Neurohormonal Mechanisms in
Insulin Resistance and Type 2 Diabetes

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ABSTRACT

Insulin resistance usually occurs early in the development of type 2 diabetes. An altered balance in the autonomic nervous system and in certain endocrine and inflammatory pathways, might contribute to the development of insulin resistance. In diabetes, hyperglycemia further aggravates insulin resistance as well as beta cell dysfunction but the mechanisms causing this phenomenon, i.e. glucotoxicity, are not fully understood.

Insulin resistance can be demonstrated in healthy first-degree relatives of type 2 diabetes patients who also have a high risk of developing type 2 diabetes. Relatives and control subjects without family history of diabetes were studied with respect to insulin sensitivity and the activity in the autonomic nervous system (ANS) and in the cortisol axis. Levels of sex hormones, leptin and cytokines were analysed. Abdominal adipose tissue distribution was determined with computed tomography.

Male relatives had decreased testosterone levels and increased leptin levels. There was an inverse relationship between insulin sensitivity and leptin levels, and in males a positive association between insulin sensitivity and testosterone levels. A tendency to lower parasympathetic reactivity was found in the relatives using heart rate variability assessment. The sympathetic/parasympathetic ratio during stress provocation was inversely correlated to insulin sensitivity, measured with glucose clamp. The insulin-resistant subjects also exhibited an overall blunted reactivity in the ANS. Cortisol reactivity after stimulation with ACTH and CRH was lower in the relatives. The amount of visceral adipose tissue (VAT) was associated with insulin resistance and with heart rate at rest and during controlled breathing and it also correlated with heart rate and sympathetic/parasympathetic ratio after an orthostatic manoeuvre.

Type 2 diabetic subjects with good and poor glycemic control, respectively, and matched healthy control subjects were examined with respect to insulin sensitivity, cortisol axis activity and blood levels of leptin, sex hormones and the adipocyte-secreted inflammatory factors interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). Bi-
opsies were taken from subcutaneous adipose tissue for determination of adipocyte size. Diabetes subjects were more insulin-resistant than controls and diabetics with poor control exhibited the highest degree of insulin resistance. This group also had the highest levels of TNF-α, morning serum cortisol and non-esterified fatty acids (NEFA). In correlation analyses, significant associations were seen between glycemic level and insulin resistance, TNF-α, IL-6 and serum cortisol levels. Insulin resistance was positively correlated to NEFA levels, TNF-α and ACTH-stimulated cortisol levels. Adipocyte size was associated with insulin resistance and levels of IL-6 and leptin.

The findings support a connection between insulin resistance and VAT amount, activity in the ANS and blood levels of hormones and adipocyte-derived molecules. Dysregulation in the complex interplay between such factors may contribute to the early pathogenesis of insulin resistance and type 2 diabetes. Adipokines and the cortisol system can also potentially aggravate hyperglycemia in patients with manifest type 2 diabetes.
LIST OF PAPERS


III  **Lindmark S, Lönn L, Wiklund U, Tufvesson M, Olsson T, Eriksson JW.** Dysregulation of the autonomic nervous system might contribute to the link between visceral adiposity and insulin resistance – A study on the interplay between insulin resistance, the cortisol axis, the autonomic nervous system and abdominal fat distribution in healthy subjects. *Submitted*

IV  **Lindmark S, Burén J, Eriksson JW.** Insulin resistance, endocrine function and adipokines in type 2 diabetes patients at different glycemic levels. *Submitted*
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>11β-HSD</td>
<td>11 beta-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BIA</td>
<td>Bio-impedance analysis</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CPT</td>
<td>Cold pressor test</td>
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<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated Hemoglobin</td>
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<tr>
<td>HF</td>
<td>High frequency</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<tr>
<td>HRV</td>
<td>Heart rate variability</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
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<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<td>IL-6</td>
<td>Interleukin 6</td>
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<tr>
<td>ISI</td>
<td>Insulin sensitivity index</td>
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<td>IRS</td>
<td>Insulin receptor substrate</td>
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<tr>
<td>LBM</td>
<td>Lean body mass</td>
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<td>LF</td>
<td>Low frequency</td>
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<tr>
<td>MCR</td>
<td>Metabolic clearance rate</td>
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<tr>
<td>MODY</td>
<td>Maturity onset diabetes of the youth</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
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<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<tr>
<td>PPAR-γ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
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<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<tr>
<td>SHBG</td>
<td>Sex hormone-binding globulin</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
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<tr>
<td>SS</td>
<td>Steady state</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
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<td>VAT</td>
<td>Visceral adipose tissue</td>
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<td>WHR</td>
<td>Waist-to-hip ratio</td>
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INTRODUCTION

Diabetes Mellitus

Diabetes Mellitus is a disease characterized by elevated blood levels of glucose and the disease has been known for more than 3000 years. In the 18th century, the presence of glucose in the urine of diabetes patients was detected and documented. Insulin was discovered and isolated 1921 by Banting and Best and it became clear that insufficient insulin delivery was the cause of diabetes mellitus. Already during the 19th century it was suggested that there were two forms of diabetes, one that was present in young and underweight persons and another form that was seen in elderly and often overweight persons but it was not until 1979 that the National Diabetes Data Group and an expert group for WHO (1980) defined the two forms, type 1 and type 2 diabetes mellitus (1), characterized by an absolute and a relative insulin deficiency, respectively. Today, many other forms of diabetes have been classified, for example MODY (maturity onset diabetes of the young), an inherited form of mild diabetes with onset in late childhood or young adulthood (1). There are at least five different forms of MODY and they occur on the basis of mutations in genes affecting insulin secretion. Other specific types of diabetes include genetic defects affecting insulin action, pancreatic diseases, endocrine disorders (e.g. Cushing’s syndrome, acromegaly), infections, drug-induced diabetes, genetic syndromes with risk of diabetes and gestational diabetes (1, 2).

The most common form of diabetes is type 2 diabetes that accounts for 85-90% of all diabetes. The prevalence of type 2 diabetes in Sweden is at least 3-4% and it is expected to increase due to increased body weight and a more sedentary life-style in the population. Globally, a doubling of the prevalence of type 2 diabetes can be expected in the next 10 years, with the largest increase of type 2 diabetes incidence in the developing countries due to changes towards a “western life-style” (3). According to WHO calculations, in the year 2025 there will be 300–350 million adults with diabetes in the world. 80% of them will be found in the developing countries (4, 5).
Type 2 diabetes is a disease with a multifactorial background. An inherited component is strongly involved in the pathogenesis of the disease but lifestyle and other environmental factors, e.g. psychosocial stress, also contribute (6-8). The risk for cardiovascular disease is markedly elevated (9, 10) and it is associated with insulin resistance, and type 2 diabetes patients often have other risk factors for cardiovascular disease such as hypertension and dyslipidemia. As in type 1 diabetes there is also a high risk for microvascular disease such as retinopathy, nephropathy and neuropathy (11).

In contrast to type 1 diabetes that is caused by absolute insulin deficiency due to an autoimmune destruction of beta cells, type 2 diabetes generally results from an imbalance between insulin sensitivity and insulin supply (Figure 1). In most cases, insulin resistance is present early in the development of the disease but is not by itself sufficient to cause type 2 diabetes. With normal beta cell function the production of insulin will rise to levels high enough to maintain normal glucose tolerance. In general, among insulin-resistant subjects, only those with impaired beta cell function will develop the disease (12, 13). Further loss of beta cell function seems to be the natural course of the disease and low levels of endogenous insulin are common in patients with a long duration of type 2 diabetes (14).
Insulin and insulin action

Insulin is one of the most important anabolic hormones in the body and it is critical for the control of carbohydrate, lipid and protein metabolism. Insulin is secreted from beta cells in the endocrine pancreas. It acts by binding to the transmembrane insulin receptor in the target cells, and this activates the tyrosine kinase domain in the intracellular part of the receptor leading to phosphorylation of insulin receptor substrates (IRS). This starts a cascade of signalling reactions in the cell leading to metabolic effects as summarized in Figure 2. The main target tissues of insulin’s metabolic action are muscle, liver and adipose tissue. Insulin stimulates glucose uptake in insulin sensitive tissues, mainly skeletal muscle, and it inhibits glucose production in the liver and promotes the storage of glycogen in liver and skeletal muscle. It promotes the delivery of non-esterified fatty
acids (NEFA) to adipose tissue where they are stored as triglycerides and lipolysis in fat cells is inhibited. In general, overall protein synthesis is increased.

Insulin resistance is a condition with impaired response to insulin in the target cells. The body attempts to compensate for this by increasing the production of insulin in the beta cells and this leads to hyperinsulinemia. Insulin resistance is usually an important component in the pathogenesis of type 2 diabetes (15, 16). It is also associated with obesity, hypertension, lipid abnormalities and microalbuminuria. These conditions are often clustered together in a syndrome called the insulin resistance syndrome or the metabolic syndrome (15-17) (Figure 3). Insulin resistance is a common condition but the underlying mechanisms are not fully understood. In rare hereditary conditions, insulin resistance may be explained by a defect in the insulin receptor or signalling proteins, e.g.
IRS (18). Insulin resistance is also seen in a few patients with antibodies directed to the insulin molecule or the insulin receptor, and this can be associated with autoimmune diseases (19). Common variants in a number of candidate genes influencing glucose and fat metabolism appear to be associated with insulin resistance seen in type 2 diabetes and the metabolic syndrome (20-25) but despite extensive studies, the exact mechanisms behind the development of insulin resistance is still not known. A recent study suggests that insulin resistance is associated with dysregulation of fatty acid metabolism in skeletal muscle, possibly due to an inherited defect in mitochondrial oxidative phosphorylation (26). There are also data suggesting that insulin resistance is not caused by primary cellular defects and that factors which are present in vivo in the tissue environment may be of importance, e.g. metabolic, hormonal or neural factors (27-29). These may include insulin-antagonistic hormones, adipokines and signals from the autonomic nervous system.

Figure 3
Definition of the metabolic syndrome (According to the National Cholesterol Education Program’s Adult Treatment panel group III report (17)).
At least 3 of the 5 listed metabolic risk factors are needed for the diagnosis metabolic syndrome.
Glucotoxicity and lipotoxicity

Acute elevations of glucose and NEFA stimulates insulin secretion but chronically elevated levels of glucose and NEFA in the blood have a negative effect on both insulin sensitivity and insulin secretion, referred to as glucotoxicity and lipotoxicity, respectively (30, 31). The cellular or biochemical mechanisms responsible for glucotoxicity and lipotoxicity are only partly understood. Hyperglycemia and elevated NEFA levels appears to impair glucose transport into the cell as well as glucose oxidation and glycogen synthesis (30, 32). One possible explanation behind glucotoxicity is that elevated glucose levels lead to increased glucose flux through the hexosamine biosynthesis pathway (33-35), leading to accumulation of glucosamine-6-phosphate, which inhibits glucose transport via activation of protein kinase C (PKC) (36). PKC has been shown to enhance serine phosphorylation of the insulin receptor and thereby decrease the tyrosine kinase activity of the receptor, in turn leading to attenuation of downstream insulin signalling (37, 38). Hyperglycemia may also increase PKC activity via increased synthesis of diacylglycerol (36, 39). A mechanism that has been suggested for lipotoxicity involves the Randle cycle, i.e. free fatty acids and glucose compete as energy substrates in the cellular energy metabolism (40). However, more recent explanations for lipotoxicity include inhibition of glucose transport and interference with steps in the insulin-signal transduction. (31, 41). For example, it has been suggested that elevated NEFA levels can lead to decreased phosphatidylinositol 3-kinase (PI3-kinase) activity and increased PKC-theta activity which would interfere with the insulin signalling cascade (42, 43).

Insulin-antagonistic hormones

Several hormones, e.g. cortisol, catecholamines, glucagone and growth hormone, have insulin-antagonistic effects. During hypoglycemia, these hormones are secreted to restore blood glucose levels by stimulating glucose release from the liver and inhibiting glucose uptake in peripheral tissues. Elevated levels of these hormones due to pharmacological treatment or endogenous overproduction can produce insulin resistance and hyperglycemia.
Cortisol is a steroid hormone synthesized from cholesterol in the adrenal cortex. The hypothalamic-pituitary-adrenal (HPA) axis involves a negative feedback loop that regulates the production of cortisol. Corticotropin releasing hormone (CRH) is secreted from the hypothalamus and stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary, which stimulates the synthesis, storage and release of cortisol from the adrenal cortex. Cortisol in turn, exerts an inhibitory effect on CRH and ACTH at both the pituitary and the brain level. The secretion of cortisol exhibits a circadian rhythm with the highest levels in early morning and the lowest at midnight. Several factors influence the activity of the HPA axis including stress, cytokines and starvation. The metabolic effects of cortisol are mainly exerted via binding to the intracellular glucocorticoid receptor (GR), which belong to the nuclear receptor super-family. The activated hormone-receptor complex binds to DNA and regulates the expression of specific genes. Cortisol has a broad spectrum of effects in many tissues aiming to maintain homeostasis under conditions of strain. The effect of cortisol in the glucose and lipid metabolism is to a large extent opposite that of insulin. Hepatic glucose production is increased, while insulin-stimulated glucose uptake in muscle and adipose tissue is impaired. Adipose tissue lipolysis appears to be stimulated and, hence, the release of free fatty acids into the circulation is increased. Protein synthesis is generally decreased and amino acid release is increased. High serum levels of cortisol of endogenous or exogenous origin can cause insulin resistance and in diabetes patients, this can lead to a deterioration of the glycemic control. It has also been indicated that glucocorticoids have a direct inhibitory effect on glucose-induced insulin release. Many authors have discussed the role of endogenous cortisol in the development of insulin resistance and obesity but previous results are not consistent.

Catecholamines are also stress hormones with insulin-antagonistic effects. They enhance glycogen breakdown, increase the release of NEFA by stimulating lipolysis. In the endocrine pancreas, insulin secretion is inhibited and glucagone production is stimulated. Adrenaline is mainly produced and stored in the adrenal medulla whereas the major part of noradrenaline appears in sympathetic nerve endings. Adrenal secretion
of catecholamines is mainly regulated by the sympathetic nervous system and is increased by exercise, hypoglycemia, acute ischemic heart conditions, anoxia, surgery and many other stressful stimuli (53). The sympathetic nervous system mainly affects glucose metabolism by increasing catecholamine secretion from the adrenals and by stimulating the release of glucose from the liver, which in turn is partly due to glucagon secretion from the pancreas (54, 55). As a response to hyperglycemia, activation of the parasympathetic nervous system promotes insulin secretion from beta cells and glycogen synthesis in the liver (55). The autonomic nervous system is also highly involved in adipose tissue metabolism. The effect of sympathetic stimulation is catabolic with enhanced lipolysis whereas parasympathetic stimulation appears to enhance glucose and lipid uptake in the adipose tissue and to improve insulin sensitivity and to affect glucose and fat metabolism in an anabolic way (56-58). Hyperinsulinemia stimulates sympathetic outflow via effects in the hypothalamus and an elevated activity of the sympathetic nervous system has been suggested as the link between insulin resistance and hypertension (59, 60). Stress and elevated levels of catecholamines impair glycemic control in type 2 diabetes and an altered activity in the sympathetic nervous system or abnormal sensitivity to sympathetic stimuli has been reported in type 2 diabetes (61-65).

The renin-angiotensin system (RAS) must also be considered in the development of insulin resistance. It has been shown that treatment with angiotensin converting enzyme inhibitors (ACE-i) and angiotensin receptor blockers (ARB) may improve insulin sensitivity (66-68) and prevent the development of type 2 diabetes (69-71). Angiotensin II exerts many effects that can be related to insulin resistance, e.g. increases hepatic glucose production, inflammation and the activity in the sympathetic nervous system (72). It has also been suggested that blockade of RAS with either ACE-i or ARB increases adiponectin production (73).
Adipose tissue and adipokines

Obesity is a strong risk factor for type 2 diabetes and the metabolic syndrome (74, 75). Moreover, the distribution of body fat is of importance. Visceral adiposity is strongly linked to insulin resistance, type 2 diabetes, hypertension and dyslipidemia whereas for subcutaneous fat there seems to be no such consistent associations (76-79). Adipose tissue is not only a metabolic organ responsible for storage of energy. It is also a biologically active and dynamic tissue with endocrine activity, producing several different substances with different endocrine or paracrine functions, e.g. leptin, tumor necrosis factor alpha (TNF-α), interleukins, adiponectin and plasminogen activator inhibitor-1 (PAI-1) (80) (Figure 4). In the adipose tissue, the biologically inactive cortisone is converted to active cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (81) and testosterone is converted to oestrogen by aromatases (82). Adipose tissue function is regulated by multiple external influences such as autonomic nervous system activity, the rate of blood flow and the delivery of a multitude of substrates and hormones from the circulation (83).

Figure 4
Adipocyte-secreted factors.
Leptin is the product of the *ob* gene and is secreted mainly from adipocytes (84, 85). It regulates energy balance by reducing food intake and increasing energy expenditure after binding to specific receptors in the hypothalamus. The production as well as the circulating levels of leptin is elevated in obese compared to lean subjects and leptin levels are decreased upon weight reduction (86, 87). Inherited defects in the leptin molecule or in the leptin receptor, respectively, have been detected in a few families with extreme obesity (88, 89). Subjects with aberrant leptin have been successfully treated with leptin substitution, but in common obesity, leptin treatment has not been effective. Moreover, leptin resistance has been suggested as a mechanism behind obesity (90, 91).

Adipose tissue synthesise cytokines, for example TNF-α and interleukin-6 (IL-6) that have effects on metabolism in the adipose tissue and probably also in other organs. Cytokine release from adipose tissue appears to be stimulated by inflammatory stimuli and also by catecholamines and β-adrenergic stimulation. Insulin and cortisol have been suggested to regulate cytokine release, but data are conflicting (92, 93). It has been suggested that serum concentrations of TNF-α and IL-6 are elevated in obesity and that weight loss results in decreased levels (94). High serum levels of TNF-α and IL-6 also seem to be associated with insulin resistance and type 2 diabetes (95-98). Knock-out experiments have shown that insulin resistance is prevented in obese mice lacking TNF-α (99). Mice lacking IL-6, however, develop obesity, which is partly reversed by IL-6 replacement (100). Furthermore, it has been shown that intracerebroventricular administration of IL-6 causes increased energy expenditure in rats (101) and that IL-6 levels in the cerebrospinal fluid is negatively correlated with fat mass in obese humans (102). In humans the effects of TNF-α appears to be mainly autocrine and paracrine whereas IL-6 is released systemically and acts for example on the hypothalamus and the liver (93). Both TNF-α and IL-6 inhibit lipoprotein lipase and TNF-α also stimulates hormone sensitive lipase leading to decreased lipid accumulation within the adipose tissue. In addition, it has been suggested that TNF-α interferes with intracellular insulin signalling and induces a down-regulation of glucose transport proteins (GLUT 4) (93, 103-105). TNF-α and IL-6 are also produced locally in the adrenal cortex where they modify adrenal steroid secretion and, in fact, they promote the secretion of cortisol. The produc-
tion of TNF-α and IL-6 in the adrenals is regulated by the same factors that regulate other adrenal hormones, e.g. ACTH and angiotensin II (106). Cytokines are also believed to have influences on the HPA axis activity via direct actions on the pituitary and the hypothalamus (107).

Adiponectin is a recently characterized, adipocyte-derived plasma protein (108) with insulin sensitizing, anti-atherogenic and anti-inflammatory properties. Plasma levels of adiponectin are negatively associated with obesity and insulin resistance (109-111) and low levels of adiponectin can predict the future risk of developing type 2 diabetes (112). Adiponectin can interact directly with endothelial cells to improve vascular function (110). Administration of adiponectin to obese or diabetic mice reduces plasma NEFA levels and also glucose excursions and enhances insulin sensitivity (113-115). Adiponectin is secreted from both subcutaneous and visceral adipose tissue but, surprisingly, secretion appears to be generally higher from visceral adipose tissue (116). Secretion of adiponectin is positively regulated by insulin and IGF-1 and negatively regulated by glucocorticoids, β-adrenergic stimulation, TNF-α and IL-6. PPAR-γ agonists appear to increase plasma levels of adiponectin (117, 118). Androgens inhibit adiponectin secretion and adiponectin levels are lower in men as compared to women (119).

In summary, insulin sensitivity is regulated and modulated by a complex interplay between several factors such as hormones, adipokines, metabolic substrates, fat distribution and neural activity. The role of such interactions in the development of insulin resistance needs to be further elucidated.
AIMS AND HYPOTHESIS

The aim of this study is to obtain further insight in the mechanisms contributing to the development of insulin resistance and type 2 diabetes in humans. Our hypothesis is that insulin resistance is not caused by a primary target cell defect and that neural or hormonal factors may contribute to the development of insulin resistance.

Specific aims:

I To explore the interplay between insulin resistance, cortisol, sex hormones and leptin.

II To investigate dysregulation of the autonomic nervous system as a potential mechanism for insulin resistance early in the development of type 2 diabetes.

III To evaluate the association between abdominal adipose distribution, regulation of the cortisol axis and the autonomic nervous system in relation to insulin resistance.

IV To elucidate mechanisms contributing to insulin resistance in association with hyperglycemia and specifically to investigate the interplay between glycemic level, insulin resistance, insulin-antagonistic hormones and adipokines in type 2 diabetes patients.

An outline of the hypothesis and the study design is presented in Figure 5.
Figure 5
Schematic illustration of the study protocol and study cohorts in relation to the different stages in and hypothetical mechanisms behind the development and progression of insulin resistance and type 2 diabetes. FDR= first-degree relatives of type 2 diabetes patients.
SUBJECTS AND METHODS

Study cohorts
Study I-III
30 non-diabetic subjects in the ages 19 to 42 years, were recruited from the Umeå area by advertisement in a local newspaper. 15 had two first-degree relatives or one first-degree and at least two second-degree relatives with type 2 diabetes (R). The remaining 15 had no family history of diabetes (C). The groups were well matched according to BMI, gender and age. 16 were males and 14 females. 2 diabetes relatives had impaired glucose tolerance. They were otherwise all in good health as determined by medical history, clinical examination and laboratory tests including hematology, serum electrolytes, creatinine and serum lipids. None of the subjects received any chronic medication.

In study I, 36 subjects from a similar study cohort in Göteborg was combined with our Umeå study cohort. The total cohort in study I was thus 66 subjects, 33 diabetes relatives and 33 control subjects (19 M/14 F in each group).

In study IV, 30 subjects were recruited by advertisement in the local newspaper or recruited among patients at the Diabetes Unit of Umeå University Hospital. 20 were type 2 diabetic patients, ten with poor and ten with good metabolic control, defined according to HbA1c levels. Ten were non-diabetic control subjects, matched for BMI, gender and age. 18 subjects in the total cohort were males and 12 females. One of the subjects in the diabetes group and one in the control group had treatment for hypertension and one subject in the diabetes group was treated for hyperlipidemia. Two diabetes subjects received thyroid hormone replacement due to hypothyroidism. Two diabetes subjects suffered from mild asthma bronchiale. All subjects were otherwise in good health as determined by medical history, clinical examination, ECG and blood tests including hematology, serum electrolytes, creatinine, serum lipids and liver enzymes. In the diabetes groups, the mean duration of diabetes was similar, 6.3 years (range 1–14) in the poorly controlled group and 5.3 years (range 2–10) in the group with good metabolic control. Of the diabetic subjects, 7 had no pharmacological treatment, seven were treated with sulfonylurea alone, four with sulfonylurea and metformin in combination.
One subject was treated with repaglinide alone and one subject with repaglinide and metformin in combination.

**Examinations**

Unless otherwise is indicated examinations were performed and blood samples were obtained at 8.00 in the morning after an overnight fast and with the subjects supine in a comfortable bed. The subjects did not take any medication before the examinations and blood samplings.

Anthropometric measurements included body weight, measured to the nearest 0.1 kg using a calibrated electronic platform scale, height to the nearest cm, and waist and hip circumference measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Body composition was determined with the bioelectrical impedance analysis (BIA) method (120).

**Oral glucose tolerance test (OGTT)**

The subjects in study I-III underwent an OGTT. Venous blood samples for determination of blood glucose and serum insulin were obtained before and 120 minutes after ingestion of 75 g glucose in a liquid solution. 2-h blood glucose values of 6.7–9.9 were defined as impaired glucose tolerance.

**Euglycemic hyperinsulinemic clamp**

This examination was performed in the entire study cohort to determine insulin sensitivity (121). The subjects received a priming dose followed by a constant infusion (56mU/m²/body surface/minute) of human insulin (Actrapid®). In parallel a glucose infusion (200mg/ml) was administered with an infusion rate adjusted to maintain blood glucose values at 6 mmol/L. The glucose infusion rate was used as a measure of insulin sensitivity, i.e. the so-called M-value that was calculated by dividing the glucose infusion rate during the last 60, out of 120 minutes (steady state) of the clamp by body weight (mg/kg/min). Blood samples for determination of plasma levels of non-esterified fatty acid and serum insulin were obtained at start and after 60, 90 and 120 minutes of
the clamp examination and for determination of serum adiponectin levels at start and after 120 minutes.

**Analysis of heart rate variability (HRV)**

Analysis of HRV was performed in study II to assess the function and activity of the autonomic nervous system. During continuous ECG recording the subjects 1) performed controlled breathing at a rate of 12 breaths/minute, 2) was tilted passively to 70° head up position and finally 3) underwent a cold-pressor test (CPT) by holding one lower arm in ice-cold water for 2 minutes. These procedures were utilised to activate the autonomic nervous system in a standardised manner. Controlled breathing stimulates parasympathetic activity whereas tilting and cold pressor test activates the sympathetic nervous system. The electrocardiographic R-R intervals were used to determine spectral activity. Total spectral power and the power of the high (HF) and low (LF) frequency components were calculated and log-transformed. The variability of the HF component mirrors respiratory activity and is mainly influenced by parasympathetic activity and the variability of the LF component is associated with vasomotor control and are influenced by both sympathetic and parasympathetic activity (122-124). The ratio of low frequency power and high frequency power was calculated and used to mirror the balance between sympathetic and parasympathetic activity.

**Assessments of the cortisol axis**

In study III and IV following procedures were used to evaluate the function and activity of the cortisol axis. *Diurnal urinary cortisol excretion:* Urine was collected for 24 hours during two separate days to determine free cortisol excretion. *Diurnal salivary cortisol measurement:* Saliva was collected in the morning, at noon, in the afternoon and early and late in the evening (in study IV in the morning, at noon and only once in the evening) and analysed to determine free cortisol concentration (125). *Oral dexamethasone suppression test:* The subjects received dexamethasone in a dose of 3.5 µg/kg at 10.00 PM day 1. Blood samples for determination of serum cortisol were obtained 8.00 AM day 1 and day 2 after an overnight fast. *Low dose ACTH stimulation test:* 1 µg tetra-cosactrin (ACTH) was injected as a bolus. Blood samples for determination of serum
cortisol were obtained in the basal state (0 min) and then at 30, 40, 60, and 120 minutes after ACTH injection (126). CRH stimulation test (study III): This test was performed at 1.00 PM with the subjects fasting during the previous 3 hours. Human CRH was injected as a single dose of 1 µg/kg. Blood samples for determination of serum cortisol and plasma ACTH were obtained in the basal state and 15, 30, 60, 90 and 120 minutes after CRH injection (127). Skin blanching (study IV): Peripheral vascular glucocorticoid sensitivity was assessed by skin blanching following a standard dermal application of beclomethasone (128, 129).

Determination of adipocyte cell size (study IV)
At 8.00 AM, following an overnight fast, a subcutaneous needle biopsy was taken from the lower part of the abdomen after dermal local anaesthesia with lidocain. Adipocytes were isolated by treatment of pieces of adipose tissue with collagenase and filtering through a nylon mesh. Cell size was determined in isolated adipocytes by measuring cell diameter in a microscope and cell volume and weight was calculated (29, 130).

Determination of abdominal adipose tissue distribution with computerized tomography (CT) (study III)
Of the 30 study participants, 18 (9 R and 9 C) accepted to participate in this part of the study. The subjects were examined in the evening and in a non-fasting state. Two CT scan sections were performed at the levels of the second lumbar vertebra (L2) and the fourth lumbar vertebra (L4), respectively. Tissue areas were determined (131) and the total adipose tissue and visceral adipose tissue volumes were calculated (132).

Statistical analyses
Statistical analyses were performed using the SPSS package (SPSS Inc., Chicago, IL, USA).
Data are means±SEM unless otherwise is indicated. All variables were tested for normality and parametric or non-parametric tests were used accordingly. Simple linear regressions were used to analyse correlations between variables and multiple linear regression analyses or partial correlation analyses were used to evaluate independent as-
sociations between variables. P-values less than 0.05 were considered as statistically significant.
SUMMARY OF RESULTS

Study I
The interplay between insulin resistance, steroid hormones and leptin was explored in 33 healthy relatives of type 2 diabetes patients and 33 age-, sex- and BMI-matched control subjects without a family history of diabetes. The diabetes relatives were more insulin resistant than control subjects, when gender was analysed separately, this was significant only in males. Male relatives displayed lower morning cortisol and testosterone levels and higher leptin levels compared to male control subjects. After adjustments for age and BMI, testosterone levels were positively associated with insulin sensitivity (M-value) in male subjects ($r=0.48$, $p=0.04$). Leptin levels were negatively associated with insulin sensitivity in male and female relatives and in male controls ($r=-0.66$, $r=-0.67$ an $r=-0.49$, respectively, $p<0.05$). In the female control group this association was nearly significant ($r=-0.51$, $p=0.063$). There were no significant associations between insulin resistance and cortisol levels.

In conclusion, male subjects predisposed for type 2 diabetes display abnormalities in leptin, cortisol and testosterone levels. Insulin resistance was associated with high leptin levels and, in males, with low testosterone levels.

Study II
The possible interplay between insulin resistance and the activity in the autonomic nervous system was investigated in 15 healthy type 2 diabetes relatives and 15 sex-, age- and BMI-matched control subject without a family history of diabetes. There was a tendency of lower parasympathetic activity (HF power) in the relatives compared to the control subjects during controlled breathing. The ratio of sympathetic/parasympathetic (LF/HF) activity during cold pressor test was negatively associated with insulin sensitivity ($r=-0.53$, $p=0.006$). When dividing the total cohort into two groups based on their M-value, the group with low M-value exhibited signs of a higher sympathetic/parasympathetic ratio ($p=0.04$) and lower parasympathetic activity during controlled breathing and cold pressor test ($p=0.01$ and $p=0.03$, respectively) compared with
the group with high M-value. In general, the insulin-resistant group displayed lower reactivity in the autonomic nervous system upon provocations.

In conclusion, an altered balance and reactivity in the autonomic nervous system appeared to be associated with insulin resistance.

Study III
Adipose tissue distribution and the regulation of the cortisol axis were examined and the association with insulin resistance and the activity in the autonomic nervous system were evaluated in 15 healthy relatives of type 2 diabetes patients and 15 age-, sex- and BMI-matched control subjects without a family history of diabetes. The relatives displayed lower peak serum cortisol levels following stimulation with ACTH and CRH. There were no differences between the groups in serum cortisol levels after inhibition with dexamethasone. There was a tendency of higher 24-h urinary cortisol excretion and of slightly lower diurnal salivary cortisol levels in the relatives. The amount of visceral abdominal adipose (VAT) tended to be greater (39%) in relatives but the difference did not reach statistical significance. The amount of subcutaneous abdominal adipose tissue (SAT) was similar in the two groups. VAT was negatively associated with insulin sensitivity and positively associated with heart rate at rest, during controlled breathing and during an orthostatic manoeuvre, and with heart rate and sympathetic/parasympathetic balance (LF/HF ratio) after an orthostatic manoeuvre. There were no significant associations between SAT and insulin resistance, the cortisol axis activity or the activity in the autonomic nervous system.

In conclusion, diabetes relatives tended to have a larger amount of visceral fat and the amount of visceral fat was associated with insulin resistance, heart rate and the sympathetic/parasympathetic balance. Moreover, diabetes relatives were less sensitive to stimulation of the HPA axis.
Study IV

Insulin sensitivity, cortisol axis activity, blood levels of leptin, steroid hormones and cytokines were examined in type 2 diabetes patients with good or poor glycemic control (HbA1<7.0% and >7.5%, Swedish standard, respectively) and healthy control subjects. Biopsies were taken from the subcutaneous abdominal adipose tissue and adipocyte size was determined. Type 2 diabetes patients were more insulin resistant than healthy control subjects. Diabetic subjects with poor glycemic control exhibited the highest degree of insulin resistance. Plasma TNF-\(\alpha\) were elevated in the diabetes group and the highest levels of morning serum cortisol and non-esterified fatty acids (NEFA) was seen in the poorly controlled diabetes patients. The degree of skin blanching after dermal application of beclomethasone was lower in the diabetes group, indicating a decreased peripheral vascular sensitivity to glucocorticoids. HOMA index for beta cell function was significantly lower in the diabetics compared to the control subjects and it was lowest in the poorly controlled diabetics. Glycemic level (HbA1c) was positively associated with insulin resistance, and also with TNF-\(\alpha\) and interleukin-6 levels and basal as well as ACTH-stimulated cortisol levels. Insulin resistance assessed with glucose clamp was positively associated with NEFA levels in the basal state and during clamp, ACTH-stimulated serum cortisol and with TNF-\(\alpha\) and SHBG levels. Adipocyte cell size was positively associated with insulin resistance and blood levels of interleukin-6 and leptin.

In a recent substudy, we also measured serum C-reactive protein (CRP) and adiponectin levels in this study cohort. The levels of C-reactive protein (CRP) were highest in the poorly controlled diabetics (P 4.21±1.31, G 1.17±0.25 and C 1.96±0.41 mg/L, respectively) but only the difference between the poorly controlled and the well-controlled diabetics were statistically significant (p=0.01). After adjustments for BMI, sex and age, there were significant associations between CRP levels and HbA1c-levels (r=0.63, p<0.001), fasting plasma NEFA levels (r=0.35, p=0.03), and morning serum cortisol levels (r=0.66, p<0.001). No association was found between CRP and insulin resistance (M-value). Adiponectin levels were measured both in the basal state and after 120 minutes of hyperinsulinemia during clamp and they were higher in female compared to male subjects in the total cohort (M 6.9±0.9, F 11.8±2.1 and M 6.1±0.8, F 11.2±1.7,
µg/mL, before and during clamp, respectively) but they did not change significantly during hyperinsulinemia. In female subjects, adiponectin levels were lower in the poorly controlled diabetes group compared to the well-controlled diabetes group and the control subjects but only the difference between the P and G groups were statistically significant. In male subjects the levels of adiponectin were similar in all three groups (Table 1). In partial correlation analyses, adjusting for BMI, sex and age, adiponectin levels were negatively associated with adipocyte size (r= -0.36, p=0.05). There were no significant associations between adiponectin levels and insulin resistance (M-value) or HbA1c-levels.

In conclusion, poor glycemic control was associated with insulin resistance and with elevated levels of TNF-α, IL-6, CRP and morning cortisol levels and cortisol levels after stimulation with ACTH.

<table>
<thead>
<tr>
<th>Adiponectin (µg/mL)</th>
<th>Poor control</th>
<th>Good control</th>
<th>Control subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>basal</td>
<td>Males</td>
<td>6.1±2.1</td>
<td>6.8±1.3</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>6.5±0.5</td>
<td>17.4±4.2</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>P vs. G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during clamp steady state</td>
<td>Males</td>
<td>5.5±1.8</td>
<td>5.8±0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>7.1±0.8</td>
<td>14.8±3.4</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>P vs. G</td>
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</tbody>
</table>

Table 1
Adiponectin levels in the basal state and during clamp steady state in diabetes patients with good and poor metabolic control and in control subjects.
DISCUSSION

Insulin resistance occurs early in the development of type 2 diabetes and it can be demonstrated in young, healthy relatives of type 2 diabetes patients suggesting a large genetic component (21, 28, 133). Male diabetes relatives were more insulin-resistant than control subjects in study I, but in the smaller cohort examined in study II and III only a tendency towards higher degree of insulin resistance was detected in the diabetes relatives. It must, however, be considered that diabetes relatives are a heterogeneous group and an overlap in insulin sensitivity can be expected in comparison to a control group (134). Moreover, the size of the study cohort was limited and it might not have enough power to detect small differences between the groups.

In the present study, in non-diabetic subjects, high leptin levels were associated with insulin resistance, partly independent of fat mass. In men, insulin resistance was also strongly and inversely associated with testosterone levels. Male diabetes relatives displayed lower morning cortisol and testosterone levels and higher leptin levels, whereas female relatives displayed a tendency to higher testosterone levels compared to female control subjects. These findings were independent of the degree of adiposity. It is established that leptin levels are strongly associated with obesity and it has also been demonstrated that leptin levels are associated with fat cell size (87, 135) and both obesity and large fat cells are associated with a high risk of developing type 2 diabetes. Various studies have been performed to explore the association between leptin levels and insulin resistance, but only a few of these studies were able to detect an association that was independent of adiposity (136-138).

Glucocorticoids are steroid hormones that exert strong insulin-antagonistic effects. Elevated glucocorticoid levels due to endogenous cortisol overproduction, i.e. Cushing’s syndrome, or due to pharmacological treatment may lead to a clinical condition that resembles the metabolic syndrome; insulin resistance and impaired glucose tolerance as well as hypertension, hypertriglyceridemia, impaired fibrinolysis and central fat deposition (50, 52). Many studies on cortisol axis activity in obesity and in the metabolic syn-
drome have been performed but without consistent conclusions. Cortisol production appears to be elevated in obesity (139) but there is no evidence for increased circulating cortisol levels in obesity or the metabolic syndrome (140, 141). An altered sensitivity to stimuli regulating the cortisol axis has been suggested in the metabolic syndrome and obesity but the results are contradictory (142-144).

In this study there were no differences in 24-h urinary excretion or diurnal salivary cortisol profile between diabetes relatives and controls. In study I, morning serum cortisol levels were lower in male diabetes relatives as compared to control subjects. Upon stimulation of the HPA-axis with ACTH and CRH, the diabetes relatives in study III, displayed lower cortisol levels compared to controls indicating a decreased sensitivity to stimuli. On the other hand, inhibition with dexamethasone yielded similar serum cortisol levels in both groups. After adjustments for BMI, no significant associations between insulin resistance and any of the cortisol axis measurements were found. Glucocorticoid action on target tissues depends not only on circulating levels but also on the intracellular hormone concentration. Enhanced local action of glucocorticoids in adipose tissue and skeletal muscle has been suggested in the metabolic syndrome (145). The enzyme 11 beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activates functionally inert cortisone to active cortisol within tissues that are targets of insulin such as adipose tissue and liver and this enzyme may thereby regulate auto- and paracrine glucocorticoid action. The activity of this enzyme appears to be elevated in fat depots from obese subjects (146, 147) and pharmacological studies have shown that inhibition of 11β-HSD1exerts a positive effect on insulin sensitivity (144, 148). Elevated local, e.g. intracellular or interstitial, levels of cortisol in the adipose tissue can thus not be excluded, but no such measurements were performed in this study. It must also be appreciated that the cohort in study III was small and therefore could fail to detect subtle differences in the HPA axis activity.

The central nervous system can affect and modulate glucose and lipid metabolism in several ways. A high sympathetic neural activity can contribute to the development of insulin resistance as well as to other features of the metabolic syndrome via increased
levels of catecholamines that have insulin-antagonistic effects (54). Sympathetic nerve fibres also directly innervate adipose tissue and stimulate lipolysis, leading to increased release of NEFA, contributing to insulin resistance (41, 57, 149). There also seem to be a functional parasympathetic innervation of adipose tissue (56, 58). An attenuated parasympathetic activity has been suggested to promote the development of insulin resistance (150, 151). A decreased parasympathetic activity without an increase in sympathetic activity obviously also leads to an altered sympathetic/parasympathetic balance with a relative predominance of sympathetic activity. An altered sympathetic activity or an abnormal sensitivity to sympathetic stimuli has been reported in type 2 diabetics and also in animal models of type 2 diabetes (61, 62, 64, 152). On the other hand, hyperinsulinemia can also trigger hyperactivity in the sympathetic nervous system (153, 154).

In the non-diabetic subjects in this study, insulin resistance was associated with the ratio of sympathetic/parasympathetic activity during stress provocations, and insulin-resistant subjects exhibited a higher sympathetic/parasympathetic ratio and a lower parasympathetic activity compared to insulin sensitive subjects. The insulin-resistant subjects also showed less reactivity during provocations performed to activate the sympathetic and parasympathetic nervous systems and possibly also a delayed restoration to baseline after provocations. The results are in agreement with previous results suggesting that an altered balance in the ANS is associated with insulin resistance (62, 151, 155, 156). An attenuated parasympathetic activity as well as an enhanced sympathetic activity could explain the findings in this study although it is not possible to establish the exact contribution of each component with our techniques.

Obesity is strongly associated with insulin resistance and it is a powerful risk factor for the development of type 2 diabetes and other components of the metabolic syndrome (74, 157). The distribution of the body fat seems to be crucial (77, 78). Visceral adipose tissue (VAT) is morphologically and functionally different from subcutaneous adipose tissue (SAT) (158, 159). Antilipolytic effects of insulin and α-adrenergic stimulation are lower in VAT than in SAT (160) but insulin effect on glucose uptake appears to be similar in the two depots (161). Glucocorticoid effects are more pronounced in VAT,
possibly due to higher expression of glucocorticoid receptors and increased levels of 11β-HSD1 (162). Insulin-antagonistic effects of glucocorticoids appear to be more important in VAT compared to SAT (161). VAT releases more NEFA and produces more angiotensinogen, IL-6 and PAI and less leptin than SAT (159), factors that might contribute to insulin resistance whereas, surprisingly, adiponectin secretion appears to be higher from VAT compared to SAT (116). It has also been shown in a prospective study that increased amount of VAT predicted the development of type 2 diabetes in a high-risk population (163). In study III, abdominal fat distribution was measured with computed tomography. There was a tendency towards a greater amount of visceral fat in the diabetes relatives compared to the control subjects, but the difference was not statistically significant. This may be due to the fact that only a subgroup of 9 relatives and 9 control subjects participated in the CT examination, and thus the study sample might be too small to detect a true difference between the groups. However, another study comparing diabetes relatives and control subjects with respect to visceral fat accumulation reported no differences in VAT amount between the groups despite a higher degree of insulin resistance in the group of diabetes relatives (164). Similar results were reported from a study examining abdominal fat distribution in Pima Indians compared with Caucasians (165). In this study, VAT was strongly associated with insulin resistance. VAT was also positively associated with heart rate, both in the resting state and after provocations, and with the sympathetic/parasympathetic ratio after an orthostatic manoeuvre. These findings may suggest that a large amount of visceral adipose tissue can promote activation of the sympathetic nervous system. This can potentially be mediated by enhanced release of free fatty acids into the portal circulation as has been suggested in previous studies (166, 167). A high sympathetic activity could also possibly promote visceral fat accumulation (168, 169), which in turn would affect insulin sensitivity.

Not only the amount of adipose tissue but also the size of the adipocytes appears to be important in the development of insulin resistance. Several studies have shown that increased adipocyte size is associated with insulin resistance (170, 171). It has also been shown that a large adipocyte size can predict type 2 diabetes (172). Enlarged fat cells secrete increased amounts of adipokines, e.g. TNF-α, IL-6, leptin, which could contrib-
ute to insulin resistance (172, 173). Interestingly, data from a recent study suggest that visceral adipocyte size may be more strongly linked to insulin resistance than is subcutaneous adipocyte size (161). In the present work, fat cell size was only determined in study IV, i.e. diabetic patients and their control subjects. Subcutaneous adipocyte size was similar in type 2 diabetes patients and non-diabetic control subjects. Nevertheless, fat cell size was strongly associated with insulin resistance, both in vivo and in vitro (29) and also with circulating leptin and IL-6 levels.

In study IV, type 2 diabetes patients were more insulin-resistant than BMI-matched non-diabetics and the diabetes subjects with poor glycemic control exhibited the highest degree of insulin resistance. There was a strong association between glycemic level and insulin resistance among the diabetes patients and also in the total cohort in study IV. This may be due to the glucotoxic effect, i.e. hyperglycemia aggravates insulin resistance but, on the other hand, insulin resistance obviously is expected to impair the control of glucose levels and hence contribute to hyperglycemia.

TNF-α levels were higher in the diabetic patients compared to control subjects and highest in the poorly controlled diabetic subjects and this was not due to adiposity. There was a tendency to higher IL-6 levels in the poorly controlled diabetes patients compared to the diabetics with good control and the non-diabetics but the difference was not significant. Moreover, glycemic level (HbA1c) was associated with both TNF-α and IL-6 levels whereas insulin resistance was associated with TNF-α, but not with IL-6 levels. These results are not quite in accordance with previous results reporting a correlation between insulin resistance and TNF-α as well as IL-6 levels (92, 174). It has also been reported that TNF-α and IL-6 levels are elevated in type 2 diabetes patients compared to non-diabetics (92, 98, 175). It has been suggested that increased adipocyte size would secrete increased amounts of adipokines and that this could mediate insulin resistance in obesity (92, 176). In the subjects in study IV there was an association between adipocyte size and IL-6 but not TNF-α levels, which again is an unexpected finding as TNF-α levels were higher in the poorly controlled diabetes patients that were also more insulin resistant than the other groups. On the other hand, there was no differ-
ence in fat cell size between the poorly controlled diabetes group and the other two
groups. This finding might therefore suggest that adipocyte size is not crucial for the
amount of TNF-α secreted. But, on the other hand, only the size of the subcutaneous
adipocytes was measured and visceral adipocyte size may be of greater importance for
TNF-α secretion.

The highest levels of morning serum cortisol were seen in the poorly controlled diabetes
group as compared to well-controlled diabetics and non-diabetic control subjects. Urin-
ary cortisol excretion did not differ between the three groups. Neither did the cortisol
levels after stimulation with ACTH and inhibition with dexamethasone. High morning
serum cortisol levels and serum cortisol levels after stimulation with ACTH were asso-
ciated with poor glycemic control. High morning cortisol levels were also associated
with insulin resistance. In study I, morning serum cortisol levels were lower in male
diabetes relatives but serum cortisol levels did not display any association with insulin
resistance. This might indicate that the elevated cortisol levels seen in the poorly con-
trolled diabetes group could be a consequence rather than a cause of hyperglycemia.
The diabetic subjects showed a lower peripheral vascular sensitivity to glucocorticoids
after dermal application of beclomethasone when compared to control subjects. Vaso-
constriction response after dermal application of glucocorticoids is a method to examine
the function of the glucocorticoid receptor and a decreased vasoconstrictor response
indicates an abnormality in the glucocorticoid receptor. An abnormal glucocorticoid
receptor function could lead to resistance to negative feedback in the cortisol axis and
this would give an over-activity in the cortisol axis (177). In this study, however, no
signs of over-activity in the cortisol axis were detected including results of the dex-
amethasone inhibition test.

Cytokines are involved in the regulation of cortisol secretion, both IL-6 and TNF-α ac-
tivates the cortisol axis at the level of the pituitary and the hypothalamus (107) and
TNF-α may enhance the conversion of cortisone to cortisol by activation of 11β-HSD1
(178). Moreover, TNF-α, IL-6 and their receptors are present in the adrenal cortex
where they modify the adrenal steroid secretion (106). Therefore it might also be sug-
gested the higher levels of morning cortisol in the poorly controlled diabetic subjects are explained by the elevated levels of cytokines in this group.

C-reactive protein (CRP) is an acute-phase protein synthesized in the liver. Several studies have shown a positive association between C-reactive protein (CRP) and features of the metabolic syndrome, in particular obesity (179, 180). CRP can also predict the future risk of diabetes (181, 182) including the risk of progression from impaired glucose tolerance (IGT) to diabetes (183). Moreover, it has been shown that CRP levels are elevated in IGT and type 2 diabetes (184, 185). CRP also appears to be associated with insulin resistance and glycemic levels in newly diagnosed type 1 diabetes (186). In our present study, CRP levels were clearly highest in the poorly controlled diabetics and lowest in the well-controlled diabetics and this difference was not explained by adiposity as the groups were matched for BMI. The difference between the poorly controlled diabetics and the control subjects did not reach statistical significance (p=0.14) and CRP levels were similar in well-controlled diabetics and control subjects. CRP levels were strongly associated with glycemic levels whereas there was no association between CRP levels and insulin resistance. These results might indicate that it is hyperglycemia that triggers the increase in CRP that previously has been reported in type 2 diabetes and IGT. There was also a strong association between CRP levels and morning serum cortisol levels and cortisol levels were also associated with glycemic level and therefore it could not be completely excluded that an increase in cortisol level causes hyperglycemia as well as an elevation in CRP levels.

In agreement with previous reports, adiponectin levels in this study were higher in women than in men, and it has been suggested that this is due to the fact that testosterone inhibits adiponectin secretion (119). When comparing the diabetes patients with the non-diabetic subjects in this study, we failed to detect any consistent differences in adiponectin levels. In female subjects, however, adiponectin levels were lowest in the poorly controlled diabetes group and highest in the well-controlled group and this difference was statistically significant. In male subjects adiponectin levels were similar in all three groups. In correlation analyses, adiponectin levels were not associated with
glycemic level (HbA1c) and neither with insulin resistance (M-value). According to previous results (111), it would be expected that adiponectin levels were lower in the diabetic patients than in non-diabetic subjects and a slight tendency in that direction was seen in the male subjects. In females, however, adiponectin levels were highest in the well-controlled diabetic subject which is a somewhat unexpected finding suggesting that the glycemic level per se may play a role in the regulation of adiponectin secretion. On the other hand, in correlation analyses there was no association between glycemic control and adiponectin levels, so this possible relationship remains to be established.
CONCLUSION

Insulin resistance is often seen in the pre-diabetic state, notably in healthy subjects genetically predisposed for type 2 diabetes. In the present study, insulin-resistant subjects display an attenuated parasympathetic response upon various provocations and there were signs of an increased ratio of sympathetic to parasympathetic activity. Male subjects genetically predisposed for type 2 exhibit lower serum testosterone and morning cortisol levels and a tendency to higher leptin levels whereas female diabetes relatives have a tendency to higher testosterone levels. Relatives of type 2 diabetes patients exhibit a lower increase in serum cortisol levels after stimulation with ACTH and CRH. The amount of visceral adipose tissue (VAT) is associated with insulin resistance and with heart rate and sympathetic/para-sympathetic balance. In type 2 diabetes patients, poor glycemic level is associated with insulin resistance and pronounced beta cell dysfunction. Moreover, high levels of adipocyte-derived cytokines and cortisol were associated with both insulin resistance and high blood glucose levels. Subcutaneous adipocyte size was associated with insulin resistance and with leptin and IL-6 levels.

Glucose and fat metabolism is carefully regulated in order to maintain a physiological balance. Insulin acts in an anabolic way, stimulating uptake and storage of energy substrates. Glucocorticoids, catecholamines and the sympathetic nervous system act in the opposite way and in recent years it has became apparent that this applies to several cytokines as well. In situations with increased need to mobilize energy substrates, e.g. starvation, physical exercise, infection, trauma or hypoglycemia, some of these insulin-antagonistic factors are activated to provide fuel to important organs. There also seem to be an intricate system regulating the interplay between all these factors. In this study, there are signs of dysregulation in the autonomic nervous system, the cortisol axis, adipose tissue distribution and adipokine production in insulin resistance associated with type 2 diabetes. An altered balance in any of these insulin-antagonistic systems due to genetic and/or environmental factors may be a contributing factor in the development of insulin resistance seen in the metabolic syndrome and type 2 diabetes. A hypothetical model for the interplay between these systems in the pathogenesis of insulin resistance
is depicted in Figure 6. From this study with a cross-sectional design it is, however, not possible to establish the causal or temporal relationships. Ongoing longitudinal studies in some of our cohorts will hopefully help to elucidate these aspects.

Figure 6
Hypothetical model of the interplay between neural, hormonal, genetic and environmental factors in the development of insulin resistance.
Insulinresistens uppträder vanligtvis tidigt i utvecklingen av typ 2-diabetes. Mekanismerna som leder till insulinresistens är ej helt klarlagda. En förändrad balans i det autonoma nervsystemet, i vissa hormonsystem eller inflammatoriska faktorer skulle kunna bidra till uppkomsten av insulinresistens. Hyperglykemi vid etablerad diabetes förvärrar dessutom såväl insulinresistens som betacells-dysfunktion men det är inte heller helt klarlagt vilka mekanismer som förklarar detta fenomen, s.k. glukotoxicitet.


Manliga diabetessläktingar uppvisade lägre nivåer av testosteron och högre nivåer av leptin jämfört med friska kontrollpersoner. Vi fann ett invers samband mellan insulinkänslighet och leptinnivåer. Hos män sågs en positiv association mellan insulininkänslighet och testosteronnivån. Diabetessläktingarna uppvisade en tendens till lägre parasympatisk nervaktivitet men i övrigt såg inga skillnader i autonom nervaktivitet mellan släktingar och kontrollpersoner. Kvoten mellan sympatisk- och parasympatiskusaktivitet vid stressprovokation var invers korrelerad till insulininkänslighet. Insulinresistenta försökspersoner företegrade högre symatikus/parasympatisk-kvot och lägre parasympatiskusaktivitet jämfört med insulininkänsliga individer. De uppvisade också generellt lägre reaktivitet i det autonoma nervsystemet i samband med provokationer. Diabetesläktingarna hade en lägre stegring av s-kortisol efter stimulering med ACTH och CRH. Mängden visceralt fett (VAT) tenderade att vara högre hos diabetessläktingarna jämfört med kontrollerna. VAT var negativt korrelerat till insulininkänslighet och positivt korrelerat till hjärtfrekvens i vila, under kontrollerad andning samt till hjärtfrekvens och sym-
patikut-parasympatikut-quot vid ortostatisk manöver. Vi fann inga korrelationer mellan mängden subcutant bukfett och insulinkänslighet, aktiviteten i det autonoma nervsystemet eller kortisolvåder.

Typ 2-diabetiker med god respektive dålig metabol kontroll samt friska kontrollpersoner undersöktes med avseende på insulinkänslighet, aktivitet i kortisolaxeln och blodnivåer av leptin, könshormoner och inflammatoriska fettvävssubstanser som interleukin-6 (IL-6) och TNF-alfa. Fettvävsbiopsi togs från subcutant bukfett för bedömning av fettcellsstorlek. Diabetikerna var mer insulinresistenta än kontroller och detta var mest uttalat hos diabetiker med dålig metabol kontroll. Dessa hade också högst nivåer av TNF-alfa, kortisol och fria fettsyror. I korrelationsanalyser sågs signifikanta associationer mellan glykemisk nivå (HbA1c) och insulinresistens, TNF-alfa, IL-6 och basalt respektive ACTH-stimulerat serum-kortisol. Graden av insulinresistens var korrelerad till nivåer av fria fettsyror, TNF-alfa och ACTH-stimulerat kortisol. Fettcellernas storlek uppvisade ett starkt samband med insulinresistens samt IL-6- och leptin-nivåer.

Våra fynd talar för ett samband mellan insulinresistens och visceral fettmängd, aktiviteten i det autonoma nervsystemet samt blodnivåer av olika hormoner samt fettcellsproducerade substanser och detta tycks vara delvis oberoende av genetisk bakgrund. Ett komplext samspel mellan dessa faktorer kan vara av betydelse för uppkomst av insulinresistens och typ 2-diabetes men också leda till försämrad metabol kontroll vid manifest typ 2-diabetes.
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