Body composition of parents and their infants
methodological, anthropometric, metabolic and genetic studies

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To Hanna
and our wonderful children
Elias and Siri
Drawing by Elias, 4 years old
1. ABSTRACT

Body composition in infancy may be of importance for later health. In particular, infant body composition may be relevant regarding obesity risk in childhood. Recent advances in body composition methodology using air displacement plethysmography (ADP) have provided possibilities to accurately measure body composition of infants in a quick and non-invasive manner. The aims of this thesis were to study associations of parental body composition using ADP, glucose homeostasis during pregnancy and infant genetics with infant body composition also using ADP. When using ADP in adults, a correction for the thoracic gas volume (TGV) is needed and TGV can be predicted using equations developed in non-pregnant adults. Thus another aim was to study the validity of using such equations during pregnancy.

Parent couples were invited to this study at a routine visit to a maternity clinic in Linköping between September 2008 and October 2010. When the mother was in gestational week 32, parental body composition using ADP and maternal glucose homeostasis variables were assessed. Size and body composition of healthy, singleton and full term (≥ 37 gestational weeks) infants were measured at 1 and 12 weeks of age and a total of 211 infants were included in the studies. Weight and length at 1 year of age were reported by parents. Saliva samples were collected from the infants to obtain DNA for genotyping of the fat mass and obesity associated (FTO) gene.

Body composition results calculated using measured and predicted TGV were compared in 27 women. Results showed that predicted TGV yields a very marginal overestimation (0.5 %) of fat mass (FM). Further, each kg increase in maternal and paternal fat-free mass (FFM) was associated with 15.6 g (\(P=0.001\)) and 9.1 g (\(P=0.007\)), respectively, more FFM in their 1-week old infants. FM of fathers was not related to infant FM. However, maternal FM was positively associated with FM of daughters (5.8 g/kg, \(P=0.007\)), but not of sons (\(P=0.79\)) at 1 week of age. Similarly, each standard deviation increase in maternal HOMA-IR (homeostatic model assessment-insulin resistance) was related to 52.7 more g of FM (\(P<0.001\)) in 1-week-old daughters, but no such relationship was found for sons (\(P=0.79\)). The number of risk alleles at the FTO locus rs9939609 was not associated with infant body mass index (BMI) or infant FM at 1 or 12 weeks of age. However, the number of risk alleles was positively associated (\(P\leq 0.033\)) with infant length at 1 and 12 weeks of age, and the results suggested that this association was stronger in boys than in girls.

The results presented in this thesis show that: i) The use of predicted TGV when applying ADP in gestational week 32 overestimated % FM only slightly. ii) Associations between parental and infant body composition are present early in life. Thus, parental FFM was positively related to FFM in 1-week-old infants. Furthermore, maternal FM and insulin resistance (HOMA-IR) were positively related to FM of 1-week-old daughters, but no such relationships were observed for sons. iii) The FTO genotype is not associated with infant body fatness at 1 or 12 weeks of age. However, the results suggested that the number of FTO risk alleles is positively associated with infant length, especially in boys.

In conclusion, parental and genetic factors were associated with infant size and body composition and these relationships may be of importance for future body composition and health.
2. LIST OF PUBLICATIONS


III. **Henriksson P**, Löf M, Forsum E. Glucose, insulin, and the insulin-like growth factor binding protein 1 in the circulation of pregnant women in relation to their own body composition and to that of their infants. (Manuscript)

IV. **Henriksson P**, Löf M, Soderkvist P, Forsum E. Variation in the fat mass and obesity-related (FTO) genotype is not associated with body fatness in infants, but possibly with their length. Pediatr Obes 2014;9:e112-5.
3. RELATED PUBLICATIONS (NOT INCLUDED IN THE THESIS)


4. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>ADP</td>
<td>Air displacement plethysmography</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BV</td>
<td>Body volume</td>
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<tr>
<td>BV\textsubscript{predTGV}</td>
<td>Body volume calculated using predicted thoracic gas volume</td>
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<tr>
<td>BV\textsubscript{measTGV}</td>
<td>Body volume calculated using measured thoracic gas volume</td>
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<td>CV</td>
<td>Coefficient of variation</td>
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<td>d</td>
<td>Body density</td>
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<tr>
<td>d\textsubscript{FM}</td>
<td>Density of fat mass</td>
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<td>d\textsubscript{FFM}</td>
<td>Density of fat-free mass</td>
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<td>FAO</td>
<td>Food and Agricultural Organization</td>
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<td>FFM</td>
<td>Fat-free mass</td>
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<td>Fat-free mass index</td>
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<td>FMI</td>
<td>Fat mass index</td>
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<td>FTO gene</td>
<td>Fat mass and obesity associated gene</td>
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<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<td>GWAS</td>
<td>Genome-wide association studies</td>
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<td>HbA\textsubscript{1c}</td>
<td>Haemoglobin A\textsubscript{1c}</td>
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<td>HAPO study</td>
<td>Hyperglycemia and Adverse Pregnancy Outcome study</td>
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<td>HOMA-IR</td>
<td>Homeostatic model assessment-insulin resistance</td>
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<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein 1</td>
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<td>IOM</td>
<td>Institute of Medicine</td>
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<td>PATHOS</td>
<td>Parents And THeir Offspring Study</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>Standard deviation score</td>
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<td>Thoracic gas volume</td>
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<td>TGV\textsubscript{meas}</td>
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5. INTRODUCTION

5.1 Background

According to the World Health Organization, childhood obesity is one of the most serious public health challenges of the 21st century (1), and the proportion of overweight and obese children is high in many countries (1, 2). This is of great concern, since obese children tend to remain obese in adulthood (1, 3), when they are more likely to develop disorders such as diabetes and cardiovascular disease (1). Data from Ng et al. suggest that there will be continued increases in the prevalence of obesity in the developing world, where today approximately two thirds of the world’s obese people live (2). Furthermore, although the increase in obesity prevalence in developed countries has attenuated in the past decade, no county has reported a substantial decrease in this prevalence (2). This is consistent with Swedish data showing that although the increase in overweight/obesity prevalence appears to have levelled off both in adults (4) and children (4, 5), it is still high.

Overweight and obesity may be established early in life (3, 6), and even factors during intrauterine life may be of importance in relation to obesity development and health (7-10). For example, Barker suggested that the intrauterine environment, reflected by a low birth weight, is associated with impaired health in adulthood (7). Indeed, recent meta-analyses have confirmed the relationships between a low birth weight, on the one hand, and high blood pressure (11), increased cardiovascular mortality (9), and increased all-cause mortality (9) later in life on the other hand. Furthermore, a high birth weight has been associated with an increased obesity risk, as defined by body mass index (BMI; \[\text{body weight (kg)/height}^2\text{ (m)}\]), later in life (12). However, when Wells et al. (13) reviewed the literature relating birth weight to body composition in childhood and in adulthood, they concluded that birth weight was strongly related to the amount of fat-free mass (FFM) in the body, whereas the corresponding association between birth weight and body fat mass (FM) was considerably weaker. Interestingly, a low FFM and a high FM have both been reported to be independent risk factors for premature mortality in adulthood (14). It has also been suggested that the positive association between birth weight and later FFM may explain the link between low birth weight and later cardiovascular disease (i.e. the Barker hypothesis) (15). It may thus be relevant to note that not only the weight at birth, but also the components of the body, i.e. the contributions of FFM and FM to birth weight, may be of relevance for adult health.
Consequently, some researchers have discussed the potential influence of infant body composition on later health. For example, Yajnik et al. speculated that the so-called thin-fat Indian infant, with a low FFM but a well-preserved FM at birth, may have an increased risk for insulin resistance later in life (16). Furthermore, Wells (10) proposed that poor intrauterine growth may permanently restrict the FFM, leading to a lower "metabolic capacity, i.e. a reduced ability to tolerate an energy dense diet, and consequently an increased risk for excessive fat retention. Finally, Hull et al. observed that infants of overweight and obese mothers had more FM than infants of normal-weight mothers (17). These authors also suggested that a high FM and a low FFM, rather than birth weight, are mediators between foetal growth and adult health (17). Although infant FM and FFM may be important for later health, very little is known about their determinants. One of the main reasons for this is that accurate body composition methodology that is applicable during infancy has not been available until recently.

5.2 Parental influences on infant body composition

Previous studies have indicated that maternal BMI is positively associated with infant FM (17-19). Interestingly, Lingwood et al. reported that the BMI in women with gestational diabetes was positively related to infant FM in infant girls, but not in infant boys (20). Also, the BMI of fathers has been suggested to influence infant birth weight (21). However, BMI is a relatively poor measure of body fatness (22), especially in pregnant women (23), and thus studies using appropriate methodology when investigating associations between body composition of parents and their infants are motivated. In such a study Butte et al. (24) reported no associations between maternal and infant body composition, although their study may have been too small (n=63) to identify such associations. Furthermore, I have not identified any studies investigating associations between body composition of fathers and their infants. Thus, further larger studies investigating relationships between parental and infant body composition are motivated.

Since very little is known regarding associations between maternal and infant body composition, there is limited knowledge regarding the mechanisms by which maternal body composition may influence infant body composition. However, variables related to the glucose homeostasis of the pregnant woman may be of interest in this respect since as early as 1952 Pedersen (25) proposed that the high blood glucose concentration in gestational
diabetes mellitus (GDM) is transferred to the foetus, with a subsequent stimulation of foetal growth and fat retention. Recently, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study concluded that pregnancy glycaemia is positively associated with infant FM, measured by skinfolds, even in women considered to be non-diabetic (25). Using dual x-ray absorptiometry, Friis et al. have also reported that higher glycaemia in pregnancy is related to more infant FM (26). Hence, variables such as concentrations of glucose and insulin in the circulation of pregnant women, as well as homeostasis model assessment-insulin resistance (HOMA-IR), may be relevant when investigating the influence of maternal metabolism on infant size and body composition. Also, the insulin-like growth factor binding protein 1 (IGFBP-1) may be of interest in this context since its concentration in serum is inversely related to that of insulin (27) as well as to infant birth weight (28, 29). Interestingly, all these variables (glucose, insulin, HOMA-IR, IGFBP-1) describing maternal glucose homeostasis have been related to the BMI (26) or FM (30, 31) of women during pregnancy. Thus, these variables may represent mechanisms by which maternal body composition influences infant body composition. Importantly, previous studies have not accurately measured body composition of mothers and infants, but have relied on maternal BMI (26, 32) and/or skinfolds as an estimate of infant body fatness (25, 33).

5.3 Genetic influences on infant body composition

It is well-established that obesity has a strong genetic component (34, 35). Despite this fact, efforts to identify genetic loci associated with obesity have been unsuccessful until recently (36, 37). However, with the introduction of large genome-wide association studies (GWAS) a number of obesity associated loci have been identified. In 2007, the fat mass and obesity associated (FTO) gene was the first such locus discovered by means of GWAS (38). In this study the addition of each risk allele at the single nucleotide polymorphism, rs9939609, was associated with an increased risk for obesity (odds ratio=1.31) and an increased body weight of ≈ 1.0 kg. Associations between the FTO genotype and BMI have been replicated in numerous studies (36, 37) and such relationships between the FTO genotype and body fatness have also been presented (39). Although FTO is the locus with the strongest association with BMI, variation in FTO explains less than 1 % of the variation in BMI (36, 37). Associations between the FTO genotype and body fatness, BMI and FM have also been found in children (38). In infancy, however, several studies report no relationship between the FTO genotype and BMI (40-42), and Sovio et al. even reported an inverse association between the number of
FTO risk alleles and the BMI of infants (43). However, BMI is a poor predictor of body fatness in infancy (44) and thus studies relating the FTO genotype to infant body fatness should be based on accurate measures of body composition. The two published studies examining associations between the FTO genotype and infant FM have reported contradictory results (42, 45). Thus, Mook-Kanamori et al. (42) found no such association at 6 months of age whereas López-Bermejo et al. (45) reported a positive association between the number of risk alleles and FM in 2-week-old infants.

5.4 Advances in human body composition assessment

Studies of human body composition have been of interest for a long period of time (46). Historically, however, in vivo studies of body composition have constituted a complex issue. Ground-breaking progress in the field was made by Behnke and colleagues in 1942 with the introduction of underwater weighing to measure the body volume of human subjects (46). This enabled calculation of body density when body weight was also assessed. By assigning densities to the fat mass (FM) and fat-free mass (FFM), Behnke et al. were also able to estimate the relative proportions of FM and FFM in the human body by means of densitometry, i.e. the procedure of estimating body composition from body density. Siri (in 1956) and Brozek (in 1961) published equations to estimate % FM based on a subject’s density, and these are widely used even today.

For a long period of time, underwater weighing was considered to be the golden standard in human body composition research (46). Currently, more advanced models dividing the human body into three or four components (fat, water, protein and mineral) are considered to be the golden standards for use when new methods are evaluated (47). There are several body composition techniques available with inherent strengths and weaknesses. In general, simpler methods such as skinfolds and bioelectrical impedance are easy to apply, but their validity may be questioned (47). Conversely, valid body composition methods may be costly, involve advanced technology with potential radiation exposure, or they may be demanding for subjects (47). The latter applies for underwater weighing, since this methodology usually involves complete submerging of the body into water (46, 47). Such a procedure may be unpleasant for many subjects and it is not applicable for infants. However, with the introduction of air displacement plethysmography (APD), body volume, and consequently density, of adults (48) and infants (49) can be easily assessed in a non-invasive manner.
5.5 Principles of densitometry

The body weight can be divided into FM and FFM (i.e. a two-component model). Main components of the FFM are water, protein and mineral, and their respective densities are relatively well-established (46). If densities of FM (dFM) and FFM (dFFM) are known and the subject’s body density (d) is measured, the following equation can be used: 1/d = x/dFM + (1-x)/dFFM where x is the proportion of FM in the body. By using appropriate densities of FM and FFM, the equation can be rearranged, enabling calculation of percent FM (46). The density of FM (0.9007 g/cm³) is well-established and considered to be constant (46), while the density of FFM is more variable. This is due to the fact that its proportions of water, protein and mineral vary depending, for example, on age and physiological status (46). For healthy non-pregnant adults, a FFM density of 1.1 g/cm³ is considered to be appropriate (50).

In infants, the water content in the FFM is substantially higher than in adults (51), and consequently their FFM density is lower than in adults. Data regarding growth and body composition, including FFM-density, from birth to 10 years of age were published by Fomon et al. in 1982 (51). These data have to some extent been confirmed by Wells et al. (52), Eriksson et al. (53) and Andersen et al. (54).

During pregnancy the composition of the FFM is altered. Thus, the water content of FFM increases while its concentrations of protein and mineral decrease slightly (55, 56) resulting in a lower FFM-density. Van Raaij et al. (56) calculated average changes in FFM-density throughout pregnancy using data by Hytten (55). The Institute of Medicine (IOM) (57) as well as the Food and Agricultural Organization (FAO) (58) have stated that body composition methods taking into account the changes in FFM-density described by van Raaij (56) are satisfactory for use with pregnant women. Studies based on densitometry have provided further support for this statement (59, 60). Thus, Hopkinson et al. (60) found the use of the FFM-density by Van Raaij (56) to yield reliable mean estimates of FM in gestational week 36 when compared to results obtained using a four-component model. Furthermore, our research group has published results showing that use of the FFM-density estimated by van Raij (56) in gestational week 32 (1.092 g/ml) gives satisfactory estimates of FM when compared to results obtained using a three-component model (59).
5.6 Air displacement plethysmography

5.6.1 Bod Pod and Pea Pod

The Bod Pod was introduced in the mid-1990s and applies ADP (air displacement plethysmography) in order to measure body volume of adults and children (50, 61). The Bod Pod consists of a scale and a chamber in which the volume of the subject is measured. The measurement starts with weighing the subject, and subsequently the subject's body volume is determined using ADP. Body composition is then calculated by means of the Bod Pod software using appropriate densities of FFM and FM. The ability of the Bod Pod to determine body volume and thus body composition has been well documented for subjects with a large variation in size and body composition (50, 61).

The introduction of the Pea Pod, also based on ADP, provided new possibilities to accurately investigate the body composition of infants (62, 63). The measurement procedure is quick, safe, non-invasive (49, 62, 63) and is therefore acceptable to parents, infants and health personnel. The Pea Pod, introduced in 2004, can measure the body composition of infants from birth until approximately 6 months of age (≈ 8 kg) (49), and it consists of a scale and a chamber with a tray that can be pulled out. The weight of the infant is measured on the scale and body volume is subsequently measured in the chamber. The complete test procedure takes about 3 minutes (62). The density of the subject is calculated using the measured body weight and volume. Body composition is then calculated by means of the Pea Pod software using appropriate densities of FFM and FM. Results obtained using Pea Pod have been found to be in good agreement with results obtained by means of a four-component model (62) as well as by isotope dilution (63).

5.6.2 Methodological principles

When applying ADP, the body volume is measured in a chamber utilizing the relationship between pressure and volume, i.e. the gas laws of Boyle and Poisson (48-50). The measured body volume needs to be corrected, since the air close to the subject's body behaves differently than the remaining air in the chamber, which results in an underestimation of the subject's volume (48-50). To correct for this a correction volume, the so-called “surface area artifact”, is calculated based on the length/height and weight and is added to the body volume of the subject (48-50).
The air in the lungs and thorax also behaves differently than air not adjacent to the body during an ADP measurement (48-50). This results in an underestimation of body volume corresponding to 40% of the thoracic gas volume (TGV), which is equivalent to the average amount of air in the lungs and thorax during an ADP measurement. Therefore, TGV is measured or predicted and then 40% is added to the measured body volume. In infants, TGV is predicted using infant age, length and weight (49). In adults, TGV can be measured as a part of the measurement procedure in Bod Pod (50, 64). However, this assessment can be difficult to perform and subjects may be unable to produce satisfactory results. A more convenient approach is to predict the TGV based on height and age using established sex-specific equations developed in non-pregnant adults (65, 66). In such subjects McCrory et al. showed that body composition results obtained using predicted TGV were in good agreement with those obtained using measured TGV (67). However, the effect of using such predicted TGV on body composition results has not been assessed in pregnancy. This is a relevant topic, since physiological and anatomical changes during pregnancy such as increased subcostal angle, dislocation of the diaphragm and the growing uterus may affect the TGV (68, 69).
6. SPECIFIC AIMS

To measure and to predict TGV, using an equation developed for non-pregnant women, in women in gestation week 32 and to compare body composition and BV results obtained using these two kinds of TGV (Paper I).

To study the body composition of women in gestational week 32 in relation to the size and body composition of their 1-week-old infants (Paper II).

To study the body composition of fathers in relation to the size and body composition of their 1-week-old infants (Paper II).

To study relationships between body composition and glucose homeostasis variables (glucose, insulin, HOMA-IR, IGFBP-1 and HbA\textsubscript{1c}) in the circulation of women in gestational week 32 (Paper III).

To study relationships between glucose homeostasis variables (glucose, insulin, HOMA-IR, IGFBP-1 and HbA\textsubscript{1c}) in the circulation of women in gestational week 32 versus infant size and body composition at 1 week of age (Paper III).

To study relationships between the number of risk alleles in the FTO gene (rs9939609) in relation to infant size and body composition (Paper IV).
7. MATERIALS AND METHODS

7.1 Participants and study outline (Papers I-IV)

Figure 1 describes the recruitment of participants. A total of 1530 parent couples, from a well-educated middle income population, received an invitation to participate in the study called PATHOS (Parents And THeir Offspring Study) at a routine visit to the “Kvinnohälsan” maternity clinic in Linköping (September 2008-October 2010) during early pregnancy. Of those, 249 couples agreed to participate and both parents were measured when the woman was in gestational week 32. A subgroup of 40 consecutively included women (non-smoking, non-asthmatic) were invited to participate in a study in which the effect of pregnancy on TGV was investigated (Paper I). Two women declined participation in this study and 11 failed to produce three acceptable estimates of TGV, and thus the sample size consisted of 27 women.

Healthy, singleton and full term (≥ 37 gestational weeks) offspring of the 249 parent couples were included in further studies. Infants born prematurely (n=11), not healthy (n=2) or born to a mother with preeclampsia (n=5) or gestational diabetes (n=1) were excluded. In addition, 19 parent couples withdraw from the study. In total, 211 infants were measured at 1 and 12 weeks of age. Body composition of two infants was not measured, but their weight and length were measured. Thus the sample size was 209 infants in Papers II and III. Parents reported the weight and length of their 1-year-old children and we were able to collect such data for 209 infants. Since weight, length and BMI were important outcomes in Paper IV, all infants with data regarding weight and length at 1 and 12 weeks (n=211) were included in this study.

7.2 Measurement of parents (Papers I-III)

Gestational age was assessed by means of a routine ultrasound examination in approximately gestational week 12-14 (70). Parents were investigated in the morning after an overnight fast when the woman was in gestational week 32 (31.4±0.3). On this occasion, a blood sample was collected from the woman and then both parents had their height measured. Subsequently their weight and body composition were assessed by means of Bod Pod. TGV was measured for women in Paper I. Additional information (maternal smoking habits, parity, weight before pregnancy, gestational weight gain, parental education level, infant birth weight and infant feeding, etc.) were obtained by means of questionnaires administered at this measurement or when infants were measured.
Figure 1. Recruitment of participants in the thesis
7.3 Measurement of infants (Papers II-IV)
Infant size and body composition were investigated at 1 week (1.0±0.3 weeks), and at 12 weeks (12.1±0.5 weeks) of age when infant length was measured and body composition was assessed using Pea Pod. The weight and length of 1-year-old infants were reported by their parents after a routine visit at a child health care centre. Saliva samples were collected from the infants, generally at the first measurement, to obtain DNA for FTO-genotyping.

7.4 Ethics
The project was conducted according to the guidelines laid down in the Declaration of Helsinki and all studies were approved by the Research Ethics Committee, Linköping, Sweden (M187-07, 2010/68-32). Informed consent, witnessed and formally recorded, was obtained from all parents.

7.5 Methods
7.5.1 Thoracic gas volume and body composition of pregnant women (Paper I)
TGV was measured while the subject was sitting in the Bod Pod using a technique comparable to that used in standard plethysmography (48, 50). Measurements were defined as acceptable according to criteria recommended by the manufacturer (50). Measured TGV (TGVmeas) was the average of three acceptable measurements. The average within - subject standard deviation (SD) for measured TGV was 0.184 litres and its corresponding coefficient of variation was 5.9 %. The technical error of measurement (71) was 0.210 litres. These figures are in agreement with those reported in previous studies (65, 72), indicating that the repeatability of the measurements is satisfactory. Predicted TGV (TGVpred) was calculated using height and age of the women by means of Bod Pod Software 4.2.4 (65, 66). Height was measured with a wall stadiometer to the nearest 0.5 cm. Body weight and volume were assessed by means of Bod Pod (48, 50). Body composition was calculated using Bod Pod software 4.2.4, and 1.092 g/cm³ was used as FFM density. Calculation of FM was based on TGVpred (FM_{TGVpred}) as well as on TGVmeas (FM_{TGVmeas}).
7.5.2 Body composition assessment of parents (Papers II and III)

Height was measured with a wall stadiometer to the nearest 0.5 cm. Body weight and volume, in tight fitting underwear or spandex-type swim suit/pants, were measured using the Bod Pod (COSMED USA, Inc., Concord, CA, USA) (48, 50). Bod Pod software 4.2.4. was used to predict TGV (65, 66) and to calculate body composition. The FFM density values used were 1.1 g/cm³ (men) (73) and 1.092 g/cm³ (women) (56).

7.5.3 Body composition assessment of infants (Papers II-IV)

The procedure used to assess body composition by means of Pea Pod has been described previously (44, 62). In brief, infant length was measured to the nearest 0.5 cm using a length board. Then infants were weighed without clothing and subsequently their body volume was measured in Pea Pod (COSMED USA, Inc., Concord, CA, USA). Body composition was calculated by means of the Pea Pod software 3.0.1. using the FFM density model published by Fomon et al. (51).

7.5.4 Analysis of glucose homeostasis variables in pregnant women (Paper III)

Blood collected in EDTA-containing vacutainer tubes was used for high-performance liquid chromatography analysis of HbA1c (74). Plasma glucose was analysed by means of the glucose hexokinase method and serum insulin using the Elecsys electrochemiluminescence immunoassay on a Cobas 602 (Roche Diagnostics Scandinavu AB, Bromma, Sweden). HOMA-IR was calculated according to Matthews et al. (75). Serum samples were stored at -70° C prior to analysis of IGFBP-1 by means of a one-step enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Inter assay coefficients of variation were 7.8 and 20.0 % for samples with high (1688 ng/ml) and low (4 ng/ml) IGFBP-1 concentrations, respectively. Plasma glucose, serum insulin and HbA1c were analysed at the Department of Clinical Chemistry, Linköping University Hospital, which is accredited for these analyses (ISO/IEC 17025).

7.5.5 Genotyping of the FTO locus rs9939609 (Paper IV)

The FTO locus (rs9939609) was genotyped as described in Paper IV. In brief, DNA from saliva samples was extracted using QuickExtract DNA Extraction Solution 1.0 (Epicentre Biotechnologies, Madison, WI, USA). Genotyping, i.e. assessing the number of risk (A) or wild type (T) alleles, was based on real-time PCR (polymerase chain reaction) using the
TaqMan Genotyping Assay (ID: C\_30090620\_10), and was analysed on a ABI Prism 7900 HT “Sequence Detection System” (Applied Biosystems, Carlsbad, CA, USA). Of the 211 subjects, saliva samples were not available for 2 subjects, and 207 (99 %) of the remaining samples were successfully genotyped.

### 7.6 Statistics

Values are given as means and SD or as n and %. For all studies \(P<0.05\) was considered statistically significant and all hypothesis tests were two-sided. Statistical analysis was performed using PASW Statistics 18 (IBM, Somers, NY, USA) or Statistica software 9.1 (StatSoft, Inc., Tulsa, OK, USA). Agreement between two methods was evaluated as suggested by Bland and Altman (Paper I) (76). Linear regression (Papers I-IV), and t-tests (Papers I and II) were performed according to Kleinbaum et al. (77) as described in these papers. Glucose homeostasis variables were log transformed to obtain normality since they were positively skewed (Paper III). For easier interpretation, internal standard deviation scores (SDS) were calculated by subtracting the sample mean from each observation and dividing the difference obtained by the SD of the sample (Paper III). Correlations for dependent observations were compared (Paper III) as described by Kleinbaum et al. (77). A chi square test was performed to test if the allele frequencies of the FTO gene were in Hardy Weinberg equilibrium (Paper IV). In order to statistically test whether relationships differed between sexes (Papers II-IV), an interaction term was added to the model as described in these papers.

### 7.7 Considerations concerning data analysis

Since maternal parity, infant sex, infant gestational age at birth and infant age are related to infant size and/or body composition (44, 78-80), results that are adjusted for these variables are presented when infants are studied (Papers II-IV). It is noteworthy that in these studies unadjusted estimates were very similar to estimates adjusted for these variables. Adjusting for additional potential confounders such as breastfeeding, maternal educational attainment and age also yielded very similar results. Furthermore, sex-differences in offspring body composition in relation to maternal BMI (20), glycaemia (33, 81, 82) and infant genetics (83) have been reported. This provided motivation to investigate sex differences in relation to parental body composition, maternal glucose homeostasis variables and infant genetics. Finally, since most previous knowledge regarding the influence of intrauterine factors on later
health and body composition is based on birth weight, weight of the infants was used as an outcome measure. Since weight is the combination of FFM and FM, the outcomes are presented in absolute values (g) and not only in percent. Relationships were observed between maternal FFM and infant length (Paper II) as well as between the FTO genotype and infant length (Paper IV). In these papers infant body composition results were also adjusted for length (Papers II and IV) and/or presented as fat-free mass index (FFMI) and fat mass index (FMI) (Paper II). FMI was \[\text{FM (kg)/height}^2 \text{ (m)}\] and FFMI was \[\text{FFM (kg)/height}^2 \text{ (m)}\].
8. RESULTS

8.1 Predicted versus measured thoracic gas volume (Paper I)

Descriptive data for the women participating in this study are presented in Table 1. As shown in Table 2, TGV\textsubscript{pred} was significantly higher, by 0.187 litres (6 %), than TGV\textsubscript{meas}. In the Bland and Altman analysis, a significant negative correlation between TGV\textsubscript{pred} minus TGV\textsubscript{meas} and the average of TGV\textsubscript{pred} and TGV\textsubscript{meas} was found (Figure 2b). Further, the limits of agreement were wide (from − 0.675 to + 1.049 litres), corresponding to − 21.0 and 32.6 % of the average of TGV\textsubscript{pred} and TGV\textsubscript{meas}.

Table 1. Characteristics of 27 women in the study (Paper I).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at measurement (years)</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Stage of gestation at measurement(^*) (weeks)</td>
<td>31.4 ± 0.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.06</td>
</tr>
<tr>
<td>Weight before pregnancy(^†) (kg)</td>
<td>66 ± 12</td>
</tr>
<tr>
<td>BMI before pregnancy(^‡) (kg/m(^2))</td>
<td>23.5 ± 3.9</td>
</tr>
<tr>
<td>Weight at measurement (kg)</td>
<td>75.3 ± 11.5</td>
</tr>
<tr>
<td>Gestational weight gain(^†§) (kg)</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Infant birth weight(^†) (kg)</td>
<td>3.48 ± 0.57</td>
</tr>
</tbody>
</table>

BMI, body mass index.
\(^*\)Calculated from a routine ultrasound examination in approximately gestational week 12 (70).
\(^†\)Self-reported data.
\(^‡\)Calculation based on self-reported weight before pregnancy and height measured in gestational week 32.
\(^§\)Calculated as the last known weight in pregnancy minus weight before pregnancy.
Table 2. Predicted* and measured thoracic gas volume as well as body volume and fat mass calculated using predicted and measured thoracic gas volume for women (n=27) in gestational week 32 (Paper I).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGV(_{\text{pred}}) (litres)</td>
<td>3.314(\dagger) ± 0.206</td>
</tr>
<tr>
<td>TGV(_{\text{meas}}) (litres)</td>
<td>3.127 ± 0.488</td>
</tr>
<tr>
<td>BV(_{\text{predTGV}}) (litres)</td>
<td>74.211(\ddagger) ± 11.919</td>
</tr>
<tr>
<td>BV(_{\text{measTGV}}) (litres)</td>
<td>74.136 ± 11.878</td>
</tr>
<tr>
<td>FM(_{\text{predTGV}}) (%)</td>
<td>35.0§ ± 5.3</td>
</tr>
<tr>
<td>FM(_{\text{measTGV}}) (%)</td>
<td>34.5 ± 5.2</td>
</tr>
</tbody>
</table>

TGV, thoracic gas volume; BV, body volume; FM, fat mass; TGV\(_{\text{pred}}\), predicted thoracic gas volume; TGV\(_{\text{meas}}\), measured thoracic gas volume; BV\(_{\text{predTGV}}\), body volume calculated using TGV\(_{\text{pred}}\); BV\(_{\text{measTGV}}\), body volume calculated using TGV\(_{\text{meas}}\); FM\(_{\text{predTGV}}\), fat mass calculated using TGV\(_{\text{pred}}\); FM\(_{\text{measTGV}}\), fat mass calculated using TGV\(_{\text{meas}}\).

* TGV was predicted using an equation developed in non-pregnant women as described in Materials and Methods.
\(\dagger\) Significantly \((P = 0.033)\) higher than TGV\(_{\text{meas}}\).
\(\ddagger\) Significantly \((P = 0.033)\) higher than BV\(_{\text{measTGV}}\).
§ Significantly \((P = 0.043)\) higher than FM\(_{\text{measTGV}}\).

Average BV\(_{\text{predTGV}}\) was very slightly (0.075 litres or 0.1 %) but significantly higher than average BV\(_{\text{measTGV}}\) (Table 2). Furthermore, the limits of agreement were small (from – 0.271 to + 0.421 litres), equivalent to – 0.4 and + 0.6 % of the average of BV\(_{\text{predTGV}}\) and BV\(_{\text{measTGV}}\) (Figure 3b).

Average FM\(_{\text{predTGV}}\) (%) was significantly, but only very slightly (0.5 % FM) higher than average FM\(_{\text{measTGV}}\) (Table 2). Furthermore, a strong significant correlation was observed between FM\(_{\text{predTGV}}\) and FM\(_{\text{measTGV}}\) (Figure 4a). There was no significant association between FM\(_{\text{predTGV}}\) and FM\(_{\text{measTGV}}\) on the one hand and the average of FM\(_{\text{predTGV}}\) and FM\(_{\text{measTGV}}\) on the other hand (Figure 4b). Finally, the limits of agreement in the Bland and Altman analysis were small (from – 1.9 to + 2.9 % FM), corresponding to – 5.5 to + 8.3 % of the average of FM\(_{\text{predTGV}}\) and FM\(_{\text{measTGV}}\).
Figure 2. (a) TGV\textsubscript{meas} (measured thoracic gas volume) (y) regressed on TGV\textsubscript{pred} (predicted thoracic gas volume) (x). The slope of the regression line ($y = -0.568 + 1.115x$, $r = 0.471$, standard error of the estimate = 0.439, $P = 0.013$) is not significantly ($P = 0.786$) different from the line of identity ($y = x$). (b) Bland-Altman scatter plot; TGV\textsubscript{pred} minus TGV\textsubscript{meas} (y) regressed on the average of TGV\textsubscript{pred} and TGV\textsubscript{meas} (x). The solid line represents the mean difference between TGV\textsubscript{pred} and TGV\textsubscript{meas} (0.187 litres) and the dashed lines are the limits of agreement (2 SD = 0.862 litres). The regression equation is $y = 3.546 - 1.043x$, $r = -0.741$ ($P < 0.001$). Data collected from 27 women in gestational week 32.
Figure 3. (a) BV\textsubscript{measTGV} (body volume calculated using measured thoracic gas volume) (y) regressed on BV\textsubscript{predTGV} (body volume calculated using predicted thoracic gas volume) (x). The slope of the regression line ($y = 0.186 + 0.996x$, $r > 0.999$, standard error of the estimate $= 0.171$, $P < 0.001$) is not significantly ($P = 0.194$) different from the line of identity ($y = x$).

(b) Bland-Altman scatter plot; BV\textsubscript{predTGV} minus BV\textsubscript{measTGV} (y) regressed on the average of BV\textsubscript{predTGV} and BV\textsubscript{measTGV} (x). The solid line represents the mean difference between BV\textsubscript{predTGV} and BV\textsubscript{measTGV} (0.075 litres) and the dashed lines are the limits of agreement (2 SD = 0.346 litres). The regression equation is $y = -0.179 + 0.003x$, $r = 0.236$ ($P = 0.235$). Data collected from 27 women in gestational week 32.
Figure 4. (a) $\text{FM}_{\text{measTGV}}$ (fat mass calculated using measured thoracic gas volume) (y) regressed on $\text{FM}_{\text{predTGV}}$ (fat mass calculated using predicted thoracic gas volume) (x). The slope of the regression line ($y = 1.139 + 0.953x$, $r = 0.974$, standard error of the estimate = 1.2, $P < 0.001$) is not significantly ($P = 0.296$) different from the line of identity ($y = x$). (b) Bland-Altman scatter plot; $\text{FM}_{\text{predTGV}}$ minus $\text{FM}_{\text{measTGV}}$ (y) regressed on the average of $\text{FM}_{\text{predTGV}}$ and $\text{FM}_{\text{measTGV}}$ (x). The solid line represents the mean difference between $\text{FM}_{\text{predTGV}}$ and $\text{FM}_{\text{measTGV}}$ (0.5 % FM) and the dashed lines are the limits of agreement (2 SD = 2.4 % FM). The regression equation is $y = -0.267 + 0.022x$, $r = 0.095$ ($P = 0.638$). Data collected from 27 women in gestational week 32.
8.2 Parental variables versus infant size and body composition (Papers II and III)

8.2.1 Characteristics of parents and infants (Papers II and III)

Descriptive data for the parents are found in Table 3.

Table 3. Characteristics of parents (n=209) at the time of investigation (Papers II and III).

<table>
<thead>
<tr>
<th></th>
<th>Mothers</th>
<th>Fathers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of gestation† (weeks)</td>
<td>31.4 ± 0.3</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 ± 4</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.1 ± 10.4‡</td>
<td>83.0 ± 12.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 6</td>
<td>182 ± 6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 3.4§</td>
<td>25.0 ± 3.7</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>49.6 ± 5.3</td>
<td>63.3 ± 6.7</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>26.5 ± 7.4</td>
<td>19.7 ± 9.7</td>
</tr>
<tr>
<td>FM (%)</td>
<td>34.3 ± 5.8</td>
<td>22.9 ± 8.6</td>
</tr>
<tr>
<td>Glucose in plasma (mmol/l)</td>
<td>4.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Insulin in serum (pmol/l)</td>
<td>66 ± 37</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>HbA1c¶</td>
<td>31 ± 3</td>
<td></td>
</tr>
<tr>
<td>IGFBP-1 in serum¶ (ng/ml)</td>
<td>108 ± 71</td>
<td></td>
</tr>
<tr>
<td>Level of education**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>0 (0 %)</td>
<td>2 (1.0 %)</td>
</tr>
<tr>
<td>High school</td>
<td>60 (28.7 %)</td>
<td>92 (44.0 %)</td>
</tr>
<tr>
<td>University degree</td>
<td>149 (71.3 %)</td>
<td>115 (55.0 %)</td>
</tr>
<tr>
<td>Parity**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>106 (50.7 %)</td>
<td></td>
</tr>
<tr>
<td>≥1</td>
<td>103 (49.3 %)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD or n (%). BMI, body mass index; FFM, fat-free mass; FM, fat mass. HOMA-IR, homeostatic model assessment-insulin resistance; HbA1c, haemoglobin A1c; IGFBP-1, insulin-like growth factor binding protein 1.

* Fathers were measured when the pregnant woman was in gestational week 32
† Based on a routine ultrasound examination in approximately gestational week 12 (70).
‡ Weight before pregnancy was 66 ± 10 kg (self-reported).
§ BMI before pregnancy was 23 ± 3 kg/m², calculated using self-reported weight.
¶ HbA1c (n=208), IGFBP-1 (n=204)
** Self-reported
Six (2.9%) women were underweight (BMI<18.5 kg/m²), 160 (76.6%) were of normal weight (BMI=18.5-24.9 kg/m²), 32 (15.3%) were overweight (BMI=25.0-29.9 kg/m²) and 11 (5.3%) were obese (BMI≥ 30.0 kg/m²) before pregnancy. Corresponding figures for fathers were: underweight (0.5%, n=1), normal weight (56.5%, n=118), overweight (34.4%, n=72) and obesity (8.6%, n=18). Maternal weight gain for the complete pregnancy was 15 ± 5 kg. Infant data are presented in Table 4. Compared to boys, girls were significantly shorter and lighter and contained significantly more FM and less FFM.

Table 4. Characteristics of infant boys and girls (Papers II and III).

<table>
<thead>
<tr>
<th>At birth</th>
<th>Girls (n=99)</th>
<th>Boys (n=110)</th>
<th>All (n=209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>3540 ± 460</td>
<td>3660 ± 450</td>
<td>3600 ± 460</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40.0 ± 1.2</td>
<td>40.1 ± 1.2</td>
<td>40.1 ± 1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At the time of measurement</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>51.0† ± 1.5</td>
<td>52.0 ± 1.5</td>
<td>51.5 ± 1.5</td>
</tr>
<tr>
<td>Length-for-age z score‡</td>
<td>0.26 ± 1.13</td>
<td>0.11 ± 1.07</td>
<td>0.18 ± 1.10</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3523† ± 445</td>
<td>3653 ± 462</td>
<td>3591 ± 458</td>
</tr>
<tr>
<td>Weight-for-age z score‡</td>
<td>-0.14 ± 1.01</td>
<td>-0.20 ± 1.03</td>
<td>-0.17 ± 1.02</td>
</tr>
<tr>
<td>FFM (g)</td>
<td>3049† ± 318</td>
<td>3243 ± 339</td>
<td>3151 ± 343</td>
</tr>
<tr>
<td>FFM (kg/m²)</td>
<td>11.61† ± 0.79</td>
<td>12.07 ± 0.74</td>
<td>11.85 ± 0.80</td>
</tr>
<tr>
<td>FM (g)</td>
<td>474† ± 171</td>
<td>409 ± 188</td>
<td>440 ± 183</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>1.79† ± 0.59</td>
<td>1.51 ± 0.66</td>
<td>1.64 ± 0.64</td>
</tr>
<tr>
<td>FM (%)</td>
<td>13.2† ± 3.6</td>
<td>10.9 ± 4.1</td>
<td>12.0 ± 4.0</td>
</tr>
<tr>
<td>Mode of feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td>94 (94.9 %)</td>
<td>104 (94.5 %)</td>
<td>198 (94.7 %)</td>
</tr>
<tr>
<td>Breast milk and formula</td>
<td>5 (5.1 %)</td>
<td>6 (5.5 %)</td>
<td>11 (5.3 %)</td>
</tr>
<tr>
<td>Formula</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
</tr>
</tbody>
</table>

Data are means ± SD or n (%). FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index.

‡ Significantly (P<0.05) different from the corresponding value for boys.

† Calculated using Swedish reference data (80).
8.2.2 Parental FFM in relation to infant body size and composition (Paper II)
Associations between parental FFM and infant size and body composition are presented in Table 5. FFM of mothers ($P=0.042$), but not of the fathers, was positively associated with infant length. Furthermore, the FFM of both mothers and fathers was positively related to the weight ($P\leq0.030$), FFM ($P\leq0.007$) and FFMI ($P\leq0.025$) of their infants. More specifically, each kg increase in FFM of mothers and fathers was associated with an increase in infant FFM of 15.6 g and 9.1 g, respectively. Parental FFM remained significantly related to infant FFM when associations were further adjusted for infant length (footnotes ‡ and § Table 5). No interactions ($P>0.10$) between infant sex and parental FFM for infant FFM or FFMI were observed.

8.2.3 Parental FM in relation to infant body size and composition (Paper II)
The results of a regression analysis between parental FM (independent variables) and infant size and body composition (dependent variables) are shown in Table 5. No significant associations between parental FM and any of the variables describing infant size and body composition were found. However, a significant ($P=0.034$) interaction between maternal FM and infant sex was identified when the infant % FM was the dependent variable (footnote †† in Table 5). As shown in Table 5 (footnotes † and ‡), corresponding $P$ values for this interaction (maternal FM x infant sex) were $P=0.050$ and $P=0.058$ when infant FM (g) and infant FMI, respectively, were dependent variables. Finally, no significant interactions between FM of fathers and infant sex were observed in any of the models with infant FM or FMI as dependent variables (Table 5).
Table 5. Size and body composition variables of 209 infants at 1 week of age regressed on body composition variables of their parents*.

<table>
<thead>
<tr>
<th>Infant variables</th>
<th>Parental variables (independent variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(dependent variables)</td>
<td>FM (kg)</td>
</tr>
<tr>
<td></td>
<td>Mothers†</td>
</tr>
<tr>
<td></td>
<td>b</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>5.1</td>
</tr>
<tr>
<td>FFM (g)</td>
<td>2.6</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>0.007</td>
</tr>
<tr>
<td>FM (g)</td>
<td>2.6</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>0.010</td>
</tr>
<tr>
<td>FM (%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index.

* Analysed using multiple regression analysis. Partial regression coefficient (b), coefficient of determination (r²) calculated from partial r, and the P value (P) are given for each relationship. Independent variables in all models are: FM (kg) and FFM (kg) of mothers and fathers, mothers’ height, fathers’ height, maternal parity (0 or ≥1), infant sex, infant gestational age at birth and age at measurement.

† Body composition (FM and FFM) was measured when mothers were in gestational week 32.

‡ Relationship remained significant (b=9.0, r²=0.03, P=0.009) when infant length was included as an additional independent variable.

§ Relationship remained significant (b=5.1, r²=0.02, P=0.039) when infant length was included as an additional independent variable.

¶ Interaction terms “mothers’ FM x infant sex” (P=0.050) and “fathers’ FM x infant sex” (P=0.20) when entered separately in regression models.

** Interaction terms “mothers’ FM x infant sex” (P=0.058) and “fathers’ FM x infant sex” (P=0.19) when entered separately in regression models.

†† Interaction terms “mothers’ FM x infant sex” (P=0.034) and “fathers’ FM x infant sex” (P=0.30) when entered separately in regression models.
The observed interaction between maternal FM and infant sex suggested that maternal FM affects the FM of girls and boys differently. Indeed, when results were stratified by infant sex (Table 6), maternal FM was positively associated with the FM and FMI of daughters ($P \leq 0.008$), but not of sons ($P \geq 0.77$).

Table 6. Fat mass of infant girls (n=99) and boys (n=110) at 1 week of age regressed on the fat mass of their mothers.

<table>
<thead>
<tr>
<th>Infant variables</th>
<th>Mothers’ FM† (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(dependent variables)</td>
</tr>
<tr>
<td></td>
<td>$b$</td>
</tr>
<tr>
<td>FM (g)</td>
<td>5.8</td>
</tr>
<tr>
<td>FMI (kg/m$^2$)</td>
<td>0.020</td>
</tr>
<tr>
<td>FM (%)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

FFM, fat-free mass; FM, fat mass; FMI, fat mass index.

* Analysed using multiple regression analysis. Partial regression coefficient ($b$), coefficient of determination ($r^2$) calculated from partial r, and the $P$ value ($P$) are given for each relationship. Independent variables in all models are: FM (kg), FFM (kg) and height of mother, maternal parity (0 or $\geq 1$) and infant gestational age at birth and age at measurement.

† Body composition (FM and FFM) was measured when mothers were in gestational week 32.

8.2.4 Glucose homeostasis variables in pregnancy in relation to body composition of mothers and infants (Paper III)

Results obtained when glucose homeostasis variables (glucose, insulin, HOMA-IR, HbA1c, and IGFBP-1) in the circulation of women in gestational week 32 were regressed on variables describing their body composition (BMI, FM, FMI and FFMI) are presented in Table 7. All these body composition variables were significantly associated with the investigated glucose homeostasis variables. The associations with variables describing body fatness (i.e. BMI, FM and FMI) versus insulin and HOMA-IR were particularly strong ($r^2=0.32-0.36$) and their correlation coefficients were significantly higher ($P \leq 0.005$) than the corresponding coefficients for FMI ($r^2=0.13-0.14$) (footnote 4 in Table 7). Furthermore, when IGFBP-1 (SDS) was regressed on insulin (SDS), a significant negative association ($b=-0.55$, $r^2=0.30$, n=204, $P<0.001$) was found.
Table 7. Glucose homeostasis variables (glucose, insulin, HOMA-IR, HbA1c and IGFBP-1) in the circulation of women, pregnant in gestational week 32, regressed on variables describing their body composition (BMI, fat mass, fat mass index and fat-free mass index) †.

<table>
<thead>
<tr>
<th>Body composition‡ (independent variables)</th>
<th>Glucose (SDS§) n 209</th>
<th>Insulin (SDS§) n 209</th>
<th>HOMA-IR (SDS§) n 209</th>
<th>HbA1c (SDS§) n 208</th>
<th>IGFBP-1 (SDS§) n 204</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>b 0.10*** 0.11</td>
<td>r² 0.18*** 0.36</td>
<td>b 0.18*** 0.36</td>
<td>r² 0.08*** 0.06</td>
<td>r² -0.13*** 0.19</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>b 0.04*** 0.07</td>
<td>r² 0.08*** 0.33</td>
<td>b 0.08*** 0.32</td>
<td>r² 0.02* 0.03</td>
<td>r² -0.05*** 0.14</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>b 0.10*** 0.07</td>
<td>r² 0.22*** 0.33</td>
<td>b 0.22*** 0.32</td>
<td>r² 0.08** 0.04</td>
<td>r² -0.14*** 0.14</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>b 0.22*** 0.10</td>
<td>r² 0.25*** 0.13</td>
<td>b 0.26*** 0.14</td>
<td>r² 0.16** 0.05</td>
<td>r² -0.26*** 0.13</td>
</tr>
</tbody>
</table>

BMI, body mass index; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; HOMA-IR, homeostatic model assessment-insulin resistance; HbA1c, haemoglobin A1c, IGFBP-1, insulin-like growth factor binding protein 1.

Glucose homeostasis variable and body composition variable was associated: * P<0.05, ** P<0.01, *** P<0.001.

† Analysed using simple regression analysis. Regression coefficient (b), coefficient of determination (r²), and the P value (P) are given for each relationship.

‡ Body composition (FM and FFM) of pregnant women was measured in gestational week 32.

§ Internal standard deviation scores (SDS), calculated as described in Materials and Methods.

¶ Correlation coefficients between variables describing body fatness (i.e. BMI, FM and FMI), on the one hand, and insulin and HOMA-IR, on the other hand, were significantly higher (P<0.005) than the corresponding values for FFMI versus insulin and HOMA-IR.
No significant ($P\geq 0.18$) relationships between any of the investigated glucose homeostasis variables and infant length were found and no significant associations were observed between HbA1c and any variable describing infant size or body composition. **Figure 5** shows associations between maternal glucose, insulin, HOMA-IR and IGFBP-1 (independent variables) and infant weight (a), infant FFM (b) and infant FM (c) (dependent variables). As shown in **Figure 5a**, significant relationships were identified between maternal glucose, HOMA-IR and IGFBP-1, on the one hand, and infant weight on the other hand. Further, none of these glucose homeostasis variables were significantly related to infant FFM, but they were all significantly associated with infant FM ($P\leq 0.009$), (**Figure 5c**).

### 8.2.5 Insulin/HOMA-IR of pregnant women in relation to fat mass of daughters and sons (Paper III)

Significant interactions between maternal insulin and infant sex ($P=0.034$) as well as between maternal HOMA-IR and infant sex ($P=0.042$) were identified when infant FM (g) was the dependent variable. No such sex interactions were observed for maternal glucose ($P=0.33$) or IGFBP-1 ($P=0.93$). The observed sex interactions provided motivation to investigate associations between maternal insulin/HOMA-IR and infant FM for girls and boys separately. As shown in **Figure 6**, maternal insulin and HOMA-IR were positively related to FM of infant girls ($P<0.001$), but not to FM of infant boys ($P\geq 0.065$).
Figure 5

(a) Infant weight

(b) Infant fat-free mass

(c) Infant fat mass

Regression coefficient, 95% CI (g/DS)

Glucose    Insulin    HOMA-IR    IGFBP-1

* * **
Legend to Figure 5. Weight (g), fat-free mass (g) and fat mass (g) of infants at 1 week of age (independent variables) regressed on glucose homeostasis variables (glucose, insulin, HOMA-IR and IGFBP-1), assessed in their mothers when pregnant in gestational week 32 (dependent variables). Glucose homeostasis variables are expressed as standard deviation scores (SDS) and estimates are presented as regression coefficients (b) in g/SDS with a 95% confidence interval (CI). Regression models were adjusted for maternal parity, infant gestational age at birth, infant sex and age at measurement. HOMA-IR (homeostasis model assessment-insulin resistance); IGFBP-1 (insulin-like growth factor binding protein-1). Differences between regression coefficient and zero: * P<0.05, ** P<0.01, *** P<0.001.

a) Infant weight
   glucose: b=61.0, r²=0.03, n=209, P=0.022
   insulin: b=51.4, r²=0.02, n=209, P=0.051
   HOMA-IR: b=55.6, r²=0.02, n=209, P=0.035
   IGFBP-1: b=-68.2, r²=0.03, n=204, P=0.011

b) Infant fat-free mass
   glucose: n=209, P=0.13
   insulin: n=209, P=0.26
   HOMA-IR: n=209, P=0.21
   IGFBP-1: n=204, P=0.13

c) Infant fat mass
   glucose: b=31.0, r²=0.04, n=209, P=0.006
   insulin: b= 29.4, r²=0.03, n=209, P=0.009
   HOMA-IR: b=31.3, r²=0.04, n=209, P=0.005
   IGFBP-1: b=-38.5, r²=0.06, n=204, P=0.001
Figure 6. Fat mass (g) of 1-week-old girls and boys (dependent variables) regressed on insulin (SDS) and HOMA-IR (SDS) of their mothers when pregnant in gestational week 32 (independent variables). Estimates are presented as regression coefficients (b) in g/SDS with a 95% confidence interval (CI). Regression models were adjusted for maternal parity, infant gestational age at birth and age at measurement. HOMA-IR (homeostasis model assessment-insulin resistance). Differences between regression coefficient and zero: * P<0.05, ** P<0.01, *** P<0.001. † Refers to the P value obtained when the interaction terms (insulin x infant sex) and (HOMA-IR x infant sex) were entered separately in the regression models as described in Materials and Methods.

Insulin
Girls: b=52.5, r²=0.12, n=99, P<0.001; Boys: b=5.4, r²=0.00, n=110, P=0.75

HOMA-IR
Girls: b=52.7, r²=0.13, n=99, P<0.001; Boys: b=7.8, r²=0.00, n=110, P=0.65
Table 8 shows an analysis where the FM of infant girls is regressed on the FM and HOMA-IR of their pregnant mothers. As shown in this table, maternal FM and HOMA-IR are both significantly related to the FM of their daughters when fitted in separate models (a and b). However, when both maternal FM and HOMA-IR were included as independent variables in a multiple regression analysis (model c), only the relationship with HOMA-IR remained significant ($P=0.017$). Using insulin instead of HOMA-IR in a corresponding analysis yielded very similar results (data not shown).

**Table 8. FM of 1-week-old girls regressed on FM and HOMA-IR of their mothers when pregnant in gestational week 32.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Maternal (independent) variables</th>
<th>b</th>
<th>$r^2$</th>
<th>$P$</th>
<th>Model adjusted $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>FM (kg)</td>
<td>5.8</td>
<td>0.09</td>
<td>0.004</td>
<td>0.27</td>
</tr>
<tr>
<td>b</td>
<td>HOMA-IR (SDS†)</td>
<td>52.7</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.30</td>
</tr>
<tr>
<td>c</td>
<td>FM (kg)</td>
<td>2.7</td>
<td>0.01</td>
<td>0.24</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>HOMA-IR (SDS†)</td>
<td>41.4</td>
<td>0.06</td>
<td>0.017</td>
<td></td>
</tr>
</tbody>
</table>

FM, fat mass; SDS, standard deviation score; HOMA-IR, homeostatic model assessment-insulin resistance.

* Analysed using multiple regression analysis. Partial regression coefficient (b), coefficient of determination ($r^2$) calculated from partial r, and the $P$ value ($P$) are given for each relationship. FM (g) of girls was the dependent variable. Model also included maternal parity, infant gestational age at birth and age at measurement as independent variables.

† Refers to internal SDS, calculated as described in Materials and Methods.
8.3 FTO genotype in relation to infant size and body composition (Paper IV)

Table 9 shows characteristics of the infants in this study. Girls were born after 40.0±1.2 gestational weeks and weighed 3530±450 g at birth. Corresponding figures for boys were 40.1±1.2 weeks and 3680±490 g, respectively. Of the girls, 33.0% (n=32) had no risk allele, 45.4% (n=44) had 1 risk allele, and 21.6 % (n=21) had 2 risk alleles (for rs9939609). Of the boys, 38.2% (n=42) had no risk allele, 44.5% (n=49) had 1 risk allele, and 17.3 % (n=19) had 2 risk alleles for rs9939609. Allele frequencies were in Hardy-Weinberg equilibrium both for girls ($P=0.73$) and boys ($P=0.53$).

Associations between the number of risk alleles (A) at the FTO locus rs9939609 (i.e. FTO genotype) and infant size and body composition are shown in Table 10. No significant relationships were observed between the FTO genotype and infant BMI at any of the investigated ages. Furthermore, the FTO genotype was not associated with infant FM at 1 or 12 weeks of age. In contrast, the number of risk alleles was positively related ($P<0.05$) to infant FFM and weight both at 1 and 12 weeks of age. However, these relationships were not significant after adjustment for infant length. This may indicate that the observed associations between the FTO genotype and infant FFM/weight were explained by an effect on infant length. Indeed, positive associations were found between the number of risk alleles and length at 1 ($P=0.033$) and 12 ($P=0.007$) weeks but not quite at 1 year of age ($P=0.052$). Also, some evidence of an interaction between the FTO genotype and infant sex (footnote ‡ in Table 10) was found when infant length was the dependent variable. Thus, the number of risk alleles was positively related to length at the age of 1 week ($P=0.004$), 12 weeks ($P=0.001$) and 1 year ($P=0.012$) for boys, but not for girls.
Table 9. Characteristics of girls and boys in the study at 1 and 12 weeks as well as at 1 year of age (Paper IV).

<table>
<thead>
<tr>
<th></th>
<th>1 week of age</th>
<th>12 weeks of age</th>
<th>1 year of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls (n=97)</td>
<td>Boys (n=110)</td>
<td>Girls (n=97)</td>
</tr>
<tr>
<td>Age (week)</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>51.0 ± 1.5</td>
<td>52.0 ± 2.0</td>
<td>60.5 ± 2.0</td>
</tr>
<tr>
<td>Length-for-age z score</td>
<td>0.24 ± 1.12</td>
<td>0.15 ± 1.11</td>
<td>0.32 ± 0.97</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3515 ± 438</td>
<td>3682 ± 497</td>
<td>5856 ± 573</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>-0.15 ± 0.99</td>
<td>-0.15 ± 1.09</td>
<td>-0.20 ± 0.87</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>13.4 ± 1.1</td>
<td>13.6 ± 1.2</td>
<td>15.9 ± 1.1</td>
</tr>
<tr>
<td>FFM (g)</td>
<td>3042 ± 312</td>
<td>3245 ± 339</td>
<td>4263 ± 364</td>
</tr>
<tr>
<td>FM (g)</td>
<td>473 ± 172</td>
<td>411 ± 190</td>
<td>1593 ± 356</td>
</tr>
<tr>
<td>FM (%)</td>
<td>13.2 ± 3.6</td>
<td>10.9 ± 4.1</td>
<td>27.0 ± 4.2</td>
</tr>
<tr>
<td>Mode of feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td>92 (94.8 %)</td>
<td>104 (94.5 %)</td>
<td>79 (81.4 %)</td>
</tr>
<tr>
<td>Breast milk and formula</td>
<td>5 (5.2 %)</td>
<td>6 (5.5 %)</td>
<td>15 (15.5 %)</td>
</tr>
<tr>
<td>Formula</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
<td>3 (3.1 %)</td>
</tr>
</tbody>
</table>

Data are Means ± SD or n (%). BMI, body mass index; FFM, fat-free mass; FM, fat mass.
* Calculated using Swedish reference data (80).
† n=108
Table 10. Results of regression analysis relating the number of FTO (rs9939609) risk alleles (0, 1 or 2) as independent variable to body size and composition (dependent variables) in a longitudinal study of infants at 1 and 12 weeks as well as at 1 year of age.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>1 week of age*</th>
<th>12 weeks of age*</th>
<th>1 year of age*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>b</td>
<td>r²</td>
</tr>
<tr>
<td>Length (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>207</td>
<td>0.31</td>
<td>0.023</td>
</tr>
<tr>
<td>Boys</td>
<td>97</td>
<td>0.01</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>110</td>
<td>0.56</td>
<td>0.078</td>
</tr>
<tr>
<td>Girls</td>
<td>207</td>
<td>93.3</td>
<td>0.030</td>
</tr>
<tr>
<td>Boys</td>
<td>97</td>
<td>0.01</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>207</td>
<td>0.18</td>
<td>0.016</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>207</td>
<td>0.18</td>
<td>0.016</td>
</tr>
<tr>
<td>FFM (g)</td>
<td>207</td>
<td>56.4</td>
<td>0.022</td>
</tr>
<tr>
<td>FM (g)</td>
<td>207</td>
<td>22.7</td>
<td>0.010</td>
</tr>
<tr>
<td>FM (%)</td>
<td>207</td>
<td>0.36</td>
<td>0.005</td>
</tr>
</tbody>
</table>

b, slope of regression line (effect per risk allele); r², coefficient of determination calculated from partial r; P, P-value; BMI, body mass index; FFM, fat-free mass; FM, fat mass.

* FTO was coded as: 0, no risk allele; 1, one risk allele; 2, two risk alleles. Thus the regression analysis assumed an additive effect of the number of risk alleles. Models were adjusted for maternal parity, infant gestational age at birth, infant age at measurement and infant sex (except when the sexes are analysed separately).

† Length and weight at the age of 1 year were reported by parents after a visit to a health clinic.

‡ P for sex-interaction (i.e. number of FTO risk alleles x infant sex) was 0.067 (1 week), 0.047 (12 week), 0.11 (1 year). Furthermore, when including a sex-interaction (together with infant sex) in unadjusted models corresponding P-values were: 0.026 (1 week), 0.024 (12 weeks), 0.048 (1 year).

§ Not significant (P ≥ 0.19) when adjusted for infant length.
9. DISCUSSION

9.1 Comments on the study population

The proportion of parents with a university degree in the studies was considerably higher as compared to Swedish national data (84). Further, the parents had similar BMIs (means and SD) as reported in a previous study conducted in a similar population in Linköping, Sweden (44). The proportion of overweight and obese men in the studies was comparable to Swedish national data whereas the proportion of overweight/obese women was slightly lower (4). Further, the average and the variation in maternal gestational weight gain were similar to those reported in the Swedish maternity care registry (85). The proportion of breastfed infants was high, as is common in Sweden (86). Finally, the average and the variation in weight and length of the infants in the studies were in agreement with Swedish reference data (80) and their body composition was similar to that reported in a previous study conducted in Linköping, Sweden (44), and in other well-nourished populations (79, 87).

9.2 Strengths and limitations

A major strength of this thesis was that the body composition of the parents and infants was measured using accurate body composition methodology (50, 62). Furthermore, mothers and infants were measured within a narrow time frame. This is especially important for infants, who grow very rapidly during the first weeks of life (44, 80). Finally, several previous studies have investigated sex differences only by stratifying the results by sex (33, 81-83). In this thesis, sex interactions were statistically tested, which can be considered to be a more satisfactory way of identifying sex differences.

A potential limitation is that the weight and composition of the foetus contribute to the weight and composition of the pregnant women, which may have influenced the results. Previous studies show that the weight and FM content of the foetus in gestational weeks 31-32 are approximately 1.65 ± 0.17 kg (29) and 6 % (88), respectively. Consequently, the contribution of FM from the foetus is small, on average circa 100 g, representing only about 0.4 % of the FM of the average women in this study. Furthermore, foetal weight is mainly FFM (88), and the variation in foetal weight (SD = 0.17 kg) (29) is small in relation to the variation in FFM (SD=5.3 kg) of our women in gestational week 32. Hence, it is unlikely that the contributions of FM and FFM from the foetus have affected the results of this thesis in any important way.
It is impossible to differentiate between the effects of preconceptional body composition and body composition changes during pregnancy. However, as indicated in Paper II, FFM (kg) and FM (% and kg) before pregnancy, in a previous study, were closely correlated (≥0.9) to the corresponding values in week 32. Furthermore, it may be relevant to consider that some of the fathers in the studies may not have been biological fathers (i.e. paternal discrepancy). However, this phenomenon is not very common in populations with high socioeconomic status such as ours (89). Also, the study included collection of DNA from both parents, who were of course informed of that before participation. Hence, it is not likely that paternal discrepancy represents a major issue in this thesis. Further, the sample size in Paper IV was relatively small for a genetic study. Hence, the findings regarding a relationship between FTO genotype and infant length, especially for boys, need confirmation. Finally, many hypothesis tests were conducted, increasing the risk of type 1 errors.

9.3 Prediction of thoracic gas volume in gestational week 32 (Paper I)

Predicting TGV, using an equation developed for non-pregnant women, overestimated TGV in gestational week 32. Further, the Bland-Altman evaluation (Figure 2b) showed that the bias introduced by using such predicted TGV was affected by the size of TGV. Similar biases have also been presented by Minderico et al. in obese non-pregnant women (72). Since only 40% of TGV is added to the measured BV (48, 50), the overestimation in BV using predicted TGV is relatively small, corresponding to 75 ml on average. Consequently, the effect on body composition results was also relatively small. Indeed, the overestimation of FM corresponded to only 0.5 % FM, and as shown in Figure 4b there was no evidence that this overestimation was affected by maternal %FM. As discussed in Paper I, the correlation between FM_{predTGV} (%) and FM_{measTGV} (%) and its standard error of the estimate in this study were very similar to corresponding figures for non-pregnant women (67, 90). It can thus be argued that predicting TGV is as applicable for pregnant women as it is for non-pregnant women. Consequently, predicting TGV when applying ADP in gestational week 32, using an equation developed for non-pregnant women, yields results for BV and FM with a very slight, and for many purposes unimportant, overestimation.
9.4 Parental body composition versus infant size and body composition (Paper II)

An important finding was that the FFM of both mothers and fathers was positively related to infant FFM and weight at 1 week of age. These results are in accordance with previous studies where maternal FFM was found to be positively related to infant birth weight (91-93). The findings in this thesis contribute additional knowledge, since it was demonstrated that it was the FFM of infants, rather than the FM, that is related to maternal FFM. Given the present results, it is possible that the reported positive association between maternal BMI and infant birth weight (91-93) may to a large extent be due to an effect of the FFM rather than the FM of the mother. Further, Starling et al. found that maternal BMI was associated with both infant FM and FFM (19). Given the association between maternal and infant FFM identified in this thesis, it is reasonable to suggest that it is the FFM component of the BMI that is related to infant FFM. To my knowledge, associations between paternal and infant FFM have not been investigated before. Thus, the finding regarding a positive relationship between the FFM of fathers and their infants is novel and needs confirmation in further studies. The effect size regarding the association between maternal FFM and infant FFM was 171% of the corresponding effect size for the association between paternal and infant FFM. This can be reconciled with findings showing that maternal weight has a larger influence on birth weight than the weight of the father (94). Such differences between mothers and fathers may be due to the intrauterine influence on foetal growth. Also, genetic factors may be of importance regarding how parental FFM influences infant FFM.

It is noteworthy that no significant relationships were identified between maternal and infant FM when girls and boys were analysed together. This is in agreement with the results of Butte et al. (24), which to my knowledge is the largest study in this area that has applied accurate body composition methodology both in mothers and infants. The finding that maternal FM was positively related to infant FM only in girls can be reconciled with the results presented by Lingwood et al. (20). Also, it may be possible that previous observations regarding a higher body fatness of infants born to women with a high BMI (17-19) can be explained by a relationship between the FM of mothers and daughters.
9.5 Glucose homeostasis variables in gestational week 32 versus maternal and infant body composition (Paper III)

All glucose homeostasis variables in the study were correlated with the FMI of the women and also with their FFMI. Interestingly, for insulin and HOMA-IR, correlations with FMI were especially strong, and stronger than corresponding correlations with FFMI. This is in agreement with the contention that body fatness (as in obesity) increases insulin resistance and the concentration of insulin in blood. The positive relationships between HOMA-IR/insulin in gestational week 32 and the FM of infant girls are of interest regarding the observed positive relationship between the FM of mothers and their infant girls. As shown in Table 8, this relationship was no longer significant when maternal HOMA-IR was included in the regression model. A possible interpretation of this observation is that the relationship between mothers' and daughters' FM is mediated by maternal insulin resistance. An increased insulin resistance is considered to be part of the physiological response to pregnancy for the purpose of directing glucose to the foetus. A previous study showed a positive relationship between the plasma glucose concentration and placental weight for women carrying a female foetus but not for women with a male foetus (95). Since the weight of the placenta is commonly associated with its capacity to transport glucose and other nutrients from mother to foetus (96), placental growth may represent a mechanism by which an increased maternal insulin-resistance results in increased FM of infant girls, but not of infant boys. Furthermore, the results of Simón-Muela et al. (33) may be of interest, since these authors found that insulin concentrations in cord blood were positively related to skinfold thickness in newborn girls, but not in newborn boys. It may also be relevant to note results from an Indian study in which girls of mothers with diabetes had larger skinfolds at 2 and 5 (81) as well as at 9.5 years of age (82) than had girls of healthy mothers. Conversely, no such difference was found for boys. These observations suggest that the observed sex difference may also persist during childhood, although it should be noted that the study by Krishnevi et al. (81, 82) was small.

The observed inverse relationship between the serum concentration of IGFBP-1 in the maternal circulation and infant birth weight is in accordance with the two previously conducted studies (28, 29) in this area. A new observation from the present study is that IGFBP-1 was related to the FM component of infant weight. Clearly, there is a need for future studies investigating how IGFBP-1 affects foetal size and body composition.
9.6 FTO genotype versus infant size and body composition (Paper IV)

The results relating variation in the FTO gene to infant size and body composition can be compared to previous studies. In the present study no associations were observed between the FTO genotype and infant body fatness, which is in agreement with results in 6-month-old infants reported by Mook-Kanamori et al. (42). However, the results disagree with those of López-Bermejo et al. (45), who found an association between the FTO genotype and FM at 2 weeks of age. Another finding in the present study was the significant association between the FTO genotype and infant length, which appeared to be particularly strong for boys. Earlier studies have shown no such relationship (45), although one study (97), which used a risk score based on several obesity-associated loci including FTO (rs9939609), showed this score to be associated with length as early as at 6 weeks of age.

9.7 General discussion

The observed associations between parental factors and infant FFM and FFM were relatively weak, explaining less than 10 % of the variation in infant size or body composition. This is rather expected given the results of comparable studies in the field (20, 98). The magnitude of the effect of variations in parental variables on infant body composition can be calculated based on the information presented in Tables 3-6 and Figures 5-6. For example, a difference in maternal and paternal FFM, both equivalent to 1 SD, corresponds to a difference of 144 g FFM (0.42 SD) in their infants. Similarly, 1 SD difference in maternal FM and HOMA-IR was associated with a difference in FM of infant girls corresponding to 43 g (0.25 SD) and 53 g (0.31 SD), respectively. Thus, although the identified associations are probably less useful on an individual level, they may well be of importance in a population perspective.

This thesis has presented several relationships involving infant body composition which may be of importance in relation to obesity development and/or health later in life. For instance, parental FFM was related to infant FFM and maternal FM was related to the FM of infant girls. These relationships may be of relevance in relation to body composition development. However, there is a lack of longitudinal data relating body composition in infancy to later body composition. To my knowledge, the longest follow-up of body composition data at birth has been presented in the Southampton Women's Survey (8). In this study (8), birth FM was positively, but quite weakly, related to FM at 6 years of age. Further, birth FFM was positively related to FFM at 6 years of age. Although the results from the
Southampton Women's Survey were unadjusted and did not take into account important covariates such as infant length (8), they suggest that body composition at birth may contribute to later body composition. Also, it is important to note that factors during foetal life may influence later obesity risk through other mechanism than the influence of infant FM on later FM. One such potential mechanism may be through developmental programing of appetite and energy balance (99).

Unpublished data from the infants in this thesis show that parental FFM is still positively associated with infant FFM at 12 weeks of age. Furthermore, the relationship between maternal FM and the FM of infant girls could still be identified at 12 weeks of age, but was then slightly weaker. Further studies are needed to confirm the relationships between parental and infant FFM as well as the relationship between maternal FM and the FM of infant girls, and to investigate if these relationships remain later in life. The body composition of children in the thesis is currently being assessed at 4.5 years of age and it may be relevant to publish this data in conjunction with the 12-week data.

The influence of infant body size and growth on later body composition has been investigated in several studies (13, 100). For example, accelerated growth during infancy is a well-known risk factor for later obesity (100), and a previous study showed that infants with an increased genetic risk for obesity grew more rapidly (97). Thus, the observed association between the FTO genotype and infant length observed in this thesis may be relevant in relation to obesity development. Another interesting finding was that parental FFM was positively related to infant weight (and FFM). Previous studies have shown that birth weight is positively related to FFM both in childhood and adulthood (13). Hence, it is conceivable that the associations between parental and infant FFM are maintained later in life. In this context it may be relevant to note that a low FFMI has been associated with higher all-cause mortality in adulthood (14). Therefore it is possible that the relationships between parental and infant FFM identified in this thesis are relevant in relation to human health.
10. CONCLUSIONS

Using TGV predicted by an equation developed for non-pregnant women when applying ADP in gestational week 32 overestimated % FM. This overestimation was small and therefore probably unimportant in many situations (Paper I).

The FFM of parents was positively related to weight and the FFM of their 1-week-old infants and this relationship may well be relevant in a population perspective (Paper II).

Maternal FM in gestational week 32 was positively related to the FM of their 1-week-old infant daughters, while no such relationship was found for sons (Paper II).

Variables describing body composition (BMI, FM, FMI, FFMI) of women in gestational week 32 were all related to glucose homeostasis variables (glucose, insulin, HOMA-IR, HbA1c, IGFBP-1) in their circulation. The associations between variables describing body fatness (BMI, FM and FMI) and insulin/HOMA-IR were positive and particularly strong (Paper III).

Glucose homeostasis variables in the circulation of the mothers, when pregnant in gestational week 32, were positively (glucose, insulin, HOMA-IR) and negatively (IGFBP-1) related to the weight and FM of their 1-week-old infants (Paper III).

Insulin resistance (measured as HOMA-IR) of the mothers in gestational week 32 was positively related to the FM of infant daughters, but not to the FM of infant sons. The results suggest that insulin resistance mediates the positive association between the FM of mothers and daughters identified in paper II (Paper III).

Variation in the FTO genotype was not related to infant FM or BMI. However, the number of risk-alleles at the FTO locus rs9939609 was positively related to infant length at 1 and 12 weeks of age, relationships which may be stronger in boys than in girls (Paper IV).
11. FUTURE PERSPECTIVES

Results from this thesis have provided new information regarding associations between body composition of parents and their infants. Also, results have been presented regarding associations of maternal glucose homeostasis and the FTO genotype with infant size and body composition. However, knowledge regarding factors that influence infant body composition and the effect infant body composition has on later body composition is limited. Thus, additional studies in this area are needed.

Studies investigating potential mechanisms explaining relationships between maternal and infant body composition are motivated. In particular, the mechanism underlying the observed association between maternal FM and the FM of infant girls may be of special interest.

The studies in this thesis were conducted to investigate determinants of infant body composition, which may be of relevance for obesity development and future health. To clarify the significance of relationships between parental factors and offspring body composition, there is a need for future longitudinal studies investigating such relationships beyond infancy. Such future studies may consider the following aspects:

Firstly, forthcoming studies should be relatively large, since associations between parental factors and infant body composition in this thesis, as well as in previous research, tend to be relatively weak (20, 98).

Secondly, an association between the FFM of fathers and their infants was observed in this thesis. This can reconciled with previous cross-sectional studies which have identified associations between fathers and their children regarding their body composition or BMI (101, 102). Thus, the investigation of paternal influences on offspring body composition should be considered in future studies.

Thirdly, evidence that maternal FM and insulin-resistance, as well as genetics, may affect size and body composition of infant girls and boys differently has been presented in this thesis and in previous studies (20, 33, 81-83). Hence, the investigation of potential sex differences may be considered in forthcoming studies.
Finally, future studies investigating associations between parental variables and offspring body composition should be conducted using valid body composition methods. ADP represents attractive methodology in this respect since it provides a possibility to measure parents (50), infants (62) and children (103) accurately. Since the measurement procedures in Bod Pod and Pea Pod are standardised and relatively robust, these devices may be suitable for multicentre studies. Also, it is possible that some of the data obtained by cohort studies using ADP (17, 19, 44, 79, 87) may be useful in meta-analyses.

To the best of my knowledge, PATHOS is the largest study so far that applies ADP in parents and their infants, and this study may provide a basis for future studies. The children in PATHOS are currently being investigated at 4.5 years of age, when their body composition is measured using ADP as previously described (104). To date (April 2015), approximately 165 children have been examined, and based on preliminary data we expect a participation rate of circa 85%. My hope is that the children in PATHOS will also be investigated in adolescence and adulthood, which may provide further knowledge regarding longitudinal relationships of body composition in parents and their offspring.
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Kroppssammansättning under spädbarnstiden kan vara av betydelse för senare hälsa och för risken att utveckla fetma under barndomen. Utrustning som tillämpar så kallad helkroppspletsmografi gör det möjligt att mäta kroppssammansättningen hos spädbarn (Pea Pod) och vuxna (Bod Pod) på ett snabbt, tillförlitligt och icke-invasivt sätt. Syftet med denna avhandling var att studera om föräldrars kroppssammansättning, moderns glukosomsättning under graviditet och barnets egen genetik har samband med nyfödda barns storlek och kroppssammansättning. Då helkroppspletonsmografi används hos vuxna görs en korrigering för lungvolym (TGV; thoracic gas volume). TGV kan predikteras med ekvationer som utvecklats för icke-gravida vuxna. Ett ytterligare syfte var att undersöka om sådana ekvationer kan användas under graviditet.


Resultat från 27 kvinnor visade att predikterad TGV ger en obetydlig överskattning (0,5 %) av fettmassan. En ökning i den fettfria massan hos föräldrarna var associerad till mer fettfri massa hos spädbarnen vid 1 veckas ålder. För varje kg mer fettfri massa hos mamman innehöll barnen 15.6 g mer fettfri massa. Motsvarande siffra för sambandet mellan pappor och barn var 9.1 g. Fettmassan hos pappor var ej associerad till spädbarnens fettmassa. Däremot motsvarade varje kg mer fettmassa hos modern 5.8 g mer fettmassa hos döttrarna vid 1 veckas ålder och en ökning av mammans insulinresistens, motsvarande en standarddeviation för hennes HOMA-IR, innebar då 52.7 g mer fettmassa hos döttrarna. Motsvarande samband saknades för söner. Det fanns inga samband mellan antalet riskanlag i FTO-genen och
spädbarns fettmassa. Däremot var detta antal relaterat till längd hos spädbarn vid 1 och 12 veckors ålder och dessa samband tycktes vara starkare hos pojkar än hos flickor.

De resultat som presenteras i denna avhandling visar att: i) Användningen av predikterad TGV vid tillämpning av helkroppspletysemografi i graviditetsvecka 32 överskattade fettmassan mycket marginellt. Denna överskattning är förmodligen oviktig i många situationer. ii) Samband mellan föräldrars och barns kroppssammansättning kan finnas tidigt i livet. Exempelvis var föräldrarnas fettfria massa relaterad till den fettfria massan hos deras spädbarn. Dessutom var moderns fettfria massa och insulinresistens relaterat till fettmassan hos döttrar, men ej hos söner. iii) FTO-genen har inget samband med fettmassan vid 1 och 12 veckors ålder men tycks då ha ett samband med barnens längd, särskilt hos pojkar.

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Papers

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