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Reduced Ovulation Rate in Adolescent Girls Born Small for Gestational Age

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FSH and insulin are key hormones involved in spontaneous ovulation. Adolescent girls born small for gestational age (SGA) are at risk for FSH and insulin resistance. We have assessed whether ovulation rate is reduced in SGA girls.

Ovulatory function was assessed by weekly filter paper progesterone measurements, obtained by finger-stick autosampling for 3 consecutive months in matched populations of asymptomatic, nonobese girls (mean age, 15.5 yr; ≥3 yr postmenarche) who were either born with an appropriate weight for gestational age (AGA; n = 24; mean birthweight, 3.3 kg) or born small for gestational age (SGA; n = 25; mean birthweight, 2.3 kg).

The prevalence of anovulation was higher among SGA than AGA girls (40% *vs.* 4%; $P = 0.002$). Moreover, in the relatively small fraction of ovulating SGA girls, the ovulation rate was lower than in AGA adolescents (average number of ovulations during the study, 1.4 *vs.* 1.9; $P < 0.01$).

In conclusion, the endocrine correlates of prenatal growth restraint are herewith extended to include oligo-ovulation and anovulation in adolescence. It remains to be verified whether this SGA-related phenomenon persists into the reproductive age range. If it does, then fetal growth restraint may prove to be one of the enigmatic components underpinning hitherto unexplained female subfertility. (*J Clin Endocrinol Metab* 87: 3391–3393, 2002)

THE MOST DYNAMIC phase of ovarian development occurs before birth (1, 2). After prenatal growth restriction, ovarian development may be impaired (3). Infant as well as adolescent girls born small for gestational age (SGA) are known to be hyporesponsive to FSH (4, 5). In addition, ultrasound studies have visualized that ovarian size tends to be reduced in adolescent SGA girls (6). We have now assessed whether ovulation rate is reduced in adolescent SGA girls.

Subjects and Methods

Subjects

The study population consisted of 49 girls (age, 15.5 ± 0.2 yr; range, 13–18 yr) recruited among healthy relatives of hospital staff or among asymptomatic girls who attended the pediatric endocrine clinic for evaluation of thyroid function, timing of pubertal development, or postmenarcheal growth status. Recruitment characteristics of the study subpopulations are detailed in Table 1.

The inclusion criteria were: 1) weight at term birth (37–41 wk) either appropriate for gestational age (AGA, between −1 SD and +1 SD) or small for gestational age (SGA, below −2 SD); 2) menarche 5 ± 2 yr before study; 3) menstrual cycles (25–35 d) with a variation of no more than 5 d (7); and 4) body mass index less than 25 kg/m^2.

The exclusion criteria were: evidence for a syndromatic, chromosomal, or infectious etiology of low birthweight; hirsutism [defined as a score of ≥8 on Ferriman and Gallwey scale (8)]; a history of precocious pubarche (9) or precocious puberty (10); thyroid dysfunction, Cushing's syndrome, hyperprolactinemia; previous or current use of oral contraceptives; and a family or personal history of diabetes mellitus.

Birthweight and gestational age data were obtained from hospital records or from the girls' pediatricians and transformed into SD scores as described (11).

Hormonal measurements

At the start of the study, in fasting state and in the early follicular phase (range, cycle d 5 ± 3), blood sampling was performed for measurement of glucose, serum insulin, lipid profile, LH, FSH, estradiol, dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, and SHBG; the free androgen index (FAI), an index of free testosterone, was calculated [FAI = testosterone (nmol/liter) × 100/SHBG (nmol/liter)] as described (12).

Serum glucose was measured by the glucose oxidase method. Immunoreactive insulin was assayed by IMX (Abbott Diagnostics, Santa Clara, CA). The mean intra- and interassay coefficients of variation (CV) were 4.7% and 7.2%, respectively. LH and FSH were measured by immunochemiluminescence (IMMULITE 2000; Diagnostic Products, Los Angeles, CA), with intra- and interassay CV of 3.5% and 5.0%, respectively, for LH and 4.6% and 6.3% for FSH. Serum estradiol, DHEAS, androstenedione, testosterone, and SHBG were assayed as previously described (13, 14). Serum samples were kept frozen at −20 C until assay.

Ovulation assessment

For 3 months, the adolescents maintained prospective diaries on menses, from which cycle characteristics were derived. Starting on d 21 of the first menstrual cycle of the study, and then once weekly until the end of month 3, the girls collected blood by finger-stick. Blood was placed on filter paper (Diagnostic Products blood specimen collection card), was allowed to dry for 2 h at room temperature, and was stored in plastic envelopes at 4 C until the end of the 3-month study phase.

Ovulation was considered to have occurred if progesterone concentration was above 1.0 ng/ml in a filter paper sample obtained 5–8 d before menses. This ambulatory ovulation assessment through progesterone monitoring on filter paper was validated as follows. In a first step, blood was sampled from 15 young women with regular menstrual cycles, in both the early follicular and the luteal phase of the cycle (5–8 d before menses); part of the blood sample was poured into filter paper;

Abbreviations: AGA, Appropriate weight for gestational age; CV, coefficient(s) of variation; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; SGA, small for gestational age.

TABLE 1. Recruitment characteristics of the AGA and SGA subgroups

	AGA (n)	SGA (n)
Healthy volunteer	14	8
Thyroid function screening; euthyroidism confirmed	3	1
Normal variation in timing of pubertal development		
Late-normal	2	0
Early-normal	1	1
Postmenarcheal evaluation of growth status, without history of precocious pubarche or precocious puberty		
Height within or above target range	4	7
Height below target range	0	8
Totals	24	25

the remaining was centrifuged, and serum was stored until assay at −20 C. Progesterone was then simultaneously assayed in filter paper and in serum by a coat-A-count RIA (Diagnostic Products). Human blood was washed three times with saline solution and spotted on a filter paper, and this was used for blanc counts. The filter paper blood samples were processed, in duplicate, in tubes containing only the paper disks, which were serially cut with a device made for this purpose. After incubation with I^{125} progesterone for 3 h at room temperature (15–25 C), the tubes were aspirated without removing the disks, and the radioactivity was measured in a γ-counter. The detection limit of the assay was 0.02 ng/ml (0.06 nmol/liter). The mean intra- and interassay CV were 4% and 5.7%, respectively. The correlation between progesterone concentrations in serum and those in filter paper was 0.946 ($P = 0.0001$). Serum and filter paper progesterone concentrations ranged in the follicular phase, respectively, between 1–4 ng/ml and 0.07–0.7 ng/ml, and in the luteal phase between 8–20 ng/ml and 1–6 ng/ml.

Statistical analysis and ethics

Results are expressed as mean ± SEM. Two-sided, unpaired *t* test was used for statistical comparisons, unless mentioned otherwise; significance level was set at *P* value less than 0.05.

The study protocol was approved by the Institutional Review Board of Barcelona University Hospital of Sant Joan de Déu. Informed consent was obtained from parents and/or study subjects, assent being obtained from minors.

Results

Table 2 summarizes the clinical characteristics, baseline endocrine-metabolic data, and ovulation results of the two study subpopulations. As expected, SGA adolescents were found to have higher fasting insulin and lower SHBG concentrations than AGA girls, as well as higher serum testosterone, androstenedione, and DHEAS concentrations (all $P \leq 0.01$).

Figure 1 depicts the fractions of AGA and SGA girls as distributed by number of detected ovulations over the study time span of 3 months. The prevalence of anovulation was higher among SGA than AGA girls (40% *vs.* 4%; $P = 0.002$). Moreover, in the relatively small fraction of ovulating SGA girls, the ovulation rate was lower than in AGA adolescents (mean number of ovulations, 1.4 *vs.* 1.9; $P < 0.01$). Ovulation results were similar in SGA adolescents who had reached a stature within target height range (n = 14) and those with a stature below target height range (n = 11); the number of anovulatory *vs.* ovulatory girls were 6 *vs.* 8 and 4 *vs.* 7, respectively.

TABLE 2. Clinical characteristics, baseline endocrine-metabolic data, and ovulation results of AGA *vs.* SGA subgroups

	AGA (n = 24)	SGA (n = 25)
Weight at term birth (kg)	3.3 ± 0.1	2.3 ± 0.1[d]
Age (yr)	15.6 ± 0.2	15.4 ± 0.3
Postmenarcheal age (yr)	3.8 ± 0.2[a]	4.1 ± 0.2
Height (cm)	159.0 ± 1.1[a]	150.7 ± 1.0[d]
Body mass index (kg/m^2)	20.4 ± 0.4	20.4 ± 0.4
LH (IU/liter)	4.4 ± 0.4	5.3 ± 0.6
FSH (IU/liter)	4.0 ± 0.2	6.7 ± 0.5[d]
Estradiol (pg/ml)	51 ± 6	71 ± 13
Testosterone (ng/dl)	44 ± 3	70 ± 6[c]
FAI	2.9 ± 0.2	5.8 ± 0.6[d]
SHBG (μg/dl)	1.6 ± 0.1	1.3 ± 0.1[b]
Androstenedione (ng/dl)	145 ± 8	219 ± 20[b]
DHEAS (μg/dl)	157 ± 14	257 ± 18[d]
Glucose (mg/dl)	85 ± 2	87 ± 2
Insulin (mU/liter)	8.3 ± 0.5	13.0 ± 0.7[c]
Total cholesterol (mg/dl)	164 ± 5	164 ± 5
LDL-cholesterol (mg/dl)	86 ± 4	91 ± 4
HDL-cholesterol (mg/dl)	65 ± 3	60 ± 3
Triglycerides (mg/dl)	54 ± 4	64 ± 7
Anovulatory *vs.* ovulatory girls	1 *vs.* 23	10 *vs.* 15[e]
Mean ovulation rate in ovulatory girls	1.9	1.4[b]

Values are mean ± SEM. LDL, Low-density lipoprotein; HDL, high-density lipoprotein.

[a] Mean age at menarche: 12.3 yr; mean height at 15.5 yr in Northern Spain: 160.5 cm (Ref. 15).

[b] $P \leq 0.01$; [c] $P \leq 0.001$; and [d] $P \leq 0.0001$ *vs.* AGA.

[e] $P = 0.002$ by χ^2.

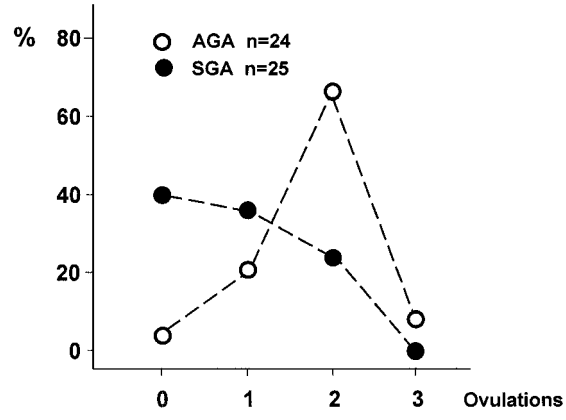

FIG. 1. Fractions of AGA and SGA subpopulations distributed by number of ovulations detected over 3 months of study.

Discussion

This is a first study assessing adolescent ovulation rate over 3 months, on an ambulatory basis, through weekly measurements of progesterone concentrations in capillary blood dried on filter paper. With this novel method, 23 of 24 participating AGA girls were found to have at least one ovulatory cycle within 3 months. This ovulation rate (96%) is, so far, the highest reported in adolescents (16, 17), suggesting that this technique has a sensitivity superior to previous methods (17). Moreover, it is unlikely that this simple method overestimates ovulation rate because the time lag between the proposed ovulation date and the onset of the following menses was uniformly consistent with the time course of a normal ovulatory cycle.

The ovulation rate in SGA girls was found to be strikingly

low; the anovulatory fraction was much larger than in the AGA girls. Moreover, in ovulatory SGA girls, the individual number of ovulations over 3 months was also reduced. Interestingly, the reduction in ovulation rate was comparable in SGA adolescents who had reached a stature within target range and in SGA girls with a postmenarcheal stature that was below target level. This observation suggests that anovulation secondary to prenatal growth restraint is a phenomenon that is essentially unrelated to completeness of spontaneous catch-up growth. Thus, in SGA girls, spontaneous recovery of linear growth during childhood does not warrant normal ovulatory function in adolescence; conversely, persistent growth failure in SGA girls will not necessarily be followed by anovulation.

That the link between reduced prenatal growth and anovulation has apparently escaped attention for so long may in part be attributable to the fact that the majority of SGA girls normalize their stature, and hereby no longer present an obvious reminder of their early growth restraint. The copresence of obesity may have been another notoriously confounding factor in ovulation research; the absence of obesity in the described study population has presumably facilitated the disclosure of the link between prenatal growth and postmenarcheal ovulation rate.

Prenatal growth restraint has previously been documented to be associated with relative hyperinsulinism, hyperandrogenism, and FSH hypersecretion in adolescent girls from Catalunya (5, 18, 19). These associations were confirmed in the present cohort and may each contribute to the reduced ovulation rate in SGA adolescents.

Insulin sensitization is becoming an approach of choice to induce ovulation in women with anovulatory hyperinsulinism-hyperandrogenism (14, 20). It would be of interest to explore whether insulin-sensitizing treatment is also capable of inducing ovulation in SGA adolescents with anovulation as part of a more subtle constellation, including limited FSH hypersecretion and mild hyperinsulinemic hyperandrogenism.

In conclusion, the endocrine correlates of prenatal growth restraint are herewith extended to include oligo-ovulation and anovulation in adolescence. It remains to be verified whether this SGA-related phenomenon persists into the reproductive age range. If it does, then fetal growth restraint may prove to be one of the enigmatic components underpinning hitherto unexplained female subfertility.

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