Effects of a High Protein/Low Carbohydrate Versus a Standard Hypocaloric Diet on Weight and Cardiovascular Risk Factors, Role of a Lys656asn Polymorphism of Leptin Receptor Gene

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Abstract

Background: The SNP of the leptin receptor (Lys656Asn) has been related with metabolic parameters. The aim of our design was to evaluate the influence of Lys656Asn variant of Leptin receptor gene on weight loss and metabolic parameters secondary to a high protein/low carbohydrate diet vs. a standard hypocaloric diet.

Design: A total of 280 obese subjects were randomly allocated to one of two diets during 9 months (Diet HP (high protein-low carbohydrate hypocaloric diet)) and Diet S (standard protein hypocaloric diet)).

Results: After both diets and in both genotypes, body mass index (BMI), fat mass, weight, systolic blood pressure and waist circumference decreased, without differences between both diets. With the diet type HP and in both genotypes, triglycerides decreased. In subjects with Lys656Lys genotype, glucose, insulin levels, HOMA-R, total cholesterol and LDL- total cholesterol decreased after HP diet. No statistical changes were detected in Asn allele carriers. After diet S and in patients with Lys656Lys genotypes, only total cholesterol, triglycerides and LDL- total cholesterol decreased, without changes in Asn allele carriers.

Conclusion: Obese subjects with Asn656 allele have a different metabolic response than Lys656Lys genotype subjects, secondary to the same weight loss with two different hypocaloric diets.

Keywords: High protein/low carbohydrate; Standard hypocaloric diet; LYS656ASN; LEPR; Leptin


Introduction

The incidence of obesity is increasing all Western Countries. This dramatically increasing in obesity prevalence has led to an important increase of investigation on adipose tissue and its role in metabolism, inflammation and other physiologic processes [1]. Obesity has a low grade systemic inflammation and adipose tissue is considered an active secretory organ of a new family of molecules called adipokines (for example, leptin). Leptin is a protein containing 167 amino acids, demonstrates structural similarities with cytokines and is mainly produced by white adipose tissue. Some studies have confirmed that other tissues express leptin, including ovaries, placenta, stomach, pituitary, skeletal muscle and liver [2]. This adipokine acts as an afferent satiety signal, regulating appetite in humans. Obese patients may be leptin resistant and are characterized by high, rather than low, levels of leptin [3]. Considering everything previously mentioned, the role of leptin in the obesity is an area of important investigation.

Some genetic variant (polymorphisms) in leptin receptor gene have been studied with contradictory results [4]. One of them is the SNP on codon 656, this change produce a modification in electric charge with a possibility to be functional. Patients with rs8179183 variant of LEPR gene have a different metabolic response than wild type after weight loss secondary to different diets [5-7]. In these previous studies, only leptin levels had a significant decrease in wild group. However, although dietary intakes are the main tool to decrease weight in obese subjects, responded to diet was heterogeneous, and a role for genetic turned determinants in the inter individual variation is postulated [8].

A recent meta-analysis with low-carbohydrate/high protein diets has shown that such diets have benefit effects on weight loss, metabolic parameters and cardiovascular risk factors [9]. As far as we know, no interventional designs have been realized in order to evaluate the effect of this polymorphism on response to diets of this type.

The aim of our study was to evaluate the effect of this polymorphism on metabolic parameters and weight loss secondary to a high protein/low carbohydrate vs. a standard hypocaloric diets (1000 kcal/day) during 9 months.

Methods and Subjects

Subjects

A sample of 280 obesity non diabetic outpatients was analyzed in a prospective way. Written informed consent was fulfilled from all subjects. Exclusion criteria included history of stroke or cardiovascular disease during the previous 12 months, total cholesterol > 275 mg/dl, triglycerides > 350 mg/dl, fasting plasma glucose >110 mg/dl, blood pressure > 140/90 mmHg, as well as the use of treatments to diabetes mellitus (insulin, sulphonilurea, dypeptidil type IV inhibitors drugs, thiazolidinedions) , glucocorticoids, antineoplastic agents, angiotensin...
receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Local Ethical Committee.

Procedure

Basal fasting glucose, total cholesterol, c-reactive protein (CRP), insulin, insulin resistance (HOMA-IR), LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration and adipokines (leptin, resistin, adiponectin) levels were determined within the start of the trial and repeated after 3 months and 9 months of both dietary intervention. Weight, height, and blood pressure measures were determined within the start of the trial and repeated 3 and 9 months of intervention. A tetrapolar bioimpedance was realized in order to measure fat mass. These measures were realized at same time of the day (morning).

Genotype of Lys656Asn variant of Leptin receptor gene was studied.

280 obese patients were randomly allocated to one of two diets. Diet HP (a hypertoric diet, high protein-low carbohydrate) consisted in a diet of 1050 cal/day, 33% of fats (39.0 g/day), 33% of carbohydrates (86.1 g/day) and 34% of proteins (88.6 g/day). The distribution of fats was 63.8% of monounsaturated fats, 23.5% of saturated fats and 12.6% of polyunsaturated fats. Diet S (hypocaloric diet, standard protein) consisted in a diet of 1093 cal/day, 27% fats (32.6 g), 53% carbohydrates (144.3 g/day) and 20% proteins (55.6 g/day). The distribution of fats was 67.4% of monounsaturated fats, 20.9% of saturated fats, and 11.6% of polyunsaturated fats. The exercise program was an aerobic physical activity at least 3 times per week (60 min each). The adherence of these diets was recorded each 7 days with a phone call in order to improve both diets. National composition food tables were used as reference [10].

Biochemical assays

Cholesterol and triglyceride concentrations were measured by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was measured enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. Low-density lipoprotein cholesterol was determined using Friedewald formula.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was determined by enzymatic colorimetric (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) [12] and the homeostasis model assessment for insulin resistance (HOMA-IR) was determined using these values [13]. CRP was determined by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensitivity 0.5 mg/dl.

Plasma hormone levels were measured using the multiplex Biorad® 10 plex assay following manufacturer's instructions (Bio-Rad®, Hercules, CA). This system lets for quantitative measurement of different molecules, while consuming a small amount of material; resistin, leptin and adiponectin. Limits of detection were as follows (pg/ml): resistin (1.4), leptin (1.8), and adiponectin (3.8).

Statistical analysis

Sample size was calculated to observed differences over 10% 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. A Chi square test was used to evaluate the Hardy–Weinberg equilibrium. Non-parametric parameters were analyzed with the Wilcoxon test. A mixed model has been used within and between subjects to test the interaction between the polymorphism groups and outcome variables, due to the repeated measures study design. Qualitative parameters were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. The statistical analysis was realized for the combined Lys656/Asn656 and Asn656/Asn656 as a group and genotype Lys656/Lys656 as other group (dominant model). A p-value under 0.05 was considered statistically significant.

Results

280 patients gave signed consent and were included in the study. The mean age was 49.5 ± 11.2 years and the mean body mass index 37.8 ± 3.2, with 75 males (26.8%) and 205 females (73.2%). One hundred and eighty eight patients (54 males/134 females) (67.1%) had the genotype Lys656/Asn656 and 92 (32.9%) patients (21 males/71 females) Lys656/Lys656 (n=81, 28.9%) or Asn656/Asn656 (n=11, 3.9%) (Asn allele carriers). The Hardy Weinberg equilibrium was fulfilled p=0.23.

Sex frequency was equal in both groups, males (22.7% vs 23.6%) and females (77.3% vs 76.4%). Age was equal in both groups (Lys656/Lys656 genotype: 49.7 ± 10.2 years vs Asn carriers group: 48.9 ± 12.7 years/n.).

In the 139 subjects (94 Lys656/Lys genotype and 45 Asn allele carriers) randomized to diet HP, basal determination of dietary intake with a 3 days written food register detected a basal calorie intake of 2099.8 ± 432.6 kcal/day, a fat intake of 90.0 ± 29.3 g/day (41.1% of calories), a carbohydrate intake of 219.38 ± 30.9 g/day (40.8% of calories) and a protein intake of 81.2 ± 59.1 g/day (27.1% of calories). During the trial, these patients attained the recommendations of diet; 1010.4 ± 911.9 calories (34.2% of carbohydrates, 32.6% of lipids and...
36.2% of proteins). The 141 subjects (94 LysLys/Lys genotype and 47 Asn allele carriers) randomized to diet S, basal assessment of dietary intake with a 3 days written food register showed a basal caloric intake a calorie intake of 2084 ± 394.1 kcal/day, a fat intake of 910.0 ± 131.0 g/day (38.4% of calories), a carbohydrate intake of 213.9 ± 28.3 g/day (42.7% of calories), and a protein intake of 90.9 ± 11.9 g/day (19.9% of calories). During the trial, these patients reached the recommendations of diet; 1008.8 ± 79.1 calories (51.8% of carbohydrates, 24.9% of lipids and 18.8% of proteins).

 Anthropometric parameters of patients at baseline and at 3-9 months of intervention are shown in Table 1. With the diet type HP (high protein hypocaloric diet) and in both genotype groups (Lys656Lys vs Lys656Asn+Asn656Asn), body mass index (BMI) (-2.2 ± 1.1 kg/m² vs -1.9 ± 1.3 kg/m²; ns), weight (-6.1 ± 2.0 kg vs -6.7 ± 4.0 kg: ns), fat mass (-5.2 ± 3.1 kg vs -5.1 ± 3.0 kg; ns), systolic blood pressure (-4.1 ± 2.0 mmHg vs -3.1 ± 1.9 mmHg) and waist circumference (-6.1 ± 5.0 cm vs -6.3 ± 4.5 cm; ns) decreased. There were not significant differences between the changes (on weight, waist circumference, BMI, systolic blood pressure and fat mass) in either genotype group. With the diet type S (Standard hypocaloric diet) and in both groups, body mass index (-1.3 ± 1.0 kg/m² vs -1.1 ± 0.9 kg/m²; ns), weight (-4.8 ± 3.0 kg vs -5.1 ± 3.2 kg; ns), fat mass (-4.3 ± 3.1 kg vs -4.0 ± 3.0 kg; ns), blood pressure (-4.0 ± 1.9 mmHg vs -4.3 ± 2.1 mmHg) and waist circumference (-5.2 ± 4.0 cm vs -6.0 ± 3.9 cm: ns) decreased. There were not statistical differences between the effects in either genotype group with diet S. Table 2 shows the metabolic parameters. With the diet type HP and in both genotypes, triglycerides (-19.0 ± 8.1 mg/dl vs -13.1 ± 7.9 mg/dl; p<0.05) decreased. Only in patients with Lys656Lys genotype, glucose (-4.1 ± 1.1 mg/dl vs -1.1 ± 2.5 mg/dl; p<0.05), insulin levels (-2.6 ± 2.0 UI/L vs -0.9±2.0 UI/L<0.05), HOMA-IR (-0.5 ± 1.0 units vs -0.1 ± 1.1 units; p<0.05), total cholesterol (-12.0 ± 8.1 mg/dl vs -5.1 ± 6.1 mg/dl; p<0.05), and LDL- total cholesterol (-6.8 ± 4.1 mg/dl vs -2.9 ± 3.1 mg/dl; p<0.05) decreased. All these parameters remained unchanged in the S diet group. There were significant changes in glucose, insulin, HOMA-IR, total cholesterol and LDL- total cholesterol in the HP diet group compared to the S diet group, where no changes were observed. Only in patients with Lys656Lys genotype, glucose (p<0.05), insulin (p<0.05), HOMA-IR (p<0.05), total cholesterol (p<0.05), and LDL- total cholesterol (p<0.05) decreased. These changes showed that the HP diet was more effective than the S diet in improving metabolic parameters.

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unchanged in patients with Asn allele carriers. With the diet S and in patients with Lys656Asn genotypes, only total cholesterol (-13.2 ± 7.1 mg/dl vs -5.1 ± 8.2 mg/dl; p<0.05), triglycerides (-12.1 ± 7.1 mg/dl vs -4.1 ± 7.9 mg/dl; p<0.05) and LDL- total cholesterol (-5.7 ± 3.1 mg/dl vs -2.1 ± 5.1 mg/dl; p<0.05) decreased. Lipid profile remained unchanged in patients with Asn allele genotype.

Table 3 reports levels of adipokines. With the diet HP and in both group of genotypes, leptin levels (-16.3 ± 12.1 ng/ml vs. -20.0 ± 11.1 ng/ml) decreased. With the diet S, leptin levels (-16.9 ± 5.2 ng/ml vs. -21.1 ± 4.3 ng/ml) decreased in both groups, too. The decrease of leptin levels was similar with diet HP and S.

Discussion

Findings from our study suggest that, in obese patients following a high protein hypocaloric diet, carriers of the Asn 656 allele of the LEPR gene do not respond with a decrease of LDL cholesterol, insulin levels and HOMA-IR after a significant weight loss. These obese patients (Asn gene do not respond with a decrease of LDL cholesterol, insulin levels and HOMA-IR after weight loss. These obese subjects showed a decrease

{\begin{table}[h]
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
Characteristics & DIET H (n=139) & DIET S (n=141) \\
\hline & LysLys(n=94) & LysAsn+ AsnAsn (n=45) & LysLys(n=94) & LysAsn+ AsnAsn (n=47) \\
\hline
Adiponectin (ng/ml) & 10.2 ± 6.0 & 9.6 ± 5.1 & 10.3 ± 8.0 & 10.8 ± 7.0 & 11.1 ± 9.0 & 10.9 ± 7.2 & 0.23 & 11.0 ± 4.1 & 10.8 ± 4.0 & 9.9 ± 4.3 & 0.34 & 0.01 & 39.1 ± 10.9 & 20.8 ± 9.2 & 18.5 ± 9.5 & 0.01 & 31.9 ± 13.0 & 20.3 ± 8.0 & 15.0 ± 6.1 & 0.01 & 41.9 ± 10.1 & 22.7 ± 9.3 & 21.6 ± 8.1 & 0.02 \\
Resistin (ng/ml) & 7.4 ± 3.2 & 7.2 ± 3.6 & 6.9 ± 4.3 & 7.3 ± 3.1 & 7.0 ± 3.2 & 7.1 ± 3.1 & 0.45 & 8.0 ± 2.5 & 7.9 ± 3.1 & 7.7 ± 2.8 & 0.45 & 0.01 & 16.1 ± 10.4 & 8.7 ± 9.3 & 9.6 ± 6.5 & 0.01 & 18.9 ± 10.4 & 9.8 ± 9.3 & 10.8 ± 6.5 & 0.01 & 20.7 ± 10.4 & 11.8 ± 9.3 & 12.7 ± 6.5 & 0.01 \\
Leptin (ng/ml) & 31.8 ± 17.4 & 21.9 ± 13.1* & 15.3 ± 10.1* & 39.1 ± 10.9 & 20.8 ± 9.2 & 18.5 ± 9.5 & 0.01 & 31.9 ± 13.0 & 20.3 ± 8.0 & 15.0 ± 6.1 & 0.01 & 41.9 ± 10.1 & 22.7 ± 9.3 & 21.6 ± 8.1 & 0.02 \\
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\end{tabular}
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(*) <0.05, in each group with basal values. No statistical differences among genotypes in each diet or in different diet groups. Quantitative variables with normal distribution were analyzed with a two-tailed, aired Student’s-t test (resistin). Non-parametric parameters were analyzed with the Wilcoxon test (leptin and adiponectin).

Only leptin levels have a significant decrease in subjects with genotype Lys656Lys secondary to weight loss with both diets. The changes in serum leptin concentration due to 2 months’ intervention with low fat are higher than with a low carbohydrate diet (low fat diet: 30.3%; p<0.05) and (low carbohydrate diet: 15.5%; p<0.05).

A limitation of previous studies is the short term intervention (2-3 months), in our present study dietary intervention has been realized during 9 months. The longest intervention study in the literature was one year after the realization of a bilipancreatic derivation [17]. In both genetic groups, BMI, weight, glucose, triglyceride, total cholesterol, LDL cholesterol, and blood pressure decreased. Initial weight percent loss at 1 year of follow-up was higher in mutant group than in wild-type group (38.9% vs 29.9%; p<0.05). In our present study, after a high protein hypocaloric diet, subjects with Lys656Lys genotype of the LEPR gene showed a decrease of LDL cholesterol, insulin levels and HOMA-IR after weight loss. These obese subjects showed a decrease of LDL cholesterol and triglyceride levels after standard hypocaloric diet. 9 months of both diets significantly reduced weight, body mass index, waist circumference, fat mass and systolic blood pressure in obese subjects with both genotypes, with poorer biochemical response in patients carrying the allele Asn. The decrease of leptin levels was similar in both diets and in both genotypes.

Other authors have obtained association between SNP on codon 656 and obesity parameters. In postmenopausal females with intolerance of glucose (ITG), relationships were found with Lys656Asn for fasting insulin, as well as in response to an oral glucose test. In premenopausal females with ITG, associations were detected with Lys656Asn for overall glucose response to the glucose load [18]. 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 [19].

These different metabolic responses could be related with inclusion criteria and heterogeneity of subjects in the interventional studies, distribution of nutrients of the hypocaloric diets, time course of dietary intervention and interaction with other polymorphisms in LEPR gene. For example Xenachis et al. [20] have study the response to a hypocaloric diet in the LEPR gene polymorphism, but in a different polymorphisms (Thr343Ser). The overweight women carried the Ser allele lost more weight in response to low calorie diet than non carriers. At last, other theories could be explained through an association of genetic variation in this receptor with the functionality of Leptin receptor and, therefore, influenced the leptin response and weight lost to energy restriction. Mars et al. [21] had observed heterogeneity of metabolic responses with the Lys109Arg, Glu223Arg and Lys656Asn SNPs in the initial decline in leptin after a hypocaloric diet with a 21.5% of fats.
In conclusion, carries of ASn656 allele have a different metabolic change than wild genotype obese, with a lack of decrease of HOMA-IR, insulin, total cholesterol and LDL cholesterol after a high protein hypocaloric diet and a lack of decrease of HOMA-IR, insulin, total cholesterol, LDL cholesterol and triglycerides after a standard hypocaloric diet. This polymorphism did not influence in terms of weight loss.

Authors Contribution
Daniel Antonio de Luis designed the study an wrote the article. R Aller realized nutritional evaluation. O Izaola realized nutritional evaluation. D Primo realized laboratory analysis. E Romero designed the study an wrote the article.

References