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Utility of Early Insulin Response and Proinsulin to Assess Insulin Resistance

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Abstract

Objective—To determine whether obesity and premature adrenarche are additive events increasing the risk of insulin resistance and β -cell failure, using early insulin response (EIR) or the insulinogenic index and proinsulin as markers.

Study design—Prospective case-control study at a tertiary care academic medical center; 81 prepubertal, predominantly Hispanic children (34 M/47 F): Lean Control [(4M, 6F) age(y), 6.5 ± 1.2 ; BMI-z, 0.08 ± 0.6], Obese Control [(20M, 10F) age(y), 7.2 ± 1.5 ; BMI-z, 2.5 ± 0.5], lean premature adrenarche [(3M, 11F) age(y), 7.1 ± 1.2 ; BMI-z, 0.09 ± 0.6], and obese premature adrenarche [(7M, 20F) age(y), 7.3 ± 1.0 ; BMI-z, 2.2 ± 0.4]. Fasting glucose (G_0), insulin (I_0), PI_0 , androgen levels, IGF-1, IGFBP-1, and lipids were obtained. OGTT was performed. EIR was calculated as $(I_{30} - I_0)/(G_{30} - G_0)$. Between group differences were assessed with two-way analysis of variance with interactions and associations explored with correlation/regression.

Results—EIR was greater in all obese patients with and without premature adrenarche. Combined analysis of the independent variables, obesity and premature adrenarche, showed that obese premature adrenarche had the greatest EIR. Obese subjects with premature adrenarche had greater

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fasting PI levels than their lean counterparts. Fasting PI/I ratio showed no statistical significance between groups.

Conclusion—We have used EIR and PI as markers to assess risk of insulin resistance and impaired insulin secretion, and have shown that obese children with premature adrenarche may be at greater risk for the development of pre-diabetes and T2DM than their lean counterparts.

Keywords

Pre-diabetes; Insulinogenic index; Overweight children

An increase in type 2 diabetes (T2DM) and pre-diabetes (impaired fasting glucose-IFG and impaired glucose tolerance-IGT) has paralleled the childhood obesity epidemic(1). Prior to the onset of T2DM, there is a phase of β -cell hyperfunction, characterized by an increase in insulin secretion(2). Over time, β -cell failure occurs. As β -cell function declines, glucose tolerance also declines, resulting in IGT and eventually T2DM.

It has been proposed that insulin secretion abnormalities, particularly during the early phase, are the basis of metabolic disorders leading to insulin resistance and T2DM. Many reports have shown that defective early insulin release is already apparent in adult patients with early stages of T2DM(3–6). In pediatric studies, there is a higher early insulin response (EIR) in obese children and adolescents with IGT and even normal glucose tolerant individuals with very low insulin sensitivity(7,8).

Fasting hyperproinsulinemia has been associated with insulin resistance. Disproportionate elevation of proinsulin (PI) to insulin (I) is a suggested marker of β -cell dysfunction or impending failure of insulin secretion(9). In adults, increased fasting PI/I ratios predict the development of IGT and T2DM, and are associated with, hypertension, low HDL, and high triglycerides in nondiabetic adults(10).

Risk factors for pre-diabetes in children include obesity, acanthosis nigricans (AN), and premature adrenarche. Girls with premature adrenarche have been shown to have decreased levels of sex hormone binding globulin (SHBG), hyperinsulinemia, insulin resistance, and unfavorable lipid profiles(11–14). After puberty, they are at increased risk for anovulation and functional ovarian hyperandrogenism, including polycystic ovary syndrome (PCOS) (15,16).

The objective of this study was to determine whether obesity and premature adrenarche are additive events increasing the risk of insulin resistance and β -cell failure, using EIR and PI as markers.

Methods

Eighty-one prepubertal children (34 boys and 47 girls, age 5–9 years) were studied. They were classified into four groups: (1) lean control subjects; (2) obese control subjects; (3) lean subjects with premature adrenarche; and (4) obese subjects with premature adrenarche.

Obese was defined as body mass index (BMI) greater than or equal to 95th percentile for age and sex. Lean was defined as BMI less than 85th percentile for age and sex. For the subjects with premature adrenarche, the criteria for entry into the study was the appearance of pubic hair with or without axillary hair before 8 years of age for girls, 9 years for boys, Tanner 1 breast for girls and testicular volume 3 cc or less for boys as well as a Δ 4-androstenedione (Δ 4-A) and/or dehydroepiandrosterone sulfate (DHEAS) level(s) in Tanner II range with no evidence of an adrenal enzyme defect or other endocrinopathy. The criteria for entry into the control group included absence of pubic hair on physical examination, Tanner 1 breast for girls

and testicular volume 3cc or less for boys, with no evidence of an adrenal enzyme defect or other endocrinopathy. None of the subjects were taking any medications at the time of the study. BMI and BMI-Z scores were based on CDC 2000 growth curves(17). Clinical characteristics of the subjects are presented in Table I. Informed consents from a legal guardian of each subject and assent from subjects over 7 years of age were obtained before participation in the study. The study was approved by the Institutional Review Board of Columbia University Medical Center.

Patients were recruited from the Pediatric Endocrinology clinic and General Pediatric clinic at Morgan Stanley Children's Hospital of New York – Presbyterian Hospital. After an overnight fast, a half day visit to the Pediatric General Clinical Research Center (GCRC) at Columbia University Medical Center was initiated. History and physical examination were performed and included height, weight, blood pressure, and the presence or absence of acanthosis nigricans (AN). Pubertal staging for breast, genitalia, and pubic hair was performed according to Tanner(18,19). Basal levels of glucose (G_0), insulin (I_0), proinsulin (PI_0), IGF-1, IGFBP-1, DHEAS, $\Delta 4$ -A, SHBG, testosterone, LH, FSH, and lipids were measured in all subjects. Patients underwent a standard 1.75g/kg glucola (maximum 75g) oral glucose tolerance test (OGTT). The subjects were instructed to maintain their usual diet until the overnight fast. Before and 30, 60, 90, and 120 min after the ingestion of oral glucose, blood was sampled for plasma glucose and serum insulin and proinsulin levels determined.

Insulin, Proinsulin, IGF-1, IGFBP-1, DHEAS, $\Delta 4$ -A, testosterone, SHBG, FSH, and LH levels were measured by Esoterix Endocrinology (Calabasas Hills, CA). Both insulin and proinsulin were measured by an immunochemiluminometric assay. The insulin assay sensitivity was 0.6 μ U/mL. The insulin intra-assay percent coefficient of variation (%CV) was 3–9% and its inter-assay %CV was 7–13%. The proinsulin assay sensitivity was 1 pM. The proinsulin intra-assay %CV was 6–12% and the inter-assay %CV was 8–14%. Plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in the Core Laboratory of the GCRC at Columbia Presbyterian Medical Center. Low-density lipoprotein (LDL) cholesterol was calculated according to the formula of Friedewald et al.(20).

The fasting plasma glucose was divided by the fasting serum insulin to calculate the fasting glucose to insulin ratio (FGIR), a measurement that has been validated in prepubertal girls with premature adrenarche using the IV and OGTT(21,22). The quantitative insulin sensitivity check index (QUICKI) and the composite whole-body insulin sensitivity index [ISI(comp)], both previously validated against the euglycemic insulin clamp, were also calculated(23,24). QUICKI was calculated as $1/(\log I_0 + \log G_0)$, and ISI(comp) was calculated according to the formula: $10,000/\text{square root of } [(G_0)(I_0)(\text{mean serum insulin during OGTT})(\text{mean blood glucose during OGTT})]$. HOMA was calculated as $(I_0)(G_0)/22.5$, with G_0 in mmol/L(25). The area under the curve for insulin and proinsulin (I_{AUC120} and PI_{AUC120}) was calculated using the trapezoidal rule(26). The EIR or the insulinogenic index was calculated as: $(I_{30} - I_0)/(G_{30} - G_0)$ (27). Fasting PI/I was calculated as a molar ratio.

Data were evaluated for normality and log-transformed prior to analysis when the distribution differed significantly from Gaussian. The following variables met this criterion: I, PI, I_{AUC120} , PI_{AUC120} , SHBG, IGF-1, IGFBP-1, FGIR, and QUICKI. All data are reported in raw units as the mean $\pm$ standard deviation unless otherwise noted.

The hypothesis that premature adrenarche and obesity additively increase insulin resistance was assessed with general linear models (SAS Proc GLM) with fixed effects for premature adrenarche status, obesity status, and their interaction for PI_0 , PI_0/I_0 , PI_{AUC120} , and EIR. When the overall model achieved significance, pair-wise comparison of obese versus lean premature adrenarche and obese premature adrenarche versus obese controls were examined. Similar

analyses were conducted for other continuous measures using Scheffé's adjustment for post-hoc comparisons when the overall model F-test was significant to display the background of insulin resistance testing for other measures in the groups. Between-group differences in within-subject response to OGTT were estimated with linear mixed models for repeated measures (SAS Proc MIXED) with fixed effects for premature adrenarche status, obesity status, sample time and their interactions with random effects for subjects and an autoregressive covariance structure. Model estimated means and between-group differences are reported. The apriori hypotheses regarding obese premature adrenarche, lean premature adrenarche, and obese controls were explored with post-hoc testing of the model estimated means by the method of simultaneous confidence intervals. For these hypotheses, p-values less than 0.05 were considered to represent statistical significance. No adjustment of the type I error rate was made for the three outcomes associated with our restricted hypotheses. The associations among PI_0 and PI_0/I_0 and lipid levels and among PI_0 or PI_0/I_0 and other commonly used measures of insulin sensitivity were explored with Pearson correlation to show the extent to which fasting PI indices are reflected in these measures.

Results

All four groups were comparable in terms of age and birth weight (Table I). There were no significant differences between the obese controls vs. obese premature adrenarche groups and lean controls vs. lean premature adrenarche groups with respect to BMI, family history of T2DM, and the presence of AN. Ethnicity reflected the demographics of our population. Acanthosis nigricans was more common in the obese subjects regardless of premature adrenarche status. A family history of T2DM was present in 70% (57/81) of the subjects. No subject was a product of a pregnancy complicated by gestational diabetes.

The premature adrenarche state of the subjects with lean and obese premature adrenarche was confirmed by significantly higher DHEAS and $\Delta 4$ -A levels in the obese premature adrenarche versus obese control groups ($p < 0.001$, $p < 0.01$) and lean premature adrenarche versus lean control groups ($p < 0.05$, $p < 0.05$). Although not statistically significant, SHBG levels were lower in premature adrenarche groups.

Triglyceride levels were significantly greater in the obese subjects with and without premature adrenarche ($p < 0.01$, $p < 0.05$). IGF-1 was significantly higher in the subjects with premature adrenarche with or without obesity ($p < 0.001$, $p < 0.001$). IGFBP-1 levels in subjects with obese premature adrenarche was almost three-fold lower and two-fold lower than the lean and obese control group, respectively, and IGFBP-1 was significantly lower in the obese subjects with and without premature adrenarche ($p < 0.05$, $p < 0.05$).

We were able to obtain complete post-Glucola samples (0, 30, 60, 90, 120 minutes) in 58 of the 81 patients whereas 63 patients completed 0 and 30 minute post-Glucola sample collection for the calculation of EIR (Table II, Figure 1).

None of the subjects had IGT or diabetes as defined by their responses of the OGTT. However, 1 lean control, 4 obese controls, and 4 subjects with obese premature adrenarche had IFG defined as a fasting glucose ≥ 100 (28). Although the obese controls, lean premature adrenarche, and obese premature adrenarche groups had greater 120 minute glucose values compared with lean controls ($p < 0.01$, $p < 0.05$, $p < 0.05$), there were no other statistically significant differences demonstrated at other OGTT time points.

In contrast, obese controls had more than double the fasting ($p < 0.001$) and 30 minute ($p < 0.05$) insulin values of lean controls and quadruple the 120 minute value ($p < 0.0001$). Subjects with obese premature adrenarche had double the fasting ($p < 0.01$), 30 minute ($p < 0.01$), and 120 minute ($p < 0.01$) insulin values of subjects with lean premature adrenarche. When comparing

obese groups, obese premature adrenarche had 1.5-fold greater 30 minute ($p<0.05$) and 120 minute ($p<0.01$) insulin values compared with obese controls, without significant difference at 0 minutes. Further, although there were no differences between subgroups in G_{AUC120} , the mean I_{AUC120} was greatest in subjects with obese premature adrenarche and obese premature adrenarche I_{AUC120} was greater than in obese controls ($p<0.05$) or lean premature adrenarche ($p<0.001$) and higher in subjects with lean premature adrenarche than lean controls ($p<0.001$).

Markers of insulin sensitivity were used to describe each subgroups' pattern of insulin resistance (Table II, Figure 2). $ISI(comp)$ was significantly lower in the obese premature adrenarche group versus the lean premature adrenarche and obese control groups ($p<0.05$, $p<0.05$) suggesting decreased insulin sensitivity in subjects with both obesity and premature adrenarche. $FGIR$ and $QUICKI$ were the lowest in the obese premature adrenarche group; they were significantly lower than that of the lean premature adrenarche group ($p<0.01$). $HOMA$ was significantly higher in overweight subjects with and without premature adrenarche ($p<0.001$, $p<0.001$).

EIR was our approximation of first-phase insulin secretion. EIR was significantly higher in subjects with obese premature adrenarche versus those with lean premature adrenarche, as well as subjects with obese premature adrenarche versus obese controls ($p<0.05$, $p<0.05$). When comparing EIR with other measures in our population, linear regression analysis demonstrated EIR was highly associated with $SHBG$ ($p<0.05$), $QUICKI$ ($p<0.05$), $FGIR$ ($p<0.05$), $ISI(comp)$ ($p<0.05$), and $HOMA$ ($p<0.05$) in subjects with obese premature adrenarche. In obese controls, EIR was highly associated with $QUICKI$ ($p<0.01$), $FGIR$ ($p<0.01$), $ISI(comp)$ ($p<0.05$), and $HOMA$ ($p<0.001$). There was a significant positive correlation between EIR and $HOMA$ ($r = 0.72$) and between EIR and I_0 ($r = 0.74$) in the cohort.

Consistent with our $HOMA$ and EIR results, we found PI_0 was more than doubled in both obese control and obese premature adrenarche groups compared with their lean controls ($p<0.01$, $p<0.01$). Interestingly, the same group differences were seen at 30 minutes ($p<0.001$, $p<0.01$), but additionally obese premature adrenarche was greater than obese controls ($p<0.05$) and lean premature adrenarche was greater than lean controls ($p<0.05$). At 120 minutes, again both obese groups (obese controls and obese premature adrenarche) had greater mean proinsulin levels than their lean counterparts ($p<0.0001$, $p<0.01$) and lean premature adrenarche was nearly twice the level of lean controls ($p<0.01$). Similar to I_{AUC120} patterns, PI_{AUC120} was greatest in subjects with obese premature adrenarche, significantly greater in obese groups when compared with lean ($p<0.01$, $p<0.001$), and both premature adrenarche groups versus the controls without premature adrenarche ($p<0.05$, $p<0.05$). The PI_0/I_0 ratio was not significant when each of the groups were compared.

Pearson correlations were analyzed in all four groups between PI_0/I_0 and PI_0 with insulin sensitivity indices as well as between PI_0/I_0 and lipid profiles to confirm the usefulness of these measures in our obese study population. PI_0 strongly correlated with all five established measures of insulin sensitivity in obese control subjects, with $HOMA$ having the strongest correlation ($r=0.76$, $p<0.0001$). In subjects with obese premature adrenarche, EIR , $QUICKI$, $ISI(comp)$, and $FGIR$ had somewhat weaker correlation with PI_0 ($r=0.47$, $p<0.05$; $r=-0.39$, $p<0.05$; $r=-0.53$, $p<0.01$; $r=-0.44$, $p<0.05$). When examining the PI_0/I_0 in subjects with obese premature adrenarche, it also moderately correlated with $QUICKI$ ($r=0.44$, $p<0.05$) and inversely with $HOMA$ ($r=-0.47$, $p<0.05$). In subjects with lean premature adrenarche, PI_0/I_0 correlated with $FGIR$ ($r=0.69$, $p<0.01$). Lastly, a significant positive correlation was seen in subjects with obese premature adrenarche between triglyceride levels and PI_0/I_0 ($r=0.43$, $p<0.05$) and a negative correlation was seen in obese controls between HDL and PI_0 ($r=-0.60$, $p<0.01$).

Discussion

We sought to investigate the utility of EIR and PI, as novel predictive markers, to assess insulin resistance in children with obesity and premature adrenarche. Further we wanted to assess if prepubertal children who are both obese and have premature adrenarche are at greater risk for altered β -cell function compared with lean control children. The ability to identify at an earlier age children at risk for insulin resistance who would develop a decline in β -cell function could allow clinicians to enact simple preventative measures before they develop overt glucose intolerance and diabetes. These simple markers could also be used to follow β -cell function over time and for monitoring clinician interventions.

The elevated triglycerides place these subjects at increased risk for metabolic syndrome. Ibanez et al has shown that Spanish girls with premature adrenarche have greater triglyceride, cholesterol, and LDL than their healthy peers(29–31). In a study by Mathew et al, they also were able to confirm the increased prevalence of metabolic syndrome markers in lean boys and girls with premature adrenarche(32). As reviewed by Shaibi et al, metabolic syndrome prevalence rates in overweight Hispanic youth between the ages of 8–13 years old with family history of T2DM have been estimated to be between 25–39% depending on definition(33). Insulin sensitivity was demonstrated to be inversely correlated with the number of criteria filled for metabolic syndrome. In our study, we did not consistently measure waist circumference or blood pressure to define metabolic syndrome, but our study further demonstrates the additional risk of metabolic syndrome in children with obesity and premature adrenarche.

Nine subjects had IFG. These fasting glucoses were minimally elevated between 100–106 mg/dl, thus we believe these values could be attributed to the stress during the initial blood draw. At 120 minutes however, glucose was significantly higher in all groups compared with lean controls, but none were diagnostic for IGT. Insulin and proinsulin values at 0, 30, and 120 minutes were significantly elevated in all obese versus lean subjects. At 30 and 120 minutes insulin and proinsulin was significantly greater in obese premature adrenarche versus obese controls. Additionally, I_{AUC120} and PI_{AUC120} was also significantly greater in obese premature adrenarche versus obese controls. We suggest this compensatory increase in insulin secretion is required to counter the insulin resistance in these subjects to maintain normal glucose homeostasis. It has also been proposed that basal hyperinsulinemia can also contribute further to insulin resistance. Thus the findings of significantly greater insulin and proinsulin levels in the subjects with obese premature adrenarche support our theory that premature adrenarche places patients at greater risk for insulin resistance.

As expected, all obese versus lean subjects had significantly greater HOMA and significantly decreased QUICKI, FGIR, and ISI(comp). We were able to demonstrate that both EIR and fasting PI are two additional reliable markers for insulin resistance because they correlated with SHBG, QUICKI, FGIR, ISI(comp), and HOMA in our obese cohort. We were able to first confirm that obese subjects had evidence of greater insulin resistance than their lean counterparts based on their HOMA values, and we secondly showed that subjects with obese premature adrenarche were in fact the most insulin resistant group. This finding was reinforced by QUICKI, ISI(comp), and FGIR analysis. Finally, when comparing obese premature adrenarche with obese controls, ISI(comp) was lower in obese premature adrenarche, suggesting further decreased insulin sensitivity in subjects who were both obese and had premature adrenarche.

We were not able to demonstrate PI_0/I_0 to be a significant marker for insulin resistance or β -cell dysfunction. Of note is a recently published study evaluating PI and PI/I ratio as predictors of metabolic syndrome in adults from Chile that reported similar results and also found PI but not the PI/I ratio to be a significant risk factor for metabolic syndrome(34). We hypothesize

that patients with insulin resistance with presumed normal β -cell function are able to compensate with overproduction of insulin to prevent an elevated fasting PI/I ratio. In addition, Retnakaran et al describes the confounding effect of reduced hepatic clearance of insulin results in decreased PI/I ratios in normoglycemic, insulin resistant individuals(35).

Limitations of this study include the unequal subject numbers in subgroups and small sample size limiting statistical power. Our obese premature adrenarche and obese control groups have a greater number of subjects because of the prevalence of obesity and the common association between premature adrenarche and obesity, as recently reinforced in a retrospective study of premature pubarche patients in New York City(36). Thus the weight factor might be more strongly represented when comparing all obese versus lean subjects. We evaluated 81 total patients, and minor effects may have escaped detection due to lack of sufficient study power. Our trends of elevated total cholesterol and decreased HDL might have been statistically significant with increased number of study subjects.

EIR is useful in following the initial defect of early insulin release and both EIR and fasting proinsulin are reliable and accurate markers for insulin resistance. We could not demonstrate a difference in the fasting proinsulin/insulin ratio, our marker of early β -cell dysfunction, in this pilot study of normoglycemic individuals. Nonetheless, we speculate that using both proinsulin and EIR as indicators of insulin resistance and first-phase β -cell function will be helpful in defining early β -cell dysfunction when following these patients for possible future progression to subsequent T2DM. Children who are both obese and have premature adrenarche have factors that are additive with respect to insulin resistance.

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Abbreviations

I	Insulin
PI	Proinsulin
T2DM	type 2 diabetes mellitus
EIR	early insulin response/insulinogenic index
IGT	impaired glucose tolerance
IFG	impaired fasting glucose

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Figure 1. Glucose, Insulin, Proinsulin OGTT Results

Δ = lean premature adrenarche vs lean controls, $\dagger$ = lean vs obese premature adrenarche, * = lean vs obese controls, Ω = obese controls vs obese premature adrenarche when $p < 0.05$

Figure 2. Markers of Insulin Sensitivity

Δ = lean premature adrenarche vs lean controls, † = lean vs obese premature adrenarche, * = lean vs obese controls, Ω = obese controls vs obese premature adrenarche when $p < 0.05$

Table I

Clinical Characteristics, Androgen Levels, SHBG, Lipid Profiles, IGF-1, and IGFBP-1 of Subjects and Controls

	Control/Lean	Control/Obese	Lean Premature Adrenarche	Obese Premature Adrenarche
No.	10	30	14	27
Age (yrs)	6.5±1.2	7.2±1.5	7.1±1.2	7.3 ±1.0
Sex	6F/4M	10F/20M	11F/3M	20F/7M
Birth weight (kg)	3.2±0.9	3.7±0.5	3.1±0.6	3.3±0.6
BMI (kg/m²)	15.6±0.8	25.9±4.2	15.8±0.9	23.7±3.4
BMI z-score	0.1±0.6	2.5±0.5	0.1±0.6	2.2±0.4
Ethnicity *	8H, 2B	23H, 1B, 1W, 1ND, 4 Other	8H, 4B, 1W, 1 Other	17H, 5B, 3W, 2 Other
FH of T2DM	3/10	21/30	10/14	23/27
Presence of Acanthosis Nigricans	1/10	19/30	4/14	17/27
Androstenedione (ng/dl)	25.5±12.0 ^c	35.4±10.9 ^d	39.4±14.2 ^c	48.4±16.1 ^d
DHEAS (µg/dl)	16.0±13.4 ^c	29.4±19.0 ^d	49.4±42.0 ^c	59.7±33.9 ^d
Testosterone (ng/dl)	3.5±0.8 ^c	4.2±2.3 ^d	5.2±2.1 ^c	6.3±3.6 ^d
SHBG (nmol/l)	40.9±62.1	41.7±50.3	24.8±43.2	24.4±35.7
Total cholesterol (mg/dl)	147.4±20.8	166.0±34.6	159.3±25.6	160.9±26.5
Triglycerides (mg/dl)	61.9±6.5 ^a	79.7±37.4 ^a	57.6±12.0 ^b	73.6±22.5 ^b
HDL (mg/dl)	51.7±7.8	48.6±12.3	50.9±13.4	49.0±9.3
LDL (mg/dl)	83.4±22.8	101.5±31.8	93.2±21.0	96.3±26.4
IGF-I (ng/ml)	115.0±37.1 ^c	148.2±60.0 ^d	203.0±71.7 ^c	221.0±77.0 ^d
IGFBP-I (ng/ml)	68.8±46.5 ^a	35.4±39.7 ^a	38.7±28.3 ^b	18.5±22.1 ^b

* H=Hispanic, B=Black, W=White, ND = not documented

^a lean vs obese controls,

^b lean vs obese premature adrenarche,

^c lean premature adrenarche vs lean controls,

^d obese premature adrenarche vs obese controls when p<0.05

Table II

OGTT Results, Markers of Insulin Sensitivity, EIR, and Proinsulin Levels

	Control/Lean	Control/Obese	Lean Premature Adrenarche	Obese Premature Adrenarche
Fasting Glucose (mg/dl)	87.9±7.7	91.0±7.9	86.4±6.0	89.9±6.5
Glucose 30 min (mg/dl)	133.7±24.2	143.7±34.7	147.4±36.6	137.0±22.8
Glucose 120 min (mg/dl)	91.6±10.1 <i>a,c</i>	111.0±12.9 <i>a</i>	104.9±17.7 <i>c</i>	109.8±12.4
Fasting Insulin (μU/ml)	4.1±2.8 <i>a</i>	10.5±7.3 <i>a</i>	5.6±4.1 <i>b</i>	11.9±6.5 <i>b</i>
Insulin 30 min (μU/ml)	23.7±11.8 <i>a</i>	68.5±79.0 <i>a,d</i>	48.9±22.4 <i>b</i>	111.0±77.7 <i>b,d</i>
Insulin 120 min (μU/ml)	9.1±6.4 <i>a,c</i>	36.7±40.7 <i>a,d</i>	28.1±15.9 <i>b,c</i>	56.3±29.5 <i>b,d</i>
G_{AUC120} (mg/dl)	216.5±28.7	241.8±33.9	236.6±35.4	227.6±30.0
I_{AUC120} (μU/ml)	31.5±13.9 <i>a,c</i>	91.2±80.8 <i>a,d</i>	68.9±20.1 <i>b,c</i>	132.2±77.7 <i>b,d</i>
QUICKI	0.41±0.06	0.35±0.03	0.40±0.07 <i>b</i>	0.34±0.03 <i>b</i>
HOMA	0.91±0.59 <i>a</i>	2.38±1.74 <i>a</i>	1.28±0.87 <i>b</i>	2.65±1.53 <i>b</i>
ISI (comp)	22.52±10.63	7.07±3.57 <i>d</i>	10.05±6.41 <i>b</i>	5.04±2.88 <i>b,d</i>
FGIR	31.8±21.3	12.4±6.5	30.3±27.2 <i>b</i>	10.0±5.3 <i>b</i>
EIR	0.59±0.31	1.46±1.53 <i>d</i>	1.13±1.49 <i>b</i>	2.50±1.80 <i>b,d</i>
Proinsulin₀ (pmol/l)	4.1±2.5 <i>a</i>	10.5±7.5 <i>a</i>	5.2±3.5 <i>b</i>	12.1±6.5 <i>b</i>
PI at 30 min (pmol/l)	16.3±13.9 <i>a,c</i>	45.2±33.1 <i>a,d</i>	28.3±12.4 <i>b,c</i>	64.3±31.3 <i>b,d</i>
PI at 120 min (pmol/l)	17.5±11.1 <i>a,c</i>	61.2±46.2 <i>a</i>	34.1±17.7 <i>b,c</i>	80.9±43.1 <i>b</i>
PI_{AUC120} (pmol/l)	32.6±15.4 <i>a,c</i>	99.1±67.0 <i>a,d</i>	53.1±21.3 <i>b,c</i>	131.2±66.5 <i>b,d</i>
PI₀/I₀ (pmol/l)	0.15±0.07	0.15±0.07	0.21±0.20	0.17±0.10

a lean vs obese controls,*b* lean vs obese premature adrenarche,*c* lean premature adrenarche vs lean controls,*d* obese premature adrenarche vs obese controls when $p < 0.05$