Insulin resistance in obese patients with type 2 diabetes mellitus : effects of a very low calorie diet

Jazet, I.M.

CHAPTER 3

The relation between leptin and insulin remains when insulin secretion is disturbed

Ingrid M. Jazet, A. Jaap Fogteloo, A. Edo Meinders.

Department of General Internal Medicine, Leiden University Medical Centre, Leiden, The Netherlands

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ABSTRACT

Serum insulin and leptin levels correlate positively. It is unknown whether this relation remains the same in cases of severely disturbed insulin secretion and after rapid weight loss. We therefore studied the relation between insulin and leptin in obese type 2 diabetic patients before and after considerable weight loss.

In 17 obese (BMI 37.6 ± 1.4 kg/m^2, mean ± SEM) type 2 diabetic patients (duration 8.0 ± 1.4 years, fasting plasma glucose [FPG 12.9 ± 0.8 mmol/L, HbA_{1c} 8.6 ± 0.4%), blood glucose-lowering medication was discontinued (day -1) and a 30-day very low calorie diet (VLCD, 450 kCal/day) was started. On days 0, 2 and 30, body weight, body fat mass (with bioelectrical impedance analysis [BIA]), fasting serum glucose, insulin and leptin were determined. Homeostatic model assessment was used to estimate insulin resistance (HOMA-IR) and β-cell function (HOMA-β). On days 2 and 30, an intravenous glucose tolerance test (IVGTT) was performed.

Fasting serum leptin levels correlated positively with fasting serum insulin levels ($r = 0.72$, $p = 0.001$ on day 2; $r = 0.78$, $p = 0.001$ on day 30) and area under the curve (AUC) of insulin ($r = 0.74$, $p = 0.001$ on day 2; $r = 0.84$, $p = 0.0001$ on day 30), as well as HOMA-β, as a measure of insulin secretion, even after correction for body mass index (BMI) and body fat mass, with which serum leptin levels were also positively correlated.

In conclusion, in a group of obese type 2 diabetic patients with a wide range of residual endogenous insulin secretion, we found a positive relation between fasting serum leptin and insulin levels (fasting as well as AUC), even after correction for BMI and body fat mass. This was true both before weight loss and during energy restriction with weight loss.

INTRODUCTION

Leptin, the product of the *ob*-gene[1], is a 16 kDa protein that is mainly synthesised by white adipose tissue[1,2]. Leptin acts on hypothalamic neuropeptide-containing regions[3] and regulates body weight by controlling energy expenditure and food intake[1,4,5]. Serum leptin levels are positively correlated with body mass index (BMI) and body fat mass in both rodents and humans[6-8]. For any given body weight, serum leptin levels are higher in women than in men. However, after correction for fat mass, these differences seem to disappear[6,7], although not all authors agree[9]. Serum leptin levels show a diurnal pattern with a nocturnal peak shortly after midnight, and a midmorning low between 10:00 AM and 12:00 noon[10,11]. Serum leptin levels fluctuate with changes in body weight. Remarkably, with weight reduction, serum leptin levels fall before significant weight loss has occurred[12,13], suggesting that factors other than body fat mass regulate serum leptin levels in the short term. Possible regulators of the early decrease in serum leptin levels are energy restriction itself and/or serum insulin levels. The latter are also positively correlated with BMI and body fat mass.

A positive relation between serum leptin and serum insulin levels has been described in normal weight and obese subjects with or without impaired glucose tolerance[9,14-18] and in type 2 diabetic patients[16,19-21]. This positive relation has also been found before and after weight loss in obese men and women[14,22-24]. However, data on the effect of weight loss in type 2 diabetic patients, especially obese type 2 diabetic patients, are scarce[15,25]. It has been postulated[26] that during progressive β-cell failure, the relation between serum insulin and serum leptin levels is lost, either because of lower serum insulin levels or because of the developing hyperglycaemia, which might have a deleterious effect on both insulin production by β-cells and leptin production by adipose tissue.

In this study, we investigated both the effect of energy restriction (2 days of 450 kCal/day, minimal weight loss) and the effect of energy restriction plus weight loss (30 days of 450 kCal/day) on the relationship between serum leptin levels and serum insulin levels. Our study group was unique in the sense that we studied a group severely obese type 2 diabetic patients with varying degrees of endogenous insulin secretion, as assessed by an intravenous glucose tolerance test (IVGTT). We were, therefore, also able to address the relation between serum leptin levels and residual endogenous insulin secretory capacity.

PATIENTS AND METHODS

In 17 obese (BMI 37.6 ± 1.4 kg/m^2, mean ± SEM) type 2 diabetic patients (duration 8.0 ± 1.4 years) who had persistent high blood glucose levels (12.9 ± 0.8 mmol/L) and HbA_{1c} percentages (8.6 ± 0.4%) despite maximal doses of oral blood glucose lowering medication and/or

insulin (66 to 400 units/day), all blood glucose-lowering medication was stopped (day -1) and a very low calorie diet (VLCD, Modifast®, 450 kCal/day) was started for 30 days.

On days 0, 2, 10 and 30, weight and length were measured, and fasting serum glucose, insulin, C-peptide and leptin levels were determined. Body fat mass was measured using bio-electrical impedance analysis (BIA, Bodystat, Bodystat®, Bodystat Ltd. Douglas, Isle of Man). An IVGTT (25 g of i.v. in 4 min with blood sampling at 0, 2, 4, 6, 8, 10, 12, 20, 30, 40, 50 and 60 min) was performed after an overnight fast on days 2 and 30 of the VLCD[27,28].

We chose day 2 instead of day 0 for the first IVGTT because most patients had used NPH insulin on the evening before the start of the study. For the same reason, we used laboratory measures taken on day 2 for baseline values of fasting plasma insulin and C-peptide. In addition, we used the data for body fat mass achieved *via* a BIA on day 0 also on day 2. The reason we did so was that the BIA was not reliable on day 2 due to fluid shifts (the natriuresis of "fasting" induces a new fluid and salt balance in the first few days of a diet). Furthermore, body fat mass would not have changed yet during 2 days of a VLCD; thus, data obtained on day 0 would be applicable on day 2 as well.

Blood Chemistry

All blood chemistry was measured at the Laboratory for Clinical Chemistry of Leiden University Medical Centre. Serum glucose was measured using a fully automated Hitachi 747 (Hitachi, Tokyo, Japan) system. Serum insulin was measured by immunoradiometric assay (Medgenix, Fleurus, Belgium) with a detection limit of 3.0 mU/L. The interassay coefficient of variation (CV) was below 6%. Serum leptin concentrations were determined by a standardised radio immunoassay (Linco Research, St. Charles, MO, USA), with a detection limit of 0.5 µg/L and a coefficient of variation of 3-5% at different levels.

Statistical analysis and mathematical calculations

Values are presented as mean ± standard error of the mean (SEM).

The glucose disappearance rate (k-value) was determined by (natural) log-linear regression of the glucose concentrations against time over the period from 10 to 60 minutes post-glucose loading[27]. The areas under the curve (AUC) of glucose and of insulin were determined over the periods from 0 to 60 and 10 to 60 min, respectively, post-glucose loading from zero level using the linear trapezoidal rule.

Estimates of insulin resistance and β-cell function by HOMA score were calculated with the formulas as described by Matthews *et al.*[29].

For comparisons between study days a Student's *t*-test for paired samples was used. The relation between serum leptin and serum insulin levels, as well as with the AUC of insulin were evaluated with a two-tailed Pearson's correlation. In addition, two-tailed partial correlations were carried out for adjustment of BMI, fat mass, age and gender. All analyses were

performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA). A p value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics are presented in Table 1. Fourteen of the 17 patients completed the 30-day VLCD; the other 3 patients were not able to adhere to the diet and stopped within just a few days. We did not have any follow-up data from these three patients; so they were left out of the analysis comparing differences in various parameters between day 2 and day 30. Therefore, data on day 2 (fasting insulin, AUC insulin) in Table 2 (n=14) may differ from data on day 2 in Table 1 (n=17). For the correlation analysis between fasting serum leptin and insulin (fasting and AUC) all available data were used, resulting in 17 patients being analysed for this relation on day 2 and 14 patients on day 30 (Table 3).

The 14 patients who completed the study showed a gradual weight loss, amounting -2.5 ± 0.2 kg on day 2 (reflecting mainly salt and fluid loss) and -12.2 ± 0.8 kg on day 30 of the diet. This is equal to a reduction in BMI from 38.3 ± 1.5 kg/m^2 on day 0 to 37.5 ± 1.5 kg/m^2 on day 2 and 34.1 ± 1.5 kg/m^2 on day 30 of the diet ($p = 0.0001$ from day 0 to day 2, and day 0 to day 30, as well as from day 2 to day 30, see also Table 2). The decline in fasting serum leptin levels from day 0 to day 2 was highly significant, with a mean of 6.8 ± 1.6 ng/mL ($p = 0.001$, n=14). On day 30, the drop in fasting serum leptin levels in the 14 patients who completed the diet was also significant (Table 2).

Table 1. Patient characteristics (n=17).

Sex (male/female)	9 : 8
Age (years)	59.0 ± 1.9
Weight (kg)	110.7 ± 4.2
BMI (kg/m^2)	37.6 ± 1.4
Fat mass (kg)	42.6 ± 3.2
Fasting plasma glucose day 0 (mmol/L)	12.9 ± 0.8
HbA_{1c} (%)	8.6 ± 0.4
Duration type 2 diabetes (years)	8.0 ± 1.4
Fasting C-peptide day 0 (ng/mL)	1.3 ± 0.16
Fasting insulin day 2 (mU/L)	21.2 ± 3.5
Fasting leptin day 0 (ng/mL)	27.3 ± 5.3
AUC of insulin day 2 (mU*50 min)	1357 ± 224
Blood glucose lowering therapy	
only insulin	n = 4 (mean 167 units/day)
oral glucose-lowering therapy	n = 6
combination therapy	n = 7 (mean 168 units of insulin/day)

Data are presented as mean ± SEM.

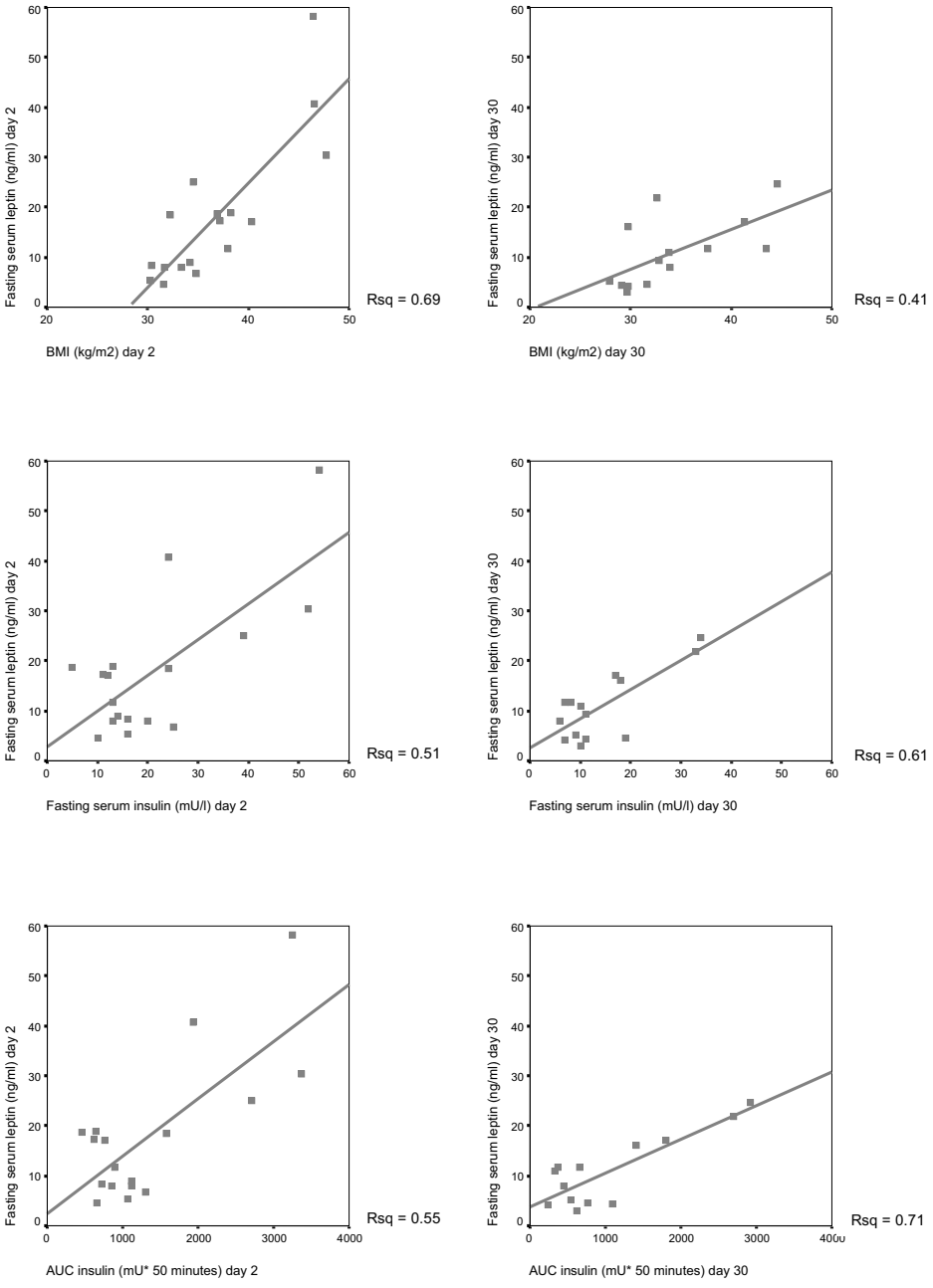

Figure 1.

Scatterplots of the correlation analysis between fasting serum leptin and BMI (top row), fasting serum insulin (middle row) and AUC of insulin (bottom row) on day 2 (left side, n=17) and day 30 (right side,n=14) of the VLCD.

Table 2. Changes in anthropometric values, fasting serum insulin and leptin concentrations and estimates of insulin secretion and insulin sensitivity.

	Day 2 (n=14)	**Day 30** (n=14)	**P**
Weight (kg)	109.3 ± 5.0	99.7 ± 4.8	0.0001
BMI (kg/m^2)	37.5 ± 1.5	34.1 ± 1.5	0.0001
Fat mass (kg)	44.5 ± 3.8	38.6 ± 3.9	0.0001
Fasting serum glucose (mmol/L)	14.9 ± 1.1	12.0 ± 1.5	0.007
Fasting serum insulin (mU/L)	23.0 ± 4.1	14.3 ± 2.4	0.010
Fasting serum leptin (ng/mL)	20.3 ± 3.9	10.9 ± 1.8	0.008
AUC of insulin (mU*50 min)	1537.5 ± 242.4	1068.5 ± 247.6	0.005
AUC of glucose (mmol*60 min)	1194.4 ± 63.6	1721.0 ± 690.8	NS
k-Value (%/min)	0.46 ± 0.03	0.48 ± 0.04	NS
HOMA-IR	13.9 ± 2.2	6.6 ± 0.9	0.002
HOMA-β	49.4 ± 10.9	55.2 ± 13.4	NS

This table shows the changes in various parameters from day 2 to day 30 of the diet. A paired Student's *t*-test was used since all patients served as their own controls. Because only 14 patients have completed the study these data represent only those 14 patients. Hence, values might differ from Table 1, because on day 0 data from 17 patients were available.
Values are presented as mean ± SEM. NS = not significant.

The decline in fasting serum leptin levels was paralleled by a decline in fasting serum insulin levels. On both day 2 and day 30 fasting serum leptin levels correlated positively with fasting serum insulin levels and AUC of insulin (Table 3 and Fig. 1). The change in fasting serum leptin levels from day 2 to day 30 (delta leptin 2-30) also correlated positively with the change in fasting serum insulin levels from day 2 to day 30 (delta insulin 2-30) ($r = 0.71$, $p = 0.005$) and the change in AUC of insulin from day 2 to day 30 (delta AUC insulin 2-30) ($r = 0.81$, $p = 0.001$).

Fasting serum leptin levels were positively correlated with body weight ($r = 0.52$, $p = 0.033$ on day 2; $r = 0.60$, $p = 0.024$ on day 30) and BMI ($r = 0.84$, $p = 0.0001$ on day 2; $r = 0.64$, $p = 0.014$ on day 30). Fasting serum insulin levels correlated positively with body weight and BMI on day 2, whereas the correlation with BMI was lost on day 30. After adjustment for BMI, gender and age, the positive correlation between fasting serum leptin and fasting serum insulin levels and AUC of insulin remained (Table 3). The decrease in fasting serum leptin levels from day 2 to day 30 was also positively correlated with the decrease in fasting serum insulin levels and the decrease in AUC of insulin from day 2 to day 30 after adjusting for BMI, gender and age. After correction for fat mass, the positive relation between fasting serum leptin and serum insulin (fasting and AUC) remained (Table 3).

No correlation was found between fasting serum leptin levels and either fasting plasma glucose (FPG) or k-values (as a measure of the glucose disposal rate). Fasting serum leptin levels also showed no correlation with the AUC of glucose during an IVGTT, HbA_{1c} levels, duration of type 2 diabetes, or fasting C-peptide levels. Fasting serum leptin levels were positively correlated with HOMA-IR ($r = 0.57$, $p = 0.017$ on day 2; $r = 0.64$, $p = 0.013$ on day 30) and HOMA-β ($r = 0.83$, $p = 0.0001$ and $r = 0.76$, $p = 0.001$ on day 2 and day 30, respectively),

however. After correcting for BMI in a partial correlation analysis, these relations remained significant with the exception of HOMA-IR on day 2.

Table 3. Partial correlation analysis of fasting serum leptin with the fasting serum insulin and AUC of insulin.

		Day 2 (n=17)	Day 30 (n=17)
Fasting serum insulin (mU/L)	Unadjusted	r = 0.72, p = 0.001	r = 0.78, p = 0.001
	Adjusted for BMI	r = 0.51, p = 0.042	r = 0.81, p = 0.001
	Adjusted for BMI and gender	r = 0.58, p = 0.024	r = 0.80, p = 0.002
	Adjusted for BMI, gender and age	r = 0.60, p = 0.023	r = 0.90, p = 0.0001
	Adjusted for fat mass, gender and age	r = 0.58, p = 0.030	r = 0.86, p = 0.003
AUC of insulin (mU*50min)	Unadjusted	r = 0.74, p = 0.001	r = 0.84, p = 0.0001
	Adjusted for BMI	r = 0.54, p = 0.030	r = 0.83, p = 0.001
	Adjusted for BMI and gender	r = 0.61, p = 0.016	r = 0.84, p = 0.001
	Adjusted for BMI, gender and age	r = 0.64, p = 0.015	r = 0.93, p = 0.0001
	Adjusted for fat mass, gender and age	r = 0.60, p = 0.023	r = 0.94, p = 0.001

DISCUSSION

This study shows that, even in patients with a severely disturbed endogenous insulin secretion, a positive relation between fasting serum insulin and fasting serum leptin levels exists, even after correcting for BMI and body fat mass. This was true both during energy restriction (day 2) and during weight loss plus energy restriction (day 30). Furthermore, fasting serum leptin levels also correlated with HOMA-β and the AUC of insulin as measures of insulin secretory capacity.

We found a sharp decline in both fasting serum leptin and fasting serum insulin levels after only 2 days of the VLCD. Other investigators have also seen a rapid decrease in serum leptin levels with energy restriction[12,13,30-32]. Since fat mass can hardly have decreased significantly in such a short period of time, this decline in fasting serum leptin and insulin levels more likely reflects a signal to the brain that the body is in negative energy balance. Support for this concept can be found in the study of Chin-chance *et al.*[33]. Six healthy normal weight subjects were included in a 12-day study with four consecutive dietary treatment periods of 3 days each. A baseline period (feeding at 100% of total energy expenditure [TEE]) was followed by random crossover periods of overfeeding (130% TEE) or underfeeding (70% TEE), separated by a eucaloric period (100% TEE). Serum leptin levels responded acutely to modest changes in energy intake (declining during 70% TEE and increasing during 130% TEE) and, remarkably, returned to baseline values only after completion of the complementary feeding periods, indicating that leptin levels were a marker of short-term cumulative energy balance. In contrast, in the long-term, when weight loss occurs, serum leptin levels once again reflect body fat stores. Wadden *et al.*[34] showed that, in the first 6 weeks of a diet, serum leptin levels were primarily determined by the degree of caloric restriction, whereas at 40 weeks weight

loss accounted for 47% of the variance in serum leptin levels. In addition, Cella *et al*.[13] found a gradual rise in serum leptin levels with further weight loss.

We also studied the relation between fasting serum leptin and fasting serum insulin levels after a period of energy restriction plus weight loss. To our knowledge, with regard to the relation between serum leptin and serum insulin levels after weight loss, few studies have been performed in obese type 2 diabetic patients. Moreover, these studies had either included very few patients[15,25] or patients with only mild diabetes and obesity[15,25].

Our study is, therefore, unique with regard to the extreme patient population (severely obese type 2 diabetic patients, inadequately regulated on maximal oral blood glucose-lowering medication and/or insulin therapy) and the fact that we performed a dynamic test in the form of an IVGTT. We were, therefore, able to demonstrate that the relation between fasting serum leptin and insulin levels, even after correction for BMI and fat mass, holds true over a wide range of residual endogenous insulin secretory capacity (as defined by the AUC of insulin).

What we were not able to demonstrate was whether this positive relationship between serum insulin and serum leptin levels is due to leptin regulating insulin levels or *vice versa*. Several facts point to the latter. Firstly, when octreotide is given to patients with an insulinoma, serum leptin levels fall within half an hour of the decline in serum insulin levels[35]. Secondly, during a prolonged hyperinsulinaemic euglycaemic clamp, serum leptin levels show a dose-dependent[36] increase[37]. Thirdly, serum leptin levels are increased by insulin therapy, both in patients with type 1 and type 2 diabetes[19,38]. Fourthly, when patients were stratified to high and low serum insulin groups, serum leptin levels were higher in the high insulin group than in the low insulin group, while BMI was the same[14]. Fifthly, conversely argued, leptin therapy does not increase serum insulin levels[39]; in fact, leptin probably diminishes insulin levels by directly inhibiting insulin secretion. To that end, functional leptin receptors are present on the cell membranes of pancreatic β-cells[40].

In conclusion, even in patients with a highly disturbed endogenous insulin secretion, a positive relation between fasting serum leptin and serum insulin levels (fasting and AUC) can be found. This relation was found during both energy restriction and weight loss. Whether insulin regulates leptin levels or *vice versa,* or, whether both are regulated in concert to reflect changes in energy balance cannot be deduced from this study. However, the evidence at hand makes it seem most likely that insulin regulates leptin.

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