Menstrual disorders in adolescence: a marker for hyperandrogenaemia and increased metabolic risks in later life? Finnish general population-based birth cohort study

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STUDY QUESTION: Are self-reported menstrual disorders associated with hyperandrogenaemia and metabolic disturbances as early as in adolescence?

SUMMARY ANSWER: Menstrual disorders at the age 16 are a good marker of hyperandrogenaemia, and an adverse lipid profile was associated with higher androgen levels.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: Hyperandrogenism per se has been suggested to be a significant metabolic risk factor in women and a cause of physical and psychological morbidity in adolescent girls. A weak positive correlation has been described between hyperandrogenaemia and obesity in adolescent girls, but the clinical consequences are still poorly understood. Hyperandrogenism and insulin resistance are also key features of polycystic ovary syndrome (PCOS), and women with PCOS are consequently at an increased risk of developing type 2 diabetes mellitus and/or metabolic syndrome, and may have increased cardiovascular morbidity.

Our findings confirm that the association between menstrual disorders, hyperandrogenism, obesity and metabolic risks is already evident in adolescence.

STUDY DESIGN: This population-based, cross-sectional study used postal questionnaires to targeting 15–16-year-old girls in the Northern Finland Birth Cohort 1986 (n = 4567).

PARTICIPANTS AND SETTING: There were 3669 girls who answered the postal questionnaire and out of 3373 girls who also underwent clinical examinations and blood tests, 2448 were included in the analyses. The questionnaire included one question about the regularity and length of the menstrual cycle: ‘Is your menstrual cycle (the interval from the beginning of one menstrual period to the beginning of the next period) often (more than twice a year) longer than 35 days?’ The girls who answered ‘yes’ to this question were considered to be suffering from menstrual disorders and were classified as ‘symptomatic’. The girls who answered ‘no’ were defined as ‘non-symptomatic’.

MAIN RESULTS AND THE ROLE OF CHANCE: There were 709 (29%) girls who reported menstrual disorders (symptomatic girls) and 1739 who had regular periods (non-symptomatic girls). In the whole population and in both study groups, there were significant correlations between body mass index (BMI) and waist-to-hip ratio, hyperandrogenaemia and metabolic parameters. Symptomatic girls exhibited significantly higher serum concentrations of testosterone (P = 0.010), lower levels of sex hormone-binding globulin (P = 0.042) and higher free androgen indices [FAIs; geometric mean 3.38 (interquartile range (IQR): 2.27, 5.18) versus 3.08 (IQR: 2.15, 4.74), P = 0.002]. The two
groups had comparable BMI and insulin sensitivity, and serum levels of glucose, insulin and lipids. There was a significant linear trend towards higher FAI values in the higher BMI quartiles in both symptomatic and non-symptomatic girls. In the whole population, there was a statistically significant linear decrease in high-density lipoprotein concentrations (P < 0.001) and higher triglyceride concentrations (P = 0.004) in the upper FAI quartile.

**Implications:** Information regarding menstrual disorders in adolescence is a good marker of hyperandrogenaemia and may be an early risk factor for the development of PCOS in adulthood. The association between obesity, hyperandrogenism and metabolic risks is already evident in adolescence, which strengthens the importance of noting menstrual disorders at an early stage.

**Bias, Limitations, Generalizability:** The cross-sectional nature of the study does not allow us to draw conclusions concerning the metabolic risks of this population in later life. The diagnosis of menstrual disorders was based on a questionnaire, suggesting a risk of information bias in reporting the symptoms. This study was not designed to diagnose PCOS, as ultrasonography was not available and there was no clinical evaluation of hyperandrogenism (i.e. hirsutism). However, we were able to take into account potential confounding factors in the analyses.

**Study Funding/Competing Interests:** This work was supported by grants from the Finnish Medical Society Duodecim, the North Ostrobothnia Regional Fund, the Academy of Finland (project grants 104781, 120315, 129269, 1114194, SALVE), University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), the European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643) and the Medical Research Council, UK (PrevMetSyn/SALVE). None of the authors have any conflict of interest to declare.

**Key words:** PCOS / hyperandrogenaemia in adolescence / menstrual cycle / androgens / oligoamenorrhoea

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**Introduction**

Hyperandrogenism per se is believed to be a significant metabolic risk factor in women. Post-menopausal women with a history of irregular menses and elevated androgen levels have more indications of coronary arterial disease verified by angiography and are more at risk of suffering cardiovascular events and death compared with a control population (Shaw et al., 2008). The clinical manifestations of hyperandrogenism are also recognized to be a significant cause of physical and psychological morbidity in adolescent girls (Fleischman and Mansfield, 2005).

Hyperandrogenism is a key feature of polycystic ovary syndrome (PCOS), a common endocrine disorder suffered by 5–10% of women of reproductive age (Franks, 1995). At least one-third of women with PCOS are overweight and some degree of insulin resistance is estimated to be present in 50–70% of all women with PCOS (Nisenblat and Norman, 2009). Consequently, these women are at an increased risk of developing type 2 diabetes mellitus (T2DM), metabolic syndrome and cardiovascular morbidity (Legro, 2002; Solomon et al., 2002). The compensatory hyperinsulinaemia is believed to increase hyperandrogenism by stimulating ovarian and adrenal androgen biosynthesis and suppressing circulating levels of sex hormone-binding globulin (SHBG; Legro et al., 2004). Decreased SHBG levels increase the free androgen index (FAI) and the amount of bioavailable testosterone. Thus women with PCOS are more exposed to biologically active testosterone (Franks, 1995; Hickey et al., 2009).

Adolescent hyperandrogenaemia is associated with hirsutism, acne and menstrual irregularity and it is an important marker of PCOS and subsequent risk of T2DM, especially when associated with obesity (Lewy et al., 2001). Previous studies have shown a weak positive correlation between hyperandrogenaemia and obesity (Van Hooff et al., 2000). The clinical consequences, however, are poorly understood, although case–control studies highlight the importance of pubertal hyperandrogenism with regard to insulin resistance, adiposity and other metabolic risk markers, with many similarities to adult PCOS (Lewy et al., 2001).

The aim of this study was to identify early endocrine and metabolic indices of PCOS by investigating the association between self-reported...
Menstrual disorders (an important surrogate marker of PCOS), hyperandrogenaemia and metabolic disturbances at age 15–16 years. Further, we wanted to establish whether a relationship exists between metabolic markers such as body mass index (BMI) and hyperandrogenism as assessed by FAI.

Materials and Methods

Study population

The prospective Northern Finland Birth Cohort 1986 (NFBC 1986), which has been followed up since the fetal period, consists of 9362 mothers and their 9479 births (9432 children born alive), who had an expected date of birth between 1 July 1985 and 30 June 1986, drawn from the two northernmost provinces of Finland, i.e. Oulu and Lapland. In 2001–2002, when the children were 15–16 years old (mean age: 15.5 years, SD: 0.37 years), the adolescents and their parents each received postal questionnaires (providing 80 and 76% response rates, respectively).

Of the adolescents (n = 9432, of these 4567 females) then living in Finland, either in the original catchment area or elsewhere, 3669 (80.3%) answered the questionnaire and 3373 (74%) underwent clinical examination and gave fasting blood samples in 2001–2002. After excluding twins and triplets, pregnant girls, oral contraceptive users and users of other forms of hormone treatment, and subjects with incomplete data, 2448 singleton females (53.6% of those eligible) were available for the analyses (Fig. 1).

The questionnaire included one question about the regularity and length of the menstrual cycle: ‘Is your menstrual cycle (the interval from the beginning of one menstrual period to the beginning of the next period) often (more than twice a year) longer than 35 days?’ The girls who answered ‘yes’ to this question were considered to be suffering from oligo- or amenorrhoea and were classified as ‘symptomatic’. The girls who answered ‘no’ were defined as ‘non-symptomatic’.

BMI was calculated by dividing the body weight in kilogram by the squared height in metres. Weight, and waist and hip circumferences (measured to the nearest centimetre with a soft tape at the level midway between the lowest rib margin and the iliac crest and at the widest part of the gluteal region) were assessed and the waist-to-hip ratio (WHR) was calculated.

The socioeconomic status (SES) of the family was obtained from the parents. A question about annual income was included in the questionnaire, and to enable comparison of households of different sizes and structures, household consumption units (The OECD List of Social Indicators, 1982 scale) were calculated by assigning the first adult in the household a value of 1 unit, with additional adults (>17 years) receiving 0.7 and each child (≤17 years) 0.5 units (United Nations. Statistical Office, 1977). The families were classified into quartiles based on their annual income per consumption unit.

The Ethics Committee of the Northern Ostrobothnia Hospital District approved this study and informed consent was obtained from all subjects.

Laboratory methods

Serum samples for assay of testosterone (T) were analysed by using Agilent triple quadrupole 6410 liquid chromatography/mass spectrometry equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE, USA). Multiple reaction monitoring was used to quantify testosterone by using trideuterated testosterone (d3-testosterone), with the following transitions: m/z 289.2→97 and 289.2→109 for testosterone and 292.2→97 and 292.2→109 for d3-testosterone. The intra-assay coefficient of variations (CVs) of the method were 5.3, 1.6 and 1.2% for testosterone at 0.6, 6.6 and 27.7 nmol/l, respectively. The inter-assay CVs were 5.3, 4.2 and 1.0% for the respective concentrations. In an adult female population, an upper limit for testosterone of 2.3 nmol/l is considered as normal.

Plasma glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and triglycerides were determined by using an automatic chemical analyser (Cobas Integra 700, Roche Diagnostics, Switzerland). Serum insulin was analysed by radioimmunossay (Pharmacia Diagnostics, Uppsala, Sweden), high-sensitive C-reactive protein (hs-CRP) by an immunoenzymometric assay (Medix Biochemica, Espoo, Finland) and SHBG by time-resolved fluorimunossay (AutoDelfia, PerkinElmer, Turku, Finland).

The FAI was calculated by using the equation 100 × T (nmol/l)/SHBG (nmol/l). To quantify the degree of insulin sensitivity, homeostasis model assessment (HOMA-S) values were calculated using the validated calculator available at http://www.dtu.ox.ac.uk.

Statistical methods

The χ² test was used to compare categorical variables between the study groups. Comparisons of continuous variables were conducted by using Student’s t-test. The distributions of laboratory measurements were skewed; therefore, they were logarithmically transformed to achieve normality. Correlations were tested using Pearson’s correlation analysis. Linear regression analysis was used to calculate percentage differences in biochemical characteristics between the study groups. The results were further adjusted for smoking, SES, alcohol consumption, BMI and age at menarche. The whole study population was also stratified into the FAI and BMI quartiles and analysis of variance was used to assess the trends in biochemical variables across these quartiles. The confidence intervals (CIs) were calculated using the CIA computer program (Gardner and Altman, 1989). Statistical analyses were performed by using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Questionnaire data show that 709 (29%) girls had menstrual disorders (symptomatic subjects) and 1739 had regular periods (non-symptomatic subjects). The symptomatic girls were statistically comparable with the non-symptomatic peers as regards alcohol consumption, smoking habits and SES (Table I). Symptomatic and non-symptomatic girls were similar with regards to BMI, serum levels of insulin, glucose, hs-CRP and lipids (serum levels of total cholesterol, HDL, LDL and triglycerides) and insulin sensitivity, measured by HOMA-S (Table II).

1873 girls recalled their menarcheal age. Menarcheal age was slightly higher in symptomatic (13.1 years) compared with non-symptomatic girls (12.9 years, P < 0.001). Gynaecological age, defined as the number of years since menarche, was therefore calculated (based on the date of the answer to the questionnaire) to be 2.52 (SD 1.10) years in the whole study population, 2.34 (SD 1.1) years in the symptomatic and 2.59 (SD 1.09) years in the non-symptomatic girls (P < 0.001 between the two groups).

In the whole population and in both study groups, there were significant correlations between BMI (and WHR) and hyperandrogenaemia (especially FAI) and metabolic parameters (such as CRP, lipids and indicators of insulin sensitivity, Table III).

Symptomatic girls exhibited significantly higher serum concentrations of testosterone (P = 0.010), lower serum levels of SHBG (P = 0.042) and higher FAI values (P = 0.002, Table II). In the group of symptomatic girls, 7.8% (55/709) exhibited testosterone levels above the upper normal limit (95th percentile), i.e. 2.68 nmol/l in
this population, which was significantly higher in comparison to non-symptomatic girls (4.9%, 86/1739, \(P = 0.007\)). In the whole study population, 3.5% (86/2448) of the girls exhibited both menstrual disorders and elevated serum testosterone levels. The adjustment neither for BMI nor WHR (data not shown) altered the above associations. In fact, after adjusting for BMI, the percentage differences between the symptomatic and non-symptomatic groups even increased for FAI (from 9.5 to 11.3%), testosterone (from 4.2 to 4.5%) and SHBG (−4.8 to −6.1%). Similarly, significant differences remained after additional adjustments for smoking, SES and alcohol consumption. Further adjustment for menarcheal or gynaecological age did not change the results (data not shown). With a threshold level of 2.68 nmol/l testosterone, the sensitivity and specificity of being ‘symptomatic’ to predict hyperandrogenaemia were 39 and 72%, respectively.

A slightly higher proportion of girls were underweight (BMI, \(<18.5\) kg/m² according the WHO’s definition) in the symptomatic (20.3%) compared with the non-symptomatic group (16.9%) (\(P = 0.054\)). In subanalyses excluding the underweight girls, the above described correlations did not change; however, the differences between the symptomatic and non-symptomatic groups became non-significant as regards WHR (\(P = 0.13\)) and metabolic

### Table I Background characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Non-symptomatic girls</th>
<th>Symptomatic girls</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N) (1228–1733*)</td>
<td>Percentages and 95%CI</td>
<td>(N) (499–706*)</td>
<td>Percentages and 95%CI</td>
</tr>
<tr>
<td>Use of alcohol(b)</td>
<td>385</td>
<td>22.2% (20.3–24.2%)</td>
<td>152</td>
</tr>
<tr>
<td>Smokers(c) (%)</td>
<td>319</td>
<td>18.5% (16.7–20.3%)</td>
<td>104</td>
</tr>
<tr>
<td>Socioeconomic status(d) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>287</td>
<td>23.4% (21.0–25.7%)</td>
<td>119</td>
</tr>
<tr>
<td>Second</td>
<td>321</td>
<td>26.1% (23.7–28.6%)</td>
<td>130</td>
</tr>
<tr>
<td>Third</td>
<td>288</td>
<td>23.5% (21.1–25.8%)</td>
<td>131</td>
</tr>
<tr>
<td>Fourth</td>
<td>332</td>
<td>27.0% (24.6–29.5%)</td>
<td>119</td>
</tr>
</tbody>
</table>

\(a\) Numbers vary due to non-response to some items.
\(b\) Once per month or more.
\(c\) 2–4 days per week or more and
\(d\) Annual income per consumption unit divided into quartile.

### Table II Anthropometric, hormonal and metabolic parameters in the girls with self-reported menstrual disorders amenorrhea and in the non-symptomatic girls of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Non-symptomatic girls (N = 1320–1739*)</th>
<th>Geometric mean (IQR(b))</th>
<th>Symptomatic girls (N = 553–709*)</th>
<th>Geometric mean (IQR(b))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI [kg/m²]</td>
<td>1735</td>
<td>21.01 (19.11, 22.67)</td>
<td>706</td>
<td>20.78 (18.82, 22.05)</td>
<td>0.080</td>
</tr>
<tr>
<td>WHR</td>
<td>1728</td>
<td>0.772 (0.74, 0.80)</td>
<td>702</td>
<td>0.768 (0.74, 0.80)</td>
<td>0.044</td>
</tr>
<tr>
<td>Age at menarche [yr]</td>
<td>1320</td>
<td>12.9 (12.25, 13.67)</td>
<td>553</td>
<td>13.1 (12.5, 13.92)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Testosterone [nmol/l]</td>
<td>1739</td>
<td>1.59 (1.28, 2.02)</td>
<td>709</td>
<td>1.65 (1.31, 2.07)</td>
<td>0.010</td>
</tr>
<tr>
<td>SHBG [nmol/l]</td>
<td>1739</td>
<td>51.49 (36.60, 68.90)</td>
<td>709</td>
<td>48.99 (35.28, 65.85)</td>
<td>0.042</td>
</tr>
<tr>
<td>FAI</td>
<td>1739</td>
<td>3.08 (2.15, 4.74)</td>
<td>709</td>
<td>3.38 (2.27, 5.18)</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin [mU/l]</td>
<td>1632</td>
<td>9.68 (7.50, 12.18)</td>
<td>666</td>
<td>9.50 (7.40, 12.20)</td>
<td>0.318</td>
</tr>
<tr>
<td>Glucose [mmol/l]</td>
<td>1566</td>
<td>5.01 (4.80, 5.30)</td>
<td>640</td>
<td>5.02 (4.80, 5.30)</td>
<td>0.736</td>
</tr>
<tr>
<td>HOMA-S</td>
<td>1545</td>
<td>80.1 (63.65, 103.00)</td>
<td>631</td>
<td>81.5 (63.40, 104.00)</td>
<td>0.352</td>
</tr>
<tr>
<td>hs-CRP [ng/l]</td>
<td>1734</td>
<td>0.25 (0.09, 0.60)</td>
<td>709</td>
<td>0.23 (0.09, 0.56)</td>
<td>0.133</td>
</tr>
<tr>
<td>Total Cholesterol [mmol/l]</td>
<td>1620</td>
<td>4.30 (3.83, 4.80)</td>
<td>664</td>
<td>4.33 (3.90, 4.80)</td>
<td>0.386</td>
</tr>
<tr>
<td>HDL [mmol/l]</td>
<td>1620</td>
<td>1.46 (1.29, 1.65)</td>
<td>664</td>
<td>1.45 (1.26, 1.67)</td>
<td>0.686</td>
</tr>
<tr>
<td>LDL [mmol/l]</td>
<td>1620</td>
<td>2.19 (1.90, 2.60)</td>
<td>664</td>
<td>2.22 (1.90, 2.60)</td>
<td>0.196</td>
</tr>
<tr>
<td>Triglycerides [mmol/l]</td>
<td>1620</td>
<td>0.74 (0.57, 0.95)</td>
<td>664</td>
<td>0.74 (0.56, 0.95)</td>
<td>0.751</td>
</tr>
</tbody>
</table>

\(a\) Numbers vary due to non-response to some items or blood sample failures.
\(b\) Interquartile range (IQR) from 25\(th\) to 75\(th\) percentile.
After stratifying the whole study population into BMI quartiles, there was a significant trend towards higher FAI values in the higher BMI quartiles, and this trend remained for both the symptomatic and non-symptomatic groups. The differences in FAI values between the symptomatic and non-symptomatic groups were significant in the two highest BMI quartiles (Fig. 2A). In the highest BMI quartile (25.4 kg/m², range 22.5–46.9 kg/m²), symptomatic girls had significantly higher BMI (P = 0.005) and FAI values (P = 0.002), lower serum levels of SHBG (P = 0.016) and HDL-cholesterol (P = 0.002), higher serum levels of insulin (P = 0.031) and lower HOMA-S values (P = 0.027, Fig. 2B) than the non-symptomatic girls, but the hs-CRP levels did not differ between the two groups. However, there was a significant linear trend towards higher CRP levels from the lowest to the highest BMI quartiles in the whole study population [from 0.16 (95% CI: 0.14–0.18) to 0.48 mg/l (0.43–0.54), P < 0.001], and in the symptomatic [from 0.15 (0.12–0.18) to 0.52 mg/l (0.42–0.65), P < 0.001] and non-symptomatic girls [from 0.17 (0.14–0.19) to 0.47 mg/l (0.41–0.53, P < 0.001)].

After stratifying the whole study population into FAI quartiles, there was a significant linear trend in the higher FAI quartiles towards lower serum HDL-cholesterol levels (from 1.48 (95% CI: 1.46–1.51) to 1.41 (1.39–1.43) mmol/l, P < 0.001) in the whole study population, and serum triglyceride levels increased significantly from the second [0.72 (0.70–0.74) mmol/l] and third FAI quartiles [0.72 (0.70–0.75) mmol/l] to the fourth [0.77 (0.75–0.80 mmol/l, P = 0.009 and P = 0.032, respectively].

### Discussion

This study shows that a single, simple question about the regularity and length of the menstrual cycle, at age 16, may help identify girls with an increased risk for hyperandrogenaemia. In the whole population and both study groups, there were significant correlations between BMI (and WHR) and hyperandrogenaemia and metabolic parameters. The girls with the highest BMI exhibited the greatest degree of hyperandrogenaemia and the most unfavourable metabolic findings, and those with the highest FAI had a more adverse lipid profile, thus supporting earlier data showing an association between obesity, hyperandrogenaemia and metabolic risks.

The strength of this study lies in the fact that it has been performed on a large population-based birth cohort of females. Furthermore, the use of the most modern and accurate testosterone assay (liquid chromatography and tandem mass spectrometry; Janse et al., 2011) is no doubt a strength as it is now generally acknowledged that measurement of serum testosterone by immunoassay in women can be unreliable. A limitation of our study is that the diagnosis of oligoamenorrhea was based on a questionnaire, suggesting an important risk of information bias in reporting the symptoms. Nevertheless, we have previously shown that self-reported menstrual disorders (by using the same questionnaire) can help to identify most women with an endocrine profile typical of PCOS (Taponen et al., 2003, 2004). Moreover, this study was not designed to diagnose PCOS, as ultrasonography was not performed and there was no clinical evaluation of hyperandrogenism (i.e. hirsutism). However, we were able to take into account potential confounding factors in the analyses. Lastly, the girls were not screened for late onset adrenal hyperplasia, but the prevalence of this disorder in our country is considerably lower than that reported for other populations (under 1/15 000; Jaakelainen et al., 1997).

In our study population, almost one-third of the girls were classified as symptomatic according to their menstrual pattern at a mean age of 15.5 years, ~2.5 years after menarche. It is generally accepted that it may take up to 5 years after menarche for the hypothalamic–pituitary–ovarian (HPO) axis to reach maturation (Apter, 1980). Studies concerning the rates of menstrual irregularities in adolescents have shown various prevalence rates from 12% at age 15 (Nwankwo et al., 2010) to almost 30% 2 years after menarche (WHO, 1986), to ~20% 5 years after menarche (McDonough and Gantt, 1982), depending on the definition used for menstrual irregularities and age at

### Table III  Correlations with BMI for hormonal and metabolic parameters in the whole population, and in the symptomatic and non-symptomatic groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole population</th>
<th>Symptomatic girls</th>
<th>Non-symptomatic girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHR</td>
<td>r = 0.45**</td>
<td>r = 0.48**</td>
<td>r = 0.44**</td>
</tr>
<tr>
<td>FAI</td>
<td>r = 0.30*</td>
<td>r = 0.33*</td>
<td>r = 0.29*</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>r = 0.35*</td>
<td>r = 0.43*</td>
<td>r = 0.32*</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>r = 0.33*</td>
<td>r = 0.36*</td>
<td>r = 0.32*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>r = 0.22*</td>
<td>r = 0.22*</td>
<td>r = 0.22*</td>
</tr>
<tr>
<td>LDL</td>
<td>r = 0.21*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>r = −0.24a</td>
<td>r = −0.29a</td>
<td>r = −0.22a</td>
</tr>
<tr>
<td>SHBG</td>
<td>r = −0.30a</td>
<td>r = −0.33a</td>
<td>r = −0.29a</td>
</tr>
<tr>
<td>HOMA-S</td>
<td>r = −0.34a</td>
<td>r = −0.43a</td>
<td>r = −0.30a</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>r = −0.24a</td>
<td>r = −0.27a</td>
<td>r = −0.22a</td>
</tr>
</tbody>
</table>

BMI, body mass index; WHR, waist-hip ratio; FAI, free androgen index; hs-CRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SHBG, sex hormone-binding globulin; HOMA-S, homeostasis model assessment.

*P-value < 0.001.
were exacerbated by obesity (Taponen et al., 2003, 2004). Similarly, a study on hormone concentrations during the first years after menarche showed that girls with irregular menstrual cycles and/or oligomenorrhea exhibit higher androgen levels than girls with regular menstrual cycles (van Hooff et al., 2000). In the present study, the differences in hormonal and metabolic parameters between the symptomatic and non-symptomatic groups were significant, yet they remained very close to each other. Therefore, the clinical significance of this finding remains to be confirmed in further follow-up studies. A Swedish prospective study of menstrual disturbances in adolescents demonstrated that after 6 years of follow-up, irregular menstruation was still present in 62%, and that 59% of those with persisting irregular menstruation fulfilled the criteria for diagnosis of PCOS (Wiksten-Almstromer et al., 2008). Follow-up of the symptomatic girls of the present study population until adulthood will reveal whether they will continue to manifest or will go on to develop all the characteristic features of PCOS and whether they will exhibit risk factors of cardiovascular diseases and T2DM. As PCOS is the most important cause of anovulatory infertility, this finding is also of great importance as regards fertility concerns for these young women in the future.

In the present study, the symptomatic girls entered menarche 3 months later than the non-symptomatic girls, which could at least partly explain the higher rate of menstrual irregularities in these girls. Gynaecological age, defined as the number of years since menarche, has been shown to be a stronger predictor of ovulatory cycles than chronological age, i.e. adolescents with earlier menarche tend to develop ovulatory cycles sooner than those with later onset of menarche (Apter and Vihko, 1983). In other studies, however, preadolescent girls who later developed PCOS had significantly earlier pubarche and thelarche (Ibanez et al., 1993; Ibanez et al., 2008), but large studies addressing the age at menarche in relation to PCOS are lacking. The gynaecological age of the girls (2.5 years) was probably too short to abolish an effect of menarcheal age on menstrual cyclicity, but should be long enough to allow most hormonal changes to have taken place (Van Hooff et al., 2000). A substantial number of the girls had probably still not reached full maturation of the HPO axis, a feature that possibly leads to underestimation of the correlation between menstrual irregularities, hyperandrogenaemia and determinants of metabolic risks. Additional adjustment for menarcheal and gynaecological age, however, did not change the results. The later menarcheal age in the symptomatic girls could also be a hallmark of already existing menstrual disorders, but whether or not a later menarcheal age is predictive of future menstrual irregularities and an early symptom of PCOS remains to be elucidated in further follow-up studies.

Surprisingly, the symptomatic girls did not exhibit other metabolic risks linked to PCOS, possibly because the subjects were very young and clinical manifestation of insulin resistance and metabolic disorders had not yet developed. Moreover, the symptomatic group also included girls with mild cycle irregularities, thus decreasing the differences between the groups. In line with this, girls with irregular menstrual cycles have been previously shown to exhibit similar insulin serum concentrations and sensitivity than girls with regular menstrual cycles (Van Hooff et al., 2000). Moreover, in our study, being extremely lean may be one possible cause of menstrual irregularities:

**Figure 2** (A and B) FAI and HOMA-S in the different BMI (kg/m²) quartiles (presented as geometric mean with the range in parentheses). Open bars represent non-symptomatic girls and filled bars symptomatic girls. The P-values represent differences between the two study groups in the different quartiles. *One outlier with an BMI of 71.5 kg/m² lies outside the shown range.*
the symptomatic girls had slightly lower BMIs and WHRs than the non-symptomatic girls, and the proportion of underweight girls was significantly higher in the symptomatic group, in keeping with results of a previous study (Van Hooff et al., 2000). A subanalysis excluding the girls with a low BMI potentiated the statistical significance of the differences between the symptomatic and non-symptomatic groups as regards T, SHBG and FAI.

Still, the symptomatic girls in the highest BMI quartile had the highest prevalence of hyperandrogenaemia and the most unfavourable metabolic findings. It is to note that mild overweight (in the highest quartile, the mean BMI was 25.4 kg/m², a level just above the overweight limit) was already associated with significantly worse hyperandrogenaemia and a more adverse metabolic profile. The girls in the highest FAI quartile had also the most unfavourable lipid profile. All these findings and those including the correlation analyses in the whole population and both study groups confirm earlier data on an association and synergistic interaction between obesity, hyperandrogenaemia and metabolic risks (Rajkhowa et al., 1997; Apridonidze et al., 2005; Fruzzetti et al., 2009). In line with these results, a previous cross-sectional study in girls during early puberty revealed a significant association between extreme obesity and hyperandrogenaemia (McCarty et al., 2006). Moreover, biochemical markers of hyperandrogenism have been correlated specifically to lower serum HDL-cholesterol and higher triglyceride concentrations, i.e. dyslipidaemia and metabolic syndrome (Rajkhowa et al., 1997; Apridonidze et al., 2005; Fruzzetti et al., 2009), and a recent study has shown that the relative risk of metabolic dysfunction, independently of insulin resistance and obesity, increased 4-fold for every quartile increase in free testosterone (Coviello et al., 2006). It remains to be determined whether, as seems likely, prospective studies of our NFBC population will reveal an increasing incidence of metabolic abnormalities, especially in relation to excessive weight gain.

Serum levels of CRP reflect the inflammatory milieu and have been shown to correlate well with the risk of cardiovascular diseases as well as related events (Wild et al., 2010). Moreover, previous studies on adolescent girls with hyperandrogenaemic phenotypes of PCOS have shown an increase in parameters of chronic inflammation (Alemezadeh et al., 2010). In the present study, however, serum CRP levels did not differ between the symptomatic and non-symptomatic girls, probably as a result of similar BMI in the two groups. This result is in line with that in a previous study showing that the parameter most strongly associated with chronic inflammation was not PCOS but obesity (Mohlig et al., 2004). Accordingly, in the present study, there were significant correlations between CRP levels, BMI and WHR, and a significant linear trend towards higher CRP levels in the highest BMI quartiles, but no such trend was observed towards the highest FAI quartiles.

We conclude that menstrual disorders, identified via a simple question at the time of adolescence, is a good marker of hyperandrogenaemia and may be a risk factor for the development of PCOS in adulthood. The presence of menstrual disorders in the highest BMI and hyperandrogenaemia quartiles is also associated with subclinical metabolic disorders as early as at age 16, suggesting an increased risk of cardiovascular diseases in later life. All these findings confirm that the association between menstrual disorders, hyperandrogenism, obesity and metabolic risks is already evident in adolescence, which strengthens the importance of recording menstrual disorders at an early stage.

Authors’ roles


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Conflict of interest

None of the authors have any conflict of interest to declare.

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