

PRO12ALA POLYMORPHISM OF THE PPAR γ 2 LOCUS MODULATES THE RELATIONSHIP BETWEEN ENERGY INTAKE AND BODY WEIGHT IN TYPE2 DIABETIC PATIENTS

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Running title: Pro12Ala polymorphism modulates the relation between weight and energy intake

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Abbreviations: BMI, Body Mass Index; PPAR γ 2, Peroxisome Proliferator-Activated Receptor- γ 2; P/S, polyunsaturated to saturated fatty acids ratio; HbA1c, Glycated hemoglobin; HPLC, high performance liquid chromatography.

Summary

Objective. We explore the relation between BMI, habitual diet and the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2).

Study design and research methods. The Pro12Ala variant was characterized in 343 unrelated type 2 diabetic patients, consecutively seen at the outpatient clinic of a health district of the province of Naples. Anthropometric and laboratory parameters were measured, habitual diet was assessed by a validated, semiquantitative, food frequency questionnaire.

Results. The overall frequency of Ala12 was 12% (n=42). BMI was significantly higher in Ala carriers than non Ala carriers, whereas total daily energy intake or macronutrient composition of the diet were similar in the two groups. For further analysis, participants were stratified according to genotype and sex-specific quartiles of energy intake. BMI increased in both genotype groups with increasing energy intake, ($p < 0.03$). BMI was similar in Ala carriers and non Ala carriers (30.0 vs 30.1 Kg/m² $p > 0.10$) in the lower quartile of energy intake, but significantly higher in Ala carriers in the upper quartile (36.0 vs 32.1 Kg/m²; $p < 0.001$). Average daily energy intake and diet composition were comparable within each quartile for carriers or non carriers of the Ala allele. Relatively to the non carriers, Ala carriers had a significantly lower energy intake per Kg body weight thus suggesting that the Ala allele is associated with a higher food efficiency. The confounding role of medications, glucose control and physical exercise was ruled out

Conclusions This study provides evidence of a differential susceptibility to fat accumulation, and hence weight gain, in response to habitual high energy intake for Ala carriers as compared to Pro/Pro homozygotes.

Introduction

Over the last two decades the prevalence of overweight and obesity has increased worldwide (1). Although the epidemic of obesity is largely caused by dietary and other lifestyle-related factors, the genetic background likely plays a role in determining the differences among individuals in gaining weight under the same environmental conditions. Studies on rodents have shown a different susceptibility to obesity induced by a high fat diet (2). Likewise, the understanding of the aetiology of complex traits such as obesity in humans requires the exploration of the combined gene-environment effect (3,4).

Among the genetic factors potentially involved in the etiology of obesity, the gene encoding for the peroxisome proliferator-activated receptor γ (PPAR γ), a nuclear receptor that regulates adipocyte differentiation, lipid storage, fat specific gene expression and insulin action, has attracted much attention .

Of the three PPAR γ isoforms, PPAR γ 2 mRNA is the most abundantly and specifically expressed in adipose tissue, which makes PPAR γ 2 a candidate gene for the regulation of body weight. Furthermore PPAR γ 2 can bind a variety of compounds including fatty acids of dietary origin and it is, therefore, an interesting gene for gene-diet interaction studies as well (3,5). A missense mutation resulting in a substitution of Proline for Alanine in codon 12 (Pro12Ala) has been found in the PPAR γ 2 isoform. This genetic variation has been extensively investigated in relation to obesity with apparently controversial results (6-8). In cross-sectional studies the Ala variant has been associated with lower or higher

BMI, whereas the few available longitudinal data indicate a tendency for the Ala carriers to gain more weight over time than non carriers.

The relation between Pro12 Ala polymorphism and environmental, lifestyle-related, factors has been little explored. The few available studies have been conducted in non diabetic people and, although not entirely consistent, they support the idea that the impact of this polymorphism on weight and metabolic features is modulated by lifestyle-related factors (9-15).

Obesity is a common feature of type 2 diabetes, and dietary treatment plays a key role in the management of these patients; it is therefore particularly relevant to explore means to identify diabetic patients who are more sensitive to weight gain / loss under given conditions. The aim of the study was to explore, in a population based sample of type 2 diabetic patients, the relation between BMI, habitual diet and the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2) .

Methods

The study design was cross sectional, observational. Participants were 343 unrelated type 2 diabetic people (144 men and 199 women), aged 40 – 70 years, consecutively seen at the outpatient diabetic clinic of a health district of the province of Naples. The study was approved by the local ethics committee; informed consent was obtained from all participants. Patients with serum creatinine \geq 2 mg/dl or cardiovascular events in the previous six months we excluded. All participants were regularly followed-up at the clinic by their own doctors, according to current guidelines for good clinical

practice. The study investigations were conducted by ad-hoc trained observers unaware of the participant's genotype status. No intervention was implemented; the prescribed diet and medications were not modified by the study investigators. Weight, height and waist circumference were measured with participants wearing light clothing and no shoes. BMI was calculated as body weight (in kilograms) divided by squared height (in meters). Waist circumference was measured at the level of the umbilicus: values ≥ 88 cm for women and ≥ 102 for men were used to define visceral adiposity. The waist/hip ratio was also calculated as an additional measure of fat distribution: hip circumference was measured at the widest part of the hip region, visceral adiposity was defined as waist/hip ratio ≥ 0.85 for women and ≥ 0.90 for men. Fasting glucose, triglycerides, total and HDL cholesterol were measured by standard laboratory methods on fresh plasma. Insulin was assayed by radioimmunoassay on frozen samples stored at -80°C for a maximum of six months. Glycated haemoglobin was measured by HPLC.

Dietary habits were investigated with the use of a 138-item semi quantitative food-frequency questionnaire administered by trained dieticians and designed on the basis of previous validity and reliability studies (16,17). Briefly, participants were asked how often, on average, they had consumed a specified portion of a given food during the previous year. Daily nutrient intake was calculated by multiplying the nutrient content of the specified portion of a food item by the frequency of its daily consumption and then summing the results of all the items. Food values for energy and nutrients were taken from the tables of the European Institute of

Oncology (18). Energy intake, (Kcalories /day), total saturated and polyunsaturated fat (g/day) were calculated; the polyunsaturated to saturated fatty acids ratio (P/S) and the glycemic load of the diet were also computed. Energy expenditure due to physical activity was evaluated by a standardized questionnaire (19). Participants were asked to fill in a questionnaire on habitual physical activity at work and during leisure time, which considered four increasing activity levels. For analytical purposes participants with the lowest activity level were considered as sedentary, those with an activity level higher than the lowest value were grouped together and defined as "physically active". Medication use was assessed by interview.

Genomic DNA was isolated from whole blood using Biorobot EZ1 Qiagen. By polymerase chain reaction (PCR) all samples were genotyped for the Pro12Ala SNP. All the oligoprimers were tested by gradient PCR to optimize melting temperature. Genotyping was performed by an allele specific amplification method using SYBR Green detection in a Realtime ABI PRISM 7000 apparatus (PE Applied Biosystem).

Statistical analysis

Data are given as means and standard deviations or percentages. For not normally distributed variables, log transformed values were used in the analyses; the original values are given in the text and tables as geometric means and interquartile ranges. Group means were compared by unpaired Student's t-test or analysis of variance, as appropriate. Proportions were compared by contingency tables and χ^2 square analysis. The separate and combined

effect of the Pro12Ala polymorphism and diet on BMI was explored across quartiles of caloric intake using the two way analysis of variance. Due to the different distribution of energy intake between men and women, sex specific quartiles were computed. Multivariate analysis was conducted by linear regression with BMI as the outcome variable; the independent variables included in the model were Pro12Ala polymorphism, estimated daily energy intake, total fat, saturated fat, P/S ratio, glycemic load, age, gender, hypoglycemic medications, glycosylated hemoglobin, physical activity. The χ^2 goodness-of-fit test was used to assess deviation from Hardy-Weinberg equilibrium of the genotypic frequency by calculating expected frequencies of genotypes. A p value of less than 0.05 (two tailed) was considered significant. All statistical analyses were conducted using Statistical Package for Social Science (SPSS) for Windows version 12.0

Results

The general characteristics of the study participants are shown in Table 1, together with the PPAR γ 2 genotype. As expected for type 2 diabetic patients, participants were middle aged and generally overweight. As for the PPAR γ 2 genotype, 301 subjects (88%) were Pro/Pro homozygotes, 41 (11.7%) Pro/Ala heterozygotes and only 1 person was homozygote for the Ala variant: therefore in subsequent analyses people with the Ala/Ala or Pro/Ala genotype are considered as one group and are referred to as "Ala carriers", whereas individuals with the Pro/Pro genotype are referred to as "non Ala carriers". The genotype distribution is in Hardy Weinberg equilibrium.

Ala carriers and non carriers were comparable with respect to age, diabetes duration, glycosylated hemoglobin, blood pressure, glucose, insulin, HOMA IR index, total and HDL cholesterol (table 1). BMI was significantly higher in carriers than non carriers ($p < 0.02$), whereas no significant differences were observed for waist circumference and waist/hip ratio between the two groups (table 1). The proportion of patients with central body fat distribution defined according to either waist circumference (≥ 102 cm in men and ≥ 88 cm in woman), or waist/hip ratio (≥ 0.90 in man and ≥ 0.85 in woman) was not significantly different in Ala carriers or non Ala carriers (64% vs 67% and 80% vs 81%). Fasting plasma triglycerides were significantly higher in carriers than non carriers: this difference was largely driven by BMI and was no longer evident after correction for BMI.

Differences in BMI were not explained by differences in dietary habits. Estimated energy intake or the macronutrient composition of the diet - i.e. intake of total fat, saturated fat, polyunsaturated fat, polyunsaturated to saturated fatty acids ratio and carbohydrates - was not significantly different between the two groups. Medications for diabetes are known to affect body weight, however we did not observe any significant difference in the proportion of patients using insulin, insulin secretagogues, or insulin sensitizers (namely metformin, as the thiazolidinediones were not marketed in Italy at the time the study was conducted) between the two genotype groups (table 2). Study participants were generally sedentary; the proportion of physically active people was low in both groups with no significant differences between Ala carriers and non Ala

carriers (13.3 % vs 21.4 % respectively, $p < 0.24$).

To explore the separate and combined effect of the Pro12 Ala polymorphism and diet on BMI, participants were stratified according to sex specific quartiles of energy intake and genotype. BMI increased progressively with increasing energy intake in both genotype groups with a significant linear trend ($p < 0.03$ for the effect of energy intake; $p < 0.016$ for the effect of genotype, with no significant interaction). Figure 1 clearly shows that in the first quartile of energy intake BMI was similar in carriers and non carriers (30.0 vs 30.1 Kg/m²; $p = 0.1$), whereas in the highest quartile of caloric intake the Ala carriers had a significantly greater BMI than Pro/Pro homozygotes (36.0 vs 32.1; Kg/m²; $p < 0.016$). Interestingly, average daily energy intake and diet composition - i.e. total fat, saturated fat, P/S ratio and carbohydrates - were comparable within each quartile for carriers or non carriers of the Ala allele (table 3). Relatively to the non carriers, Ala carriers had a significantly lower energy intake per Kg body weight (table 3), thus suggesting that the Ala allele is associated with a higher food efficiency (i.e. for the same body weight a lower energy intake is required to maintain body weight stable). Possible confounders such as glycemic control, physical activity and proportion of patients on insulin, sulphonylureas or metformin were comparable between Ala carriers and non Ala carriers within quartiles (appendix table).

Multivariate regression analysis (table 4) was performed to explore the independent effect on BMI of energy intake, diet composition and genotype (presence / absence of the Ala allele); since age and gender are associated with both BMI and energy intake, these two

variables were included in the model. Among the variables included in the model only energy intake and presence of the Pro12Ala polymorphism were significantly and independently associated with BMI. This finding did not change when type of hypoglycemic medications, glycated hemoglobin and physical activity were also included in the model.

Discussion

The study shows that type 2 diabetic patients carrying the Pro12Ala polymorphism of the PPAR γ 2 have a significantly higher BMI than non carriers despite a similar energy intake. As a matter of fact, BMI increases progressively with increasing energy intake in both groups, however Ala carriers had a significantly lower energy intake per Kg body weight, thus suggesting that the Ala allele is associated with a higher food efficiency. The confounding effect of hypoglycemic medications, glycemic control, and physical activity was ruled out, thus conferring consistency to the finding.

Very few studies have assessed the impact of genetic polymorphisms and diet on weight and none of these was performed in diabetic people. PPAR γ 2 is one of the most promising candidate genes of common obesity, although so far results of association studies have been somewhat inconsistent. Cross sectional studies have shown either no difference, or a lower BMI, or a modestly greater BMI in Ala carriers compared to non carriers; the few available prospective studies suggest that the Pro12 Ala polymorphism is associated with higher insulin sensitivity and may confer increased susceptibility to weight gain over time, particularly in obese people (8); however no information on habitual

diet was collected in these studies. Results of intervention studies in non diabetic patients indicate that the Pro12Ala polymorphism may modulate physiological responses to dietary fat intake in humans (9-15). In the Quebec Family Study the Ala carriers had higher BMI, waist circumference and fat mass than non carriers, but were more resistant to weight gain and metabolic deterioration when exposed to a high fat intake (10). At least three other studies have confirmed that the weight response to the amount and the type of dietary fat differs according to the PPAR γ 2 genotype (9,12,15). In our study, the Pro12Ala polymorphism did not seem to modulate the impact of the fat content of the diet on BMI. It is relevant to note in this regard that the present study was conducted in a Mediterranean region where on average the habitual dietary fat intake is lower than in Northern European and American countries. Furthermore the study was conducted in diabetic patients who are usually prescribed, as part of their treatment, a diet reduced in both total and saturated fat. All study participants were regularly attending a diabetic clinic and, although most patients were not fully compliant with the prescribed diet, nonetheless the average intake of total fat and saturated fat was substantially lower in this sample than in previous studies (i.e. average total fat intake was 60 g in our study, 90 g in the Canadian study (10) and 72 g in the Finnish study (11). Likewise the polyunsaturated to saturated fatty acids ratio was higher in our study than in others. It is possible that the modifying effect of the Pro12Ala variation on the relationship between dietary fat intake and BMI may be not evident for a low total fat intake, predominantly of the unsaturated type. Alternative explanations for not

confirming previous findings include the difficulty to accurately assess fat intake based on food frequency records.

Our finding of a differential susceptibility to fat accumulation by genotype is in keeping with a study in which women carriers of the Ala variant were shown to be more susceptible to weight regain when resuming spontaneous energy intake after a weight loss treatment (20). These results are compatible with the hypothesis that Ala carriers have a higher food efficiency (i.e. for the same body weight they need a lower energy intake to keep their weight stable).

As to mechanisms responsible for the effects of the Ala variant on individual weight regulation we can only make speculations. The cellular and molecular mechanisms by which PPAR γ affects adipogenesis are not entirely clear; it has been suggested that the Pro12Ala polymorphism is associated with greater insulin sensitivity and this could be linked to a greater increase in body weight (21-24). This hypothesis would also be in line with results of functional studies showing that one likely mechanism through which PPAR γ reduces insulin resistance is via up-regulation of several adipocyte genes and metabolic pathways which favor adipocyte uptake of circulating fatty acids and promote a net flux of fatty acids from the blood and other tissues into adipocytes. A more efficient suppression of lipolysis by insulin in the Ala carriers could also contribute to a greater weight gain in this group by further tilting towards lipogenesis the minutely regulated balance between lipogenesis and lipolysis (24-25). Consistent with this hypothesis is the finding of a 50% reduction in circulating levels of FFA during euglycemic hyperinsulinemic clamp in Ala carriers

reported by Tschitter (26), although no association between the Pro12 Ala allele and fasting FFA levels was found in a large population studied by our group (27).

Among the study limitations there is indeed the small sample size; we calculated that the study has a 80% power of detecting a difference of 1 unit BMI between carriers and non carriers with a $p < 0.05$. Furthermore, having focused on diabetic people may limit the generalizability of our findings, although the genotype distribution in this population is in Hardy Weinberg equilibrium and similar to that observed in other Caucasian populations (28). Limitations are also built in the assessment of habitual diet: nutrients intake was assessed retrospectively using a questionnaire validated against seven day food record. The major pitfalls of this method are recall bias and errors in the estimate of portion size by visual inspection of pictures; however, we do not see why these limitations should apply differently to the two genotype groups, thus introducing a systematic errors in the estimate of dietary habits. In any case expected relations, such as that between energy intake and body weight, is confirmed in this study thus conferring consistency to the findings.

Overall results of this study suggest a differential susceptibility to fat accumulation, and hence weight gain, for Ala carriers and non Ala carriers when exposed to habitual excess energy intake. Based on this and other findings the hypothesis can be formulated that Ala carriers are more prone to weight gain when exposed to an obesogenic environment, but may benefit more from energy restriction, or increased energy expenditure. The role of a combined gene-environment effect in the etiology of complex traits such as obesity and insulin resistance needs to be further explored, in that it may provide a basis for identifying at risk individuals at a young age and selecting the most responders to preventive measures based on life style modifications.

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References

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL : Prevalence and trends in obesity among US adults, 1999-2000. *JAMA*, 288: 1723-1727, 2002.
2. West DB, York B: Dietary fat, genetic predisposition, and obesity: lessons from animal models. *Am J. of Clin. Nutr* 67 : 505S- 512S , 1998.
3. Uusitupa M: Gene–diet interaction in relation to the prevention of obesity and type 2 diabetes: Evidence from Finnish Diabetes Prevention Study. *NMCD* 56: 1-9 , 2005.
4. Cocozza S: Methodological aspects of the assessment of gene-nutrient interactions at the population level *NMCD* ,2006 (In press)
5. Knouff C, Auwerx J: Peroxisome Proliferator activated receptor gamma calls for activation in moderation: Lessons from genetics and pharmacology. *Endocrine Reviews* 25: 899-918, 2003.
6. Masud S, Ye S, on behalf of the SAS group: Effect of the Peroxisome Activated Receptor γ gene Pro12 Ala variant on body mass index: a meta-analysis. *J Med Genet* 40: 773-780, 2003.
7. Stefanski A, Majkowska L, Ciechanowicz A, Frankow M, Safranow K, Parczewski M, Pilarska K: Lack of association between the Pro12Ala polymorphism in PPAR γ 2 gene and body weight changes, insulin resistance and chronic diabetic complications in obese patients with type 2 diabetes. *Archives of Medical Research* 37: 736-743, 2006.
8. Ek J, Urhammer TIA, Sorensen T, Auwers J, Pedersen O: Homozygosity of the Pro12Ala variant of the peroxisome proliferator-activated receptor- γ 2 (PPAR- γ 2):divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* 42: 892-895,1999
9. Luan J, Browne PO, Harding A-H , Halsall DJ, O' Rahilly, S, Chatterjee VKK , Wareham NJ: Evidence for gene-nutrient interaction at PPAR γ locus. *Diabetes* 50 :686-689, 2001.
10. Robitaille J, Despres JP, Perusse L, Vohl MC: The PPAR γ P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. *Clin Genet* 63: 109-116 , 2003.
11. Lindi VI , Sivenius K, Niskanen L, Laakso M, Uusitupa M I : Effect of the Pro12Ala polymorphism of the PPAR γ 2 gene on long term body weight change in Finnish non diabetic subjects. *Diabetologia* 44: 925-926, 2001.
12. Franks PW, Luan J , Browne PO , Harding AH , O' Rahilly S, Chatterjee VK , Wareham NJ : Does Peroxisome Proliferator Activated receptor γ genotype (Pro12Ala) modify the association of physical activity and dietary fat with fasting insulin level ? *Metabolism* 53 :11-16 , 2004.
13. Pisaballo R, Sanguetti C, Stoll M, Prendez D : High incidence of type 2 diabetes in Peroxisome Proliferator-activated receptor γ 2 Pro12Ala carriers exposed to a high chronic intake of trans fatty acids and saturated fatty acids. *Diabetes Care* 27 : 2251-2252, 2004.
14. Memisoglu A, Hu FB , Hankinson SE , Manson J E, De Vivo I, Willet WC, Hunter D-J: Interaction between a peroxisome proliferator–activated

- receptor γ gene polymorphism and dietary fat intake in relation to body mass. *Human Molecular Genetics*, 12: 2923- 2929, 2003.
15. Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K, Storlien L, Riccardi G, Rivellese AA., Uusitupa MIJ : Impact of the Pro12Ala polymorphism of PPAR γ 2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. *Mol Genet Metab* 79:52-60, 2003.
 16. Panico S, Dello Iacovo R, Celentano E, et al : Progetto ATENA, a study on the etiology of major chronic diseases in women: design, rationale and objectives. *Eur J Epidemiol* 8:601-608, 1992.
 17. Trevisan M, Ferro-Luzzi A, Krogh V, et al.: Questionario alimentare semiquantitativo sviluppato per indagini epidemiologiche in Italia. In: Panico S. et al (eds). *La Malattia Cardiovascolare Arteriosclerotica nella Donna: Una nuova generazione di studi epidemiologici*. Ann Ist Sup San 28: 397- 402, 1992.
 18. Salvini S: A food composition database for epidemiological studies in Italy. *Cancer Lett* 19 ;114(1-2) :299-300, 1997.
 19. Saltin B, Grimby G: Physiological analysis of middle-aged men and former athletes. *Circulation* 38: 1104-1105, 1968.
 20. Nicklas BJ, Van Rossum EF, Berman DM, Ryan AS, Dennis KE, Shuldiner AR: Genetic variation in the peroxisome proliferators- activated receptor γ 2 gene affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50 :2172-2176, 2001.
 21. Lazar MA: PPAR γ ,10 years later. *Biochimie* 87 : 9-13, 2005.
 22. Stumvoll M, Haring H: Reduced lipolysis as possible cause for greater weight gain in subjects with the Pro12Ala polymorphism in PPAR γ 2. *Diabetologia* 45: 152-153, 2002.
 23. Kao L, Coresh J, Shuldiner AR, Boerwinkle E, Bray MS, Brancati FL: Pro12Ala of the Peroxisome Proliferator-Activated Receptor- γ 2 gene is associated with lower serum insulin levels (PPAR- γ 2): in non obese african americans. The Atherosclerosis Risk in Communities Study . *Diabetes* 52:1568-1572,2003
 24. Tremblay A, Boulè N, Doucet E and Woods SC: is the insulin resistance syndrome the price to be paid to achieve body weight stability? *International Journal of Obesity* 29:1295-1298,2005
 25. Lehrke M , Lazar M: The many faces of PPAR γ .*Cell* 123 : 999, 2005.
 26. Tschritter O, Fritsche A, Stefan N, Haap M, Thamer, Bachmann O, Dahl D, Maerker E, Machicao F, Haring H, Stumvooll M: Increased insulin clearance in Peroxisome Proliferator-ctivated Receptor γ 2 Pro12Ala. *Metabolism* 52 :778-783, 2003.
 27. Vaccaro O, Mancini FP, Ruffa G, Sabatino L, Iovine C, Masulli M, Colantuoni V, Riccardi G : Fasting plasma free fatty acid concentrations and Pro12Ala polymorphism of the peroxisome proliferator activated receptor PPAR γ 2 gene in healthy individuals. *Clin Endocrinol* 57 : 481-486, 2002.
 28. Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, Riccardi G, Colantuoni V: Pro12Ala substitution in the Peroxisome Proliferator-

activated Receptor- γ 2 is not associated with type 2 diabetes. *Diabetes*
48:466-1468,1999

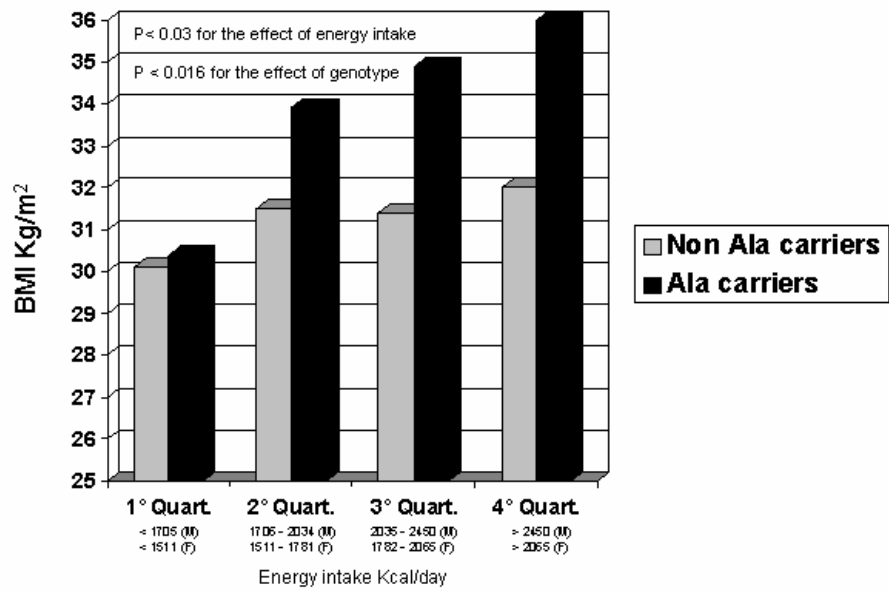


TABLE 1: Characteristics of the study participants by genotype			
	<i>Non Ala carriers (301)</i>	<i>Ala carriers (42)</i>	p
Males (%)	128 (42,5%)	16 (39.0%)	0.738
Diabetes duration (years)	15.8 ± 8.9	14.6 ± 7.73	0.375
Age (years)	57.9 ± 8.6	58.9 ± 6.7	0.463
BMI (kg/m²)	31.3 ± 5.8	33.6 ± 7.1	0.022
Waist (cm)	99.8 ± 11.8	102.5 ± 14.5	0.192
Waist/hip	0.93 ± 0.7	0.92 ± 0.6	0.411
Glucose (mg/dl) *	169 (130-168)	180 (141-248)	0.356
Insulin (μU/ml) *	8.3 (5.6-9.3)	8.4 (5.1-13.4)	0.910
Total cholesterol (mg/dl)	209 ± 44	210 ± 48	0.872
Hdl- chol (mg/dl)	48.15 ± 12.81	47.45 ± 13.76	0.761
Triglycerides (mg/dl) *	133 (93-180)	148 (97 -211)	0.020
HB1Ac (%)	7.4 ± 1.8	7.5 ± 2	0.795
HOMA IR	4.58 ± 3.83	4.96 ± 2.91	0.562
*Geometric mean; () interquartile range			

TABLE 2: Nutrient intake, hypoglycemic treatment and physical activity by genotype

	<i>Non Ala carriers (301)</i>	<i>Ala carriers(42)</i>	p
<u>Nutrients intake</u>			
Total fat (g)	60.35 ± 20.84	60.2 ± 19.72	0.967
Saturated fat (g)	20.42 ±9.18	20.39 ± 8.20	0.986
Glycemic load	131.30 ± 65.95	121.26 ± 30.1	0.172
P/S	0.53 ± 0.20	0.56 ± 0.24	0.440
<u>Hypoglycemic treatments (%)</u>			
Diet only	77 (25.6)	11 (26.2)	0.453
Acarbose	5 (1.7)	3 (7.1)	
Metformin only	77 (25.6)	9 (21.4)	
Sulphonylureas only	73 (24.2)	9 (21.4)	
Metformin + Sulphonylureas	18 (6.0)	2 (4.8)	
Insulin alone or in combination	51 (16.9)	8 (19.1)	
<u>Physically active (%)</u>	40 (13.3)	9 (21.4)	0.239

TABLE 3 Average daily intake of energy and macronutrients by sex specific quartiles of energy intake and genotype					
Energy intake quartiles (K calories/day)	Q1 <1705 M <1511 F	Q2 1706–2034 M 151 – 1781 F	Q3 2035 –2450 M 1782–2065 F	Q4 > 2450 M > 2065 F	p
Number					
<i>Non Ala carriers</i>	75	75	77	74	
<i>Ala carriers</i>	11	11	9	11	
Energy (kcal/day)					
<i>Non Ala carriers</i>	1382±202	1730±132	2023±171	2671±449	Quart= 0.001; Genotype= 0.787
<i>Ala carriers</i>	1250±276	1761±154	2102±116	2595±517	
Energy (kcal /kg body)					
<i>Non Ala carriers</i>	19.2±3.9	22.6.0±4.2	26.7±5.0	33.6±7.5	Quart = 0.001; Genotype= 0.04
<i>Ala carriers</i>	17.7±4.0	22.0±4.3	23.9±4.7	30.7±8.2	
Total fat (gr/day)					
<i>Non Ala carriers</i>	42±9	53±7	61±10	83±17	Quart = 0.001; Genotype= 0.909
<i>Ala carriers</i>	41±14	54±9	64±7	79±19	
Saturated fat (gr/day)					
<i>Non Ala carriers</i>	12±3	17±3	20±5	30±8	Quart = 0.001; Genotype= 0.903
<i>Ala carriers</i>	12±4	18±4	21±4	28±8	
P/S					
<i>Non Ala carriers</i>	0.62±0.24	0.53±0.17	0.51±0.17	0.44±0.16	Quart= 0.01; Genotype= 0.317
<i>Ala carriers</i>	0.66±0.22	0.54±0.33	0.56±0.16	0.47±0.16	
Glycemic load					
<i>Non Ala carriers</i>	103±37	120±24	138 ±63	164±99	Quart= 0.05; Genotype= 0.298
<i>Ala carriers</i>	76 ±19	117±16	134 ±36	156 ±29	

TABLE 4 Multivariate regression analysis of the association between genotype, diet variables and BMI			
	Beta	Se Beta	p
Energy (Kcal)	0.004	0.001	0.004
Sex (m,f)	4.967	0.667	0.000
Genotype (<i>Ala carriers, Non Ala carriers</i>)	2.356	0.954	0.014
Age (years)	-0.014	0.039	0.709
Total fat (g)	0.025	0.070	0.721
Saturated fat (g)	-0.194	0.162	0.230
P/S	0.761	2.203	0.730
Glycemic load	-0.002	0.006	0.661