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Research Report

Polymorphism in the PPARgamma2 and beta2-adrenergic genes and diet lipid effects on body composition, energy expenditure and eating behavior of obese women ☆

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Abstract

In order to evaluate the effect of polymorphism in the PPARgamma2 and beta2-adrenergic genes and diet lipids on body composition, energy expenditure and eating behavior of obese women, 60 subjects were submitted to anthropometric, biochemical, dietary, molecular, basal and postprandial metabolism (indirect calorimetry) and eating behavior (visual analog scale) evaluation. Fat and saturated fatty acid (SFA) high diet was used to assess postprandial metabolism. The frequency of Pro12Pro/Gln27Gln, Pro12Pro/Gln27Glu, Pro12Pro/Glu27Glu and Pro12Ala/Gln27Glu genotypes was 35.71%, 30.37%, 23.21% and 10.71%, respectively. These values were not significant ($p>0.05$) for the dietary, anthropometric, biochemical and metabolic parameters. The Pro12Ala/Gln27Glu group was found to present greater energy used in postprandial period (EUPP). The presence of the PPARgamma2 gene variant, independent of beta2-adrenergic gene polymorphism, resulted in fat oxidation increase. Also, this group presented higher satiety, compared to the Pro12Pro/Gln27Gln group. The presence of the variant alleles in the PPARgamma2 gene suggests benefits in food intake control.
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Keywords: Obesity; PPARgamma2; Beta2-adrenergic receptor; Diet; Energy metabolism; Eating behavior

Introduction

The prevalence of overweight and obese people is increasing worldwide in both developed and developing countries (Document of Latin American Consensus on Obesity, 1999). In Brazil, overweight rates for the population over 20 is 40.6%, with obesity rates of 8.8% for men and 12.7% for women (POF, 2004). In Spain, 11.9% men and 13.6% women are obese (Martínez, Moreno, & Martínez-Gonzales, 2004).

Low-energy expenditure and increase in food intake are involved in obesity, with the most important factors being difficult to determine (Bray, 1976; Monteiro & Halpern, 2000; Wolff, 1997).

The risk of developing obesity has a genetic component and many studies have quantified *loci* and candidate genes involved in its etiology. Human genome sequencing has offered numerous benefits to the study of genetic polymorphism, helping identify possible risks caused by the alleles, in addition to the development of genomic technologies that favor the study of diagnosis and treatment of obesity (Kowalski, 2004).

According to Perusse et al. (2001), this number is increasing, with a total of 54 new *loci* being added to the genetic map of human obesity last year, and the number of

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marked genes and chromosomal regions that have been associated with human obesity being presently around 250. Additionally, an increase in DNA sequence alterations in genes specific to the obesity phenotype has become evident, with 130 studies reporting positive association with 48 candidate genes. The genetic map of obesity reveals that the *loci* affecting obesity-related phenotypes may be found in all chromosomes, except Y.

Efforts to identify candidate genes for obesity have concentrated on adipose tissue, since thermogenesis regulation through the symphatic nervous system carried out by the brown adipose tissue is mediated by the beta-adrenergic receptors. Also, the PPARgamma (peroxisome proliferators-activated receptor gamma) plays an important role in adipogenesis and may control 30 genes responsible for the environmental signs linked to nutrients, such as fatty acids (Froguel & Boutin, 2001).

The PPARgamma2 gene is expressed preferably in differentiated adipocytes (Medina, Sewter, & Vidal-Puig, 2000) and mediates the expression of specific fat tissue cell genes (Spiegelman, Castillo, Hauser, & Puigservar, 1999) that codify proteins directly related to the lipogenic pathways (Desvergne & Wahli, 1999). Hence, this gene affects the fatty acid stock in fat tissue, participating in adipocyte differentiation by inducing pre-adipocyte maturation in fat cells. It acts by stimulating hydrolysis of the circulating triglycerides (TG) and subsequent entry of the fatty acids in the adipose cells. It also stimulates binding and activation of cytosol fatty acids, events required for TG synthesis (Gregoire, Smas, & Sul, 1998; Kersten, Desvergne, & Wahli, 2000) and participates also in adipocyte hypertrophy (Kubota et al., 1999).

Fat tissue accumulation occurs by means of three mechanisms: proliferation of pre-adipocytes found in fat deposits, its differentiation in adipocytes capable of storing fat, and through imbalance between lipogenesis and lipolysis, favoring the former. All these events are dependent on environmental and genetic factors. Adipogenesis persists throughout life and may be influenced by diet size, frequency and composition. Certain adipogenic transcription factors, such as PPARgamma, which interacts with cell-cycle regulating proteins, once modified, result in changes in gene expression related to adipogenesis (Palou, Bonet, & Rodríguez, 2001).

Malczewska-Malec et al. (2004) evaluated the relation among risk factors associated with obesity, including insulin-resistance, lipid tolerance, arterial hypertension, endothelial function and genetic polymorphisms; with appetite regulation, adipocyte differentiation and insulin-sensitivity, thermogenesis and fatty acid catabolism. The relation between obesity and certain genetic polymorphisms was observed.

Polymorphism of the PPARgamma2 gene is characterized by proline-to-alanine substitution at codon 12 (Deeb et al., 1998).

Studies on obese men and women show conflicting results when considering the relation between the variant in PPARgamma2 gene and obesity (Bearmer et al., 1998; Valve et al., 1999). Deeb et al. (1998) suggest that polymorphism of the PPARgamma2 gene has been associated with reduction of body mass index (BMI) (Deeb et al., 1998) and alteration of gene function. Kubota et al. (1999) evaluated mice with one function allele in the PPARgamma gene (Pro12Ala), and verified resistance to obesity development. However, Valve et al. (1999) observed that obese women with the Ala12Ala genotype had increased BMI, lean body mass, fat mass, and waist and hip circumferences compared with the women with the Pro12Pro or Pro12Ala genotypes.

There are various ligands of this gene (Houseknecht, Cole, & Steele, 2002; Mori et al., 1998), including polyunsaturated fatty acids (PUFA) (Azcárate, Moral, & Hernández, 2000; Houseknecht et al., 2002). Thus, change in the PUFA content in the dietary content could increase lipogenesis in subjects without the variant allele in the PPARgamma2 gene.

Dietary fatty acids influence various metabolic routes in an array of organs. Part of this influence causes alteration in mRNA activation. Under intense energetic restriction or fasting, activation of TG removal from the fat tissue occurs and large amounts of fatty acids are released into the liver, with the events occurring in this organ being influenced by PPAR (Kersten et al., 2000). On the other hand, high-fat diets promote adipocyte differentiation (Margareto, Larrarte, Marti, & Martínez, 2001; Vidal-Puig et al., 1996). Low fatty acid levels in animals fed high fat diets may occur because such diets could induce fat accumulation, using the circulating fatty acids (Berraondo, Marti, Duncan, & Martínez, 2000). With a high-fat diet adipocyte increased hypertrophy and insulin resistance are produced, while in rodents with polymorphism of the PPARgamma gene, the same effect has not been observed (Kubota et al., 1999).

In contrast, the beta2-adrenergic receptors are mediators of the lipolytic effects of the catecholamines (Meirhaeghe et al., 2001), participating in energetic homeostasis, since they stimulate the reduction of glycogen use and the increase of lipid mobilization (Arner, 2001). The beta2-adrenergic receptors (β2-AR) gene was found to be expressed in subcutaneous fat tissue (Large et al., 1997). However, abdominal fat has a higher density and sensitivity to the beta-adrenergic receptor gene, and in obese individuals with increase waist circumference (WC), beta2-adrenergic gene activity is higher (Meirhaeghe et al., 2001). The presence of the variant in this gene, characterized by a glutamine-to-glutamic acid substitution at codon 27, is associated with body weight gain (Hellström, Large, Reynisdottir, Wahrenberg, & Arner, 1999).

Thus, when considering the functions of the evaluated genes, it is suggested that polymorphism of the beta2-adrenergic receptor gene promotes body weight gain in subjects on high fat diets, since it could reduce oxidation of the ingested fat. On the other hand, polymorphism of the PPARgamma2 gene would facilitate body weight loss in the presence of a high fat diet.

In addition to the genetic factor, Ravussin and Bogardus (2000) suggest that, in different populations, obesity is greatly influenced by environmental factors, considering that subjects of the same community living in the same environment vary in body size and composition genetically determined as a response to the environment.

Eating behavior becomes an important factor when environmental factors are taken into account in obesity genesis.

Fats are known to have a lower satiation effect than carbohydrates, generating a passive over consumption of energy (Blundell, Burley, Cotton, & Lawton, 1993).

Dietary fat and oil differ in fatty acid chain length, their degree of saturation, and the position and stoichiometric double-bound configuration, affecting their oxidation rates (Piers, Walker, Stoney, Soares, & O'Dea, 2002), due to their relation with insulin resistance (Borkman et al., 1993). The oxidation speed of certain fatty acids is also related to satiety. Considering that saturated fatty acids (SFA) have slower oxidation speed than PUFA, the former favor fat deposition. Monounsaturated fatty acids (MUFA) favor lipid deposition as energy source in fat tissue, when compared with PUFA, thus exerting less control on appetite. It should be considered that oxidized lipids promote satiety whereas stored lipids do not (Friedman, 1998; Monteiro & Mourão, 2002). Lawton, Delargy, Brockman, Smith, and Blundell (2000) reported also that PUFA offer better appetite control compared to PUFA and SFA.

In addition to lipids, carbohydrates have been associated with appetite control. They seem to be efficient in inhibiting appetite on a short-term basis, compared to fat, due to their oxidative priority, which reflects in satiety duration (Monteiro & Mourão, 2002). Mourão, Monteiro, Hermsdorff, and Leite (2004) evaluated the effect of sucrose and its substitute (sucralose) on appetite and satiety in lean and overweight men, maintaining the fatty acid profile and the caloric density of the diets. It was verified that sucrose substitution and the increase of complex carbohydrates in the diet reduced hunger, and increased satiety, thermogenesis, resting respiratory quotient (RRQ), resting metabolic rate (RMR) and carbohydrate oxidation (CHOX), regardless of the group. However, overweight men showed higher energy expenditure and lower thermogenesis, when compared to lean men, regardless of diet.

Although evidence shows that obesity is a multifactor disease and that genes and the environment are related, few studies have associated the effect of one or more genes, and gene–environment interactions on energy expenditure and eating behavior. Hence, further and more detailed evaluations of these factors are required to clarify the differences in energy expenditure and eating behavior of obese subjects.

The purpose of this paper is to assess the effect of fat diet and polymorphism of the PPARgamma2 and β2-AR genes on energy expenditure and eating behavior of overweight women. Our aim is to acquire a deeper understanding of the possible interactions among the PPARgamma2 and beta2-adrenergic genes, dietary and metabolic factors in body weight variation.

Methods

Subjects

Fifty-six obese women (WHO, 1998) were selected, aged 20–49 yr (34.6 $\pm$ 7.6) and BMI between 30.00 and 56.46 kg m^{-2} (37.7 $\pm$ 6.2). The women were submitted to nutritional evaluation to meet inclusion criteria, which considered women with BMI $\geqslant$ 30 kg m^{-2}, absence of diabetes, weight loss under 3 kg in the past 3 months, absence of menopause symptoms and chronic diseases.

Experimental protocol

The women were recruited in Pamplona (Spain). The study protocol (Fig. 1) was established and approved by the Department of Physiology and Nutrition of the School of Pharmacy of Navarra University.

Dietary evaluation was followed by a clinical assay, in which anthropometric, metabolic, biochemical and

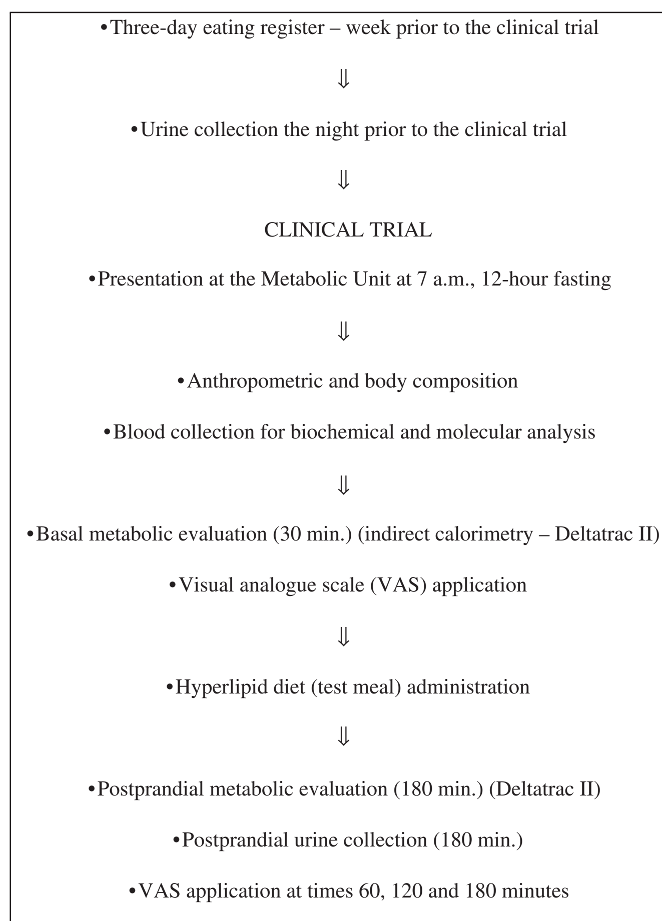

Fig. 1. Experimental protocol.

molecular parameters and eating behavior were assessed. Groups were divided according to the genotypes of the evaluated genes.

During the trial, the women remained sedentary and were fed only the test meal and water.

Assessment of habitual diet

Usual diet intake was evaluated in the week prior to the clinical trial by means of a detailed food registry, 3 days per week, two typical and one atypical. Registry analysis was performed using the Medisystem (Sanocare SL) software.

Anthropometric and body composition assessment

The women were weighed using a micro digital electronic balance (Seca), with capacity of 150 kg and 100 g precision. Height was determined using a 0.5 cm scale vertical anthropometer (Geissler, Miller, & Shah, 1987; Gibson, 1990). BMI was calculated (Bray & Gray, 1988), and classified according to WHO (1998).

The bio-electrical impedance (BI) (Biodynamics model 310) method was used to evaluate total body water, and indirectly the fat-free mass (FFM) and total body fat (TBF) (Lukaski, Johnson, Bolonchuk, & Lykken, 1985; Valtueña, Blanch, Barenys, Solá, & Salas-Salvadó, 1995; Valve et al., 1999).

The relation between the WC and hip circumference was determined (Després, Lemieux, & Prud′homme, 2001; Weinsier et al., 1995). The WC was also evaluated alone, due to its relation with co-morbidity risks (NCEP, 2001; WHO, 1998).

Assessment of energy metabolism

Energy metabolism was evaluated by indirect calorimetry using the Deltatrac II® (Datex-Engstrom, Finland) monitor (Ferrannini, 1988). Oxygen (O_2) consumption and carbon dioxide (CO_2) production (mL/min) (Ferrannini, 1988; Valve et al., 1999); fasting (8 h) and postprandial (3 h) urine nitrogen; and high-fat diet composition were used to calculate:

- Basal energy expenditure (BEE) (kcal/min)-$(16.4 \times VO_2) + (4.5 \times VCO_2) - (O_2)3.4 \times N$ (g/min))/4.18 (N = urine nitrogen).
- Non-protein respiratory quotient (NPRQ) and postprandial non-protein respiratory quotient (PPNPRQ) = $NPVCO_2$ (L/min)/$NPVO_2$ (L/min). $NPVCO_2 = VCO_2$ (L/min)$-PVCO_2$ (L/min). $PVCO_2$ (L/min) = (N (g/min) $\times 6.25 \times 0.774$). $NPVO_2 = VO_2$ (L/min)$-PVO_2$ (L/min). PVO_2 (L/min) = (N (g/min) $\times 6.25 \times 0.966$).
- Basal lipid oxidation (BLOX) and postprandial lipid oxidation (PPLOX) (g/min) = $NPVO_2 \times (1^a - NPRQ)/(0.293^b \times 2.019^c)$. [a]Quotient of complete oxidation of lipids, [b]difference between 1 and 0.707, [c]VO_2 (L)/oxidized lipids (g).
- Basal carbohydrate oxidation (BCHOX) (g/min)-$NPVO_2 \times (NPRQ - 0.707)/(0.293 \times 0.829^d)$. [d]$VO_2$ (L)/oxidized glucose (g).
- Postprandial carbohydrate oxidation (PPCHOX) (g/min) = $NPVO_2 \times (NPRQ - 0.707^e)/(0.293^b \times 0.746^f)$. [e]Quotient of the complete oxidation of carbohydrate, [b]difference between 1 and 0.707, [f]VO_2 (L)/oxidized glucose (g).
- Thermic effect of food (TEF) (%) = {[BMR (kcal/kg/min) postprandial$-$BMR (kcal/kg/min) in fast] $\times$ 30 $\times$ 100}/kcal of diet.
- Energy used in postprandial period (EUPP) = (PPMR in 30 min) + [(PPMR in 60 min $\times$ 30) + (PPMR in 90 min $\times$ 30) + PPMR in 120 min $\times$ 30) + (PPMR in 180 min $\times$ 30)].

Liquid protein oxidation was calculated to obtain non-protein oxidation of other macronutrients (Ferrannini, 1988; Labayen, Forga, & Martínez, 1999).

In order to evaluate postprandial energy metabolism a high-fat diet (95%) and SFA (Fraîche cream) were used. The amount of the diet offered corresponded to 50% of the subject's total energy expenditure (TEE) (WHO, 1985).

Fasting urea and postprandial urine concentrations, used to calculate urinary nitrogen, were determined through the enzymatic method with urease and dehydrogenase glutamate.

Assessment of eating behavior

A visual analog scale (VAS) of 10 cm was used to evaluate eating behavior (appetite, satiety, satiation, urge to eat and thirst) (Alonso, Prieto, & Antó, 1995). VAS was applied at times 0 (fasting), and 60, 120 and 180 min after test diet intake. The questionnaire answers were not discussed or compared among the study volunteers.

Biochemistry assessment

Leptin, insulin, and TG levels were analyzed using blood samples collected during the clinical trial.

Serum levels of insulin (Boden, Chen, Kolacznski, & Polansky, 1997) were determined by radioimmunoassay (RIA) technique (Valve et al., 1999) using the Coat-A-Count® Insulin (Diagnostic Products Corporation) kit.

Serum levels of leptin were determined by immunoradiometric assay (IRMA) (Miles, Lipschitz, Bieber, & Cook, 1974), using the Active™ Human Leptin IRMA (DSL-23100) (Diagnostic Systems Laboratories, Inc) kit.

Serum levels of TG were determined by means of a colorimetric enzymatic reaction using the GPO/PAP method (Valve et al., 1999) and measured photometrically (Cobas Mira).

Molecular analysis

Genomic DNA was isolated from the white cells in blood samples by organic extraction (phenol/chloroform)

(Hellström et al., 1999), based on density gradient centrifugation. The samples were quantified in a spectrophotometer at 260, 270, 280 and 310 nm and kept at −20 °C at 100 ng/μL concentration.

The detection of alleles Pro12Pro, Pro12Ala and Ala12Ala in the PPARgamma2 gene was carried out by means of DNA polymerase chain reaction (PCR) (Mullis & Falooma, 1987), available in the GenBank DNA AB005520 (2003). The primers used were 5′-GCCAATT-CAAGCCCAGTC-3′ and 5′-GATATGTTTGCAGA-CAGTGTATC AGTGA AGGAATCGCTTTCC G-3′. The cycling conditions were curling temperature of 59 °C, denaturation temperature and time of 94°C/5 min, and extension temperature and time of 72 °C/30 s, totaling 35 cycles. The generated fragment was of 267 pb (pairs of bases). After sample enzymatic digestion (60 °C/180 min) using restriction enzyme Bst UI, the following fragments were generated: 267 pb (Pro12Pro); 267, 224 and 43 pb (Pro12Ala); and 224 and 43 pb (Ala12Ala).

The detection of alleles Gln27Gln, Gln27Gluxc and Glu27Glu in the β2-AR gene was carried out by means of PCR (Mullis & Falooma, 1987), available in the GenBank DNA Y00106 (2003). The primers were 5′-CCGC-CGTGGGTCCGCC-3′ and 5′-CCATGACCAGATCAG-CAGCAC-3′. The cycling conditions were curling temperature of 65 °C, denaturation temperature and time of 94 °C/5 min and extension temperature and time of 72 °C/30 s, in a total of 35 cycles. The generated fragment was 310 pb. After enzymatic digestion of the samples (37 °C/180 min) using restriction enzyme ITA I, the following fragments were generated: 171, 84 and 55 pb (Gln12Gln); 226, 171, 84 and 55 pb (Gln12Glu); and 226, 84 pb (Glu12Glu).

Experimental design and data analysis

The dietary, anthropometric, metabolic and biochemical parameters were analyzed using a completely randomized design with four treatments (A: Pro12Pro allele in PPARgamma2 gene and Gln27Gln in β2-AR gene; B: Pro12Pro in the PPARgamma2 gene and Gln27Glu in β2-AR gene, C: Pro12Pro allele in PPARgamma2 gene and Glu27Glu allele in β2-AR gene, and D: Pro12Ala and Gln27Glu in PPARgamma2 and β2-AR genes, respectively). The data were analyzed with ANOVA. Differences between the group means were evaluated by the Duncan test at 5% probability.

The data obtained from the VAS were analyzed using a split-plot scheme, with the groups constituting the plots (A, B, C and D) and the times constituting the split-plots (0, 60, 120 and 180 min), in a completely randomized design. Data interpretation was performed using ANOVA, Duncan test at 5% probability, and regression analysis. The models were chosen based on regression coefficient significance, using the *t*-test at 1% probability, and on the coefficient of determination.

Results

Frequency of genetic polymorphism

Following molecular evaluation of the PPARgamma2 and β2-AR genes, it was observed that 35.71%, 30.37%, 23.21% and 10.71% of the women showed the genotypes Pro12Pro/Gln27Gln (group A), Pro12Pro/Gln27Glu (group B), Pro12Pro/Glu27Glu (group C) and Pro12Ala/Gln27Glu (group D), respectively.

Assessment of polymorphism effect on dietary, anthropometric, biochemical and metabolic parameters

The phenotype of each genotype, characterized for the anthropometric and biochemical parameters did not differ among groups ($p > 0.05$) (Table 1).

No differences were observed among groups ($p > 0.05$) regarding energy and macronutrient intake and habitual diet lipid profile.

Considering the metabolic parameters, the EUPP was higher in group D, compared to groups A, B and C ($p < 0.05$), whereas the other parameters did not differ among groups ($p > 0.05$) (Table 2).

Table 1
Anthropometric and biochemical characteristics of groups Pro12Pro/Gln27Gln (group A), Pro12Pro/Gln27Glu (group B), Pro12Pro/Glu27Glu (group C) and Pro12Ala/Gln27Glu (group D)

Parameters/groups	Group A ($n = 20$)	Group B ($n = 17$)	Group C ($n = 13$)	Group D ($n = 6$)
Age (years)	33.5±6.6 a	34.4±8.3 a	35.5±7.9 a	36.7±9.0 a
BMI ($kg\,m^{-2}$)	38.0±7.0 a	35.9±3.5 a	38.3±6.5 a	40.4±9.0 a
Waist/hip ratio	0.82±0.06a	0.85±0.09 a	0.86±0.05 a	0.83±0.04 a
WC (cm)	102.3±18.3 a	100.2±9.8 a	106.2±11.9 a	107.7±19.8 a
TFB (%)	47.0±5.9 a	45.8±3.8 a	46.8±5.4 a	49.2±7.2 a
FFM (%)	53.5±6.4 a	54.4±3.8 a	54.0±6.1 a	51.8±7.9 a
Leptin ($ng\,mL^{-1}$)	82.7±26.7 a	79.4±23.4 a	77.7±26.2 a	65.8±19.5 a
Insulin ($MIL\,mL^{-1}$)	11.1±7.5 a	13.0±10.0 a	12.3±5.9 a	11.6±9.6 a
Triglycerides ($mg\,dL^{-1}$)	97.8±44.4 a	84.8±35.9 a	93.1±34.8 a	77.7±22.7 a

Means followed by the same letter in the line do not differ at 5% probability by the Duncan test.*Abbreviations:* BMI—body mass index; WC—waist circumference; TBF—total body fat; FFM—fat-free mass.

Table 2
Basal and postprandial metabolic characteristics of groups Pro12Pro/Gln27Gln (group A), Pro12Pro/Gln27Glu (group B), Pro12Pro/Glu27Glu (group C) and Pro12Ala/Gln27Glu (group D)

	Group A	Group B	Group C	Group D
BEE (kcal min^{-1})	1.20 ± 0.22 a	1.15 ± 0.12 a	1.22 ± 0.14 a	1.31 ± 0.25 a
BNPRQ (VCO_2/VO_2)	0.81 ± 0.05 a	0.81 ± 0.07 a	0.83 ± 0.06 a	0.79 ± 0.04 a
BLIPOX (g min^{-1})	0.07 ± 0.02 a	0.07 ± 0.03 a	0.06 ± 0.03 a	0.09 ± 0.03 a
BCHOX (g min^{-1})	0.09 ± 0.04 a	0.08 ± 0.06 a	0.10 ± 0.05 a	0.08 ± 0.03 a
PPNPRQ (VCO_2/VO_2)	0.78 ± 0.04 a	0.74 ± 0.08 a	0.78 ± 0.04 a	0.75 ± 0.04 a
PPLIPOX (%)	15.0 ± 3.2 a	16.8 ± 6.0 a	15.7 ± 4.4 a	19.7 ± 6.0 a
PPCHOX (%)	204.2 ± 113.9 a	136.3 ± 100.2 a	206.6 ± 115.0 a	139.1 ± 113.2 a
EUPP (%)	27.3 ± 1.9 a	27.4 ± 2.5 a	28.2 ± 2.3 a	30.6 ± 2.00 b
TEF (%)	2.4 ± 1.3 a	2.6 ± 1.3 a	2.1 ± 1.00 a	2.9 ± 1.3 a

Means followed by the same letter in the line do not differ at 5% probability, Duncan test.
Abbreviations: BEE—basal energy expenditure; BNPRQ—basal non-protein respiratory quotient; BLIPOX—basal lipid oxidation; BCHOX—basal carbohydrate oxidation; PPNPRQ—postprandial non-protein respiratory quotient; PPLIPOX—postprandial lipid oxidation; PPCHOX—postprandial carbohydrate oxidation; EUPP—energy used in postprandial period; TEF—thermic effect of food.

Table 3
Mean values of hunger, satiety, satiation, urge to eat, and thirst sensations in Pro12Pro/Gln27Gln (group A), Pro12Pro/Gln27Glu (group B), Pro12Pro/Glu27Glu (group C) e Pro12Ala/Gln27Glu (group D) women

Groups	Hunger	Satiety	Satiation	Urge to eat	Thirsty
A	28.0 ± 24.7 a	41.4 ± 32.9 b	48.7 ± 29.6 a	40.5 ± 28.6 a	59.0 ± 30.7 a
B	28.4 ± 27.5 a	52.7 ± 35.7 ab	58.4 ± 32.5 a	36.4 ± 32.5 a	60.6 ± 32.0 a
C	34.9 ± 29.3 a	42.1 ± 34.9 ab	46.8 ± 33.8 a	45.8 ± 31.4 a	54.2 ± 28.3 a
D	23.4 ± 20.9 a	65.7 ± 28.0 a	59.7 ± 32.6 a	26.7 ± 24.8 a	65.6 ± 30.2 a

Means followed by the same letter in the line do not differ at 5% probability, Duncan test.

Assessment of eating behavior

Table 3 shows that satiety did not differ among groups B, C and D. However, group D showed higher values ($p < 0.05$), compared to group A.

It was observed that high-fat diet resulted in a reduced feeling of hunger and urge to eat during the first 60 min, and subsequent increase after 120 min. The opposite was observed in the satiety and satiation curves, which increased the following intake and began reducing 120 min after intake. The feeling of thirst showed a linear increase from time 0. No interaction was found between evaluation time and the groups (Fig. 2).

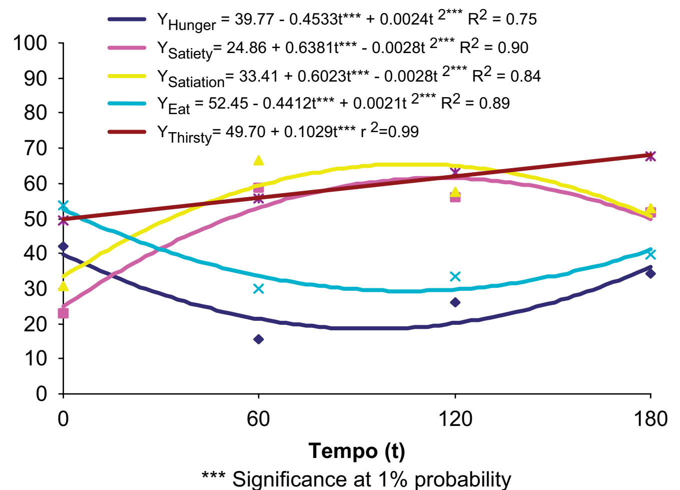

Fig. 2. Estimates of sensations of hunger, satiety, satiation, urge to eat and thirsty, in time (minutes), according to the Visual Analog Scale (VAS) in Pro12Pro/Gln27Gln (group A), Pro12Pro/Gln27Glu (group B), Pro12Pro/Glu27Glu (group C) and Pro12Ala/Gln27Glu (group D) women. (***) Significance at 1% probability.

Discussion

The susceptibility to obesity is partly determined by genetic factors, but an "obesity-promoting environment" is typically necessary for its phenotypic expression.

The polymorphism frequency of PPARgamma2 and β2-AR genes is not observed in other studies.

Mori et al. (1998) verified a low frequency of the polymorphism Pro12Ala in the gene PPARgamma2 (3%) in Japanese men. Deeb et al. (1998) also observed frequency of 12% in the allele alanine in non-diabetic men and women. Ek et al. (1999) verified a frequency of 14% of the polymorphism Pro12Ala in obese men. van Rossum et al. (2002) verified that 75.4%, 23.1% and 1.5% of the obese men and 79.7%, 20.3% and 0% of the non-obese men presented the genotypes Pro12Pro, Pro12Ala and Ala12Ala, respectively. Also, 76.7%, 22.6% and 0.7%

of the obese women and 73.7%, 25.7% and 0.6% of the non-obese women presented the genotypes Pro12Pro, Pro12Ala and Ala12Ala, respectively. No difference was observed among the groups.

Meirhaeghe, Helbecque, Cottel, and Amouyel (1999) verified that the frequency of the genotypes Gln27Gln, Gln27Glu and Glu27Glu was 33.1%, 51.0% and 15.9%, respectively. van Rossum et al. (2002) verified that 30.7%, 53.0% and 16.3% of the obese men; 26.7%, 55.8% and 17.5% of the non-obese men; 34.4%, 45.7% and 19.9% of the obese women; and 31.5%, 49.1% and 19.4% of the non-obese women presented the genotypes Gln27Gln, Gln27Glu and Glu27Glu, respectively. No differences were observed among the groups.

According to Bearmer et al. (1998) and Valve et al. (1999), studies on obese men and women show conflicting results when considering the relation between the variant in PPARgamma2 gene and obesity, since some verified a significant increase of BMI and WC in subjects with Pro12Ala polymorphism, whereas others reported that the variant is associated with the lowest BMI.

Deeb et al. (1998) evaluated lean adult men and women and verified that the presence of the variant in the PPARgamma2 gene was related to reduced fasting insulin concentration.

When evaluating lean subjects, Luan et al. (2001) verified that the presence of the allele alanine in the PPARgamma2 gene led to adiposity reduction following intake of PUFA-rich diets, and considering that some fatty acid derivatives are ligands of PPAR, they are expected to more effectively stimulate adipogenesis in Pro12Pro than in Pro12Ala. The authors confirmed the relation between the type of fatty acid and Pro12Ala polymorphism in fasting insulin, since increase in the diet's PUFA:SFA ratio caused BMI and insulin reduction, whereas PUFA:SFA ratio reduction resulted in higher BMI, compared to Pro12Pro subjects. This finding could reflect also an increase in hormone resistance.

In the present study, intake of a high-fat and SFA diet promoted higher lipid oxidation, in the short term, in women with the alanine variant, due to EUPP increase in group D.

In view of the lack of data showing a possible interaction between the two evaluated genes, it is important to propose a study on the effect of fatty acid type on different allele combinations of the different obesity candidate genes, as well as an increase in the sample size.

It is known that genetic factors also affect the parameters related to eating behavior such as appetite and satiety, which influence obesity pathogenesis.

The presence of the alanine allele in the PPARgamma2 gene led to satiety increase, since groups B and C, which had variant alleles in the β2-AR gene, did not differ in relation to it. There seems to be no difference in gene expression between the alleles of the PPARgamma2 and β2-AR genes. This result suggests that satiety is determined by an association of the two genes.

A smaller insulin concentration in women with the variant alanine in the PPARgamma2 gene could result in anabolism reduction and consequent increase in plasma nutrient concentration, what explains higher satiety in women with the allele alanine.

As previously discussed, Lawton et al. (2000), Monteiro and Mourão (2002), and Friedman (1998) reported the existence of variation in the oxidation velocity of certain fatty acids, which influences satiety. PUFA would favor satiety, compared with MUFA and SFA. However, no data are available on evaluation of appetite variation and satiety in obese subjects with polymorphism of PPARgamma2 and β2-AR genes. Considering that group D showed a higher EUPP and that the ingested diet was high fat and SFA, it is suggested that there is a tendency for higher lipid oxidation in this group. Thus, increased lipid oxidation would favor satiety.

Houseknecht et al. (2002) reported the function of PPARgamma in various physiological and physiopathological events, including stimulating insulin action and lipid metabolism regulation. Activation of the gene improves insulin action, which is reduced during adipocyte differentiation. In humans, the presence of the alanine allele is related to reduction of fasting insulin concentration; however, these subjects may show less amount of fat tissue, leading to insulin sensitivity (Deeb et al., 1998).

The adrenergic receptor genes regulate lipid mobilization, energy expenditure and glycogen depression (Barber, Millet, Galitzki, Lafontan, & Berlan, 1996). The β2-AR family mediates the lipolytic effects of catecholamines (Meirhaeghe et al., 2001), which stimulate lipid mobilization, having an opposite effect of insulin (Flatt, 1996). Hellström et al. (1999) believe that polymorphism in the β2-AR gene could lead to obesity.

Hence, the glutamic acid variant in codon 27 of the β2-AR gene would cause the lipolytic effects of catecholamines, possibly leading to a potential insulin effect and appetite increase.

Valet et al. (2000) demonstrated how the two genes for alpha 2 and beta 3-adrenergic receptors and diet interact to influence fat mass.

van Rossum et al. (2002) investigated the association between DNA polymorphism in several candidate genes for obesity and weight gain. Polymorphisms in these genes may contribute to weight gain through effects on energy intake, energy expenditure or adipogenesis. This study compared 286 subjects aged 20–40 years who gained an average of 12.8 kg during a mean follow-up of 6.8 years with 296 subjects who remained relatively constant over the same period with respect to occurrence of several polymorphisms in candidate genes of obesity and some lifestyle factors. Only variations in the β2-AR gene (Gly16Arg and Gln27Glu) and leptin genes appeared to susceptibility to weight gain. The study suggests further research was necessary to establish the role of lifestyle factors, or interactions between genes or between genes and lifestyle factors on weight gain with age.

The eating behavior of obese subjects may be explained by some mechanisms. According to group D results, one should consider the fact that obese subjects with a high percentage of TBF, almost always insulin-resistant, have a high yield and secretion of the tumoral necrosis factor (TNFα). TNFα reduces the expression of the lypogenic genes, including PPARgamma2, which already shows altered functions in the presence of the allele alanine. In addition, it reduces appetite and promotes leptin secretion by adipocytes, which has been related to energy homeostasis, in comparison to insulin. The variant allele in the β2-AR gene would also lead to increased insulin production but resistance to hormone action and the more pronounced function of the circulating leptin would reduce the effect of insulin. On the other hand, a high fat diet may reduce insulin levels and increase food intake. However, fatty acid type influences nutrient metabolism. Other results obtained by the researchers showed that women in group D with higher BMI and higher intake of habitual SFA had also higher fasting insulin levels.

Kubota et al. (1999) evaluated mice with one function allele in the PPARgamma gene (Pro12Ala), and verified resistance to obesity development, in part due to hyperleptinemia and subsequent hypophagia. It was also verified that a high-fat diet produces increased insulin resistance in subjects with the Pro12Pro allele in the PPARgamma2 gene. In view of this, differences in eating behavior were found to depend on the subject's genotype.

Further studies are needed to elucidate the effect of the interaction of different genes related to obesity with environmental factors such as diet, anthropometric, biochemical and metabolic parameters, and eating behavior. This knowledge will be of great importance in the dietary planning for obese subjects with different genetic characteristics, helping them lose and maintain body weight.

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