VILNIUS UNIVERSITY

Lina Zabulienė

PECULIARITIES OF CLINICAL MANIFESTATION, BODY SIZE AND BODY BUILD IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Summary of Doctoral Dissertation
Biomedical sciences, Medicine (06 B)

Vilnius, 2011

VILNIUS UNIVERSITY

Lina Zabulienė

PECULIARITIES OF CLINICAL MANIFESTATION, BODY SIZE AND BODY BUILD IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Summary of Doctoral Dissertation
Biomedical sciences, Medicine (06 B)

Vilnius, 2011

The dissertation was prepared from 2007 to 2011 at Vilnius University Medical Faculty Anatomy, Histology and Anthropology Chair.

Scientific supervisor

prof. dr. Janina Tutkuvienė (Vilnius University, Biomedical sciences, Medicine – 06B)

The dissertation will be defended in the Medical Research Council of Vilnius University:

Chairman

prof. dr. Jolanta Dadonienė (Vilnius University, Biomedical sciences, Medicine – 06B)

Members:

prof. dr. Janina Didžiapetrienė (Vilnius University Institute of Oncology, Biomedical sciences, Medicine – 06B)

prof. dr. Gražina Stanislava Drąsutienė (Vilnius University, Biomedical sciences, Medicine – 06B)

prof. habil. dr. Vaidutis Kučinskas (Vilnius University, Biomedical sciences, Medicine – 06B)

prof. dr. Rasa Verkauskienė (Lithuanian University of Health Science, Biomedical sciences, Medicine – 06B)

Opponents:

prof. dr. Virgilijus Beiša (Vilnius University, Biomedical sciences, Medicine – 06B)

prof. dr. Rūta Jolanta Nadišauskienė (Lithuanian University of Health Science, Biomedical sciences, Medicine – 06B)

The dissertation will be defended at the open session of the Medical Research Council of Vilnius University on 17th of November, 2011 at 2 p.m. in the large auditorium of Vilnius University Medical Faculty: M.K. Čiurlionio g-vė, LT-03101, Vilnius, Lithuania.

The summary of the doctoral thesis has been sent on 17th of October 2011. The dissertation is available in the Library of Vilnius University.

INTRODUCTION

The human height, weight, body build and proportions are the result of a long evolution process and adaptation to the environment [Tutkuvienė, 2010]. The relation between the female body build, health and fertility has been known since ancient times. The symbol of beauty and fertility of Palaeolithic period, Venus of Willendorf, is obese, however, the fatty tissue is accumulated in the lower part of the body, and this type is called gynoid (feminine) figure [Kirchengast, 2005; Dasgupta and Reddy, 2008]. It is interesting to note that first the allusion associating the body size and health was found by a French scientist and philosopher Rabbi Levi ben Gershon in the Bible in 1328 concerning the fact that excess fatty tissue in women and obesity could cause infertility [Ben-Shlomo et al., 2008]. Rubens period artists portrayed the female ideal as a woman with a relatively slender upper torso but wide hips and thick thighs. In many cultures the gynoid adipose tissue accumulation in the body is more attractive as associating with woman's health and fertility until nowadays [Thornhill and Grammer, 1999; Weeden and Sabini, 2005; Tutkuvienė, 2010].

Polycystic ovary syndrome (PCOS) is one of the most serious and frequent endocrine and metabolic diseases among reproductive age women occurring in 4–17.8% women of various nationalities, i.e. involving over 100 million women globally [Chen et al., 2008; March et al., 2010; Padmanabhan, 2009]. PCOS is a heterogeneous clinical syndrome characterized by the presence of hyperandrogenism and (or) excess androgen in blood, anovulation, menstrual cycle disturbance as well as polycystic ovary morphology [Franks, 1995; Fauser et al., 2004; Azziz et al., 2009]. It is difficult to detect PCOS, besides, the diagnostic criteria are still under dispute, i.e. the number of features (hyperandrogenism, anovulation and polycystic ovary morphology) and their possible combinations needed to confirm the diagnosis are still being debated [Fauser et al., 2004; Azziz et al., 2009]. According to the different combinations of the features there are four main phenotypes of PCOS distinguished: type I classic, type II classic, normoovulatory and normoandrogenic one [Guastella et al., 2010], however, the frequency of these PCOS phenotypes has not yet been studied in Lithuania.

Every PCOS component has its own differentiating features. The hyperandrogenism cut-off values are specific, applicable to a certain region only. It has

been agreed that in order to confirm the hyperandrogenism diagnosis it is necessary to identify the androgen level in the healthy women's population and set the 95th percentile as the cut-off value [Knochenhauer et al., 1998; Fauser et al., 2004; Azziz et al., 2006; Barth et al., 2007; Yildiz et al., 2008; Azziz et al., 2009; Barth et al., 2010]. The main clinical feature of hyperandrogenism in women is hirsutism, i.e. excessive thick and dark hair growth in women in androgen-depending body areas. The hair growth intensity in women is also known to differ depending on the country and geography, predetermined by age, ethnic and inherited factors (e.g. 5-α reductase activity). [Rosner et al., 2007; Barth et al., 2007; Lujan et al., 2008]. Most frequently hirsutism (which measured by a Ferriman-Gallwey score) is identified having a score of 6 or greater; in some populations even a score of 9 and more [Ferriman ir Gallwey, 1961; Hatch et al., 1981, Rosenfield, 2005, Yildiz et al., 2009]. The choice of hyperandrogenism cut-off values has influence on identification of PCOS phenotypes. [Guastella et al., 2010; Chae et al., 2008]. Hyperandrogenism cut-off values have not yet been determined in Lithuania.

PCOS pathogenesis has not been fully researched and clarified so far. Disturbance of relations among hypothalamus, hypophysis and ovaries, increased luteinizing hormone secretion, continuous estrogenemia, excess androgen and excess insulin as well as insulin resistance are typical features of this syndrome [Franks, 1995; Speroff and Fritz, 2004; Azziz et al., 2009]. PCOS may cause not only hirsutism, menstrual cycle disturbances, infertility, but also increase the risk of obesity, type 2 diabetes mellitus, endothelium dysfunction, early atherosclerosis, cardiovascular diseases, dyslipidemia, hypertension, excess androgen-related tumours (especially those of uterus mucous membrane and ovaries) or even depression, therefore, it makes strong impact on women's health and quality of life [Franks, 1995; Speroff and Fritz, 2004; Fauser et al., 2004; Ehrmann, 2005; Azziz et al., 2009]. The metabolic disturbances are more pronounced and more frequent metabolic syndrome is in women with classical PCOS phenotypes comparing with normoovulatory and normoandrogenic PCOS [Barber et al., 2007; Welt et al., 2006; Belosi et al., 2006; Hsu et al., 2007]. However, body mass index (BMI), waist circumference, lipid metabolism, insulin resistance and metabolic syndrome frequency are similar in PCOS women with normal androgen level and the controls [Barber et al., 2007; Kauffman et al., 2006; Dewailly et al., 2006; Guastella et

al., 2010]. Due to multiple phenotypical manifestations of the syndrome and treacherous PCOS course the majority of women (up to 70%) are not aware of being ill with PCOS [March et al., 2010]. To make matters worse, there is no unanimous opinion as to who and how should follow and treat these women, therefore, they seek help of many specialists (internists or family doctors, paediatricians, gynaecologists, dermatologists as well as endocrinologists). There was no research carried out concerning clinical PCOS manifestation in Lithuania so far.

Body size and build changes well reflect many inner processes of the organism, metabolic peculiarities, functioning of sex hormones as well as other hormones. Besides, anthropometric measurements are informative, simple, easily applied and repeated in everyday clinical practice. The following main anthropometric body size and build indices are generally used: longitudinal and transverse skeletal measurements, body mass and its composition, BMI, body circumference, skinfolds and body proportions [Tutkuvienė and Jakimavičienė 2004; Jakimavičienė and Tutkuvienė, 2004]. The change of certain indices often brings about the changes in others as well. The BMI is mostly dependent upon the active body mass and skeleton size, while the adipose tissue changes BMI relatively less: the BMI of two people with the same adipose tissue amount may differ because of the different active mass, in addition, the BMI of people with long legs is lower than that of people with shorter legs, and the body mass is relatively lower in the same height people whose body is slender; besides, they are likely to have less muscle and connective tissue [Frisancho, 2008; Henneberg et al. 2010; Tutkuvienė, 2010].

Many anthropometric indices are used to assess the risk of metabolic, cardiovascular and other diseases or even mortality [Tutkuvienė and Jakimavičienė, 2004; Jakimavičienė and Tutkuvienė, 2004]. Greater weight and visceral obesity are related to CV diseases, diabetes, insulin resistance, metabolic syndrome and earlier mortality risk [Gambineri et al., 2002; Zhu et al., 2002; Koster et al., 2007; Engeland et al., 2003]. Higher stature, greater weight, abdominal obesity and wider elbow were observed to be associated with higher breast cancer risk at young age [Freni et al., 1996; Chumlea et al., 2002; Lahman et al., 2004; Schouten et al., 2008, Amaral et al., 2010]. Low stature may be the precursor of cardiovascular diseases, myocardial infarction, stroke, diabetes, insulin resistance and early (earlier than 65 years of age) mortality [Davey Smith et al., 2000; Engeland et al., 2003; Batty et al., 2009; Paajanen et al.,

2010]. The level of cholesterol, fibrinogen and glucose as well as insulin resistance, CV risk is higher in people whose legs length and the ratio between the leg length and waist height is lower. [Li et al., 2007; Bogin and Varela-Silva, 2010; Gunnell et al., 2003; Whitley et al., 2010; Dangour et al., 2007; Lawlor et al., 2004; Velasquez-Melendez et al., 2005].

PCOS, determined by complex pathogenetic mechanisms, is also related to body size and build changes [Azziz et al., 2009; Allahbadia and Merchant, 2011]. According to various research data, 33–88% PCOS women complain of overweight or are obese [Knochenhauer et al., 1998; Barr et al. 2008; Balen et al., 1995; Legro, 2000, Li and Lin, 2005; Jones et al., 2004; Sam et al., 2008]. The anthropometric indices showing overweight, adipose tissue accumulation site and adipose tissue amount were currently studied in women with PCOS, namely: body mass, BMI, waist, chest, hip circumference, waist-to-hip ratio, waist-to-height ratio, waist conicity index, visceral obesity indices and skinfods [Crosignani et al., 2003; Hassa et al., 2006; Toscani et al., 2007, Cascella et al., 2008; Carmina et al., 2007; Penaforte et al., 2011; Pasquali et al., 1999; Manneras-Holm et al., 2011; Wehr et al., 2009a; Huang et al., 2010; March et al., 2010; Barth et al., 2010; Sterner-Victorin et al., 2010; Azziz et al., 2004b; Goverde et al., 2009]. While researching the adipose tissue topography, the strongest sexual dimorphism feature, the adipose tissue mass of the internal organs, waist and arms was found to be greater in women with PCOS than that in the control women, i.e. over two thirds of adipose tissue in women of different weight (even of low one) is accumulated in the upper part of the torso [Kirchengast and Huber, 2001; Li and Lin, 2005; Kirchengast, 2005; Hashimoto et al., 2003; Penaforte et al., 2011; Snijder et al., 2004; March et al., 2010; Moran and Teede, 2009; Toscani et al., 2007; Gennarelli et al., 2000; Crosignani et al., 2003]. On the other hand, the adipose tissue topographic peculiarities exist in women of different nationalities (even in case of norm the body build differs depending on ethnicity). So far the peculiarities of body size and build in women with PCOS have not been researched in Lithuania.

The skeleton size and proportion indices in women with PCOS have not been described until now in the literature available, however, certain features could be assessed as hyperandrogenization morphological indices, namely: shoulder and pelvis width ratio, hand and foot size, peculiarities of facial features and hand grip [Tutkuvienė,

2010; La Velle, 1995; Coquerelle et al., 2011; Volgyi et al., 2010; Berger et al., 2011]. The relation between the second and fourth finger length ratio and early androgenization have been researched so far [Cattral et al., 2005; Lutchmaya et al., 2004; McIntyre, 2006; Lujan et al., 2010a; Lujan ME et al., 2010b; Dressler and Voracek, 2011].

It is evident that morphological body parameters, physiological and biochemical indices make a complex interrelated system. A question arises as to which anthropometric indices are most important and most closely related to PCOS, which indices should be measured to observe the first signs of body build changes in women with PCOS and differences from the healthy women? The early changes in physical condition would help predict certain metabolic changes, the course of PCOS and allow a more rational choice of prevention, means of treatment; prevent the patient from the PCOS consequences.

AIM AND OBJECTIVES OF RESEARCH

Aim of research

To identify the peculiarities of clinical signs and symptoms, body size and build in women with polycystic ovary syndrome and assess their interrelations.

Objectives of research

1. To determine the cut-off values of hyperandrogenemia and hirsutism in order to identify objectively the women with PCOS syndrome.
2. To assess the peculiarities of PCOS clinical manifestation in young women (reproductive health, endocrine and metabolic disturbances).
3. To study the peculiarities of body size (skeleton, body circumferences and proportions, skinfolds and body composition) in women with PCOS and the controls and their relation to the general health condition.
4. To compare the peculiarities of clinical manifestation, body size and build in women with different PCOS phenotypes.
5. To determine the body size and build indices in women allowing to suspect PCOS.

INNOVATIVENESS AND RELEVANCE OF RESEARCH

Currently, the number of women with PCOS is increasing worldwide, the phenomenon related to obesity pandemic, spread of metabolic syndrome as well as other still undisclosed factors. The work under discussion provides for the first time a comprehensive analysis of the physical and health condition in Lithuania's women with PCOS. The cut-off values of hyperandrogenism and androgens have not been determined in Lithuania so far. We have determined the cut-off values of hyperandrogenism in young women to be of use to both practitioners and scientists.

Currently there is no unanimous opinion concerning the criteria to be used as guidelines in diagnosing PCOS. Our research is dealing with and comparing various PCOS physical condition and health indices, characterizing various PCOS phenotypes as well as the phenotype of women with PCOS having normal androgen concentration which differs insignificantly from the healthy women by clinical manifestation.

The research assesses for the first time the metabolic, reproductive health as well as other endocrine changes in women with PCOS, shows their close association with obesity and other morphological parameters. A comprehensive combined analysis of body size and build relation to PCOS has been carried out, morphological parameters of significance in confirming the PCOS diagnosis have been determined. The research results under discussion are important and useful not only to anthropologists but to all clinicians: endocrinologists, gynaecologists, family doctors in early detection of PCOS, its treatment and prevention.

Statements to be defended

1. The hyperandrogenism cut-off values of our population differ from that of other populations, and they are of importance in order to identify the PCOS.
2. Certain reproductive and endocrine health disturbances are typical of women with PCOS, the latter depending upon the PCOS phenotypical manifestation: women of classic PCOS phenotypes have most reproductive and endocrine health disturbances, whereas differences between various physical parameters of women with normoandrogenic PCOS and those of the controls are insignificant.

3. Women with PCOS have specific body size and build peculiarities which possess PCOS predictive properties.

STUDY POPULATION AND RESEARCH METHODOLOGY

Study population

The study was carried out in 2007–2011; the dissertation was prepared at the Anatomy, Histology and Anthropology Chair of the Faculty of Medicine of Vilnius University in cooperation with the Outpatient Clinics of Antakalnis, Central and Karoliniškių in Vilnius city. The study population included women aged 20–35, residents of Vilnius and Vilnius region who were referred to endocrinologist. All the women, who were referred to the above outpatient clinics because of suspected PCOS (hirsutism, menstrual cycle disturbance, infertility, body weight gain) were offered to take part in the study. The control group involved the healthy women consecutively, who agreed to take part in the study.

The study was approved by the Lithuanian Bioethics Committee. In total, 235 women (135 women with PCOS and a 100 control women without hyperandrogenism or menstrual cycle disturbances) were studied (Fig.1).

The criteria for including women into PCOS group were: (1) subject consent to participate; (2) women aged 20–35; (3) PCOS detected according to the Rotterdam criteria, i.e. no less than two out of three components, namely: (a) hyperandrogenism and/or hyperandrogenemia, (b) anovulation; (c) polycystic ovary morphology [Fauser et al., 2004; Azziz et al., 2009].

The criteria for including women into the control group were: (1) subject consent to participate; (2) women aged 20–35; (3) no evidence of androgen excess or having no hyperandrogenism of any other origin; (4) normal menses; (5) no family history in a first-degree relatives of PCOS.

The following subjects were excluded from the study: (1) women who suffered from acute (during the three months prior to the study) or severe chronic diseases; (2) increased serum 17-OH-progesterone level >6 nmol/L during follicular phase or women who had been diagnosed with other causes of androgen excess such as congenital adrenal hyperplasia, adrenal or ovary androgen-secreting tumours, Cushing's syndrome; (3) who

had FSH outside the normal ranges (2–10 IU/l) during follicular phase or premature ovarian failure; (4) who had hyperprolactinemia; (5) who had noncompensated hypothyreosis; (6) who were ill with other metabolic or endocrine diseases; (7) who were excluded if they had used any form of estrogens, androgens, contraceptive pills, antiepileptic drugs, clomiphene citrate, weight or lipid reducing drugs, metformin, phytoestrogens, homeopathic and other preparations known to affect the body size, hairiness, lipid, carbohydrate, protein metabolism or amount of hormones in the last 6 month before signing the consent; (8) who suffered from mental diseases, nervous anorexia or bulimia; (9) who were pregnant, breastfeeding or having given birth in less than a year; (10) who could not undergo all the study procedures due to other reasons.

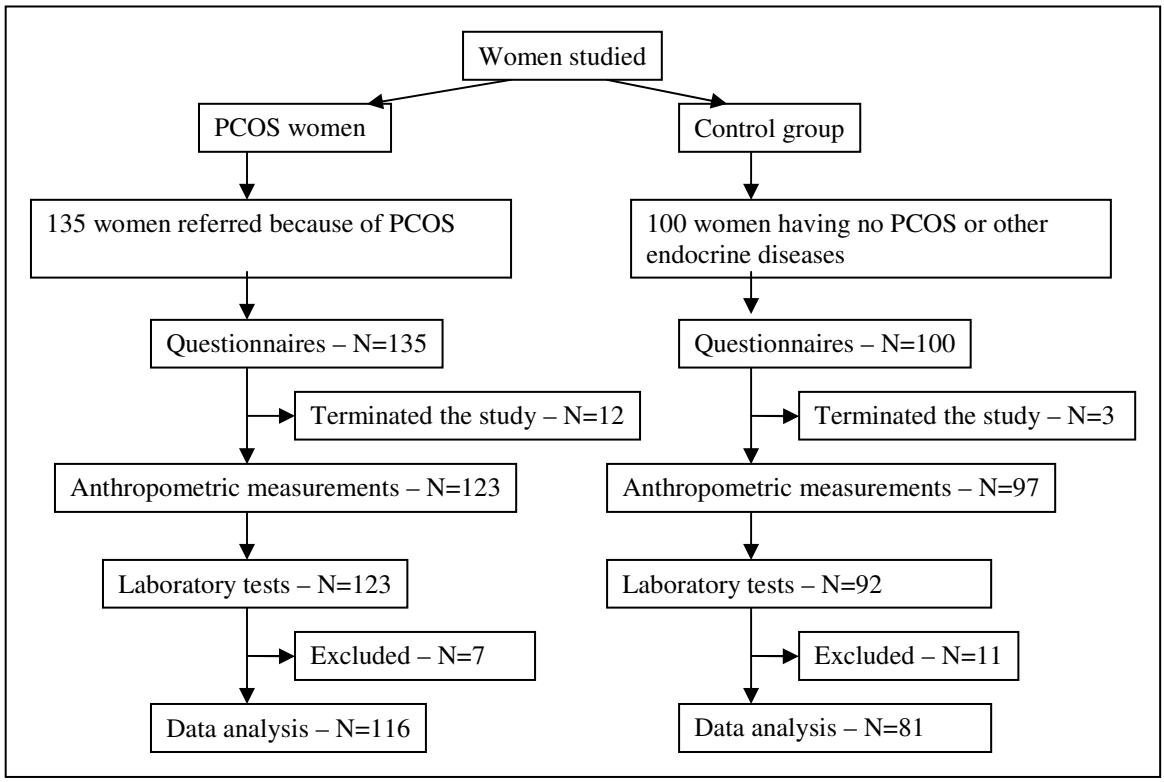

Figure 1. Inclusion into the study

The data of 38 women were excluded from further analysis. 197 composed the final study sample: 116 PCOS women and 81 controls (Fig.1).

Research methodology

The women's data were collected by questioning and by the records in the questionnaire filled in by the participants. The questionnaire included the following parts: (1) demographic data: age, nationality, education, social status; (2) growth anamnesis; (3) health condition; (4) family history; (5) menstruation cycle condition (menarche age, regularity, cycle length); (6) gynaecological anamnesis and infertility; (7) hairiness and acnes, their changes.

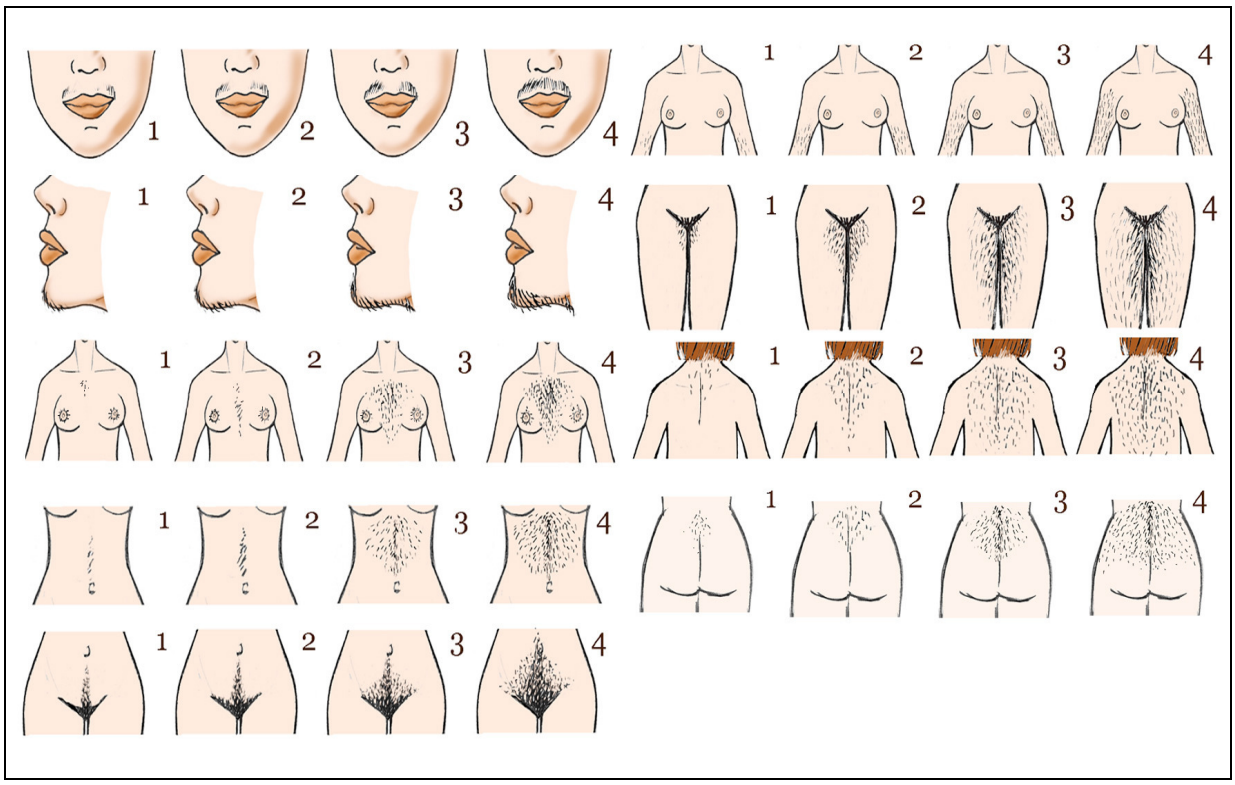

Figure 2. Detection of hirsutism (pictures are presented according to the modified D. Ferriman et J.D. Gallwey scale composed according to Ferriman and Gallwey, 1961, Hatch et al., 1981, Rosenfield, 2005, Yildiz et al., 2009)

On physical examination the following signs were determined: acnes, *Acanthosis nigricans* of the neck, armpits, area below the breasts, elbows, knuckles and skin, abdominal stretch marks and hairiness. Hairiness in women under the study was determined by the research author according to the modified D. Ferriman et J. D. Gallwey scale (1961), by assessing the nine androgen-dependant body areas, i.e. the hairiness of the upper lip, chin, breast, upper spine, lower spine, upper abdomen, lower abdomen, upper arms and thighs (Fig. 2).

Instrumental tests

Arterial blood pressure (BP) was measured according to the arterial BP measuring guidelines of the European Hypertension Society of 2007 [Mancia et al., 2007]. Systolic and diastolic BP was measured twice after having a rest and sitting quietly for 5–10 min, in both arms (arm being in the position of 45 degrees to the waist) using standard quicksilver aneroid sphygmomanometer. Heart rate was measured two times. Handgrip was determined by dynamometer, the grip was measured two times with a 5 minutes break and in addition, relative handgrip – handgrip and body mass ratio – was calculated. The means of all measurements were used for statistical analysis.

Anthropometrics

The same anthropometric instruments were used to measure all the women, all the measurements were done trice, and the mean obtained was used for statistical calculations. All the measurements were performed by the author. The instruments' precision was checked at the beginning and during the study periodically once a month. The standard anthropometric instruments and standard anthropometric methods were used [Martin et Saller, 1959; Knussmann et al., 1988; Anthropometrica, 2002; Tutkuvienė et Jakimavičienė, 2004; Jakimavičienė et Tutkuvienė, 2004].

In total 69 body size and build measurements were performed: body mass, 16 longitudinal skeletal measurements (height, sitting height, chin, shoulder, iliac, pubic and finger height, hand and foot length, plantar and back lengths of the second and fourth fingers, the lengths of the second and fourth toes), 10 transverse skeletal measurements (shoulder breadth, chest breadth and depth, widths of pelvis, elbow, wrist, hand, knee, ankle, foot), 12 head and facial measurements (head length and width, width of the face and mandible, physiognomic, morphological and middle face height, upper and lower face depth, lips width, thickness of the upper and lower lips), 16 body circumferences (circumferences of shoulder, of chest above the breasts, under the breasts and the greatest, of upper arm under tension and relaxed, forearm, wrist, waist at most narrow area, at umbilicus level and at iliac crest level, hip, the greatest and middle thigh, calf) and 13 skinfolds (subgenial, thoracic I and II, midaxillary, subscapular, triceps, biceps, forearm, abdominal, suprailiac, thigh, knee and calf skinfolds) and abdominal high. To determine body size, proportional and compositional changes there were 66 derivative

indices obtained. The mass of skeleton, skin and subcutaneous layer, muscle and the rest organs were calculated according to J. Matiegka (1921) formula, while the relative and absolute passive and absolute active mass were obtained using J. V. G. A. Durnin et M. M. Rahaman (1967) and J. V. G. A. Durnin et J. Womersley (1974) formulae.

Laboratory blood tests

Laboratory tests were done applying standard laboratory methods. Venous blood samples were collected from each individual participating in the study after an overnight fasting for 12–14 hours, except water. Blood samples were taken in the morning (between 7 and 9 a.m.) three times: (1) during follicular phase (days 3–6) of spontaneous or progestin-induced bleeding in anovulatory patients; (2) in the middle of the menstrual cycle; (3) on days 21–25 of the menstrual cycle. 9 ml of venous blood were collected in total. Intra- and inter-assay coefficients of variation for the tests were less than 5%.

Glucose tolerance test was carried out according the methodology suggested by the World Health Organization (WHO) in 2006, and the results were assessed following the WHO and American Diabetes Association (ADA) guidelines [World Health Organization, 2006; ADA, 2011; Bartoli et al., 2011].

Free androgen index (FAI) was defined according to the formula suggested by A. Vermeulen et al., 1999: *FAI = (total testosterone (nmol/l)×100) /SHBG (nmol/l)* [Vermeulen et al., 1999]. In order to evaluate insulin resistance several indeces were calculated: HOMA-IR index was calculated by homeostasis model of assessment-insulin resistance formula *HOMA-IR = (insulin (μIU/ml)) × (fasting plasma glucose (mmol/l)) / 22.5*) [Matthews et al. 1985], glucose to insulin ratio (GI) [Legro et al, 1998] and Quantitative Insulin Sensitivity Check Index *QUICKI = 1/(log(insulin concentration (μIU/ml)) + log(plasma fasting glucose (mmol/l)/0,0555))* [Katz et al., 2000]. Insulin resistance was determined if HOMA-IR was higher that 2.5 (level of 95th percentile of controls)

Grouping of the study participants

Women with diagnosis of PCOS according to Rotterdam criteria were divided into 4 well-characterized phenotypes: 1) type I classic PCOS (with biochemical hyperandrogenaemia and/or clinical hyperandrogenism, chronic anovulation and

polycystic ovaries); 2) type II classic PCOS (with biochemical hyperandrogenaemia and/or clinical hyperandrogenism and chronic anovulation but normal ovaries on ultrasound); 3) normoovulatory PCOS (with biochemical hyperandrogenaemia and/or clinical hyperandrogenism and with polycystic ovaries on ultrasound and confirmed regular ovulation); 4) normoandrogenic PCOS (chronic anovulation and polycystic ovaries but no evidence of clinical hyperandrogenism or biochemical hyperandrogenaemia) [Diamanti-Kandarakis et Panidis, 2007; Guastella et al., 2010].

Women were divided into 2 groups: with metabolic syndrome and subjects without metabolic syndrome. To determine metabolic syndrome criteria of three institutions were applied: (1) 2001 yrs. National Cholesterol Education Program and III criteria of the Guidelines of Treating Adults (NCEP, 2001) [Alexander CM et al., 2005]; (2) 2005 yrs. International Diabetes Federation (IDF, 2005) [International Diabetes Federation, 2005]; (3) 2009 yrs. International Diabetes Federation, National Heart, Lungs and Blood Institute, American Heart Association, World Heart Association, International Atherosclerosis Society, International Obesity Research Association (AHA, 2009) [Alberti et al., 2009].

Statistical data analysis

The number of subjects participating in the study (sample size) was calculated using open source epidemiological toolkit OpenEPI v. 2.3.1. To calculate sample size method for unmatched case -control study was used. It was assumed that the statistical power of the criteria applied should be 95%, and the significance level equal to 0.05. We have calculated the need to study at least 122 individuals (61 women in each group, PCOS and the control), when the control and case ratio is 1:1.

Microsoft Excel program was used for the initial (descriptive) data analysis. Statistical analysis was carried out using the statistical software package SPSS (v.19). Normal distribution was confirmed by the Kolmogorov-Smirnov test for all the interval variables. More than half of the data passed the normality test[1]. Descriptive statistics for categorical and discrete variables are presented by absolute and relative frequencies, as

[1] Non-parametric *Mann-Whitney U* or *Kruskal-Wallis* criteria were also applied for interval variables, for which normality assumption was not valid. For the homogeneity of the results the work includes parametric criteria results in the data tables, bearing no difference in significance.

for continuous variables, by the mean values, standard deviation (SD), median, minimal (Min) and maximal (Max) index meanings, 5^{th}, 10^{th}, 25^{th}, 75^{th}, 90^{th} and 95^{th} percentiles, mean confidence intervals (95% CI).

To assess test of independency of categorical variables Pearson's χ^2-test or, in case the number of observations was low (lower than 3), Fisher's exact test were conducted. The differences between the means of the continuous variables were tested using Student's t-test, one-way analysis of variance (ANOVA), covariance analysis (ANCOVA), when comparing mean of the groups additional variables possibly influencing interval variables were considered. The Bonferroni correction was applied for multiple comparisons in *post hoc* tests. To evaluate the relationship between continuous variables Pearson's correlation coefficient (r) was calculated. Correlation was ranked as very weak if r was lower than 0.2, weak if r=0.2–0.39, moderate if r=0.4–0.69, strong when r=0.7–0.79 and very strong if r was more than 0.8. In order to assess the impact of independent variables on the dependent variables single and multiple regression models were produced.

ROC curves were constructed to examine the diagnostic test performance and AUROC (area under the receiver operating characteristics) was assessed; Youden index was computed to estimate variables cut-off values. To evaluate the appropriateness of the cut-off values positive and negative likelihood ratios were calculated. To test statistical hypotheses the significance level chosen was 0.05.

RESULTS

Characteristics of the subjects

197 women have been studied and their data analysed. The studied women's mean age was 27.48±3.79 years; the youngest participant was 20, while the oldest was 35. There was no significant difference between the PCOS and control women's average age: PCOS women were 27.16±3.87 years of age, the control group – 27.95±3.65 years ($p>0.05$).

Lithuanians dominated in both the groups - 148 women (75.13%); there also were 23 (11.68%) Polish, 18 (9.14%) Russian and 8 (4.06%) women of other nationalities (Byelorussians, Jews and those who have not chosen nationality) studied.

The PCOS group included 116 women, whereas the controls numbered 81 women. The distribution of the PCOS group was as follows: 76 (65.51%) had type I classic PCOS phenotype, 10 (8.62%) had type II classic PCOS phenotype, 15 (12.93%) normoovulatory PCOS phenotype, and 15 (12.93%) had normoandrogenic PCOS phenotype (Fig. 3).

Determination of hyperandrogenism

In order to identify hyperandrogenemia in women with PCOS the cut-off values based on 95th percentile of androgens of controls were defined. The cut-off value of testosterone (T) was 1.68 nmol/l, free androgen index (FAI) – 2.94, dehydroepiandrosterone sulphate (DHEAS) – 10.42 μmol/l. According to the hairiness 95th percentile it was determined that score 6 and greater hairiness is considered as hirsutism.

We have assessed the application of different hyperandrogenism cut-off values in clinical practice: if in determining PCOS according to the Rotterdam criteria we had used the hyperandrogenism cut-off values of other populations (e.g. T – 2.74 nmol/l, FAI – 4.5, DHEAS – 9.0 μmol/l, hairiness – score 8), 14% type I classic PCOS phenotype women with hyperandrogenism according to the androgen cut-off values of the Lithuanian population would have been ascribed to normoandrogenic PCOS phenotype (Fig. 3).

If the hyperandrogenism cut-off values of other populations were applied in determining PCOS according to the Androgen Excess Society criteria, one-sixth of all the women with PCOS would not be diagnosed with PCOS. Therefore, our determined cut-off values of hyperandrogenism would be of use in clinical practice as well as evaluating women's reproductive health.

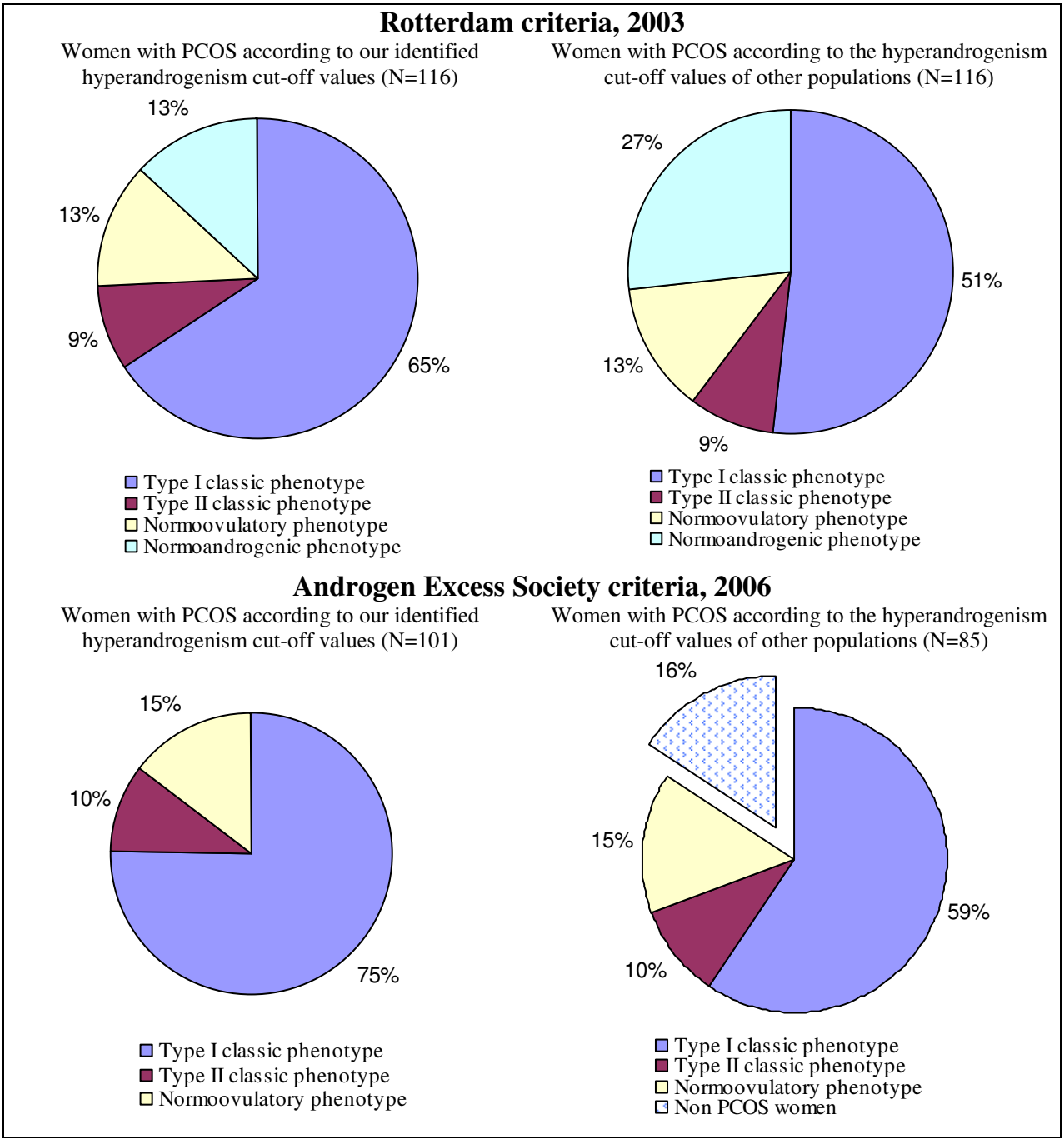

Figure 3. Distribution of women with PCOS into phenotypes

Health condition peculiarities in women with PCOS

In order to assess the PCOS clinical manifestation we studied the peculiarities in changes of the women's endocrine functioning, reproductive health and metabolism.

Peculiarities of endocrine functioning

There was statistically significant difference found concerning gonadotropin and androgen levels between women with PCOS and those in the controls, i.e. FSH and SHBG were lower in women with PCOS, while LH, LH and FSH ratio (LH/FSH), T level, DHEAS and FAI were greater than those in the control (Table 1).

Endocrine functioning indices in women with type I classic PCOS phenotype differed from the rest PCOS phenotypes: LH and LH/FSH were statistically significantly greater than those of the controls and normoovulatory phenotype women, whereas FSH was lower than in the controls ($p<0.01$) group ($p<0.0001$).

Table 1. Gonadotropin and androgen concentration in the women studied (mean ± SD)

Parameter	PCOS (N=116)	Control (N=81)	p
FSH, U/l	5.47±1.48	6.21±2.19	<0.0001
LH, U/l	7.85±4.09	6.26±2.2	<0.0001
LH/ FSH	1.48±0.77	0.98±0.31	<0.0001
T, nmol/l	1.98±0.69	0.99±0.36	<0.0001
SHBG, nmol/l	45.1±27.02	73.59±23.26	<0.0001
DHEAS, µmol/l	8.16±2.95	6.76±2.19	<0.0001
FAI	6.14±4.1	1.49±0.72	<0.0001
SD – Standard deviation, PCOS – women with polycystic ovary syndrome, FSH – follicle-stimulating hormone, LH – liuteinizing hormone, LH/FSH – LH and FSH ratio, T – testosterone, SHBG – sex hormone-binding globulin, DHEAS - dehydroepiandrosterone sulphate, FAI – free androgen index			

FAI was highest in women with type II classic PCOS phenotype. Women with androgen excess had T level statistically significantly almost twice higher, SHBG almost twice lower, and FAI exceeded that of normoandrogenic PCOS phenotype women and the controls almost three times. There was no difference observed concerning T, SHBG, FAI and DHEAS in normoandrogenic PCOS phenotype women and the controls ($p>0.05$) (Fig. 4).

In total, 82.75 % PCOS and one-tenth of the control women ($p<0.0001$) were diagnosed with hyperandrogenemia (increased T, DHEAS and FAI levels). Almost half of PCOS women had SHBG lower than the 5th percentile in the controls. The majority (94.8%) of PCOS women with hyperandrogenemia had increased FAI, 76% had increased T level, 27.1 % – DHEAS, whereas one-fifth of PCOS women had all three indices increased. Average hairiness in women with PCOS scored 6.44±4.91, and over half of women with hyperandrogenism had score 6 and greater hirsutism, including over

two-thirds with hirsutism score 8 or greater. Hirsutism in the control women ranged from 0 to score 5. There was no hairiness difference observed in women with androgen excess, however, it was statistically significantly about 4 times higher than that of PCOS women with normal androgen level and the controls ($p<0.0001$). There was no statistically significant difference between the hairiness of the PCOS women with normal androgen level and the controls ($p>0.05$). 44.83% of PCOS women and almost one-fifth (19.75%) of the controls suffered from acne ($p<0.0001$). The frequency of acne was more statistically significant in type II classic PCOS phenotype women than in the women with normoandrogenic PCOS phenotype ($p<0.05$).

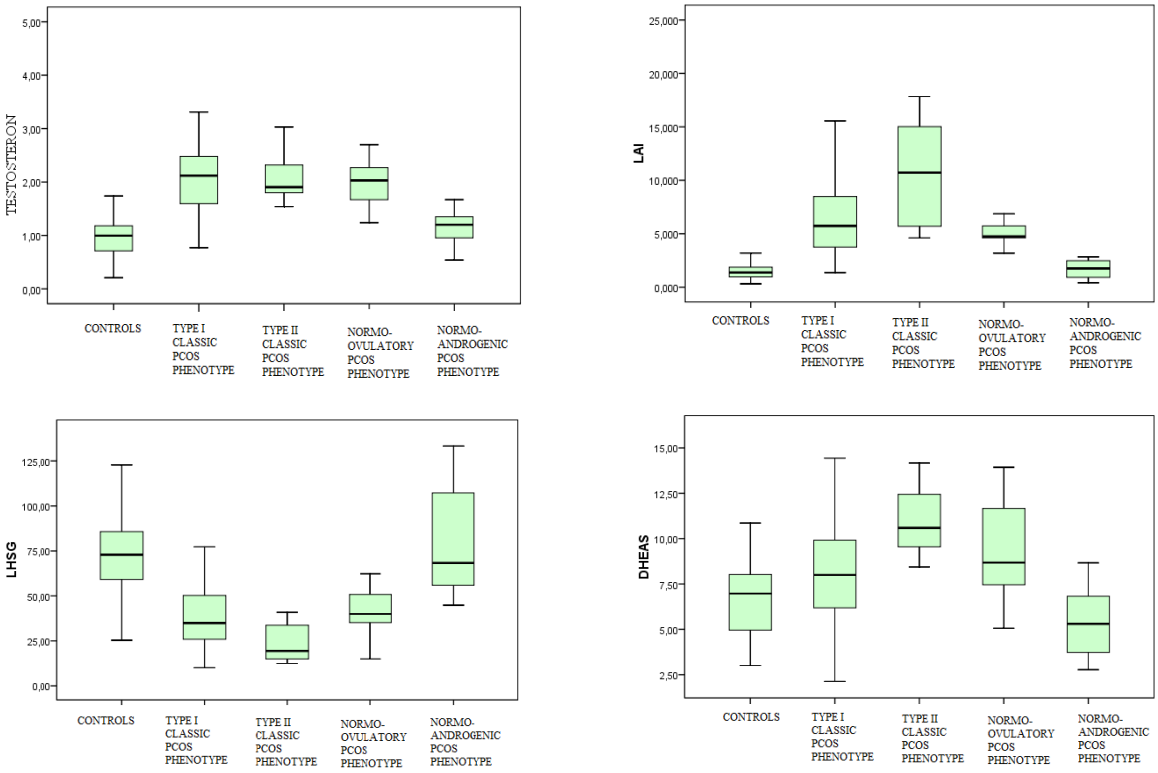

Figure 4. Androgen parameters of the women studied. T – testosterone, SHBG – sex hormone-binding globulin, DHEAS - dehydroepiandrosterone sulphate, FAI – free androgen index

FAI, testosterone and LH/FSH demonstrated the best PCOS predictive properties (AUROC was 0.920, 0.906 and 0.716, respectively) (Fig. 5). The cut-off values of these indices were as follows: FAI – 2.25 (sensitivity 87.8%, specificity 88%), testosterone – 1.33 nmol/l (sensitivity 84.3%, specificity 88.4%), LH/FSH – 1.39 (sensitivity 46.1%,

specificity 91.7%). We have calculated that 87.9% of PCOS women had FAI higher than 2.25, 84.5% had T level greater than 1.33 nmol/l and 46.6% had LH/FSH exceeding 1.39, while only one-sixth of the controls had such indices ($p<0.0001$).

Thus, the changes in the endocrine function of women with PCOS are evident. The most apparent endocrine disturbances and clinical hyperandrogenism symptoms are present in women with classic PCOS phenotypes with hyperandrogenemia and ovulatory dysfunction. The determined critical cut-off values of endocrine indices are suitable for identification of PCOS women.

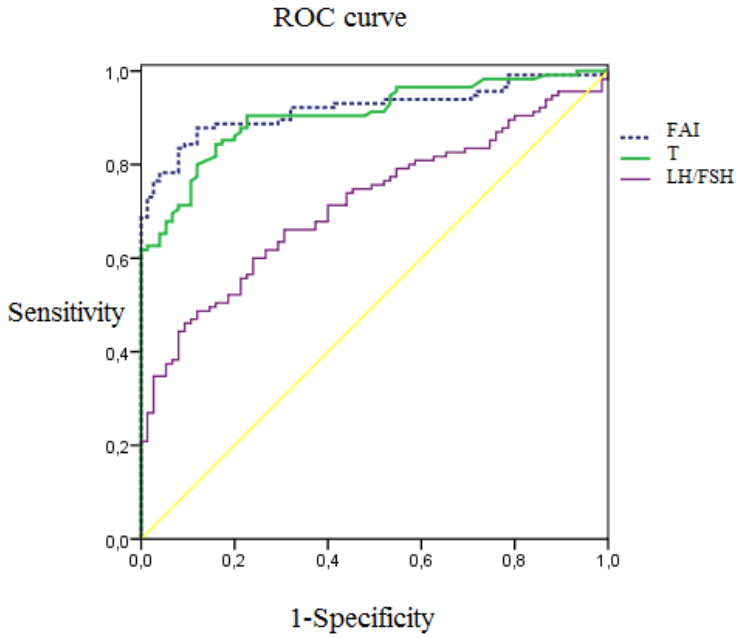

Figure 5. FAI, T level and LH/FSH ROC curves to predict PCOS. FAI – free androgen index, T – testosterone, LH/FSH – LH and FSH ratio

Reproductive health

Over 87% PCOS women suffered from menstrual dysfunction, whereas all the controls had regular menstrual cycle (Table 2). The shortest cycle in women with PCOS was 28 days, the longest lasted over 360 days. On average, PCOS women went through 8 menstrual cycles every year. About one-tenth (9.48%) of PCOS women suffered from amenorrhea. After distributing the women studied into the PCOS phenotypes it was found that amenorrhea occurred in women with type I and II classic PCOS phenotype.

Table 2. Reproductive health of the women studied

Feature	Type I classic PCOS (N=76)	Type II classic PCOS (N=10)	Normo-ovulatory PCOS (N=15)	Normo-androgenic PCOS (N=15)	Control (N=81)	p (between PCOS and control)
Oligomenorrhea	66 (86.8%)	9 (90 %)	0	15 (100%)	0	<0.0001
Amenorrhea	10 (13.2%)	1 (10 %)	0	0	0	0.004
No of childbirths	13 (17.1%)	1 (10 %)	4 (26.7%)	0	22(27.2%)	0.101
Impossible to conceive	33 (43.4%)	0	4 (26.7%)	6 (40.0%)	0	<0.0001
Morphology of polycystic ovaries	76 (100%)	0	15 (100%)	15 (100%)	0	

Correlative analysis showed that number of menstrual cycles in women with PCOS had statistically significant weak negative correlation with LH (r = –0.360, $p<0.0001$) and average negative one with LH/FSH (r = –0.400, $p<0.0001$). There were no other correlations between menstrual cycle dysfunction and endocrine indices detected.

There was no difference concerning the menarche age among all the women studied as well as in the PCOS groups (the controls – 13.20±1.39 yrs., PCOS women – 13.41±1.68 yrs., $p>0.05$). 71.3% PCOS women complained of menstrual cycle dysfunction since menarche, 28.7% after 8.69±5.59 yrs. on average (from 2 to 21 yrs.) following menarche, i.e. at age of 21.76±4.85 yrs. 91.37% PCOS women had polycystic ovary morphology.

In total, 15.5% women with PCOS and 27% controls gave birth ($p>0.05$) (Table 2). Almost half of the PCOS women had unfavourable obstetric and gynaecological anamnesis: failed pregnancies or infertility. Eight pregnancies (6.9%) of PCOS women failed: spontaneous miscarriage occurred in six (5.17%), one (0.86%) had ectopic pregnancy and there was one (0.86%) stillbirth. Nine (11.11%) controls and seven (6.35%) PCOS women became pregnant but terminated it. 37.1% PCOS women could not conceive at all: about 40% type I classic, one-third normoovulatory and 40% normoandrogenic PCOS phenotype women, however, there was no statistically significant difference found among the groups. PCOS women unable to conceive were statistically significantly 2.8 yrs. older (mean age 27 yrs.) compared to those who had no such problems. Only half of the women unable to get pregnant underwent examination

concerning the causes of infertility. As many as 63% of women with ovulatory dysfunction could not conceive, while 90.7% PCOS women unable to get pregnant were diagnosed with anovulation. Body mass, BMI, systolic and diastolic BP, glucose, TG, HOMA-IR in women with PCOS unable to conceive were statistically confidently higher than those of the PCOS women, who had no such problem. We detected the dependence of infertility upon obesity: the probability of obese PCOS women to be infertile was 2.73 times higher than not obese PCOS women. On the other hand, longitudinal study of these women should be conducted to determine the impact of weight decrease on the reproductive health, metabolism and hormone balance.

Peculiarities of metabolism

In order to assess the metabolic status and CV risk factors of women with PCOS, we have analysed the lipid and glucose metabolism, insulin, BP, HR, handgrip, CRP and white blood cells (WBC) count. Since there was statistically significant difference between the body mass in women with PCOS and the controls, we took into consideration the impact of body mass index while comparing the results as well.

Dyslipidemia

There was no difference in total cholesterol level between the PCOS and the controls as well as among the PCOS phenotypes ($p>0.05$). The HDL in women with PCOS was statistically significantly lower, while LDL and TG higher than those of the controls, besides, there also remained statistically significant difference between HDL and TG after adjustment for BMI ($p<0.05$) (Table 3). There was higher total cholesterol-to-HDL as well as TG-to-HDL ratio statistically significantly observed in women with PCOS, and the difference remained after adjustment for BMI impact either ($p<0.05$). We determined that HDL in obese women with PCOS was statistically significantly lower, and cholesterol/HDL higher compared to that in normal weight and overweight PCOS women ($p<0.05$).

68.3% women with PCOS and 31.7% controls had dyslipidemia, defined as total cholesterol higher than 5 mmol/l and (or) LDL higher than 3 mmol/l and (or) HDL lower than 1 mmol/l and TG higher than 1.7 mmol/l, ($p<0.05$). Dyslipidemia was diagnosed for over a half of androgen excess women with PCOS. 70% PCOS women suffering from

dyslipidemia had BMI higher than 25 kg/m², 48% were obese, 84% had large skeleton, and 52% picnomorphic somatotype.

The metabolic syndrome component - HDL lower than 1.3 mmol/l, - occurred in 5 control and one-third of women with PCOS ($p<0.0001$). Such a HDL level was statistically significantly more frequent in androgen excess women with PCOS compared to the controls ($p<0.01$), however, there was no difference observed between the women with normoandrogenic PCOS phenotype and controls. TG higher that 1.7 mmol/l, another metabolic syndrome component, occurred in 17 (14.66%) PCOS women and two controls ($p<0.01$).

Table 3. Lipid metabolism parameters in the women studied

Parameter	Mean ± SD			Adjusted mean*		
	PCOS (N=116)	Control (N=81)	p	PCOS (N=116)	Control (N=81)	p
Total chol., mmol/l	4.84±0.87	4.71±0.73	0.278	4.81	4.76	0.699
HDL, mmol/l	1.51±0.40	1.82±0.31	<0.0001	1.58	1.71	0.012
LDL, mmol/l	2.83±0.76	2.55±0.71	0.009	2.76	2.64	0.326
TG, mmol/l	1.15±0.63	0.77±0.34	<0.0001	1.06	0.89	0.034
Total chol/HDL	3.43±1.10	2.65±0.57	<0.0001	3.12	2.97	0.046
TG/LDL	0.89±0.70	0.45±0.24	<0.0001	0.77	0.61	0.041
SD – standard deviation, PCOS – women with polycystic ovary syndrome, chol – cholesterol, HDL – high density lipoprotein cholesterol, LDL – low density lipoprotein cholesterol, TG- triacylglycerol's, Chol./HDL – cholesterol and high density lipoprotein cholesterol ratio, TG/HDL – triacylglycerol and high density lipoprotein cholesterol ratio; *Means adjusted for BMI equal to 25.12 kg/m^2						

Disturbance of lipid metabolism is typical of all the women with PCOS, however, total cholesterol analysis is not sufficient in looking for CV risk factors, thus, all the lipidogram is to be evaluated. About half of PCOS women with dyslipidemia were obese and had picnomorphic somatotype, while over two-thirds had large skeleton, therefore, our recommendation is to examine PCOS women with these symptoms for dyslipidemia.

Glucose metabolism impairment

Glucose metabolism parameters in women with PCOS - fasting glucose and glucose after glucose tolerance test (GTT1 and GTT2) as well as glycosylated haemoglobin A1c (HbA1c) were statistically significantly higher in PCOS women than in the controls ($p<0.0001$). The glucose metabolism difference (except HbA1c) remained statistically significant after adjustment for BMI ($p<0.01$) (Table 4). Glycemia in type I

classic PCOS phenotype women was greater than in the controls (p<0.0001), however, did not differ among the phenotypes. Glucose tolerance test revealed fasting glycemia higher in all PCOS phenotypes than that in controls (p<0.01). Glycemia in 2 hours after intake of 75g glucose was higher in type I classic, normoovulatory and normoandrogenic PCOS than in controls (p<0.05).

Impaired fasting glycemia (5.6 mmol/l or higher) occurred in 59.5% PCOS women, over one-third had 6.1 mmol/l glycemia or higher, while among the controls it constituted only 12.3% (p<0.001). Impaired glucose tolerance was detected in about one-tenth women with PCOS, diabetes mellitus in three PCOS women, whereas controls had no such disorders. BMI exceeded 25 kg/m^2 in 73% PCOS women with impaired glycemia, 48% were obese, 78% had large skeleton, and 57% had picnomorphic somatotype.

Table 4. Carbohydrates metabolism parameters in the women studied

Parameter	Mean ± SD			Adjusted mean*		
	PCOS (N=116)	Control (N=81)	p	PCOS (N=116)	Control (N=81)	P
Glucose, mmol/l	5.12±0.53	4.74±0.47	<0.0001	5.04	4.82	0.004
GTT 1, mmol/l	5.69±0.69	4.78±0.44	<0.0001	5.57	4.88	<0.0001
GTT 2, mmol/l	5.68±1.61	4.15±1.04	<0.0001	5.48	4.45	<0.0001
HbA1c,%	5.43±0.38	5.23±0.26	<0.0001	5.37	5.30	0.191
SD – standard deviation, PCOS – women with polycystic ovary syndrome, GTT1 – fasting glucose prior to glucose tolerance test, GTT2 – glucose 2 hours. after glucose tolerance test, HbA1c – glycosylated haemoglobin A1c. *Means adjusted for BMI equal to 25.12 kg/m^2						

It is mandatory to carry out glucose tolerance test in order to determine glucose metabolism disorders and prevent diabetes mellitus for women with PCOS, since sometimes fasting glucose may appear to be normal, besides, special attention is to be paid to obese, women with large skeleton and picnomorphic somatotype.

Insulin concentration and insulin resistance

We have determined HOMA-IR in women with PCOS was statistically significantly higher, and QUICK and GI indices were lower than those of the controls (p<0.0001). After adjustment for BMI, it was only QUICKI index in PCOS women that appeared to be statistically significantly lower than that of the controls (Table 5). Insulin and HOMA-IR were statistically significantly higher in women with types I and II

classic PCOS phenotypes, whereas QUICKI and GI lower than those of the controls ($p<0.01$).

Table 5. Insulin and insulin resistance indices in the women studied

Index	Mean ± SD			Adjusted mean*		
	PCOS (N=116)	Control (N=81)	p	PCOS (N=116)	Control (N=81)	p
Insulin, µU/ml	12.57±10.29	6.39±2.8	<0.0001	10.31	9.63	0.491
HOMA-IR	3.00±2.78	1.36±0.63	<0.0001	2.40	2.22	0.497
GI	10.87±5.93	15.52±5.71	<0.0001	12.17	13.66	0.067
QUICKI	0.34±0.03	0.37±0.03	<0.0001	0.35	0.36	0.007

HOMA-IR – homeostasis model of assessment-insulin resistance, GI – glucose-to- insulin ratio, QUICKI – Quantitative Insulin Sensitivity Check Index.
* Means adjusted for BMI equal to 25.12 kg/m^2

Insulin resistance was statistically significantly more frequent in women with PCOS than in the controls (39.7% and 4.9%, respectively, $p<0.0001$). It was more frequent in PCOS women with hyperandrogenism than in the controls, and in women with type I and II classic PCOS phenotypes than in women with normoandrogenic PCOS phenotype ($p<0.05$), however, there was no significant difference between the PCOS women with normal androgen level and the controls concerning the latter data. Over two-thirds (71.8%) of obese, one-third (37%) overweight and 16% normal weight women with PCOS were insulin-resistant. On the other hand, 60.9% insulin-resistant women with PCOS were obese, 67.4% had hirsutism, almost all (93.5%) had large skeleton, 34.8% complained of impaired fasting glycemia, 19.6% had impaired glucose tolerance, 6.5% were ill with type II diabetes mellitus.

About half (46.55%) women with PCOS and only two (2.5%) controls had insulin resistance feature - *Acanthosis nigricans* - in different body areas: neck, armpits, groin and elbows, below the breasts ($p<0.0001$). *Acanthosis nigricans* is statistically significantly more frequent in PCOS women with hyperandrogenism compared to the PCOS women with normal androgen level and the controls ($p<0.05$). As many as one-fourth of women with PCOS had *Acanthosis nigricans*, visceral obesity and large skeleton.

Insulin resistance in women with PCOS closely correlated with *Acanthosis nigricans*, obesity and large skeleton, thus, screening and follow-up of women with

mentioned signs as well as with menstrual cycle disturbance and (or) hirsutism are important for women's reproductive health and prevention of metabolic disorders.

Cardiovascular status

Systolic, diastolic, mean and pulse arterial blood pressure (BP) and the heart rate in women with PCOS was statistically significantly higher than in the controls, moreover, WBC count and C-reactive protein (non-traditional CV risk factors) were higher, however, relative handgrip was statistically significantly lower ($p<0.01$) (Table 6).

Systolic and diastolic arterial BP, WBC count and CRP in women with types I and II classic PCOS phenotypes was statistically significantly higher than that of the controls ($p<0.05$). Relative handgrip was statistically significantly lower in PCOS women with androgen excess compared to the controls ($p<0.05$), whereas there was no difference observed between the normal androgen level women and the controls ($p<0.05$).

One-tenth of PCOS women were diagnosed with primary arterial hypertension, i.e. arterial BP exceeding 140/90 mmHg, however, it was not found in the controls ($p<0.01$). 34.5% PCOS women and 29.6% controls belonged to medium CV risk group according to CRP (CRP = 1–3 mg/l) and 22.4% made up high risk group with CRP higher than 3 mg/l.

Table 6. Risk factors of CV diseases in the women studied

Index	Mean ± SD			Adjusted mean*		
	PCOS (N=116)	Control (N=81)	p	PCOS (N=116)	Control (N=81)	p
Systolic BP, mmHg	118.4±13.33	109.86±8.52	<0.0001	115.60	113.87	0.263
Diastolic BP, mmHg	79.15±9.81	74.36±6.96	<0.0001	77.34	76.96	0.761
Pulse BP, mm Hg	39.25±6.73	35.50±5.41	<0.0001	38.27	36.91	0.149
Mean BP, mmHg	92.24±10.64	86.19±7.07	<0.0001	90.09	89.26	0.515
HR, times/min	79.22±13.01	74.86±9.05	<0.01	75.21	78.47	0.083
WBC, 10^9/l	6.31±1.48	5.53±1.05	<0.0001	6.14	5.77	0.076
CRP, mg/l	2.40±3.04	0.78±0.52	<0.0001	1.89	1.50	0.241
Right handgrip	29.28±4.79	28.85±5.34	0.553	28.74	29.63	0.263
Left handgrip	26.81±4.99	26.59±5.53	0.769	26.39	27.20	0.336
Relative right handgrip	0.40±0.09	0.48±0.09	<0.0001	0.43	0.45	0.113
Relative left handgrip	0.37±0.10	0.44±0.10	<0.0001	0.39	0.41	0.202
BP – arterial blood pressure, HR – heart rate, WBC – white blood cells count; CRP – C-reactive protein; *Means corrected considering mean BMI equal to 25.12 kg/m^2						

Hence, young women with PCOS had higher CV risk: not only dyslipidemia, more frequent insulin resistance and impaired glucose metabolism but also higher arterial blood pressure and heart rate (reflecting the activation of sympathetic nervous system), impaired indices of inflammation (CRP, WBC) and lower relative physical strength. Therefore, these women should be followed-up because of possible CV diseases.

Metabolic syndrome

Metabolic syndrome was determined for almost one-fifth of women with PCOS according to the NCEP, 2001, criteria, one-fourth according to the IDF, 2005 and AHA, 2009, criteria, however, there was no metabolic syndrome found in the controls (Table 7). Over one-fourth women with type I and II classic PCOS phenotypes had metabolic syndrome according to all the criteria, and it was statistically significantly different from the controls ($p<0.0001$). There was no metabolic syndrome determined in women with normoandrogenic PCOS phenotype.

Table 7. Metabolic syndrome frequency in the women studied

Metabolic syndrome criteria	PCOS (N=116)	Control (N=81)	p
According to NCEP, 2001	22 (19%)	0	<0.0001
According to IDF, 2005	30 (25.9%)	0	<0.0001
According to AHA, 2009	28 (24.1%)	0	<0.0001
NCEP - National Cholesterol Education Program and III criteria of the Guidelines of Treating Adults; IDF - International Diabetes Federation; AHA – International Diabetes Federation, National Heart, Lungs and Blood Institute, American Heart Association, World Heart Association, International Atherosclerosis Society, International Obesity Research Association			

BMI of all the women with metabolic syndrome exceeded 25 kg/m^2. More than half of obese PCOS women had metabolic syndrome. Large skeleton was indicative of all the women with metabolic syndrome, and almost one-third of large skeleton women had metabolic syndrome. Frame index (FI) cut-off value in determining metabolic syndrome was calculated to be FI = 48.1 and more that 90% of women with metabolic syndrome had a higher FI. Over 80% of PCOS women with metabolic syndrome had picnomorphic somatotype, and about half of these women suffered from metabolic syndrome. The cut-off value of the metric index (MI) in predicting metabolic syndrome was calculated to be MI = 0.3 and it was exceeded in more than 70% women with

metabolic syndrome. The cut-off values of the area under the ROC curve, sensitivity, specificity, positive and negative correlations as well as probability to have metabolic syndrome are presented in Table 8.

Table 8. Waist circumference and skeletal size characteristics in predicting metabolic syndrome

Index	AUROC	Sensitivity, %	Specificity, %	Positive likelihood ratio	Negative likelihood ratio	MetS probability
Waist circumference >83.5 cm	0.966	100	86.4	7.41	0.135	55%
Skeleton index > 48.1	0.926	92.9	81.7	5.06	0.198	46%
Metric index >0.3	0.884	75	90	14.08	0.071	70%
MetS – metabolic syndrome; AUROC - area under ROC curve						

Undoubtedly, metabolic syndrome helps identify individuals having higher risk of developing CV diseases and type II diabetes mellitus. Waist circumference is a usual clinical sign for metabolic syndrome selection, which is more closely related to the syndrome than BMI, however, we have found that skeleton size and somatotype indices – FI and MI – are also very important in identifying the metabolic syndrome.

Peculiarities of body size and build in women with polycystic ovary syndrome

Skeleton size and proportions, body circumferences and ratios, skinfolds thickness and body composition of women with PCOS and controls as well as interrelations of these variables were assessed in order to evaluate body size and build in women with polycystic ovary syndrome. Skeleton frame size is characterized by longitudinal and transverse measurements and their proportions as well as certain facial characteristics.

Longitudinal skeletal measurements

Having compared the height of women with PCOS and the controls no statistically significant difference was found (Table 9). The neck of women with PCOS was determined to be 0.76 cm shorter, trunk statistically significantly higher by 2 cm, middle pelvis height was 2.04 cm higher, hand 0.3 cm longer than those of the controls ($p<0.05$), meanwhile the leg and arm of the controls was statistically significantly longer by 1cm than those of women with PCOS ($p<0.05$). Moreover, relative trunk and hand

length in women with PCOS was statistically significantly greater and relative arm and leg length lower than that of the controls ($p<0.05$).

The trunk of women with type I and II classic PCOS phenotypes was statistically significantly higher (2.5 cm and 3.9 cm, respectively), and the middle pelvis height was greater (2.08 cm and 3.67 cm, respectively) that in the controls ($p<0.05$).

Comparison of 2nd and 4th finger length (both palmar and dorsal) and their ratio showed no statistically significant difference either between women with PCOS and controls or among women with certain PCOS phenotypes. It was determined that 1st, 2nd and 4th toes in women with PCOS were statistically significantly longer by 0.3 cm compared to the controls, and this difference was especially noticeable between women with type I classic PCOS phenotype and the controls ($p<0.05$).

Table 9. Longitudinal skeletal measurements in the women studied (mean±SD)

Parameter	PCOS (N=116)	Control (N=81)	p
Height, cm	167.10±6.91	166.71±6.91	0.679
Neck length, cm	8.02±2.55	8.78±2.02	0.027
Trunk height, cm	51.42±3.87	48.86±3.63	<0.0001
Middle pelvis height, cm	11.89±3.41	9.85±2.87	<0.0001
Arm length, cm	73.08±5.03	74.72±4.33	0.018
Leg length, cm	86.37±5.07	87.84±5.03	0.047
Hand length, cm	17.38±0.73	17.07±1.01	0.021
Foot length, cm	24.14±1.12	24.18±1.33	0.801
Height sitting-to-height ratio	0.53±0.02	0.53±0.01	0.812
Trunk height-to-height ratio	0.31±0.02	0.29±0.02	<0.0001
Arm length-to-height ratio	0.44±0.03	0.45±0.01	<0.0001
Leg length-to-height ratio	0.52±0.02	0.53±0.02	<0.0001
Height sitting-to-arm ratio	1.22±0.1	1.19±0.06	0.011
Height sitting-to-leg length ratio	1.03±0.05	1.01±0.05	0.011
Intermembrane index	79.24±5.16	80.59±2.73	0.018
SD – Standard deviation, PCOS – women with polycystic ovary syndrome			

There was statistically significant weak correlation among trunk height, middle pelvis height, foot length, 4th toe length and lipid, glucose, insulin resistance and inflammatory (CRP) indices in women with PCOS, while leg length and its correlations were negative, middle pelvis height and androgens correlated statistically significantly weakly, and correlation between length of extremities and FAI was negative and weak. No such correlation was observed among the controls.

There was statistically significant weak correlation among trunk height, middle pelvis height, foot length, 4th toe length and lipid, glucose, insulin resistance and inflammatory (CRP) indices in women with PCOS, while leg length and its correlations were negative, middle pelvis height and androgens correlated statistically significantly weakly, and correlation between length of extremities and FAI was negative and weak. No such correlation was observed among the controls.

Hence, longitudinal skeletal measurements and their proportions in women with PCOS (especially with hyperandrogenism) differ from those of the controls and correlate strongly with many metabolism parameters, whereas middle pelvis height and length of extremities correlate with androgens.

Transverse skeletal measurements

Transverse skeletal measurements (shoulder and chest width, chest depth, width of pelvis, knee, ankle, foot, elbow and hand) in women with PCOS were statistically significantly greater than those in the controls ($p<0.01$), while there was no difference found concerning wrist width (Table 10).

Table 10. Transverse skeletal measurements in the women studied

Parameter	Mean ± SD			Adjusted mean*		
	PCOS (N=116)	Control (N=81)	p	PCOS (N=116)	Control (N=81)	p
Shoulder width, cm	40.98±3.14	39.06±1.73	<0.0001	40.15	40.25	0.741
Chest width, cm	28.15±3.38	25.13±1.44	<0.0001	27.21	26.47	0.005
Chest depth, cm	19.78±3.13	17.43±1.47	<0.0001	18.93	18.65	0.292
Pelvis width, cm	32.49±3.37	30.94±2.32	<0.0001	31.53	32.32	0.011
Elbow width, cm	8.1±1.00	7.05±0.69	<0.0001	7.80	7.47	<0.0001
Wrist width, cm	5.22±0.41	5.19±0.57	0.663	5.15	5.30	0.035
Knee width, cm	10.68±1.55	9.96±0.84	<0.0001	10.27	10.54	0.055
Ankle width, cm	6.21±0.51	5.61±0.55	<0.0001	6.11	5.75	<0.0001
Hand width, cm	7.73±0.51	7.37±0.39	<0.0001	7.65	7.50	0.028
Foot width, cm	9.06±0.58	8.74±0.46	<0.0001	8.95	8.90	0.463
Akromiocristal index	79.29±5.55	79.26±5.65	0.971	78.54	80.33	0.040
Thoracic index	70.3±7.24	69.45±5.66	0.352	145.08	142.95	0.311
Metric index	-0.29±1.05	-1.2 ±0.37	<0.0001	-0.61	-0.750	0.025
Skeletal index	50.49±5.85	42.32±3.88	<0.0001	0.47	0.45	<0.0001
Chest-to-pelvis ratio	0.87±0.06	0.82±0.06	<0.0001	0.86	0.82	<0.0001
Hand size	44.54±2.97	43.26±2.38	<0.0001	44.08	43.92	0.689
Foot size	37.57±2.26	36.20±1.85	<0.0001	37.24	36.69	0.085

SD – Standard deviation, PCOS – women with polycystic ovary syndrome
*Mean adjusted for BMI to be equal to 25,12 kg/m^2

Frame size derivative indices in women with PCOS – metric and frame indices, chest-to-pelvis ratio, hand and foot size – were statistically significantly greater than those of the controls ($p<0.05$).After adjustment for BMI there appeared to be no difference between shoulder width, chest depth and knee and foot widths in women with PCOS and the controls ($p<0.05$), chest, elbow, ankle and hand in women with PCOS were statistically significantly wider that those in the controls ($p<0.05$), however, pelvis and wrist were narrower compared to the controls ($p<0.05$). Transverse skeletal measurements in PCOS women with hyperandrogenism were statistically significantly greater than in the controls ($p<0.05$). It was ankle width (the only skeletal measurement) in PCOS women with normal androgen level that statistically significantly exceeded that of the controls ($p<0.05$).

Large frame size was statistically significantly three times more frequent among the women with PCOS than the controls ($p<0.0001$). More than two-thirds of women with hyperandrogenism PCOS phenotype had large frame size, whereas small frame size was prevalent in less than one-tenth PCOS women. Picnic and especially picnomorphic somatotype was statistically significantly more frequent among the women with PCOS than the controls (39.6% and 1.2%, respectively), half of women with PCOS and hyperandrogenism had picnic or picnomorphic somatotype, whereas 39.6% women with PCOS and even 85% of the controls had gracile and especially gracile somatotype ($p<0.0001$). Distribution of normoandrogenic PCOS phenotype women in the groups according to frame size and somatotype was very similar to that of the controls ($p>0.05$).

There was statistically significant weak or average correlation among the transverse skeletal measurements, frame size indices and FAI, lipid, glucose, insulin resistance and CRP in women with PCOS. Shoulder, chest, pelvis, knee and ankle width as well as metric index correlated weakly negatively with LH, SHBG and HDL cholesterol. Metric index and frame index had the strongest correlation with markers of endocrine function and metabolism in PCOS women group, however, no correlation among these indices was found in the controls or its was weaker. Thus, women with PCOS have more massive frame size, their chest is wider, pelvis is narrower, elbow, and ankle and hand breadth is higher compared with healthy women.

Head and face measurements

Face and mandible of women with PCOS was statistically significantly wider, face depth greater, and lower facial part higher than those of the controls ($p<0.05$) (Table 11).

There was no difference determined concerning head length and width, forehead height, lip width and thickness among the groups. Calculation of facial indices showed that mandibular facial index and facial depths ratio in women with PCOS statistically significantly exceeded those of the controls ($p<0.05$). After adjustment for BMI the mandible remained statistically significantly greater ($p<0.05$).

Table 11. Head and facial measurements of the women studied

Parameter	Mean ± SD			Adjusted mean*		
	PCOS, N=116	Control, N=81	p	PCOS, N=116	Control N=81	p
Head length, cm	18.63±0.78	18.49±0.62	0.161	18.65	18.46	0.100
Head width, cm	14.95±0.69	14.80±0.61	0.126	14.87	14.91	0.699
Face width, cm	13.28±0.62	13.01±0.55	0.002	13.19	13.14	0.595
Mandible width, cm	10.34±0.73	9.83±0.53	<0.0001	10.20	10.00	0.025
Physiognomic face height, cm	17.52±0.95	17.18±0.80	0.010	17.46	17.28	0.209
Morphological face height, cm	11.07±0.75	10.74±0.58	0.001	11.00	10.84	0.145
Middle face height, cm	6.95±0.54	6.81±0.37	0.047	6.95	6.81	0.073
Forehead height, cm	6.45±0.79	6.44±0.71	0.923	6.46	6.44	0.866
Lower face height, cm	4.12±0.57	3.94±0.37	0.010	4.05	4.04	0.813
Upper face depth, cm	11.04±0.56	10.68±0.41	<0.0001	13.31	13.22	0.323
Lower face depth, cm	13.07±0.61	12.82±0.54	0.004	12.99	12.93	0.493
Lip width, cm	4.62±0.38	4.54±0.36	0.137	4.59	4.59	1.000
Upper lip, cm	0.78±0.15	0.74±0.14	0.064	0.78	0.75	0.278
Lower lip, cm	0.96±0.17	0.93±0.15	0.194	0.95	0.94	0.747
Mandibular facial index	93.68±7.23	91.7±5.75	0.041	93.11	92.51	0.570
Upper facial index	52.41±4.74	52.38±3.17	0.962	52.77	51.87	0.179
Facial depths ratio	83.40±3.4	84.56±4.33	0.045	102.62	102.45	0,859

SD – Standard deviation, PCOS – women with polycystic ovary syndrome
*Mean adjusted for BMI to be equal to 25,12 kg/m^2

Facial width, mandible width, lower facial height, upper and lower facial depth in women with type I classic PCOS phenotype appeared to be statistically significantly greater than those in the controls ($p<0.05$). There were no statistically robust head and facial measurements differences observed among the phenotypes.Head and facial measurements in women with PCOS correlated more with transverse skeletal measurements, while correlation between head and facial measurements and longitudinal skeletal measurements was stronger in the controls.

Thus, the face in women with PCOS is more massive, mandible wider, lower facial part higher and deeper, and facial parameters correlate more with skeletal size than with longitudinal measurements.

Predictive abilities of skeletal size

In order to find out PCOS discriminating abilities of the skeletal measurements and indices ROC curves were made. Chest width and depth, width of elbow, ankle, hand and mandible as well as frame index and metric index (AUROC were 0.703–0.800) had the best PCOS predictive properties among longitudinal, transverse and facial measurements, trunk height, 1st toe length, shoulder, knee and face widths had slightly less marked predictive properties (AUROC 0.625–0.681), whereas height, arm length, other transverse skeletal and facial measurements did not demonstrate prognostic abilities (AUROC lower than 0.5) (Fig. 6, Table 12).

Table 12. Predictive characteristics of skeletal measurements and indices for PCOS

Parameter	AUROC	Sensi-tivity, %	Speci-ficity, %	Positive likelihood ratio	Negative likelihood ratio	PCOS proba-bility
Trunk height > 51.75cm	0.677	46.6	82.7	2.69	0.371	0.8
Middle pelvis height > 13.05	0.690	38.8	90.1	3.93	0.255	0.9
Relative leg length < 0.52	0.642	77.8	49.1	2.21	0.452	0.8
1st toe length > 4.65cm	0.679	63.8	69.1	2.07	0.484	0.7
Chest width > 26.45cm	0.788	65.5	84	4.08	0.245	0.9
Elbow width > 7.75cm	0.800	60.3	86.4	4.44	0.225	0.9
Ankle width >5.85 cm	0.791	78.5	66.7	2.35	0.425	0.8
Hand width > 7.55 cm	0.703	60.3	71.6	2.13	0.471	0.8
Skeletal index > 45.92	0.800	63.8	88.9	5.74	0.174	0.9
Metric index >–0.94	0.774	67.2	82.7	3.89	0.257	0.8
Mandible width > 10.45cm	0.704	46.6	88.9	4.19	0.239	0.9
PCOS – women with polycystic ovary syndrome; AUROC – area under ROC curve						

The cut-off values of skeletal measurements to identify PCOS were calculated according to ROC curves data and Youden index (Table 12). The probability of PCOS for having frame index higher than 45.92 was 5.74 times greater when that in the controls.

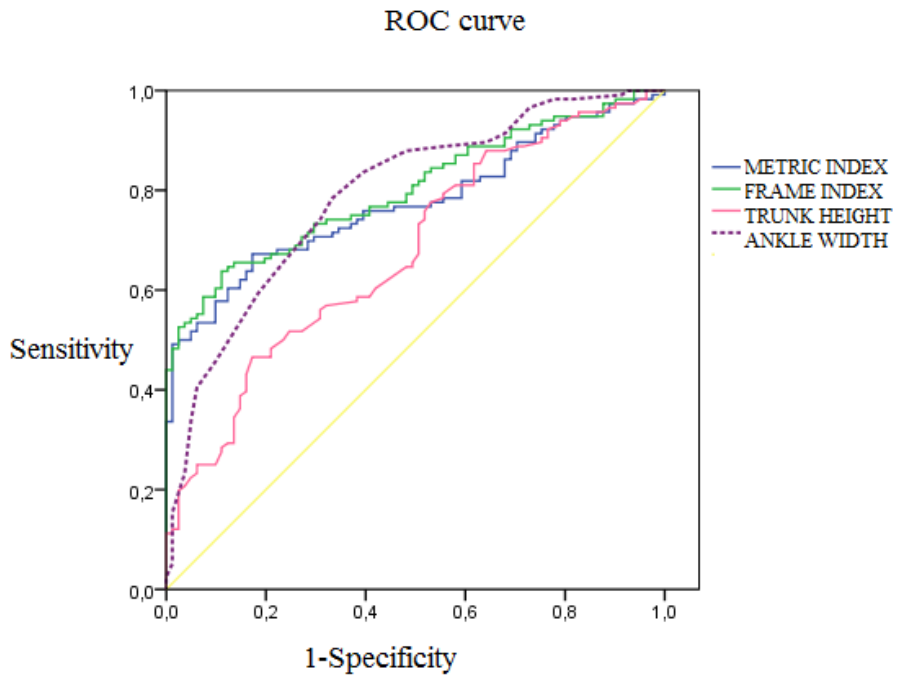

Figure 6. ROC curves of metric index, skeletal index and ankle width and trunk height in predicting PCOS

Elbow width, chest and mandible width, metric index as well as other skeletal measurements showed lower positive likelihood ratios. The highest probability of PCOS (90%) was in women whose chest width, elbow width, frame index and mandible were greater than the cut-off values.

Peculiarities of body mass and BMI

The height of women with PCOS was similar, however, average body mass statistically significantly 16 kg greater, and BMI exceeded that of the controls by almost 6 kg/m^2 ($p<0.0001$) (Table 13). Statistically significantly overweight or obesity was more frequently observed in women with PCOS than in the controls ($p<0.05$). 56.9% PCOS women had greater than 25 kg/m^2 BMI, and more than half (59%) of them were obese.

Body mass and BMI of types I and II classic PCOS phenotypes were statistically significantly greater (15-21 kg and 6 kg/m^2) compared to those of the controls and normoandrogenic PCOS phenotype women. Type II classic PCOS phenotype women weighing statistically significantly 20 kg more had the greatest body mass and BMI, and BMI exceeded that of the controls and women with normal androgen level by 8 kg/m^2

(p<0.05). There was no significant difference observed between the body mass and BMI of normoandrogenic PCOS phenotype women and the controls (p<0.05).

Table 13. Body mass and BMI of the women studied (mean ± SD)

Parameter	PCOS (N=116)	Control (N=81)	p
Height, cm	167.10±6.91	166.71±6.91	0.679
Body mass, kg	76.68±20.15	60.57±8.42	<0.0001
BMI, kg/m²	27.47±7.02	21.76±2.40	<0.0001
Normal weight (BMI < 25 kg/m²)	50(43.1%)	70 (81.4%)	<0.0001
Overweight (BMI 25–29.9 kg/m²)	27(23.3%)	11 (13.6%)	0.026
Obesity (BMI ≥ 30 kg/m²)	39(33.6%)	0	<0.0001
SD – Standard deviation, PCOS – women with polycystic ovary syndrome, BMI – body mass index			

One-tenth controls and one-fifth PCOS women complained of overweight since childhood (6-7 yrs. of age, before starting school), however, the difference was insignificant (p>0.05). Women with PCOS had overweight since adolescence more frequently than the controls and it was statistically significant: one-fourth (26.7%) of PCOS women and 2.5% controls had overweight since adolescence (p<0.0001).

97.4% obese women with PCOS had large frame size, 92% were of picnomorphic somatotype, on the other hand, 78% picnomorphic and 48.7% women with large frame size were obese.

One-third of PCOS women were obese, the majority of endocrine and metabolic parameters of these women were impaired comparing with women of lower weight. It should be noted that the majority of obese women with PCOS also had larger frame size and picnomorphic somatotype.

Body circumferences

All the body circumferences of women with PCOS, except breasts size, were statistically significantly greater than those of the controls (p<0.0001). (Table 14). The greatest difference was observed between the mean waist circumference at its narrowest (16.79%) and mean waist circumference at umbilicus (15.14%). Body circumference over 80 cm (visceral obesity) was found in half of PCOS women (including two-thirds with circumference larger than 88 cm) and 7.4% controls (p<0.0001).

Shoulder circumference was statistically significantly shorter in normal weight women with PCOS compared to the controls (p<0.05), while other body circumferences

showed no difference. Body circumferences of overweight PCOS women and the controls did not differ significantly. After adjustment for BMI, it became evident that shoulder circumference, upper arm circumference at rest, largest chest circumference at breasts and hip circumference were statistically significantly smaller in women with PCOS ($p<0.05$), and there was no statistically significant difference observed concerning other circumferences compared to the controls.

Most circumferences in women with type I and II classic PCOS phenotypes were the largest and statistically significantly differed from the those of women with normal androgen level as well as the controls ($p<0.05$). Breasts of normoandrogenic PCOS phenotype women were the smallest and statistically significantly smaller by 3.2 cm compared to those of classic PCOS phenotype women ($p<0.05$).

Table 14. Body circumferences of the women studied

Parameter, cm,	Mean ± SD				Adjusted mean*		
	PCOS (N=116)	Control (N=81)	Diffe-rence, %	p	PCOS (N=116)	Control (N=81)	p
Neck circumference	33.66±3.33	31.42±1.51	7.13	<0.0001	32.86	32.56	0.354
Shoulder circumference	107.39±10.89	102.28±4.78	5.00	<0.0001	104.26	106.76	0.002
Upper arm circumference (relaxed)	29.87±4.86	26.68±2.84	11.98	<0.0001	28.30	28.93	0.031
Upper arm circumference (strained)	31.46±5.02	27.92±2.48	12.67	<0.0001	29.86	30.22	0.187
Forearm circumference	24.98±2.80	22.98±1.51	8.68	<0.0001	24.11	24.23	0.483
Wrist circumference	15.71±1.19	15.04±0.82	4.49	<0.0001	15.40	15.49	0.434
Chest circumference (above breasts)	93.37±11.86	83.94±5.06	11.24	<0.0001	89.67	89.24	0.503
Chest circumference (below breasts)	85.94±13.10	76.36±5.43	12.55	<0.0001	81.78	82.31	0.408
Largest chest circumference	97.59±15.29	87.45±6.51	11.60	<0.0001	92.75	94.38	0.034
Size of breasts	11.65±4.25	11.09±3.45	5.02	0.313	10.96	12.07	0.060
Waist circumference 1	82.04±14.82	70.24±5.86	16.79	<0.0001	77.35	76.96	0.581
Waist circumference 2	91.61±15.72	79.57±6.58	15.14	<0.0001	86.69	86.61	0.923
Waist circumference 3	98.03±14.80	86.15±6.75	13.79	<0.0001	93.26	92.98	0.685
Hip circumference	103.97±11.38	96.66±5.81	7.56	<0.0001	100.39	101.79	0.040
Largest thigh circumference	60.95±7.53	55.88±4.54	9.07	<0.0001	58.57	59.29	0.159
Hip middle circumference	53.16±6.62	49.06±4.49	8.35	<0.0001	51.18	51.90	0.201
Calf circumference	38.32±4.90	35.16±2.55	8.99	<0.0001	36.87	37.25	0.289

SD – Standard deviation, PCOS – women with polycystic ovary syndrome;
Waist circumference 1 – slenderest looking waist line was measured in horizontal plane; waist circumference 2 – circling waist around umbilicus; waist circumference 3 – circling abdomen around frontal upper iliac crests.
*Means adjusted for BMI to be equal to 25,12 kg/m^2

There was no difference in body circumferences between PCOS and controls women with small and medium frame size, whereas all body circumferences (except

wrist circumference) of PCOS women with large frame size statistically significantly differed from those of the controls ($p<0.05$). Breast size was about 1 cm smaller in women with PCOS of all frame sizes, however, not significantly.

Significant correlations were found between the body circumferences and metabolism parameters in women with PCOS, with the strongest correlations between chest and waist circumferences. There was statistically significant average and strong negative correlation of body circumferences with SHBG, HDL-cholesterol, GI and QUICKI indices. Breast size of women with PCOS correlated averagely with BMI and weakly with testosterone, FAI, lipid and insulin resistance indices. Breasts size correlated weakly with BMI, and there was weak negative correlation with FSH, SHBG and GI index in control group.

After adjustment for BMI body circumferences in PCOS women defining active mass and muscle volume indirectly (shoulder and upper arm circumferences), defining amount of protective "healthy" subcutaneous adipose tissue (hip circumference) and serving as a marker of fertility and health (breast size) appeared to be smaller than those in control group. Possibly, due to frame size and abounding adipose tissue other PCOS women body circumferences were greater than those in controls.

Parameters of body proportions

It was found that some of the indices showing picnomorphic somatotype in women with PCOS were statistically significantly higher than those of the controls ($p<0.01$), except shoulder-to-hip as well as shoulder-to-waist ratios as being lower than those of the controls ($p<0.01$), as well as chest circumference index showing no difference between the groups (Table 15).

There were means of waist-to-height ratios found to be most different between women with PCOS and the controls (assessing by absolute values and per cent). One-sixth of women with PCOS and one control woman were determined with waist-to-hip ratio higher than 0.85 (WHO considers it to be a CV risk factor) ($p<0.01$). Waist-to-height ratio exceeding 0.5 was found in 50 (43.1%) women with PCOS and two (2.5%) controls ($p<0.0001$).

Table 15. Body proportions of the women studied

Parameter	Mean ± SD				Adjusted mean*		
	PCOS (N=116)	Control (N=81)	Diffe-rence, %	p	PCOS (N=116)	Control (N=81)	p
Shoulder-to-waist ratio	1.33±0.12	1.46±0.08	-9.10	<0.0001	1.36	1.41	<0.0001
Shoulder-to-hip ratio	1.04±0.07	1.06±0.05	-2.25	0.004	1.04	1.05	0.244
Chest-to-hip ratio	0.94±0.08	0.91±0.05	3.45	<0.0001	0.92	0.93	0.736
Chest circumference index	1.14±0.04	1.15±0.05	-0.91	0.120	1.13	1.148	0.056
Waist-to-height ratio	0.49±0.09	0.42±0.03	16.54	<0.0001	0.46	0.46	0.841
Waist-to-breasts ratio	0.84±0.04	0.80±0.04	4.34	<0.0001	0.83	0.81	0.002
Waist and hip circumference	0.79±0.08	0.73±0.05	8.01	<0.0001	0.77	0.75	0.065
Conicity index	1.12±0.07	1.07±0.05	4.05	<0.0001	1.10	1.09	0.514
Waist-to-thigh ratio	1.34±0.13	1.26±0.08	6.59	<0.0001	1.32	1.29	0.120
Waist-to-thigh median ratio	1.54±0.15	1.44±0.20	6.63	<0.0001	1.51	1.49	0.446
SD – Standard deviation, PCOS – women with polycystic ovary syndrome *Means adjusted for BMI to be equal to 25,12 kg/m^2							

There was statistically significant difference observed only between two ratios, namely, shoulder-to-waist ratio was lower, while waist-to-breasts ratio was greater in normal weight women with PCOS compared to the controls ($p<0.05$). After adjustment for BMI shoulder-to-waist ratio was found to be statistically significantly lower, and waist-to-breasts ratio greater in women with PCOS than in the controls ($p<0.05$).

Waist-to-height ratio was significantly greater in women with hyperandrogenic PCOS phenotype, while shoulder-to-waist ratio lower than those of the controls ($p<0.05$). Waist-to-hip ratio in women with type I and II classic PCOS phenotypes was greater than that in the controls. Chest-to-hip ratio, conicity index, waist-to-hip indices in women with type I classic PCOS phenotype were significantly greater than in the controls ($p<0.05$). There was no difference observed in proportions between the normoandrogenic PCOS phenotype women and the controls.

Body proportions in the small and medium frame size subjects did not differ significantly between women with PCOS and the controls. However, the majority of indices in large frame size women with PCOS differed statistically significantly from the controls: shoulder-to-waist, shoulder-to-hip ratios were lower, and waist-to-height, waist-to-hip, waist-to-thigh ratios, conicity index were greater than those in the large frame size controls ($p<0.05$).

Most body proportions in women with PCOS correlated weakly or averagely with FAI, lipid, glycemia, insulin resistance and CRP, and the strongest correlation of the parameters above was with waist-to-height ratio, whereas in the controls proportions

correlated weakly with LH and FSH; weak correlation of waist-to-height ratio with lipid and CRP was observed.

Table 16. Predictive properties of body proportions in identifying PCOS

Parameter	AUROC	Sensi-tivity, %	Speci-ficity, %	Positive likelihood ratio	Negative likelihood ratio	PCOS proba-bility
Shoulder-to-waist ratio < 1.45	0.814	86.2	61.7	2.18	0.458	0.8
Waist-to-height ratio > 0.46	0.735	60.3	86.4	4.38	0.228	0.9
Waist-to-breasts ratio > 0.83	0.736	60.3	76.5	2.52	0.397	0.8
Waist-to-hip ratio > 0.77	0.736	55.2	87.7	4.06	0.246	0.9
AUROC - area under ROC curve; PCOS – women with polycystic ovary syndrome						

ROC curve was produced involving four predictive indices and the area under ROC curve was evaluated, while Youden index was applied in calculating the cut-off values. Shoulder-to-waist ratio appeared to be most sensitive (86.2%) in identifying PCOS (Table 16).

Hence, increase in relative waist circumference (especially in respect to shoulder and the largest chest circumference) and decrease in shoulder girdle muscle volume and breast size are the most evident features distinguishing women with PCOS from the controls.

Visceral obesity

All the visceral obesity parameters in women with PCOS (abdominal height, abdominal diameter index, visceral adiposity index, lipid accumulation product and abdominal volume index) were statistically significantly greater than those of the controls, and the lipid accumulation product differed greatest ($p<0.0001$) (Table 17).

Table 17. Visceral obesity indices in the women studied

Parameter	Mean ± SD				Mean corrected*		
	PCOS (N=116)	Control (N=81)	Diffe-rence %	p	PCOS (N=116)	Control (N=81)	p
Abdominal height, cm	21.73±3.97	18.52±1.57	17.33	<0.0001	20.53	20.24	0.219
Abdominal diameter index	0.41±0.04	0.38±0.05	7.90	<0.0001	0.40	0.39	0.205
Abdominal obesity index	2.32±0.46	1.95±0.17	18.97	<0.0001	2.18	2.16	0.368
LAI	31.52±30.38	9.91±7.81	218.06	<0.0001	23.79	20.97	0.255
Abdominal volume index	14.27±5.12	10.44±1.63	36.69	<0.0001	12.68	12.72	0.861
SD – Standard deviation, PCOS - women with polycystic ovary syndrome; LAI – lipid accumulation product; *Means adjusted for BMI equal to 25,12 kg/m^2							

There was no difference observed between normal weight women with PCOS and the controls concerning visceral obesity, however, abdominal height of overweight women with PCOS was statistically significantly greater by 1.77 cm than that of the controls ($p<0.05$). After adjustment for BMI, visceral obesity parameters in women with PCOS appeared to be insignificantly greater (except abdominal volume index) ($p>0.05$).

Visceral obesity parameters in women with type I and II classic PCOS phenotypes were statistically significantly greater compared to the controls and normoandrogenic PCOS phenotype women, whereas in normoovulatory PCOS phenotype women the above parameters were significantly greater than in the controls ($p<0.05$). There was no difference found between normoandrogenic PCOS phenotype women and the controls concerning visceral obesity ($p>0.05$).

There was no difference observed between small and medium frame size PCOS women and the controls, however, visceral obesity of large frame size PCOS women exceeded that of the controls ($p<0.0001$). Visceral obesity parameters in women with PCOS correlated averagely and strongly with the FAI, lipids, glycemia, insulin resistance and CRP, and the strongest correlation was found to be with lipid accumulation product, whereas visceral obesity parameters in the controls correlated weakly with LH, FSH, lipid and insulin resistance.

Thus, visceral obesity parameters could be of importance not only in predicting diabetes mellitus and the risk of CV diseases but also in screening of women with PCOS for metabolic disorders.

Skinfolds and their ratios

The adipose skinfolds in women with PCOS were found to be significantly thicker by 3.56–11.45 mm than in the controls ($p<0.0001$) (Table 18).

The greatest difference was found in the thickness of iliac and biceps skinfolds. Skinfolds sums of arms, legs, upper and lower trunk and extremities as well as upper body fat signs – central index and adipose tissue distribution indices in women with PCOS were greater than those in the controls, in addition, arm and trunk skinfolds in women with PCOS were relatively thicker than legs which could indicate accumulation of adipose tissue in the upper part of the body in women with PCOS.

Table 18. Adipose skinfolds and their ratios in the women studied

Parameter	Mean ± SN				Adjusted mean*		
	PCOS (N=116)	Control (N=81)	Difference, %	p	PCOS (N=116)	Control (N=81)	p
Adipose skinfolds							
Subscapular skinfold	12.85±4.82	8.87±2.99	44.88	<0.0001	11.56	10.73	0.070
Chest skinfold 1	15.30±7.29	8.85±4.08	72.93	<0.0001	13.40	11.57	0.008
Chest skinfold 2	21.94±9.27	14.81±5.53	48.16	<0.0001	19.53	18.25	0.154
Midaxillary skinfold	21.65±8.44	14.68±6.20	47.54	<0.0001	19.51	17.75	0.056
Abdominal skinfold	28.56±8.54	20.52±7.48	39.16	<0.0001	26.33	23.72	0.010
Suprailiac skinfold	22.62±9.52	11.18±5.16	102.4	<0.0001	20.16	14.70	<0.0001
Subscapular skinfold	25.63±10.91	15.08±6.92	69.99	<0.0001	22.41	19.69	0.003
Triceps skinfold	27.70±8.25	19.76±5.97	40.12	<0.0001	25.44	22.99	0.003
Biceps skinfold	18.21±7.14	8.20±3.80	122.09	<0.0001	16.51	10.64	0.0001
Forearm skinfold	13.96±6.05	7.82±3.24	78.51	<0.0001	12.40	10.06	<0.0001
Thigh skinfold	35.41±6.38	31.85±7.54	11.17	<0.0001	33.87	34.06	0.834
Knee skinfold	20.90±9.32	13.34±5.98	56.66	<0.0001	18.80	16.35	0.019
Calf skinfold	27.80±10.05	18.72±7.26	48.47	<0.0001	25.19	22.46	0.011
Sums of adipose skinfolds							
Trunk skinfold	148.55±52.81	93.97±34.135	58.07	<0.0001	132.89	116.40	<0.0001
Arm skinfolds	59.86±19.85	35.78±11.86	67.29	<0.0001	54.34	43.69	<0.0001
Leg skinfolds	84.11±22.77	63.92±17.75	31.59	<0.0001	77.86	72.87	0.037
Skinfolds of limbs	143.97±40.94	99.70±28.33	44.4	<0.0001	132.19	116.56	<0.0001
Upper skinfolds	157.23±54.82	98.06±33.60	60.34	<0.0001	140.74	121.68	<0.0001
Lower skinfolds	135.29±37.49	95.61±28.049	41.49	<0.0001	124.34	111.29	<0.0001
All skinfolds sum	292.51±90.32	193.67±59.72	51.04	<0.0001	265.08	232.96	<0.0001
Indices of skinfolds and ratios of skinfolds sums							
Central index	0.91±0.26	0.76±0.21	20.30	<0.0001	0.86	0.82	0.261
Adipose tissue distribution index	0.50±0.06	0.48±0.06	5.60	0.003	0.49	0.49	0.986
Trunk-to-arms skinfolds ratio	2.50±0.58	2.65±0.51	3.52	0.061	2.47	2.70	0.009
Trunk-to-legs skinfolds ratio	1.75±0.41	1.48±0.35	18.79	<0.0001	1.69	1.57	0.035
Trunk-to-all skinfolds ratio	0.5±0.05	0.48±0.05	4.16	0.009	0.70	0.58	<0.0001
Arm-to-leg skinfolds ratio	0.71±0.14	0.56±0.11	26.57	<0.0001	1.12	10.73	0.071
Upper-to-lower skinfolds ratio	1.15±0.18	1.03±0.17	11.9	<0.0001	0.52	1.07	0.064
Upper-to-all skinfolds ratio	0.53±0.04	0.50±0.04	5.56	<0.0001	0.86	0.51	0.070

SD – standard deviation, PCOS – women with polycystic ovary syndrome; Thickness of skinfolds is presented in mm, skinfolds ratio in units; *Means adjusted for BMI equal to 25.12 kg/m^2.

Even in normal weight women with PCOS all the folds (except legs adipose skinfolds) were significantly larger than in the controls, and the skinfolds' ratios confirmed extensive accumulation of adipose tissue in the upper part of the body in women with PCOS once more. Similar results were obtained after adjustment for BMI. Thickness and sums of skinfolds (except abdominal and thigh folds) in women with type

I and II classic as well as normoovulatory PCOS phenotypes were statistically significantly greater than in the controls, moreover, in women with type I classic PCOS phenotype adipose tissue is significantly more abundant in the upper part of the body (trunk and arms), while in type II classic PCOS phenotype women it is in the arms, compared to the controls ($p<0.05$). There was no difference found concerning thickness of skinfolds between normoandrogenic PCOS phenotype women and controls, however, adipose tissue accumulations in arms in PCOS women with normal androgen level appeared to be perhaps the only sign allowing discerning them from the controls.

Adipose tissue was found to accumulate in certain areas of the upper part of the trunk in PCOS women with small and medium frame size (above iliac crest and in arms), whereas in large frame size PCOS women adipose tissue accumulates in all the trunk area, and less of it is found in thighs.

Best PCOS predictive properties demonstrated of iliac, subscapular and triceps and biceps skinfolds, sum of arms skinfolds as well as arms-to-legs skinfolds ratio according to the area under ROC curve and Youden index (Table 19).

Table 19. Predictive properties of adipose skinfolds for PCOS

Parameter	AUROC	Sensi-tivity, %	Speci-ficity, %	Positive likelihood ratio	Negative likelihood ratio	PCOS proba-bility
Suprailiac skinfold >18.8 mm	0.829	64.7	92.6	8.73	0.115	0.9
Subscapular skinfold >21.7 mm	0.776	57.8	86.4	4.25	0.235	0.9
Triceps skinfold >30.2 mm	0.772	49.1	98.8	39.80	0.025	1
Biceps skinfold > 11.7 mm	0.887	77.6	85.2	5.24	0.191	0.9
Arm skinfolds > 56.2 mm	0.835	59.5	96.3	22.34	0.045	1
Arm-to-leg skinfolds ratio >0.62	0.801	75.0	72.8	2.67	0.375	0.8
AUROC - area under ROC curve; PCOS - PCOS – women with polycystic ovary syndrome						

Adipose skinfolds thickness of almost all the women with PCOS correlated weakly or averagely with FAI, lipid, glucose metabolism, insulin resistance and CRP, however, there were no correlations found between thigh skinfold and glycemia parameters. Some adipose skinfolds of the controls correlated significantly weakly with FAI, lipid and insulin resistance indices, CRP, however, no correlations between skinfolds and glucose metabolism parameters were detected.

It is evident that adipose tissue in women with PCOS tends to accumulate mostly in the arms (especially in upper arms area) and trunk (especially in the waist and above

iliac crest), meanwhile adipose tissue amount in the thigh area (the so-called “safe” zone) of women with PCOS is relatively lower than that of the controls, which may be not only the sign of metabolic diseases but the distinguishing feature of PCOS women as well.

Body composition

All the body composition parameters in women with PCOS were found to be statistically significantly greater than those of the controls ($p<0.05$) (Table 20). Both normal weight and overweight women with PCOS had statistically significantly greater adipose tissue mass and lower muscle mass compared to the controls. After adjustment for BMI it appeared that passive (both absolute and relative) mass in PCOS women was significantly greater than that of the controls, however, muscle mass and relative muscle mass, absolute active mass were lower ($p<0.05$).

Body composition parameters of hyperandrogenic PCOS phenotype women were statistically significantly greater than those of the controls, while body composition between normoandrogenic PCOS phenotype and the controls did not differ.

Table 20. Body composition of the women studied

Parameter	Mean ± SD				Adjusted mean*		
	PCOS (N=116)	Control (N=81)	Difference, %	p	PCOS (N=116)	Control (N=81)	p
Body composition according to J. Matiegka formula							
Skeletal mass, kg	11.55±2.38	9.73±1.42	18.72	<0.0001	10.88	10.70	0.379
Skin and subcutaneous mass, kg	32.58±11.49	20.89±6.75	55.97	<0.0001	28.91	26.13	<0.0001
Muscle mass, kg	26.59±6.00	24.40±3.22	8.97	0.001	24.88	26.85	<0.0001
Relative muscle mass (kg/m)	15.9±3.45	14.61±1.59	8.83	0.001	14.86	16.03	<0.0001
Rest of the mass, kg	15.80±4.15	12.48±1.73	26.59	<0.0001	14.45	14.40	0.758
Body composition according to J. V. G. A. Durnin and M. M. Rahaman formula							
Relative passive mass, %	34.98±5.02	28.38±4.28	23.26	<0.0001	33.48	30.52	<0.0001
Absolute passive mass, kg	27.56±10.29	17.42±4.67	58.17	<0.0001	24.16	22.30	<0.0001
Absolute active mass, kg	47.74±9.40	42.95±4.44	13.84	<0.0001	46.01	47.61	0.013
Body composition according to J. V. G. A. Durnin and J. Womersley formula							
Relative passive mass, proc.	36.58±6.04	28.63±5.14	27.76	<0.0001	34.77	31.21	<0.0001
Absolute passive mass, kg	28.94±11.30	17.63±5.20	64.20	<0.0001	25.20	22.98	<0.0001
Absolute active mass, kg	49.12±10.24	43.15±4.60	11.16	<0.0001	44.96	46.93	0.003

SD – standard deviation, PCOS – women with polycystic ovary syndrome
*Means adjusted for BMI equal to 25.12 kg/m^2.

Relative passive mass of small frame size women with PCOS was significantly greater than that of the controls, and all the passive mass parameters of large frame size women with PCOS exceeded the controls ($p<0.05$), while medium frame size women’s

parameters did not differ. Muscle mass of small and medium frame women with PCOS appeared to be relatively lower than that of the controls.

Table 21. Predictive properties of body composition indices

Parameter	AUROC	Sensitivity, %	Specificity, %	Positive likelihood ratio	Negative likelihood ratio	PCOS probability
Skin and subcutaneous mass according to J. Matiegka >24.2 kg	0.795	75.0	74.1	2.89	0.346	0.8
Relative passive mass according to Durnin–Rahaman > 34.97%	0.832	58.6	95.1	11.87	0.084	0.9
Absolute passive mass according to Durnin–Rahaman > 23.64 kg	0.806	57.8	90.1	5.85	0.171	0.9
Relative passive mass according to Durnin–Womersley > 35.56%.	0.832	58.6	95.1	11.87	0.084	0.9
Absolute passive mass according to Durnin–Womersley > 24.14 kg	0.809	60.3	90.1	4.89	0.205	0.9
AUROC - area under ROC curve; PCOS - women with polycystic ovary syndrome						

Both passive and active body mass of women with PCOS correlated significantly averagely and strongly with the androgens, metabolism parameters, insulin resistance indices and CRP, whereas no correlation was indentified between the above parameters and active mass of the controls.

Skin and subcutaneous mass as well as absolute and relative passive mass showed good PCOS predictive properties (area under ROC curve and Youden index were assessed), and positive likelihood ratio exceeded 2 (Table 21).

Analysis of body composition showed women with PCOS to possess both absolutely and relatively plumper body composition: adipose tissue level is greater in women with PCOS, therefore, insulin resistance may increase. On the other hand, muscle mass of women with PCOS is lower, which could associate with lower insulin sensitivity, worse general health condition and lower staying-power).

Summing-up

26 body size and build parameters were determined, the identification of which had probability of PCOS ranging from 0.8 to 1. Women with PCOS studied had from 0 to 26 signs (15.9 signs on average), and the controls had from 0 to 17 signs (4.23 signs on average). Elbow width, chest width, frame index and metric index, shoulder-to-waist ratio, subscapular, biceps, triceps and iliac skinfolds, sum of arm skinfolds, arm-to-leg

skinfold ratio, relative and absolute body mass were determined as having the greatest areas under ROC curve (more than 0.750). The cut-off values of relative leg length, ankle width, shoulder-to-waist ratio, biceps, arm-to-leg skinfolds ratio, skin and subcutaneous mass according to J. Matiegka formula ranked as having the highest sensitivity (more than 70%). In order to learn the number of signs identified sufficient in predicting PCOS, we carried out ROC curve analysis and determined that 8 and more of the above parameters lead to 88.5% probability of PCOS presence. The area under ROC curve for build of 8 signs was 0.889, sensitivity 76.7%, specificity 84%, positive likelihood ratio 5.4, negative one 0.185. The majority (73.3%) of women with PCOS had 8 and more morphological body size and build parameters, while the same number of parameters was present only in 13.6% controls ($p<0.0001$).

To identify hierarchical correlations between clinical and morphological parameters factor analysis was performed. Several main factors (metabolic parameters, plumpness, frame size and androgen excess, height and longitudinal measurements) characterised the clinical manifestation of PCOS, evidencing the fact that PCOS is a complex non-homogenous disease conditioned by many different factors.

CONCLUSIONS

1. The cut-off values of hyperandrogenemia in our population are lower than those in other populations: testosterone level of 1.68 nmol/l, FAI - 2.94, DHEAS - 10.42 μmol/l. Hairiness score 6 and more are considered hirsutism. Application of the above cut-off values provides precise identification of PCOS as well as distinguishing the PCOS phenotypes.
2. Endocrine function, reproductive and metabolism parameters of women with PCOS differ from the controls: impaired gonadotropin ratio, increased androgen level and hirsutism, higher probability of infertility, more frequent dyslipidemia, impaired fasting glycemia, insulin resistance and metabolic syndrome are attributed to PCOS.
3. Women with PCOS have larger frame size, longer trunk, higher and narrower pelvis, shorter extremities and more massive face in comparison with healthy women. Body mass, body circumferences, skin and subcutaneous mass, relative and absolute passive mass of women with PCOS are higher, whereas relative

muscle mass of women with PCOS is lower than that of the controls. Subcutaneous adipose tissue in PCOS women accumulates in upper part of the body: on trunk, waist and arms. These parameters closely correlate with insulin resistance and obesity.

4. Disturbances of endocrine, reproductive function and metabolism, changes of body size and build in women with type I and type II classic PCOS phenotypes are the most, whereas clinical manifestation of PCOS in women with normal androgen concentration is mild: there are no endocrine, lipid metabolism disorders, insulin resistance or metabolic syndrome, but impaired glucose tolerance detected. There is minor difference in body size and build parameters in PCOS women with normal androgen level compared to healthy women – ankle is wider and relatively more adipose tissue is accumulated on PCOS women upper arm.
5. Morphological parameters of body size and build and their combinations possess properties in identifying PCOS in young women: relative leg length, chest, elbow and ankle width, frame index and metric index, shoulder-to-waist ratio, subscapular, biceps, triceps and iliac skinfolds, sum of arm skinfolds, arm-to-leg skinfolds ratio, parameters of adipose tissue mass demonstrate the best PCOS predictive ability.

LIST OF PUBLICATIONS

1. L. Zabulienė, J Tutkuvienė. Kūno sandara ir policistinių kiaušidžių sindromas (Body composition and polycystic ovary syndrome). Medicina (Kaunas) 2010; 47(2):142–57.
2. L. Zabulienė, Z.A. Kučinskienė, J. Ališauskas, J. Tutkuvienė. Policistinių kiaušidžių sindromo kriterijų įvairovė ir ištyrimo metodai (Variety in criteria and diagnostic methods of polycystic ovary syndrome). Laboratorinė medicina. 2010; 12–4(48):208–18.
3. L. Zabulienė, J. Tutkuvienė. Policistinių kiaušidžių sindromas: skeleto dydžio kitimai (Polycystic ovary syndrome: changes of skeleton size). Sveikatos mokslai. 2011; 21(1)S:46–54.

4. L. Zabulienė, J. Tutkuvienė. Policistinių kiaušidžių sindromas: medžiagų apykaitos, endokrininės veiklos ir reprodukcinės sveikatos kitimai. (Polycystic ovary syndrome: endocrine, reproductive and metabolic disturbances). Sveikatos mokslai. 2011; 21(1)S:55–66.

About the author

Lina Zabulienė graduated from Medical faculty of Vilnius University, Lithuania, in 1995. In 2000 she completed residency of endocrinology at Vilnius University. Since 1998 she has been working as consultant in endocrinology at Department of Endocrinology of Karoliniškių, Antakalnio and Centro Out-patient Clinics. She also lectures and conducts seminars on health assessment, prevention of the internal and family health at the Medical Faculty of Vilnius University.

Since 2007 she has been involved in doctoral PhD studies at Department of Anatomy, Histology and Anthropology, Vilnius University, her current research interest is dedicated to the investigation of polycystic ovary syndrome and body size and build relationship.

Lina Zabiulienė is a member of the Lithuanian Society of Physicians, Morphology Society, Lithuanian Society of Endocrinology, Lithuanian Society of Ultrasound specialists, as well as European Anthropological Association, European Society of Endocrinology, European Association for the Study of Diabetes, Federation of the International Danube, Endocrine Society, Society of Androgen Excess and PCOS.

Lina Zabulienė has authored or co-authored over 30 articles published in various medical journals. She has been awarded for contributing in implementing science into practice by the Vilnius Medical Society. Her fields of interest are PCOS, physical anthropology, diabetes, its complications and prevention, thyroid diseases and prolactinomas.