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Relative hypoleptinemia in patients with type 1 and type 2 diabetes mellitus

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OBJECTIVE: To determine the relation between plasma leptin concentrations and metabolic control in human diabetes mellitus.
DESIGN AND SUBJECTS: Cross sectional study consisting of 156 patients with diabetes mellitus type 1 ($n=42$), type 2 ($n=114$), and non-diabetic subjects ($n=74$).
RESULTS: Plasma leptin concentrations were lower ($P<0.05$) in type 1 (8.3 ± 1.7 ng/ml) and type 2 diabetic (14.9 ± 1.8 ng/ml) than in non-diabetic humans (18.3 ± 1.9 ng/ml). Only female type 1 and type 2 diabetic subjects also had decreased leptin/BMI ratios ($P<0.05$ *vs* non-diabetic females). The log rank test identified age-adjusted correlation of plasma leptin concentration with sex ($P<0.0004$) and body mass index ($P<0.0218$), but not with glycosylated haemoglobin A_{1c} ($P>0.5$) in all groups. Plasma leptin was correlated with age ($P<0.0058$) and serum triglycerides ($P<0.0199$) in type 1 diabetic patients, and with serum cholesterol ($P<0.0059$) and LDL ($P<0.0013$) in type 2 diabetic patients.
CONCLUSIONS: Defective leptin production and/or secretion might be present independently of metabolic control in female patients with type 1 or type 2 diabetes mellitus.
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Keywords: leptin; obesity; diabetes mellitus; cholesterol; triglycerides; human

Introduction

Lack of leptin in *ob/ob* mice results in obesity followed by hyperinsulinaemia and hyperglycaemia which can be reversed by leptin treatment.[1] On the other hand, *db/db* mice display elevated leptin concentrations due to defective leptin receptor function and do not respond to leptin treatment.[2] Human obesity is not likely related to either relative hypoleptinaemia or leptin resistance,[3] although recently mutations of the *ob* gene have been detected in extreme obesity.[4,5] Nevertheless, plasma leptin concentrations are closely correlated with body mass index (BMI) and several indicators of body fat content in humans.[6,7]

Since obesity is frequently associated with insulin resistance eventually resulting in type 2 diabetes mellitus,[8] it is of interest whether or not plasma leptin concentration is also related to the diabetic metabolic state. Evidence has been provided that leptin is involved in the regulation of peripheral glucose metabolism at the level of the pancreatic β-cell,[9,10] liver,[11] and fat cells.[12] Of note, insulin has been shown to stimulate *ob* gene expression and leptin secretion in cultured human adipocytes.[13] Hyperinsulinaemia characteristic for insulin resistant states could be therefore associated with hyperleptinaemia. Such a relationship has been demonstrated in lean insulin resistant men[14] and hyperinsulinaemic hypothyroidism,[15] but not in subjects with impaired glucose tolerance,[16] overt type 2 diabetes mellitus[17] or polycystic ovary syndrome.[18,19] Moreover, conflicting results have been reported as to the effect of hyperinsulinaemia on leptin expression and circulating plasma leptin levels in humans.[20–22] Thus, the interrelation between leptin and insulin/insulin resistance is still unclear and no data are available at present on plasma leptin concentrations in type 1 diabetic patients.

The aim of this cross sectional study was therefore to compare plasma leptin concentrations of patients with type 1 as well as type 2 diabetes mellitus under daily conditions with that of non-diabetic subjects in order to delineate a potential role of leptin deficiency or resistance to leptin action for human diabetes mellitus.

Methods

Subjects

We examined 235 subjects who consecutively visited the outpatient clinics for diabetes mellitus and endocrine and metabolic diseases. Complete data sets were

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obtained in 230 patients who were further divided into three groups based on the patients' history and clinical examination: (i) patients with insulin dependent diabetes mellitus (type 1 diabetes; $n=42$); (ii) patients with non-insulin dependent diabetes mellitus (type 2 diabetes; $n=114$); and (iii) non-diabetic subjects (control; $n=74$). Obesity was defined by a body mass index (BMI) of $\geq 27.3\,kg/m^2$ for male and a BMI $\geq 27.8\,kg/m^2$ for female subjects.[6] Type 1 diabetic patients (age of manifestation: 21 ± 2 y) were on an intensified insulin regimen with multiple daily insulin injections allowing near normoglycaemic control. Type 2 diabetic patients were on therapy with oral hypoglycaemic agents (sulphonylurea and/or metformin) or conventional insulin treatment with two daily injections. Control subjects were taking no medication on a regular basis and displayed no evidence of impaired glucose tolerance or any other endocrine dysfunction that may contribute to leptin dysregulation.[15] Subjects were weight stable for at least 2 months without recent changes in diet and lifestyle. Blood was drawn after overnight fasting for ~ 12 h in type 2 diabetic and nondiabetic subjects. Type 1 diabetic subjects were examined at least 2 h after insulin injection and meal ingestion which should not affect plasma leptin concentration.[15,23] This was confirmed in five type 1 diabetic subjects body mass index (BMI), $24.3\pm0.2\,kg/m^2$) presenting with no difference in plasma leptin concentrations between the 12 h fasted state (10.4 ± 3.3 ng/ml) and 2 h after insulin administration and meal ingestion (9.4 ± 3.6 ng/ml). The study was performed according to the Declaration of Helsinki.

Assays

Plasma leptin concentrations were measured by a double-antibody radioimmunoassay for human leptin (Linco, St Charles, MO, USA) with intra-assay and interassay coefficients of variance of 4.1% and 5.5%, respectively.[15] Glycosylated haemoglobin A_{1c} (HbA_{1c}, normal $\leq 5.8\%$) was determined following HPLC-separation (C-R4A Chromatopac, Shimadzu, Kyoto, Japan) by using cation exchange columns and elution with increasing sodium gradients with intraassay and interassay coefficients of 3%. Measurements of serum cholesterol (normal ≤ 200 mg/dl), HDL (≥ 45 mg/dl), LDL (≤ 150 mg/dl) and triglycerides (≤ 172 mg/dl) were performed by automated enzymatic assays (CHOD-Pap and GPO-Pap, Hitachi, Japan) in the routine laboratory.

Calculations and statistics

All data are given as means $\pm$ s.e. The Wilcoxon rank sum test was used to analyse differences of plasma leptin concentrations and other metabolic parameters according to sex, BMI (grouped into obese and non-obese subjects) and diagnosis. Analysis of variance was used for comparison of data. Multiple regression analyses between leptin and BMI or metabolic parameters were also performed after adjustment for age. In addition, the rate of increase in plasma leptin with BMI was assessed from leptin/BMI ratios[24] and the results presented as a Box–Whiskers plot and differences between groups compared with Tukey's studentized range test. For all statistical analyses the SAS software package version 6.12 (SAS Institute, Cary, NC) was used. *P*-values less than 0.05 were considered statistically significant.

Results

Age and BMI of all groups and subgroups are presented in Table 1. Patients with type 2 diabetes mellitus were older ($P<0.0001$) compared with type 1 diabetic patients and non-diabetic subjects serving as control group. Type 1 diabetic patients presented with lower ($P<0.0001$) mean BMI compared to type 2 diabetic patients as well as to control subjects.

According to their HbA_{1c} all diabetic subgroups presented with good metabolic control (Table 2). Type 1 diabetic patients who were on an intensified insulin

Table 1 Patients' characteristics

		Age	*Body mass index*	
	n	*Means± s.e. (y)*	*Means± s.e. (kg/m²)*	*Range (kg/m²)*
Type 1 diabetes				
Total	42	37.2±2.2	24.8±0.5**	(19.2–38.2)
Female	23	38.3±3.1	24.6±0.8	(19.2–38.2)
Male	19	35.8±3.2	25.1±0.6	(20.6–30.6)
Type 2 diabetes				
Total	114	60.9±1.3*	29.0±0.5	(17.2–46.5)
Female	64	62.9±2.1	28.9±0.6	(17.2–46.5)
Male	50	59.1±1.6	29.2±0.7	(20.6–42.6)
Control				
Total	74	43.3±1.7	28.4±0.8	(17.6–53.5)
Female	54	44.4±2.7	36.1±1.4	(17.6–53.5)
Male	20	41.4±1.9	23.6±0.3	(21.0–43.8)

*$P<0.0001$ *vs* type 1 diabetes and *vs* control.
**$P<0.0001$ *vs* type 2 diabetes and *vs* control.

Table 2 Parameters of glucose and lipid metabolism of the subjects described in Table 1. Glycosylated haemoglobin A_{1c} (HbA_{1c}) serum concentrations of cholesterol (S-Chol), high density lipoproteins (S-HDL), low density lipoproteins (S-LDL), and triglycerides (S-TG) are given as means ± s.e.

	HbA1c (%)	*S-Chol (mg/dl)*	*S-HDL (mg/dl)*	*S-LDL (mg/dl)*	*S-TG (mg/dl)*
Type 1 diabetes					
Total	7.5 ± 0.2	199.8 ± 7.1**	62.3 ± 2.7†	106.6 ± 5.6‡	144.8 ± 13.9§
Female	7.4 ± 0.2	206.1 ± 9.8	63.9 ± 3.4	112.2 ± 6.2	132.5 ± 19.9
Male	7.6 ± 0.3	192.3 ± 10.5	60.3 ± 4.3	100.0 ± 9.5	159.6 ± 19.1
Type 2 diabetes					
Total	8.0 ± 0.2	228.7 ± 3.9	49.2 ± 1.4	137.7 ± 3.6	228.1 ± 13.2
Female	8.0 ± 0.2	233.7 ± 5.0	54.8 ± 2.2	140.2 ± 5.8	201.1 ± 14.7
Male	8.1 ± 0.2	223.7 ± 5.5	43.6 ± 1.8	135.1 ± 5.3	255.1 ± 36.9
Control					
Total	5.3 ± 0.05*	211.7 ± 5.7	51.9 ± 1.9	128.4 ± 4.5	160.8 ± 21.0
Female	5.3 ± 0.06	215.3 ± 5.9	54.7 ± 2.5	132.9 ± 4.9	152.6 ± 24.4
Male	5.3 ± 0.08	211.3 ± 9.9	44.9 ± 2.6	129.3 ± 9.2	206.0 ± 44.3

*$P < 0.0001$ *vs* type 1 and type 2 diabetes.
**$P < 0.05$ *vs* type 2 diabetes and control.
†$P < 0.05$ *vs* type 2 diabetes and control.
‡$P < 0.05$ *vs* type 2 diabetes and control.
§$P < 0.0001$ *vs* type 2 diabetes and control.

regimen had gradually, but not significantly ($P = 0.09$) lower HbA_{1c} levels than type 2 diabetic patients (Table 2). They also displayed lower, total serum cholesterol levels along with higher HDL and lower LDL compared with the other groups (Table 2). Serum triglycerides were lower ($P < 0.0001$) in type 1 diabetic patients compared with type 2 diabetic as well as control subjects.

Despite no difference in mean BMI (all females, $27.3 \pm 0.5\,kg/m^2$; all males, $27.5 \pm 0.3\,kg/m^2$), mean plasma leptin concentrations of women were almost doubled compared with that of men (all females, 18.4 ± 0.9 ng/ml; all males, 9.2 ± 0.5 ng/ml). That difference in plasma leptin concentrations holds true within the groups of type 1, type 2 and non-diabetic subjects (Table 3). Regardless of sex, there were also differences between the diabetic and non-diabetic subjects: type 1 diabetic patients exhibit lower mean plasma leptin concentrations compared with type 2 diabetic patients and healthy controls. Obese type 2 diabetic and non-diabetic subjects had higher mean plasma leptin concentrations than the respective lean subgroups (Table 3).

Linear regression analysis of age-corrected leptin concentrations *vs* BMI revealed lower slopes of the fitted line for type 1 (slope = 0.7) and type 2 diabetic patients (slope = 1.2) compared with non-diabetic controls (slope = 2.1) (Figure 1A). Age-corrected regression analyses within all female subgroups (Figure 1B) showed a steeper increase in plasma leptin with rising BMI as compared with male subgroups (Figure 1C). The correlation in female type 1 diabetic patients failed to reach statistical significance ($P = 0.0748$), which is most likely due to the impact of age in this subgroup ($P = 0.0395$).

In order to adjust for BMI, leptin/BMI ratios are presented for all subgroups in Figure 2. Leptin/BMI ratios in female subgroups were higher than in corresponding male subgroups ($P < 0.0001$). Non-diabetic females had higher leptin/ratios than type 1and type 2 diabetic patients, while leptin/ratios between non-diabetic and diabetic males were not different.

The log rank test revealed a strong association of plasma leptin with sex ($P < 0.0005$) and BMI (diabetes mellitus type 1, $P = 0.0218$; other groups, $P < 0.0005$) in all three diagnosis groups, whereas correlation between plasma leptin and age ($P = 0.0058$) as well as serum triglycerides ($P = 0.0199$) could be only detected in patients with diabetes mellitus 1. In patients with type 2 diabetes mellitus plasma leptin was correlated with total serum cholesterol ($P = 0.0059$) as well as LDL ($P = 0.0013$), while its association with serum triglycerides ($P = 0.1976$) did not reach statistical significance.

Table 3 Plasma leptin concentrations (means ± s.e.; in ng/ml) in subgroups of the subjects described in Table 1

	Total	*Female*	*Male*	*Lean*	*Obese*
Type 1 diabetes	8.3 ± 1.7*	12.4 ± 1.8	4.2 ± 0.5**	8.0 ± 1.3	13.6 ± 3.4
Type 2 diabetes	14.9 ± 1.8	18.8 ± 1.3	11.7 ± 1.3†	10.1 ± 1.0	19.0 ± 1.3§
Control	18.3 ± 1.9	24.7 ± 3.3‡	11.8 ± 1.9†	11.6 ± 1.2	35.2 ± 4.6§

*$P < 0.05$ type 1 *vs* type 2 diabetes *vs* control.
**$P < 0.0003$ *vs* respective female subgroup.
†$P < 0.0001$ *vs* respective female subgroup.
‡$P < 0.05$ type 1 *vs* type 2 diabetes *vs* control.
§$P < 0.0001$ *vs* respcetive lean subgroup.

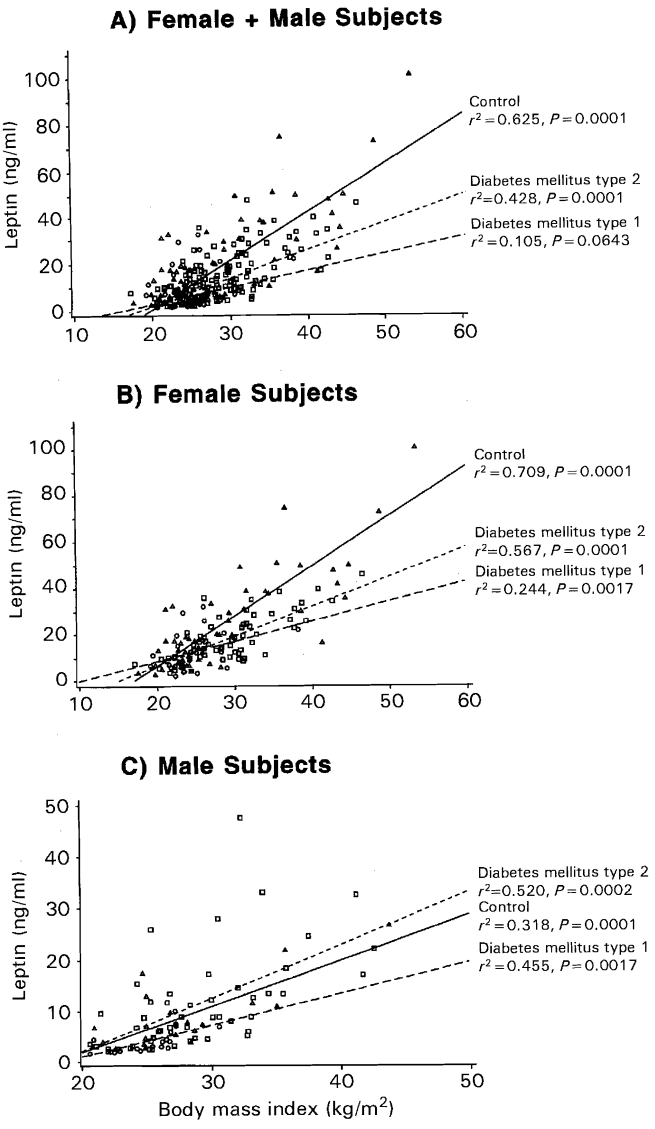

Figure 1 Age-adjusted correlation between plasma leptin concentration and body mass index **(A)** in patients (females and males combined) with type 1 ($n=42$, ○), type 2 diabetes mellitus ($n=114$, □), and non-diabetic subjects ($n=72$, △); **(B)** female patients with type 1 ($n=23$, ○) and type 2 diabetes mellitus ($n=64$, □), and non-diabetic subjects ($n=54$, △), and **(C)** in male patients with type 1 ($n=19$, ○), and type 2 diabetes mellitus ($n=50$, □), and non-diabetic subjects ($n=20$, △).

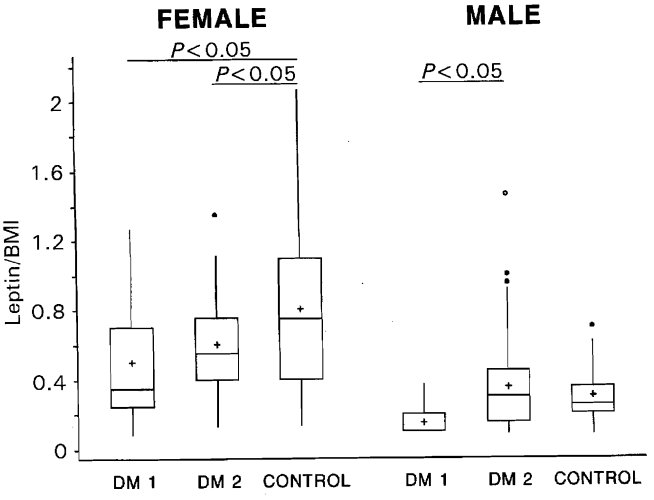

Figure 2 Ratios of plasma leptin (ng/ml) to body mass index (BMI, kg/m^2) in type 1 diabetic (23f/19m), type 2 diabetic (64f/50m), and non-diabetic subjects (54f/20m) grouped according to sex. Data were compared using Tukey's studentized range test and are presented as Box–Whiskers plots.

However, there was no association ($P>0.5$) between plasma leptin and HbA_{1c} in any of the tested subgroups.

Discussion

In addition to the known correlation of plasma leptin with BMI and sex, the present study found that both type 1 and type 2 diabetic patients in good metabolic control display lower basal plasma leptin concentrations than non-diabetic humans. Plasma leptin concentrations were also correlated with serum triglycerides in type 1 diabetes mellitus and with serum cholesterol and LDL levels, in type 2 diabetes mellitus.

The strong relation between body mass index and plasma leptin previously reported for non-diabetic[6] and insulin resistant[16] or type 2-diabetic subjects[7,25] holds true in part for patients with type 1 diabetes mellitus. After adjustment for age, this correlation became weaker and did not obtain statistical significance ($P=0.0748$) in female type 1 diabetic patients, while it remained strong in male type 1 diabetic patients ($P=0.0017$). Female type 1 diabetic patients presented with ~3-fold higher plasma leptin concentrations than the respective male groups confirming the findings in female non-diabetic and type 2 diabetic patients.[7,17] Despite a similarly high correlation between plasma leptin and sex, plasma leptin concentrations were only ~1.6-fold increased in the female type 2 diabetic patients and ~2-fold increased in the control group. Despite the good correlation of leptin with either BMI or other indices of body fat mass,[6,7,15] it cannot be excluded that the observed sex difference might be a result of increased subcutaneous fat mass in females. Recently, evidence was provided that plasma leptin correlates with subcutaneous femoral fat, but not with intraabdominal fat mass.[26] Using plasma leptin/BMI ratios the increases in circulating leptin concentrations relative to BMI were still ~38% and ~26% lower in female type 1 and type 2 diabetic patients than in non-diabetic females, respectively.

We also found that plasma leptin correlates with age in type 1, but not type 2 diabetic or non-diabetic subjects. This was primarily the result of a weak albeit significant ($P=0.0395$) association between age and leptin in the female type 1 diabetic subgroup. Negative correlation of age with plasma leptin has been reported in healthy subjects.[22,27] The lack of such

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association in our type 2 diabetic patients could be explained by the small number of young patients in this group, although the control group comprising of a broad range of age also did not exhibit a significant relation between age and plasma leptin. Nevertheless, it has been shown recently that the relationship between circulating leptin and relative fat mass can be disrupted in elderly subjects,[28] which supports our findings in diabetic patients.

The main finding of the present study was that female type 1 and type 2 diabetic patients displayed lower basal plasma leptin concentrations than non-diabetic subjects. In particular, this was accounted for by a $\sim$61% and a $\sim$46% decrease of plasma leptin concentrations in the obese type 1 and type 2 diabetic subgroups, respectively. In the lean subgroups, only type 1 diabetic patients presented with $\sim$31% lower plasma leptin concentrations than the respective control group. These results could be seen in contrast to the elevation of basal plasma leptin concentrations reported for lean insulin-resistant men.[14] Nevertheless, it is conceivable that plasma leptin concentrations could parallel the initial increase and late decrease in plasma insulin concentrations characteristic for the pathogenesis of diabetes mellitus type 2.[29] Of note, plasma leptin concentrations are significantly correlated with fasting insulin levels in healthy subjects[30] and increased in insulin-treated type 2 diabetic patients[25] as well as hyperinsulinaemic hypothyroidism.[15] Nevertheless, other authors found no interaction between insulin secretion and plasma leptin.[7,16,30]

A moderate decrease of plasma leptin concentration has been demonstrated in poorly controlled type 2 diabetic subjects[23] suggesting a potential role of glycaemia for leptin secretion. Similarly, plasma leptin was significantly reduced in the diabetic subgroup of a study in morbidly obese subjects who were also in poor metabolic control.[31] The present study found clearly lower plasma leptin concentrations in both type 2 and type 1 diabetic patients who were in relatively good metabolic control ($\sim$7.4% and $\sim$8.5%) which argues against a correlation between diabetic metabolic control and plasma leptin.

The effect of insulin on plasma leptin levels has been discussed controversially. *In vitro*, insulin increases leptin secretion by adipocytes[13] and leptin *per se* may modulate insulin secretion.[9] *In vivo*, an oral glucose tolerance test[15] or euglycaemic-hyperinsulinaemia[20] do not affect circulating plasma leptin concentrations, whereas long-term hyperinsulinemia[32] or 72 h hyperglycemia may increase plasma leptin.[33] Thus, the long-term loss of the physiological pulsatility of insulin secretion[34] could be responsible for the decreased plasma leptin levels in diabetes mellitus observed in the present study. Even more there is evidence that hyperinsulinemia with concomitant hypoglycaemia, that usually occurs in well controlled diabetic patients, leads to a reduction of plasma leptin levels.[35] While no defects of the *ob* gene could be detected in human type 2 diabetes mellitus[36] or insulin resistant women with polycystic ovary syndrome,[18] central or humoral changes may negatively affect leptin action and secretion.[37] In amenorrheic women athletes hypoleptinaemia and absence of the normal diurnal leptin cycle have been observed, indicating a link between leptin secretion and chronic nutritional status.[19] Alternatively, the decrease in total plasma leptin concentrations could be also accounted for by a reduction of synthesis or secretion of leptin binding proteins in plasma secretion.[37] Finally, since the calculated apparent K_m of $\sim$10 ng/ml for renal leptin uptake is close to physiological plasma leptin levels, small alterations of plasma leptin concentrations may result in major changes of leptin elimination.[38] Thus, increased renal leptin elimination by glomerular filtration and/or tubular metabolism might contribute to the observed decrease of plasma leptin concentrations in our diabetic patients who did not suffer from renal insufficiency.

The correlation of triglycerides, total serum cholesterol, and LDL with plasma leptin indicate an interaction of leptin with lipid metabolism, although other studies could not find such association.[7,23,26] Only Rainwater *et al*[39] reported a correlation of specific HDL phenotype (HDL-TG) and large HDL-apo A-I with leptin, suggesting a possible aggregation of atherogenic phenotypes such as diabetes, obesity and dyslipoproteinemia. By employing a hyperleptinaemic rat model, leptin has been recently shown to decrease triglyceride concentrations in liver, skeletal muscle and pancreatic β-cells by elevation of mitochondrial oxidation of free fatty acids.[10] Low leptin concentrations could be therefore lead to triglyceride accumulation in pancreatic islets which contributes to β-cell dysfunction[40] as well as to elevation of plasma concentrations of free fatty acids which in turn induce insulin resistance in skeletal muscle.[41] Although short-term changes in plasma FFA induced by lipid infusion and Acipimox did not affect plasma leptin concentrations,[42] it cannot be ruled out that long-term alterations of lipolysis could modulate leptin secretion.

In conclusion, the present results suggest that a defect in leptin production and/or secretion might be present independently of metabolic control in female patients with diabetes mellitus type 1 and type 2 and may be linked to diabetes-related lipid disorders.

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