Insulin Sensitivity and Lipid Profiles in Girls with Central Precocious Puberty before and during Gonadal Suppression

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Context: Early menarche is associated with increased risk of cardiovascular disease in adulthood. It is unknown whether metabolic risk factors are adversely affected in girls with central precocious puberty (CPP) already at time of diagnosis.

Objective: The objective of the study was to evaluate metabolic profiles in girls with early normal puberty (EP) and CPP.

Design and Setting: This was a combined cross-sectional and longitudinal study at a tertiary center of pediatric endocrinology.

Patients and Intervention: Twenty-three girls with EP or CPP and 115 controls with normal pubertal timing were evaluated by oral glucose tolerance test, dual-energy x-ray absorptiometry scan, and fasting blood samples. Fifteen girls (13 CPP) were treated with GnRH agonists (GnRHa) and re-evaluated after 12 and 52 wk of treatment.

Main Outcome Measures: Insulin and glucose levels during oral glucose tolerance test and fasting lipid levels were measured.

Results: At the time of diagnosis, girls with CPP had higher fasting insulin, triglyceride, and low-density lipoprotein-cholesterol levels as well as lower insulin sensitivity and high-density lipoprotein/total cholesterol ratios (all $P < 0.05$) compared with controls after adjustment for pubertal stage and body fat percentage. Age at pubertal onset positively predicted insulin sensitivity for a given pubertal stage ($P = 0.04$) in girls with EP and CPP. Insulin sensitivity decreased significantly during 1 yr of GnRHa treatment ($P = 0.04$).

Conclusions: Girls with CPP had adverse metabolic profiles at the time of diagnosis compared with puberty-matched controls. In addition, those with the earliest onset of puberty had the most adverse metabolic profiles. Surprisingly, metabolic profiles deteriorated even further after withdrawal of sex steroids by GnRHa treatment. **(*J Clin Endocrinol Metab* 95: 3736–3744, 2010)**

During the last decades, a decline in age at puberty in girls has been reported worldwide (1–3) paralleled by an increase in the incidence of central precocious puberty (CPP) (4). Although the potential long-term consequences of the recent advancement toward earlier age at puberty are unknown, a growing body of epidemiological evidence raises concern for future public health.

Early maturational timing in girls is associated with increased all-cause, cancer, and cardiovascular mortality (5, 6). In accordance, early age at menarche is associated with in-

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Abbreviations: AUC, Area under the curve; CPP, central precocious puberty; CV, coefficient of variation; DI, disposition index; EP, early normal puberty; GnRHa, GnRH agonist; HDL-C, high density lipoprotein-cholesterol; ISI, insulin sensitivity index; LDL-C, low density lipoprotein-cholesterol; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglyceride.

creased risk of obesity (7–10), hypertension (5), and type 2 diabetes (10) as well as ischemic heart disease and stroke events (5) in adulthood. A recent study indicated that the effect of early menarcheal age on adult cardiovascular risk may be ascribed to high childhood adiposity (11). In accordance, high adiposity in childhood predicts early menarcheal age (12, 13) as well as high adiposity and increased cardiovascular risk in adulthood (14, 15). On the other hand, larger gains in adiposity during puberty and in early adulthood are seen in women with a history of early menarche (7, 12). Thus, adverse metabolic programming by early maturation *per se* does seem to play an additional role.

Girls with CPP have increased adiposity at time of diagnosis (16, 17). In addition, inhibition of pubertal progression by GnRH agonist (GnRHa) treatment is associated with a continuous gain in adiposity despite suppression of gonadotropin and gonadal hormone secretion in such girls (16, 18). Thus, GnRHa treatment does not seem to reverse the adverse body compositional changes associated with early maturation.

Insulin is a well-known contributor to the development of cardiovascular disease, and its implication for pubertal timing is increasingly recognized (19). High insulin levels in childhood is associated with early age at menarche (12, 13) and adverse cardiovascular risk in early adulthood independent of the degree of adiposity (15, 20). In addition, high insulin secretion and low insulin sensitivity predict future gain in fat mass in normal birth weight children (21) as well as in girls born small for gestational age (22). In accordance, insulin-sensitizing therapy has been shown to reduce adiposity, delay age at pubertal onset, and decrease the pace of progression of puberty in small-for-gestational-age girls (23, 24). Thus, low insulin sensitivity could be a central risk factor for early pubertal timing and subsequent for increased cardiovascular morbidity and mortality. Low levels of SHBG levels are associated with low insulin sensitivity and adverse metabolic risk profiles during puberty (25). In girls with CPP, SHBG levels are reduced at diagnosis and decreases further after withdrawal of sex steroids by GnRHa treatment (17). Thus, indirect evidence indicates that girls with CPP have low insulin sensitivity and adverse lipid profiles at diagnosis and that these cardiovascular risk factors do not respond favorably to gonadal suppression. However, to what extent this reflects true alterations in insulin sensitivity and lipid levels have not been previously evaluated prospectively in girls with CPP before and during GnRHa treatment.

In the present study, we evaluated body composition, insulin sensitivity, and lipid profiles in girls with early normal and precocious puberty before and during gonadal suppression with GnRHa treatment.

Patients and Methods

Patients and controls

Patients with reported onset of breast development before the age of 9 yr were recruited consecutively from our outpatient clinic at the Department of Growth and Reproduction, Copenhagen University Hospital, from May 2008 to September 2009. In total, 32 girls volunteered to participate. All patients presented with breast budding as first sign of puberty and all were premenarcheal. Patients were diagnosed with idiopathic central precocious puberty (CPP, n = 15) if age at onset of breast development was less than 8 yr, peak LH level was greater than 5 IU/liter (n = 13) and/or peak LH to peak FSH ratio was greater than 0.66 (n = 15) (26) in response to rapid-acting GnRHa (0.1 mg Relefact LHRH, Sanofi-Aventis, Frankfurt am Main, Germany) and a nonpathological brain magnetic resonance imaging. Girls' onset of breast budding between 8 and 9 yr and a peak LH level greater than 5 mU/liter (n = 8) and/or peak LH to peak FSH ratio greater than 0.66 (n = 8) on GnRH test were diagnosed with early normal puberty (EP; n = 8). The remaining girls (n = 9) were not biochemically pubertal and excluded for further analysis. The mean age at pubertal onset was 7.6 yr (7.2–7.9) and 8.3 yr (8.0–8.6) in girls with CPP and EP, respectively. Fifteen girls (13 with CPP and two with EP) were initiated on long-acting GnRHa treatment (leuprolide acetate 3.75 mg) with sc injections every 28th day and reexamined after 11.9 (10.2–12.3, n = 15) and 51.9 (48.9–55.1, n = 9) wk of treatment. All reexaminations were done on days of injection. Bone age was evaluated at baseline as well as after 1 yr by the computer-based bone age analyzer BoneXpert (27).

Control subjects were recruited as a part of The Copenhagen Puberty Study from four primary schools in the Copenhagen community (1, 28). One hundred fifteen healthy (premenarcheal, n = 85) girls were included. No prior medical history of conditions associated with altered pubertal timing or intake of medications was reported. Other aspects from this study has previously been published (25).

Pubertal development was described according to Tanners criteria. Body composition was evaluated in all subjects by a whole-body dual-energy x-ray absorptiometry scan using a Hologic CDR 1000/W densitometer (Hologic Inc., Bedford, MA) with software version 6.2 [pediatric normative data are not included in this software package but has previously been collected (25, 29)].

Blood sampling

An iv cannula was inserted into an antecubital vein, from which venous fasting blood samples were drawn into standard vacuum tubes. Blood was centrifuged (3000 g at 10 min) within 30 min and plasma immediately stored at −20 C until analyses. A standard 2-h oral glucose tolerance test (OGTT) with an oral glucose load of 1.75 g of glucose per kilogram body weight (maximum 75 g glucose) was carried out. Blood samples were drawn with 30 min intervals for determination of glucose and insulin. Full OGTT data were available on 12 girls with CPP, six with EP, and 112 controls. The area under the curve (AUC) for plasma glucose (AUC_{glu}) and insulin (AUC_{ins}) was calculated by the trapezoidal rule. Whole-body insulin sensitivity index (ISI) was calculated by the formula developed by Matsuda and DeFronzo (30) and subsequently converted to picomolar insulin per millimolar glucose. Insulin secretion was calculated as first-phase

insulin release by the formulas developed by Stumvoll *et al.* (31). β-Cell compensatory capacity was evaluated by the disposition index (DI) as the product of the insulin sensitivity and first-phase insulin release (25, 32).

Analyses

Insulin was determined by an electrochemiluminescent immunoassay (Elecsys insulin reagents kit; Roche Diagnostics, Mannheim, Germany) on automated Roche modular analytics module E170 (Roche, Mannheim, Germany). The detection limit was 2 pmol/liter and intra- and interassay coefficients of variation (CVs) less than 5%, respectively. Glucose and lipids [total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG)] were determined on a Roche Modular Analytics (SWA) Module P (Roche Diagnostics). Glucose was determined by enzymatic absorption photometry (GLU; Roche) with intra- and interassay CV less than 2%, respectively. TC, LDL-C, HDL-C, and TG were determined by enzymatic colorimetric analyses (CFAS TC/LDL/HDL plus, TG GPO-PAP; Roche). For all lipids the intra- and interassay CVs was less than 3%, respectively. SHBG was determined by a time-resolved immunofluorescence assay (Delfia; Wallac Oy, Turku, Finland) with intra- and interassay CV less than 7%, respectively. Serum FSH and LH were measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer, Boston, MA) with detection limits of 0.06 and 0.05 IU/liter for FSH and LH, respectively. Intra- and interassay CV was less than 5% in both gonadotropin assays. Estradiol levels were determined by RIA (Pantex, Santa Monica, CA) with detection limit of 18 pmol/liter and intra- and interassay CVs of 7.5 and 12.3%, respectively (1).

Statistics

Baseline characteristics and changes during GnRHa treatment are presented as medians as well as the fifth and 95th percentiles or range, respectively. Mann-Whitney *U* tests were used for comparison between groups at baseline. Adjusted analyses were done using general linear models in which insulin and lipid metabolic parameters were adjusted for pubertal stage and body fat percentage. ISI, insulin secretion, DI, all lipids, and lipid ratios were log transformed to obtain approximate normal Gaussian distribution of the residuals as well as to obtain a residual variance that did not depend on the level. No interactions between breast stage and diagnostic groups (CPP/EP/controls) were observed in any of these models. Changes in main outcome variables during GnRHa treatment in girls with CPP were evaluated by Wilcoxon signed rank tests.

Ethics

The study was in accordance with the ethnical principles of the Helsinki II declaration. The study protocol was approved by the local ethics committee (references KF 01 282214 and KF 11 2006-2033). All children and parents gave their informed written consent.

Results

Baseline characteristics

Baseline characteristics of patients and controls are shown in Table 1. All patients with CPP and EP were in breast stage II or III at baseline. The median bone age was 9.8 (8.9–11.0) and 10.2 (7.7–11.0) yr for patients with EP and CPP, respectively. Patients with EP and CPP had higher FSH, LH, testosterone and estradiol levels than age-matched controls, respectively (all $P < 0.05$, Table 1). Median peak LH and peak FSH levels were 6.1 (3.9–22.7) and 6.7 (4.6–10.6) in patients with EP and 9.4 (4.5–61.7) and 9.4 (5.2–34.2) in patients with CPP, respectively.

Early normal puberty

Except for differences in anthropometrics, body composition or metabolic outcome variables did not differ between patients with EP and age-matched prepubertal and puberty-matched controls, respectively (Table 1). Due to slightly skewed distribution in stages of breast development between the puberty-matched control and EP groups, all analyses were rerun adjusted for breast stage. Except for higher glucose levels during OGTT [13.8% (0.9–26.7), $P = 0.03$], no significant differences were found between girls with EP and controls after adjustment for pubertal stage.

CPP

Comparison of main outcome variables between patients with CPP and age-matched prepubertal and puberty-matched controls, respectively, are shown in Table 1. As for the EP group, the distribution in stages of breast development between the puberty-matched control and CPP groups was slightly skewed and all analyses therefore rerun adjusted for breast stage. Significantly higher fasting and oral glucose-stimulated insulin levels, higher TC, LDL-C, and TG levels and lower ISI and HDL to TC ratios were found in girls with CPP compared with controls after adjustment for breast stage (Fig. 1A). No significant difference was found in first-phase insulin release, unadjusted as well as adjusted for insulin sensitivity, despite lower insulin sensitivity in girls with CPP compared with controls (Fig. 2). The DI was not significantly different between girls with CPP and controls ($P = 0.18$).

The higher total body fat percentage in the CPP group compared with puberty-matched controls was still present after adjustment for pubertal stage (Fig. 1A). To determine whether the adverse metabolic parameters found in the CPP group were attributed to the greater degree of adiposity, we reanalyzed all models after additional adjustment for total body fat percentage. With the exception of TC, all differences in insulin or lipid levels between girls with CPP and controls remained statistically significantly different after adjusting for total body fat percentage (Fig. 1B). In addition, when analyses on lipid levels were adjusted for both pubertal stage and insulin sensitivity, all, but TC, became insignificantly different between girls with CPP and controls, although the

TABLE 1. Baseline characteristics

	CPP (B2-3) (n = 15)	EP (B2-3) (n = 8)	Control (B1) (n = 24)	Control (B2-3) (n = 40)	Control (B1-5) (n = 115)
B1–B5 (%)	−/53/47/−/−	−/50/50/−/−	100/−/−−/−−/−	−/38/62/−/−	21/13/22/34/10
Age (yr)	8.6 (7.5–9.9)[a]	9.1 (8.5–9.4)[a]	8.9 (7.4–11.4)	11.3 (9.41–12.7)	11.8 (8.6–15.4)
Height (cm)	137 (121–147)[a]	141 (133–150)[a]	134 (122–149)	150 (133–163)	153 (129–174)
Weight (kg)	33.5 (22.7–45.7)[a,b]	34.9 (28.9–38.2)[a,b]	29.6 (20.9–38.7)	39.1 (29.2–56.1)	42.3 (2.9–60.7)
BMI	17.9 (15.5–22.3)[b]	17.2 (15.5–18.1)	16.1 (13.6–18.9)	17.8 (15.3–21.8)	18.0 (14.5–22.6)
TBF (%)	23.8 (19.1–34.9)[a,b]	21.0 (20.1–25.9)	20.0 (15.9–27.9)	21.8 (15.6–25.5)	21.1 (16.1–29.7)
FSH (U/liter)	4.35 (1.32–11.80)[b]	2.09 (1.20–7.59)[b]	1.31 (0.39–6.27)	3.89 (0.98–6.90)	3.95 (0.87–7.43)
LH (U/liter)	0.84 (0.06–2.79)[b]	0.21 (0.07–2.60)[b]	0.05 (<dL–0.43)	0.39 (<dL–3.69)	1.32 (<dL–9.40)
E2 (pmol/liter)	48 (<dL–205)[b]	27 (<dL–204)[b]	<dL (<dL–44)	40 (<dL–187)	65 (<dL–449)
SHBG (mmol/liter)	70 (28–158)	92 (45–140)	94 (53–176)	92 (27–140)	85 (31–156)
FI (pmol/liter)	58 (40–121)[a,b]	40 (22–83)	33 (9–64)	44 (11–101)	47 (17–94)
FG (mmol/liter)	4.8 (4.1–5.3)	4.8 (4.6–5.1)	5.0 (3.5–6.4)	4.9 (3.7–5.7)	4.9 (3.8–5.5)
MPI (pmol/liter)	338 (177–569)[a,b]	276 (161–439)	210 (84–314)	246 (154–429)	249 (115–508)
MPG (mnol/liter)	6.3 (5.2–7.6)	6.5 (5.9–8.4)	6.2 (4.8–7.9)	5.7 (4.3–8.0)	5.7 (4.4–7.5)
WBISI	1.4 (0.8–2.4)[a,b]	2.3 (1.2–2.7)	2.7 (1.4–6.2)	1.9 (1.0–4.7)	1.9 (0.9–4.4)
First IR (pmol/liter)	1508 (820–2289)[b]	999 (345–1861)	945 (362–1587)	1160 (712–2004)	1233 (599–2550)
DI	1996 (1010–2635)	2163 (915–2743)	2474 (1016–3871)	2244 (947–4306)	2447 (1140–4181)
TG (mmol/liter)	0.83 (0.45–1.51)[a,b]	0.63 (0.38–1.05)	0.71 (0.47–1.18)	0.69 (0.43–1.36)	0.75 (0.46–1.51)
TC (mmol/liter)	4.5 (2.8–6.3)[a]	3.8 (3.1–4.1)	4.2 (2.7–4.9)	3.8 (3.1–4.9)	3.8 (2.8–4.8)
HDL-C (mmol/liter)	1.4 (0.9–1.8)	1.4 (1.1–1.6)	1.5 (0.9–2.1)	1.4 (0.7–2.1)	1.5 (1.0–2.1)
LDL-C (mmol/liter)	2.5 (1.4–4.4)[a]	2.2 (1.5–2.5)	2.2 (1.4–3.7)	2.2 (1.1–3.0)	2.2 (1.2–3.1)

Baseline characteristics in girls with CPP, EP, age-matched prepubertal controls, puberty-matched controls, and all controls. Results are presented as medians (fifth and 95th percentile). To convert glucose from nanomoles per liter to milligrams per deciliter, multiply by a factor 18. To convert insulin from picomoles per liter to microunits per milliliter, divide by a factor 6. To convert ISI into the original scale calculated by glucose in milligrams per deciliter and insulin in microunits per milliliter, multiply by a factor 3. BMI, Body mass index; TBF, total body fat; E2, estradiol; FI, fasting insulin; FG, fasting glucose; MPI, mean plasma insulin (OGTT); MPG, mean plasma glucose (OGTT); WBISI, whole-body ISI (30); first IR, first-phase insulin release (31); < dL, below detection limit.

[a] $P < 0.05$ *vs.* puberty-matched controls.

[b] $P < 0.05$ *vs.* age-matched control.

same tendencies were still present (all $P < 0.10$). All the patients and controls had normal glucose tolerance (2 h glucose level <7.8 mmol/liter).

Exclusion of the two girls who meet only the LH to FSH ratio greater than 0.66 criteria for precocious puberty did not alter any of the above results.

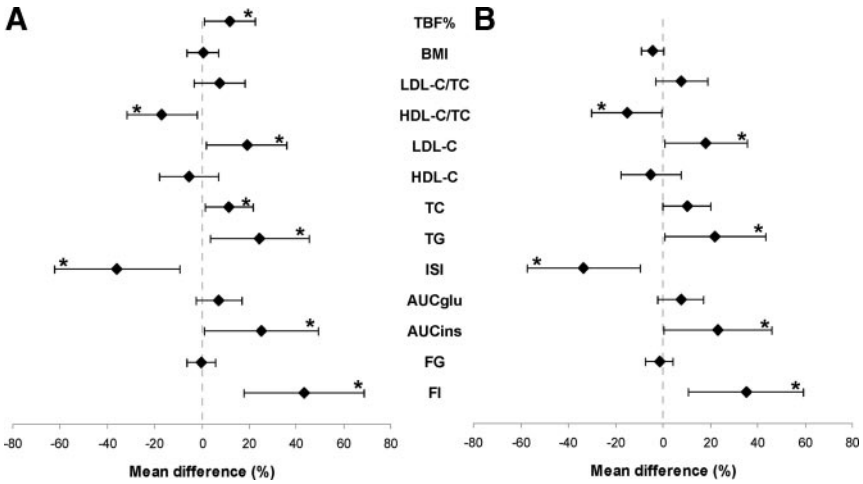

FIG. 1. Main outcome measures in girls with CPP. Data are shown as mean differences and 95% confidence intervals compared with controls after adjustment for pubertal stage (A) and pubertal stage and total body fat percentage (TBF%) (B). *, $P \leq 0.05$ *vs.* controls. BMI, Body mass index; FI, fasting insulin; FG, fasting glucose; AUCins, area under curve for insulin; AUCglu, area under curve for glucose. (30).

Age at puberty

We explored the possible relationship between pubertal timing and metabolic risk factors during early puberty by evaluating associations between age at onset of breast development and the metabolic parameters for a given pubertal stage. We found that age at pubertal onset was a significant negative predictor of fasting insulin levels, first-phase insulin release, and TG levels and a positive predictor of insulin sensitivity for a given stage of breast stage (Fig. 3). No other metabolic or body composition parameters were associated with age at pubertal onset.

Effects of GnRHa

Longitudinal changes in reproductive hormone levels, body composition, and metabolic parameters during 1 yr of GnRHa treatment are shown in Table 2. The advancement in bone age decreased significantly over the treatment period [−0.34 yr (−0.69 to 0.00), $P = 0.05$]. All patients were adequately suppressed in basal and GnRH-stimulated

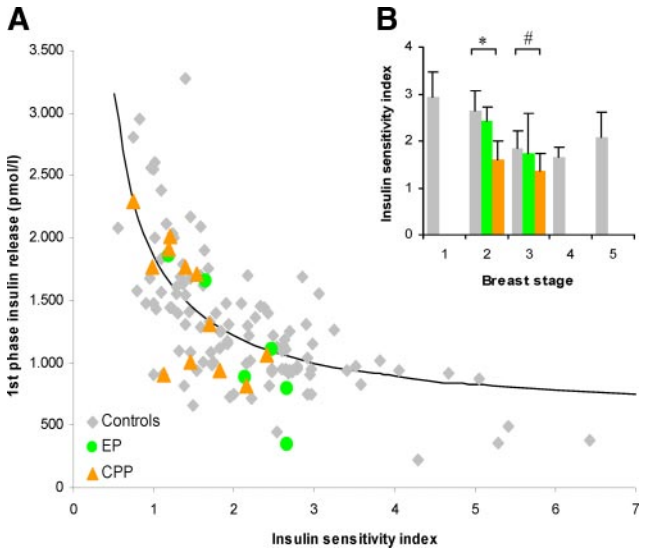

FIG. 2. Insulin sensitivity and insulin secretion in 18 patients with EP or idiopathic CPP and 115 controls. A, Insulin secretion in relation to insulin sensitivity. *Solid black line* represents the inverse relationship on controls ($R^2 = 0.43$, $P < 0.001$). No difference was found in insulin secretion after adjustment for insulin sensitivity between EP or CPP *vs.* controls ($P > 0.10$). B, Mean insulin sensitivity levels according to breast stages. *Error bars* represent 95% confidence interval. Significant lower insulin sensitivity was found in girls with CPP *vs.* controls in breast stage 2 (*, $P = 0.003$) but not in breast stage 3 (#, $P = 0.26$). In a total analysis, insulin sensitivity was significantly lower in girls with CPP *vs.* controls [35.8% (9.7–61.9), $P = 0.008$] but not in girls with EP *vs.* controls after adjustment for stage of breast development.

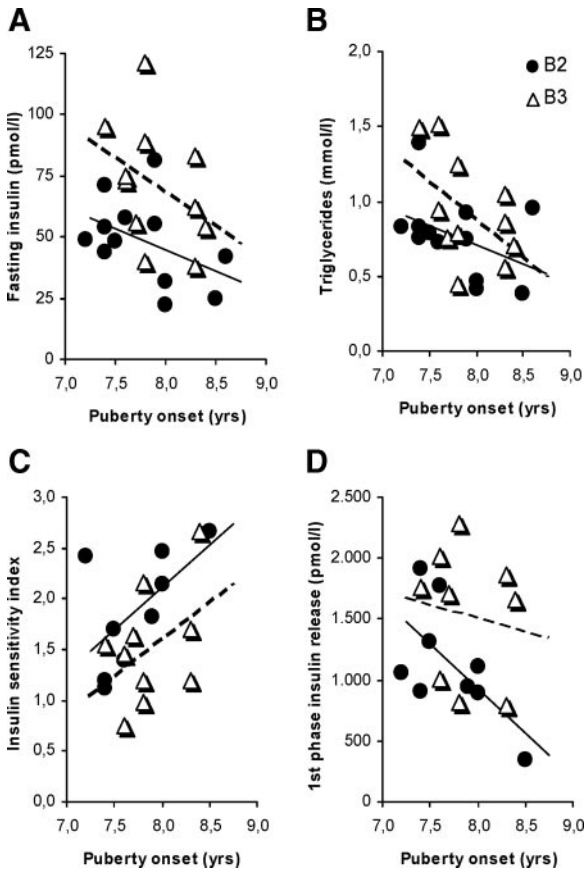

FIG. 3. Insulin parameters in relation to timing of pubertal onset in 23 patients with EP or CPP. *Solid black circles* (●) and *open triangles* (△) represent breast stages 2 and 3, respectively. Linear regression lines are shown as *solid lines* for breast stage 2 and *punctuated lines* for breast stage 3. A, Fasting insulin levels. B, TG levels. C, ISI (30). D, First-phase insulin secretion (31). Age at pubertal onset was positively associated with fasting insulin levels, TG levels, and insulin secretion and negatively associated with insulin sensitivity after adjustment for pubertal stage (all $P \leq 0.04$). To convert insulin levels from picomoles per liter to microunits per milliliter, divide by a factor 6. To convert ISI into the original scale calculated by glucose in milligrams per deciliter and insulin in microunits per milliliter, multiply by a factor 3.

gonadotropin levels as well as estradiol levels after the initial 12 wk of treatment. No significant changes in metabolic or body compositional parameters were observed during this period (Fig. 4). From 12 to 52 wk of treatment, fasting insulin, TG levels, and total body fat percentage increased significantly, whereas insulin sensitivity decreased significantly (Fig. 4). No significant changes were observed in the DI. All patients remained glucose tolerant (2 h glucose level < 7.8 mmol/liter) during treatment. A large absolute gain in body fat percentage from 12 to 52 wk of treatment was significantly correlated with a low absolute increase in fasting insulin levels ($r = -0.67$, $P = 0.049$) and first-phase insulin release ($r = -0.72$, $P = 0.045$) but not with insulin sensitivity or SHBG levels.

Discussion

In the present study, we have shown that girls with CPP have low insulin sensitivity and adverse lipid profiles at the time of diagnosis compared with normally timed pubertal controls. These differences were not solely accounted for by the concomitant higher adiposity found in girls with CPP. In addition, age at pubertal onset positively predicted insulin sensitivity during the early stages of puberty. Interestingly, during the 1-yr follow-up period on GnRHa treatment, insulin sensitivity decreased even further, and total body fat percentage increased in girls with CPP, despite complete gonadal suppression.

Early menarcheal age is associated with increased cardiovascular morbidity and mortality in adulthood (5, 6, 10). However, recent evidence suggests that this association may be primarily ascribed to higher childhood adiposity (11). Nevertheless, early maturational timing *per se* also seems to contribute to this adverse metabolic programming (7, 12). Metabolic risk profiles have not previously been evaluated in girls with CPP before and during GnRHa treatment, and a recent consensus statement on the use of GnRHa in children have urgently requested such data to be collected (33).

Girls with CPP had similar body mass index but significantly increased total body fat percentage by dual-energy x-ray absorptiometry, compared with normally

TABLE 2. Changes in reproductive hormone levels and metabolic parameters during 1 yr of GnRHa treatment

	Baseline visit (n = 15)	12 wk visit (n = 15)	52 wk visit (n = 9)
Breast stage	III (II–III)	II (I–III)[a]	I (I–III)[a]
BMI (kg/m^2)	18.1 (15.5–22.4)	17.4 (14.7–23.8)	18.6 (15.9–20.7)[a,b]
TBF (%)	23.8 (19.1–34.9)	24.1 (19.3–39.1)	29.2 (21.1–37.1)[a,b]
FSH (U/liter)	4.2 (0.4–11.8)	0.6 (0.2–1.5)[a]	1.1 (0.2–2.2)[a,b]
LH (U/liter)	0.4 (0.1–4.2)	0.2 (0.1–0.9)	0.2 (0.1–1.1)
Peak FSH (U/liter)	10.1 (5.2–34.2)	0.8 (0.3–5.0)[a]	1.2 (0.2–2.8)[a,b]
Peak LH (U/liter)	12.5 (3.9–61.7)	0.6 (0.3–1.9)[a]	0.5 (0.1–1.9)[a]
E2 (pmol/liter)	36 (<dL–205)	<dL (<dL–48)[a]	<dL (<dL–35)[a]
SHBG (mmol/liter)	70 (28–158)	64 (28–103)[a]	51 (27–132)[a]
FI (pmol/liter)	58 (38–121)	63 (24–157)	85 (46–103)[a,b]
FG (mmol/liter)	4.9 (4.1–5.3)	4.8 (4.2–5.2)	4.8 (4.4–5.8)
MPI (pmol/liter)	308 (161–569)	321 (121–742)	472 (185–545)[a]
MPG (mnol/liter)	6.4 (5.2–7.6)	6.3 (4.6–7.5)	6.2 (4.9–8.3)
WBISI	1.45 (0.75–2.67)	1.47 (0.63–4.13)	1.00 (0.85–2.51)[b]
First IR (pmol)	1309 (794–2289)	1223 (839–2559)	1590 (1004–2527)[a]
DI	2110 (1010–2635)	1926 (1298–3890)	1858 (1027–2835)
TG (mmol/liter)	0.79 (0.45–1.51)	0.60 (0.50–1.52)	0.88 (0.61–1.50)[b]
TC (mmol/liter)	4.2 (2.8–5.4)	4.4 (2.7–5.1)	4.0 (3.0–4.9)
HDL-C (mmol/liter)	1.5 (0.9–1.8)	1.5 (1.0–1.7)	1.5 (1.2–2.0)
LDL-C (mmol/liter)	2.4 (1.4–3.8)	2.5 (1.5–3.4)	2.3 (0.8–3.1)

Patients were evaluated at baseline and after 11.9 (10.2–12.3) and 51.9 (48.9–55.1) wk of treatment. Results are presented as medians (range). BMI, Body mass index; TBF, total body fat; E2, estradiol; FI, fasting insulin; FG, fasting glucose; MPI, mean plasma insulin (OGTT); MPG, mean plasma glucose (OGTT); WBISI, whole-body ISI (30); first IR, first-phase insulin release (31); <dL, below detection limit. To convert glucose from nanomoles per liter to milligrams per deciliter, multiply by a factor 18. To convert insulin from picomoles per liter to microunits per milliliter, divide by a factor 6. To convert ISI into the original scale calculated by glucose in milligrams per deciliter and insulin in microunits per milliliter, multiply by a factor 3.

[a] $P < 0.05$ *vs.* baseline visit.

[b] $P < 0.05$ *vs.* 12-wk visit. *P* values by Wilcoxon signed rank tests.

timed pubertal controls. Increased adiposity in girls with CPP is consistent with previous studies (16, 17). To what extent the increased adiposity is the cause or the result of early pubertal onset could not be determined in our study due to the fact that puberty was already in progress at the initial visit. High adiposity in childhood is associated with earlier onset of puberty (34) as well as earlier menarche (12, 13). In addition, greater gain in adiposity markers has been shown in adolescence (12, 13) and adulthood (7) in girls with early compared with late menarche. Thus, the relationship between adiposity and pubertal timing seems to be causally bidirectional.

Although the association between early maturation and increased cardiovascular risk may be primarily ascribed to greater adiposity, this does not adequately explain the association (5, 6). Thus, additional factors influencing both pubertal timing and cardiovascular disease risk, beyond the effect of adiposity, may be involved. Insulin could be such a factor. High levels of insulin predict adverse cardiovascular risk factors independent of adiposity already in childhood and adolescence (20). In addition, early menarche is associated with higher insulin levels in childhood (13) as well as with a greater rate of change in insulin levels in early adolescence (12) compared with late menarche. Thus, the relationship between insulin and pubertal timing may also be causally bidirectional. In the present study, insulin sensitivity was decreased in girls with CPP compared with puberty-matched controls. In addition, earlier age at pubertal onset predicted lower insulin sensitivity for a given stage of puberty in girls with CPP and EP. Thus, the earlier the pubertal onset, the lower the insulin sensitivity going into puberty or the steeper the fall in insulin sensitivity during puberty (or a combination of both). The lower insulin sensitivity in the earliest maturing girls was adequately compensated for by higher insulin secretion. In addition, the lower insulin sensitivity in girls with CPP was not solely accounted for by the concomitantly higher adiposity compared with puberty-matched controls. Thus, insulin sensitivity may play a central and independent role in the relationship between early pubertal timing and adverse metabolic risk.

Although OGTT for determination of insulin sensitivity is less accurate than performing hyperinsulinemic-euglycemic clamps, our findings are consistent across different estimates of glucose metabolism. In addition, the use of OGTTs is potential applicable to general clinical practice.

Although early maturational timing is associated with adverse body composition and insulin levels, the influence on lipid profiles is more ambiguous (12, 13). In the present study, the majority of the lipid parameters were adversely

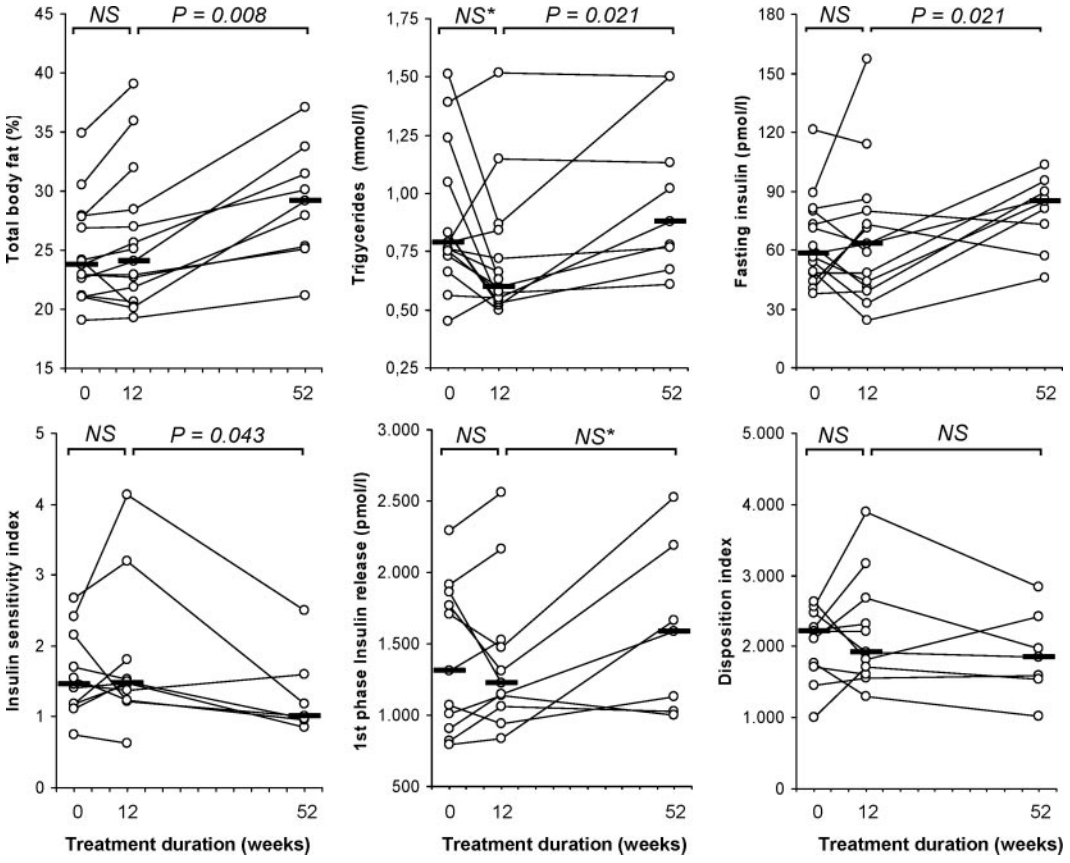

FIG. 4. Longitudinal changes in total body fat percentage, TG levels, and glucose homeostasis during 1 yr of GnRHa treatment. Patients were evaluated at baseline and after 11.9 (10.2–12.3) and 51.9 (48.9–55.1) wk of treatment. Measurements are presented as *open black circles* (○) and connected by *thin black lines* for each patient. Medians are shown as *black bars*. *P* values are by Wilcoxon signed ranks tests. NS, Nonsignificant ($P > 0.10$). NS*, $P = 0.06$. To convert glucose from nanomoles per liter to milligrams per deciliter, multiply by a factor 18. To convert insulin from picomoles per liter to microunits per milliliter, divide by a factor 6. To convert ISI into the original scale calculated by glucose in milligrams per deciliter, and insulin in microunits per milliliter multiply by a factor 3.

affected in girls with CPP compared with puberty-matched controls. In addition, these adverse differences were not accounted for by differences in total body fat percentage. Although adjustment for insulin sensitivity weakened most of the differences in lipid levels, the tendencies were still present, indicating that additional factors is most likely involved.

Changes in body composition and glucose homeostasis are tightly linked to sexual maturation. Total body fat percentage is relatively stable in childhood but increases in girls during adolescence (29). In addition, insulin sensitivity follows a curvilinear pattern with nadir around mid-puberty (25, 35). To determine the influence of gonadal suppression on metabolic risk profile, we reevaluated all the metabolic parameters 12 and 52 wk after initiation of GnRHa treatment. Basal and stimulated gonadotropin levels as well as estradiol levels were markedly suppressed beyond 12 wk of treatment. Thus, if sex steroids were implicated in the adverse metabolic programming of early puberty, one would intuitively expect a regression toward prepubertal levels or at least arrest in the metabolic parameters during treatment beyond this point. By contrast, we found that the increased adiposity at time of diagnosis worsened after sex steroid withdrawal, which is in accordance with previous studies (16, 17). Concomitantly, fasting insulin and triglyceride levels increased and insulin sensitivity decreased. Thus, several of the adversely affected metabolic parameters evident at time of diagnosis deteriorated even further despite sex steroid withdrawal and regression in clinical signs of puberty. This speaks against a strong influence of sex steroids, neither directly nor through central feedback mechanism, on changes in glucose homeostasis or body composition during puberty, in accordance with previous longitudinal studies (16, 35, 36). In addition, the lack of metabolic changes during the initial 12 wk of treatment also speaks against a direct effect of the GnRH agonists. However, significant changes in body composition may take longer than 12 wk to be fully manifested.

The observed changes are consistent with pubertal progression in regard to glucose homeostasis and body composition not suppressed by GnRHa treatment. GnRHa primarily desensitizes the pituitary gonadotrophs to the increasing hypothalamic GnRH stimulation during puberty, thereby decreasing gonadotropin secretion, which

in turn decreases gonadal sex steroid secretion (37). Thus, with the exception of altered sex steroid feedback, GnRHa treatment may not influence upstream neural networks responsible for the coordination of the reproductive and metabolic aspects of pubertal maturation. In this respect, hypothalamic neurons expressing neuropeptide Y and kisspeptin are both critically involved in central modulation of GnRH secretion as well as modulation of adiposity and glucose homeostasis in response to peripheral metabolic clues such as insulin and leptin (19, 38). Relieving the metabolic pressure on these neural networks by increasing insulin sensitivity and thereby lowering insulin and leptin levels may prove beneficial as an adjuvant to GnRHa treatment in girls with CPP. In accordance, prepubertal and pubertal insulin-sensitizing therapy has been associated with favorable effects on pubertal onset and progression and adiposity and adipocytokine profiles both during and after completion of puberty in girls at risk of early puberty (23, 24, 39). However, data on metabolic risk factors and body composition in girls previously treated with GnRHa are sparse and ambiguous (18, 40). Thus, further studies are needed to accurately determine whether these metabolic alterations persist after discontinuation of GnRHa treatment and completion of pubertal maturation.

In summary, we presented novel data on metabolic risk profiles in girls with CPP and found that these girls have lower insulin sensitivity and adverse lipid levels at the time of diagnosis compared with normally timed pubertal controls. In addition, we found that the younger the age at onset of puberty, the more adverse insulin sensitivity and TG levels were affected during the early stages of puberty. During 1 yr follow-up on GnRHa treatment, the adverse metabolic profiles deteriorated even further in the girls with CPP despite suppression of gonadotropin and sex steroid levels. Thus, treatment with GnRH agonists does not seem to prevent the adverse metabolic state of puberty in girls with CPP, and it remains to be seen whether other adjunct treatment strategies (such as insulin sensitizing therapy) may prove beneficial in these early maturing girls.

Acknowledgments

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