The Estimated Adolescents'Carbohydrate Metabolism and Insulin Resistance in PCOS

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The Polycystic Ovary Syndrome (PCOS) represents a recognised entity within the female medical conditions today. The multidisciplinary aspects refer to various and variable symptomatology, which mainly associates the reproductive problems with clinical signs of hyper-androgyny and metabolic disorders, frequently associated with obesity. The present study demonstrates the existence of some carbohydrate metabolic anomalies in adolescents diagnosed with PCOS which had remained asymptomatic and undiagnosed prior to the analysis. It is thus suggested that metabolic testing is necessary for adolescents diagnosed with PCOS, even if they are neither overweight nor part of the definition criteria. It is important for the therapeutic interventions to analyse the DM2 intermediary risk markers, as the oral glucose tolerance test (OGTT) repeated 3 hours after glucose powder dose ingestion is informative of the persistent hyperglycaemia and the decrease in pancreatic function.

Keywords: Polycystic Ovary Syndrome; adolescents; carbohydrate metabolism

Obesogenic environment and *globesity* are new terms introduced to reflect the extent and the significance of the problems due to obesity on Earth, including those in underdeveloped countries [1]. The lack of physical activity, the sedentary work style, unhealthy eating and use of forbidden substances in childhood, adolescence and adulthood characteristic of people in present days overlap with genetic predisposition to some non-communicable diseases as aggravating factors and are important in their evolution [2]. At the same time, the relation between these conditions and Polycystic Ovary Syndrome (PCOS), as well as the consistency of specialists in elucidating the causes and mechanism behind it and its early diagnosis will likely result in more frequent reports of PCOS within the female population [3].

In the past years PCOS has been increasing in epidemiological significance, now reportedly between 6 and 12% women in reproductive age [4]. Most research is mainly limited to the reproductive age, as the typical signs of hyper-androgyny (hirsutism and acne) are usually associated with reproductive system problems (infertility, menstruation problems) or metabolic anomalies (disglycaemia, dislipidemia) and frequently obesity [5].

The etiopathogeny is not yet completely discovered, although it has been demonstrated that a multitude of factors are involved: genetic, environmental and behavioural, all under the influence of time. All these factors, in various associations, account for the phenotypes described in different studies and the multidisciplinary aspect of the condition [6]. There are also various standardised definitions of the condition. The *Rotterdam criteria* by ESHRE/ASRM defining PCOS, refer to adult women, have been accepted by most subsequent studies from various medical specialties and are supported by this group until today: oligo/anovulation and/or hyper-androgyny and/or specific ultrasound ovary image changes in vaginal examination (high ovarian volume and small subcapsular follicles) – two of three criteria being necessary [7]. The multi-phenotypic aspect of PCOS has been explained in detail by AES-PCOS (Androgen Excess Society). In fact, PCOS is considered one of the most frequent causes of hyper-androgyny [8], the ovary morphology and ovulation characteristics not being specific in adolescence [6]. The disputes concern the symptoms which overlap with the physiological changes specific to adolescents: transient hyper-androgyny (acne, moderate signs of hirsutism and high values of testosterone), possible menstrual cycle disorders and inconclusive ovary ultrasound images (only by transabdominal access, multi-follicular aspect or unilateral changes) [9].

Although it has been proved that insulin resistance (IR) and countervailing hyperinsulinemia are at the root of the metabolic dysfunctions associated with PCOS, they have not been included among the criteria defining the disease so far. In the past two or three decades, many studies have associated IR to PCOS, even for adult women and adolescents of normal weight or underweight, but this has remained an alternative diagnostic criterion for teenagers, although oral anti-diabetic treatment was included in PCOS therapy [10,11].

A careful monitoring of children with abnormal glucose metabolism or diabetes reduces the risk of cardiovascular disease [12]. Thus, the aim of this study was to evaluate the glucose metabolism in teenagers with PCOS in order to discover the frequency of the early disorders.

Experimental part

Materials and methods

The study was conducted as a prospective one, over a three-year period. It included 122 adolescents between 14 and 19 years old, diagnosed with PCOS according to the *Amsterdam 2010* criteria (the Rotterdam ESRE/AMSR criteria adapted to the age groups). Informed consent was obtained according to the active European Union and World Health Organisation regulations on the research on human subjects and the study was approved by the Ethics Committee at the Dunarea de Jos University of Galati, Romania. The evaluation of the studied parameters was performed against a control group of 102 people chosen at random, without specific PCOS symptoms, other disorders

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or hormonal treatments in the past 3 months, with a similar age group structure to that of the PCOS girls.

Anamnesis data, anthropometric measurements, clinical examination, imaging examination of the reproductive system and laboratory data concerning glucose metabolism and insulin resistance were collected: fasting glucose (FG), fasting insulin (FI), the oral test for glucose tolerance at 1 hour (OGTT-1), 2 hours (OGTT-2) and 3 hours (OGTT-3). The reference range of FG values for children are the same as those for adults, with a cut-off value of 100 mg/dL. FI was determined by chemi-luminescence immunoassay (CLIA), with a Sonolite apparatus. The interpretation of the glucose tolerance test was made followng the recommendations of IDF (International Diabets Federation) and ADA (American Diabetes Association): a result between 140 and 200 mg/dL after 2 hours was considered to indicate impaired glucose tolerance (IGT) and any values over 200 mg/dL were considered indicative of diabetes mellitus (DM). Values between 100 and 126 mg/dL were considered to assign a pacient to the group of glucose disorder termed impaired fasting glucose (IFG). Normal values of glucose after 3 h were considered to be those under 100 mg/dL, as well as those of basal glucose [13]. Postprandial hypoglycemia was considered in the case of glucose levels under 55 mg/dL.

The homeostatic model assessment (HOMA) was used for quantification of insulin resistance (IR), according to the accepted formula: *FG×FI/22.5*. The updated version HOMA-2 (HOMA Calculator, Diabetes Unit Trial of Oxford University, https://www.dtu.ox.ac.uk/ homacalculator/) gives the correlation referring to peripheral glucose and the hepatic fluctuations and shows the insulin-resistance index, as well as the functional percent of β-pancreatic cells. The HOMA values increase in direct proportion to insulin resistance, and IR was defined at a HOMA–IR value of over 2.2. Values of the HOMA index under 2.0 were considered normal; values between 2.0 and 2.2 were cut-off values; values between 2.2 and 3.0 indicated moderate IR and values over 3.0 were considered to indicate severe IR. The assessment of the β-pancreatic cells function (HOMA-â) was made depending on the unit value.

Mean values and standard deviations were calculated for all the variables. Data-Analysis and Analysis Toolspack of *Microsoft Exel 2010* software were used for analysis of variance (ANOVA), Student's *t*-test, and the *Minitab 19* programme. The results from analytical methods were considered statistically significant at a $p \leq 0.05$.

Results and discussions

The distribution of adolescent girls among age ranges (PCOS and control) is shown in table 1.

The laboratory results showed statistically significant differences of the FI values ($p = 0.017$) and FG ($p = 0.016$) between the PCOS group and the control group, for all age ranges. The OGTT 1 hour after glucose ingestion showed no significant increase of FG values in 59 cases (49.36%), with a flat curve aspect, but with no statistically significant differences between the groups ($p = 0.115$), not even between the age groups ($p = 0.128$). In non-diabetic persons, elevated FG and glycated hemoglobin are correlated with increased risk of cardiovascular disease [14]. There were significant differences between the PCOS age ranges and the control group for the OGTT values after 2 hours ($p= 0.05$) and after 3 h ($p = 0.045$) and HOMA scores ($p = 0.028$ for HOMA-ß and $p = 0.049$ for HOMA-IR) (table 2).

AGE / N	PCOS		CONTROL	
	Urban	Rural	Urban	Rural
14-<15 Y	8	4	6	4
15-<16 Y	15	4	10	6
16-<17 Y	20	7	16	6
17-<18 Y	21	10	18	6
18-<19 Y	23	10	22	8
TOTAL	87	35	74	28

Table 1
AGE SERIES(n) OF PCOS SAMPLE AND CONTROL

Variable		n	14-15y	n	15-16 y	n	16-17 y
FI	PCOS	12	6.497±6.859	19	15.274±12.41	27	8.582±8.387
	Control	10	10.060±4.027	16	8.338±4.519	22	7.145±3.962
FG	PCOS	12	92.100±12.47	19	92.950±14.50	27	84.850±9.85
	Control	10	84.800±7.820	16	86.000±3.960	22	81.090±4.70
OGTT-2	PCOS	12	96.500±22.30	19	118.370±35.4	27	94.96±28.18
	Control	10	93.600±17.52	16	86.380±8.330	22	85.36±10.85
OGTT-3	PCOS	12	72.170±18.97	19	94.890±28.46	27	75.70±16.56
	Control	10	84.000±12.88	16	71.880±6.940	22	76.00±12.65
HOMA-IR	PCOS	12	0.858±0.781	19	1.658±1.548	27	1.041±0.967
	Control	10	2.164±1.050	16	1.794±0.952	22	1.459±0.841
HOMA ß	PCOS	12	0.802±0.702	19	1.837±1.505	27	1.553±1.258
	Control	10	1.650±0.218	16	1.445±1.059	22	1.356±0.625
BMI	PCOS	12	22.266±4.194	19	25.638±4.869	27	23.922±5.05
	Control	10	22.688±2.701	16	20.865±3.250	22	21.179±2.45

Table 2
MEAN VALUES WITH STANDARD DEVIATION FOR FG, FI, OGTT, HOMA AND BMI SCORES FOR AGE RANGES IN BOTH GROUPS (PCOS AND CONTROL)

Variable		n	17-18 y	n	18-19 y	p-age group	p general
FI	PCOS	31	9.284±8.931	33	8.123±9.838	0,078	0,339
	Control	24	8.850±4.467	30	8.340±4.095		
FG	PCOS	31	87.70±10.07	33	91.690±18.40	0,016	0,001
	Control	24	83.750±5.85	30	80.000±8.86		
OGTT-2	PCOS	31	97.94±28.01	33	101.76±31.63	0,05	0,018
	Control	24	93.92±20.31	30	92.930±16.94		
OGTT-3	PCOS	31	80.29±21.61	33	76.940±21.41	0,045	0,457
	Control	24	75.17±14.53	30	76.470±13.24		
HOMA-IR	PCOS	31	1.053±0.892	33	1.144±1.302	0,059	0,194
	Control	24	1.794±0.917	30	1.690±10.96		
HOMA ß	PCOS	33	1.429±1.206	33	0.978±0.933	0,028	0,064
	Control	24	1.664±0.902	30	2.179±1.330		
BMI	PCOS	31	23.507±4.74	33	23.557±5.166	0,069	0,002
	Control	24	22.559±5.77	30	20.546±2.492		

Table 2
Continuted

Legend: FI-fastum insuline; FG-fastum glucose;
The oral test for glucose tolerance at 2 hours (OGTT-2) and 3 hours (OGTT-3);
The homeostatic model assessment forinsulinresistance study (HOMA-IR) and for â-pancreatic cells function (HOMA-ß);
BMI-body mass index

The early examination of the glucose metabolism is recommended to start from adolescence, also considering the fact that glucose disorders have been reported in family members of the women affected by PCOS [15]. Considered to be the *gold standard* test for women adolescents, OGTT is recommended by specialists in observing the early changes in the glucose metabolism of girls with PCOS [16]. According to the mean values, we observed differences between the PCOS group age ranges for FG, OGTT-2 and HOMA scores, which increased with age (table 2). This phenomenon could be interpreted in the light of increased insulin resistance, which can lead to prediabetes and DM2.

A selectivity of the insulin-resistance in various tissues has been demonstrated [17]. This phenomenon increases the frequency of DM2 in women with high body mass index (BMI) with PCOS as opposed to that in obese women without PCOS, due to progresive dysfunction of pancreatic β-cells [17].

For more than 25 years, the high level of insulin and insulin resistance have been intensely correlated with PCOS pathogenesis and have been considered as an important risk factor for its evolution, especially due to their role in triggering DM2, metabolic syndrome and cardiovascular diseases [18]. On the other hand, with age, healthy women of reproductive age present an aggravation of IR, while the circulating androgens decrease [19].

Particularly in PCOS in insulin resistant women, the pituitary gland, the ovaries and the adrenal glands are stimulated by a considerably higher level of insulin, triggering a higher level of luteinising hormone and androgens [20, 21].

The risk of DM2 associated with PCOS is estimated to be 7-fold higher than in the rest of the population [22], and subclinical forms of modified glucose tolerance may appear in a third of the cases, with a 10-fold higher risk of developing gestational diabetes and a more rapid evolution to DM2, even in women with normal weight [23]. The beneficial effect of treatment with insulin-lowering agents in improving the specific symptoms was reported; it resulted in correction of the ovulation and restoration of fertility especially in cases accompanied by obesity [24]. Women with PCOS may develop DM2 later in life, perhaps after 20–30 years from the disease diagnosis, and worsening of the simptoms has been reported in 15–35% of the cases [25].

The diagnosis of diabetes in PCOS is reported by many studies. Some suggest that the indication for OGTT is restricted to the cases with fasting glucose (FG) between 6.1 and 7.0 mmol/L [26], contrary to the recommendations of ESRE/AMSR and AES-PCOS, which suggest OGTT of women with PCOS, especially in overweight cases [27].

Depending on the category of glucose metabolism disorders, there were significant differences between the PCOS group and the control group, with more frequent anomalies in the PCOS girls. Thus, impaired fasting glucose (IFG) was identified in 13.91% of PCOS group, whereas 1.67% of the girls were diagnosed with DM2 (table 3).

The values obtained at 30 min and 60 min showed flat curves (small increase in the glucose values) in 48.36% of the adolescents in the PCOS group, compared to 19.61% of the girls in the control group. OGTT was made for all the PCOS non-diabetic cases and the results indicated subclinical disorders of the glucose metabolism. Compared to the FG results, OGTT identified more PCOS girls with IGT and DM2, a supplementary 0.82% rate for DM2 and a total 16.39% rate for IGT.

The test results for glycaemia 3 h after glucose powder ingestion showed a higher percent of metabolic anomalies in group I. Thus, the percentage of girls with persistent glycaemia above the normal value was 12.30%, whereas those with defined DM2 were 3.28%. Persistent hyperglycaemia affects the secretion ability of the pancreas by damaging the beta cells and further inducing higher levels of blood glycaemia [28].

OGTT at 3 hours showed a 22.95% rate of hypoglycaemia in the PCOS group (with a minimum value of 46 mg/dL) compared to a rage of 9.8% in the control group (with a single minimum value of 52 mg/dL). Reactive hypoglycaemia, as a postprandial hypoglycaemic status, may appear after 2-5 h from food ingestion and is correlated in PCOS with the degree of insulin resistance and the β-pancreatic cell function [29]. In 2005, a study made on 64 young women with PCOS and BMI less than 25 kg/m^2 showed the presence of postprandial hypoglycaemia in 50% of the cases, an aspect correlated with reduced values of dehydro-epiandrosterone sulphate (DHEA-S) [29]. That study acknowledges that underweight state with postprandial hypoglycaemia and family history of diabetes need to be taken into consideration for further risk of developing diabetes mellitus [30].

The Minitab surface plot for BMI and the values of glycaemia obtained by OGTT 2 and OGTT 3 indicated direct association between high OGTT-2 values and high BMI values. Reduced values of OGTT-3 were especially observed in subjects with low BMI, but there were also cases with normal or high BMI with low values of OGTT-3 glycaemia correlated with postprandial hypoglycaemia (fig. 1). The statistical analysis revealed significant differences for OGTT-2 between the PCOS group and the control group (p = 0.018)(table 3).

The Minitab graph for BMI, FI and FG showed a good association between high BMI cases and high FG values, also between cases with high FG and high FI values (fig. 2).

The association between BMI and the level of insulin in the blood has been well demonstrated, but in recent years, there have been reports of insulin resistance in people with normal weight or low BMI [31]. In this study, statistical analysis of the HOMA-IR score values was also performed to study the insulin resistance. Values between 0.34 and 9.15, with a mean of 2.06 ± 2.28 were obtained in the PCOS group. The normal HOMA-IR values of healthy girls

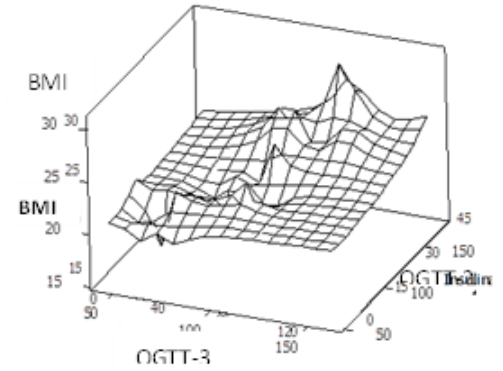

Fig. 1. Surface plot for BMI and glycaemic indices obtained by OGTT 2 and OGTT 3.

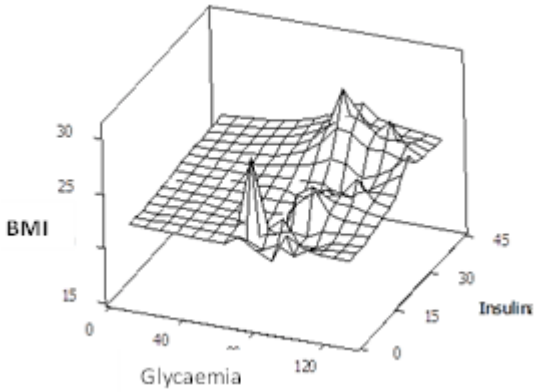

Fig. 2. Surface plot for BMI and the values of FG and FI.

Table 3

THE FREQUENCY OF MAIN GLUCOSE METABOLISM VARIABLES IN PCOS GROUP AND CONTROL GROUP (FG, FI, OGTT, HOMA)

		PCOS %	*CONTROL%*
Fasting G	FG normal	84.43	98.04
	IFG	13.93	1.96
	DM	1,64	0,.00
Fasting I	FI normal	37.70	78.43
	FI ↗	11.48	0.00
	FI↘	50.82	21.57
OGTT-1	Normal	51.64	80.39
	Smooth curve	48.36	19.61
OGTT- 2	Normal	82.79	94.12
	IGT	16.39	5.88
	DM	0.82	0.00
OGTT -3	Normal	61.48	86.27
	IFG	12.30	3.92
	DM	3.28	0.00
	Hypoglicaemia	22.95	9.80
HOMA -IR	Normal IR	50.82	92.16
	IR ≥ 3	49.18	7.84
HOMA -ß	Normal ß	65.57	84.31
	ß ≤ 1	26.23	15.69

are in the range of 1.7–2.0. Moderate IR was considered for HOMA-IR values between 2.2 and 3, in 4.10% of PCOS cases and 3.92% of control subjects. IR is considered severe at HOMA-IR values higher than 3, which was observed in 26.23% of PCOS adolescents and in 3.92% of subjects in the control group.

The HOMA-2 calculator program was used to analyse the pancreatic function; this program evaluates the pancreatic function through the HOMA-ß parameter. HOMA-2 values below one unit are considered a decrease in pancreatic function. In our study, the percentage of PCOS cases with impaired pancreatic function was 34.43%, compared to 9.80% of the cases considered as being normal.

For over three decades, various disorders of glucose metabolism have been reported in women with PCOS. Some studies have reported a frequency higher than 30% for decreasing the glucose tolerance (IGT) in such PCOS cases, while 7.5% have been linked to DM2; this has been demonstrated in the past even in PCOS women with normal or reduced BMI, 10.3% of these being reported with IGT and 1.5% with DM2 [31], while untreated cases have developed diabetes at the age of menopause [19]. Compared with the results from these studies, in our study, 5.74% of all the PCOS cases had DM2; 13.93%, modified basal glycaemia (IFG) and 28.69%, impaired glucose tolerance (IGT).

However, although the American Diabetes Association (ADA) recommends screening for DM in obese children with high risk, the International Diabetes Federation (IDF) does not recommend turning OGTT into a routine practice in healthy young people. Still, it is acknowledged that determining the basal glycaemia as a single test for

diagnosis of diabetes will not identify approximately 70% of the children with high risk of glucose metabolism disorders [32].

Conclusions

Our study provides evidence suggesting the existence of some anomalies in the glucose metabolism in PCOS adolescents. These anomalies are asymptomatic and remain undiagnosed until analysis, even if there is lack of obesity. For therapeutic interventions, the analysis of intermediary risk markers for DM2 is important. The oral test for glucose tolerance needs to be repeated three hours after ingestion, as this is relevant for the persistent *dysglycemia* and reduced pancreatic functions. Thus, it is believed that a collaboration of specialists of various medical disciplines who study PCOS will be beneficial to reaching an agreement concerning a more suggestive name for this condition, its causes and underlying mechanisms, definition criteria on age groups, early diagnosis and proper management with a view to reducing its evolution risks.

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