Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults

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Genetic Variation in the Leptin Receptor Gene, Leptin, and Weight Gain in Young Dutch Adults

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Abstract

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Objective: To investigate the association between leptin levels, polymorphisms in the leptin receptor (*LEPR*) gene, and weight gain.

Research Methods and Procedures: From two large prospective cohorts in The Netherlands (n = 17,500), we compared the baseline leptin of 259 subjects who had gained an average of 12.6 kg (range 5.5 to 33 kg) with 277 subjects who kept stable weight (range −2.6 to 3.1 kg) after a mean follow-up of 6.8 years. Three polymorphisms in the *LEPR* gene (Lys109Arg, Gln223Arg, and Lys656Asn) were determined.

Results: Weight gainers had significantly higher baseline leptin levels than those who kept stable weight (odds ratio = 1.27, 95% confidence interval 1.1 to 1.5, per SD increase in $\log_e$-transformed leptin). Weight gainers with the Arg109 or the Arg223 alleles had higher leptin levels compared with the noncarriers of these alleles. Only among men, the association between leptin and weight gain tended to be stronger among those with an Arg223 allele compared with those without this mutation.

Discussion: Relatively high leptin levels predict weight gain, suggesting that leptin resistance plays a role in the development of obesity in the general population. Higher leptin levels for those with a Lys109Arg or Gln223Arg mutation (or a linked other marker) may imply that these subjects have a modified functional leptin receptor. However, the role of these mutations on weight gain is limited.

Key words: weight gain, leptin, polymorphisms, leptin receptor, prospective study

Introduction

Leptin is a hormone that is mainly produced by adipose tissue and binds to receptors in the hypothalamus (1). The discovery of mutations in the leptin gene and its receptor in rodent models of obesity (e.g., *ob/ob* and *db/db* mice) and in rare cases of human morbid obesity indicates that leptin functions as an afferent signal in a negative feedback loop that regulates energy balance (2–6). However, the role of leptin and its receptor in the development of obesity in the general population is less clear. The etiology of common obesity is more complex, because many genetic, metabolic, and environmental factors might interact, but it remains possible that leptin or its receptor is involved.

Particular mutations in the leptin gene in animal models and humans lead to leptin deficiency and extreme obesity (2–6). For common obesity, a similar, but weaker, negative association between leptin and weight might be found. Common obesity, however, is characterized by high, rather than low, levels of leptin, and it has been suggested that obese subjects may be leptin resistant (7). Several mechanisms may contribute to leptin resistance. Diminished leptin receptor signaling due to a mutant leptin receptor might be one of the explanations (8). This has been demonstrated in *db/db* mice and some severely obese humans who are homozygous for a mutation in the leptin receptor, who have extremely high leptin levels (6). Such mutations are extremely rare and cannot be responsible for obesity in the general population. However, this observation suggests that other, less severe disruptions of the normal leptin-signaling pathway might be involved in the development of common obesity (9). Therefore, it is conceivable that more common

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mutations in the leptin receptor (*LEPR*)[1] gene could modify the function of the leptin receptor in such a way that they affect leptin levels in the general population.

We selected, from a large population study, subjects who gained weight in a follow-up period of, on average, 7 years and subjects who kept stable weight. We investigated whether the baseline leptin levels predicted weight gain. In addition, we investigated the association between three polymorphisms in the *LEPR* gene and leptin levels and the modification of the association between leptin and weight gain by these polymorphisms.

Research Methods and Procedures

Subjects were selected from participants in cardiovascular monitoring projects that have been carried out in two towns of The Netherlands, Maastricht and Doetinchem. The design and rationale of the selection of the participants has been described in more detail elsewhere (10). In short, the baseline measurements occurred between 1987 and 1997 at the Municipal Health Services (11). In Doetinchem, a second measurement occurred 6 years after the baseline measurement (1993 to 1997), again at the Municipal Health Service. In Maastricht, the follow-up measurements were ascertained in 1998 by means of a self-administered questionnaire. To exclude, as much as possible, other potential influences on weight change, subjects from these two cohorts ($n = 17{,}743$) who were dieting, were high alcohol consumers, were pregnant, had changed their smoking status recently, suffered from serious illnesses, and those of 40 years and above were excluded. Furthermore, those with a follow-up of less than 4 years, those without an informed consent, a blood sample, or data on weight were also excluded. From the remaining group (19% of the original sample), we selected "high weight gainers" by taking the top decile of the distribution of average weight gain per year ($\geq$1.4 and $\geq$1.3 kg/year for the cohorts of Doetinchem and Maastricht, respectively). We randomly selected an equal sized group of subjects (=stable weight) whose weight remained relatively constant (range: -0.3 to $+0.3$ kg/year). The non-weight gainers were frequency matched for town, sex, age, and smoking status with the weight gainers. Because menopause can be a confounder of the association between leptin and weight gain (12,13), we also excluded in these analyses seven female subjects who had an operation on the womb or ovaries or did not have a period in the last 12 months. Data on leptin levels and DNA polymorphisms were available for 259 weight gainers and 277 non-weight gainers.

Measurements

The examinations at baseline and the second measurement included physical examinations, e.g., anthropometric measurements, a self-administered questionnaire, and blood sampling. However, for those in Maastricht, the second measurement included only a self-administered questionnaire.

Anthropometric Measures. Weight at baseline was measured without shoes and wearing light indoor clothing. For all participants in Doetinchem, weight was remeasured after 6 years in the same season as in the initial measurement. For the participants from Maastricht, it is likely that subjects wore less clothing when measured at home compared with the measurement at the health center. To allow for the weight of clothing, we added 1.5 kg at the self-reported weight. This amount was based on some measurements of the clothes by investigators at the health center. Body mass index (BMI) was calculated as weight divided by squared height. Weight gain was defined as the difference between the weight at baseline and the weight at the second examination. Because the period of follow-up varied (6 years in Doetinchem and 4.0 to 11.3 years in Maastricht), we calculated the average weight gain per year.

Characteristics of the Study Population. The questionnaires at baseline and/or follow-up provided information about history of chronic diseases, alcohol consumption, cigarette smoking, pregnancy, and educational level. Smoking history was categorized into "nonsmokers" (those who had never smoked or stopped smoking more than 5 years before the start of the study), "smokers" (subjects who reported to be a current smoker at baseline as well as at the second measurement), and the remaining group (=subjects who had changed their smoking habits during the two measurements or stopped smoking $<$5 years before the start of the study). This last category was an exclusion category. The educational level, a measure for socioeconomic status, was classified into three categories: low (primary school, lower vocational/general education), medium (intermediate/higher general education, intermediate vocational education), and high (higher vocational education, university).

Leptin. Leptin concentrations were measured in the baseline nonfasting plasma samples, which had been stored at -20 °C for 6 to 14 years. These measurements were done in duplicate by radioimmunoassay (HL-81K kit, Linco Research Inc., St. Charles, MO).

Genotyping. We determined three polymorphisms in the *LEPR* gene: a lysine to alanine substitution at codon 109 (Lys109Arg), a glutamine to arginine substitution at codon 223 (Glu223Arg), and lysine to asparagine at codon 656 (Lys656Asn) (14). Genotyping was done using polymerase chain reaction-restriction fragment length polymorphism analyses. To increase the power of the statistical analyses, for each polymorphism, heterozygotes were combined with the smallest group of homozygotes. For example, subjects with the Lys109/Arg109 or the Arg109/Arg109 genotype of the *LEPR* gene were combined and compared with those with the Lys109/Lys109 genotype.

[1] Nonstandard abbreviations: LEPR, leptin receptor gene; BMI, body mass index.

Table 1. Characteristics of the study population [means (SD) or percentages]

	Men			Women		
	High weight gain (n = 126)	**Stable weight (n = 134)**	***p* value**	**High weight gain (n = 133)**	**Stable weight (n = 143)**	***p* value**
At baseline						
Age (years)	28.2 (5.9)	28.9 (5.6)	*	30.1 (5.9)	29.9 (6.0)	*
Weight (kg)	79.4 (10.9)	76.6 (10.0)	0.03	66.2 (11.1)	63.4 (8.1)	0.02
Height (cm)	1.82 (0.07)	1.80 (0.07)	0.02	1.68 (0.07)	1.67 (0.06)	0.11
BMI (kg/m^2)	24.0 (3.0)	23.7 (2.8)	0.40	23.5 (3.7)	22.9 (2.8)	0.12
Smoking (%)	36.5	35.1	*	33.8	34.3	*
Socioeconomic status (%)						
Low	46.8	41.8		54.9	46.9	
Medium	42.1	38.8		33.1	32.9	
High	11.1	19.4	0.17	12.0	20.3	0.15
At end of follow-up						
Follow-up time (years)	6.9 (1.7)	7.2 (1.9)	0.24	6.7 (1.5)	6.8 (1.6)	0.51
Weight (kg)	92.0 (11.6)	77.0 (10.0)	*	79.1 (12.4)	63.9 (8.1)	*
Weight gain (kg/year)	1.86 (0.54)	0.06 (0.17)	*	1.90 (0.46)	0.08 (0.17)	*
BMI (kg/m^2)	27.9 (3.3)	23.9 (2.8)	*	28.0 (4.0)	23.1 (2.8)	*

* p Values not given; criteria for group selection.

Data Analyses

Differences in characteristics between high weight gainers and non-weight gainers were analyzed with chi-square tests and Student's t tests. Because leptin concentrations and weight gain were not normally distributed, these variables were natural logarithmically transformed ($\log_e$). The association between leptin and weight gain was performed in three ways. First, leptin levels adjusted for baseline BMI were evaluated according to weight gain (yes/no) using analyses of covariance. The (adjusted) logarithmically transformed leptin values were transformed back to normal values. Second, logistic regression analyses were carried out to investigate the association between leptin (per SD increase in the logarithm) and weight gain (yes/no). In addition, among weight gainers, regression analyses were performed to investigate the BMI-adjusted association between leptin and weight gain per year.

Geometric means of leptin levels according to each polymorphism in the *LEPR* gene were also calculated on the basis of analysis of covariance. For each polymorphism separately, we also analyzed these levels according to combinations of two polymorphisms. These analyses were performed separately in weight gainers and those with stable weight.

Finally, we tested whether the association between leptin and weight gain (yes/no) was modified by the genotyping of the *LEPR* gene, by adding the genotype and the interaction term between these genetic factors and leptin to the logistic regression models.

All these associations were adjusted for baseline BMI. Furthermore, all analyses were done separately in men and women and in men and women combined (adjusted for sex), and they were performed using the statistical package SAS (version 8.1; SAS Institute, Cary, NC).

Results

The characteristics of the study population are shown in Table 1. Subjects were, on average, 29.3 years (range 20 to 40) old and had a baseline BMI of 23.5 kg/m^2 (SD $\pm$ 3.1). Subjects with high weight gain increased, on average, 12.6 kg (range 5.5 to 32.9 kg) corresponding with an increase in BMI of 4 units, whereas those with "stable" weight gained, on average, 0.5 kg (range -2.6 to 3.1 kg) during a mean follow-up of 6.8 years. At baseline, weight gainers already had a higher body weight and height (in men) compared with the nonweight gainers. BMI was not significantly different between the two groups (p = 0.40 and 0.12 for men and women, respectively). Weight gainers did not significantly differ in educational level from non-weight gainers.

Table 2. Leptin levels for weight gainers and those who kept stable weight

	Total median (range)	High weight gain, geometric mean (95% confidence interval)	Stable weight, geometric mean (95% CI)	*p* value
Men	$n = 260$	$n = 126$	$n = 134$	
Leptin (μg/L)	3.0 (1.1 to 15.5)	3.4 (3.1 to 3.7)	3.0 (2.7 to 3.3)	0.06
Leptin, adjusted for baseline BMI (μg/L)		3.3 (3.1 to 3.6)	3.0 (2.8 to 3.3)	0.08
Women	$n = 276$	$n = 133$	$n = 143$	
Leptin (μg/L)	9.7 (1.9 to 74)	10.6 (9.6 to 11.8)	9.2 (8.3 to 10.2)	0.05
Leptin, adjusted for baseline BMI (μg/L)		10.2 (9.5 to 10.9)	9.6 (8.9 to 10.3)	0.25
Men and women	$n = 536$	$n = 259$	$n = 277$	
Leptin (μg/L)*	5.8 (1.1 to 74)	6.0 (5.6 to 6.5)	5.3 (4.9 to 5.6)	0.007
Leptin, adjusted for baseline BMI (μg/L)*		5.8 (5.5 to 6.1)	5.4 (5.1 to 5.7)	0.04

* (Also) adjusted for sex.

Leptin and Weight Gain

Leptin levels of women were higher than those of men (median 9.7 and 3.0 μg/L, respectively). These levels were strongly correlated with baseline BMI levels ($r = 0.60$ and 0.72 for men and women, respectively). Table 2 shows the geometric means of the leptin levels for the weight gainers and those who kept stable weight. At baseline, weight gainers had significantly higher leptin levels than non-weight gainers, 3.4 vs. 3.0 and 10.6 vs. 9.2 μg/L for men and women, respectively. These differences could partly be explained by the variation in BMI as an indicator for fat mass. For men, the difference in leptin levels remained borderline statistically significant after adjustment for BMI. The odds ratio for weight gain according to one SD increase in the logarithm of leptin levels was 1.27 (95% confidence interval, 1.07 to 1.51) for the total group of subjects. After adjustment for BMI, this was 1.28 (1.01 to 1.61) for the total group (see Table 3). Table 4 shows the association between leptin and weight gain among the weight gainers. For the male weight gainers, higher leptin levels tended to be associated with more weight gain ($p = 0.02$), whereas among female weight gainers, no association was observed.

Table 3. Odds ratios for the risk of weight gain (yes/no) from baseline to follow-up for SD increase in $\log_e$-transformed leptin levels

	Men		Women		Total
	SD of the natural logarithm	Odds ratio (95% confidence interval)	SD of the natural logarithm	Odds ratio (95% confidence interval)	Odds ratio (95% confidence interval)*
		$n = 260$		$n = 276$	$n = 536$
Risk of weight gain per SD increase in $\log_e$-transformed leptin levels	0.54	1.27 (0.99 to 1.63)	0.62	1.27 (1.00 to 1.61)	1.27 (1.07 to 1.51)
Risk of weight gain per SD increase in $\log_e$-transformed leptin levels, adjusted for baseline BMI		1.32 (0.97 to 1.80)		1.23 (0.87 to 1.74)	1.28 (1.01 to 1.61)

* Also adjusted for sex.

Table 4. Average $\log_e$-transformed weight gain per year from baseline to follow-up per SD increase in $\log_e$-transformed leptin levels among weight gainers

	Men (n = 126)		Women (n = 133)	
	β (95% CI)	p value	β (95% confidence interval)	p value
Average $\log_e$-transformed weight gain per year	0.048 (0.007, 0.089)	0.02	−0.002 (−0.041 to 0.037)	0.90
Average $\log_e$-transformed weight gain per year, adjusted for baseline BMI	0.053 (0.002, 0.104)	0.04	−0.001 (−0.063, 0.062)	0.98

Leptin and Polymorphisms in LEPR *Gene.* Table 5 shows the geometric means of leptin according to the polymorphisms in the *LEPR* gene. In addition, the contrast in leptin between carriers and noncarriers of combinations of two mutations is shown. Statistically significant differences in leptin levels were observed among only the weight gainers. Carriers of the Arg109 allele or the Arg223 allele tended to have higher leptin levels compared with noncarriers of these alleles, $p = 0.02$ and 0.03, respectively. For carriers of the Arg109 and Arg223 alleles, leptin levels were 10% to 20% higher compared with the noncarriers of these two alleles (6.6 vs. 5.6 μg/L, $p = 0.02$). Differences in leptin levels according to the polymorphism at position 656 of the *LEPR* gene were not statistically significant. However, male weight gainers with either the Arg109 allele or the Arg223 allele and the Asn656 allele have (borderline) significantly higher leptin levels compared with those with none of these alleles (4.0 vs. 3.0 μg/L, $p = 0.06$; or 3.6 vs. 2.6 μg/L, $p = 0.02$).

Leptin and Polymorphism in LEPR *Gene and Weight Gain.* Table 3 showed that the risk of being a weight gainer increases with higher baseline levels of leptin. If polymorphisms in the leptin receptor play a role in the leptin signaling, it is likely that the association between leptin and weight gain is modified by mutations in the *LEPR* gene. However, Table 6 shows that the associations between leptin and risk for weight gain were not clearly modified by these mutations. Only among men, the association tended to be stronger among those with an Arg223 allele compared with those without this mutation.

Discussion

In our study population, leptin levels adjusted for BMI were positively associated with weight gain; those with higher leptin levels had a higher risk for gaining weight. Furthermore, among those who gained weight, baseline leptin levels were higher for carriers of the Arg109 or Arg223 mutations in the leptin receptor. The associations were clearer among men than among women. Among men, the association between baseline leptin and weight gain was modified by the Gln223Arg mutation in the *LEPR* gene.

To appreciate these findings, certain aspects of the study must be considered. An advantage of our study was our prospective design to study the association between leptin and weight gain. Because leptin might affect weight gain, and a higher weight could, in turn, influence leptin levels, cross-sectional and longitudinal studies on the role of leptin in the etiology of obesity can generate completely opposite conclusions (15).

Secondly, the issue of potential selection bias must be considered. The participation rate in the original cohort was ~50% (11). It is unlikely, however, that there was selective participation by baseline leptin level or genotype among the weight gainers and non-weight gainers. This is supported by the fact that the distributions of the genotypes were in Hardy-Weinberg Equilibrium.

Thirdly, our findings could be affected by misclassification problems. For the subjects from Maastricht, the weights at the end of the follow-up were self-reported. Because errors in self-reported weight increase with the magnitude of overweight, this misclassification was probably not randomly distributed (16). However, we assumed that the distinction between weight gainers and those who kept stable weight and the ranking in weight gainers were hardly affected by this.

Leptin levels were measured in nonfasting blood samples that were not taken for all subjects at the same time of the day. Because leptin concentrations in humans show a clear diurnal pattern, and this pattern is entrained to meal timing (17), leptin levels measured in our study might be affected by this pattern. However, it is unlikely that these factors differed for weight gainers and weight keepers, or that they were associated with the genotype of the *LEPR* gene. This is confirmed in the follow-up measurements of a subsample of 286 subjects. In this subsample, time of blood sampling or time of last eating was not significantly associated with leptin levels, nor did the weight gainers and weight keepers differ in these factors. Because several studies found that

Table 5. Leptin levels (μg/L) according to polymorphisms in the *LEPR* gene, adjusted for baseline BMI

	Men				Women				Total			
	High weight gain		Stable weight		High weight gain		Stable weight		High weight gain		Stable weight	
	n	Geometric mean (95% confidence interval)	*n*	Geometric mean (95% confidence interval)	*n*	Geometric mean (95% confidence interval)	*n*	Geometric mean (95% confidence interval)	*n*	Geometric mean (95% confidence interval)*	*n*	Geometric mean (95% confidence interval)*
LEPR Lys109Arg												
Lys109/Lys109	70	3.2 (2.9 to 3.6)	73	3.0 (2.7 to 3.3)	70	10.0 (9.1 to 11.1)	70	9.1 (8.3 to 10.1)	140	5.8 (5.4 to 6.2)	143	5.3 (5.0 to 5.7)
Arg109/+	56	3.7 (3.3 to 4.1)	61	3.0 (2.7 to 3.3)	63	11.3 (10.3 to 12.6)	73	9.3 (8.4 to 10.2)	119	6.5 (6.0 to 7.1)	134	5.4 (5.0 to 5.8)
p value		0.11		0.99		0.11		0.86		0.02		0.87
LEPR Gln223Arg												
Gln223/Gln223	43	3.1 (2.7 to 3.5)	43	3.0 (2.6 to 3.4)	38	10.0 (8.7 to 11.4)	42	8.9 (7.8 to 10.1)	81	5.6 (5.1 to 6.2)	85	5.2 (4.8 to 5.7)
Arg223/+	83	3.6 (3.3 to 4.0)	91	3.0 (2.8 to 3.3)	95	10.9 (10.0 to 11.9)	101	9.3 (8.6 to 10.1)	178	6.4 (6.0 to 6.8)	192	5.4 (5.1 to 5.7)
p value		0.05		0.84		0.27		0.55		0.03		0.53
LEPR Lys656Asn												
Lys656/Lys656	77	3.3 (3.0 to 3.7)	89	2.9 (2.7 to 3.1)	95	10.6 (9.7 to 11.6)	93	8.7 (7.7 to 9.8)	172	6.0 (5.6 to 6.4)	182	5.3 (4.9 to 5.7)
Asn656/+	49	3.6 (3.1 to 4.0)	45	3.2 (2.9 to 3.6)	38	10.6 (9.2 to 12.2)	50	9.5 (8.7 to 10.3)	87	6.3 (5.7 to 6.9)	95	5.4 (5.0 to 5.8)
p value		0.40		0.14		0.97		0.23		0.49		0.89
Combination of two polymorphisms†												
LEPR Lys109Arg and Gln223Arg												
Lys109/Lys109 & Gln223/Gln223	42	3.1 (2.7 to 3.5)	43	3.0 (2.6 to 3.4)	38	10.0 (8.7 to 11.4)	40	8.7 (7.7 to 10.0)	80	5.6 (5.1 to 6.2)	83	5.2 (4.7 to 5.7)
Arg109/+ & Arg223/+	55	3.7 (3.3 to 4.1)	61	3.0 (2.7 to 3.3)	63	11.1 (10.2 to 12.6)	71	9.2 (8.3 to 10.1)	118	6.6 (6.1 to 7.1)	132	5.3 (5.0 to 5.7)
p value		0.05		0.90		0.05		0.54		0.02		0.59
LEPR Lys109Arg and Lys656Asn												
Lys109/Lys109 & Lys656/Lys656	33	3.0 (2.6 to 3.5)	42	2.7 (2.4 to 3.1)	43	9.8 (8.6 to 11.1)	35	9.9 (8.6 to 11.4)	76	5.5 (5.0 to 6.1)	77	5.3 (4.8 to 5.8)
Arg109/+ & Asn656/+	12	4.0 (3.1 to 5.2)	14	2.9 (2.4 to 3.6)	11	11.0 (8.5 to 14.2)	15	9.2 (7.4 to 11.4)	23	6.9 (5.7 to 8.2)	29	5.3 (4.6 to 6.2)
p value		0.06		0.63		0.43		0.59		0.04		0.92
LEPR Gln223Arg and Lys656Asn												
Gln223/Gln223 & Lys656/Lys656	19	2.6 (2.1 to 3.2)	18	2.5 (2.1 to 3.0)	23	9.7 (8.1 to 11.6)	16	10.1 (8.2 to 12.4)	42	5.1 (4.5 to 5.9)	34	5.0 (4.4 to 5.8)
Arg223/+ & Asn656/+	25	3.6 (3.1 to 4.3)	20	3.0 (2.5 to 3.6)	23	10.8 (9.1 to 129)	24	9.2 (7.7 to 10.9)	48	6.4 (5.6 to 7.2)	44	5.4 (4.8 to 6.1)
p value		0.02		0.12		0.41		0.50		0.02		0.46

* Also adjusted for sex.

† Based on analyses in which all four combinations of these two polymorphisms were included, only the results of these two extreme groups are shown.

leptin concentrations fluctuate according to the phase of the menstrual cycle (18,19), and blood samples were not taken for all women at the same time in the cycle, it is likely that this variation has diluted our results. This might explain the clearer findings in men compared with those among women.

Furthermore, the matching factors could have confounded our results. Fortunately, these biases were of minor importance because similar results were found with and without adjustment for these factors.

At baseline, female weight gainers had slightly higher BMI values compared with those who kept stable weight. Because persons with more fat mass have higher leptin levels, and fat mass also independently affects the risk of weight gain due to e.g., environmental factors, we adjusted the associations by taking BMI into the analyses to control

Table 6. Odds ratio for the risk of weight gain from baseline to follow-up per SD increase in $\log_e$-transformed leptin levels according to polymorphisms in the *LEPR* gene, adjusted for baseline BMI

	Men		Women		Total	
	n	Odds ratio (95% confidence interval)	*n*	Odds ratio (95% confidence interval)	*n*	Odds ratio (95% confidence interval)
LEPR Lys109Arg						
Lys109/Lys109	143	1.00 (0.49 to 2.04)	140	1.25 (0.66 to 2.37)	283	1.26 (0.87 to 1.83)
Arg109/+	117	1.49 (0.98 to 2.28)	136	1.23 (0.80 to 1.90)	253	1.30 (0.99 to 1.70)
p interaction		0.39		0.97		0.89
LEPR Gln223Arg						
Gln223/Gln223	86	0.58 (0.24 to 1.38)	80	1.04 (0.46 to 2.39)	166	0.98 (0.63 to 1.54)
Arg223/+	174	1.61 (1.11 to 2.33)	196	1.26 (0.87 to 1.83)	370	1.36 (1.06 to 1.74)
p interaction		0.046		0.68		0.18
LEPR Lys656Asn						
Lys656/Lys656	166	1.71 (0.84 to 3.48)	188	1.12 (0.61 to 2.04)	354	1.50 (1.05 to 2.14)
Asn656/+	94	1.14 (0.72 to 1.78)	88	1.28 (0.79 to 2.09)	182	1.15 (0.86 to 1.54)
p interaction		0.41		0.75		0.26
Combinations of two polymorphisms†						
LEPR Lys109Arg and Gln223Arg						
Lys109/Lys109 & Gln223/Gln223	85	0.87 (0.56 to 1.36)	78	1.25 (0.78 to 1.99)	163	1.03 (0.75 to 1.42)
Arg109/+ & Arg223/+	116	1.43 (0.98 to 2.09)	134	1.31 (0.93 to 1.85)	250	1.36 (1.06 to 1.76)
p interaction		0.10		0.87		0.18
LEPR Lys109Arg and Lys656Asn						
Lys109/Lys109 & Lys656/Lys656	75	1.28 (0.79 to 2.06)	78	1.06 (0.65 to 1.73)	153	1.17 (0.84 to 1.65)
Arg109/+ & Asn656/+	26	1.54 (0.66 to 3.60)	26	0.92 (0.44 to 1.92)	52	1.17 (0.65 to 1.94)
p interaction		0.71		0.75		0.90
LEPR Gln223Arg and Lys656Asn						
Gln223/Gln223 & Lys656/Lys656	37	0.95 (0.44 to 2.08)	39	0.88 (0.46 to 1.70)	76	0.94 (0.57 to 1.54)
Arg223/+ & Asn656/+	45	1.48 (0.79 to 2.76)	47	1.14 (0.67 to 1.92)	92	1.26 (0.85 to 1.88)
p interaction		0.39		0.55		0.36

p Interaction, test of *p* value of the interaction term between leptin and the specific genotype.
* Also adjusted for sex.
† Subjects with other genotypes were excluded from these analyses.

for the confounding. BMI is not a perfect measure for the amount of body fat percentage, but in Dutch adults they are highly correlated ($r \approx 0.75$) (20). Therefore, it is possible that the observed differences between weight gainers and weight keepers are still partly caused by residual confounding of body fat percentage. Although this confounding may have affected the association between leptin and weight gain, it is unlikely that it has affected our conclusion that leptin predicts weight gain. In addition, it remains possible that our association can be explained by other unknown and confounding factors that influence both weight gain and leptin.

Rodent models of obesity (e.g., *ob/ob* mice) and some human cases of severe obesity are leptin deficient due to a rare mutation in the leptin gene (2,5). Treatment with leptin results in a decrease in food intake and body weight (21–24). Therefore, we expected that for common obesity, a similar, but weaker, negative association between leptin and

weight would be observed. However, we found no evidence for the hypothesis that relatively low leptin levels predispose to weight gain. In contrast, we observed that those with higher leptin levels tended to have an increased risk for gaining weight compared with those with low levels.

Our finding is in contrast with a study in 36 obese Pima Indians; those with relatively low leptin levels were more prone to gain weight compared with those with higher leptin levels (25). Also, Lindroos et al. found a negative association between leptin and weight gain, but only in a small subgroup of 24 very obese Swedes without a history of parental obesity (26). However, other prospective studies in more moderately obese populations found no association (27–29) or also a positive (30,31) association, as in our study, between leptin and weight gain. There are several possible explanations for these differences. First, it is possible that the association between leptin and weight gain is modified by the degree of obesity. Secondly, in the study in Pima Indians, adjustments were made for more accurate measures of body fat percentage (assessed by hydrodensitometry) than BMI. Thirdly, other population characteristics that were different across studies may explain the inconsistencies.

Because *db/db* mice and some humans with leptin resistance due to a defect in their leptin receptor are severely obese and have high plasma leptin levels (6), we expected a similar, but weaker, association between leptin and weight gain in our population. Our observation that weight gainers had significantly higher baseline leptin concentrations than weight keepers is consistent with this hypothesis that leptin resistance contributes to weight gain in a general white population.

Some other studies reported leptin levels according to mutations in the *LEPR* gene (32–34). Only Chagnon et al. also reported higher leptin levels after adjustment for fat mass among middle aged men with an Arg223 allele (34). Most of the other associations found were not independent of BMI and were performed in very small groups, in overweight and obese, nonwhite, or postmenopausal women, which may explain the inconsistency in these results (33). Our findings of increased leptin levels in carriers of the Arg109 allele or the Gln223 allele of the *LEPR* gene compared with those in the noncarriers of these alleles suggest that for these variants, the signaling of leptin is impaired. However, so far as we know, no studies have been performed on the affected functionality of this protein due to these mutations or other linked markers. Whether its functionality is indeed impaired needs further investigation.

A defective leptin receptor due to mutations in the gene is one of the explanations for a positive association between leptin and weight gain. Arch et al. suggested that in most cases, leptin concentrations are raised not only because of genetic variability, but also because of other opposing forces that promote obesity (35). Our results support this idea, because weight gainers differed in some aspects of dietary habits and physical activity from non-weight gainers (10), and weight gainers had higher leptin levels. It is likely that variation in genetic factors and lifestyle factors, such as physical activity and metabolic factors (36–38), explains the inconsistency in the reported associations between leptin and weight gain.

Nevertheless, our data suggest that persons with the Arg109 allele or the Gln223 allele are more prone to this influence of other leptin-influencing factors. Seeing the different associations found among men between leptin and weight gain across the genotypes, it is likely that these polymorphisms in the *LEPR* gene have some small impact on weight gain. That this is a small impact is not surprising because weight gain is influenced by several factors and pathways. However, in the same study population, a weak association between these polymorphisms and weight gain was observed among women for only the Lys109Arg genotype; women with the Lys109/Lys109 polymorphism had a lower risk to gain weight (10). These contrasting findings suggest that the role of these polymorphisms on weight gain is very complicated and not yet clear. Also, meta-analyses have shown that there is no clear evidence that these polymorphisms are associated with BMI (39). They also argue that these alleles can still have an impact on weight, but these effects can be compensated by other (genetic) factors and pathways. Therefore, further studies on the influence of these alleles on intermediate phenotypes instead of weight could help to elucidate the role of these alleles on the development of obesity.

Thus, we conclude that in white subjects, relatively high leptin levels seem to predict subsequent weight gain. Furthermore, Lys109Arg or Gln223Arg polymorphisms in the *LEPR* gene are associated with higher leptin levels, suggesting that these polymorphisms (or markers that are in linkage disequilibrium with these polymorphisms) lead to leptin resistance. However, the role of these mutations on weight gain is limited.

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References

1. **Friedman JM, Halaas JL.** Leptin and the regulation of body weight in mammals. *Nature*. 1998;395:763–70.
2. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372:425–32.
3. **Lee GH, Proenca R, Montez JM, et al.** Abnormal splicing of the leptin receptor in diabetic mice. *Nature*. 1996;379:632–5.
4. **Chen H, Charlat O, Tartaglia LA, et al.** Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell*. 1996;84:491–5.
5. **Montague CT, Farooqi IS, Whitehead JP, et al.** Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*. 1997;387:903–8.
6. **Clément K, Vaisse C, Lahlou N, et al.** A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. 1998;392:398–401.
7. **Considine RV, Sinha MK, Heiman ML, et al.** Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334:292–5.
8. **Schwartz MW, Woods SC, Porte D Jr., Seeley RJ, Baskin DG.** Central nervous system control of food intake. *Nature*. 2000;404:661–71.
9. **Clément K.** Leptin and the genetics of obesity. *Acta Paediatr*. 1999;88(suppl):51–7.
10. **van Rossum CTM, Hoebee B, Seidell JC, et al.** Genetic factors and weight gain in Dutch men and women. *Int J Obes Relat Metab Disord*. 2002;26:517–28.
11. **Verschuren WMM, van Leer EM, Blokstra A** *et al*. Cardiovascular disease risk factors in The Netherlands. *Neth J Cardiol*. 1993;6:205–10.
12. **Brann DW, Wade MF, Dhandapani KM, Mahesh VB, Buchanan CD.** Leptin and reproduction. *Steroids*. 2002;67: 95–104.
13. **Astrup A.** Physical activity and weight gain and fat distribution changes with menopause: current evidence and research issues. *Med Sci Sports Exerc*. 1999;31:S564–7.
14. **Chung WK, Kehoe LP, Chua M, et al.** Exonic and intronic sequence variation in the human leptin receptor gene (*LEPR*). *Diabetes*. 1997;46:1509–11.
15. **Ravussin E, Gautier J-F.** Metabolic predictors of weight gain. *Int J Obes Relat Metab Disord* 1999;23(suppl):37–41.
16. **Rowland ML.** Self-reported weight and height. *Am J Clin Nutr*. 1990;52:1125–33.
17. **Schoeller DA, Cella LK, Sinha MK, Caro JF.** Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest*. 1997;100:1882–7.
18. **Hardie L, Trayhurn P, Abramovich D, Fowler P.** Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clin Endocrinol*. 1997;47:101–6.
19. **Quinton ND, Laird SM, Okon MA, et al.** Serum leptin levels during the menstrual cycle of healthy fertile women. *Br J Biomed Sci*. 1999;56:16–9.
20. **Deurenberg P, Weststrate JA, Seidell JC.** Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr*. 1991;65:105–14.
21. **Halaas JL, Gajiwala KS, Maffei M, et al.** Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*. 1995;269:543–6.
22. **Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P.** Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science*. 1995;269:546–9.
23. **Pelleymounter MA, Cullen MJ, Baker MB, et al.** Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*. 1995;269:540–3.
24. **Farooqi IS, Jebb SA, Langmack G, et al.** Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*. 1999;341:879–84.
25. **Ravussin E, Pratley RE, Maffei M, et al.** Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med*. 1997;3:238–40.
26. **Lindroos AK, Lissner L, Carlsson B, et al.** Familial predisposition for obesity may modify the predictive value of serum leptin concentrations for long-term weight change in obese women. *Am J Clin Nutr*. 1998;67:1119–23.
27. **Haffner SM, Mykkänen LA, Gonzalez CC, Stern MP.** Leptin concentrations do not predict weight gain: the Mexico City Diabetes Study. *Int J Obes Relat Metab Disord*. 1998; 22:695–9.
28. **Folsom AR, Jensen MD, Jacobs DR Jr., Hilner JE, Tsai AW, Schreiner PJ.** Serum leptin and weight gain over 8 years in African American and Caucasian young adults. *Obes Res*. 1999;7:1–8.
29. **Hodge AM, de Courten MP, Dowse GK, et al.** Do leptin levels predict weight gain? A 5-year follow-up study in Mauritius. Mauritius Non-communicable Disease Study Group. *Obes Res*. 1998;6:319–25.
30. **Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS.** Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes*. 1998;47: 239–43.
31. **Chu NF, Spiegelman D, Yu J, Rifai N, Hotamisligil GS, Rimm EB.** Plasma leptin concentrations and four-year weight gain among US men. *Int J Obes Relat Metab Disord*. 2001; 25:346–53.
32. **Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI.** A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women. *Hum Genet*. 2001;108:233–6.
33. **Mammes O, Aubert R, Betoulle D, et al.** LEPR gene polymorphisms: associations with overweight, fat mass and response to diet in women. *Eur J Clin Invest*. 2001;31:398–404.

34. **Chagnon YC, Chung WK, Pérusse L, Chagnon M, Leibel RL, Bouchard C.** Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. *Int J Obes Relat Metab Disord*. 1999;23:278–86.
35. **Arch JR, Stock MJ, Trayhurn P.** Leptin resistance in obese humans: does it exist and what does it mean? *Int J Obes Relat Metab Disord*. 1998;22:1159–63.
36. **Hodge AM, Westerman RA, de Courten MP, Collier GR, Zimmet PZ, Alberti KG.** Is leptin sensitivity the link between smoking cessation and weight gain? *Int J Obes Relat Metab Disord*. 1997;21:50–3.
37. **Raben A, Astrup A.** Leptin is influenced both by predisposition to obesity and diet composition. *Int J Obes Relat Metab Disord*. 2000;24:450–9.
38. **van Aggel-Leijssen DPC, van Baak MA, Tenenbaum R, Campfield LA, Saris WHM.** Regulation of average 24h human plasma leptin level: the influence of exercise and physiological changes in energy balance. *Int J Obes Relat Metab Disord*. 1999;23:151–8.
39. **Heo M, Leibel RL, Boyer BB, et al.** Pooling analysis of genetic data: the association of leptin receptor (*LEPR*) polymorphisms with variables related to human adiposity. *Genetics*. 2001;159:1163–78.