

Insulin resistant phenotype is associated with high serum leptin levels in offspring of patients with non-insulin-dependent diabetes mellitus

Ilkka Vauhkonen, Leo Niskanen, Steven Haffner¹, Sakari Kainulainen², Matti Uusitupa³ and Markku Laakso

Departments of Medicine, ²Clinical Radiology and ³Clinical Nutrition, Kuopio University Hospital and University of Kuopio, Kuopio, Finland and ¹Department of Medicine, The University of Texas, San Antonio, Texas, USA

(Correspondence should be addressed to L Niskanen, Department of Medicine, University of Kuopio, PO Box 1777, FIN-70211 Kuopio, Finland)

Abstract

Objective: To investigate whether there are differences in serum leptin levels between the offspring of non-insulin-dependent diabetes mellitus (NIDDM) patients representing different phenotypes of NIDDM, and furthermore to investigate the role of different fat tissue (subcutaneous fat area (SCFAT) and intra-abdominal fat area (IAFAT)) and insulin sensitivity on serum leptin levels.

Design: Twenty non-diabetic offspring of NIDDM patients with insulin secretion deficient phenotype (IS-group), 18 non-diabetic offspring of NIDDM patients with insulin resistant phenotype (IR-group) and 14 healthy control subjects without a family history of diabetes were studied.

Methods: Serum leptin levels were measured by RIA. SCFAT and IAFAT were measured by computed tomography, the total fat mass (TFM) by bioelectrical impedance and the whole body glucose uptake (WBGU) by the euglycemic hyperinsulinemic clamp technique.

Results: Subjects of the control group ($P = 0.003$) and the IS-group ($P < 0.001$) had lower serum leptin levels than subjects of the IR-group even after adjustment for gender ($P < 0.001$), TFM ($P = 0.009$), fasting plasma insulin ($P = 0.003$) and for IAFAT ($P < 0.001$). The differences weakened after adjustments for SCFAT ($P = 0.028$) or WBGU ($P = 0.040$) and disappeared after adjustment for both SCFAT and WBGU ($P = 0.058$). In the stepwise multiple regression analyses SCFAT, WBGU and gender explained 58% of the variation of serum leptin levels whereas IAFAT failed to be a significant determinant of serum leptin levels.

Conclusions: The higher serum leptin levels in the IR-group was markedly, but not solely, explained by lower rates of WBGU and higher SCFAT. SCFAT was shown to be a more important determinant of serum leptin levels than IAFAT among these study groups.

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Introduction

Leptin, the *obese (ob)* gene product, has been shown to be expressed in adipocytes in humans and animals (1–6). It has been proposed to act as a satiety factor between adipose tissue and the central nervous system (7–9). Both human and animal studies have indicated a strong positive relationship between the amount of adipose tissue and serum leptin levels (7). There is, however, a wide variation in serum leptin levels among individuals with similar body mass index (BMI) or fat mass (10–12) indicating that there are also other modifiers of serum leptin levels. Long-term exogenous hyperinsulinemia leads to increase in *ob* gene expression and plasma leptin concentrations in both humans and animals (13–17). Furthermore, insulin resistance is associated with elevated serum leptin levels (18–20). Both subcutaneous and intra-abdominal adipose tissues express leptin mRNA (2, 5, 6, 21), but their relative importance for leptin secretion has remained controversial. Furthermore,

serum leptin levels have been found to be higher in women than in men (21–23) and this could be explained partly by divergent overall adiposity and/or regional body fat distribution.

In the present study, we investigated whether there are differences in serum leptin in well-characterized non-diabetic offspring of non-insulin-dependent diabetes mellitus (NIDDM) patients with both insulin secretion deficient phenotype (IS-group) and insulin resistant phenotype (IR-group) and in control subjects without a family history of diabetes. Furthermore, we studied the association of insulin sensitivity, fat distribution and body fat mass with serum leptin levels.

Methods

Subjects

The subjects for the present study were offspring of the patients with newly diagnosed NIDDM who were

originally studied in 1979–1981. The formation and representativeness of the study population have been described earlier in detail (24–27). We have followed these patients for over 10 years and performed repeated oral glucose tolerance tests (baseline, 5 years, 10 years). The index patients were subdivided into two groups on the basis of fasting C-peptide value at the 10-year follow-up study: (i) NIDDM patients with low fasting C-peptide level (<450 pmol/l) reflecting deficient insulin secretion capacity; and (ii) NIDDM patients with high fasting C-peptide level (>880 pmol/l) reflecting insulin resistance. Proband with glutamic acid decarboxylase and/or islet cell antibody positivity (11 patients altogether) were excluded. Additional exclusion criteria for the selection of the offspring were: (i) diabetes mellitus in both parents or in the offspring; (ii) dyslipidemia (serum total triglycerides >2.5 mmol/l); (iii) drug treatment or any disease that could potentially disturb carbohydrate metabolism; (iv) pregnancy; (v) overt psychiatric disease; and (vi) age under 30 or over 55 years.

Offspring of the probands with low fasting C-peptide (<450 pmol/l) (IS-group) The IS-group consisted of 20 subjects (15 women and 5 men) who were offspring of 11 probands (28). One to three subjects from each family were included. The mean age of the probands was 68.4 years, their mean BMI was 28.1 kg/m² and their mean fasting C-peptide was 350 pmol/l at the 10-year follow-up.

Offspring of the probands with high fasting C-peptide (>880 pmol/l) (IR-group) The IR-group consisted of 18 subjects (11 women and 7 men) who were offspring of nine probands (28). One to three offspring from each family were included. The mean age of the probands was 65.7 years, their mean BMI was 32.0 kg/m² and their mean fasting plasma C-peptide was 980 pmol/l.

Control group The control subjects had to fulfill the following inclusion criteria: (i) age from 30 to 55 years; (ii) no diabetes; (iii) first degree relatives without a history of diabetes; (iv) no drug treatment or any disease that could potentially disturb carbohydrate metabolism; (v) BMI within the range of mean \pm 2 s.d. of the BMI in the IS- and IR-groups; and (vi) no history of hypertension. Altogether 17 subjects met the inclusion criteria. Three of the 17 subjects had dyslipidemia in the examination and were therefore excluded (28). Thus, the control group consisted of 14 offspring (5 men and 9 women) of eight probands. One to three subjects from each family were examined. The mean age of the probands was 64.7 years, their mean BMI was 26.0 kg/m² and their mean fasting plasma C-peptide was 480 pmol/l.

Study protocol

The subjects were admitted to the metabolic ward of the Department of Medicine of the Kuopio University Hospital for 2 days. On the first day, after 12 h, the bioelectric impedance measurement was performed followed by an oral glucose tolerance test. On the second day the hyperinsulinemic euglycemic clamp test was performed. Within 1 month after these examinations a computed tomography (CT) of the abdominal fat was performed.

The protocol was approved by the Ethics Committee of the Kuopio University Hospital and the University of Kuopio. Informed consent was given by all the subjects studied.

Body composition and fat distribution

Body composition was determined by bioelectrical impedance (RJL Systems, Detroit, MI, USA) in the supine position after 12 h fast (29).

Abdominal fat distribution was evaluated by CT (Somatom Plus S, Siemens, Erlangen, Germany) according to the method of Sjöström *et al.* (30). Briefly, the scanning was performed with 120 kV and the slice thickness was 10 mm. The subjects were examined in the supine position with their arms stretched above their heads. The fourth lumbar vertebra (L4) was mapped with a radiograph of the vertebral spine and one scan from that level was obtained. Total and visceral fat areas were calculated by delineating the area by graph pen and then computing the adipose tissue surfaces with an attenuation range of –30 to –190 HU (30–31). Visceral fat area was calculated by drawing a line within the muscle wall delineating the abdominal cavity. Subcutaneous fat area (SCFAT) was measured by subtracting the amount of visceral fat from the total fat area. The radiologist (SK) evaluated the amount of visceral and abdominal fat without knowledge of the group to which the subjects belonged.

Euglycemic clamp

The degree of insulin resistance was evaluated with the euglycemic hyperinsulinemic clamp technique (32). A priming dose of insulin infusion (Actrapid 100 IU/ml, Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to acutely raise plasma insulin to the desired level, where it was maintained by continuous insulin infusion at a rate of 80 mU/m² body surface area per min. Blood glucose was clamped at 5.0 mmol/l for the next 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals (mean coefficient of variation of blood glucose was <4% in both study groups and control group). The data were calculated for each 20-min interval; the mean value for the period from 120 to 180 min was used to

calculate the rates of whole body glucose uptake (WBGU).

Assays and calculations

Blood glucose in the fasting state and during clamp were measured by the glucose oxidase method (Glucose & Lactate Analyzer 2300 Stat Plus, Yellow Springs Instrument Co. Inc., Yellow Springs, OH, USA). For the determination of plasma insulin, blood was collected into EDTA tubes. After centrifugation, the plasma for the determination of insulin was stored at -20°C until analysis. Plasma insulin was determined by RIA (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden). Leptin was measured by a commercial RIA (Linco Research Inc., St Louis, MO, USA) (33).

Statistical analysis

All calculations were performed with the SPSS for Windows program (SPSS Inc., Chicago, IL, USA). Data are shown as means \pm s.e.m. The differences among the three groups were tested by one-way ANOVA. Only in cases of $P < 0.05$ were two groups compared. The differences between the two groups were analyzed by the Student's *t*-test for unpaired samples or by the χ^2 -test when appropriate. Pearson and adjusted (partial) correlation coefficients were calculated for selected variables. Determinants of serum leptin levels were evaluated by multiple linear regression analysis.

Results

Clinical and biochemical characteristics of the study subjects

Table 1 shows the clinical and biochemical characteristics of the study subjects. The groups were comparable with respect to age and gender. The IR-group had higher BMI and total fat mass (TFM) than the control group ($P = 0.008$ and $P = 0.032$ respectively) or the IS-group ($P < 0.001$ and $P = 0.031$ respectively). Fasting blood glucose did not differ among the study groups. The IR-group had higher fasting plasma insulin than the control group ($P = 0.015$) or the IS-group ($P = 0.006$). The IR-group had lower rates of WBGU than the control group ($P < 0.001$) and the IS-group ($P < 0.001$). Furthermore, the IR-group had higher SCFAT and intra-abdominal fat area (IAFAT) than the control group ($P = 0.020$ and $P = 0.035$ respectively) or the IS-group ($P = 0.004$ and $P = 0.058$ respectively).

The IR-group had higher fasting serum leptin levels than the control group ($P < 0.001$) or the IS-group ($P < 0.001$). The differences between the serum leptin levels persisted after adjustments for gender ($F = 17.1$; $P < 0.001$), TFM ($F = 5.2$; $P = 0.009$), fasting blood glucose ($F = 9.1$; $P < 0.001$), fasting plasma insulin ($F = 5.9$; $P = 0.003$), glucose tolerance ($F = 7.9$;

$P < 0.001$) and for IAFAT ($F = 9.4$; $P < 0.001$). However, the differences weakened after adjustments for SCFAT ($F = 3.9$; $P = 0.028$), WBGU ($F = 3.4$; $P = 0.040$) and for both (SCFAT = WBGU) ($F = 3.0$; $P = 0.058$).

Correlations between body composition, abdominal fat distribution, insulin sensitivity and serum leptin levels

Control group TFM was correlated with serum leptin levels after adjustment for gender ($r = 0.97$, $P < 0.001$). A highly significant correlation between SCFAT and serum leptin levels was observed after adjustment for gender ($r = 0.83$, $P < 0.001$), but it vanished when adjusted for both gender and TFM ($r = 0.14$, $P = \text{NS}$). Furthermore, there was a correlation between IAFAT and serum leptin levels after adjustment for gender ($r = 0.67$, $P = 0.011$), but it vanished after adjustment for both gender and TFM ($r = 0.28$, $P = \text{NS}$). WBGU correlated inversely with serum leptin levels after adjustment for gender ($r = -0.77$, $P = 0.002$), but vanished when adjusted for both gender and TFM ($r = 0.08$, $P = \text{NS}$).

IS-group Interestingly, there was no correlation between TFM and serum leptin levels even after adjustment for gender ($r = 0.11$, $P = \text{NS}$). A significant correlation between SCFAT and serum leptin levels was found after adjustment for gender ($r = 0.52$, $P = 0.022$) and further, for both gender and TFM ($r = 0.54$, $P = 0.022$). There was a trend of correlation between IAFAT and serum leptin levels after adjustment for gender ($r = 0.42$, $P = 0.07$) or for both gender and TFM ($r = 0.41$, $P = 0.09$). WBGU correlated inversely with serum leptin levels after adjustment for gender ($r = -0.53$, $P = 0.019$) and for both gender and TFM ($r = -0.52$, $P = 0.025$).

IR-group Interestingly, no significant correlations between TFM, SCFAT, IAFAT or WBGU and serum leptin levels were found even after adjustment for gender ($r = 0.11$, $r = 0.13$, $r = 0.03$ and $r = -0.17$ respectively; $P = \text{NS}$ for all correlations) or other adjustments.

Pooled population TFM correlated with serum leptin levels after adjustment for gender ($P < 0.001$) (Fig. 1). SCFAT was correlated with serum leptin after adjustment for gender or for both gender and TFM ($P < 0.001$ and $P = 0.006$) (Fig. 1). IAFAT correlated with serum leptin levels after adjustment for gender ($P = 0.002$), but not after adjustment for gender and TFM ($P = \text{NS}$). WBGU was correlated with serum leptin levels after adjustment for gender and for both gender and TFM ($P < 0.001$ and $P < 0.001$ respectively) (Fig. 1).

Multiple linear regression analyses

In the stepwise multiple linear regression analyses including all subjects, gender and WBGU were associated with serum leptin levels ($r^2 = 0.54$, $P < 0.001$) when

Table 1 Clinical and biochemical characteristics (means \pm S.E.M.) of the study subjects.

	Controls (n = 14)	IS-group (n = 20)	IR-group (n = 18)	P value (ANOVA)
Age (years)	40.1 \pm 1.5	41.3 \pm 1.4	40.5 \pm 0.9	NS
Sex (M/F)	5/9	5/15	7/11	NS
Body mass index (kg/m ²)	25.0 \pm 1.0	24.6 \pm 0.5	28.8 \pm 0.99 ^{*,†††}	<0.001
Total fat mass (kg)	19.0 \pm 2.6	20.5 \pm 1.2	26.4 \pm 2.1 ^{*,†}	<0.05
Fasting blood glucose (mmol/l)	4.5 \pm 0.1	4.5 \pm 0.1	4.7 \pm 0.1	NS
Fasting insulin (pmol/l)	48.6 \pm 4.8	49.2 \pm 3.6	72.6 \pm 7.2 ^{*,††}	<0.01
Serum leptin (ng/l)	13.0 \pm 2.4	13.4 \pm 1.6	23.2 \pm 2.1 ^{***,†††}	<0.001
Men	5.7 \pm 2.1	7.9 \pm 1.8	14.9 \pm 2.3	–
Women	17.0 \pm 2.7	15.3 \pm 1.8	28.8 \pm 1.8	–
Subcutaneous fat area (cm ²)	214 \pm 37	215 \pm 15	303 \pm 25 ^{*,††}	<0.01
Men	161 \pm 65	216 \pm 22	303 \pm 41	–
Women	231 \pm 41	214 \pm 19	303 \pm 31	–
Intra-abdominal fat area (cm ²)	74 \pm 12	84 \pm 12	120 \pm 16 [*]	<0.05
Men	77 \pm 30	137 \pm 33	183 \pm 24	–
Women	72 \pm 13	64 \pm 6	80 \pm 9	–
Whole body glucose uptake (μ mol/kg per min)	62.2 \pm 4.4	56.1 \pm 2.2	41.6 \pm 3.3 ^{***,†††}	<0.001

* $P < 0.05$ vs controls. ** $P < 0.01$ vs controls. *** $P < 0.001$ vs controls. † $P < 0.05$ vs IS-group. †† $P < 0.01$ vs IS-group. ††† $P < 0.001$ vs IS-group.

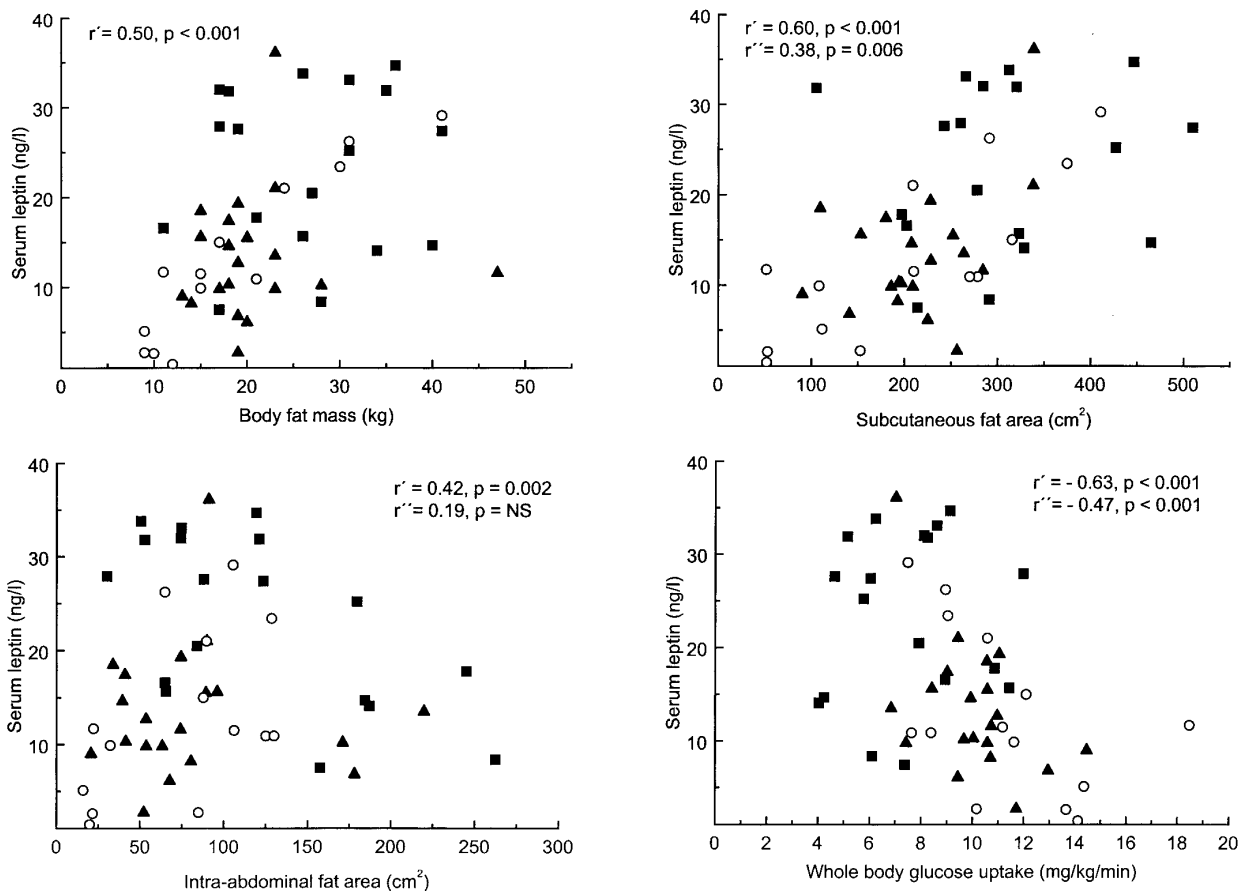


Figure 1 Correlations between serum leptin and body fat mass, SCFAT, IAFAT and WBGU. ○, control group; ▲, IS-group; ■, IR-group. r' = correlation coefficient adjusted for gender; r'' = correlation coefficient adjusted for gender and TFM.

Table 2 Factors associated with serum leptin levels in stepwise multiple analyses.

	RC ^c	Beta	P value
Model 1 ^a			
Whole body glucose uptake ($\mu\text{mol/kg per min}$)	-1.8 ± 0.3	-0.55	<0.001
Gender	11.4 ± 2.0	0.57	<0.001
R ²	0.538	-	-
Model 2 ^b			
Subcutaneous fat area (cm^2)	0.02 ± 0.01	0.28	0.03
Gender	11.4 ± 2.0	0.57	<0.001
Whole body glucose uptake ($\mu\text{mol/kg per min}$)	-1.8 ± 0.3	-0.55	<0.001
R ²	0.580	-	-

^a Factors included in the model: age, gender, TFM, WBGU, fasting plasma insulin, fasting blood glucose, glucose tolerance (NGT/IGT), study group (control/IS/IR) and the interaction term of TFM and study group.

^b Factors included in the model: age, gender, SCFAT, IAFAT, WBGU, fasting plasma insulin, fasting blood glucose, glucose tolerance (NGT/IGT), study group (control/IS/IR) and the interaction term of TFM study group.

^c Values are the regression coefficients (RC) \pm S.E.M. for the linear model of parameters.

other variables in the model were age, TFM, fasting plasma insulin, fasting blood glucose, glucose tolerance (normal glucose tolerance/impaired glucose tolerance), the study group (control/IS/IR) and interaction term of TFM and the study group (Model 1; Table 2). When SCFAT and IAFAT were included in the model instead of TFM, SCFAT, gender and WBGU explained 58% of the variation of serum leptin levels ($P < 0.001$) (Model 2; Table 2). When men and women were analyzed separately using Model 1, only SCFAT explained the variation of serum leptin levels in men ($r^2 = 0.47$, $P = 0.002$) whereas in women SCFAT and WBGU explained 48% of the variation of serum leptin levels ($P < 0.001$).

Regression analyses were also performed in each of the study groups. In the control group, by using Model 1, TFM and gender explained 96% of the variation of the serum leptin levels ($P < 0.001$). Furthermore, using Model 2 SCFAT and gender explained 81% of the variation of serum leptin levels ($P < 0.001$). In the IS-group, only gender and WBGU explained 43% of serum leptin levels ($P = 0.026$) in both models used. In the IR-group, by using the same models, only gender had a significant contribution, explaining 59% of the variation of serum leptin levels.

Discussion

The novel finding of this study was that the non-diabetic offspring of NIDDM patients with insulin resistant phenotype had higher serum leptin levels than the control subjects and the offspring of NIDDM patients with insulin secretion deficient phenotype. This was mainly due to a higher degree of insulin resistance and greater amount of subcutaneous fat tissue in the offspring of NIDDM patients with insulin resistant phenotype. Moreover, subcutaneous fat tissue was shown to be a more important determinant of serum leptin levels than intra-abdominal fat tissue among these study subjects.

Insulin resistance, i.e. decreased rates of WBGU, has been associated with elevated serum leptin levels (18–19) and it is thought to be at least partly a consequence of the trophic effect of compensatory hyperinsulinemia on the fat tissue (13). Nyholm *et al.* (18) reported increased serum leptin levels in non-diabetic offspring of NIDDM patients and it was explained by a higher degree of insulin resistance in these subjects compared with control subjects. However, in that study the offspring of NIDDM patients were not classified according to the phenotype of NIDDM. In the present study insulin resistance was the strongest independent factor explaining high serum leptin levels in the offspring of NIDDM patients. Interestingly, however, the insulin resistant offspring showed no correlation between WBGU and serum leptin levels, whereas the two other groups did. This could indicate that the offspring of NIDDM patients with the insulin resistant phenotype have some factors which lead to an additional increase of serum leptin levels and interfere with the relationship between serum leptin levels and insulin sensitivity. It could be that these subjects are resistant to the biological function of leptin (19, 22), which may be related to leptin's action to inhibit hypothalamic neuropeptide Y expression (34–36). Alternatively, effects of other hormones, e.g. corticosteroids (37), differences in leptin binding to transport proteins in serum and/or leptin clearance (12, 38) could account for increased serum leptin levels and blurred association of insulin sensitivity and serum leptin levels in the offspring of NIDDM patients with insulin resistant phenotype.

Previous studies have shown that both subcutaneous and intra-abdominal adipose tissues express leptin mRNA (2, 5, 6, 21), but their relative importance for leptin secretion in humans has remained controversial (5, 6, 39–43). The study of Montague *et al.* (21) suggested that leptin mRNA was expressed predominantly in subcutaneous fat tissue in humans. This is in line with our present study showing that subcutaneous

fat tissue together with gender and WBGU explained almost 60% of the variation in serum leptin levels, whereas intra-abdominal fat tissue failed to have a significant contribution to serum leptin levels in multiple linear regression analyses. The intra-abdominal fat tissue, however, weakly associated with serum leptin levels in non-diabetic control subjects without a family history of diabetes, suggesting that in non-diabetic healthy subjects both subcutaneous and intra-abdominal fat contribute to serum leptin levels. In addition, a trend for association of intra-abdominal fat tissue and serum leptin levels was found in the offspring of NIDDM patients with insulin secretion deficient phenotype in whom the fat distribution and insulin sensitivity were similar to those of the control subjects. Interestingly, we did not observe any correlation between subcutaneous fat tissue and serum leptin levels in the offspring of NIDDM patients with insulin resistant phenotype. In fact, those subjects even showed an inverse correlation with intra-abdominal fat tissue and serum leptin levels. However, men of the insulin resistant group were more intra-abdominally obese than were the women, which could explain the inverse correlation between intra-abdominal fat and serum leptin levels.

In the present study the subjects consisted of two groups of offspring from well-characterized patients with both insulin secretion deficient and insulin resistant phenotypes of NIDDM. The classification of the phenotype of the diabetic patients was based on the long follow-up during which the patients were both clinically and metabolically well evaluated. On the other hand, the offspring of control subjects in the present study were healthy offspring of non-diabetic controls. Previous studies have shown strong associations of serum leptin levels with BMI and especially with TFM, although at a given fat mass wide inter-individual variation occurs (10–12). In line with previous studies the body fat mass emerged as a strong determinant of serum leptin levels in the control group. In both groups of offspring of diabetic patients, however, body fat mass was not associated with serum leptin levels, and this could at least partly be explained by large inter-individual variation in serum leptin levels and by a small sample size in subgroup analyses. However, these results may suggest that in the prediabetic stage still unidentified factors may interfere with the relationship of body fat mass with serum leptin levels. Furthermore, we emphasize that because this study consisted of three highly selected groups these results cannot directly be compared with population-based studies. However, when the study groups were pooled in statistical analyses, TFM, SCFAT, insulin sensitivity and to a lesser extent also IAFAT showed associations with serum leptin levels consistent with the view that these factors are determinants of serum leptin levels.

In conclusion, the present study shows that the offspring of NIDDM patients with the insulin resistant phenotype have higher serum leptin levels than the

control subjects without a family history of diabetes and the offspring of NIDDM patients with the insulin secretion deficient phenotype. This was mostly, but not solely, explained by a higher degree of insulin resistance and greater amount of subcutaneous fat tissue among the offspring of the insulin resistant phenotype patients. Therefore, some other factors, e.g. concomitant leptin resistance, are involved in the increase of serum leptin levels in these subjects. The present study also suggests that subcutaneous fat tissue is a more important determinant of serum leptin levels than is intra-abdominal fat tissue.

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