

PPAR- γ 2 Pro12Ala Variant, Insulin Resistance and Plasma Long-chain Polyunsaturated Fatty Acids in Childhood Obesity

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ABSTRACT: Pro12Ala variant of peroxisome-proliferator-activated receptor-gamma2 (PPAR- γ 2) may be linked to insulin sensitivity. This study examined whether an association of PPAR- γ 2 Pro12Ala with insulin resistance and plasma LCPUFAs may exist in obese children. One hundred and forty Italian normolipidemic obese children (58 girls and 82 boys, mean age [SD], 10.2 [2.7] y) entered the study. Obesity was defined according to International Obesity Task Force. BMI Z-scores were calculated. Fasting blood glucose, insulin, lipids and plasma fatty acids were measured. Insulin resistance was estimated by the homeostatic model assessment (HOMA-IR). The frequency of Ala allele was 9%. Mean [SD] values of fasting insulin and HOMA-IR in Pro/Pro versus Pro12Ala groups were: 19.3 [10.6] versus 14.1 [10.4] μ U/mL ($p = 0.017$) and 4.2 [2.3] versus 3.0 [2.3] ($p = 0.022$). Mean [SD] values of plasma C20:3n-9 and of C20:4n-6, C20:5n-3, C22:6n-3 and n-6/n-3 LCPUFA in phospholipids in Pro/Pro versus Pro12Ala groups were: 0.15 [0.07] versus 0.12 [0.08] % ($p = 0.014$), 8.9 [1.9] versus 10.2 [2.6] % ($p = 0.023$), 0.34 [0.15] versus 0.42 [0.11] % ($p = 0.005$), 2.1 [0.9] versus 2.6 [0.9] % ($p = 0.032$) and 4.8 [1.2] versus 4.2 [0.7] ($p = 0.017$). Pro12Ala may be associated with higher insulin sensitivity and higher LCPUFAs, particularly n-3, levels in plasma phospholipids of obese children. (*Pediatr Res* 60: 485–489, 2006)

Childhood obesity is an increasing condition in the general population, possibly associated with later onset of chronic disturbances and clinical complications, such as type 2 diabetes mellitus and cardiovascular disease (1), and represents an emerging health problem (2). Insulin resistance, glucose intolerance and hyperinsulinemia are major components of the metabolic syndrome (3), highly prevalent among children and adolescents with marked obesity (4). Although the mechanisms underlying insulin resistance are not fully elucidated, both genetic factors and environmental factors leading to obesity play relevant roles in its etiology (5).

Among genetic factors, peroxisome-proliferator-activated receptor-gamma (PPAR- γ 2), a nuclear transcription factor, is involved in the metabolic disposal of the adipose tissue (6) including adipocyte differentiation, adipogenesis and the pathogenesis of insulin resistance states (7–9). The Pro12Ala variant of PPAR- γ 2, highly prevalent in Caucasians (10), has

been studied for the association with obesity-related or insulin resistance-related traits (11–13). Some studies suggest that the Pro12Ala genotype may be associated with improved insulin sensitivity (12,13), especially in obese subjects, but this matter is still controversial (14–16).

Among environmental factors, the quality of dietary fats may be also linked to the metabolic syndrome, particularly in adults, with excess saturated fats enhancing, and unsaturated fats preventing, the development of the major complications of the metabolic syndrome through several independent mechanisms including the blood lipid picture and other mechanisms linked to the atherogenic process (<http://www.healthierus.gov/dietaryguidelines/>).

Within this context, polyunsaturated fatty acids (PUFAs) are able to activate PPAR γ , leading to a decreased insulin resistance status (17). PUFA-enriched diets (such as the Eskimo diet) have been associated with increased insulin sensitivity and decreased triglyceride levels in rodents and humans (18), two effects that are shared by the synthetic PPAR γ agonists. Also the main PUFA derivatives, arachidonic and docosahexaenoic acids, are effective PPAR activators (19).

Despite the potential clinical relevance of the association between Pro12Ala variant of PPAR- γ 2 and insulin resistance with the long-chain polyunsaturated fatty acid (LCPUFA) status, there is lack of studies in the pediatric population. Aim of the present study was to examine whether an association of PPAR- γ 2 Pro12Ala variant might exist with measures of insulin resistance and the plasma LCPUFA profile in obese white children.

MATERIALS AND METHODS

The present observational study included 140 obese children (mean age [SD], 10.2 [2.7] y, 58 girls and 82 boys), consecutively admitted to our department from January, 2003 to October, 2005.

Inclusion criteria were: weight at birth \geq 2500 g, gestational age 37–42 wk inclusive, singleton birth, obesity, and having white parents. Children exhibiting diabetes mellitus and/or endocrine diseases, chronic liver diseases, and infectious disorders were excluded. Additionally, children having hyperlipidemia according to Italian guidelines (20) were excluded to prevent possible bias effects on plasma fatty acid status.

Abbreviations: FA, fatty acid; HOMA-IR, homeostatic model assessment; LCPUFA, long chain polyunsaturated fatty acid; PL, phospholipids; PPAR- γ 2, peroxisome-proliferator-activated receptor-gamma; PUFAs, polyunsaturated fatty acids

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All children were born in Lombardy, central-northern Italy, where they are currently residing. A pediatrician described the investigation and written informed consent was obtained from both parents. The Institutional Ethical Committee approved the study.

A medical history was obtained from parents by a standardized questionnaire at a personal interview conducted by the same pediatrician before the child's hospital discharge. Anthropometrics (height, weight), blood pressure, fasting biochemical features (glucose, insulin levels, lipid and fatty acids profile) of children were evaluated during the hospital stay, within three days of recruitment. The same pediatrician performed the Tanner staging of puberty.

Two experienced pediatricians, according to standardized procedures, performed anthropometric measurements. The body weight (kg) was measured with an electronic scale accurate to 0.1 kg and height (cm) was measured to the nearest 0.5 cm with a Harpenden stadiometer. For each anthropometric variable, three measurements were taken and the mean value was considered for the analysis. The coefficient of variation of the measurements ranged from 0.8–1.4% (observer 1) and from 0.7–1.4% (observer 2). This small and comparable degree of inter-observers measurement variation was deemed to yield reliable anthropometric data. The body mass index (BMI) was calculated from the ratio of weight to length/height² (kg/m²). In accordance with the International Obesity Task Force, a child was defined obese if her/his BMI was above the age- and sex-adjusted BMI Cole's curve passing through the cut-off of 30 kg/m² at 18 y (21). Compared with Italian age- and sex-adjusted growth charts for BMI (22), all recruited eligible children exhibited BMI over 97th percentile. BMI Z-scores were calculated and adjusted for age and gender by using the LMS-method of Cole (23) and Italian reference data (22).

At the hospital, parents had body weight and height measured. A parent was defined overweight if her/his BMI was equal or greater than 25 kg/m², obese if BMI was equal or greater than 30 kg/m². Parental overweight/obesity was defined when at least one parent was overweight/obese.

Blood samples were collected after overnight fasting. Serum insulin levels (μ U/mL) were determined by an electrochemiluminescence immune assay analyzer, and plasma glucose levels (mg/dL) by enzymatic method. Flavored glucose in a dose of 1.75 g/kg of body weight (up to a maximum of 75 g) was given orally, and blood samples were obtained for measurements of plasma glucose and serum insulin at baseline and plasma glucose at 120 min. Insulin resistance was estimated by the homeostatic model assessment (HOMA-IR) (24). HOMA-IR is calculated as the product of fasting glucose (mmol/L) and fasting insulin (μ U/mL), divided by 22.5.

Total cholesterol (TC) and triacylglycerol (TG) plasma levels were measured using a dry multiplate enzymatic method (Ectachem DT-60; Eastman Kodak Co., Rochester, NY). LDL-cholesterol (LDL-C) serum levels were calculated according to the Friedewald formula (LDL-C = TC - [HDL-C + TG/5]) (25). Plasma fatty acid (FA) analyses (expressed as % total FA) were performed by means of capillary gas chromatography after lipid extraction according to Folch *et al.* (26) and the phospholipid fraction of plasma fatty acids has been separated by thin-layer chromatography as described (27).

Genetic assessment was based on 200 base pairs of sequence surrounding PPAR γ 2 Pro12Ala, provided to Applied Biosystems (Foster City, CA) to develop Taqman Allelic Discrimination (AD) Assays using their assay by design platform. Genotyping of the Pro12Ala AD was performed using primers (0.9 μ mol/L each). Forward 5'-TTATGGGTGAACTCTGG-GAGATT-3' and reverse 5'-TGCACAGATGTATCAGTGAAGGA-3' and the Taqman MGB probes Fam-TTCTGGGTCAATAGG and Vic-CTTCT-GCGTCAATAG (0.1 μ mol/L each; Applied Biosystems). Four microliters of a 10 ng/ μ L stock of DNA was dispensed into 384-well PCR plates using a Biomek FX robot (Beckman Coulter, Fullerton, CA, USA), to which 6 μ L of a mix containing primers, MGB probes, and TaqMan Universal PCR Master Mix (Applied Biosystems) and incubated at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min before analysis on a 7900 HT plate reader (Applied Biosystems). Individual genotypes were determined using SDSv2.1 software (Applied Biosystems).

The same experienced radiologist performed liver ultrasonography by a Hitachi H21 (Hitachi High Technology Corporation Ltd., Tokio, Japan) using a 3.5-MHz convex array transducer. Longitudinal, subcostal, ascending, and oblique scans were performed (28). Liver echogenicity was evaluated independently on videotape by three radiologists unaware of the subject, and a consensus was established. Absolute agreement among judgements before discussion occurred in 134/140 (95.7%) cases.

Finally, dietary habits of children were assessed at recruitment by means of an age-adjusted Food Frequency Questionnaire made up of 116 items and designed according to Block (29). The same experienced dietician, unaware of the obesity status of children, interviewed mothers for approximately 50 min and each meal was analyzed to find out which food was eaten and how often. Usual portion sizes were estimated using household measures, the

weight of purchase (*e.g.*, pasta) or unit (*e.g.*, fruit juice). A 24-h recall on the day before the hospital admission was further recorded at the end of the interview to standardize the usual serving size. Quantification and analysis of the energy intake and nutrient composition were performed with an ad hoc PC software program developed at our department and based on the Food and Nutrient Data Base issued by the National Institute of Nutrition (30).

Statistical analysis. Descriptive data are shown as mean (SD) or number of observations (percentage). The χ^2 test was used for comparing discrete variables. Comparison between groups for continuous variables was performed by the *t* test for unpaired data or the Mann-Whitney test, as appropriate. Triglycerides, insulin and HOMA-IR were not normally distributed and log₁₀-transformation was used for analysis. For clarity of interpretation, results are expressed as untransformed values. Adjustments were made for potential confounders (infant's gender, age, body weight at birth, Tanner stage, BMI Z-score, duration of obesity and BMI* PPAR γ 2 interaction). The association of the Pro12Ala polymorphism with indices of insulin resistance (fasting insulin levels, HOMA-IR) and plasma FA was tested using multiple linear regression. All *p*-values <0.05 were considered to indicate statistical significance (two-tailed test).

RESULTS

The frequency of Ala allele was 9% (Pro/Pro 81.4%, Pro/Ala 18.6%). No child was carrier of Ala/Ala genotype. Table 1 reports clinical and biochemical variables of children according to Pro12Ala PPAR- γ 2 genotype. Ala carriers, compared with non-carriers, showed lower fasting insulin levels (*p* < 0.05) and lower HOMA-IR (*p* < 0.05). These association remained significant (fasting insulin, odds ratio [OR] 0.94, 95% confidence interval [CI] 0.88-1.00; HOMA-IR, OR 0.74, 95%CI 0.55-0.99) also at a multiple logistic regression analysis fitted for PPAR γ 2 and including variables listed in Table 1 as covariates. Pro12Ala genotype was not associated with any other cardiovascular traits listed in Table 1 both before (Table 1) and after adjusting for either fasting insulin or HOMA-IR (any *p* > 0.413). No significant interaction effect

Table 1. Clinical and biochemical parameters of children according to Pro12Ala PPAR- γ 2 genotype

Characteristic	Pro/Pro (N = 114)	Pro/Ala (N = 26)	<i>p</i> -value†
Gender (boys/girls)	64/50	18/8	0.336
Age (y)	10.2 ± 2.7	10.0 ± 2.7	0.743
BMI Z-score	2.23 ± 0.43	2.30 ± 0.32	0.436
Duration of obesity (y)	4.9 ± 2.5	5.1 ± 2.4	0.466
Systolic BP (mm Hg)	113 ± 10	116 ± 10	0.143
Diastolic BP (mm Hg)	69 ± 7	70 ± 9	0.670
Fasting glucose (mmol/L)	4.81 ± 0.32	4.75 ± 0.32	0.620
120min OGTT glucose (mmol/L)	6.15 ± 1.26	6.16 ± 0.89	0.792
Fasting insulin (mU/mL)	19.3 ± 10.5	14.1 ± 10.4	0.024*
HOMA-IR	4.2 ± 2.3	3.0 ± 2.3	0.040*
Total cholesterol (mmol/L)	4.19 ± 0.67	4.35 ± 0.65	0.288
HDL cholesterol (mmol/L)	1.29 ± 0.27	1.29 ± 0.28	0.994
LDL cholesterol (mmol/L)	2.35 ± 0.63	2.46 ± 0.55	0.430
Triglycerides (mmol/L)	1.14 ± 0.64	1.29 ± 0.83	0.470
Increased liver echogenicity (yes/no)	18/96	6/20	0.375

Values are mean ± SD or number of observations.

* Statistically significant.

† *P*-values were adjusted for infant's gender, age, body weight at birth, Tanner stage, BMI Z-score, duration of obesity and BMI* PPAR γ 2 interaction.

SI conversion factors: to convert glucose to mg/dL, divide values by 0.0555; to convert cholesterol, divide values by 0.0259; to convert triglycerides, divide values by 0.0113.

was observed between BMI and Pro12Ala polymorphism in modulating insulin sensitivity (HOMA- IR p -value interaction = 0.480; fasting insulin p -value interaction = 0.396).

Increased liver echogenicity was found in 24 out of 140 cases (17%). The distribution of Tanner stage did not differ ($p = 0.655$) between carriers and noncarriers: stage I, 46.2% versus 42.7%; stage II-III, 38.5% versus 33.6%; stage IV-V, 15.4% versus 23.6%, respectively. Familial overweight was found in 38.5% and 36.8% of Ala carriers and noncarriers, respectively ($p = 0.997$), familial obesity was 40.4% versus 53.8% ($p = 0.273$).

No significant differences were found between Ala carriers and non-carriers for total dietary energy or any macronutrients intake: mean (SD) total energy (kcal/die), 2467 (910) versus 2765 (935) ($p = 0.188$); protein (%), 15 (2) versus 15 (2) ($p = 0.487$); carbohydrate (%), 57 (7) versus 56 (6) ($p = 0.165$); fat (%), 31 (5) versus 32 (5) ($p = 0.433$). No inter-group differences were found with respect to the dietary intake of the main FA families, including PUFAs (%), 4.1 (0.5) versus 4.2 (0.5) ($p = 0.327$), or polyunsaturated to saturated fatty acid ratio, 0.39 (0.11) versus 0.39 (0.10) ($p = 0.391$).

Table 2 shows the relationship of the plasma FA profile of total lipids with Pro12Ala PPAR- γ 2 genotype. The Pro12Ala polymorphism was associated with lower plasma C20:3n-9 ($p < 0.05$). The FA composition of plasma phospholipids (PL) according to PPAR- γ 2 genotype is reported in Table 3. The Pro12Ala polymorphism was significantly associated with lower plasma PL n-6/n-3 PUFA, n-6/n-3 LCPUFA and higher levels of n-3 PUFA, n-6 LCPUFA, n-3 LCPUFA, C20:4n-6, C20:5n-3 and C22:6n-3.

A logistic regression model was further fitted for PPAR γ 2 genotype, and including HOMA-IR and % plasma phospholipids and confounders as covariates, to assess the independent association of Pro12Ala with LCPUFA and HOMA-IR. This analysis showed that phospholipid n-6/n-3 PUFA was the only

Table 2. LCPUFA as FA % of plasma total lipids according to Pro12Ala PPAR- γ 2 genotype

Variable	Pro/Pro (N = 114)	Pro/Ala (N = 26)	p -value
Saturated FA	35.9 \pm 2.6	36.5 \pm 2.6	0.069
Monounsaturated FA	26.4 \pm 3.3	26.4 \pm 2.2	0.566
Polyunsaturated FA	37.6 \pm 4.7	37.0 \pm 3.3	0.496
n-6 PUFA	34.8 \pm 4.5	34.2 \pm 3.1	0.397
n-3 PUFA	2.6 \pm 0.7	2.7 \pm 0.5	0.125
n-6/n-3 PUFA	13.9 \pm 3.3	12.9 \pm 2.1	0.132
n-6 LCPUFA	10.0 \pm 1.6	10.4 \pm 1.4	0.243
n-3 LCPUFA	2.3 \pm 0.7	2.4 \pm 0.4	0.092
n-6/n-3 LCPUFA	4.5 \pm 1.0	4.3 \pm 0.7	0.513
C18:2n-6	24.7 \pm 4.2	23.8 \pm 2.6	0.156
C20:4n-6	7.2 \pm 1.3	7.3 \pm 1.3	0.822
C18:3n-3	0.29 \pm 0.10	0.27 \pm 0.08	0.668
C20:5n-3	0.44 \pm 0.19	0.44 \pm 0.11	0.446
C22:6n-3	1.6 \pm 0.6	1.6 \pm 0.4	0.274
C20:3n-9	0.15 \pm 0.07	0.12 \pm 0.08	0.014*
C20:5n-3/C20:4n-6	0.059 \pm 0.024	0.059 \pm 0.024	0.939
C20:3n-9/ C20:4n-6	0.022 \pm 0.016	0.018 \pm 0.013	0.061
C22:6n-3/C20:4n-6	0.21 \pm 0.06	0.22 \pm 0.04	0.402

Values are mean \pm SD.

* Statistically significant.

Table 3. LCPUFA as FA % of plasma phospholipids according to Pro12Ala PPAR- γ 2 genotype

Variable	Pro/Pro (N = 114)	Pro/Ala (N = 26)	p -value
Saturated FA	48.3 \pm 2.7	46.7 \pm 6.1	0.700
Monounsaturated FA	16.9 \pm 2.3	16.6 \pm 2.5	0.250
Polyunsaturated FA	34.7 \pm 3.5	36.6 \pm 5.5	0.340
n-6 PUFA	31.5 \pm 2.8	32.7 \pm 4.6	0.415
n-3 PUFA	3.1 \pm 1.1	3.7 \pm 1.1	0.006*
n-6/n-3 PUFA	11.6 \pm 4.2	9.0 \pm 1.3	0.001*
n-6 LCPUFA	13.1 \pm 2.5	15.0 \pm 2.9	0.002*
n-3 LCPUFA	2.9 \pm 1.1	3.6 \pm 1.1	0.004*
n-6/n-3 LCPUFA	4.8 \pm 1.2	4.2 \pm 0.7	0.017*
C18:2n-6	18.4 \pm 2.3	17.7 \pm 2.2	0.200
C20:4n-6	8.9 \pm 1.9	10.2 \pm 2.6	0.023*
C18:3n-3	0.09 \pm 0.04	0.10 \pm 0.11	0.342
C20:5n-3	0.34 \pm 0.15	0.42 \pm 0.11	0.005*
C22:6n-3	2.1 \pm 0.9	2.6 \pm 0.9	0.032*
C20:3n-9	0.16 \pm 0.06	0.16 \pm 0.03	0.972
C20:5n-3/C20:4n-6	0.038 \pm 0.013	0.044 \pm 0.018	0.409
C20:3n-9/ C20:4n-6	0.018 \pm 0.007	0.017 \pm 0.006	0.272
C22:6n-3/C20:4n-6	0.23 \pm 0.07	0.25 \pm 0.05	0.079

Values are mean \pm SD.

* Statistically significant.

variable independently associated with PPAR γ 2 genotype (OR 0.75, 95% CI 0.61–0.90).

Lastly, a correlation analysis showed that in the present study HOMA-IR was negatively associated with plasma n-3 LCPUFA ($p = 0.002$), n-6 LCPUFA ($p = 0.022$), C20:4n-6 ($p = 0.016$) and C22:6n-3 ($p = 0.012$). Considering the FA composition of plasma phospholipids, HOMA-IR was negatively associated with n-3 LCPUFA ($p = 0.021$), C22:6n-3 ($p = 0.046$) and C22:6n-3 to C20:4n-6 ratio ($p = 0.029$), and positively with the n-6/n-3 LCPUFA ratio ($p = 0.003$).

DISCUSSION

This study examined the association of plasma fatty acids with obesity and insulin resistance in normolipidemic obese children focusing on the differences between carriers of the more common PPAR- γ 2 Pro/Pro genotype and those carrying the possibly protective Pro12Ala variant. Indeed, because obesity is one of the most important risk factors for cardiovascular diseases and type 2 diabetes, obese children, who are presumably at a higher risk, may be protected from these diseases by the phenotypic effect of the Ala allele on insulin resistance since the Pro12Ala polymorphism of PPAR- γ 2 gene is associated with greater insulin sensitivity (14,15). Through the years the PPAR- γ 2 Pro12Ala polymorphism beneficially influences insulin resistance and its tracking from childhood to adulthood (31). Also the rates of skeletal muscle glucose uptake are higher in subjects carrying the Ala allele than in subjects carrying the Pro12Pro genotype, at least within the non-obese population (32). The question whether Pro12Ala polymorphism modulates the skeletal muscle glucose uptake differently in non-obese and obese subjects is still open.

Skeletal muscle is a major site of insulin action, and insulin sensitivity may be related to the fatty-acid composition of phospholipids within the muscle membranes. LCPUFAs, in-

cluding docosahexaenoic acid (C22:6 n-3, [DHA]), are major components of cell membranes. Low levels of DHA and other LCPUFAs, including arachidonic acid (C20:4 n-6 [AA]), and a high n-6/n-3 PUFA ratio in skeletal muscle membrane phospholipids are associated with insulin resistance in human adults (33,34).

In young infants, breast-feeding increases LCPUFA levels in skeletal muscle membranes and early development of relatively higher levels of LCPUFAs in skeletal muscle phospholipids, influenced by either the type of feeding and/or genetic-related pathways, is associated with lower fasting plasma glucose levels (35). Accordingly, early changes in skeletal muscle membrane phospholipid FA saturation index may play a role in the subsequent development of diseases associated with insulin resistance. The less unsaturated muscle membranes in children whose mothers have higher fasting insulin and triglyceride levels may as well reflect a genetic reluctance to incorporate PUFAs into membranes, thus predisposing them to insulin resistance syndromes (36).

Since hypercholesterolaemia and hypertriacylglycerolaemia might substantially figure in defining plasma total fatty acid profiles, we have selected obese children showing blood lipid levels within a range of normality to prevent misinterpretations of data on the individual fatty acid status. Indeed the normal distribution of fatty acids differs significantly in cholesterol esters and triacylglycerols from those seen in phospholipids or free FA (37). Moreover in our sample, obese children with different PPAR- γ 2 genotype were comparable for plasma total cholesterol and triacylglycerol values.

The results of the present study suggest a possible link and explanation of the pathways leading to increased insulin sensitivity in carriers of the Pro12Ala genotype. Obese children with the Pro12Ala variant exhibited lower values of fasting insulin and HOMA-IR. In our study the average difference in Ala carriers compared with non carriers was 28% for fasting insulin levels and 30% for HOMA-IR. Other authors (15) found an average difference of around 15% (fasting insulin) and 17% (HOMA-IR). However, we would point out that in the population studied by Buzzetti et al.(15), the mean HOMA-IR was 5.3 and 4.4 in Pro/Pro and Ala carriers, compared with 4.2 and 3.0 found in the present study. Values found in Buzzetti et al. (15) were therefore around 26–46% higher. Accordingly, the two populations might be not directly comparable for variables possibly related to insulin sensitivity. It may be not excluded that children with different insulin sensitivity might differently respond to the PPAR γ 2 genotype Pro12Ala variant for these variables.

Obese children with the Pro12Ala variant showed higher levels of the major PUFAs (n-3 PUFA, n-6 LCPUFA, n-3 LCPUFA, C20:4n-6, C20:5n-3 and C22:6n-3), in plasma phospholipids. Plasma phospholipids may reflect the FA composition of red blood cell lipids since they are in continuous exchange (38). In turn, red blood cell FA composition may reflect the FA (particularly PUFA) composition of muscle membrane phospholipids, and it has been shown that the type of feeding shows similar effect on the LCPUFA composition of erythrocytes and muscles (39). Therefore our findings are consistent with the previous data of a lower representation of

C-20 and C-22 LCPUFA in membrane muscles of subjects with increased insulin resistance (33,35). Furthermore, the higher levels of C:20:3 n-9 in total plasma lipids of obese children with the Pro/Pro genotype might indicate a homeostatic mechanism leading to an increased synthesis of the endogenous LCPUFAs derived from the n-9 series, in absence of the highly unsaturated n-6 and n-3 compounds (40).

Since no major dietary differences between Ala carriers and non-carriers have been found, it may be hypothesized that either a preferential channeling of dietary LCPUFAs and/or a major LCPUFA endogenous synthesis from the parental n-6 and n-3 precursors could take place in obese children with the Pro12Ala variant. Whichever the case, and given a major protection from complications in the carriers of the Ala allele (14,15), raising levels of LCPUFAs in membranes of Pro/Pro genotype carriers might be beneficial for insulin sensitivity in obese children. Finally, a logistic regression model disclosed that a lower n-6/n-3 PUFA ratio in plasma phospholipids might be an indicator through which Pro12Ala exerts its effects on HOMA-IR, more than individual LCPUFA concentrations.

A limitation of the present study is the unfeasibility to estimate the dietary intake of the single FAs and then to evaluate the correlations between plasma FA values and dietary FA intakes. Indeed, this would be an important analysis as plasma FA levels are, presumably, a function of dietary intake and biologic processing of fats.

In conclusion, within the limitations of the present study, obese children carriers of the Pro12Ala variant seem to be protected from metabolic complications by pathways leading to a major representation of C-20 plus C-22 n-6 and n-3 LCPUFA in plasma phospholipids, presumably affecting in a parallel fashion the muscle membrane composition. Large longitudinal studies need to clarify the relationship between plasma PUFA and metabolic conditions with respect to PPAR γ 2 genotype in obese children and to study the dietary modifications able to improve the metabolic conditions in the carriers of the most common Pro/Pro genotype.

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