Perinatal Energy Substrate Metabolism

Glucose Production and Lipolysis in Pregnant Women and Newborn Infants with Particular Reference to Intrauterine Growth Restriction (IUGR)

BARBRO DIDERHOLM
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**Abstract**


Glucose is the most important fetal nutrient and the production of this substrate increases in the pregnant woman. In the last trimester the increased insulin resistance directs energy substrates to the fetus. Fetal growth is sometimes disturbed, often without an obvious explanation.

After birth the newborn infant must produce its own glucose, primarily for the brain. Fatty acids from lipolysis are also important energy substrates. Hypoglycaemia can be a problem, occurring frequently in preterm infants and infants born small for gestational age (SGA). In addition, SGA infants are at risk of developing the metabolic syndrome in adulthood. Neonatal medication can influence energy metabolism. One such medication is theophylline, administered in preterm infants to prevent apnoea.

We investigated energy substrate production in women with normal and IUGR pregnancies, in preterm neonates, before and after theophylline treatment and in newborn SGA infants, using stable isotope-labelled compounds and gas chromatography-mass spectrometry.

We found that late pregnancy was associated with an almost twofold increase in the rate of lipolysis. This provides substrates for maternal energy metabolism, which may spare glucose for the fetus. Even though glucose production was comparable in the two groups of pregnant women, those with IUGR had a lower rate of lipolysis. A reduced supply of energy substrates could be one factor underlying IUGR. In spite of the insulin resistance of late pregnancy, insulin still had a regulatory role in energy substrate production in the women with normal pregnancies, but not in those with IUGR.

Although infants born preterm and/or SGA have limited energy stores, we demonstrated that they are capable of both lipolysis and glucose production. Theophylline had no adverse effects on energy substrate production. Data on insulin and IGFBP-1 in the SGA infants indicate that in such infants insulin sensitivity is increased peripherally but reduced in the liver.

**Keywords**: Lipolysis, Glucose, Newborn infant, Pregnant women, IUGR, Insulin, Stable isotopes

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urn:nbn:se:uu:diva-4842 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-4842)
To my,
beautiful, beloved daughter Agnes,
being born both preterm and small,
now growing, arguing and understanding.
List of papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I  Diderholm B, Stridsberg M, Lindeberg-Nordén S, Ewald U, Gustafsson J. Increased lipolysis in non-obese pregnant women studied in the third trimester. BJOG 2004 (online publication date 17-Dec-2004).

II Diderholm B, Stridsberg M, Nordén-Lindeberg S, Gustafsson J Decreased maternal lipolysis in intrauterine growth restriction (IUGR) in the third trimester. Submitted


IV Diderholm B, Ewald U, Ahlsson F, Gustafsson J Energy substrate production in infants born small for gestational age (SGA). Manuscript
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Cover: An embryo in the womb, 1512 by Leonardo da Vinci.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic AMP</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>FABP</td>
<td>Fatty acid binding protein</td>
</tr>
<tr>
<td>GCMS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GH</td>
<td>Pituitary growth hormone</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transporter 1</td>
</tr>
<tr>
<td>GPR</td>
<td>Glucose production rate</td>
</tr>
<tr>
<td>HCG</td>
<td>Human chorionic gonadotrophin</td>
</tr>
<tr>
<td>hPGH</td>
<td>Human placental growth hormone</td>
</tr>
<tr>
<td>HPL</td>
<td>Human placental lactogen</td>
</tr>
<tr>
<td>IDM</td>
<td>Infant of diabetic mother</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein 1</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
</tr>
<tr>
<td>kcal</td>
<td>Kilocalories 1 kcal=4.2 kilojoule</td>
</tr>
<tr>
<td>MJ</td>
<td>Megajoule</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>Ra</td>
<td>Rate of appearance</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age (birth weight &lt;-2 SD)</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Transforming growth factor-α</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
</tr>
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</table>
Introduction

During pregnancy the growth of the fetus is dependent on a continuous flow of nutrients from the mother. Glucose is the most important energy substrate in the growing fetus, followed by amino acids, lactate and to a lesser extent non-esterified fatty acids (NEFA). Most of the glucose is used by the relatively large fetal brain. Glucose is transported across the placental barrier through facilitated diffusion mediated by glucose transporter 1 (GLUT1). Pregnant women increase their glucose production during pregnancy to meet the demands of the growing fetus. The third trimester is characterised by increasing insulin resistance. This together with the insulin antagonistic and lipolytic effects of pregnancy-specific hormones promotes the utilisation of fatty acids as energy substrates for the mother, saving glucose and amino acids for the fetus. Fetal growth is sometimes disturbed. Factors underlying intrauterine growth restriction (IUGR) can be of maternal, placental or fetal origin. Often, however, there is no obvious explanation.

Birth terminates the flow of energy substrates from the mother. Thus, mobilisation of endogenous energy sources in the infant is necessary until breastfeeding is established. Immediately after birth the infant’s glucose concentration falls, resulting in a decrease in the insulin level and an increase in counter-regulating hormones, mainly catecholamines and glucagon. These changes stimulate glycogenolysis and gluconeogenesis and also lipolysis (Fig.1). In addition, the increase in the circulating level of thyroid-stimulating hormone (TSH) directly after birth is a strong stimulus for lipolysis.

Preterm infants have small substrate stores and may have immature hormone and enzyme systems. Together, this can result in insufficient energy substrate production. The immature glucose homeostasis can also lead to hyperglycaemia. Recurrent perinatal hypo- or hyperglycaemia increases the risk of adverse neurological development.

There are drugs used neonatally which influence the metabolism of glucose and lipids. One such drug is theophylline, which is often administered in preterm infants to prevent apnoea of prematurity.

Small for gestational age (SGA) infants have reduced stores of glycogen and fat, with a consequently increased risk of hypoglycaemia. There is also evidence for increased glucose consumption due to the relatively large brain:body mass ratio. In addition, infants born SGA may, like preterm
infants, have delayed maturation of the gluconeogenic pathways. There are also reports of a defective counter-regulation of hypoglycaemia and occurrence of hyperinsulinism. The association between being born SGA and the development of the metabolic syndrome later in life raises questions about perinatal energy metabolism and its regulation.

Figure 1. Pathways for glucose metabolism (NEFA = non-esterified fatty acids).
Background

Fetal growth

During the first phase of pregnancy the growth of the embryo is autonomous and is controlled by oncogenes and growth factors such as, transforming growth factor-α (TGF-α) and epidermal growth factor (EGF).\textsuperscript{26} Embryonic growth is achieved mainly by an increase in the number of cells. Fetal growth first takes place by cell division and also by hyperplasia and later mainly by an increase in cell size.\textsuperscript{27} The growth of the fetus relies upon the nutrient and oxygen supply as well as on insulin, insulin-like growth factor-I (IGF-I) and other growth factors.\textsuperscript{26} In the second trimester differentiation of the organs takes place. The growth in length is most pronounced during this period and has its peak in the 20\textsuperscript{th} week of gestation (Fig. 2). During the third trimester there is a marked increase in weight, mainly through accretion of fat and proteins.\textsuperscript{28} The size of the fat depots, which depends on the availability of maternal energy substrates and the fetal response to their transfer, is one of the determinants of birth weight.\textsuperscript{28}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Weight and crown-rump length during human development. (Data from Moore KL, Persaud TVN: The developing human: Clinically oriented embryology, 6\textsuperscript{th} ed. Philadelphia, Saunders, 1998)}
\end{figure}
Energy metabolism in pregnancy

Energy balance

Pregnancy is dependent on maternal adaptation of nutrient metabolism as well as on anatomical and physiological changes. The estimated energy cost of pregnancy, including the resting metabolic rate, gain in fat and fat-free mass, is approximately 336 MJ (80 000 kcal), but there are large inter-individual differences. These differences depend on the food intake, intensity of physical activity and the amount of fat deposition. During pregnancy a healthy woman gains about 13 kg, which is accounted for by the fetus, placenta, growth of the uterus including the amnion, fat accumulation and an increased amount of body fluid (Table 1).

There is an anabolic phase early in pregnancy in which maternal deposition of fat takes place to prepare for the supply to the fetus both in late pregnancy and during lactation. In late pregnancy the maternal lipid metabolism changes to a catabolic phase, which parallels the maximal growth of the fetus. During pregnancy energy expenditure increases gradually as a result of the augmented metabolism of the uterus, the fetal metabolism and the increased maternal work load from the circulation and breathing.

Table 1. Mean energy cost of pregnancy

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Energy Cost (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>3500</td>
</tr>
<tr>
<td>Placenta</td>
<td>600</td>
</tr>
<tr>
<td>Uterus, fluids, breasts</td>
<td>5000</td>
</tr>
<tr>
<td>Maternal fat</td>
<td>4000</td>
</tr>
<tr>
<td>Basal metabolic rate</td>
<td>—</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13100</td>
</tr>
</tbody>
</table>

Modified from Durnin

Hormonal changes

Hormones which maintain pregnancy and influence maternal metabolism are secreted initially from the corpus luteum and subsequently from the placenta. Human chorionic gonadotrophin (HCG) preserves the corpus luteum until the placenta is able to support the pregnancy. Human placental lactogen (HPL), a hormone similar in structure to growth hormone, has effects on glucose and lipid metabolism and increases the maternal appetite. Oestrogens influence the reproductive organs, including the uterus and the mammary glands, and enhance the blood flow in the uterine artery, leading to an increased nutritional flow to the fetus. In addition, oestrogens influence the lipid and carbohydrate metabolism.

The role of progesterone is mainly to relax smooth muscle and modulate the immune response, but it also influences energy metabolism. Placen-
tal growth hormone (hPGH) replaces pituitary growth hormone (GH) in mid-gestation.\textsuperscript{35} In contrast to GH, hPGH is not secreted in a pulsatile manner.\textsuperscript{36} The effects of hPGH are only exerted in the mother, and result in stimulation both of glucose production and lipolysis. These effects are most pronounced in late gestation.\textsuperscript{35, 37}

In spite of normal or improved insulin sensitivity the insulin secretion is increased following a glucose load in early pregnancy.\textsuperscript{4, 38} This increase is at least partly due to the influence of oestrogen.\textsuperscript{39} Together with an increased appetite and the influence of cortisol and progesterone, the enhanced insulin secretion results in stimulation of lipogenesis and deposition of maternal body fat.\textsuperscript{39, 40}

As pregnancy proceeds, insulin resistance gradually develops both peripherally and in the liver.\textsuperscript{7} Euglycaemic-hyperinsulinaemic clamp studies have shown a reduction of the peripheral insulin sensitivity in late pregnancy of up to 50\%.\textsuperscript{4} Many possible factors underlying the insulin resistance have been investigated. It has been suggested, for example that increasing levels of hormones including HPL, hPGH, prolactin, cortisol, progesterone and oestrogens decrease insulin sensitivity.\textsuperscript{34, 39, 41} Other contributory factors could be elevated levels of NEFA\textsuperscript{12, 42} and tumour necrosis factor-\alpha (TNF-\alpha).\textsuperscript{43} The decreased insulin sensitivity in late pregnancy directs glucose to the fetus.\textsuperscript{6, 7}

Animal studies have shown that, the fetal production of IGF-I is stimulated by insulin and indirectly by the availability of glucose.\textsuperscript{44} There is a correlation between IGF-I levels in cord blood and infant size at birth.\textsuperscript{45} The effect of IGF-I is modulated by insulin-like growth factor binding protein-1 (IGFBP-1), the production of which is inhibited by insulin.\textsuperscript{46, 47} There is placental production of IGF-I directed towards the maternal circulation, with increasing levels during progression of pregnancy.\textsuperscript{37, 48} Leptin levels increase during pregnancy, with a peak between 22 and 27 weeks of gestation. These levels are correlated to gestational weight gain, but not to birth weight.\textsuperscript{40}

**Energy substrates**

The most important energy substrate in the fetus is glucose (Fig. 3).\textsuperscript{27} Most of the fetal carbon comes from glucose\textsuperscript{49} and the fetal brain is an obligate glucose user.\textsuperscript{1} The estimated fetal glucose requirement in the third trimester will be covered by a 14\% increase in maternal glucose production.\textsuperscript{39} Several studies have shown that the rate of maternal glucose production in late gestation increases by 16-30\%\textsuperscript{3-5} in order to meet the needs of the fetus and placenta.\textsuperscript{1} Studies in sheep have shown that of the glucose consumed by the placenta, one-third is metabolised to lactate,\textsuperscript{50} part of which can be efficiently used by the fetus.\textsuperscript{1, 51}
The insulin resistance of late pregnancy is responsible for the increase in glycogenolysis and gluconeogenesis. Kalhan et al.\(^5\) reported that total glucose production and gluconeogenesis increase with advancing gestation. The most important gluconeogenic substrates are alanine, pyruvate, lactate and, though to a smaller extent, glycerol. In non-pregnant women only \(~4\%\) of the plasma glucose pool comes from glycerol.\(^52\)

Amino acids are used mainly for protein synthesis, but can also be utilised for gluconeogenesis.

The hormones underlying the insulin resistance in late pregnancy also enhance lipolysis.\(^34\) During fasting in the third trimester there is a more rapid shift from the use of carbohydrates to lipids as compared to that in non-pregnant women.\(^10\) The increased rate of lipolysis provides additional substrates for maternal energy metabolism, saving glucose and other substrates for the fetus.\(^12, 53\) In the fasting situation, ketone bodies are formed by \(\beta\)-oxidation of NEFA. The ketone bodies can be used both as energy substrates and for lipid synthesis by the fetus.\(^31\)

**Figure 3.** Energy substrates transported to and deposited in the fetus (NEFA=non-esterified fatty acids) Modified from ref.\(^27\)

**Placental transport of energy substrates**

Placental transport is dependent on the maternal-fetal gradient of energy substrates, the blood flow in the uterine artery and the density of specific
transport molecules, as well as on the area of the maternal-fetal interface.\textsuperscript{51} Glucose is transported from the mother to the fetus by facilitated diffusion via GLUT1.\textsuperscript{2} The rate of glucose transport via GLUT1 is insulin-independent and is determined by the gradient between the mother and the fetus. The fetal glucose level corresponds to \(~75\%\) of that in the mother.\textsuperscript{54}

Amino acids are actively transported through transport proteins, resulting in a fetal-maternal gradient.\textsuperscript{55}

The transport of lipids across the placenta is limited. NEFA in general cross the placenta by simple diffusion, but essential fatty acids are transported actively via fatty acid binding proteins (FABPs).\textsuperscript{56} In early gestation embryonic and fetal lipids originate in maternal NEFA, which are transported across the placenta, but in late pregnancy most of the fetal fat stores are synthesised from glucose.\textsuperscript{28} The transport of glycerol (a product of lipolysis) is also limited, but this can be used as a substrate for gluconeogenesis in the mother.\textsuperscript{31} In the fasting situation maternal ketone bodies are readily transported to the fetus.\textsuperscript{57}

Factors modifying the growth of the fetus

Constitutional factors such as parental size and intergenerational effects of maternal birth weight have been found to be related to fetal growth.\textsuperscript{58} Further, studies have shown that socio-economic factors, for example poor surroundings, are associated with low birth weight.\textsuperscript{27, 59} Demographic factors are also known to influence the growth of the fetus.\textsuperscript{60} Maternal factors with an impact on fetal growth may include nutritional status, parity and chronic disease.\textsuperscript{27} Fetal factors are often related to chromosomal anomalies or genetic syndromes leading to growth restriction. However, some rare syndromes lead to increased fetal growth (e.g. Beckwith-Wiedemann and Sotos’s syndrome). In pregnancies complicated by diabetes mellitus, increased placental transport of glucose may lead to fetal macrosomia.\textsuperscript{61}

Intrauterine growth restriction

The concepts intrauterine growth restriction and small for gestational age are related but not synonymous. In IUGR there is a reduction in the expected fetal growth rate as judged by repeated ultrasound examinations. However, restricted fetal growth may not always result in an infant born SGA. Small for gestational age is a description of an infant whose birth weight is lower than normal in relation to the length of gestation. Different definitions have been used in the literature (<2SD, <5\% or <10\% percentile). In this thesis the definition used is <2 SD of the Swedish fetal growth chart,\textsuperscript{62} corresponding to approximately <3\% percentile. This definition is used clinically for the diagnosis of SGA in Sweden.
The incidence of IUGR is dependent on the population examined. Approximately 4-8% of newborn infants in industrialised countries and 6-30% in developing countries are judged as growth-restricted. Of all infants weighing less than 2500 g approximately one third are considered to have IUGR.

Among factors proven to cause IUGR, maternal malnutrition is the most common worldwide, but there are many other factors that can explain the condition (Table 2). In 40% of the cases no apparent underlying factor is found.

Table 2. Factors underlying fetal growth restriction

**Maternal**
- Demographic
- Socio-economic
- Intergenerational (low maternal birth weight)
- Malnutrition
- Chronic disease
- Hypoxaemia (high altitude)
- Drugs
- Uterine constraint

**Placental**
- Defective placentation
- Placental dysfunction (pre-eclampsia, infarctions, bleeding, reduced area)
- Placental infection

**Fetal**
- Chromosomal disorders
- Congenital anomalies
- Fetal infections

**Birth**
Birth terminates the continuous supply of nutrients from the mother and for the infant there is a change to intermittent feeding. Before breastfeeding is established, the newborn infant has to mobilise its energy depots. A term infant with a birth weight appropriate for gestational age (AGA) has glycogen sufficient for 10-12 hours of fasting and the fat stores can theoretically last for several weeks, but in preterm infants and infants born SGA the energy depots are diminished. Reduced energy depots may also be a consequence of anaerobic metabolism following asphyxia.
Postnatal metabolic adaptation

The fall in blood glucose in the newborn infant immediately after birth alters the insulin/glucagon ratio and this, together with the catecholamine surge at birth, mobilises stored energy substrates (Fig. 4). A healthy newborn infant produces $\approx 4 - 6 \text{mg kg}^{-1} \text{min}^{-1}$ of glucose (adult $\approx 2 \text{mg kg}^{-1} \text{min}^{-1}$), of which the major part is used by the brain. At first, glucose is provided from liver glycogen, but later, additional glucose is produced by gluconeogenesis mainly from lactate, pyruvate and alanine. Lipolysis is stimulated by catecholamines and the postnatal rise of TSH, together with an increasing level of cortisol and a decrease in the insulin level. The products of lipolysis support gluconeogenesis; glycerol as a precursor of glucose and NEFA by providing energy through $\beta$-oxidation. In addition, NEFA is a precursor of ketone bodies, which are important alternative energy substrates for the brain of the newborn infant.

Human milk has a high fat and low carbohydrate content, thus contributing to the higher levels of ketone bodies seen in breast-fed infants. Hence, there is a shift in the use of energy substrates from predominantly glucose in the fetus to lipids in the newborn infant.

Preterm infants

Most of the energy depots of the newborn infant are accumulated in late gestation. This period of intrauterine life is shortened or even absent in infants born preterm. In these infants the regulation of energy substrate production may be immature. Further, the energy utilisation is attenuated in response to increased thermoregulatory demands and possibly also to enhanced breathing efforts due to respiratory distress. An adequate energy supply is therefore critical in the immediate postnatal period. Even though these infants are at risk of hypoglycaemia, parenteral nutrition often results in hyperglycaemia. Factors that may promote hyperglycaemia include an immature response to a glucose load, with reduced insulin levels or failure to inhibit hepatic glucose production. Stress induced by respiratory failure and sepsis increases the circulating levels of catecholamines, which further stimulate glucose release. Previous studies by our group have shown that extremely preterm infants are capable both of glucose production and lipolysis. In addition they have a capacity for gluconeogenesis.
Drugs with metabolic effects used in the perinatal period

Several drugs used in the perinatal period may have potential effects on energy metabolism. Maternal antihypertensive treatment such as α- and β-blockers, as well as antenatal steroids given to increase lung maturation, have such effects.\(^ {75, 76}\) Theophylline is a drug commonly used in preterm infants as prophylaxis and treatment of apnoea.\(^ {79}\) Besides having effects on bronchial smooth muscle\(^ {77}\) and the ventilatory drive,\(^ {78}\) theophylline also influences lipolysis\(^ {79}\) and metabolism of glucose.\(^ {18}\) Toxic doses given to dogs resulted in hyperglycaemia, an effect which was probably mediated through stimulation of β-adrenergic receptors, since it could be blocked by administration of propranolol.\(^ {78}\) In addition, theophylline has a stimulatory effect on glycogenolysis, gluconeogenesis and lipolysis by inhibition of cAMP-phosphodiesterase, thus increasing the levels of cAMP.\(^ {80}\) However, to achieve this stimulatory effect the concentration has to be 3 to 10 times higher than that normally found in preterm infants treated with theophylline.\(^ {81, 82}\) Administration of theophylline in adults has been reported to increase lipolysis without affecting the level of plasma glucose, insulin, catecholamines or growth hormone.\(^ {79}\) In a study of preterm infants receiving chronic theophylline treatment, no effect on energy substrate production was observed.\(^ {53}\)
Small for gestational age infants

Infants born SGA are at increased risk of developing hypoglycaemia neonatally. Several reasons for this have been suggested, including small energy stores, a high brain:body weight ratio, delayed maturation of the gluconeogenic pathways and/or neonatal hyperinsulinaemia. Studies on the rate of glucose production (GPR) in infants born SGA have shown that they have rates in the lower normal range, as compared to AGA infants, but data on the inhibitory effect of glucose infusion on GPR differ between the studies. Further, low levels of glucose and lack of a relation between glucose and insulin have been found in newborn SGA infants. However, Bazaes et al. observed a correlation between glucose and insulin in a large cohort of infants born SGA, studied at 48 hours of age.

Data related to lipolysis in newborn SGA infants are somewhat contradictory and there is only limited information available on the rate of glycerol production in these particular infants. Several studies have shown that IGF-I levels are reduced and IGFBP-1 levels are increased in these infants, probably as a result of the reduced levels of insulin and glucose.

Consequences of disturbed perinatal energy metabolism

Intrauterine growth restriction is associated with an increased risk of fetal and neonatal mortality and morbidity, including prenatal still-birth and intrapartum asphyxia, with later neurological sequelae. Neonatal morbidity also includes hypo- and hyperglycaemia, thermoregulatory problems and an increased risk of infections.

In addition to neonatal morbidity, infants born SGA are at risk of developing disease later in life. During childhood 10% of children born SGA will fail to have a catch-up growth, resulting in short adult stature. There is also an increased risk of developing mild cognitive and behavioural problems, as well as learning difficulties.

Much interest has been focused on long-term effects of fetal growth restriction during the last decade. The “fetal programming” hypothesis states that the fetus adapts to limited nutritional support. These adjustments, however, may have metabolic consequences later in life. First an association was found between being born SGA and having cardiovascular disease in adulthood. Later, relations between SGA and insulin resistance, glucose intolerance, dyslipidaemia and hypertension, all parts of the metabolic syndrome, were described. Recent data have shown that some of these metabolic alterations already occur at early school age. However, little is known about the metabolic situation of the newborn SGA infant.
Aims of the studies

The overall aim of these studies was to investigate the energy substrate production in the perinatal period.

The specific aims were:

- to study lipolysis and glucose production during the third trimester in women with normal pregnancies (study I).
- to determine whether the maternal energy substrate production is impaired in pregnancies complicated by IUGR (study II).
- to study glucose production and lipolysis in preterm infants (≤ 32 weeks of gestation) and the effect of initiation of theophylline therapy (study III).
- to assess the capacity for glucose production and lipolysis in newborn SGA infants in relation to regulating hormones (study IV).
Subjects

The studies in this thesis were approved by the Human Ethics Committee of the Medical Faculty of the University of Uppsala, Sweden. Consent to participation was obtained from the pregnant women and from the parents of the infants after they had received oral and written information. Gestational ages were estimated by ultrasound examination (week 16 – 18). Study I was performed at Uppsala University Children’s Hospital, between the years 2001 and 2002. Study II was carried out at the Department of Obstetrics and Gynaecology at Uppsala University Hospital, from 2001 to 2004. All infants in studies III and IV were admitted to the neonatal unit at Uppsala University Children’s Hospital. The infants in study III were recruited from 1995 to 1998 and those in study IV from 2000 to 2004.

Pregnant women, studies I and II

**Study I**
Eight healthy, non-smoking women, mean age 33±4.8 years, with normal pregnancies were investigated following an overnight fast. The women were recruited among pregnant women in the staff working at the Uppsala University Hospital (Table 3). The length of gestation at the time of the study was 34.6 ± 0.9 weeks.

**Study II**
Ten healthy, non-smoking pregnant women with diagnosed IUGR were studied following an overnight fast. The women had a mean age of 31 ± 4.9 years. They were recruited from consecutive patients attending the antenatal care centre at the Uppsala University Hospital because of diagnosed IUGR. The diagnosis was made by repeated ultrasound measurements; these were considered normal at the first routine examination at 16 weeks, but later showed reduced fetal weight gain. The length of gestation at the time of the study was 35.4 ± 1.6 weeks (Table 3).
Table 3. Maternal, fetal and infant characteristics in studies I and II

<table>
<thead>
<tr>
<th>Preganancies</th>
<th>Study I</th>
<th></th>
<th>Study II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal n=8</td>
<td>IUGR n=10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (y)</td>
<td>33.3</td>
<td>4.8</td>
<td>31.4</td>
<td>4.9</td>
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<tr>
<td>Height (cm)</td>
<td>167.9</td>
<td>4.2</td>
<td>165.8</td>
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<tr>
<td>Pre-pregnancy weight (kg)</td>
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<td>7.8</td>
<td>57.4</td>
<td>4.7</td>
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<tr>
<td>Pre-pregnancy BMI</td>
<td>22.3</td>
<td>2.3</td>
<td>20.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Weight gain at study (kg)</td>
<td>12</td>
<td>3.2</td>
<td>9</td>
<td>4.7</td>
</tr>
<tr>
<td>BMI at study</td>
<td>26.5</td>
<td>2.9</td>
<td>24.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Length of gestation at study (w)</td>
<td>34.6</td>
<td>0.9</td>
<td>35.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Fetal weight deviation (%)</td>
<td>3</td>
<td>10</td>
<td>-32***</td>
<td>7.3</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3708</td>
<td>535</td>
<td>2250***</td>
<td>340</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>51.3</td>
<td>1.8</td>
<td>45.9***</td>
<td>2.1</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.9</td>
<td>1.9</td>
<td>32.2**</td>
<td>1.5</td>
</tr>
<tr>
<td>Gestational age at birth (w)</td>
<td>40.4</td>
<td>1.2</td>
<td>38.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>614</td>
<td>150</td>
<td>387**</td>
<td>73</td>
</tr>
</tbody>
</table>

** p<0.01, *** p<0.001

Newborn infants, studies III and IV

Study III

In this study ten preterm infants were investigated. Their gestational ages at birth were ≤32 weeks and their birth weights >900g. The postnatal age at the time of the study was 39±23 hours (Table 4). The mothers were healthy before pregnancy, but two of them developed pre-eclampsia, one ablatio placentae, one placenta praevia and one endometritis. Eight of the infants were delivered by caesarean section and two vaginally. Enteral feeds with breast milk, 2 mL · kg⁻¹, were given every second to third hour, in some cases combined with parenteral nutrition.
Table 4. *Infant characteristics in study III*

<table>
<thead>
<tr>
<th>Study III</th>
<th>Preterm infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=10)</td>
<td>mean</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1276</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>39</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>29</td>
</tr>
<tr>
<td>Gestational age at birth (w)</td>
<td>29</td>
</tr>
<tr>
<td>Postnatal age at study (h)</td>
<td>39</td>
</tr>
<tr>
<td>Duration of fast (h)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Study IV**

Eleven healthy newborn SGA infants with a gestational age at birth of 35±3 weeks and a birth weight of 1800±500 g were studied at a mean age of 24 h. Nine of the eleven infants were also short for gestational age (Table 5). The mothers were healthy prior to pregnancy and none of them smoked. Five mothers developed pre-eclampsia with hypertension in late pregnancy. Ten of the infants were born by caesarean section and one by vaginal delivery. The infants were fed 3.6 mL kg⁻¹ h⁻¹ of breast milk every second to third hour.

Table 5. *Infant characteristics in study IV*

<table>
<thead>
<tr>
<th>Study IV</th>
<th>Preterm and term infants born SGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=11)</td>
<td>mean</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1804</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>43</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>30.6</td>
</tr>
<tr>
<td>Gestational age at birth (w)</td>
<td>35.4</td>
</tr>
<tr>
<td>Postnatal age at study (h)</td>
<td>24.4</td>
</tr>
<tr>
<td>Duration of fast (h)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

SGA=small for gestational age
Methods

Rates of glycerol and glucose production were determined by the use of stable isotope-labelled compounds and analysis of isotope dilution by gas chromatography-mass spectrometry – GCMS. The rate of glycerol production reflects the rate of lipolysis. The products of lipolysis from triacylglycerols (triglycerides) are one molecule of glycerol and three molecules of fatty acids (NEFA) (Fig. 5). There is no re-esterification of glycerol and NEFA in adipose tissue.52

\[
\begin{align*}
  &\text{Triacylglycerol} \\
  \rightarrow &\text{Lipase} \\
  \rightarrow &\text{Glycerol} + 3 \text{H}_2\text{O} \\
  \rightarrow &\text{NEFA}
\end{align*}
\]

**Figure 5.** Lipolysis of triacylglycerol to glycerol and NEFA (NEFA=non-esterified fatty acids).

Stable isotope dilution technique

Isotopes are chemically identical atoms with different numbers of neutrons, resulting in changed atomic weights. Stable isotopes are not radioactive and occur naturally in small amounts. A molecule labelled with a stable isotope is usually metabolised in the same way as the corresponding unlabelled molecule. Hence, the labelled compound can be used to trace the unlabelled compound of interest.104 After a constant-rate infusion of a stable isotope-labelled compound (tracer), this will gradually equilibrate with the corresponding endogenous compound (tracee). When the plasma concentration
and the isotopic enrichment of the compound are close to constant, an approximate steady state situation prevails.

GCMS can be used to identify labelled and unlabelled forms of a compound in biological samples. The GCMS technique is a sensitive, specific and precise method that is applicable for metabolic research. Small amounts of a substrate can be measured, making small sample sizes possible. Since stable isotope tracers are non-radioactive, studies of this kind are ethically acceptable in humans irrespective of age.

Analysis by gas chromatography-mass spectrometry

The isotopic enrichment of compounds labelled with stable isotopes can be analysed by GCMS. After precipitation of plasma proteins in the sample, “derivatisation” results in the formation of a volatile complex of the molecule of interest. The derivatised molecule is injected into the gas chromatograph (GC) and vaporised at a high temperature and is then transported by a carrier gas through the GC column to the mass spectrometer. In the capillary column, the derivatised molecule is separated from other molecules in the sample by temperature-regulated interaction between the stationary phase and the molecule of interest. After the separation in the GC column the molecules are transferred to the ion source of the mass spectrometer. The neutral molecule is then ionised by bombardment either with electrons (EI – electron impact) or by protonation in a gas phase (CI – chemical ionisation). Depending on the properties of the molecule and the ionisation method, the ionised molecule either remains intact or disintegrates into fragments. This is followed by separation, by a magnetic field based on mass over charge ratio (m/z). A detector then records the amount of ions corresponding to labelled and unlabelled compounds (Fig. 6). From this ratio the isotopic enrichment of the compound is calculated.

Figure 6. Diagram of a GCMS computer system (modified after Smith R M, Busch K L: Understanding mass spectra : A basic approach, New York, Wiley, 1999.)
Materials

Stable isotope labelled compounds

The tracers used in studies I and II were [6,6-²H₂]-glucose (isotopic purity 98 atom %) and [1,1,2,3,3-²H₅]-glycerol (isotopic purity 98 atom %). In studies III and IV the tracers used were [6,6-²H₂]-glucose (isotopic purity 98 atom %) and [2-¹³C]-glycerol (isotopic purity 98 atom %). In addition, an internal standard of [1,1,2,3,3-²H₅]-glycerol (isotopic purity atom 98%) was used to quantify the plasma glycerol concentration in studies III and IV. All tracers were purchased from Cambridge Isotope Laboratories, Woburn, MA, USA. The solutions were sterile in microbiological cultures and pyrogen-free when tested by the Limulus lysate method.¹⁰⁶

Experimental design

The tracers were administered intravenously as primed constant rate infusions by a volumetric pump (IMED 965 micro, IMED, Oxford, England). The blood samples were obtained from a second peripheral vein catheter or in some of the infants in study III from an umbilical artery catheter. All patients in study III and 6/11 patients in study IV received a glucose infusion in order to avoid hypoglycaemia. First, a blood sample for analysis of natural isotopic abundance was taken. After 60 min of tracer infusion blood samples were obtained every 10 or 15 min. In studies I, II and IV 7 samples were collected, and in study III 9 samples were taken. Plasma was immediately separated by centrifugation and frozen at -70°C. In study III theophylline, 6 mg · kg⁻¹, was administered in the middle of the sampling period.

Chemical procedures

In studies I and II plasma glucose and in study IV blood glucose concentrations were determined by the glucose oxidase method (ABL 735, Radiometer, Denmark). In study III plasma glucose concentrations were measured directly in each sample by the glucose oxidase/peroxidase method in an Ames Minilab 1 glucose analyser (Bayer AG, Leverkusen, Germany). Hormonal analyses in studies I and II were performed with commercial kits (Autodelfia, Wallac Oy, Turku, Finland and Modular, Roche Diagnostics, Bromma, Sweden), and Chromogranin A was measured by a competitive radioimmunoassay¹⁰⁷ at the Department of Clinical Chemistry, University Hospital, Uppsala. A radioimmunoassay technique was used to measure insulin¹⁰⁸ and glucagon (kit RB 310, Euro-diagnostica AB, Medeon, Malmö, Sweden) in study IV. The analyses were performed at the Department of
Endocrinology, Karolinska University Hospital, Stockholm, Sweden. Plasma glycerol was quantitated by a UV method (Enzytec Glycerol no. 1 002 809, Diffchamb AB, Gothenburg, Sweden) in studies I and II, and by GCMS using an internal standard of [1,1,2,3,3-2H5]-glycerol in studies III and IV.

Gas chromatography-mass spectrometry
The pentaacetate derivative of glucose and the triacetate derivative of glycerol were prepared after precipitation of plasma proteins with acetone. The analyses were made on a Finnigan SSQ 70 mass spectrometer (Finnigan MAT, San José, CA, USA) equipped with a Varian 3400 gas chromatograph (Varian Associates Inc, Sunnyvale, CA, USA) with a non-polar (DB 1) capillary column (15,000 x 0.25 mm, film thickness 0.25 µm), later changed to an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with the same kind of capillary column. Chemical ionisation was performed with methane. The ions monitored had an m/z of 331 (M), 332 (M+1) and 333 (M+2) for glucose and m/z 159 (M), 160 (M+1) and 164 (M+5) for glycerol.

Calculations
The isotopic enrichments of glucose and glycerol obtained during periods of approximate steady state can be used to calculate the turnover rate of these substrates. Glucose production rate and rate of glycerol production are calculated as follows: GPR = (i x 100/IR) - glucose infusion rate; rate of glycerol production = (i x 100/IR), where i is the infusion rate of the tracer, and IR is the isotopic ratio of the tracer in plasma [given as labelled (tracer)/unlabelled substrate in %].109 Glucose Rate of appearance (glucose Ra) = GPR + rate of administration of unlabelled and labelled glucose. The fraction of glycerol converted to glucose and the fraction of glucose derived from glycerol were calculated from 13C-enrichment of glucose as described by Bougnères et al.110 and Patel and Kalhan.91

Statistical analyses
The data are presented as mean ± standard deviation (SD) or, if not normally distributed, as median and range. Independent samples t-test and correlation analyses with Pearson’s correlation two-tailed test were performed with the SPSS program (LEAD Technologies, Inc, Chicago, IL). Correlations and differences were considered significant at p <0.05.
Results

Pregnant women, studies I and II

There were no differences in anthropometric characteristics between the women with normal pregnancies and those with IUGR. However, there were differences in placental, fetal and infant size (Table 3). Plasma concentrations of glycerol and glucose averaged $110\pm2 \text{ µmol} \cdot \text{L}^{-1}$ and $4.2\pm0.16 \text{ mmol} \cdot \text{L}^{-1}$ in the women with normal pregnancies and $170\pm49 \text{ µmol} \cdot \text{L}^{-1}$ and $4.0\pm0.3 \text{ mmol} \cdot \text{L}^{-1}$ in the women with IUGR. The plasma level of glycerol was significantly higher in the IUGR group ($p=0.008$).

In the women with normal pregnancies the mean rate of glycerol production, reflecting lipolysis, was $3.06\pm0.66 \text{ µmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and in those with IUGR $2.36\pm0.58 \text{ µmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($p=0.030$). GPR did not differ between the groups (Table 6). In the women with normal pregnancies there was a correlation between the rates of glycerol and glucose production ($r=0.75$, $p=0.033$) (Fig.7). This relationship was not found in the women with IUGR.

Table 6. Concentrations and production rates of glucose and glycerol in the women of studies I and II

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Study I</th>
<th></th>
<th>Study II</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean SD</td>
<td>mean SD</td>
<td>mean SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-glucose (mmol \cdot \text{L}^{-1})</td>
<td>4.20 0.16</td>
<td>4.40 0.30</td>
<td>4.00 0.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose production rate (µmol \cdot \text{kg}^{-1} \cdot \text{min}^{-1})</td>
<td>13.2 1.5</td>
<td>12.1 1.5</td>
<td>13.2 1.5</td>
<td>0.120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-glycerol (µmol \cdot \text{L}^{-1})</td>
<td>110 2</td>
<td>170 5</td>
<td>110 2</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol production rate (µmol \cdot \text{kg}^{-1} \cdot \text{min}^{-1})</td>
<td>3.06 0.66</td>
<td>2.36 0.58</td>
<td>3.06 0.66</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 7. Correlation between rates of glycerol and glucose production in women with normal pregnancies after a 12-hour fast in the third trimester ($r = 0.75$, $p = 0.033$).

The maternal height and weight both prior to pregnancy and at the time of the study correlated inversely with GPR in the women with normal pregnancies. No such correlations were seen in the women with IUGR. There were no correlations between the maternal production of glycerol or glucose and the anthropometric data of the newborn infant, with the exception of GPR and birth length in the IUGR group.

Glucoregulatory and other analysed hormones did not differ between the two groups of women (Table 7). Thus, plasma insulin averaged 4.8±2.2 mU · L⁻¹ in the women with normal pregnancies and 5.6±1.7 mU · L⁻¹ in the women with IUGR.

In the women with normal pregnancies the fasting insulin levels correlated inversely both with the rate of glycerol production ($r = -0.85$, $p = 0.008$) and GPR ($r = -0.78$, $p = 0.021$). Except for this correlation, no hormones correlated with rates of glycerol or glucose production in the two groups.
Table 7. Hormone levels in the women in studies I and II

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal n=8</td>
<td>IUGR n=10</td>
</tr>
<tr>
<td></td>
<td>mean  SD</td>
<td>mean  SD</td>
</tr>
<tr>
<td>Insulin (mU·L⁻¹)</td>
<td>4.8  2.2</td>
<td>5.6  1.7</td>
</tr>
<tr>
<td>Glucagon (ng·L⁻¹)</td>
<td>53   11</td>
<td>62   21</td>
</tr>
<tr>
<td>hPGH (ng·mL⁻¹)</td>
<td>22   13</td>
<td>30   16</td>
</tr>
<tr>
<td>GH (mU·L⁻¹)</td>
<td>0.45  0.31</td>
<td>0.92  1.59</td>
</tr>
<tr>
<td>IGF-I (μg·L⁻¹)</td>
<td>183  32</td>
<td>209  77</td>
</tr>
<tr>
<td>IGFBP-I (ng·mL⁻¹)</td>
<td>156  50</td>
<td>183  91</td>
</tr>
<tr>
<td>Oestradiol (nmol·L⁻¹)</td>
<td>73   33</td>
<td>75   39</td>
</tr>
<tr>
<td>Progesterone (nmol·L⁻¹)</td>
<td>478  100</td>
<td>575  318</td>
</tr>
<tr>
<td>Cortisol (nmol·L⁻¹)</td>
<td>584  102</td>
<td>592  139</td>
</tr>
<tr>
<td>Chromogranin A (nmol·mL⁻¹)</td>
<td>3.2  0.7</td>
<td>3.4  0.7</td>
</tr>
</tbody>
</table>

Newborn infants, studies III and IV

Study III

In the preterm infants, plasma concentrations of glycerol and glucose, the rate of appearance of glycerol, and GPR, were calculated during two periods of steady state, before and after administration of theophylline (Table 8). Compared to the values before theophylline administration, both plasma glucose (p=0.0006) and GPR (p=0.002) differed after this treatment.

Table 8. Glucose and glycerol kinetics before and after administration of theophylline

<table>
<thead>
<tr>
<th>P-glucose</th>
<th>Ra (glucose)</th>
<th>GPR</th>
<th>P-glycerol*</th>
<th>Glycerol production</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol·L⁻¹</td>
<td>μmol·kg⁻¹·min⁻¹</td>
<td>μmol·kg⁻¹·min⁻¹</td>
<td>μmol·L⁻¹</td>
<td>μmol·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>before</td>
<td>4.0 ± 1.9</td>
<td>51.7 ± 13.9</td>
<td>33.3 ± 13.9</td>
<td>67 (7.5-785)</td>
</tr>
<tr>
<td>after</td>
<td>4.7 ± 2.1</td>
<td>42.2 ± 10.6</td>
<td>23.9 ± 10.6</td>
<td>122 (44-803)</td>
</tr>
</tbody>
</table>

n=8, *= presented as median and range, Ra=rate of appearance, GPR=glucose production rate
Glucose production rate correlated with plasma glucose both before and after theophylline administration ($r=0.90$ and $r=0.84$, $p<0.001$, respectively) (Fig. 8).

![Graph showing correlations between glucose production rate (GPR) and plasma glucose before and after theophylline administration.](image)

*Figure 8. Correlations between glucose production rate (GPR) and plasma glucose before and after theophylline administration.*

Gluconeogenesis from glycerol could be calculated in 8/10 infants. The median fraction of glycerol converted to glucose was 21 (6.2-61) % before and 38 (11-92) % after administration of theophylline. The median proportion of glucose derived from glycerol was 1.8 (0.3-7.8) % before and 5.8 (1.1-29) % ($p=0.04$) after theophylline administration.

**Study IV**

The plasma glucose concentration in the infants born SGA was $4.1\pm1.1$ mmol $\cdot$ L$^{-1}$. The rate of glucose appearance averaged $30.3\pm8.2$ $\mu$mol $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ and that of glucose production $21.1\pm6.1$ $\mu$mol $\cdot$ kg$^{-1} \cdot$ min$^{-1}$. The infants receiving (n=6) and those not receiving (n=5) additional glucose infusion differed in the rate of glucose appearance ($35.5\pm7.4$ vs. $24.1\pm3.3$ $\mu$mol $\cdot$ kg$^{-1}$).
min⁻¹, p=0.012), but not with regard to GPR or to the plasma glucose concentration.

The mean plasma concentration of glycerol in the SGA infants of study IV was 224±79 µmol L⁻¹. The rate of appearance of glycerol averaged 6.1±1.6 µmol kg⁻¹ min⁻¹ and that of glycerol production 5.6±1.6 µmol kg⁻¹ min⁻¹. Neither the concentration nor the rate of production of glycerol differed between the infants receiving and those not receiving additional glucose. In all infants, the fraction of glycerol converted to glucose was 55±22 %. This represented 8±3 % of the glucose produced. The infants receiving glucose infusion showed reduced conversion of glycerol to glucose in comparison with those who were not given extra glucose (43±23 vs. 70±7 %, p=0.036), resulting in a lower relative contribution to the glucose production (6±4 vs. 10±1 %, p=0.03). The concentrations of insulin, glucagon, IGF-I and IGFBP-1 (n=9), measured during approximate steady state, are shown in Table 9. There were no differences between the infants with and without glucose infusion except in the case of IGFBP-1, the infants given glucose showing higher levels (404±66 vs. 209±52 µg L⁻¹, p=0.004) of this binding protein.

Table 9. Glucose and glycerol kinetics and hormone levels in newborn small for gestational age infants

<table>
<thead>
<tr>
<th></th>
<th>mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glucose (mmol L⁻¹)</td>
<td>4.1±1.1</td>
</tr>
<tr>
<td>GPR (µmol kg⁻¹ min⁻¹)</td>
<td>21.1±6.1</td>
</tr>
<tr>
<td>Ra (glucose) (µmol kg⁻¹ min⁻¹)</td>
<td>30.3±8.2</td>
</tr>
<tr>
<td>P-glycerol (mmol L⁻¹)</td>
<td>224±79</td>
</tr>
<tr>
<td>Glycerol production (µmol kg⁻¹ min⁻¹)</td>
<td>5.6±1.6</td>
</tr>
<tr>
<td>Glycerol to glucose (%)</td>
<td>55±22</td>
</tr>
<tr>
<td>Glucose from glycerol (%)</td>
<td>8±3</td>
</tr>
<tr>
<td>Insulin (mU L⁻¹) †</td>
<td>6.7±1.7</td>
</tr>
<tr>
<td>Glucagon (pmol L⁻¹) † (median, range)</td>
<td>71(48-169)</td>
</tr>
<tr>
<td>IGF-I (µg L⁻¹) †</td>
<td>17±6</td>
</tr>
<tr>
<td>IGFBP-1 (µg L⁻¹) †</td>
<td>306±117</td>
</tr>
</tbody>
</table>

GPR=rate of glucose production, Ra=rate of appearance, † n=9
There was a strong correlation between birth weight and rate of glycerol production ($r=0.904$, $p<0.001$) (Fig. 9). The rate of glycerol production also correlated strongly with the fraction of glucose formed from glycerol ($r=0.88$, $p<0.001$).
Discussion

An efficient maternal energy metabolism is a prerequisite for adequate fetal weight gain and for the metabolic adaptation of the newborn infant. This thesis addresses questions concerning perinatal energy substrate production in normal pregnancy and in pregnancies complicated by IUGR, as well as in SGA and preterm infants.

The use of stable isotope dilution technique makes it possible to perform studies on energy substrate kinetics in humans. The technique provides information on substrate production from small sample volumes, which makes it particularly suitable for studies in newborn infants.

In the pregnant woman and in the newborn infant several adaptive mechanisms provide substrates for energy production. In late gestation there is an accelerating fetal weight gain. To meet the demand for energy substrates, the pregnant woman has to adjust her metabolism. There is a net energy requirement of approximately 80 000 kcal during a normal pregnancy. We could show that in addition to an augmented glucose production there was also a marked increase in lipolysis in women with normal pregnancies as compared to reported data for non-pregnant women. We also found that the rate of lipolysis was lower in women with pregnancies complicated by IUGR. Even though infants born preterm and/or SGA have limited energy stores, we demonstrated that they are capable of lipolysis as well as glucose production.

Kinetic data on lipolysis in pregnancy are limited. Sivan et al. reported on rates of lipolysis in women with overweight or obesity studied in late pregnancy and post partum. In the third trimester they found a lower basal glycerol production than that observed in our study on non-obese healthy pregnant women. This discrepancy may be due to the large difference in BMI between the groups, since earlier data on non-pregnant subjects have shown that excess fat mass may be associated with a decreased rate of lipolysis.

In the study on women with pregnancies complicated by IUGR we found that the rate of lipolysis was decreased in comparison with that in normal pregnancies. Although the number of women was limited, the group was uniform in that all were healthy and non-smokers. Intrauterine growth restriction was diagnosed by repeated ultrasound measurements and the
women delivered infants with birth weights <-2SD. The reduced lipolysis might influence the total energy substrate production and lead to an increased maternal glucose consumption, in turn reducing the amount of glucose available for the fetus.

In keeping with others,3-5 we demonstrated that late normal pregnancy is associated with an increased GPR compared to reported data for non-pregnant women.52 Since the rates of glucose production did not differ between the two groups of pregnant women, impaired maternal glucose production does not seem to be a factor underlying intrauterine growth restriction.

The finding of an inverse correlation between maternal anthropometric data and the rate of glucose production indicates that it is mainly the requirements of the brain and the feto-placental unit,113 and not primarily maternal size, that determine glucose turnover at rest.

The plasma levels of glycerol were higher in the women with IUGR than in those with normal pregnancies. In view of the fact that NEFA and glycerol, products of lipolysis, only cross the placental barrier to a limited extent,51 it would seem that the increased lipolysis in normal pregnancy mainly provides substrates for maternal energy metabolism.12, 53 This saves glucose and amino acids for the fetus.10-12 Most of the energy derived from lipolysis comes from the subsequent β-oxidation of NEFA. The energy from this promotes gluconeogenesis114 and the fetus can benefit from this de novo synthesis of glucose. The elevated levels of glycerol in the women with IUGR could reflect a low conversion of this compound into glucose. Gluconeogenesis from glycerol is increased during fasting in animals.57 Accordingly, the increased levels of glycerol in the women with IUGR could reflect a low conversion of this compound into glucose. However, gluconeogenesis from glycerol only seems to contribute to a minor extent to endogenous glucose production in adults.52 It may be questioned whether a decrease in gluconeogenesis from glycerol is of any importance, since alanine, lactate and pyruvate are the important precursors of glucose.5

Fasting during normal pregnancy leads to a more rapid use of fat instead of carbohydrates as compared to the non-pregnant state.115 This adaptation saves glucose for the fetal needs.116, 117 During maternal fasting, ketone bodies formed by β-oxidation of NEFA easily cross the placenta and act as energy substrates for the fetus.57

We found an inverse correlation between levels of insulin and rates of appearance of glycerol and glucose in the women with normal pregnancies. This indicates a regulatory role for insulin, in spite of the reduced insulin sensitivity reported in late pregnancy. Maternal glycerol production and GPR correlated, indicating that lipolysis and the ensuing β-oxidation of fatty
acids support gluconeogenesis by formation of NADH, ATP and acetyl-CoA.\textsuperscript{114} In contrast to the situation in normal pregnancy, in women with IUGR we did not find any correlation between insulin and rates of energy substrate production, nor between lipolysis and glucose production. This indicates an altered regulation of energy substrate production in this group of pregnant women.

Several of the studied hormones act during pregnancy by decreasing insulin sensitivity.\textsuperscript{8} We found no difference between the two groups of women with regard to levels of glucagon, cortisol, GH, hPGH, oestradiol, progesterone, thyroxine, triiodothyronine, thyrotropin or chromogranin A. None of these hormones correlated with production of glycerol or glucose, nor explained the decreased rate of lipolysis in the women with IUGR.

Concerning the question whether IUGR is associated with low or normal levels of maternal IGF-I, reports in the literature are contradictory.\textsuperscript{37, 48, 118, 119} In our studies there was no difference in IGF-I levels between the two groups of women. Plasma IGFBP-1, modulating IGF-I activity,\textsuperscript{120} was increased in both groups. This could reflect decreased hepatic insulin sensitivity during pregnancy.\textsuperscript{121} The fact that the levels of insulin and IGFBP-1 were comparable between the two groups indicates that IUGR is not associated with altered hepatic insulin sensitivity.

The postnatal adaptation in the infant involves activation of several metabolic pathways. Energy from lipolysis is a major contributor to the requirements during the immediate postnatal period. Earlier studies on preterm and term AGA and SGA infants have shown a considerable variation with regard to the rate of lipolysis.\textsuperscript{74, 87, 91, 110} We found that lipolysis in the preterm infants and in the infants born SGA was lower than in most term infants studied.\textsuperscript{87, 91, 110} This is not surprising considering the limited amount of stored fat in these groups. In the SGA infants birth weight correlated with the rate of glycerol production, indicating that lipolysis depends on the amount of stored fat.

Our group has previously reported data on lipolysis in extremely preterm infants.\textsuperscript{74} The median rate of glycerol production in these infants was in fact similar to the mean rates of lipolysis in the two groups of infants in the present studies. However, there was a very large variation between the individual rates in the extremely preterm infants. This was not seen in the more mature infants of studies III and IV.

Because of the relatively large size of the brain, the glucose requirement of a newborn infant is more than twice as high as that of an adult. Our data on the preterm infants showed that they were capable of an efficient glucose production on the second day of life. During the study periods they were normoglycaemic when given a low-rate infusion of glucose. Although newborn
infants, particularly those born preterm or SGA, are at risk for hypoglycaemia, parenteral nutrition can induce hyperglycaemia. The data showing a high glucose Ra in the preterm infants are in line with earlier results from our group, indicating that the suppression of glucose production during glucose infusion is incomplete. This suggests a disturbance of hormonal glucose regulation. In addition, preterm infants have less tissue that can handle surplus glucose by an insulin-dependent uptake.

In the infants born SGA the glucose production was in the lower normal range as compared to data reported earlier for term AGA infants. When the SGA infants were divided into two groups, one preterm group given additional glucose and one more mature group without glucose infusion, the groups only differed in rate of appearance of glucose. Kalhan et al. compared rates of glucose production before and after a glucose infusion in infants born SGA. The basal GPR in these infants was somewhat higher than that in the infants without glucose infusion in our study. The reason for this may be that the infants studied by Kalhan et al. were more mature and had higher birth weights (SGA was defined as <10th percentile in that study). In addition, these authors found that GPR was inhibited after glucose infusion. No such inhibition was seen in our study. Although the number of infants was small, the results indicate that our infants in fact needed extra energy support. The interpretation of the data related to additional glucose infusion is made difficult by the small sample size both in our study and in that of Kalhan et al.

In the preterm group gluconeogenesis from glycerol varied markedly between the individual infants and contributed only to a small extent to glucose production. In the infants born SGA approximately half of the glycerol was converted to glucose and this contributed to 8% of the total GPR. This input is in the same range as that in most studied AGA infants. The infants born SGA, studied without glucose infusion, showed a higher conversion of glycerol to glucose, indicating a supportive role of gluconeogenesis from glycerol under these conditions.

Acute administration of theophylline in the preterm infants did not result in metabolic alterations of clinical importance. The plasma glucose concentration rose following a bolus dose. Srinivasan et al. also observed an increase in plasma glucose in preterm infants after a bolus dose of theophylline. The rate of glucose production decreased somewhat, whereas the rate of lipolysis was not influenced by the medication. Even though plasma glucose increased, no episodes of hyperglycaemia occurred during the study periods. A proposed relation between glucose utilisation and plasma glucose was supported by the correlations between GPR and plasma glucose before and after theophylline. After treatment, there was a new higher regulatory set-
point between GPR and the glucose level, as indicated by the change in regression line (Fig. 8).

The only previous study on energy substrate production in connection with theophylline therapy in infants is that by Fjeld et al., who concluded that chronic administration of theophylline did not influence energy substrate production in preterm infants 2-5 weeks old.

The risk of later development of the metabolic syndrome in infants born SGA has put focus on the regulation of energy metabolism in such infants and children. The concept of “fetal programming” refers to an adaptation to reduced nutritional support during fetal life with life-long consequences. The nutritional situation of the preterm infant in the intensive care unit partly mimics the intrauterine circumstances of IUGR. Recently, Hoffman et al. reported that children who were born preterm had reduced insulin sensitivity, a risk factor for later development of type 2 diabetes mellitus.

Little is known about the hormonal regulation of energy substrate production in the newborn infant born SGA. The levels of insulin in our infants were comparable with those of a large cohort of SGA infants investigated 48 hours postnatally, but lower than levels reported for AGA infants. The results contradict the possibility that hyperinsulinaemia may occur already at birth, as suggested before. Our data on the glucose/insulin ratio are compatible with the occurrence of increased neonatal peripheral insulin sensitivity in infants born SGA. This increase indicates that insulin resistance does not occur until later in life.

There were no correlations between the level of insulin or the insulin/glucagon ratio and GPR in the SGA infants. The lack of such a correlation is in line with a relative insensitivity of the neonatal hepatocyte to insulin, as suggested by Hawdon et al. The occurrence of hepatic insensitivity is also supported by the finding of an increased IGFBP-1 level as well as an increased IGFBP-1/insulin ratio in SGA infants in this and other studies. The reduced insulin levels in the SGA infants may serve to protect from hypoglycaemia when energy stores are scarce. Fasting insulin levels may increase during the first year since data reported by Soto et al. showed that at 1 year of age the fasting insulin levels in children born SGA were similar to those in children born AGA. Studies of SGA children between 4 and 10 years of age indicate that a development towards insulin resistance may occur, particularly in those who have a rapid weight gain.
Summary and conclusions

This thesis concerns energy substrate production, particularly lipolysis, in pregnant women in late gestation and in newborn infants during the first days of life, and the principal findings and conclusions are as follows:

- Lipolysis in the third trimester was markedly increased in women with normal pregnancies studied after an overnight fast. The energy produced can be used for maternal energy requirements, thus saving glucose and amino acids for the fetus.
- Lipolysis supports maternal gluconeogenesis.
- Late normal pregnancy is associated with increased glucose production.
- In spite of the insulin resistance in late pregnancy, insulin still exerted a regulatory effect on glucose production and lipolysis in normal pregnancy.
- Healthy, non-smoking women, with pregnancies complicated by IUGR of unknown aetiology, had a decreased rate of lipolysis. This decrease could reduce the amount of glucose available for the fetus.
- The glucose production rate did not differ between the women with normal and those with IUGR pregnancies. Thus, a difference in glucose production does not seem to explain the fetal growth restriction.
- Newborn preterm infants are capable of lipolysis and glucose production, as well as of gluconeogenesis from glycerol.
- Acute administration of theophylline in the newborn preterm infant had no adverse metabolic effects.
- Newborn preterm and term SGA infants have a capacity for lipolysis and glucose production, but at somewhat lower rates than those reported for AGA infants.
- Newborn infants born SGA appear to have increased peripheral, but decreased hepatic insulin sensitivity.

New questions related to perinatal energy metabolism are raised from the results presented in this thesis. Studies of energy substrate production in pregnancies resulting in infants born large for gestational age, could give further insight into mechanisms regulating fetal growth. In addition, studies on placental glucose transport could add important knowledge concerning other possible mechanisms underlying altered growth of the fetus.
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