

Early metformin therapy to delay menarche and augment height in girls with precocious pubarche

Lourdes Ibáñez, M.D., Ph.D.,^a Abel Lopez-Bermejo, M.D., Ph.D.,^b Marta Diaz, Ph.D.,^a Maria Victoria Marcos, M.D.,^c and Francis de Zegher, M.D., Ph.D.^d

^a Endocrinology, Hospital Sant Joan de Déu, University of Barcelona, Barcelona, and CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid; ^b Pediatric Endocrinology, Dr. Trueta Hospital, Girona; and ^c Endocrinology Unit, Hospital de Terrassa, Terrassa, Spain; and ^d Department of Woman and Child, University of Leuven, Leuven, Belgium

Objective: To study the effects of early metformin treatment on menarche, height, and polycystic ovary syndrome (PCOS) markers. Low-birthweight (LBW) girls with precocious pubarche (PP) are at risk for an early menarche (<12 years), an adult stature below target level, and PCOS. Hyperinsulinemic insulin resistance is thought to be a key factor.

Design: Open-label, randomized study.

Setting: University hospital.

Patient(s): Thirty-eight LBW-PP girls.

Intervention(s): At age 8 years, girls were randomly assigned to remain untreated or to receive metformin for 4 years; subsequently, both subgroups were followed without treatment until each girl was postmenarcheal.

Main Outcome Measure(s): Age at menarche, height, weight, endocrine-metabolic state (fasting blood), body composition (by absorptiometry), abdominal fat (subcutaneous vs. visceral), and hepatic adiposity (by magnetic resonance imaging).

Result(s): At last assessment, girls in each subgroup were on average 2 years beyond menarche; the mean growth velocity was below 2 cm/years. Age at menarche was 11.4 ± 0.1 years in untreated girls and 12.5 ± 0.2 years in metformin-treated girls; the latter girls were taller and much leaner (with less visceral and hepatic fat) and had more favorable levels of circulating insulin, androgens, and lipids.

Conclusion(s): Early metformin therapy (age ~8–12 years) suffices to delay menarche; to augment postmenarcheal height; to reduce total, visceral, and hepatic adiposity; and to curb the endocrine-metabolic course of LBW-PP girls away from adolescent PCOS. (Fertil Steril® 2010; ■:■–■. ©2010 by American Society for Reproductive Medicine.)

Key Words: Metformin, menarche, insulin, IGF-I, DHEAS, puberty, pubarche, birthweight, epigenetic, androgen excess, polycystic ovary syndrome, PCOS, obesity, height, adrenarche, lipids, abdominal fat, subcutaneous fat, adiposity, hepatic fat, fatty liver, visceral fat, body composition, lean mass

Girls with precocious pubarche (PP; pubic hair <8 years), in particular those with a birthweight below average for gestational age, tend to develop an early and rapidly progressive puberty that leads to an early menarche (<12 years), to an adult height below target level, and to features of polycystic ovary syndrome (PCOS) (1–4). Hyperinsulinemic insulin resistance with total, visceral, and hepatic adiposity is thought to be a major driver of such early maturation (5, 6), and we therefore tested whether an early insulin-sensitizing intervention (for 4 years, starting at an average age of 8 years) can delay the onset of puberty (7), attenuate body adiposity (8, 9), and normalize the menarcheal timing as well as the postmenarcheal stature and endocrine-metabolic state of such PP girls. For this purpose, we used metformin, an old biguanide whose actions seem to be largely exerted either directly by reducing energy charge (decrease of cellular ATP and concomitant increase of AMP

and/or indirectly by activation of AMP-activated protein kinase, a conserved regulator of the cellular response to low energy (10, 11). Here we report the final results on menarcheal timing along with data on height, endocrine-metabolic state, and body composition in a late stage of adolescent maturation.

MATERIALS AND METHODS

Subjects and Ethics

The study population consisted of 38 PP girls with a birthweight Z-score between -1.0 and -3.0 , distributed in two subgroups of 19 girls, whose baseline characteristics were comparable (Table 1) (7). In the total population, birthweight (mean \pm SEM) was 2.4 ± 0.1 kg after 38.6 ± 0.4 weeks of gestation, age at diagnosis of PP 6.8 ± 0.2 years, age at study start 7.9 ± 0.1 years, bone age 9.0 ± 0.1 years, height 129.4 ± 1.2 cm, weight 31.0 ± 0.9 kg, body mass index (BMI) 18.4 ± 0.3 kg/m², dehydroepiandrosterone sulfate (DHEAS) at PP diagnosis 102 ± 6 μ g/dL, and post-ACTH 17-hydroxyprogesterone 274 ± 16 ng/dL.

As described (7–9), the inclusion criteria were [1] PP due to exaggerated adrenarche, as judged by high serum DHEAS and/or androstenedione levels; [2] low birthweight (LBW) defined as body weight below 2.9 kg at term birth (38–41 weeks) or below -1 SD for gestational age at preterm birth (33–37 weeks); [3] BMI <22 kg/m², which corresponds to the $+2$ SD cutoff in girls aged ~8 years; [4] prepuberty (Tanner B1). None of the girls had a family or personal history of diabetes or presented evidence for thyroid dysfunction, glucose intolerance, or adrenal hyperplasia; none was

Received July 19, 2010; revised August 20, 2010; accepted August 24, 2010.

L.I., M.D., and M.V.M. are Clinical Investigators of CIBERDEM, Madrid, Spain). A.L-B. is an Investigator of the Fund for Scientific Research I3 (Ministry of Education and Science, Spain). F.d.Z. is an Investigator of the Clinical Research Council of the University Hospital Leuven.

Reprint requests: Lourdes Ibáñez, M.D., Ph.D., Endocrinology Unit, Hospital Sant Joan de Déu, University of Barcelona, Passeig de Sant Joan de Déu, 2, 08950 Esplugues, Barcelona, Spain (E-mail: libanez@hsjdbcn.org).

TABLE 1

Clinical, endocrine-metabolic, and body composition indices in girls with a history of LBW-PP who were randomized either to remain untreated or to receive metformin for 4 years and to remain thereafter untreated.

	Untreated (n = 19)			Early Metformin (n = 19)		
	Start ^a	Postmenarche	Change	Start ^a	Postmenarche	Change
Time postmenarche, y	—	1.9 ± 0.2	—	—	1.7 ± 0.3	—
Height gain in past year, cm	—	1.8 ± 0.4	—	—	1.7 ± 0.4	—
Age	8 ± 0.2	13 ± 0.2	5 ± 0.1	8 ± 0.2	14 ± 0.2 ^b	6 ± 0.1 ^c
Distance to target height, ^d cm	32.6 ± 1.3	4.8 ± 1.4	27.8 ± 0.8	32.0 ± 1.9	0.6 ± 1.2 ^b	31.4 ± 1.2 ^e
BMI Z-score	1.2 ± 0.3	1.6 ± 0.4	0.4 ± 0.3	1.4 ± 0.4	0.7 ± 0.3 ^b	-0.7 ± 0.2 ^c
IGF-I, ng/mL	215 ± 10	549 ± 27	334 ± 31	197 ± 11	471 ± 38	275 ± 40
Fasting insulin, μU/mL	8.2 ± 0.6	14.8 ± 1.2	6.5 ± 1.2	8.6 ± 0.9	11.7 ± 1.3 ^b	3.1 ± 1.2 ^e
SHBG, μg/dL	1.6 ± 0.1	0.9 ± 0.1	-0.7 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	-0.5 ± 0.1
T, ng/dL	28 ± 3	66 ± 6	38 ± 7	32 ± 3	46 ± 4 ^f	14 ± 5 ^c
Free androgen index	1.9 ± 0.2	8.4 ± 1.1	6.6 ± 1.2	2.5 ± 0.4	5.2 ± 0.6 ^f	2.7 ± 0.7 ^c
DHEAS, μg/dL	95 ± 9	240 ± 20	145 ± 18	104 ± 10	221 ± 25	117 ± 20
Androstenedione, ng/dL	90 ± 5	326 ± 20	236 ± 21	98 ± 7	293 ± 16	195 ± 16
LDL cholesterol, mg/dL	102 ± 6	95 ± 5	-7 ± 6	107 ± 7	86 ± 6	-21 ± 3 ^e
HDL cholesterol, mg/dL	61 ± 3	49 ± 2	-12 ± 3	60 ± 3	54 ± 2 ^b	-6 ± 2 ^e
LDL-to-HDL cholesterol	1.8 ± 0.2	2.0 ± 0.1	0.2 ± 0.2	1.9 ± 0.2	1.7 ± 0.1 ^b	-0.2 ± 0.1 ^c
Triglycerides, mg/dL	63 ± 7	85 ± 12	22 ± 10	74 ± 10	67 ± 7	-7 ± 8 ^e
Absorptiometry						
BMD, g/cm ²	0.73 ± 0.02	1.11 ± 0.02	0.36 ± 0.02	0.75 ± 0.02	1.17 ± 0.02 ^b	0.41 ± 0.03
Lean mass, kg	19.6 ± 0.5	33.5 ± 1.0	14.0 ± 0.7	19.7 ± 0.7	35.3 ± 0.9	15.6 ± 0.5
Fat mass, kg	10.3 ± 0.9	21.9 ± 1.8	11.5 ± 1.3	10.8 ± 1.0	18.8 ± 1.4	8.0 ± 1.2 ^e
Fat-to-lean mass	0.53 ± 0.04	0.65 ± 0.05	0.12 ± 0.04	0.55 ± 0.05	0.54 ± 0.04 ^b	-0.01 ± 0.04 ^c
Abdominal fat, kg	2.8 ± 0.3	6.7 ± 0.4	3.9 ± 0.4	3.0 ± 0.4	5.3 ± 0.3 ^c	2.3 ± 0.4 ^c
Abdominal MRI						
SC fat, cm ²	—	136 ± 16	—	—	138 ± 17	—
Visceral fat, cm ²	—	49 ± 5	—	—	33 ± 2 ^f	—
Intrahepatic lipid content, %	—	17 ± 2	—	—	11 ± 2 ^b	—

Note: Results are shown at study start and at a comparable stage of postmenarcheal maturation, when adult stature is almost attained. Values are mean ± SEM. To convert SEM into SD, multiply the SEM values by 4.36. SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone-sulfate; BMD, bone mineral density; MRI, magnetic resonance imaging. Free androgen index was calculated as T × 100/SHBG.

To convert units into SI, divide the concentrations of SHBG by 0.0288, those of LDL cholesterol and HDL cholesterol by 38.7, and those of triglycerides by 88.5; multiply the concentrations of DHEAS by 0.02714 and those of T by 0.03467.

^a No significant differences between randomized subgroups at start.

^b $P < .05$, ^c $P \leq .01$ between subgroups for changes from study start until postmenarche.

^d Target height was calculated as midparental height, adjusted for gender (paternal height - 13 cm) and secular trend (+ 2 cm).

^e $P < .05$, ^f $P \leq .01$ between postmenarcheal subgroups.

Ibáñez. Metformin to delay menarche and augment height. *Fertil Steril* 2010.

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 was obtained from girls. The results after 2, 4, and 5 years of study have been reported (7–9).

Study Design and Assessments

Girls were randomly assigned (12) to remain untreated for 4 years or to receive metformin for 4 years, once daily at dinner time (425 mg for 2 years, then 850 mg for 2 years) (7, 8). Thereafter, all girls were followed without intervention until all the girls of their subgroup had experienced menarche, for a total duration of 5 years in control girls and 6 years in metformin-treated girls (see below).

Clinical examination, including height measurement with a Harpenden stadiometer, was performed every 6 months by the same investigator (LI). Age at menarche was derived by 6-month history.

Assessment of fasting insulin, IGF-I, sex hormone binding globulin (SHBG), DHEAS, androstenedione, T and lipid profile, and body composition were each performed every 6 months for 2 years and yearly thereafter.

Body composition was assessed by dual-energy X-ray absorptiometry with a Lunar Prodigy coupled to Lunar software (Lunar Corp., Madison, WI) (13).

From year 5 of the study onward, SC and visceral fat 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) (9). MRI was then also used to assess intrahepatic lipid content by comparing the relative intensity of the liver to that of SC fat and spleen, assuming that the latter is fat-free (9). All MRI scans were performed by the same operator (blinded to treatment allocation), and all images were analyzed by the same radiologist (also blinded to treatment allocation).

Hormone Assays and Statistics

Serum immunoreactive insulin, IGF-I, SHBG, DHEAS, androstenedione, and T were assayed by immunochemiluminiscence (IMMULITE 2000, Diagnostic Products, Los Angeles, CA) (5); the intra- and interassay

coefficients of variation were between 4% and 8%. Samples from randomized subgroups were kept frozen until assay and were assayed concomitantly.

Statistical analyses were performed with SPSS 12.0. T-tests were used to compare outcomes between subgroups, and the general linear model for repeated measures was used to compare changes between subgroups. For uniformity, all results are expressed as mean \pm SEM. The level of significance was set at $P < .05$.

RESULTS

Age at menarche was 11.4 ± 0.1 years in untreated girls (9) and 12.5 ± 0.2 years in metformin-treated girls ($P < .0001$). In our study population of LBW-PP girls, early metformin therapy for 4 years was thus accompanied by an average delay of menarche of about 1 year and by a more normal distribution of menarcheal age (Fig. 1) (1).

Table 1 compares the clinical, endocrine-metabolic, and body-composition indices between untreated and metformin-treated girls who have reached, after, respectively, 5 and 6 years of study, a similarly late stage of adolescent maturation, as judged by time postmenarche and by near-adult height velocity. The untreated subgroup is unlikely to reach target height, a prospective finding that confirms retrospective evidence (1). In contrast, the metformin-treated subgroup is close to reaching target height. An apparently persisting feature of transiently metformin-treated girls is their reduced adiposity relative to untreated girls (Fig. 2). Nearly 2 years postmenarche, the untreated girls have $\sim 50\%$ more visceral and hepatic fat than metformin-treated girls (Table 1). Two years after stopping metformin, the formerly treated girls still had ~ 3 kg less fat and ~ 2 kg more lean mass and thus a much lower ratio of fat-to-lean mass than the untreated girls in a comparably late stage of maturation. The metformin-treated girls also had lower levels of fasting insulin and circulating T, as well as a more favorable lipid

profile, suggesting that early metformin curbs their endocrine-metabolic course away from hyperinsulinemic androgen excess, at least in the first postmenarcheal years.

DISCUSSION

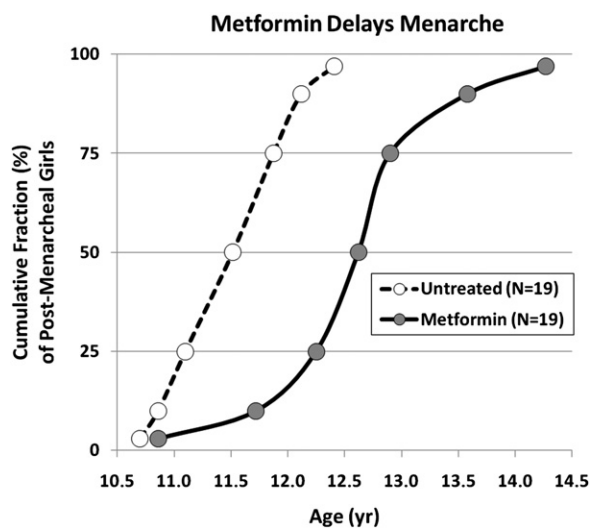
Early metformin intake (mean age, 8–12 years) appears to delay the menarche of LBW-PP girls by ~ 1 year toward normal and to augment their nearly adult stature toward normal. The effects of early metformin intake in LBW-PP girls compare thus to those previously observed in LBW girls with an early-normal onset of puberty (without PP) (6). The findings from these two studies should no longer come as a surprise since precocious/early puberty and late/delayed puberty have recently been associated to states of respectively low and high insulin sensitivity (14, 15).

Menarche before age 12 is an established risk factor for adult morbidity, including for breast cancer, cardiovascular disease, and type 2 diabetes (16–18). The vast majority ($>75\%$) of untreated LBW-PP girls experienced menarche before age 12, as expected (1), whereas the vast majority of the metformin-treated girls experienced menarche after age 12 (Fig. 1). One of the next challenges will be to verify whether early metformin intervention can partially prevent the adult morbidity associated with early menarche.

LBW-PP girls are at risk for developing hyperinsulinemic and anovulatory androgen excess (PCOS) by late adolescence (2–4). In the present study, the metformin-treated LBW-PP girls still had, 2 years after stopping such treatment, a leaner body composition (particularly in viscera and liver) than the untreated girls in a comparably late stage of adolescent maturation. Metformin-treated girls also had lower fasting levels of circulating insulin and T and had a more favorable lipid profile. Collectively, these markers suggest that the SC adipose tissue of metformin-treated girls is in a less hyperexpanded state perhaps because metformin therapy across puberty has increased the expandability of adipose tissue and/or because metformin has reduced the need to store fat. Regardless of the precise mechanisms that will prove to be involved, the

FIGURE 1

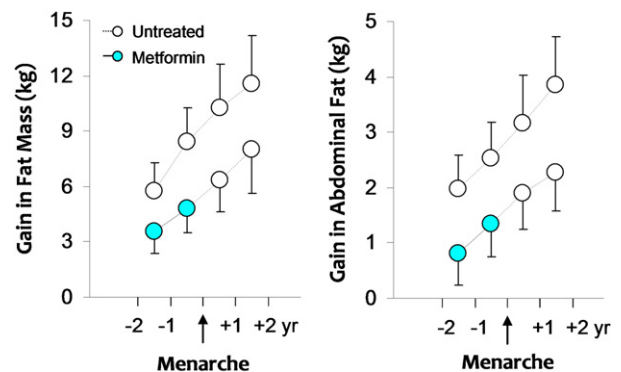
Cumulative fractions (%) of postmenarcheal LBW-PP girls who were at a mean age of 8 years randomized either to remain untreated or to receive metformin for 4 years. All girls were subsequently untreated at least until menarche. Menarcheal percentiles 3, 10, 25, 50, 75, 90, and 97 of each subgroup are plotted.



Ibáñez. Metformin to delay menarche and augment height. *Fertil Steril* 2010.

FIGURE 2

Cumulative gain in total and abdominal fat mass in prepubertal girls (age ~ 8 years) with a history of LBW-PP who were randomized to remain untreated ($n = 19$; open circles, dotted line) or to receive metformin ($n = 19$; colored circles, continuous line) for 4 years followed by 2 years without intervention. Mean gains and 95% confidence intervals are here plotted at comparable stages of pre- and postmenarcheal maturation.



Ibáñez. Metformin to delay menarche and augment height. *Fertil Steril* 2010.

endocrine-metabolic and body-composition profiles of the metformin-treated girls suggest that early metformin therapy holds potential as an approach to delay, attenuate, or even prevent the development of this subtype of PCOS (19).

In conclusion, early metformin intake (age ~8–12 years) slows down the rapid maturation of LBW-PP girls, so that their menarche is delayed by about 1 year toward normal; their postmenarcheal

stature is augmented to target level; their total body, visceral, and hepatic adiposity is reduced; and their endocrine-metabolic course is curbed away from dyslipidemia and from hyperinsulinemic androgen excess and thus away from adolescent PCOS. It remains to be studied whether metformin treatment yields similar results, for example, in LBW girls without PP and in adipose and/or hyperinsulinemic girls without LBW or PP.

REFERENCES

- Ibáñez L, Jiménez R, de Zegher F. Early puberty-menarche after precocious pubarche: relation to prenatal growth. *Pediatrics* 2006;117:117–21.
- Ibáñez L, Potau N, Francois I, de Zegher F. Precocious pubarche, hyperinsulinism and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab* 1998;83:3558–662.
- Ibáñez L, de Zegher F, Potau N. Anovulation after precocious pubarche: early markers and time course in adolescence. *J Clin Endocrinol Metab* 1999;84:2691–5.
- Ibáñez L, Valls C, Potau N, Marcos MV, de Zegher F. Polycystic ovary syndrome after precocious pubarche: ontogeny of the low birthweight effect. *Clin Endocrinol* 2001;55:667–72.
- Ibáñez L, Ferrer A, Ong K, Amin R, Dunger D, de Zegher F. Insulin sensitization early post-menarche prevents progression from precocious pubarche to polycystic ovary syndrome. *J Pediatr* 2004;144:23–9.
- Ibáñez L, Valls C, Ong K, Dunger D, de Zegher F. Metformin therapy during puberty delays menarche, prolongs pubertal growth, and augments adult height: a randomized study in low-birthweight girls with early-normal onset of puberty. *J Clin Endocrinol Metab* 2006;91:2068–73.
- Ibáñez L, Ong K, Valls C, Marcos MV, Dunger DB, de Zegher F. Metformin treatment to prevent early puberty in girls with precocious pubarche. *J Clin Endocrinol Metab* 2006;91:2888–91.
- Ibáñez L, López-Bermejo A, Diaz M, Marcos MV, de Zegher F. Metformin treatment for 4 yr to reduce total and visceral fat in low-birthweight girls with precocious pubarche. *J Clin Endocrinol Metab* 2008;93:1841–5.
- Ibáñez L, Lopez-Bermejo A, Diaz M, Marcos MV, de Zegher F. Pubertal metformin therapy to reduce total, visceral and hepatic adiposity. *J Pediatr* 2010;156:98–102.
- Reitman ML, Schadt EE. Pharmacogenetics of metformin response: a step in the path toward personalized medicine. *J Clin Invest* 2007;117:1226–9.
- Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* 2010;120:2355–69.
- Ibáñez L, Valls C, Marcos MV, Ong K, Dunger D, de Zegher F. Insulin sensitization for girls with precocious pubarche and with risk for polycystic ovary syndrome: effects of prepubertal initiation and postpubertal discontinuation of metformin. *J Clin Endocrinol Metab* 2004;89:4331–7.
- Ibáñez L, Suárez L, López-Bermejo A, Diaz M, Valls C, de Zegher F. Early development of visceral fat excess following spontaneous catch-up growth in children with low birthweight. *J Clin Endocrinol Metab* 2008;93:925–8.
- Sørensen K, Mouritsen A, Mogensen SS, Aksglaede L, Juul A. Insulin sensitivity and lipid profiles in girls with central precocious puberty before and during gonadal suppression. *J Clin Endocrinol Metab* 2010;95:3736–44.
- Wilson DA, Hofman PL, Miles HL, Sato TA, Billett NE, Robinson EM, et al. Enhanced insulin sensitivity in prepubertal children with constitutional delay of growth and development. *J Pediatr* 2010;156:308–12.
- Trivers KF, Gammon MD, Abrahamson PE, Lund MJ, Flagg EW, Kaufman JS, et al. Association between reproductive factors and breast cancer survival in younger women. *Breast Cancer Res Treat* 2007;103:93–102.
- Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, et al. Early age at menarche associated with cardiovascular disease and mortality. *J Clin Endocrinol Metab* 2009;94:4953–60.
- He C, Zhang C, Hunter DJ, Hankinson SE, Buck Louis GM, Hediger ML, et al. Age at menarche and risk of type 2 diabetes: results from 2 large prospective cohort studies. *Am J Epidemiol* 2010;171:334–44.
- de Zegher F, Lopez-Bermejo A, Ibáñez L. Adipose tissue expandability and the early origins of polycystic ovary syndrome. *Trends Endocrinol Metab* 2009;20:418–23.