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ORIGINAL CONTRIBUTION

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Lipolysis, fatness, gender and plasma leptin concentrations in healthy, normal-weight subjects

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Summary *Background:* Relationship between plasma leptin and adiposity and gender has been reported in adults. Effect of age on plasma leptin is unclear and regulation of leptin production by white adipose tissue is still poorly understood.
Objectives: To study if age and parameters of lipolysis are related to plasma leptin concentrations.
Methods: Seventy-seven healthy, normal-weight subjects (age range 19-82 y.) had measurements of body composition (^{18}oxygen dilution technique) and of fasting plasma levels of leptin, glycerol, and nonesterified fatty acids (NEFA).
Results: Plasma leptin was correlated to NEFA (r = 0.28) and glycerol (r = 0.48) concentrations. The relationship between %fat and plasma leptin was best fitted by an exponential (r^2 = 0.82). In multiple regression %fat, body mass index, glycerol, and gender, but not fat mass, age or NEFA contributed independently to the variation in log plasma leptin. Log plasma leptin was higher in women than in men for a given glycerol concentration.
Conclusion: Adiposity, lipolysis, and gender are related to plasma leptin in healthy humans.

Key words Leptin – %fat, ^{18}O-labelled water – glycerol – NEFA

Introduction

The *ob* gene encodes a protein, leptin, which is only produced in fat cells and secreted into the blood (44). Administration of leptin to *ob/ob* mice (obese mice with a deficient *ob* gene) appears to correct metabolic disturbances, i.e., increased food intake and reduced energy expenditure (24).

In human obesity significant mutations of the *ob* gene are rare (22) and resistance to the action of leptin is supposed (6) possibly because of the deficient functioning of leptin receptors in the brain (4).

In lean humans leptin is believed to signal the brain about how much fat is on board. Leptin being a hormone involved in the regulation of human energy balance (27), its production by white adipose tissue has to be regulated. A positive correlation between fatness and plasma leptin has been reported several times in lean and obese humans (3, 6, 15, 20, 46). However, such a relationship remains to be studied in elderly subjects since age-induced changes in body composition involve an increase in fat mass and in %fat. Ostlund et al. (23) have shown that plasma leptin decreases with age despite the increase in fat mass. The signal(s) for a feedback loop are not yet understood. Insulin is an obvious candidate but the evidence is inconsistent (8, 17, 19, 26, 31, 39, 40, 42). Other metabolic parameters, especially those arising from white adipose tissue lipolysis, i.e., glycerol and nonesterified fatty acids (NEFA), have not been considered.

Therefore, the aim of this study was to investigate the relationship between plasma leptin concentration and age, parameters of body composition, and lipolysis in healthy subjects.

Material and methods

Volunteers

Seventy-seven healthy volunteers (47 men, 30 women), 19-82 yr. old, whose physical characteristics are given in Table 1 were recruited for participation in the study. All volunteers gave written informed consent for participation, and the study was approved by the local medical school ethical committee.

Protocol

Fasting volunteers were admitted to the metabolic unit at 8:00 a.m. They had been instructed to consume their usual diet for the 3 d prior to the measurements. Baseline plasma samples were collected for the measurement of leptin, glycerol, and NEFA concentrations. A baseline saliva sample was collected with a cotton ball left for 5 min in the mouth and then squashed in a syringe. Volunteers then received the labeled water dose and had measurements of body composition.

Methods

Body composition

Body weight was measured in light clothing and in the fasting state on a SECA 709 scale (SECA, Les Mureaux, France) with a precision of ± 0.1 kg. Height was measured barefoot with a SECA microtoise to ± 0.2 cm.

Fat free mass was calculated from measured total body water assuming a 73.2% hydration coefficient (32) which appears not to differ between adults and elderly subjects (14, 29). Fat mass was calculated as body weight minus fat free mass and expressed in absolute and relative to body weight (%fat) values. Total body water was measured by dilution of ^{18}O enriched water as described by Vaché et al. (41). Briefly, after collection of the baseline saliva sample, an accurately weighed dose of 50.0 g $H_2{}^{18}O$ (2 Atm%, Enritech Technologies Ltd, Rehovot, Israel) was taken orally. Saliva samples were collected again 4, 5, and 6 h after the dose, when isotopic equilibrium was reached. Samples were kept frozen at -20 °C until analyzed mass spectrometrically as described elsewhere (41). ^{18}O dilution space was calculated from post-dose saliva isotopic enrichments net of baseline (41). Total body water was considered 1% smaller than ^{18}O dilution space to account for exchanges with non-aqueous compounds (30).

Leptin concentrations

Fasting plasma leptin concentrations were measured with a human leptin radio immunoassay (^{125}I human leptin, Linco Research, St Charles, MO, USA) according to manufacturer's recommendations. It is a homologous assay with the antibody being raised against highly purified human leptin and both standard and tracer being prepared with human leptin. The coefficients of variation ranged from 3.4 to 8.3% (within-run) and between 3.6 to 6.2% (between-run). All procedures were performed in duplicates (standards, quality controls, and samples) and radioactivity was measured on a gamma counter (Packard, model 5530).

NEFA and glycerol concentrations

NEFA concentrations were measured using a specific enzymatic assay (Unipath, NEFAC*, Dardilly, France), and absorbances were read at 550 nm on a Uvikon 810 spectrophotometer (Kontron, France). Glycerol concentrations were measured using a specific enzymatic assay (Glycerol, Boehringer Mannheim, France), and absorbances were read at 340 nm on the same spectrophotometer.

Statistical analyses

Results are presented as mean ± SEM. Partial correlation matrices and stepwise regressions were performed to de-

Table 1 Physical and metabolic characteristics of the subjects

	Mean	SEM	r	P
Body weight (kg)	68.4	1.13	0.194	n.s.
Body Mass Index ($kg.m^{-2}$)	24.4	0.43	0.670	<0.001
Age (yr)	52.6	2.11	0.379	<0.001
Fat mass (kg)	18.7	0.84	0.785	<0.001
%fat	27.1	1.02	0.827	<0.001
NEFA ($\mu mol.l^{-1}$)	539	24.7	0.280	<0.05
Glycerol ($\mu mol.l^{-1}$)	90	4.7	0.483	<0.001
Plasma leptin ($ng.ml^{-1}$)	7.3	0.96		

r is the Pearson correlation coefficient between plasma leptin concentration and the variable

termine which variables contributed independently to variation in plasma leptin concentrations. Analyses of covariance were used to compare regression lines between sexes. Statistical analyses were performed with Statview 4 statistical package (Abacus Concept, Inc, CA). Significance was considered at the 5% level.

Results

Table 1 displays body composition data and metabolic parameters, and the correlation coefficient between plasma leptin concentration and each variable considered. A significant relationship was observed between plasma leptin and both glycerol and NEFA (Fig. 1). Plasma leptin concentration was better correlated with %fat (r = 0.83) than with fat mass (r = 0.79) or BMI (r = 0.67). However, as shown in Fig. 1 the plasma leptin concentration-%fat plot was best fitted with an exponential (r^2 = 0.82).

Individual relationships between BMI, fat mass, %fat, age, glycerol or NEFA, and log plasma leptin concentrations had an R^2 equal or superior to the R^2 of the equivalent relationship with plasma leptin. Since most of these variables were correlated with each other (for example, fat mass, %fat, and age....) a partial correlation was performed which established that 4 variables contributed independently to the variation in log plasma leptin concentration: BMI, %fat, sex, and glycerol concentration. The corresponding model is displayed in Table 2 with an adjusted R^2 of 0.895. Absolute fat mass, age, and NEFA were no longer independent predictors.

The slope of the relationship between log plasma leptin concentration and %fat was steeper in women than in men (0.091±0.010 vs. 0.072±0.007, p<0.05, Fig. 2 top panel).

The slopes of the relationships between log plasma leptin and glycerol concentrations are not significantly different between women and men. However, the mean log plasma leptin concentration adjusted for differences in glycerol concentrations was higher in women than in men (2.24±0.16 vs. 0.94±0.08, P<0.001, analysis of covariance, Fig. 2 bottom panel).

Discussion

The present study shows that across a wide range of ages, plasma leptin concentration can be predicted by 4 independent parameters, i.e., BMI, %fat, sex, and plasma glycerol concentration. These results are important since there is variability in leptin levels at each level of BMI or %fat, suggesting that factors other than BMI or %fat may regulate leptin concentrations.

The relationship between %fat or BMI and plasma leptin concentration has already been mentioned, both in

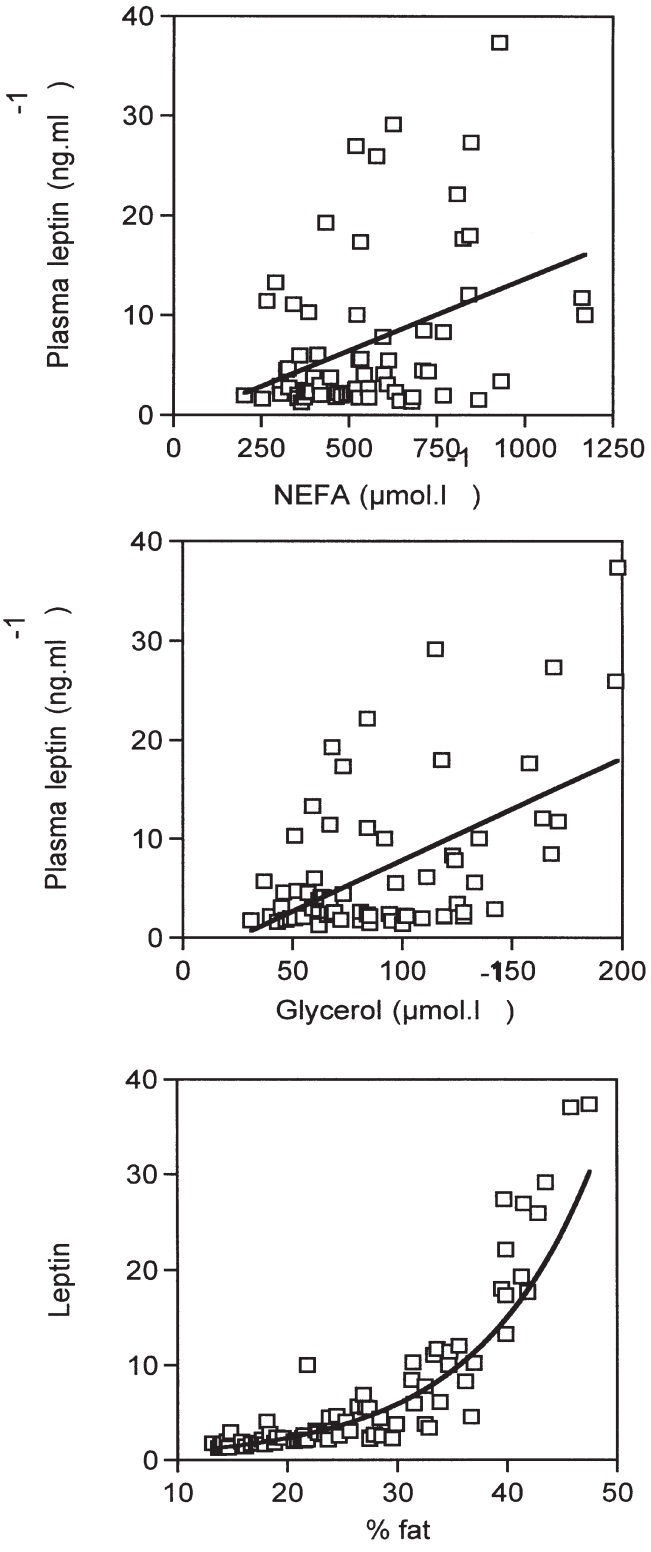

Fig. 1 Relationship between plasma leptin concentration and nonesterified fatty acids concentration (NEFA, top panel), glycerol concentration (middle panel), and %fat (bottom panel). The thick lines correspond to the best fit.

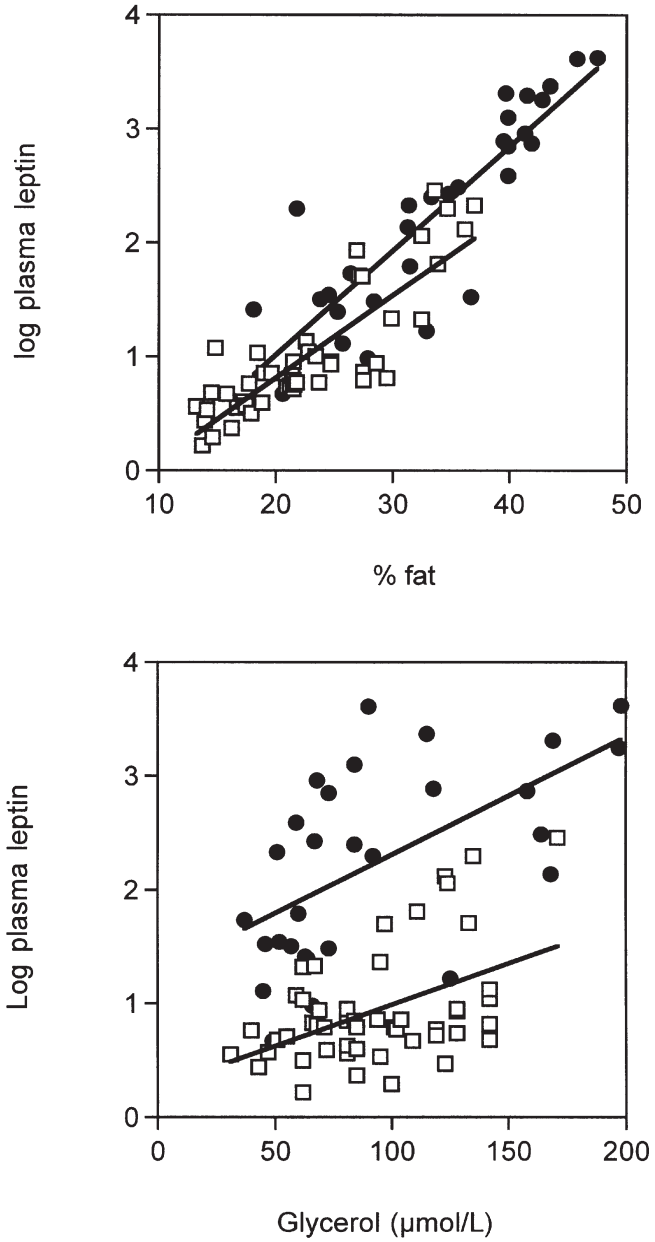

Fig. 2 Relationship between log plasma leptin concentration and %fat (top panel), and glycerol concentration (bottom panel) in men (□) and women (●). The thick lines correspond to the linear fits.

obese (20, 46) and lean subjects (6, 15), both in children (1, 3, 27) and adults (23, 25). However, although it appears that plasma leptin concentration is better correlated to %fat than to BMI (6, 20), the present study shows that both BMI and %fat contribute independently to the variation in plasma leptin concentration. This might be because ^{18}O labeled water for the measurement of body composition is a very accurate and precise probe to measure total body water, hence body composition (30). The present study suggests that parameters related to adiposity (%fat and BMI) are involved in the between-subject variance in plasma leptin concentration and that fat mass per se does not contribute independently of %fat to the variation.

Table 2 Model for log leptin concentrations

Variables	R^2	Residual SD
%fat (1)	0.816	0.175
(1) + sex (2)	0.843	0.163
(2) + BMI (3)	0.885	0.140
(3) + glycerol	0.895	0.135

Glycerol concentration, i.e., a parameter of lipolysis, is positively correlated to plasma leptin concentration. Although in simple regressions both NEFA and glycerol are correlated with plasma leptin, NEFA is no longer an independent parameter in multiple regressions. This can be because glycerol is a better indicator of lipolysis than NEFA (16). It is unlikely that glycerol could have a direct stimulatory effect on leptin secretion since lipolysis implies a state of negative energy balance, while leptin is known to decrease energy intake in animals (24), increase energy expenditure (24, 27), hence promoting negative energy balance. There would be no possible regulation along this loop. It is more likely that lipolysis is the consequence of a leptin-induced negative energy balance, unless leptin directly stimulates lipolysis. Leptin was shown to increase the rate of lipolysis in isolated white adipocytes from Zucker rats (38) and mices (10, 11). However, there is still no evidence that hormone-sensitive lipase is regulated by leptin concentration. Moreover, Shimabukuro et al. (33) have shown that Wistar rats made chronically hyperleptinemic failed to display increased lipolysis and/or ketogenesis despite a rapid lipid depletion. Lipolysis could therefore be the metabolic consequence on adipocytes of a factor or hormone involved in a feedback loop regulating leptin secretion. Hormone-sensitive lipase is regulated by cAMP-dependent protein kinase activity (12). The latter is the result of a balance between stimulatory ß-adrenergic influences and inhibitory influences arising from insulin, adenosine or α-adrenergic compounds (7).

Insulin has been proposed as a good signal to regulate plasma leptin concentration since it stimulates the expression of the *ob* gene in adipocytes (12, 28). However, although correlations are often found between leptin and fasting insulin concentrations (8, 19, 31), controversy remains as to whether insulin is capable of influencing plasma leptin concentration and the *ob* gene expression in vivo (8, 17, 19, 26, 31, 40, 42). For the feedback loop, leptin inhibits insulin binding in isolated white adipocytes (43). Leptin also inhibits insulin secretion both directly at

the pancreatic islet level (9) and indirectly by reducing neuropeptide-Y concentrations in the brain (36).

Norepinephrine could be a signal in the loop. Gettys et al. (12) have shown on isolated white adipocytes that insulin-stimulated leptin secretion was inhibited by β3-adrenergic receptor agonists. Norepinephrine and isoproterenol decrease the level of leptin mRNA in adipocytes (18, 38) while β3-adrenergic receptor agonists reduce the *ob* gene expression and circulating leptin levels (5, 21). However, decreased sympathetic output by using an inhibitor of catecholamine synthesis was not associated with decreased leptin secretion in men and women (45). For the feedback loop, plasma leptin concentration was positively correlated with muscle sympathetic nerve activity in men (35). Moreover, leptin stimulates sympathetic outflow from brown adipose tissue (5) and increases sympathetic activity by reducing neuropeptide-Y secretion (2). Therefore, increased glycerol concentration could be the metabolic consequence of the regulatory action of norepinephrine and insulin on leptin secretion.

Although a higher plasma leptin concentration has been demonstrated in women compared to men (13, 23) no satisfactory explanation has been forwarded. Blum et al. (1) have shown that plasma leptin concentration is inversely related to testosterone concentration in boys but not in girls. Moreover, they have shown that gender difference in plasma leptin concentration appears in late puberty and adolescence, plasma leptin concentration increasing in girls and decreasing in boys after Tanner stage 2. However, plasma leptin concentration was inversely related to testosterone concentration in diabetic men but not in healthy men (39). This sex-related difference cannot be explained by a difference in body fat since the relationship between %fat and plasma leptin concentration was steeper in women.

Finally, age does not appear to be an independent predictor of log plasma leptin concentration; this is in agreement with the findings from Perry et al. (25) but not with those from Ostlund et al. (23).

In conclusion, the present study shows that across a wide range of age, plasma leptin concentration is correlated with parameters of fatness, sex, and lipolysis. Sympathetic activity and insulin should be considered as potential signals for a leptin feedback loop.

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