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Are menstrual disorders in adolescent girls related to metabolic disorders?

Czy zaburzenia miesiączkowania u dorastających dziewcząt są związane z zaburzeniami metabolicznymi?

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Abstract

Introduction: Menstrual disorders in adolescent girls are a common clinical problem. They are often accompanied by lipid and glucose metabolism disturbances. The aim of the study was to investigate to what extent the metabolic profile of adolescent girls relates to the severity of their menstrual disorders.

Material and methods: The study included 165 girls with menstrual disturbances and 49 regularly menstruating girls (REG) without clinical hyperandrogenism, matched for age and BMI. The subjects from the study group were divided into 2 subgroups: OLIGO – 111 girls with oligomenorrhea and SA – 54 girls with secondary amenorrhoea. In all girls, hormonal, lipid, and carbohydrate metabolism profiles were assessed.

Results: In the SA subgroup concentrations of total cholesterol (TC) and LDL were significantly higher than in the REG and OLIGO groups. Triglyceride (TG) concentration was also the highest in the SA group and significantly higher than in the REG group. The prevalence of lipid metabolism disorders was higher in the SA group (65%) vs. the REG (40%) and OLIGO (51%) groups. The subgroups did not differ significantly in terms of fasting and OGTT glucose and insulin as well as HOMA-IR. TyG index was significantly higher in the OLIGO and SA groups than in the REG group. BMI z-score correlated with TG, LDL, fasting and 120' OGTT glucose and insulin, HOMA-IR, and TyG and negatively with HDL. No relationship between hormonal concentration and metabolic disturbances was found.

Conclusions: Adolescent girls with menstrual disorders are insulin resistant, regardless of PCOS diagnosis. The severity of menstrual disorders may be related to the incidence of lipid disorders in adolescent girls.

Key words:
metabolic disorders, adolescent girls, oligomenorrhea, secondary amenorrhea.

Streszczenie

Wprowadzenie: Zaburzenia miesiączkowania u dorastających dziewcząt są istotnym problem klinicznym i często wiążą się z zaburzeniami gospodarki lipidowej i węglowodanowej. Celem pracy była ocena profilu metabolicznego u nastoletnich dziewcząt w zależności od ciężkości zaburzeń miesiączkowania.

Materiał i metody: Badaniem objęto 165 dziewcząt z zaburzeniami miesiączkowania i 49 regularnie miesiączkujących dziewcząt bez cech hiperandrogenizmu (REG) dobranych pod względem wieku i BMI. Pacjentki grupy badanej podzielono na 2 podgrupy: OLIGO – 111 dziewcząt z oligomenorrhea i SA – 54 dziewczęta z wtórnym brakiem miesiączki. U wszystkich dziewcząt dokonano oceny gospodarki hormonalnej, lipidowej i węglowodanowej.

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Wyniki: U dziewcząt z grupy SA stężenia cholesterolu całkowitego (TC) i LDL (LDL) były istotnie wyższe niż w grupie REG i OLIGO. Stężenie triglicerydów (TG) było również najwyższe w SA i istotnie wyższe niż u dziewcząt regularnie miesiączkujących. Częstość występowania zaburzeń gospodarki lipidowej była wyższa w SA (65%) w porównaniu z REG (40%) i OLIGO (51%). Podgrupy nie różniły się istotnie pod względem stężenia glukozy i insuliny na czczo i w OGTT oraz HOMA-IR. Wskaźnik TyG był istotnie wyższy w OLIGO i SA niż w REG. BMI Z-*score* dodatnio korelowało z TG, LDL, glikemią i insuliną na czczo, stężeniem glukozy i insuliny w 120' OGTT, HOMA-IR, TyG oraz ujemnie ze stężeniem HDL. Nie stwierdzono związku między stężeniem hormonów a zaburzeniami metabolicznymi.
Wnioski: Dziewczęta z zaburzeniami miesiączkowania, niezależnie od diagnozy PCOS, charakteryzują się występowaniem insulinooporności. Ciężkość zaburzeń miesiączkowania może być związana z zaburzeniami gospodarki lipidowej.
Key words:
zaburzenia metaboliczne, nastoletnie dziewczęta, rzadkie miesiączki, wtórny brak miesiączki.

Introduction

Menstrual disorders in adolescent girls are a significant clinical problem. The normal cycle duration in teenage girls is 21–45 days, but in the first 2 years after menarche, cycles are usually irregular and many of them are anovulatory, due to the immaturity of the hypothalamic-pituitary-ovary axis. The mean age of the first menstruation is 12–13 years, and most often, 2–3 years after menarche the cycles become ovulatory, resulting in their normalization [1, 2].

However, in some girls, disturbances in the menstrual cycle, such as rare menstruation, are not an expression of immaturity of the hypothalamic-pituitary-ovary axis, but a symptom of disease. It is believed that menstrual cycles lasting more than 90 days in the first year after menarche or longer than 45 days in subsequent years, and amenorrhoea up to the age of 15 or secondary amenorrhoea, require diagnostics, with particular emphasis on polycystic ovary syndrome (PCOS). It is estimated that approximately 50% of adolescent girls with oligomenorrhea will develop full-blown PCOS in the future. The syndrome is characterized by a wide spectrum of symptoms associated with ovulation disorders and androgen excess [3–7]. Additionally, patients with PCOS tend to be overweight and obese. Dyslipidaemia, insulin resistance (IR), impaired glucose tolerance, and type 2 diabetes occur more often than in the general population, and the level of metabolic disturbances is associated with the severity of PCOS phenotype [8, 9]. Da Silva Bouzas *et al.* [10] found that also adolescent girls with irregular cycles are at higher risk of glucose and lipid disturbances as well as metabolic syndrome (MS). We hypothesized that in adolescent girls the severity of menstrual disturbances can be related to the lipid and glucose metabolism disturbances.

The aim of the study was to investigate the metabolic profile in adolescent girls with menstrual disorders with respect to their severity.

Material and methods

The study was retrospective and included 165 adolescent girls with menstrual disturbances (chronological age: 16.4 ±1.2 years, gynaecological age: 4.0 ±2.0 years) and 49 regularly menstruating girls without clinical symptoms of hyperandrogenism (chronological age: 16.2 ±1.3 years, gynaecological age: 4.4 ±1.4 years) in whom hormonal disorders were excluded. The girls were recruited from the patients of the Department of Paediatrics and Paediatric Endocrinology and matched for chronological and gynaecological age. All participants were Caucasian.

According to menstrual disturbances the subjects from study group were divided into 2 subgroups. The OLIGO group comprised 111 girls with oligomenorrhoea (chronological age:16.4 ±1.3 years, gynaecological age: 4.2 ±1.7 years), and the SA group comprised 54 girls with secondary amenorrhoea (chronological age:16.4 ±1.1 years, gynaecological age: 3.6 ±2.4 years). Oligomenorrhoea was defined as menstrual cycles ≥ 45 days; secondary amenorrhoea was defined as absence of menstruation within the last 3 months in a patient who previously had normal menstruation [11]. Patients with eating disorders, thyroid dysfunction, disorders of the adrenal cortex, hyperprolactinemia, and gynaecological age below 2 years were excluded from the study. No subject was using medication known to influence endocrine, glucose, and lipid profiles. PCOS diagnosis was made on the basis of the criteria of Pena AS *et al.* [12, 13].

The study was approved by the Local Bioethics Committee (no. PCN/CBN/0022/KB/201/21).

In all patients, the following data were analysed:

1. Anthropometric measurements (body weight, height). Body weight was measured with an accuracy of 0.1 kg; body height was determined with a Harpenden stadiometer with a precision of 0.1 cm. Body mass index (BMI) and standard deviation of the mean BMI value (BMI z-score; calculated using the Paediatric Z-score Calculator based on percentile grids) were calculated [14].
2. Biochemical tests results – fasting total cholesterol (TC), HDL cholesterol (HDL), LDL-cholesterol (LDL), triglycerides (TG), as well as fasting and at 120 minutes glucose in oral glucose tolerance test (OGTT) after a load of 75 g glucose. Hyperlipidaemia was diagnosed when the TC, LDL-cholesterol, or TG level were above, and/or HDL-cholesterol below, the age and gender normal values [15]. Elevated glucose concentration was considered when fasting glucose was > 99 mg/dl and/or glucose in 120' of OGTT was > 139 mg/dl, and respectively hyperinsulinaemia was diagnosed when fasting insulin was > 15 mIU/l and/or insulin in 120' of OGTT was >75 mIU/l [16].
3. Hormonal tests results: total testosterone (T), oestradiol (E2), luteinizing hormone (LH), follicle stimulating hormone

(FSH), dehydroepiandrosterone sulphate (DHEAS), 17-hydroxyprogesterone (17OHP), androstenedione (A), fasting and 120-minute OGTT insulin (INS). The assessment of the homeostasis model of insulin resistance (HOMA-IR), defined as fasting blood glucose (mg/dl) and INS (μU/ml) divided by the constant (22.5), was used to calculate the insulin resistance index. Additionally, TyG index, known to be a good and reliable index of insulin resistance, was calculated as Ln (fasting triglycerides [mg/dl] $\times$ fasting glucose [mg/dl]/2) [15]. Hyperandrogenaemia was defined as a total T concentration greater than 45 ng/dl (standard given by the laboratory). Hyperinsulinaemia was diagnosed when fasting INS was >15 mIU/l and/or INS in 120' of OGTT was >75 mIU/l [16].

To determine the biochemical and hormonal parameters, a venous blood sample was drawn during the follicular phase of the menstrual cycle (day 2–5 of the cycle) or 3 months after the last menstruation, in the morning, in the fasting state (12 hours after the last meal).

The basal plasma concentration of LH, FSH, INS, and DHEAS was determined using the chemiluminescence method (Immulite 2000XPi; Siemens, Germany), and the concentration of E2 and T using the electrochemiluminescence method (Cobas e601, Roche, Germany). To determine the concentration of 17OHP and A, enzyme immunoassays (ELISA) were used (DS2). The lipid profile was done by enzymatic calorimetric test.

Auxological data and hormonal results were compared using Statistica 13.3 PL software. All values were expressed as mean (standard deviation) for normal distribution or median (interquartile range) for skewed distribution. Differences between 3 groups were assessed by one-way ANOVA or Kruskal-Wallis test, followed by the least significant difference (LSD) test for multiple comparisons when applicable. Correlation analysis was performed using the Pearson correlation coefficient for normally distributed samples and the Spearman correlation coefficient for non-normally distributed data. Gamma correlation was used for non-normal distributions with many tied ranks. $P < 0.05$ was considered statistically significant.

Results

The anthropometric, clinical, biochemical, and hormonal characteristics of the adolescent girls are presented in Tables 1 and 2. The differences in chronological age, and BMI z-score, between the groups were insignificant ($p > 0.05$). Although the age of menarche was significantly younger in the REG group than in the OLIGO ($p = 0.03$) and SA ($p < 0.001$) groups. The prevalence of obesity was higher in the SA (52%) group than in the REG (31%) ($p = 0.045$) group and OLIGO (43%) ($p > 0.05$) group. According to the criteria of Pena *et al.*, PCOS could be diagnosed in 78 girls from the OLIGO (70.3%) group and

Table I. Clinical and hormonal characteristics of adolescent girls with menstrual disorders: with oligomenorrhoea (OLIGO, $n = 111$) and secondary amenorrhoea (SA, $n = 54$), and control group of regularly menstruating girls (REG, $n = 49$)

Parameter	OLIGO ($n = 111$)	SA ($n = 54$)	REG ($n = 49$)
Chronological age [years]	16.4 1.3	16.4 1.1	16.2 1.3
Gynaecological age [years]	4.2 1.7	3.7 2.4[a]	4.4 1.4[b]
Age of menarche [years]	12.3 1.6	13.0 1.3[c]	11.9 1.5[d]
BMI Z-score	2.1 9.5	1.1 1.4	0.8 1.1
Ferriman-Gallwey score	6.2 6.2	2.9 5.2[e]	1.9 2.7f
LH [mIU/ml]	9.6 ±7.6	9.8 ±6.8	7.2 ±8.5[g,h]
FSH [mIU/ml]	4.8 ±1.8	7.5 ±12.8[i]	5.1 ±2.2
Testosterone [ng/dl]	59.8 23.2	53.7 27.6	44.3 19.0[j]
Androstenedione [ng/ml]	4.6 2.3	4.1 1.7	4.1 1.7
DHEAS [g/dl]	303.5 126.0	274.9 114.5	294.4 115.7
17OHprogesteron [ng/ml]	2.5 1.5	2.2 1.5	2.2 0.9
Oestradiol [pmol/l]	214.3 175.0	174.0 109.8	225.1 225.3

[a]OLIGO vs. SA, $p = 0.04$; [b]SA vs. REG, $p = 0.01$; [c]OLIGO vs. SA, $p = 0.03$; [d]SA vs. REG, $p < 0.001$; [e]OLIGO vs. SA, $p < 0.001$; [f]SA vs. REG, $p < 0.001$; [g]OLIGO vs. REG, $p = 0.03$; [h]SA vs. REG, $p = 0.009$; [i]OLIGO vs. SA, $p = 0.007$; [j]OLIGO vs. REG, $p < 0.001$

Table II. Metabolic characteristics of adolescent girls with menstrual disorders: with oligomenorrhoea (OLIGO, $n = 111$) and secondary amenorrhoea (SA, $n = 54$), and control group of regularly menstruating girls (REG, $n = 49$)

Parameter	OLIGO ($n = 111$)	SA ($n = 54$)	REG ($n = 49$)
Total cholesterol [mg/dl]	164.5 ±31.2	188.7 ±40.5[a]	163.3 ±30.4[b]
HDL cholesterol [mg/dl]	50.9 ±11.8	53.2 ±12.3	54.0 ±10.1
LDL cholesterol [mg/dl]	90.2 ±26.3	109.7 ±34.8[c]	91.8 ±25.6[d]
Triglycerides [mg/dl]	116.1 ±51.1	120.8 ±54.0[e]	88.4 ±33.1[f]
Fasting glucose [mg/dl]	88.0 ±7.6	88.4 ±8.4	87.0 ±9.1
Glucose at 120 min of OGTT [mg/dl]	106.1 ±28.0	110.4 ±30.3	105.7 ±27.7
Fasting insulin [μIU/ml]	14.3 ±8.9	16.2 ±11.4	13.9 ±7.2
Insulin at 120 minutes of OGTT [μIU/ml]	87.4 ±63.8	94.9 ±66.0	93.9 ±65.3
HOMA-IR	3.2 ±2.0	3.6 ±2.7	3.1 ±1.8
Triglyceride-glucose index (TyG index)	8.5 ±0.42	8.5 ±0.47[g]	8.2 ±0.36[h]

[a]OLIGO vs. SA, $p < 0.001$; [b]SA vs. REG, $p < 0.001$; [c]OLIGO vs. SA, $p < 0.001$; [d]SA vs. REG, $p = 0.009$; [e]SA vs. REG, $p = 0.001$; [f]OLIGO vs. REG, $p = 0.002$; [g]SA vs. REG, $p < 0.001$; [h]OLIGO vs. REG, $p = 0.001$;

25 (46.3%) from the SA group ($p = 0.003$) [16]. In the OLIGO group the T concentration was significantly higher than in the REG group ($p < 0.001$). There were no differences in DHEAS, 17OHP, and A levels between the groups. In the SA group the FSH concentration was the highest and significantly higher than in the OLIGO group ($p = 0.007$). Girls with regular menstruation had a significantly lower LH concentration compared to the SA group ($p = 0.009$) and OLIGO group ($p = 0.03$). The highest value of the LH/FSH ratio was observed in the REG group, and the lowest in the OLIGO group. These differences were statistically significant ($p < 0.001$).

Mean ovarian volumes were the highest in the SA group, while the lowest was observed among patients without menstrual disorders ($p = 0.012$). In the OLIGO group, 78 patients (70.3%) showed polycystic ovarian morphology, while in the SA group such a structure was found in 25 girls (46.3%). These were statistically significant differences ($p = 0.003$).

We found significant differences between the examined subgroups in the parameters of lipid metabolism (Table 2). The highest mean concentrations of TC, LDL, and TG were found in the SA group as compared to controls ($p < 0.001$; $p = 0.009$; $p = 0.001$, respectively). TC and LDL levels in the SA group were also significantly higher than in the OLIGO group ($p < 0.001$; $p < 0.001$, respectively). In the OLIGO group the mean TG concentration was significantly higher than in the REG group ($p = 0.002$).

The prevalence of lipid metabolism disorders was highest in the SA group (65%) compared to the REG (40%) ($p = 0.01$) and OLIGO (51%) ($p > 0.05$) groups. The prevalence of hypertriglyceridaemia (TG > 130 mg/dl) was highest in the SA group (39%) compared to the REG (12 %) ($p = 0.003$) and OLIGO (24%) ($p > 0.05$) groups. Moreover, the prevalence of level LDL > 110 mg/dl was highest in the SA group (41%) compared to the REG (20%) ($p = 0.03$) and OLIGO (17%) ($p = 0.002$) groups. The highest prevalence of HDL<40 mg/dl was found in the OLIGO group (15%) compared to the REG group (6%) ($p > 0.05$), and it was similar to the prevalence in the SA group (13%) ($p > 0.05$).

All 3 subgroups did not differ significantly in terms of fasting and OGTT glucose and INS concentration as well as HOMA-IR (Table 2). However, the TyG index was significantly higher in the study groups than in the control group (OLIGO: $p = 0.001$, SA: $p < 0.001$). The prevalence of increased glucose level (SA: 7 girls [13%], OLIGO: 17 girls [15%], REG: 8 girls [17%], $p > 0.05$) and INS level (SA: 28 girls [54%], OLIGO: 59 girls [53%], REG: 24 girls [51%], $p > 0.05$) did not differ between the groups.

In girls with menstrual disturbances the BMI z-score was positively correlated with TG, LDL, fasting and OGTT glucose and INS level, HOMA-IR, and TyG and negatively with HDL concentration (Table 3).

There was no relationship between the lipids and glucose disturbances and E2, LH, and DHEAS in girls with menstrual disorders. However, the DHEAS level was related to glucose and INS in 120 min of OGTT ($r = 0.2$, $p = 0.02$, $r = 0.2$, $p = 0.02$, respectively). Whereas 17OHP correlated with fasting hyperglycaemia ($r = 0.26$, $p = 0.03$). Moreover, we found that the T and 17OHP level were related negatively with the LDL level

($r = –0.16$, $p = 0.04$, $r = –0.20$, $p = 0.02$, respectively) as well as LDL>110 mg/dl ($r = –0.19$, $p = 0.03$, $r = –0.29$, $p = 0.002$, respectively). Also, E2 negatively correlated with the TC and LDL concentration ($r = –0.16$, $p = 0.04$, $r = –0.19$, $p = 0.02$, respectively). FSH concentration was related positively with TC and LDL ($r = 0.27$, $p < 0.001$, $r = 0.25$, $p = 0.002$, respectively) and LDL > 110 mg/dl ($r = 0.18$, $p = 0.05$).

Because of the high prevalence of PCOS in groups with menstrual disturbances, we decided to look at the adolescent girls with menstrual disturbances and their lipid and carbohydrate metabolism in respect to PCOS diagnosis. We divided the study group into PCOS and non-PCOS subgroups. In the PCOS group we found the highest (significantly higher than in the non-PCOS group) hirsutism score ($p < 0.001$), TTE ($p < 0.001$), DHEAS ($p < 0.001$), 17OHP ($p = 0.007$), and A ($p < 0.001$) level (Table 4). In terms of metabolic characteristics, the TG concentration (PCOS vs. REG, $p = 0.002$; non-PCOS vs. REG, $p < 0.001$) and TyG index were significantly higher in both study groups than in the control group. The other lipids, glucose, INS concentration, and HOMA-IR did not differ significantly between the groups.

Table III. Correlation between BMI z-score and metabolic parameters in the study group

BMI Z-score	R	*p*
Total cholesterol	0.13	0.06
HDL cholesterol	–0.44	< 0.001
LDL cholesterol	0.20	0.003
Triglycerides	0.42	< 0.001
Fasting glucose	0.17	0.016
Glucose at 120 min of OGTT	0.24	< 0.001
Fasting insulin	0.53	< 0.001
Insulin at 120 min of OGTT	0.33	< 0.001
HOMA IR	0.53	< 0.001
Triglyceride-glucose index (TyG index)	0.42	< 0.001

Table IV. MHormonal and metabolic characteristics of adolescent girls with menstrual disorders: with PCOS diagnosis (PCOS, $n = 103$) and without PCOS diagnosis (non-PCOS, $n = 62$), and control group of regularly menstruating girls (REG, $n = 49$)

Parameter	PCOS ($n = 103$)	non-PCOS ($n = 62$)	REG ($n = 49$)
Ferriman-Gallwey score	7.8 ±6.2[a]	0.7 ±1.8[b]	1.9 ±2.7
Testosterone [ng/dl]	69.2 ±23.2[c]	37.4 ±10.5[d]	44.3 ±19.0
Androstenedione [ng/ml]	4.9 ±2.3[e]	3.3 ±1.1	4.1 ±1.7
DHEAS [g/dl]	328.3 ±128.1[f]	254.0 ±99.0	294.4 ±115.7
17OHprogesteron [ng/ml]	2.6 ±1.5[g]	2.0 ±1.3	2.2 ±0.9
Total cholesterol [mg/dl]	166.7 ±30.8	181.9 ±42.2	163.3 ±30.4
HDL cholesterol [mg/dl]	50.3 ±12.4	53.5 ±11.0	54.0 ±10.1
LDL cholesterol [mg/dl]	92.3 ±25.7	103.8 ±36.7	91.8 ±25.7
Triglycerides [mg/dl]	116.3 ±51.9[h]	119.9 ±52.5[i]	88.5 ±33.1
Fasting glucose [mg/dl]	88.9 ±8.4	86.8 ±6.6	87.0 ±9.1
Glucose at 120 min of OGTT [mg/dl]	109.6 ±30.4	103.9 ±25.6	105.7 ±27.7
Fasting insulin [μIU/ml]	15.5 ±9.8	14.0 ±9.7	13.9 ±7.3
Insulin at 120 min of OGTT [μIU/ml]	96.9 ±71.1	77.8 ±49.53	93.9 ±65.3
HOMA-IR	3.5 ±2.3	3.1 ±2.2	3.1 ±1.8
Triglyceride-glucose index (TyG index)	8.5 ±0.43[j]	8.5 ±0.44[k]	8.2 ±0.36

[a]PCOS vs. non-PCOS, $p < 0.001$; [b]PCOS vs. REG, $p < 0.001$; [c]PCOS vs. non-PCOS, $p < 0.001$; [d]PCOS vs. REG, $p < 0.001$; [e]PCOS vs. non-PCOS, $p < 0.001$; [f]PCOS vs. non-PCOS, $p < 0.001$; [g]PCOS vs. non-PCOS, $p = 0.007$; [h]PCOS vs. REG, $p = 0.002$; [i]non-PCOS vs. REG, $p < 0.001$; [j]PCOS vs. REG, $p = 0.001$; [k]non-PCOS vs. REG, $p < 0.001$

Discussion

In our study we evaluated metabolic profiles in adolescent girls with menstrual disorders with respect to symptomatic severity (oligomenorrhoea and secondary amenorrhoea) and compared them to regularly menstruating patients. Despite similar BMI z-score, we found that in girls with secondary amenorrhoea the concentrations of TC and LDL cholesterol were the highest and significantly higher than in girls with regular menses and oligomenorrhoea. The TG level was also the significantly higher in girls with menstrual disorders than in regularly menstruating peers. The prevalence of lipid metabolism disorders was the highest in girls with secondary amenorrhoea. When we divided the study group into PCOS and non-PCOS subgroups, we found higher TG level and TyG index in the study group that in the control group. Metabolic abnormalities in the study group were mainly related to BMI-score. Based on our study, we conclude that girls with menstrual disorders are insulin resistant, but girls with SA have the highest risk of lipid disturbances.

PCOS is closely related to the occurrence of metabolic disorders such as obesity or insulin resistance (IR) with accompanying compensation hyperinsulinaemia [19]. Moreover, PCOS is associated with higher risk of developing other metabolic disorders, such as type 2 diabetes (T2D), hypertension, dyslipidaemia, and cardiovascular diseases [20]. Previous data suggest that the pattern of menstrual cycle irregularities may correlate with metabolic disorders [21, 22]. It is well established that insulin resistance is directly related to obesity and BMI, playing an important role not only in the pathophysiology of MS, but also PCOS. Also, hyperinsulinaemia may promote menstruation disorders, altering the gonadotropin-releasing hormone (GnRH) pulse secretion pattern, suppressing sex hormone-binding globulin (SHBG), and stimulating ovarian androgenesis. Hyperandrogenaemia has been reported to be an independent risk factor for MS in adult women without PCOS [23–25]. There are still limited data in the literature concerning the metabolic features of different PCOS phenotypes in adolescence. Coviello *et al.* [24] confirmed that the prevalence of MS was significantly higher in patients with menstrual disorders. Many other authors also found the relationship between high cholesterol and LDL levels and low HDL concentration in patients with long cycles. Recent studies suggested that the severity of lipid and carbohydrate disorders may depend on the presented phenotype [21, 26, 27]. Fruzzetti *et al.* [21] evaluated the endocrine and metabolic characteristic of adolescents with PCOS showing different phenotypes. Their study indicates an increased concentration of androgens as a critical factor for the development of lipid disorders. Similarly to other studies, our data show that adolescents with irregular menstruation present a higher prevalence of lipid alterations, with high TC, LDL, and TG levels. The lipid metabolism disorders were most pronounced in the group of patients with secondary amenorrhoeaAs is commonly known IR is one of the most common metabolic disorders in women with PCOS, and it concerns 65–70% of all patients [28]. IR and hyperinsulinaemia are considered important components in the pathogenesis of this endocrinopathy [29]. Unexpectedly, the subgroups also did not differ significantly in terms of carbohydrate and insulin parameters. The hyperinsulinaemic euglycaemic clamp remains a gold standard for accurate evaluation of insulin resistance. However, due to its complexity it cannot be implemented on a routine basis [30]. The great diversity of insulin concentration in the population, both fasting and post-loaded (OGTT), different assay methods, and the contribution of multiple confounding factors that can significantly affect the result, make it impossible to clearly define the cut-off point for hyperinsulinism. Moreover, studies indicate that fasting insulin is not a good marker of insulin resistance [31, 32]. Alternatively, in clinical research to estimate insulin action, several surrogate indexes are widely used. These indexes are derived from plasma glucose and insulin levels at fasting or after oral glucose load [30–32]. In our study differences between the groups in regard to fasting and OGTT glucose and INS concentration as well as HOMA-IR were not statistically significant. But higher values of the HOMA-IR index were observed in the group of patients with menstrual disorders, especially in the SA group. In addition, when we used the TyG index, we found it significantly higher in the study groups than in the control group. TyG is considered as a simple, available surrogate for identifying IR, and it showed an association with diabetes, hypertension, non-alcoholic fatty liver disease, and atherosclerosis [17, 33]. Moreover, it is not based on the fasting INS concentration, which is considered not to be a reliable index of IR assessment.

Recent studies show that adolescent girls with irregular menstruation present a higher prevalence of IR and clinical/laboratory disorders related to IR, including higher waist circumference, higher glucose level after 2 hours of OGTT, fasting, and post-overload insulin, HDL suppression, and TG elevation, compared to the subgroup with regular menstrual cycles. Panidis *et al.* [29] suggested that amenorrhoea is associated with more severe IR in PCOS. This association can be explained by the relationship between IR and anovulation. Despite the lack of statistically significant differences in the assessment of carbohydrate metabolism between the groups, patients with SA presented higher blood glucose levels in 120 'OGTT and higher insulin levels, both fasting and 120' OGTT and HOMA-IR. We also observed the highest concentrations of TG in this group. The accumulation of triglyceride content has been associated with the insulin-resistant state. Also, when we looked at our patients with respect to PCOS diagnosis, HOMA-IR was highest in the PCOS group without reaching statistical significance, but TyG and TG were similar in the non-PCOS and PCOS groups and significantly higher than in the control group.

Moreover, we found many significant correlations between metabolic parameters and BMI Z-score, whereas only a few with hormones levels. In our study, all 3 subgroups did not differ significantly in terms of androgens concentrations. We only noticed that in the group with menstrual disturbances androgens as well as oestradiol levels were negatively related with high TC and LDL levels. 17OHP was the only hormone related to high TG concentration. The main factor associated with elevated TG levels and decreased HDL levels was BMI Z-score. Excess body weight seems to be the key factor worsening the

metabolic profile of patients with secondary amenorrhoea and oligomenorrhoea since adolescence.

Our study weakness is its retrospective approach, which is related with the lack or incompleteness of some data. The analysis would be more valuable if we were able to analyse the relationship between metabolic disturbances and some anthropometric data (waist and hip circumference), family interview regarding the occurrence of lipid metabolism disorders, or SHBG concentration. The strength of our manuscript is the biochemical and hormonal analysis of a high number of patients matched for gynaecological age.

In conclusion, adolescent girls with menstrual disorders are insulin resistant, regardless of fulfilling PCOS diagnostic criteria. Moreover, girls with the most severe menstrual disturbances (secondary amenorrhoea) have the least favourable lipid profile. Metabolic disturbances in girls with menstrual disorders are associated mainly with body weight, and only some weak relationship with hormonal level can be considered. We concluded that all girls with menstrual disturbances should be closely monitored for metabolic parameters and that the severity of menstrual disorders may be related to the incidence of lipid disorders.

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