled water method. 2010. (Manuscript)

3. ABBREVIATIONS

AEE	Activity energy expenditure
BMI	Body mass index
BMR	Basal metabolic rate
CO_2	Carbon dioxide
CV	Coefficient of variation
DLW	Doubly labelled water
FFM	Fat-free mass
HOMA-IR	Homeostatic model assessment of insulin resistance
FFMD-Fomon	Fat-free mass density according to Fomon
FFMD-Butte	Fat-free mass density according to Butte
FM	Fat mass
FMI	Fat mass index
HF	Hydration factor
O_2	Oxygen
PAL	Physical activity level
PAL_{BMR}	PAL obtained as TEE divided by BMR
PAL_{SMR}	PAL obtained as TEE divided by SMR
PI	Ponderal index
SD	Standard deviation
SMR	Sleeping metabolic rate
TBF	Total body fat; in this thesis equivalent to “body fat”.
TEE	Total energy expenditure
TBW	Total body water

4. INTRODUCTION

4.1 Background

In response to a positive energy balance the human body retains an excessive amount of fat. From an evolutionary perspective the physiological capacity to store energy in the form of fat meant a greater chance of survival in times of starvation. It is therefore hardly surprising that human beings with a sedentary lifestyle encounter problems with overweight and obesity when living in surroundings where highly energy dense foods are plentiful and easily accessible.

Obesity is associated with an increased risk for several diseases such as diabetes, heart disease and some forms of cancer (1) and it also has psychosocial consequences. In recent decades the number of children with overweight and obesity (1, 2) has increased worldwide. This trend is especially pronounced in the U.S. (3). Since 2000, the rate of obesity and overweight in children and adolescents has decreased in France, Germany and England, while an increasing rate has been observed in many other European countries (4). Childhood obesity represents a serious health problem since it tends to persist into adulthood (1). However, available knowledge is limited regarding the specific mechanisms responsible for the recent increase in childhood obesity. For example, there is insufficient knowledge regarding the normal development of body composition during infancy and childhood, and the lack of appropriate methodology for studying this area is most likely an important explanation for this situation.

Studies conducted in recent years have demonstrated that nutrition in early life may be associated with body composition and health later in life (5). For example, poor nutrition during pregnancy is associated with low birth weight and a high risk of obesity later in life (5). Furthermore, birth weight and growth rate have both been identified as variables correlated with fatness in childhood and adulthood (1, 6, 7). A high birth weight has been related to a high body mass index (BMI), and a low birth weight has been related to central adiposity (1, 5, 8). However, the complexity of these relationships emerges when body composition is considered. Studies of fat and lean mass in the body at childhood, adolescence and adulthood in relation to size at birth have shown that a high birth weight tends to be associated with a large fat-free mass (FFM) rather than with a high fat mass (FM), while low birth weight is associated with central fatness (5, 9). Further, in Western populations the infant growth rate has been linked to adiposity later in life, while research in Brazil,

Guatemala and India has shown an early growth rate to be associated with lean mass rather than with FM at adolescence and young adulthood (5).

The foetus is influenced by the nutritional situation of its mother. Studies have shown that body composition of the mother before pregnancy is related to growth of her foetus (1, 10) and there are indications that a high body fat content in women leads to general augmentation in foetal growth rather than to stimulation of adipose tissue growth (10). However, other studies (11, 12) have reported that the offspring of overweight and obese women have a higher body fat content than the offspring of lean mothers. Thus, although it is clear that the nutritional status of a mother influences the growth of her foetus, available results are contradictory. Consequently there is a need for more studies in this area to clarify if and how the composition of the newborn is affected by the body composition of the mother.

It has been argued that some periods during human growth and development are more critical than others when it comes to the risk of obesity. Such periods are foetal life, early infancy and the periods of adiposity rebound, i.e. between five and seven years of age as well as adolescence (1). Early adiposity rebound, parental obesity, high birth weight, and rapid early weight gain have all been independently associated with obesity in childhood (13).

The role of physical activity in establishing overweight and obesity early in life has attracted interest. For example, the relationship between total body fat (TBF, %) and physical activity level (PAL) has been investigated in several studies and although it is not a universal finding (14), a significant negative relationship between these two variables has often been identified (15-17). This may indicate that as children become fatter, they tend to be less physically active, resulting in an increased risk for a positive energy balance and possibly leading to even more fat retention, thereby creating a vicious cycle. However, it has been pointed out (15) that the correlation between PAL and TBF (%) may be spurious if estimates of PAL are calculated using values for resting energy metabolism obtained by means of prediction equations based on body weight. It is also conceivable that the amount of energy expended in response to physical activity is influenced by environmental and genetic mechanisms. For example, the tendency to expend a high amount of such energy may be biologically determined (18) or influenced by the child's stage of motor development.

4.2 Adipokines

It has been hypothesized that some kind of chemical signal may be responsible for transferring information regarding the mother's nutritional status to her foetus (1). In fact, pregnancy affects the metabolism and physiology of a woman in several ways that may influence the nutritional needs of herself as well as of her foetus. Such metabolic and physiological effects that are well-known to occur during pregnancy are changes in food intake and energy expenditure as well as increases in body fatness and insulin resistance. In this context the so-called adipokines (i.e. leptin, adiponectin and resistin), which are released from adipose tissue, are of interest due to evidence suggesting that these hormones are involved in regulating the kind of processes mentioned above (19-22).

4.2.1 Leptin

Leptin is a hormone that regulates appetite and energy expenditure and published data indicate that leptin is involved when insulin resistance is established (20). The concentration of leptin in serum increases during pregnancy and has been shown to correlate with the TBF content in pregnant (23) and in non-pregnant individuals (23-25) as well as with insulin resistance in non-pregnant women (24). A correlation between leptin in serum and insulin resistance has also been found in a group of pregnant women, including women with gestational diabetes (26) and in healthy women in the first trimester (27).

4.2.2 Adiponectin

Adiponectin is known to be involved in the regulation of blood glucose levels and insulin sensitivity (19). Results from studies regarding relationships between serum concentrations of adiponectin and body fatness are contradictory in pregnant (28-30) and non-pregnant (24, 31) women, probably because body fatness is not an independent predictor of adiponectin in serum (31). An inverse correlation between serum adiponectin and insulin resistance has been found in non-pregnant women (24, 31, 32) and in groups of pregnant women including subjects with gestational diabetes (30, 33).

4.2.3 Resistin

Resistin is produced by adipose tissue, but is not a true adipokine since it is not produced by adipocytes. It has been suggested that resistin is associated with insulin resistance (19, 34), but its role in this context is uncertain. Silha *et al*. (24) found no correlation between body

fatness and serum concentrations of resistin in non-pregnant women. No data regarding relationships between resistin in serum and TBF or HOMA-IR (homeostatic model assessment of insulin resistance) in pregnant women have been reported.

4.3 Body composition

4.3.1 Principles of body composition assessment

Assessing the composition of the human body requires a model of its components. The simplest model is the so-called two-component model where fat represents one part, the fat mass (FM) and the remaining body mass the other part, the so-called fat-free mass (FFM). This latter part can be further divided into water and solids, thereby creating a three-component model consisting of fat, water and solids. (35, 36).

Body composition can be assessed based on body density, i.e. body weight divided by body volume. The density of the human body is a function of the proportions of its components and their densities (37). Assuming that the body is composed of FM and FFM and that the densities of these components are known, the proportions of FFM and FM in the body can be calculated using the equation: $1/d = x / d_{FM} + (1-x)/d_{FFM}$. In this equation x is the proportion of fat in the body while d, d_{FM} and d_{FFM} are the densities of the body, FM and FFM, respectively (37). Body composition can thus be calculated from body density if FM and FFM densities are known. The density of fat is 0.9007 (37) and it is quite constant and well established (38), while the density of FFM is more variable since FFM consists of several components with different densities in proportions that may vary in response to age and physiological status. Values for FFM density in infants and children have been published by Fomon *et al.* (39) and by Butte *et al.* (40).

Previously, assessment of body density was often used as a reference body composition method. Assessing body weight presents no problems, while measuring body volume is more difficult. Traditionally, body volume was measured by means of underwater weighing (37), which involves completely submerging the subject in water. For obvious reasons this is not possible in infants and small children. However, in 2004 the so-called PeaPod (Life Measurement, Inc., Concord, CA, USA) (41) device was launched, making it possible to measure the body volume of infants in a quick, convenient, accurate and safe way.

The models described above make it possible to measure the body composition of humans *in vivo*. Most commonly, total body water (TBW) is assessed and used to calculate FFM and then FM is calculated by subtracting FFM from body weight. Calculating FFM from TBW requires that the amount of water in FFM, the so-called hydration factor (HF), is known.

4.3.2 Hydration factor

As indicated above, assessing body composition *in vivo* using the two-component model assumes that HF is known. Another condition which has to be fulfilled is that the variability of HF between subjects, the so-called biological variability,is low. Hydration of FFM undergoes considerable changes during the life cycle. In healthy adults HF is about 0.73, while it is considerably higher in newborns and declines during infancy and childhood (42). Furthermore, the ratio between extracellular and intracellular water changes rapidly in the early postnatal period (43). The biological variability of HF has been found to be low (about 2 % or less of the average HF) in adults (44), in 8 to 12-year-old children (36) and in pregnant women in gestational week 32 (45). Butte *et al.* (40) assessed HF in two-week-old infants and found this estimate to have a low coefficient of variation (CV), 1.8-1.9 %. This figure, which includes the variability associated with the methods used to assess HF, indicates that the biological variability of HF is also low in young infants. However, no study investigating the biological variability of HF in newborns is available.

4.3.3 PeaPod

PeaPod consists of a scale and a chamber with a tray that can be pulled out. A computer is connected to the system. PeaPod uses air displacement plethysmography to measure the volume of the infant body. This technique is based on relationships between pressure and volume in gases as expressed by Boyle's and Poisson's laws (41). The measured body volume is adjusted for thoracic gas volume by appropriate equations. From the subject's weight and length a correction for the so-called "surface area artifact" is made using an appropriate equation (41). The figures for weight and volume of the infant obtained in PeaPod are used to calculate body density. Body composition is then calculated using data for FFM density published by Fomon or Butte (39, 40) and assuming an FM density of 0.9007 (g/ml). When results obtained using the air-displacement plethysmography technique, as used in PeaPod, have been compared with results obtained using a 4-component body-composition model (46), it has been shown that the PeaPod system is able to provide accurate assessments of infant body composition. Results obtained in fullterm neonates by means of PeaPod have

been published by Hull *et al.* (12), Moyer-Mileur *et al.*(47) and Carberry *et al.* (48). Gilchrist has reported preliminary reference data, assessed using PeaPod, for 80 fullterm breastfed infants at the following ages: 1 and 2 weeks and 1, 2, 3, 4, and 5 months (49). Results obtained in premature infants have been published by Roggero *et al.* (50, 51) and Gianni *et al.* (52). The study included in this thesis is the first to provide longitudinal data on body composition, obtained by means of PeaPod, in a group of healthy Swedish infants.

4.3.4 Reference data on fat-free mass density

Values for FFM density in infants and children have been published by Fomon *et al.* (39) and by Butte *et al.* (40). Fomon's values represent reference data for boys and girls from birth to age 10 years, while Butte's data were collected in a longitudinal study of children of both sexes between two weeks and two years of age. Fomon constructed his model using data obtained during his own investigations in combination with data from the literature (39, 53). This model provides data regarding the contents of protein, water and minerals in FFM for each month of life until the age of 9 months, thereafter at the ages of 12, 18 and 24 months, and for each year until 10 years of age. Butte's model is based on measurements of body weight, body water, body protein and bone minerals in 76 children at the ages of 0.5, 3, 6, 12, 18 and 24 months (40). An important difference between the two models is the estimated amount of water in FFM (HF), which is higher in Butte's model, especially during the very beginning of life (40). More studies of HF during early infancy are therefore motivated.

4.4 Doubly labelled water method

4.4.1 Theoretical background

During recent decades the doubly labelled water (DLW) method has been used extensively to assess the total energy expenditure (TEE) of human subjects during free-living conditions. Such estimates were previously not possible. In this technique an accurately weighed amount of the stable isotopes ^{2}H and ^{18}O are given to the subject *per os* after collecting one or several background urine samples. Additional urine samples are then collected during one to two weeks after dosing. Subsequently, isotope enrichments of dose and urine samples are assessed using an isotope-ratio masspectrometer. The principle is that deuterium mixes with body water, while ^{18}O mixes both with body water and CO_2. Consequently, ^{2}H is lost from the body as water, while ^{18}O is lost both as water and as CO_2. The difference between the disappearance rates of ^{2}H and ^{18}O is therefore a measure of the CO_2- production rate, which can be

converted to energy expenditure using the Weir equation (54) with appropriate assumptions regarding the so-called "food quotient" (55). Calculating CO_2 production also requires an estimate of TBW, which can be obtained using either of the isotopes by means of two different modes of calculation, the plateau approach or the back extrapolation approach. The capacity of the DLW method to provide valid estimates of TEE in humans is well-documented (56).

4.4.2 Application

The DLW method can be used to assess the amount of energy expended by a human subject in response to physical activity during free-living conditions. This requires an estimate of the resting energy expenditure assessed, for example, as the basal metabolic rate (BMR). BMR can be estimated by means of prediction equations (57) or measured using indirect calorimetry during defined conditions (resting, thermoneutrality, and in the post absorptive state). In adults, BMR is generally measured after an overnight fast when the subject is awake, but in small children the measurement is generally performed in the non-fasting state when the child is asleep and is then called the sleeping metabolic rate (SMR). The energy expended in response to physical activity can be assessed either as the PAL, calculated as TEE (assessed by means of the DLW-method), divided by BMR or SMR, or as activity energy expenditure (AEE), i.e. TEE minus BMR or TEE minus SMR. The DLW-method also provides data on TBW, information that is useful in body composition studies (section 4.3).

5. SPECIFIC AIMS

To explore relationships before, during and after pregnancy between TBF, insulin resistance and serum concentrations of leptin, adiponectin and resistin.

To study women's BMI before pregnancy and weight gain during pregnancy in relation to the body composition of their infants.

To assess the HF and its biological variability in fullterm newborns aged 10 days or younger.

To compare results obtained when values for FFM density published by Fomon *et al.* (39) and by Butte *et al.* (40) are used to calculate body composition from body density in young infants.

To use PeaPod, a device based on air displacement plethysmography, to study body composition of healthy, fullterm infants born to well-nourished women with a Western life-style.

To study the development of body composition during infancy and early childhood.

To compare relationships in 1.5-year-old children between PAL and TBF (%) obtained when predicted versus measured values for resting energy expenditure are used to calculate PAL.

To relate changes in TBF (%) between 12 weeks and 1.5 years of age to energy expended in response to physical activity at the age of 1.5 years.

6. MATERIALS AND METHODS

6.1 Pregnancy study (Paper I)

6.1.1 Study design

Serum samples from 23 women were analysed for their concentrations of leptin, adiponectin and resistin. The samples were collected at seven measurement occasions: before pregnancy, in gestational weeks 8, 14, 20, 32 and 35, as well as 2 weeks after delivery. Blood glucose, serum insulin concentrations and body composition were assessed at four measurement occasions: before pregnancy, in gestational weeks 14 and 32, as well as 2 weeks after delivery. The women were asked to come to the hospital in a fasting state, and upon arrival they were asked when they had last eaten any food. A subgroup of 17 women were in the fasting state at all four measurements: before pregnancy, in gestational weeks 14 and 32, as well as 2 weeks after delivery. The characteristics including body composition of the 23 women are reported in paper I. Before pregnancy, all women were healthy with normal blood glucose (4.2 ± 0.5 mmol/L) and serum insulin (7.4±2.4 mU/l) concentrations. None of the women developed gestational diabetes. They all delivered one healthy, fullterm infant. The study was approved by the Ethics Committee of the University of Linköping, Sweden.

6.1.2 Analyses of blood and serum samples

Blood was collected from a cubital vein and the glucose content was measured colorimetrically using the HemoCue analyser (HemoCue AB, Ängelholm, Sweden). The blood was kept at + 4° C for 4 h and thereafter centrifuged at 1500 g for 10 minutes. Serum was harvested and stored at -70° C. Serum insulin was measured by means of radioimmunoassay (Pharmacia Insulin RIA 100, Pharmacia & UpJohn Diagostics, Uppsala, Sweden). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as HOMA-IR=[fasting levels of insulin in plasma (mU/L) x fasting levels of glucose in plasma (mmol/L)] / 22.5 according to Matthews *et al*. (58, 59). Serum glucose concentration was calculated by multiplying the blood glucose concentration by 1.11. Adipokines were analysed using kits from Linco Research, St. Charles, MO, USA, i.e. the human leptin ELISA kit (Cat. # EZHL-80SK), the human adiponectin ELISA kit (Cat. # EZHADP-61K) and the human resistin ELISA kit (Cat. # EZHR-95K). According to the manufacturer these kits measure leptin in free and bound forms, all multimeric forms of adiponectin, and both hexameric and trimeric forms of resistin, respectively. However, for resistin it has not been possible to definitely confirm which isoforms the assay actually measures.

6.2 HF-study (Paper II)

6.2.1 Study design

The HF and its biological variability were assessed in 12 infants at or before their 10th day of life (Table 1). Their volumes and weights (without clothing) were estimated using PeaPod (Life Measurement, Inc., Concord, CA, USA) (41) at a measurement session with two repeated assessments two hours apart. TBW was assessed using stable isotopes. The study was approved by the Ethics Committee of the University of Linköping, Sweden.

6.2.2 Recruitment and characteristics of the infants

Parent couples were recruited by means of advertising in the local press. Inclusion criteria were singleton birth and a healthy infant born after at least 37 weeks of gestation with a birth weight above 2500 g. Gestational age was assessed using an ultrasound scan in gestational week 12-14 (60). Weight and length at birth were measured in the delivery room and reported to us by the parents. At the measurement session the infant's length, to the nearest cm, was recorded using a length board. At the time of this session all infants except two were completely breastfed.

6.2.3 Assessment of total body water

Each infant was given an accurately weighed dose of stable isotopes (0.07g $^{2}H_2O$ and 0.18 $H_2{}^{18}O$ per kg body weight) using a piece of tubing attached to a syringe. The tubing was attached to a finger of one of the researchers and placed in the mouth of the infant. The infant was stimulated to suck by touching its palate. The tubing and syringe were weighed before and after administration of the dose and the difference between the two recorded weights represented the weight of the dose. The parents were asked to collect two background urine samples at home and bring them to the measurement session. Further, they were instructed to collect urine samples on the evening of the day of dosing and 3, 6 and 9 days after that day and to note the time of sampling carefully. Urine samples were obtained using baby urine collector bags (Coloplast, Humlebaeck, Denmark). Dose and urine samples were analysed to assess TBW and CO_2-production as described in section 6.4.

Table 1. Description of infants in the HF-study

		At birth		At measurement session		
Infant	Sex	gestational age (wks+ds)	weight (g)	age (ds)	weight* (g)	length (cm)
A	boy	39 + 0	3 780	8	3 717	51
B	boy	39 + 6	3 265	8	3 404	51
C	boy	40 + 5	4 600	8	4 637	57
D	boy	41 + 3	4 340	8	4 081	56
E	boy	40 + 0	3 320	8	3 267	51
F	boy	40 + 3	3 460	9	3 494	53
G	boy	38 + 6	3700	8	3 697	52
H	boy	40 + 1	3830	9	4 240	53
I	girl	40 + 5	4570	7	4 319	53
J	girl	41 + 1	4680	10	4 664	55
K	girl	39 + 0	3670	9	3 707	51
L	boy	41 + 6	4230	8	4 502	56
Mean			3955	9	3 973	53
SD			510	1	488	2

* Average of first and second weighing.

6.2.4 Calculation of the hydration factor

HF was calculated (38) as:

TBW x 100 / [Body weight – (2.118 x Body volume – 0.78 x TBW -1.354 x Body weight)]

6.2.5 Propagation of error analysis

The methodological error associated with the assessment of TBW (0.026 kg, 0.95 %) was calculated from estimates obtained using $^{2}H_2O$ and $H_2{}^{18}O$, respectively. The corresponding error obtained when assessing body weight (0.0004 kg, 0.01 %) was estimated by weighing objects twice that had a mass between 1.9 and 6.3 kg. For body volume the error (0.04 %) reported by Urlando (41) when measuring metal cylinders in PeaPod was used. The methodological error associated with the assessment of HF was calculated using propagation of error analysis (61). The biological variability (Vb) was calculated using the following equation: $(Vt)^2 = (Vb)^2 + (Vm)^2$ where Vt is the observed SD (standard deviation) of the estimate of HF and Vm is the propagated methodological error.

6.3 Longitudinal study (Papers II, III, IV)

6.3.1 Study design

The body composition of 108 infants was studied before the age of 10 days (first measurement) and between 77 and 91 days of age (second measurement). Forty-five of the 108 parent couples consented to participate in a follow-up study, and in 20 (Table 2) of these 45 children body composition and TEE were assessed at the age of 1.5 years (third measurement). At this measurement the SMR was also measured. The 20 children were randomly selected from the 45 children.

The group of 108 infants were also used to compare results obtained when body composition was calculated using the FFM density model presented by Fomon *et al.* (39) (FFMD-Fomon) versus the corresponding model published by Butte *et al*. (40) (FFMD-Butte). The longitudinal study was approved by the Ethics Committee of the University of Linköping, Sweden.

Table 2. Weight, length and gestational age at birth of children in the longitudinal study. Data are shown for all subjects and for the 20 subjects participating in the third measurement.

All subjects

	Girls	Boys	Boys and girls
n	53	55	108
Weight, g	3530 ± 457	3803 ± 540	3669 ± 517
Length, cm	51 ± 2	51 ± 2	51 ± 2
Gestational age, weeks	40.3 ± 1.1	40.0 ± 1.3	40 ± 1.2

Subjects participating in third measurement

	Girls	Boys	Boys and girls
n	9	11	20
Weight, g	3930 ± 490	3890 ± 510	3910 ± 490
Length, cm	52 ± 2	51 ± 2	52 ± 2
Gestational age, weeks	40.6 ± 1.3	39.7 ± 1.5	40.1 ± 1.5

Data given as means (SD).

6.3.2 Recruitment and characteristics of the children

Seven hundred and ninety-eight pregnant women, living in an area with a well-educated, middle income population, were contacted by mail and asked to participate in the study. The women's names and addresses were obtained from a maternity clinic. The inclusion criteria were singleton birth and a healthy infant, born after at least 37 weeks of gestation. One hundred and seventy-seven parent couples consented to participate. Five of these were excluded due to birth before week 37, four due to a sick infant, 42 couples withdraw their consent and 16 infants participated only in the first measurement. Thus 108 infants were

included in the study. Gestational age was assessed using an ultrasound scan in gestational week 12-14 (60). Weight and length at birth were measured in the delivery room and reported to us by the parents. A description of the characteristics at birth for infants in the longitudinal study is presented in Table 2. BMI was calculated as body weight (kg) / length^2 (m). Ponderal index (PI) was calculated as 100 x body weight (g) / length^3 (cm). Fat mass index (FMI) was TBF (kg)/ length^2 (m). At the first measurement 85 % of the 108 infants were exclusively breast fed while 12 % received breast milk in combination with formula. At the second measurement 79 % were still exclusively breast fed while 15 % were breast and formula fed in combination. At the third measurement all 20 children were weaned.

6.3.3 Assessment of body fat

At the first and second measurements the volume and weight of the infant (without clothing) were measured using PeaPod (Life Measurement, Inc., Concord, CA, USA) (41). Length was measured to the nearest cm using a length board. Body composition was calculated from body density based on the model presented by Fomon or Butte (39, 40) using PeaPod software 3.0.1. At the third measurement FFM was calculated as TBW/0.784 (39). TBW was assessed as described in section 6.4. To obtain TBF, FFM was deducted from body weight.

6.3.4 Studies in parents

The pregnant women were instructed to record their body weight regularly two to three weeks before the expected date of delivery, and the weight closest to the day when delivery occurred was recorded in the questionnaire. Height was measured using a wall stadiometer (Tillquist, Spånga, Sweden). Body weight (in light clothing) was recorded using a scale (KCC 150; Mettler-Toledo, Albstadt, Germany). For fathers who never brought their baby to a measurement session, values for weight and height were self-reported. Information regarding the infant's weight, length and health at birth, the infant's mode of feeding, the mother's weight before pregnancy and her parity, and the parents´ health, smoking habits, education and occupation was recorded in a questionnaire the parents received before their child's birth. This questionnaire was brought to us at the first measurement. Additional information regarding the child's health and mode of feeding at the second and third measurements was also obtained using a questionnaire.

6.3.5 Application of the doubly labelled water method at 1.5 years of age

The parents were asked to collect two urine samples at home and to bring those to the measurement session. At this session the child was given an accurately weighed dose of stable isotopes (0.14 g ^2H_2O and 0.35 $H_2{}^{18}O$ per kg body weight) mixed with fruit juice. The container was rinsed twice with juice and the child also consumed the washings. The parents were instructed to collect urine samples on days 1, 5, 10 and 15 after the day of dosing and to note the time of sampling carefully. Urine samples were obtained using baby urine collector bags (B. Braun Medical, Boulogne Cedex, France), or using cotton balls in the diaper. In the latter case a syringe was used to recover the urine. To assess TEE and TBW, dose and urine samples were analysed as described in section 6.4.

6.3.6 Energy expenditure in response to physical activity

SMR was measured using a ventilated hood system (Deltatrac Metabolic Monitor; Datex Instrumentarium Corp, Helsinki, Finland). Carbon dioxide (CO_2) production and oxygen (O_2) consumption were measured for 20 minutes. The recordings obtained after 10 minutes were used to calculate SMR by means of the Weir equation (54). TEE during 14 days was assessed using the DLW method according to the description in section 6.4. AEE was TEE – SMR. BMR was calculated using body weight by means of equations for boys and girls below three years of age (57). PAL was calculated as TEE/SMR (PAL_{SMR}) or as TEE/BMR (PAL_{BMR}).

6.4 Doubly labelled water method (Papers II and IV)

Each subject was given an accurately weighed dose of isotopes. Urine samples were stored in glass vials with an internal aluminium-lined screw cap sealing at +4° C until sample collection was completed, after which they were stored at -20° C until analysed. ^2H and ^{18}O enrichments of dose and urine samples were analysed using an isotope ratio masspectrometer fitted with a $CO_2/H_2/H_2O$ equilibrium device (Deltaplus XL, Thermoquest, Bremen, Germany). The procedure described by Thielecke and Noack (62) was followed, except that equilibration times for H_2 and CO_2 were 360 min and 840 min, respectively. The mass spectrometric response was standardized using Vienna standard mean ocean water and standard water samples with known enrichments. Dose and urine samples from each subject were always analysed simultaneously within the same equilibrium device, when a linear mass spectrometric response was also confirmed. ^2H_2O dilution space (N_D) and $H_2{}^{18}O$ dilution space (N_O) were calculated using zero time enrichments obtained from the exponential isotope disappearance curves that provided estimates for the elimination rates for ^2H and ^{18}O,

respectively (k_D and k_O). CO_2 production was calculated according to Davies & Coward (63) assuming that 25% of total water losses were fractionated. TEE was calculated from CO_2 production using the Weir formula (54) assuming the food quotient to be 0.85 (55). TBW was the average of N_D/1.041 and N_O/1.007. Analytic precision for results expressed in ppm was 0.22 for ^2H and 0.03 for ^{18}O. When samples from one adult subject were analysed nine times the following coefficients of variation were obtained: TEE (1.2 %), TBW (0.3%) and N_D/ N_O (0.15%).

6.5 Statistics

Values are given as means, SD and CV. Significance was accepted when $P < 0.05$. Statistica Softwear, version 7.1 (STAT SOFT, Scandinavia AB, Uppsala, Sweden) was used for linear regression analysis including Pearson's correlation coefficient, for Student´s t-test for independent and dependent means and for analysis of variance followed by Tukey´s test. Non-normally distributed variables were log-transformed before analysis of variance. When neither the dependent nor the independent variable was normally distributed, Spearman´s r is given. Otherwise Pearsons´s r is reported. In the pregnancy study, Minitab, version 15 (Minitab Inc., State College, PA, USA) was used to identify significant differences between "slopes of regression lines" using multiple regression analysis with TBF (%) and individual variables for stage of gestation as indicator variables. Interaction between TBF (%) and stage of gestation was used to test if the effect of TBF (%) on the dependent variable differed significantly between different stages of gestation. This test was followed by a Boniferroni correction to adjust for multiple comparisons. In the study of infants and young children, slopes of regression lines were compared using "method I" described by Kleinbaum *et al.* (64) or by means of a linear regression model using SAS (Institute Inc., Cary, NC, USA).

7. RESULTS

7.1 Pregnancy study (Paper I)

7.1.1 Serum concentrations of adipokines

Serum concentrations of leptin, adiponectin and resistin before, during, and after pregnancy for the women in the study are shown in Table 3. The serum concentrations of leptin were significantly higher at all measurements during pregnancy when compared to the corresponding concentrations before pregnancy. The serum concentrations of adiponectin were significantly lower in gestational weeks 32 and 35 when compared to the corresponding concentrations before pregnancy. Serum resistin increased during pregnancy and was significantly higher than the prepregnant value in gestational weeks 14, 32 and 35.

7.1.2 Leptin, total body fat and HOMA-IR

As shown in Figure 1, TBF (%) of the women was significantly correlated with serum leptin before pregnancy, in gestational weeks 14 and 32, as well as *postpartum*. Furthermore, when serum leptin (ng/ml) was regressed on TBF (%) the slopes (2.11-2.66) of the regression lines obtained varied only slightly between measurements and were not significantly different. In addition, leptin in serum was significantly correlated with HOMA-IR before pregnancy as well as in gestational weeks 14 and 32 ($n=17$, $r=0.53$-0.70, $0.002<P<0.05$), but not *postpartum*.

7.1.3 HOMA-IR and total body fat

Regression analysis with HOMA-IR as the dependent variable and TBF (%) as the independent variable before pregnancy, in gestational weeks 14 and 32, and *postpartum* is shown in Figure 2. These variables were significantly correlated before pregnancy, in gestational weeks 14 and 32, but not *postpartum*. In gestational week 32 the slope (0.111) of the regression line was significantly steeper than the corresponding value (0.046) obtained before pregnancy.

Table 3. Serum concentrations of leptin, adiponectin and resistin before and during pregnancy and postpartum in 23 Swedish women.

	Before conception		Gestational week 8		Gestational week 14		Gestational week 20		Gestational week 32		Gestational week 35		Two weeks postpartum	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leptin[*] (ng/ml)	23.9	23.6	28.1[†]	18.0	28.6[†]	17.1	32.6[†]	18.5	36.7[†‡§]	19.4	35.8[†]	21.7	19.5[‡§‖**††]	16.5
Adiponectin (μg/ml)	12.6	5.8	12.0	4.2	12.4	4.3	10.9	4.3	9.0[†‡§‖]	4.1	9.1[†‡§‖]	4.7	8.0[†‡§‖]	3.2
Resistin (ng/ml)	12.7	4.1	14.7	6.5	18.6[†‡]	5.8	16.0	7.1	19.8[†‡‖]	6.6	17.5[†]	5.8	14.7[§***]	6.7

[*] Values were log-transformed before analysis of variance.

[†] Significantly different ($P<0.05$) from the corresponding value before pregnancy using analysis of variance and Tukey´s test.

[‡] Significantly different ($P<0.05$) from the corresponding value in gestational week 8 using analysis of variance and Tukey´s test.

[§] Significantly different ($P<0.05$) from the corresponding value in gestational week 14 using analysis of variance and Tukey´s test.

[‖] Significantly different ($P<0.05$) from the corresponding value in gestational week 20 using analysis of variance and Tukey´s test.

[***] Significantly different ($P<0.05$) from the corresponding value in gestational week 32 using analysis of variance and Tukey´s test.

[††] Significantly different ($P<0.05$) from the corresponding value in gestational week 35 using analysis of variance and Tukey´s test.

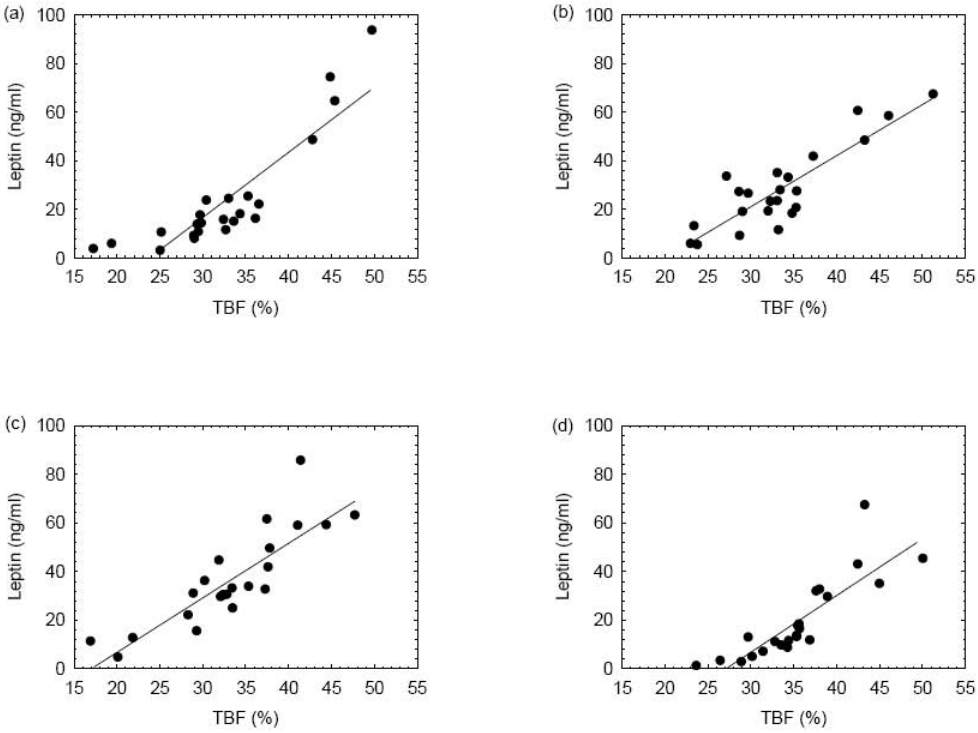

Figure 1. Relationships between leptin in serum (ng/ml) (y) and TBF (%) (the percentage of total body fat) (x) before pregnancy (a), in gestational weeks 14 (b) and 32 (c) as well as two weeks *postpartum* (d) in 23 Swedish women. The linear regressions are: y=2.66 x – 62.9, r=0.88 (*P*<0.001) (a); y=2.11 x – 42.1, r=0.87 (*P*<0.001) (b); y=2.26 x – 38.4, r=0.85 (*P*<0.001) (c); y=2.33 x – 63.0, r=0.85 (*P*<0.001) (d).

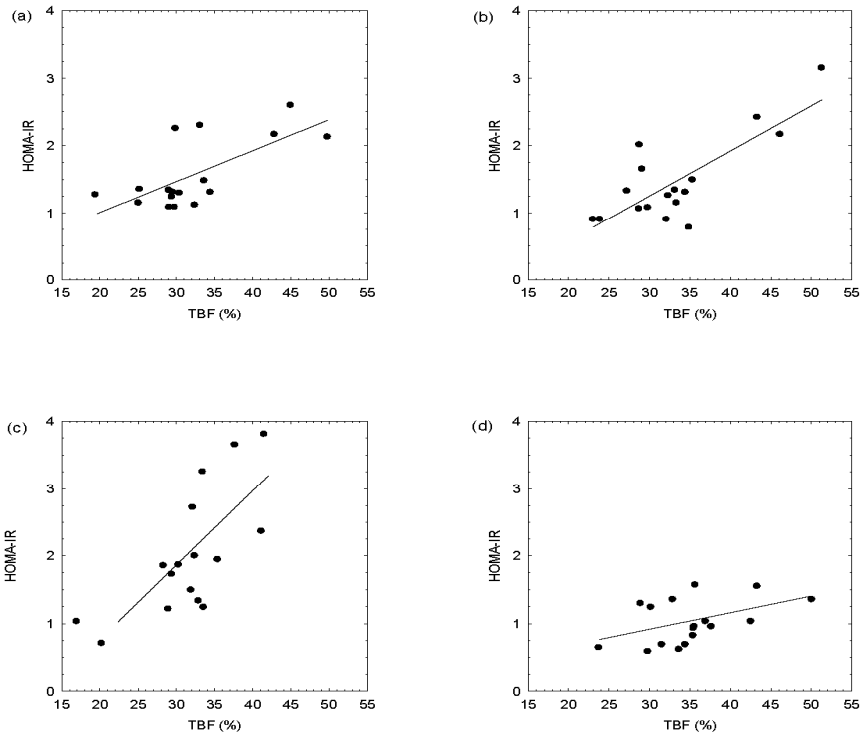

Figure 2. Relationships between HOMA-IR (homeostasis model assessment of insulin resistance) (y) and TBF (%) (the percentage of total body fat) (x) before pregnancy (a), in gestational weeks 14 (b) and 32 (c) as well as two weeks *postpartum* (d) in 17 Swedish women. The linear regressions are: y=0.046 x + 0.10, r=0.678 (*P*<0.01) (a); y=0.067 x – 0.75, r=0.788 (*P*<0.001) (b); y=0.111* x – 1.46, r=0.789 (*P*<0.001) (c); y=0.024 x + 0.17, r=0.456 (*P*>0.05) (d); * significantly (*P*<0.05) different from the corresponding values obtained before pregnancy and two weeks *postpartum* using multiple regression analysis followed by a Boniferroni correction.

7.1.4 Adiponectin, total body fat and HOMA-IR

Neither TBF (%) nor TBF (kg) correlated with adiponectin in serum at any of the measurement occasions before, during or after pregnancy. However, adiponectin (x) in serum was negatively correlated with HOMA-IR (y) in gestational week 32 (n=17, r=-0.52, $P<0.05$).

7.1.5 Resistin, total body fat and HOMA-IR

Serum concentrations of resistin did not correlate with TBF (%, kg) either before pregnancy or in gestational weeks 14 or 32, or *postpartum*. Resistin in serum did not correlate with HOMA-IR (n=17) either before pregnancy or in gestational weeks 14 or 32, or *postpartum*.

7.2 HF-study (Paper II)

7.2.1 The hydration factor and its biological variability

The TBW of the infants in the HF study was 2.73 ± 0.29 kg representing 68.6 ± 3.4 % of body weight. During the 7-10 days following dosing, these infants gained 47 ± 18 g body weight per day. Their body density was 1.038 ± 0.008 g/ml at the two assessments at the measurement session. HF was 80.99 ± 0.74 % (first assessment) and 80.85±0.70 % (second assessment), average value 80.9 %. Using propagation of error analysis the methodological variability when assessing HF was found to be 0.303 (0.37 % of average HF). The biological variability was calculated to be 0.678 (0.84%) (first assessment) and 0.627 (0.78 %) (second assessment), average value 0.653 (0.81%).

7.3 Longitudinal study

7.3.1 Comparison of fat-free mass density models (Paper II)

As shown in Table 4, estimates of body fat (g,%) were always significantly lower when calculated using FFMD-Butte than when using FFMD-Fomon. For five of the 108 infants invalid results were obtained when FFMD-Butte was used at one week of age. These five infants were then significantly lighter (2692±210 vs 3693±210 g, $p<0.001$) and shorter (48±2 vs 52±2 cm, $p<0.001$) than the remaining 103 infants in the study. Their body fat content calculated using FFMD-Fomon was 4.0±1.7%, which was significantly ($p<0.001$) lower than the corresponding figures for the remaining 103 infants (Table 4).

Table 4. Weight, length and body composition calculated using fat-free mass density models presented by Butte *et al.* (5) (FFMD-Butte) and by Fomon *et al.* (4) (FFMD-Fomon) in infants in the longitudinal study at one and 12 weeks of age (means±SD).

	One week		12 weeks
	n=108	n=103	n=108
Weight, g	3646 ± 526	3693 ± 492	6133 ± 701
Length, cm	52 ± 2	52 ± 2	61 ± 2
Fat-free mass density model			
FFMD-Butte			
Fat-free mass, g	*	3290 ± 572**	4659 ± 454†
Fat, g	*	402 ± 459**	1473 ± 408†
Fat, %	*	9.0 ± 3.5**	23.7 ± 4.8†
FFMD-Fomon			
Fat-free mass, g	3162 ± 390	3191 ± 375	4498 ± 454
Fat, g	484 ± 188	502 ± 173	1634 ± 412
Fat, %	12.9 ± 3.9	13.4 ± 3.4	26.4 ± 4.7

* Data not given since "invalid results" were obtained when 5 of the 108 infants were measured in PeaPod.

** Significantly different ($p<0.05$) compared to the corresponding values obtained when using FFMD-Fomon.

† Significantly different ($p<0.01$) compared to the corresponding values obtained when using FFMD-Fomon.

Figure 3 shows results obtained for girls (A) and for boys (B) when the increase in body fat (%) between one and 12 weeks of age, assessed using FFMD-Butte (y), was regressed on the same increase calculated using FFMD-Fomon (x). The increase according to FFMD-Butte minus the increase according to FFMD-Fomon was 2.00 ± 0.25 % (girls, n=50) and 1.49 ± 0.23 % (boys, n=53). This difference (0.51 %) between boys and girls was significant ($p<0.001$).

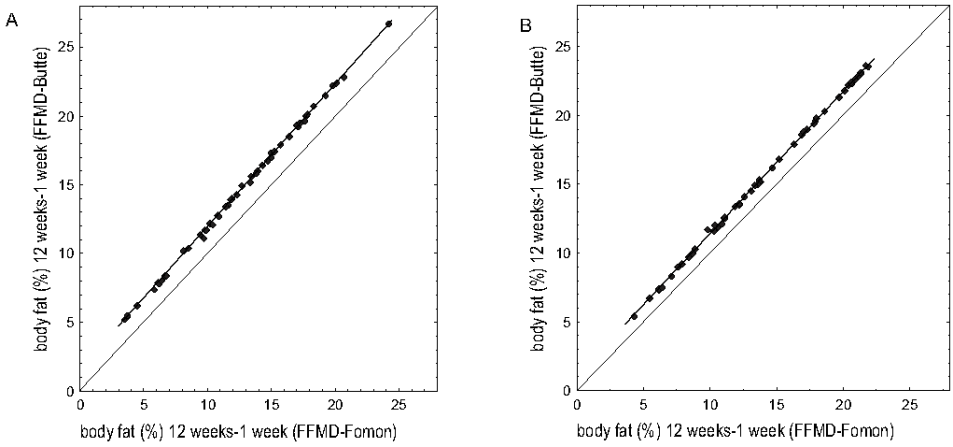

Figure 3. The increase in body fat (%) between one and 12 weeks calculated using the fat-free mass density model developed by Butte *et al.* (5) (FFMD-Butte) (y) regressed on the same increase calculated using the fat-free mass density model developed by Fomon *et al.* (4) (FFMD-Fomon) (x). The line of identity (x = y) is also shown.

A. Girls (n=50)

Regression equation: $y=1.042x + 1.476$, $r=1.000$, $p<0.0001$

B. Boys (n=53)

Regression equation: $y=1.037x + 0.993$, $r=1.000$, $p<0.0001$

7.3.2 Parents (Paper III)

Ninety-eight percent of the parents had finished secondary school and 63 % had a university degree. Age and anthropometric data for the mothers (n=108) and fathers (n=108) of the infants in the study are shown in Table 5. Before pregnancy 22 % of the women were overweight (BMI=25.0-29.9) while 5 % were obese (BMI = >30). Five percent of the mothers smoked before and during pregnancy. The parity of the mothers varied between 0 and 4.

Table 5. Age and anthropometric data for mothers (n=108) and fathers (n=108) of infants in the longitudinal study**.**

	Mothers	Fathers
Age, years	31 (4)	33 (5)
Weight, kg	65.6 (9.7)*	82.6 (11.7)†
Height, m	1.67 (0.06)‡	1.81 (0.07) †
BMI, kg/m^2	23.4 (3.3) **	25.0 (3.2) †
Total weight gain during pregnancy, kg	14.4 (5.2) ††	

Data given as means (SD).

BMI = body mass index.

* Before pregnancy, self-reported

† Recorded value (n=53) or self-reported (n=55)

‡ Recorded value

** Calculated using the self-reported weight before pregnancy and the recorded height.

†† A weight recorded as close to delivery as possible minus the self-reported weight before pregnancy.

7.3.3 Body composition and anthropometrics of the infants (Paper III)

Table 6 shows age, length, weight, body fat (calculated according to FFMD-Fomon), FFM, BMI and PI for the infants (n=108) in the longitudinal study. At both measurements, age was the same for the two sexes, i.e. 1.1 weeks (first measurement) and 12.1 weeks (second measurement). At the first measurement girls contained 13.4 ± 3.7 % and boys 12.5 ± 4.0 % body fat. The corresponding figures at the second measurement were 26.3 ± 4.2 % (girls) and 26.4 ± 5.1 % (boys). These figures did not differ significantly between the sexes at either of the measurements. The proportion of fat in the amount of weight gained between the first and second measurements (g/g) was 45 ± 9 % for both boys and girls. Furthermore, body fat (%) at the first and second measurements was not correlated in girls or in boys or in the sexes combined. However, in all infants BMI at the first measurement correlated significantly ($r=0.26$, $p<0.01$) with BMI at the second measurement.

Table 6. Age, anthropometric variables, body composition (calculated according to FFMD-Fomon), BMI and PI for girls (n=53) and boys (n=55) in the longitudinal study at the first and second measurements and the increase in some variables between the two measurements.

First measurement	Girls	Boys
- Age, weeks	1.1 (0.3)	1.1 (0.3)
- Length, cm	51 (2)	52 (2)
- Weight, g	3520 (472)	3768 (551)
- Body fat, %	13.4 (3.7)	12.5 (4.0)
- Body fat, g	484 (173)	484 (203)
- Fat-free mass, g	3036 (340)	3285 (398)
- BMI kg/m^2	13.5 (1.2)	13.8 (1.3)
- PI, $100 \cdot g/cm^3$	2.6 (0.2)	2.6 (0.2)

Continued

Table 6, continued

Second measurement		
- Age, weeks	12.1 (0.5)	12.1 (0.4)
- Length, cm	60 (2)	62 (2)
- Weight, g	5842 (667)	6413 (619)
- Body fat, %	26.3 (4.2)	26.4 (5.1)
- Body fat, g	1551 (357)	1714 (447)
- Fat-free mass, g	4291 (429)	4698 (364)
- BMI, kg/m^2	16.0 (1.4)	16.7 (1.5)
- PI, $100 \cdot g/cm^3$	2.7 (0.2)	2.7 (0.3)
Increase between first and second measurements		
- Length, cm	9 (1)	10 (1)
- Weight, g	2322 (490)	2644 (576)
- Body fat, %	12.9 (5.3)	14.0 (5.8)
- Body fat, g	1067 (355)	1231 (434)
- Fat-free mass, g	1255 (264)	1414 (270)

Data given as means (SD). BMI = body mass index, PI = ponderal index.

Figure 4a shows body fat (%) versus weight for girls at the first and second measurements. The corresponding figures for boys are shown in Figure 4b. The regression lines shown in these figures were significant for both sexes at both measurements. The slopes of the regression lines were not significantly different between boys and girls at either of the measurements. Furthermore, no significant difference either for boys or for girls was found between the slopes of the regression lines obtained at the first and at the second measurements.

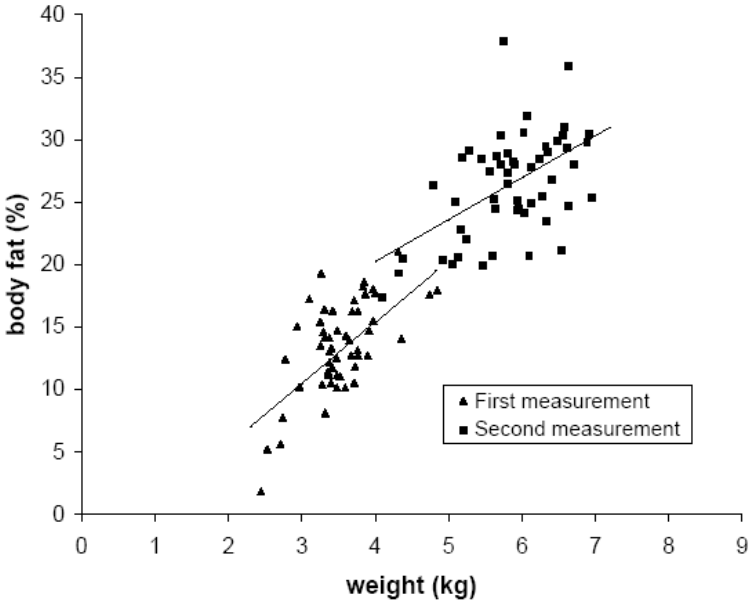

Figure 4a. Body fat (%) regressed on body weight (kg) for girls in the longitudinal study (n=53) at the first measurement (body fat (%) = -4.601 + 5.124 · body weight (kg), r = 0.65, p<0.001) and at the second measurement (body fat (%) = 6.8024 + 3.338 · body weight (kg), r = 0.53, p<0.001.

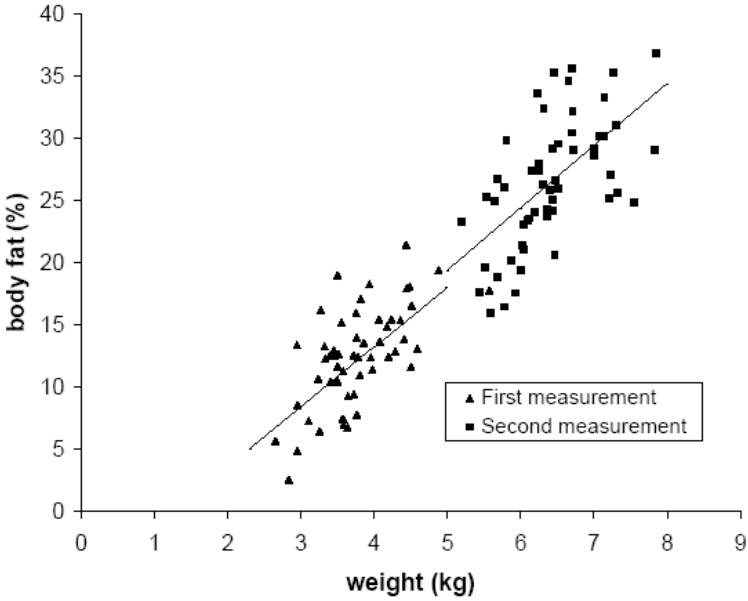

Figure 4b. Body fat (%) regressed on body weight (kg) for boys in the longitudinal study (n=55) at the first measurement (body fat (%) = -5.035 + 4.644 · body weight (kg), r = 0.64, p<0.001) and at the second measurement (body fat (%) = -5.787 + 5.025 · body weight (kg), r = 0.60, p<0.001).

Figure 5a illustrates how body fat (%) increased with age for individual infant girls in the study. The corresponding data for infant boys are shown in Figure 5b. In all infants, body fat at the first measurement (12.9 ± 4.0 %) was negatively correlated with the increase in body fat (13.4 ± 5.5 %) between the first and second measurements ($r = -0.56$, $p < 0.0001$). For girls the correlation coefficient for this relationship was - 0.61 ($p<0.0001$) and for boys it was - 0.50 ($p<0.001$).

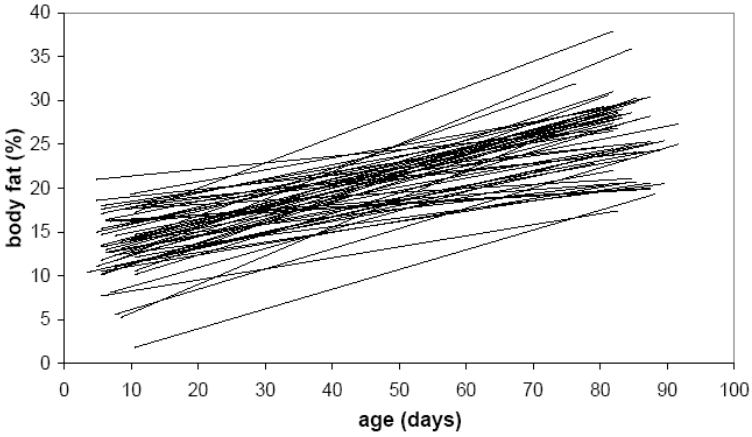

Figure 5a. Body fat (%) versus age (days) in girls (n=53) in the longitudinal study. Each line represents one infant.

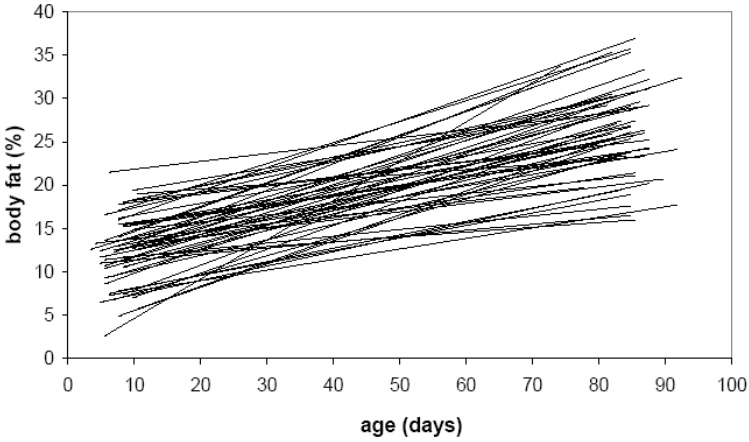

Figure 5b. Body fat (%) versus age (days) in boys (n=55) in the longitudinal study. Each line represents one infant.

7.3.4 Maternal BMI and infant variables (Paper III)

Table 7 shows correlations for maternal BMI before pregnancy versus infant variables. Positive significant linear relationships were found between maternal BMI values and birth weight as well as between such BMI values and weight of the infants at the first measurement. The mothers' BMI values before pregnancy were not correlated with the body fat (%, g) or the FFM (g) of their infants at the first measurement. However, the mothers' BMI values before pregnancy were correlated with the BMI and PI of their infants at this measurement. There was a significant correlation ($r=0.21$, $p<0.05$) between the mothers' total weight gain during pregnancy and the birth weight of their infants, but no correlation between this weight gain and the body fat (%, g) of the infants at the first measurement. The mothers' BMI values before pregnancy were not correlated with the weight (g), body fat (% or g), FFM (kg), BMI or PI of their infants at the second measurement (data not shown).

Table 7. Correlation coefficients (r) for linear relationships between maternal BMI before pregnancy and infant variables.

Infant variable	r	p
At birth		
- Weight (g)	0.25	0.0094
At first measurement		
- Weight (g)	0.20	0.044
- BMI, (kg/m^2)	0.26	0.0068
- PI, ($100 \cdot g/cm^3$)	0.27	0.0055
- Body fat (%)	0.14	0.15
- Body fat (g)	0.17	0.086
- Fat-free mass (g)	0.18	0.059

BMI= body mass index, PI = ponderal index

7.3.5 Longitudinal changes in body composition (Papers III, IV)

As mentioned above, TBF (%) increased for all children between the first and second measurements. However, as shown in Figure 6, increases (13 children) as well as decreases (7 children) in TBF (%) were observed between the second and third measurements. Such results were also obtained when TBF (%) was replaced by FMI as an estimate of body fatness. Between the first and second measurements TBF of the 20 children increased significantly ($p<0.000\ 001$) from 14.5 to 26.6 %. However, the corresponding increase between the second and third measurements was small (from 26.9 to 28.2 %) and was not significant. The change in TBF (%) [TBF (%) at 1.5 years minus TBF (%) at 12 week] was 1.6 ± 4.3 %, range -6.3 to

11.2 %. Using data for 20 children, no significant correlations for TBF (kg, %) between the first and third measurements or between the second and third measurements were found. For FMI we found no significant correlation between the first and third measurements, while the correlation between the second (x) and third (y) measurements was significant (r=0.45, p=0.047, n=20).

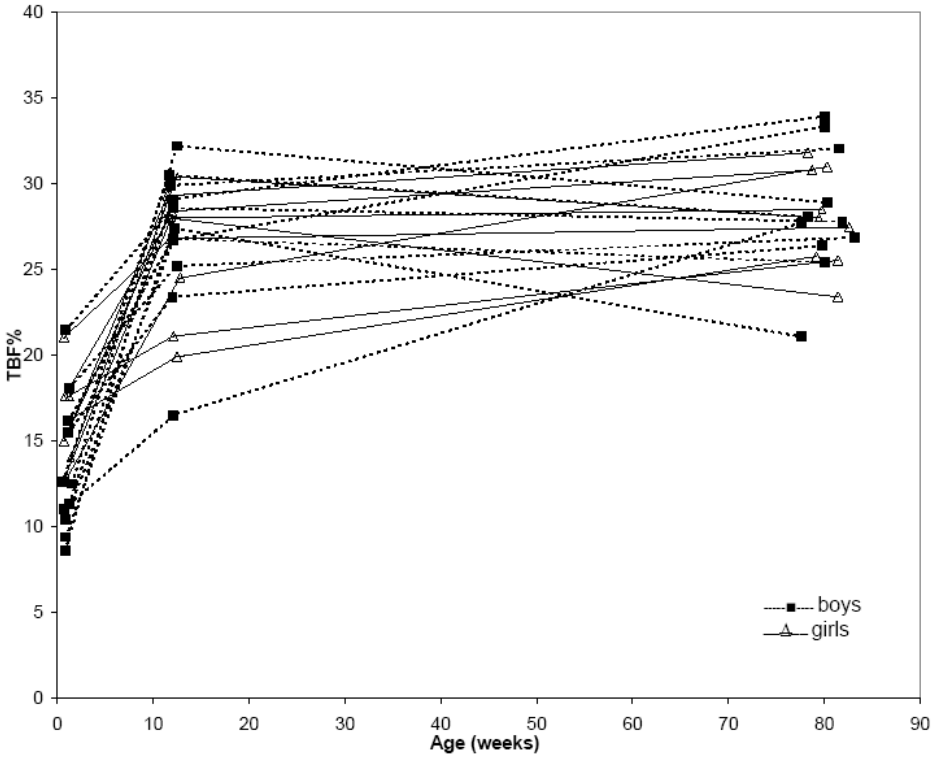

Figure 6. Total body fat (TBF) at first, second and third measurements of children (n=20) in the longitudinal study in relation to age. Each line represents one child.

7.3.6 Energy expenditure results (Papers III, IV)

Table 8 shows N_D and N_O, N_D/ N_O, k_D, k_O, k_D/k_O, CO_2 production and TBW for the 20 children in the longitudinal study, as well as for boys and girls separately, at the third measurement. Table 9 shows age, body weight, length, TBF (kg, %), FFM, BMI, FMI, SMR, BMR, AEE, PAL_{SMR} and PAL_{BMR} for all children as well as for boys and girls separately. A significant correlation was found between PAL_{BMR} (x) and TBF (%) (y) (r=-0.48, p=0.03, n=20) while the correlation between PAL_{SMR} (x) and TBF (%) (y) was not significant (r=-0.36, p=0.12, n=20). AEE ($MJ \cdot day^{-1}$) (x) was correlated with body weight (kg) (y) (r=0.51, p=0.02). The correlation between AEE ($MJ \cdot day^{-1} \cdot kg^{-1}$) (x) and TBF (%) (y) did not quite reach significance (r=-0.43, p=0.06, n=20). FMI did not correlate with AEE ($MJ \cdot day^{-1}$), AEE ($MJ \cdot day^{-1} \cdot kg^{-1}$), PAL_{SMR} or PAL_{BMR}.

Table 8. Deuterium and oxygen-18-dilution spaces (N_D and N_O), N_D/ N_O, elimination rates for ^2H (k_D) and ^{18}O (k_O), k_D/k_O, carbon dioxide (CO_2) production and total body water (TBW) for children in the longitudinal study at the third measurement. Means ± SD

	Girls	Boys	All
	(n=9)	(n=11)	(n=20)
N_D (mol)	383.4±35.5	385.4±32.7	384.5±33.1
N_O (mol)	373.6±33.7	378.6±32.1	376.3±32.0
N_D/ N_O	1.026±0.0121	1.018±0.013	1.022±0.013
k_D (day^{-1})	0.152±0.022	0.155±0.028	0.154±0.025
k_O (day^{-1})	0.199±0.024	0.201±0.029	0.200±0.026
k_D/k_O	0.764±0.025	0.767±0026	0.766±0.025
CO_2 production ($mol \cdot day^{-1}$)	7.19±0.84	7.52±0.77	7.37±0.80
TBW (kg)	6.66±0.61	6.72±0.57	6.70±0.57
TBW (%)	56.5±2.2	56.7±3.4	56.6±2.8

Table 9. Age, body weight, length, total body fat (TBF), fat-free mass (FFM), body mass index (BMI), fat mass index (FMI), total energy expenditure (TEE), sleeping metabolic rate (SMR), basal metabolic rate (BMR), activity energy expenditure (AEE), and physical activity level (PAL) of children in the longitudinal study at the third measurement. Means ± SD

	Girls	Boys	All
	n=9	n=11	n=20
Age (days)	561±10	560±12	560±11
Body weight (kg)	11.8±0.88	11.9±0.86	11.8±0.85
Length (m)	0.84±0.02	0.83±0.02	0.84±0.02
TBF (%)	28.0±2.8	28.3±3.7	28.2±3.3
TBF (kg)	3.30±0.38	3.37±0.54	3.34±0.46
FFM (kg)	8.49±0.77	8.51±0.71	8.50±0.72
BMI	16.8±0.8	17.1±1.1	17.0±1.0
FMI *	4.72±0.58	4.85±0.81	4.79±0.70
TEE ($MJ \cdot day^{-1}$)	3.86 ± 0.45	4.04 ± 0.41	3.96 ± 0.43
SMR ($MJ \cdot day^{-1}$)	2.68 ± 0.21	2.95 ± 0.30	2.83 ± 0.29
BMR ($MJ \cdot day^{-1}$)	2.76 ± 0.22	2.84 ± 0.22	2.80 ± 0.21
AEE * ($MJ \cdot day^{-1}$)	1.18 ± 0.39	1.09 ± 0.38	1.13 ± 0.38
AEE ($kJ \cdot day^{-1} \cdot kg^{-1}$)	98.7 ± 28.7	91.5 ± 31.2	94.8 ± 29.5
PAL_{SMR} †	1.44 ± 0.15	1.38 ± 0.16	1.41 ± 0.15
PAL_{BMR} ‡	1.40 ± 0.12	1.42 ± 0.10	1.41 ± 0.11

* Calculated as TEE-SMR

† Calculated as TEE/SMR

‡ Calculated as TEE/BMR

As shown in Figure 7, PAL_{SMR} (x) was correlated with the change in TBF (%) between 12 weeks and 1.5 years (y), r=-0.52, p=0.02, n=20. The corresponding results when this change [TBF (%) at 1.5 years minus TBF (%) at 12 weeks] (y) was regressed on AEE (x) ($MJ \cdot day^{-1} \cdot kg^{-1}$) were: r=-0.51, p=0.02, n=20 and y= 8.68 - 0.31 · x.

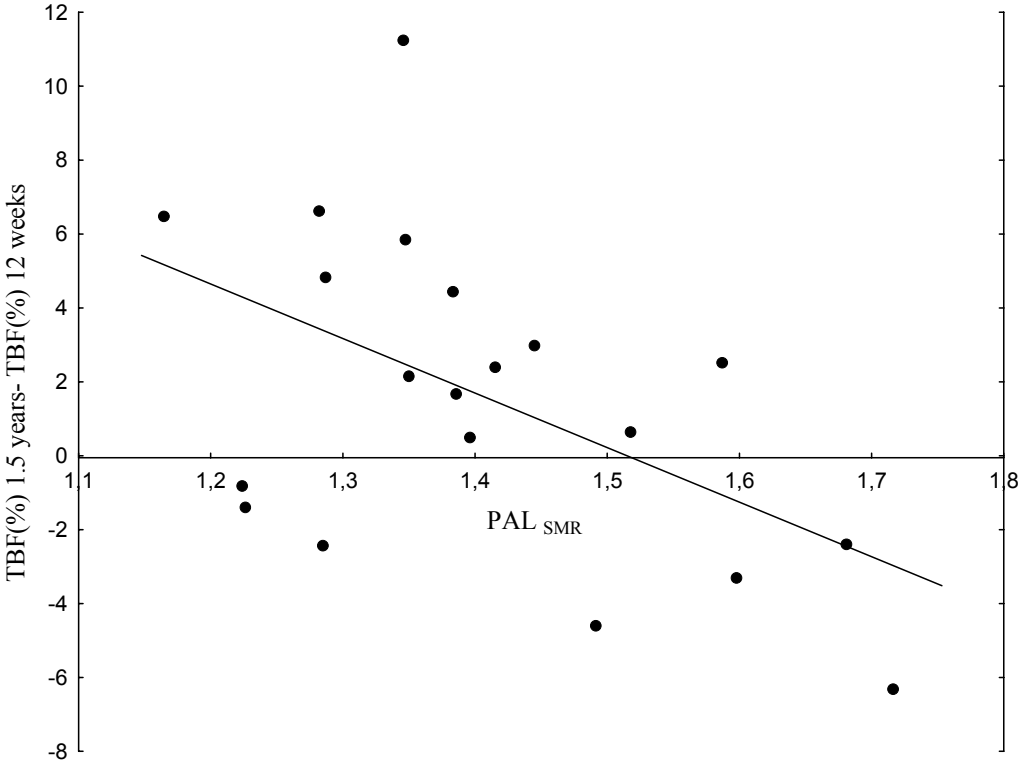

Figure 7. [TBF (%) at 1.5 years of age minus TBF (%) at 12 weeks of age] (y) regressed on PAL_{SMR} at the age of 1.5 years (x). Results obtained in 20 children in the longitudinal study. Regression equation: y= 22.4 – 14.8 · x, r=-0.52, p=0.02.

8. DISCUSSION

The results obtained in the pregnancy study show that body fatness and insulin resistance are related in pregnant and non-pregnant healthy women, and indicate that pregnancy affects the slope of the regression line obtained when HOMA-IR is regressed on TBF (%). Thus, a woman in the second half of pregnancy tends to be more insulin resistant than a non-pregnant woman with the same TBF content (%). A high insulin resistance during pregnancy tends to direct glucose to the foetus rather than to maternal tissues. Thus, it may be advantageous for the foetus if the mother gains fat during pregnancy, since this helps it to compete favourably for available nourishment. Note that the data demonstrate this enhancing effect of pregnancy in healthy non-diabetic women. This result is considered to be a new and interesting observation. Previous investigators (27) have pointed out that body fat retention may be a causal factor for the decrease in insulin sensitivity during normal pregnancy, and the relationship between body fat and insulin resistance is well established. The new finding in this thesis is that healthy pregnancy enhances this relationship. The mechanism behind this enhancing effect is unknown. Published data (20) indicate that leptin is involved when insulin resistance is established. Therefore it is interesting to note that serum concentrations of leptin correlated with HOMA-IR in these healthy women before and during pregnancy. This information, together with the well-established relationship between serum leptin and body fatness, makes it relevant to speculate that leptin may have a role regarding the enhancing effect of pregnancy on the relationship between body fatness and insulin resistance as identified in this study. Based on an extensive search of the literature, it was concluded that the present study is the first to identify a correlation between serum adiponectin and HOMA-IR in healthy pregnant women. This result suggests that adiponectin also has a role regarding insulin resistance during pregnancy. No significant relationships were identified before, during or after pregnancy between serum concentrations of resistin and TBF or between resistin and HOMA-IR. Cortelazzi *et al.* (34) were of the opinion that resistin has a minor role in relation to insulin resistance during pregnancy and the present results support this conclusion.

Data in this thesis show a correlation between maternal BMI before pregnancy and weight of the offspring at birth and at one week of life, while no correlations between such maternal BMI values and the body fat (%) of their infants were found. These results are consistent with the results from a study by Moyer-Mileur *et al.* (47) where no correlation between pre

pregnant maternal BMI and TBF (%) of the infants, measured within 72 h after birth, was found. These findings tend to suggest that the body fat content of a woman has a stimulating effect on the growth, rather than on the fat retention, of her foetus, in agreement with results of previous studies (10, 65).

The results obtained when comparing FFM density models during body composition calculation clearly show that FFMD-Butte (40) produced values for body fat that were significantly lower than those obtained when FFMD-Fomon (39) was used. Further, increases in body fat (%) between one and 12 weeks of age were greater when using FFMD-Butte than when using FFMD-Fomon. The different figures for FFM hydration in the two models are undoubtedly an important explanation for this discrepancy. According to Butte *et al*. (40), HF is 82.7 % (boys) and 83.1 % (girls) at two weeks of age and 81.0 % (boys) and 81.1 % (girls) at three months of age. The corresponding figures in the Fomon model (39) are 80.6 % (boys and girls) at birth and 80.0 % (boys) and 79.9 % (girls) at three months of age. Obviously, the difference in FFM hydration between the two models is especially pronounced during the first days/weeks of life. According to the HF-study, FFM contains 81 % water during this period of life, which is in better agreement with Fomon's (39) data than with Butte's (40). Furthermore, these results are in agreement with data published by Wang *et al*. (42) who reported that FFM in newborns of different species contains 81 % water. The observation in the HF-study that the biological variability of HF is very small (0.8 % in newborns) is important. A high biological variability in HF would lead to variations in fat-free mass (FFM) density and consequently impair accuracy when assessing body composition according to the procedure used in the PeaPod device.

The results presented in this thesis suggest that a low body fat content (TBF %) in newborns is associated with a high fat retention during the first three months after birth. The relationships between body fat (%) and weight, as illustrated in Figures 4a and 4b, demonstrate that infants of the same sex with similar age and body weight may well contain very different amounts of fat. For children in the longitudinal study the proportion of fat in the amount of weight gained between the first and second measurements (g/g) was 45 % in both sexes. This figure can be compared to the corresponding figures calculated using data from Fomon (34% for males and 38% for females) and from Butte (57% for both sexes) (53). The data in this thesis thus indicate a fat retention during early life that falls between the figures previously estimated in the two reference studies so far available.

Available reference data regarding body composition during infancy and childhood (39, 40) demonstrate that healthy infants gain body fat during the first months of life. The TBF (%) content reaches a maximum between 3 and 6 months and decreases slowly during the second half of the first year of life. The strong tendency of human infants to increase their body fat content during the first few months of life is clearly demonstrated by the data in this thesis. Between 12 weeks and 1.5 years of age the children's average change in TBF (%) was an increase but, as indicated in Figure 6, the variation between individual children was considerable. The results obtained suggest that the change in body fatness (% TBF) during this period of life is related to the physical activity of the child. Furthermore, it was observed that values for body fatness, TBF (%) and FMI obtained at the first measurement were not correlated with the corresponding values at the second measurement. A possible explanation for this lack of correlation is that body fat content (%) at one and 12 weeks reflects different nutritional situations. When the infant is one week old its body composition is still a reflection of the nutritional conditions *in utero*, while after 12 weeks its body composition has also been influenced by other factors such as the amount and kind of feeding and the infant's ability to regulate its energy intake.

Tennefors *et al.* (15) observed a strong negative correlation between PAL and TBF (%) in children as early as at the ages of 9 and 14 months. These authors considered the possibility that this finding was due to a vicious cycle where high body fatness tends to limit physical activity thereby promoting further retention of body fat. However, Tennefors *et al.* (15) also pointed out that the observed correlation may be a spurious one, since the PAL-values of their children were calculated using prediction equations based on body weight. The findings in this thesis demonstrate that such PAL-values may well result in spurious correlations between PAL and TBF (%). However, the findings may also indicate that a vicious cycle, such as that described above, is in fact present in young children, since a possible correlation between TBF (%) and AEE (MJ/day/kg) was observed in this study. This correlation was, however, not significant, which may be due to the small number of subjects.

In this study it is assumed that the difference between TEE and SMR represents a valid estimate of the amount of energy expended in response to physical activity. However, this difference also includes energy expended in response to tissue synthesis as well as the thermic effect of food (66). The weight gain of children in our study was on average 5 g per day

during the two-week metabolic period. This figure was used to calculate the average energy expenditure associated with synthesis of new tissue (67), which was found to be as low as 1.7 % of TEE. The thermic effect of food also represents a low proportion of TEE at only about 5-10 % (68). Thus our estimates of AEE tend to be slightly too high. Meaningful corrections of AEE-values in individual children for energy expended in response to tissue synthesis and the thermic effect of food are unfortunately not possible. In adults, the best available procedure for estimating physical activity energy expenditure is considered to be calculation of the difference between TEE measured using the DLW-method and BMR assessed using indirect calorimetry (69). In small children, such as the subjects in this thesis, it is not possible to measure BMR. However, SMR can be measured, and Ganpule *et al.* (70) have shown good agreement between such values and BMR.

9. CONCLUSIONS

The results indicate that pregnancy in healthy women has an enhancing effect on the relationship between body fatness and insulin resistance. Leptin may possibly have a role in this enhancing effect of pregnancy.

A correlation between adiponectin in serum and insulin resistance was found in healthy pregnant women in gestational week 32.

Resistin in serum was not correlated with TBF (%, kg) or with insulin resistance in healthy pregnant women.

The findings can be reconciled with previously published data suggesting that the body fat content of a woman has a stimulating effect on the growth, rather than on the fat retention, of her foetus.

The HF-value assessed in this thesis is in agreement with that presented in the "Fomon model" and the results show that this is the best available model when calculating body composition from body density in newborn fullterm infants.

The biological variability of HF was found to be low, only 0.8 % of average HF, in healthy fullterm newborns.

The study demonstrates that infants of the same sex with similar age and body weight may well contain very different amounts of fat.

A low TBF content in the newborn period was found to be associated with a high fat retention during the first three months of life.

The strong tendency by human infants to increase their TBF content during the first few months of life was clearly demonstrated.

The results showed that between the ages of 12 weeks and 1.5 years some children increased their TBF (%) while in others it was instead decreased.

The level of physical activity at 1.5 years of age was found to be associated with the change in TBF (%) content during the period between 12 weeks and 1.5 years of age.

Valid estimates of resting energy metabolism are required when studying the relationship between PAL and body fat (%) in young children. Estimates obtained using prediction equations based on body weight may give misleading results.

10. GENERAL DISCUSSION

Obesity, including childhood obesity, is the result of both environmental and genetic factors. It has long been known that obesity has a strong genetic component (71). Recent studies have shown that the genetics of obesity is complicated and is presently far from understood (72). Furthermore, environmental conditions such as low physical activity and high energy intake are certainly important (73). In addition, the concepts of early programming of metabolic- and hormonal systems as well as epigenetic modification of DNA-expression are of interest in this context (74). How these different factors interact to promote childhood obesity is not well understood. However, based on the fact that the proportion of overweight and fat children in many countries has increased considerably within a few decades (2, 73), it is likely that environmental factors are important.

Obesity is defined as an excess of body fat (73). However, it is not always possible to measure the fat content of human subjects, and therefore there is a need for simple estimates of obesity. The most commonly used estimate is BMI. Other estimates are PI (in infants), percentage of fat in the body, waist circumference and FMI. The optimal estimate would be one that is valid, easily assessed, and includes a clear definition of values for overweight and obesity with references to future health risks. The estimates in use today lack one or more of these desired qualities. However, more longitudinal studies assessing the true body fat content, adjusted for the size of the body, with results related to future health risks, together with more widespread clinical use of appropriate techniques for assessing body fatness, will hopefully help to provide clarification in this matter.

When assessing body composition of humans *in vivo* we are limited to methodology that indirectly measures the amount of body fat. Philosophically one could ask how we can state that a method does in fact measure body fat, since this variable cannot be measured *in vivo* and therefore reference estimates of body fat cannot be obtained. However, we can develop models on the basis of measurable variables with known characteristics such as water solubility and density. The capacity of such models to produce reasonable results can then be tested. Such an approach was applied in paper II in this thesis and the results can be regarded as a small step towards establishing methodology for assessment of body composition during infancy. Such methodology is certainly needed in order to study early life origins of obesity.

This thesis presents some interesting observations in relation to the biology of body fat in humans during reproduction and early growth. The observation regarding an enhancing effect of pregnancy on the relationship between body fatness and insulin resistance may represent an important explanation for the strong tendency by pregnant women to retain body fat. It is conceivable that the maternal body fat content has a role in the regulation of foetal growth. This could be very useful biologically, since fat mothers tend to live in an environment where food is plentiful, while the opposite is true for lean mothers. Body size is an important factor determining the amount of dietary energy required by man and other mammals. This makes it reasonable to speculate that the present results represent a mechanism by which offspring size is regulated in response to the availability of dietary energy in the environment in which the mother is living. Birth weight has been shown to be associated with overweight and obesity later in life (8, 75). As shown in this thesis, infants of similar weight and sex at the age of one week may contain very different amounts of fat. This raises the question as to whether it is the size or the fatness at birth that is crucial for the development of overweight and obesity. More studies considering body composition at birth are needed to clarify such questions.

The general role of physical activity in energy balance and its potential to counteract obesity is well recognized. However, the role of physical exercise versus more spontaneous kinds of physical activity is less well understood. Levine *et al.* (18) have published results suggesting that lean subjects expend more energy as NEAT (non-exercise activity thermogenesis) than obese subjects. NEAT refers to changes in posture and movements associated with the routines of daily life. Furthermore, Levine *et al.* (18) presented results indicating that this discrepancy between lean and obese subjects is biologically determined, possibly due to a difference in their genetic or epigenetic make-up. In early childhood, physical activity in the routines of daily life is probably characterized by mostly spontaneous movements. Therefore, it can be speculated that the relationship presented in Figure 7 may be explained by the kind of biological variation proposed by Levine *et al.* The possible role of such a variation in establishing childhood obesity needs further studies.

An interesting question when studying mechanisms promoting overweight and obesity in childhood is whether a high level of body fat in infancy is maintained into childhood and later life. In the present study it was observed that FMI at three months of age was correlated with the corresponding value at the age of 1.5 years. The significance of this finding in relation to

childhood obesity is not possible to assess at the present time since this study is small and limited to very young children. A longitudinal study in 30 children by Wells *et al.* (66) found a correlation between TBF (%) at 12 weeks and TBF (%) at 2.5 years of age. It is obviously important to conduct further longitudinal studies within this area. The study in paper IV is part of an ongoing longitudinal study providing a larger material for the purpose of investigating relationships between body fatness in infancy and childhood.

As indicated above, environmental factors, i.e. physical activity and energy intake, are likely to be important when overweight and obesity are established during childhood. This thesis indicates in a number of ways that the mechanisms involved are complicated and far from understood. Nevertheless, it is important to keep in mind that it is possible to change environmental factors. This provides hope regarding the possibility of defeating the epidemic of childhood obesity in the future through appropriate environmental changes at an early stage.

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12. SAMMANFATTNING PÅ SVENSKA

Övervikt och fetma bland barn har under senare år blivit allt vanligare i många delar av världen. Studier av nutrition, metabolism och fysiologi under graviditet och de tidiga barnaåren är av vikt för att förstå vilka faktorer som ligger bakom denna utveckling. Speciellt viktigt är att studera hur kroppssammansättningen förändras tidigt i livet. Den här avhandlingen innehåller tre studier som berör detta ämnesområde. I en **studie på gravida** analyserades serumprover, insamlade från 23 kvinnor innan, under och efter deras graviditet, med avseende på halter av leptin, adiponektin och resistin. Via serumproverna fastställdes också kvinnornas insulinresistens (HOMA-IR). Dessa resultat relaterades sedan till mängden kroppsfett hos dessa kvinnor. Mängden kroppsfett (%) och leptin visade, före och under graviditet, en signifikant korrelation med HOMA-IR. En regressionsanalys av HOMA-IR (y) och % kroppsfett (x) i graviditetsvecka 32 gav ett k-värde (lutning) på 0,111, vilket i jämförelse med motsvarande k-värde före graviditet 0,046 var signifikant högre ($p<0.05$). Detta resultat visar att hos friska kvinnor potentierar graviditeten sambandet mellan kroppsfett och insulinresistens. I en **studie av hydreringsgrad i fettfri kroppsvikt** (HF) fastställdes HF i 12 nyfödda med hjälp av dubbelmärkt vatten och helkroppsplethysmografi (PeaPod). HF uppmättes till 80,9% med en låg biologisk variation (0,81 % av genomsnittlig HF). I en **longitudinell studie** mättes kroppsdensiteten med PeaPod hos 108 friska fullgångna spädbarn (53 flickor, 55 pojkar) när de var en respektive tolv veckor gamla. Deras kroppssammansättning beräknades med två olika modeller (Fomons och Buttes). Uppgift om mödrarnas pregravida BMI samlades in. Vid 1,5 års ålder mättes kroppssammansättning och total energiomsättning hos 20 av de 108 barnen. Vid detta tillfälle mättes även viloomsättningen med indirekt kalorimetri under sömn. Viloomsättningen predikterades även med en formel. Buttes modell gav signifikant ($p<0.05$) lägre nivå av kroppsfett (%) jämfört med Fomons modell och i fem fall erhölls inga resultat alls. Beräkningar med Fomons modell visade att vid en veckas ålder innehöll flickorna 13.4 ± 3.7 % och pojkarna 12.5 ± 4.0 % kroppsfett. Motsvarande värden vid 12 veckors ålder var 26.3 ± 4.2 % och 26.4 ± 5.1 %. Mödrarnas BMI innan graviditet korrelerade med kroppsvikt men inte med kroppsfett (g,%) eller fettfri vikt (g) hos deras barn vid en veckas ålder. Vid 1,5 års ålder innehöll flickorna (n=9) 28.0±2.8 % och pojkarna (n=11) 28.3±3.7 % kroppsfett. Mellan en och 12 veckors ålder ökade alla barnen sin kroppsfetthalt. Mellan 12 veckor och 1,5 år ökade kroppsfetthalten hos 13 barn medan den minskade hos 7. Resultat visar att predikterad viloomsättning ökar risken för att få en falsk korrelation mellan kroppsfetthalt och fysisk aktivitetsnivå jämfört med om

man använder uppmätt viloomsättning. Den fysiska aktivitetsnivån vid 1,5 års ålder (x), var negativt korrelerad till förändring i kroppsfetthalt [kroppsfett (%) vid 1.5 år minus kroppsfett (%) vid 12 veckor] (y), r=-0.52, p=0.02. Sammanfattningsvis tyder resultaten på att kvinnors kroppsfetthalt har en stimulerande effekt på fostrets på totala tillväxt men inte på dess retention av kroppsfett. Dessutom visar resultaten att Fomons modell är den bästa tillgängliga när det gäller att beräkna kroppssammansättningen hos spädbarn från kroppsdensitet. Slutligen tyder resultaten på att den fysiska aktivitetsnivån vid 1,5 års ålder har betydelse för hur fort den höga kroppsfetthalten, som är typisk för spädbarnsperioden, sjunker under tidig barndom.

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