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lek. Katarzyna Paczkowska

Markers of metabolic disturbances in polycystic ovary syndrome

Markery zaburzeń metabolicznych w zespole policystycznych jajników

ROZPRAWA NA STOPIEŃ DOKTORA
W DZIEDZINIE NAUK MEDYCZNYCH I NAUK O ZDROWIU
W DYSCYPLINIE NAUKI MEDYCZNE

PROMOTOR
Prof. dr hab. n. med. Jacek Daroszewski

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Rozprawę dedykuję **Mamie**

w podziękowaniu za stworzenie mi wszelkich możliwości rozwoju,
nieskończoną wiarę w to, że osiągnę wyznaczone cele,
naukę wytrwałości i odpowiedzialności,
ale przede wszystkim – przekazanie, co w życiu jest naprawdę ważne.

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   IF: 4.7; MEiN score: 100

2. Paczkowska K, Rachoń D, Berg A, Rybka J, Kapczyńska K, Bolanowski M, Daroszewski J. *Alteration of Branched-Chain and Aromatic Amino Acid Profile as a Novel Approach in Studying Polycystic Ovary Syndrome Pathogenesis*. Nutrients. 2023 Sep 26;15(19):4153. doi: 10.3390/nu15194153

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The total Impact Factor (IF) of the publications constituting the dissertation is 12.7.

The total number of points for the publications constituting the dissertation, according to the list of scoring provided by the Ministry of Education and Science (MEiN) is 310.

II. ABBREVIATIONS

AAA – aromatic amino acids

AA - amino acids

AbO – abdominal obesity

AIP – Atherogenic Index of Plasma

BCAA – branched-chain amino acids

BMI – Body Mass Index

CRP – C-reactive protein

DHEA-S – dehydroepiandrosterone sulfate

FAI – free androgen index

FSH – follicle-stimulating hormone

HOMA-IR – homeostatic model assessment of insulin resistance

Ile – isoleucine

IR - insulin resistance

LAP – Lipid Accumulation Product

Leu – leucine

LH – luteinizing hormone

Metrnl – meteorine-like protein

PCOS – Polycystic Ovary Syndrome

Phe – phenylalanine

SHBG – sex hormone-binding globulin

Trp – tryptophan

TSH – thyroid stimulating hormone

Tyr – tyrosine

WBC – white blood cells

WC – waist circumference

Val – valine

III. ABSTRACT IN ENGLISH

The dissertation consists of a series of three articles published in international scientific journals listed in the Journal Citation Reports by Web of Science and in the list of scientific journals provided by the Ministry of Education and Science in Poland (MEiN).

The series of papers addresses metabolic disorders in polycystic ovary syndrome (PCOS), focusing on alteration of secretory function of muscle tissue and early markers of metabolic disturbances.

Polycystic ovary syndrome (PCOS) is one of the most common endocrinological disorders affecting women of reproductive age worldwide. Although it is conventionally linked mostly to childbearing age, it influences patients' health throughout their lifespan. Clinical manifestation of PCOS varies among patients and includes hyperandrogenism, anovulation, polycystic morphology of the ovaries (PCOM) and a wide spectrum of metabolic, dermatological and psychological health issues.

The first and second publications present a scientific project conducted in a cooperation with the Medical University of Gdansk on the abnormalities in the amino acid profile of patients with PCOS and the link between anthropometric, biochemical and hormonal parameters and the concentrations of branched-chain amino acids (BCAA) and aromatic amino acids (AAA).
The study was conducted in a group of 208 patients diagnosed with PCOS and 118 healthy women. PCOS individuals had significantly higher concentrations of all BCAA and AAA in comparison to the control group. Additionally, in the population of PCOS patients, those presented with insulin resistance had significantly higher concentrations of almost all analyzed AA, except for tryptophan, in comparison to individuals without IR.
The study revealed that PCOS is connected to various metabolic disorders, including alteration in amino acid profile; additionally, disturbances in glucose metabolism observed in PCOS are suggested to be linked not only to insulin resistance but also to the misfunction of other molecules involved in maintaining glucose metabolism, such as leucine.

The third publication reports results from the study concerning assessment of the myokines: Irisin and Meteorin-like protein (Metrnl) in a group of patients with polycystic ovary syndrome and the connection between the concentrations of these molecules and the severity of metabolic disorders.

The study comprised 31 women with PCOS and 18 healthy individuals. Metrnl and irisin concentrations did not differ significantly between PCOS women and the control group. On the other hand, Irisin level was significantly higher in a group of overweight and obese women in comparison to normal weight individuals, while Metrnl concentration was similar in both groups.

The findings suggest that although irisin and Metrnl seem to have similar physiological functions, they have individual mechanisms in physiological adaptation to metabolic disease.

In conclusion, skeletal muscles have an influence on essential physiological processes and their secretory function might be affected in metabolic disorders. Moreover, alteration in amino acid profile might be used as a biomarker in the assessment of early metabolic disturbances.

IV. ABSTRACT IN POLISH (STRESZCZENIE W JĘZYKU POLSKIM)

Rozprawa doktorska składa się z cyklu trzech artykułów opublikowanych w międzynarodowych czasopismach naukowych znajdujących się na liście Journal Citation Reports by Web of Science oraz w wykazie czasopism naukowych Ministerstwa Edukacji i Nauki (MEiN).

Cykl prac podejmuje tematykę zaburzeń metabolicznych w zespole policystycznych jajników (PCOS), skupiając się na nieprawidłowościach funkcjonowania wydzielniczego tkanki mięśniowej oraz wczesnych markerach zaburzeń metabolicznych.

Zespół policystycznych jajników jest jednym z najczęstszych zaburzeń endokrynologicznych diagnozowanych wśród kobiet w wieku rozrodczym na całym świecie; mimo że jest to schorzenie wiązane głównie z wiekiem rozrodczym, wpływa na zdrowie pacjentek przez całe ich życie. Prezentacja kliniczna PCOS różni się między chorymi, obejmując hiperandrogenizm, cykle anowulacyjne, policystyczną morfologię jajników (PCOM) oraz szerokie spektrum problemów metabolicznych, dermatologicznych i psychologicznych.

Publikacja pierwsza oraz druga omawia wyniki projektu naukowego przeprowadzonego we współpracy z Uniwersytetem Medycznym w Gdańsku; badanie dotyczyło zaburzeń w profilu aminokwasowym pacjentek ze zdiagnozowanym zespołem policystycznych jajników oraz wpływu czynników antropometrycznych, biochemicznych i hormonalnych na obserwowane zmiany w stężeniach aminokwasów rozgałęzionych (BCAA) oraz aromatycznych (AAA).

Badanie prowadzono w grupie 208 pacjentek ze zdiagnozowanym PCOS oraz 118 zdrowych kobiet. Osoby ze stwierdzonym PCOS miały istotnie wyższe stężenia wszystkich aminokwasów rozgałęzionych i aromatycznych w porównaniu do grupy kontrolnej. Dodatkowo, w populacji pacjentek z PCOS, osoby z insulinoopornością miały istotnie wyższe stężenia prawie wszystkich analizowanych aminokwasów, z wyjątkiem tryptofanu, w porównaniu do osób bez IR.
Wykazano, że PCOS jest związany z różnymi zaburzeniami metabolicznymi, w tym ze zmianami w profilu aminokwasów; dodatkowo, obserwowane w PCOS nieprawidłowości metabolizmu glukozy mogą być związane nie tylko z insulinoopornością, ale także z nieprawidłowym funkcjonowaniem innych cząsteczek zaangażowanych w utrzymanie prawidłowego poziomu glukozy, takich jak np. leucyna.

Trzecia publikacja przedstawia wyniki z badania dotyczącego oceny miokin: iryzyny oraz peptydu podobnego do meteoryny (Metrnl) w grupie pacjentek z zespołem policystycznych jajników oraz zależności między stężeniami tych cząsteczek a nasileniem zaburzeń metabolicznych. Do badania włączono 31 kobiet z rozpoznanym PCOS oraz 18 zdrowych kobiet. Stężenia Metrnl i iryzyny nie różniły się istotnie między kobietami z PCOS i grupą kontrolną. Jednocześnie, poziom iryzyny był istotnie wyższy w grupie kobiet z nadwagą i otyłością w porównaniu do osób o prawidłowej masie ciała, podczas gdy stężenia Metrnl były podobne w obu grupach.
Wyniki badań sugerują, że chociaż iryzyna i Metrnl wydają się mieć podobne funkcje fizjologiczne, odgrywają one indywidualne role w fizjologicznej adaptacji w zaburzeniach metabolicznych.
Podsumowując, mięśnie szkieletowe są zaangażowane w podstawowe mechanizmy fizjologiczne, a ich funkcja wydzielnicza może być upośledzona w zaburzeniach metabolicznych. Dodatkowo, zmiany w profilu aminokwasów mogą posłużyć jako biomarker w ocenie wczesnych zaburzeń metabolicznych.

V. DISSERTATION SUMMARY

1. INTRODUCTION

1.1 Overview of Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the most common endocrinological disorders among women in reproductive age worldwide [1]. Although this disease is conventionally linked mostly to childbearing age, it affects patients' health throughout their lifespan [2]. The pathogenesis remains unclear; however, the connection between insulin resistance and the PCOS development is strongly suspected [3].

Clinical manifestation of PCOS varies among patients and includes hyperandrogenism, anovulation and polycystic morphology of the ovaries (PCOM), which serve as diagnostic criteria, and vide spectrum of metabolic, dermatological and psychological health issues [4]. Heterogeneity in symptomatology resulted in the distinction of several diagnostic criteria and different phenotypes based on presence of specific features [1].

Taking into account vide spectrum of possible metabolic complications that affects PCOS women from a young age, they might be considered as a biological model of early metabolic disease. Among diagnosed health issues, the most frequent findings in PCOS are: impaired glucose metabolism, including insulin resistance, prediabetes and diabetes mellitus, obesity, abdominal obesity, atherogenic dyslipidemia, hypertension and non-alcoholic fatty liver disease [5; 6]. Insulin resistance (IR) affects roughly 50 to 80% of women with PCOS [7]; hyperinsulinemia, which results from insulin resistance, is connected to androgen excess observed in PCOS. Elevated levels of insulin stimulate secretion of ovarian androgens [8] and suppress the synthesis of sex hormone-binding globulin [9]. Moreover, androgen excess is related to the reduction of insulin sensitivity in skeletal muscles [10] and promotes visceral adiposity development [11;12].

Abdominal (central) obesity is caused by an accumulation of visceral fat tissue and it has been proven that waist circumference has stronger association with other metabolic disturbances than BMI [13]. Within groups of people with correct BMI and overweight, abdominal obesity increased the frequency of occurrence of cardiovascular disease risk factors [14]. PCOS women face a 2-3 times greater likelihood of being overweight or obese and the prevalences of obesity and central obesity in PCOS individuals were estimated at 49% and 54%, respectively [15].

Moreover, it was reported that PCOS women have an increased risk of developing various mental health issues and they manifest lower quality of life than healthy individuals [16;17]. The occurrence of anxiety and depressive symptoms in PCOS patients ranges from 14% to 67%, in contrast to the prevalence in age-matched population, which was assessed at 4% to 6% [18;19]. Additionally, the risk of developing depression for individuals suffering from PCOS remains significantly increased throughout the lifetime [20].

1.2 Amino acid profile

Amino acids (AA) are molecules crucial for protein synthesis and maintaining homeostasis; they are involved in numerous vital processes, in which they serve as regulatory factors directly or through their metabolites. They participate in the regulation of metabolic pathways and gene expression, cell signaling, enhancing immune system and synthesis of hormones [21]. The most important reservoir for AA in both forms, free and bonded in the proteins, are skeletal muscles [22].

AA may be categorized based on chemical structure and there are distinguished branched-chain amino acids (BCAA), such as valine, leucine and isoleucine, and aromatic amino acids (AAA): phenylalanine, tyrosine and tryptophan.

Numerous studies reported an alteration of circulating BCAA and AAA in metabolic disorders, including insulin resistance, diabetes mellitus and cardiovascular disease, suggesting a link between them and activity of enzymes involved in AA metabolism [23-25]; previous research from our department reported disturbances in amino acid profile in a group of man with metabolic syndrome [26].

1.3 Myokines: Irisin and Meteorin-like protein

The primary role of skeletal muscles was thought to be connected mainly to locomotion and maintaining body posture; however, recent studies have uncovered that muscle tissue is involved in the synthesis of various molecules, called “myokines”, which have para- and endocrine functions [27]. More than 650 muscle-derived factors have been identified as myokines and this group includes Irisin and Meteorin-like protein (Metrnl) [28].

Irisin was found to have an influence on numerous metabolic pathways, including glucose metabolism, through the AMPK pathway, and potential enhancement of energy expenditure.

This particle is also suspected to be involved in exercise-induced neuroprotection [29] and its increased expression in breast cancer was recognized as a positive prognostic marker [30].

Metrnl secretion is stimulated by physical activity and exposure to cold, and it was reported this myokine plays a role in an improvement of glucose tolerance via AMP-activated protein kinase (AMPK) or peroxisome proliferator-activated receptor δ (PPARδ) [31], enhancement of energy expenditure and promoting the expression of genes involved in thermogenic processes [32].

Evaluation of Irisin and Metrnl concentrations has been investigated in various disorders, including diabetes mellitus, obesity and cardiovascular disease; however, the results are inconclusive [33-35]. In the previous research from our department, a link between circulating irisin and obesity was found [36]; what is more, decreased irisin level was reported in acromegaly [37].

2. AIM OF THE STUDY

The aim of the study was to evaluate secretory function of muscle tissue and understand its role in the pathogenesis of metabolic disturbances in polycystic ovary syndrome.

Detailed aims:

2.1 Evaluation of BCAA and AAA as a differentiating factor between patients with PCOS and control group in a general population and in a subpopulation of women with obesity, abdominal obesity or insulin resistance.

2.2 Assessment of circulating concentrations of two myokines: Irisin and Meteorin-like protein in a group of women with PCOS.

2.3 Determination of an influence of metabolic parameters and hyperandrogenemia on amino acid profile.

2.4 Specification of connections between anthropometric, metabolic and hormonal parameters and circulating Irisin and Metrnl concentrations.

2.5 Assessment of BCAA, AAA, Irisin and Metrnl as early markers of metabolic disturbances.

3. METHODOLOGY

3.1 Methodology of the study described in the Publication 1 and Publication 2

The study population comprised 326 women: 208 diagnosed with PCOS and 118 healthy individuals. PCOS was diagnosed according to the revised 2003 Rotterdam criteria. Participants were included based on the following criteria: age between 18 and 40 years, no previous hypoglycemic or hypolipemic treatment and cessation of hormonal contraception at least 6 months prior to tests. Additionally, women in the control group had normal morphology of ovaries, assessed in transvaginal ultrasound examination, and regular menstrual cycles.

Anthropometric, biochemical and hormonal measurements were performed using standard techniques and commercially available methods. Blood samples were obtained for analyses after overnight fasting, in a follicular phase of the menstrual cycle. Body composition parameters were evaluated using bioelectrical impedance.

Assessment of branched-chain and aromatic amino acids levels was performed with a gas-liquid chromatography combined with tandem mass spectrometry (GLC-MSMS Focus GC – IonTrap ITQ700 (Thermo) system).

3.2 Methodology of the study described in the Publication 3

31 women diagnosed with PCOS based on the revised 2003 Rotterdam criteria and 18 healthy individuals were comprised to the study. All women met inclusion criteria, such as: age between 18 and 40 years, no history of bariatric surgery, no weight loss exceeding 5% within 3 months before tests, no previous history of hypoglycemic or hypolipemic therapy and refraining from using hormonal contraception for 6 months before tests. Women assessed as the control group had normal morphology of ovaries and regular menses. Patients were instructed to perform their typical physical activity and food intake for one week prior to tests.

Based on BMI criterion, in accordance to WHO definition, the study population was additionally divided into two groups: normal-weight (NW; BMI<25) and overweight and obese (OW; BMI ≥25).

Multiple anthropometric, biochemical and hormonal parameters were evaluated. Blood samples for all tests were collected after overnight fasting, in follicular phase, between 3rd and 6th day of the menstrual cycle. An oral glucose tolerance test was carried out with glucose and insulin measurement before the test, after 60 and 120 minutes. Moreover, body composition evaluation was performed with dual-energy X-ray absorptiometry (DXA). Circulating irisin and Metrnl concentrations were assessed using ELISA assays.

Statistical analyses presented in all three papers were carried out with the Statistica (TIBCO), version 13.3; p-value < 0.05 was determined as statistically significant.

4. SUMMARY OF THE RESULTS

4.1 Summary of the results described in Paper 1 and Paper 2.

Various anthropometric, biochemical and hormonal parameters were compared between PCOS women and control group; results are presented in Table 1. Women from both groups were similar according to BMI, waist circumference, fasting glucose, cholesterol, LAP, AIP and CRP. On the other hand, patients with PCOS were significantly younger, had higher LH to FSH ratio, prolactin, DHEA-S, testosterone, FAI and fasting insulin concentration; SHBG and estradiol concentration were significantly lower in this group.

Table 1. Comparison of anthropometric, biochemical and hormonal parameters between PCOS women and control group [38]

Parameter	PCOS	Control	p-value
age	25.86 ± 5.38	31.08 ± 6.99	<0.001
BMI [kg/m^2]	26.09 ± 6.37	25.39 ± 5.22	0.67
waist circumference [cm]	89.75 ± 15.16	87.17 ± 14.59	0.12
fasting glucose [mg/dl]	87.22 ± 6.56	86.97 ± 8.37	0.41
HDL [mg/dl]	65.69 ± 18.44	66.97 ± 15.64	0.40
triglycerides [mg/dl]	95.39 ± 60.55	86.79 ± 42.61	0.31
total cholesterol [mg/dl]	188.23 ± 35.60	188.89 ± 35.71	0.86
non-HDL [mg/dl]	122.65 ± 37.94	121.91 ± 35.18	0.83

Tablet 1 cont.

Parameter	PCOS	Control	p-value
CRP [mg/l]	1.78 ± 3.28	1.72 ± 2.54	0.88
WBC	6.26 ± 1.57	5.67 ± 1.35	0.001
TSH [mU/l]	2.49 ± 1.55	2.10 ± 1.30	0.02
LH [mIU/ml]	9.57 ± 7.40	7.09 ± 6.03	<0.001
FSH [mIU/ml]	6.85 ± 4.05	6.76 ± 2.30	0.85
LH/FSH	1.46 ± 1.04	1.13 ± 0.98	<0.001
estradiol [pg/ml]	232.79 ± 151.47	391.13 ± 179.84	<0.002
prolactin [uIU/l]	433.11 ± 194.11	372.35 ± 152.38	0.006
DHEA-S [ug/dl]	314.53 ± 125.92	204.22 ± 74.25	<0.001
testosterone [nmol/l]	1.86 ± 0.67	1.05 ± 0.33	<0.001
SHBG [nmol/l]	65.75 ± 38.41	76.77 ± 37.17	0.001
fasting insulin [mU/ml]	11.83 ± 6.89	8.91 ± 4.80	<0.001
FAI	3.99 ± 3.29	1.75 ± 1.28	<0.001
androstenedione [ng/ml]	3.28 ± 1.31	2.13 ± 0.82	<0.001
HOMA-IR	2.55 ± 1.53	1.96 ± 1.15	<0.001
LAP	38.13±35.39	29.35 ± 21.85	0.14
AIP	0.14±0.28	0.1±0.2	0.36

BMI – Body Mass Index, CRP – C-reactive protein, WBC – white blood cells, TSH – thyroid stimulating hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEA-S – dehydroepiandrosterone sulfate, SHBG – sex hormone-binding globulin, FAI – free androgen index, HOMA-IR – homeostatic model assessment of insulin resistance, LAP – Lipid Accumulation Product, AIP – Atherogenic Index of Plasma

PCOS women, in contrast to healthy individuals, had significantly increased concentrations of branched-chain and aromatic amino acids: valine 331.02±64.75 vs 305.26±55.90 nmol/ml ($p<0.001$), leucine 131.83±22.12 vs 124.15±19.89 nmol/ml ($p<0.001$), isoleucine 77.73±15.76 vs 71.68±14.20 ($p<0.001$), phenylalanine 47.37±7.0 vs 45.4± 6.09 nmol/ml ($p=0.01$), tyrosine 61.69±9.56 vs 58.08±8.89 nmol/ml ($p<0.01$) and tryptophan 53.66±11.42 vs 49.81±11.18 nmol/ml ($p<0.01$), respectively. In a group of women with insulin resistance or abdominal obesity, significant differences were observed in the most of analyzed amino acids levels, except for phenylalanine in IR+ group and phenylalanine and tyrosine in AbO+ group, as presented in Table 2.

Table 2 Amino acid profile in the subpopulations of women with insulin resistance, abdominal obesity and obesity. [38;39]

Women with insulin resistance (IR+)			
	PCOS	Control	p
Valine [nmol/ml]	356.9 ± 67.2	325.9 ± 65.7	<0.05
Leucine [nmol/ml]	139.42 ± 23.8	128.4 ± 25.4	<0.05
Isoleucine [nmol/ml]	83.5 ± 17.7	75.9 ± 17.2	<0.05
BCAA [nmol/ml]	579.82 ± 102.66	530.21 ± 105.22	<0.05
Phenylalanine [nmol/ml]	48.36±6.72	45.86±6.27	0.08
Tyrosine [nmol/ml]	65.97±9.65	60.17±8.73	<0.01
Tryptophan [nmol/ml]	53.62±11.12	48.82±15.0	0.03
AAA [nmol/ml]	167.95±22.64	154.85±24.17	<0.01
Women with abdominal obesity (AbO+)			
Valine [nmol/ml]	343.48 ± 65.69	314.29 ± 52.68	<0.001
Leucine [nmol/ml]	136.09 ± 22.83	126.41±17.83	<0.001
Isoleucine [nmol/ml]	80.66 ± 16.26	72.79 ± 13.59	<0.001
BCAA [nmol/ml]	558.13±100.51	514.22±79.76	<0.05
Phenylalanine [nmol/ml]	47.52±6.41	46.33±5.79	0.15
Tyrosine [nmol/ml]	62.56±9.90	60.78±8.57	0.20
Tryptophan [nmol/ml]	53.03±10.40	49.55±12.34	<0.01
AAA [nmol/ml]	163.11±21.41	156.66±20.13	0.01
Women with obesity (Ob+)			
Valine [nmol/ml]	365.59 ± 71.27	335.11 ± 53.77	0.06
Leucine [nmol/ml]	143.86 ± 25.99	134.87 ± 16.79	0.10
Isoleucine [nmol/ml]	86.86 ± 17.91	79.37 ± 14.54	0.12
BCAA [nmol/ml]	596.31 ± 109.08	549.34 ± 78.81	0.08
Phenylalanine [nmol/ml]	48.79±6.97	48.14±6.33	0.74
Tyrosine [nmol/ml]	67.57±9.54	61.68±7.84	0.02
Tryptophan [nmol/ml]	53.03±9.69	48.63±14.59	0.02
AAA [nmol/ml]	169.38±21.57	158.44±24.53	0.03

AAA – aromatic amino acids, BCAA – branched-chain amino acids

In the population of PCOS patients, those presented with insulin resistance had significantly higher concentrations of almost all analyzed amino acids, except for tryptophan, when compared to individuals without IR; the results of statistical analysis are presented in Table 3.

Table 3 Differences in AA concentrations between women with and without insulin resistance among PCOS group [38]

	IR+ (86)	IR- (123)	p
Valine [nmol/ml]	356.48±66.91	313.14±56.72	<0.001
Leucine [nmol/ml]	139.19±23.77	126.58±19.29	<0.001
Isoleucine [nmol/ml]	83.35±17.69	72.84±12.87	<0.001
BCAA [nmol/ml]	579.01±102.32	513.47±83.48	<0.001
Phenylalanine [nmol/ml]	48.41±6.70	46.68±7.14	0.01
Tyrosine [nmol/ml]	65.94±9.60	58.74±8.32	<0.001
Tryptophan [nmol/ml]	53.70±11.08	53.68±11.66	0.80
AAA [nmol/ml]	168.04±22.53	159.10±21.98	<0.001

BCAA – branched-chain amino acids, AAA – aromatic amino acids

Assessment of "tryptophan ratio" revealed no differences between PCOS and healthy individuals (p=0.88). On the other hand, in the study population, significantly decreased tryptophan ratio was found in a group of obese women in contrast to non-obese (0.075±0.018 vs 0.086±0.018; p<0.001) as well as in a group of women with IR when compared to IR- individuals (0.078±0.019 vs 0.087±0.018; p<0.001).

4.2 Summary of the results described in the Paper 3

Women from both groups, PCOS and control, were age- and BMI-matched; additionally, they do not differ significantly according to WHR, body fat and lean mass, fasting glucose, HOMA-IR, total cholesterol, triglycerides, SHBG, estradiol, testosterone and androstenedione concentrations. Dissimilarity between groups was observed in relation to waist circumference and LH to FSH ratio.

Table 4. Comparison of anthropometric, biochemical and hormonal parameters between PCOS and control group [40]

Parameter	PCOS	Control	p-value
Age [years]	26.0±3.6	28.0±5.6	0.14
BMI [kg/m^2]	26.17±7.41	28.92±7.10	0.23
Waist circumference [cm]	82.0±15.6	93.9±16.7	0.04
WHR	0.80±0.08	0.83±0.06	0.24
Fasting glucose [mg/dl]	84.5±8.0	86.1±7.0	0.49
HOMA-IR	2.02±1.63	2.50±2.1	0.42
Body fat mass [kg]	26.74±11.52	32.88±12.23	0.13
Body lean mass [kg]	46.73±10.4	49.98±10.84	0.36
Total cholesterol [mmol/l]	4.54±0.83	4.26±1.29	0.65
HDL [mmol/l]	1.35±2.07	1.36±0.53	0.88
LDL [mmol/l]	2.62±0.88	2.46±0.88	0.85
Triglycerides [mmol/l]	0.95±0.49	0.96±0.74	0.58
TSH	1.50±1.18	1.58±0.67	0.82
LH/FSH	1.42±0.76	0.77±0.35	0.002
Estradiol	49.59±38.35	39.64±16.43	0.34
Testosterone	0.38±0.16	0.32±0.12	0.25
Androstenedione	3.37±1.37	2.94±2.15	0.41
SHBG	50.17±26.19	44.96±24.03	0.56

BMI — body mass index; WHR — waist-to-hip ratio; HOMA-IR — homeostasis model assessment of insulin resistance; TCh — total cholesterol; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TG — triglycerides; TSH — thyroid-stimulating hormone; LH — luteinising hormone; FSH — follicle-stimulating hormone; SHBG — sex hormone-binding globulin

Metrnl and irisin concentrations did not differ significantly between PCOS women and control group. Although irisin level was not connected to hyperandrogenemia, weak, positive correlation between Metrnl and androstenedione levels was found.

Irisin level was significantly higher in a group of overweight and obese women in comparison to normal weight individuals, while Metrnl concentration was similar in both groups. Moreover, negative correlations between irisin level and fasting glucose, HOMA-IR and body fat mass were found. On the other hand, Metrnl level was not related to biochemical or anthropometric parameters.

5. CONCLUSIONS

5.1 PCOS is a complex disorder with various metabolic complications, including disturbances in amino acid profile.

5.2 Alteration in amino acid profile in PCOS women is connected to metabolic disturbances; however, it is not entirely triggered by them.

5.3 PCOS is suspected to be related not only to insulin resistance but to the misfunction of other regulatory molecules involved in maintaining glucose homeostasis, including potential “leucine resistance”.

5.4 Hyperandrogenemia, which is an integral feature of PCOS, has an influence on various metabolic parameters, including amino acid profile.

5.5 Dysfunction of tryptophan metabolism introduce an additional insight into pathogenesis of polycystic ovary syndrome and should be further investigated.

5.6 Research on myokines’ role in metabolic disorders and PCOS is a promising concept; however, inconclusive results from various studies limit their clinical application.

5.7 Although Irisin and Meteorin-like protein seem to have similar physiological functions, results from the present study suggest individual mechanisms of those molecules in physiological adaptation to metabolic disease.

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Specific Alteration of Branched-Chain Amino Acid Profile in Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in reproductive age women; it is a complex health issue with numerous comorbidities. Attention has recently been drawn to amino acids as they are molecules essential to maintain homeostasis. The aim of the study was to investigate the branch chain amino acid (BCAA) profile in women with PCOS. A total of 326 women, 208 diagnosed with PCOS and 118 healthy controls, participated in the study; all the patients were between 18 and 40 years old. Anthropometrical, biochemical and hormonal parameters were assessed. Gas-liquid chromatography combined with tandem mass spectrometry was used to investigate BCAA levels. Statistical analysis showed significantly higher plasma levels of BCAAs (540.59 ± 97.23 nmol/mL vs. 501.09 ± 85.33 nmol/mL; $p < 0.001$) in women with PCOS. Significant correlations ($p < 0.05$) were found between BCAA and BMI, HOMA-IR, waist circumference and total testosterone levels. In the analysis of individuals with abdominal obesity, there were significant differences between PCOS and controls in BCAA (558.13 ± 100.51 vs. 514.22 ± 79.76 nmol/mL) and the concentrations of all the analyzed amino acids were higher in the PCOS patients. Hyperandrogenemia in PCOS patients was associated with significantly higher leucine, isoleucine and total BCAA levels. The increase of BCAA levels among PCOS patients in comparison to healthy controls might be an early sign of metabolic alteration and a predictive factor for other disturbances.

Keywords: PCOS; BCAA; hyperandrogenemia; insulin resistance; abdominal obesity

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1. Introduction

Polycystic ovary syndrome (PCOS) is a one of the most common endocrinopathies in reproductive age women, affecting 6 to 20% of them according to different criteria [1,2], with a wide range of clinical manifestations, including menstrual irregularity, impaired fertility and cutaneous signs. However, PCOS is not only a reproductive disorder but also a complex health issue with numerous comorbidities; it is associated with a higher ratio of admissions to hospitals from various causes [3] and decreased work ability [4]. Several metabolic disturbances were found in PCOS, affecting carbohydrate, fat and protein metabolism [5]. Women with PCOS have an increased risk of insulin resistance (IR), type 2 diabetes, obesity and cardiovascular disease [3] and they are predisposed to develop metabolic syndrome with a rate of progression as high as 50% [6]. The mechanisms underlying the connection between PCOS and metabolic disorders are still not well understood [7]; however, PCOS patients might be considered as a biological model using research to study complex metabolic disturbances.

Insulin resistance is found in approximately 50–80% of PCOS individuals [8], and a connection between IR and hyperandrogenism is still being investigated. It was found that increased levels of insulin enhance the secretion of ovarian androgens [9] and inhibit the production of sex hormone-binding globulin [10]. Additionally, androgen excess seems to be correlated with the reduction of insulin sensitivity in skeletal muscles [11].

Attention has recently been drawn to amino acids (AAs), as they are molecules essential to maintain homeostasis [12]. Skeletal muscles account for approximately 40% of body mass and they are the most significant depository of free and bonded in the proteins amino acids in the body [13]. Amino acids are the precursors of polypeptides and proteins, but an increasing amount of evidence in the literature shows that they are regulating factors in a variety of metabolic pathways as well [14]. The link between glucose and amino acid metabolism is widely described; AAs might be the substrates for gluconeogenesis, but glucose can be a substrate for non-essential AA synthesis as well [15].

The amino acids involved in the regulation of key metabolic processes are called "functional AA" and this group includes leucine, tryptophan, glutamine, proline, cysteine and arginine [13]. Amino acids may be categorized by the chemical structure as well; such classification has been used in the present study, in which branched-chain AAs (BCAA) were analyzed.

Structurally similar BCAA groups consist of valine (VAL), leucine (LEU) and isoleucine (ILE) and they are included in the group of essential AAs that cannot be synthesized in the human body and must be provided with food [16]. Recently, BCAAs have been found to link several physiological processes, regardless of protein synthesis; [17] it was suggested that BCAAs participate in the regulation of glucose, lipid and protein metabolism [18] and also play a role in mitochondrial processes [19]. Leucine has been found to activate the mTOR—a metabolic pathway that connects nutrition with aging. [20,21] Several studies revealed higher levels of BCAA in patients with insulin resistance or type 2 diabetes mellitus and the correlation of BCAAs with HOMA-IR [22].

The alteration in the AA profile has been proposed as an early sign of developing metabolic disturbances. Simultaneously, PCOS patients develop numerous metabolic disorders at a younger age than other women. Taking into account those facts, PCOS individuals might be considered as the biological model of early metabolic disturbances. Therefore, in the present study, the BCAA profile in PCOS patients was analyzed to look for a link between AAs and anthropometrical, biochemical and hormonal parameters, including known markers of affected metabolism.

2. Material and Methods

2.1. Study and Control Groups

A total of 326 women, 208 diagnosed with PCOS and 118 healthy controls, participated in the study. All patients were between 18 and 40 years old, and they had no history of diabetic or hypolipemic therapy. If they had taken hormonal contraceptives, the treatment had been discontinued at least 6 months before blood tests were performed. The control group had regular menses and normal ovarian morphology assessed in ultrasound examination. Polycystic ovary syndrome was diagnosed according to the revised 2003 Rotterdam criteria [23]. The study was approved by the Bioethics Committee of the Medical University of Gdańsk (permission number NKBBN/27/2018) and all the subjects gave written consent to participate.

2.2. Anthropometrical Parameters

Anthropometrical parameters such as weight, height and waist circumference were assessed with standard techniques. Body mass index (BMI) was calculated as: BMI = weight [kg]/height^2 [m^2].

Obesity was diagnosed in patients with BMI $\geq$ 30 in accordance with WHO criteria [24]. Applying the BMI criterium, for further analysis, the study population was categorized as obese (Ob+) or non-obese (Ob−).

Abdominal (central) obesity was defined as waist circumference (WC) greater or equal to 80 cm [25]. Therefore, in analysis, the subgroup of patients with abdominal obesity (AbO+) was separated and included 143 PCOS patients and 74 controls.

Body composition (percentage of fat, fat-free and muscle mass) was measured with bioelectrical impedance.

2.3. Biochemical and Hormonal Assessment

Blood samples for biochemical and hormonal assessment were collected after overnight fasting and measurements were performed using commercially available methods.

Insulin resistance was assessed with homeostatic model assessment of insulin resistance (HOMA IR) and HOMA IR was calculated using the formula: HOMA IR = insulin (μU/mL) × glucose (mmol/L)/22.5. The upper range for HOMA IR was taken with a value of 2.5 [26–28]. Our study population was divided into groups, based on whether they had insulin resistance (IR+) or not (IR−), according to values of HOMA-IR. The IR+ group was classified as 85 PCOS women and 30 controls, while the IR− group was classified as 123 PCOS and 88 control.

Free androgen index (FAI) was calculated by the following formula: FAI = total testosterone [nmol/L] ∗ 100/SHBG [nmol/L] and reference interval 0.6–4.4 was applied as it was determined for immunoassay [29]. In the present study, hyperandrogenemia was diagnosed when level of total testosterone or androstenedione was above the upper laboratory range or FAI was greater than 4.4. Among PCOS patients, 121 of them were diagnosed with hyperandrogenemia (HA+) and 87 without (HA−).

2.4. Branched-Chain Amino Acids Profile Assessment

Gas-liquid chromatography combined with tandem mass spectrometry (GLC-MSMS Focus GC—IonTrap ITQ700 (Thermo) system) was used to assess AA levels. The methodology was consistent with the one described in a previous study [30].

2.5. Statistical Analysis

Statistical analyses were conducted using Statistica (TIBCO), version 13.3. Comparisons of anthropometrical, biochemical, hormonal and BCAA profiles were assessed using *t*-test and the Mann–Whitney U test for normally and non-normally dispersed parameters, respectively. The correlations were calculated with the Spearman correlation. $p < 0.05$ was taken as statistically significant in all analyses.

3. Results

A wide spectrum of anthropometrical parameters, endocrinological and biochemical blood test results, were compared between PCOS women and healthy women; these are presented in Table 1.

Table 1. Analysis of anthropometrical, biochemical and hormonal parameters in PCOS and control groups.

	PCOS	Control	*p*
age	25.86 ± 5.38	31.08 ± 6.99	<0.001
BMI	26.09 ± 6.37	25.39 ± 5.22	0.67
waist circumference (cm)	89.75 ± 15.16	87.17 ± 14.59	0.12
fasting glucose (mg/dL)	87.22 ± 6.56	86.97 ± 8.37	0.41
HDL	65.69 ± 18.44	66.97 ± 15.64	0.40
triglycerides	95.39 ± 60.55	86.79 ± 42.61	0.31
total cholesterol	188.23 ± 35.60	188.89 ± 35.71	0.86

Table 1. *Cont.*

	PCOS	Control	*p*
LDL	104.47 ± 33.37	104.46 ± 32.52	0.81
albumin	48.09 ± 2.85	47.35 ± 2.62	0.03
non-HDL	122.65 ± 37.94	121.91 ± 35.18	0.83
CRP	1.78 ± 3.28	1.72 ± 2.54	0.88
WBC	6.26 ± 1.57	5.67 ± 1.35	0.001
TSH	2.49 ± 1.55	2.10 ± 1.30	0.02
LH	9.57 ± 7.40	7.09 ± 6.03	<0.001
FSH	6.85 ± 4.05	6.76 ± 2.30	0.85
LH/FSH	1.46 ± 1.04	1.13 ± 0.98	<0.001
estradiol	232.79 ± 151.47	391.13 ± 179.84	<0.002
prolactin	433.11 ± 194.11	372.35 ± 152.38	0.006
DHEA-S	314.53 ± 125.92	204.22 ± 74.25	<0.001
testosterone	1.86 ± 0.67	1.05 ± 0.33	<0.001
SHBG	65.75 ± 38.41	76.77 ± 37.17	0.001
fasting insulin	11.83 ± 6.89	8.91 ± 4.80	<0.001
FAI	3.99 ± 3.29	1.75 ± 1.28	<0.001
androstendione	3.28 ± 1.31	2.13 ± 0.82	<0.001
HOMA-IR	2.55 ± 1.53	1.96 ± 1.15	<0.001
percentage of fat mass (%)	31.96 ± 10.41	30.32 ± 9.27	0.53
percentage of fat-free mass (%)	68.05 ± 10.41	69.68 ± 9.27	0.53
percentage of muscle mass (%)	64.95 ± 8.87	66.95 ± 8.83	0.23

BMI—Body Mass Index, CRP—C-reactive protein, WBC—white blood cells, TSH—thyroid-stimulating hormone, LH—luteinizing hormone, FSH—follicle-stimulating hormone, DHEA-S—dehydroepiandrosterone sulfate, SHBG—sex hormone-binding globulin, FAI—free androgen index, HOMA-IR—homeostatic model assessment of insulin resistance.

Several parameters were significantly higher in PCOS patients, including LH, LH/FSH ratio, prolactin, DHEA-S, testosterone, androstenedione, FAI, HOMA-IR and fasting insulin, while SHBG was lower in comparison to controls. The control group was accurately recruited and, apart from age and HOMA-IR, there were no significant differences between groups in the crucial metabolic parameters, especially BMI, waist circumference, lipid profile and percentage of body fat, fat-free and muscle mass, as well as in the level of the inflammatory marker—C-reactive protein (CRP).

Total BCAA concentrations were a differentiating factor between the PCOS and healthy individuals. Significant differences were observed both when BCAAs were analyzed as a group or separately. The results are presented in the Table 2.

Table 2. Comparison of BCAA plasma concentration between PCOS and control group.

	PCOS	Control	*p*
VAL (nmol/mL)	331.02 ± 64.75	305.26 ± 55.90	<0.001
LEU (nmol/mL)	131.83 ± 22.12	124.15 ± 19.89	<0.001
ILE (nmol/mL)	77.73 ± 15.76	71.68 ± 14.20	<0.001
BCAA (nmol/mL)	540.59 ± 97.23	501.09 ± 85.33	<0.001

VAL—valine, LEU—leucine, ILE—isoleucine, BCAA—branched-chain amino acids.

Biochemical, hormonal and anthropometrical parameters were analyzed as the potential factors that affect the BCAA profile. The Spearman coefficients are shown in Table 3; all the presented correlations were statistically significant. Positive correlations were found between BCAA and BMI, HOMA-IR, waist circumference, testosterone level, FAI and percentage of fat mass. Negative correlations were found between BCAA and estradiol, percentage of muscle and fat-free body mass.

Table 3. Correlation of BCAA level with selected parameters.

	BCAA
BMI	0.32
HOMA-IR	0.36
waist circumference	0.36
Estradiol	−0.25
Testosterone	0.20
FAI	0.34
percentage of fat-free mass	−0.37
percentage of fat mass	0.37
percentage of muscle mass	−0.39

BMI—Body Mass Index, HOMA-IR—homeostatic model assessment of insulin resistance, FAI—free androgen index.

The classification of the study population according to insulin resistance (IR+, IR−) showed significant differences in the IR+ subgroup between PCOS and control in total BCAA (579.82 ± 102.66 vs. 530.21 ± 105.22 nmol/mL, $p < 0.05$), as well as in the LEU, ILE and VAL concentrations when analyzed separately (respectively: 139.42 ± 23.8 vs. 128.4 ± 25.4 nmol/mL, $p < 0.05$; 83.5 ± 17.7 vs. 75.9 ± 17.2 nmol/mL, $p < 0.05$; 356.9 ± 67.2 vs. 325.9 ± 65.7 nmol/mL, $p < 0.05$). In this subpopulation, there were no significant differences in HOMA-IR between PCOS and control women (3.96 ± 1.36 vs. 3.52 ± 0.95; $p = 0.12$), while differences were observed in FAI, testosterone and androstenedione levels (respectively: 5.63 ± 4.09 vs. 2.50 ± 1.97, $p < 0.001$; 2.01 ± 0.77 vs. 1.04 ± 0.33 nmol/l, $p < 0.001$; 3.36 ± 1.34 vs. 2.01 ± 0.88 ng/mL, $p < 0.001$)

In the IR- subgroup, all the BCAA levels were higher in the PCOS group (total BCAA 513.47 ± 83.48 nmol/mL vs. 491.16 ± 75.56 nmol/mL; $p = 0.05$; VAL 313.14 ± 56.72 nmol/mL vs. 298.22 ± 50.65 nmol/mL; $p = 0.07$; LEU 126.59 ± 19.29 nmol/mL vs. 122.70 ± 17.53 nmol/mL; $p = 0.12$) but significant difference was only observed in the ILE levels (73.7 ± 12.9 vs. 70.2 ± 12.8 nmol/mL; $p = 0.04$).

The differences in AAs levels between PCOS and controls were further analyzed in the Ob+ and Ob− subgroups. The significant changes in amino acid profile continued to be observed in the non-obese subgroup, while in the obese subgroup there were no significant differences as is presented in Table 4.

Table 4. Comparison of BCAA profile between PCOS and control groups in the Ob− and OB+ subgroups.

	Non- Obese Individuals (Ob−)			**Obese Individuals (Ob+)**		
	PCOS	**Control**	***p***	**PCOS**	**Control**	***p***
VAL (nmol/mL)	321.21 ± 59.44	299.46 ± 54.38	0.003	365.59 ± 71.27	335.11 ± 53.77	0.06
LEU (nmol/mL)	128.42 ± 19.68	121.86 ± 19.91	0.002	143.86 ± 25.99	134.87 ± 16.79	0.10
ILE (nmol/mL)	75.14 ± 14.11	70.19 ± 13.36	0.002	86.86 ± 17.91	79.37 ± 14.54	0.12
BCAA (nmol/mL)	524.76 ± 87.69	491.51 ± 83.43	0.001	596.31 ± 109.08	549.34 ± 78.81	0.08

VAL—valine; LEU—leucine, ILE—isoleucine, BCAA—branched-chain amino acids.

The study population was subsequently divided into two subgroups with and without abdominal obesity (AbO+, AbO−). Total BCAA levels significantly differed between PCOS and the control group in the AbO+ subgroup (558.13 ± 100.51 vs. 514.22 ± 79.76 nmol/mL; $p < 0.05$) and concentrations of all the analyzed AAs were higher in PCOS patients (VAL 343.48 ± 65.69 vs. 314.29 ± 52.68 nmol/mL, $p < 0.001$; LEU 136.09 ± 22.83 vs. 126.41 ± 17.83 nmol/mL, $p < 0.001$; ILE 80.66 ± 16.26 vs. 72.79 ± 13.59 nmol/mL, $p < 0.001$). In the AbO- subgroup, the significant differences between PCOS and the control group were found only in the VAL level (311.67 ± 57.70 vs. 292.13 ± 57.30 nmol/mL $p < 0.05$). Total BCAA levels tended to be higher in PCOS patients but without statistical significance (509.02 ± 83.09 vs. 483.42 ± 92.37 nmol/mL; $p = 0.053$). It is worth mentioning that in the AbO+ subgroup, as in the whole study population, the waist circumference did not vary significantly ($p = 0.20$) between PCOS and the control group. However, the AbO+ PCOS women differed from healthy individuals in HOMA-IR ($p < 0.001$), FAI ($p < 0.001$), testosterone ($p < 0.001$) and androstenedione levels ($p < 0.001$).

The BCAA levels were analyzed in PCOS patients according to whether they had hyperandrogenemia (HA+, HA−), and the results are shown in Table 5. Hyperandrogenemia in PCOS patients was associated with significantly higher LEU, ILE and total BCAA.

Table 5. Differences in BCAA among PCOS patients with and without hyperandrogenemia.

	With Hyperandrogenemia (HA+)	**Without Hyperandrogenemia (HA−)**	***p***
VAL (nmol/mL)	338.54 ± 71.61	320.56 ± 52.41	0.10
LEU (nmol/mL)	136.47 ± 23.33	125.38 ± 18.59	<0.001
ILE (nmol/mL)	79.83 ± 16.89	74.81 ± 13.60	0.014
BCAA (nmol/mL)	554.85 ± 106.38	520.75 ± 79.24	0.02

VAL—valine; LEU—leucine, ILE—isoleucine, BCAA—branched-chain amino acids.

4. Discussion

Analyzing AA profiles in a large group of PCOS patients, major differences in comparison to healthy individuals were found. The most important finding was a significant increase in plasma BCAA, especially leucine, in PCOS patients with hyperandrogenemia. Additionally, more severe disturbances in BCAA were shown in PCOS patients when the analysis was made in a subgroup with abdominal obesity.

A significant difference in patients' ages between the PCOS and control groups was observed in this study. In order to search for PCOS-specific differences related to BCAA metabolism, one of the basic conditions for the recruitment to the control group was a similarity with PCOS patients in most metabolic parameters already known as cardiovascular risk factors. Polycystic ovary syndrome individuals develop an altered metabolic profile at a younger age than healthy ones. Finally, there were no differences between those groups in LDL, TG, BMI, waist circumference, fasting glucose or body fat mass; however, significantly higher levels of fasting insulin and HOMA-IR were found.

A difference in CRP levels between the groups was not observed in this study. In various research, a higher CRP level in PCOS women was explained by chronic inflammation due to metabolic alterations connected with an increased level of free radicals and oxidative stress [6]. Patients with PCOS are predisposed to more severe metabolic disturbances, which is why chronic inflammation is usually more strongly marked in this group [31]. A similarity in the CRP level between the groups suggests a comparable severity of metabolic derangements in the present study. Moreover, hormonal analysis did not show important differences apart from LH, testosterone and prolactin concentrations and the LH/FSH ratio, which is commonly known in PCOS women and has been confirmed in numerous studies [32,33].

The results from the present study showed significantly higher levels of BCAA in PCOS women compared with healthy ones, which is consistent with the previously published

research [34–38], but the mechanism underlying this increase remains unknown. Although a high amount of protein in the diet and the over-nutrition of women with PCOS might be one of the causes, it was previously shown among men that the correlation of IR with a higher BCAA level did not arise from increased protein intake [39]. An increased BCAA concentration is considered the result of an altered catabolism due to the decreased activity of BCAA catabolic enzymes in adipose tissue [40,41].

While higher total BCAA concentrations in PCOS women were reported in several studies, the assessment of LEU levels in this group is inconsistent. In the current study, LEU concentration was significantly higher in PCOS women—both in the whole study group and in the subgroups with abdominal obesity or insulin resistance. Similar results have been presented in a few studies [34,35,41,42]. but opposite results have been published as well [43].

Leucine is one of the functional amino acids [14]; it may activate the mTOR pathway and by this mechanism enhance protein synthesis and, indirectly, also insulin signaling. [44] This process can be energetically costly, as it is usually connected with an increase in glucose uptake in tissues such as skeletal muscle [19]. What is more, LEU is suspected to stimulate insulin secretion from pancreatic b-cells [45] and to improve insulin sensitivity through various mechanisms. This potent insulin-sensitizing capability was shown in mice model research, in which leucine supplementation was given to one group and led to a reduction in HOMA-IR and the fasting insulin level [46].

The LEU function in metabolism regulation seems to not only be connected with insulin secretion and signaling. In vitro and animal studies revealed that LEU activates BCKDC, which limits the enzymes responsible for BCAA catabolism; it is speculated that LEU works as a nutritional signal that promotes BCAA disposal [47]. Our findings of LEU and the total BCAA concentration increase suggest a more complex regulation of BCAA clearance.

The results from the present study and the literature data lead us to the hypothesis that PCOS might be connected not only with IR but with resistance to different molecules involved in glucose homeostasis, including "leucine resistance". A detailed explanation of this phenomenon is worth looking for to understand the mechanism of PCOS development; however, further studies are needed to assess if the alteration in LEU metabolism might be a part of PCOS pathogenesis or, rather, a secondary disfunction arising from impaired insulin signaling.

An association of increased BCAA levels with the risk of IR regardless of obesity in normoglycemic middle-aged women [48] and in the PCOS population [34] was previously reported. It was suggested that BCAA excess might be a predictive factor of the development of IR [49,50] and type 2 diabetes mellitus [51,52], and it might promote IR by interference with insulin signaling in muscles [22].

In this study, BCAA concentrations were positively correlated with HOMA-IR. Among the subgroup of patients with insulin resistance, significant differences in BCAA concentrations were present between PCOS women and the control group, despite no differences in the IR parameters between those groups. These results support the hypothesis that metabolic disturbances, including alterations of the AA profile found in IR patients, are more severe in PCOS independently from the severity of IR. The underlying reasons for this PCOS-specific phenomenon should still be investigated.

The comparison of BCAA in the subpopulation of IR- PCOS patients revealed increased levels of all the BCAAs but only the ILE level was significantly changed, which is in contradiction to results from another study [34] They reported only an elevated level of VAL among PCOS women without IR. The inconsistency between studies might derive from differences in study populations, especially the ones in BMI and the severity of co-existing metabolic disturbances.

The present analysis revealed positive correlations of BCAA with BMI and the percentage of body fat mass. In the previous studies, significantly increased levels of circulating branched-chain amino acids were observed in obese patients and the BCAA levels were

positively correlated with HOMA-IR [22]. The association of obesity and insulin resistance with BCAA was stronger than with lipid metabolites [22,53]. On the other hand, Chang et al. did not find a correlation between BCAA levels, BMI and the percentage of body fat mass [38]. The inconsistency might correspond to differences in the study design and the inclusivity criteria for the study cohort. The women from both the PCOS and control groups were older than our study subjects, and only overweight or obese women were included; that is why they presented a more severe metabolic phenotype. The difference between Asian and European populations in BMI impact on cardio-metabolic risk should also be kept in mind when comparing results from various studies.

Separating an Ob− subgroup revealed a similarity to the whole study population in an alteration of the BCAA profile between PCOS and control; however, differences were not observed in the Ob+ subgroup. This might support the hypothesis that an alteration in BCAA levels is an early sign of metabolic disturbances and distinguish lean PCOS patients and controls but the differences are less marked in obese individuals. This finding is inconsistent with another study, in which it was found that a higher BCAA level seems to distinguish PCOS women from women with metabolic syndrome [38].

As far as we know, we found for the first time an association of the BCAA profile with waist circumference. Abdominal obesity increases the incidence of cardiovascular disease risk factors in women with proper BMI [54] and it was proven in several studies that WC has a stronger association with other metabolic disturbances than BMI [55]. In the present study, central obesity, as a harm negative factor affecting the cardiovascular system, was correlated with BCAA alteration: a positive correlation of waist circumference with BCAA was found and the correlation was stronger than the one observed with BMI. In the AbO+ subgroup, concentrations of VAL, LEU and ILE were significantly increased in PCOS patients. In this subgroup, there was no difference in mean waist circumferences. However, the results might be affected by the differences in HOMA-IR to some extent.

Hyperandrogenemia is connected to IR and might be another factor that influences the AA profile but it has not yet been widely investigated in women. The literature data are very limited and the analysis of BCAA and hyperandrogenism in PCOS women was not discussed in most previous studies. The comparison of HA+ and HA− PCOS women in our study did not show significant differences in anthropometric parameters (BMI, waist circumference, percentage of body fat and muscle mass). Simultaneously, significantly increased LEU and ILE levels in HA+ in comparison to HA− PCOS women were observed. Moreover, BCAA levels were positively correlated with circulating total testosterone concentrations. The positive correlation of BCAA with testosterone was also observed in one study [37], but on the contrary no correlation was previously suggested. [38] In another analysis, androgen excess was connected to disturbances in lipid metabolism and not affecting LEU levels [34]. These inconsistences might result from a difference in hyperandrogenism definition; in our study only laboratory hyperandrogenemia was taken into account when patients were categorized as HA+ or HA−, while in other studies women with only clinical signs of hyperandrogenism were included in the HA+ group.

5. Conclusions

Polycystic ovary syndrome is a heterogenous disorder that is also connected with changes in the serum BCAA profile. An increase in BCAA levels among PCOS women in comparison to healthy controls might be an early sign of metabolic alteration and a predictive factor for other disturbances. Moreover, an assessment of the influence of androgens on BCAA levels in the female population should be more closely investigated in future studies.

6. Limitations

The presented research also has some limitations. First of all, we were not able to assess the influence of diet and protein intake on the BCAA level. Additionally, body composition was assessed with bioelectrical impedance analysis, not with the gold standard of this

measurement: dual-energy X-ray absorptiometry. Finally, it remains unclear whether an alteration in the BCAA profile is a part of pathogenesis or rather a result of other disturbances observed in PCOS women.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the ethical reasons.

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Abbreviations

AA—amino acid; AbO—abdominal obesity; BCAA—branched-chain amino acids; BMI—Body Mass Index; CRP—C-reactive protein; DHEA-S—dehydroepiandrosterone sulfate; FAI—free androgen index; FSH—follicle-stimulating hormone; HA—hyperandrogenemia; HOMA-IR—homeostatic model assessment of insulin resistance; ILE—isoleucine; IR—insulin resistance; LEU—leucine; LH—luteinizing hormone; Ob—obesity; PCOS—polycystic ovary syndrome; SHBG—sex hormone-binding globulin; TSH—thyroid-stimulating hormone; VAL—valine; WBC—white blood cells.

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VII. PUBLICATION 2

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Alteration of Branched-Chain and Aromatic Amino Acid Profile as a Novel Approach in Studying Polycystic Ovary Syndrome Pathogenesis

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects reproductive-age women and predisposes them to the development of metabolic disturbances. Recent research has shown that several metabolic factors may play a role in PCOS pathogenesis, and it has been suggested that an alteration in the amino acid profile might be a predictive sign of metabolic disorders. Metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) are concepts that have attracted scientific attention; however, a universal definition has not been established yet for these terms. Already existing definitions of MHO involve the coexistence of obesity with the absence or minimal presence of other metabolic syndrome parameters. A group of 326 women, 209 diagnosed with PCOS and 117 healthy individuals, participated in this study. Multiple parameters were assessed, including anthropometrical, biochemical, and hormonal ones, and gas–liquid chromatography, combined with tandem mass spectrometry, was used to investigate the amino acid profile. Statistical analysis revealed noticeably higher levels of all aromatic amino acids in PCOS women compared to the control group: phenylalanine 47.37 ± 7.0 vs. 45.4 ± 6.09 nmol/mL ($p = 0.01$), tyrosine 61.69 ± 9.56 vs. 58.08 ± 8.89 nmol/mL ($p < 0.01$), and tryptophan 53.66 ± 11.42 vs. 49.81 ± 11.18 nmol/mL ($p < 0.01$); however, there was no significant difference in the "tryptophan ratio" between the PCOS and control group ($p = 0.88$). A comparison of MHO and MUO PCOS women revealed that LAP, leucine, and isoleucine concentrations were significantly higher among the MUO subgroup: respectively, 101.98 ± 34.74 vs. 55.80 ± 24.33 ($p < 0.001$); 153.26 ± 22.26 vs. 137.25 ± 25.76 nmol/mL ($p = 0.04$); and 92.92 ± 16.09 vs. 82.60 ± 18.70 nmol/mL ($p = 0.02$). No significant differences in BMI, fasting glucose, and HOMA-IR between MHO and MUO were found: respectively, 35.0 ± 4.8 vs. 36.1 ± 4.6 kg/m^2 ($p = 0.59$); 88.0 ± 6.0 vs. 87.73 ± 6.28 mg/dL ($p = 0.67$); and 3.36 ± 1.70 vs. 4.17 ± 1.77 ($p = 0.1$). The identification of altered amino acid profiles in PCOS holds potential clinical implications. Amino acids may serve as biomarkers for diagnosing and monitoring the metabolic status of individuals with PCOS. The alteration of BCAAs and AAAs may be involved in PCOS pathogenesis, but the underlying mechanism should be further investigated.

Keywords: PCOS; amino acid profile; branched-chain amino acids; aromatic amino acids; metabolically healthy obesity

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1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by hyperandrogenism, anovulation, and a polycystic morphology of the ovaries. It is

a heterogenous disorder with different clinical presentations; a diversity in phenotypes, such as the presence of hyperandrogenemia, is connected to the severity of its health implications [1]. Apart from the well-known reproductive manifestations, PCOS has been associated with an increased risk of metabolic disorders, including insulin resistance, central obesity, and cardiovascular disease [2,3]. Additionally, several studies reported that PCOS patients have an increased risk of various psychiatric disorders, including, but not limited to, depression [4,5]. Taking into consideration the diversity in PCOS phenotypes, there are differences in its treatment, and therapy should be individualized [1].

Beyond the classical metabolic disorders, women with PCOS also manifest a disruption in amino acid metabolism [6]. Amino acids (AAs) are molecules that are essential in polypeptide and protein synthesis. Additionally, they are involved in various physiological processes as regulating factors and play an important role in maintaining homeostasis [7]. Among amino acids, there are molecules which participate directly or through their metabolites in neurotransmission, the regulation of gene expression, or antioxidation [7]. On the other hand, it has also been suggested that an alteration in the amino acid profile might be a predictive sign of other metabolic disturbances [8,9]. "Functional AAs" is a term used for a group of amino acids which are involved in the regulation of metabolic pathways; leucine, tryptophan, glutamine, proline, cysteine, and arginine are included in this category [7]. Another classification of AAs is based on the chemical structure of the molecule, which includes branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs), both analyzed in the present study.

Increased serum BCAA concentrations were previously described in patients with obesity, insulin resistance, or type 2 diabetes [10], as well as, recently, in women with PCOS [5]. Interestingly, BCAA concentrations in obese individuals tend to decrease following weight loss due to both lifestyle modifications and gastric bypass surgery [11,12]. An elevated BCAA level was also associated with a susceptibility to IR irrespective of obesity in normoglycemic women [13]. An increase in BCAAs was additionally suspected to potentially promote IR through the disruption of insulin signaling in myocytes [10]. Simultaneously, significant changes in AAA level were found in PCOS women [14], as well as in patients diagnosed with metabolic syndrome [15]. Tryptophan is one of the AAAs, mainly metabolized in humans through the kynurenine pathway [16]. An overly activated tryptophan–kynurenine pathway was already shown to exist in cardiovascular disease, obesity, type 2 diabetes mellitus, and PCOS [17–20], and the up-regulation of enzymes from this pathway was found to be connected to metabolic disturbances and might play a physiological, protective role [19].

PCOS women have a significantly higher risk of becoming overweight and developing obesity when compared to age-matched non-PCOS individuals [21], and the body mass index (BMI) is a simple formula calculated based on an individual's weight and height that allows the classification of overweight and obesity, according to WHO recommendations [22]. The BMI does not directly assess body fat mass, but there is evidence indicating a relationship between BMI level and adverse effects on health [23]. From the individual point of view, BMI is not a prognostic marker to evaluate potential comorbidities as it does not indicate heterogeneity in the distribution of body fat [24,25]; this limitation results in a notable number of people classified as overweight and obese who do not develop metabolic and cardiovascular complications. As a consequence, metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) are concepts that have attracted scientific attention; however, a universal definition has not been established yet [26] for these terms. Already existing definitions of MHO involve the coexistence of obesity with the absence or minimal presence of other metabolic syndrome parameters [25,26].

Individuals assessed as having MHO tend to present decreased levels of systemic inflammation markers, although their fat mass does not differ compared to people with MUO [27]. Moreover, there are studies describing a lower risk of developing type 2 diabetes mellitus or cardiovascular disease among MHO patients, but, on the other hand, it is still higher than in metabolically healthy, normal-weight people [28]. Assessment of MHO and

MUO PCOS women revealed differences not only in classic metabolic parameters between those groups but also in novel markers, such as the Visceral Adiposity Index (VAI), and Fatty Liver Index (FLI) as well [29].

An alteration in the amino acid profile was suggested as having a potential role in distinguishing PCOS women from controls in both normal-weight and obese populations [30]; however, the underlying mechanism has not been elucidated yet.

In the present study, we evaluated the plasma concentrations of BCAAs and AAAs as potential biomarkers of early metabolic disorders connected to PCOS and assessed a relationship between the amino acid profile and metabolic disturbances.

2. Material and Methods

The methodology of this study is consistent with the one presented in the previous study [31].

2.1. Study and Control Groups

The study population of Caucasian ethnicity included 208 patients diagnosed with PCOS and 118 individuals in the control group. Inclusion criteria were age between 18 and 40 years old, no history of diabetic or hypolipemic therapy, and not taking hormonal contraception within 6 months before tests were performed. Women classified as control group had regular cycles, and no abnormalities in ovarian morphology assessed in ultrasound examination. The diagnosis of PCOS was made based on the revised 2003 Rotterdam criteria [32]. The Bioethics Committee of the Medical University of Gdańsk (permission number NKBBN/27/2018) approved the study, and all women provided written consent.

2.2. Anthropometric Parameters

Weight, height, and waist circumference were assessed with standard techniques. BMI was calculated with a formula in which patient weight in kilograms was divided by height in meters squared. Obesity was assessed as BMI of 30 or above, according to the WHO classification [22]. In the study population, 67 women were diagnosed with obesity; in this group, there were 46 PCOS and 21 control individuals.

Abdominal obesity was diagnosed based on waist circumference (WC) greater or equal to 80 cm. Subsequently, a group of women with abdominal obesity (AbO+) was separated and included 143 PCOS and 74 control individuals.

MUO was defined based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definition of metabolic syndrome as the presence of at least two out of the four diagnostic criteria, excluding waist circumference [33], and 19 PCOS individuals were included in this group; 27 women with PCOS who met fewer than two criteria were classified as MHO.

2.3. Biochemical and Hormonal Assessment

Blood samples were collected after overnight fasting; biochemical and hormonal measurements were performed using commercial methods.

Homeostatic model assessment of insulin resistance (HOMA IR) was used to assess insulin resistance and IR was defined as HOMA IR above 2.5 [34–36], and, among the study population, 115 women met this criterion, including 85 PCOS patients and 30 controls.

Lipid accumulation product (LAP) was calculated based on following: LAP = (waist circumference [cm] $-$ 58) $\times$ (triglyceride concentration [mmol/L]);

Atherogenic Index of Plasma (AIP) was calculated with the formula: AIP = lg(TG [mmol/L]/HDL [mmol/L])

Free androgen index (FAI) was assessed based on the formula FAI = total testosterone [nmol/L] $\times$ 100/SHBG [nmol/L], and reference range 0.6–4.4 was applied [37]. Hyperandrogenemia was defined as a concentration of total testosterone and/or androstenedione and/or FAI above the reference range.

PCOS patients were evaluated for hyperandrogenemia using the criteria mentioned above and divided into subgroups with hyperandrogenemia (HA+), including 121 women, and without hyperandrogenemia (HA−), including 87 individuals.

2.4. Branched-Chain and Aromatic Amino Acid Profile Assessment

BCAA and AAA concentrations were assessed using gas chromatography combined with mass spectrometry (GC/MS). Commercially available EZ:faast amino acid analysis kit (Phenomenex) was used for amino acid concentration determination according to manufacturer's manual as published previously [38]. Briefly, serum samples after addition of norvaline as internal standard were subjected to solid phase extraction, washing with n-propanol, and eluting with a mixture of NaOH, n-propanol, and 3-picoline, followed by a derivatization of amino acids with propyl chloroformate. After the reaction and liquid/liquid extraction using isooctane, samples were analyzed using GC-MS system from ThermoFisher (ThermoFisher Scientific, Austin, TX, USA): focus gas chromatograph equipped with the column ZB-AAA GC (10 m, 0.25 mm ID, Phenomenex) and connected to mass spectrometer ITQ 700. For quantitation purposes, three level calibration standard curves were used and calculations were based on internal standard method (internal standard: norvaline). Peak identifications were carried out by retention time and MS spectra matching to Calibration Solution Mix supplied with EZ:faast Kit. All measurements (samples and standards) were performed in triplicate.

Tryptophan ratio is defined as the concentration of tryptophan divided by the sum of valine, leucine, isoleucine, phenylalanine, and tyrosine levels.

2.5. Statistical Analysis

Statistica (TIBCO), version 13.3, was used for data analysis. Differences between the groups were calculated using *t*-test and the Mann–Whitney U test for normally and non-normally dispersed parameters, respectively. The Spearman correlation was used to assess correlation between parameters. In all analyses, $p < 0.05$ was considered statistically significant.

3. Results

The baseline information (anthropometric, hormonal, and biochemical parameters) is presented in Table 1 as previously published [3]. Compared with healthy individuals, several parameters were significantly higher in PCOS patients, including LH, prolactin, testosterone, DHEA-S, FAI, androstenedione, HOMA-IR, and albumin. On the other hand, anthropometric and metabolic parameters, excluding age and HOMA-IR, do not differ noticeably between study groups.

Table 1. Anthropometric, biochemical, and hormonal data of the study subjects.

	PCOS	Control	*p*
age	25.86 ± 5.38	31.08 ± 6.99	<0.001
BMI [kg/m^2]	26.09 ± 6.37	25.39 ± 5.22	0.67
waist circumference [cm]	89.75 ± 15.16	87.17 ± 14.59	0.12
fasting glucose [mg/dL]	87.22 ± 6.56	86.97 ± 8.37	0.41
HDL [mg/dL]	65.69 ± 18.44	66.97 ± 15.64	0.40
triglycerides [mg/dL]	95.39 ± 60.55	86.79 ± 42.61	0.31
total cholesterol [mg/dL]	188.23 ± 35.60	188.89 ± 35.71	0.86
LDL [mg/dL]	104.47 ± 33.37	104.46 ± 32.52	0.81
albumin [mg/mL]	48.09 ± 2.85	47.35 ± 2.62	0.03
non-HDL [mg/dL]	122.65 ± 37.94	121.91 ± 35.18	0.83
CRP [mg/L]	1.78 ± 3.28	1.72 ± 2.54	0.88

Table 1. *Cont.*

	PCOS	Control	*p*
WBC	6.26 ± 1.57	5.67 ± 1.35	0.001
TSH [mU/L]	2.49 ± 1.55	2.10 ± 1.30	0.02
LH [mIU/mL]	9.57 ± 7.40	7.09 ± 6.03	<0.001
FSH [mIU/mL]	6.85 ± 4.05	6.76 ± 2.30	0.85
LH/FSH	1.46 ± 1.04	1.13 ± 0.98	<0.001
estradiol [pg/mL]	232.79 ± 151.47	391.13 ± 179.84	<0.002
prolactin [uIU/L]	433.11 ± 194.11	372.35 ± 152.38	0.006
DHEA-S [ug/dL]	314.53 ± 125.92	204.22 ± 74.25	<0.001
testosterone [nmol/L]	1.86 ± 0.67	1.05 ± 0.33	<0.001
SHBG [nmol/L]	65.75 ± 38.41	76.77 ± 37.17	0.001
fasting insulin [mU/mL]	11.83 ± 6.89	8.91 ± 4.80	<0.001
FAI	3.99 ± 3.29	1.75 ± 1.28	<0.001
androstenedione [ng/mL]	3.28 ± 1.31	2.13 ± 0.82	<0.001
HOMA-IR	2.55 ± 1.53	1.96 ± 1.15	<0.001
LAP	38.13 ± 35.39	29.35 ± 21.85	0.14
AIP	0.14 ± 0.28	0.1 ± 0.2	0.36

BMI—Body Mass Index, CRP—C-reactive protein, WBC—white blood cells, TSH—thyroid stimulating hormone, LH—luteinizing hormone, FSH—follicle-stimulating hormone, DHEA-S—dehydroepiandrosterone sulfate, SHBG—sex-hormone-binding globulin, FAI—free androgen index, HOMA-IR—homeostatic model assessment of insulin resistance, LAP—lipid accumulation product, AIP—Atherogenic Index of Plasma. In our study, PCOS women had significantly higher BCAA concentration in comparison to the control group. The differences between study groups were still observed in the subpopulations of patients with insulin resistance or abdominal obesity but not among obese women.

Current statistical analysis revealed noticeably higher levels of all aromatic amino acids in PCOS women compared to the control group: phenylalanine 47.37 ± 7.0 vs. 45.4 ± 6.09 nmol/mL ($p = 0.01$), tyrosine 61.69 ± 9.56 vs. 58.08 ± 8.89 nmol/mL ($p < 0.01$), and tryptophan 53.66 ± 11.42 vs. 49.81 ± 11.18 nmol/mL ($p < 0.01$).

Table 2 presents the differences in AA concentrations between PCOS and healthy individuals in the study subpopulations: women with insulin resistance, abdominal obesity, or obesity. It was found that, in all listed subpopulations, the tryptophan concentration and level of aromatic amino acids analyzed as a group were significantly increased.

In the study subpopulation of women without insulin resistance, it was found that only tryptophan and isoleucine concentrations differ significantly between PCOS and healthy individuals, with $p = 0.04$ and $p = 0.02$, respectively.

The amino acid profile was assessed in the PCOS group between women with (IR+) and without insulin resistance (IR-). All analyzed amino acids, except for tryptophan, had significantly higher concentrations in the IR+ group. Results, including HOMA-IR and FAI, are presented in Table 3.

As shown in Table 4, similar results were observed in the PCOS group between patients with and without obesity. It is worth noting that obese women had significantly higher HOMA-IR (3.69 ± 1.75 vs. 2.22 ± 1.28; $p < 0.001$) and FAI (6.72 ± 4.71 vs. 3.21 ± 2.19; $p < 0.001$).

Table 2. Comparison of AAA level between PCOS and control groups in the subpopulations of women with insulin resistance, abdominal obesity, and obesity.

Women with Insulin Resistance (IR+)			
	PCOS	**Control**	***p***
Phenylalanine [nmol/mL]	48.36 ± 6.72	45.86 ± 6.27	0.08
Tyrosine [nmol/mL]	65.97 ± 9.65	60.17 ± 8.73	<0.01
Tryptophan [nmol/mL]	53.62 ± 11.12	48.82 ± 15.0	0.03
AAA [nmol/mL]	167.95 ± 22.64	154.85 ± 24.17	<0.01
Women with abdominal obesity (AbO+)			
Phenylalanine [nmol/mL]	47.52 ± 6.41	46.33 ± 5.79	0.15
Tyrosine [nmol/mL]	62.56 ± 9.90	60.78 ± 8.57	0.20
Tryptophan [nmol/mL]	53.03 ± 10.40	49.55 ± 12.34	<0.01
AAA [nmol/mL]	163.11 ± 21.41	156.66 ± 20.13	0.01
Women with obesity (Ob+)			
Phenylalanine [nmol/mL]	48.79 ± 6.97	48.14 ± 6.33	0.74
Tyrosine [nmol/mL]	67.57 ± 9.54	61.68 ± 7.84	0.02
Tryptophan [nmol/mL]	53.03 ± 9.69	48.63 ± 14.59	0.02
AAA [nmol/mL]	169.38 ± 21.57	158.44 ± 24.53	0.03

AAA—aromatic amino acid.

Table 3. Differences in AA concentrations, HOMA-IR, and FAI between women with and without insulin resistance among PCOS group.

	IR+ (86)	**IR− (123)**	***p***
BCAA [nmol/mL]	579.01 ± 102.32	513.47 ± 83.48	<0.001
Val [nmol/mL]	356.48 ± 66.91	313.14 ± 56.72	<0.001
Leu [nmol/mL]	139.19 ± 23.77	126.58 ± 19.29	<0.001
Ile [nmol/mL]	83.35 ± 17.69	72.84 ± 12.87	<0.001
AAA [nmol/mL]	168.04 ± 22.53	159.10 ± 21.98	<0.001
Phe [nmol/mL]	48.41 ± 6.70	46.68 ± 7.14	0.01
Tyr [nmol/mL]	65.94 ± 9.60	58.74 ± 8.32	<0.001
Trp [nmol/mL]	53.70 ± 11.08	53.68 ± 11.66	0.80
HOMA-IR	3.94 ± 1.36	1.56 ± 0.56	<0.001
FAI	5.63 ± 4.06	2.83 ± 1.85	<0.001

BCAA—branched-chain amino acid, Val—valine, Leu—leucine, Ile—isoleucine, AAA—aromatic amino acid, Phe—phenylalanine, Tyr—tyrosine, Trp—tryptophan, HOMA-IR—homeostatic model assessment of insulin resistance, FAI—free androgen index.

There were no significant differences in the "tryptophan ratio" between the PCOS and control group ($p = 0.88$); however, in the study population, the tryptophan ratio was significantly lower in obese individuals when compared to non-obese subjects (0.075 ± 0.018 vs. 0.086 ± 0.018; $p < 0.001$), and in women with insulin resistance compared to those without IR (0.078 ± 0.019 vs. 0.087 ± 0.018; $p < 0.001$). A positive correlation was found between the tryptophan ratio and percentage of body muscle mass (S = 0.38; $p < 0.05$), and a negative correlation with the percentage of body fat mass (S = −0.39; $p < 0.05$), BMI (S = −0.38; $p < 0.05$), HOMA-IR (S = −0.28; $p < 0.05$), and FAI (S = −0.21; $p < 0.05$).

Table 4. Differences in AA concentrations between obese and non-obese women among PCOS group.

	PCOS Ob+ (46)	PCOS Ob− (163)	*p*
BCAA [nmol/mL]	596.31 ± 109.08	524.68 ± 87.43	<0.001
Val [nmol/mL]	365.59 ± 71.27	321.21 ± 59.26	<0.001
Leu [nmol/mL]	143.86 ± 25.99	128.36 ± 19.63	<0.001
Ile [nmol/mL]	86.86 ± 17.91	75.11 ± 14.08	<0.001
AAA [nmol/mL]	169.38 ± 21.57	160.92 ± 22.58	0.007
Phe [nmol/mL]	48.79 ± 6.97	47.0 ± 6.97	0.03
Tyr [nmol/mL]	67.57 ± 9.54	60.04 ± 8.88	<0.001
Trp [nmol/mL]	53.03 ± 9.69	53.88 ± 11.85	0.53

BCAA—branched-chain amino acid, Val—valine, Leu—leucine, Ile—isoleucine, AAA—aromatic amino acid, Phe—phenylalanine, Tyr—tyrosine, Trp—tryptophan.

The next step in the statistical analysis was to assess differences in the amino acid profile between metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) PCOS patients. As presented in Table 5, it was found that MUO PCOS women had higher concentrations of leucine and isoleucine; there were no significant differences in the levels of other AAs.

Table 5. Comparison of amino acid concentrations between PCOS patients with metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO).

	MHO (27)	MUO (19)	*p*
BCAA [nmol/mL]	578.41 ± 114.60	621.75 ± 98.03	0.15
Val [nmol/mL]	358.56 ± 74.26	375.58 ± 67.47	0.45
Leu [nmol/mL]	137.25 ± 26.76	153.26 ± 22.26	0.04
Ile [nmol/mL]	82.60 ± 18.17	92.92 ± 16.09	0.02
AAA [nmol/mL]	166.92 ± 16.82	172.89 ± 27.06	0.57
Phe [nmol/mL]	47.29 ± 5.63	50.90 ± 8.23	0.12
Tyr [nmol/mL]	67.30 ± 8.22	67.94 ± 11.38	0.95
Trp [nmol/mL]	52.32 ± 8.23	54.04 ± 11.63	0.88

BCAA—branched-chain amino acid, Val—valine, Leu—leucine, Ile—isoleucine, AAA—aromatic amino acid, Phe—phenylalanine, Tyr—tyrosine, Trp—tryptophan.

An analysis of the correlation between the biochemical and anthropometrical parameters and amino acid profile showed significant relationships between AIP, BMI, LAP, FAI, and HOMA-IR, and both BCAA and AAA. The Spearman correlation coefficient is presented in Table 6.

Table 6. Correlation of biochemical, hormonal, and anthropometrical parameters with BCAA and AAA plasma concentrations presented as Spearman correlation coefficient.

	BCAA	AAA	*p*
AIP	0.28	0.16	<0.05
BMI	0.32	0.14	<0.05
LAP	0.39	0.24	<0.05
FAI	0.34	0.18	<0.05
HOMA-IR	0.36	0.21	<0.05

AIP—Atherogenic Index of Plasma, BMI—Body Mass Index, LAP—lipid accumulation product, FAI—free androgen index, HOMA-IR—homeostatic model assessment of insulin resistance.

The AAA levels were subsequently analyzed in the PCOS population between women with (HA+) and without (HA−) hyperandrogenemia, and no significant differences were found. The results are presented in Table 7.

Table 7. Comparison of aromatic amino acid concentrations in PCOS population between women with (HA+) and without hyperandrogenemia (HA−).

	PCOS HA+ (122)	PCOS HA− (87)	*p*
AAA [nmol/mL]	162.80 ± 21.63	162.75 ± 24.0	0.62
Phe [nmol/mL]	47.65 ± 6.81	47.04 ± 7.28	0.20
Tyr [nmol/mL]	61.64 ± 9.90	61.79 ± 9.05	0.73
Trp [nmol/mL]	53.52 ± 10.43	53.93 ± 12.69	0.87

BCAA—branched-chain amino acid, Val—valine, Leu—leucine, Ile—isoleucine, AAA—aromatic amino acid, Phe—phenylalanine, Tyr—tyrosine, Trp—tryptophan.

4. Discussion

In the current study, several alterations in the amino acid profile have been found in patients diagnosed with PCOS; concentrations of aromatic amino acids, analyzed as a group or separately, were increased in PCOS women compared to healthy individuals. Moreover, in the subpopulations of women with obesity (Ob+), abdominal obesity (AbO+), or insulin resistance (IR+), there was observed a tendency towards an increased level of all aromatic amino acids in individuals with PCOS; however, only an increase in tryptophan concentration was statistically significant.

The results are in agreement with the previous research [30,39–41], in which higher AAA serum concentrations were reported in the PCOS population. However, the partial differences appear with regard to the AAA level in women with obesity. It was reported that, among obese women diagnosed with metabolic syndrome, tyrosine level was associated with PCOS and the sum of phenylalanine and tyrosine was higher in PCOS patients [42], whereas, in our study, the differences in tryptophan level were the most significant. A lack of conformity in the results might be caused by different inclusion criteria and the severity of metabolic disturbances in the study groups; in our study, not all obese patients were diagnosed with metabolic syndrome.

Among our PCOS group, individuals with insulin resistance had significantly higher concentrations of all BCAAs, phenylalanine, and tyrosine; however, tryptophan level does not vary between the groups. Similar results were observed in the subgroup of obese PCOS patients when compared to non-obese ones. Subsequently, an analysis of the AA profile of obese PCOS women revealed that patients presenting with MUO had significantly higher leucine and isoleucine levels and no differences in the concentrations of other analyzed amino acids. These results suggest an important correlation between metabolic health and average plasma levels of leucine and isoleucine. The underlying mechanisms behind the changes in serum BCAA concentrations among the obese population is not well understood but it is suggested that it might be connected to the altered metabolism of BCAAs in adipocytes of the white adipose tissue [43]. Leucine accounts for approximately 10% of tissue protein and its plasma level may increase rapidly during catabolic states [44]; its concentration was significantly associated with muscle mass and strength and also negatively correlated with sarcopenic risk [45]. The severe accumulation of all BCAAs due to branched-chain α-ketoacid dehydrogenase (BCKD) deficiency is a cause of maple syrup urine disease (MSUD). The clinical picture of this rare genetic disorder includes neuropsychiatric disorders, such as ataxia, as well as mental and psychomotor retardation, liver insufficiency, and altered carbohydrates metabolism [46], which is a biological presentation of the toxic effect of massive BCAA excess. A mild increase in the BCAA level found in PCOS is connected with much less intense adverse effects; however, a negative impact on metabolic health is observed. While analyzing the disturbances in BCAA metabolism,

individuals with PCOS and those diagnosed with MSUD represent opposite extremes of the continuum of BCAA accumulation in the human body.

A significant positive correlation of BCAA and AAA levels with surrogate markers of visceral adiposity (LAP) and plasma atherogenic activity (AIP) was found, and, as far as we know, these correlations have not been reported previously. AIP was introduced as one of the biomarkers to predict cardiovascular disease as it is negatively correlated with the size of the lipoprotein particle [47]. Moreover, a relationship between AIP and metabolic disorders, such as obesity, diabetes, and metabolic syndrome, was reported [48–50]. The LAP index represents impaired fat distribution, and a value equal or higher than 34.5 was suggested as indicative of a possible risk factor for cardiovascular disease in PCOS [51]. The associations between both BCAAs and AAAs with the mentioned surrogate markers of cardiometabolic issues requires further investigations.

Hyperandrogenemia is a part of the clinical picture of PCOS and has a great impact on the metabolism. Sexual dimorphism is observed in the testosterone influence on metabolic processes. On the one hand, a low concentration of testosterone is connected to hyperinsulinemia and insulin resistance in men, and testosterone replacement therapy improves the insulin sensitivity in hypogonadal men [52,53]; on the other hand, in women, androgen excess is correlated with insulin resistance, and free testosterone level in adolescent girls was suggested as a risk factor of metabolic syndrome development [54].

In the present study, concentrations of aromatic amino acids in the PCOS group do not differ significantly between women with and without hyperandrogenemia, but, on the other hand, a weak positive correlation between AAA level and total testosterone, as well as FAI, was found. In the literature, levels of all tryptophan–kynurenine metabolites in urine or plasma measurements of PCOS women were positively correlated with FAI and plasma testosterone level [20,55].

A significantly increased concentration of BCAAs was previously reported in PCOS [30,39–41], and our data are consistent [31]. The chronic elevation of BCAA levels was suspected to impair the transport of aromatic amino acids into cells and, as a result, to reduce the production of particular neurotransmitters [10]. That may also lead to an imbalance between Trp level and its neutral amino acids competitors (Phe, Leu, Ile, Val, and Tyr); a decreased "tryptophan ratio" was observed in obesity [10,56,57] and depression [58,59].

Although, in the present study, the median tryptophan level was significantly higher in PCOS patients, there was no difference in the tryptophan ratio between PCOS and healthy women. On the other hand, in the study population, there was a significant reduction in the tryptophan ratio in obese when compared to non-obese individuals and in insulin-resistant women when compared to those with a normal insulin sensitivity, which is consistent with the literature data [10,57]. The increase in tryptophan level was previously reported in PCOS women in plasma [20] and ovarian follicular fluid [60], but, as far as we know, the tryptophan ratio has not been assessed previously in the PCOS population and it was first counted in PCOS in the present study.

The increased tryptophan concentration and, surprisingly, no change in the tryptophan ratio in PCOS women might suggest a mechanism for developing this disturbance independent of insulin resistance and in a manner not related to the BCAA and AAA transport system, which is important in regulating neurotransmitter biosynthesis. This finding supports the hypothesis that metabolic disturbances observed in PCOS have a complex etiology and not all of them simply arise from insulin resistance and obesity; additionally, it indicates a possibility that an impaired tryptophan metabolism might be involved in PCOS pathogenesis. This observation is consistent with the literature data that tryptophan metabolism is affected in PCOS regardless of body mass [20].

Tryptophan metabolism involves two pathways: one for serotonin and, subsequently, melatonin synthesis; and the kynurenine pathway, which is responsible for the catabolism of approximately 95% of tryptophan [61]. The kynurenine pathway includes intermediate metabolites, such as kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, or kynurenic acid [62]. It was reported that hypertension, obesity, and

diabetes are associated with higher levels of metabolites from the tryptophan–kynurenine pathway [63]. Additionally, there is evidence suggesting that tryptophan metabolism might be dysregulated in PCOS. In the present study, tryptophan catabolites were not assessed but metabolomic research showed disturbances in the kynurenine pathway in PCOS manifested by the up-regulation of tryptophan and its catabolites [20,47]. Moreover, modifications in the enzymatic activity of indoleamine 2,3-dioxygenase (IDO), the rate-limiting enzyme of the kynurenine pathway, were reported in PCOS, which may lead to important imbalances between metabolites of the two pathways engaged in tryptophan metabolism [20].

The prevalence of depressive and anxiety symptoms is approximately 14 to 67% among PCOS women, whereas, in the age-matched general population, it is typically 4–6% [64,65]. It was also found that the prevalence of depression remains significantly higher during the life-time in PCOS patients [66]. Although the psychosocial aspects of a PCOS diagnosis are involved in the development of depressive symptoms, the dysregulation of tryptophan metabolism may be potentially connected with the higher incidence of depression among PCOS individuals. First of all, the increased concentration of metabolites in the kynurenine pathway seems to be associated with a neurotoxic effect in CNS; it was found that quinolinic acid induces the production of reactive oxygen species and pro-inflammatory cytokines [67]. Additionally, the alteration in tryptophan metabolism may be connected with an imbalance in serotonin synthesis in the central nervous system and, as a consequence, affects mood regulation.

5. Limitations

The present study has several limitations. First of all, the subgroups of PCOS women with MHO and MUO included a small number of subjects, and that may affect the statistical calculations. Moreover, only amino acid concentrations were measured, without an assessment of their metabolites, which could have an additional impact. Finally, it still remains unclear whether the reported alteration of the AA profile is a consequence of other metabolic and hormonal disturbances, or if it is a part of PCOS pathogenesis.

6. Conclusions

The identification of altered amino acid profiles in PCOS holds potential clinical implications. Amino acids may serve as biomarkers for diagnosing and monitoring the metabolic status of individuals with PCOS. Furthermore, targeting amino acid metabolism could provide new therapeutic approaches for the management of PCOS and associated metabolic complications. The alteration of BCAAs and AAAs may be involved in PCOS pathogenesis but the underlying mechanism should be further explored. An investigation of the enzymes involved in the regulation of amino acid metabolism is one of the promising approaches in future studies.

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VIII. PUBLICATION 3

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Circulating levels of irisin and Meteorin-like protein in PCOS and its correlation with metabolic parameters

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Abstract

Introduction: Research on obesity, which results from excessive food consumption and sedentary lifestyle, has focused on increasing energy expenditure. Recently, muscle tissue is being investigated as an endocrine active organ, secreting molecules called myokines. Multiple studies have been performed to assess myokine levels in various disorders, including polycystic ovary syndrome (PCOS) and metabolic syndrome. Irisin and Meteorin-like protein (Metrnl) are particles which, among others, are suggested to play an important role in adipose tissue browning and improving insulin sensitivity.

Material and methods: The study population consisted of 31 women with PCOS and 18 healthy individuals. PCOS was diagnosed based on revised 2003 Rotterdam criteria. Multiple anthropometrical, hormonal, and biochemical parameters were assessed, including oral glucose tolerance test and body composition with dual energy X-ray absorptiometry. Serum levels of irisin and Metrnl were measured by enzyme-linked immunosorbent assay (ELISA).

Results: There were no differences between the PCOS and control groups according to age, body mass index (BMI), waist-to-hip ratio (WHR), fasting glucose, homeostasis model assessment of insulin resistance (HOMA- IR), or body mass composition. Assessment of Metrnl and irisin concentrations revealed no significant differences between PCOS and healthy women. The irisin level was negatively correlated with BMI, body fat mass, fasting glucose, and insulin concentrations. No relationship between Metrnl level and metabolic parameters was found.

Conclusions: Although irisin seems to be a promising biomarker, inconsistent research limits its value in clinical use in the assessment or treatment of obesity. Metrnl level was not affected in the study population, but it might be connected to the severity of metabolic disturbances.

Key words: *irisin; Meteorin-like protein; Metrnl; myokines; metabolic disturbances; obesity; PCOS*

Introduction

The global prevalence of obesity has nearly tripled dur-ing the past 4 decades, and this trend has been observed in different regions worldwide [1]. As a result, between 1990 and 2015 an increase in deaths and disability rates related to overweight and obesity was estimated of approximately 28% and 35%, respectively [2]. Obesity is associated with numerous health issues, including diabetes mellitus, cardiovascular disease, sleep apnoea, depression, and bone metabolism disturbances [3, 4], which are related to changes in adipose metabolism [5]. It was found that among overweight and obese women, approximately a quarter of them suffer from polycystic ovary syndrome (PCOS) [6].

PCOS is one of the most common endocrinopa-thies among reproductive-age women [7]. Clinical presentation varies between patients; however, reproductive, dermatological, metabolic, and psychological issues may be present [8]. Polycystic ovary syndrome, as a condition affecting women's metabolic health from young age, might be a biological model of insulin resis-tance and early metabolic disturbances.

In the past, skeletal muscle function was thought to be mainly associated with locomotion and body posture. Recently, studies revealed that myofibers, in response to exercise, express and release different factors that have paracrine and endocrine effects [9]. This group of molecules, called "myokines", includes irisin and Meteorin-like protein (Metrnl). Research on obesity, resulting from excessive food consumption and sedentary lifestyle, has focused on increasing energy expenditure, and muscle tissue is being inves-tigated as an endocrine active organ.

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Irisin was firstly described by Boström et al. in 2012. It is a product of the proteolytic cleavage of fibronectin type III domain containing 5 (FNDC5) by as-yet-un-known enzymes. [10]. It was revealed that FNDC5 expression was higher in muscles and organs composed of muscles, such as the tongue, rectum, or heart and was significantly lower in the adipose tissue [11].

Irisin was found to be involved in various meta-bolic pathways; it also has an influence on glucose metabolism in skeletal muscle cells through the AMPK pathway [12], induces the translocation of GLUT4 to the cell membrane, stimulates glucose uptake [13], and downregulates the expression of genes involved in gluconeogenesis and glycogenolysis. [14] Moreover, a positive influence of irisin on the nervous system was suggested, and this myokine is suspected to be an important particle in exercise-induced neuroprotec-tion [15, 16]. One of the main possible roles of irisin is promoting energy expenditure through influencing the differentiation of white adipose tissue (WAT) into brown adipose tissue (BAT) [17]. Circulating irisin concentration has a day-night secretion rhythm [**18a**] and increases shortly after acute exercise [14]; however, no statistical differences were reported in regard to iri-sin levels between people with low, moderate, or high physical activity [**18a**]. Additionally, it was reported that the irisin level is not related to dietary habits and does not change after meal consumption [18a].

Expression in various tissues and circulating levels of irisin have been investigated in numerous disorders, including obesity, diabetes mellitus, neuropsychiatric disorders, and cancers. Circulating levels of irisin tend to be decreased in several types of cancer [19]; however, higher expression of irisin in breast cancer tissue was reported as a good prognostic marker [20]. Although the results from studies related to metabolic disorders are inconclusive, some research suggests higher concentrations of irisin in individuals with obesity or DM, and this phenomenon was explained as possible "irisin resistance" [21].

Meteorin-like protein (Metrnl), also called subfa-tin, is a particle with approximately 40% similarity to Meteorin, according to amino acid sequence [22], but the physiological functions of those proteins differ. Meteorin is a factor that plays a role in neurogen-esis [23], while Metrnl was suggested to be involved in maintaining metabolic homeostasis [24]. Expres-sion of Metrnl was found in various organs, including liver, spleen, heart, skin, fat, and muscle tissue [25]. Similarly to Irisin, the expression of Metrnl in adipose and muscle tissue is enhanced by physical exercise and exposure to cold. Moreover, there is evidence linking Metrnl with an improvement in insulin sensi-tivity, an increase in energy expenditure, and positive regulation of thermogenic gene expression associated with promoting browning of adipose tissue. Moreover, an anti-inflammatory function of Metrnl was reported [26]. In the muscle tissue, Metrnl improves insulin sensitivity through AMP-activated protein kinase (AMPK) or peroxisome proliferator-activated receptor δ (PPARδ) [27]. On the other hand, it was suggested that Metrnl concentration is altered in several disorders, including obesity, diabetes mellitus type 2, and cardiovascular disease [28–30]. Additionally, Metrnl has been investi-gated as a diagnostic biomarker or therapeutical target in cardiometabolic disorders [31].

The aim of the study was to assess circulating levels of irisin and Meteorin-like protein, 2 myokines with potentially similar physiological functions, in women with PCOS in relation to severity of metabolic disturbances.

Material and methods

Description of the study population

The study population included 49 Caucasian women: 31 with PCOS and 18 healthy individuals. PCOS was diagnosed based on the re-vised 2003 Rotterdam criteria after excluding related disorders [32]. Among inclusion criteria for whole study population were as follows: age between 18 and 40 years, no history of hypoglycaemic or hypolipidaemic treatment, and not using hormonal contraception for at least 6 months prior to tests. Exclusion criteria were as follows: preg-nancy, diabetes mellitus (DM), history of bariatric surgery, chronic disease with therapy that has an influence on hormonal secretion, loss of 10% or more body weight in the 3 months prior to the tests. Besides those mentioned above, an additional criterion for the control group was no history of menstrual irregularity in the past 3 years. Patients were asked to perform their usual daily physical activity for the 7 days prior to tests.

The research was conducted under the Declaration of Helsinki, and approval was obtained from the Ethical Committee of Wroclaw University of Medical Sciences (approval no KB-566/2020). All individuals who participated in the study provided written consent.

Anthropometrical parameters

Weight, height, and waist and hip circumferences were measured with standard techniques. Body mass index (BMI) was calculated with the formula:

$$BMI = weight\ [kg]/height_2\ [m_2].$$

Overweight was defined as BMI ≥ 25, and obesity was diagnosed in patients with BMI equal to or higher than 30, in accordance with World Health Organisation (WHO) criteria [33]. Taking into consideration the BMI criterion, the study population was divided into 2 groups: normal-weight (NW) or overweight/obese (OW).

Waist circumference (WC) greater than or equal to 80 cm was a criterium to define central obesity. Consequently, the group of women with abdominal obesity (AbO+) was separated from the study population.

Hormonal and biochemical assessment

All participants were examined, and all tests were performed in follicular phase, between the third and sixth day of the menstrual cycle. Blood samples were taken after overnight fasting; biochemi-cal and hormonal assessments were performed with commercially available methods.

An oral glucose tolerance test with 75 g of glucose was performed, and glucose and insulin levels were measured before the test,

and after 60 and 120 minutes. Homeostatic model assessment of insulin resistance (HOMA-IR) was used to assess insulin resistance, and HOMA IR was calculated with the standard formula:

$$HOMA\text{-}IR = insulin\ (\mu U/mL) \times glucose\ (mmol/L)/22.5.$$

Insulin resistance was defined as HOMA-IR higher than 2.5 [34–36].

Body composition

Body composition was assessed with dual-energy X-ray absorpti-ometry (DXA). Hologic Discovery QDR Series densitometer (Ho-logic Incorp. USA, APEX 4.5.2.1, Windows 7 Professional system) was used for the measurements.

Irisin and Metrnl levels

Concentrations of serum irisin and Metrnl were measured in duplicate by ELISA using commercially available assays. The fol-lowing kits were used: Irisin ELISA Kit (BioVendor, Czech Repub-lilc, Catalogue No. RAG018R; sensitivity 1.0 ng/ml) and Human Meteorin-like protein (METRNL) ELISA Kit (Assay Genie, Ireland, Catalogue No. HUEB2525; sensitivity 7.3 pg/ml). Assays were car-ried out according to the manufacturers' protocols.

Statistical analysis

Statistical analyses were performed with Statistica (TIBCO), version 13.3. The T-test and the Mann-Whitney U test were used to compare anthropometrical, biochemical, and hormonal parameters with irisin and Metrnl levels for normally and non-normally dispersed data, respectively. The correlations between parameters were as-sessed with Spearman correlation. In all analyses a p-value less than 0.05 was considered as statistically significant.

Results

Comparison of basic anthropometrical, biochemi-cal, and hormonal parameters between the PCOS and control groups is shown in Table 1. There were no differences in terms of age, BMI, WHR, fasting glucose, HOMA-IR, or body mass composition. In the study population PCOS women had significantly higher LH/FSH ratio and lower waist circumference.

Statistical analysis revealed no significant differenc-es in Metrnl or irisin levels between PCOS and healthy individuals as shown in Figures 1 and 2. However, irisin concentrations were in the narrow range of higher values.

The study population was divided into 2 groups: normal-weight and overweight or obese patients. The Metrnl level did not differ between those groups, as shown in Figure 3. Conversely, irisin concentrations differed between groups and were significantly higher in normal-weight women (Fig. 4).

Correlations between irisin and Metrnl levels and other parameters are presented in Table 2.

The irisin level was negatively correlated with BMI, body fat mass, android-to-gynoid ratio, and fasting glucose and positively correlated with HDL. There were

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Table 1. ***Comparison of anthropometric, biochemical, and hormonal parameters between polycystic ovary syndrome (PCOS) and control group***

Parameter	PCOS	Control	p-value
Age [years]	26.0 ± 3.6	28.0 ± 5.6	0.14
BMI [kg/m$_2$]	26.17 ± 7.41	28.92 ± 7.10	0.23
Waist circumference [cm]	82.0± 15.6	93.9± 16.7	0.04
WHR	0.80± 0.08	0.83± 0.06	0.24
Fasting glucose [mg/dL]	84.5 ± 8.0	86.1 ± 7.0	0.49
HOMA-IR	2.02± 1.63	2.50 ± 2.1	0.42
Body fat mass [kg]	26.74± 11.52	32.88± 12.23	0.13
Body lean mass [kg]	46.73 ± 10.4	49.98± 10.84	0.36
TCh [mmol/L]	4.54± 0.83	4.26± 1.29	0.65
HDL [mmol/L]	1.35± 2.07	1.36± 0.53	0.88
LDL [mmol/L]	2.62± 0.88	2.46± 0.88	0.85
TG [mmol/L]	0.95± 0.49	0.96± 0.74	0.58
TSH [µIU/mL]	1.50± 1.18	1.58± 0.67	0.82
LH/FSH	1.42± 0.76	0.77± 0.35	0.002
Oestradiol [pg/mL]	49.59± 38.35	39.64± 16.43	0.34
Testosterone [ng/mL]	0.38± 0.16	0.32± 0.12	0.25
Androstenedione [ng/mL]	3.37± 1.37	2.94± 2.15	0.41
SHBG [nmol/L]	50.17± 26.19	44.96± 24.03	0.56

BMI — body mass index; WHR — waist-to-hip ratio; HOMA-IR — homeostasis model assessment of insulin resistance; TCh — total cholesterol; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TG — triglycerides; TSH — thyroid-stimulating hormone; LH — luteinising hormone; FSH — follicle-stimulating hormone; SHBG — sex hormone-binding globulin

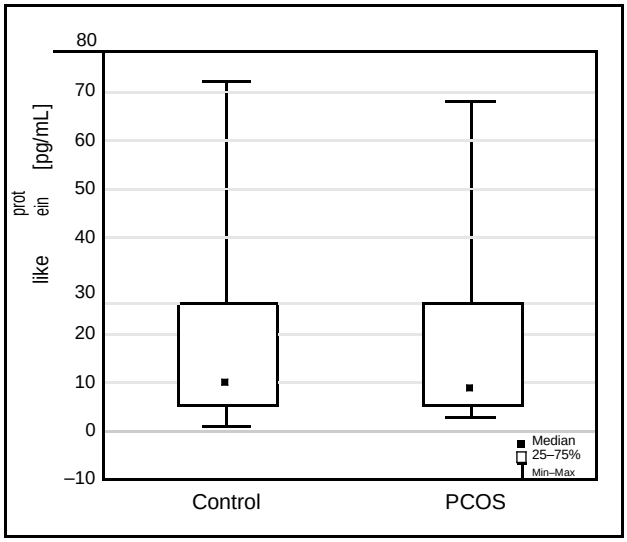

Figure 1. *Comparison of circulating Meteorin-like protein (Metrnl) level between polycystic ovary syndrome (PCOS) and healthy individuals*

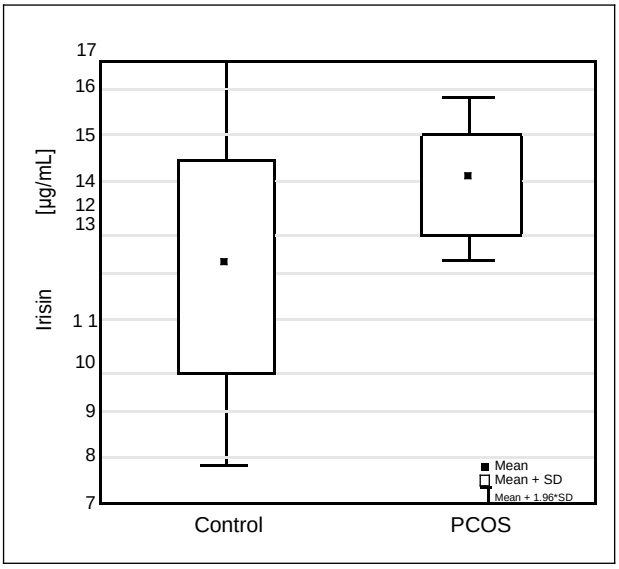

Figure 2. *Comparison of circulating irisin levels between polycystic ovary syndrome (PCOS) women and control group*

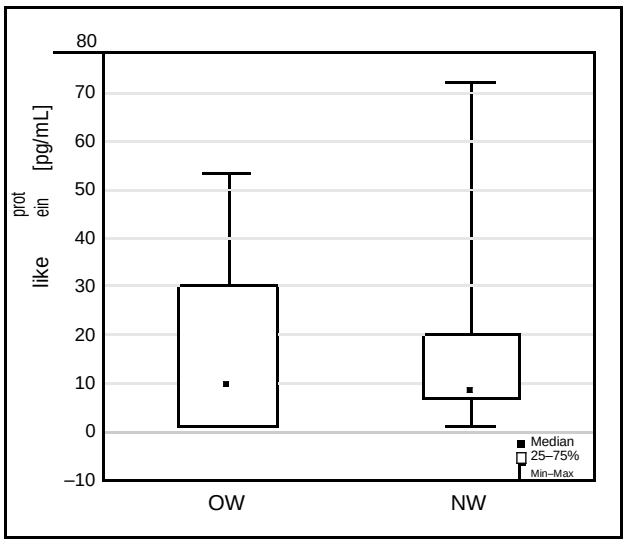

Figure 3. *Comparison of circulating Meteorin -like protein (Metrnl) levels between women with body mass index (BMI) equal or greater than 25 (OW) and normal-weight individuals (NW); p = 0.77*

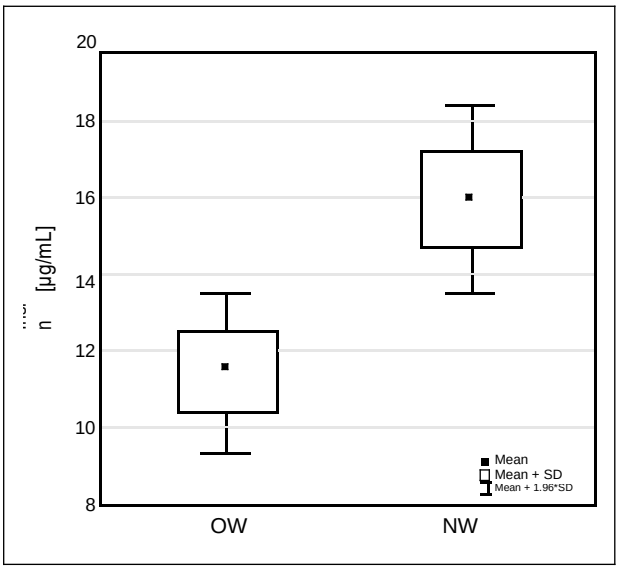

Figure 4. *Comparison of circulating irisin levels between overweight or obese women (OW) and normal-weight individuals (NW); p < 0.05*

no significant relationships between LH/FSH ratio, testosterone, or androstenedione and irisin concentrations.

The Metrnl concentration was not significantly associated with anthropometrical and biochemical parameters; the only correlation was a positive one with androstenedione level.

Discussion

In the current study, irisin and Meteorin-like protein, 2 myokines engaged in regulation of energy metabolism, were measured in a PCOS population in comparison to healthy individuals. The myokines level was assessed with reference to various parameters, including body composition, to better understand influencing factors.

Analysis of irisin showed significantly higher concentrations in normal-weight women when compared to the group of overweight and obese individuals. Moreover, there were negative correlations between irisin level and BMI, fat mass, and the proportion between fat mass and whole body mass. A negative relationship was also found between circulating irisin

Table 2. ***Correlation coefficients between circulating irisin and Metrnl levels and anthropometrical, biochemical, and hormonal parameters***

	Irisin	Metrnl
BMI	–0.538*	0.208
Androstenedione	–0.219	0.341*
Testosterone	–0.209	0.122
LH/FSH	0.059	0.27
Trunk fat mass	–0.567*	0.212
Whole body fat mass	–0.592*	0.200
Android/gynoid ratio	–0.428*	0.102
Trunk/limb fat mass ratio	–0.443*	0.154
Whole body fat/whole body mass	–0.444*	0.231
HOMA–IR	–0.580*	–0.093
Fasting glucose	–0.492*	0.169
Total cholesterol	–0.073	–0.071
HDL	0.445*	–0.057
Triglycerides	–0.204	–0.162
Irisin		–0.115

BMI — body mass index; LH — luteinising hormone; FSH — follicle-stimulating hormone; HOMA-IR — homeostatic model assessment of insulin resistance; HDL — high-density lipoprotein; *p < 0.05

and markers of carbohydrate metabolism: fasting glu-cose and HOMA-IR.

Literature data regarding a link between patients' BMI and irisin concentrations are inconclusive. There are studies suggesting positive, negative, as well as no corre-lation between those parameters; however, more studies reported higher irisin levels in individuals with higher BMI. [37] At the same time, comparison of OW women to NW individuals in the PCOS population showed significantly higher irisin levels in the first group [38].

On the other hand, elevated levels of irisin in obese population were reported to be connected with lower risk of complications related to cardiometabolic disease [39]. It was also suggested that increased irisin concentration might improve insulin sensitivity and is connected to lower risk of insulin resistance [40]. PCOS is strongly associated with altered glucose metabolism and insulin secretion, and the coexistence of both factors in our study population, obesity and insulin resistance, might have an impact on irisin level.

Results from the present study did not show sig-nificant differences in irisin levels between the PCOS and control groups. Moreover, irisin and androgen concentrations were not correlated.

Literature data regarding irisin levels in PCOS are conflicting: higher, lower, and comparable concentra-tions were reported in the PCOS group when com-pared to the control group [41–45]. A meta-analysis summarising 8 studies suggested increased circulating irisin level in PCOS patients; however, differences were not seen when PCOS women were compared to BMI-matched healthy controls [46]. Results from our study are in agreement with this conclusion [46]; there were no differences in circulating irisin, but the PCOS and control groups were similar according to BMI and other anthropometrical parameters, including WHR and total fat mass, but not for WC. Comparable irisin concentrations between the study and control groups suggest an alteration of irisin level as a result of metabolic disturbances accompanying PCOS.

Research assessing the relationship between circulat-ing androgens and irisin levels is limited, and the results are contradictory. On the one hand, it was reported that increased FAI corresponded with higher irisin levels, and FAI was assessed as the main prognostic factor of an elevated irisin level [47]; on the other hand, a nega-tive correlation between circulating irisin level and LH, testosterone, and FAI was also presented in adolescents and adults with PCOS [43, 48], or no relationship was found in adult PCOS women [45].

An inconsistency between research regarding hy-perandrogenaemia and irisin concentration might be partially caused by physiological connection of hyper-androgenaemia to insulin resistance. Increased levels of insulin enhance secretion of ovarian androgens [49] and inhibit the production of sex hormone-binding globulin [50]; what is more, androgen excess is cor-related with the reduction of insulin sensitivity in skeletal muscles [51]. Our results did not show any relationship between androgen and irisin levels; how-ever, significant correlation with the surrogate marker of insulin resistance was present.

In the current study there were no differences in Metrnl concentrations between women with and with-out PCOS or between normal-weight and obese or overweight individuals. Additionally, we found a cor-relation only between Metrnl and androstenedione level; there were no statistically significant relationships between Metrnl level and other hormonal, biochemical, or anthropometrical parameters.

As far as we know, Metrnl levels were analysed in PCOS only in 2 independent studies [52, 53]. In both studies significantly lower Metrnl levels were found in PCOS women when compared to healthy controls. In the first study, PCOS patients were divided into 2 groups: infertile patients and patients with recurrent pregnancy loss (RPL; defined as 2 or more losses of pregnancy before 20 weeks of gestation). Women with RPL have higher Metrnl serum levels when compared to infertile patients [52].

Inconsistency between our results and the data presented in the literature might be connected to dis-

similarities in study design as well as to differences in the study population. In contrast to the first study [52], infertility or RPL were not an inclusion criterion for PCOS women in our study. Additionally, individuals who participated in our research did not take me-dicaments influencing glucose metabolism. In both the studies mentioned above, PCOS women had more strongly expressed insulin resistance, and a negative correlation between Metrnl and HOMA-IR was found [52, 53]. In the second study, the association between Metrnl and HOMA-IR was only observed in PCOS, not in the control group [53]. In our study, the PCOS women had insignificantly lower BMI and HOMA-IR when compared to controls. The differences in meta-bolic parameters between individuals participating in the studies might be related to the observed inconsis-tency in Metrnl levels in PCOS.

Existing findings regarding relationships between Metrnl concentrations and anthropometrical or bio-chemical parameters are inconclusive. Higher Metrnl expression was found in adipose tissue of obese chil-dren in comparison to lean individuals [54]; however, the studies related to the circulating level of Metrnl as a biomarker of obesity are inconsistent. Some research reported higher levels of Metrnl in overweight or obese patients when compared to a normal-weight popula-tion [55, 56], but conversely lower levels of Metrnl [57, 58] or no significant differences were also shown [52, 59]. At the same time, variations in circulating Metrnl were observed according to the presence of additional comorbidities, such as DM; higher levels of Metrnl in a diabetic population were not found in a non-diabetic group [56].

Moreover, studies are contradictory in terms of the relationship between circulating Metrnl level and metabolic parameters such as BMI, waist circum-ference, percentage of fat tissue, or insulin sensitivity. Negative correlations between Metrnl concentration and HOMA-IR [57], BMI [58, 60], visceral fat area, and TG and TCh levels [58, 61] were reported. On the other hand, a positive association between circulat-ing Metrnl and BMI, TCh, TG, or HOMA-IR [55] and no relationship between Metrnl level and BMI was also described [61].

Taking into consideration the results from the pres-ent study and the literature data mentioned above, changes in circulating Metrnl might be connected to the severity of the metabolic disorders and hence not observed in milder disturbances of carbohydrate me-tabolism. What is more, duration of metabolic disease and number of complications or additional health is-sues might have an impact as well.

To the best of our knowledge, this is the first study comparing circulating irisin and Metrnl in the PCOS population; our study did not show a significant correlation between those myokines in this group.

Correlation between irisin and Metrnl levels was observed in one study of a group of patients with DM2 in the male population; this association was not confirmed in non-diabetic individuals and the female population of the study [56]. What is more, in a study comparing diabetic to non-diabetic individuals, Metrnl and irisin concentrations were not correlated when assessed in the whole study population [55].

Bearing in mind the similar physiological functions of irisin and Metrnl, no correlation between circulating levels of those myokines suggests individual roles rather than synergetic actions in physiological adaptation to metabolic disease. It is possible that different regula-tory factors trigger the secretion of both myokines, and, as a result, their concentrations are unevenly affected by metabolic disturbances.

The most important limitation of the study was a small size of the groups. However, the results pre-sented in this paper are the first phase of the study; the research continues and we intend to provide more comprehensive data of larger groups in the close future.

Conclusions

Although irisin seems to be a promising biomarker, inconsistent research limits its value in clinical use in the assessment or treatment of obesity. However, the correlation of irisin concentration with metabolic parameters suggests its important role in maintaining homeostasis. Metrnl level was not affected in the study population, but it might be connected to the severity of metabolic disturbances.

Data availability statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available.

Ethics statement

The research was conducted under the Declaration of Helsinki and obtained approval from the Ethical Committee of Wroclaw University of Medical Sciences (approval no. KB-566/2020).

Author contributions

Conceptualisation: K.P. and J.D.; methodology: K.P., A.Z., J.D.; validation: K.P. and J.D.; formal analysis: K.P., J.K., and J.D.; investigation: K.P., J.S., K.Z., D.J., A.Z.; resources: K.P., J.S., K.Z., D.J., D.K-W., and J.D.; data curation: K.P. and J.K.; writing—original draft preparation: K.P. and J.D.; writing—review and editing: K.P., J.D., D.J., A.Z., and M.B.; visualisation: K.P.; supervision: J.D.; project administration: K.P. and J.D.; funding acquisition: KP, JD, and MB. All authors have carefully read and accepted the manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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IX.CURRICULUM VITAE

KATARZYNA PACZKOWSKA

Experience

04/2022 – present	Clinical Validation Team Infermedica, Wrocław, Poland
11/2021 – present	Endocrinological Ambulatory Care
10/2019 – 03/2024	PhD student, Doctoral School, Wroclaw Medical University
04/2016 – 07/2021	Residency program in Endocrinology, Department of Endocrinology, Diabetes and Isotope Therapy , University Clinical Hospital, Wrocław
10/2014 – 10/2015	Internship, Raszeja Hospital, Poznań
13–26/08/2012	Summer practice in Republici Spitatul in Chisinau, Republic of Moldova

Education

10/2019 – 03/2024	PhD studies; Department of Endocrinology, Diabetes and Isotope Therapy, Doctoral School, Wroclaw Medical University
10/2014 – 06/2016	Postgraduate studies in Clinical Sexology; Adam Mickiewicz University, Poznan
10/2008 – 06/2014	Doctor of Medicine Poznan University of Medical Sciences, Faculty of Medicine
09/2013 – 12/2013	Studies within Socrates-Erasmus Program, Cerrahpasa Tip Facultesi, Istanbul University

Additional Experience

01/2023 – present	Member of the European Academy of Andrology
03/2020 – present	Member of the European Society of Endocrinology
2018 – present	Member of the Polish Society of Endocrinology
10/2012 – 06/2014	Member of the Faculty of Medicine Council, Poznan University of Medical Sciences
06/2011 – 06/2014	Editor-in-chief in “PulsUM” – Poznan University of Medical Sciences students’ newspaper

X. Scientific achievements

Publications

1. Paczkowska K, Rachoń D, Berg A, Rybka J, Kapczyńska K, Bolanowski M, Daroszewski J. *Specific Alteration of Branched-Chain Amino Acid Profile in Polycystic Ovary Syndrome*. Biomedicines. 2023 Jan 1;11(1):108. doi: 10.3390/biomedicines11010108
2. Paczkowska K, Rachoń D, Berg A, Rybka J, Kapczyńska K, Bolanowski M, Daroszewski J. *Alteration of Branched-Chain and Aromatic Amino Acid Profile as a Novel Approach in Studying Polycystic Ovary Syndrome Pathogenesis*. Nutrients. 2023 Sep 26;15(19):4153. doi: 10.3390/nu15194153
3. Paczkowska K, Sobczuk J, Zawadzka K, Jędrzejuk D, Zembska A, Konieczny J, Kaszubkiewicz-Wardęga D, Bolanowski M, Daroszewski J. *Circulating levels of irisin and meteorin-like protein in PCOS and their correlation with metabolic parameters*. [Ahead of print in Endokrynologia Polska; doi: 10.5603/ep.99111]
4. Misan N, Korszun P, Gruca-Stryjak K, Paczkowska K, Nowak A, Wozniak P, Ropacka-Lesiak M. *Does predelivery body mass index really matter in pregnancy?* Ginekol Pol. 2022;93(11):922-929. doi: 10.5603/GP.a2022.0005
5. Paczkowska K, Otlewska A, Loska O, Kolačkov K, Bolanowski M, Daroszewski J. *Laboratory interference in the thyroid function tests.* Endokrynol Pol 2020;71(6):551-560. doi: 10.5603/EP.a2020.0079
6. Misan N, Paczkowska K, Szmyt M, Kapska K, Tomczak L, Bręborowicz GH, Ropacka-Lesiak M. *Nutritional behavior in pregnancy*. Ginekol Pol. 2019;90(9):527-533. doi: 10.5603/GP.2019.0090
7. Daroszewski J, Paczkowska K, Jawiarczyk-Przybyłowska A, Bolanowski M, Jeleń M. *Anaplastic thyroid carcinoma with rapid thyrotoxicosis - a case report and the literature review*. Endokrynol Pol. 2018;69(1):28-31. doi: 10.5603/EP.a2018.0010
8. Katulski K, Czyżyk A, Podkowa N, Podfigurna-Stopa A, Ignaszak N, Paczkowska K, Sławek S, Szpurek D, Męczekalski B *Clinical and hormonal features of women with polycystic ovary syndrome living in rural and urban areas*. Ann Agric Environ Med. 2017 Sep 21;24(3):522-526. doi: 10.5604/12321966.1227642
9. Paczkowska K, Rzymski P, Kubasik M, Opala T. *Sonoelastography in the evaluation of capsule formation after breast augmentation – preliminary results from a follow-up study*. Arch Med Sci. 2016 Aug 1;12(4):793-8. doi: 10.5114/aoms.2015.49935
10. Katulski K, Sławek S, Czyżyk A, Podfigurna-Stopa A, Paczkowska K, Ignaszak N, Podkowa N, Męczekalski B *Bone mineral density in women with polycystic ovary syndrome*. J Endocrinol Invest. 2014 Dec;37(12):1219-24.

Awards and Grants

1. FCR Scholarship and oral presentation at World Congress of Gyneacological Endocrinology, Florence, 2022
2. ESE Young Investigator Award granted at 22nd European Congress of Endocrinology (e-ECE 2020) for the presentation *Congenital isolated follicle-stimulating hormone deficiency due to the FSHB gene mutation in a female patient – a rare case report*
3. FCP Scholarship and oral presentation at World Congress of Gyneacological Endocrinology, Florence, 2020
4. RID (Regional Initiative of Excellence) Grant from the Medical University of Wroclaw for the period 09/2020 – 07/2022
5. Scientific Grant for Young Scientists at Wroclaw Medical University 2020 for the period 01/2020 – 12/2021
6. Lower Silesian leaders in medicine – scholarship to support PhD students clinical and scientific development; granted 10/2019
7. The award from Polish Thyroid Association granted during 7th Congress of the Polish Thyroid Association held in Bydgoszcz, in 2019 for the presentation *Sick patient or abnormal laboratory results? Interferences in laboratory diagnosis of thyroid diseases*
8. Ist prize at 24th European Students' Conference held in Berlin in 2013, in Gyneacology and Obstetrics Session, for the presentation *Cardiovascular risk factors in patients diagnosed with polycystic ovary syndrome*
9. Ist prize at 14th International Congress of Young Medical Scientists held in Poznań in 2014, in Radiology Session, for the presentation *Usefulness of the sonoelastography in the evaluation of the capsular formation after breast augmentation*.
10. Graduating with honors and receiving a medal from Poznan University of Medical Sciences for academic achievement and community service (2014).
11. Scholarship of the Minister of Science and Higher Education in the academic year 2013/2014

Oral and poster presentations at International and National Congresses

13-16/05/2023	European Congress of Endocrinology, Istanbul 2023; poster presentation *Early markers of metabolic disorders, such as leucin and isoleucine level and lipid accumulation product, should be considered as the potential additional criterion in PCOS classification*
21-24/05/2022	European Congress of Endocrinology, Milan 2022; presentation in Rapid Communications Session and poster *Plasma amino acid profile in women with polycystic ovary syndrome and its correlation with metabolic disturbances*
11-14/05/2022	20th World Congress of Gynecological Endocrinology; oral presentation *Disturbances in the amino acid profile in women diagnosed with polycystic ovary syndrome.*
02-05/12/2020	19th World Congress of Gynecological Endocrinology; oral presentation *Novel FSHB mutation leading to isolated FSH deficiency – a rare case report*
05-09/09/2020	e-ECE 2020; oral presentation *Congenital isolated follicle-stimulating hormone deficiency due to the FSHB gene mutation in a female patient – a rare case report*
05-09/09/2020	e-ECE 2020 Poster presentation *Large „forgotten goiter" in the thoracic cavity – a case report*
03-05/10/2019	7th Congress of the Polish Thyroid Association, Bydgoszcz, 2019; oral presentation *Sick patient or abnormal laboratory results? Interferences in laboratory diagnosis of thyroid diseases*
17-20/10/2018	18th Congress of the ENEA, Wroclaw 2018; Poster presentation *Is the diagnosis of multiple endocrine neoplasia type 1 always obvious? Case study and literature review*
28-30/05/2015	15th International Congress of Young Medical Scientists, Poznan; oral presentation Pregnancy complicated by the placental abruption and the Couvelaire uterus – a case report
17-19/05/2014	24th International Medical Sciences Student Congress, Istanbul; oral presentation *Usefulness of the sonoelastography in the evaluation of the capsular formation after breast augmentation*
17-19/05/2014	24th International Medical Sciences Student Congress, Istanbul; oral presentation *Chromosomal abnormalities in fetuses with abnormal heart image in prenatal screening*

09-10/05/2014 14th International Congress of Young Medical Scientists, Poznan; oral presentation *Usefulness of the sonoelastography in the evaluation of the capsular formation after breast augmentation*

09-10/05/2014 14th International Congress of Young Medical Scientists, Poznan; oral presentation *Differences in the metabolic features among women diagnosed with polycystic ovary syndrome living in urban and rural areas of Poland*

09-10/05/2014 14th International Congress of Young Medical Scientists, Poznan; oral presentation *Is there a possibility to predict the capsular contracture after breast augmentation?*

09-10/05/2014 14th International Congress of Young Medical Scientists, Poznan; oral presentation *Chromosomal abnormalities in fetuses with abnormal heart image in prenatal screening*

10-12/04/2014 International Medical Students' Conference 2014, Kraków; oral presentation *Quadriplet monochorionic pregnancy complicated by the coexistence of TRAP and sIUGR*

10-12/04/2014 International Medical Students' Conference 2014, Kraków; oral presentation *Usefulness of the sonoelastography in the evaluation of the capsular formation after breast augmentation*

05-08/03/2014 16th World Congress of Gynecological Endocrinology; oral presentation *The severity of the cardiovascular risk factors among patients diagnosed with polycystic ovary syndrome (PCOS)*

04-07/09/2013 24th European Students' Conference, Berlin; oral presentation *Cardiovascular risk factors in patients diagnosed with polycystic ovary syndrome*

10-11/05/2013 13th International Congress of Young Medical Scientists; oral presentation *Metabolic disorders of patients diagnosed with polycystic ovary syndrome*

10-11/05/2013 13th International Congress of Young Medical Scientists; oral presentation *A rare coexistence of TRAP and sIUGR in quadriplet monochorionic pregnancy – a diagnostic challenge*

11-12/05/2020 12th International Congress of Young Medical Scientists; oral presentation *Multiple cardiac tumors in the fetus – a case report*

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 566/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Ocena stężenia miokiny meterin-like (METRNL) u pacjentek z zespołem policystycznych jajników"

będącego częścią projektu badawczego pt:

„Badanie polimorfizmów genu FNDC5 w aspekcie funkcji mięśni i zaburzeń metabolicznych u pacjentek z zespołem policystycznych jajników"

zgłoszonym przez **prof. dr hab. Jacka Daroszewskiego** zatrudnionego w Katedrze i Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy projektu badawczego realizowanego w ramach Regionalnej Inicjatywy Doskonałości – Grant dla Wybitnych Doktorantów.
Nr rejestrowy CWN UMW: STM.C10.20.031

Wrocław, dnia 28 września 2020 r.
BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący

prof. dr hab. Jan Kornafel

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 24.03.2024 r.

Lek. Katarzyna Paczkowska
Studentka Szkoły Doktorskiej
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIE

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potwierdzam

Podpis

Katarzyna Paczkowska

Gdańsk, 05.03.2024 r.

Prof. dr hab. Dominik Rachoń
Zakład Endokrynologii Klinicznej i Doświadczalnej,
Uniwersytet Medyczny w Gdańsku

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Podpis

Dominik Rachoń

potwierdzam

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Gdańsk, 05.03.2024 r.

mgr Andrzej Berg
Katedra i Zakład Chemii Farmaceutycznej,
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Podpis

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Wrocław, 06.03.2024 r.

Prof. dr hab. Jacek Rybka
Instytut Immunologii i Terapii Doświadczalnej
PAN im. Ludwika Hirszfelda we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: **Paczkowska K, Rachoń D, Berg A, Rybka J, Kapczyńska K, Bolanowski M, Daroszewski J. *Specific Alteration of Branched-Chain Amino Acid Profile in Polycystic Ovary Syndrome*. Biomedicines. 2023 Jan 1;11(1):108. doi: 10.3390/biomedicines11010108** mój udział polegał na współtworzeniu metodologii badania, walidacji metody laboratoryjnej, przeprowadzeniu oznaczenia profilu aminokwasowego oraz edycji manuskryptu.

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Podpis

Wrocław, 06.03.2024 r.

Dr Katarzyna Kapczyńska
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PAN im. Ludwika Hirszfelda we Wrocławiu

OŚWIADCZENIE

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Podpis

Katarzyna Kapczyńska

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UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 06.03.2024 r.

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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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potwierdzam

Podpis

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UNIWERSYTET MEDYCZNY
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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Wrocław, 21.03.2024 r.

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Podpis

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 25.03.2024 r.

Lek. Katarzyna Paczkowska
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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIE

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potwierdzam

Podpis

Katarzyna Paczkowska

Gdańsk, 05.03.2024 r.

Prof. dr hab. Dominik Rachoń
Zakład Endokrynologii Klinicznej i Doświadczalnej,
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Podpis

Dominik Rachoń

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Podpis

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Wrocław, 06.03.2024 r.

Prof. dr hab. Jacek Rybka
Instytut Immunologii i Terapii Doświadczalnej
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OŚWIADCZENIE

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Podpis

Wrocław, 06.03.2024 r.

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Podpis

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UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 06.03.2024 r.

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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
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potwierdzam

Podpis

UNIWERSYTET MEDYCZNY
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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Wrocław, 21.03.2024 r.

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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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Podpis

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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 20.03.2024 r.

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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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potwierdzam

Katarzyna Paczkowska

Podpis

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Podpis

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UNIWERSYTET MEDYCZNY
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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Podpis

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UNIWERSYTET MEDYCZNY
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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Wrocław, 20.03.2024 r.

Dr hab. n. med. Diana Jędrzejuk
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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

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Wrocław, 20.03.2024 r.

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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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potwierdzam

prof. dr hab. n. med. Jacek Daroszewski
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Podpis

Zembska Agnieszka

Wrocław, 20.03.2024 r.

Dr inż. Jacek Konieczny
CodeAI, Poznań

OŚWIADCZENIE

Oświadczam, że w pracy: **Paczkowska K, Sobczuk J, Zawadzka K, Jędrzejuk D, Zembska A, Konieczny J, Kaszubkiewicz-Wardęga D, Bolanowski M, Daroszewski J. Circulating levels of irisin and meteorin-like protein in PCOS and their correlation with metabolic parameters,** przyjętej do druku w czasopiśmie Endokrynologia Polska; doi: 10.5603/ep.99111, mój udział polegał na opracowaniu danych.

Podpis

Jacek Konieczny

podwrotem

prof. dr hab. n. med. Jacek Daroszewski
lekarz specjalista chorób wewnętrznych
ENDOKRYNOLOGII i DIABETOLOGII
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Wrocław, 20.03.2024 r.

lek. Dorota Kaszubkiewicz-Wardęga
II Klinika Ginekologii i Położnictwa
Uniwersytecki Szpital Kliniczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: **Paczkowska K, Sobczuk J, Zawadzka K, Jędrzejuk D, Zembska A, Konieczny J, Kaszubkiewicz-Wardęga D, Bolanowski M, Daroszewski J. Circulating levels of irisin and meteorin-like protein in PCOS and their correlation with metabolic parameters,** przyjętej do druku w czasopiśmie Endokrynologia Polska; doi: 10.5603/ep.99111, mój udział polegał na gromadzeniu danych klinicznych.

Podpis

Kaszubkiewicz-Wardęga

potwierdzam

Daroszewski

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 20.03.2024 r.

Prof. dr hab. Marek Bolanowski
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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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Podpis

potwierdzam

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami

kierownik: prof. dr hab. Marek BOLANOWSKI

Wrocław, 21.03.2024 r.

Prof. dr hab. n. med. Jacek Daroszewski
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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: **Paczkowska K, Sobczuk J, Zawadzka K, Jędrzejuk D, Zembska A, Konieczny J, Kaszubkiewicz-Wardęga D, Bolanowski M, Daroszewski J. Circulating levels of irisin and meteorin-like protein in PCOS and their correlation with metabolic parameters,** przyjętej do druku w czasopiśmie Endokrynologia Polska; doi: 10.5603/ep.99111, mój udział polegał na współtworzeniu koncepcji oraz metodologii badania, interpretacji i analizie danych, pozyskaniu środków finansowych na realizację projektu, korekcie manuskryptu oraz nadzorze merytorycznym projektu.

Podpis

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