Effects of Body Weight Reduction on Plasma Leptin and Adiponectin/Leptin Ratio in Obese Patients With Type 1 Diabetes Mellitus

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Summary

The aim of this study was to explore the changes in the adipokines leptin and adiponectin in obese patients with type 1 diabetes mellitus (T1DM) who underwent seven days of fasting and 21 days of low-calorie diet (LCD). The plasma leptin and adiponectin concentrations were measured in 14 obese patients with T1DM at baseline, immediately after 7 days of fasting, and after 21 days of LCD. 13 non-obese patients with T1DM were studied only after an overnight fasting. Bioimpedance technique was used for determination of body composition. Obese T1DM patients lost 6.0 kg (6.0; 6.8) (median, 25 %; 75 %) and decreased their fat tissue after fasting and LCD. Plasma leptin in obese T1DM was significantly higher than in non-obese T1DM patients: 9.10 (5.06; 25.89) vs. 1.71 (1.12; 7.08) $\mu g \cdot l^{-1}$ and transiently decreased immediately after fasting: 3.45 $\mu g \cdot l^{-1}$ (1.47; 7.00), ($P<0.05$). Adiponectin/leptin ratio in obese T1DM was significantly lower than in non-obese T1DM patients: 0.67 (0.57; 1.49) vs. 3.50 (2.46; 6.30) $\cdot 10^3$ and transiently increased immediately after fasting: 2.22 (1.26; 3.24) $\cdot 10^3$, ($P<0.05$). We conclude that obese patients with T1DM are characterized by hyperleptinemia that is reduced by prolonged fasting, but only slightly affected by low calorie diet.

Key words

Type 1 diabetes mellitus • Obesity • Fasting • Leptin • Adiponectin

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Introduction

There is evidence that the prevalence of obesity in T1DM patients is growing (Libman *et al.* 2003). Although insulin deficiency is the main pathogenic factor in T1DM, obese subjects with T1DM can also be insulin resistant (Pambianco *et al.* 2007), and the pathogenesis of their micro- and macrovascular complications is now attributed partly to insulin resistance (IR) (Kilpatrick *et al.* 2007, Thorn *et al.* 2005, Orchard *et al.* 2003) as well as classical factors, such as metabolic control, body mass index, smoking, dyslipidemia, and hypertension. The pathophysiological link between obesity and IR remains to be elucidated, but several recent studies indicate a central role for adipose tissue and its endocrine dysfunction (Mora and Pressin 2002, Aldhahi and Hamdy 2003, Ferroni *et al.* 2004). Obesity is typically associated with a more proinflammatory endocrine profile of adipose tissue that contributes to the development of its metabolic complications, including IR and type 2 diabetes mellitus (Mráz *et al.* 2011). It is thus conceivable

that obesity in patients with type 1 diabetes mellitus could also contribute to the development of subclinical inflammation and IR.

The adipokines leptin and adiponectin have been most extensively studied in patients with obesity and type 2 diabetes mellitus. Plasma leptin concentration increases in proportion to body fat mass, and regulates food intake and energy expenditure to maintain body fat stores (Campfield *et al*. 1995). Leptin is secreted into the bloodstream and reaches the brain through the blood-brain barrier and blood-cerebrospinal fluid barrier. Leptin acts on the hypothalamus, where it induces anorexia (Elmquist *et al*. 1999, Housa *et al.* 2006, Huml *et al*. 2011). Hyperleptinemia, which either manifests gradually in association with age-related obesity or is generated rapidly by the consumption of energy-enriched diets, is associated with decreased brain leptin concentrations (Kalra 2011). Increased blood levels of leptin fail to reduce feeding or to be reflected in increased levels of leptin in the cerebrospinal fluid. If leptin is delivered directly into the brain, it reduces feeding, but delivery into the blood is essentially without effect (Kastin and Pan 2006). The mechanism of leptin resistance has been elucidated. The first cause of leptin resistance is impaired leptin transport across the brain-blood barrier. The second cause of leptin resistance is impaired leptin signal transduction in hypothalamic neurons (Burguera *et al*. 2000).

In healthy leptin-sensitive individuals, leptin inhibits insulin biosynthesis and secretion from pancreatic β-cells (Amitani *et al.* 2013). By contrast, insulin stimulates leptin secretion from adipose tissue (Tsai *et al.* 2012). Leptin stimulates hepatic gluconeogenesis and hepatic insulin sensitivity *via* the hepatic branch of the vagus nerve. Additionally, leptin increases glucose uptake in skeletal muscle, heart, and brown adipose tissue *via* the sympathetic nervous system. In leptin-resistant overweight individuals, insufficiency of leptin signaling in the hypothalamus (induced in obese subjects by hyperleptinemia) causes hyperglycemia and hyperinsulinemia (German *et al*. 2009).

Adiponectin is another insulin-sensitizing hormone produced by adipocytes, which has also anti-inflammatory and anti-atherogenic properties. In obese subjects, plasma adiponectin levels are reduced. It has been suggested that adiponectin levels in plasma are inversely correlated with visceral adiposity (Matsuzawa *et al*. 2004, Nedvídková *et al.* 2005) and IR (Weyer *et al*. 2001). Mutations in the gene encoding for adiponectin are associated with type 2 diabetes mellitus and features of metabolic syndrome (MS) including hypertension, dyslipidemia, and atherosclerosis (Kondo *et al*. 2002). It has been reported that the adiponectin/leptin (A/L) ratio was more effective as a parameter of IR than was adiponectin or leptin alone, and was a more sensitive and reliable marker of IR than was homeostasis model assessment (HOMA)-IR in subjects without hyperglycemia, as well as in type 2 diabetes patients (Inoue *et al*. 2005, 2006). The A/L ratio is low in subjects with MS, and gradually decreases according to the number of MS components, suggesting A/L ratio as a predictive marker for MS (Jung *et al*. 2010).

We hypothesized that increased leptin and low A/L ratio would be associated with IR in obese, Caucasian T1DM patients. A reduction of body weight in obese T1DM patients might be a crucial step in reducing their IR, but this is associated with a risk of hypoglycemia (Kemmer 1992). In this pilot study we aimed to characterize the clinical phenotype in obese patients with type 1 diabetes during body weight reduction with respect to changes of leptin and adiponectin concentrations and their relationship to metabolic and anthropometric parameters.

Material and Methods

Subjects

The details of the study groups and study design have been recently reported (Musil *et al*. 2013).

Briefly, fourteen obese, C-peptide-negative patients with T1DM were included in the study: 9 men and 5 females, aged 41.0 years (23.0; 47.0), body mass index (BMI) 33.2 kg · m^{-2} (32.33; 33.63). The median glycated hemoglobin concentration was 7.4 % (7.1; 8.1). For controls, we recruited 13 non-obese, C-peptide-negative patients with T1DM: 8 men and 5 females, aged 36.0 years (22.5; 47.0), BMI 22.50 kg · m^{-2} (20.78; 24.13). The median glycated hemoglobin concentration was 8.9 % (6.8; 10.0). There was no significant difference between the two groups of patients in age, gender ratio, duration of diabetes, or glycated hemoglobin.

Study design

Briefly, the patients were admitted to the diabetes ward and one day prior to the beginning of fasting, we performed a hyperinsulinemic-euglycemic clamp. After the clamp, patients fasted for 7 days. On the eighth day of the testing period, we repeated the

hyperinsulinemic-euglycemic clamp, after which patients were placed on a standardized, low-calorie, diabetic diet containing 150 g of carbohydrates and a total of 5 000 kJ. Twenty-one days after the fasting period, patients were admitted to the diabetes ward again, and the third hyperinsulinemic-euglycemic clamp was performed. In the group of non-obese T1DM patients, only one hyperinsulinemic-euglycemic clamp was performed without any further intervention. In both groups of patients waist circumference was measured halfway between the ribs and iliac crest.

Body composition monitor

Bioimpedance spectroscopy (Fresenius Medical Care, Bad Homburg, Germany) was used to determine body composition. Total body fat mass (kg), relative fat mass (%), lean tissue mass (kg), relative lean tissue mass (%), and fat tissue index – adipose tissue mass · height^{-2} (kg · m^{-2}) – were measured.

Hyperinsulinemic-euglycemic clamp

All studies were performed after an 8 to 10-h overnight fast. The two-step hyperinsulinemic-euglycemic clamp, lasting 6 h (period 1: 0 to 120 min; period 2: 120 to 360 min) was conducted as previously described (DeFronzo *et al*. 1979). Insulin action was estimated as glucose disposal (M) calculated between 320 and 360 min. IR was calculated as IR=1/M.

Analytical methods

Free fatty acids were analyzed with a FFA-HR kit (Wako chemicals GmbH, Neuss, Germany) using a UV-VIS spectrophotometer (Shimadzu Pharma Spec 1700 UV Probe, Kyoto, Japan). Plasma leptin and adiponectin were analyzed with a Bio-Rad Luminex system (Millepore, Billerica, Massachusetts, United States). We analyzed plasma leptin concentration at 360 min of hyperinsulinemic-euglycemic clamp to evaluate the effect of hyperinsulinemia on plasma leptin concentration.

Statistical methods

The numerical data were tested according to distribution. Mann-Whitney test was used to assess the differences between obese T1DM patients vs. non-obese T1DM patients, and Wilcoxon test for the evaluation of differences between the individual phases of observation of obese T1DM patients. For correlation analysis, Spearman's correlation coefficient and the associated p-value were calculated. Statistical significance was determined based on a probability level of less than 0.05. The statistical evaluation was performed using SigmaStat (Systat Software, Chicago IL, USA).

All data are expressed as median (lower quartile, upper quartile).

Results

Clinical and biochemical characteristics of the obese T1DM and non-obese T1DM patients are summarized in Table 1. All obese T1DM patients tolerated the period of weight reduction: they lost 6.0 kg (6.0; 6.8) of body weight (median, 25 %; 75 %) and 7.0 cm (4.4; 10.0) in waist circumference after fasting and maintained this reduction in body weight and waist circumference after 21 days on the low-calorie diet. There was a significant decrease in body fat immediately after fasting and after 21 days on the low calorie diet in obese T1DM patients ($p<0.05$), as measured by bioimpedance spectroscopy. Insulin resistance as estimated by 1/M during hyperinsulinemic-euglycemic clamp was significantly increased in obese T1DM patients compared to non-obese T1DM subjects ($p<0.05$) (Musil *et al*. 2013). Plasma leptin concentration in obese T1DM was significantly higher than in non-obese T1DM patients and transiently decreased immediately after fasting ($p<0.05$). Plasma leptin concentration during hyperinsulinemic-euglycemic clamp significantly increased in obese T1DM ($p<0.001$) and in non-obese T1DM ($p<0.05$). A/L ratio in obese T1DM was significantly lower than in non-obese T1DM patients and transiently increased immediately after fasting ($p<0.05$).

Significant positive Spearman's correlation was found in obese T1DM subjects before fasting and in non-obese T1DM between leptin and body fat, relative body fat, and fat tissue index ($p<0.05$). Significant negative Spearman's correlation was found in obese T1DM subjects before fasting and in non-obese T1DM between A/L ratio and body weight, BMI, body fat, relative body fat, and fat tissue index ($p<0.05$). Significant negative Spearman's correlation was found in obese T1DM subjects before fasting and in non-obese T1DM between adiponectin and IR (1/M) ($p<0.05$) (Table 2).

Discussion

This study provides a comparative analysis of the clinical phenotype of type 1 diabetic subjects with

Table 1. Characteristics of the obese T1DM and non-obese T1DM patients.

Obese T1DM (n=14)	**Before fasting**	**After fasting**	**21 days after low calorie diet**	**Non-obese T1DM (n=13)**
Body mass index	33.2	31.4	31.4	22.50
(kg · m^{-2})	(32.33; 33.63) #	(29.9; 31.6) **	(29.0; 31.2) **	(20.78; 24.13)
Body weight (kg)	98.5	92.5	91.5	71.0
	(93.8; 101.1) #	(88.5; 95.1) **	(86.0; 95.8) **	(61.5; 76.5)
Waist circumference (cm)	108.0	100.0	101.0	80.0
	(103.3; 110.5) #	(90.0; 102.0) **	(93.5; 103.0) **	(73.8; 88.3)
Body fat (kg)	36.0	32.2	32.5	13.6
	(33.3; 41.7) #	(30.3; 35.6) *	(29.1; 36.1) *	(9.0; 18.0)
Relative body fat (%)	36.1	35.3	34.4	20.9
	(33.3; 39.4) #	(31.6; 39.7)	(31.8; 38.1)	(12.7; 27.7)
Fat tissue index (kg · m^{-2})	16.1	13.8	14.4	5.9
	(14.7; 17.7) #	(13.0; 16.7) *	(13.1; 16.2) *	(3.70; 9.30)
Glucose disposal (M)	9.50	6.68	9.65	12.1
(mg min^{-1} kg^{-1})	(9.04; 10.3) #	(6.35; 7.69) **	(8.63; 10.1)	(10.7; 13.1)
1/Glucose disposal (M)	0.11	0.15	0.10	0.08
(mg^{-1} · min · kg)	(0.10; 0.11) #	(0.13; 0.16) **	(0.10; 0.11)	(0.08; 0.09)
FFA (mmol · l^{-1})	0.46	0.99	0.4	0.27
	(0.34; 0.58) #	(0.78; 1.36) *	(0.36; 0.68)	(0.17; 0.61)
Leptin (ug · l^{-1})	9.10	3.45	9.55	1.71
	(5.06; 25.89) #	(1.47; 7.00) *	(6.06; 16.86)	(1.12; 7.08)
Leptin (μg · l^{-1}) in 360 min	22.7	12.4	20.6	4.15
of clamp procedure	(15.3; 39.2) [a]	(3.91; 16.4) [a]	(13.3; 27.9) [a]	(2.20; 13.0) [b]
Adiponectin	7.98	6.49	7.65	11.45
(mg · l^{-1})	(5.82; 11.36)	(5.25; 8.51)	(5.66; 9.76)	(6.85; 15.45)
Ratio adiponectin/leptin	0.67	2.22	0.73	3.50
· 10^3	(0.57; 1.49) #	(1.26; 3.24) *	(0.49; 0.83)	(2.46; 6.30)

** p<0.001, before fasting vs. the indicated time in obese T1DM subjects (Wilcoxon test). * p<0.05, before fasting vs. the indicated time in obese T1DM subjects (Wilcoxon test). # p<0.05 between obese T1DM subjects before fasting and non-obese T1DM (Mann-Whitney test). [a] p<0.001, leptin before clamp procedure vs. leptin at the indicated time of clamp procedure (Wilcoxon test). [b] p<0.05, leptin before clamp procedure vs. leptin at the indicated time of clamp procedure (Wilcoxon test). Data in Table 1 are expressed as median (lower quartile, upper quartile).

regard to obesity, leptin and adiponectin concentrations, and insulin resistance. Our study confirms that type 1 diabetes in obese subjects shares many characteristics with previous data in insulin-resistant obese individuals with type 2 diabetes or metabolic syndrome, but retains differences fundamental to their respective pathophysiologies. In spite of having a different type of diabetes, obese T1DM patients with increased BMI, body fat and waist circumference share features of IR with patients with type 2 diabetes. The obese patients with T1DM in our study were characterized by hyperleptinemia. Plasma leptin was identified as having a positive association with adiposity and IR (Mente *et al*. 2010). The T1DM patients in our study showed significant positive correlation between leptin and BMI and adiposity. During the study, leptin concentrations transiently fell after seven days of fasting. In previous studies in obese T2DM, the reduction of fat mass occurring during weight loss resulted in decreased leptin concentrations (Monzillo *et al*. 2003, Pasarica *et al.* 2009, Mráz *et al.* 2011, Urbanavičius *et al.* 2013, Palikhe *et al*. 2014). After weight loss, individuals maintaining a reduced weight have reduced energy expenditure (Rosenbaum *et al*. 2008, Tremblay and Chaput 2009), decreased sympathetic nervous system tone, lower circulating concentrations of leptin, thyroxine and

Table 2. Spearman's correlation analysis in obese T1DM subjects before fasting and non-obese T1DM.

	Correlation coefficient	P value
Leptin ($\mu g \cdot l^{-1}$) and Fat tissue index ($kg \cdot m^{-2}$)	0.52	0.007
Leptin ($\mu g \cdot l^{-1}$) and Body fat (kg)	0.51	0.008
Leptin ($\mu g \cdot l^{-1}$) and Relative body fat (%)	0.48	0.013
Adiponectin ($mg \cdot l^{-1}$) and 1/M ($mg^{-1} \cdot min \cdot kg$)	−0.40	0.044
Ratio adiponectin/leptin $\cdot 10^3$ and Body mass index ($kg \cdot m^{-2}$)	−0.39	0.049
Ratio adiponectin/leptin and Body weight (kg)	−0.46	0.020
Ratio adiponectin/leptin $\cdot 10^3$ and Fat tissue index ($kg \cdot m^{-2}$)	−0.61	0.001
Ratio adiponectin/leptin and Body fat (kg)	−0.62	<0.001
Ratio adiponectin/leptin and Relative body fat (%)	−0.56	0.002

triiodothyronine, and increased parasympathetic nervous system tone. Thus, metabolic, autonomic, and neuroendocrine systems act in conjunction to favor weight regain after otherwise successful weight loss (Rosenbaum *et al*. 2005, Lips *et al.* 2013). In previous clinical studies, most of these metabolic, autonomic, and neuroendocrine changes favoring weight gain *via* decreased energy expenditure were reversed by the administration of leptin to weight-reduced individuals (Farooqi *et al*. 1999, Rosenbaum *et al*. 2005, Galgani *et al.* 2010). In our study, plasma leptin after 21 days of low calorie diet remained high despite maintained reduced body weight in obese T1DM patients. The explanation for this difference may be the fact that the fasting itself and not the adipose mass reduction is the primary regulator of leptin levels in T1DM. This regulatory mechanism was described in a previous study with normal weight and obese volunteers (Boden *et al.* 1996, Mantzoros *et al.* 2011). The previous studies did not include T1DM patients treated by insulin. Insulin stimulates leptin secretion from adipose tissue (Tsai *et al.* 2012). By this mechanism, insulin treatment in obese T1DM patients during the LCD period could also contribute to hyperleptinemia. Plasma leptin concentration during hyperinsulinemic-euglycemic clamp in our study significantly increased in both groups of patients. Another explanation for hyperleptinemia after the LCD period in obese T1DM patients is the fact that the LCD did not lead to further weight loss. A reduction of body weight in obese T1DM patients is associated with a risk of hypoglycemia (Kemmer 1992). We explain the absence of further weight loss after LCD by problems in adherence to LCD amongst obese T1DM patients who were treated with insulin.

Another interesting finding from our study is the fact that serum adiponectin concentrations in obese type 1 diabetes patients did not differ from the lean control type 1 diabetes group nor were they significantly affected by the weight loss. This finding is in contrast to previous results in patients with obesity and type 2 diabetes mellitus where long-term weight loss significantly increased serum adiponectin concentrations (Pasarica *et al.* 2009, Urbanavičius *et al.* 2013, Palikhe *et al.* 2014). The reason for the unchanged adiponectin levels in our study may be the relatively short term duration of the study and rather modest weight loss. Several previous studies showed that moderate weight loss in the obese does not result in the elevation of adiponectin levels (Blesso *et al*. 2013, Mediano *et al.* 2013). Our study did confirm a negative association of A/L ratio with BMI, adiposity and IR in obese T1DM before fasting and in non-obese T1DM, as reported in previous studies (Vega and Grundy 2013), and a negative association between adiponectin and IR (Weyer *et al*. 2001). The high lipolytic activity during massive weight loss after seven days of fasting in our obese T1DM patients, who had IR before the weight reduction program, was associated with significant increase of FFA, which paralleled a temporary decrease in insulin sensitivity caused by the reduction of glucose oxidation. This decline in glucose oxidation reflects a metabolic adaptation to non-stress fasting and is in agreement with previous publications (Awad *et al.* 2009, Duška *et al.* 2005, Féry and Balasse 1994).

Despite bringing some novel data, our study has several limitations that need to be taken into account when interpreting the results. The major limitation of this study is the small number of patients (14 obese patients with T1DM and IR and 13 non-obese patients with T1DM). However, obese patients were investigated three times during the observation period of 30 days to obtain a

detailed impression of the serum leptin levels during pronounced weight loss. The concentrations of leptin and adiponectin were measured up to day 30 of the experimental protocol but no longer, which might have underestimated the association between the investigated factors and IR. Additionally, we cannot exclude the presence of any genetic variations in the leptin gene associated with the concentrations of leptin, although the contribution of these variants might be small compared to other clinical features such as BMI (Becer *et al*. 2013, Schwenk *et al.* 2013).

We conclude that obese patients with type 1 diabetes mellitus are characterized by insulin resistance, hyperleptinemia and unchanged adiponectin concentrations. The combination of 7-days fasting with 3-weeks low calorie diet did not significantly affect serum leptin or adiponectin concentrations despite a significant weight loss, suggesting that mechanisms other than modulation of endocrine function of adipose tissue might be involved in the adaptation of metabolism to body weight reduction.

Conflict of Interest

There is no conflict of interest.

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