

Dysregulation of the Autonomic Nervous System Can Be a Link between Visceral Adiposity and Insulin Resistance

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Abstract

LINDMARK, STINA, LARS LÖNN, URBAN WIKLUND, MAGNUS TUFVESSON, TOMMY OLSSON, AND JAN W. ERIKSSON. Dysregulation of the autonomic nervous system can be a link between visceral adiposity and insulin resistance. *Obes Res.* 2005;13:717–728.

Objective: To evaluate the interplay among abdominal adipose tissue distribution, the cortisol axis, the autonomic nervous system, and insulin resistance.

Research Methods and Procedures: Two age-, sex-, and BMI-matched groups were studied. Fifteen subjects were first-degree relatives of patients with type 2 diabetes (R), and 15 had no family history of diabetes (controls, C). A hyperinsulinemic euglycemic clamp, cortisol measurements, and analysis of heart rate variability (HRV) were performed. Computed tomography was performed in a subgroup ($n = 9 + 9$) to determine abdominal adipose tissue distribution.

Results: R tended to be less insulin-sensitive than C (M value 9.2 ± 1.0 vs 10.3 ± 0.7 mg/kg per minute, not significant). Stimulation with tetracosactin or corticotropin releasing hormone yielded lower peak serum cortisol levels in R ($p = 0.03$ and $p = 0.06$, respectively). The amount of visceral abdominal fat (VAT) tended to be greater in R. In all subjects, VAT was negatively correlated to insulin sensitivity ($r = -0.93$, $p < 0.001$). There was a positive association between VAT and resting heart rate ($r = 0.70$,

$p = 0.003$) and sympathetic/parasympathetic ratio in HRV assessment after tilt ($r = 0.53$, $p = 0.03$). Subcutaneous abdominal tissue was not associated with insulin sensitivity or any of the hormonal or HRV assessments.

Discussion: Subjects genetically predisposed for type 2 diabetes had a tendency toward a larger amount of VAT and to lower insulin sensitivity compared with control subjects. The amount of visceral fat was strongly associated with insulin resistance and signs of a high ratio of sympathetic vs. parasympathetic reactivity. A large amount of visceral fat may act in concert with sympathetic/parasympathetic imbalance to promote the development of insulin resistance, and this may be partly independent of genetic background.

Key words: type 2 diabetes, family history, insulin resistance, cortisol axis, visceral fat

Introduction

Type 2 diabetes is associated with insulin resistance and impaired insulin secretion. Insulin resistance occurs early in the development of the disease and can predict the risk of future diabetes. Healthy relatives of type 2 diabetes patients have an increased risk of developing type 2 diabetes later in life, and they are prone to insulin resistance, albeit with an overlap in insulin sensitivity vs. subjects without any family history of diabetes (1–3). The primary mechanisms causing insulin resistance are unknown, but the involvement of candidate genes governing cellular insulin action have been suggested (1,3,4). Notably, cellular insulin resistance may be reversible (5,6); therefore, the in vivo milieu of insulin's target cells might be of importance in the development of insulin resistance, e.g., through hormonal and/or neural factors (7–9).

Stress hormones such as glucocorticoids and catecholamines oppose the effects of insulin. Increased levels of glucocorticoids due to endogenous overproduction or glucocorticoid treatment cause insulin resistance and may impair the metabolic control in diabetic subjects (10–12). A

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correlation between low birth weight and features of the metabolic syndrome and an increased activity in the cortisol axis has been demonstrated and might support a link between the cortisol axis and insulin resistance (13–15). In addition, stress and exogenous administration of catecholamines have been reported to increase glucose levels and worsen metabolic control in type 2 diabetes patients (16,17).

Enhanced activity in the sympathetic nervous system (SNS)¹ can produce insulin resistance (18). Type 2 diabetes is often associated with hypertension and lipid abnormalities, and these conditions often cluster in the so-called insulin resistance syndrome (19,20). Elevated activity of the SNS has been proposed as a link between insulin resistance and hypertension but as a consequence rather than a cause of insulin resistance and hyperinsulinemia (21,22). Some human and animal studies provide results that are compatible with abnormalities of the autonomic nervous system contributing to the etiology of type 2 diabetes (23–26). We recently reported an association between insulin resistance and an imbalance between the sympathetic vs. parasympathetic reactivity of the autonomic nervous system (9).

Obesity has proven to be a strong risk factor for type 2 diabetes and for other features of the metabolic syndrome (27–29). Not only the degree of obesity but also the distribution of body fat is of importance (30). It has been demonstrated that upper body fat distribution is a stronger marker for the metabolic syndrome in humans than obesity per se (31–34). Studies using computed tomography (CT) or magnetic resonance imaging to distinguish between visceral abdominal tissue (VAT) and subcutaneous abdominal tissue (SAT) indicate that VAT, compared with SAT, is more strongly linked to insulin resistance, type 2 diabetes, hypertension, and dyslipidemia (35–40). It has also been shown that VAT accumulation precedes the development of type 2 diabetes (41). Experimental work in animals also support a specific, unfavorable biological impact of visceral as opposed to subcutaneous fat depots with respect to metabolic regulation (42,43).

Several studies have focused on neuroendocrine activity in obesity, and they suggest a link between abdominal obesity and cortisol axis dysregulation (44–50). It is also well-known that patients with Cushing's syndrome have visceral fat accumulation (51). Abdominal obesity may also be associated with an altered activity in the SNS (52–55).

The aim of this study was to evaluate the interplay among abdominal adipose tissue (AT) distribution, regulation of the cortisol axis, and the autonomic nervous system in

relation to insulin resistance. Our main hypothesis was that dysregulation of the cortisol axis and the autonomic nervous system would be associated with visceral fat mass and that such neuroendocrine dysfunction could contribute to insulin resistance in individuals with central obesity. We studied healthy subjects either with or without a family history of type 2 diabetes. Data on basal hormonal levels and on the autonomic nervous regulation of the heart have recently been reported in this study population (8,9).

Research Methods and Procedures

Study Population

Thirty healthy, nondiabetic subjects were enrolled in the study. They were previously investigated with respect to insulin resistance, leptin, sex hormones, and the activity of the autonomic nervous system (8,9). In the present study, cortisol axis activity and body fat distribution were addressed, and these results were related to previously reported findings on autonomic nervous activity and insulin resistance. Fifteen of the subjects had either two first degree or one first degree plus at least two second degree relatives with type 2 diabetes, and 15 subjects did not have any known family history of diabetes. The subjects were recruited by advertisement in the local newspaper. Written and oral informed consent was obtained from all subjects, and the study protocol was approved by the Ethical Committee of Umeå University.

The diabetes relatives and control subjects were matched according to age, sex, and BMI. The study subjects had no chronic medication and were all in good health, as determined by medical history, clinical examination, and laboratory analyses including blood cell counts, serum electrolytes, creatinine, and lipids. None of the subjects suffered from depression or any other psychiatric disorder. Anthropometric and laboratory characteristics of the study groups are shown in Tables 1 and 2. Systolic blood pressure was near significantly higher in the diabetes relatives, but the other variables were very similar between the groups. To specifically address the associations between insulin resistance and other assessments, the combined groups were used because both groups comprised subjects with high and low insulin sensitivity, respectively. All subjects were invited to an examination with CT to evaluate abdominal fat distribution, and 18 subjects (nine relatives and nine control subjects) agreed to participate in this part of the study. These two subgroups of relatives and control subjects were also very similar with respect to age, sex, and BMI (data not shown).

Examinations

Unless otherwise indicated, the examinations were performed in the morning after an overnight fast (>10 hours) and with the subjects in the supine position.

¹ Nonstandard abbreviations: SNS, sympathetic nervous system; CT, computed tomography; VAT, visceral abdominal tissue; SAT, subcutaneous abdominal tissue; AT, adipose tissue; OGTT, oral glucose tolerance test; ACTH, tetracosactin; CRH, corticotropin releasing hormone; HRV, heart rate variability; CPT, cold pressor test; L2, second lumbar vertebra level; L4, fourth lumbar vertebra level; NEFA, nonesterified fatty acid; AUC, area under the curve.

Table 1. Anthropometric characteristics of relatives of type 2 diabetes patients and control subjects

	Relatives (N = 15)	Controls (N = 15)	p
Sex (men/women)	8/7	8/7	NS
Age (years)	32.5 ± 1.9	32.7 ± 1.9	NS
BMI (kg/m ²)	24.2 ± 0.9	23.6 ± 0.7	NS
Waist-to-hip ratio	0.85 ± 0.02	0.86 ± 0.02	NS
Systolic blood pressure	128 ± 4	118 ± 3	0.06
Diastolic blood pressure	76 ± 2	76 ± 2	NS
Smokers/nonsmokers	1/14	1/14	NS
Fat mass %*	19.2 ± 1.8	18.0 ± 1.8	NS
Lean mass %*	80.8 ± 1.8	82.0 ± 1.8	NS
Visceral adipose tissue (cm ²)*†	120 ± 27	86 ± 20	NS
Subcutaneous adipose tissue (cm ²)*†	182 ± 35	172 ± 28	NS
Visceral/subcutaneous adipose tissue ratio*†	0.74 ± 0.19	0.50 ± 0.08	NS
Total adipose tissue (cm ²)*†	301 ± 58	258 ± 44	NS
Sagittal diameter (cm)*†	23.0 ± 1.4	22.0 ± 1.1	NS

Data are means ± SEM or number of subjects. NS, not significant.

* Only subjects participating in the CT examination (N = 9 + 9).

† Calculated values obtained from the CT scans at the L2 and L4 levels.

BMI was calculated as weight (kilograms) divided by height squared (meters squared). Body composition was determined with the bioelectrical impedance analysis

method (BIA 101-Fitness; RJL Systems, Detroit, MI) (56). For the subjects undergoing CT, the anthropometric measurements were performed on the same occasion.

Table 2. Blood chemistry in study participants

	Relatives (N = 15)	Controls (N = 15)	p
Hemoglobin A _{1c} (%)(normal range 3.6 to 5.0)	3.9 ± 0.1	3.9 ± 0.1	NS
Fasting blood glucose (mM)	4.5 ± 0.1	4.4 ± 0.1	NS
OGTT 2-hour blood glucose (mM)	4.6 ± 0.2	4.2 ± 0.2	NS
Fasting serum insulin (mU/L)	8.4 ± 1.2	7.8 ± 0.9	NS
OGTT 2-hour serum insulin (mU/L)	30.4 ± 8.9	25.7 ± 6.0	NS
Fasting serum C-peptide (pM)	700 ± 80	629 ± 32	NS
Serum cholesterol (mM)	4.8 ± 0.2	4.9 ± 0.2	NS
Serum high-density lipoprotein-cholesterol (mM)	1.4 ± 0.1	1.4 ± 0.1	NS
Serum low-density lipoprotein-cholesterol (mM)	2.8 ± 0.2	2.9 ± 0.2	NS
Serum triglycerides (mM)	1.3 ± 0.2	1.3 ± 0.2	NS
M value (mg/kg per minute)*	9.2 ± 1.0	10.3 ± 0.7	NS
Plasma NEFA (μM)			
Basal	461 ± 61	354 ± 40	NS
During Clamp*	43 ± 6	35 ± 4	NS

Data are means ± SE.

* M value and NEFA were obtained during steady state (60 to 120 min) of the glucose clamp.

Oral Glucose Tolerance Test (OGTT). The OGTT was performed with the subjects in sitting position by ingestion of 75 grams of glucose in a liquid solution. Venous blood samples were obtained in the fasting state and after 120 minutes and were analyzed for blood glucose and serum insulin. Two-hour blood glucose values of 6.7 to 9.9 mM were defined as impaired glucose tolerance (57).

Euglycemic Hyperinsulinemic Clamp. The protocol and the results have previously been described in detail (9). In brief, semisynthetic human insulin (Actrapid; Novo-Nordisk Industry, Copenhagen, Denmark) was infused as a priming dose, followed by a constant infusion at 56 mU/m² per minute for 120 minutes. In parallel, a glucose infusion (200 mg/mL) was started, and the infusion rate was adjusted to maintain a steady state blood glucose level at 6.0 mM. The rate of glucose infusion served as a measure of insulin sensitivity, and the M value was calculated by dividing the glucose infusion rate during the last 60 minutes (steady state) of the clamp by body weight (milligrams per kilogram per minute).

Low-Dose Tetracosactin (ACTH) Stimulation Test. The subjects received one intravenous cannula (Venflon) in an antecubital vein for administration of the test substance and for blood sampling. ACTH (1 µg; Synacthen; Novartis, Basel, Switzerland) was injected as a bolus (58). Blood samples for determination of serum cortisol were obtained in the basal state (0 minutes) and then at 30, 40, 60, and 120 minutes after ACTH injection. The samples were centrifuged within 30 minutes and stored at -20 °C until analyzed.

Corticotropin Releasing Hormone (CRH) Stimulation Test. This test was performed at 1 PM with the subjects fasting during the previous 3 hours. The subjects were positioned in a comfortable bed. One intravenous cannula (Venflon) was inserted into an antecubital vein for administration of the test substance and for blood sampling. After 30 minutes of rest, human CRH (Ferring Arzneimittel GmbH, Kiel, Germany) was injected as a single dose of 1 µg/kg (59,60). Blood samples for determination of serum cortisol and plasma ACTH were obtained in the basal state (-15 and 0 minutes) and 15, 30, 60, 90, and 120 minutes after CRH injection. The samples for cortisol determination were centrifuged within 30 minutes and stored at -20 °C until analyzed. The samples for ACTH determination were collected in chilled tubes containing EDTA and Trasyol 1125 KIE/tube (Bayer AG, Leverkusen, Germany) and were kept cold before and during centrifugation and then stored at -80 °C until analyzed.

Oral Dexamethasone Suppression Test. Blood samples for determinations of serum cortisol were obtained at 8 AM. At 10 PM the same day, the subjects were given dexamethasone orally as a liquid solution in a dose of 3.5 µg/kg. Blood samples for determinations of serum cortisol were obtained at 8 AM the next day. The samples were centrifuged

within 30 minutes and stored at -20 °C until analyzed. On both occasions, the subjects were fasting since 10 PM the day before.

Sampling for Diurnal Salivary and Urinary Cortisol. A sampling device (Salivette; Sarstedt, Rommelsdorf, Germany) was used to collect saliva. The Salivette consists of a small cotton swab inside a centrifugation tube. The cotton swab was chewed for 1 minute and then placed back into the tube. Samples were obtained at 7:30 AM, 11:30 AM, 5:00 PM, 8 PM, and 10 PM on 2 different random days. The tubes were then centrifuged, and the saliva was stored at -20 °C until analyzed for cortisol concentration (61,62). Urine was collected during two separate days (2 × 24 hours) for measurements of free cortisol excretion. Urinary creatinine was assessed concomitantly, and all subjects displayed creatinine excretion within the normal range, indicating adequate urine collection.

Heart Rate Variability (HRV) during Rest, Controlled Breathing, Tilting, and Cold Pressor Test (CPT)

The basic data were previously reported for the 27 participants that could be evaluated, and the procedures and analyses are described in detail in that publication (9). In brief, electrocardiogram was recorded continuously while the subject performed controlled breathing at a rate of 12 breaths/min for 1 minute, was tilted passively to 70° head-up position (4 minutes), and, finally, underwent a CPT with one lower arm put in ice-cold water for 2 minutes. The different procedures were utilized to activate the autonomic nervous system in a standardized manner. Controlled breathing stimulates parasympathetic nerve activity, whereas tilting and CPT stimulate sympathetic nerve activity. The electrocardiographic R-R intervals were transformed to a heart rate time series by cubic spline interpolation. The power spectral density was estimated by autoregressive modeling of linearly detrended heart rate data. The total spectral power and the power of the low frequency (0.04 to 0.15 Hz) and high-frequency (0.15 to 0.40 Hz) components were calculated and log-transformed. The variability of the HF component is mainly mediated by parasympathetic activity, whereas the LF fluctuations are mediated by both sympathetic and parasympathetic activity. The LF-to-HF ratio mirrors the balance between the sympathetic and parasympathetic activity. Calculations were performed by use of the Matlab Software (MathWorks, Natick, MA).

CT

The subjects were all examined in the nonfasting state, and the CT scans were obtained in the evening. Examinations were made with a Siemens Somatom Plus 4 CT system, version VB 40c (Siemens, Erlangen, Germany) with the following settings: 140 kV, FoV 352, mAs 180, slice thickness 10 mm, and fixed filtration. Scans were

obtained at the second lumbar vertebra level (L2) and at the fourth lumbar vertebra level (L4). The images were transferred to a separate UNIX-based analyzing unit. Tissue areas were determined as previously described (63) with the following precision errors calculated from double determinations: SAT, 0.5%; and VAT, 1.2% (63). The total AT and VAT volumes were calculated from predictive equations according to Kvist et al. (64). One control subject was only examined at L4 and was, therefore, excluded from the calculations. Thus, nine relatives and eight control subjects were actually included in these analyses.

Assays

Blood glucose concentrations were determined by the HemoCue glucose system (HemoCue AB, Ängelholm, Sweden). Hemoglobin A_{1c} was measured by high-pressure liquid chromatography (Integral 4000; BioRad, Anaheim, CA). Serum insulin concentrations were measured by microparticle immunoassay (Abbot Imx; Abbot Laboratories, Abbot Park, IL). Serum cortisol was measured by chemiluminescent immunometric assay (IMMULITE; BioRad). Coefficient of variation within-run was 7.4% at level 82 nM and 6.0% at level 967 nM. Coefficient of variation between-run was 9.8% at level 82 nM and 3.5% at level 967 nM. Serum C-peptide and plasma ACTH were measured by chemiluminescent immunometric assay (IMMULITE; BioRad). Nonesterified fatty acid (NEFA) concentrations were determined using a commercial enzymatic kit (Wako Chemicals GmbH, Neuss, Germany). Salivary and urinary cortisol was measured by radioimmunoassay (Orion Diagnostica Cortisol test; Orion, Espoo, Finland). With this method for free urinary cortisol, glucocorticoid metabolites are also included; therefore, the assay probably reflects total cortisol production (65). All other analyses of blood and urine chemistry were performed according to routine methods at the Department of Clinical Chemistry, Umeå University Hospital.

Statistical Analysis

Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL). Data are means \pm SEM unless otherwise indicated. All variables were tested for normality, and parametric or nonparametric tests were used accordingly. Simple linear regressions were used to analyze correlations between variables, and multiple linear regression analyses (the enter and step-wise statistics in SPSS) were used to evaluate independent associations among data. $p < 0.05$ was considered statistically significant. At a power level of 80% for the linear regression analyses, we would detect association with $r^2 = 0.22$ when $n = 30$ and with $r^2 = 0.36$ when $n = 18$. These calculations are based on the noncentral F distribution and a significance level = 0.05.

Results

Blood Chemistry and OGTT

The results are shown in Table 2. Two diabetes relatives but no control subject displayed impaired glucose tolerance. Fasting serum insulin, serum C-peptide, blood glucose, and hemoglobin A_{1c} did not differ significantly between the groups. Neither were there any differences in serum lipids between the groups.

Euglycemic Hyperinsulinemic Clamp

Data from the euglycemic clamps were mainly reported previously (9). The M value and insulin sensitivity index (data not shown) did not differ significantly between the groups, although there was a tendency toward lower insulin sensitivity in the relatives (Table 2). Plasma NEFA concentrations in the basal state were slightly but not significantly higher in relatives. During steady state, hyperinsulinemia NEFA levels were suppressed, and there was no consistent difference between the groups (Table 2).

Cortisol Axis Assessments

The results are shown in Table 3. There were no differences in basal morning serum cortisol levels between the groups. After injection of 1 μ g of ACTH, the serum cortisol concentration increased in both groups, with the maximum peak at 30 or 40 minutes after injection. The cortisol level at 30 minutes was significantly lower in relatives compared with controls ($p = 0.03$). Area under the curve (AUC) for serum cortisol during the ACTH test was slightly but not significantly lower in relatives than control subjects (data not shown). Serum cortisol and plasma ACTH levels were markedly increased after CRH injection in both groups. Serum cortisol at 30 minutes was lower in relatives than in controls ($p = 0.06$), whereas ACTH levels did not differ. There was no significant difference in AUC for serum cortisol or plasma ACTH during the CRH test between the groups. Serum cortisol levels after dexamethasone did not differ between groups. Twenty-four-hour urinary cortisol excretion was measured as the average of two 24-hour urine collections. It was somewhat higher in relatives than in controls, but this difference was not significant. The salivary cortisol levels were higher in the morning and then decreased during the day in both groups. The relatives tended to have slightly lower salivary cortisol levels throughout the day compared with controls, but the difference was not statistically significant (data not shown). AUC for salivary cortisol did not differ significantly between the groups.

Body Fat Distribution Determined with CT (Table 1)

The mean value of the amount of AT calculated from the L2 and L4 were used for all statistical analyses. The amount of VAT was greater in relatives, but the difference was not

Table 3. Cortisol levels in study participants

	Relatives (N = 15)	Controls (N = 15)	p
24-Hour urinary cortisol (nmol)	96 ± 11	77 ± 11	NS
Serum cortisol (nM)			
Before ACTH	460 ± 35	497 ± 29	NS
After ACTH (30 min)	650 ± 36	750 ± 26	0.03
Before CRH	232 ± 18	205 ± 22	NS
After CRH (30 min)	456 ± 15	520 ± 29	0.06
Before dexamethasone	464 ± 34	531 ± 30	NS
After dexamethasone (10 hours)	318 ± 41	330 ± 30	NS

Samples before ACTH and before and after dexamethasone were obtained at 8 AM in the fasting state. Samples before CRH were obtained at 1 PM after 3-hour fasting
Data are means ± SE.

statistically significant. The predicted values of total body fat and total body visceral fat were similar in the two groups (data not shown).

Regression Analyses

Associations among measures of insulin resistance, HRV, cortisol axis activity, and AT distribution were evaluated with simple linear regression analyses, and when significant associations were found, multiple regression analyses including BMI, sex, and age as independent variables were performed. The results from the simple regression analyses, using VAT as dependent variable, are shown in Table 4 and Figures 1 and 2, and the results of multiple regression analyses are shown in Table 5. M value, serum insulin levels, and heart rate remained as significant predictors of VAT.

In step-wise multiple regression analyses (Table 6), entering VAT, M value, BMI, and the parameters from the HRV examination as dependent and/or independent variables, M value was the strongest independent predictor of VAT, followed by BMI (not shown), and also of LF/HF during CPT. VAT displayed the strongest independent association with M value and with LF-to-HF ratio after tilt. VAT displayed the strongest association with heart rate.

In contrast to VAT, SAT did not show any significant associations with insulin resistance, cortisol axis measurements, or the HRV variables in multiple regression analyses. Moreover, no consistent independent associations were seen in any of the regression analyses for cortisol axis measurements vs. HRV variables, AT distribution, or insulin sensitivity. The association between HRV measures and insulin sensitivity was reported previously (9).

Discussion

The mechanisms behind insulin resistance are not fully understood. Genetic factors are of importance, and they

interact with lifestyle factors; thus, for example, obesity, physical inactivity, and tobacco use can contribute to insulin resistance. The aim of this work was to study the interplay between neuroendocrine factors and fat distribution in the development of insulin resistance. Abdominal AT distribution, activity in the cortisol axis, and the autonomic nervous system were evaluated, and the relationship among such measures and insulin sensitivity was examined. The main finding was an association between the amount of visceral fat and signs of high sympathetic vs. parasympathetic reactivity, which might be an important link between visceral obesity and insulin resistance and type 2 diabetes.

As previously reported (34,36,37), we demonstrated that VAT is strongly associated with insulin resistance. There were also significant associations between VAT and heart rate and between VAT and the sympathetic/parasympathetic

Table 4. Results from simple linear regression analyses with VAT as dependent variable

	r	p
M value (mg/kg per minute)	-0.93	<0.001
Fasting serum insulin (mU/L)	0.85	<0.001
2-Hour serum insulin during OGTT (mU/L)	0.84	<0.001
Fasting serum C-peptide (pM)	0.87	<0.001
Heart rate (bpm)		
At rest	0.70	0.003
After tilt	0.72	0.002
After CPT	0.69	0.003
During controlled breathing	0.62	0.01
LF-to-HF ratio after tilt	0.53	0.03
LF power during tilt	0.56	0.03

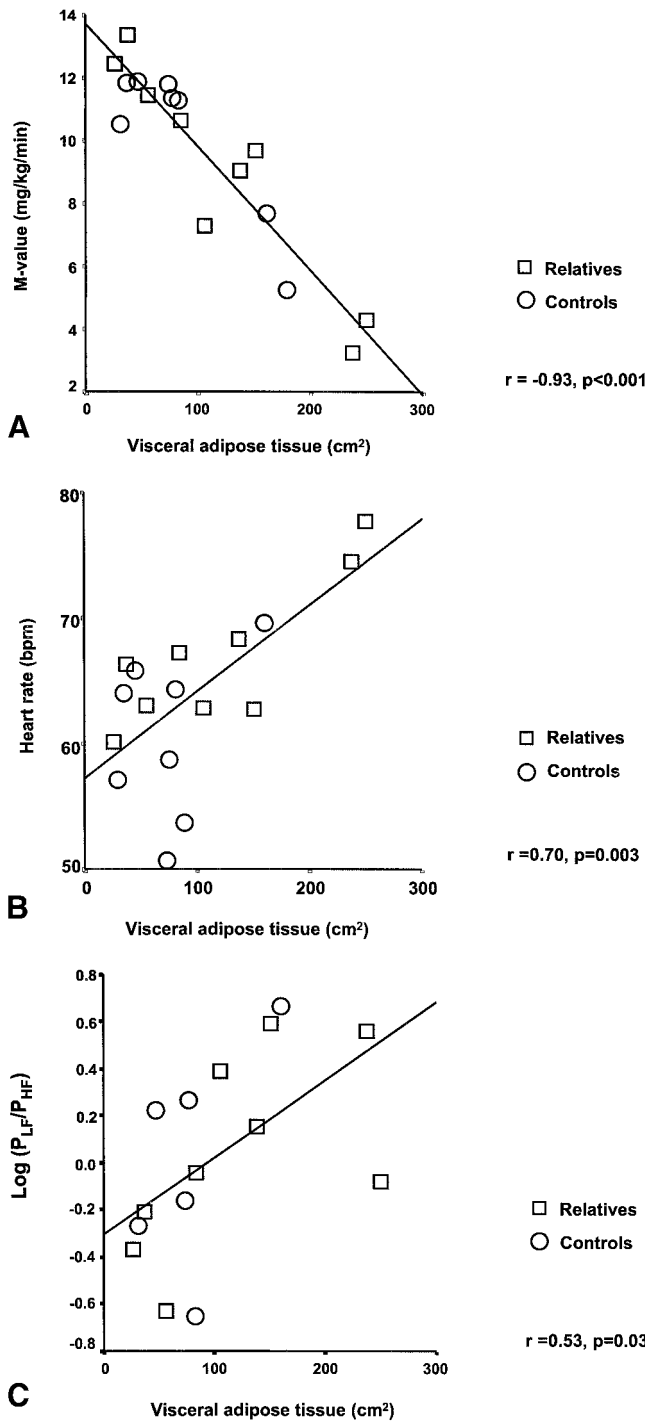


Figure 1: (A) The amount of VAT vs. insulin sensitivity (M value). (B) The amount of VAT vs. heart rate in the resting, supine state. (C) The amount of VAT versus sympathetic/parasympathetic balance in the supine position directly after 70° tilt (LH/HF power in HRV analyses).

balance. These findings might suggest that a large amount of visceral fat can promote activation of the SNS. This can potentially be mediated by enhanced release of NEFA to the

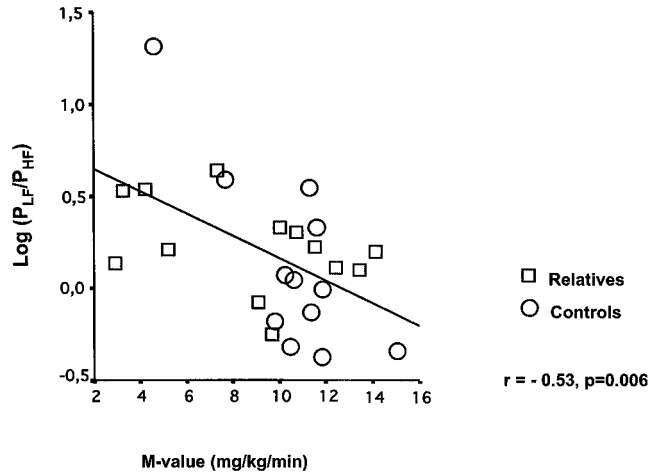


Figure 2: Insulin sensitivity (M value) versus sympathetic/parasympathetic balance during standardized stress (CPT).

portal circulation as has been suggested in previous studies (66,67). An altered balance in the autonomic nervous system can also potentially affect AT distribution. Some authors have suggested that a high sympathetic activity due to psychosocial stress may promote visceral fat accumulation (68,69). Recently, it was elegantly demonstrated that there is parasympathetic innervation of VAT and SAT that consists of separate pathways, including their central nervous system nuclei (70). It can be speculated whether parasympathetic signaling to the AT would be beneficial, due to inhibition of lipolysis and fatty acid release, or unfavorable through enhanced fat storage. Several authors have none-

Table 5. Results from multiple linear regression analyses with VAT as dependent variable

Independent variables	Standardized coefficient β	p
M value	-0.60	<0.001
Fasting serum insulin	0.51	0.001
2-Hour serum insulin during OGTT	0.52	<0.001
Fasting serum C-peptide	0.49	0.001
Heart rate (basal)	0.40	0.009
Heart rate (after tilt)	0.35	0.058
Heart rate (after CPT)	0.37	0.03
Heart rate (during controlled breathing)	0.31	0.056

Each independent variable entered together with BMI, sex, and age.

Table 6. Results from multiple linear regression analyses

Dependent	Independent	Standardized coefficient β	<i>p</i>
M value	VAT	-0.84	<0.001
	LF-to-HF ratio during CPT	-0.22	0.05
LF-to-HF ratio after tilt	VAT	0.50	0.03
LF-to-HF during CPT	M value	-0.56	0.03
Heart rate (basal)	VAT	0.70	0.003

Factors entered as independent variables: BMI, M value, VAT, and LF-to-HF ratio after tilt and during CPT. Only significant associations are shown.

theless suggested that an attenuated parasympathetic activity may promote insulin resistance (70–73). Moreover, it has been speculated that compartment-specific alterations in the balance of sympathetic/parasympathetic outflow would be a factor contributing the metabolic syndrome including visceral fat accumulation, i.e., a high sympathetic-to-parasympathetic ratio to skeletal muscle, vasculature, and thoracic organs and a low sympathetic-to-parasympathetic ratio to the abdomen including AT (74). There are previous data suggesting a low sympathetic tone in obesity (75), and this is seemingly at variance with our results. However, the results may not be contradictory because the elevated activity in the sympathetic vs. parasympathetic system reported here was specifically associated with visceral adiposity, and, moreover, it was found mainly in response to stressful provocations.

Multiple regression analyses showed significant associations between VAT and metabolic variables such as insulin resistance (low M value), fasting C-peptide, and serum insulin. In contrast, SAT displayed no significant associations with any of these metabolic parameters. This indicates that the amount of VAT as compared with SAT is more predictive for insulin resistance. On the contrary, AT distribution did not seem to be of importance for the regulation of the cortisol axis activity.

Heart rates in the resting state, directly after tilt and CPT, and during controlled breathing were all independently and significantly associated with the amount of abdominal visceral fat. There was also a positive association between VAT and the sympathetic/parasympathetic balance after an orthostatic maneuver, reflected by the LF-to-HF ratio. The SAT did not exhibit any association with heart rate or HRV measurements.

There were no significant differences between the relatives of type 2 diabetes patients and the control subjects in abdominal fat distribution. It must, however, be appreciated that the sample size was small; hence, the power to detect group differences was limited. There were tendencies toward a greater amount of VAT in the relatives, which might have become significant with a larger study sample. Nonetheless, another study comparing diabetes relatives and control subjects did not demonstrate any difference in AT distribution (76), and similar findings were reported in Pima Indians compared with whites (77). Although first degree relatives of type 2 diabetes subjects have a high lifetime risk of developing type 2 diabetes, it is a heterogeneous group, and we found a large overlap between relatives and control subjects with respect to insulin sensitivity as was also previously seen (78). There was about a 10% nonsignificant reduction in M values in the first-degree relatives; again, a larger sample size might have demonstrated a significant difference.

There were only slight differences between the relatives and the controls in cortisol axis measurements, but again, the limited study sample must be taken into consideration, and, possibly, our study has failed to demonstrate some true group differences. Surprisingly, however, the relatives had a slightly but significantly attenuated adrenocortical response, i.e., cortisol release, after stimulation with ACTH and CRH but a normal response to CRH at the pituitary level, i.e., ACTH release. This finding may suggest a subtle alteration of the adrenocortical capacity to secrete cortisol in subjects with a genetic predisposition for type 2 diabetes, but the underlying mechanism is not known. ACTH is the major stimulating factor for cortisol release from the adrenal cortex. However, other humoral factors such as MSH, growth hormone, prostaglandins, interferons, and cytokines (e.g., tumor necrosis factor- α and interleukins) have been suggested as possible regulators of the adrenal cortex (79). The SNS might also promote cortisol release through direct innervation of the adrenal cortex. In prediabetic subjects, perturbations in these factors per se or in their interaction with the adrenal cortex could lead to changes in the regulation of cortisol release. However, there is no reason to believe that the demonstrated attenuation in adrenocortical response would directly contribute to insulin resistance.

Previous studies concerning the cortisol axis activity in obesity suggest changes in cortisol metabolism and an increased urinary excretion of cortisol and cortisol metabolites (47,49,80). These studies indicate tissue-specific alterations in glucocorticoid exposure, due to variability in prereceptor glucocorticoid metabolism (49,80). Cytokines could constitute a putative link between our finding of altered cortisol axis reactivity and changes in glucocorticoid metabolism. Cytokines are produced in adipocytes, mononuclear blood cells, and microglia, and they may influence cortisol secretion, glucocorticoid receptor sensitivity, and

the activity of the glucocorticoid-activating enzyme 11- β -HSD-1, and they may also interfere with insulin action through mechanisms in the central nervous system and in the insulin-sensitive tissues (81,82).

Taken together, the present results suggest a link between the amount of visceral fat and the balance of sympathetic/parasympathetic reactivity of the autonomic nervous system. Insulin resistance is strongly associated with VAT amount, and it also appears to be associated with dysregulation of the autonomic nervous system (9,27–29). However, it is not clear at present what the mechanisms are that explain these associations. Thus, it is not possible to establish any temporal or causal relationship based on this cross-sectional investigation. Furthermore, the study cohort is limited, and these results should be confirmed in larger, longitudinal studies. Nevertheless, the demonstrated association between the balance of sympathetic vs. parasympathetic nervous activity and VAT can suggest that a large amount of visceral fat may activate the sympathetic and/or inactivate the parasympathetic nervous system. One potential mechanism could be exerted through an enhanced release of free fatty acids from VAT into the portal circulation that will act on the liver and secondarily lead to sympathetic activation (66,67). A high sympathetic/parasympathetic balance, in turn, would then lead to insulin resistance (18). Conversely, it is also possible that dysregulation of the autonomic nervous activity is a primary event that promotes adipose distribution to the visceral depots, which in turn can be detrimental for insulin action through other mechanisms, e.g., local production of adipokines, cortisol, and fatty acids (68,69,83). Our main hypothesis is that visceral fat accumulation is an early phenomenon caused by environmental and genetic factors. Visceral adiposity might then (e.g., through portal delivery of NEFA or adipokines) lead to an altered reactivity in the autonomic nervous system that in turn will promote insulin resistance.

We demonstrated subtle perturbations in adrenocortical function among the subjects that were genetically predisposed to type 2 diabetes. It is since long known that hypothalamo-pituitary-adrenal axis dysregulation can contribute to insulin resistance, but our results suggest that it is not part of the circuit—or, perhaps, vicious circle—formed by the autonomic nervous system, adipose distribution, and insulin resistance. The measures of the three latter factors did not differ consistently between subjects with and subjects without a family history of type 2 diabetes and neither did the interactions between these factors. Therefore, perturbations in the interplay among the autonomic nervous system, AT, and glucose metabolism leading to insulin resistance probably are partly of nongenetic origin.

In conclusion, our data suggest that the association between visceral adiposity and insulin resistance can be partly mediated by an altered reactivity in the sympathetic and/or parasympathetic nervous system.

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References

1. **Beck-Nielsen H, Groop LC.** Metabolic and genetic characterization of prediabetic states: sequence of events leading to non-insulin-dependent diabetes mellitus. *J Clin Invest.* 1994; 94:1714–21.
2. **Bonadonna RC, Del Prato S, Bonora E, et al.** Roles of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. *Diabetes.* 1996;45:915–25.
3. **Eriksson J, Franssila-Kallunki A, Ekstrand A, et al.** Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *N Engl J Med.* 1989;321:337–43.
4. **Vestergaard H, Bjoerbaek C, Andersen PH, Bak JF, Pedersen O.** Impaired expression of glycogen synthase mRNA in skeletal muscle of NIDDM patients. *Diabetes.* 1991;40:1740–5.
5. **Zierath JR, Galuska D, Nolte LA, Thorne A, Kristensen JS, Wallberg-Henriksson H.** Effects of glycaemia on glucose transport in isolated skeletal muscle from patients with NIDDM: in vitro reversal of muscular insulin resistance. *Diabetologia.* 1994;37:270–7.
6. **Buren J, Lindmark S, Renstrom F, Eriksson JW.** In vitro reversal of hyperglycemia normalizes insulin action in fat cells from type 2 diabetes patients: is cellular insulin resistance caused by glucotoxicity in vivo? *Metabolism.* 2003;52:239–45.
7. **Eriksson JW, Smith U, Waagstein F, Wysocki M, Jansson PA.** Glucose turnover and adipose tissue lipolysis are insulin-resistant in healthy relatives of type 2 diabetes patients: is cellular insulin resistance a secondary phenomenon? *Diabetes.* 1999;48:1572–8.
8. **Jansson PA, Eliasson B, Lindmark S, Eriksson JW.** Endocrine abnormalities in healthy first-degree relatives of type 2 diabetes patients: potential role of steroid hormones and leptin in the development of insulin resistance. *Eur J Clin Invest.* 2002;32:172–8.
9. **Lindmark S, Wiklund U, Bjerle P, Eriksson JW.** Does the autonomic nervous system play a role in the development of insulin resistance? A study on heart rate variability in first-degree relatives of type 2 diabetes patients and control subjects. *Diabet Med.* 2003;20:399–405.
10. **Andrews RC, Walker BR.** Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Colch).* 1999;96: 513–23.
11. **Perley M, Kipnis DM.** Effect of glucocorticoids on plasma insulin. *N Engl J Med.* 1966;274:1237–41.

12. **Friedman TC, Mastorakos G, Newman TD, et al.** Carbohydrate and lipid metabolism in endogenous hypercortisolism: shared features with metabolic syndrome X and NIDDM. *Endocr J.* 1996;43:645–55.
13. **Reynolds RM, Walker BR, Syddall HE, et al.** Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab.* 2001;86:245–50.
14. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM.** Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia.* 1993;36:62–7.
15. **Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C.** Thinness at birth and insulin resistance in adult life. *Diabetologia.* 1994;37:150–4.
16. **Ortiz-Alonso FJ, Herman WH, Zobel DL, Perry TJ, Smith MJ, Halter JB.** Effect of epinephrine on pancreatic beta-cell and alpha-cell function in patients with NIDDM. *Diabetes.* 1991;40:1194–202.
17. **Wales JK.** Does psychological stress cause diabetes? *Diabet Med.* 1995;12:109–12.
18. **Surwit RS, Feinglos MN.** Stress and autonomic nervous system in type II diabetes: a hypothesis. *Diabetes Care.* 1988; 11:83–5.
19. **DeFronzo RA, Ferrannini E.** Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* 1991;14:173–94.
20. **Reaven GM.** Banting lecture 1988: role of insulin resistance in human disease. *Diabetes.* 1988;37:1595–607.
21. **Daly PA, Landsberg L.** Hypertension in obesity and NIDDM: role of insulin and sympathetic nervous system. *Diabetes Care.* 1991;14:240–8.
22. **Lembo G, Vecchione C, Iaccarino G, Trimarco B.** The crosstalk between insulin and the sympathetic nervous system: possible implications in the pathogenesis of essential hypertension. *Blood Press.* 1996;1(suppl):38–42.
23. **Laitinen T, Vauhkonen IK, Niskanen LK, et al.** Power spectral analysis of heart rate variability during hyperinsulinemia in nondiabetic offspring of type 2 diabetic patients: evidence for possible early autonomic dysfunction in insulin-resistant subjects. *Diabetes.* 1999;48:1295–9.
24. **Kuhn CM, Cochrane C, Feinglos MN, Surwit RS.** Exaggerated peripheral responses to catecholamines contributes to stress-induced hyperglycemia in the ob/ob mouse. *Pharmacol Biochem Behav.* 1987;26:491–5.
25. **Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW, Smythe GA.** The effects of sympathetic nervous system activation and psychological stress on glucose metabolism and blood pressure in subjects with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia.* 1992;35:835–43.
26. **Surwit RS, Schneider MS.** Role of stress in the etiology and treatment of diabetes mellitus. *Psychosom Med.* 1993;55:380–93.
27. **Hubert HB, Feinleib M, McNamara PM, Castelli WP.** Obesity as an independent risk factor for cardiovascular disease: a 26- year follow-up of participants in the Framingham Heart Study. *Circulation.* 1983;67:968–77.
28. **Lillioja S, Mott DM, Spraul M, et al.** Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med.* 1993;329:1988–92.
29. **Manson JE, Colditz GA, Stampfer MJ, et al.** A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med.* 1990;322:882–9.
30. **Vague J.** The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease: 1956. *Obes Res.* 1996; 4:204–12.
31. **Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH.** Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. *Metabolism.* 1984;33:68–75.
32. **Haffner SM, Stern MP, Hazuda HP, Pugh J, Patterson JK.** Do upper-body and centralized adiposity measure different aspects of regional body-fat distribution? Relationship to non-insulin-dependent diabetes mellitus, lipids, and lipoproteins. *Diabetes.* 1987;36:43–51.
33. **Larsson B, Seidell J, Svardsudd K, et al.** Obesity, adipose tissue distribution and health in men: the study of men born in 1913. *Appetite.* 1989;13:37–44.
34. **Ohlson LO, Larsson B, Svardsudd K, et al.** The influence of body fat distribution on the incidence of diabetes mellitus: 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes.* 1985;34:1055–8.
35. **Despres JP, Nadeau A, Tremblay A, et al.** Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. *Diabetes.* 1989;38:304–9.
36. **Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S.** Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism.* 1987;36:54–9.
37. **Gautier JF, Mourier A, de Kerviler E, et al.** Evaluation of abdominal fat distribution in noninsulin-dependent diabetes mellitus: relationship to insulin resistance. *J Clin Endocrinol Metab.* 1998;83:1306–11.
38. **Gray DS, Fujioka K, Colletti PM, et al.** Magnetic-resonance imaging used for determining fat distribution in obesity and diabetes. *Am J Clin Nutr.* 1991;54:623–7.
39. **Shuman WP, Morris LL, Leonetti DL, et al.** Abnormal body fat distribution detected by computed tomography in diabetic men. *Invest Radiol.* 1986;21:483–7.
40. **Sparrow D, Borkan GA, Gerzof SG, Wisniewski C, Silbert CK.** Relationship of fat distribution to glucose tolerance: results of computed tomography in male participants of the Normative Aging Study. *Diabetes.* 1986;35:411–5.
41. **Bergstrom RW, Newell-Morris LL, Leonetti DL, Shuman WP, Wahl PW, Fujimoto WY.** Association of elevated fasting C-peptide level and increased intra-abdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes.* 1990;39:104–11.
42. **Gabriely I, Barzilai N.** Surgical removal of visceral adipose tissue: effects on insulin action. *Curr Diab Rep.* 2003;3:201–6.
43. **Masuzaki H, Paterson J, Shinyama H, et al.** A transgenic model of visceral obesity and the metabolic syndrome. *Science.* 2001;294:2166–70.

44. **Björntorp P.** Obesity and adipose tissue distribution as risk factors for the development of disease: a review. *Infusions-therapie*. 1990;17:24–7.
45. **Jessop DS, Dallman MF, Fleming D, Lightman SL.** Resistance to glucocorticoid feedback in obesity. *J Clin Endocrinol Metab*. 2001;86:4109–14.
46. **Ljung T, Andersson B, Bengtsson BA, Björntorp P, Marin P.** Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. *Obes Res*. 1996;4:277–82.
47. **Marin P, Darin N, Amemiya T, Andersson B, Jern S, Björntorp P.** Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism*. 1992;41:882–6.
48. **Pasquali R, Cantobelli S, Casimirri F, et al.** The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. *J Clin Endocrinol Metab*. 1993;77:341–6.
49. **Rask E, Olsson T, Soderberg S, et al.** Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab*. 2001;86:1418–21.
50. **Rosmond R, Dallman MF, Björntorp P.** Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab*. 1998;83:1853–9.
51. **Lonn L, Kvist H, Ernest I, Sjöström L.** Changes in body composition and adipose tissue distribution after treatment of women with Cushing's syndrome. *Metabolism*. 1994;43:1517–22.
52. **Abate NI, Mansour YH, Tuncel M, et al.** Overweight and sympathetic overactivity in black Americans. *Hypertension*. 2001;38:379–83.
53. **Corry DB, Tuck ML.** Obesity, hypertension, and sympathetic nervous system activity. *Curr Hypertens Rep*. 1999;1:119–26.
54. **Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G.** Sympathetic nervous system and insulin resistance: from obesity to diabetes. *Am J Hypertens*. 2001;14:304S–309S.
55. **Sondergaard SB, Verdich C, Astrup A, Bratholm P, Christensen NJ.** Obese male subjects show increased resting forearm venous plasma noradrenaline concentration but decreased 24-hour sympathetic activity as evaluated by thrombocyte noradrenaline measurements. *Int J Obes Relat Metab Disord*. 1999;23:810–5.
56. **Lukaski HC, Bolonchuk WW, Hall CB, Siders WA.** Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol*. 1986;60:1327–32.
57. **Alberti KG, Zimmet PZ.** Definition, diagnosis and classification of diabetes mellitus and its complications: I. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15:539–53.
58. **Rasmuson S, Olsson T, Hagg E.** A low dose ACTH test to assess the function of the hypothalamic-pituitary-adrenal axis. *Clin Endocrinol (Oxf)*. 1996;44:151–6.
59. **Roy MS, Roy A, Gallucci WT, et al.** The ovine corticotropin-releasing hormone-stimulation test in type I diabetic patients and controls: suggestion of mild chronic hypercortisolism. *Metabolism*. 1993;42:696–700.
60. **Nasman B, Olsson T, Fagerlund M, Eriksson S, Viitanen M, Carlstrom K.** Blunted adrenocorticotropin and increased adrenal steroid response to human corticotropin-releasing hormone in Alzheimer's disease. *Biol Psychiatry*. 1996;39:311–8.
61. **Guehot J, Fiet J, Passa P, et al.** Physiological and pathological variations in saliva cortisol. *Horm Res*. 1982;16:357–64.
62. **Hagg E, Olsson T, Grankvist K.** Salivary cortisol during an overnight dexamethasone suppression test using a simple saliva collection device. *Horm Metab Res*. 1990;22:553–4.
63. **Chowdhury B, Sjöström L, Alpsten M, Kostanty J, Kvist H, Lofgren R.** A multicompartiment body composition technique based on computerized tomography. *Int J Obes Relat Metab Disord*. 1994;18:219–34.
64. **Kvist H, Chowdhury B, Grangard U, Tylene U, Sjöström L.** Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. *Am J Clin Nutr*. 1988;48:1351–61.
65. **Meikle AW, Takiguchi H, Mizutani S, Tyler FH, West CD.** Urinary cortisol excretion determined by competitive protein-binding radioassay: a test of adrenal cortical function. *J Lab Clin Med*. 1969;74:803–12.
66. **Bergman RN, Ader M.** Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2000;11:351–6.
67. **Bentham L, Keizer K, Wiegman CH, et al.** Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. *Am J Physiol Endocrinol Metab*. 2000;279:E1286–93.
68. **Björntorp P.** Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev*. 2001;2:73–86.
69. **Marniemi J, Kronholm E, Aunola S, et al.** Visceral fat and psychosocial stress in identical twins discordant for obesity. *J Intern Med*. 2002;251:35–43.
70. **Kreier F, Fliers E, Voshol PJ, et al.** Selective parasympathetic innervation of subcutaneous and intra-abdominal fat: functional implications. *J Clin Invest*. 2002;110:1243–50.
71. **Takayama S, Sakura H, Katsumori K, Wasada T, Iwamoto Y.** A possible involvement of parasympathetic neuropathy on insulin resistance in patients with type 2 diabetes. *Diabetes Care*. 2001;24:968–9.
72. **Lautt WW.** The HISS story overview: a novel hepatic neurohumoral regulation of peripheral insulin sensitivity in health and diabetes. *Can J Physiol Pharmacol*. 1999;77:553–62.
73. **Bartness TJ.** Dual innervation of white adipose tissue: some evidence for parasympathetic nervous system involvement. *J Clin Invest*. 2002;110:1235–7.
74. **Kreier F, Yilmaz A, Kalsbeek A, et al.** Hypothesis: shifting the equilibrium from activity to food leads to autonomic imbalance and the metabolic syndrome. *Diabetes*. 2003;52:2652–6.
75. **Bray GA.** Obesity, a disorder of nutrient partitioning: the MONA LISA hypothesis. *J Nutr*. 1991;121:1146–62.
76. **Johanson EH, Jansson PA, Lonn L, et al.** Fat distribution, lipid accumulation in the liver, and exercise capacity do not explain the insulin resistance in healthy males with a family history for type 2 diabetes. *J Clin Endocrinol Metab*. 2003;88:4232–8.

77. **Gautier JF, Milner MR, Elam E, Chen K, Ravussin E, Pratley RE.** Visceral adipose tissue is not increased in Pima Indians compared with equally obese Caucasians and is not related to insulin action or secretion. *Diabetologia*. 1999;42:28–34.
78. **Carvalho E, Jansson PA, Axelsen M, et al.** Low cellular IRS 1 gene and protein expression predict insulin resistance and NIDDM. *FASEB J*. 1999;13:2173–8.
79. **Vinson GP, Hinson JP, Toth IE.** The neuroendocrinology of the adrenal cortex. *J Neuroendocrinol*. 1994;6:235–46.
80. **Rask E, Walker BR, Soderberg S, et al.** Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. *J Clin Endocrinol Metab*. 2002;87:3330–6.
81. **Arzt E, Kovalovsky D, Igaz LM, et al.** Functional cross-talk among cytokines, T-cell receptor, and glucocorticoid receptor transcriptional activity and action. *Ann N Y Acad Sci*. 2000;917:672–7.
82. **Tomlinson JW, Moore J, Cooper MS, et al.** Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology*. 2001;142:1982–9.
83. **Walker BR.** Is “Cushing’s disease of the omentum” an affliction of mouse and men? *Diabetologia*. 2004;47:767–9.