Effect of Lys656Asn Polymorphism of Leptin Receptor Gene on Cardiovascular Risk Factors and Serum Adipokine Levels after a High Polyunsaturated Fat Diet in Obese Patients

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Background: Human obesity is characterized by high levels of leptin, and leptin levels may change with weight loss and dietary restriction. The aim of our study was to investigate the influence of Lys656Asn polymorphism in the leptin receptor gene on cardiovascular risk factors, weight loss, and serum leptin levels to a high polyunsaturated fatty acid (PUFA) hypocaloric diet in obese patients. **Design:** A sample of 132 obese patients was analyzed in a prospective way with a dietary intervention. The enriched PUFAs hypocaloric intervention consisted in a diet of 1,459 kcal, 45.7% of carbohydrates, 34.4% of lipids, and 19.9% of proteins. **Results:** In wild-type group, BMI (-1.9 ± 1.4 kg/m²), weight (-4.4 ± 3.2 kg), fat mass (-4.2 ± 3.8 kg), waist circumference (-4.1 ± 3.1 cm), systolic blood pressure ($-7.0 \pm$

12.1 mmHg), diastolic blood pressure (-3.9 ± 6.8 mmHg), insulin (-1.8 ± 5.6 mU/l) and HOMA-IR (-0.5 ± 1.5 Units) decreased. In mutant genotype group, BMI (-2.0 ± 2.1 kg/m²), weight (-3.6 ± 4.1 kg), waist circumference (-3.1 ± 4.1 cm), total cholesterol (-25.2 ± 19.6 mg/dl), LDL cholesterol (-16.6 ± 25.6 mg/dl), and triglycerides (-26.6 ± 39.1 mg/dl) decreased. Only leptin levels have a significant decrease in wild genotype group (-6.6 ± 10.2 ng/ml) (25.1%). **Conclusion:** Carriers of ASn656 allele have a different response than wild-type obese, with a lack of decrease in insulin levels, leptin levels, and HOMA-IR. However, obese patients with this mutant allele have a better lipid profile after weight loss. J. Clin. Lab. Anal. 29:432–436, 2015. ©

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Saturated fatty acids consumption has been associated with a higher risk of cardiovascular disease (CVD), whereas unsaturated fatty acids have protective effects against CVD (1). However, although nutritional recommendations are targeted to the whole population with the objective to reduce CVD, response to diet turned out to be heterogeneous, and a role for genetic turned determinants in the interindividual variation is admitted (2).

Obesity is characterized by a low-grade systemic inflammation and the current view of adipose tissue is that of an active secretory organ, sending out and responding to signals (such as leptin) that modulate appetite, insulin sensitivity, and inflammation. Leptin, a protein containing 167 amino acids, demonstrates structural similarities with cytokine family and is mainly produced by adipocytes (3). However, the role of leptin and its receptor in the development of obesity in the general population is less clear. For

example, human obesity is characterized by high, rather than low, levels of leptin, and it has been suggested that obese patients may be leptin resistant (3).

Different polymorphisms in leptin receptor gene (LEPR) have been described (4). Patients with Asn656 allele of LEPR gene have a different metabolic response than wild type with a standard hypocaloric diet (5). In the previous study, leptin levels did not change in carriers of

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Asn656 allele after weight loss. Obesity treatments are focused on hypocaloric diets, but quality of dietary fat is an important factor to modulate cardiovascular risk factors associated with obesity.

The aim of our study was to investigate the influence of Lys656Asn polymorphism in the LEPR on cardiovascular risk factors, weight loss, and serum leptin levels to a high polyunsaturated fatty acid (PUFA) hypocaloric diet in obese patients.

A population of 132 obesity (body mass index >30) patients was analyzed in a prospective way. These patients were studied in a nutrition clinic unit and signed an informed consent. The enriched PUFA hypocaloric intervention consisted in a diet of 1,459 kcal, 45.7% of carbohydrates, 34.4% of lipids, and 19.9% of proteins. The distribution of fats was as follows: 21.8% of saturated fats, 55.5% of monounsaturated fats, and 22.7% of polyunsaturated fats (7 g per day of w-6 fatty acids, 2 g per day of w-3 fatty acids, and a ratio w6/w3 of 3.5). Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood, and adipokine levels (leptin, adiponectin, resistin, IL-6, and TNF α) were measured at basal time and after 2 months of hypocaloric diet. Genotype of leptin receptor (LEPR) gene polymorphism (Lys656Asn) was studied.

Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol >300 mg/dl, triglycerides >400 mg/dl, blood pressure >140/90 mmHg, fasting plasma glucose >110 mg/dl, as well as the use of sulfonilurea, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blocker, angiotensin converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit.

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA). The polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 μ l of each oligonucleotide primer (primer forward: 5'-GCA GTT CCT ATG AGA GGA CC-3'; primer reverse: 5'-AAA TTG GGA ATA CCT TCC AAA GT-3'), and 0.25 μ l of each probes (wild probe: 5'-Fam-AGT GAC ATT TTT CTC CTT TTT CAT AGT ATC-Tamra-3') and (mutant probe: 5'-Hex-AGT GAC ATT TTT CTC GTT TTT CAT AGT AT-Tamra-3') in a 25 μ l final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA). The Hardy-Weinberg equilibrium was examined with *P* value >0.05.

Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer, and averaged. Body weight was measured to an accuracy of 0.5 kg and body mass index computed as body weight/(height²). Waist (narrowest diameter be-

tween xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to-hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition (6) (Biodynamics Model 310e, Seattle, WA, USA).

Plasma glucose levels were determined using an automated glucose oxidase method (Glucose analyzer 2, Beckman Instruments, Fullerton, California). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values (7). CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0–7 mg/dl and analytical sensitivity of 0.5 mg/dl.

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4–12 ng/ml. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10–100 ng/ml. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis) with a sensitivity of 0.246 ng/ml and a normal range of 8.65–21.43 ng/ml. Interleukin 6 and TNF α were measured by ELISA (R&D Systems, Inc.) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 were 1.12–12.5 pg/ml and TNF α were 0.5–15.6 pg/ml.

Patients received prospective serial assessment of nutritional intake with 3-day written food records. All enrolled subjects received instruction to record their daily dietary intake for 3 days including a weekend day. National composition food tables were used as reference (8). Regular aerobic physical activity (walking was allowed, no other exercises) was maintained during the period study (three times per week).

Sample size was calculated to detect differences over 15% in leptin levels with 90% power and 5% significance (*n* = 130). The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's *t*-test. Nonparametric variables were analyzed with the Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. The statistical analysis was performed for the combined *Lys656/Asn656* and

TABLE 1. Changes in Anthropometric, Cardiovascular Risk Factors, and Adipokines

Characteristics	Lys656/Lys 656		Lys656/Asn656 and Asn656/Asn656	
	0 time	At 2 months	0 time	At 2 months
BMI	38.6 ± 5.1	36.6 ± 5.5 ^a	37.2 ± 6.3	35.2 ± 3.5 ^a
Weight (kg)	98.3 ± 17.7	93.9 ± 17.5 ^a	95.0 ± 15.5	91.3 ± 15.9 ^a
Fat free mass (kg)	55.2 ± 13.1	54.9 ± 10.3	55.9 ± 12.6	55.2 ± 11.8
Fat mass (kg)	40.9 ± 12.7	36.8 ± 12.3 ^a	38.9 ± 10.5	35.6 ± 10.6 ^a
Waist circumference	113.8 ± 15.3	109.7 ± 14.6 ^a	112.9 ± 10.810	9.5 ± 10.4 ^a
Waist-to-hip ratio	0.91 ± 0.08	0.91 ± 0.1	0.92 ± 0.09	0.91 ± 0.1
Systolic BP (mmHg)	130.5 ± 14.4	123.6 ± 11.6 ^a	124.9 ± 10	122.2 ± 10.3
Diastolic BP (mmHg)	84.4 ± 9.6	80.5 ± 8.2 ^a	81.9 ± 7.7	80.0 ± 7.6
Glucose (mg/dl)	95.2 ± 10.2	92.9 ± 11.8	94.2 ± 9.1	91.3 ± 8.8
Total ch. (mg/dl)	207.6 ± 47.4	202.2 ± 31.8	208.3 ± 45.9	183.1 ± 30.8 ^a
LDL-ch. (mg/dl)	133.9 ± 42.1	129.6 ± 30	132.5 ± 37.1	115.1 ± 28.4 ^a
HDL-ch. (mg/dl)	51.4 ± 11.9	49.5 ± 11.9	49.1 ± 12.4	49.9 ± 17.2
TG (mg/dl)	136.2 ± 71.8	131.8 ± 41.9	128.2 ± 42.7	101.6 ± 45.9 ^a
Insulin (mUI/L)	12.9 ± 8.7	11.1 ± 5.1 ^a	13.4 ± 6.8	12.9 ± 6.1
HOMA	3.3 ± 1.8	2.8 ± 1.4 ^a	3.7 ± 2.2	3.3 ± 1.7
CRP (mg/dl)	6.4 ± 4.5	6.7 ± 3.1	5.3 ± 3.1	5.7 ± 4.2
IL 6 (pg/ml)	1.7 ± 1.1	1.4 ± 1.0	2.1 ± 0.8	2.4 ± 1.1
TNF-α (pg/ml)	2.6 ± 0.9	2.9 ± 2.2	1.9 ± 0.9	2.1 ± 1.2
Adiponectin (ng/ml)	10.3 ± 5.3	9.6 ± 4.3	9.4 ± 4.8	9.0 ± 4.1
Resistin (ng/ml)	4.6 ± 1.9	4.7 ± 1.9	5.2 ± 2.8	5.7 ± 3.3
Leptin (ng/ml)	32.3 ± 25.5	25.7 ± 17.3 ^a	24.9 ± 14.8	20.5 ± 15.8

CRP, C reactive protein; Ch, Cholesterol; TG, Triglycerides; IL-6, interleukin 6.

^a*P* < 0.05, in each group with basal values.

Asn656/Asn656 as a mutant group and type *Lys656/Lys656* as wild group. A *P*-value under 0.05 was considered statistically significant.

One hundred and thirty-two patients gave informed consent and were enrolled in the study. The mean age was 46.7 ± 16.6 years and the mean BMI 37.3 ± 6.1, with 36 males (27.3%) and 96 females (72.7%). Eighty-eight patients (23 males/65 females) (66.7%) had the genotype *Lys656/Lys656* (wild genotype group) and 44 patients (13 males/31 females) *Lys656/Asn656* (*n* = 3,929.5%) or *Asn656/Asn656* (*n* = 53.8%) (mutant genotype group).

Table 1 shows changes in anthropometric parameters. In wild-type group, BMI (−1.9 ± 1.4 kg/m²), weight (−4.4 ± 3.2 kg), fat mass (−4.2 ± 3.8 kg), waist circumference (−4.1 ± 3.1 cm), systolic blood pressure (−7.0 ± 12.1 mmHg) and diastolic blood pressure (−3.9 ± 6.8 mmHg) decreased. In mutant genotype group, BMI (−2.0 ± 2.1 kg/m²), weight (−3.6 ± 4.1 kg), waist circumference (−3.1 ± 4.1 cm) decreased.

Table 1 shows changes in classic cardiovascular risk factors. In wild genotype group, insulin (−1.8 ± 5.6 mUI/l) and HOMA-IR (−0.5 ± 1.5 Units) decreased. In mutant genotype group, total cholesterol (−25.2 ± 19.6 mg/dl), LDL cholesterol (−16.6 ± 25.6 mg/dl), and triglycerides (−26.6 ± 39.1 mg/dl) decreased.

Table 1 shows differences between basal and after treatment levels of adipokines. Only leptin levels have a signifi-

cant decrease in wild genotype group (−6.6 ± 10.2 ng/ml) (25.1%). The mutant genotype group did not reach statistical differences −2.8 ± 10.7 ng/ml (12.3%).

Our results show that obese patients following an enriched PUFA hypocaloric diet, carriers of the Asn 656 allele of the LEPR receptor gene responded with an improvement of total cholesterol, LDL-cholesterol, and triglycerides after weight loss. Patients without Asn 656 allele responded with a decrease of insulin levels and HOMA-IR after a significant weight loss.

Some population-based studies have been conducted to explore the effects of this polymorphism in the LEPR on body mass index (9–11). Our results show that there is no relationship between these polymorphism and anthropometric variables, baseline data of patients with wild and mutant genotype were similar.

Studies that have evaluated the effect of this polymorphism on the metabolic changes that produce weight loss in obese patients are scarce. One study (10) demonstrated the different responses in weight loss and leptin decrease secondary to a hypocaloric diet with a 25% of calories from fats in relation with Lys656Asn polymorphism. As our results showed, the lack of leptin decrease in patients with Lys656 allele was observed. This different response of leptin changes has been described in other study (11), the changes in serum leptin concentration due to a hypocaloric intervention with low fat were higher than

with a low carbohydrate diet. Lakka et al. (12) showed in White patients that exercise increased sensitivity to insulin and decreased fasting glucose in the LEPR Asn656Asn homozygotes but not after dietary intervention. Mars et al. (9) had observed heterogeneity of metabolic responses with the Lys109Arg, Gln223Arg, and Lys656Asn polymorphisms in the acute decline in leptin after energy restriction with a 21.55 of fats. In the literature, the most important variable that determines circulating leptin concentration is body fat mass (13). The decrease in serum leptin after weight loss is variable. Some authors have reported a decrease of 14% in serum leptin (11) until 45%. Our results are therefore similar to those in the literature.

In our study, carriers of the Asn 656 allele of the LEPR gene do not respond with a decrease of insulin levels and HOMA-IR after a significant weight loss. Other authors have detected association between polymorphism on codon 656 and obesity phenotypes. In postmenopausal women with intolerance of glucose (ITG), associations were found with Lys656Asn for fasting insulin, as well as in response to an oral glucose tolerance test (OGGT). In premenopausal women with ITG, associations were found with Lys656Asn for overall glucose response to the glucose load (14). In contrast, no associations with insulin or glucose were found in women with normal glucose tolerance. In other studies, differences in substrate oxidation rates were found. In fasting conditions, Lys656Lys showed a trend to oxidize more carbohydrates and less fat than Asn656 carriers (15).

Another result obtained in our study is the significant decrease in levels of LDL cholesterol and triglycerides in relation to weight loss in carriers of allele As656. We have no clear explanation for this fact, but high intake of polyunsaturated fats in the diet of our patients interacts with the carriers of this allele producing a better lipid profile or different changes in leptin levels. Animal cell and human studies have demonstrated that fatty acids also influence leptin expression and concentration. Dietary PUFA intake negatively correlates with leptin concentrations (16), whereas reduced leptin concentration has been reported following intervention with fish oil (17). Recently, Phillips et al. (18) have demonstrated that homozygosity for the LEPR polymorphism rs3790433 G allele was associated with insulin resistance, with a deleterious effect more evident against a background of low w3 for high w6 PUFA.

Perhaps, these different metabolic responses could be explained by inclusion criteria and heterogeneity of subjects in the studies of the literature and the distribution of macronutrients of the hypocaloric diets. For example, Yannakoulia et al. (19) have demonstrated that leptin levels and their changes appeared to be related to energy and macronutrient intakes. The amount of weight loss could

be other factor, for example, the weight loss was higher in Asn656 allele carriers than wild-type group after a massive weight loss with bariatric surgery (20).

In conclusion, carriers of ASn656 allele have a different response than wild-type obese, with a lack of decrease in insulin levels, leptin levels, and HOMA-IR. However, obese patients with this mutant allele have a better lipid profile after weight loss.

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