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Postprandial plasma adiponectin response is reduced in prepubertal premature pubarche girls

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Abstract

The association between premature pubarche (PP) and metabolic syndrome is controversial and not supported by some authors. The aim of this study was to determine insulin resistance syndrome, plasma adiponectin, and fatty acid profile in PP girls to discern potential confounder variables and markers of metabolic disturbances. We studied 22 prepubertal girls with a diagnosis of PP and 20 healthy controls who differed in body mass index (BMI) (19.33 ± 0.71 vs 17.30 ± 0.60). We evaluated insulin resistance syndrome components and postprandial response of adiponectin, nonesterified fatty acids, and fatty acid profile after consumption of a standardized breakfast. No lipid disturbances were detected in the PP group. High-density lipoprotein to low-density lipoprotein cholesterol ratio tended to be lower in PP girls (P = .052), but this effect disappeared when data were adjusted for both BMI and age (P = .480). Insulin levels tended to be higher at 2 hours in PP girls, who showed significantly higher C-peptide area under the curve. In contrast, adiponectin at 3 hours after the meal and postprandial adiponectin area under the curve were significantly lower. The PP girls showed significantly higher percentages of eicosapentaenoic acid in total plasma and plasma phospholipids. No differences were found in the postprandial fatty acid clearance rate. In conclusion, PP girls and controls differed in postprandial plasma adiponectin response and in postprandial plasma C-peptide response after both BMI and age adjustment. Cholesterol plasma disturbances were mainly attributable to their higher BMI, although n-3 polyunsaturated fatty acids were higher because of the PP.

1. Introduction

Premature pubarche (PP) is defined as the development of pubic hair before the age of 8 years in girls and 9 years in boys [1]. It is rarely observed in children under the age of 2 years old, when it probably has a different etiology than at later ages [2]. It can be associated with slightly higher dehydroepiandrosterone sulfate levels and some signs of androgen function (eg, acne and sweating), but the mechanisms underlying the initiation of adrenal androgen secretion at adrenarche are still not well understood.

Although PP has been considered a benign normal variant of puberty, some authors have described it as an early clinical feature of the metabolic syndrome (obesity, hypertension, dyslipidemia, and insulin resistance) in some girls [3,4]. Premature pubarche, the main clinical manifestation of premature adrenarche (PA), has been associated with insulin resistance and dyslipidemia, especially when the child has both intrauterine growth restriction and postnatal catch-up growth [5,6]. Young girls with PP are also at increased risk of developing polycystic ovary syndrome because insulin resistance is a key factor in the pathogenesis of this sequence [7]. It has been claimed that many effects reported in lean PP girls disappear when the data are adjusted for height [8] or for body mass index (BMI) [9], suggesting that the disturbances observed might be mainly related to the overweight condition of the subjects.

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Obesity is a common feature of prepubertal and pubertal girls with a history of PP [10,11]. Girls with PP have hyperandrogenism, with higher levels of serum dehydroepiandrosterone sulfate and androstenedione; and girls with obesity also show a greater degree of hyperandrogenism with insulin resistance [12]. It has been postulated that insulin and insulin-like growth factor–1 might stimulate ovarian and adrenal steroidogenesis and that a dysregulation in these systems could cause PP and, in puberty, a polycystic ovary syndrome [13]. Although data have been published on alterations in metabolic syndrome components in PP girls, it must be established whether these results are independent of the girls' weight and whether they are related to fat distribution and adipocytokine alterations associated with the metabolic syndrome in obesity.

The aim of the present study was to investigate metabolic abnormalities associated with insulin resistance syndrome in PP and control girls and to identify potential markers specifically linking these alterations to PP.

2. Patients and methods

Twenty-two prepubertal girls with PP and 20 healthy age-matched controls were selected from the Pediatric Endocrinology Unit of the Hospital Reina Sophia of Córdoba (Spain). The minimum sample size to detect an effect due to PP (type I error $\alpha = 0.05$ and type II error $\beta = 0.2$; power, 80%) was estimated to be 20 children per group based on a difference of at least 20% between groups in mean values of main outcome variables (main plasma fatty acids; oleic and linoleic acid) and on variances previously obtained in fatty acid analyses of children's plasma measured by gas-liquid chromatography [14], and from adiponectin levels in the blood of prepubertal lean Spanish children [6].

Inclusion criteria were absence of disease as verified by clinical examination and classification as prepubertal (Tanner I) based on Tanner criteria and the presence of pubic hair before the age of 8 years. Participants were 6-12 years old (control subjects, 6.7-12 years old; PP, 5.7-10.5 year old). We measured fasting gonadotropins and sex hormones (follicle stimulant hormone [FSH], lutein hormone [LH], estradiol, and testosterone) to validate that selected children classified as prepubertal by clinical signs were truly at this stage. Control children were selected within those who consulted to exclude potential endocrinologic disorders. Exclusion criteria were presence of pubertal development, disease, or malnutrition; being on a restrictive diet; use of medication that alters blood pressure or glucose or lipid metabolism; and history in the past year of a long rest period or consumption of hypocaloric diet. Girls were classified as normal weight if their BMI was between the 25th and the 75th percentile. Girls with low height or low weight or any pathology were excluded from the normal group. Four PP girls and one control girl were obese, with a BMI that exceeded the 97th percentile for their age and sex according to Spanish standards (BMI *z* score ≥2.0). Patient characteristics are shown in Table 1. The study was approved by the Ethics Committee of the hospital, and all parents signed an informed consent form before their child's participation in the study.

Table 1
Clinical and metabolic parameters in control and PP girls at prepubertal age

	Control (n = 20)	PP (n = 22)	P^a
Age (y)	9.12 ± 0.37	8.34 ± 0.29	.107
Height (cm)*	127.4 ± 2.1	131.5 ± 1.6	.135
Weight (kg)*	28.40 ± 1.50	33.59 ± 1.57	.027
BMI (kg/m^2)	17.30 ± 0.60	19.33 ± 0.71	.043
BMI *z* score	-0.52 ± 0.30	0.45 ± 0.31	.031
Estradiol (ng/L)	22.60 ± 2.43	24.85 ± 3.32	.594
Testosterone (ng/L)*	0.30 ± 0.06	0.53 ± 0.04	.002
LH (U/L)	0.17 ± 0.07	0.33 ± 0.12	.238
FSH (U/L)	2.07 ± 0.30	2.21 ± 0.37	.765
Total cholesterol (mg/dL)	162.7 ± 5.83	171.7 ± 6.52	.313
LDL-c (mg/dL)	86.53 ± 4.47	99.06 ± 4.42	.061
HDL-c (mg/dL)	63.50 ± 3.61	61.38 ± 3.24	.672
LDL-c/HDL-c ratio	1.45 ± 0.13	1.64 ± 0.10	.052
Triglycerides (mg/dL)	65.55 ± 9.44	77.81 ± 9.91	.377
Uric acid (mg/dL)	3.80 ± 0.21	4.20 ± 0.24	.213
Fasting NEFA (mmol/L)	0.53 ± 0.04	0.53 ± 0.05	.953
NEFA 1 h (mmol/L)	0.22 ± 0.05	0.17 ± 0.03	.364
NEFA 2 h (mmol/L)	0.23 ± 0.04	0.17 ± 0.03	.264
NEFA 3 h (mmol/L)*	0.31 ± 0.06	0.18 ± 0.03	.046
AUC NEFA (mmol/L)	0.87 ± 0.12	0.67 ± 0.09	.180

Mean ± SEM.

[a] *P* values by Student *t* test.

* $P < .05$ by univariate linear model adjusted for both BMI *z* score and age (weight, $P = .006$; height, $P = .016$; testosterone, $P = .002$; NEFA 3 hours, $P = .038$).

2.1. Sampling

Baseline blood samples were obtained from children during fasting, using an indwelling venous line, to measure levels of glucose; insulin; adiponectin; sex hormones FSH, LH, estradiol, and testosterone to confirm prepubertal clinical stage; lipids; total fatty acids; and fatty acid profile in lipid fractions.

Blood samples were also taken postprandially at 1, 2, and 3 hours after a standardized breakfast to determine total plasma fatty acids, nonesterified fatty acids (NEFA), and adiponectin response. Glucose, insulin, and C-peptide were measured during fasting and at 2 hours after breakfast. The composition of the breakfast was described by Larqué et al [15]. All samples were processed within 2 hours of sampling and divided into aliquots for immediate analysis or long-term storage at −80°C until their analysis.

2.2. Breakfast composition for postprandial study

Both control and PP received a standardized breakfast at 9:00 AM. The composition of the meal was based on the typical breakfast for children in Spain containing 200 mL milk, 10 g sugar, 18 g cocoa, 10 g butter, 30 g toasted bread, and 20 g jam; this typical breakfast provided 259.2 kcal

259.2 kcal (1084.5 kJ) from carbohydrates (59% of the breakfast energy), 39.2 kcal (163.9 kJ) from proteins (9% of energy), and 139.5 kcal (583.1 kJ) from lipids (32% of the energy) [15].

2.3. Biochemical analysis

Glucose was analyzed by the glucose oxidase method using an automatic analyzer (coefficient of variance [CV], 1%) (Roche-Hitachi Modular PyD Autoanalyzer; Roche Laboratory Systems, Mannheim, Germany). Plasma insulin (CV, 2.6%) and C-peptide were measured by radioimmunoassay using an automatic analyzer for microparticles (Axsym; Abbott Laboratories, Chicago, IL). Plasma levels were determined during fasting and at 2 hours after the breakfast. Insulin resistance and tissue insulin sensitivity were calculated by using the homeostasis model assessment (HOMA) index, as defined by the equation HOMA = [fasting glucose (in millimoles per liter) × fasting insulin (in microunits per milliliter)/22.5] [16], and the quantitative insulin sensitivity check index (QUICKI), as defined by the equation QUICKI = 1/(log fasting insulin + log fasting glucose) [17]. Sex hormones (FSH: CV, 3.6%; LH: CV, 3.1%; testosterone: CV, 2%; estradiol: CV, 1.8%) were measured by chemiluminescence using an automatic analyzer (Architect I4000, Abbott Laboratories). Plasma TG (CV, 1.5%), high-density lipoprotein cholesterol (HDL-c) (CV, 0.8%), low-density lipoprotein cholesterol (LDL-c), and uric acid were measured by means of an automatic analyzer (Roche-Hitachi Modular PyD Autoanalyzer, Roche Laboratory Systems). Nonesterified fatty acids were analyzed by spectrophotometry using a commercial kit (Randox, Barcelona, Spain). Plasma adiponectin (CV, 4.4%) was measured by radioimmunoassay (Human Adiponectin RIA Kit, catalog no. HADP-61HK; Linco Research, St Charles, MO).

2.4. Quantitation of fatty acids in total plasma lipids and plasma lipid fractions

For analysis of the total plasma fatty acid profile, 0.1 mL of plasma was extracted into hexane/isopropanol (4:1) after addition of 250 μL of 0.2 g/L tridecanoyl acid (13:0) as internal standard. After centrifugation for 10 minutes (4°C) at 1500*g*, the organic layer was separated and evaporated to dryness under nitrogen. Synthesis of fatty acid methyl esters (FAME) from total plasma lipids was performed with 3 mol/L methanolic HCl (Supelco, Bellafonte, PA) at 85°C for 45 minutes; derivatives were extracted into hexane and stored at −20°C until gas chromatographic analysis [15].

The fatty acids from plasma lipid fractions were extracted from 0.5 mL of plasma using 100 μL of internal standard (0.625 g/L tripentadecanoyl glycerol [TG 15:0], 0.857 g/L diheptadecanoyl-phosphatidylcholine [PL 17:0], 1.0 g/L of heptadecanoyl cholesterol [CE 17:0], and 0.05 g/L of pentadecanoic acid [15:0]) [18]. Four milliliters of hexane/2-propanol (3:2) with 25 mg/L of butylated hydroxytoluene (BHT) was added and centrifuged for 10 minutes (4°C) at 1500*g*. The organic layer was evaporated to dryness under vacuum. The isolated lipids were dissolved into 200 μL hexane/methyl *tert*-butyl ether/acetic acid (100:3:0.3 vol/vol/vol) for isolation of each lipid fraction using aminopropyl columns (SepPak Cartridges; Waters, Milford, MA). Fractions obtained from the columns were evaporated to dryness under vacuum, and 100 μL hexane was added to each tube. Fatty acid methyl ester from each plasma lipid fraction was formed as previously reported [18]. Briefly, hexane extracts were dissolved into 2 mL of methanol/benzene (4:1, vol/vol); and BHT (9 μmol/L) was added to samples as antioxidant. Two hundred microliters of acetyl chloride was slowly added, and tubes were then closed and subjected to methanolysis at 100°C for 1 hour. After tubes were cooled in water, 5 mL of 0.43-mol/L K_2CO_3 solution was slowly added to stop the reaction and neutralize the mixture. Tubes were then shaken and centrifuged, and the benzene upper phase was removed and transferred to another glass tube to be dried under nitrogen and resuspended to 100 μL with hexane until gas chromatographic analyses.

Quantitation of FAME obtained from total plasma lipids or each lipid fraction was performed by gas-liquid chromatography using a Hewlett-Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) as previously described [18]. Peaks were identified by comparison of their retention times with appropriate FAME standards purchased from Sigma Chemical, Urbana, IL.

2.5. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Variables that did not follow a normal distribution (TG, insulin, HOMA, and fatty acid percentages) were log-transformed before analysis. Sociodemographic and clinical variables were compared both by unpaired 2-tailed Student *t* test and by univariate linear model adjusted for both BMI *z* score and age. Linear correlations were performed by Pearson test. Multivariable logistic regression models were performed to identify parameters significantly related to the likelihood of PP. Associations of insulin resistance syndrome were analyzed by multivariable linear regression models. A backward stepwise method was used to identify the independent variables responsible for adiponectin levels. Mean differences among different postprandial times and specific variables were evaluated by means of 2-way analysis of variance with post hoc comparisons using Bonferroni tests. $P < .05$ was considered significant. SPSS version 13.0 (SPSS, Chicago, IL) was used for the data analyses.

3. Results

Prepubertal girls diagnosed with PP had significantly higher weight and BMI vs controls (Table 1). Because these variables can influence the results for PP effects, the clinical and metabolic data of participants were adjusted for BMI *z* score and even for age. As expected, PP group had

significantly higher testosterone levels (hyperandrogenism) vs controls; and this difference remained significant when data were adjusted for both BMI *z* score and age ($P = .002$). In addition, a tendency for the LDL-c/HDL-c ratio to be higher in the PP group ($P = .052$) disappeared when the data were adjusted for BMI *z* score ($P = .480$), indicating that it was mainly influenced by the BMI (Table 1). Both TG and NEFA were similar between the groups, detecting lower NEFA levels in the PP group only at 3 hours after consumption of the standardized breakfast (Table 1).

No differences were found between groups in fasting insulin and glucose levels (Fig. 1) or in HOMA (1.86 ± 0.32 control vs 1.88 ± 0.09 PP) and QUICKI (0.36 ± 0.01 control vs 0.36 ± 0.01 PP). C-peptide levels tended to be higher in the PP group at 2 hours ($P = .088$) after the breakfast (Fig. 1); and its area under the curve (AUC) was significantly higher in the PP group, even after adjusting for both BMI *z* score and age in a general linear model ($P = .047$, $R^2 = 0.410$), indicating that almost 41% of the C-Peptide response was associated to the PP condition (Fig. 1). Adiponectin, which modulates insulin action, was significantly lower in PP girls (Fig. 2). Adiponectin during fasting ($P = .027$) as well as at each postprandial hour and AUC ($P = .02$) were all significantly lower in the PP group by *t* test. However, when the data were adjusted for the BMI *z* score and age, adiponectin at 1 hour ($P = .008$) and adiponectin AUC remained significant ($P = .044$, $R^2 = 0.252$), with adiponectin at 3 hours showing also a trend toward lower values in PP girls ($P = .080$) (Fig. 2). Using Pearson correlations, adiponectin was inversely correlated with insulin AUC ($R = -0.362$, $P = .022$) and C-peptide AUC ($R = -0.348$, $P = .037$); but these correlations absolutely disappeared after adjustment of the data to both age and BMI *z* score. A significant multiple regression model was constructed with adiponectin AUC as dependent variable and NEFA AUC, C-peptide AUC, and uric acid as independent variables ($P = .027$, $r = 0.502$). Using a backward stepwise model, only uric acid remained independently associated with adiponectin AUC in these subjects ($P = .022$, $r = 0.469$, odds ratio = −0.376 [−24.42, −2.048]).

Regarding the fasting plasma fatty acid profile, percentages of n-3 polyunsaturated fatty acids (PUFA) were significantly higher in PP girls (Table 2). This difference was mainly due to the higher proportions of 20:5 n-3 eicosapentaenoic acid (EPA) in their total plasma (Table 2) and plasma phospholipids (0.31% ± 0.1% in PP vs 0.03% ± 0.02% in controls, $P = .030$). 22:6 n-3 docosahexaenoic acid (DHA) was also significantly higher in PP girls than in controls. The proportion of EPA was positively correlated with estradiol levels ($r = 0.545$, $P = .003$) but not with testosterone levels ($r = 0.203$, $P = .309$). The postprandial response of fatty acids did not differ between PP and controls, and the differences were mostly in the timing of the fatty acid plasma peak (Fig. 3). However, EPA AUC was significantly higher in PP girls than in controls (5.41 ± 1.07 mg/dL vs 2.64 ± 0.19 mg/dL, $P = .046$).

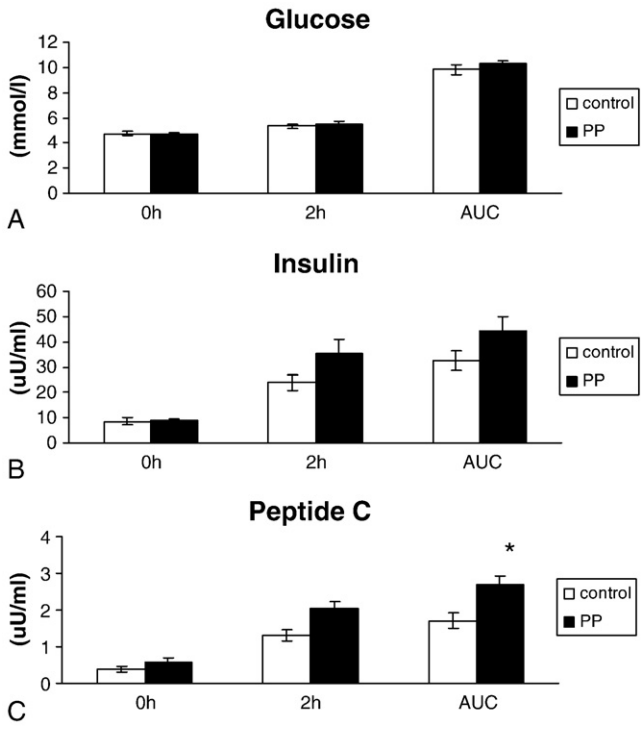

Fig. 1. Plasma glucose (A), insulin (B), and peptide C (C) responses 2 hours after intake of a standardized breakfast in control (n = 20) and prepubertal PP (n = 22) girls. Univariate linear model adjusted for both BMI *z* score and age. Values with different superscripts are significantly different ($P < .05$). Results are expressed as mean ± SEM.

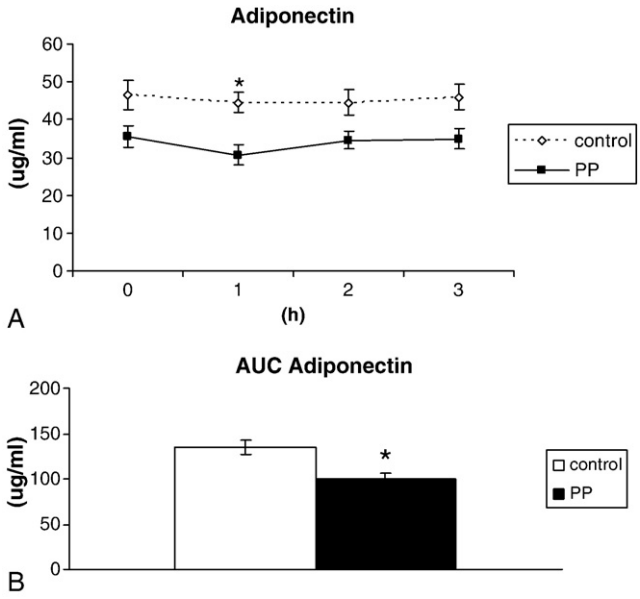

Fig. 2. A, Postprandial response of plasma adiponectin in control and PP girls after receiving a standardized breakfast. B, Adiponectin AUC in this postprandial test. Univariate linear model adjusted for both BMI *z* score and age. Values with different superscripts are significantly different ($P < .05$). Results are expressed as mean ± SEM.

Table 2
Fasting fatty acid composition (% wt/wt) in total plasma of control and PP girls

Fatty acids	Control (n = 20)	PP (n = 22)	P^a
12:0	0.11 ± 0.03	0.14 ± 0.02	.365
14:0	0.87 ± 0.12	1.11 ± 0.09	.207
15:0*	0.19 ± 0.01	0.22 ± 0.01	.064
16:0	22.63 ± 0.29	22.89 ± 0.44	.617
17:0	0.25 ± 0.01	0.28 ± 0.01	.055
18:0	8.96 ± 0.01	9.23 ± 0.25	.224
20:0	0.22 ± 0.01	0.21 ± 0.01	.500
22:0	0.79 ± 0.09	0.60 ± 0.07	.136
23:0	0.31 ± 0.04	0.28 ± 0.03	.435
24:0	0.62 ± 0.04	0.55 ± 0.04	.289
16:1 n-9	0.32 ± 0.02	0.34 ± 0.02	.755
16:1 n-7	1.10 ± 0.05	1.27 ± 0.09	.749
16:1 *trans*	0.08 ± 0.01	0.12 ± 0.01	.068
17:1 n-7	0.13 ± 0.02	0.09 ± 0.02	.192
18:1 *trans*	0.42 ± 0.09	0.57 ± 0.13	.402
18:1 n-9	17.00 ± 0.62	17.72 ± 0.48	.310
18:1 n-7	1.19 ± 0.08	1.26 ± 0.05	.889
18:1 *cis*	0.05 ± 0.01	0.07 ± 0.02	.417
20:1 n-9	0.23 ± 0.03	0.25 ± 0.04	.937
22:1 n-9	0.10 ± 0.04	0.20 ± 0.08	.406
24:1 n-9	0.70 ± 0.07	0.59 ± 0.08	.367
18:2 n-6*	30.66 ± 0.58	28.97 ± 0.52	.222
18:2 *trans**	0.24 ± 0.02	0.19 ± 0.02	.093
20:2 n-6	0.17 ± 0.01	0.25 ± 0.04	.194
18:3 n-6	0.29 ± 0.04	0.33 ± 0.08	.963
18:3 n-3	0.17 ± 0.01	0.28 ± 0.05	.166
20:3 n-6	1.16 ± 0.09	1.43 ± 0.10	.165
18:4 n-3	0.14 ± 0.02	0.16 ± 0.02	.284
20:4 n-6	7.19 ± 0.36	7.17 ± 0.45	.795
22:4 n-6	0.37 ± 0.07	0.41 ± 0.07	.759
20:5 n-3*	0.27 ± 0.04	0.40 ± 0.04	.041
22:5 n-6	0.23 ± 0.02	0.21 ± 0.03	.621
22:5 n-3	0.27 ± 0.02	0.30 ± 0.02	.123
22:6 n-3	1.66 ± 0.13	1.90 ± 0.12	.044
SFA	34.96 ± 0.37	35.52 ± 0.58	.349
MUFA	21.64 ± 0.73	21.65 ± 0.61	.380
PUFA	42.58 ± 0.70	41.81 ± 0.80	.896
trans FA	0.74 ± 0.10	0.88 ± 0.12	.418
PUFA n-6	40.06 ± 0.63	38.77 ± 0.73	.434
n-3 PUFA*	2.51 ± 0.15	3.04 ± 0.15	.006

Mean ± SEM. SFA indicates saturated fatty acids; MUFA, monounsaturated fatty acids.

[a] P values by univariate linear model adjusted for both BMI z score and age.

* $P < .05$ by Student t test.

4. Discussion

Lipid metabolic disturbances detected in this series of PP girls were mainly attributable to their higher BMI. Some signs of insulin resistance were observed, and both age- and BMI-adjusted results showed higher C-peptide levels after a standardized breakfast in the PP vs control group. We present the first report of a lower adiponectin response due to PP that was independently associated with uric acid levels. Higher plasma EPA levels detected in the PP girls might be important for the further development of lipid metabolism in these children.

The plasma LDL/HDL ratio showed a trend to higher values in the PP girls; but this ratio was strongly affected by BMI, and this trend disappeared when the data were adjusted for BMI. This result is in agreement with findings in girls with biochemical PA (with or without PP), who showed similar BMI-adjusted blood pressure and lipid values to those of normal controls [9]. Güven et al [19] reported an increased LDL/HDL ratio and altered blood pressure in PA children, but they did not take account of the significantly higher BMI of the children. However, Ibañez et al [5] found higher TG levels and LDL/HDL ratios in PP girls than in BMI-matched controls, which they attributed to their hyperinsulinemia [20].

In the present study, the insulin response tended to be higher in PP children than in controls; and the difference was significant for the C-peptide AUC. Utrianen et al [9] reported a significantly higher BMI-adjusted serum insulin concentration in PP children, confirming the association between PP and insulin resistance. Premature pubarche has also been associated with higher insulin-like growth factor–1 concentrations, which in turn have been related to a polymorphism in children with PP [13]. Insulin resistance might enhance hepatic very low-density lipoprotein synthesis, which could contribute to the increased TG and LDL-c values. However, our PP series did not show hyperinsulinemia; and the differences in insulin response were not sufficient to disturb the lipid profile. The relatively small sample size of this study could be a limitation on these outcomes. Nevertheless, the C-peptide response emerged as a more sensitive marker than insulin response to evaluate glucose disturbances in these children. C-peptide could generally be a better parameter for detecting hyperinsulinemia after a standardized meal.

Plasma adiponectin is strongly associated with obesity, and insulin sensitivity has been negatively correlated with BMI and fat content [21]. The present study contributes the first description of a significant and BMI-independent decrease in adiponectin response in PP children. Serum androgen levels have been negatively associated with plasma adiponectin during male puberty development [22,23], and hyperandrogenism might explain the reduced adiponectin levels in our PP girls. When especially hyperandrogenic women gain weight, excess mass tends to localize intraabdominally, which could explain the results; however, we did not measure waist circumference in this study. Lower adiponectin levels have also been reported in women with polycystic ovarian syndrome, who have increased levels and bioavailability of testosterone [24]. It has been reported that pubertal stage was a stronger predictor of adiponectin levels in lean boys compared with BMI [23].

Adiponectin and BMI together have been reported to explain 73% of the variance in insulin sensitivity among adolescents [25]. In the present study, adiponectin was inversely correlated with insulin AUC and C-peptide AUC, pointing toward its insulin-sensitizing role; but these correlations absolutely disappeared after adjustment of the

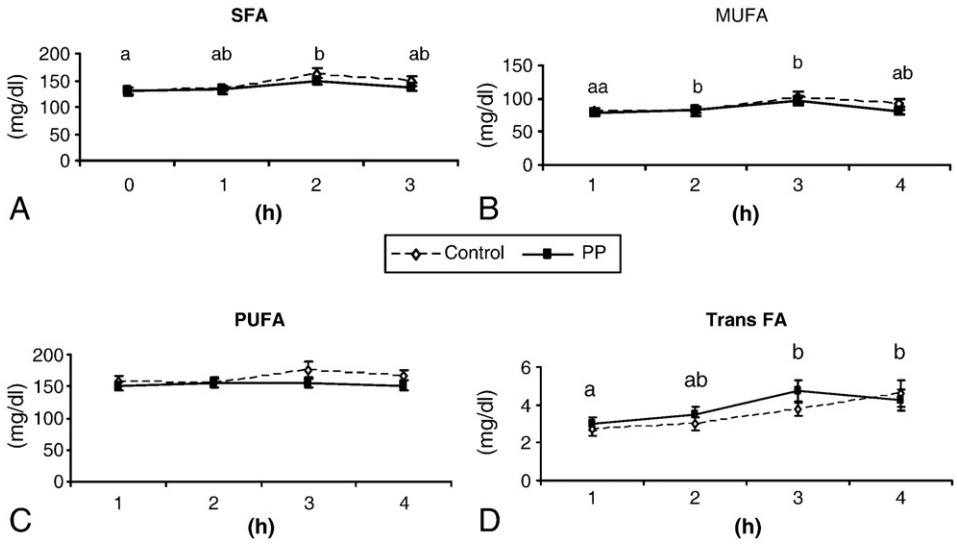

Fig. 3. Postprandial clearance of plasma saturated fatty acids (A), monounsaturated fatty acids (B), PUFA (C), and *trans* fatty acid concentration (D) in control and PP prepubertal girls after receiving a standardized breakfast. Results are expressed as mean ± SEM. Analysis of variance general linear model for repeated measurements was used to evaluate the 2 sources of variation in the study (pubarche and postprandial time) followed by post hoc Bonferroni multiple *t* test. Different letters indicate significant differences as a function of time ($P < .05$).

data to both age and BMI *z* score. It is possible that insulin resistance could be a main determinant of reduced adiponectin levels in PP children with higher insulinemia than in our series. We constructed a model with C-peptide AUC, NEFA AUC, and uric acid as independent variables of adiponectin response in these children. When C-peptide and NEFA were eliminated following a backward stepwise model, only uric acid remained significantly associated with adiponectin levels.

Uric acid concentrations were only slightly higher in the PP girls and at levels that were far from hyperuricemic. Nevertheless, uric acid was the most significant independent predictor of adiponectin level. Hyperuricemia is considered by some investigators to be a metabolic syndrome component that reflects insulin resistance [26,27], and some epidemiologic research has demonstrated a positive relationship between serum uric acid levels and the prevalence of metabolic syndrome [28,29]. It has been postulated that elevated uric acid may not only result from insulin resistance but also promote or exacerbate it [30]. Hyperuricemia has been also linked to visceral fat accumulation and hypoadiponectinemia by several authors [31,32]. However, it is the first time that this association between adiponectin and uric acid in PP girls is reported, even when our subjects did not present hyperuricemia (>7 mg/dL serum uric acid) or hyperinsulinemia. Another mechanism involved could be an effect acting via increased oxidative stress and tissue hypoxia, known in turn to lead to the release of purine intermediates and the accumulation of uric acid.

We also report the first analysis of the fatty acid profile and postprandial fatty acid response in PP children, demonstrating significantly higher percentages of n-3 PUFA (EPA and DHA) in PP girls than in controls. This finding was confirmed by analysis of the fatty acid profile in lipid fractions. This difference was not related to different fish consumption of the participants, which was recorded by calling them and asking for their fish consumption per week (EPA intake, 0.17 ± 0.07 g/d in controls vs 0.23 ± 0.06 g/d in PP; DHA, 0.48 ± 0.10 g/d in controls vs 0.42 ± 0.11 g/d in PP girls). Furthermore, the proportion of EPA was positively correlated with estradiol levels but not with DHA levels. Human and animal studies have reported that women have a greater ability to synthesize EPA and DHA from α-linolenic acid compared with their male counterparts [33-35]. Plasma and tissue concentrations of n-3 LC-PUFA were positively associated with circulating concentrations of estradiol and progesterone in rats [35]. In transsexual subjects under hormone replacement therapy, estrogens produced higher DHA concentrations in women than in men [36]. Although plasma estradiol levels did not significantly differ between our groups, the PP girls showed a trend to higher values. Plasma estradiol rather than testosterone would have been largely responsible for the effect on plasma EPA levels in the PP girls. An increase in plasma EPA may prevent further metabolic disturbances associated with PP because of its hypotriglyceridemic and anti-inflammatory properties.

We and other authors have reported positive associations of adiponectin plasma levels with n-3 fatty acids [37,38]. It is important to mention that, in our study, plasma DHA percentage was positively correlated with plasma adiponectin levels at 3 hours ($P = .348$, $P = .047$) and inversely correlated with plasma uric acid levels ($P = -.348$, $P = .047$); however, all these correlations disappeared when the correlations were adjusted by both BMI *z* score and age. Eicosapentaenoic acid was not correlated in our study with adiponectin but only with estradiol levels, and this

relationship was even stronger when the correlations were adjusted to both variables. As mentioned above, EPA is a precursor of anti-inflammatory eicosanoids; and its content could be essential for metabolism in PP girls.

The groups did not differ in plasma clearance of the different dietary fatty acids after intake of the standardized breakfast. Our group previously reported a lower plasma clearance of *trans* fatty acids in obese children vs controls [15]. This was not the case with the present group, who only showed a delay in the clearance of *trans* fatty acids with respect to *cis* or saturated fatty acids, which was not attributable to their PP condition.

In conclusion, PP was associated with reduced postprandial plasma adiponectin in these prepubertal girls independently of their BMI, although they did not exhibit lipid disturbances or insulin resistance syndrome.

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