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Being Born Large for Gestational Age

Metabolic and Epidemiological Studies

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Abstract

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Obesity is a major health problem in the Western world. Mean birth weight has increased during the last 25 years. One explanation is that the proportion of large for gestational age (LGA) infants has increased. Such infants risk developing obesity, cardiovascular disease and diabetes later in life. Despite the risk of neonatal hypoglycemia, their postnatal metabolic adaptation has not been investigated. Our data, obtained with stable isotope labeled compounds, demonstrate that newborn LGA infants have increased lipolysis and decreased insulin sensitivity. After administration of glucagon, the plasma levels of glucose and the rate of glucose production increased. The simultaneous increase in insulin correlated with the decrease in lipolysis, indicating an antilipolytic effect of insulin in these infants.

We also demonstrated an intergenerational effect of being born LGA, since women born LGA, were at higher risk of giving birth to LGA infants than women not born LGA. Further, the LGA infants formed three subgroups: born long only, born heavy only, and born both long and heavy. Infants born LGA of women with high birth weight or adult obesity were at higher risk of being LGA concerning weight alone, predisposing to overweight and obesity at childbearing age. In addition we found that pregnant women with gestational diabetes were at increased risk of giving birth to infants that were heavy alone. This could explain the risk of both perinatal complications and later metabolic disease in infants of this group of women.

To identify determinants of fetal growth, 20 pregnant women with a wide range of fetal weights were investigated at 36 weeks of gestation. Maternal fat mass was strongly associated with insulin resistance. Insulin resistance was related to glucose production, which correlated positively with fetal size. The variation in resting energy expenditure, which was closely related to fetal weight, was largely explained by BMI, insulin resistance, and glucose production. Lipolysis was not rate limiting for fetal growth in this group of women. Consequently, high maternal glucose production due to a high fat mass may result in excessive fetal growth.

Keywords: large for gestational, glucose production, lipolysis, insulin resistance, intergenerational, gestational diabetes mellitus, stable isotopes, pregnant women, newborn infant

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To the miracles of my life, who mean the
world to me, my three wonderful children,
Anton, Ebba and Agnes.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-IV.

- I. F Ahlsson, B Diderholm, U Ewald, J Gustafsson. Lipolysis and insulin sensitivity at birth in infants who are large for gestational age. *Pediatrics*. 2007; 120(5):958-65.
- II. F Ahlsson, J Gustafsson, T Tuvemo, M Lundgren. Females born large for gestational age have a doubled risk of giving birth to large for gestational age infants. *Acta Paediatr*. 2007; 96(3):358-62.
- III. F Ahlsson, M Lundgren, T Tuvemo, J Gustafsson, B Haglund. Gestational diabetes and offspring body disproportion. Manuscript
- IV. F Ahlsson, B Diderholm, B Jonsson, S Nordén-Lindeberg, U Ewald, A Forslund, M Stridsberg, J Gustafsson. Maternal glucose production and resting energy expenditure determine fetal size. Manuscript

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Abbreviations

ADA	American Diabetes Association
AGA	appropriate for gestational age
ATP	adenosine triphosphate
BIA	bioimpedance
BMI	body mass index
BMR	basal metabolic rate
CI	confidence interval
CV	coefficient of variation
EGF	epidermal growth factor
EI	electron impact
FFM	fat-free mass
FGF-2	fibroblast growth factor 2
FM	fat mass
GCMS	gas chromatography-mass spectrometry
GDM	gestational diabetes mellitus
GH	growth hormone
GPR	glucose production rate
hCG	human chorionic gonadotropin
HOMA	homeostasis model assessment
ICD	International Classification of Diseases
IDMs	infants of diabetic mothers
IE	isotopic enrichment
IGFBP	insulin-like growth factor binding protein
IGF-I	insulin-like growth factor-I
IGF-II	insulin-like growth factor-II
IR	insulin resistance
IUGR	intrauterine growth restriction
kcal	kilocalories
kDa	kilodalton
LGA	large for gestational age
LGA1w	LGA for length and weight

LGAol	LGA for length only
LGAow	LGA for weight only
m/z	mass over charge ratio
MJ	megajoule
NEFA	non-esterified fatty acid
OGTT	oral glucose tolerance test
OR	odds ratio
PAPPA	pregnancy-associated plasma protein A
PC	pyruvate carboxylase
PD	pyruvate dehydrogenase
PEPCK	phosphoenol pyruvate carboxy kinase
PMA	post menstrual age
PRL	prolactin
REE	resting energy expenditure
RIA	radioimmunoassay
SD	standard deviation
SDS	standard deviation score
SGA	small for gestational age
TGF- α	transforming growth factor- α
TNF- α	tumor necrosis factor- α
TSH	thyroid stimulating hormone
WHO	World Health Organization

Introduction

Obesity is one of the greatest challenges of the western world in the 21st century. More than 60 % of the adult American population are overweight and more than 30 % are obese and the numbers are still increasing.¹ During the past three decades the prevalence of overweight has doubled among US children 6 to 11 years of age and tripled among those aged 12 to 19 years.² In 2006 the prevalence of overweight in European children was estimated to be 20 %. The rise in obesity has created a global increase of associated conditions, such as cardiovascular disease, type 2 diabetes, hypertension, stroke, dyslipidemia, osteoarthritis, and certain cancers.³⁻⁵

The mean birth weight has increased markedly in several countries during the last twenty-five years,⁶⁻¹⁰ despite an increase in preterm birth.^{11, 12} One explanation is that an increasing proportion of large for gestational age (LGA) infants are being born.¹³ Children born LGA have a higher prevalence of overweight in adolescence and an increased risk of developing cardiovascular disease, type 1 and type 2 diabetes, prostate cancer, and breast cancer.¹⁴⁻²⁵

There are several maternal anthropometric characteristics that may be associated with increased fetal growth, such as a high maternal body mass index (BMI), tall height, high weight, and excessive weight gain during pregnancy.²⁶⁻²⁸ Paternal birth weight predicts the birth weight of the offspring to some extent.²⁹ It has been demonstrated in intergenerational studies that women who themselves were born small for gestational age (SGA), are at increased risk of giving birth to SGA infants.³⁰ Nevertheless, the question of whether an intergenerational effect occurs regarding being born LGA has not been studied.

In pregnancy several physiologic alterations occur, one of which is a metabolic adaptation to secure the supply of glucose and amino acids to the fetus. During the first trimester maternal energy stores are formed³¹ for mobilization in later stages of pregnancy.³²⁻³⁴

Pregnant women have higher rates of glucose production and lipolysis compared with non-pregnant women.^{32, 35-37} Pregnancy has also been shown to result in an increased basal metabolic rate (BMR),³⁸ and this increase is related to fetal growth.³⁹ However, only limited information is available on metabolic mechanisms underlying excessive fetal growth in the non-diabetic pregnant woman.

Size at birth is influenced by several factors. Fetal growth is initially autonomous but is later dependent on the flow of nutrients across the placenta. Diseases such as viral infections, maternal diabetes and maternal hypertension may also influence the weight of the fetus. The relation between birth weight and adult metabolic disease has been discussed extensively in recent years.⁴⁰ It has been demonstrated that infants born SGA have increased insulin sensitivity at birth,^{41,42} even though they may develop insulin resistance already in childhood.⁴³ The postnatal adaptation of infants born SGA has been investigated,⁴² but so far there is only little information on insulin sensitivity and production of energy substrates in infants born LGA.

Offspring of women with gestational diabetes mellitus (GDM) constitute a particular subgroup of LGA infants. Over fifty years ago, Pedersen et al.⁴⁴ postulated that gestational diabetes leads to an intrauterine hyperinsulinemic environment that in turn causes macrosomia. Women with GDM have been shown to have a more than three times higher risk of giving birth to an LGA infant compared to women without GDM.¹³ In addition, gestational diabetes increases the risk of perinatal complications,⁴⁵ possibly as a result of infant disproportion with regard to birth weight versus birth length.

In the light of the above considerations, the current research project was undertaken to investigate metabolic mechanisms underlying excessive fetal growth in non-diabetic pregnant women and to address the question of whether there is an intergenerational effect of being born LGA. Other questions emerging within the project are whether body disproportion in the newborn infant is one of the reasons behind perinatal complications in GDM and to investigate the postnatal metabolic adaptation in the newborn LGA infant.

Background

Energy metabolism during pregnancy

During pregnancy a metabolic adaptation takes place in order to secure the supply of glucose and amino acids for the growing fetus. The maternal glucose-stimulated insulin secretion increases and the glucose stimulation threshold decreases.^{46,47} It has also been demonstrated that the volume of the pancreatic islets increases during pregnancy.⁴⁸ In normal pregnancy there is an approximately 50 % decrease in insulin-mediated glucose disposal, and to maintain euglycemia a 200-250% increase in insulin secretion is necessary.^{49,50} In the first trimester the insulin sensitivity is similar to that in nonpregnant women.³¹ Later during pregnancy the insulin sensitivity decreases and the insulin levels rise,³²⁻³⁴ partly as an effect of pregnancy specific hormones, e.g., prolactin (PRL), placental lactogen, progesterone, and placental growth hormone, which have insulin-antagonistic and lipolytic effects.⁵¹ One suggested pathway by which prolactin induces insulin resistance is by inducing a decrease in adiponectin, an insulin sensitizing hormone.⁵² Placental lactogen increases insulin secretion from β -cells.⁵³ Progesterone may also have an effect on glucose metabolism through the progesterone receptor. When this receptor is activated, β -cell hyperplasia is downregulated, resulting in decreased insulin secretion.⁵⁴ Recently it has been demonstrated that the cytokine tumor necrosis factor- α (TNF- α) correlates well to insulin resistance during late gestation.⁵⁵ This finding indicates an additional mechanism responsible for insulin resistance during pregnancy. Another factor contributing to the insulin resistance during pregnancy is the rising levels of non-esterified fatty acids (NEFA) seen in the pregnant state.^{56,57} Proposed mechanisms of induction of insulin resistance by NEFA are impairment of muscle glycogen synthase activity and a reduction of glucose transport or phosphorylation, or both.⁵⁸ The insulin resistance promotes the mobilization of fatty acids as energy substrates in the pregnant woman. This enables the pregnant woman to save glucose and amino acids for the growing fetus.^{36,59} It has been demonstrated that maternal insulin resistance is positively associated with fetal growth.⁶⁰ Further, we³⁷ and others^{32,35} have established that pregnant women have a higher rate of glucose production in the third trimester compared with non-pregnant women. Pregnancy is also associated with an increased rate of lipolysis.^{36,37} Data from our group show that pregnant women carrying

fetuses with intrauterine growth restriction (IUGR) have a decreased rate of lipolysis compared to women giving birth to AGA infants. The reduced amount of energy substrates from lipolysis may lead to a situation where glucose and amino acids aimed for the growing fetus must instead be consumed by the woman herself, to meet the metabolic demands associated with pregnancy.⁶¹

Energy requirements during pregnancy consist of requirements for deposition of maternal and fetal tissue and the increased energy expenditure due to maintenance and physical activity. The estimated total energy cost of a pregnancy in a woman with a weight gain of approximately 12 kg is between 321 and 325 MJ (corresponding to 76 400 to 77 400 kcal), distributed as 375, 1200 and 1950 kJ/day in the first, second and third trimesters, respectively.³⁸ The energy is distributed as follows: the development of a 3.4 kg infant requires 8300 kcal, the placenta with a weight of 0.6 kg requires 730 kcal, the increase in size of uterus, breasts and fluids corresponds to 3490 kcal, accumulation of maternal fat requires another 26 000 kcal, and the increasing BMR requires 30 000 kcal.⁶² Differences in the reported total energy costs are probably due to differences in methodology and study populations.

The increase in BMR is assumed to be due to the increase in oxygen consumption, which in turn is related to the work associated with maternal circulation, and respiration and the increased tissue mass during pregnancy. The increase in BMR varies markedly among different pregnant women, but the reasons for this are not fully understood.^{63, 64} Thus, gestational weight gain, pre-pregnancy fat mass, nutritional status, and cardiac output are all factors, that are related to the increase in BMR during pregnancy.^{39, 65} There is also a relationship between maternal BMR and fetal growth.³⁹

Protein accretion takes place mainly in late pregnancy. Of the total protein accretion during pregnancy, which is estimated to be 925 g, approximately 42% is deposited in the fetus, 17% in the uterus, 14% in the blood, 10% in the placenta and 8% in the breasts.³⁸

Fat buildup during pregnancy contributes substantially to the total energy cost of pregnancy. Fat accretion in well nourished pregnant women in developed countries measured with corrected two-component or three- or four-component body composition models averaged 4.3 kg in combination with a total weight gain of 13.8 kg.³⁸ The rate of fat buildup changed during pregnancy, and averaged 8 g/ day in the first trimester and 26 g/day in the second trimester, but varied between -7 and 23 g/day in the third trimester.⁶⁶ In contrast to the situation in developed countries, Lawrence et al.⁶⁴ reported that in un-supplemented Gambian women there was a decrease in fat mass, since they lost 0.3 kg fat during pregnancy, although they gained 7.2 kg in weight. Adipose tissue becomes more active during pregnancy. Thus, lipolysis increases³⁷ and the amount of fat mass becomes closely related to the basal metabolic rate.⁶⁷ This contrasts to the situation in the non-pregnant state,

where fat free mass but not fat mass is associated with the basal metabolic rate.⁶⁸

Leptin and adiponectin are adipokines that play important roles in the complex signaling system of adipose tissue. Leptin is a 16 kDa protein that is produced and secreted by adipose tissue and there is a strong relation between leptin levels and body weight.⁶⁹ One of the major sites of action of leptin is the hypothalamus, and leptin modulates the hypothalamic-pituitary-gonadal axis⁷⁰ and effectively reduces appetite.⁷¹ It has been suggested that leptin increases lipolysis.⁷² During pregnancy leptin levels increase by the action of pregnancy hormones such as estrogen and human chorionic gonadotropin (hCG)⁷³ and by development of central leptin resistance. Leptin levels reach their peak during mid pregnancy and have been shown to be associated with maternal weight and BMI. Highman et al.⁷⁴ reported a strong correlation between maternal fat mass and leptin levels in early, mid and late pregnancy. They also found that leptin levels rose in early pregnancy and that the increase was more pronounced than the early-pregnancy increase in body fat and resting energy expenditure. These findings indicate that other mechanisms than an increased fat mass and an elevated BMR also contribute to this early leptin increase, possibly including effects of cortisol and pregnancy specific hormones, as well as development of central leptin resistance.^{74, 75} Since leptin is a lipolytic hormone, a relation between the increased leptin levels and the increased lipolysis in pregnancy might be anticipated.³⁷ Although maternal fat mass is associated with birth size, however Grisaru-Granovsky et al.⁷⁶ found no correlation between maternal levels of leptin and birth weight. But, umbilical cord levels of leptin are closely related to infant weight as well as to infant fat mass.^{77, 78}

Adiponectin is a 244 amino acid (28 kDa) protein that is expressed and secreted only by adipose tissue.^{79, 80} The gene encoding for adiponectin is located on chromosome 3q27, a region linked to type 2 diabetes and obesity.⁸¹ Adiponectin increases insulin sensitivity and decreased levels of the protein are seen in type 2 diabetes and obesity, conditions associated with insulin resistance.⁸² In rodents administration of adiponectin increases glucose uptake and fat oxidation in muscle and in the liver it reduces fatty acid uptake and glucose production.⁸³ The decrease in glucose production occurs through a decreased enzymatic action of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Further, adiponectin improves whole body insulin sensitivity.⁸⁴ Adiponectin is inversely related to body weight, fat mass and insulin levels, and its level is increased by weight reduction in obese humans.⁸⁵ Adiponectin and its receptors have been detected in the placenta.⁸⁶ However, information concerning adiponectin and human pregnancy is still limited.

Although cord blood levels of insulin-like growth factor-I (IGF-I) are related to the size of the newborn infant, maternal IGF-I levels do not correlate with fetal size.⁸⁷ However, maternal plasma levels of fibroblast growth factor 2

(FGF-2) have been shown to be associated with the size of the placenta as well as with infant size. FGF-2 is an 18 kDa peptide, which can act as a mitogen and also is a potent angiogenic agent. It has been demonstrated that the levels of FGF-2 in sera obtained during pregnancy from women delivering SGA infants are lower than those in such sera from women delivering AGA infants.⁸⁸

Fetal growth and nutrition

Initially, the growth of the embryo is mediated mainly by cell division. Later during pregnancy fetal growth also occurs by an increase in cell size. Fetal growth is dependent on nutrient and oxygen supply as well as on growth factors such as epidermal growth factor (EGF), transforming growth factor- α (TGF- α), insulin, IGF-I, IGF-II and FGF-2, and oncogenes.⁸⁹ Insulin, the major regulator of glucose, protein and lipid metabolism, is important for the growth of the fetus. Thus, hyperinsulinemia is associated with excessive fetal growth, which can be seen in familial hyperinsulinemia,⁹⁰ Beckwith-Wiedemann syndrome,⁹¹ and gestational diabetes mellitus.²⁸ In a hyperinsulinemic rhesus monkey model it was documented that insulin infusion for 3 weeks resulted not only in an increased fetal weight but also in enlargement of the fetal liver, heart and spleen.⁹² Insulin exerts its effects by several mechanisms. It supports the uptake and utilization of nutrients in insulin sensitive tissues, it has mitogenic actions, and influences release of secondary hormones, such as IGFs and their binding proteins.⁹³⁻⁹⁵ Insulin receptors are present in all fetal tissues.⁹⁶ In pancreatectomized fetal sheep the level of IGF-I was reduced and fetal growth was restricted. Following treatment with insulin, the growth rate increased.⁹⁷ The results confirm that insulin is necessary for fetal growth even when the nutritional status is optimal. Insulin exerts some of its effects through IGFs, which are molecules structurally related to insulin. There are two isomers of IGFs, IGF I and II. Their molecular size is approximately 7.6 kDa and they are principally formed in the liver, although virtually all tissues in human and animal fetuses can synthesize these peptides.^{98, 99} In the fetus the levels of IGF- II are higher than those of IGF-I, but since the IGF-I receptor has a strong affinity for IGF-I, this growth factor is a more effective mitogen than IGF-II. IGFs are only found to a small extent in free form, and otherwise bound to one of six binding proteins, IGFBP-1 to IGFBP-6. The IGFBPs are carrier proteins which prolong the half-life of the IGFs and modulate their action.¹⁰⁰ The IGF system consists of at least four receptors, the insulin receptor, the type-I insulin-like growth factor receptor, the mannose 6-phosphate/ IGF- II receptor and the hybrid insulin/ IGF-I receptor. The IGFBPs are regulated by proteases which cleave the binding proteins to low affinity fragments. The proteases thus act as co-mitogens.¹⁰¹ The levels of IGFs and their binding proteins are influ-

enced by fetal nutrition and insulin levels.¹⁰² The IGFs play an important role in fetal growth, and *Igf1* and *Igf2* null mice are severely growth retarded with a birth weight corresponding to 40 % of the expected.^{103, 104} Double knockout of *Igf1* and *Igf2* in mice results in 80% growth restriction, and inactivation of the *Igf1* receptor gives a 55% reduction of birth weight.¹⁰⁵ IGF-I deficiency in humans leads to severe growth restriction, mental retardation and sensorineural deafness.^{106, 107} Infants born IUGR have lower circulating levels of IGF-I¹⁰⁸ and macrosomic infants of diabetic mothers have elevated levels of IGF-I in umbilical cord serum.¹⁰⁹ In the fetus IGF-I expression is regulated primarily by genetic factors, whereas the levels of IGF-II are controlled by epigenetic mechanisms (stable alterations of gene expression through DNA methylation and histone modifications).¹¹⁰ There is growing evidence that maternal nutrition can alter the epigenetic state of the fetal genome causing both excessive growth and growth restriction which may lead to consequences in adult life.¹¹¹ It has been reported that IGFBP-1 acts as an inhibitor of fetal growth,¹⁰² probably by decreasing the level of free IGF-1. In addition, there are several reports of elevated IGFBP-1 levels in cord blood in IUGR infants.^{112, 113} Overexpression of IGFBP-1 in transgenic mice has been associated with fetal growth restriction.¹¹⁴ In contrast, the level of IGFBP-3, which circulates with IGF-I and IGF-II in a complex with the acid labile subunit and is the primary binding protein that extends the half-life of the IGFs, was reduced by 50 % in fetal cord serum of IUGR infants.¹¹⁵ In the LGA infant the level of IGFBP-3 was increased compared to that in the AGA infant.¹¹⁵ There are a few other known molecules that regulate the bioavailability of the IGFs. One is the pregnancy-associated plasma protein A (PAPP-A), which is a metalloproteinase that regulates cleavage of IGFBP-4, thus increasing the levels of bioactive IGFs.¹¹⁶ Defective PAPP-A leads to growth restriction. Rho-GAP, a small G-protein, is also involved in the regulation of IGFs; defects in this molecule are associated with growth restriction.¹¹⁷

During childhood, growth hormone (GH) regulates statural growth, mainly by stimulating production of IGF-I, but in fetal life GH is less important. However, it has some impact on growth in the third trimester and infants with congenital GH deficiency have birth lengths approximately 1 standard deviation (SD) below the mean.¹¹⁸

During the second trimester organ differentiation takes place and the increase in length is pronounced.¹¹⁹ In the third trimester there is a marked increase in weight, mostly due to buildup of fat and proteins. The fat depot is known to be an important determinant of birth weight.¹²⁰

Glucose, amino acids, and lactate are the most important energy substrates for the fetus. Glucose alone stands for about half of the total energy required. Glucose crosses the placenta by facilitated diffusion along a concentration gradient between maternal and fetal plasma. The fetus has a plasma glucose concentration that is 70-80% of that in maternal blood. Fetal glucose con-

sumption averages 7 g/kg fetal weight/ day (5 mg/kg/min), which is in the same range as the rate of endogenous glucose production in the newborn infant. The enzymes necessary for glycogenolysis and gluconeogenesis are present in the fetus, but are inactive unless provoked by extreme maternal starvation.¹²¹

During fetal life fat oxidation probably is less important than glucose oxidation and amino acid metabolism. Accordingly, ketone body production is limited in the fetus.¹²¹

Insulin is an important metabolic hormone in the fetal endocrine milieu. Since insulin cannot cross the placenta, the fetal insulin concentration is determined by the levels of glucose and amino acids in the fetal plasma. The fetal pancreatic β -cells develop responsiveness to glucose in a rather late stage of pregnancy. It is possible that in the fetus the growth and insulin secretion of the β -cells are regulated by separate mechanisms. Insulin secretion is mainly generated by the levels of glucose, whereas β -cell growth is dependent on the nutritional status,¹²² and on several growth factors, such as platelet-derived growth factor, EGF, TGF- β , IGF-I and IGF-II. Since both IGF-I and IGF-II act as β -cell mitogens,^{123, 124} over-expression of IGF-II in fetal life can lead to an increased islet cell mass.¹²⁵ The IGF axis may also be involved in prevention of neonatal apoptosis and the compensatory neogenesis of β -cells occurring around partum. This process may determine the function of islets in adult life. The last trimester could be a critical period during which substrate provision and growth factor levels programme the pancreatic islet development irreversibly, determining the metabolic response and susceptibility to disease later in life.¹²⁶

Much interest has been focused on long-term consequences of fetal growth restriction during the last decades. According to the “developmental origin of adult disease” hypothesis, the fetus adapts to a poor nutritional milieu. However, such adjustments may have metabolic consequences later in life.¹²⁷ There are several reports which link being born SGA to cardiovascular disease,¹²⁸ insulin resistance, glucose intolerance, dyslipidemia, and hypertension.¹²⁷ Recently, Kaijser et al.¹²⁹ reported that the relation between low birth weight and adult risk of ischemic heart disease appears to be mediated entirely by poor fetal growth. So far, however only limited data are available concerning the relation between excessive fetal growth and metabolic disease in childhood or adult life.

Postnatal metabolic adaptation

At birth the continuous placental flow of nutrients, mostly glucose and amino acids, is terminated. Before breastfeeding is established, the newborn infant has to produce glucose, mainly to meet the needs of the central nervous system.^{130, 131} Glucose is an important energy substrate for the brain and

during rest the central nervous system consumes the major part of the glucose produced by the liver in the newborn infant. Glucose is initially produced by glycogenolysis,¹³² but the hepatic glycogen depots are limited and will only last for 10-12 hours.¹³³ Thus, gluconeogenesis soon becomes an important source of glucose production. The most important gluconeogenic precursor is lactate, which in turn is generated by glycolysis. Among the amino acids alanine is the major precursor in gluconeogenesis. In addition, lipolysis is induced immediately after birth to secure the energy supply of the newborn infant.¹³⁴ Glycerol formed from depot fat during lipolysis may also be converted to glucose in the gluconeogenic process (*Figure 1*).

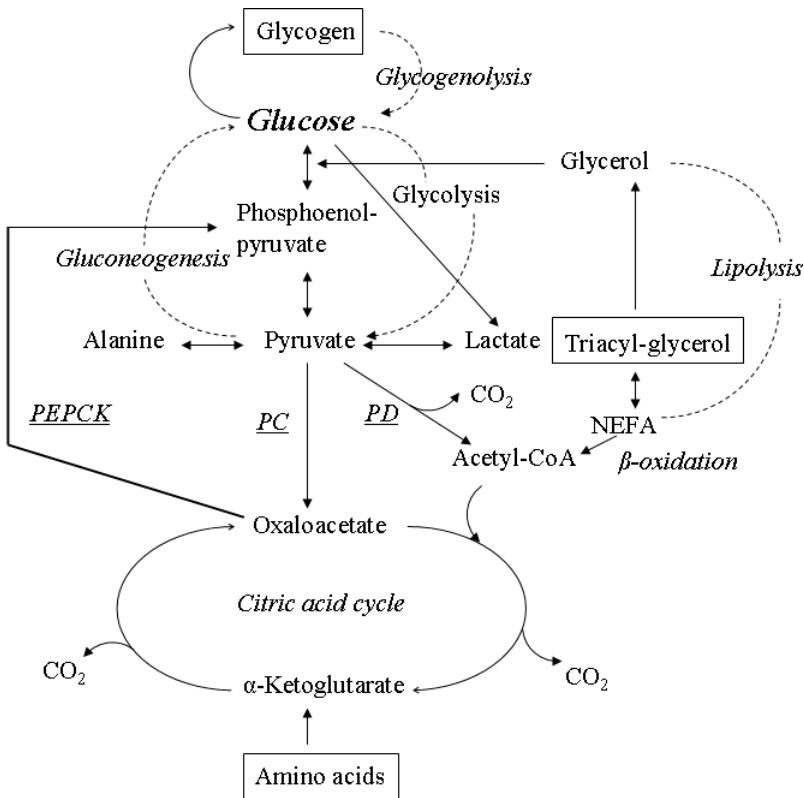


Figure 1. Glucose metabolism and synthesis. NEFA, non-esterified fatty acids; PC, pyruvate carboxylase; PD, pyruvate dehydrogenase; PEPCK, phosphoenol pyruvate carboxy kinase.

Neonatal energy substrate production has been extensively studied both in appropriate for gestational age (AGA) infants and in infants belonging to risk groups, i.e., those born preterm or SGA and infants of diabetic mothers (IDMs).¹³⁵⁻¹³⁷

Postnatal glucose production and lipolysis are under hormonal regulation. Both insulin and glucagon are important in the regulation of glucose production immediately after birth. Glucagon is produced and secreted by the α -

cells in the pancreas. It has previously been reported that glucagon can normalize blood glucose levels during hypoglycemia in normosomic and SGA infants.^{138, 139} Endogenous hepatic glucose production is stimulated by a decreased insulin/glucagon ratio, and lipolytic hydrolysis of depot fat is enhanced by the marked increase in thyroid-stimulating-hormone (TSH) that occurs during the first day of life.¹⁴⁰ In the newborn AGA infant the glucose production rate (GPR) and rate of lipolysis are in the ranges of 21.1-32.2 $\mu\text{mol/kg/min}$ and 4.4-9.5 $\mu\text{mol/kg/min}$, respectively.^{134, 135, 141} Infants born SGA, as well as those born extremely preterm, have lower rates of energy substrate production.^{42, 136} Infants of diabetic mothers have increased neonatal levels of insulin, resulting in a decreased glucose production (Table 1).¹³⁷

Table 1. Glucose production rates (GPR), and lipolysis (glycerol rate of appearance [Gly R_a]) in AGA, SGA and extremely preterm infants, and in infants of diabetic mothers (IDM).

	No.	Birth weight kg	GPR $\mu\text{mol/min}$	Gly R _a $\mu\text{mol/min}$
AGA ^{134, 135, 141}	25	3.1±0.3	21.1-32.2	4.4-9.5
IDM ¹³⁷	8	4.0±0.5	20.0±5.4	8.9±2.3
SGA ⁴²	11	1.8 ± 0.5	21.1±6.1	5.6±1.6
Preterm < 28 w ¹³⁶	10	0.9 ± 0.1	17.5 ± 5.4	2.4 -21.6

The fact that lipolysis is unimpaired in IDMs may be due to lack of a regulatory effect of insulin and/or the stimulatory effect on lipolysis of the postnatal increase in TSH. Thus, in contrast to the situation later in life, it has not been established whether insulin has a role in the regulation of lipolysis in newborn infants.

The relation between fetal/neonatal nutrition and adult metabolic disease has been studied extensively in recent years.⁴⁰ It has been reported that infants born SGA have increased insulin sensitivity^{41, 42} at birth even though they may develop insulin resistance as early as in childhood.⁴³ It is well known that infants born LGA, irrespective of the etiology, are at risk of developing neonatal hypoglycemia,^{91, 142, 143} but there is only little information on neonatal insulin sensitivity and formation of energy substrates in such infants.

Large for gestational age

The term large for gestational age is generally based on a statistical definition of size at birth. There is no general consensus concerning the definition of being born LGA. In Sweden, an infant is considered LGA when the birth weight is more than 2 SD above the mean weight for gestational age according to the Swedish standard. In many countries centiles are used for cut-off, most commonly the 90th percentile.¹⁴⁴ The number of LGA infants is in-

creasing in the western world. It is important to study factors underlying this increase, since being born LGA is a risk factor both for perinatal complications and for diseases later in life, such as obesity, diabetes, and cardiovascular disease. One well known complicating condition in LGA infants is neonatal hypoglycemia,¹⁴³ which is associated with neonatal seizures.¹⁴⁵ It has been reported that glucagon can normalize blood glucose levels during hypoglycemia in infants born AGA and SGA.^{138, 139} However, this treatment strategy has not been evaluated in LGA infants, even though it appears physiological considering the increased stores of fat¹⁴⁶ and liver glycogen¹⁴⁷ in these infants.

Gestational diabetes

One subgroup of LGA infants of particular interest is the group of offspring of women with GDM. GDM is defined as glucose intolerance of variable severity with onset or first recognition during pregnancy.¹⁴⁸

GDM is associated with increased perinatal complications, including preeclampsia, macrosomia, birth trauma, and perinatal death.^{149, 150} Women with GDM have a more than three times higher risk of giving birth to LGA infants compared to mothers without GDM.¹³ In addition, GDM is a strong risk factor for later type 2 diabetes as well as cardiovascular disease.^{151, 152}

Internationally, different criteria are used for the diagnosis of GDM following a 75 g oral glucose tolerance test (OGTT). In the U.S. the American Diabetes Association (ADA) has defined GDM on the basis of a 2-h 75 g OGTT (fasting glucose ≥ 5.3 mmol/L or 1-h glucose ≥ 10.0 mmol/L or 2-h glucose ≥ 8.6 mmol/L).¹⁴⁸ The World Health Organization (WHO) characterizes GDM with a 2-h 75 g OGTT as the joint category of diabetes and impaired glucose tolerance (fasting glucose ≥ 7.0 mmol/L or 2-h glucose ≥ 7.8 mmol/L).¹⁵³

In Sweden the incidence of GDM has been reported to be between 1 and 2 %.^{154, 155} The explanation for this inconsistency in incidence in Sweden could be that there are local differences in the definition of GDM.

Pedersen et al.⁴⁴ postulated as early as in the fifties that gestational diabetes leads to an intrauterine hyperinsulinemic environment, which causes fetal macrosomia. Interventions to improve glucose control in pregnancies complicated by gestational diabetes have been found to reduce perinatal complications.¹⁵⁶ Independent associations of maternal glucose concentrations in the third trimester and pre-pregnancy BMI with infant birth weight have been shown. The fact that only 18 % of the variation in birth weight was explained by these two variables indicates a need for further investigations of other factors related to fetal growth.¹⁵⁷ McFarland et al.¹⁵⁸ have reported the occurrence of asymmetrical macrosomia in infants of mothers with GDM. They defined this condition as a decreased head-to-shoulder ratio and increased shoulder and extremity circumferences in addition to increased

body fat. The asymmetry may explain the propensity to shoulder dystocia in these infants.¹⁵⁸ Hence, this particular group of infants needs special consideration with regard to the risk of an adverse perinatal outcome.

Boney and colleagues¹⁴⁴ have demonstrated that children exposed to gestational diabetes mellitus and born LGA may develop the metabolic syndrome as early as in childhood. Infants, born large for gestational age of women with GDM, display at birth increased abdominal, suprailiac and medial calf skin subcutaneous fat depots. At one year of age the anthropometric deviations also include increases in BMI and waist circumference.¹⁵⁹ The abnormalities become more pronounced up to the age of 4-7 years in children born LGA of women with gestational diabetes mellitus, compared to infants born LGA of non-diabetic mothers.¹⁶⁰ It has also been demonstrated that offspring exposed to maternal diabetes are at increased risk of having impaired glucose tolerance as teenagers.¹⁶¹ Additionally Rizzo et al.¹⁶² found that there was an association between poor metabolic control during pregnancy and low IQ in children of mothers with diabetes during pregnancy.

Aims of the studies

The overall aim of these studies was to identify mechanisms underlying excessive fetal growth and to study the postnatal metabolic adaptation in infants born LGA.

The specific aims were:

to estimate rates of glucose production and lipolysis and to assess insulin sensitivity in newborn LGA infants of non-diabetic mothers (study I);

to investigate the effect of glucagon on production of energy substrates in this group of infants (study I);

to determine whether non-diabetic women who themselves were born LGA are at increased risk of giving birth to LGA infants (study II);

to determine, in a large cohort, whether women with GDM are at increased risk of giving birth to infants who are LGA with respect to weight alone (study III);

to investigate to what extent fetal weight in non-diabetic pregnant women is determined by maternal BMI, insulin resistance, glucose production, and lipolysis (study IV).

Subjects

The Human Ethics Committee of the Medical Faculty of the University of Uppsala approved all studies included in this thesis. Study I was carried out at the Uppsala University Children's Hospital, between 2001 and 2005. Study IV was performed at the post delivery ward, of the Department of Obstetrics and Gynecology at Uppsala University Hospital, between 2005 and 2007. The pregnant women participating in study IV and the parents of the infants participating in study I received oral and written information before they consented to participate.

Study I

Newborn infants

The study comprised ten healthy newborn term LGA infants (four girls) of non-diabetic mothers, born at a mean gestational age of 40 ± 1.6 (SD) weeks with a mean birth weight of 4734 ± 487 g (Table 2).

Table 2. Characteristics of the infants of study I.

Infant no.	Birth weight (g)	Birth length (cm)	Gestational age at birth (wk)	Postnatal age (h)
1	4960	56.5	42.3	24
2	5020	56	39.3	28
3	5390	57	41.4	19
4	4180	50	38.9	7
5	5225	55	41.3	25
6	5030	56	41.1	19
7	4200	53	38	10
8	4930	56	41.1	9
9	4350	50	38.7	7
10	4050	51	38	16
Mean \pm SD	4734 ± 487	55 ± 2.8	40 ± 1.6	16.4 ± 7.8

The pre-pregnancy BMI of their mothers averaged 29.5 ± 7 kg/m². LGA was defined as a birth weight >2 SD for gestational age as compared to the

Swedish fetal growth chart.¹⁶³ Gestational age was determined by ultrasound examination in weeks 16-18 of pregnancy. The infants were studied at a mean postnatal age of 16 ± 8 (SD) hours. The interval between the last feed and the commencement of the study was at least 3 hours.

Study II

Study population

Information obtained from the Swedish Birth Register on birth characteristics of 47 783 women was used. The women were included in the register both as newborn infants and as mothers. The women were born from 1973 through 1983, and delivered their first infant between 1989 and 1999.

Women born in multiple births, women with congenital malformations or type 1 diabetes mellitus, women younger than 16 years at delivery (n=282), those with a very low final height (<130 cm) or weight (<39 kg) (n=27) and those born outside the Nordic countries (n=3602) were excluded. Similarly, offspring born in multiple births or with congenital malformations (n=1238) were excluded. Thus, 5149 women were excluded from the study. Of the remaining 42 634 women, 2066 (4.9%) were born large for gestational age according to the reference data of Niklasson et al.¹⁶³ (Figure 2).

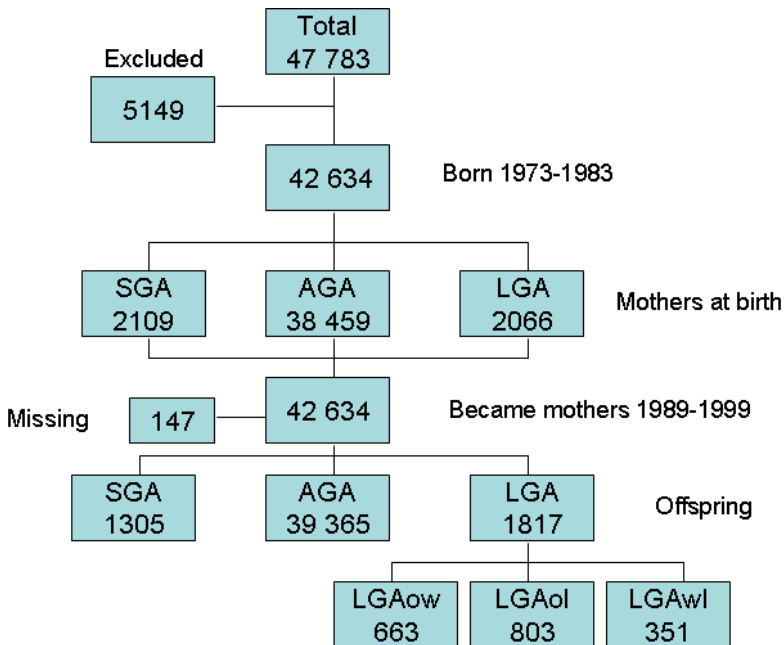


Figure 2. The subjects in study II. LGAow, LGA only weight; LGAol, LGA only length; LGAwl, LGA for weight and length.

Study III

Study population

Information from the Swedish Medical Birth Register on birth characteristics of all infants born alive at term between 1992 and 2004, who were included in the Register, had a correct identification number, and whose birth length and birth weight were recorded was used in study III. In order to increase the homogeneity of the population, we included only infants born of Nordic mothers aged 15 to 44 years at the time of delivery and with a pre-pregnancy weight of between 35 and 140 kg and a height of between 140 and 200 centimeters. We excluded infants born in multiple births. Since the principal aim was to investigate the impact of gestational diabetes, all mothers with a previous history of diabetes were excluded. The cohort comprised a total of 892 084 infants (*Figure 3*).

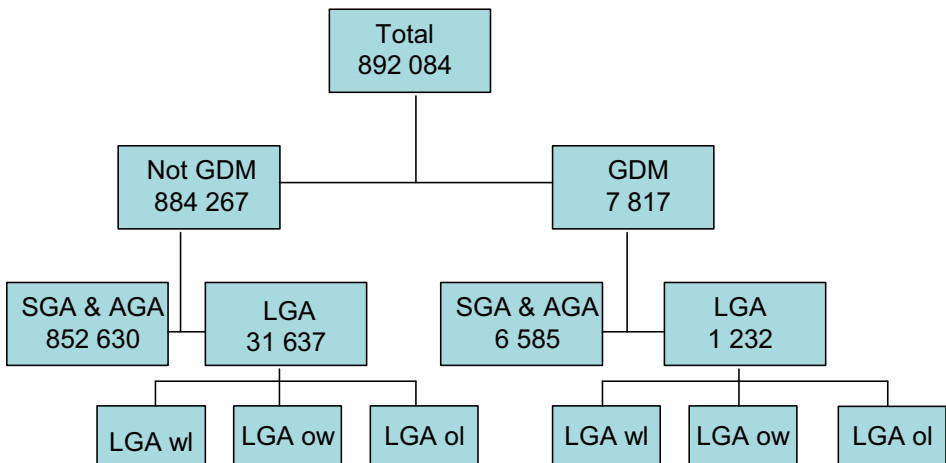


Figure 3. The infants of study III, GDM, exposed to gestational diabetes mellitus; SGA, small for gestational age; AGA, appropriate for gestational age and LGA, large for gestational age. LGAwl, LGA for weight and length; LGAow, LGA only weight; LGAol, LGA only length.

Study IV

Pregnant women

Twenty non-smoking healthy pregnant women were recruited for the study. Ten of the women had previously given birth to an LGA infant, whereas the other ten women had no history of giving birth to a macrosomic infant. This approach was used to create a study population comprising women giving birth to infants with a wide range of weights. The women who had no prior history of giving birth to an LGA infant were recruited among pregnant women working at the University Hospital of Uppsala, and those who had previously given birth to an LGA infant were recruited from consecutive patients attending the antenatal care center at the Uppsala University Hospital for ultrasonic estimation of gestational age. The characteristics of the women are presented in Table 3. Six of the women had a pre-pregnancy BMI in the range corresponding to overweight or obesity. For screening of the pregnant population, random non-fasting blood glucose levels are measured routinely four times during the pregnancy (weeks 10-14, 20-24, 28-32, and 32-36). Because of high screening blood glucose values, two of the women were submitted to glucose tolerance tests, which were normal. The women had normal HbA1c levels, 4.3 ± 0.4 %. All pregnancies were free from complications. The infants were delivered vaginally except in two cases, where cesarean sections were performed at the mothers' request.

Table 3. Pre-pregnancy characteristics of the women (N=20).

<i>Maternal</i>	<i>Mean±SD</i>	<i>Median</i>	<i>Range</i>
Age (years)	33.0 ± 4.8		
Height (cm)	167.7 ± 4.6		
Parity		1	0-3
Pre-pregnancy weight (kg)		66.5	55-112
Pre-pregnancy BMI		23.9	20.0-39.7

Methods

Stable isotope dilution technique and gas chromatography-mass spectrometry (GCMS) were used to determine rates of glucose and glycerol production. The production of glycerol reflects the rate of lipolysis. Lipolysis of depot fat results in the formation of one molecule of glycerol and three molecules of fatty acids. Glycerol is not re-esterified in adipose tissue.¹⁶⁴

Stable isotope dilution technique

Research on neonatal metabolism is limited by several factors. Preferably it should be minimally invasive and the amount of blood sampled must be small. Further, each study has to give maximal information, because of the difficulties in recruiting newborn infants. By use of stable isotope labeled (i.e., non-radioactive) compounds and analysis by GCMS these requirements are fulfilled. Isotopes are chemically identical atoms with different atomic weights due to different numbers of neutrons in the nucleus. Stable isotopes are non-radioactive. Many of them occur naturally in small amounts, natural abundance (*Figure 4*).

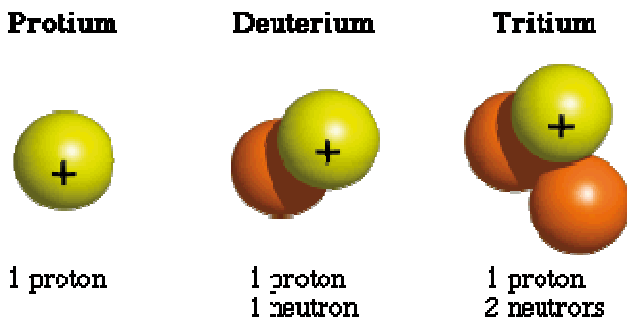


Figure 4. The three isotopes of hydrogen: protium, deuterium (stable isotope) and tritium (radioactive isotope). With permission from The Jefferson Lab, Newport, VA.

Stable isotopes can be used to trace movements of unlabeled molecules of interest, since the metabolism of a molecule labeled with a stable isotope usually does not differ from that of a matching unlabeled molecule.¹⁶⁵ During constant rate infusion of a stable isotope labeled compound (tracer) into

the bloodstream it will gradually become distributed in the extracellular compartment and equilibrate with the corresponding unlabeled compound (tracee) entering the same compartment as a result of production and feeding.¹³³ Calculations of energy substrate production are usually performed during approximate steady state with regard to absolute concentration and isotopic enrichment. GCMS can be used to analyze isotopic enrichment of a compound. GCMS is a technique which is sensitive, specific and precise. With high sensitivity means that small fractions (picogram or less) of a substance can be measured with high precision, i.e. the variation of replicate analyses of a given sample is small (<1%). Labeled and unlabeled molecules can be analyzed simultaneously and an isotopically labeled analogue of the compound under analysis can be added as an internal standard. Further, studies of interrelations between substrates are possible, since several stable isotopes can be used simultaneously. Additionally, since stable isotope tracers are non-radioactive, they are ethically accepted for use in pediatric research.¹³³

Analysis by gas chromatography-mass spectrometry

To prepare a compound for analysis by GCMS, deproteinized plasma containing the labeled and unlabeled molecules of the compound of interest is subjected to chemical derivatization in order to generate a complex volatile molecule.¹⁶⁶ In the injector part of the gas chromatograph the derivatized molecule is vaporized at high temperature, and then carried through the column of the gas chromatograph to the mass spectrometer (*Figure 5*).¹⁶⁶ In the column the derivatized molecules in the sample are separated from other components. This separation is achieved by temperature synchronized interaction between the derivatized molecule and the stationary phase, which coats the inner surface of the column. Through the interface between the gas chromatograph and the mass spectrometer, the purified compound of interest is transferred to the ion source of the mass spectrometer. By bombardment the neutral molecule is then ionized either with electrons (EI- electron impact) or by protonation in a gas phase (CI- chemical ionization).¹⁶⁶ Depending on which ionization method is used and the properties of the molecules, the ionized molecule will either remain intact or disintegrate into fragments. The fragments are separated in the quadrupole, by a magnetic field on the basis of mass over charge ratio (m/z). A detector in the mass spectrometer records the amount of ions corresponding to labeled and unlabeled compounds (*Figure 5*). The ratio is then used to calculate the isotopic enrichment of the compound of interest.

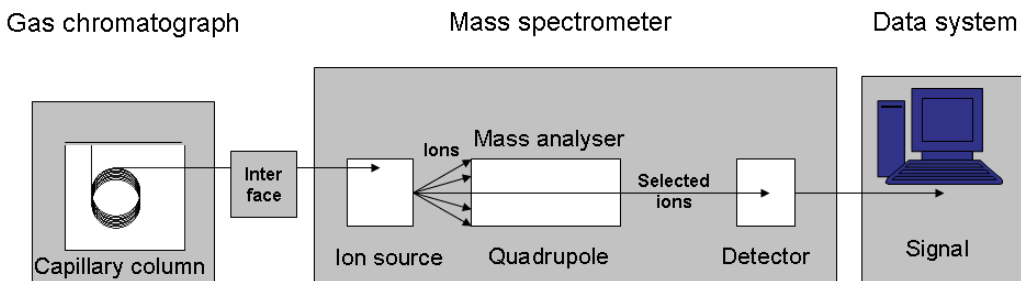


Figure 5. Schematic diagram of GCMS computer system (modified after Smith RM, Busch KL: Understanding mass spectra: A basic approach, New York, Wiley, 1999.)

Isotope tracers

In study I, [6,6-²H₂]-glucose (isotopic purity 98 atom %) and [2-¹³C]-glycerol (isotopic purity 98 atom %) were used. The compounds were purchased from Cambridge Isotope Laboratories, Woburn, MA, USA. The [6,6-²H₂]-glucose and [2-¹³C]-glycerol were each dissolved in 0.9% saline in concentrations of 4.5 and 1.2 mg/mL, respectively. In study IV, the tracers used were [6,6-²H₂]-glucose (isotopic purity 98 atom %) and [1,1,2,3,3-²H₅]-glycerol (isotopic purity 98 atom %), also purchased from Cambridge Isotope Laboratories, Woburn, MA, USA. The [6,6-²H₂]-glucose and [1,1,2,3,3-²H₅]-glycerol were each dissolved in 0.9% saline solution at concentrations of 4.5 and 1.2 mg/ml, respectively. The solutions were sterile in microbiological cultures and pyrogen free when tested by the limulus lysate method.¹⁶⁷ For the administration of tracers calibrated volumetric pumps (IMED 965 micro, IMED, Oxford, England) were used.

Chemical procedures

The blood from the EDTA tubes was instantly centrifuged and the plasma was frozen at -70°C until analyzed. To measure plasma glycerol in study I, an internal standard of [1,1,2,3,3-²H₅]-glycerol was added to the plasma samples.⁴² For the analysis of isotopic enrichment, plasma proteins were precipitated with acetone, and the triacetate derivative of glycerol and the pentaacetate derivative of glucose were prepared by addition of equivalent amounts of pyridine and acetic anhydride.

In study I the isotopic enrichments of [6,6-²H₂]-glucose, [2-¹³C]-glycerol and [1,1,2,3,3-²H₅]-glycerol were determined by GCMS. The standard curves used were prepared by gradually increasing the amounts of labeled glucose and glycerol in relation to the corresponding unlabeled compounds.¹³⁷ The

ions monitored had m/z ratios of 331, 332, and 333, corresponding to unlabeled, ^{13}C -labeled (M+1), and dideuterated glucose (M+2). For glycerol, the ions with m/z ratios of 159, 160, and 164 were monitored, reflecting unlabeled glycerol, ^{13}C -labeled glycerol (M+1) and the 5-deuterated internal standard (M+5). The contribution of $^{13}\text{C}_2$ -glucose to M+2 was determined in two of the infants (nos. 4 and 5) by GCMS of the saccharic acid tetraacetate derivative of glucose with monitoring of ions 347 (M) and 349 (M+2).¹⁶⁸ It was shown that $^{13}\text{C}_2$ -glucose contributed less than 10% to the M+2 enrichment of plasma glucose in both cases.

In study IV the isotopic enrichments of $[1,1,2,3,3\text{-}^2\text{H}_5]$ -glycerol and $[6,6\text{-}^2\text{H}_2]$ -glucose were used to calculate productions of glycerol and glucose respectively, as described above.

In study I, blood glucose was measured directly by the glucose oxidase method (ABL 735, Radiometer, Denmark). The mean coefficients of variation (CVs) for plasma glucose concentration during approximate steady state before and after administration of glucagon were 7% and 5%, respectively. The radioimmunoassay (RIA) technique was used to measure insulin,⁴² IGF-I,¹⁶⁹ IGFBP-1,¹⁷⁰ and glucagon (kit RB 310, Euro-diagnostica AB, Medeon, Malmö, Sweden).

In study IV, the biochemical analyses were performed at the certified laboratory of the Department of Clinical Chemistry at the University Hospital in Uppsala. The samples were frozen until analyzed. Measurements of routine clinical chemistry analytes and hormones were performed on an Architect Ci8200® analyzer (Abbott, Abbot Park, IL, USA) or with an automated immunoassay system (Modular E170, Roche Diagnostics GmbH, Mannheim, Germany). IGF-1 was measured with an automated immunoassay system (IMMULITE® 2500; Siemens, Los Angeles, CA, USA).

Gas chromatography-mass spectrometry

A Finnigan SSQ 70 mass spectrometer (Finnigan MAT, San José, CA, USA) equipped with an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a non-polar (DB1) capillary column (15m x 0.25 mm) was used. The temperatures in the oven were changed according to a program, from 180° to 250° and from 100° to 140° for glucose and glycerol, respectively. Methane was used for chemical ionization with selective monitoring of ions.

Calculations

In study I, isotopic enrichments of [6,6-²H₂]-glucose and [2-¹³C]-glycerol were used to calculate appearance rates (R_a) of glucose and glycerol during periods of approximate steady state before and after injection of glucagon.¹³⁷ The CVs were 4% and 2% respectively, for glucose (m/z 333/331) and 6% and 4%, respectively for glycerol (m/z 160/159). The glucose production rate and the rate of glycerol production were calculated as follows: Production rate = (i x 100/IE), where i is the infusion rate of the tracer and IE is the isotopic enrichment of the tracer in plasma [given as molar ratio, i.e., labeled (tracer)/unlabeled substrate in %]. The fraction of glycerol converted to glucose and the fraction of glucose derived from glycerol were calculated from ¹³C-enrichment of glucose reflected by m/z 332/331 before and after glucagon injection during approximate steady state as described by Patel and Kalhan et al.¹³⁵ The concentrations of plasma glycerol were calculated after addition of an internal standard of [1,1,2,3,3-²H₅]-glycerol into the blood samples obtained during periods of approximate steady state before and after injection of glucagon. The ion current ratio 159/164 was used and compared with data of a standard curve. The mean CVs were 8% and 10%, respectively.

Insulin sensitivity was evaluated by use of the homeostasis model assessment (HOMA). The HOMA index correlates with more complex measures of insulin resistance in adults.¹⁷¹

Insulin sensitivity was also assessed by calculating the plasma glucose (mg/dL)/insulin (mU/L) ratio. This ratio and the HOMA index were recently used by Bazaes et al.⁴¹ for calculating insulin sensitivity in infants born AGA and SGA.

In study IV, rates of production of glycerol and glucose were calculated from isotopic enrichments of [1,1,2,3,3-²H₅]-glycerol and [6,6-²H₂]-glucose attained during periods of approximate steady state. The mean CVs for enrichments of glycerol and glucose were 10±5% and 2±1%, respectively. Glycerol and glucose production rates were calculated as in study I. Insulin resistance was assessed by The HOMA Calculator 2.2 program (Diabetes Research Laboratory, Oxford, United Kingdom).

Fetal weight estimation

For fetal weight estimation biparietal diameter, abdominal diameter and femoral length were measured. The values obtained were inserted in the formula developed by Persson and Weldner and estimated weight was calculated.¹⁷²

Resting energy expenditure

Indirect calorimetry makes it possible to measure the metabolic free energy conversion. By indirect calorimetry the metabolic rate is estimated from consumption of oxygen and production of carbon dioxide. To extract the chemical energy of a substrate the substrate is oxidized, since the ultimate common pathway of all cellular fuels is oxidation. By indirect calorimetry the total energy production in the body is measured. Assuming that all oxygen is used to oxidize fuels and that all carbon dioxide is recovered, makes it possible to calculate the total quantity of energy created. The term “energy production” means conversion of energy from nutrients to chemical energy in the form of adenosine triphosphate (ATP), plus the loss of energy during the process.¹⁷³

For measurements of oxygen consumption and carbon dioxide production an open ventilated system was used with a face hood connected to an ergospirometer (Sensormedics 2900Z, Anaheim, CA, USA). Before every test a calibration with two gas mixtures (one with 16.0% O₂ and 4.06% CO₂ in nitrogen and the other with 26.2% O₂ in nitrogen, AGA, Stockholm, Sweden) was performed. The women were awake lying in a resting position during the measurement, which lasted for 30 minutes. Respiratory gas exchange was recorded at 60 s intervals. During the last 15 min, the women were considered to be at rest¹⁷⁴ and the mean (resting energy expenditure/day [REEday]) values during this period were used to calculate resting energy turnover expressed in kcal/day, kcal/min (REEmin), kcal/kg/day (REE-weight) and kcal/kg FFM/day (REEFFM) according to the Weir equation.¹⁷⁵

Body composition measurements

Body composition can be assessed by several methods. Skinfold measurement alone lacks the precision to estimate fat mass changes accurately during pregnancy, since fat accretion is not equally distributed in the pregnant woman. Owing to the increased hydration in fat-free mass (FFM) during pregnancy, two-compartment body composition methods based on total water, body density, and total potassium is not reliable. It is not adequate to use unadjusted FFM constants for hydration, density, and potassium content, since these are not applicable in pregnant women. Two-compartment models that use corrected constants for FFM, which are available, are acceptable.¹⁷⁶ However, in pregnant women it is appropriate to use a three- or four-compartment model in which the hydration or density of FFM is measured.¹⁷⁷

In study IV body composition was assessed with a three-compartment model combining measurements of multi-frequency bioimpedance (BIA) and skin fold thickness. The BIA measurement (Xitron Hydra, San Diego, USA) was

performed with electrode placement over the right wrist and ankle and skin-fold thickness was obtained by means of two measurements at four different locations (biceps, triceps, subscapular and suprailiac folds) with a Harpenden caliper (John Bull, British Indicators, St Albans, England). The three-compartment model has been evaluated against underwater weighing in combination with dual energy x-ray absorptiometry.¹⁷⁸

The Swedish Medical Birth Register

Data from the Swedish Medical Birth Register, kept by the National Board of Health and Welfare, was used in studies II and III. The register was started in 1973, and contains data on more than 99% of all births in Sweden.¹⁷⁹ Beginning with the first antenatal visit, information is collected prospectively during pregnancy. The data include maternal demographic factors, reproductive history, complications during pregnancy, and information on the delivery and the neonatal period. The women are interviewed by a midwife concerning their current health, current smoking habits, and family situation. Information is also collected about diseases in the family. Weight is measured and current height is self-reported or measured (if unknown). Since 1983 the register has included information on smoking habits and maternal illnesses, such as diabetes mellitus. Since 1991 information on maternal weight at registration for antenatal care has also been recorded. The data are then forwarded to the Swedish Medical Birth Register. All records on births and deaths are validated every year against the Register of the Total Population (kept by Statistics Sweden), using the mother's unique personal identification number, a number that is assigned to each Swedish resident at birth.

Study design

Study I

Two peripheral vein catheters were inserted in the newborn infant, one for tracer infusion and the other for collection of blood samples (*Figure 6*).

The tracers were infused in 140 min.¹³⁷ Blood samples were obtained before the start of the tracer infusion and then every ten minutes between the 60th and 140th min (a total of 8-9 samples; 1 mL/sample corresponded to approximately 2.2 % of the estimated blood volume).

The effect of an i.v. injection of glucagon (Glucagon Novo Nordisk, 1.0 mg/mL), 0.2 mg/kg, given 90 minutes after the start of the isotope infusion, was analyzed in eight of the infants. The results of this study were compared with earlier data from our own laboratory or with literature data.



Figure 6. Large for gestational age infant during the study. Consent to publication was obtained from the parents.

Studies II and III

In study II, maternal age was defined as completed years at delivery and categorized into three subgroups, 16-19, 20-22, and 23-26 years. In study III, age was defined as completed years at delivery and categorized into six subgroups, 15-19, 20-24, 25-29, 30-34, 35-39, and 40-44 years. Smoking habits at the first visit to the antenatal clinic were categorized into non-smoking (i.e., non-daily smoking), smoking 1-9 cigarettes/day and smoking ≥ 10 cigarettes/day. Gestational diabetes was defined in accordance with the Swedish versions of ICD-9 (89-97) or ICD-10 (97-04), using the codes 648A (ICD-9) and O24.4 (ICD-10), respectively. There was no possibility of separating insulin-treated women from those treated with diet alone. We did not include pre-gestational diabetes in the analyses. Women with the diagnoses ICD 9: 250 A-X and ICD 10: E10-14 and O24.0-O24.3 before pregnancy were excluded. Body mass index was calculated at the first visit for antenatal care as the ratio between weight and squared adult height (kg/m^2). Lean weight was defined as BMI < 18.5 , normal weight as BMI between 18.5 and 24.9, overweight as BMI 25.0-29.9, and obesity as BMI $\geq 30 \text{ kg}/\text{m}^2$.¹⁸⁰ Gestational age was estimated from an ultrasonic examination in the second trimester, if available, otherwise from the last menstrual period. Adult height standard deviation score (SDS) was calculated as individual height minus mean cohort height/cohort SDS for height, and stratified into short adult stature (< -2 SDS, < 154 cm), normal adult stature (-2 to 2 SDS, 154-179 cm) and tall adult stature (> 2 SDS, > 179 cm).¹⁸¹

In study II, birth weight for gestational age was categorized into three subgroups. Light for gestational age was defined as more than 2 SDS below the mean birth weight for gestational age, appropriate for gestational age as birth weight between -2 and $+2$ SDS, and heavy for gestational age was defined as birth weight more than 2 SDS above the mean.¹⁶³ Birth length for gestational age was categorized analogously. Albertsson-Wikland et al.¹⁸² divided children born SGA into three subgroups: those born short only, those born light only, and those born both short and light for gestational age. In accordance with this categorization, LGA was defined as > 2 SDS in either birth length, birth weight, or both in relation to gestational age, and the LGA subjects were divided into three subgroups: those born long only, those born heavy only, and those born both long and heavy for gestational age. In study II, identical definitions of large for gestational age were used for mothers and children. Information about the women's age, smoking habits, height, and weight at the time when they gave birth (1989-1999) was used in the analyses in study II.

In study III, birth weight and birth length for gestational age were categorized in the same way as in study II, but instead of using > 2 SDS according to Niklasson et al.¹⁶³ the cohort was used as its own reference.

Study IV

The pregnant women were studied at a mean gestational age of 36.4 ± 1.0 weeks and they were fasted and rested in bed from 10 pm the evening before the investigation. At 5 am the following morning two peripheral vein catheters were inserted, one for infusion of the tracers and one for collection of blood samples. The catheter used for sampling was rinsed with saline during the study. The infusions of stable isotope tracers were started at 6 am. Bolus doses of tracers were given in five minutes, followed by a constant rate infusion for six hours. At 8.30 am the body composition was assessed. Resting energy expenditure was measured by indirect calorimetry at 9 am, i.e., after 11 hours of fasting (*Figure 7*). Subsequently, fetal ultrasound was performed to estimate fetal weight. At 11.20 am, after 5 hours and 20 minutes of tracer infusion, five blood samples were taken at ten-minute intervals. Immediately after sampling, plasma was separated by centrifugation and frozen pending analysis. Enteral contributions of glycerol and glucose were considered to be negligible in view of the long fasting period before the examination. It was not possible to recruit a control group in this study for several reasons. The results are therefore compared with earlier data from our own laboratory or in the literature.



Figure 7. A pregnant woman during, the study of resting energy expenditure. A research nurse and an ergospirometer (Sensormedics 2900Z, Anaheim, CA, USA) in the background.

Statistical analysis

In study I, the results are presented as mean \pm SD or as median and range when the variables were not normally distributed. Correlation analyses were performed with Pearson's correlation two-tailed test. Comparisons between measurements before and after glucagon injection were made with the paired Student's t-test. Differences and correlations were considered significant at p values <0.05.

In study II, the standard statistical package (SPSS) for Windows, V12.0 (SPSS Inc., Chicago, Illinois, USA) was used in the statistical calculations. Regression analyses were performed to investigate the effects of maternal birth characteristics, age, smoking habits, and maternal diabetes on the infants' birth characteristics. The outcomes are presented as odds ratios (OR) with confidence intervals (CI).

In study III, multiple logistic regression analysis was used to evaluate the association between gestational diabetes and the outcome in the three LGA subgroups. Crude and adjusted analyses were performed. Adjustments were made for maternal age, parity, pre-pregnancy BMI, and smoking. Risk estimates are presented as OR with 95% CI. In the logistic regression analyses observations with insufficient information for the calculation of BMI and mothers for whom information about smoking habits was lacking were excluded. The SAS program was used for statistical analyses.

In study IV, the results are presented as mean \pm SD or as median and range if the variable could not be considered normally distributed. Correlation analyses were performed using Spearman's rho. Further, forward stepwise multiple linear regression analyses were performed. Deviation from normality was tested using the Kolmogorov-Smirnov test. The statistical package SPSS, version 15 (LEAD Technologies, Chicago, Illinois, USA) was used in the data analyses. Results were considered significant at p values of less than 0.05.

Results

Study I

The mean plasma glucose concentration in the LGA infants was 3.8 ± 0.5 mmol/L and the mean GPR was 30.2 ± 4.6 $\mu\text{mol/kg/min}$.

The mean plasma concentration of glycerol was 384 ± 183 $\mu\text{mol/L}$ and the mean rate of glycerol production was 12.7 ± 2.9 $\mu\text{mol/kg/min}$. The fraction of glycerol converted to glucose averaged 59 ± 20 %. This corresponded to $13 \pm 5\%$ of the total glucose production.

Serum concentrations of insulin, glucagon, IGF-1 and IGFBP-1 were 10.8 ± 2.8 mU/L, 52 (range 34-107) pmol/L, 38 ± 17 $\mu\text{g/L}$, and 231 ± 79 $\mu\text{g/L}$, respectively.

The glucose/insulin ratio was 6.6 ± 1.6 mg/dL/mU/L. As calculated according to HOMA, insulin sensitivity (S%) was 82 ± 19 %, beta cell function (B%) 221 ± 73 %, and insulin resistance (IR) 1.3 ± 0.3 .

Following injection of glucagon the mean rate of glucose production increased by 13.3 ± 8.3 $\mu\text{mol/kg/min}$ ($p < 0.05$) (*Figure 8*) and the mean blood glucose level by 1.4 ± 0.5 mmol/L ($p < 0.05$), i.e., by 44% and 37 %, respectively. Concomitantly the mean rate of glycerol production decreased by 16% from 12.8 ± 3.2 to 10.7 ± 2.9 $\mu\text{mol/min/kg}$ ($p < 0.05$) (*Figure 9*). There was also a decrease in the proportion of glucose generated from glycerol ($p < 0.05$). The mean serum insulin concentration increased from 10.9 ± 3.0 to 30.9 ± 10.3 mU/L.

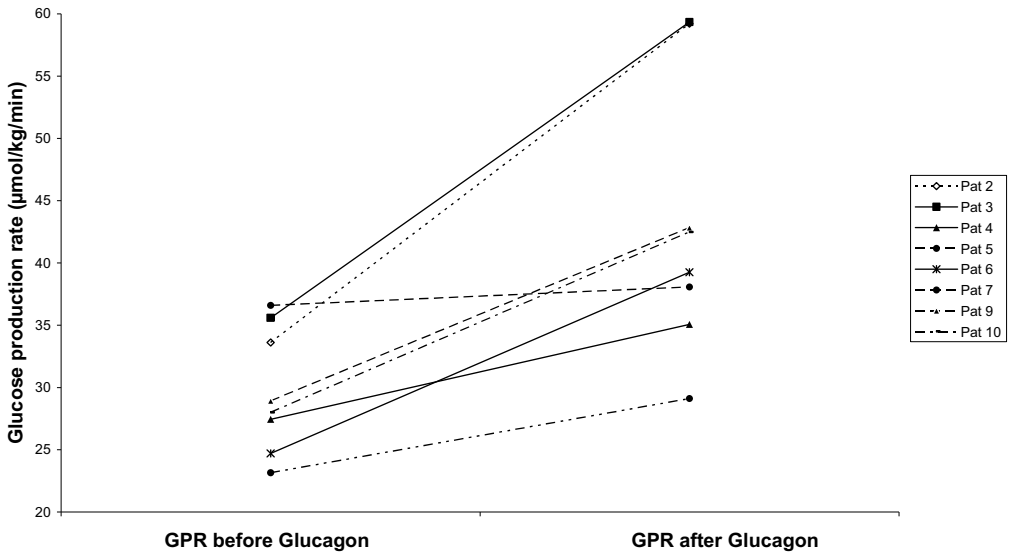


Figure 8. Effect of glucagon on rate of glucose production (GPR) (N=8) ($p < 0.05$)

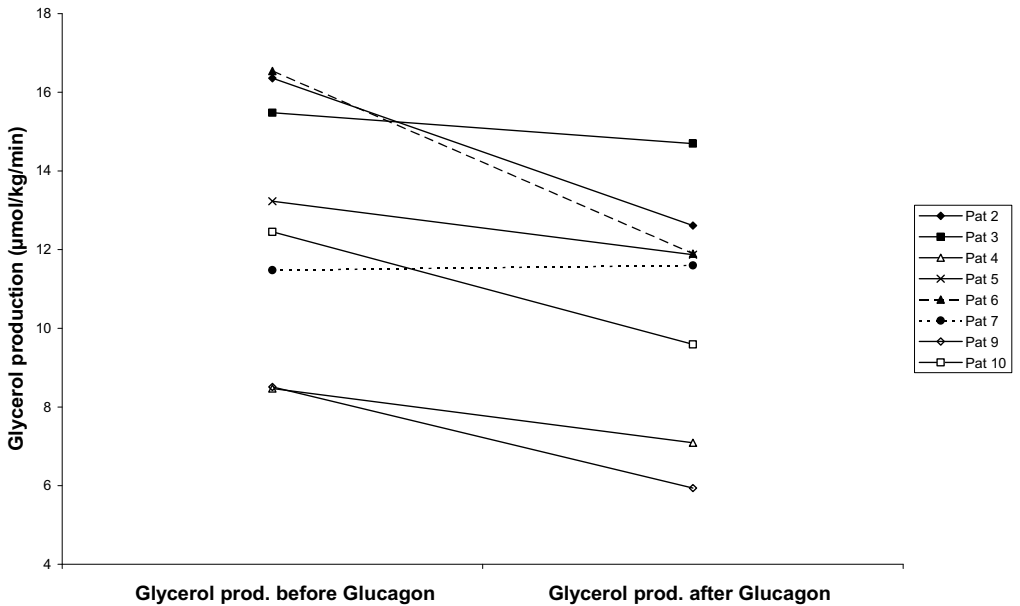


Figure 9. Effect of glucagon on rate of glycerol production (lipolysis) (N=8) ($p < 0.05$).

Both the rate of lipolysis and that of glucose production correlated with birth weight ($r=0.680$, $p < 0.05$, and $r=0.680$, $p < 0.05$, respectively). In addition

GPR correlated with the blood glucose level ($r= 0.716, p<0.05$). There was a strong inverse correlation between the decrease in lipolysis and the increase in serum insulin after administration of glucagon ($r=-0.808, p=0.015$) (Figure 10).

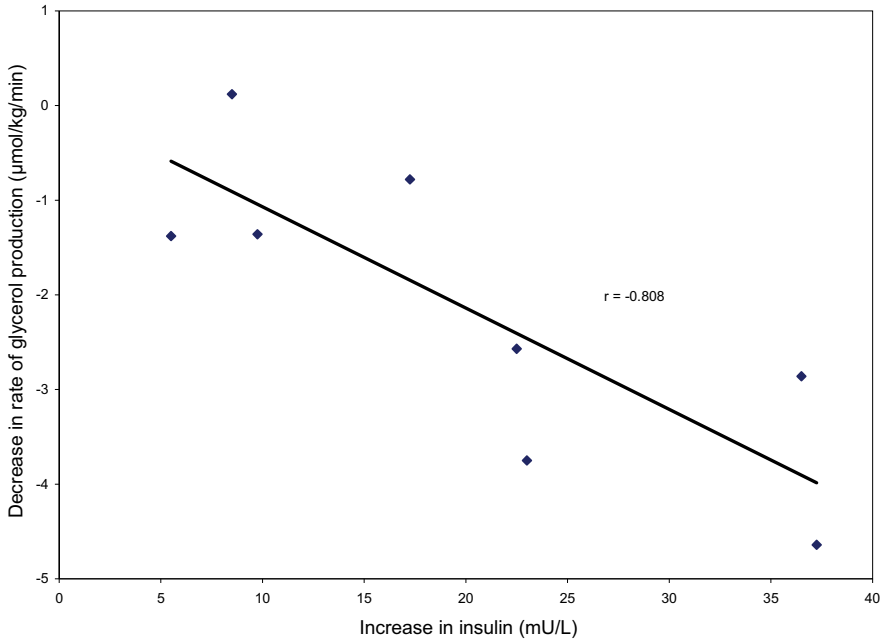


Figure 10. Correlation between the increase in serum insulin and the decrease in rate of glycerol production following glucagon administration (N=8) ($r=-0.808, p=0.015$)

Study II

In the total cohort of 42 634 infants, 1817 (4.3%) were born LGA, 39 365 (92.3%) were born AGA and 1305 (3.1%) were born SGA. Corresponding information on 147 infants (0.3%) was missing. Maternal characteristics at birth and in adulthood in relation to birth characteristics of the infants are presented in (Table 4). Mothers with a birth weight above 2 SDS gave birth to infants that were almost 500 g heavier than those of mothers with a birth weight below -2 SDS. A tall final height and high BMI among the women were associated with an increased mean birth weight and birth length of the offspring. However, the difference between the subgroups with BMI 25-29.9 and BMI > 30 was small.

Table 4. Maternal characteristics in relation to mean birth length (SD) and mean birth weight (SD) of the offspring.

Mother	N	Offspring Birth length (cm)	Offspring Birth weight (g)
Birth length (SDS)			
<-2	1 363	49.0 (2.7)	3239 (543)
-2 to 2	39 528	50.2 (2.4)	3490 (533)
>2	1 403	51.3 (2.4)	3710 (540)
Missing	340	50.1 (2.4)	3447 (535)
Birth weight (SDS)			
<-2	1 427	49.3 (2.6)	3236 (550)
-2 to 2	39 757	50.2 (2.4)	3490 (532)
>2	1 135	51.2 (2.4)	3775 (535)
Missing	315	50.0 (2.4)	3453 (550)
Age (years)			
16 to 19	10 644	50.0 (2.5)	3452 (550)
20 to 22	18 428	50.2 (2.4)	3493 (531)
23 to 26	13 562	50.4 (2.4)	3513 (534)
Height (SDS)			
<-2	525	48.8 (2.5)	3192 (518)
-2 to 2	37 875	50.2 (2.4)	3493 (531)
>2	739	51.3 (2.1)	3733 (501)
Missing	3 495	49.9 (2.7)	3434 (586)
BMI (kg/m²)			
<18.5	1 632	49.6 (2.4)	3308 (505)
18.5-24.9	23 643	50.2 (2.4)	3474 (517)
25-29.9	7 445	50.5 (2.4)	3563 (546)
>30	2 998	50.5 (2.7)	3589 (592)
Missing	6 916	50.1 (2.6)	3460 (558)

Analysis performed by multivariate logistic regression demonstrated that women born LGA with regard to weight or to length had a doubled risk of giving birth to an LGA infant, compared to women born AGA (Table 5). Being obese (BMI >30) was associated with the greatest risk of having an LGA infant, as compared to non-obese women.

Table 5. Maternal characteristics in relation to rates and odds ratio (95% confidence interval) of giving birth to a large for gestational age infant (>2SDS).

Mother	Rate (%)	LGA Crude	LGA Adjusted*
Birth length (SDS)			
<-2	1.5	0.34 (0.22-0.54)	0.59 (0.37-0.96)
-2 to 2	4.2	1.00**	1.00**
>2	10.7	2.77 (2.32-3.3)	2.01 (1.61-2.51)
Missing	3.0		
Birth weight (SDS)			
<-2	1.9	0.45 (0.30-0.66)	0.60 (0.38-0.95)
-2 to 2	4.2	1.00**	1.00**
>2	11.9	3.11 (2.58-3.75)	1.96 (1.54-2.48)
Missing	3.5		
Age (years)			
16 to 19	3.5	0.73 (0.64-0.83)	0.94 (0.81-1.10)
20 to 22	4.4	0.91 (0.82-1.02)	1.04 (0.93-1.17)
23 to 26	4.8	1.00**	1.00**
Height (SDS)			
<-2	1.5	0.35 (0.17-0.71)	0.44 (0.22-0.89)
-2 to 2	4.3	1.00**	1.00**
>2	8.1	1.99 (1.52-2.61)	1.54 (1.14-2.09)
Missing	4.2		
BMI (kg/m²)			
<18.5	2.1	0.61 (0.43-0.86)	0.65 (0.45-0.92)
18.5-24.9	3.4	1.00**	1.00**
25-29.9	6.1	1.86 (1.65-2.09)	1.82 (1.61-2.05)
>30	8.4	2.64 (2.28-3.06)	2.56 (2.20-2.98)
Missing	4.2		

*Adjusted for all other variables included in the table.

**Reference group

The impacts of maternal birth characteristics and maternal adult size on offspring anthropometry were further analyzed by dividing the LGA infants into three subgroups, those born tall only, those born heavy only and those born both tall and heavy for gestational age (Table 6). These data showed that high maternal birth weight (above 2 SDS) for gestational age was associated with a 2.6 times higher risk of giving birth to an offspring that was LGA for weight only (LGAow). However, there was no increase in the risk that the offspring would be born LGA for length alone (LGAol). Mothers who were born tall (above 2 SDS) for gestational age had a doubled risk of giving birth to an offspring that was long alone (LGAol) but did not have an increased risk of giving birth to an infant born heavy only.

Table 6. Adjusted odds ratios with 95% confidence interval for giving birth to subgroups of large for gestational age infants by maternal birth and adult characteristics.

Mother	LGAol	LGAow	LGAlw
Birth length (SDS)			
<-2	0.62 (0.32-1.23)	0.61 (0.27-1.34)	0.53 (0.16-1.74)
-2 to 2	1.00**	1.00**	1.00**
>2	2.27 (1.65-3.14)	1.34 (0.91-1.97)	2.45 (1.60-3.75)
Birth weight (SDS)			
<-2	0.73 (0.39-1.36)	0.53 (0.24-1.18)	0.47 (0.14-1.54)
-2 to 2	1.00**	1.00**	1.00**
>2	1.17 (0.78-1.75)	2.63 (1.85-3.75)	2.21 (1.41-3.49)
Age (years)			
16 to 19	0.78 (0.61-0.98)	1.08 (0.85-1.38)	1.11 (0.78-1.58)
20 to 22	0.99 (0.83-1.17)	0.97 (0.80-1.18)	1.33 (1.02-1.72)
23 to 24	1.00**	1.00**	1.00**
Height (SDS)			
<-2	0.65 (0.27-1.58)	0.30 (0.08-1.23)	0.29 (0.04-2.06)
-2 to 2	1.00**	1.00**	1.00**
>2	1.37 (0.86-2.17)	1.56 (0.96-2.54)	1.68 (0.92-3.06)
BMI (kg/m²)			
<18.5	0.87 (0.56-1.36)	0.55 (0.28-1.07)	0.23 (0.06-0.93)
18.5-24.9	1.00**	1.00**	1.00**
25-29.9	1.38 (1.15-1.65)	2.17 (1.78-2.64)	2.19 (1.68-2.85)
>30	1.69 (1.33-2.16)	3.21 (2.54-4.05)	3.16 (2.31-4.33)

**Reference group

(LGAol, LGA only length; LGAow, LGA only weight; LGAlw, LGA length and weight.)

In overweight and in obese women the risk of giving birth to infants heavy only (LGAow) was increased twofold and threefold, respectively, but there was only a slightly increased risk of giving birth to an infant that was long only (LGAol).

In the further analyses the mothers were classified into four groups, those not born LGA, those born long only, those born heavy only, and those born both long and heavy for gestational age (Tablezz 7). Women born heavy only had a 40 % higher risk of being overweight and a 2.5 times higher risk of being obese at childbearing age compared to women not born LGA.

Table 7. Adjusted odds ratios with 95% confidence interval of having an adult BMI of 25-30 and BMI > 30, according to being born as a non-LGA infant or an LGA infant regarding only length (mLGAol), regarding only weight (mLGAow) or regarding both length and weight (mLGAlw).

Women at birth	Adult BMI 25-30	Adult BMI >30
Not LGA	1.00 **	1.00 **
mLGAol	1.12 (0.94-1.32)	1.22 (0.96-1.55)
mLGAow	1.41 (1.17-1.72)	2.49 (1.99-3.12)
mLGAlw	1.07 (0.84-1.37)	1.53 (1.11-2.09)

Adjusted for height, smoking, and diabetes.

** Reference group

Study III

The number of infants born between 1992 and 2004 and fulfilling the inclusion criteria was 892 084. Of these, 7 817 infants were born of mothers with gestational diabetes. This represents an incidence of GDM of 0.9% in the studied population. Maternal characteristics are presented in Table 8. The mean birth weight and length of infants of mothers with diabetes were 3 793 g and 50.9 cm, respectively. Corresponding data for the infants of mothers without gestational diabetes were 3 609 g and 50.6 cm, respectively. In both groups birth weight increased with increasing maternal parity and with increasing BMI. Smoking mothers gave birth to smaller children than non-smoking mothers. Non-smoking women with GDM, high parity, and obesity gave birth to the heaviest infants.

Table 8. Infant anthropometry in relation to maternal characteristics and exposure to maternal gestational diabetes mellitus

	<i>Gestational Diabetes</i>					
	<i>No</i>			<i>Yes</i>		
	<i>N (%)</i>	<i>Birth weight Mean (SD)</i>	<i>Birth length Mean (SD)</i>	<i>N (%)</i>	<i>Birth weight Mean (SD)</i>	<i>Birth length Mean (SD)</i>
<i>Maternal age, yrs</i>						
15-19	15 758 (1.8)	3 495 (470)	50.2 (2.1)	71 (0.9)	3 645 (602)	50.6 (2.3)
20-24	135 920 (15.4)	3 567 (471)	50.5 (2.0)	817 (10.5)	3 817 (555)	50.8 (2.2)
25-29	320 540 (36.2)	3 608 (475)	50.6 (2.0)	2 329 (29.8)	3 804 (564)	51.0 (2.2)
30-34	281 503 (31.8)	3 630 (485)	50.7 (2.1)	2 562 (32.8)	3 790 (569)	50.9 (2.2)
35-39	111 651 (12.6)	3 629 (500)	50.7 (2.1)	1 632 (20.9)	3 786 (574)	50.9 (2.2)
40-44	18 895 (2.1)	3 601 (519)	50.5 (2.2)	406 (5.2)	3 757 (593)	50.9 (2.4)
<i>Parity</i>						
1	366 322 (41.4)	3 514 (464)	50.4 (2.1)	2 760 (35.3)	3 635 (537)	50.6 (2.2)
2-3	467 367 (52.9)	3 676 (480)	50.8 (2.0)	4 218 (54.0)	3 868 (562)	51.1 (2.2)
4-	50 578 (5.7)	3 685 (514)	50.7 (2.1)	839 (10.7)	3 933 (591)	51.2 (2.3)
<i>BMI</i>						
<18.5	19 516 (2.2)	3 377 (447)	49.8 (2.1)	82 (1.0)	3 554 (578)	50.4 (2.1)
18.5 - < 25	501 695 (56.7)	3 574 (465)	50.5 (2.0)	2 710 (34.7)	3 687 (539)	50.6 (2.1)
25 - <30	166 602 (18.8)	3 692 (492)	50.9 (2.1)	1 863 (23.8)	3 824 (562)	51.0 (2.2)
>30	61 413 (6.9)	3 746 (527)	51.0 (2.1)	1 832 (23.4)	3 915 (576)	51.2 (2.2)
Missing	135 041 (15.3)	3 609 (485)	50.6 (2.1)	1 330 (17.0)	3 811 (583)	50.9 (2.2)
<i>Smoking habits</i>						
Non-smoker	709 300 (80.2)	3 638 (477)	50.8 (2.0)	6 122 (78.3)	3 817 (560)	51.0 (2.1)
1-9 cig/day	84 240 (9.5)	3 471 (481)	50.0 (2.1)	800 (10.2)	3 693 (587)	50.5 (2.3)
≥10 cig/day	44 399 (5.0)	3 417 (478)	49.7 (2.1)	507 (6.5)	3 648 (574)	50.3 (2.3)
Missing	46 328 (5.2)	3 600 (484)	50.5 (2.1)	388 (5.0)	3 809 (618)	50.9 (2.3)

The infants in the cohort were divided into nine subgroups (Table 9). Those who were AGA both for weight and length represented by far the largest subgroup, in all comprising 833 755 infants. Among the LGA infants, the subgroup that was LGA concerning weight alone was by far the largest (Table 10).

Table 9. Infants divided according to birth weight and birth length. N = number of infants in each group, (%) = percentage of infants in each group compared to the total number of infants in the cohort.

<i>Length</i>	<i>SGA</i> <i>N (%)</i>	<i>AGA</i> <i>N (%)</i>	<i>LGA</i> <i>N (%)</i>	<i>Total</i> <i>N (%)</i>
<i>Weight</i>				
<i>SGA</i> <i>N (%)</i>	5 947(0.7)	12 865 (1.4)	12 (0.0)	18 824 (2.1)
<i>AGA</i> <i>N (%)</i>	6 648 (0.7)	833 755 (93.5)	6 206 (0.7)	846 609 (94.9)
<i>LGA</i> <i>N (%)</i>	17 (0.0)	21 187 (2.4)	5 447 (0.6)	26 651 (3.0)
<i>Total</i> <i>N (%)</i>	12 612 (1.4)	867 807 (97.3)	11 665 (1.3)	892 084 (100.0)

Table 10. Number of infants in LGA subgroups and number of SGA and AGA infants. N= number, % = percentage of whole cohort.

	<i>Gestational Diabetes</i>				<i>Total</i>	
	<i>no</i>		<i>yes</i>		<i>N</i>	<i>%</i>
	<i>N</i>	<i>%</i>	<i>N</i>	<i>%</i>		
<i>LGA, weight and length</i>	5 136	0.6	311	4.0	5 447	0.6
<i>LGA, only weight</i>	20 366	2.3	838	10.7	21 204	2.4
<i>LGA, only length</i>	6 135	0.7	83	1.1	6 218	0,7
<i>SGA & AGA</i>	852 630	96.4	6 585	84.2	859 215	96.3
<i>Total</i>	884 267	100.0	7 817	100.0	892 084	100.0

Analysis performed by multiple logistic regression demonstrated that infants of GDM mothers had a more than five times higher risk (OR 5.25, 95% CI 4.84-5.70) of being born LGA concerning weight alone, than infants born of non-diabetic mothers (Table 11). The risk of being born both long and heavy was somewhat higher (OR 7.06, 95% CI 6.16-8.08). However, this latter group was considerably smaller than the group of LGA infants born heavy

only. The risk that a woman with GDM would give birth to an infant that was long but not heavy for gestational age was much lower than the risk of giving birth to an infant heavy alone (OR 1.55, 95% CI 1.20-2.00). After adjustments for possible confounding maternal factors such as age, parity, BMI, and smoking, there was a risk reduction in all three LGA subgroups. However, GDM was still associated with a markedly increased risk of giving birth to an LGA infant, particularly with a high birth weight (Table 11). Further, in the population of women giving birth to LGA infants, the odds ratio for giving birth to an LGA infant that was heavy alone was 1.19 (95% CI 1.03-1.37) for a woman with GDM compared to a non-diabetic woman.

Table 11. Crude and adjusted odds ratios [OR] with 95% confidence intervals [CI] for giving birth to subgroups of LGA infants when exposed to maternal gestational diabetes. N= number included in the analysis, N of events = number of LGA infants included in the analysis.

<i>Outcome</i>	<i>N</i>	<i>N of events</i>	<i>OR (95% CI)</i>	
			<i>Crude</i>	<i>Adjusted*</i>
<i>LGA, weight and length</i>	717 314	4 497	7.06 (6.16-8.08)	4.65 (4.05-5.35)
<i>LGA, only weight</i>	730 320	17 476	5.25 (4.84-5.70)	3.71 (3.41-4.04)
<i>LGA, only length</i>	718 001	5 157	1.55 (1.20-2.00)	1.36 (1.05-1.75)

*Adjusted for maternal age, parity, BMI, and smoking.

Study IV

Maternal, fetal and infant characteristics are shown in Table 12. Six of the 20 infants were born LGA for weight (>2 standard deviations). Estimated fetal weights at 36 weeks' post menstrual age (PMA) were distributed between 2670 and 4175 g (mean 3193 ± 454 g), corresponding to -0.2 to 2.7 SDS. The birth weights were between 3180 and 5040 g (mean 4092 ± 406 g), corresponding to -0.3 to 2.8 SDS.

Table 12. Maternal, fetal, and infant outcome variables.

<i>N=20</i>	<i>Mean ± SD</i>	<i>Median</i>	<i>Range</i>
<i>Maternal</i>			
Post menstrual age at study (weeks)	36.4±1		
Weight at study (kg)		83.5	73-117
BMI at study (kg/m ²)		29.4	26-41
Fat-free mass (kg)	55.8±6.3		
Fat mass (kg)	30.5±7.5		
Resting energy expenditure (kcal/day)	1908±307		
<i>Fetal</i>			
Estimated fetal weight (g)	3193±454		2670-4175
Estimated fetal weight SDS			-0.2 to 2.7
<i>Infant</i>			
Gestational age at birth (weeks)	40.6±1		
Birth weight (g)	4092±406		3180-5040
Birth weight SDS			-0.3 to 2.8
Birth length (cm)	52.5±1.7		
Head circumference (cm)	36.5±1.1		

The median rate of total body glycerol production, reflecting lipolysis, was 214 (110-576) $\mu\text{mol}/\text{min}$ and the median relative rate was 2.5 (1.3-7.3) $\mu\text{mol}/\text{kg}/\text{min}$. Plasma glycerol concentration averaged 58.2 ± 14.7 $\mu\text{mol}/\text{L}$. The median total rate of glucose production was 805 (653-1337) $\mu\text{mol}/\text{min}$ and the median relative rate was 9.7 (7.8-15.9) $\mu\text{mol}/\text{kg}/\text{min}$. The median concentration of plasma glucose was 4.5 (3.5-6.4) mmol/L and HOMA insulin resistance averaged 1.5 ± 0.75 . The mean level of IGF-1 was 391 ± 146 $\mu\text{g}/\text{L}$. Maternal fat mass (FM) at study averaged 30.5 ± 7.5 kg. The mean REE/day was 1908 ± 307 kcal/day (Table 12). Maternal weight, BMI and fat mass correlated positively with the maternal insulin resistance (Table 13).

Table 13. Bivariate Spearman correlations on total glucose production rate (GPR) and HOMA insulin resistance (IR).

	<i>Total GPR $\mu\text{mol}/\text{min}$</i>	<i>IR HOMA.</i>
Pre-pregnancy weight (kg)	$r=0.52, p<0.019$	$r=0.65, p<0.002$
Pre-pregnancy BMI (kg/m^2)		$r=0.70, p<0.001$
Weight at study (kg)	$r=0.48, P<0.031$	$r=0.62, p<0.004$
BMI at study (kg/m^2)		$r=0.72, p<0.000$
Fat mass (kg)		$r=0.66, p<0.002$
IR with HOMA calc.	$r=0.78, p<0.000$	

However, when these variables were used as predictors in a multiple forward stepwise regression analysis, only maternal fat mass was found to be significant (Table 15, model 1) and explained 36 % of the variation in insulin resistance. Both maternal insulin resistance and weight at study correlated positively with total glucose production (Table 13). When these variables were used as predictors in stepwise regression analysis on glucose production, only insulin resistance entered significantly, explaining 62 % of the variation in total glucose production (Table 15, model 2). Correspondingly, total glucose production explained 31% of the variation in estimated fetal weight at 36 weeks PMA (Table 15, model 3).

In a model in which total glucose production, maternal insulin resistance, fat mass, weight, and BMI at study were all used as independent variables, total glucose production and BMI entered significantly and explained 44% of the variation in estimated fetal weight (Table 15, model 4).

Additionally, 51% of the variation in estimated fetal weight and 41% of the variation in birth weight were explained by REE alone in models where maternal insulin resistance, BMI, weight at study, and total glucose production did not enter significantly (Table 15, models 5-6). There were also bivariate positive correlations between REE and maternal FM, weight, BMI at study, insulin resistance and glucose production (Table 14).

Table 14. Bivariate Spearman correlations with resting energy expenditure (REE).

	<i>REE per day</i>
Weight at study (kg)	r=0.77, p<0.000
BMI at study (kg/m ²)	r=0.62, p<0.005
Maternal fat mass (kg)	r=0.53, p<0.019
Total GPR μ mol/min	r=0.58, p<0.009
IR with HOMA calc.	r=0.46, p<0.046
Estimated fetal weight (g)	r=0.69, p<0.001
Birth weight (g)	r=0.65, p<0.003

Finally, in a multiple forward stepwise regression analysis in which REE was the dependent variable and maternal BMI at investigation and total glucose production were the independent variables, 72% of the variation in REE was explained by the included variables (Table 15, model 7). Further, when insulin resistance was added to the model, 87% of variation was explained (Table 15, model 8). Owing to the strong correlation between total glucose production and insulin resistance the sign of the slope changes and becomes negative for insulin resistance when total glucose production entered the regression model.

Table 15. Results from multiple forward stepwise linear regression analyses. Adjusted R-square (R^2 Adj), and slopes (95% CI). Predictors included in the models are denoted by slope or non-significant (NS).

Model:	Dependent variable	R^2 Adj.	Maternal fat mass at study (kg)	Weight study (kg)	BMI at study (kg/m^2)	Insulin resistance	Glucose production ($\mu\text{mol}/\text{min}$)	Resting energy expenditure (kcal/day)
1	Insulin resistance	0.36	0.06 (0.03 to 0.10)	NS	NS			
2	Glucose production ($\mu\text{mol}/\text{min}$)	0.62		NS		175 (110 to 241)		
3	Estimated fetal weight (g)	0.31					1.6 (0.5 to 2.7)	
4	Estimated fetal weight (g)	0.44	NS	NS	47 (4.3 to 89)	NS	1.2 (0.1 to 2.2)	
5	Estimated fetal weight (g)	0.51		NS	NS	NS	NS	1.1 (0.6 to 1.6)
6	Birth weight (g)	0.41		NS	NS	NS	NS	0.9 (0.4 to 1.4)
7	Resting energy expenditure (kcal/day)	0.72			42 (22 to 63)		0.9 (0.3 to 1.4)	
8	Resting energy expenditure (kcal/day)	0.87			61 (43 to 78)	-290 (-436 to -144)	1.7 (1.2 to 2.3)	

Discussion

Much of the interest on the developmental origin of adult disease has been focused on the long-term metabolic consequences of being born SGA,⁴⁰ although earlier data have shown that being born LGA may also predispose to overweight, diabetes, and cardiovascular disease in adult life.^{14, 22, 23, 25} The relative number of infants born LGA is increasing in the western world,¹³ and one probable reason for this is that overweight and obesity among pregnant women have become more frequent during recent decades.¹³

Maternal energy substrate production and its relation to fetal growth

We have investigated a group of pregnant non-diabetic women within a wide range of BMIs, from normal weight to obesity, in an attempt to find explanations for the relationship between maternal anthropometry and fetal growth. One possible mechanism lies in the fact that in the overweight pregnant women the production of glucose, the most important energy substrate for the growing fetus, is high. We found that glucose production explained one third of the variation in estimated fetal weight in the studied group comprising overweight and normal weight non-diabetic women. This is in line with the previous finding by Rohl et al.,¹⁸³ who studied a mixed group of diabetic and non-diabetic pregnant women. Regarding possible mechanisms for the regulation of glucose production in pregnancy, an increasing insulin resistance, which is generally ascribed to the action of pregnancy-specific hormones, is part of the natural course of pregnancy.³⁶ Our data in study IV showed that maternal insulin resistance explained a considerable part of the increase in glucose production. In addition, the maternal insulin resistance itself was related to the fetal weight, a finding in agreement with data reported by Catalano et al.⁶⁰ and Rohl et al.¹⁸³

Another mechanism contributing to insulin resistance in pregnancy is the increased production of non-esterified fatty acids, resulting from increases in fat mass and lipolysis during pregnancy.⁵⁷ One of the principal findings in this study was that the variation in insulin resistance could be explained to a large extent by the maternal fat mass. This indicates that maternal fat mass, which is abundant in the overweight and obese pregnant woman,¹⁸⁴ promotes fetal growth by increasing the availability of substrates for the growing fetus.

The total rate of lipolysis in the studied pregnant women was close to that in women delivering AGA infants^{36, 37} but higher than that in pregnant women giving birth to SGA infants.⁶¹ There was no correlation between maternal lipolysis and estimated fetal weight or birth weight in the present study, showing that despite its role in energy supply, variations in maternal lipolysis do not seem to explain differences in weight gain among the fetuses of the studied group of women. Since glucose is considered to be the most important fuel for the growing fetus, the strong positive association between glucose production and insulin resistance and the fact that insulin resistance increased with increasing maternal fat mass might explain the relation between maternal fat mass and fetal weight gain. The variation in estimated fetal weight is explained to an even greater extent if both maternal BMI and glucose production are included in the regression model. This further strengthens the hypothesis that parameters related to maternal overweight and obesity are important factors explaining excessive fetal growth.

Resting energy expenditure in pregnant women

Another factor associated with fetal growth is the resting energy expenditure of the pregnant woman. This corresponds to the basal metabolism and represents the sum of several metabolic events such as oxidation of glucose, protein and fat.¹⁷⁵ During pregnancy extra energy is needed for the build-up of maternal and fetal tissues as well as for the increased basal metabolism and physical work load of the pregnant woman. Several of the women in this study had pre-pregnancy BMIs corresponding to overweight or obesity and their energy expenditure during pregnancy was increased compared to that of other pregnant women.¹⁸⁵⁻¹⁸⁷ The fact that the studied women gave birth to relatively large infants could thus be related to their increase in REE. The studied women were not subjected to specific food regimens but were otherwise studied under identical conditions following 11 hours of fasting. Forsum et al.⁶³ demonstrated that birth weight is related to the cumulative increase in maternal resting metabolic rate during pregnancy. The present data concerning REE extend this relationship even further, since 51% of the variation in estimated fetal weight and 41% of that in birth weight were explained by REE in this study. It has been demonstrated previously that approximately 60 % of the variation in BMR during pregnancy can be accounted for by the increase in maternal body weight in combination with fetal weight.³⁹ We showed that almost 90% of the variation in REE was accounted for by maternal BMI, insulin resistance, and glucose production. This indicates that resting energy expenditure in pregnancy, which is important for fetal growth, is closely related to factors associated with maternal overweight and obesity.

In non-pregnant women BMR is associated with fat-free mass but not with fat mass, probably for the reason that adipose tissue consumes less oxygen

than other tissues. However, in accordance with data on pregnant women in the third trimester,^{39, 67} we found a strong correlation between fat mass and REE, indicating substantial metabolic activity in adipose tissue. There was no association between lipolysis and REE, even though lipolysis increases during pregnancy in order to secure the supply of energy to the mother and fetus.^{36, 37} In agreement with the finding by Horowitz et al.¹⁸⁸ in non-pregnant women, the relative lipolysis was inversely associated with the fat mass in the pregnant women. Since lipolysis is more sensitive to insulin than is production of glucose,¹⁸⁹ the increase in the insulin level, which is associated with maternal overweight, may explain the inhibition of lipolysis related to body weight. However, because of their larger fat stores, total lipolysis was higher in the women with overweight or obesity. No relation was found between maternal weight gain during pregnancy and REE, possibly because of the rather limited increase in weight in the women who were obese before pregnancy. It has also been demonstrated earlier that a high BMR may be protective against excessive fat gain in overweight pregnant women.⁶⁷ Our results indicate that maternal overweight and obesity should be prevented if possible in order to reduce the number of LGA infants, since fetal growth is dependent on maternal factors closely associated with overweight and obesity.

IGF-1 levels in pregnancy and fetal growth

We found that the maternal IGF-1 levels were higher in the group of pregnant women who gave birth to relatively large infants, than in those reported by Diderholm et al.^{37, 61} in IUGR and AGA pregnancies. However, there was no association between maternal IGF-1 levels and fetal size or birth weight in this investigation, a finding in line with earlier data.⁸⁷

Intergenerational effects of being born LGA

It is not only maternal anthropometry during pregnancy that is important for the size of the offspring. Our epidemiological data demonstrate that there is an intergenerational relationship in being born LGA, since the birth weight and birth length of the woman herself influences the birth characteristics of her infant, regardless of her anthropometrical data at childbearing age. Even though it is well known that the first-born infant generally is smaller than the following child and that adolescence constrains the size of the newborn,^{190, 191} the relatively young group of primipara women, born LGA, had a twofold increased risk of giving birth to an LGA infant, compared to primipara women not born LGA. Previous studies have shown that high maternal age predisposes to impaired glucose tolerance and increased BMI,¹⁹² which in turn result in a higher birth weight of the offspring. Accordingly one would expect that the risk of giving birth to an LGA infant would be even more

pronounced if a group of older women, born LGA, was studied. The fact that all data from the medical birth register were collected prospectively eliminated the risk of recall bias. Further, it is not reasonable to believe that possible misclassification concerning birth characteristics and maternal anthropometry might differ between the groups. One potential confounding factor on which information was lacking was the socioeconomic status. However, in Sweden socioeconomic differences are quite small and the impact on childhood growth is insignificant.¹⁹³

Disproportions in LGA infants and risk of later disease

Only few studies have addressed the heterogeneity of fetal growth in pregnancies leading to birth of infants with asymmetrical macrosomia.^{194, 195} Infants born LGA according to weight but not to length (heavy only), represent asymmetrical macrosomia. The reliability of birth length measurements has been questioned. However, birth length is a strong predictor of final height.^{196, 197}

It is evident that LGA infants born of women who themselves had a high birth weight (> 2 SDS) are at higher risk of being disproportionate (heavy only) than LGA infants born of women with a birth weight below 2 SDS. We also provide evidence that in the cohort of women included in study II those who were born heavy only had an increased risk of becoming overweight or obese even at an early childbearing age. To be born heavy only is a substantial risk factor for obesity later in life. In fact, the risk of becoming obese was considerably higher than that of becoming just overweight. One possible reason for this later overweight and obesity may be that infants born LGA concerning only weight have already built up considerable amounts of fat depots at birth compared to infants born with a symmetrical body proportion. This is a reasonable assumption, since the variation of birth weight is determined to a greater extent by the amount of depot fat than by the fat free mass.¹²⁰ It would be of interest to study whether this particular group of LGA infants also represents those who develop cardiovascular disease and type 2 diabetes in adult life. Since LGA infants already have an increased insulin resistance at birth (see study I), it is reasonable to assume that they may be predisposed to type 2 diabetes. The fact that no increased risk of being overweight in childbearing age was found, in infants born heavy and long or just long further strengthens the hypothesis that being born LGA concerning weight alone is a predictor of adult disease. Additionally, our data show that maternal obesity is associated with the greatest risk of having an LGA newborn and that obese women have a higher risk of giving birth to infants that are heavy only, i.e., asymmetrical offspring. It is well known that asymmetrical macrosomia is associated with perinatal complications.¹⁵⁸ The fact that obesity leads to disproportionate offspring could thus be one explanation for the higher risk of perinatal complications in obese pregnant

women compared to non-obese pregnant women. In addition, it would explain why infants of such mothers are at increased risk of being admitted to neonatal care units after delivery.¹⁹⁸ This understanding is important for the obstetrical management of women with obesity.

Gestational diabetes and infant body proportions

Maternal gestational diabetes is another risk factor for birth of asymmetrical LGA infants. We found that infants of such mothers had an almost four times higher risk of being born heavy but not long for gestational age compared to infants of mothers without gestational diabetes. Additionally, among women giving birth to an LGA infant those with GDM have a 20% higher risk of giving birth to an LGA infant that is heavy alone, compared to non-diabetic women. A weakness of the study is the lack of a uniform national definition of GDM, even though virtually all pregnant women in Sweden attend maternal health care, which includes measurements of random non-fasting blood glucose four times during pregnancy. Aberg et al.¹⁵⁵ reported an incidence of GDM of 1.2 % in a Swedish county. One explanation for the lower incidence of GDM found in the present study could be local differences in the definition of GDM in Sweden. Our data demonstrate that the risk of being born LGA concerning both length and weight was slightly higher than the risk of being born heavy for gestational age alone. However, the latter group represented by far the largest subgroup of LGA infants. In addition, we previously demonstrated that female infants born both heavy and long for gestational age were not at increased risk of being overweight at a later age and had a considerably decreased risk of becoming obese compared to female infants born heavy only.¹⁹⁹ Accordingly, being born heavy only represents the most common subgroup of LGA infants in GDM pregnancies and appears to be associated with the highest risk of disease later in life. Since variations in birth weight are explained by differences in fat mass to a greater extent than by differences in lean body mass,¹²⁰ it is reasonable to believe that infants born heavy but not long for gestational age have a proportionately larger fat mass than other newborn infants. This increase in fat mass could thus be one explanation for the increased insulin resistance seen in LGA infants already at birth. Our data suggest that fetal hyperinsulinemia during GDM has a stronger impact on birth weight than on birth length. The lack of a marked effect of maternal gestational diabetes on the birth length of the infant is noteworthy in view of the assumed role of insulin as a fetal/neonatal growth factor.⁸⁹ Infants of mothers with GDM are at risk of having an adverse neonatal outcome.⁴⁵ McFarland et al.¹⁵⁸ explained the predisposition to shoulder dystocia in infants of diabetic mothers by anthropometric differences between macrosomic infants of diabetic and non-diabetic mothers. They found a decreased head to shoulder ratio and an increased abdominal circumference in the former group. Additionally, results

obtained in a clinical setting by Vohr et al.¹⁵⁹ show that maternal GDM may lead to birth of infants with a high BMI, an increased waist circumference, and an increased fat mass. Our data confirm these findings on a nation-wide population-based level. One possible explanation for the increased risk of developing the metabolic syndrome as early as in childhood among LGA infants of mothers with gestational diabetes¹⁴⁴ might be the predisposition to disproportionate anthropometry in these infants. Being born heavy but not long indicates fat accumulation in this particular group of infants, which in turn may predispose them to metabolic disease later in life. Our findings put emphasis on the importance of optimal blood glucose regulation in GDM mothers.¹⁵⁶ Further, our epidemiological studies clearly demonstrate that it is crucial to take both birth length and birth weight of the infant into account when attempting to predict morbidity in later life. In summary, high maternal birth weight, overweight and obesity during pregnancy, and maternal diabetes predispose to a high infant birth weight.

Postnatal metabolic adaptation in LGA infants

Only limited information is available on neonatal metabolism in LGA infants. It is well known that such infants have an increased risk of developing hypoglycemia immediately after birth.¹⁴³ This and the fact that infants born LGA are at risk of developing metabolic disease later in life make it essential to investigate their postnatal metabolic adaptation. We investigated energy substrate production and insulin sensitivity during the first day of life in a group of healthy term LGA infants of non-diabetic mothers. The effect of glucagon administration on energy substrate production was also studied. One principal finding was that LGA infants have a high rate of lipolysis. As compared to AGA infants, studied previously in our laboratory at a postnatal age of 4 hours,¹³⁴ the LGA infants had an almost 50% increase in lipolysis, as reflected by production of glycerol. The same was found when the data were compared with those of Patel and Kalhan,¹³⁵ who studied full term AGA infants at a postnatal age of 21 hours, i.e., close to the age of the infants in the present investigation. It has previously been demonstrated that LGA infants have an increased proportion of body fat compared to AGA and SGA infants,^{146, 200} and this could be one factor explaining the high lipolysis. Additionally, there was a strong correlation between rate of lipolysis and infant birth weight. This is in line with recent results from our laboratory concerning infants born SGA, indicating that lipolysis depends on the amount of depot fat available in the infants.⁴² The mean glucose production rate in our study cohort was at the high end of the range reported for AGA infants.^{134, 201, 202} The rather high GPR could reflect brain size, since the mean head circumference corresponded to +1.5 SD. Glycerol is one precursor of glucose in gluconeogenesis. The proportion of glycerol that was converted to glucose accounted for only approximately 10 % of the total GPR, indicating

that glycogenolysis is probably still an important source of glucose 16 hours post partum in LGA infants. The contribution made by glycerol to glucose was smaller than that reported by Patel et al.¹³⁵ in AGA infants of the same post-natal age, but close to that found by our group in AGA infants with a postnatal age of 4 hours.¹³⁴

Insulin sensitivity at birth in LGA infants

The mean insulin level was higher in our infants than that reported earlier for term AGA infants,^{41, 134, 135, 203, 204} but lower than the level found in infants of mothers with diabetes.¹³⁷ The increased insulin levels and the comparison of the glucose/insulin ratio and HOMA indices in our infants with corresponding data for AGA infants⁴¹ indicate that LGA infants have increased insulin resistance on their first day of life, analogously to the increase in older children with overweight or obesity. In addition, the β -cell activity was higher in the LGA infants than in the AGA infants studied by Bazaes et al.⁴¹ This could be one possible explanation behind the finding that infants born LGA have an increased risk of developing type 1 diabetes.²² The underlying mechanism could be that the high β -cell activity makes the β -cell more susceptible to an auto-immune attack.

Insulin is a well known inhibitor of lipolysis in adults, but this role has been questioned in the newborn. In an earlier study from our laboratory lipolysis has been found to be unimpaired despite increased insulin concentrations in infants of diabetic mothers.¹³⁷ In contrast to this finding,¹³⁷ the present data indicate that insulin actually has a regulatory role with regard to lipolysis in newborns, since a fairly strong correlation was noted between the increase in insulin and the decrease in lipolysis following glucagon administration.

The production of IGF-I is dependent on insulin activity. It is well known that IGF-1 is important for fetal growth. Several studies have shown that the level of IGF-1 in cord blood²⁰⁵⁻²⁰⁷ correlates with birth weight, but data on IGF-1 levels during the first 24-48 hours of life are limited. Nevertheless, our LGA infants had higher IGF-1 levels than those found in AGA infants at a postnatal age of 24 hours by de Zegher et al.²⁰⁸ and Giudice et al.¹¹⁵ Further, the inverse correlation between the IGF-1 level and postnatal age observed in our study is in line with the reported decline in IGF-1 during the first days of life.^{115, 208}

Glucagon treatment of newborn LGA infants

LGA infants risk developing neonatal hypoglycemia^{143, 145} even though they have increased stores of depot fat¹⁴⁶ and liver glycogen.¹⁴⁷ Taking this into consideration, glucagon administration could be an appropriate treatment of hypoglycemia in these infants. Following administration of glucagon the blood glucose level and endogenous glucose production increased in all in-

fants studied. Data on the conversion of glycerol to glucose indicate that under these conditions glycogenolysis is stimulated more than gluconeogenesis. This is in agreement with recent findings by van Kempen et al.²⁰⁹ who studied the effect of glucagon in moderately premature (30 weeks) AGA and SGA infants. However, if glucagon is used for treatment of neonatal hypoglycemia, the risk of nausea in the treated infants has to be considered.

Conclusions

Women, who themselves are born LGA, are at increased risk of giving birth to LGA infants, compared to women not born LGA. Maternal overweight increases this risk even further.

Infants, born LGA with regard to weight alone, are at increased risk of being obese or overweight at early childbearing age.

Women with GDM have an almost four times higher risk of delivering an infant that is LGA concerning weight alone, compared to women without GDM.

In the population of women giving birth to LGA infants, those with GDM are at increased risk of delivering an LGA infant that is heavy alone, compared to women without GDM.

When predicting adult morbidity for LGA infants, it is crucial to consider both the birth length and the birth weight of the infant.

Maternal BMI and fat mass determine fetal weight. High maternal fat mass results in insulin resistance, which increases hepatic glucose production, in turn providing substrate for fetal growth.

Resting energy expenditure, which is closely related to fetal weight, was largely explained by BMI, insulin resistance, and total glucose production.

Lipolysis does not seem to be rate limiting for fetal growth in the studied group of women.

Infants born LGA have a high rate of lipolysis and a propensity to decreased insulin sensitivity at birth, as compared to AGA infants.

Glucagon administration increases blood glucose and glucose production in newborn LGA infants. The correlation between the increase in plasma insulin and the decrease in lipolysis following administration of glucagon indicates an antilipolytic effect of insulin in newborn LGA infants.

Future studies

The findings presented in this thesis give rise to several new questions related to fetal growth. Further studies of energy substrate production, insulin resistance, and resting energy expenditure in other groups of pregnant women, e.g. women with gestational diabetes mellitus (GDM), may provide additional information on important determinants of fetal growth. Such information might have implications in neonatology, for example by indicating ways of improving growth of the preterm infant.

Another important issue is whether treatment of women with GDM protects their infants from perinatal complications and later metabolic disease. This question is being further addressed in an ongoing study.

In this thesis female infants, born LGA for weight alone, were shown to be at risk of developing overweight or obesity at early childbearing age. This finding gives reason to investigate the relation between neonatal body composition and later metabolic disease.

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