Metformin Treatment for Four Years to Reduce Total and Visceral Fat in Low Birth Weight Girls with Precocious Pubarche

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Context and Objective: A low birth weight (LBW) tends to be followed by overweight due to an excess of fat, including visceral fat. LBW girls with precocious pubarche (PP) (pubic hair < 8 yr) are at high risk for developing an adipose state of hyperinsulinemic androgen excess that leads toward early menarche. We explored the effects of insulin sensitization with metformin in LBW-PP girls.

Setting, Design, Patients, Intervention: Prepubertal LBW girls with PP (mean body weight 2.4 kg; age 7.9 yr; body mass index 18.4 kg/m^2) were studied. Girls were randomly assigned to remain untreated (n=19) or receive metformin for 4 yr (n = 19; 425 mg/d for 2 yr, then 850 mg/d for 2 yr).

Main Outcomes: At the start and after 4 yr, height, weight, fasting insulin, glucose, IGF-I, testosterone, lipids, leptin, high molecular weight adiponectin, body composition by absorptiometry, abdominal fat partitioning (only 4 yr) by magnetic resonance imaging, and menarcheal status were determined.

Results: Metformin-treated girls gained on average 5.5 kg (or ~50%) less fat, after 4 yr were less insulin resistant and less hyperandrogenic, had lower IGF-I levels and a less atherogenic lipid profile, and were less likely to be post-menarcheal than untreated girls, whereas their gain in height, lean mass, and bone mineral density were similar. After 4 yr, untreated girls had more visceral fat, a higher ratio of visceral-to-sc fat, and a higher leptin-to-high molecular weight adiponectin ratio (all ~50% higher) than metformin-treated girls.

Conclusion: Long-term metformin treatment appears to reduce total and visceral fat in LBW-PP girls, and to delay menarche without attenuating linear growth, thereby opening the perspective that adult height may be increased. (***J Clin Endocrinol Metab*** **93: 1841–1845, 2008)**

In the human species, prenatal growth restraint is usually followed by a postnatal catch-up of growth. Longitudinal studies disclosed that prenatal growth restraint, as judged by a low birth weight (LBW) for gestational age, is in early childhood often followed by an exaggerated weight gain (1) due to an excessive gain of fat (2), including visceral fat (3).

LBW girls with precocious pubarche (PP) (pubic hair < 8 yr) are known to be at particular risk for developing an adipose state of hyperinsulinemic androgen excess that leads toward an early menarche with growth arrest (4–6). After using metformin in post-PP adolescents (7, 8) and in post-menarcheal LBW-PP girls (9), we initiated a first randomized study exploring the early and prolonged use of metformin in LBW-PP girls, thereby focusing on body composition and on pubertal growth and maturation. We reported the prepubertal results after 6-month study (10) and the first pubertal results after 2 yr (11); here, we unveil the first post-menarcheal results after a 4-yr study.

Subjects and Methods

Subjects and ethics

The study population consisted of 38 LBW-PP girls, distributed into two well-matched subgroups of 19 girls each, whose baseline characteristics

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Abbreviations: BM, Body mass index; DHEAS, dehydroepiandrosterone sulfate; HMW, high molecular weight; HOMA, homeostasis model assessment; LBW, low birth weight; MRI, magnetic resonance imaging; PP, precocious pubarche.

were reported (10). In the total population, birth weight (mean ± SEM) was 2.4 ± 0.1 kg after 38.6 ± 0.4 wk, age at diagnosis of PP was 6.8 ± 0.2 yr, age at study start 7.9 ± 0.1 yr, bone age 9.0 ± 0.1 yr, height 129.4 ± 1.2 cm, weight 31.0 ± 0.9 kg, body mass index (BMI) 18.4 ± 0.3 kg/m^2, dehydroepiandrosterone sulfate (DHEAS) at PP diagnosis 102 ± 6 μg/dl, and post-ACTH 17-hydroxyprogesterone 274 ± 16 ng/dl.

As described (10), the inclusion criteria were: 1) PP due to exaggerated adrenarche, as judged by high-serum DHEAS and/or androstenedione levels; 2) weight less than 2.9 kg at term birth (38–41 wk) or below −1 SD for gestational age at preterm birth (33–37 wk); 3) BMI less than 22 kg/m^2, which corresponds to the +2 SD cutoff in girls aged approximately 8 yr (12); and 4) prepuberty (Tanner B1). None of the girls had a family or personal history of diabetes mellitus, or presented evidence for thyroid dysfunction, glucose intolerance, or late-onset congenital adrenal hyperplasia; none was receiving a medication known to affect gonadal function or carbohydrate metabolism.

The study was registered as ISRCTN84749320 and was approved by the Institutional Review Board of Barcelona University, Hospital of Sant Joan de Déu. Informed consent was obtained from parents and assent from the girls.

Study design and assessments

Girls were randomly assigned, as described (10, 11), to remain untreated or receive metformin, once daily at dinnertime (425 mg for 2 yr, then 850 mg for 2 yr).

Clinical examination was performed six monthly by the same investigator (L.I.); assessment of serum insulin, fasting blood glucose, SHBG, testosterone, DHEAS, a lipid profile, and body composition were each performed six monthly for 2 yr, and yearly thereafter. Serum leptin was measured at 0, 2, and 4 yr, and high molecular weight (HMW) adiponectin [the physiologically most relevant isoform of this adipokine (13)] was assessed at 0 and 4 yr. Height was measured with a Harpenden stadiometer (Holtain Ltd., Crosswell, Crymych, UK). Menarcheal timing was derived by six-monthly history.

Body composition was assessed by dual-energy x-ray absorptiometry with a Lunar Prodigy coupled to Lunar software (Lunar Corp., Madison, WI), as described (11).

After 4 yr, sc and visceral adipose tissue areas in the abdominal region were assessed by magnetic resonance imaging (MRI) using a multiple-slice MRI 1.5 Tesla scan (Signa LX Echo Speed Plus Excite; General Electric, Milwaukee, WI), as described (3). All scans were performed by

TABLE 1. Clinical, endocrine-metabolic, and body composition indices in prepubertal girls (age ~8 yr) with a combined history of LBW and PP

	Untreated			Metformin		
	Start[a]	4 yr	Δ 0–4 yr	Start[a]	4 yr	Δ 0–4 yr
Height (cm)	130 ± 2	156 ± 2[b]	26 ± 1	129 ± 2	155 ± 2[b]	26 ± 1
Weight (kg)	31 ± 1	55 ± 3[b]	24 ± 2	31 ± 1	50 ± 2[b]	19 ± 1[c]
BMI (Z score)	1.2 ± 0.3	1.3 ± 0.4[b]	0.1 ± 0.3	1.4 ± 0.4	0.6 ± 0.3[b]	−0.8 ± 0.2[d]
IGF-I (ng/ml)	215 ± 10	555 ± 22[b]	340 ± 25	197 ± 11	443 ± 20[b]	247 ± 24[c]
Fasting insulin (μU/ml)	8.2 ± 0.6	18.3 ± 1.9[b]	10.1 ± 2.0	8.6 ± 0.9	13.4 ± 1.3[b]	4.8 ± 1.0[c]
HOMA-IR	1.8 ± 0.1	4.2 ± 0.5[b]	2.5 ± 0.5	1.9 ± 0.2	3.2 ± 0.3[b]	1.3 ± 0.2[c]
SHBG (μg/dl)	1.6 ± 0.1	1.0 ± 0.1[b]	−0.6 ± 0.1	1.5 ± 0.1	1.0 ± 0.1[b]	−0.5 ± 0.1
DHEAS (μg/dl)	95 ± 9	185 ± 14[b]	91 ± 14	104 ± 10	168 ± 18[b]	64 ± 14
Testosterone (ng/dl)	28 ± 3	70 ± 10[e]	42 ± 10	32 ± 3	47 ± 4[e]	16 ± 4[c]
LDL-cholesterol (mg/dl)	102 ± 6	97 ± 6	−5 ± 6	107 ± 7	91 ± 5[b]	−17 ± 3
HDL-cholesterol (mg/dl)	61 ± 3	49 ± 2[e]	−12 ± 3	60 ± 3	57 ± 2	−3 ± 2[c]
Triglycerides (mg/dl)	63 ± 7	88 ± 13[f]	25 ± 9	74 ± 10	76 ± 11	1 ± 4[c]
Leptin (ng/ml)	19.5 ± 1.4	16.2 ± 1.3[f]	−3.2 ± 1.6	20.7 ± 2.0	13.4 ± 1.6[e]	−7.3 ± 1.8
HMW adiponectin (mg/liter)	9.1 ± 1.0	4.8 ± 0.4[b]	−4.3 ± 0.8	9.0 ± 1.0	6.9 ± 1.2	−2.1 ± 1.3
Leptin-to-HMW adiponectin	2.8 ± 0.5	4.0 ± 0.5[b]	1.2 ± 0.6	2.8 ± 0.4	2.9 ± 0.6	0.2 ± 0.7[c]
BMD (g/cm^2)	0.73 ± 0.02	1.01 ± 0.03[b]	0.28 ± 0.02	0.75 ± 0.02	1.05 ± 0.03[b]	0.30 ± 0.03
Lean mass (kg)	19.6 ± 0.5	31.6 ± 1.1[b]	12.0 ± 0.8	19.7 ± 0.7	31.9 ± 1.1[b]	12.2 ± 0.5
Fat mass (kg)	10.3 ± 0.9	20.6 ± 1.7[b]	10.3 ± 1.2	10.8 ± 1.0	15.7 ± 1.1[b]	4.8 ± 0.7[g]
Subcutaneous fat (cm^2)		120 ± 12			129 ± 13	
Visceral fat (cm^2)		41 ± 4			28 ± 2[h]	
Visceral-to-sc fat		0.38 ± 0.03			0.24 ± 0.02[i]	
Menarche		18/19			11/19[j]	

Girls were randomized to remain untreated (n = 19) or receive treatment with metformin (n = 19) for 4 yr. Values are mean ± SEM. To convert units to International System of Units: multiply the concentrations of testosterone by 0.03467 and of DHEAS by 0.02714; and divide the concentrations of SHBG by 0.0288, those of triglycerides by 88.5, and those of high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol by 38.7. BMD, Bone mineral density; HDL, high-density lipoprotein; HOMA-IR, HOMA insulin resistance; LDL, low-density lipoprotein.

[a] No significant differences between randomized subgroups at the start.

[b] $P \leq 0.0001$ *vs.* start.

[c] $P < 0.05$ for 0- to 4-yr change (Δ) *vs.* untreated.

[d] $P \leq 0.01$ for 0- to 4-yr change (Δ) *vs.* untreated.

[e] $P \leq 0.001$ *vs.* start.

[f] $P < 0.05$ *vs.* start.

[g] $P \leq 0.001$ for 0- to 4-yr change (Δ) *vs.* untreated.

[h] $P < 0.05$ *vs.* untreated after 4 yr.

[i] $P < 0.0001$ *vs.* untreated after 4 yr.

[j] $P < 0.01$ *vs.* untreated after 4 yr.

the same operator (blinded to treatment allocation), and all images were analyzed by the same radiologist (also blinded to treatment allocation).

Variations in dietary intake and physical activity level were assessed yearly with validated questionnaires (14, 15). Frequency and quantity of food intake were recorded by each girl, under parental guidance, for 7 d before the annual control visit (14). Assisted by another questionnaire, parents reported the daily activities of their child during the week before the annual control visit. Girls were classified into one of four activity levels (15): 1, low (no regular physical activity); 2, moderate (sporadically involved in physical activities); 3, high (recreational activity $\geq$ 3 times per week, 30–60 min per session); and 4, vigorous (intense physical activity $\geq$ 4 times per week, $>$ 60 min per session).

Hormone assays, calculations, and statistics

Serum glucose was measured by the glucose oxidase method. Serum immunoreactive insulin, SHBG, testosterone, DHEAS, and IGF-I were assayed as described (8, 9). Fasting insulin sensitivity was estimated from fasting insulin and glucose levels using the homeostasis model assessment (HOMA) (HOMA-CIGMA Calculator program v2.00; Diabetes Research Laboratory, Oxford, UK). Leptin was measured by RIA (LINCO Research, Inc., St. Charles, MO), and HMW adiponectin was measured by sandwich ELISA (LINCO Research), as described (16, 17). Samples from both subgroups were kept frozen until assay and were assayed concomitantly.

Statistical analyses were performed with SPSS 12.0 (SPSS, Inc., Chicago, IL). *t* tests were used to compare the changes within each subgroup and the 0- to 4-yr changes between subgroups. Differences in longitudinal data between subgroups were tested by repeated measures ANOVA. The between-subgroup difference in menarcheal prevalence was assessed by χ^2. For uniformity, all results are expressed as mean $\pm$ SEM. The level of statistical significance was set at $P < 0.05$.

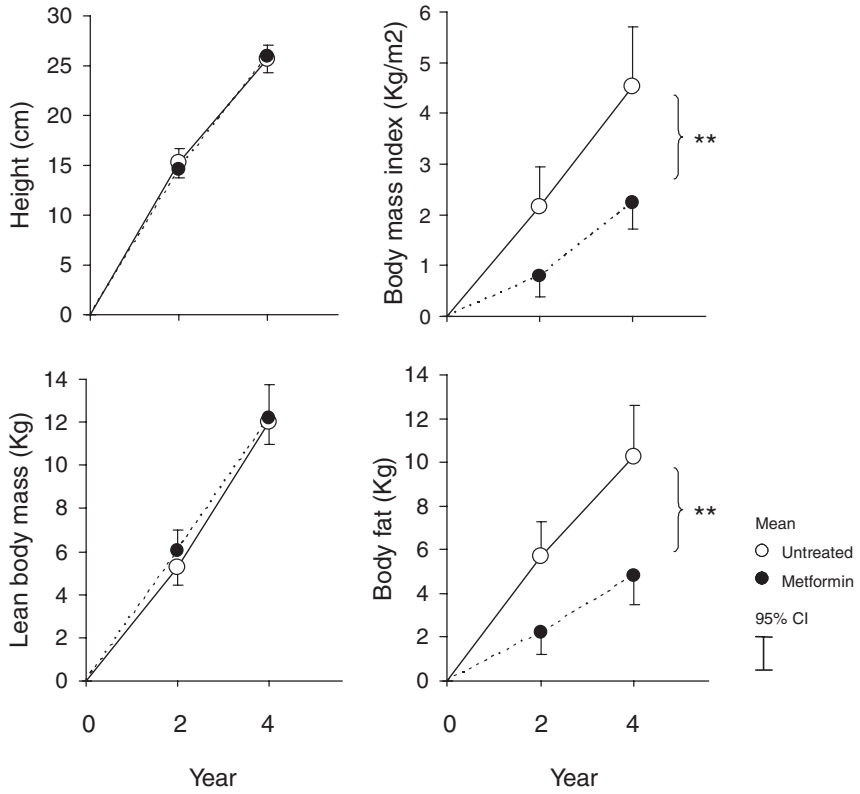

FIG. 1. Gain in height (*top left*), BMI (*top right*), lean mass (*bottom left*), and body fat (*bottom right*) in LBW-PP girls who were randomized to remain untreated (n = 19) or receive metformin (n = 19) for 4 yr. Metformin treatment was accompanied by a lower increment of body fat. Means $\pm$ 95% confidence interval (CI) are shown. **, $P < 0.005$ for longitudinal differences between subgroups.

Results

Table 1 summarizes the main results. Metformin-treated girls gained approximately 50% less fat (Fig. 1), after 4 yr were less insulin resistant and hyperandrogenic, had lower IGF-I levels and a less atherogenic lipid profile, and were less likely to be post-menarcheal than untreated girls, whereas their gain in height, lean mass, and bone mineral density were similar. After 4 yr, untreated girls had more visceral fat, a higher ratio of visceral-to-sc fat (Fig. 2), and a higher leptin-to-HMW adiponectin ratio (all ~50% higher) than metformin-treated girls. Between 42 and 48 months, annualized height velocity started to be lower in untreated (and mostly post-menarcheal) girls than in treated girls (4.0 $\pm$ 0.8 cm/yr *vs.* 5.0 $\pm$ 0.6 cm/yr; $P = 0.04$), an observation corroborating the perspective that metformin treatment may be followed by a taller stature in adulthood.

All girls had a so-called Mediterranean diet, their meal frequency and food quantity being comparable in the two subgroups and not varying detectably over the 4-yr study. The physical activity levels were similar in the untreated and metformin-treated girls, both at study start (2.4 $\pm$ 0.1 *vs.* 2.5 $\pm$ 0.1) and after 4 yr (2.5 $\pm$ 0.1 *vs.* 2.6 $\pm$ 0.1), and remained essentially unchanged during the study.

Metformin was well tolerated. Pill counts at each study visit indicated that the treatment compliance was good, the mean number of missed doses being 1 per month. Indices of hepatic and renal function remained unchanged throughout treatment. None of the patients dropped out of the study.

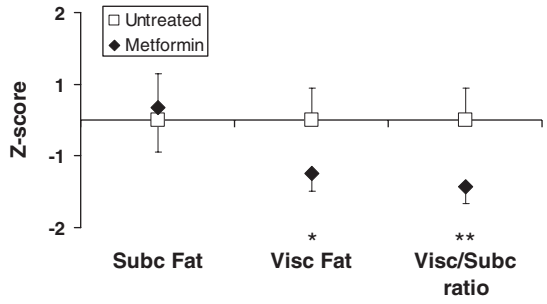

FIG. 2. Abdominal fat partitioning, assessed by abdominal MRI, in LBW-PP girls who were randomized to remain untreated (n = 19) or receive metformin (n = 19). Results after 4 yr are shown as Z scores, calculated by dividing the individual values by the corresponding baseline SD in the untreated subgroup. Metformin treatment was accompanied by a lower amount of visceral fat and by a strikingly lower ratio of visceral-to-sc fat. *Plots* represent means $\pm$ 95% confidence interval. *, $P \leq 0.05$ and **, $P \leq 0.0001$ for differences between subgroups. Subc, sc; Visc, visceral.

Discussion

LBW-PP girls tend to gain too much fat; their fat excess being a reflection of their hyperinsulinemia and hyperandrogenemia (5). In the present study, after 4 yr the increases of the insulin and testosterone levels in the untreated girls were about twice as high as in metformin-treated girls, as was their mean rate of fat deposition: 7.0 g/d in untreated girls *vs.* only 3.2 g/d in metformin-treated girls. So far, metformin had no detectable effect on BMD increase, lean mass accretion, or cumulative height gain over 4 yr. However, 18 of 19 untreated girls have already reached a postmenarcheal stage of maturation, whereas this is the case for only 11 of 19 metformin-treated girls. In addition, untreated girls started to have a lower height velocity than metformin-treated girls between 42 and 48-month study. Therefore, metformin treatment in LBW-PP girls may still prove to increase adult stature, just as pubertal metformin treatment (for 3 yr) in LBW girls with early-normal puberty proved to delay menarche, prolong growth, and augment adult height (16, 18).

After a 4-yr study, untreated LBW-PP girls had an abdominal ratio of visceral-to-sc fat that was elevated, certainly when compared with such a ratio in 12-yr-old girls with simple overweight or obesity (19, 20). Reassuringly, after 4 yr, metformin treatment was accompanied by a lower amount of visceral fat and by a strikingly lower ratio of visceral-to-sc fat in the abdomen, and, thus, by an evolution toward a more favorable metabolic phenotype (20).

The mechanisms underpinning the apparent effects of metformin in LBW-PP girls are poorly understood. Among the long-known actions of metformin are an improvement in insulin sensitivity in muscle and liver, a decrease in hepatic glucose production via gluconeogenesis, an increase in peripheral glucose use (mainly via a stimulation of insulin-mediated muscle glucose uptake and glycogen synthesis), and positive effects on insulin receptor expression and tyrosine kinase activity; in addition, metformin suppresses the gluconeogenic effects of glucagon and increases the translocation of glucose transporters to the cell surface (21, 22). Metformin also appears to have a direct effect on ovarian steroidogenesis, specifically to reduce both androgen and estradiol production (23, 24). LKB1 and AMP-activated protein kinase were recently identified as being among the prime targets of metformin's actions (25, 26). Finally, microarray studies showed that metformin is capable of inducing a hepatic gene expression pattern that mimics the profile induced by calorie restriction (27).

Our long-term study (spanning about one third of the girls' lifetime, so far) had no placebo-controlled design. This limitation implies that the observed reductions in fat gain are attributable either to metformin and/or some placebo effect that, in turn, could be conferred directly by the medication or indirectly, for example, by unreported lifestyle changes in diet and/or exercise.

In conclusion, long-term metformin treatment appears to reduce total and visceral fat markedly in LBW-PP girls and to delay menarche without attenuating linear growth, thereby opening the perspective that adult height may be increased.

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