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An age-dependent diet-modified effect of the *PPARγ* Pro12Ala polymorphism in children

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Abstract

Variation in the peroxisome proliferator–activated receptor γ gene alters the risk for adiposity in adults, with evidence of interaction with diet. We investigated the age-related association between the Pro12Ala variant (rs1801282) and diet in obesity-related traits in children. The Pro12Ala variant was assayed in 2102 young children aged 1 to 6 years and in 794 periadolescent children aged 10 to 12 years of Greek origin. In both cohorts, no differences were found in obesity traits between the Ala allele carriers and Pro/Pro homozygotes. Sex-stratified analysis showed that, in periadolescent boys, Ala carriers exhibited lower measures of skinfolds (triceps: 16.9 ± 6.9 vs 19.4 ± 7.9 mm, P = .01; subscapular: 9.6 ± 4.5 vs 11.2 ± 5.4 mm, P = .02). On the other hand, young girls who were Ala carriers presented higher measures of triceps skinfold thickness (10.5 ± 3.0 vs 9.9 ± 2.8 mm, P = .04). Nominal gene-diet interactions were revealed in periadolescents for saturated fatty acid (SFA) intake and skinfolds (P for interaction = .05). In Pro/Pro homozygous young girls, SFA and total fat (TF) intake was positively associated with higher body mass index (BMI) (P = .01), waist circumference (P = .02), and skinfold thickness (triceps-SFA: $P = 10^{-5}$, triceps-TF: $P = 10^{-9}$, subscapular-SFA: $P = 10^{-6}$, subscapular-TF: $P = 10^{-4}$). For Pro/Pro homozygotes, unsaturated fat intake was inversely associated with BMI (P = .04) in young girls, and with BMI (P = .03), waist circumference (P = .03), and triceps (P = .02) in periadolescent boys. Our results suggest that adiposity in children is influenced by the Pro12Ala polymorphism in a sex-specific and age-dependent manner. We also demonstrate evidence of an age-dependent gene-diet (SFA, TF) interaction, suggesting that the type of fat intake modifies the effect of the Pro12 allele on obesity-related measures.

1. Introduction

Childhood obesity is a rapidly growing health problem worldwide [1]. It affects numerous body systems; most notably, overweight has been related to hypertension, dyslipidemia, increased blood clotting tendency, and insulin resistance. The factors that regulate body fat content and distribution are not fully understood. With regard to dietary intake, there is no scientific consensus on whether macronutrient composition affects energy metabolism and body fat distribution beyond energy content. For example, it has been postulated that dietary fat intake promotes obesity; but it has also been suggested that high-fat diets do not constitute the primary cause of the increasing prevalence of excess body fat in Westernized societies [2,3]. On the other hand, specific macronutrient composition of the diet may be one of the important environmental factors that control nutrient partitioning to specific adipose tissue depots without affecting total body weight [4]. In fact, high-fat diets enriched with monounsaturated fatty acid (MUFA) have been shown to improve anthropometric and metabolic

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parameters [5,6]; but there is little evidence as to the molecular signals responsible for this effect.

The human peroxisome proliferator–activated receptor γ (PPARγ) plays a key role in the regulation of lipid and glucose homeostasis, in the differentiation of adipocytes, and in fatty acid storage. It functions as a heterodimer with a retinoid X-receptor, and it is activated mainly by fatty acids [7,8]. A critical role for PPARγ in the development of mammalian adipose tissue has been confirmed by the absence of adipose tissue in PPARγ-null murine embryos [9]. The Pro12Ala polymorphism has been widely investigated in relation to various disorders including type 2 diabetes mellitus [10-12], insulin sensitivity [13,14], ischemic stroke [15], lipid variations [16,17], and obesity [18-20]. However, the association of the Ala allele with a higher risk of obesity has not been consistent in all studies, suggesting heterogeneity of the effect. Furthermore, the association of the Ala allele with higher risk for obesity has been found to be modified by dietary fat intake. Luan et. al [21] reported that a low dietary polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio is associated with higher body mass index (BMI) in Ala carriers compared with Pro homozygotes, with the opposite effect for a high PUFA/SFA ratio. A direct relationship was found between transfatty acid intake and type 2 diabetes mellitus in Ala carriers [22]. It has been shown that PPARγ expression is attenuated in visceral adipose tissue in lean subjects [23,24], which may suggest a link between the Pro12Ala polymorphism and regulation of regional adiposity and body weight.

In light of the rising prevalence in pediatric obesity, it is important to assess the potential effect of the Pro12Ala polymorphism in the *PPARγ* gene at an earlier life stage. This has been attempted by several groups with largely inconsistent findings [25-28]. It has recently been shown that, in young children [28], the Pro12Ala polymorphism is associated with increased adiposity only in girls. The fact that research findings from adult and pediatric cohorts are not consistent may be due to the influence of unmeasured factors, such as diet and energy expenditure, or due to age-specific effects [29]. We investigate the association between the Pro12Ala variant of the *PPARγ* gene and obesity traits with consideration of fat intake and activity in 2 different pediatric cohorts (periadolescents and young children) in an attempt to find the true effect of this polymorphism on the development of obesity.

2. Subjects and methods

2.1. Subjects

The Gene-Diet Attica Investigation on childhood obesity (GENDAI) target population was composed of children attending fifth and sixth grades and living in the Attica region of Greece as previously described [30]. The GENDAI cohort comprised 1138 adolescent children (53% girls; mean age, 11.2 ± 0.7 years) randomly selected from elementary schools of Attica. The Growth, Exercise, and Nutrition Epidemiological Study in preSchoolers (GENESIS) study [30,31] comprised 2374 healthy children aged 1 to 6 years attending public and private day care centers in 5 geographical districts of Greece: Athens, Aitoloakarnania, Thessaloniki, Halkidiki, and Helia. The sampling of the day care centers was random, multistage, and stratified by the total population of children. Both studies were approved by the Institutional Review Board of Harokopio University and the Greek Ministry of Education, and a volunteer consent form was always included.

2.2. Dietary methodology

Dietary information was collected by 2 nonconsecutive 24-hour recalls 3 to 10 days apart [32]. Dietary information was provided by caregivers in the GENESIS study. Staff reviewed all 24-hour recalls during regular weekly meetings to resolve issues on missing foods or unrealistic quantities reported. The 24-hour recalls were analyzed using the Nutritionist Pro software, version 2.2 (Axxya Systems–Nutritionist Pro , Stafford, TX). The Nutritionist Pro food database was expanded by adding analyses of traditional Greek foods and recipes [33], and nutrient information of local processed food items (mainly snack foods, sweets, and fast foods) as shared by industry. The mean nutrient intake from the 2 recalls was used for the estimation of usual nutrient intake.

2.3. Physical activity assessment

Coinciding with the dates of the dietary recalls, participants or caregivers completed a physical activity checklist recall twice for the GENDAI study [34]. This instrument queried the participants' time spent on mild, moderate, and strenuous exercise plus sedentary pursuits (such as time spent on viewing TV or playing computer/video games) during the previous 24 hours. Afterward, the mean metabolic cost of activity (MET) scores were calculated from the children's reported history of physical activity, taking into account minutes of activity as well as minutes of inactivity. In the GENESIS study, information on the young children's physical activity was obtained by the parent/guardian during the interview conducted with the assistance of a valid, structured questionnaire [35]. Emphasis was placed on activities with intensity higher than 4 METs. Typical activities for the younger age groups were playground recreational activities and taking walks with parents, whereas sports participation, such as soccer, swimming, ballet, gymnastics, etc, were more commonly reported for the older age group.

2.4. Sexual maturity assessment

In the GENDAI study, sexual maturity, according to Tanner criteria for breast, pubic hair, and genital development [36], was self-evaluated: specifically, girls rated breast and pubic hair development and boys rated genital development

and pubic hair development using a series of standardized photos in the presence of the team's pediatrician [37,38].

2.5. Anthropometry assessment

Physical measurements of body weight and height were obtained in light clothing without shoes. Body mass index was computed as weight (in kilograms)/height (in meters)^2 and was used for participants' classification as normal weight, overweight, or obese according to the cutoff points adopted by the International Obesity Task Force [39]. In addition, a soft tape measure was used to record waist circumference (in centimeters) and hip circumference (in centimeters); and the waist-to-hip ratio was calculated as an index of central adiposity. Two measurements of right side triceps and subscapular skinfolds were done with Lange skinfold calipers (Cambridge Scientific Instruments, Cambridge, MA) to obtain a mean measurement with precision of 0.2 mm.

2.6. Blood sampling

Ten milliliters of venous blood was collected after an overnight fast (≥10 hours). The samples were placed in plain tubes as well as EDTA-containing tubes as separate aliquots for serum and plasma. In GENDAI, DNA extraction was performed by the salting-out procedure [40]; for 344 subjects, DNA samples were of limited amount or quality. Thus, we present data on 794 children (420 girls and 374 boys) for which complete information and high-quality genotype data were available. In GENESIS, DNA was extracted from buccal cells [28]; in total, 1913 children (984 boys and 912 girls) were included after excluding subjects with missing phenotypic data or DNA sample.

2.7. Genotyping

Genotyping of the Pro12Ala polymorphism (rs1801282) in the GENDAI cohort was performed using the iPLEX MassARRAY platform (Sequenom, San Diego, CA) (http://www.sequenom.com/Assets/pdfs/appnotes/8876-006.pdf). In GENESIS, the genotyping was determined by polymerase chain reaction amplification and restriction fragment length polymorphism analysis [28]. The genotyping success rate was 100% in GENDAI and 93% in GENESIS.

2.8. Statistical methods

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL). The normal distribution of the investigated variables was assessed through the Kolmogorov-Smirnov criterion. In all analyses, we used log-transformed values for measures lacking normality, namely, BMI and waist in periadolescent children. None of the dependent variables of the young children were lacking normality. However, in tables, untransformed means are presented. Categorical data were provided as frequencies or proportions (percentage). Distributions of frequencies of categorical variables were analyzed by the χ^2 test of independence. Single nucleotide polymorphism association testing was performed by applying the PLINK software (http://pngu.mgh.harvard.edu/purcell/plink) under a dominant gene action (1df) model. The Pro12Ala genotype frequency was consistent with Hardy-Weinberg equilibrium. The association of genotypes with adiposity outcomes was tested using multiple linear regressions after controlling for the effects of several potential confounders including sex and minutes of sedentary activity. Interaction terms between genotypes and sex were also tested in each model; and when results were significant, the analysis was then stratified by sex. All reported P values are based on 2-sided tests.

3. Results

The overall frequency of the Ala allele in periadolescents was 8%. A similar frequency of the minor allele (7%) was observed in young children. The genotype frequencies were in Hardy-Weinberg equilibrium in each cohort. Because of the small number of Ala12Ala homozygotes, all analyses were undertaken with a dominant genetic model. The distribution of Tanner stage (percentage) (I-V) in periadolescents did not differ between Pro12Pro (9.3/33.7/39.9/14.6/2) and Ala carriers (14.1/26.6/50/9.4) ($P > .05$) either in girls or in boys with the Pro12Pro (12.5/45.4/31/8.9/1.9) and Ala genotype (14.8/44.3/34.4/6.6) ($P > .05$). There were no differences in obesity traits between the Ala and Pro genotype groups until sex-stratified analysis was completed. A gene to sex interaction was detected in young children for triceps (P for interaction = .02) and for subscapular (P for interaction = .023) skinfold thickness, whereas nominal significances were observed in periadolescents. The anthropometric and obesity measures for individual genotype groups stratified by sex are summarized in Table 1. A lower SFA fat intake was estimated for periadolescent girls with the Ala allele (13.5 ± 2.9 vs 14.8 ± 3, $P = .002$), and young girls with 2 copies of the Pro allele had lower triceps measures ($P = .04$). The opposite effect was seen in periadolescent boys, where carriers of the Ala allele exhibited lower measures of skinfolds (triceps: 16.9 ± 6.9 vs 19.4 ± 7.9, $P = .01$; subscapular: 9.6 ± 4.5 vs 11.2 ± 5.4 mm, $P = .02$).

After adjustment for age and minutes of inactivity, nominal significance was found for gene-diet interactions in periadolescent girls for SFA intake and subscapular skinfolds (P for interaction = .04) and in periadolescent boys for SFA intake and triceps skinfolds (P for interaction = .06). Furthermore, in young boys, nominal gene-diet interactions were revealed for SFA and total fat (TF) intake with waist-to-hip ratio (P for interactions = .04 and .05, respectively). Table 2 shows the results from the multiple linear regression analyses for girls, including obesity-related covariates of BMI, skinfolds (triceps, subscapular), and waist circumference (adjusted for age and minutes of sedentary activity). In periadolescent girls, there was no

Table 1
Anthropometric and adiposity outcomes stratified by the Pro12Ala genotype (data are presented as means ± SD)

	Periadolescents			Young children		
	Pro/Pro (n = 669)	Pro/Ala and Ala/Ala (n = 125)	*P* value	Pro/Pro (n = 1648)	Pro/Ala and Ala/Ala (n = 265)	*P* value
Girls	(n = 356)	(n = 64)		(n = 792)	(n = 120)	
Weight (kg)	44.2 ± 9.4	43.6 ± 10.3	.57	16.8 ± 3.5	17.4 ± 3.2	.13
BMI (kg/m^2)	19.9 ± 3.4	19.7 ± 3.9	.55	16.2 ± 1.6	16.3 ± 1.6	.38
Obesity (%)	7.8	6.7	1.00	4.4[a]	5.6[a]	.58
Skinfolds						
Triceps (mm)	19.8 ± 7.2	20 ± 8.0	.92	9.9 ± 2.8	10.5 ± 3.0	**.04**
Subscapular (mm)	11.7 ± 5.3	12.3 ± 6.1	.94	6.9 ± 2.2	7.4 ± 2.7	**.05**
Waist circumference (cm)	67.3 ± 9.1	68.9 ± 9.6	.67	51.3 ± 4.7	52.1 ± 4.6	.08
TF (% of total energy)	40 ± 6.5	38.5 ± 5.8	**.05**	40.0 ± 5.6	39.8 ± 5.6	.71
SFA (% of total energy)	14.8 ± 3	13.5 ± 2.9	**.002**	16.5 ± 3.6	16.4 ± 3.7	.86
MUFA (% of total energy)	16.2 ± 4.3	16.2 ± 4.2	.99	16.4 ± 3.3	16.3 ± 3.4	.60
PUFA (% of total energy)	4.7 ± 1.5	4.7 ± 1.6	.73	4.2 ± 1.3	4.3 ± 1.3	.49
Boys	(n = 313)	(n = 61)		(n = 842)	(n = 142)	
Weight (kg)	44.8 ± 9.4	42.9 ± 9.3	.13	17.1 ± 3.2	17.3 ± 3.2	.55
BMI (kg/m^2)	20.4 ± 3.4	19.7 ± 3.4	.11	16.3 ± 1.6	16.3 ± 1.5	.72
Obesity (%)	8.8	7.0	.80	3.5[a]	3.7[a]	.90
Skinfolds						
Triceps (mm)	19.4 ± 7.9	16.9 ± 6.9	**.01**	9.2 ± 2.6	9.0 ± 2.2	.57
Subscapular (mm)	11.2 ± 5.4	9.6 ± 4.5	**.02**	6.3 ± 1.9	6.3 ± 1.9	.06
Waist circumference (cm)	70.7 ± 9.6	68.7 ± 9.5	.10	51.2 ± 4.3	51.2 ± 3.6	.96
TF (% of total energy)	40.2 ± 7	41 ± 7	.41	39.9 ± 5.5	40.8 ± 5.0	.11
SFA (% of total energy)	14.7 ± 3.6	15 ± 3.7	.52	16.4 ± 3.7	16.6 ± 3.0	.57
MUFA (% of total energy)	16.4 ± 4.2	16.8 ± 4.4	.53	16.5 ± 3.2	17.1 ± 3.6	.08
PUFA (% of total energy)	4.9 ± 1.5	4.6 ± 1.4	.23	4.3 ± 1.1	4.2 ± 1.1	.80

[a] Only for children ≥2 years old, as there are no International Obesity Task Force obesity cutoff points for younger ages.

detectable effect of any of the investigated variables. However, in young girls homozygous for the Pro allele, higher intakes of TF or SFA were related with higher values of the obesity-related variables. There was an association between SFA intake for Pro/Pro girls with triceps skinfold (standardized β = 0.223, $P = 10^{-9}$) and with subscapular skinfold (standardized β = 0.186, $P = 10^{-6}$). A corresponding inverse correlation was found between PUFA intake and BMI within the Pro/Pro homozygotes (standardized β, P = .04). In young boys (Table 3), the associations between TF and SFA intake with skinfolds or with BMI were similar to those for young girls, with less evidence of effect (in triceps-

Table 2
Obesity-related outcomes in girls adjusted for dietary fat intake (in grams) stratified by *Pro12Ala* polymorphism

Outcome	Predictor	Periadolescents				Young children			
		Pro/Pro		Pro/Ala and Ala/Ala		Pro/Pro		Pro/Ala and Ala/Ala	
		Standardized β	*P* value	Standardized β	*P* value	Standardized β	*P* value	Standardized β	*P* value
BMI (kg/m^2)	TF	0.010	.83	0.096	.47	0.049	.21	−0.008	.94
	SFA	−0.089	.11	0.188	.16	0.096	**.01**	−0.019	.85
	MUFA	0.040	.42	0.021	.88	−0.002	.95	−0.033	.76
	PUFA	−0.069	.26	0.053	.70	−0.082	**.04**	0.056	.60
Triceps skinfold thickness (mm)	TF	0.013	.96	0.071	.60	0.159	**10^{-5}**	0.115	.26
	SFA	−0.029	.69	0.192	.18	0.223	**10^{-9}**	0.098	.34
	MUFA	0.020	.63	−0.019	.89	0.028	.47	0.081	.44
	PUFA	−0.079	.09	−0.102	.47	−0.037	.35	0.136	.20
Subscapular skinfold thickness (mm)	TF	0.023	.51	0.062	.65	0.150	**10^{-4}**	0.134	.20
	SFA	−0.038	.59	0.278	.06	0.186	**10^{-6}**	0.190	.07
	MUFA	0.043	.45	−0.034	.80	0.038	.34	0.062	.56
	PUFA	−0.068	.52	−0.078	.61	−0.002	.95	0.001	.99
Waist circumference (cm)	TF	0.047	.39	0.042	.75	0.033	.33	−0.006	.99
	SFA	−0.078	.15	0.203	.13	0.079	**.02**	−0.016	.86
	MUFA	−0.096	.07	−0.044	.75	−0.014	.69	0.001	.99
	PUFA	−0.058	.56	−0.100	.60	−0.051	.14	0.082	.39

Multivariate linear regression models were adjusted for potential confounders: age and minutes of sedentary activities.

Table 3
Obesity-related outcomes for boys adjusted for dietary fat intake (in grams) stratified by *Pro12Ala* polymorphism

Outcome	Predictor	Periadolescents				Young children			
		Pro/Pro		Pro/Ala and Ala/Ala		Pro/Pro		Pro/Ala and Ala/Ala	
		Standardized β	*P* value	Standardized β	*P* value	Standardized β	*P* value	Standardized β	*P* value
BMI (kg/m^2)	TF	−0.081	.17	−0.024	.87	0.090	**.02**	0.006	.95
	SFA	0.03	.62	0.110	.45	0.062	.09	0.003	.97
	MUFA	−0.128	**.03**	−0.048	.75	0.036	.07	0.018	.85
	PUFA	−0.095	.08	−0.128	.37	0.034	.36	0.033	.73
Triceps skinfold thickness (mm)	TF	−0.078	.19	0.095	.51	0.080	**.04**	0.100	.31
	SFA	0.017	.54	0.287	**.05**	0.093	**.01**	0.215	**.02**
	MUFA	−0.135	**.02**	0.002	.99	0.036	.34	−0.021	.83
	PUFA	−0.048	.40	−0.176	.22	−0.009	.80	0.029	.76
Subscapular skinfold thickness (mm)	TF	0.025	.67	0.072	.67	0.051	.18	0.101	.29
	SFA	0.119	**.04**	0.125	.40	0.067	.07	0.189	**.04**
	MUFA	−0.044	.41	0.075	.62	0.012	.76	−0.023	.81
	PUFA	−0.059	.30	−0.126	.13	−0.010	.79	0.026	.78
Waist circumference (cm)	TF	−0.070	.23	0.057	.69	0.040	.24	−0.074	.40
	SFA	0.027	.64	0.106	.46	0.032	.34	−0.021	.81
	MUFA	−0.125	**.03**	0.094	.53	0.052	.12	−0.049	.58
	PUFA	−0.022	.62	−0.072	.62	0.034	.32	−0.069	.43

The multivariate linear regression models were adjusted for potential confounders: age and minutes of sedentary activities.

TF: standardized β = 0.080, P = .04; in triceps-SFA: standardized β = 0.093, P = .01; and in BMI-TF: standardized β = 0.090, P = .016). In periadolescent boys homozygous for the Pro allele, a higher MUFA intake was associated with lower values of BMI (standardized β = −0.128, P = .03), triceps skinfold thickness (standardized β = −0.135, P = .02), and waist circumference (standardized β = −0.125, P = .03).

The finding of TF and SFA with skinfolds in younger children motivated further investigation of the seemingly increased risk effect for carriers of the Pro allele. This was done by stratification by age groups in the young boys and girls (Table 4). The results suggest that the obesogenic effect of the fat intake is detectable in girls until the age of 48 months. On the other hand, in boys, it seems that the obesogenic effect begins at the age of 48 months. However, the SFA-gene interaction in boys is significant only at an earlier stage (24-36 months) (Table 4).

4. Discussion

Our study extends previous findings in children [28] and further shows that the age- and sex-specific association of the Pro12Ala polymorphism with adiposity is modified by fat intake. The use of 2 Greek cohorts, including both young children and periadolescents, allows for the detection of effects in broader age range. Associations between *PPARγ* variants and anthropometric indices were identified in either cohort only when considering each sex separately. Periadolescent boys with the Ala allele had lower adiposity measures compared with those with the Pro allele, with lower body fatness, smaller skinfolds, and smaller waist circumference. A seemingly opposite effect was seen in the young children, as young girls with the Pro allele presented lower values of triceps skinfold thickness. In addition, in periadolescent girls, Ala carriers had a significantly lower SFA intake. It has been previously

Table 4
Gene-diet modification in Pro/Pro homozygotes by age group in young children from the GENESIS cohort

Outcome	Age groups (mo)	Girls				Age groups (mo)	Boys			
		TF		SFA			TF		SFA	
		Standardized β	*P* value	Standardized β	*P* value		Standardized β	*P* value	Standardized β	*P* value
Triceps skinfold thickness (mm)	12-24 (n = 59)	0.306	**.03**	0.416	**.003**	12-24 (n = 69)	−0.170	.19	−0.076	.57
	24-36 (n = 150)	0.271	**.001**	0.297	**10^{-4}**	24-36 (n = 173)	0.113	.16	0.224	**.005**
	36-48 (n = 297)	0.153	**.01**	0.249	**10^{-4}**	36-48 (n = 334)	0.059	.31	0.104	.08
	48-60 (n = 254)	0.078	.250	0.127	.06	48-60 (n = 234)	0.168	**.02**	0.033	.63
Subscapular skinfold thickness (mm)	12-24 (n = 59)	0.291	**.05**	0.340	**.02**	12-24 (n = 69)	−0.186	.15	−0.105	.43
	24-36 (n = 150)	0.248	**.004**	0.270	**.001**	24-36 (n = 173)	0.013	.87	0.179	**.03**
	36-48 (n = 297)	0.162	**.009**	0.186	**.003**	36-48 (n = 334)	0.049	.40	0.092	.12
	48-60 (n = 254)	0.059	.39	0.116	.09	48-60 (n=234)	0.168	**.02**	0.013	.85

All models were adjusted for minutes of sedentary activities.

shown in type 2 diabetes mellitus patients that carriers of the Ala allele had a lower energy intake per kilogram body weight, thus supporting that the Ala allele was associated with higher food efficiency [20].

The Pro12Ala polymorphism has been reported to be associated with lower BMI and improved insulin sensitivity in adults [41], as well as with lower fasting blood insulin levels and lower homeostasis model assessment of insulin resistance index in Italian obese children [26]. Recent results from an 11- to 24-year-old cohort from Mexico (1210 students) indicated no association between the polymorphism and BMI [27]. In the present study, the screening analysis using BMI in each cohort did not reveal any significant association with the genotype in either age group. Stratification by sex suggested an association with adiposity in periadolescent boys and motivated further investigation on the possible risk effect of the Pro allele. Because previous reports had shown that homozygous Pro/Pro individuals in the highest quintile of TF intake had significantly higher mean BMI compared with those in the lowest quintile [24], we stratified our analysis by the presence of the Ala allele. Adiposity measures in periadolescent boys with the Pro allele varied by subtype of dietary fat intake, revealing fat intake as a modifying effect. Periadolescent boys homozygous for the Pro allele who had reported consuming higher amounts of MUFA had a lower BMI, waist circumference, and triceps skinfolds compared with those with lower intakes; yet this MUFA-modified effect was not observed in girls. Similarly, the young girls homozygous for the Pro allele who had reported consuming higher amounts of PUFA had lower BMI, in contrast to those with higher intakes of TF or SFA who had higher values across all adiposity measures. These findings are consistent with the report by Luan et al [21] who found that a low dietary PUFA/SFA ratio was associated with higher BMI in Ala carriers compared with Pro homozygotes, whereas a high PUFA/SFA ratio showed the opposite effect.

Data collection in the 2 pediatric cohorts was performed using similar assessment methods. Genotyping was done with different methods, a fact that might introduce a bias; but quality control measures ensured accuracy of genotyping, and the allele frequencies were consistent with Hardy-Weinberg equilibrium. The cross-sectional design of both studies may limit the potential to reveal causal relationships; therefore, the collection of representative and longitudinal information from large cohorts is necessary to further explore the impact of key gene-diet interactions in growth of boys and girls.

The detection of these gene-nutrient effect modifications, varying with age, emphasizes the importance of examining the effect of common polymorphisms with appropriate environmental exposure data, which may account for the heterogeneity of findings in previous studies. In support of the genotype-diet interaction of the Pro12Ala polymorphism, it has been recently shown that high fish intake was associated with low fasting serum free FA only in male Pro12Pro genotype carriers, whereas no association was seen in the Ala allele carriers [42].

In conclusion, when taking into account the dietary fat intake, it seems that the Pro allele homozygotes are at higher risk for increased adiposity; and this is expressed even at a very young age as evidenced by the findings in the young children. These results suggest that there is an age-dependent gene-diet effect modification and that the effect may pivot at the age of 48 months when the gene-diet effect is differentiated in the 2 sexes. In accordance with reports on MUFA and PUFA interaction with variation in PPARγ in adults, our findings provide the first evidence of this interaction in children.

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