Elina Malo

THE ROLE OF LOW BIRTH WEIGHT AND RESISTIN IN METABOLIC SYNDROME
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University of Oulu, P.O. Box 8000, FI-90014 University of Oulu, Finland

Abstract

Metabolic syndrome is a constellation of metabolic abnormalities including abdominal obesity, glucose intolerance, insulin resistance, hypertension and dyslipidemia. Metabolic syndrome increases the risk of cardiovascular diseases and type 2 diabetes mellitus. A unifying pathophysiological mechanism behind these abnormalities has not been detected. The prevalence of cardiovascular diseases and type 2 diabetes mellitus is increasing all over the world. It is important to identify the factors contributing to these diseases. The aim of this thesis was to study how the peptide hormone resistin is associated with metabolic syndrome and find out whether intrauterine growth restriction predisposes to adverse changes in lipid and glucose metabolism and peptide hormones in a rat model.

Resistin is secreted mainly from macrophages in humans. It possesses proinflammatory properties. Controversial results about its role on obesity, insulin resistance and metabolic syndrome have been reported. In the first study of this thesis, resistin levels were measured from 1500 Finnish subjects in the cross-sectional Health 2000 study. Higher resistin levels were detected in subjects fulfilling the criteria for metabolic syndrome compared to subjects without metabolic syndrome. Resistin was associated with several components of metabolic syndrome.

Data derived from epidemiological studies show that low birth weight is associated with an increased risk for chronic diseases in adulthood. A rat model of intrauterine growth restriction was created. In the second study, unfavorable changes in the peptide hormones resistin and adiponectin were detected that may predispose rats to subsequent insulin resistance. In addition to intrauterine growth restriction, the effect of postnatal fructose-rich diet was explored in the third study. Intrauterine growth restriction and postnatal fructose diet decreased body weight and induced adverse changes in lipid and glucose metabolism in offspring. However, fetally growth-restricted rats were not more susceptible to the adverse effect of fructose diet.

In conclusion, this study demonstrates that resistin is associated with metabolic syndrome and is increased by intrauterine growth restriction. Restricted maternal diet during pregnancy influences weight and lipid metabolism in rat offspring.

Keywords: cardiovascular diseases, diabetes mellitus type 2, fetal growth retardation, fructose, metabolic syndrome X, obesity, resistin
Malo, Elina, Alhaisen syntymäpainon ja resistiinin merkitys metabolisessa oireyhymässä. 
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Sisäaudit; Biocenter Oulu; Kliinisen tutkimuksen keskus; Oulun yliopistollinen sairaala
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä


Epidemiologisissa tutkimuksissa on havaittu, että alhainen syntymäpaino on yhteydessä korkeampaan riskiin sairastua moniin moniin kuin sydän- ja verisuonitautiin aiheutavaan sairauksiin. Toisessa ja kolmannessa osatyössä tutkittiin rottamallissa raskauden aikaisen ravintoarvojen vaikutusta jälkeläisten kasvuun, rasva- ja sokerimetaboliaan sekä peptidihormoneihin. Toisessa osatyössä todettiin, että rajoitetun ravintoa aiheuttaa korkeampaa riskiä sairastua moniin moniin kuin sydän- ja verisuonitautiin aiheutavaan sairauksiin. Toisessa ja kolmannessa osatyössä tutkittiin korkeampaa riskiä sairastua moniin moniin kuin sydän- ja verisuonitautiin aiheutavaan sairauksiin.


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Asiakastetut: aikuisesta diabeteseen, fruktoosi, lihavuuteen, metabolinen oireyhymä, resistiini, sikiönkehitys, sydän- ja verisuonitaudit
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Abbreviations

ADCY5 adenylate cyclase type 5
ADRB1 adrenergic receptor beta 1
ANCOVA analysis of covariance
ANOVA analysis of variance
CDKAL1 CDK5 regulatory subunit associated protein 1-like 1
CHD coronary heart disease
CI confidence interval
CETP cholesteryl ester transfer protein
CRP C-reactive protein
CVD cardiovascular disease
DNA deoxiribonucleic acid
EGIR European Group for the Study of Insulin Resistance
ELISA enzyme-linked immunosorbent assay
FABP4 fatty acid binding protein 4
FFA free fatty acid
HD harmonized definition of metabolic syndrome
HDL-C high-density lipoprotein cholesterol
HMGA2 high mobility group AT-hook 2
HOMA-IR homeostasis model assessment of insulin resistance
ICAM-1 intercellular adhesion molecule-1
IDF International Diabetes Federation
IHD ischemic heart disease
IL-6 interleukin-6
IUGR intrauterine growth restriction
LCORL ligand dependent nuclear receptor corepressor-like
MCP-1 monocyte chemoattractant protein-1
mRNA messenger ribonucleic acid
miRNA micro ribonucleic acid
MRI magnetic resonance imaging
MS metabolic syndrome
NCEP ATP III National Cholesterol Education Program’s Adult Treatment Panel III
PAI-1 plasminogen activator inhibitor-1
PPARγ peroxisome proliferator-activated receptor gamma
RNA ribonucleic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>sdLDL-C</td>
<td>small, dense low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>triglyceride</td>
</tr>
<tr>
<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low-density lipoprotein</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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List of original articles

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:


*Equal contribution
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1 Introduction

The current lifestyle involving excess energy intake and sedentary work with low physical activity during leisure time has led to a world-wide epidemic of obesity-related diseases. Obesity is one of the factors constituting metabolic syndrome (MS), which is a combination of metabolic abnormalities occurring in an individual at the same time more frequently than expected by chance. Other major abnormalities include insulin resistance, glucose intolerance, dyslipidemia and hypertension. MS increases the risk for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). CVDs are the leading cause of mortality in the world and the prevalence of T2DM has increased strikingly in the Western world. These chronic diseases create a massive economic burden, increase morbidity and mortality and decrease the quality of life of individuals (Eckel et al. 2005).

Massive resources have been invested to explore the pathophysiology of obesity-related diseases. It is known that these disorders are multifactorial and that genetics together with environmental factors play an important role. In addition to conventional risk factors, other factors contributing to the development of the disease are still likely to emerge and they are studied from various aspects. In addition, not all obese individuals have metabolic abnormalities which are commonly associated with obesity and the mechanisms behind this ‘healthy obesity’ are poorly understood and need further investigation (Primeau et al. 2011).

Obesity has an inflammatory component and adipose tissue is considered an active endocrine organ, especially when low-grade inflammation is present in body (Greenberg & Obin 2006). Resistin is a peptide hormone which is mainly secreted by adipocytes in rodents and macrophages in humans and it may appear as one link between inflammation and insulin resistance. Controversial information about the association of resistin and MS has been presented and the role of resistin in MS needs further research (Schwartz & Lazar 2011).

Conditions in fetal life and early postnatal development have been under intensive research since low birth weight was connected to increased risk for several chronic diseases in adulthood, including CVD (Barker 2004). It is hypothesized that suboptimal environment in utero alters growth and metabolism to ensure immediate survival of the fetus. These adaptations may be permanent and result in deleterious changes in the structure and function of several organs increasing the possibility of a disease outcome in adulthood. In addition to
intrauterine growth restriction (IUGR), early postnatal development has been demonstrated to affect later health status (Hales & Barker 1992).

The present study was carried out to explore the association of resistin and IUGR in MS and its components. The association of resistin with MS was studied in a large Finnish cross-sectional study. Rat models were used to explore whether fetal caloric restriction affects future lipid, glucose and insulin values as well as obesity-related peptide hormones. The following literature review presents the concept of the MS and the current knowledge on the association of low birth weight with MS, CVD and T2DM.
2 Review of the literature

2.1 Metabolic syndrome (MS) predisposes to type 2 diabetes (T2DM) and cardiovascular diseases (CVD)

MS is a cluster of metabolic abnormalities, such as glucose intolerance, insulin resistance, central obesity, dyslipidemia (high triglyceride (TG), high small, dense lipoprotein cholesterol (sd-LDL-C) and low high-density lipoprotein cholesterol (HDL-C) level) and hypertension. These conditions are known risk factors of CVD, stroke and T2DM and tend to co-occur in individuals more frequently than expected by a chance. They share underlying mediators, mechanisms and features (Eckel et al. 2005). Individuals with MS have 2-fold increased risk for CVD events and mortality (Gami et al. 2007). For T2DM the risk is almost 5-fold (Ford et al. 2008). MS is also documented to be associated with functional, abnormal changes in the kidney, liver, brain and cancers of the breast, pancreas and colon, for example (Braun et al. 2011, Frisardi et al. 2010, Thomas et al. 2011, Vanni et al. 2010).

The prevalence of MS is increasing worldwide. The phenomenon is parallel with the rise of prevalence of obesity. About one fourth of the adult populations in Europe, Northern and Southern America and even India carry MS. In developing countries, the prevalence is still lower, but will certainly increase as population ages, wealth increases and dietary habits change (Grundy 2008). The prevalence of obesity (body mass index (BMI) ≥30 kg/m²) in Finnish adults aged ≥30 years in 2007 was 23% in men and 21% in women (Vartiainen et al. 2010). In the United States, more than one third of adults were obese in 2004 (Ogden et al. 2006).

There has been discussion about the clinical relevance of MS as a disease entity. It is still unclear whether MS explains the risk of CVD more than what is the contribution of each of the individual components to CVD. There are studies showing that fasting glucose concentration was as good as, or better predictor of T2DM or myocardial infarction than MS itself (Reaven 2011). It is recognized that MS as a concept is useful in research by providing a network when unifying, underlying pathophysiological features behind the components of MS are explored. In addition, in public health it helps to focus attention on severe metabolic health problems. MS also reminds clinicians to concentrate on other related risk factors if one risk factor of MS is identified (Simmons et al. 2010).
2.1.1 Factors contributing to MS

There are two major risk factors for metabolic syndrome: abdominal obesity and insulin resistance (Carr et al. 2004, Reaven 1988). Other relevant conditions are aging, genotype, hormonal changes and lack of exercise.

Obesity is undoubtedly associated with numerous metabolic complications and usually the severity of the complication is proportional to the degree of obesity. Genetics and its interaction with behavioral and environmental factors influence the development of obesity. Especially physical inactivity and a Western style, high-fat, as well as high-sugar diet contributes to obesity. However, being obese does not always mean that the person has metabolic perturbations. It has been reported that 30% of obese individuals are metabolically normal (Primeau et al. 2011). The major characteristics of ‘healthy obesity’ are preserved insulin sensitivity, normal function of adipose tissue and relatively low visceral fat mass (Bluher 2010). The pathophysiology behind this phenomenon is not known. For instance, it has been shown that childhood growth has an impact on the later health of obese individuals. In the Helsinki Birth Cohort Study, attenuated weight and BMI gain during the first seven years was seen in obese individuals who developed MS compared to obese persons not developing MS (Salonen et al. 2009). The effect of postnatal weight gain on future health will be discussed further.

Since it is clearly demonstrated that insulin resistance is an inducer of hyperglycemia in T2DM, one might speculate that insulin resistance is the essential cause of MS. An abnormal fat distribution characterized by predominant upper body fat is a common feature of insulin-resistant people, whether they were obese or not (Grundy et al. 2005). The upper body fat can accumulate intraperitoneally or subcutaneously and it commonly releases increased amounts of non-esterified fatty acids via induced hormone sensitive lipase function, which can then accumulate as ectopic fat at other sites of body than adipose tissue. In muscle and liver ectopic fat predisposes to insulin resistance and dyslipidemia, and also to malfunction of these tissues such as fatty liver or non-alcohol steatohepatitis. In addition, adipose tissue of obese individuals exhibits increased secretion of inflammatory cytokines and reduced amounts of protective adipokine adiponectin, for instance (Grundy et al. 2005).

Chronic, low-grade inflammation could be the underlying cause of metabolic syndrome according to some studies (Ritchie & Connell 2007). Increased levels of pro-inflammatory cytokines induce insulin resistance in muscle and adipose
tissue. This is documented especially in obese individuals. However, even in non-obese individuals, insulin resistance and low-grade inflammation are simultaneously detected (Grundy et al. 2005).

Individual and ethnic variation is also documented to be present in the pattern of metabolic risk factors in obese or insulin-resistant people. Family and twin studies show that genetics play an important role in MS development. However, several linkage, candidate gene and genome-wide association studies have been performed and so far it has been postulated that common and rare variants explain only 30% of the heritability of MS (Joy et al. 2008).

2.1.2 Pathogenesis of MS

There is no single, consistent pathophysiological mechanism found behind MS. Obesity and insulin resistance play a central role in the pathogenesis of MS, which is overviewed in Figure 1. When adipose tissue mass is increased due to positive energy balance, it releases free fatty acids (FFA) into blood stream via induced lipolytic machinery in adipose tissue. Also other tissues undergo lipolysis releasing FFAs by lipoprotein lipase (Wang & Eckel 2009). After being internalized by hepatic cells FFAs contribute to the production of glucose and TG-rich very low-density lipoprotein (VLDL) particles in the liver. Insulin is continuously secreted from pancreatic β-cells to inhibit the production of glucose, but FFAs attenuate the action of insulin resulting in hyperinsulinemia. This compensatory action for increased insulin secretion by β-cells causes enhanced proliferation and expansion of the cells. In mice, this is induced by a newly identified hormone betatrophin, which may be a potential therapeutic agent against T2DM (Yi et al. 2013). A novel mechanism in adipose tissue – liver crosstalk was introduced in a recent study by Cao and co-workers (Cao et al. 2013). They proposed that fatty acid binding protein 4 (FABP4), which is secreted from adipocytes, is an important player in glucose metabolism by stimulation glucose production in liver.

Simultaneously, the amount of HDL-C is reduced and sdLDL particle production and –associated cholesterol are increased. This dyslipidemic triad formation is mostly regulated via cholesteryl ester transfer protein (CETP) and hepatic lipase function (Yamashita et al. 2000, Zambon et al. 2003). sdLDL-C is atherogenic since it is toxic to the endothelium, able to move through the endothelial basement membrane because of its small size and is easily oxidized. In muscle, insulin sensitivity is reduced since overabundance of FFAs inhibits
insulin-mediated glucose uptake. Muscle cells are not able to produce glycogen from glucose. FFAs accumulate intramuscularly as TG droplets. Circulating FFAs and hyperinsulinemia contribute to hypertension since they increase the activity of the sympathetic nervous system (Eckel et al. 2005).

Insulin resistant state is worsened by the release of proinflammatory markers from adipocytes and inflammatory cells, especially macrophages. The levels of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), other cytokines and resistin are increased and the levels of anti-inflammatory adiponectin reduced (Eckel et al. 2005). In addition, prothrombotic state is created when fibrinogen and plasminogen activator inhibitor-1 (PAI-1) is released from liver. Also the secretion of PAI-1 is enhanced from adipose tissue. Blood coagulation is impaired and the risk of thrombosis increased (Durina & Remkova 2007).

Fig. 1. Schematic overview of the pathophysiology of metabolic syndrome. FABP4, fatty acid binding protein 4; FFA, free fatty acid; HDL-C, high-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor-1; sdLDL, small, dense low-density lipoprotein cholesterol; SNS, sympathetic nervous system; VLDL, very low-density lipoprotein cholesterol. Modified from (Eckel et al. 2005).
2.1.3 Definitions of MS

Various consensus groups have established definitions for MS with differences in essential criteria and cutpoints of components. Reaven introduced Syndrome-X in 1988. He proposed that patients with non-insulin-dependent diabetes, high blood pressure and CHD have the same underlying syndrome consisting of a cluster of changes in resistance to insulin-mediated glucose uptake (Reaven 1988, Reaven 1993). After that, the World Health Organization (WHO) attempted to launch the first internationally accepted, clinical definition of MS in order to identify persons at increased risk of T2DM or CVD. According to the definition, insulin resistance either measured by hyperinsulinemic euglycemic clamp or occurring in T2DM, impaired glucose tolerance or impaired fasting glucose is an essential component. In addition, two of the following components need to be present: overall or abdominal obesity, hypertension, dyslipidemia (high TG and/or low HDL-C) or microalbuminuria (Alberti & Zimmet 1998).

The European Group for the Study of Insulin Resistance (EGIR) modified the WHO definition. They used the term insulin resistance syndrome and defined insulin resistance as increased plasma insulin levels (the upper quartile of the population). Patients with T2DM were excluded (Balkau & Charles 1999). The National Cholesterol Education Program’s Adult Treatment Panel III (NCEP ATP III) introduced an alternative criterion of MS in 2001 trying to identify persons with increased risk for atherosclerotic CVD who need lifestyle intervention. The definition has perhaps been most widely used by researchers and clinicians since the criteria are fulfilled when an individual has any three of the components (abdominal obesity, elevated TGs, reduced HDL-C, elevated blood pressure and elevated fasting glucose as impaired fasting glucose or T2DM) and is therefore simple to use. Insulin resistance was no longer included (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001).

The International Diabetes Federation (IDF) modified the NCEP ATP III definition by deciding that insulin resistance and abdominal obesity are so highly correlated that measuring insulin resistance is not necessary. According to the IDF, abdominal obesity was an essential component of MS and two other components were needed. In addition, they proposed thresholds for abdominal obesity according to ethnicity (Alberti et al. 2005). NCEP ATP III, IDF and other organizations joined and published a harmonized definition (HD) of MS in 2009 in order to unify the definition (Alberti et al. 2009). The agreement was that no obligatory component is needed and waist circumference cut points are national-
and region-specific. The detailed criteria are presented in Table 1. There are also other organizations that have launched their definitions for MS, but they are not discussed further here.

Markers of inflammation, oxidative stress and prothrombotic factors have also been suggested to be included in the definition of metabolic syndrome (Reilly & Rader 2003). In addition, vascular endothelial cell dysfunction has been proposed to be a candidate included in definition of MS, since it is clearly associated with hyperglycemia, hypertension and dyslipidemia (Bonora et al. 2003). There are valid standardized methods available for the analysis of endothelial function (Poredos & Jezovnik 2013).
Table 1. Definitions of metabolic syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WHO</th>
<th>EGIR</th>
<th>NCEP ATP III</th>
<th>IDF</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Required component</strong></td>
<td>Insulin resistance(^1)</td>
<td>Hyperinsulinemia(^2) (plasma</td>
<td>Central obesity: WC(^3) ≥94 cm (m), ≥80 cm (w)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>insulin &gt;75th percentile)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Criteria</strong></td>
<td>Insulin resistance or T2DM + two of the following</td>
<td>Hyperinsulinemia + two of the following</td>
<td>Three of the following</td>
<td>Central obesity + two of the following</td>
<td>Three of the following</td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td>WHR &gt;0.90 (m), &gt;0.85 (w); or BMI &gt;30 kg/m(^2)</td>
<td>WC ≥94 cm (m), ≥80 cm (w)</td>
<td>WC ≥102 cm (m), ≥88 cm (w)</td>
<td>ALREADY INVOLVED</td>
<td>WC(^3) ≥94 cm (m), ≥80 cm (w)</td>
</tr>
<tr>
<td><strong>Hyperglycaemia</strong></td>
<td>Already involved</td>
<td>Fasting glucose ≥6.1 mmol/L</td>
<td>Fasting glucose ≥5.6 mmol/L or medication</td>
<td>Fasting glucose ≥5.6 mmol/L or medication</td>
<td>Fasting glucose ≥5.6 mmol/L or medication</td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td>TG ≥1.7 mmol/L or HDL-C &lt;0.9 mmol/L (m), &lt;1.0 mmol/L (w)</td>
<td>TG ≥2.0 mmol/L or HDL-C &lt;1.0 mmol/L</td>
<td>TG ≥1.7 mmol/L or medication</td>
<td>TG ≥1.7 mmol/L or medication</td>
<td>TG ≥1.7 mmol/L or medication</td>
</tr>
<tr>
<td><strong>Dyslipidemia (second criteria)</strong></td>
<td>-</td>
<td>HDL-C &lt;1.03 mmol/L (m), &lt;1.29 mmol/L (w) or medication</td>
<td>HDL-C &lt;1.03 mmol/L (m), &lt;1.29 mmol/L (w) or medication</td>
<td>HDL-C &lt;1.03 mmol/L (m), &lt;1.29 mmol/L (w) or medication</td>
<td>HDL-C &lt;1.03 mmol/L (m), &lt;1.29 mmol/L (w) or medication</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>≥140/90 mmHg</td>
<td>≥140/90 mmHg or medication</td>
<td>≥130/85 mmHg or medication</td>
<td>≥130/85 mmHg or medication</td>
<td>≥130/85 mmHg or medication</td>
</tr>
<tr>
<td><strong>Other criteria</strong></td>
<td>Microalbuminuria</td>
<td></td>
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</tr>
</tbody>
</table>

\(^1\)Fourth quartile of fasting plasma insulin level, \(^2\)only in patients without type 2 diabetes, \(^3\)values specific for people of European origin. EGIR, European Group for the Study of Insulin Resistance; HD, Harmonized definition; HDL-C, high-density lipoprotein cholesterol; IDF, International Diabetes Federation; NCEP ATP III, National Cholesterol Education Program’s Adult Treatment Panel III; T2DM, Type 2 diabetes mellitus; TG, triglycerides; WC, Waist circumference; WHO, World Health Organization; WHR, Waist-to-hip-ratio. (I, published by the permission of Mary Ann Liebert Inc.)
2.2 Low birth weight as a potential risk factor for adult diseases

Neonatal and child mortality is strongly predicted by birth weight. It is important to note that the concepts ‘preterm’ and ‘low birth weight’ are not synonymous. It has been shown that term babies, who weigh less than 2,500 g have higher risk of mortality compared to heavier babies (Wilcox 2001).

However, being born as preterm baby does not cause this higher risk. The definition of IUGR is used to explain this higher risk which is not due to preterm delivery. Currently, there is no clinical measure for separating babies with similar birth weight who were growth-restricted \textit{in utero} or not. However, IUGR is usually defined as ‘small for gestational age’, which means that the baby belongs to the lightest 10% in gestational age class. The majority of IUGR children are born at term (Wilcox 2001).

Epidemiological studies have shown that IUGR, manifesting as low birth weight, is associated with an increased risk of several chronic diseases. In addition to metabolic syndrome, CVD and T2DM (Barker 2004), low birth weight is associated with cancers (McCormack \textit{et al.} 2005), depression (Cheung \textit{et al.} 2002) and autoimmune diseases (Kajantie \textit{et al.} 2006), for instance. Higher birth weight is also associated with later disorders such as obesity (Curhan \textit{et al.} 1996). These issues are not discussed in this literature review.

2.2.1 Fetal programming hypothesis

Developmental origins hypothesis, fetal programming and thrifty phenotype hypothesis are terms which involve stimulus or insult during a critical period of early development \textit{in utero} that is beneficial at the moment for the survival of the fetus, but can lead to long-term or permanent, disadvantageous changes in metabolism and growth in postnatal life (Barker 2007). This is essential if the adult environment does not match the environment \textit{in utero} when early life undernutrition-programmed ‘thrifty phenotype’ is switched on and plenty of food is then available after birth (Hales & Barker 1992). Loss of structural units like nephrons, pancreatic β-cells and cardiomyocytes is detected after inadequate fetal nutrition while the optimal growth and development of central nervous system is protected (McMillen & Robinson 2005). The effect of programming is tissue-, time- and challenge-specific since different cells and tissues are sensitive to various factors at different times (Hales & Barker 1992).
Originally, this concept was put forward by David Barker and colleagues in the United Kingdom when they showed that environmental factors during pregnancy affect a child’s risk of cardiovascular and metabolic diseases in adulthood. They found out that poor living conditions in the 1920s relate to the increased mortality to ischemic heart disease (IHD) in the 1970s and were the first to show in a large study population of 3,200 adults that birth weight and adult systolic blood pressure had an inverse relation (Barker & Osmond 1986, Barker et al. 1989). Since then the effect of small birth weight on the risk of adult chronic diseases has been under intensive investigation.

The fetal insulin hypothesis created by Hattersley and Tooke is an alternative explanation for the association of low birth weight and adult insulin-related diseases. They propose that genetically determined insulin resistant state in utero, including defects in glucose sensing, insulin secretion, or response to insulin of insulin-sensitive tissues, can result in delayed growth in utero as well as insulin resistance in childhood and adulthood. In other words, phenotypes of the same insulin-resistant genotype are low birth weight, insulin resistance, hypertension, glucose intolerance and T2DM. Primarily, this hypothesis was supported by results from studies concerning monogenic forms of diabetes (Hattersley & Tooke 1999). A recent genome-wide association study supporting this hypothesis is also referred to later in chapter 2.2.4 (Horikoshi et al. 2013). Genetic insulin resistance could also impair fetal angiogenesis and result in increased risk of hypertension and vascular disease. The mechanism behind this is probably impaired endothelial function in vessel walls (Hattersley & Tooke 1999).

2.2.2 Low birth weight and MS

It is challenging to define a short detailed time period during pregnancy when stress in utero can be analyzed. In addition to animal studies which are referred to in chapter 2.2.5, there are only a few exceptions in human medicine. During the Dutch famine, which occurred during the 4-month period between December 1944 and April 1945, the daily caloric intake was 400–800 calories. The famine started and came to an end suddenly and affected a previously well-nourished population. Glucose intolerance in adulthood was associated with exposure to Dutch famine during any stage of gestation. Coronary heart disease (CHD), atherogenic lipid profile, obesity and increased stress responsiveness were observed after exposure to famine in early gestation. Mid-gestation exposure was
associated with obstructive airways disease and microalbuminuria (Roseboom et al. 2006).

The association of low birth weight and metabolic syndrome components is widely reviewed by McMullen and Robinson (McMillen & Robinson 2005). One of the strongest supports for the fetal origins hypothesis is provided by the association between birth weight and blood pressure levels in adulthood. A negative association between birth weight and systolic blood pressure in childhood and adulthood has repeatedly been reported (Lenfant 2008). The estimation is that a one kg higher birth weight is associated with a 2–4 mmHg lower systolic blood pressure (Huxley et al. 2000, Law & Shiell 1996). This result has been criticized by Huxley et al. (2002), who consider that in most studies the bias from the adjustments for current weight and confounding factors overestimates the association. In addition, birth weight values should be obtained from birth records, but this is not the case in larger cohorts where the values are obtained from parental recall or self-reports, which may cause bias. The reduction of systolic blood pressure is around 0.4 mmHg/kg, which is of little clinical relevance (Huxley et al. 2002).

One example of developmental plasticity is genetic studies in the Helsinki Birth Cohort. One genotype can influence a variety of different phenotypes depending of the surrounding conditions during development. Systolic blood pressure was increased and antihypertensive medication more frequently used by persons having a combination of low birth weight and peroxisome proliferator-activated receptor gamma 2 (PPARγ 2) gene variant Pro12Pro (Yliharsila et al. 2004).

Low birth weight was associated with many markers of metabolic syndrome including insulin resistance, hypertension, dyslipidemia and abdominal obesity in a cohort of European adolescents (Vielwerth et al. 2008). Fetal growth velocity during the third trimester was measured in this cohort, but did not explain metabolic changes in adulthood. It was concluded that all other time periods during early development, except the third trimester, or postnatal catch-up growth are the most important developmental periods relevant to programming of metabolic syndrome (Vielwerth et al. 2008). Impaired glucose tolerance has also been connected to low birth weight in many studies (McMillen & Robinson 2005).

Results from studies analyzing the association of IUGR to MS as a whole entity have been more contradictory and the association is not that often documented. In the Dutch famine cohort, the prevalence of MS in adulthood defined by IDF criteria was not significantly greater in those exposed to famine
Similarly, low birth weight was not associated with MS in 7,400 young adults in Norway (Euser et al. 2010). However, several opposite results have been reported. In 1993, Barker and colleagues reported that syndrome-X was more common in men with low birth weight (Barker et al. 1993). In addition, postmenopausal women in the lowest birth weight tertile living in the United States had 2.41-fold higher risk (95% CI 1.06–5.51) of having metabolic syndrome compared to the women in the highest birth weight tertile (Yarbrough et al. 1998). A similar result was obtained in study populations of young Dutch adults aged 26–31 years (Ramadhani et al. 2006), 40-year-old Nigerians who had survived the famine in Biafra (Hult et al. 2010), Chinese adults (Li et al. 2011, Xiao et al. 2010) and nondiabetic, middle-aged Finnish men (Laaksonen et al. 2003).

2.2.3 Low birth weight, CVD and T2DM

The association of IUGR to T2DM and CVD has been studied intensively. In addition to the results obtained by Barker and colleagues (Barker & Osmond 1986, Barker et al. 1989), several other studies have been published. Prenatal exposure to the Dutch famine during early gestation contributed to the occurrence of CHD (Roseboom et al. 2000). Results from the Leningrad siege study, which lasted about 900 days, showed that intrauterine malnutrition did not influence glucose intolerance and CVD in adulthood, but this study has been criticized due to the lack of reliable birth weight data and exposure status of individuals (Stanner et al. 1997). Huxley et al. (Huxley et al. 2007) reviewed systematically 17 published studies of birth weight and subsequent IHD and concluded that one kg higher body weight at birth is associated with a 10–20% lower risk of IHD. The authors state that the relevance of this result to public health is rather small.

A meta-analysis involving 14 studies and 132,000 persons revealed that low birth weight (≤2.500 g) was associated with increased risk for T2DM (odds ratio 1.32, 95% confidence interval (CI) 1.06–1.64). Birth weight and diabetes risk have a U-shaped relation since a birth weight ≥4,000 g was associated with increased risk of the same extent (Harder et al. 2007). A systematic review by Whincup and colleagues (Whincup et al. 2008) obtained the same results when adjusted with current BMI and socioeconomic status.

A recent meta-analysis and follow-up study of birth weight of nearly 70,000 Europeans from 43 studies highlights genetic links between fetal growth and adult metabolism. Variants in the genes adenylate cyclase type 5 (ADCY5) and CDK5
regulatory subunit associated protein 1-like 1 (CDKAL1) are associated with T2DM by reducing fetal insulin concentration, adrenergic receptor beta 1 (ADRB1) with adult blood pressure and high mobility group AT-hook 2 (HMGA2) and ligand dependent nuclear receptor corepressor-like (LCORL) with adult height. It also reveals that some T2DM risk alleles are associated with either higher or lower birth weight, which brings complexity to the fetal origins of T2DM (Horikoshi et al. 2013).

Low birth weight is common in developing countries and it is hypothesized that this fetal programming hypothesis plays a role in the diabetes epidemics in these low-income regions of the world. In 2001, nearly 80% of deaths associated with cardiometabolic diseases in the whole world occurred in developing countries (Lopez-Jaramillo 2009). Recently, WHO included low birth weight as a risk factor of CVD (World Health Organization et al. 2011).

Socio-economic factors have been argued to be a confounder of fetal origin hypothesis studies. They affect fetal growth, offspring birth weight and are associated with increased risk of CVD and T2DM. Therefore, the association between low birth weight and adult health can partly be explained by social factors (Bergvall & Cnattingius 2008).

2.2.4 Low birth weight, early postnatal growth and the risk of future diseases

The effect of IUGR on MS in adulthood might be exacerbating if there is a strong contrast between fetal and postnatal environment. Scarce diet in utero and abundant diet after birth may not be the optimal combination when considering the risk of future chronic diseases. In conditions like that some, but not all, infants born with low birth weight show catch-up growth (Singhal et al. 2003). Association between increased future disease risk and slower growth of infants with low birth weight has also been reported (Barker et al. 2005, Eriksson et al. 2006).

It has been shown in animal studies that nutrient restriction increases longevity in rats and decreases cardiovascular risk factors in primates. On the contrary, overfeeding during suckling period increases the risk of metabolic syndrome and T2DM in rats (Singhal et al. 2003). The effect of postnatal growth has been thoroughly tested in experimental animals and in epidemiological human studies. In animal studies, it is usually stated that rapid weight gain following IUGR has adverse effects on the later health status (Morrison et al. 2010).
From the famine studies referred to earlier it can be concluded that a strong contrast between fetal and postnatal life increases the risk of developing adult diseases (Roseboom et al. 2006). However, it is unclear as to what kind of postnatal growth pattern is harmful for later health after IUGR. Adolescents born preterm whose growth was accelerated postnatally with nutrient-enriched diet had an increased tendency to insulin resistance measured by proinsulin levels (Singhal et al. 2003) and increased blood pressure (Singhal et al. 2007). Also children who displayed catch-up growth between birth and 2 years were more obese at the age of 5 (Ong et al. 2000).

Opposite results concerning immediate postnatal weight development and future glucose and insulin metabolism have also been reported. Low birth weight and low weight at one year of age was associated with later development of glucose intolerance and T2DM in an English cohort (Hales et al. 1991). In Finnish subjects born between 1934 and 1944, it was found that low weight gain during the first two years of life increased the risk of impaired glucose tolerance and T2DM. This association was greatest in subjects with low birth weight. The authors also stated that the most critical periods for the development of insulin resistance in adulthood were low growth in the first 6 months and rapid increase in BMI between age 2 and 11 years (Eriksson et al. 2006). The same growth pattern in childhood was detected in individuals having CHD events in adulthood. The effects of body size were independent of the socioeconomic status in adulthood (Barker et al. 2005). One explanation behind these observations may be that children who are thin or short at birth lack muscle, but disproportionately high fat mass in relation to muscle mass may develop when these children gain weight rapidly (Barker et al. 2005, Eriksson et al. 2006). IUGR may also result in a reduced number of β-cells in the pancreas, and subsequent weight gain during childhood leads to immoderate pressure on these cells with attenuated insulin production and secretion resulting in T2DM (Forsen et al. 2000). This data implies that optimizing early growth is an important way of preventing future CHD and T2DM.

### 2.2.5 Mechanisms behind intrauterine growth restriction (IUGR)

The mechanisms altering intrauterine conditions that affect the later health of a child are currently not fully known. Genetics may have an important role, but there are also other mechanisms involved. For instance, it is documented that existing genetic markers account for only around 10% of the origin of T2DM.
Lifestyle determinants for T2DM are more relevant and it should be kept in mind that lifestyle factors may also be under genetic control (Ahlqvist et al. 2011). The missing heritability of T2DM and other diseases may partly be accounted for by epigenetics, a very important field to be considered.

Twin studies are often used to study the fetal origin hypothesis since they are less prone to confounding factors compared to studies involving singletons. Twins share a similar environment before birth and during childhood. In addition, studies with monozygotic twins allow elimination of genetic effects on the association of low birth weight and diseases in adulthood. For example, lower birth weight was detected in a twin with T2DM in adulthood compared to the birth weight of the non-diabetic, identical or non-identical co-twin (Poulsen et al. 1997). However, criticism towards the use of twin studies has been presented. There are often random errors due to the small number of twins involved. Huxley et al. (Huxley et al. 2002) showed that there is no difference in the strength of association of low birth weight and subsequent blood pressure between twin and singleton studies. This suggests that there are similar causal pathways involved.

Placental insufficiency

The placenta is involved in several roles during pregnancy: providing an immunological barrier between the mother and fetus, transportation of nutrients, oxygen, amino acids and waste products between the mother and fetus and production of hormones that regulate fetal development and metabolism (Gude et al. 2004). If placental blood flow is not increased normally during pregnancy, it leads to placental insufficiency. In Western societies, placental insufficiency is proposed to be the main underlying cause of IUGR. It is suggested that one mechanism behind the insufficiency is impairment in the development of placental vessels, and those vascular changes may alter the supply of nutrients to the fetus. Other placental factors that can cause fetal growth restriction include dysfunctions such as abnormal placentation, chronic abruption, chronic inflammatory conditions, for instance (Sankaran & Kyle 2009).

There are also several maternal influences which are linked to changes in intrauterine environment and therefore to altered metabolic long-term health of offspring: maternal pregestational and gestational diabetes, age, smoking and other drug use, low socio-economic status, nutritional factors, hypertension and pre-eclampsia, for instance. Among fetal factors, chromosomal abnormalities,
genetic conditions, congenital malformations, infections in the uterus and multiple pregnancies can restrict the growth of the fetus (Sankaran & Kyle 2009).

**Epigenetics**

Epigenetics is defined as changes in gene function that occur without a change in the nucleotide sequence. Some of these changes can be heritable. Environmental factors such as IUGR can modify epigenetic states and affect the phenotype of the adult. Deoxiribonucleic acid (DNA) methylation means addition of a methyl group on the cytosine within cytosine-guanine dinucleotides located in genomic CpG islands. This epigenetic modification is stable and heritable across generations. It is usually associated with repression of gene transcription, so that high methylation allows a lower level of expression of a protein, and vice versa (Jones & Takai 2001). IUGR was associated with increased DNA methylation of the Pdx1 promoter in pancreatic islets in rats developing T2DM (Park et al. 2008).

Other epigenetic processes are covalent histone modifications such as methylation, acetylation, phosphorylation and ubiquitination. DNA methylation and histone acetylation control the imprinting of genes. Maternally imprinted genes suppress fetal growth, whereas paternally imprinted genes enhance it (Myatt 2006). Very early development is a crucial period for constructing epigenetic marks. During the Dutch famine, periconceptionally exposed individuals had less DNA methylation of imprinted IGF2 gene in adulthood compared to unexposed, same-sex siblings. There was no difference in the methylation status when exposure happened during late gestation (Heijmans et al. 2008). Post-transcriptional mechanisms are also involved in developmental programming. Expression of microRNAs (miRNA) in offspring was affected by changes in maternal diet both in humans and rats resulting in limited storage of lipids in adipose tissue, causing insulin resistance and increasing the risk of metabolic diseases (Ferland-McCollough et al. 2012).

**Glucocorticoid exposure**

Prenatal overexposure to glucocorticoid hormones is one of the major mechanisms believed to be behind developmental programming. Maternal stress or exogenous transportation from mother to fetus is a consequence of glucocorticoid excess in the fetus and is correlated with reduced birth weight (Khulan & Drake 2012). Normally, these hormones are important in many
physiological functions such as metabolism, immunology, blood pressure maintenance and electrolyte homeostasis. They are the main hormonal mediators of stress in body. Glucocorticoids alter gene expression and act as transcription factors by binding to glucocorticoid and mineralocorticoid receptors. Glucocorticoid receptors are expressed in several fetal tissues and also in the placenta from early embryonic stages. Mineralocorticoid receptor expression is more limited and present during later stages of development (Harris & Seckl 2011). In animal studies it has been documented that prenatal overexposure to glucocorticoids is associated with increased blood pressure in adulthood. The mechanism behind this association is probably that glucocorticoids can influence the hypothalamus-pituitary-adrenal axis, which plays a role in developing hypertension. Also changes in kidney and cardiovascular system structure and function, such as reduced nephron number, increased renal glucocorticoid sensitivity, altered vascular responsiveness to vasoconstrictors are detected in experimental animals (Khulan & Drake 2012).

In addition, prenatal glucocorticoid overexposure is associated with altered hepatic glucose and lipid metabolism in offspring. In pancreas, the overexposure alters β-cell development in rats and non-human primates. β-cell mass is reduced if overexposure to glucocorticoids happens during the last week of gestation. However, β-cell proliferation and islet vascularization are impacted if exposure happens throughout the pregnancy (Khulan & Drake 2012). Glucocorticoid administration to pregnant women has been used in clinical practice since the 1980s to prevent neonatal respiratory distress syndrome in preterm infants and the long-term effects of the treatment on adult outcomes are not yet fully known. In a cohort of 534 individuals exposed to antenatal betamethasone the subjects had higher insulin levels, suggesting insulin resistance at the age of 30, and may thus have increased risk of T2DM and CVD in later life (Dalziel et al. 2005). Stressful life events have been shown to be associated with subsequent diabetes. Individuals exposed to maternal stress caused by traumatic bereavement of a child during the prenatal period had increased risk of type 1 and type 2 diabetes mellitus in a study including 1,878,000 Danes (Li et al. 2012, Virk et al. 2010).

**Oxidative stress**

Oxidative stress may initiate the process of developmental programming of adult metabolism. There is relatively low oxygen environment *in utero*, which is vulnerable to injury caused by oxidative molecules. Oxidative stress happens
when the production of reactive oxidative species (ROS) exceeds the capacity of antioxidant defense mechanisms in cell. Nutritional deficiency or excess, prenatal hypoxia or glucocorticoid exposure may generate excessive ROS levels. The major ROSs are hydroxyl radical, superoxide anion and hydrogen peroxide (Thompson & Al-Hasan 2012).

The targets of ROSs in cells are mainly phospholipid membranes, proteins and nucleic acids, and mitochondria are the predominant sites of oxidative stress. ROSs can react directly with DNA causing genetic and epigenetic changes. Hypoxia \textit{in utero} and IUGR can alter the methylation status of DNA in selected genes in different tissues including heart, pancreas and liver (Thompson & Al-Hasan 2012). Epidemiologic and animal studies support the association of oxidative stress, IUGR and adult diseases. Children with low birth weight for gestational age display increased lipid peroxidation and increased blood pressure (Nuyt & Alexander 2009).

2.2.6 Animal models of IUGR

Experimental animals are used to study the developmental hypothesis of adult diseases. Animals have a shorter life span and genetic and environmental factors can be thoroughly controlled. Naturally, there are differences between animal and human development, which restricts the extrapolation of data obtained from experimental animals to humans. Rodents have multiple pregnancies and fetal nutrient supply may vary between offspring within the same litter. Sheep pregnancies are singletons or twins and thus bear more resemblance to human pregnancy (Armitage \textit{et al.} 2004). However, several different animal models have been created and they are discussed below.

Maternal dietary manipulation can be created by global diet restriction, protein content reduction, micronutrient (iron or zinc, for example) restriction or fat supplementation. Proportions between macronutrients and the timing and duration of maternal diet manipulation vary widely between studies. Postnatally dam and offspring are maintained on the same diet as prenatally or weaned to the normal chow. The rat is the most often used species in these models (Fernandez-Twinn & Ozanne 2006).

Dietary restriction models range from mild (30% reduction in caloric intake) and moderate (50% reduction) to severe (70% reduction). Low birth weight of offspring is usually achieved. Offspring phenotypes after IUGR induced by caloric restriction are summarized in Table 2 since the caloric restriction was the
method used in the original articles II and III of this thesis. In Table 2, only the changed parameters are listed. It can be concluded that blood pressure of offspring was increased if caloric restriction was induced at the beginning of pregnancy. Changes in pancreatic β-cells were detected when restriction was started in mid-pregnancy.

In protein restriction models the birth weight is lower, unchanged or even higher compared to controls depending on the study design. Central adiposity is rarely increased. Altered structure and function of fetal pancreas, reduced mitochondrial copy number in kidney and liver and glucose intolerance are induced by maternal protein restriction in rat. Also endothelial dysfunction is produced (Armitage et al. 2004).

Global energy restriction or protein restriction in the diet of laboratory animals mimics circumstances in many developing countries or individuals of limited means in Western societies. However, a maternal diet which is rich in fat or cholesterol models Western diet and is also used to induce IUGR. Fat supplementation may cause low birth weight, insulin resistance, increased blood pressure, dyslipidemia and susceptibility to the development of vascular lesions in rat offspring, depending on the timing and amount of the supplement. In addition, increased weight, adiposity of offspring in adulthood is usually present after maternal fat feeding (Armitage et al. 2004).
Table 2. Overview of rat studies on the aspect of metabolic syndrome in caloric restriction models of intrauterine growth restriction.

<table>
<thead>
<tr>
<th>Caloric restriction (of ad libitum)</th>
<th>Intervention period during gestation (days)</th>
<th>Age at measure (weeks)</th>
<th>Changed phenotype</th>
<th>Strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%</td>
<td>0-22</td>
<td>25 w</td>
<td>Plasma insulin ↑, leptin ↑, blood pressure ↑, fat pad mass ↑</td>
<td>Wistar</td>
<td>(Vickers et al. 2001)</td>
</tr>
<tr>
<td>30%</td>
<td>0-22</td>
<td>50 w</td>
<td>Blood pressure ↑</td>
<td>Wistar</td>
<td>(Woodall et al. 1996)</td>
</tr>
<tr>
<td>50%</td>
<td>1-22</td>
<td>4-16 w</td>
<td>14 w: Plasma glucose ↑; 4-14 w: blood pressure ↑</td>
<td>SHR</td>
<td>(Franco Mdo et al. 2002)</td>
</tr>
<tr>
<td>50%</td>
<td>1-22</td>
<td>16 w</td>
<td>Blood pressure ↑, oxidative stress ↑</td>
<td>Wistar</td>
<td>(Franco Mdo et al. 2003)</td>
</tr>
<tr>
<td>50%</td>
<td>10-22</td>
<td>3, 36 w</td>
<td>3 w: Weight ↑, plasma glucose ↓, insulin ↓, TG ↓; 36 w: Weight ↑, plasma glucose ↑, insulin ↑, TG ↑</td>
<td>SD</td>
<td>(Desai et al. 2005)</td>
</tr>
<tr>
<td>50%</td>
<td>11-22</td>
<td>60 w</td>
<td>Weight ↑, hepatic glucose production ↓, GFC ↓</td>
<td>SD</td>
<td>(Garg et al. 2006)</td>
</tr>
<tr>
<td>50%</td>
<td>11-22</td>
<td>3, 11 w</td>
<td>11 w: Weight ↓, plasma insulin ↓, glucose ↑</td>
<td>Wistar</td>
<td>(Holemans et al. 1999)</td>
</tr>
<tr>
<td>50%</td>
<td>14-22</td>
<td>8 w</td>
<td>Plasma insulin ↓</td>
<td>Wistar</td>
<td>(Berlin et al. 1999)</td>
</tr>
<tr>
<td>50%</td>
<td>15-22</td>
<td>3 w</td>
<td>Insulin content in islets ↓, β-cell mass ↓</td>
<td>Wistar</td>
<td>(Garcifano et al. 1997)</td>
</tr>
<tr>
<td>65%</td>
<td>14-22</td>
<td>10 w</td>
<td>Insulin content in islets ↓</td>
<td>Wistar</td>
<td>(Martin et al. 1997)</td>
</tr>
<tr>
<td>70%</td>
<td>0-18</td>
<td>8, 14, 28 w</td>
<td>All age groups: Blood pressure ↑, 28w: Plasma glucose ↑</td>
<td>Wistar</td>
<td>(Ozaki et al. 2001)</td>
</tr>
</tbody>
</table>

GFC; glucose futile cycling, HDL-C; high-density lipoprotein cholesterol, SD; Sprague-Dawley, SHR; spontaneously hypertensive rat, TG; triglycerides, w; weeks.
Placental insufficiency can be created by uni- or bilateral uterine artery ligation, uterine or umbilical artery embolism or carunclectomy (Armitage et al. 2004). These models are appropriate for human pregnancy especially in developed countries where IUGR is more often caused by placental disorders than maternal food restriction. Bilateral uterine artery ligation in pregnant rat induces diabetes in offspring at 15–26 weeks of age. The secretory capacity of β-cells is defected and insulin resistance is detected in these animals. There was also genome-wide DNA hypomethylation and alterations in histone modifications affecting the expression of genes acting in β-cell development (Simmons 2007).

2.3 Role of fructose-rich diet in MS

Monosaccharide fructose can be obtained from sucrose, fruits, honey and from sweeteners such as high fructose corn syrup, which is a commonly used sweetener especially in North America. Fructose has several metabolic effects that may predispose to T2DM and CVD, such as increased visceral adiposity and de novo lipogenesis, dyslipidemia, increased non-alcoholic steatohepatitis, decreased glucose tolerance and insulin sensitivity when compared to glucose (Stanhope 2012).

It has been suggested that the increased use of food and drinks sweetened with fructose is associated with the increasing epidemics of obesity. Since the food intake regulatory system including leptin and insulin is bypassed by fructose and lipogenesis is favored, this association is possible. Naturally, the effect of dietary fructose on health is dependent on the amount consumed. From epidemiological studies it can be concluded that moderate fructose consumption of ≤50g/day or approximately 10% of energy has no harmful effects on lipid and glucose values and ≤100g/day does not induce weight gain (Rizkalla 2010).

Comparison of studies concerning the health effects of fructose is challenging since the fructose used is either pure fructose, in fructose-sweetened beverages or in high fructose corn syrup (containing 55% fructose and 45% glucose) and it is compared with the effects of glucose or sucrose. Features of fructose are different in terms of gastrointestinal effect and absorption depending on whether it is supplemented alone or as a mixture of glucose and fructose: absorption increases if fructose is in mixture with glucose. The amounts used and the durations of diet also vary substantially (Rizkalla 2010).
Metabolic syndrome has been induced in experimental animals by high doses of fructose and the evidence that fructose can induce lipogenesis comes mainly from animal studies (Panchal & Brown 2011). Fructose may increase hepatic TG production and reduce TG clearance by adipose tissue. Lipogenesis is activated in the liver and muscle, but not in the adipose tissue of rats resulting in lipid accumulation in muscle fibers and hepatocytes (Rizkalla 2010). Increased lipid depositions in liver and muscle and VLDL-TG have also been observed in humans after 7-day hypercaloric high-fructose diet (Le et al. 2009). In acute and chronic human studies, high fructose (>15% of daily energy intake) caused increased TG levels in healthy, diabetic, overweight and obese subjects (Rizkalla 2010). Two meta-analyses state that moderate (<50g/day) fructose use has no harmful effects on TG postprandially or after fasting (Livesey & Taylor 2008, Sievenpiper et al. 2009).

Animal models have clearly shown that high fructose feeding induces hepatic and peripheral insulin resistance. However, in humans only very high doses of fructose (25% of energy for 10 weeks) reduce insulin sensitivity. Moderate use has no harmful effects on insulin sensitivity when compared to the same amount of sucrose. Fructose has been used in the diet of diabetic patients since the metabolism does not stimulate insulin secretion and it has low glycemic index. Fructose-induced hypertension is detected in animals, but there is no evidence for that relation in humans (Rizkalla 2010).

### 2.4 Resistin as a linking factor between inflammation and insulin resistance

Adipose tissue is an active organ secreting several endocrine mediators of insulin resistance. The best-known adipocyte-derived proteins are leptin and adiponectin. Leptin regulates metabolism in the whole body by inhibiting food intake, stimulating energy consumption and normalizing blood glucose level (Campfield et al. 1995, Halaas et al. 1995, Pellemounter et al. 1995). Although obese patients have increased leptin secretion, they usually exhibit leptin resistance which limits the biological effects of leptin (Westerterp-Plantenga et al. 2001). Adiponectin has several functions: it increases energy expenditure, insulin sensitivity and fatty acid oxidation (Fruebis et al. 2001, Qi et al. 2004, Yamauchi et al. 2001). It also induces HDL synthesis in the liver (Matsuura et al. 2007). Glucose production in the liver is also reduced by adiponectin (Berg et al. 2001).
In contrast to leptin, adiponectin secretion is diminished in obese patients (Arita et al. 1999, Hu et al. 1996).

Resistin is an adipokine which was discovered in 2001 when the effect of an antidiabetic thiazolidinedione (TZD) drug on gene expression of mouse adipocytes was studied (Steppan et al. 2001a). TDZs enhance the insulin sensitivity of target tissues *in vivo* by acting as a ligand for PPARγ in adipocytes (Lehmann et al. 1995). Resistin is a 12.5 kDa secretory polypeptide belonging to a family of resistin-like molecules (RELMs) or found in inflammatory zone (FIZZ) proteins, which have a unique spacing of 10–11 cysteine-rich residues (Steppan et al. 2001b). In mice it is downregulated by TZDs and its circulating levels are increased in many animal models of obesity (Schwartz & Lazar 2011).

After the first reports about resistin in rodents were published, a strong interest arose towards the potential of resistin being a link between T2DM, obesity and insulin resistance. In contrast to adipocyte-specific expression of resistin in rodents, in humans it is mainly expressed in macrophages, which can accumulate in obesity into adipose tissue and increase inflammation and the secretion of resistin and other non-protecting molecules (Savage et al. 2001). It is also expressed in human placenta, skeletal muscle, small intestine, adipose tissue, spleen, stomach, thymus, thyroid gland and uterus, for example (Nohira et al. 2004). Resistin plays a diverse role in the development of metabolic abnormalities and this is overviewed in Figure 2.
Fig. 2. The role of resistin in metabolic abnormalities. The secretion of resistin from macrophages is regulated by genetics and other factors. Proinflammatory markers affect the secretion of resistin and vice versa. Resistin has proatherogenic effects on smooth muscle cells, endothelial cells and monocytes. It induces insulin resistance in hepatocytes and adipocytes. In liver, resistin impairs gluconeogenesis and decreases glucose transport. IL-6, interleukin-6; LPS, lipopolysaccharide; TDZ: thiazolidinedione; TNF-α, tumor necrosis factor-α.

2.4.1 Resistin and inflammation

The mechanisms of how the biological activity of resistin is mediated are still unclear since the receptor of resistin has not been found. There are suggestions that the receptor might be the endotoxin receptor Toll-like receptor 4, which is shown to mediate the proinflammatory effects of resistin (Tarkowski et al. 2010). Inflammatory stimulus strongly increases the expression and secretion of resistin. This linkage exists also vice versa, since resistin increases the secretion of several cytokines, mitogens and endotoxins (Pang & Le 2006). Cell adhesion molecules, like vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) production in human macrophages is induced when incubated with exogenously added resistin. In addition, vascular smooth muscle cell proliferation and endothelial dysfunction are promoted by resistin. In studies with obese, diabetic
and lean subjects, correlations of serum resistin to markers of inflammation, such as CRP, TNF-α and IL-6, have been detected (Schwartz & Lazar 2011).

Increased resistin expression has been detected in many inflammatory diseases such as chronic liver diseases, lung diseases, atherosclerosis, rheumatoid arthritis and kidney disease and it has a clear correlation to other markers of inflammation (Tarkowski et al. 2010).

2.4.2 Resistin and insulin resistance

Animal studies have provided irrefutable evidence linking resistin to insulin resistance. In mice overexpressing resistin have decreased insulin sensitivity and altered glucose homeostasis have been demonstrated. Same phenomena are observed after administrating exogenous recombinant resistin and opposite reactions happen when resistin activity is blocked or its levels are genetically decreased (Schwartz & Lazar 2011).

In cell culture studies, resistin induces insulin resistance in myocytes (Sheng et al. 2013) and hepatocytes (Zhou et al. 2007). Epidemiological studies have attempted to show a correlation between resistin and T2DM leading to conflicting results. High levels of resistin in insulin-resistant or diabetic subjects have been reported in some studies, but not in all (Lazar 2007).

2.4.3 Resistin and obesity

In an analogous manner to the above-mentioned results concerning insulin resistance, contradictory results have been published about the connection between resistin and obesity. In cell culture studies it has been observed that resistin induces lipolysis process in adipocytes causing elevated free fatty acid secretion from the cells (Ort et al. 2005). In several studies, obese, non-diabetic subjects had higher resistin levels and resistin was associated with the levels of visceral and subcutaneous fat mass. Also weight loss by diet and exercise or bariatric surgery reduced resistin levels, but opposite results or no change in resistin after weight loss are also reported. Quite small and different study populations, variable comorbidities and medications and assays used for measuring resistin may partly be among the reasons for these discrepancies between studies. However, there is an implication that resistin could further strengthen the association between obesity and inflammation (Schwartz & Lazar 2011).
3 Aims of the present study

The aim of this thesis was to examine the role of intrauterine growth restriction as well as the peptide hormone resistin in the development of metabolic syndrome and its components.

The specific aims of the study are as follows:

1. To examine whether resistin has a biomarker role for metabolic syndrome in a Finnish cross-sectional study using five different definitions of metabolic syndrome.
2. To study the effect of fetal growth restriction on obesity-related peptide hormones, specifically resistin, in rat offspring.
3. To investigate the effects of fetal growth restriction and postnatal fructose diet on the growth, lipid and glucose metabolism of rat offspring.
4 Subjects, animals and methods

4.1 Subjects (I)

We studied subjects from a subsample of the large Finnish cross-sectional Health 2000 Health Examination Survey carried out in 2000–2001 (Aromaa & Koskinen 2004). The overall study cohort consisted of a two-stage stratified cluster sample (8,028 persons) representing the entire Finnish population aged 30 years and above.

In order to study CVD and diabetes in more detail, a subpopulation study was carried out. The substudy (n=1,867) was executed in five Finnish University Hospitals. The measurement of resistin levels was carried out on 1,508 subjects (687 men and 821 women; mean age, 57 years; range, 45–74 years).

4.2 Experimental animals (II, III)

The experimental designs were approved by the Animal Care and Use Committee of the University of Oulu (II) and the National Animal Experiment Board (III).

4.2.1 Pilot study (II)

Daily food intake during gestation was determined first by weighing the consumed food of pregnant Sprague-Dawley rats (n=4) daily throughout the gestation. Then virgin rats were mated and given 50% (n=9) or 75% (n=9) of ad libitum food intake from the fourth day of gestation until delivery. The control group (n=6) received food ad libitum. Litter sizes were equalized to six offspring, three male and three female pups, per dam. Dams and offspring received food ad libitum after delivery. The weight of the offspring was followed until one month of age, when the offspring were killed and blood was taken by cardiac puncture.

4.2.2 IUGR and postnatal fructose diet (III)

Schematic presentation of the study design is shown in Figure 3. From day 4 of gestation, a group of dams received standard laboratory chow (Harlan Teklad Global 18% Protein Rodent Diet, energy density 3.1 kcal/g, 24% of calories from protein, 18% from fat and 58% from carbohydrate, Harlan Teklad, Indianapolis,
Indiana, US) ad libitum whereas the other group received only 50% of the ad libitum food intake until delivery. The normal daily food intake during pregnancy was determined previously in study II.

![Experimental design of study III](image)

After delivery, all dams and offspring received standard chow ad libitum. On postnatal day 1, the litter sizes were equalized to 8 pups (4 males and 4 females) per litter and the following study groups were assigned: ad libitum fed dams with their own pups (CC), ad libitum fed dams with pups born from food-restricted dams (RC) and food-restricted dams with their own pups (RR). The offspring were weighed three times a week and weaned on postnatal day 23.

At the age of 1 month, seven CC litters, five RC litters and six RR litters were studied in the following manner: Two male and two female pups from each litter were anesthetized by isoflurane inhalation, blood was obtained by cardiac puncture after which the rats were killed by decapitation. Blood was collected into heparinized vacutainers and centrifuged. Plasma was removed and stored at -70 °C for later use. From one month of age onwards, half of the litters of each
study group received a fructose-rich diet (Harlan Teklad TD89247 60% Fructose Diet, energy density 3.6 kcal/g, 20.2% of calories from protein, 12.9% from fat and 66.8% from carbohydrate, Harlan Teklad) while the other half continued receiving the standard diet. At the age of 6 mo, the same procedures as described above were conducted and samples taken as previously except that the rats were fasted for 12 hours.

4.3 Methods

4.3.1 Metabolic syndrome

We used five different definitions of MS (Table 1): the definitions devised by the World Health Organization (Alberti & Zimmet 1998), the European Group for the Study of Insulin Resistance (Balkau & Charles 1999), the National Cholesterol Education Program’s Adult Treatment Panel III (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002), the International Diabetes Federation (Alberti et al. 2005) and the latest definition by IDF Task Force on Epidemiology and Prevention, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity (Alberti et al. 2009). In this study this definition is called Harmonized Definition (HD). The prevalence of MS in the present study population was 23.5%, 22.0%, 30.0%, 45.2% and 46.7% by the five definitions, respectively. There were two exceptions from the definition devised by WHO in the present study: Urinary albumin excretion was not measured and insulin resistance was not determined by the clamp technique. Instead, the fourth quartile of fasting insulin level was used as a variable to describe insulin resistance.

4.3.2 Clinical methods (I)

The measurements of waist circumference, height, weight and blood pressure were conducted by trained physicians and nurses. Blood pressure was measured from the right arm after at least 10 minute’s rest by automatic, oscillometric device. The measurement was taken three times with 1- to 2-min intervals and the average of these measurements was used in the analysis.
4.3.3 Blood sample analysis (I-III)

Blood sample analyses were measured from serum (study II) or from plasma (I, III). Concentrations of TG (I-III), total cholesterol (I-III) and HDL-C were determined by the enzymatic colorimetric method (Roche Diagnostics, Mannheim, Germany). LDL-C (I-III) was calculated by using Friedewald’s formula (Friedewald et al. 1972).

Insulin concentration was measured by commercial radioimmunoassay (I) (Phadeseph Insulin RIA, Pharmacia Sweden, Sweden) or enzyme-linked immunosorbent assay (ELISA) (II-III) (Merck Millipore, Billerica, MA, USA). Glucose (I, III) was determined by the glucose dehydrogenase method (Diagnostica Merck, Darmstadt, Germany). Adiponectin (II, III) (Merck Millipore, Billerica, MA, USA), leptin (II) (Merck Millipore, Billerica, MA, USA) and total ghrelin (II) (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) concentrations were determined by commercial ELISA kits. High-sensitivity CRP (I), IL-6 (I) and TNF-α (I) concentrations were determined by commercial chemiluminescent immunometric assays (Immulite, Diagnostic Products Corporation, Siemens Healthcare Diagnostics, USA).

Human resistin concentration was measured with an in-house assay based on the Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA®, PerkinElmer, Waltham, MA, USA) (I). The intra- and interassay coefficients of variation for the in-house assay were 8.6% and 11.0%, respectively. The in-house assay was compared to a commercial ELISA assay with separate human plasma samples and the methods correlated strongly ($r=0.754$, $P<0.001$). The in-house assay gave approximately 10% higher concentrations. Rat resistin was measured by a commercial ELISA kit (II, III) (B-Bridge International, Cupertino, CA, USA).

4.3.4 Magnetic resonance imaging (III)

Magnetic resonance imaging (MRI) was performed on six-month-old rats (n=4 males and females per group from CC and RR study groups). MRI was conducted using a 4.7 T horizontal magnet. The lipid content was analyzed from the 3D images from retroperitoneal, intra-abdominal and subcutaneous areas of adipose tissue. All data analysis was done using the home-built image processing software Aedes (aedes.uku.fi) and another in-house MATLAB application. More detailed information about MRI and image analysis is given in the original article III.
4.3.5 Statistical methods (I-III)

Statistical analysis software SPSS for Windows (versions 15.0 & 16.0, Spss Inc., Chicago, USA) was used for the statistical analyses. Analysis of variance (ANOVA) and Analysis of covariance (ANCOVA) were used when variables were normally distributed and the variances between groups were approximately equal. A P-value of less than 0.05 was considered statistically significant.

Logarithmic transformation was performed if data was not normally distributed. In the case of skewed distribution the non-parametric Mann-Whitney test was used. Categorical data were compared using the chi-square test. Correlations were tested with the Pearson correlation. Logistic regression was used to find out independent relationships among MS, its components and resistin (I).

In publication III, three-way ANOVA was used when the effect of fetal undernutrition (IUGR or normal conditions during pregnancy), suckling period (lactating dam food-restricted during pregnancy or not), postnatal diet (fructose-rich or standard chow from one month to six month of age) and their interactions were studied between the study groups. Two-way ANOVA was used only when the effect of IUGR and suckling period on the body weight of one-month-old rats was studied and when analyzing results obtained from MRI.
5 Results

5.1 High plasma resistin is associated with MS (I)

The aim of this study was to explore the association of resistin with MS in a Finnish cross-sectional study. The median resistin concentration in the study population was 62.9 ng/mL (interquartile range 45.3). The main characteristics of the subjects according to the resistin tertiles are presented in Table 3. The prevalences of MS in resistin tertiles increased with increasing resistin level: When MS was defined by the WHO criteria the prevalences were 18.1% in the first tertile and 28.7% in the third tertile, by EGIR 16.6% and 26.4%, by NCEP ATP III 26.0% and 33.7%, by IDF 40.3% and 48.8%, and by HD 41.3% and 50.4%, respectively. Resistin correlated significantly with waist circumference, total cholesterol, LDL-C, HDL-C, homeostasis model assessment of insulin resistance (HOMA-IR) and TNF-α after adjusting for age, sex and BMI.

![Resistin levels in subjects with or without MS](image)

Fig. 4. Resistin in study subjects with or without metabolic syndrome defined by different criteria. Statistically significant differences were observed in resistin levels adjusted for age and sex in the study subjects with MS when compared to the subjects without MS. Black bars: subjects with MS, white bars: subjects without MS. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Data are means (±SEM). MS, metabolic syndrome; WHO, the World Health Organization; EGIR, European Group for the Study of Insulin Resistance; ATP III, The National Cholesterol Education Program's Adult Treatment Panel III; IDF, International Diabetes Federation and HD, Harmonized Definition. (I, published by the permission of Mary Ann Liebert Inc.)
Plasma resistin concentrations were statistically significantly higher in subjects with MS compared to subjects without MS irrespective of the definition of MS that was used (Figure 4). A logistic regression model was used to examine the influence of resistin tertiles, sex and age on metabolic syndrome as independent variables. A high resistin level was a statistically significant determinant of MS ($P<0.05$ for every five MS definitions). Subjects in the third resistin tertile had a higher risk of having MS compared to the subjects in the first tertile. The highest OR was obtained using the WHO definition for MS (1.70, 95% CI 1.26–2.31, $P=0.001$). The IDF definition resulted in the lowest OR (1.31, 95% CI 1.00–1.71, $P=0.047$).

Table 3. Main characteristics of the study subjects according to the resistin tertiles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>P</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>503</td>
<td>503</td>
<td>502</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resistin (ng/mL)$^1$</td>
<td>38.1 (13.1)</td>
<td>62.9 (14.4)</td>
<td>102.1 (34.4)</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001^*$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.4 (55.7-57.1)</td>
<td>56.6 (56.0-57.4)</td>
<td>57.9 (57.2-58.6)</td>
<td>0.007</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>26.6 (26.2-26.9)</td>
<td>27.4 (26.9-27.8)</td>
<td>27.7 (27.3-28.1)</td>
<td>0.001</td>
<td>0.002$^*$</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.2 (91.0-93.4)</td>
<td>93.8 (92.6-95.1)</td>
<td>95.1 (93.9-96.3)</td>
<td>0.004</td>
<td>0.036$^*$</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.7 (5.6-5.8)</td>
<td>5.6 (5.5-5.7)</td>
<td>5.5 (5.4-5.6)</td>
<td>0.007</td>
<td>0.005$^b$</td>
</tr>
<tr>
<td>HDL-C (mmol/L)$^1$</td>
<td>1.6 (0.60)</td>
<td>1.5 (0.58)</td>
<td>1.5 (0.51)</td>
<td>$&lt;0.001$</td>
<td>0.015$^b$</td>
</tr>
<tr>
<td>TG (mmol/L)$^1$</td>
<td>1.1 (0.80)</td>
<td>1.2 (0.70)</td>
<td>1.3 (0.80)</td>
<td>0.004</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.4 (3.3-3.5)</td>
<td>3.4 (3.3-3.5)</td>
<td>3.3 (3.2-3.4)</td>
<td>NS</td>
<td>0.019$^b$</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)$^{1,2}$</td>
<td>7.4 (4.7)</td>
<td>7.8 (5.1)</td>
<td>8.2 (5.6)</td>
<td>0.024</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)$^{1,2}$</td>
<td>5.6 (0.80)</td>
<td>5.6 (0.80)</td>
<td>5.6 (0.80)</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>HOMA-IR$^{1,2}$</td>
<td>1.5 (1.2)</td>
<td>1.6 (1.6)</td>
<td>1.6 (1.6)</td>
<td>0.003</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>TNF-α (ng/L)$^1$</td>
<td>5.3 (2.4)</td>
<td>5.7 (2.6)</td>
<td>6.0 (2.4)</td>
<td>$&lt;0.001$</td>
<td>0.001$^b$</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>1.4 (1.2)</td>
<td>1.5 (1.4)</td>
<td>1.6 (1.3)</td>
<td>NS</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>2.7 (2.4-3.1)</td>
<td>2.9 (2.5-3.2)</td>
<td>3.2 (2.8-3.6)</td>
<td>NS</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.5 (135.8-139.3)</td>
<td>139.1 (137.1-141.1)</td>
<td>140.2 (138.2-142.1)</td>
<td>NS</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.1 (83.2-84.0)</td>
<td>84.5 (83.6-85.5)</td>
<td>84.7 (83.7-85.6)</td>
<td>NS</td>
<td>NS$^b$</td>
</tr>
</tbody>
</table>

Data are expressed as means (95% confidence intervals) or medians (interquartile range)$^1$. $^a$Adjusted for age and sex; $^b$adjusted for age, sex and BMI; $^c$adjusted for age, sex, BMI and smoking. $^d$Subjects with type 2 diabetes mellitus are excluded. BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; TG, triglyceride; TNF-α, tumour necrosis factor-α. (I, published by the permission of Mary Ann Liebert Inc.)
The subjects were grouped according to the presence of increasing number of components of MS. The resistin levels tended to increase in relation to the number of components (Figure 5).

![Graph showing resistin levels in relation to MS components](image)

**Fig. 5.** The mean resistin concentrations in relation to the number of components of metabolic syndrome. *P*≤0.05, ***P*≤0.001. MS, metabolic syndrome; WHO, the World Health Organization; EGIR, European Group for the Study of Insulin Resistance; ATPIII, The National Cholesterol Education Program’s Adult Treatment Panel III; IDF, International Diabetes Federation and HD, Harmonized Definition. (I, published by the permission of Mary Ann Liebert Inc.)

5.2 IUGR affects adipokine levels of rat in early adulthood (II)

The aim of this study was to investigate the effect of IUGR on obesity-related peptide hormones in rat offspring. Secondarily, the purpose was to investigate whether the observed changes in peptide hormones are connected to changes in lipid and insulin metabolism.

On postnatal day 1, the weight of the pups from 50% and 75% energy restricted dams was significantly lower compared to the control pups (*P*<0.001). The rate of weight gain did not differ between the groups during the first month. No catch-up growth in weight was detected even though the mean weight of the 75% food-restricted group was slightly higher compared to the control group; however, this did not reach statistical significance.
The levels of serum peptide hormones were measured in the one-month-old pups. Adiponectin was lower in the pups of both food-restricted groups in comparison with the control pups (Figure 6A) with a statistically significant difference observed between the 50% food-restricted group and control group ($P=0.019$). The mean concentration values for resistin were significantly higher in the 75% and 50% food-restricted groups compared to controls ($P=0.037$ and $P=0.040$, respectively) (Figure 6B). There was no statistically significant difference in serum leptin or ghrelin concentrations, although ghrelin levels tended to be lower in the pups of food-restricted dams (Figure 6C-D).

Serum total cholesterol at one month of age was significantly higher in the 50% food-restricted group compared to either the control group ($P<0.000$) or the 75% group ($P=0.001$). Serum TG levels were higher in the 50% food-restricted group compared to the control group ($P=0.049$) (Table 4). No statistically significant differences were observed between the groups in insulin concentrations (data not shown).

![Figure 6. The mean concentrations of peptide hormones in rat offspring fed ad libitum compared to offspring from dams fed either 75% or 50% of ad libitum food intake. The error bars indicate standard deviations. Serum adiponectin (panel A), resistin (panel B), leptin (panel C) and ghrelin (D) were measured from one-month-old offspring. Control n=12, 75% n=24, 50% n=18. (II, published by the permission of Elsevier.)](image-url)
Table 4. The mean concentrations of serum lipids in the one-month-old rat offspring.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>75%</th>
<th>50%</th>
<th>P control vs. 50%</th>
<th>P control vs. 75%</th>
<th>P 75% vs. 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>2.9 (0.3)</td>
<td>3.0 (0.4)</td>
<td>3.6 (0.6)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.1 (0.4)</td>
<td>1.1 (0.6)</td>
<td>1.1 (0.7)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.5 (0.1)</td>
<td>0.5 (0.3)</td>
<td>0.6 (0.2)</td>
<td>0.049</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are means (standard deviation). The control group (n=12) was fed ad libitum and IUGR groups 75% (n=21) or 50% (n=18) of ad libitum food intake during gestation. HDL, high-density lipoprotein; NS, not significant; TC, total cholesterol; TG, triglyceride. (II, published by the permission of Elsevier.)

5.3 Effect of IUGR and postnatal fructose diet on plasma lipids, adiposity and body weight (III)

The aim of study III was to investigate the effect of IUGR and postnatal fructose diet on plasma lipids, body weight and fat content measured by MRI.

One-day-old fetally undernourished offspring were smaller than control offspring (males’ mean weight in CC litters 6.61 (SD 0.36) g and in RR litters 5.76 (0.41) g, t-test \( P < 0.001 \), females’ mean weight in CC litters 6.22 (0.50) g and in RR litters 5.37 (0.90) g, t-test \( P < 0.001 \). At the age of one month, the weight of the IUGR pups did not differ from the weight of control pups. At the age of six months, both male and female IUGR pups were smaller than control pups according to three-way ANOVA analysis (\( P < 0.001 \) and \( P < 0.001 \), respectively). Fructose diet did not induce weight gain in control or fetally undernourished rats. In contrast, the fructose-fed six-month-old male rats were smaller than the rats which received standard diet (\( P = 0.012 \)).

5.3.1 Plasma lipid, glucose and insulin values

The effects of fetal undernutrition on the plasma lipid levels of one- and six-month-old offspring were examined. At the age of one month, total cholesterol levels were higher in IUGR female rats (CC mean 2.64 (SD 0.36), RR 2.94 (0.30) and RC 2.99 (0.59) mmol/L, \( P = 0.013 \)). The increase in total cholesterol was also seen in fetally undernourished male rats, but the difference was not statistically significant (CC 2.77 (0.29), RR 3.00 (0.27) and RC 2.86 (0.29) mmol/L, \( P = 0.305 \)). The difference in HDL cholesterol in IUGR female rats also reached
statistical significance (CC 0.90 (0.24), RR 1.04 (0.26) and RC 1.20 (0.69) mmol/L, $P=0.045$).

The effects of fetal undernutrition on plasma lipid levels in the six-month-old male and female rats are shown in Table 5. Fetally undernourished rats had significantly higher LDL cholesterol levels (for males $P<0.001$ and females $P=0.001$). In one-month-old rats, there were no differences in fasting glucose and insulin levels between the study groups (data not shown). Fasting glucose and insulin levels of six-month-old rats are shown in Table 5. Due to IUGR, male offspring were more insulin-sensitive based on HOMA-IR ($P=0.041$). The fructose diet increased plasma total cholesterol, triglyceride and HDL cholesterol levels in female rats ($P=0.004$, $P<0.001$ and $P=0.001$, respectively). It increased triglyceride level and decreased LDL level in male rats ($P<0.001$, and $P<0.001$, respectively).

Fasting glucose was lower in female rats who had eaten a fructose-rich diet (Table 5, $P=0.008$) but not in male rats ($P=0.841$). Fasting insulin was higher in male and female rats on fructose diet ($P=0.012$ and $P=0.030$, respectively). Fructose impaired insulin sensitivity in six-month-old male rats ($P=0.026$) but did not have any effect on insulin sensitivity in females ($P=0.382$).
Table 5. Lipid and glucose profile of six-month-old rats exposed to IUGR and standard or fructose diet in adult life.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard diet</th>
<th>Fructose diet</th>
<th>IUGR effect</th>
<th>Suckling period effect</th>
<th>Fructose diet effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC RR RC CC RR RC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>2.23 (0.34)</td>
<td>3.44 (1.13)</td>
<td>2.58 (0.76)</td>
<td>2.87 (0.98)</td>
<td>2.90 (0.45)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.65 (0.21)</td>
<td>0.78 (0.33)</td>
<td>0.52 (0.30)</td>
<td>1.68 (0.75)</td>
<td>1.76 (0.61)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.17 (0.27)</td>
<td>1.67 (0.66)</td>
<td>1.18 (0.49)</td>
<td>1.50 (0.36)</td>
<td>1.49 (0.57)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.79 (0.21)</td>
<td>1.32 (0.47)</td>
<td>1.20 (0.30)</td>
<td>0.65 (0.42)</td>
<td>0.56 (1.06)</td>
</tr>
<tr>
<td>f-glucose (mmol/L)</td>
<td>6.15 (1.33)</td>
<td>6.35 (1.05)</td>
<td>5.60 (1.18)</td>
<td>5.50 (2.73)</td>
<td>7.00 (2.25)</td>
</tr>
<tr>
<td>f-insulin (ng/ml)</td>
<td>0.81 (0.39)</td>
<td>0.73 (0.36)</td>
<td>0.56 (0.28)</td>
<td>1.28 (1.84)</td>
<td>1.33 (1.58)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.24 (0.15)</td>
<td>0.22 (0.12)</td>
<td>0.15 (0.08)</td>
<td>0.33 (0.70)</td>
<td>0.35 (0.40)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>2.60 (0.56)</td>
<td>2.66 (0.97)</td>
<td>2.73 (0.76)</td>
<td>3.12 (0.53)</td>
<td>2.82 (0.76)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.56 (0.45)</td>
<td>0.56 (0.27)</td>
<td>0.51 (0.28)</td>
<td>1.01 (0.90)</td>
<td>0.84 (0.72)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.36 (0.34)</td>
<td>1.63 (0.77)</td>
<td>1.46 (0.52)</td>
<td>1.87 (0.50)</td>
<td>1.78 (0.56)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.81 (0.40)</td>
<td>1.00 (0.46)</td>
<td>1.06 (0.36)</td>
<td>0.72 (0.36)</td>
<td>0.69 (0.23)</td>
</tr>
<tr>
<td>f-glucose (mmol/L)</td>
<td>5.60 (0.88)</td>
<td>6.20 (1.30)</td>
<td>5.40 (1.63)</td>
<td>4.75 (1.05)</td>
<td>5.30 (1.23)</td>
</tr>
<tr>
<td>f-insulin (ng/ml)</td>
<td>0.51 (0.27)</td>
<td>0.46 (0.15)</td>
<td>0.41 (0.07)</td>
<td>0.55 (0.19)</td>
<td>0.60 (0.36)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.12 (0.09)</td>
<td>0.12 (0.04)</td>
<td>0.11 (0.03)</td>
<td>0.11 (0.04)</td>
<td>0.13 (0.09)</td>
</tr>
</tbody>
</table>

Data are means (standard deviation) or medians (interquartile range). CC, offspring from control dams; RR, IUGR rats raised by their own mothers; RC, IUGR rats raised by control dams; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IUGR, intrauterine growth restriction; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerol. N=12-14 rats per group, except in glucose and insulin measurements N=6-7 rats per group. According to the univariate model, there were statistically significant interactions between suckling period and fructose diet in LDL-C in male and female offspring (P=0.004 and P=0.013, respectively).
5.3.2 Lipid analyses of adipose tissue

Magnetic resonance imaging was performed on six-month-old CC and RR rats. Lipid content (as %) of retroperitoneal, intra-abdominal and subcutaneous adipose tissue according to MRI is shown in Table 6. IUGR did not have any effect on the lipid content percentages of the tissues between the CC and RR groups. The postnatal fructose diet increased the amount of lipids in retroperitoneal and intra-abdominal adipose tissues in male rats ($P=0.008$ and $P=0.024$, respectively). There were no differences in lipid content in female rats.

Table 6. Comparison of lipid content (%) of different adipose tissues of six-month-old rats exposed to IUGR and standard or fructose diet in adult life.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Retroperitoneal adipose tissue</th>
<th>Subcutaneous adipose tissue</th>
<th>Intra-abdominal adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>CC standard</td>
<td>88.6</td>
<td>90.1</td>
<td>87.5</td>
</tr>
<tr>
<td>RR standard</td>
<td>88.6</td>
<td>88.8</td>
<td>85.6</td>
</tr>
<tr>
<td>CC fructose</td>
<td>91.5</td>
<td>88.6</td>
<td>88.6</td>
</tr>
<tr>
<td>RR fructose</td>
<td>90.9</td>
<td>91.1</td>
<td>88.5</td>
</tr>
<tr>
<td>IUGR+lactation period effect$^a$</td>
<td>$P=0.752$</td>
<td>$P=0.589$</td>
<td>$P=0.472$</td>
</tr>
<tr>
<td>Fructose diet effect</td>
<td>$P=0.008$</td>
<td>$P=0.718$</td>
<td>$P=0.169$</td>
</tr>
</tbody>
</table>

Data are means (standard deviation). CC, rat offspring from control dams; RR IUGR offspring lactated by their own dams. N=4 male and female offspring per group. $^a$IUGR+lactation period effect refers to combined effect of both IUGR and lactation by a dam which was food-restricted during pregnancy. (III, published by the permission of Nature Publishing Group.)

5.3.3 Plasma resistin and adiponectin

Plasma resistin and adiponectin concentrations were measured from plasma derived from one- and six-month-old rats. At the age of one month there were no changes in resistin or adiponectin induced by IUGR or suckling period.

Plasma resistin and adiponectin levels in rats at the age of six months are presented in Figure 7. Resistin levels were significantly increased by IUGR ($P=0.006$ in males and $P=0.017$ in females). Fructose diet decreased resistin in female rats ($P=0.020$). There were no differences in adiponectin levels between the groups.
Fig. 7. Plasma resistin and adiponectin levels in six-month-old rats. Panel A and C represent resistin and adiponectin levels in male rats, panel B and D resistin and adiponectin levels in female rats. White boxes represents standard diet fed animals, grey boxes fructose diet fed animals. Boxes represent median and middle quarters of resistin and adiponectin concentrations. Whiskers indicate the range from minimum to maximum value. Differences between study groups were examined by three-way ANOVA. Resistin levels were significantly increased by IUGR ($P=0.006$ in males and $P=0.017$ in females). Fructose diet decreased resistin in female rats ($P=0.020$). There were no differences in adiponectin levels between the groups. N=12–14 per group.
6 Discussion

6.1 Methodological consideration

6.1.1 Study population

The Health 2000 Health examination survey is a large, population-based, cross-sectional study. Resistin was measured from subpopulation of Health 2000 that consisted of 1,508 subjects. Health 2000 is clinically very well defined. At the time these measurements were performed this population was one of the largest from which resistin concentrations were obtained. The Health 2000 is a cross-sectional study; the causal relationship between resistin and MS cannot therefore be stated.

6.1.2 Resistin measurements

There is no international, cross-standardized method available for measurement of resistin concentration. Due to large variety of methods, it is impossible to say what the limits of low, normal and high concentration of resistin are and each method has its own limits. The reported normal resistin concentrations in healthy humans vary approximately between 2–200 ng/mL in literature (Aquilante et al. 2008, Fargnoli et al. 2010, Fehmann & Heyn 2002, Osawa et al. 2009, Silha et al. 2004). This large variation is explained by different methods, antibodies, standards used in the immunoassays and the lack of international quality control trials. Diurnal variation of resistin is reported to be quite low (Reilly et al. 2005).

Plasma samples of the Health 2000 study were stored at -70 °C and were thawed and freeze twice at the most. Resistin has disulfide bridges, which connect the monomer polypeptide chains via cysteine residues both intra- and intermolecularly. These bonds are quite resistant and therefore the molecule structure may not suffer from thawing and freezing (Aruna et al. 2003).

There were 687 men and 821 women in the study population. Gender differences in resistin concentrations have been reported in some studies, women having higher resistin levels which may result from the effects of female sex hormones (Lee et al. 2003, Silha et al. 2004). However, in study I the association of gender with resistin level was not significant as a determinant of MS, so all analyses were performed for the whole population.
6.1.3 Choice of the animal model

The choice of animal model to study the effects of IUGR is evidently very important. Eventually, the goal is to apply the information obtained from animal studies to humans. The genes, biochemical pathways, organs and physiology of rodents are highly related to humans. However, there is one major difference in the development between rodents and humans: rodents are born with an underdeveloped brain and endocrine system and maturation happens during the weaning period (Vuguin 2007). The benefit of using rat as a model animal is that collecting different samples as blood is appropriate for a variety of analyses to be performed.

The cholesterol metabolism of rat differs from that in humans in that rats lack a CETP. The function of CETP is the transport of cholesteryl ester from HDL to LDL and VLDL with concomitant transfer of TG to HDL. Rats have less LDL-like lipoproteins and HDL is the major cholesterol-carrying lipoprotein. Therefore the species is resistant to atherosclerosis (Charlton-Menys & Durrington 2008). This difference in cholesterol metabolism may weaken the value of the observed effects of IUGR and postnatal fructose diet on LDL-C of rats in our study. In addition, LDL is calculated by Friedewald’s formula instead of using ultracentrifugation. Ultracentrifugation would be a more reliable method for estimation of LDL cholesterol in rats but the procedure could not be performed in our study. However, the LDL-C estimated by Friedewald’s formula has been used before in rat studies. The use of the formula in rats has been evaluated in a publication of Sanchez-Muniz and Bastida (Sanchez-Muniz & Bastida 2008) where the authors compared the LDL-C obtained by Friedewald’s formula and by ultracentrifugation. The conclusion was that the use of Friedewald’s formula is suitable if serum total cholesterol is <2.6 mmol/L. In our study there were 14 male offspring from group CC, 4 from RR and 11 from RC whose total cholesterol was <2.6 mmol/L. Among these offspring the IUGR effect, postnatal diet effect and interaction effect on LDL-C was statistically significant, such as in our total offspring population. The main interests in this study design were the possible differences in LDL-C between the study groups, not trying to obtain absolute values for LDL-C. So in this study the use of the Friedewald formula is justified in the absence of LDL determined by ultracentrifugation.

In this thesis, IUGR was created by reducing the food intake of pregnant rats to 75% or 50% of *ad libitum* from the fourth day of gestation until the delivery.
The common cause of IUGR in Western societies, placental insufficiency, is represented in our study by the RC group where the food restriction lasts for the whole pregnancy while birth the nutrition of the child is usually generous after birth. In order to confirm successful beginning of pregnancies we did not induce IUGR immediately after the pregnancy was detected by vaginal plug. However, due to early induction of IUGR, it has effects on the development of the placenta. It can be hypothesized that IUGR throughout pregnancy modulates the development of the placenta. Thus it might be that the fetus does not need to adapt in such a drastic manner since the effects of IUGR on glucose and insulin metabolism were not that evident in the present study. In the study by Desai and colleagues, IUGR was started on day 10 of pregnancy and it caused more harmful changes such as increased plasma glucose, insulin and TG levels in 9-month-old offspring (Desai et al. 2005). In another study, β-cell mass and insulin content was significantly reduced already after birth in one-day-old offspring after IUGR which was induced on pregnancy day 15. These changes were retained until 21 days of age (Garofano et al. 1997). Pancreatic β-cells were not studied in the present thesis.

One limitation of the present rat model was the lack of a study group where offspring of control dams would be reared by dams that were food-restricted during pregnancy (CR group). Inclusion of the CR group would have enabled better determination of the effects of the suckling period on later health of the offspring.

6.2 Can resistin be used as a biomarker of MS?

The aim of study I was to find out whether resistin is associated with MS as defined by five different definitions. During 2011, this was the first publication about resistin and MS where the harmonized definition of MS was used. Contradictory results about the association between resistin and MS have been reported in earlier studies, the majority containing significantly smaller numbers of subjects (n=120–1,000) compared to our study. In the cases where association was found, subjects with MS had higher resistin levels (Aquilante et al. 2008, Gupta et al. 2011, Norata et al. 2007). The association was not found in all studies (Stenholm et al. 2010, Utzschneider et al. 2005, Won et al. 2009). Previously, IDF and NCEP ATP III definitions of MS have usually been used. In addition to these, the present study focussed on other definitions to explore if the subjects with MS
identified by different definitions have high resistin levels. All the five definitions performed similarly in that sense.

In the present study, resistin concentration was positively correlated with waist circumference after adjusting for sex, age and BMI. Subjects in the third resistin tertile also had higher BMI compared to subjects in the first tertile. BMI as a measure of obesity has some limitations. It is calculated by dividing weight (in kilograms) by height (in meters) squared. As it is based on height and weight, it does not directly measure body fat. BMI does not take into account age, sex, bone mass, fat distribution or muscle mass. Muscle mass, for instance, decreases and fat mass increases with age, but the weight and height does not necessarily reflect the changes and BMI value may stay the same (Rothman 2008). Waist circumference correlates with abdominal mass and is associated with increased risk of CVD. BMI may remain unchanged when waist circumference and risk of CVD are reduced. For T2DM risk, waist circumference is a stronger predictor than BMI (Klein et al. 2007). Of the MS definitions used in this study, all definitions except WHO included waist circumference as a measure of obesity. WHO defined obesity as increased waist-to-hip-ratio or BMI.

Resistin was negatively correlated with plasma HDL-C and positively correlated with plasma TG levels, which are components of MS. The same findings were reported among 1,000 Italian subjects, where resistin was also associated with increased waist circumference and systolic blood pressure, decreased HDL-C and ApoA1 level and increased risk of CVD calculated by Framingham algorithm, mainly in women (Norata et al. 2007).

Resistin was also positively correlated with HOMA-IR, which is a rather relevant estimate of insulin resistance. Based on results obtained from rodent studies, including acute resistin administration, hepatic resistin gene delivery and its transgenic expression, resistin was initially considered to be associated with insulin resistance also in humans similarly to murine and rat resistin (Lazar 2007, Steppan et al. 2001a). Similar positive associations with insulin resistance have also been reported by others. However, several attempts to find connection between resistin and insulin resistance or T2DM have failed (Lazar 2007). The correlation with insulin resistance, that we found, does not tell anything about the causality of high resistin levels and insulin resistance, since this study was cross-sectional. In addition, translating the rodent data into a human context is not simple. Human and mouse resistin sequences share approximately 60% identity on amino acid and messenger ribonucleic acid (mRNA) level and only 45% on
genomic sequence level, which is less than in most conserved hormones across the species (Schwartz & Lazar 2011).

The association of resistin and MS could be largely explained by inflammatory markers. Metabolic syndrome is associated with low-grade inflammation (Grundy et al. 2005). Kunnari and colleagues reported that resistin is positively correlated with leukocytes and hsCRP in Finnish middle-aged subjects (Kunnari et al. 2006). Inflammatory chemokine monocyte chemoattractant protein 1 (MCP-1) and white blood cell count are associated with resistin after multivariable adjustments in non-diabetic subjects without CVD (Aquilante et al. 2008). MCP-1 is known to be involved in atherosclerotic disease process in vessel wall from the early fatty streak to advanced fibrous plaques (Braunersreuther et al. 2007). In a study by Reilly et al. (2005), plasma resistin was associated with inflammatory markers CRP, soluble TNF-α receptor-2 and IL-6, but not with insulin resistance in 880 asymptomatic subjects with a family history of premature coronary artery disease and in 215 subjects with type 2 diabetes. Resistin was also associated with coronary artery calcification and its risk factors (Reilly et al. 2005). A positive correlation between resistin and TNF-α was observed in the present study, but not between resistin and IL-6 or hsCRP, although there was a trend that both IL-6 and hsCRP levels were increased according to increasing resistin tertiles. Due to the cross-sectional design of our study, the causal relationship between resistin and the associated parameters and MS cannot be examined. It could, however, be considered that resistin may influence MS development via inflammatory markers acting at different stages of the process. The observed correlation with blood lipids, glucose and obesity might be secondary. In addition, the mRNA and protein activity of the receptor candidate of resistin, Toll-like receptor 4, is shown to be increased in monocytes from subjects with MS (Jialal et al. 2012).

6.3 The effect of IUGR on resistin and adiponectin

High resistin and low adiponectin levels were observed after IUGR in one-month-old rats in study II and high resistin levels also in six-month-old rats in study III. The aim was also to measure fat mass or fat distribution of the rats by MRI, but the method was not suitable for that analysis, which is a drawback, since the observed differences in resistin and adiponectin could be explained by increased relative amount of adipose tissue. Instead, the lipid content of adipose depots was
obtained, but IUGR had no effect on lipid content in retroperitoneal, intra-abdominal or subcutaneous adipose tissue of six-month-old rats.

It has been demonstrated in a study by Ibanez and colleagues that visceral adipose tissue is favored over subcutaneous adipose tissue in IUGR children. Low levels of circulating high molecular weight adiponectin and increased visceral adiposity were detected in 6-years-old children with low birth weight, even in the absence of overweight (Ibanez et al. 2008). Fat distribution determined by dual-energy x-ray absorptiometry was altered in young, healthy and lean adult men with low birth weight. These men had higher total abdominal fat mass and a shift in fat distribution observed as higher proportion of trunk and abdominal fat mass and less leg fat relative to total fat mass. These differences could precede and underlie the development of T2DM (Rasmussen et al. 2005). Small-for-gestational age children exhibited decreased adiponectin levels compared to obese children in an Italian study, and the levels were even lower if catch-up growth had occurred (Cianfarani et al. 2004). In a study by Tamakoshi and colleagues, reduced total adiponectin level was associated with low birth weight in a Japanese, adult population independently of current BMI (Tamakoshi et al. 2006). Despite limited data, these results indicate that adiponectin insufficiency may affect metabolic changes that are observed in IUGR children and adults.

The mRNA level of resistin was increased in visceral white adipose tissue of 15-month-old male IUGR rats having hyperglycemia and glucose intolerance (Garg et al. 2013). In those rats, the amount of white adipose tissue was increased, but plasma adiponectin or its mRNA levels in white adipose tissue were not changed. Resistin and adiponectin mRNA levels in one- or six-month-old rats were not measured in the present study. However, a microarray analysis from 13- and 17-day-old fetuses and one-day-old offspring from the pilot study were conducted, but no changes in genes encoding for adipokines were detected (Hietaniemi et al. 2009). Instead, altered expression of genes encoding for cell growth, communication and adhesion and protein metabolism were detected. In that analysis, it should be noted that ribonucleic acid (RNA) was extracted from the whole fetuses/pups and some tissue-specific gene expression changes are therefore not detected.

Increased serum level of resistin and decreased level of adiponectin in one-month-old IUGR rat in study II may indicate insulin resistance and predispose rats to T2DM later in life. In study III, such differences were not detected at the age of one month. However, in six-month-old rats, IUGR significantly increased
resistin levels in male and female rats, although the differences were not particularly prominent. It should be noted that in study II male and female rats were pooled and in study III they were examined separately, although even when combined there were no statistically significant differences. Low adiponectin levels are associated with insulin resistance in humans (Hotta et al. 2000) and mice (Yamauchi et al. 2001). Increased levels of resistin are strongly associated with insulin resistance in rodents (Schwartz & Lazar 2011). Altogether, it can be concluded that high resistin and low adiponectin levels may mediate part of the association of IUGR with elevated risk for metabolic and vascular disease in adulthood.

6.4 IUGR and fructose diet affects the features of MS

In study III, the influence of IUGR on glucose and lipid profile in adulthood was investigated using the rat model. Half of the rats received high fructose diet from one month of age onwards. Fructose-rich diet is known to induce MS in rats (Panchal & Brown 2011). This is the first study examining whether rats that experienced IUGR caused by food restriction are more prone to the harmful effects of a fructose diet adopted later.

According to the previous reports, as shown in Table 2, the changes in metabolic syndrome components and related parameters in rats are variable depending on the timing and duration of IUGR. In the present study there were only minor changes in the components of MS in study III. Weight of the adult rats was not increased. On the contrary, IUGR male and female rats were smaller, and fructose diet also decreased the weight of male rats. IUGR had no effect on plasma TG, HDL-C, fasting insulin and glucose levels. Instead, a signal of insulin sensitivity indicated by a decrease in HOMA-IR was detected in male IUGR rats. Fructose diet increased TG concentrations, fasting insulin and HOMA-IR, as expected. There were no interactions between IUGR and fructose diet on these parameters. Blood pressure of the rats was not measured in this study.

In addition to the components of MS, some other changes were detected in the rats. LDL-C was increased in IUGR rats. The method for the determination of LDL was discussed in the previous chapter. It is widely recognized that low birth weight is associated with CVD in adulthood (Barker 2004). However, associations of low birth weight with risk factors of CVD such as fasting lipid concentrations are weaker than expected (Huxley et al. 2007). Elevated postprandial changes may predict CVD more strongly than fasting levels
It is indicated that the association of low birth weight and subsequent CVD are strongly explained by differences in postprandial responses. According to the study of Perälä and colleagues (Perala et al. 2011), small birth size and slow early growth predicted elevated postprandial TG and insulin responses. The authors state that this may indicate that liver development may have been abnormal in fetal and early postnatal life and the liver does not function properly in adult life. Elevated postprandial leptin and insulin levels, but not TG or glucose levels, have also been observed in adult, fetally protein-restricted rats. In that study, there were no differences in TG, but there was an increase in insulin and a decrease in glucose in fasting state (Coupe et al. 2009).

In the present study IUGR rats were not more susceptible to metabolic changes during the high fructose diet. The only interaction between IUGR and fructose diet was detected in the adult weight of male rats, control offspring receiving standard diet being the heaviest compared to other groups. Similar results were achieved in a study by Cambri and colleagues (Cambri et al. 2010), where low protein diet during pregnancy and immediate postnatal fructose diet for 90 days did not result in further changes in the markers of MS. It would be interesting to find out whether IUGR rats were more prone to increased postprandial responses after fructose-rich diet, since fructose is reported to increase postprandial TG levels (Rizkalla 2010).

The changes caused by IUGR that were observed in this thesis were not particularly prominent. It can be speculated that since IUGR was induced at the beginning of pregnancy and lasted until delivery, the development of the placenta was adjusted and fetal development was quite normal, although the offspring had small birth weight and were smaller also in adulthood. It would have been interesting to find out whether IUGR had affected the distribution of adipose tissue, but that information was not available.
7 Conclusions

The major conclusions of this thesis are as follows:

1. Resistin levels were higher in the subjects with MS defined by several criteria compared to the subjects without MS. High resistin was associated with several components of MS. The controversial association of resistin in obesity and MS was clarified and the data suggest that high resistin level is linked to metabolic disturbances.

2. High resistin and low adiponectin levels were detected in rat offspring who were intrauterine growth-restricted. Serum cholesterol levels were also increased. These changes may expose to insulin resistance and increase the risk of cardiovascular disease in later life.

3. Intrauterine growth restriction and postnatal fructose-rich diet evoked adverse changes in lipid and glucose metabolism. IUGR rats had lower body weight and increased LDL-C levels. Fructose feeding induced hypertriglyceridemia and hyperinsulinemia. There were no interactions between IUGR and fructose diet in the parameters of lipid and glucose metabolism.
8 Clinical relevance and future aspects

The prevalence of metabolic syndrome and its components is increasing worldwide, as is the prevalence of T2DM and CVD. Individuals who are affected are younger than before. In addition, it is not known why some people are obese but metabolically healthy and why some lean individuals exhibit the same metabolic perturbations as most obese people display. Therefore, it is important to find out the contributing factors and mechanisms behind these metabolic abnormalities.

The National Institute for Health and Welfare in Finland has conducted a follow-up study for Health 2000 called Health 2011. It would be interesting to find out whether resistin levels measured in the Health 2000 study predict future MS, T2DM or CVD. Discovery of the receptor for resistin would help to define the function of resistin in metabolic perturbations. It would be interesting to measure the levels of the current receptor candidate Toll-like receptor 4 in the Health 2000.

In addition to resistin, other adipokine measurements from the Finnish study population would be interesting. Especially in T2DM research, the role of adipocyte hormones in insulin sensitivity and glucose tolerance are considered important. For instance, investigating the roles of the recently identified adipokines visfatin and chemerin in metabolic syndrome, T2DM and CVD is among the possible future projects. Visfatin is a recently identified adipokine acting in insulin secretion. Contradictory results have been obtained on its levels in obesity (Al-Suhaimi & Shehzad 2013). Chemerin may play a role in metabolism, adipogenesis and inflammation. Both pro- and anti-inflammatory effects of chemerin have been reported (Rourke et al. 2013). Determination of FABP4 from Health 2000 and 2011 surveys would also be an interesting future project, since FABP4 was demonstrated to be an important factor that regulates hepatic glucose metabolism and may be a potential therapeutic target against diabetes (Cao et al. 2013).

In developing countries where the economy is accelerating and culture is changing, the number of individuals with normal body weight but increased abdominal obesity is increasing. It can be speculated that the epidemics of MS and T2DM will develop in these countries and one important contributing factor will be poor intrauterine growth and overnutrition in later life (Rinaudo & Wang 2012). In addition, an interesting question is whether IUGR and fructose diet have
affected the liver composition of the rats, and therefore the fatty acid composition and level in the liver will be among important targets in studies in the near future.
References


**Original publications**


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Original publications are not included in the electronic version of the dissertation.
1201. Huusko, Tuija (2013) Genetic and molecular background of ascending aortic aneurysms
1202. Pisto, Pauliina (2013) Fat accumulation in liver and muscle : association with adipokines and risk of cardiovascular events
1203. Yannopoulos, Fredrik (2013) Remote ischemic preconditioning as a means to protect the brain against hypothermic circulatory arrest : an experimental study on piglets
1204. Arvonien, Mika (2013) Intestinal immune activation in juvenile idiopathic arthritis
1206. Penttäli, Matti (2013) Duration of untreated psychosis : association with clinical and social outcomes and brain morphology in schizophrenia
1209. Hietikko, Elina (2013) Genetic and clinical features of familial Meniere’s disease in Northern Ostrobothnia and Kainuu
1212. Tikkanen, Jari (2013) Early repolarization in the inferolateral leads of the electrocardiogram : prevalence, prognosis and characteristics
1214. Kaakinen, Pirjo (2013) Piskäkaisaairaideen aikuisten ohjauksen laatu sairaalassa
1215. Pasanen, Anna Kaija (2013) A translational study on the roles of redox molecules, cell cycle regulators and chemokine receptors as prognostic factors in diffuse large B-cell lymphoma

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The Role of Low Birth Weight and Resistin in Metabolic Syndrome

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