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ORIGINAL ARTICLE

# Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients

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**Abstract** Elevated plasma free fatty acid (FFA), inflammatory marker, and altered adipokine concentrations have been observed in obese type 2 diabetes patients. It remains unclear whether these altered plasma concentrations are related to the diabetic state or presence of obesity. In this cross-sectional observational study, we compare basal plasma FFA, inflammatory marker, and adipokine concentrations between obese and non-obese type 2 diabetes patients and healthy, non-obese controls. A total of 20 healthy, normoglycemic males (BMI <30 kg/m<sup>2</sup>), 20 non-

obese (BMI <30 kg/m<sup>2</sup>) and 20 obese (BMI >35 kg/m<sup>2</sup>) type 2 diabetes patients were selected to participate in this study. Groups were matched for age and habitual physical activity level. Body composition, glycemic control, and exercise performance capacity were assessed. Basal blood samples were collected to determine plasma leptin, adiponectin, resistin, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP) and FFA concentrations. Plasma FFA, inflammatory marker (hsCRP, IL-6, TNF $\alpha$ ), adipokine (adiponectin, resistin, leptin), and triglyceride concentrations did not differ between non-obese diabetes patients and healthy, normoglycemic controls. Plasma FFA, IL-6, hsCRP, leptin, and triglyceride levels were significantly higher in the obese diabetes patients when compared with the healthy normoglycemic controls ( $P < 0.05$ ). Furthermore, plasma hsCRP and leptin levels were significantly higher in the obese versus non-obese diabetes patients ( $P < 0.05$ ). Significant correlations between plasma parameters and glycemic control were observed, but disappeared after adjusting for trunk adipose tissue mass. Elevated plasma leptin, hsCRP, IL-6, and FFA concentrations are associated with obesity and not necessarily with the type 2 diabetic state.

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## Introduction

Obesity, and especially visceral adipose tissue accumulation, increases the risk of developing type 2 diabetes (Jensen 2008). The greater risk of type 2 diabetes in the obese can, at least partly, be explained by changes in adipose tissue function (Bastard et al. 2006; Hajer et al. 2008;

Rasouli and Kern 2008). The classical perception of adipocytes merely as a storage site for excess lipid has changed dramatically over the last decade. This is attributed to the discovery that adipose tissue can function as an active endocrine organ, co-regulating whole-body metabolism. An increase in adipose tissue mass is generally accompanied by an increase in adipocyte size and macrophage infiltration (Otto and Lande 2005). Greater adipose tissue mass, adipocyte hypertrophy, and/or macrophage infiltration strongly modulate adipose tissue lipolysis and the secretion of numerous cytokines and adipokines (Hajer et al. 2008; Wellen and Hotamisligil 2003). In accordance, elevated plasma free fatty acid (FFA), leptin, resistin, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin-6 (IL-6) concentrations have been reported in the obese (Bastard et al. 2006; Hajer et al. 2008; Rasouli and Kern 2008). In contrast, circulating plasma adiponectin concentrations have been reported to be reduced in the obese state. Furthermore, an increase in circulating IL-6 concentration stimulates C-reactive protein (CRP) synthesis in the liver (Bastard et al. 2006). Eventually, altered plasma adipokine, inflammatory factor, and/or FFA levels can contribute to the development and/or progression of liver and skeletal muscle insulin resistance, and induce pancreatic  $\beta$ -cell apoptosis (Hajer et al. 2008).

The proposed impact of altered endocrine function of adipose tissue on the development and/or progression of type 2 diabetes has generally been studied in vivo in human cross-sectional studies. In these studies, the above-mentioned plasma parameters have been assessed and compared between type 2 diabetes patients and normoglycemic controls (Al-Daghri et al. 2003; Al-Harithy and Al-Ghamdi 2005; Bastard et al. 2002; Bullo et al. 2002; Carey et al. 2004; Haffner et al. 1996; Heilbronn et al. 2004; Kim et al. 2006; Liu et al. 1999; Miyazaki et al. 2003; Putz et al. 2004; Rieusset et al. 2004; Sivitz et al. 2003; Tatti et al. 2001; Merwe van der et al. 2001; Yu et al. 2002). In general, most of the type 2 diabetes patients included in these studies were obese. Therefore, it remains unclear whether the observed alterations in plasma FFA's, adipokines, and/or inflammatory parameters in type 2 diabetes patients are due to excess adipose tissue mass and/or directly associated with the type 2 diabetic state. To differentiate between the impact of obesity and type 2 diabetes on the altered plasma FFA, adipokine, and/or inflammatory profiles several groups have compared plasma profiles between lean and obese type 2 diabetes patients (Abdelgadir et al. 2005; Bahceci et al. 2007; Chanchay et al. 2006; Hasegawa et al. 2005; Hotta et al. 2000; Marita et al. 2005; Sayeed et al. 2003; Yang et al. 2006). Elevated plasma FFA and IL-6, but unaltered TNF $\alpha$ , levels have been reported in non-obese T2DM patients, when compared with body mass index matched normoglycemic controls (Bahceci et al. 2007; Yang et al. 2006). Whether plasma C-reactive protein,

adiponectin, leptin, and/or resistin concentrations are altered in non-obese type 2 diabetes patients when compared with healthy controls, remains unclear (Abdelgadir et al. 2005; Bahceci et al. 2007; Chanchay et al. 2006; Hasegawa et al. 2005; Hotta et al. 2000; Marita et al. 2005; Sayeed et al. 2003; Yang et al. 2006). So far, these data have only been obtained in Asian and Mid-Eastern populations. Due to apparent differences in adipose tissue distribution (Banerji et al. 1999) and the level of insulin resistance (Chandalia et al. 1999) between ethnic groups, these findings do not necessarily apply to a Caucasian population. Moreover, in none of these studies habitual physical activity was controlled between groups. Different levels of habitual physical activity might affect plasma adipokine and inflammatory marker levels (You and Nicklas 2008). Clearly, proper control of habitual activity is warranted when comparing such plasma parameters between groups.

We hypothesize that altered plasma adipokine, inflammatory factor, and/or FFA levels are related to the obese state only and are not prevalent in non-obese type 2 diabetes patients. The present study compares basal plasma FFA's, adipokines, and inflammatory markers between obese and non-obese type 2 diabetes patients versus non-obese, normoglycemic controls. This comparison increases our insight in the proposed impact of obesity in the development and/or progression of type 2 diabetes.

## Methods

### Subjects

A total of 60 Caucasian males were selected to participate in this study: 20 non-obese (BMI <30 kg/m<sup>2</sup>) and 20 obese (BMI >35 kg/m<sup>2</sup>) type 2 diabetes patients, and 20 healthy, non-obese subjects (BMI <30 kg/m<sup>2</sup>; see Table 1). Type 2 diabetes patients had been diagnosed for at least 12 months prior to investigation and were all treated with oral blood glucose lowering medication. All subjects were sedentary and had not participated in any regular exercise and/or caloric intake restriction program for at least 5 years. Subjects were informed about the nature and risks of the experimental procedures before their written informed consent was obtained. This study was approved by the local medical ethical committee of the Jessa Hospital.

### Study design

Overall, groups were matched for age and habitual physical activity. The latter was estimated by the use of the IPAQ questionnaire. Additionally, non-obese type 2 diabetes patients and normoglycemic controls were matched for body mass index. Non-obese and obese type 2 diabetes

**Table 1** Subjects' characteristics

	Healthy controls	Non-obese diabetes	Obese diabetes
Number	20	20	20
Age (years)	55 ± 1	58 ± 1	56 ± 1
Body mass index (kg/m <sup>2</sup> )	26.1 ± 0.4	26.6 ± 0.4	35.6 ± 0.6*‡
Body weight (kg)	79.9 ± 1.7	81.2 ± 1.5	108.4 ± 2.8*‡
Glycemic control			
Fasting glucose (mmol/L)	5.8 ± 0.2	9.7 ± 0.6‡	10.9 ± 0.8‡
AUC OGTT (mol min/L)	0.92 ± 0.04	1.76 ± 0.09‡	1.86 ± 0.09‡
Disease duration (years)	0 ± 0	6.2 ± 1.2‡	4.5 ± 0.9‡
Insulin (mU/L)	10.3 ± 0.9	12.1 ± 1.2	21.6 ± 2.6*‡
HOMA index	2.6 ± 0.2	5.2 ± 0.7‡	9.6 ± 0.9*‡
HbA <sub>1c</sub> (%)	5.4 ± 0.1	7.0 ± 0.2‡	8.0 ± 0.4*‡
Blood lipid profile			
Total cholesterol (mmol/L)	5.6 ± 0.2	5.0 ± 0.2	5.3 ± 0.2
HDL cholesterol (mmol/L)	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1‡
LDL cholesterol (mmol/L)	3.7 ± 0.2	3.3 ± 0.2	3.6 ± 0.1
Triglycerides (mmol/L)	1.4 ± 0.1	1.2 ± 0.2	2.2 ± 0.2*‡
Body composition			
Adipose tissue mass legs (kg)	5.2 ± 0.4	4.6 ± 0.3	9.2 ± 0.6*‡
Adipose tissue mass trunk (kg)	13.2 ± 0.7	14.4 ± 0.6	26.2 ± 1.2*‡
Adipose tissue mass total body (kg)	20.9 ± 1.1	21.8 ± 0.9	-
Fat free mass legs (kg)	18.3 ± 0.4	17.3 ± 0.4	19.4 ± 0.5
Fat free mass trunk (kg)	27.7 ± 0.6	29.0 ± 0.5	32.8 ± 0.8*‡
Exercise performance capacity			
VO <sub>2peak</sub> (L/min)	2.5 ± 0.1	2.3 ± 0.1	2.1 ± 0.1
VO <sub>2peak</sub> (mL/kg leg fat free mass/min)	135 ± 6	131 ± 7	107 ± 5*‡
W <sub>max</sub>	221 ± 10	174 ± 30‡	155 ± 37‡
Habitual physical activity (min/week)	118 ± 33	72 ± 24	80 ± 33
Smokers (n)	2	2	3

Data represent means ± SEM

Fasting glucose, insulin, HOMA index, and AUC OGTT were all determined from an OGTT performed after 3 days of discontinuation of habitual use of oral blood glucose and lipid lowering medication

HOMA homeostasis model assessment, HbA<sub>1c</sub> glycosylated hemoglobin, AUC area under the curve, OGTT oral glucose tolerance test, HDL high-density lipoprotein, LDL low-density lipoprotein, VO<sub>2peak</sub> peak whole-body oxygen uptake capacity, W<sub>max</sub> maximal workload capacity

Leg fat free and adipose tissue mass are expressed as average of the two legs

\* Significantly different between non-obese and obese T2DM patients ( $P < 0.05$ )

‡ Significantly different compared with healthy controls ( $P < 0.05$ )

patients were matched for basal fasting glucose concentrations. Fasting blood samples were collected to compare glycosylated hemoglobin (HbA<sub>1c</sub>) content, blood lipid profile, insulin, adiponectin, resistin, leptin, interleukin-6, high-sensitivity C-reactive protein, TNF $\alpha$ , and FFA concentrations between groups. Moreover, HOMA index, fat free mass and whole-body oxygen uptake and workload capacity were compared between groups. All measurements were performed at the same time during the day (between 8.00 and 12.00 AM).

#### Medication, diet and physical activity prior to testing

Three days prior to testing subjects abstained from taking oral blood glucose and/or lipid lowering medication. All subjects maintained normal physical activity and dietary patterns, and refrained from any sort of heavy physical exercise/labor for at least 3 days prior to testing. The evening prior to the test day, all subjects received the same

standardized meal ( $45 \pm 1 \text{ kJ kg}^{-1}$ , consisting of 33 energy% (En%) carbohydrate, 47 En% fat, and 20 En% protein). Daily habitual physical activity over the last 2 weeks was estimated by the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003).

#### Blood analysis

Subjects arrived at the hospital by car or public transportation and reported at the laboratory at 08.00 AM after an overnight fast. After 20 min of rest a venous blood sample was collected. Thereafter, a 2 h oral glucose tolerance test (OGTT) was performed. Plasma samples were immediately centrifuged at 1,000 g and 4°C for 5 min, after which aliquots of plasma were frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis. Blood samples were analyzed for glucose (Beckman Synchron LX 20 Analyser, Beckman Coulter Inc., USA), insulin (Advia Centaur Immunoassay System, Bayer Diagnostics Inc., USA), total cholesterol,

high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and total plasma triglycerides (Beckman Synchron LX 20 Analyser, Beckman Coulter Inc., USA). HOMA index was calculated to estimate whole-body insulin sensitivity:  $[(\text{insulin}_{(\text{mU/L})} \times \text{glucose}_{(\text{mmol/L})}) / 22.5]$ . Plasma adiponectin concentrations were assessed using a commercially available Human Adiponectin ELISA assay kit (HADP-61K, Linco Research Inc., St. Charles, MO, USA). TNF $\alpha$  concentrations were determined using a solid-phase, chemiluminescent immunometric assay (IMMULITE TNF- $\alpha$ , DPC Biemann GmbH, Bad Nauheim, Germany). Plasma high-sensitivity C-reactive protein (hsCRP) concentrations were measured by means of immunoprecipitation (Cardiophase, Dade Behring GmbH, Marburg, Germany). Resistin concentrations were assessed by enzyme-substrate reaction with a commercially available assay kit (ELISA, Phoenix Pharmaceuticals, USA). Plasma free fatty acid (FFA) levels were quantified by enzymatic method using a commercially available assay kit from Wako Chemicals GmbH, Germany. Plasma interleukin-6 (IL-6) levels were assessed by a solid-phase, enzym-labeled, chemiluminescent sequential immunometric assay (IMMULITE 1000 analyzer, EURO/DPC Ltd, UK). In addition, a small blood sample was used to determine blood HbA $_{1c}$  content (Hi-Auto A1c Analyser, Menarini Diagnostics Inc., Italy).

#### Whole-body oxygen uptake capacity

Peak whole-body oxygen uptake capacity ( $\text{VO}_{2\text{peak}}$ ) and workload capacity ( $W_{\text{max}}$ ) was assessed during an exhaustive incremental exercise test on a calibrated cycle ergometer (Ergo 1500 cycle, Ergofit GmbH, Pirmasens, Germany) using a 3-min work stage protocol (Fletcher et al. 2001). Oxygen uptake ( $\text{VO}_2$ ) measurements were performed continuously (CS 200, Schiller AG, Switzerland). The heart was monitored using a 12-lead electrocardiogram with heart rate being recorded continuously.

#### Body composition

Body mass was measured using a calibrated analogue weight scale (Tanita model TBF-300, Tanita Corp., Tokyo, Japan). Segmental and whole-body adipose tissue mass and fat free mass were determined using whole-body dual X-ray absorptiometry (Lunar DPXL, Wisconsin, USA) (Glickman et al. 2004). In healthy subjects and non-obese type 2 diabetes patients, whole-body as well as segmental (legs and trunk) adipose tissue and fat free mass were assessed. However, in the obese diabetes patients, only segmental (legs and trunk) adipose tissue and fat free mass were assessed, as the arms did not fit properly under the scanner.

#### Statistical analysis

Data are expressed as means  $\pm$  SEM. To compare three groups simultaneously, multiple one-way analysis of variance (MANOVA), with Tukey's post hoc test, were applied. In addition, relations between parameters were analyzed by Pearson correlation coefficients or by multiple regression analysis. Statistical significance was set at  $P < 0.05$ . All calculations were performed using the Statistical Package for the Social Sciences v. 15.0 (SPSS).

## Results

#### Subjects

A total of 20 Caucasian, non-obese and obese type 2 diabetes patients, and 20 non-obese normoglycemic controls were included in this study (Table 1). The diabetes patients were all using oral blood glucose lowering medication (sulfamides 25%, biguanides 70%,  $\alpha$ -glucosidase inhibitors 2%, thiazolidinediones 7%, and glinides 10%) and had been diagnosed with type 2 diabetes for  $5.4 \pm 0.7$  years. Non-obese type 2 diabetes patients and normoglycemic controls were matched for body mass index. Trunk and whole-body adipose tissue mass were similar between groups ( $P > 0.05$ ). In addition, non-obese and obese type 2 diabetes patients were matched for fasting blood glucose. In accordance, oral glucose tolerance and time since diagnosis did not differ between groups ( $P > 0.05$ ). Habitual physical activity patterns did not differ between normoglycemic controls, non-obese and obese type 2 diabetes patients ( $P > 0.05$ ).

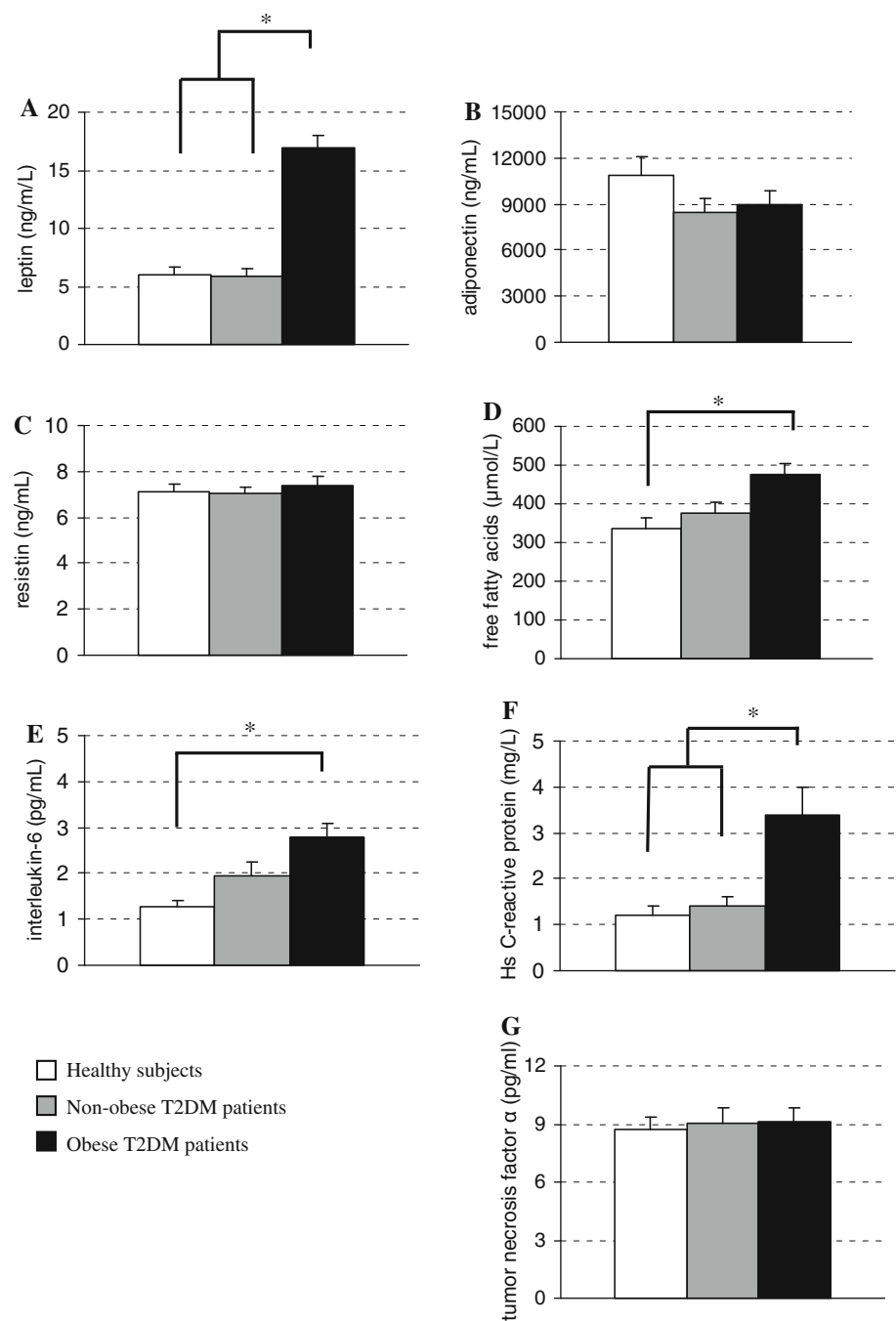
#### Glycemic control

Glycemic control was significantly impaired in non-obese and obese diabetes patients when compared with healthy, normoglycemic controls (as indicated by AUC during OGTT;  $P < 0.05$ ). In addition, blood HbA $_{1c}$  content, fasting insulin, and HOMA index were significantly elevated in the obese compared with non-obese type 2 diabetes patients (Table 1;  $P < 0.05$ ).

#### Blood lipid profile

Plasma HDL cholesterol levels were significantly lower in obese diabetes patients when compared with normoglycemic controls ( $P < 0.05$ ). Plasma triglyceride levels were significantly higher in the obese when compared with non-obese patients and normoglycemic controls ( $P < 0.05$ ).

**Fig. 1** Plasma FFA, inflammatory marker and adipokine concentrations. Data are expressed as means  $\pm$  SEM. \*Significantly different between obese T2DM patients and other subgroups ( $P < 0.05$ ). No significant differences were found between healthy subjects and non-obese T2DM patients ( $P > 0.05$ ). Plasma leptin (a), adiponectin (b), resistin (c), free fatty acid (d), interleukin-6 (e), high-sensitivity C-reactive protein (f), and tumor necrosis factor  $\alpha$  (g) concentrations



Exercise performance capacity

Whole-body oxygen uptake capacity ( $VO_{2peak}$ ) did not differ significantly between groups (Table 1). When expressed per kg leg fat free mass,  $VO_{2peak}$  was significantly lower in the obese when compared with non-obese type 2 diabetes patients and normoglycemic controls ( $P < 0.05$ ). Maximal workload capacity ( $W_{max}$ ) was significantly greater in normoglycemic controls when compared with obese and non-obese diabetes patients ( $P < 0.05$ ).

Plasma adipokines and free fatty acids

Basal plasma adipokine (adiponectin, leptin, resistin), FFA, and inflammatory marker (hsCRP, IL-6) concentrations are presented in Fig. 1. No significant differences were observed between non-obese diabetes patients and normoglycemic controls ( $P > 0.05$ ). Plasma FFA, IL-6, hsCRP, and leptin levels were significantly higher in obese diabetes patients when compared with normoglycemic controls ( $P < 0.05$ ). Furthermore, plasma hsCRP and leptin levels

**Table 2** Univariate correlations of blood parameters, adipose tissue mass, and  $VO_{2peak}$  with glycemic control

	Glycemic control			
	AUC OGTT	Fasting glucose (mmol/L)	HbA <sub>1c</sub> (%)	HOMA index
<b>Blood parameters</b>				
Free fatty acids ( $\mu$ mol/L)	0.50	0.48	0.53	0.46
Adiponectin (ng/mL)				
Leptin (ng/mL)	0.46	0.51	0.62	0.72
Resistin (ng/mL)				
High-sensitivity C-reactive protein (mg/L)	0.45	0.44	0.39	0.56
Interleukin-6 (pg/mL)	0.50	0.47	0.44	0.37
Tumor necrosis factor $\alpha$ (pg/mL)				
<b>Body composition</b>				
Trunk adipose tissue mass (kg)	0.51	0.54	0.63	0.69
Leg adipose tissue mass (kg)	0.38	0.44	0.50	0.56
<b>Exercise performance capacity</b>				
$VO_{2peak}$ (L/min)	-0.29	-0.33	-0.26	-0.31
$VO_{2peak}$ (mL/min/kg leg fat free mass)	-0.29	-0.36	-0.31	-0.48

Values provided in this table represent  $R$  values; only significant correlations are mentioned in this table ( $P < 0.05$ )

AUC area under the curve, OGTT oral glucose tolerance test, HOMA homeostasis model assessment,  $VO_{2peak}$  peak whole-body oxygen uptake capacity, HbA<sub>1c</sub> glycosylated hemoglobin

were significantly elevated in obese versus non-obese diabetes patients ( $P < 0.05$ ).

#### Correlations and multivariate regression analysis

Univariate correlations between plasma factors, adipose tissue mass,  $VO_{2peak}$ , and glycemic control are displayed in Table 2. In total subject population, plasma FFA, leptin, hsCRP, and IL-6 concentrations correlated significantly with measures of glycemic control ( $P < 0.05$ ). These correlations were no longer evident when plasma concentrations were adjusted for trunk adipose tissue mass in multivariate regression analysis ( $P > 0.05$ ).

#### Discussion

The present study compares basal plasma adipokine, inflammatory marker, and FFA concentrations between non-obese and obese type 2 diabetes patients versus non-obese normoglycemic controls. We show that basal plasma FFA, high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), leptin, and triglyceride levels are elevated in obese type 2 diabetes patients, as opposed to healthy, normoglycemic controls (Fig. 1; Table 1). On the other hand, no such differences were found between non-obese type 2 diabetes patients and healthy normoglycemic controls. Consequently, altered plasma FFA, IL-6, hsCRP, and leptin levels seem to be more related to adipose tissue mass than to the presence or absence of the type 2 diabetic state and/or the level of glycemic control. This is further supported by the observation that parameters that assess glycemic control

were no longer correlated with plasma FFA, IL-6, hsCRP and leptin levels when data were adjusted for trunk adipose tissue mass (Table 2). Therefore, our findings suggest that elevated basal plasma FFA, IL-6, hsCRP, and leptin concentrations are not necessarily related to the type 2 diabetic state.

Our findings seem to be in contrast with findings from some, but not all, studies which show that in non-obese type 2 diabetes patients, plasma FFA, IL-6, and CRP concentrations are higher, and adiponectin and leptin levels are lower, when compared with matched normoglycemic controls (Abdelgadir et al. 2005; Bahceci et al. 2007; Chanchay et al. 2006; Hasegawa et al. 2005; Hotta et al. 2000; Marita et al. 2005; Sayeed et al. 2003; Yang et al. 2006). This might be attributed to differences in the ethnic background of the volunteers in the different studies (Carroll et al. 2009; Lee and Jensen 2009; Reimann et al. 2007), which show large differences in adipose tissue mass distribution and/or the level of insulin resistance (Banerji et al. 1999; Chandalia et al. 1999). For example, Asian and Mid-Eastern populations show a relatively greater truncal adipose tissue mass when compared with BMI-matched Caucasians (Banerji et al. 1999; Chandalia et al. 1999). This might be accompanied by differences in adipokine and inflammatory marker secretion between populations of various ethnic backgrounds. Additionally, in none of these studies habitual physical activity was controlled for between groups. Different levels of habitual activity are likely to affect plasma adipokine and inflammatory marker levels, complicating the interpretation of such findings (You and Nicklas 2008).

Recent in vitro studies examined the relation between adipocyte size and adipokine/inflammatory factor release/

expression derived from Caucasian human subcutaneous adipose tissue biopsies (Jernas et al. 2006; Skurk et al. 2007). These studies indicate that leptin and IL-6 secretion were significantly greater in enlarged adipocytes. No such relation was observed for adipocyte size and TNF $\alpha$  and/or adiponectin release. Moreover, a correlation between serum amyloid A (SAA) gene expression in human adipocytes and adipocyte size has previously been reported (Jernas et al. 2006). SAA is an acute-phase protein involved in inflammation, and contributes to adipocyte-derived CRP and IL-6 release. A correlation between adipocyte-derived FFA release and adipocyte size has been well-established. These findings seem to agree with our observation that plasma hsCRP, FFA, leptin and IL-6 are no longer significantly correlated with the level of glycemic control when adjusted for trunk adipose tissue mass. Consequently, we conclude that alterations in plasma FFA, IL-6, hsCRP, and leptin levels in obese type 2 diabetes patients are attributed to the greater adipose tissue mass, and not necessarily to the presence of the type 2 diabetic state.

Data from the present study imply that systemic low-grade inflammation, a disturbed blood lipid profile, and lowered oxygen uptake capacity are more prevalent in obese, as opposed to non-obese, type 2 diabetes patients. The development of type 2 diabetes in subjects with excess adipose tissue mass is currently believed to be related to many factors. Besides genetic predisposition and ethnicity, a low skeletal muscle oxidative capacity and/or altered protein/cytokine secretion from adipocytes is linked to impairments in whole-body carbohydrate metabolism. However, we failed to observe any significant differences in whole-body oxygen uptake capacity and/or blood plasma adipokine and inflammatory markers levels in the non-obese type 2 diabetes patients when compared to the matched normoglycemic controls. Therefore, other factors are likely to play a more prominent role in the etiology of insulin resistance and type 2 diabetes in the non-obese population.

One of the more challenging features of this work was the inclusion of non-obese type 2 diabetes patients. Recent studies report that only ~10% of all Caucasian type 2 diabetes patients have a normal lean phenotype, whereas more than 60% is obese (Kramer et al. 2009). Nonetheless, the comparison of plasma adipokine and inflammatory marker concentrations in non-obese and obese type 2 diabetes patients generates the possibility to differentiate between the impact of type 2 diabetes and obesity.

This study has some important clinical implications. The presented data confirm that obese type 2 diabetes patients are clearly at a high risk of developing cardiovascular disease (based on body composition, blood lipid profile and inflammation parameters, oxygen uptake capacity). Based on these parameters, the non-obese type 2 diabetes patients seem to be at a lower risk of developing cardiovascular

complications. Since strong correlations have been reported between low-grade inflammation, disturbed blood lipid profile, reduced whole-body oxidative capacity, and the risk of developing cardiovascular complications, obese type 2 diabetes patients should be stimulated to adhere to effective exercise, nutritional, and pharmaceutical intervention to reduce excess adipose tissue mass and improve glycemic control. Interestingly, our data show that the proposed link between plasma adipokines, inflammation parameters, FFA and type 2 diabetes are more related to the level of adiposity, as opposed to insulin sensitivity and/or glycemic control. Further study remains warranted to elucidate the proposed role of these plasma parameters in the etiology of obesity and/or type 2 diabetes.

In conclusion, elevated plasma FFA, IL-6, hsCRP, leptin, and triglyceride concentrations are observed in obese, as opposed to non-obese, type 2 diabetes patients. Elevated plasma leptin, hsCRP, IL-6, and FFA concentrations are attributed to the prevalence of obesity and not necessarily associated with the type 2 diabetic state.

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**Conflict of interest statement** None.

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