IL6 GENE PROMOTER POLYMORPHISM (-174G/C) INFLUENCES THE ASSOCIATION BETWEEN FAT MASS AND CARDIOVASCULAR RISK FACTORS.

A. Moleres[1], T. Rendo-Urteaga[1], C Azcona[2], JA. Martínez[1], S. Gómez-Martínez[3], JR. Ruiz[4], LA. Moreno[5], A. Marcos[3], A. Marti[1] on behalf of the AVENA group.

[1]Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Spain

[2]Pediatric Endocrinology Unit, Department of Pediatrics, University of Navarra Hospital, University of Navarra, Pamplona, Spain

[3]Immunonutrition Research Group, Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN), Instituto del Frío, Spanish National Research Council (CSIC), Madrid, Spain

[4]Unit of Preventive Nutrition, Department of Biosciences and Nutrition at Novum, Karolinska Intitutet, Huddinge, Sweden.

[5]Growth, Exercise, Nutrition and Development (GENUD) Research Group, School of Health Sciences, University of Zaragoza, Zaragoza, Spain

Correspondence to: A. Marti (Tel. +34 948 425 600; Fax. +34 948 425 649; e-mail: amarti@unav.es

INFLUENCE OF THE -174G/C SNP OF IL6 ON ADIPOSITY

ABSTRACT

During the last decades, the prevalence of obesity has increased rapidly among young people. A polymorphism in the promoter region of the IL6 gene (-174G/C), has been previously reported to be involved in obesity and metabolic syndrome development. Therefore, the aim of the study was to examine whether the IL6 -174G/C polymorphism influence the association of body fat with low-grade inflammatory markers and blood lipids and lipoproteins in Spanish adolescents. 504 Spanish adolescents participating in the AVENA study were genotyped for the -174G/C polymorphism of the IL6 gene. Anthropometric and body composition measurements were taken and blood samples were collected for plasma molecules determinations. No differences between genotypes were observed in anthropometric values, body composition measurements and plasma markers concentration. Physical activity level differ between genotypes with subjects carrying the C allele of the polymorphism being significantly ($p<0.05$) more active than GG subjects. The association between body fat mass and plasma glucose was influenced by the -174G/C polymorphism of the IL6 gene. Subjects carrying the C allele of the mutation seem to have higher values of lipoprotein (a) and C-reactive protein as their percentage of body fat mass increase. Our results suggest that this promoter polymorphism influences the association between adiposity and some plasma markers.

Key words: adolescents, obesity, IL6, polymorphism.

INTRODUCTION

The prevalence of obesity and the metabolic syndrome is rapidly increasing among young people, especially throughout the last decades (19,37). Obesity is accompanied by a body fat mass increase that leads to serious physiopatological and psychological disorders in adolescents (15), being involved in hypertension, diabetes or dyslipidemia that accentuate obese subject cardiovascular risk. Causes of obesity include genetic factors that predisposes to obesity, children and adolescents lifestyle as well as possible interactions between genetics and environmental factors (18).

Concerning genetic influences, recent data support that between 40-70% of obesity phenotypes variability is genetically mediated (20). Most often, obesity has a polygenic origin with multiple genes being involved, thus, single-nucleotide polymorphisms (SNPs) in these genes could affect adiposity and obesity-related traits (3).

There is evidence that excessive growth of adipose tissue is accompanied by an underlying low-grade inflammation state (23). In this sense, IL6 gene is codifying for IL6 that is a pro-inflammatory cytokine involved in obesity. It is considered as an adiposity signal, since it is produced by adipose tissue and its plasma levels correlate with fat depots in humans (36). The most common polymorphism of this gene is the -174G/C (rs1800795) variant, located in the promoter region of the gene. It influences transcriptional regulation and plasma cytokine levels. Data concerning the effects of this polymorphism has led to contradictory results, with both G and C alleles of the SNP being associated with obesity comorbidities (10). Indeed, several studies showed that the G allele was associated with obesity traits (14), whereas others reported the C allele was a factor increasing the risk of developing type 2 diabetes mellitus (22), hypertension, and cardiovascular disease (13).

There is a low-grade inflammation state associated to obesity which is characterized by increased cytokines and acute-phase reactants production such as C-reactive protein (CRP) and lipoprotein (a) (38). CRP synthesis is regulated by cytokines, being the most part attributed to IL6 (30). Elevated CRP concentrations have been associated with increased risk of cardiovascular diseases (2). Elevated plasma lipoprotein (a), a LDL-like protein, has been described as an independent risk factor for vascular disease already in childhood/adolescence (5,35) and youth (16).

Therefore, the aim of this study was to investigate whether the IL6 -174G/C polymorphism influence the association of body fat with low-grade inflammatory markers and blood lipids and lipoproteins in Spanish adolescents.

SUBJECTS AND METHODS

Study subjects consisted of a subsample of 504 adolescents participating in the cross sectional AVENA Survey (total number of AVENA participants 2278). This study was designed to assess the nutritional status, dietary and leisure time habits as well as physical activity and fitness of Spanish adolescents between 13 and 18 years old, and also to identify risk factors for chronic diseases in adulthood. Data collection of this study took place from 2000 to 2002 in five Spanish cities (Granada, Madrid, Murcia, Santander and Zaragoza). The complete methodology of this multicenter cross-sectional study was described in detail elsewhere (9,25).

Written consent to participate was obtained from both parents and adolescents. The complete study protocol was conducted in accordance with the ethical rules of the Helsinki Declaration (as revised in Hong-Kong in 1989, and in Edinburgh in 2000), following the European Community´s guidelines for Good Clinical Practice (document EEC 111/3976/88 of July 1990) and current Spanish law regulating clinical research in

humans (Royal Decree 561/1993 regarding clinical trials). The study protocol was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain)

Anthropometric and laboratory measurements

Familial medical history was collected by means of a questionnaire. Weight and height were measured with an electronic SECA scale and with a telescopic height measuring SECA instrument respectively. Criteria described by Cole *et al. (4)* were used to classify the subjects as overweight, obese or normal weight. Skinfolds were measured with a Holtain skinfold calliper and waist and hip circumferences with a circumference measuring band (Type SECA 200), as previously described (24,26). The sum of six skinfold thicknesses (Σ6 skinfolds) was used as an index of total adiposity while body fat mass percentage calculated by the formulas described by Slaughter *et al*. (34).

Physical Activity

Leisure-time physical activity was assessed by mean of questionnaires (27). Based on these questionnaires subjects were assigned to one of the following groups: no activities, one activity and more than one activity practice per week.

Blood sampling

Overnight fasting venous blood samples were collected by venipuncture. The serum was separated by centrifugation, divided into aliquots and stored at -80ºC. Serum C-reactive protein (CRP), C3 and C4 were determined by immunoturbidymetry as described elsewhere (39). IL6 and TNFα concentrations were analyzed by flow citometry while data on glucose, cholesterol, HDL and LDL cholesterol and

triglycerides were obtained by enzymatic colorimetric assay via an Hitachi 911 analyzer (Roche Diagnostics, Basel, Switzerland) (8).

Genotyping

DNA was extracted from the buffy coat fraction using the Quiagen procedure described by Higuchi (12). All the subjects were genotyped for the -174G/C promoter polymorphism (rs1800795) of the IL6 gene using Taqman SNP allelic discrimination (ABI PRISM 7900 HT). The probes and the primers for these assays were designed by Applied Biosystems (Madrid, Spain). Replicate quality control samples were included in every genotyping plate with more than 99% of concordance.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 15.0 (SPSS INC., Chicago, IL).

A Chi-square test was used to evaluate the Hardy-Weinberg equilibrium. The Kolmogorov-Smirnov test was used to determine variable distribution. Mean values of anthropometric measurements and plasma markers according to genotype were analyzed by one-way ANOVA.

To assess the associations between body fat mass and plasma markers multiple regression analyses adjusted by age, gender, Tanner stage and leisure time physical activity level, were performed for each parameter separately by genotype. We tested the interaction between the IL6 polymorphism and body fat mass in some metabolic syndrome risk markers.

The level of probability was set at $p<0.05$ as statistically significant.

RESULTS

The frequency of the -174C allele of the IL6 gene was 0.34. 43% of the studied adolescents carried the GG genotype (wildtype), 46.8% of the sample were heterozygous for the mutation (-174GC) and 10.2% carried the -174C homozygous genotype. The allele distribution was in Hardy-Weinberg equilibrium. There were no differences in the C minor allele frequency between normal weight, overweight and obese adolescents ($p=0.840$).

When analyzing the main anthropometric values, body composition measurements, physical activity levels and plasma markers concentration, no differences were found between genotypes. Therefore, further analyses were done taking into account a dominant model for the IL6 mutation (GG vs GC/CC).

Differences between genotypes were only observed in relationship with leisure time physical activity, with carriers of the C allele of the -174G/C polymorphism being significantly ($p=0.013$) more active than GG subjects (Table 1).

Body fat mass was positively associated with increased triglycerides, LDL-cholesterol, apolipoprotein B, C3 and C4 ($p<0.001$) as well as total cholesterol, lipoprotein (a) and CRP ($p<0.05$) serum levels. An inverse correlation between body fat mass and HDL-cholesterol was also observed ($p<0.001$). After adjusting for age, gender, pubertal stage and physical activity practice, body fat mass remains positively associated with triglycerides, C3 and C4, lipoprotein (a) and glucose plasma levels, and negatively associated with HDLc in this adolescent population (Table 2).

To analyze whether the IL6 gene -174G/C polymorphism interacts between body fat mass values and cytokines, plasma lipids, and body composition measurements, multiple regression models were performed. All tests were adjusted for age, gender, pubertal stage based on Tanner stage and physical activity level.

When the analyses were performed according to genotype, a significant relationship between body fat mass and some body composition indicators was observed both in the GG subjects group and in the GC/CC carriers group. After adjusting for confounding variables, body fat mass was positively associated with waist circumference and waist to hip ratio (WHR) ($p<0.001$), as well as truncal/total skinfolds ratio ($p=0.001$) in both genotype groups.

Considering the relationship between body fat mass and cytokines and plasma markers levels, in both genotype groups a positive association with complement C3 and C4 and total cholesterol/HDLc ratio ($p<0.001$) was observed in the whole sample as well as a negative association with HDLc ($p<0.05$) as shown in table 3.

The -174G/C polymorphism of the IL6 gene influences the association between body fat mass and plasma glucose, CRP and lipoprotein (a). Among GG subjects a significant positive relationship between body fat mass and plasma glucose ($p=0.001$) was observed whereas this association was not statistically significant in C allele carriers (GC and CC groups) (Table 3). On the other hand, there was a positive relationship between CRP and lipoprotein (a) with body fat mass in C allele carriers ($p<0.05$), but not in GG homozygous subjects. Subjects carrying the allele C (GC/CC) showed an increase of about 0.44 mg/l C-reactive protein and 1.4 mg/dl lipoprotein (a) *per* 1% of body fat mass increase.

IL6 genotype groups were divided into tertiles of body fat mass percentage (low, medium and high). The analysis of covariance after adjusting for gender, age, pubertal status and leisure activity level, evidenced that IL6 -174C allele carriers with the lowest body fat mass percentage (first tertile) had significantly lower values of circulating lipoprotein (a) than those with higher body fat mass (second and third tertiles). This association was not shown in -174GG subjects (Figure 1). In the same way, C allele

carriers with the lowest body fat mass percentage showed significantly lower triglycerides concentration than those with the highest body fat mass (figure 2), while -174GG subjects did not show differences between body fat mass percentage tertiles.

DISCUSSION

Increasing evidence suggests the role of proinflammatory cytokines on obesity and metabolic related complications (39). In this sense, as IL6 is secreted by adipose tissue, we have studied the effect of the IL6 promoter -174G/C polymorphism on the risk of developing obesity associated comorbidities in a healthy Spanish adolescent population.

Genotype distribution was similar of that previously observed by other authors in European and Spanish adult and adolescents populations (10,28,31).

Our results show that there were no differences in anthropometric measurements, body composition and physical activity when the analyses were performed according to genotype. Neither cytokines nor lipid plasma levels differ between homozygous subjects (GG) and C allele carriers (GC/CC). Similar results have been previously reported by Panoulas *et al.* (28), where no differences in some cardiovascular disease risk factors as BMI and plasma lipids concentration were observed according to -174G/C polymorphism of the IL6 gene. Goyenechea *et al.* (10), also showed that the polymorphism did not seem to have any effect on body weight, glucose concentration or IL6 circulating levels. However, contradictory data about the polymorphism effects on metabolic traits can be found in the literature. Some studies suggest that the -174G allele is associated with insulin resistance (11), increased triglycerides and decreased HDL cholesterol (7), while other authors support that the -174C allele correlates with higher glucose (21) and insulin levels (17). Therefore, we evaluated the influence of the polymorphism on the association between body fat mass as a measure of obesity and

some plasma markers. We showed that body fat mass was positively associated with inflammatory markers C3 and C4 component fractions, as well as triglyceride concentrations and inversely associated with HDL-cholesterol independently of the genotype. These results are in agreement with those previously observed by Puchau *et al.* (29) and Ruiz *et al.* (32), and suggest an increased risk of developing cardiovascular disease, insulin resistance and metabolic syndrome (29).

Our results also showed that the IL6 -174G/C promoter polymorphism play a role on the association of body fat mass with glucose, lipoprotein (a) and CRP plasma levels. Subjects carrying the C allele of the polymorphism had a greater increase in lipoprotein (a) and CRP plasma concentration. Lipoprotein (a) has been reported as a risk factor for cardiovascular disease (1) and CRP is an inflammatory marker that has been described as a predictor risk factor for insulin resistance (6) and atherosclerosis (33) in children and adolescents: therefore, our results suggest that in our adolescent population, subjects carrying the C allele of the IL6 -174G/C polymorphism could be on a greater risk of developing obesity related diseases as they increase their percentage of body fat mass.

In conclusion, our data show that the IL6 -174G/C promoter polymorphism influences the association between body fat mass percentage and some plasma markers. Subjects carrying the C allele of the mutation seem to have higher lipoprotein (A) and CRP concentrations as their percentage of body fat mass increase.

AKNOWLEDGEMENTS

The AVENA study has been supported by the Spanish Ministry of Health, FEDER-FSE (FIS00/0015), the Spanish Ministry of Health Instituto de Salud Carlos III (FIS

PI021830). A. Moleres was supported by a grant from the Departamento de Educación del Gobierno de Navarra. We gratefully acknowledge all adolescents participants and their families as well as Ana Lorente for her excellent technical assistance.

REFERENCES

1. Anuurad, E., Boffa, M.B., Koschinsky, M.L. and Berglund, L. (2006): Lipoprotein(a): a unique risk factor for cardiovascular disease. Clin Lab Med, **26**, 751-772.
2. Buckley, D.I., Fu, R., Freeman, M., Rogers, K. and Helfand, M. (2009): C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive Services Task Force. Ann Intern Med, **151**, 483-495.
3. Clement, K., Boutin, P. and Froguel, P. (2002): Genetics of obesity. Am J Pharmacogenomics, **2**, 177-187.
4. Cole, T.J., Bellizzi, M.C., Flegal, K.M. and Dietz, W.H. (2000): Establishing a standard definition for child overweight and obesity worldwide: international survey. Bmj, **320**, 1240-1243.
5. Charakida, M., Tousoulis, D., Skoumas, I., Pitsavos, C., Vasiliadou, C., Stefanadi, E., Antoniades, C., Latsios, G., Siasos, G. and Stefanadis, C. (2009): Inflammatory and thrombotic processes are associated with vascular dysfunction in children with familial hypercholesterolemia. Atherosclerosis, **204**, 532-537.
6. Eyzaguirre, F. and Mericq, V. (2009): Insulin resistance markers in children. Horm Res, **71**, 65-74.
7. Fernandez-Real, J.M., Broch, M., Vendrell, J., Richart, C. and Ricart, W. (2000): Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. J Clin Endocrinol Metab, **85**, 1334-1339.
8. Garcia-Artero, E., Ortega, F.B., Ruiz, J.R., Mesa, J.L., Delgado, M., Gonzalez-Gross, M., Garcia-Fuentes, M., Vicente-Rodriguez, G., Gutierrez, A. and Castillo, M.J. (2007): [Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study)]. Rev Esp Cardiol, **60**, 581-588.
9. Gonzalez-Gross, M., Castillo, M.J., Moreno, L., Nova, E., Gonzalez-Lamuno, D., Perez-Llamas, F., Gutierrez, A., Garaulet, M., Joyanes, M., Leiva, A. and Marcos, A. (2003): [Feeding and assessment of nutritional status of spanish adolescents (AVENA study). Evaluation of risks and interventional proposal. I.Methodology]. Nutr Hosp, **18**, 15-28.
10. Goyenechea, E., Parra, D. and Martinez, J.A. (2007): Impact of interleukin 6 -174G>C polymorphism on obesity-related metabolic disorders in people with excess in body weight. Metabolism, **56**, 1643-1648.
11. Hamid, Y.H., Rose, C.S., Urhammer, S.A., Glumer, C., Nolsoe, R., Kristiansen, O.P., Mandrup-Poulsen, T., Borch-Johnsen, K., Jorgensen, T., Hansen, T. and Pedersen, O. (2005): Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia, **48**, 251-260.

12. Higuchi, R. (1989): "Simple and rapid preparation of samples for PCR". Erlich H,
13. Humphries, S.E., Luong, L.A., Ogg, M.S., Hawe, E. and Miller, G.J. (2001): The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. Eur Heart J, **22**, 2243-2252.
14. Illig, T., Bongardt, F., Schopfer, A., Muller-Scholze, S., Rathmann, W., Koenig, W., Thorand, B., Vollmert, C., Holle, R., Kolb, H. and Herder, C. (2004): Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. J Clin Endocrinol Metab, **89**, 5053-5058.
15. Isnard, P., Michel, G., Frelut, M.L., Vila, G., Falissard, B., Naja, W., Navarro, J. and Mouren-Simeoni, M.C. (2003): Binge eating and psychopathology in severely obese adolescents. Int J Eat Disord, **34**, 235-243.
16. Knoflach, M., Kiechl, S., Penz, D., Zangerle, A., Schmidauer, C., Rossmann, A., Shingh, M., Spallek, R., Griesmacher, A., Bernhard, D., Robatscher, P., Buchberger, W., Draxl, W., Willeit, J. and Wick, G. (2009): Cardiovascular risk factors and atherosclerosis in young women: atherosclerosis risk factors in female youngsters (ARFY study). Stroke, **40**, 1063-1069.
17. Kubaszek, A., Pihlajamaki, J., Punnonen, K., Karhapaa, P., Vauhkonen, I. and Laakso, M. (2003): The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. Diabetes, **52**, 558-561.
18. Marti, A., Martinez-Gonzalez, M.A. and Martinez, J.A. (2008): Interaction between genes and lifestyle factors on obesity. Proc Nutr Soc, **67**, 1-8.
19. Martinez, J.A. (2000): Obesity in young Europeans: genetic and environmental influences. Eur J Clin Nutr, **54 Suppl 1**, S56-60.
20. Martinez, J.A., Moreno, M.J., Marques-Lopes, I. and Marti, A. (2002): [Causes of obesity]. An Sist Sanit Navar, **25 Suppl 1**, 17-27.
21. McKenzie, J.A., Weiss, E.P., Ghiu, I.A., Kulaputana, O., Phares, D.A., Ferrell, R.E. and Hagberg, J.M. (2004): Influence of the interleukin-6 -174 G/C gene polymorphism on exercise training-induced changes in glucose tolerance indexes. J Appl Physiol, **97**, 1338-1342.
22. Mohlig, M., Boeing, H., Spranger, J., Osterhoff, M., Kroke, A., Fisher, E., Bergmann, M.M., Ristow, M., Hoffmann, K. and Pfeiffer, A.F. (2004): Body mass index and C-174G interleukin-6 promoter polymorphism interact in predicting type 2 diabetes. J Clin Endocrinol Metab, **89**, 1885-1890.
23. Moreno-Aliaga, M.J., Campión, J., Milagro, F.I. and Berjón, A. (2005): Adiposity and proinflammatory state: The chicken or the egg. Adipocytes, **1**, 1-9.
24. Moreno, L.A., Joyanes, M., Mesana, M.I., Gonzalez-Gross, M., Gil, C.M., Sarria, A., Gutierrez, A., Garaulet, M., Perez-Prieto, R., Bueno, M. and Marcos, A. (2003): Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanish adolescents. Nutrition, **19**, 481-486.
25. Moreno, L.A., Mesana, M.I., Fleta, J., Ruiz, J.R., Gonzalez-Gross, M., Sarria, A., Marcos, A. and Bueno, M. (2005): Overweight, obesity and body fat composition in spanish adolescents. The AVENA Study. Ann Nutr Metab, **49**, 71-76.
26. Moreno, L.A., Mesana, M.I., Gonzalez-Gross, M., Gil, C.M., Ortega, F.B., Fleta, J., Warnberg, J., Leon, J., Marcos, A. and Bueno, M. (2007): Body fat distribution reference standards in Spanish adolescents: the AVENA Study. Int J Obes (Lond), **31**, 1798-1805.

27. Ortega, F.B., Tresaco, B., Ruiz, J.R., Moreno, L.A., Martin-Matillas, M., Mesa, J.L., Warnberg, J., Bueno, M., Tercedor, P., Gutierrez, A. and Castillo, M.J. (2007): Cardiorespiratory fitness and sedentary activities are associated with adiposity in adolescents. Obesity (Silver Spring), **15**, 1589-1599.
28. Panoulas, V.F., Stavropoulos-Kalinoglou, A., Metsios, G.S., Smith, J.P., Milionis, H.J., Douglas, K.M., Nightingale, P. and Kitas, G.D. (2009): Association of interleukin-6 (IL-6)-174G/C gene polymorphism with cardiovascular disease in patients with rheumatoid arthritis: the role of obesity and smoking. Atherosclerosis, **204**, 178-183.
29. Puchau, B., Zulet, M.A., Gonzalez de Echavarri, A., Navarro-Blasco, I. and Martinez, J.A. (2009): Selenium intake reduces serum C3, an early marker of metabolic syndrome manifestations, in healthy young adults. Eur J Clin Nutr, **63**, 858-864.
30. Qureshi, M.M., Singer, M.R. and Moore, L.L. (2009): A cross-sectional study of food group intake and C-reactive protein among children. Nutr Metab (Lond), **6**, 40.
31. Ruiz, J.R., Buxens, A., Artieda, M., Arteta, D., Santiago, C., Rodriguez-Romo, G., Lao, J.I., Gomez-Gallego, F. and Lucia, A. (2009): The -174 G/C polymorphism of the IL6 gene is associated with elite power performance. J Sci Med Sport,
32. Ruiz, J.R., Ortega, F.B., Warnberg, J., Moreno, L.A., Carrero, J.J., Gonzalez-Gross, M., Marcos, A., Gutierrez, A. and Sjostrom, M. (2008): Inflammatory proteins and muscle strength in adolescents: the Avena study. Arch Pediatr Adolesc Med, **162**, 462-468.
33. Santos, M.G., Pegoraro, M., Sandrini, F. and Macuco, E.C. (2008): Risk factors for the development of atherosclerosis in childhood and adolescence. Arq Bras Cardiol, **90**, 276-283.
34. Slaughter, M.H., Lohman, T.G., Boileau, R.A., Horswill, C.A., Stillman, R.J., Van Loan, M.D. and Bemben, D.A. (1988): Skinfold equations for estimation of body fatness in children and youth. Hum Biol, **60**, 709-723.
35. Sorensen, K.E., Celermajer, D.S., Georgakopoulos, D., Hatcher, G., Betteridge, D.J. and Deanfield, J.E. (1994): Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. J Clin Invest, **93**, 50-55.
36. Vozarova, B., Weyer, C., Hanson, K., Tataranni, P.A., Bogardus, C. and Pratley, R.E. (2001): Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res, **9**, 414-417.
37. Warnberg, J. and Marcos, A. (2008): Low-grade inflammation and the metabolic syndrome in children and adolescents. Curr Opin Lipidol, **19**, 11-15.
38. Warnberg, J., Moreno, L.A., Mesana, M.I. and Marcos, A. (2004): Inflammatory mediators in overweight and obese Spanish adolescents. The AVENA Study. Int J Obes Relat Metab Disord, **28 Suppl 3**, S59-63.
39. Warnberg, J., Nova, E., Moreno, L.A., Romeo, J., Mesana, M.I., Ruiz, J.R., Ortega, F.B., Sjostrom, M., Bueno, M. and Marcos, A. (2006): Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. Am J Clin Nutr, **84**, 505-512.

Table 1: Adolescents characteristics according to the -174G/C promoter polymorphism of the IL6 gene. Mean ± SD.

	GG (n= 211)	GC/CC (n=293)	p
Age (years)	14.5 ± 1.05	14.6 ± 1.07	0.716
Weight (kg)	59.3 ± 12.32	60.5 ± 12.01	0.571
Z score BMI	0.01 ± 1.06	0.08 ± 1.04	0.811
Waist circumfernce (cm)	74.2 ± 9.34	74.0 ± 8.66	0.685
WHR	0.79 ± 0.06	0.79 ± 0.05	0.495
Body Composition			
Body Fat Mass (%)	22.2 ± 6.53	23.0 ± 6.58	0.602
Sum 4 Skinfolds (mm)	46.9 ± 22.45	49.7 ± 25.00	0.414
Sum 6 Skinfolds (mm)	83.8 ± 35.90	87.6 ± 39.33	0.399
Truncal/Total SF	53.7 ± 5.43	53.4 ± 5.55	0.910
Physical Condition			
Physical Activity Index	0.64 ± 0.48	0.58 ± 0.49	0.505
Leisure time activity (act/wk)	0.86 ± 0.70	1.04 ± 0.79	0.012
Citokines			
TNFα (pg/ml)	2.09 ± 1.56	2.42 ± 2.51	0.119
IFNγ (pg/ml)	17.01 ± 17.52	20.52 ± 21.59	0.060
IL6 (pg/ml)	35.16 ± 21.25	36.28 ± 23.27	0.603
CRP (mg/L)	1.85 ± 0.44	1.37 ± 2.40	0.175
C3 (mg/ml)	1.37 ± 0.26	1.35 ± 0.22	0.378
C4 (mg/ml)	0.27 ± 0.09	0.27 ± 0.10	0.456
Lipids			
Triglycirides (mg/dl)	68.9 ± 29.9	69.1 ± 32.4	0.938
Total Cholesterol (mg/dl)	162.6 ± 23.9	163.5 ± 28.5	0.701
HDLc (mg/dl)	54.7 ± 11.1	55.4 ± 11.4	0.523
Cholesterol/HDL	3.1 ± 0.69	3.0 ± 0.71	0.731
Lipoprotein (a) (mg/dl)	29.7 ± 34.8	30.8 ± 38.5	0.743
Glucose (mg/dl)	93.3 ± 9.1	93.3 ± 8.7	0.993

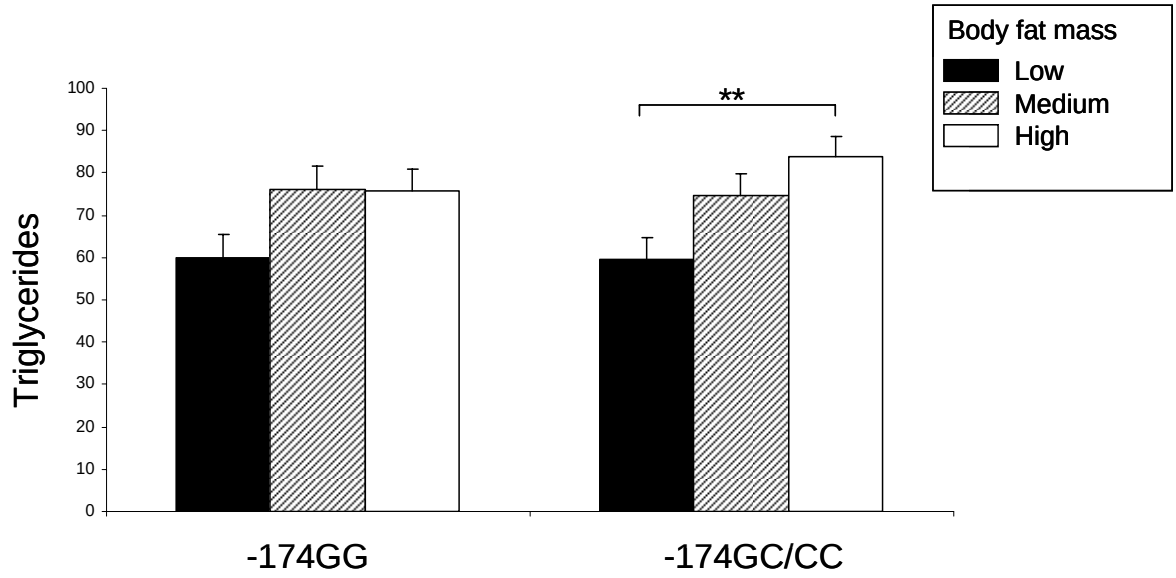
Table 2: Regression coefficients, SEM and R^2 showing the association between body fat mass percentage and inflammatory plasma markers in the whole sample
Controlling by age, gender, pubertal status and physical activity

	Body fat mass adjusted for age, gender, Tanner stage and mets			
	β	SEM	R2	p
TNFα (pg/ml)	0.049	0.000	0.035	0.332
IFNγ (pg/ml)	0.049	0.000	0.034	0.333
IL6 (pg/ml)	0.054	0.000	0.037	0.292
CRP (mg/L)	0.060	0.106	0.041	0.248
C3 (mg/ml)	0.449	1.286	0.233	<0.001
C4(mg/ml)	0.302	3.240	0.128	<0.001
Tryglicerides (mg/dl)	0.208	0.010	0.076	<0.001
Total cholesterol (mg/dl)	0.086	0.012	0.040	0.100
HDLc (mg/dl)	-0.294	0.029	0.108	<0.001
Cholesterol/HDL	0.319	0.437	0.132	<0.001
Lipoprotein (a) (mg/dl)	0.128	0.008	0.049	0.010
Glucose (mg/dl)	0.082	0.037	0.045	0.028

Table 3: Regression coefficients, SEM and R^2 showing the association between body fat mass percentage and inflammatory markers according to the -174G/C polymorphism of the IL6 gene.
Controlling by age, gender, pubertal status and physical activity

	Body fat mass adjusted for age, gender, Tanner stage and mets			
	β	SEM	R^2	p
GG (n=211)				
TNFα (pg/ml)	0.053	0.000	0.046	0.501
IFNγ (pg/ml)	0.127	0.000	0.058	0.104
IL6 (pg/ml)	0.093	0.000	0.054	0.245
CRP (mg/L)	0.009	0.126	0.051	0.917
C3 (mg/ml)	0.487	1.821	0.283	<0.001
C4 (mg/ml)	0.358	5.181	0.177	<0.001
Tryglicerides (mg/dl)	0.206	0.016	0.091	0.007
Total cholesterol (mg/dl)	0.133	0.021	0.066	0.089
HDLc (mg/dl)	-0.205	0.047	0.086	0.011
Cholesterol/HDL	0.295	0.737	0.131	<0.001
Lipoprotein (a) (mg/dl)	0.030	0.014	0.050	0.700
Glucose (mg/dl)	0.294	0.055	0.126	<0.001
GC/CC (n= 285)				
TNFα (pg/ml)	0.034	0.000	0.048	0.607
IFNγ (pg/ml)	-0.012	0.000	0.045	0.863
IL6 (pg/ml)	0.015	0.000	0.047	0.823
CRP (mg/L)	0.138	0.216	0.078	0.043
C3 (mg/ml)	0.406	1.824	0.216	<0.001
C4 (mg/ml)	0.258	4.183	0.124	<0.001
Tryglicerides (mg/dl)	0.213	0.012	0.089	0.001
Total cholesterol (mg/dl)	0.063	0.015	0.047	0.372
HDLc (mg/dl)	-0.355	0.037	0.147	<0.001
Cholesterol/HDL	0.333	0.544	0.152	<0.001
Lipoprotein (a) (mg/dl)	0.191	0.010	0.080	0.003
Glucose (mg/dl)	-0.014	0.051	0.044	0.845

A.


Body fat mass
Low
Medium
High
Triglycerides
100
90
80
70
60
50
40
30
20
10
0
**
-174GG
-174GC/CC

B.

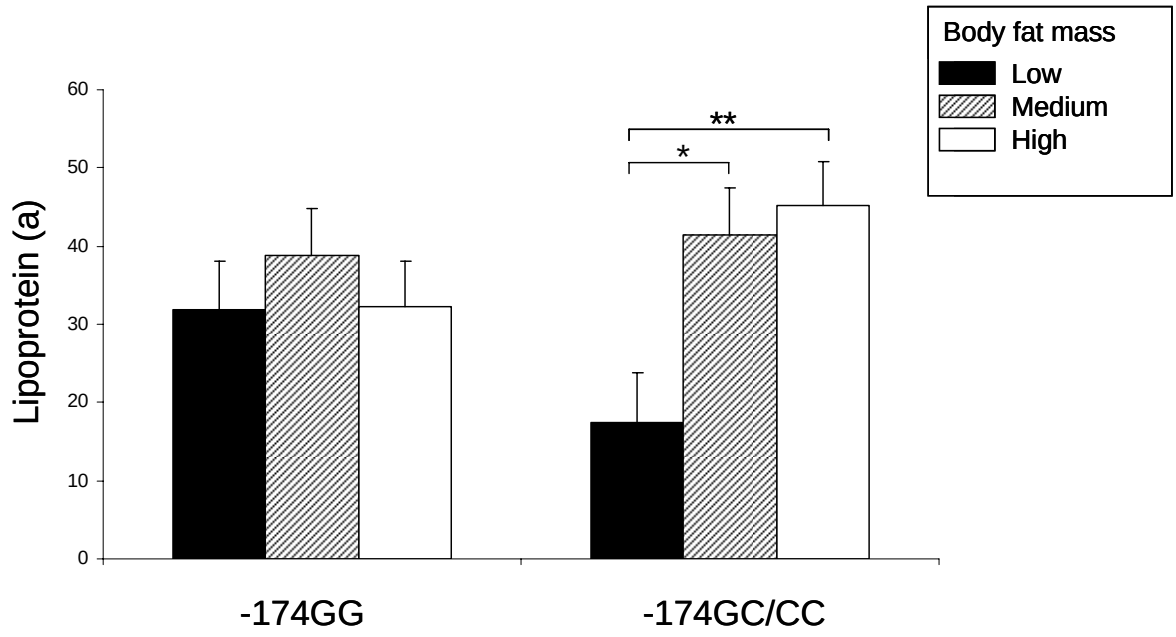

Body fat mass
Low
Medium
High
Lipoprotein (a)
60
50
40
30
20
10
0
**
*
-174GG
-174GC/CC

FIGURE LEGENDS

Fig. 1.A) Differences on triglycerides plasma concentration (mg/dl) according to body fat mass tertiles and the -174G/C polymorphism of the IL6 gene. **p<0.01

Fig. 1.B) Differences on lipoprotein (a) concentration (mg/dl) according to body fat mass tertiles and the -174G/C polymorphism of the IL6 gene. *p<0.05; **p<0.01