Leptin—A Regulator of Islet Function?: Its Plasma Levels Correlate With Glucagon and Insulin Secretion in Healthy Women

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It has previously been demonstrated that plasma leptin correlates to body fat content. It has also been demonstrated that in subjects with normal glucose tolerance, circulating leptin correlates to circulating insulin and to insulin secretion and that these relations are independent of body fat. However, whether leptin also covaries with other islet hormones is not known. We therefore studied the relation between plasma levels of leptin and glucagon secretion and circulating pancreatic polypeptide (PP) in healthy humans. Arginine was injected intravenously (5 g) at fasting and at 14 and 28 mmol/L glucose in 71 postmenopausal women with normal glucose tolerance. In a multivariate analysis controlling for the influence of the body mass index, we found that circulating leptin correlated significantly to fasting insulin ($r = .38$, $P = .002$), and to circulating insulin at 14 mmol/L glucose ($r = .29$, $P = .0019$) and 28 mmol/L glucose ($r = .32$, $P = .009$), as well as to the insulin response to arginine at all three glucose levels ($r > .30$, $P < .013$). Circulating leptin, independently of the body mass index, also correlated to fasting glucagon ($r = .31$, $P = .012$) and to the glucagon response to arginine at all three glucose levels ($r > .28$, $P < .038$). In contrast, circulating leptin did not correlate to plasma glucagon at 14 or 28 mmol/L glucose or to plasma levels of PP. We conclude that circulating leptin correlates to the secretory capacity of both glucagon and insulin but not to the reduction of plasma glucagon during hyperglycemia or to PP in a large group of postmenopausal women. This suggests that islet function is related to circulating leptin in humans.

LEPTIN, the product of the ob gene, reduces food intake and increases energy expenditure when administered to mice.[1] Previous studies have shown that circulating leptin levels correlate to body fat content.[2-5] It has therefore been suggested that leptin is a sense parameter for adipose tissue, perhaps reducing food intake in a negative-feedback pattern.[6,7] However, the physiological role of the protein is largely unknown.

We showed previously that circulating leptin, independently of the body mass index, correlates to fasting insulin levels and to insulin secretion in humans with normal glucose tolerance, whereas no such correlation was evident in impaired glucose tolerance.[4] This would suggest that leptin and islet function are related, due to insulin's stimulation of leptin expression and secretion, as inferred from studies in vitro,[8,9] or to leptin's affect on insulin secretion, as inferred from the demonstration of Ob receptors in islet endocrine cells.[10,11] However, whether leptin also covaries with islet hormones other than insulin is not known. In the present study, we have therefore examined the relation between circulating leptin and circulating glucagon and pancreatic polypeptide (PP), as well as between leptin and glucagon secretion, in a large group of healthy postmenopausal women.

SUBJECTS AND METHODS

Study Population and Research Design

The study was performed in 71 postmenopausal Caucasian women, who constituted a stratified random sample of a population in which the prevalence of impaired glucose tolerance is 27.9%.[12] All of the women were 58 or 59 years of age (mean ± SD, 58.6 ± 0.4). They were healthy, and all had normal glucose tolerance as judged by a 75-g oral glucose tolerance test. None were taking any drugs known to affect carbohydrate metabolism. The study was approved by the Ethics Committee at Lund University. All subjects provided written informed consent before entrance to the study.

Anthropometric Measurements

Height and weight were determined with the participants in light clothing without shoes. Body weight was measured to the nearest 0.1 kg in the morning after an overnight fast. Height was determined to the nearest centimeter. The body mass index was defined as the weight in kilograms divided by the squared height in meters. Waist circumference was measured at the level of the umbilicus, and hip circumference at the level of the greater trochanters; both measures were determined with the subjects standing.

Glucose-Dependent Arginine Stimulation

Insulin and glucagon secretion were determined with intravenous arginine stimulation at three blood glucose levels (fasting, 14 mmol/L, and 28 mmol/L) in the morning after an overnight fast.[13] Intravenous catheters were inserted into antecubital veins in both arms. One arm was used for infusion of glucose, and the other arm for intermittent sampling. The sampling catheter was kept patent by slow infusion of 0.9% saline when not used. Baseline samples were taken at −5 and −2 minutes. Arginine hydrochloride (5 g) was then injected intravenously over 45 seconds. Samples were taken at +2, +3, +4, and +5 minutes. A variable-rate 20% glucose infusion was then initiated to increase and maintain blood glucose at 13 to 15 mmol/L. Blood glucose was determined every 5 minutes at bedside, and the glucose infusion was adjusted to reach the desired blood glucose level of 13 to 15 mmol/L in 20 to 25 minutes. New baseline samples were taken, and then arginine (5 g) was again injected and +2-, +3-, +4-, and +5-minute samples were taken. This was followed by a 2.5-hour resting period without glucose infusion. After the rest, a high-speed (900 mL/h) 20% glucose infusion was administered for 25 to 30 minutes to increase and maintain blood glucose at 28 mmol/L or greater. At this blood glucose level, −5- and −2-minute samples were obtained, 5 g arginine was once more injected, and final samples were taken at +2, +3, +4, and +5 minutes. Glucose, insulin, and glucagon levels were determined in all samples in all subjects, and the circulating leptin level was measured in the −2-,

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+2-, and +4-minute samples at each glucose level in a subset of 12 subjects. In addition, in all subjects, plasma leptin and PP were determined in the −5-minute sample, ie, the first sample after the overnight fast.

Analyses

Samples for analysis of leptin were taken in prechilled tubes containing 0.084 mL EDTA (0.34 mol/L). The analysis was performed with a double-antibody radioimmunoassay using rabbit anti-human leptin antibodies, ^{125}I-labeled human leptin as tracer, and human leptin as standard[14] (Linco, St Louis, MO). Blood glucose concentration was determined at bedside by the glucose dehydrogenase technique with an Accutrend (Boehringer Mannheim Scandinavia, Bromma, Sweden) during the arginine test. Samples for analysis of insulin were immediately centrifuged at 5°C, and the serum was separated. Serum insulin concentrations were analyzed with a double-antibody radioimmunoassay technique. Guinea pig anti-human insulin antibodies, ^{125}I-Tyr-human insulin as tracer, and human insulin standard (Linco) were used. Glucagon samples were obtained in prechilled test tubes containing 0.084 mL EDTA (0.34 mol/L) and aprotinin (250 kallikrein-inhibiting U/mL blood; Bayer, Leverkusen, Germany). Glucagon levels were measured with a double-antibody radioimmunoassay in duplicate using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, ^{125}I-glucagon as tracer, and glucagon standard (Linco). Plasma glucose concentrations were analyzed using the glucose oxidase method. Samples for analysis of PP were taken in prechilled tubes containing 0.084 mL EDTA (0.34 mol/L). PP was determined with a double-antibody radioimmunoassay using rabbit anti-human PP antibodies, ^{125}I-labeled human PP, and human PP standard[15] (Linco). Serum or plasma were stored at −20°C until analyses, and all determinations were performed in duplicate.

Calculations

Data are presented as the mean ± SEM unless otherwise noted. The acute insulin and glucagon response to arginine (AIR and AGR, respectively) were calculated as the mean of the +2- to +5-minute samples minus the prestimulus insulin and glucagon concentration at fasting blood glucose and after increasing blood glucose to 14 mmol/L and 28 mmol/L. Previous studies have shown that the glucose potentiation of the AIR to arginine is linear between blood glucose levels of 3.5 and 18 mmol/L.[13] The slope between the AIR at fasting blood glucose and at blood glucose 14 mmol/L ($slope_{AIR}$ = ΔAIR/Δglucose) was therefore calculated as a measure of glucose potentiation of β-cell secretion. $Slope_{AGR}$ (representing glucose inhibition of α cells) was calculated in the same manner.

Statistics

Statistical analyses were performed with the SPSS (SPSS, Chicago, IL) for Windows system. Differences in plasma leptin in the glucose-dependent arginine stimulation test were determined by Student's *t* test for paired observations. Pearson's product-moment correlation was used to estimate linear relationships between variables.

RESULTS

In this study population of healthy postmenopausal women (N = 71), fasting plasma leptin levels were 19.1 ± 1.6 ng/mL (range, 3.3 to 59.2) and the body mass index was 25.1 ± 0.4 kg/m^2 (range, 17.0 to 33.1). Plasma leptin correlated significantly to the body mass index (r = .71, P < .001; Fig 1). Also, plasma leptin per unit body mass index correlated significantly to the body mass index (r = .60, P < .001; Fig 1). In contrast, plasma leptin did not correlate to the waist to hip ratio (r = .14, NS). Table 1 shows baseline levels of insulin, glucagon, glucose, and PP and anthropometric measurements in these women.

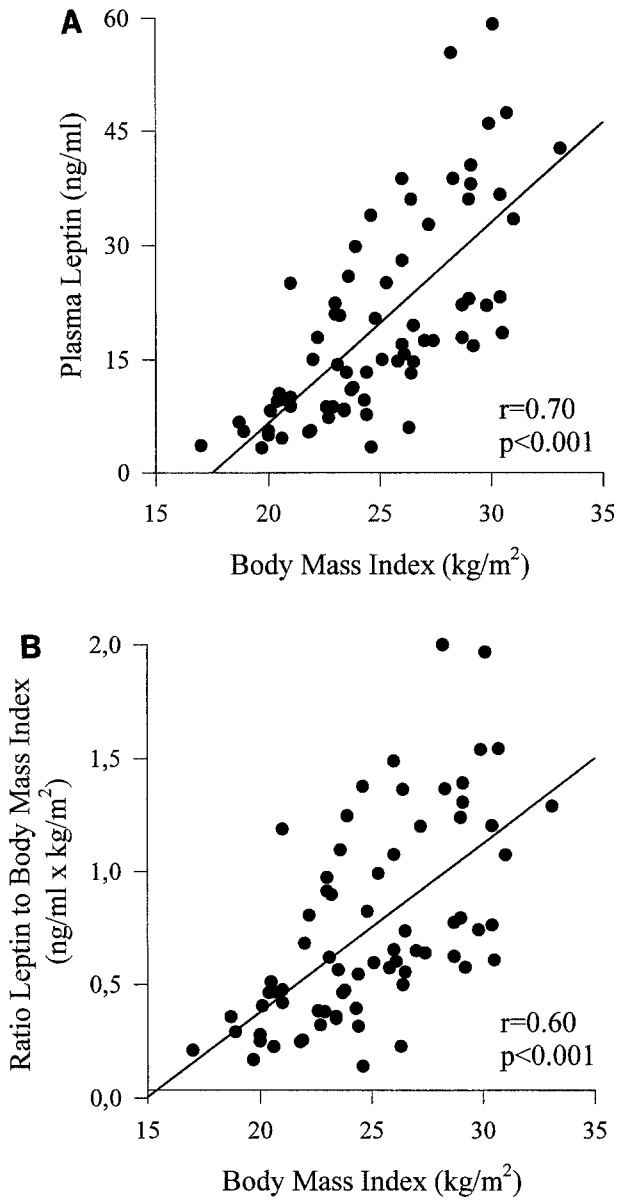

Fig 1. Correlation between (A) body mass index and fasting plasma leptin and (B) body mass index and the ratio of plasma leptin to body mass index (ie, circulating leptin per unit body mass index) in 71 postmenopausal women.

Following arginine injection, circulating levels of both insulin and glucagon increased. Increasing the blood glucose level potentiated arginine-stimulated insulin secretion and inhibited glucagon secretion (Fig 2). To study whether circulating leptin levels correlated to insulin and glucagon secretion, we performed partial correlation analyses to study the independent correlation between plasma leptin and variables of insulin and glucagon secretion after controlling for body mass index (Table 2). It was thereby found that plasma leptin significantly correlated both to fasting insulin and to the insulin secretory responses to arginine and glucose. Plasma leptin also correlated

significantly to $slope_{AIR}$. Also, the fasting glucagon level and the glucagon secretory responses to arginine at all three glucose levels correlated to circulating leptin, whereas no significant correlations were observed between circulating leptin and the reduced circulating glucagon after increasing glucose to 14 and 28 mmol/L or $slope_{AGR}$ (Table 2).

After controlling for both body mass index and fasting glucagon in the multivariate analysis, circulating leptin still correlated significantly to the insulin response to arginine at fasting glucose ($r = .27$, $P = .028$), 14 mmol/L glucose ($r = .28$, $P = .025$), and 28 mmol/L glucose ($r = .26$, $P = .039$). Similarly, after controlling for both body mass index and fasting insulin in the multivariate analysis, circulating leptin correlated significantly to the glucagon response to arginine at fasting glucose ($r = .25$, $P = .049$), 14 mmol/L glucose ($r = .29$, $P = .020$), and 28 mmol/L glucose ($r = .26$, $P = .042$).

In 12 subjects, plasma leptin was determined before and at 2 and 4 minutes after arginine injection at the three glucose concentrations in the glucose-dependent arginine stimulation test. Figure 3 shows the leptin level immediately before arginine injection and the mean of the 2- and 4-minute leptin values after arginine injection at each glucose level. Plasma leptin in the samples immediately before arginine injection was marginally lower at both 14 mmol/L glucose (1.4 ± 0.4 ng/mL, or 8.7% ± 1.8%, $P = .003$) and 28 mmol/L glucose (1.8 ± 0.4 ng/mL, or 10.6% ± 1.5%, $P = .001$) than at fasting glucose. Furthermore, at fasting glucose, plasma leptin was already reduced at 2 to 4 minutes after arginine injection (by 0.7 ± 0.3 ng/mL, or 3.7% ± 1.5%, $P = .016$), whereas at the higher glucose levels, no significant influence of arginine was evident on circulating leptin.

Plasma PP was 171 ± 14 pg/mL (range, 39 to 408) in the study group. It did not correlate significantly to the fasting insulin ($r = .01$, NS), fasting glucagon ($r = .02$, NS), body mass index ($r = -.11$, NS), or circulating leptin ($r = .07$, NS).

DISCUSSION

In this study of 71 healthy postmenopausal women with normal glucose tolerance, we confirm several previous reports that circulating leptin correlates to the body mass index in humans.[2-5,16-18] This correlation is due to the covariation of circulating leptin with body fat content[2-5] and is explained by a high production of leptin in fat tissue rather than a diminished leptin clearance in subjects with a high body mass index.[17] The r value for the correlation between circulating leptin and the body mass index in our study was .70, similar to other studies.[2-5,16-18] However, it is of interest that leptin levels displayed a 20-fold difference between the highest and lowest value, yet the body mass index differed only twofold. This suggests that although the body mass index is an important determinant of circulating leptin, other factors are also involved. Identification of these factors would be of interest for a better understanding of the physiological role of leptin.[16] By relating the plasma leptin level divided by the body mass index to the body mass index (Fig 1), it is evident that subjects with a higher body mass index have a relatively larger contribution by each unit of body mass index to

Table 1. Baseline (fasting) Serum Insulin, Plasma Leptin, Glucagon, PP, and Glucose, and Body Mass Index in 71 Postmenopausal Women Aged 58.6 ± 0.4 Years

Parameter	Mean ± SEM
Leptin	19.1 ± 1.6 ng/mL
Insulin	58.8 ± 2.6 pmol/L
Glucagon	69.1 ± 2.6 ng/L
Glucose	4.9 ± 0.1 mmol/L
BMI	25.1 ± 0.4 kg/m²
WHR	0.78 ± 0.05

Abbreviations: BMI, body mass index; WHR, waist to hip ratio.

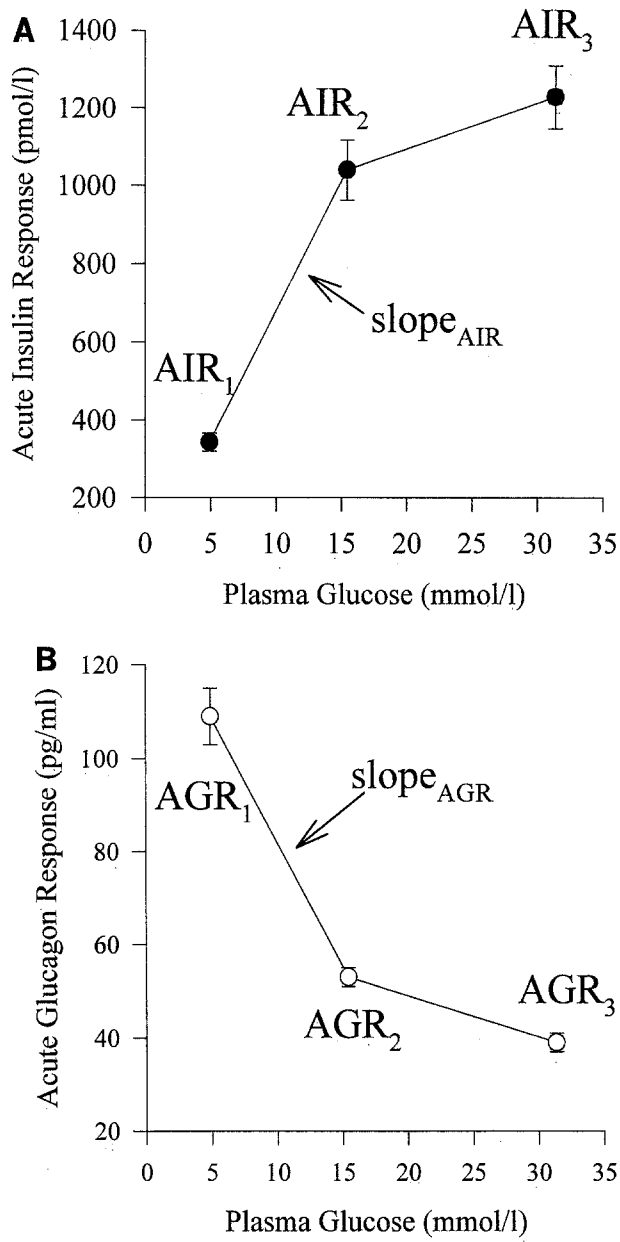

Fig 2. Calculated (A) AIR and (B) AGR after intravenous injection of arginine (5 g) at fasting glucose and after increasing blood glucose to 14 and 28 mmol/L in 71 postmenopausal women. Values are the mean ± SEM. AIR_1 and AGR_1, responses to arginine at fasting glucose; AIR_2 and AGR_2, responses at glucose 14 mmol/L; AIR_3 and AGR_3, responses at high glucose. Slope $_{AIR}$ and $slope_{AGR}$ show the linear relation used for calculation of the glucose sensitivity of insulin and glucagon secretion, respectively.

Table 2. Correlation Coefficients (*r*) Between Plasma Leptin and Variables of Glucagon and Insulin Secretion in 71 Postmenopausal Women in a Multivariate Analysis After Controlling for the Influence of Body Mass Index

Variable	Glucagon		Insulin	
	r	*P*	*r*	*P*
Fasting glucagon or insulin	.31	.012	.38	.002
Glucagon or insulin level				
BG 14 mmol/L	.11	NS	.29	.019
BG 28 mmol/L	−.08	NS	.32	.009
Glucagon or insulin response to arginine				
Fasting BG	.28	.038	.33	.007
BG 14 mmol/L	.33	.008	.32	.008
BG 28 mmol/L	.29	.018	.30	.013
$Slope_{AIR}$ or $slope_{AGR}$	.12	NS	.28	.020

Abbreviations: BG, blood glucose; NS, not significant.

circulating leptin. This confirms previous results on leptin kinetics in humans suggesting that leptin production per 100 g fat/min is higher the higher the body mass index[17] and previous in vitro results that the ob gene is overexpressed in isolated adipocytes from individuals with obesity compared with lean subjects.[19,20] This relatively higher leptin production and secretion and therefore circulating leptin concentrations in subjects with a high body mass index (also per unit fat) might be explained by a reduced leptin sensitivity (leptin resistance) in these subjects, as previously suggested.[3] In our study, we also found that circulating leptin did not significantly correlate to the ratio of waist circumference (a measure of upper-body fat distribution) to hip circumference (a measure of lower-body fat distribution), ie, to central adiposity, yet leptin correlated to the body mass index in this group of subjects. This would suggest that at least in postmenopausal women, circulating leptin is related more to the overall degree of obesity than to central adiposity.

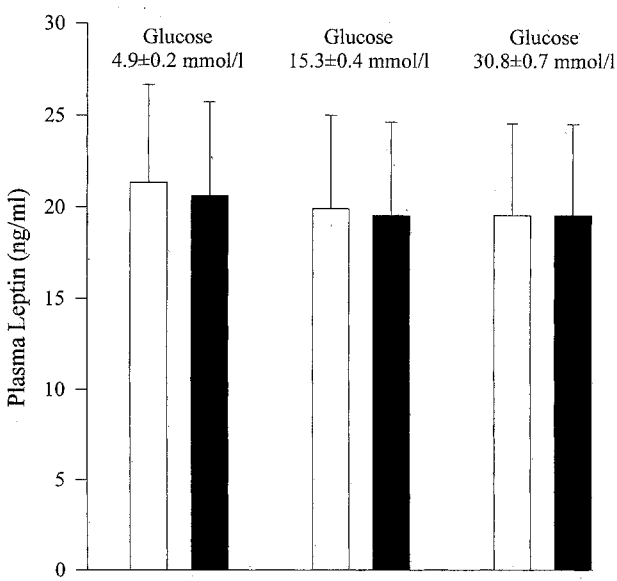

Fig 3. Plasma leptin level immediately before (□) and mean of plasma leptin levels at 2 and 4 minutes after (■) intravenous injection of arginine (5 g) at 3 different plasma glucose levels (4.9 ± 0.2, 15.3 ± 0.4, and 30.8 ± 0.7 mmol/L) in 12 postmenopausal women. Values are the mean ± SEM.

We previously showed that circulating leptin correlates to fasting insulin and to insulin secretion independently of the body mass index in a group of postmenopausal women with normal glucose tolerance.[4] In this study, we examined whether this finding is an illustration of a more general correlation between leptin and islet function. We then found that circulating leptin covaries not only with fasting insulin and the insulin secretory response to arginine and glucose, but also with fasting glucagon and the glucagon secretory response to arginine. These relations were also evident independently of the body mass index. Furthermore, the correlation between leptin and insulin secretion was independent of glucagon, and conversely, the correlation between leptin and glucagon secretion was independent of insulin. Thus, the circulating leptin level seems related to the secretory rate of the two glucoregulatory hormones of the islets, and it may be speculated that leptin is involved in the regulation of both insulin and glucagon secretion in healthy subjects.

The relation between leptin on one hand and glucagon and insulin secretion on the other may be due to leptin's stimulation of secretion of these islet hormones. This can be inferred from studies demonstrating that the leptin receptor is expressed in pancreatic islets.[10,11] However, long-term leptin administration has been found not to affect circulating insulin levels in normal mice and to actually reduce circulating insulin in ob/ob mice.[21] In addition, leptin was recently suggested to inhibit stimulated insulin secretion from islets isolated from the ob/ob mouse and its wild-type controls.[11] Another possibility is that the correlation between the circulating leptin level and glucagon and insulin secretion is due to a glucagon- or insulin-induced increase in plasma leptin. Although not yet studied for glucagon, this would be supported for insulin by recent studies showing that insulin administration increases adipose tissue leptin mRNA in rats.[8,9] However, circulating insulin at physiological levels does not seem to affect circulating leptin.[4,8]

An intriguing observation from the present study is that the circulating leptin level correlated significantly to the glucagon secretory capacity after arginine administration but not to the reduced glucagon levels after increasing blood glucose levels. This suggests that leptin levels are related to secretion from glucagon cells, which is inhibited by glucose. However, more studies are required to elucidate the mechanism of the relation between leptin and islet function.

In contrast to the correlation of leptin with glucagon and insulin, leptin did not correlate to PP, the third hormone from the pancreatic islets. The function of PP has not been established, although PP is increased during hypoglycemia and has been regarded as mirroring vagal nerve activity.[22] If that were also true in this group of postmenopausal women, it is unlikely that leptin is related to vagal activity.

The design of the present study also allowed some conclusions on the potential rapid influence of the insulin secretagogues, glucose and arginine, on plasma leptin concentrations in humans. It was found that increasing the glucose concentra-

tion did marginally, albeit significantly, reduce circulating leptin, and similarly, rapid intravenous injection of arginine at fasting glucose marginally reduced plasma leptin levels. It is known that circulating leptin displays a circadian rhythm, and following a peak during the night, leptin is reduced during the morning hours.[23] This could explain the reduction in circulating leptin during the entire glucose-dependent arginine stimulation test, which has a 3.5-hour duration. However, it is also possible that impairment of leptin secretion by glucose and subsequent reduction of plasma leptin levels would be detectable, since the half-life of circulating leptin in humans has been assumed to be approximately 25 minutes.[17] Inhibition of leptin secretion by glucose thus cannot be excluded at present. However, with arginine, this explanation is unlikely, since the sampling was performed at 2 and 4 minutes after arginine injection. Instead, a marginal increase in leptin clearance induced by arginine might contribute to the decreased concentrations. In any case, these results deserve further consideration.

In conclusion, our results suggest that in postmenopausal women, circulating leptin correlates to insulin and glucagon secretion independently of the body mass index, whereas circulating leptin does not correlate to PP. The results suggest that leptin is related to islet function in humans.

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