European Review for Medical and Pharmacological Sciences 2013; 17: 2810-2815

Relation of –55CT polymorphism of UCP3 gene with weight loss and metabolic changes after a high monounsaturated fat diet in obese non diabetic patients

D.A. DE LUIS, R. ALLER, O. IZAOLA, B. DE LA FUENTE, R. CONDE, J.M. EIROS BOUZA

Institute of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain

Abstract. – AIMS: The aim of our study was to investigate the influence of -55CT polymorphism of UCP3 gene on metabolic response, weight loss and serum adipokine levels to a high monounsaturated fat hypocaloric diet in obese patients.

PATIENTS AND METHODS: A sample of 128 obese patients was analyzed in a prospective way during 3 months.

RESULTS: Eighty eight patients (21 males/67 females) (68.8%) had the genotype 55CC (wild genotype group) and 40 patients (8 males/32 females) (31.3%) 55CT (mutant genotype group). In wild genotype group, BMI (–1.6±1.3 kg/m^2), weight (–4.3±3.7 kg), fat mass (–3.5±3.3 kg), waist circumference (–5.1±2.9 cm), total cholesterol (–7.2±10.6 mg/dl), LDL cholesterol (–5.3±12.8 mg/dl) and leptin (–4.7±10.1 ng/ml) decreased. In mutant genotype group, BMI (1.3±2.2 kg/m^2), weight (–3.0±1.4 kg), fat mass (–2.5±1.1 kg), waist circumference (–2.8±3.1 cm) and leptin (–5.8±10.7 decreased.

CONCLUSIONS: In patients with –55CC UCP3 genotype, a high mono-unsaturated hypocaloric diet reduced BMI, weight, waist circumference, waist to hip ratio, fat mass, LDL-cholesterol, total cholesterol and leptin levels. Carriers of T allele had a different response than –55CC patients, with a significant decrease of the same antropometric parameters, but lower than in the wild genotype group, and without significant changes in cholesterol levels.

Key Words:
55CT polymorphism of UCP3 gene, Monounsaturated fatty acids, Obesity.

Abbreviations

CRP = C reactive protein; HDL = high density lipoprotein; HOMA = homeostasis model assessment; LDL = low density lipoprotein; PCR = polymerase chain reaction; Ucp = uncoupling protein WHR = waist-to hip ratio

Introduction

An accumulating body of evidence shows that modest weight loss (5%) through dietary changes and exercise is an effective means fo managing obesity-associated disorders[1]. The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity and energy expenditure[2].

Obesity may result from interactions between different factors controlling the environment, eating behavior, and energy expenditure. The genetics behind common obesity is likely to be dominated by several polymorphisms each with moderate effect. Uncoupling protein 3 (UCP3) belongs to a family of mitochondrial transporters that could uncouple the oxidative phosphorylation by increasing the proton leak of the inner mitochondrial membrane[3]. Decreased expression or function of UCP3 could reduce energy expenditure and increase the storage of energy as fat[4]. Some studies have pointed to a role of UCP3 in the regulation of whole body energy homeostasis[5], lipids as metabolic substrates diet induced obesity[6], and regulation of lipids as metabolic substrates[7]. The C/C genotype of a polymorphism in the UCP3 promotor (–55C->T) is associated with increased BMI and interacts with physical activity[8]. It was shown that –55 T/T genotype is associated with an atherogenic lipid profile in French Caucasians and with a decreased risk of type 2 diabetes[9]. Our group has demonstrated that weight loss is associated with different changes depending of –55C/T UCP3 genotype. Carriers of T allele have a different response secondary to a standard hypocaloric diet than wild type obese[10,11] and the distribution of macronutrient and type of dietary fat could be implied in this different metabolic response[11].

Corresponding Author: Daniel A. De Luis, MD; e-mail:dadluis@yahoo.es

Another interesting topic area is the active metabolic role of adipose tissue in obese patients. The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity[5,11-13].

Furthermore, in light of previous findings, the aim of our study was to investigate the influence of –55CT polymorphism of UCP3 gene on metabolic response, weight loss and serum adipokine levels to a high monounsaturated fat hypocaloric diet in obese patients.

Patients and Methods

Patients

A population of 128 obese non diabetic outpatients was analyzed in a prospective way. These patients were studied in a Nutrition Clinic Unit and signed an informed consent. 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 sulphonylurea, thiazolidinediones, insulin, glucocorticoids, inhibitors of dipeptidyl peptidase IV, analogues of GLP-1, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit.

Procedure

All patients with a 3 weeks weight-stabilization period before recruitment were enrolled. The high monounsaturated fat hypocaloric intervention consisted in a diet of 1342 kcal, 46.6% of carbohydrates, 34.1% of lipids and 19.2% of proteins). The distribution of fats was; a 21.7% of saturated fats, a 67.5% of monounsaturated fats and a 10.8% of polyunsaturated fats. Weight, blood pressure, basal glucose, C-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and adypocitokines (leptin, adiponectin, resistin, TNF alpha, and interleukin 6) levels were measured at basal time and at three months, after life style modification. Genotype of UCP3 gene polymorphism was studied.

Genotyping of UCP3 Gene Polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, Los Angeles, CA, USA). The polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 µL of each oligonucleotide primer (primer forward: 5'-GAT CTG GAA CTC ACT CAC CTC-3'; primer reverse: 5'-CTG TTG TCT CTG CTG CTT CT-3'), and 0.25 µL of each probes (wild probe: 5'-Fam-TAT ACA CAC GGG CTG ACC TGA-Tamra-3') and (mutant probe: 5'-Hex-CTT ATA CAC ACA GGC TGA CCT GA-Tamra-3´) in a 25 µL final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA, USA). DNA was denaturated at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95ºC for 15 sec, and annealing at 59.3° for 45 sec). The PCR were run in a 25 µL final volume containing 12.5 µL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase. The Hardy Weinberg equilibrium was examined with p value > 0.05.

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), 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.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyzer 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L)[14] and the homeostasis model assessment for insulin sensitivity (HOMA-IR) was calculated using these values[15]. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl.

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[16]. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml[17]. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml [18]. Interleukin 6 and TNF alpha were measured by ELISA (R&D systems, Inc., Minneapolis,

MN, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNFalpha (0.5-15.6 pg/ml)[19-20].

Anthropometric Measurements

Body weight was measured to an accuracy of 0.5 kg and body mass index (BMI) computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition[21] (Biodynamics Model 310e, Seattle, WA, USA). Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Dietary Intake and Habits

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference[21]. Regular aerobic physical activity (walking was allowed, no other exercises) was mantained during the period study (3 hours per week).

Statistical Analysis

Sample size was calculated to detect differences over 3% in weight loss with 90% power and 5% significance. The results were expressed as means ± standard deviation. 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. Non-parametric variables were analyzed with the W-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 *–55CT* and *–55TT* as a mutant group and wild type *–55CC* as second group. A *p*-value under 0.05 was considered statistically significant.

Results

One hundred and twenty eight patients gave informed consent and were enrolled in the study. The mean age was 48.8±10.3 years and the mean BMI 36.9±6.4, with 29 males (22.7%) and 99 females (77.3%). All patiens completed the follow up during 3 months, with a weight loss of 4.1±1.0 kg (3.9%). All patients reach dietary intake recommendations.

Eighty eight patients (21 males/67 females) (68.8%) had the genotype *55CC* (wild group) and 40 patients (8 males/32 females) (31.3%) *55CT* (mutant group). Sex distribution was similar in wild and mutant type groups (wild type: 23.9% males vs 76.1% females and mutant: 20.0% males vs 80.0% females)

Table I shows the differences in anthropometric variables. In wild group, BMI (–1.6±1.3 kg/m^2), weight (–4.3±3.7 kg), fat mass (–3.5±3.3 kg) and waist circumference (–5.1±2.9 cm) decreased. In mutant group, BMI (1.3±2.2 kg/m^2), weight (–3.0±1.4 kg), fat mass (–2.5±1.1 kg),

Table I. Changes in anthropometric variables.

Characteristics	55CC Basal time	55CC at 3 months	55CT Basal time	55CT at 3 months
BMI	37.7 ± 6.0	36.1 ± 5.6*	37.3 ± 6.7	36.1 ± 5.9*
Weight (kg)	98.1 ± 17.5	93.7 ± 16.1*	93.4 ± 20.4	90.5 ± 19.6*
Fat free mass(kg)	56.4 ± 13.9	55.7 ± 13.6	51.0 ± 6.6	50.3 ± 9.8
Fat mass (kg)	42.5 ± 11.9	38.8 ± 10.3*	39.0 ± 12.2	37.5 ± 8.1*
WC (cm)	114.3 ± 14.1	109.3 ± 11.6*	108.1 ± 13.2	105.2 ± 12.1*
WHR	0.92 ± 0.09	0.91 ± 0.08	0.93 ± 0.2	0.91 ± 0.1
SBP (mmHg)	124.9 ± 11.7	123.3 ± 11.4	129.5 ± 18.4	125.3 ± 19.8
DBP (mmHg)	80.6 ± 7.4	78.5 ± 8.1	80.7 ± 4.2	77.8 ± 8.4

DBP: Diastolic blood pressure. SBP: Systolic blood pressure. WC: waist circumference. WHR: Waist to hip ratio. *$p < 0.05$, in each group with basal values.

waist circumference (–2.8±3.1 cm) decreased. No differences were detected between basal values of these values in both groups.

The decrease of BMI, weight, fat mass and waist circumference were higher in wild type group than mutant type group.

Table II shows the differences in classic cardiovascular risk factors. In wild group, total cholesterol (–7.2±10.6 mg/dl) and LDL cholesterol (–5.3±2.8 mg/dl) decreased. In mutant group, biochemical parameters did not change in a significant way.

Table III shows differences between basal and after treatment levels of adipokines. Only leptin levels had a significant decrease in wild group (–4.7±10.1 ng/ml) and mutant group reach statistical differences (–5.8±10.7 ng/ml), too. Il6, TNF alpha, resistin and adiponectin remained unchanged after weight loss.

No differences in basal parameters (biochemical, anthropometric and dietary) were detected between heterocygous patients *–55C/T* and homocygous patients *–55C/C*.

Discussion

In wild group of –55CC UCP3 gene patients, a high mono-unsaturated hypocaloric diet reduced BMI, weight, waist circumference, waist to hip ratio, fat mass, LDL-cholesterol, total cholesterol and leptin levels. Our study showed that carriers of T allele had a different response than wild type obese, with a significant decrease of the same antropometric parameteres, but lower than in the wild group, and without significant changes in cholesterol levels.

The ubiquitous expression of UCP2, the expression of UCP3 in skeletal muscle, and their homology with UCP1 made UCP3 attractive targets for interventions aimed at manipulating energy expenditure[23]. One remarkable finding was that caloric restriction for 5 days resulted in a 2 to 3 fold increase in UCP mRNA levels in lean and obese patients[24], this fasting induced increase in UCP3 mRNA was completely reversed within 2 hours of reffeding.

Genetic polymorphisms in UCP genes have been variably associated with metabolic and obesity-related phenotypes. For example, Dalgaard et al[25] tested whether variation of the UCP3 promoter is associated with juveline or maturity onset obesity or body weight change over a 26 year follow up among Danish subjects, without a statistical association. In other studies, no difference in genotype frequencies was observed between obese and lean subjects in a French cohort[9], too. However, other study[26] has demonstrated that UCP3 –55CT polymorphism carriers have apparentely a lower risk of obesity. Liu et al[27] found statistically association and linkage between –55CT and BMI, and subjects carrying the T allele had an average of 3.5% lower BMI than those without it. Our study did not show statistical differences among basal anthopometric parameters in both genotype groups. As we can see, it is, therefore, unclear that the –55C/T variant has an effect on basal BMI or body fat content.

Some studies have analyzed the effect of the variant –55CT of UCP3 gene on weight loss with different hypocaloric diets. In a previous investigation[10], weigth, fat mass, LDL cholesterol, leptin and IL-6 decreased in the wild group with a hypocaloric diet with a 25% of dietary fat. Nevertheless, in the mutant group, only weight decreased without improvements in metabolic parameters. Other study[11], with two different hypocaloric diets (low fat vs low carbohydrate

Table II. Clasical cardiovascular risk factors.

Characteristics	55CC Basal time at 3 months		55CT Basal time at 3 months	
Glucose (mg/dl)	98.1 ± 11.5	96.2 ± 10.2	99.1 ± 16.1	98.1 ± 12.2
Total ch. (mg/dl)	203.1 ± 35.1	196.8 ± 32.1*	216.5 ± 33.7	212.1 ± 29.9
LDL-ch. (mg/dl)	124.7 ± 51.6	118.4 ± 31.1*	138.5 ± 31.9	136.3 ± 22.2
HDL-ch. (mg/dl)	52.2 ± 12.6	50.5 ± 9.6	53.2 ± 11.8	52.6 ± 10.8
TG (mg/dl)	114.5 ± 51.4	116.7 ± 53.6	134.9 ± 51.2	130.4 ± 56.9
Insulin (mUI/L)	13.4 ± 7.6	12.4 ± 8.2	11.3 ± 8.2	11.4 ± 6.9
HOMA	3.3 ± 1.1	3.1 ± 2.4	2.9 ± 2.4	3.0 ± 1.4
CRP (mg/dl)	5.5 ± 6.9	5.5 ± 7.5	5.1 ± 4.1	4.8 ± 2.6

Chol: Cholesterol; CRP: C reactive protein; TG: Triglycerides. *$p < 0.05$, in each group with basal values.

Table III. Circulating adypocitokines.

Characteristics	55CC Basal time at 3 months		55CT Basal time at 3 months	
IL 6 (pg/ml)	1.07 ± 1.1	1.84 ± 1.6	1.22 ± 1.5	1.62 ± 0.9
TNF-α (pg/ml)	2.4 ± 1.2	2.3 ± 1.1	3.8 ± 2.6	3.3 ± 3.6
Adiponectin (ng/ml)	9.6 ± 3.5	9.2 ± 3.7	7.6 ± 3.6	8.6 ± 5.3
Resistin (ng/ml)	4.7 ± 2.2	4.1 ± 1.7	4.2 ± 1.1	4.5 ± 1.3
Leptin (ng/ml)	24.0 ± 15.8	19.2 ± 11.2*	33.8 ± 23.1	27.8 ± 16.1*

Chol: Cholesterol; CRP: C reactive protein; TG: Triglycerides. *$p < 0.05$, in each group with basal values.

diet), showed a significant decrease of weight and fat mass in carriers of the T variant without metabolic changes with both diets, the distribution of saturated and unsaturated dietary fat were similar in both diets. In carriers of -55CC genotype improved weight, fat mass, leptin and insulin levels with both diets[11]. In this study, the weight loss was higher in CC patients than CT patients and the first group had a significant improvement in LDL-cholesterol levels. It is difficult to understand how a silent mutation could influence phenotypes such as fat mass loss or adipocytokines levels modifications. Perhaps, the genetic variation in the promotor of UCP3 gene is associated with the functionality of mitochondrial oxidation and, therefore, influences the LDL cholesterol response and weight loss secondary to energy restriction. Other hypothesis could be the type of intervention, for example the weight loss secondary to a biliopancreatic diversion was not affected by –55CT genotype[28] and the studies with restriction of calory and modifications in distribution of macronutrient have demonstrated different genotype's responses[10,11]. Finally, the type of dietary fat could interact with the weight loss response and –55CT polymorphism and monounsaturated rich diets showed this type of metabolic response.

Two studies have confirmed this interesting interaction between this polymorphism and weight loss induced by caloric restriction[29-30]. For example, two intronic single nucleotide polymorphisms (SNPs) of UCP3 gene, Int3-47G/A and Tyr210Tyr, both of them were significantly ssociated with changes in body weight secondary to a very low calory diet (VLCD), and no aminoacid changes have been described with these SNPs[29]. Other study[30] revealed that two SNPs in UCP2-3 gene cluster were associated with the changes of BMI induced by VLCD. As we can see the heterogeneity of this complex family of uncoupling proteins (UCPs) and the potential interaction with environment (diet) made difficult to explain the results of weight modification and variation of energy expenditure in subjects with genetic variations of UCP. However, this area of investigation has a huge interest in order to initiate treatments in obese subjects.

Conclusions

In patients with –55CC UCP3 genotype, a high mono-unsaturated hypocaloric diet reduced BMI, weight, waist circumference, waist to hip ratio, fat mass, LDL-cholesterol, total cholesterol and leptin levels. Carriers of T allele had a different response than wild type obese, with a significant decrease of the same antropometric parameters, but lower than in the wild group, and without significant changes in cholesterol levels. Additionals studies will be needed to clarify this interesting interaction between environment intervention and genes.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Finnish Diabetes Prevention Study Group: prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001; 344: 1343-1350.

2) Matsuzawa Y. Adipocytokines: Emerging therapeutic targets. Curr Atheroscler Rep 2005; 7: 58-62.

3) Vidal Puig A, Solanes G, Grujic D, Flier J, Lowell BB. UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 1997; 235: 79-82.

4) Saltzman E, Roberts SB. The role of energy expenditure in energy regulation: findings from a decade research. Nutr Rev 1995: 53: 209-220.

5) Bouchard C, Perusse L, Chagnon YC, Warden C, Ricquier D. Linkage between markers in the vicinity of uncoupling protein 2 gene and resisting metabolic rate in humans. Hum Mol Genet 1997; 6:1887-1889.

6) Clapham J, Arch J, Chapman H. Mice overexpressing human uncoupling protein 3 in skeletal muscle are hyperphagic lean. Nature 2000; 406: 415-418.

7) Samec S, Seydoux J, Dullo A. Role of UCP homologues in skeletal muscle and free fatty acids in obesity. Lancet 1998; 351: 1933-1934.

8) Otabe S, Clement K, Dina C, Pelloux V, Guy-Grand B, Froguel P, Vasseur F. A genetic variation in the 5´flanking region of the UCP3 is associated with body mass index in humans in interaction with physical activity. Diabetologia 2000; 43: 245-249.

9) Meirrhaeghe A, Amoyel P, Helbecque N, Cottel D, Otabe S, Froguel P, Vasseur F. An uncoupling protein 3 gene polymorphism associated with a lower risk of developing type 2 diabetes and with atherogenic lipid profile in a French cohort. Diabetologia 2000; 43: 1424-1428.

10) De Luis DA, Aller R, Izaola O, Sagrado M, Conde R. Modulation of adipocytokines response and weight loss secondary to a hypocaloric diet in obese patients by -55ct polymorphism of UCP3 gene. Horm Metab Res 2008; 40: 214-218.

11) De Luis DA, Aller R, Izaola O, Gonzalez M, Conde R. Modulation of adipocytokines response and weight loss secondary to a hypocaloric diet in obese patients by -55ct polymorphism of UCP3 gene. Horm Metab Res 2009; 41: 62-66.

12) Okasaki T, Himeno E, Nanri H, Ogata H, Ikeda M. Efects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. Clin Exp Pharmacol 1999; 26: 415-420.

13) Xenachis C, Samojlik E, Raghuwanshi MP, Kirschner MA. Leptin, insulin and TNF-alpha in weight loss. J Endocrinol Invest 2001; 24: 865-870.

14) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

15) Duart MJ, Arroyo CO, Moreno JL. Validation of an insulin model for the reactions in RIA. Clin Chem Lab Med 2002; 40: 1161-1167

16) Pfutzner A, Langenfeld M, Kunt T, Lobig M, Forst T. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin Lab 2003; 49: 571-576.

17) Meier U, Gressner AM. Endocrine regulation of energy metabolis: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50: 1511-1525.

18) Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem 2004; 50: 219-221.

19) Lubrano V, Cocci F, Battaglia D, Papa A, Marracini P, Zucchelli GC. Usefulness of high-sensitivity IL6 measurment for clinical characterization of patients with coronary artery disease. J Clin Lab Anal 2005; 19: 110-114.

20) Khan SS, Smith MS, Reda D, Suffredini AF, Mc Coy JP. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin Cytom 2004; 61: 35-39.

21) Lukaski H, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985; 41: 810-817.

22) Mataix J, Mañas M. Tablas de composición de alimentos españoles. Ed: University of Granada, 2003.

23) Hesselink MKC, Mensink M, Schrauwen P. Human uncoupling protein-3 and obesity: an update. Obes Res 2003; 11: 1429-1443.

24) Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M. Increased uncoupling protein 2 and 3 mRNA expression during fasting in obese and lean humans. J Clin Invest 1997; 100: 2665-2670.

25) Dalgaard LT, Sorensen TIA, Drivshom T, Borch Johnsen K, Andersen T, Hansen T. A prevalent polymorphism in the promoter of the UCP3 gene and its relationship to body mass index and long term body weight change in the Danish population. J Clin Endoc Metab 2001; 86: 1398-1402.

26) Alonso A, Marti A, Corbalan MS, Martinez Gonzalez MA, Forga L, Martinez JA. Association of UCP3 gene -55CT polymorphism and obesity in a Spanish population. Ann Nutr Metab 2005; 49: 183-188.

27) Liu YJ, Liu PY, Long J, Lu Y, Elze L, Recker RR, Deng HW. Linkage and association analyses of the UCP3 gene with obesity phenotypes in Caucasian families. Physiol Genomics 2005; 22: 197-203.

28) De Luis DA, Pacheco D, Aller R, Goznalez M, Izaola O, Terroba MC, Cuellar L, Conde R, Martin T, Perez CAstrillon JL. Influence of -55CT polymorphism of UCP3 gene on surgical results of biliopancreatic diversion. Obes Surg 2010; 20: 895-899.

29) Cha MH, Shin HD, Soo Kim KS, Lee BH, Yoon Y. The effects of uncoupling protein 3 haplotypes on obesity phenotypes and very low-energy diet-induced changes among overweight Korean female subjects. Metabolism 2006; 55: 578-586.

30) Yoon Y, Lae Park B, Cha MH, Soo Kim K, Sub Cheong H, Hyun Choi Y, Doo Shin H. Effects of genetic polymorphisms of UCP2 and UCP3 on very low calorie diet-induced body fat reduction in Korean female subjects. Biochem Biophys Res Commun 2007; 359: 451-456.