Associations of Leptin With Body Fat Distribution and Metabolic Parameters in Non–Insulin-Dependent Diabetic Patients: No Effect of Apolipoprotein E Polymorphism

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Leptin levels have been shown previously to be associated with anthropometric parameters such as the body mass index (BMI), total body fat, and subcutaneous fat. Since apolipoprotein E (apoE) polymorphism is known to be a genetic marker affecting the relationship between certain anthropometric and metabolic parameters, we evaluated whether the leptin level and/or associations between the leptin level and body composition in non–insulin-dependent diabetic patients could be determined by apoE polymorphism. In 171 type 2 diabetic patients (105 male and 66 female), body composition (BMI, waist to hip ratio [WHR], fat mass, and visceral fat) was measured and fasting blood samples were obtained to determine the apoE genotype, leptin, glucose, and insulin levels, and the lipid profile. The mean leptin level for the whole group was 11.7 ± 9.3 ng/mL, with a significant difference ($P < .001$) between men (7.1 ± 4.9 ng/mL) and women (19.0 ± 10.1 ng/mL). No difference was found for leptin levels or anthropometric variables between the 3 different apoE genotypes (E3/E3 homozygotes, E2 carriers, and E4 carriers). Only low-density lipoprotein (LDL) cholesterol was significantly different between the 3 apoE subgroups. The correlations of leptin with anthropometric variables, especially visceral fat, tended to be different between the 3 apoE groups, but this was not independent and no effect was found after controlling for the other parameters in the model. A multiple regression model containing gender, subcutaneous fat, fasting glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol explained 81% of the variance in leptin levels. We conclude that apoE polymorphism has no effect on the leptin level or its associations with other anthropometric and metabolic parameters.
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LEPTIN, the product of the *ob* gene, is mainly an adipocyte-secreted hormone with an important role in the regulation of body weight in humans.[1] Serum leptin levels are strongly associated with parameters of body composition like the body weight, body mass index (BMI), and total body fat.[2] Despite this strong association, large variations in leptin levels are observed for a certain BMI or fat mass which cannot be explained by weight or body composition.[2] Leptin concentrations were previously reported not to differ in diabetics compared with matched nondiabetics, and the associations with obesity parameters are also similar in diabetics.[3] In type 2 diabetic patients, elevated serum and very–low-density lipoprotein (VLDL) triglycerides are the most frequent lipid abnormality.[4] Apolipoprotein E (apoE) has a central role in the metabolism of triglyceride-rich lipoproteins (VLDL and chylomicron remnants), where it acts as a receptor-binding ligand. ApoE in humans exhibits a genetic polymorphism, with 3 major isoforms, E2, E3, and E4, of which apoE3 is the most prevalent. The 6 most common apoE genotypes are E2/E2, E3/E3, and E4/E4 homozygotes, and E2/E3, E3/E4, and E2/E4 heterozygotes. It has been reported that these various genotypes carry a different risk profile for atherosclerosis, because of differences in the affinity for lipoprotein receptors in the liver (low-density lipoprotein [LDL] and remnant receptor) between the apolipoprotein types.[5] ApoE4 carriers show increased triglycerides, total serum cholesterol, and LDL cholesterol and are predisposed to coronary heart disease.[5] The apoE2 isoform is also associated with hypertriglyceridemia but lower cholesterol values.[5]

With a higher BMI and fat mass, the risk for dyslipidemia and the development of atherosclerosis increases. Especially, a predominantly abdominal fat distribution is associated with hypertriglyceridemia and hyperinsulinemia.[6] In type 2 diabetic patients, who are generally overweight, an increased risk for the development of coronary and peripheral atherosclerotic disease is often observed, especially in the case of predominantly abdominal fat accumulation.[7]

It has been shown previously that apoE polymorphism can influence the associations between certain anthropometric and metabolic variables. Pouliot et al[8] showed that the relation of abdominal obesity to plasma triglycerides was altered by apoE polymorphism. Després et al[9] found different relationships between insulin and plasma lipoproteins for women with the apoE ϵ4 allele compared with apoE3 homozygotes. Uusitupa et al[10] showed that apoE polymorphism could modify central obesity–induced changes in serum lipids, insulin, and blood pressure. It may be expected that apoE polymorphism can also influence the relationship of body fat distribution to other anthropometric variables. We hypothesized that such a genetic polymorphism could be a factor in altering the associations between anthropometric or metabolic variables and leptin levels.

Since body composition and fat distribution are important measures for assessing the metabolic risk in non–insulin-dependent diabetic patients, and since leptin levels are associated with anthropometric variables, especially body fat, we investigated the possibility that apoE might determine the associations between leptin and body composition, specifically

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visceral fat, in type 2 diabetic patients. We also wanted to evaluate whether it is possible to use apoE polymorphism as a genetic marker to identify certain subgroups of patients in whom leptin could be more predictive for certain diabetes-associated biological parameters such as insulin, visceral fat, and triglycerides.

SUBJECTS AND METHODS

Subjects

Non–insulin-dependent diabetic patients known to have type 2 diabetes for at least 2 years were recruited from the outpatient clinic of the Department of Endocrinology at University Hospital Antwerp, Belgium. A total of 171 patients were included in this study, 105 males and 66 females. Most of them were treated with oral hypoglycemic agents (sulfonylurea or metformin, or a combination of both). Eleven subjects used no medication and were on dietary treatment only. Insulin-treated patients were excluded. Patients were seen by a physician for a physical examination, and a medical history was recorded. None of the subjects were taking any medication known to influence appetite behavior or were treated for other specific endocrine diseases such as Cushing's disease or hypothyroidism. The subjects' body weight was stable for at least 3 months before the study measurements.

The study protocol was approved by the Medical-Ethical Committee of the University Hospital Antwerp, and patients provided informed consent.

Anthropometric Measurements

All anthropometric measurements were performed in the morning with the patients in the fasting condition and undressed. Height was measured to the nearest 0.5 cm, and body weight was measured with a digital scale to the nearest 0.1 kg. The BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in the upright position at the midpoint between the lower rib margin and the iliac crest, and hip circumference at the level of the trochanter major. The waist to hip ratio (WHR) was calculated from these 2 circumferences. Fat mass (in kilograms and percent total body fat) was determined by a bioimpedance measurement with a body composition analyzer (BIA-101, RJL Systems, Detroit, MI) as described by Lukaski et al[11] and calculated using the formula of Deurenberg et al.[12] A computed tomographic (CT) scan was performed in 157 subjects at the L4-L5 level as described previously to determine visceral and subcutaneous fat.[13,14]

Blood Sampling

A fasting blood sample was obtained to determine leptin levels and metabolic parameters. For leptin measurement, a serum sample was frozen and stored at −80°C until the samples were analyzed in batch. Serum leptin levels were measured using a radioimmunoassay (RIA) as described previously.[15] The antibody used in the RIA was a polyclonal antibody raised in rabbits against highly purified recombinant human leptin. The limit of detection of this assay was 0.5 ng/mL.

To evaluate glucose metabolism, fasting glucose, insulin, and C-peptide levels were measured in 163 subjects. Serum insulin and C-peptide levels were determined by a RIA using the Pharmacia (Uppsala, Sweden) Insulin RIA 100 and the Behring (Marburg, Germany) RIA-gnost assays, respectively. Hemoglobin A_{1c} (HbA_{1c}) was detected by photometry after cation-exchange high-performance liquid chromatography (Bio-Rad, Richmond, CA). For lipid measurements, another fasting serum sample was taken in 165 subjects to measure total, LDL, and high-density lipoprotein (HDL) cholesterol and triglyceride levels as described previously.[16]

ApoE genotype was determined on a DNA sample using a polymerase chain reaction technique. Amplification was achieved with the primers described by Wenham et al.[17]

Statistical Analysis

Subjects were classified into 3 groups for data analysis according to apoE genotype: apoE2 group, all subjects with the apoE2/E3 genotype (there were no subjects homozygous for E2); apoE3 group, only subjects homozygous for apoE3/E3; and apoE4 group, all E4 carriers, ie, subjects who either were homozygous for apoE4/E4 or had the apoE3/E4 genotype. One patient with the apoE2/E4 genotype was not included for analysis. Comparisons between the 3 apoE groups were performed by ANOVA, with the Bonferroni test for multiple comparisons. The homogeneity of variances was tested with the Levene test, and variables with heterogenous variances were tested with the nonparametric Kruskal-Wallis test. Differences were considered significant at a P level less than .05.

Differences between men and women were tested with a t test. Studentized residuals of leptin after linear regression were used to test differences after correction for fat mass. Pearson's correlation coefficients were calculated between leptin and anthropometric variables. Since leptin levels were not normally distributed, leptin values were logarithmically transformed before these analyses, which produced a normal distribution. Since leptin is strongly determined by fat mass, partial correlations controlling for fat mass were calculated between leptin and metabolic parameters (lipids and glucose).

Multiple regression analysis was performed to identify the most important determinants of leptin. ApoE genotype was factorized for this analysis, using 2 "dummy" variables to account for the 3 categories. Only variables with a P level less than .1 are represented in the tables. All statistical analyses were performed using STATISTICA, 1999 edition (StatSoft, Tulsa, OK).

RESULTS

Subject Characteristics

Characteristics of the patients are listed in Table 1. The data are presented as the mean ± SD. The mean age of this study population was 59 ± 10 years and the mean known duration of diabetes 8 ± 7 years. The BMI was 28.9 ± 5.1 kg/m^2.

The mean serum leptin level reached 11.7 ± 9.3 ng/mL.

Table 1. Characteristics of 171 Type 2 Diabetic Men and Women in the Study

Characteristic	Men (n = 105)	Women (n = 66)	P
Leptin (ng/mL)	7.1 ± 4.9	19.0 ± 10.1	.000
Age (yr)	58 ± 11	60 ± 9	.639
Weight (kg)	84.3 ± 15.1	80.2 ± 14.6	.742
BMI (kg/m^2)	27.7 ± 4.5	30.8 ± 5.3	.035
Waist (cm)	101 ± 13	104 ± 14	.520
WHR	1.01 ± 0.10	0.99 ± 0.11	.198
Fat mass (%)	26.8 ± 9.0	42.7 ± 7.3	.001
Visceral adipose tissue (cm^2)	207 ± 86	212 ± 78	.505
Subcutaneous adipose tissue (cm^2)	287 ± 130	430 ± 126	.001
Subcutaneous/visceral fat	1.52 ± 0.69	2.21 ± 0.87	.004
HbA_{1c} (%)	8.3 ± 1.7	8.1 ± 1.6	.657
Fasting glucose (mg/dL)	184 ± 50	173 ± 57	.891
Fasting insulin (μU/mL)	15.7 ± 9.0	19.4 ± 11.7	.173
Fasting C-peptide (nmol/L)	1.08 ± 0.43	1.23 ± 0.54	.452
Total cholesterol (mg/dL)	225 ± 39	229 ± 41	.633
LDL cholesterol (mg/dL)	165 ± 38	168 ± 40	.201
HDL cholesterol (mg/dL)	38 ± 11	46 ± 14	.138
Triglycerides (mg/dL)	199 ± 135	188 ± 137	.350

Women had significantly higher leptin levels than men (19.0 ± 10.1 *v* 7.1 ± 4.9 ng/mL). This difference remained significant ($P < .001$) after correction for total fat mass. Anthropometric parameters were also different for men and women; especially the percent fat mass and amount of subcutaneous fat were significantly higher in women. The mean WHR reached 1.00, and 71% of the study group had an abdominal fat distribution (defined by WHR ≥ 1 for men and ≥0.85 for women): 91% of the women and 58% of the men showed this type of fat partitioning.

ApoE Genotypes

The apoE genotype frequencies were, respectively, 22 in the apoE2 group, 109 apoE3 homozygotes, and 40 subjects in the apoE4 group. The allele frequencies were 0.80 for the ε3 allele, 0.06 for the ε2 allele, and 0.14 for the ε4 allele.

Leptin levels did not differ among the 3 apoE groups. Most anthropometric variables like weight, BMI, waist circumference, fat mass, abdominal fat, and subcutaneous fat tended to be lower in the apoE4 group, but these differences were not significant (Table 2). In men only, a borderline value was found ($P = .056$) for differences in fat mass between the 3 groups (data not shown).

LDL cholesterol was significantly different in the 3 groups ($P = .004$), with apoE2 showing the lowest value and apoE4 the highest. This was only significant in women ($P = .0002$), not in men. Total cholesterol was also significantly different among the 3 groups in women only ($P = .006$). No differences were found in glucose and insulin levels.

Table 2. Characteristics of the Three Groups Classified on the Basis of the ApoE Polymorphism

Characteristic	ApoE2 (n = 22)	ApoE3 (n = 109)	ApoE4 (n = 40)	ANOVA (*P*)
Leptin (ng/mL)	13.5 ± 10.8	11.4 ± 8.9	11.3 ± 9.8	.46*
Age (yr)	61 ± 9	58 ± 11	58 ± 9	.98†
Weight (kg)	86.4 ± 18.6	83.0 ± 15.2	80.1 ± 11.8	.73†
BMI (kg/m²)	30.5 ± 5.8	28.9 ± 5.0	27.9 ± 4.6	.16
Waist (cm)	105 ± 16	103 ± 14	99 ± 11	.20
WHR	1.01 ± 0.12	1.01 ± 0.10	0.99 ± 0.10	.46
Fat mass (%)	36.9 ± 10.1	32.8 ± 11.6	31.0 ± 11.5	.16
Visceral fat (cm²)	220 ± 76	209 ± 86	203 ± 76	.75
Subcutaneous fat (cm²)	371 ± 154	346 ± 146	316 ± 140	.38
HbA_{1c} (%)	8.3 ± 1.6	8.4 ± 1.6	7.9 ± 1.6	.33
Fasting glucose (mg/dL)	171 ± 44	180 ± 53	182 ± 59	.72
Fasting insulin (μU/mL)	17.5 ± 10.5	17.4 ± 10.4	16.2 ± 10.2	.82
Fasting C-peptide (nmol/L)	1.15 ± 0.55	1.16 ± 0.47	1.09 ± 0.46	.77
Total cholesterol (mg/dL)	211 ± 41	228 ± 41	231 ± 35	.15
LDL cholesterol (mg/dL)	141 ± 36	168 ± 40	174 ± 31	.004
HDL cholesterol (mg/dL)	43 ± 12	40 ± 12	44 ± 14	.20
Triglycerides (mg/dL)	246 ± 175	196 ± 140	165 ± 85	.85†

*ANOVA on log-leptin because of skewed distribution.
†Kruskal-Wallis test because of heterogeneity of variances.

Table 3. Correlations of Log-Leptin With Anthropometric and Metabolic Parameters

Parameter	All Patients (N = 171)	ApoE2 (n = 22)	ApoE3 (n = 109)	ApoE4 (n = 40)
Weight	.43‡	.36	.44‡	.43†
BMI	.71‡	.48*	.75‡	.74‡
Waist	.62‡	.42	.67‡	.62‡
WHR	.36‡	.23	.42‡	.28
Percent fat mass	.82‡	.65†	.84‡	.80‡
Visceral fat	.45‡	.02	.58‡	.20
Subcutaneous fat	.82‡	.74‡	.82‡	.85‡
Subcutaneous/visceral fat	.38‡	.67†	.25*	.55†
HbA_{1c}	−.02	−.35	.03	−.02
Fasting glucose	−.03	−.24	−.01	.03
Fasting insulin	.50‡	.26	.49‡	.64‡
Fasting C-peptide	.51‡	.13	.55‡	.63‡
Total cholesterol	.07	−.13	.17	−.06
LDL cholesterol	.06	−.18	.22*	−.22
HDL cholesterol	−.03	.14	−.04	−.08
Triglycerides	.16*	.01	.12	.45†

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Leptin and Anthropometric Variables

For all patients, significant correlations were found between leptin and all anthropometric variables measured (Table 3). These were strongest for the percent fat mass and subcutaneous abdominal fat (both $r = .82$). Although correlations with both subcutaneous and visceral fat were found in men and women, the relation with visceral fat remained significant after correction for total body fat only in men ($P = .01$), not in women, whereas the relation with subcutaneous fat remained significant in both sexes ($r = .39$, $P < .001$ in men and $r = .43$, $P = .001$ in women).

These correlations were slightly different among the 3 apoE genotypes (Table 3). In the apoE3 group, significant correlations with leptin were found for all anthropometric variables. In contrast, in the apoE2 and apoE4 groups, correlations with leptin were only present for the BMI, percent body fat, and subcutaneous fat. The associations of leptin with visceral fat were only significant in the apoE3 homozygotes. The heterogeneity between the apoE groups is illustrated in Fig 1 for the relationship of leptin with visceral fat and with subcutaneous fat in men and in women.

Leptin and Metabolic Parameters

Metabolic regulation as measured by HbA_{1c} was not correlated with leptin levels, nor were glucose levels (Table 3). Significant correlations were found with insulin and C-peptide in all subjects, as well as the apoE3 and E4 groups separately. These persisted after adjustment for total fat mass, and remained especially high in the apoE4 group (with correlation coefficients of .58 and .57, respectively, *P* for both = .001).

For lipids, a weak correlation between leptin and LDL cholesterol was found in the apoE3 homozygotes ($r = .22$, $P < .05$), while a stronger correlation with triglycerides was found in the apoE4 carriers only ($r = .45$, $P < .01$). After adjustment for fat mass, an inverse relation with LDL choles-

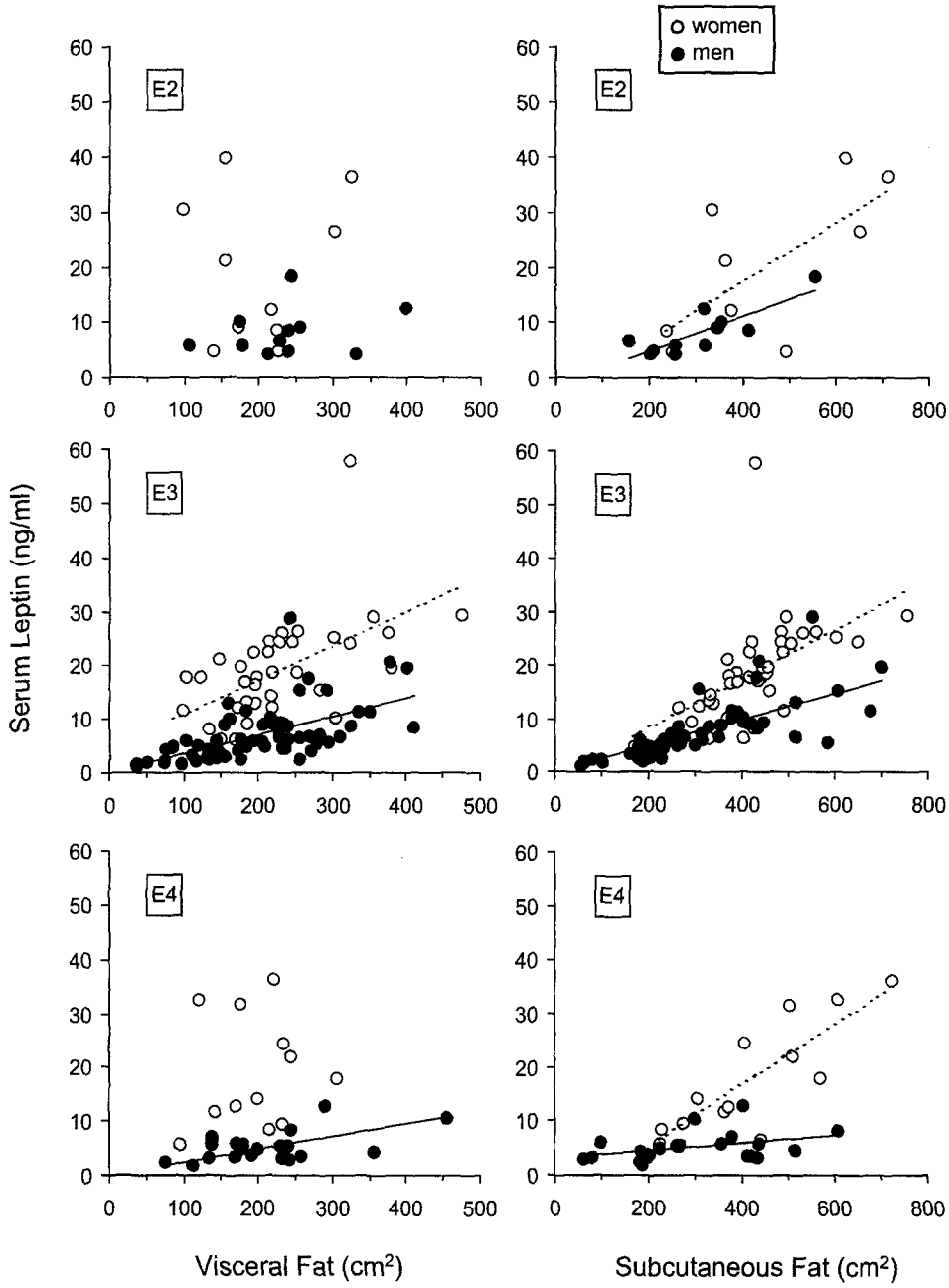

Fig 1. Relationship between the serum leptin level (expressed as log-leptin) and visceral and subcutaneous fat area, measured by CT scan in 157 type 2 diabetic patients, shown by apoE genotype.

terol became significant in the apoE4 group ($r = .38$, $P < .05$), while the correlation in the apoE3 group was attenuated ($r = .18$, $P = .08$). The association with triglycerides in the apoE4 group was dependent on fat mass, and thus disappeared after correction for fat mass.

Multiple Regression Analyses

In a multiple regression analysis, 81% ($R^2 = .81$) of the variance in leptin was explained by a model containing gender, the amount of subcutaneous fat, HDL cholesterol, triglycerides, fasting glycemia, and percent fat mass (Table 4). ApoE polymorphisms did not contribute to this. In a backward regression analysis, gender and intraabdominal fat distribution, both subcutaneous fat and visceral fat, proved to be the most important determinants for leptin, together explaining 76% of the variance.

Effect of Diabetes Treatment

The distribution of treatment was different in the apoE2 group compared with the apoE3 and E4 groups (Table 5). However, we found no differences in leptin between the groups receiving different oral antidiabetic treatment: the mean leptin levels were, respectively, 10.0 ng/mL in the group treated with sulfonylurea, 10.9 ng/mL with metformin, and 10.3 ng/mL with combined therapy. Only pharmacotherapy-naive patients had higher leptin levels (17.4 ng/mL, P for ANOVA with 4 treatment groups, including this last group on diet = .096). When treatment was included as an additional parameter in

Table 4. Multiple Regression Analysis With Log-Leptin as a Dependent Variable (N = 136, R^2 of the model = .81)

Parameter	Beta	T	P
Gender	−0.363	−4.67	.000008
Subcutaneous fat mass	0.374	3.39	.000948
HDL cholesterol	−0.183	−2.70	.008
Triglycerides	−0.201	−2.61	.010
Fasting glycemia	−0.136	−2.17	.032
Percent fat mass	0.180	1.95	.053
ApoE3	−0.043	−0.68	.50
ApoE4	−0.041	−0.63	.53

multiple regression analysis, there was no significant effect on leptin levels.

DISCUSSION

The main question of this study was whether apoE polymorphism could have an effect on leptin levels, and whether this genetic factor could influence the relationship between leptin and several anthropometric and metabolic parameters, mainly visceral fat, insulin, and lipids. No effect of apoE polymorphism on leptin levels was found. Leptin levels were not significantly different among the 3 apoE subgroups, and apoE was not a significant parameter for leptin in multiple regression analysis.

Our second objective was to evaluate whether the apoE genotype could influence the relations between leptin and anthropometric or metabolic parameters. Leptin has been shown to be associated with body composition. High correlations with the BMI and body fat were found in all human studies.[2,18-21] Among our cohort of obese and non-obese type 2 diabetics, very strong correlations were found between leptin and the BMI, body fat, and abdominal fat measured by CT scan. We measured visceral fat by CT scan at the L4-L5 level, a method that gives an almost correct measure of visceral adipose tissue.[13] With this method, we previously found a very strong correlation between leptin and subcutaneous fat in obese women, while the association of leptin with visceral fat was less strong and not independent of total body fat.[22] Our data in type 2 diabetics confirm these findings: we also found a strong relationship between the serum leptin level and the subcutaneous fat mass rather than visceral fat, which was confirmed by the results of multiple regression analyses. In addition, a gender difference was found, since the association of leptin with visceral fat remained significant in men even after adjustment for fat mass, whereas it disappeared in women.

Despite the fact that apoE had no effect on leptin levels per se, the correlations of leptin with anthropometric variables seemed different when compared among the 3 apoE groups. For the apoE3 homozygotes, correlations were similar to those found for all subjects. This could partly be attributed to the high prevalence of apoE3. While a significant correlation with measurements of body composition (total body fat and total abdominal fat) is found in all 3 groups, correlations with measures of central fat accumulation like the WHR and visceral fat were only significant in apoE3 homozygotes. Although comparisons among the different apoE groups are hampered by the smaller number of subjects in the apoE2 and E4 carriers, some relationships are clearly different among the groups. This is especially the case for the waist circumference, WHR, and visceral fat, for which significant correlations with leptin are found in the apoE3 group, but not in the apoE2 and apoE4 groups. Since abdominal fat, and visceral fat in particular, is considered a risk factor for cardiovascular disease, leptin levels could be an indication of cardiovascular risk in subjects with apoE3 (most frequent), but not apoE2 (negative correlation with visceral fat, while correlation with subcutaneous fat is very high) or apoE4 (very low correlation with waist circumference). This is consistent with the analyses of the lipid data, where leptin is only associated with LDL cholesterol in the apoE3 group.

The distribution of apoE genotypes in type 2 diabetics seems similar to that in nondiabetic populations.[4] Our data show a similar distribution to those previously reported for our population.[23] This implies that a relatively low number of subjects with $\epsilon 4$ and especially $\epsilon 2$ alleles were included, since the natural occurrence of these alleles is low. This could be a partial explanation for the lower correlations in these subgroups.

Evidence for a physiological relationship between leptin and insulin is increasing. Correlations between both biological parameters are observed in human studies. In vitro, an increase in leptin secretion by isolated adipocytes is found when insulin is added to the medium.[24-27] In rats, hyperinsulinemia induces increased mRNA expression and plasma leptin levels.[28,29] In humans, leptin levels increase during a long-term hyperinsulinemic clamp.[25,30] On the other hand, leptin seems to exert insulin- and glucose-lowering effects. In mice treated with leptin, a decrease in glucose and insulin is found, which can only partially be explained by the weight loss.[31,32] However, the exact mechanisms are not yet clear. An important finding in this matter is the identification of leptin receptors on pancreatic β cells,[33] by which leptin can influence insulin secretion.[34-38] The leptin receptor was also found on human hepatocytes, where leptin can cause attenuation of several insulin-induced activities.[39] Després et al[9] showed that apoE polymorphism can modify the relationship of hyperinsulinemia to hypertriglyceridemia. Since leptin and insulin are associated, we evaluated whether apoE could also have an influence on this association.

Serum leptin levels were reported previously to be associated with fasting insulin levels in normal healthy subjects, but not in subjects with impaired glucose tolerance.[40] In our study group of type 2 diabetic patients treated with oral antidiabetics or with diet alone, we found strong correlations between leptin levels and fasting serum insulin and C-peptide, but no relation at all with glucose or HbA_{1c} levels as a measure of metabolic regulation. These correlations were independent of fat mass, and no difference was found between the 3 apoE groups. Glucose and insulin levels were also significant predictors of

Table 5. Distribution of Treatment Groups According to ApoE Genotype

Treatment	ApoE2 Carriers	ApoE3/E3	ApoE4 Carriers
No oral antidiabetics	—	10%	6%
Sulfonylurea	53%	26%	26%
Metformin	26%	15%	18%
Metformin + sulfonylurea	21%	49%	50%

leptin in a multiple regression analysis, while a trend was found for HbA_{1c}.

It has also been suggested that leptin levels in diabetic patients are affected by diabetes treatment, due to the differential effects of metformin and sulfonylureas on insulin, the former improving insulin sensitivity and the latter stimulating insulin secretion. We have therefore checked whether a possible confounding effect of diabetes treatment could have influenced our results. Although the treatment groups were not evenly distributed over the 3 apoE groups, we presume that this should not have an effect on our results firstly because in each group about 75% of the patients received sulfonylureas, and secondly because treatment did not seem to influence leptin levels in our group. Leptin levels were comparable in the 3 treatment groups receiving oral antidiabetic medication. Only the group on diet showed higher leptin levels, partly explained by a higher BMI and probably also by a better β-cell function in this group of patients.

The apoE genotype is known to be associated with the lipid profile. ApoE4 carriers show increased cholesterol levels and are therefore at greater risk for coronary heart disease, while the apoE2 isoform is associated with hypertriglyceridemia but lower cholesterol values.[5] This was also found in this diabetic population, although the apoE4 group showed the lowest triglyceride levels, probably due to slightly lower weight and fat mass, although these differences were not significant. The only significant difference found among the 3 groups was LDL cholesterol, with apoE4 carriers showing the highest LDL levels. It was also in this apoE4 group that associations with leptin were found: a strong correlation was found with triglycerides, but it disappeared after correction for body fat; after adjustment for body fat, an inverse relation with LDL was found in this group.

Not much is known about the relation between leptin and lipids. In some studies, a positive correlation with triglyceride levels was found, and a negative association with HDL cholesterol.[41] Recently, a possible role for leptin in triglyceride storage in fat cells was proposed.[42] Leptin was shown to be an independent predictor for adipose tissue lipoprotein lipase activity.[41] Since apoE also plays a role in the metabolism of triglyceride-rich particles, one could imagine its possible effect on the relation between leptin and lipids, especially LDL and triglycerides. We previously found an association for leptin receptor polymorphisms and LDL cholesterol levels, suggesting a possible role for leptin and its receptor in the lipoprotein metabolism.[43]

Altogether, in this study in type 2 diabetic subjects, we found that 81% of the variability in leptin levels can be explained by the following parameters: gender, percent fat mass and subcutaneous fat, HDL cholesterol and triglycerides, and fasting glycemia. The most important parameters determining leptin in humans are gender and the amount of subcutaneous and visceral fat, together explaining 75% of its variability.

Conclusion

In summary, we conclude that leptin levels are not influenced by apoE genotype. However, leptin is only associated with abdominal fat accumulation in apoE3 homozygotes, in contrast to total body fat, which is associated with leptin in all subjects.

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REFERENCES

1. Caro JF, Sinha MK, Kolaczynski JW, et al: Leptin: The tale of an obesity gene. Diabetes 45:1455-1462, 1996

2. Considine RV, Sinha MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996

3. Haffner SM, Stern MP, Miettinen H, et al: Leptin concentrations in diabetic and nondiabetic Mexican-Americans. Diabetes 45:822-824, 1996

4. Taskinen M-R: Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. Diabetes 41:12-17, 1992 (suppl 2)

5. Davignon J, Gregg RE, Sing CF: Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1-21, 1988

6. Kissebah AH, Vydelingum N, Murray R, et al: Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 54:254-260, 1982

7. Van Gaal LF, Rillaerts E, Creten W, et al: Relationship of body fat distribution pattern to atherogenic risk factors in NIDDM. Preliminary results. Diabetes Care 11:103-106, 1988

8. Pouliot M-C, Després J-P, Moorjani S, et al: Apolipoprotein E polymorphism alters the association between body fatness and plasma lipoproteins in women. J Lipid Res 31:1023-1029, 1990

9. Després J-P, Verdon M-F, Moorjani S, et al: Apolipoprotein E polymorphism modifies relation of hyperinsulinemia to hypertriglyceridemia. Diabetes 42:1474-1481, 1993

10. Uusitupa MIJ, Karhunen L, Rissanen A, et al: Apolipoprotein E phenotype modifies metabolic and hemodynamic abnormalities related to central obesity in women. Am J Clin Nutr 64:131-136, 1996

11. Lukaski HC, Johnson E, Bolonchuk WW, et al: Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 41:810-817, 1985

12. Deurenberg P, Weststrate J, Hautvast J: Changes in fat-free mass during weight loss measured by bioelectrical impedance and by densitometry. Am J Clin Nutr 49:33-36, 1989

13. Van der Kooy K, Seidell JC: Techniques for the measurement of visceral fat: A practical guide. Int J Obes 17:187-196, 1993

14. Degryse H, Kerschot E, De Schepper A, et al: Planimetry on a CT section by means of a histogram. Radiology 165:68, 1987 (suppl, abstr)

15. Ma Z, Gingerich RL, Santiago JV, et al: Radioimmunoassay of leptin in human plasma. Clin Chem 42:942-946, 1996

16. Rillaerts E, Van Gaal L, Xiang DZ, et al: Blood viscosity in human obesity: Relation to glucose tolerance and insulin status. Int J Obes 13:739-745, 1989

17. Wenham PR, Price WH, Blundell G: Apolipoprotein E genotyping by one stage PCR. Lancet 337:1158-1159, 1991

18. Maffei M, Halaas J, Ravussin E, et al: Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155-1161, 1995

19. Dua A, Hennes MI, Hoffmann RG, et al: Leptin: A significant indicator of total body fat but not of visceral fat and insulin insensitivity in African-American women. Diabetes 45:1635-1637, 1996

20. Haffner SM, Gingerich RL, Miettinen H, et al: Leptin concentrations in relation to overall adiposity and regional body fat distribution in Mexican Americans. Int J Obes 20:904-908, 1996

21. Lönnqvist F, Wennlund A, Arner P: Relationship between circulating leptin and peripheral fat distribution in obese subjects. Int J Obes 21:255-260, 1997

22. Wauters M, Mertens I, Considine R, et al: Are leptin levels dependent on body fat distribution in obese men and women? Eat Weight Disord 3:124-130, 1998

23. Muls E, Kempen K, Vansant G, et al: The effects of weight loss and apolipoprotein E polymorphism on serum lipids, apoproteins A1 and B, and Lp(a). Int J Obes 17:711-716, 1993

24. Leroy P, Dessolin S, Villageois P, et al: Expression of *ob* gene in adipose cells; regulation by insulin. J Biol Chem 271:2365-2368, 1996

25. Kolaczynski JW, Nyce MR, Considine RV, et al: Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes 45:699-701, 1996

26. Gettys TW, Harkness PJ, Watson PM: The β_3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 137:4054-4057, 1996

27. Wabitsch M, Jensen PB, Blum WF, et al: Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45:1435-1438, 1996

28. Saladin R, De Vos P, Guerre-Millo M, et al: Transient increase in *obese* gene expression after food intake or insulin administration. Nature 377:527-529, 1995

29. Cusin I, Sainsbury A, Doyle P, et al: The *ob* gene and insulin: A relationship leading to clues to the understanding of obesity. Diabetes 44:1467-1470, 1995

30. Boden G, Chen X, Kolaczynski JW, et al: Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 100:1107-1113, 1997

31. Pelleymounter MA, Cullen MJ, Baker MB, et al: Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. Science 269:540-543, 1995

32. Schwartz MW, Baskin DG, Bukowski TR, et al: Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. Diabetes 45:531-535, 1996

33. Kieffer TJ, Heller RS, Habener JF: Leptin receptors expressed on pancreatic β-cells. Biochem Biophys Res Commun 224:522-527, 1996

34. Emilsson V, Liu Y-L, Cawthorne MA, et al: Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46:313-316, 1997

35. Kieffer TJ, Heller RS, Leech CA, et al: Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β-cells. Diabetes 46:1087-1093, 1997

36. Tanizawa Y, Okuya S, Ishihara H, et al: Direct stimulation of basal insulin secretion by physiological concentrations of leptin in pancreatic β cells. Endocrinology 138:4513-4516, 1997

37. Pallett AL, Morton NM, Cawthorne MA, et al: Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. Biochem Biophys Res Commun 238:267-270, 1997

38. Ookuma M, Ookuma K, York DA: Effects of leptin on insulin secretion from isolated rat pancreatic islets. Diabetes 47:219-223, 1998

39. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. Science 274:1185-1188, 1996

40. Larsson H, Elmstahl S, Ahrén B: Plasma leptin levels correlate to islet function independently of body fat in postmenopausal women. Diabetes 45:1580-1584, 1996

41. Berman DM, Rogus EM, Busby-Whitehead MJ, et al: Predictors of adipose tissue lipoprotein lipase in middle-aged and older men: Relationship to leptin and obesity, but not cardiovascular fitness. Metabolism 48:183-189, 1999

42. Unger RH, Zhou Y-T, Orci L: Regulation of fatty acid homeostasis in cells: Novel role of leptin. Proc Natl Acad Sci USA 96:2327-2332, 1999

43. Wauters M, Mertens I, Van Gaal L, et al: Associations between leptin receptor gene polymorphisms and obesity-related phenotypes in obese women. Int J Obes 23:S30 (O67), 1999 (suppl 5, abstr)