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Wydział Lekarski z Oddziałem Stomatologii i Oddziałem Nauczania
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Rozprawa doktorska
Ocena stężeń androgenów, leptyny i greliny we krwi pacjentek z zespołem policystycznych jajników w korelacji z parametrami antropometrycznymi i dietą

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Spis treści:

1. Wykaz publikacji stanowiących rozprawę doktorską

Prace oryginalne:

1. **Aleksandra Maria Polak,** Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: *Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes*. Journal of Clinical Medicine, 2020. 9(3), 732. Doi: 10.3390/jcm9030732

IF = 3,303, MNiSW = 140

2. **Aleksandra Maria Polak**, Anna Krentowska, Agnieszka Łebkowska, Angelika Buczyńska, Marcin Adamski, Edyta Adamska-Patruno, Joanna Fiedorczuk, Adam Jacek Krętowski, Irina Kowalska, Agnieszka Adamska: *The Association of Serum Levels of Leptin and Ghrelin with the Dietary Fat Content in Non-Obese Women with Polycystic Ovary Syndrome.* Nutrients, 2020. 12(9), 2753. Doi: 10.3390/nu12092753

IF = 4,546, MNiSW = 140

2. Zestawienie publikacji doktoranta

Rodzaj publikacji	**Liczba**	**Impact Factor**	**Punktacja MNiSW**
Prace włączone do rozprawy doktorskiej	2	7,849	280
Prace, które nie zostały włączone do rozprawy doktorskiej	3	6,236	205
Razem	**5**	**14,085**	**485**

3. Wstęp

Zespół policystycznych jajników (Polycystic Ovary Syndrome, PCOS*)* jest najczęstszą endokrynopatią wśród kobiet w wieku rozrodczym, z częstością występowania 6–20%, w zależności od zastosowanych kryteriów [1]. Zgodnie z obecnie obowiązującymi kryteriami rotterdamskimi (European Society of Human Reproduction and Embryology, ESHRE/ American Society for Reproductive Medicine, ASRM) PCOS rozpoznaje się na podstawie spełnienia co najmniej dwóch z trzech następujących kryteriów: hiperandrogenizm kliniczny i/lub biochemiczny, oligoowulacja i/lub brak owulacji oraz policystyczna morfologia jajników w badaniu ultrasonograficznym metodą transwaginalną (Polycystic Ovarian Morphology, PCOM) [2]. Diagnoza PCOS może być postawiona po wykluczeniu innych jednostek chorobowych o podobnym obrazie klinicznym, takich jak guzy wydzielające androgeny, późno ujawniający się przerost nadnerczy, zespół Cushinga, hiperprolaktynemia. W oparciu o kryteria rotterdamskie wyodrębniono cztery kliniczne fenotypy PCOS (Tabela 1). Najbardziej powszechny fenotyp A [3], określany jako „klasyczny" spełnia wszystkie trzy kryteria PCOS: kliniczny/biochemiczny hiperandrogenizm, zaburzenia miesiączkowania i policystyczna morfologia jajników. Fenotyp B, w którym występuje kliniczny/biochemiczny hiperandrogenizm i zaburzenia miesiączkowania, oraz fenotyp C, charakteryzujący się klinicznym/biochemicznym hiperandrogenizmem i PCOM, są spotykane nieco rzadziej, natomiast najmniej powszechny jest fenotyp D, w którym obserwuje się zaburzenia miesiączkowania i PCOM oraz prawidłowe stężenia androgenów w surowicy [4].

Tabela 1. Fenotypy PCOS

	Fenotyp A	**Fenotyp B**	**Fenotyp C**	**Fenotyp D**
hiperandrogenizm kliniczny/biochemiczny	+	+	+	-
oligoowulacja/brak owulacji	+	+	-	+
PCOM	+	-	+	+

Większość kobiet z PCOS charakteryzuje występowanie zaburzeń metabolicznych, takich jak otyłość brzuszna i insulinooporność [5], które predysponują do rozwoju nieprawidłowej tolerancji glukozy, a w konsekwencji do ujawnienia się cukrzycy typu 2, a także dyslipidemii wiążącej się ze zwiększonym ryzykiem rozwoju chorób układu krążenia [6]. Dotychczas przeprowadzone badania wykazały, że insulinooporność odgrywa istotną rolę w patogenezie PCOS [7], a poszczególne fenotypy różnią się między sobą pod względem występowania insulinooporności [4]. W insulinooporności, której towarzyszy hiperinsulinemia, insulina działa synergistycznie z hormonem luteinizującym (luteinizing hormone, LH), prowadząc do zwiększonej produkcji androgenów w komórkach tekalnych jajnika [8]. Hiperandrogenizm biochemiczny obejmuje podwyższone stężenia w surowicy całkowitego i wolnego testosteronu, androstendionu oraz siarczanu dehydroepiandrosteronu (dehydroepiandrosterone sulfate, DHEA-S). Dane z piśmiennictwa wskazują, że zwiększone stężenie całkowitego i wolnego testosteronu w surowicy krwi u kobiet z PCOS jest związane z nadmiarem trzewnej tkanki tłuszczowej [9], a także insulinoopornością i częstszym występowaniem zaburzeń tolerancji glukozy [10], jednak nie badano tej zależności w poszczególnych fenotypach PCOS (fenotyp A, B, C, D).

Jednocześnie wykazano że nieprawidłowo zbilansowana dieta pacjentek z PCOS może prowadzić do otyłości androidalnej oraz insulinooporności [11]. Istnieją sprzeczne dane na temat tego, który makroskładnik odżywczy zawarty w diecie (białka, tłuszcze, węglowodany) jest najbardziej powiązany z rozwojem otyłości [12-17]. Tkanka tłuszczowa jest głównym miejscem magazynowania nadmiaru energii, ale także organem wydzielania wewnętrznego. Adipocyty syntetyzują i wydzielają substancje biologicznie czynne, w tym leptynę [18-19]. Istniejące doniesienia sugerują związek między przyrostem masy ciała a zaburzeniem równowagi w stężeniach leptyny i greliny w osoczu kobiet z PCOS [20-21]. Leptyna wpływa na równowagę energetyczną organizmu, powodując zmniejszenie spożycia pokarmu i wzrost wydatku energetycznego [22]. Wykazano, że hormon ten, będący produktem genu leptyny, wydzielany jest proporcjonalnie do ilości tkanki tłuszczowej [23], a otyłość jest związana ze zwiększonym stężeniem leptyny w surowicy [24-25]. Jednakże u osób otyłych, pomimo podwyższonych stężeń leptyny w surowicy, jej działanie ulega osłabieniu ze względu na współwystępowanie oporności na leptynę [26-27]. Do tej pory opublikowano kilka badań mających na celu ustalenie związku pomiędzy składnikami diety a stężeniem leptyny w osoczu, a istniejące doniesienia przedstawiają sprzeczne wyniki. Niektórzy autorzy zaobserwowali, że posiłki wysokowęglowodanowe zwiększają stężenie leptyny w osoczu u osób o prawidłowej masie ciała [25]. Z kolei Pourghassem i współautorzy, w badaniu przeprowadzonym na grupie kobiet z PCOS wykazali, że dieta o wysokiej zawartości tłuszczu obniża stężenie leptyny w osoczu [28]. Podobne wyniki uzyskali Kong i współautorzy, którzy dodatkowo zaobserwowali ujemną korelację pomiędzy stężeniem leptyny w osoczu a odsetkiem energii pochodzącej ze spożycia węglowodanów [29]. W przeciwieństwie do wyników cytowanych powyżej badań,

Yannakoulia i współautorzy wykazali związek pomiędzy stężeniem leptyny w osoczu a spożyciem tłuszczu w diecie [30]. Kolejnym hormonem, który ma znaczący wpływ na bilans energetyczny, przyjmowanie pokarmów oraz masę ciała jest grelina [31]. Odgrywa ona ważną rolę w krótkoterminowej regulacji apetytu poprzez stymulację przyjmowania pokarmu, a jej stężenie we krwi rośnie przed posiłkiem i maleje po spożyciu pokarmu [31]. Niskie stężenia greliny w osoczu stwierdzono w stanach dodatniego bilansu energetycznego, takich jak otyłość, a zatem są odwrotnie powiązane z insulinoopornością i cukrzycą typu 2 [32]. Wcześniejsze badania wykazały niższe stężenia greliny w osoczu krwi kobiet z PCOS w odniesieniu do grupy kontrolnej przy porównywalnym wskaźniku masy ciała (Body Mass Index, BMI) [33]. Badania nad związkiem pomiędzy składnikami odżywczymi a stężeniem greliny i leptyny w osoczu pacjentek z PCOS są ograniczone i przedstawiają sprzeczne wyniki.

4. Omówienie prac składających się na pracę doktorską

4.1. Cel rozprawy doktorskiej

Celem pracy była ocena stężeń androgenów, leptyny i greliny we krwi pacjentek z zespołem policystycznych jajników w korelacji z parametrami antropometrycznymi i dietą.

4.2. Praca pt. „Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes"

Celem pracy była ocena zależności pomiędzy stężeniami androgenów w surowicy, insulinoopornością i rozkładem tkanki tłuszczowej ocenianej za pomocą dwuwiązkowej absorpcjometrii promieniowania rentgenowskiego (dual-energy X-ray absorptiometry, DXA) w różnych fenotypach PCOS.

Szczegółowe informacje dotyczące celu pracy, materiałów i metod, wyników oraz wniosków zostały zaprezentowane w pracy oryginalnej wchodzącej w skład rozprawy doktorskiej:

Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: *Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes.* Journal of Clinical Medicine, 2020. 9(3), 732. Doi: 10.3390/jcm9030732

4.2.1 Materiał i metody

4.2.1.1 Grupa badana

Do badania włączono 146 kobiet: 89 pacjentek z PCOS przydzielonych do jednego z czterech fenotypów (fenotyp A, B, C, D) oraz 57 kobiet stanowiących grupę kontrolną. Kobiety z PCOS były pacjentkami hospitalizowanymi w Klinice Endokrynologii, Diabetologii i Chorób Wewnętrznych oraz Klinice Chorób Wewnętrznych i Chorób Metabolicznych Uniwersytetu Medycznego w Białymstoku. Diagnoza PCOS oraz podział na fenotypy (A, B, C, D) zostały przeprowadzone w oparciu o kryteria rotterdamskie opisane we Wstępie [2]. Kryteria wykluczenia obejmowały: choroby przebiegające z zaburzeniami miesiączkowania i/lub hiperandrogenizmem tj. hiperprolaktynemia, zespół Cushinga, późno ujawniający się wrodzony przerost nadnerczy (w tym celu oznaczyliśmy stężenie 17-hydroksyprogesteronu w surowicy), niedoczynność i nadczynność tarczycy, ciąża (wykonano test ciążowy), karmienie piersią, a także cukrzyca typu 1 lub 2, przewlekła lub ostra infekcja (w ciągu ostatnich 30 dni), antykoncepcja hormonalna i/lub terapia antyandrogenowa (w ciągu ostatnich 6 miesięcy) oraz stosowanie leków wpływających na masę ciała, hiperglikemię, dyslipidemię lub nadciśnienie tętnicze. Grupę kontrolną rekrutowano spośród studentek spełniających następujące kryteria: brak hirsutyzmu, w wywiadzie regularne, owulacyjne cykle miesiączkowe oraz prawidłowa morfologia jajników w przezpochwowym badaniu ultrasonograficznym.

Zgoda na przeprowadzenie badań w ramach przygotowania niniejszej pracy została wydana przez Komisję Bioetyczną Uniwersytetu Medycznego w Białymstoku (numer zgody: R-I-002/127/2018). Wszystkie kobiety wzięły udział w badaniu dobrowolnie i wyraziły pisemną świadomą zgodę na włączenie do badania. Wszystkie procedury

były szczegółowo wyjaśnione uczestniczkom przed rozpoczęciem badania.

4.2.1.2 Protokół badania

U wszystkich kobiet zostało wykonane pełne badanie lekarskie oraz przeprowadzono pomiary antropometryczne. Wskaźnik masy ciała (BMI) obliczono poprzez podzielenie masy ciała wyrażonej w kilogramach przez kwadrat wzrostu podanego w metrach (kg/m^2). Obwód talii mierzono w pozycji stojącej, w najmniejszym obwodzie pomiędzy klatką piersiową i grzebieniem biodrowym. U każdej uczestniczki badania wykonano pomiar skurczowego i rozkurczowego ciśnienia tętniczego krwi. Kliniczny hiperandrogenizm określono na podstawie oceny występowania hirsutyzmu (zdefiniowanego jako więcej niż osiem punktów w zmodyfikowanej skali Ferrimana-Gallwey'a) i/lub obecności trądziku. Zaburzenia miesiączkowania zdefiniowano jako mniej niż sześć miesiączek w przeciągu ostatniego roku. Transwaginalne badanie USG zostało wykonane u wszystkich kobiet przez tego samego ginekologa aparatem o częstotliwości 5–9 MHz (Voluson 730 Expert GE Healthcare) we wczesnej fazie folikularnej. Próbki krwi do badania pobierano na czczo pomiędzy 3. a 6. dniem cyklu bądź niezależnie od fazy cyklu w przypadku braku miesiączki dłuższego niż 3 miesiące. W grupie kobiet z PCOS i grupie kontrolnej wykluczono cukrzycę na podstawie doustnego testu tolerancji glukozy z 75g glukozy.

4.2.1.3 Analizy biochemiczne

U wszystkich kobiet wyliczono wskaźnik insulinooporności (Homeostasis Model Assessment of Insulin Resistance, HOMA-IR), między innymi oznaczono stężenia w surowicy testosteronu całkowitego, androstendionu i DHEA-S metodą radioimmunologiczną (DIAsource

ImmunoAssays S.A., Belgia), natomiast stężenia w surowicy globuliny wiążącej hormony płciowe (sex hormone-binding globulin, SHBG) oznaczono metodą radioimmunometryczną (ZenTech, Angleur, Belgium), a także obliczono współczynnik wolnych androgenów (free androgen index, FAI).

4.2.1.4 Analiza składu ciała

Analizę składu ciała przeprowadzono za pomocą badania DXA (GE Healthcare, Chicago, IL, USA, Lunar iDXA). Oszacowano masę trzewnej tkanki tłuszczowej (visceral adipose tissue, VAT) w regionie androidalnym oraz obliczono wskaźnik tkanki tłuszczowej androidalnej (A) do gynoidalnej (G) (wskaźnik A/G).

4.2.1.5 Analiza statystyczna

Analiza statystyczna została przeprowadzona przy użyciu oprogramowania pakietu Statistica (Statistica 13.3, Statsoft, Kraków). Zmienne zbadano pod kątem normalności rozkładu za pomocą testu Shapiro–Wilka. Ze względu na brak normalności rozkładu wszystkie wartości wyrażono jako medianę i przedział międzykwartylowy. Różnice pomiędzy badanymi grupami oceniono nieparametrycznym testem Kruskala–Wallisa z wielokrotnymi porównaniami (post-hoc) średnich rang dla wszystkich par obu grup. Analiza korelacji została wykonana za pomocą testu Spearmana. Następnie przeprowadzono wieloczynnikową analizę regresji celem zbadania niezależnych korelacji. Wartość $p<0,05$ uznano za istotną statystycznie.

4.2.2 Wyniki

4.2.2.1 Charakterystyka grupy badanej

W grupie pacjentek z PCOS 34 (38%) kobiety prezentowały fenotyp A, 20 (23%) kobiet fenotyp B, 20 (23%) kobiet fenotyp C oraz 15 (16%) kobiet fenotyp D. Grupy nie różniły się pod względem wieku i BMI ($p>0,05$). Stężenia całkowitego testosteronu w surowicy były istotnie wyższe w fenotypie A ($p<0,01$) i fenotypie C ($p<0,01$) w porównaniu do grupy kontrolnej. Ponadto, w fenotypach A, B i C zaobserwowano wyższe stężenie całkowitego testosteronu w porównaniu z fenotypem D (odpowiednio; $p<0,01$; $p=0,01$; $p<0,01$). W fenotypie A i B stwierdzono niższe stężenie SHBG w surowicy w odniesieniu do grupy kontrolnej (w obydwu przypadkach $p<0,01$). W fenotypach A, B i C współczynnik FAI był istotnie wyższy w porównaniu z grupą kontrolną (wszystkie $p<0,01$). Jednocześnie wykazano wyższe wartości współczynnika FAI w fenotypie A w porównaniu z fenotypem D ($p<0,01$). Stężenia w surowicy androstendionu i DHEA-S były istotnie wyższe w fenotypach A i C w porównaniu z grupą kontrolną (wszystkie $p<0,01$). Nie obserwowano różnic w wartościach wskaźnika HOMA-IR pomiędzy badanymi grupami ($p=0,25$), jednakże glikemia na czczo była wyższa w fenotypie A w porównaniu z fenotypem C ($p=0,04$). Analiza składu ciała za pomocą badania DXA wykazała większą ilość VAT ($p=0,01$) oraz większy wskaźnik A/G ($p<0,01$) w fenotypie A w odniesieniu do grupy kontrolnej.

4.2.2.2 Zależność pomiędzy HOMA-IR a VAT oraz wskaźnikiem A/G oszacowanymi za pomocą DXA w badanych grupach

We wszystkich fenotypach PCOS wykazano dodatnią korelację HOMA-IR z VAT (wszystkie $p<0,05$) oraz ze wskaźnikiem A/G (wszystkie $p<0,05$).

4.2.2.3 Zależność pomiędzy HOMA-IR a stężeniem androgenów w surowicy w badanych grupach

Wykazano korelację pomiędzy FAI i HOMA-IR w fenotypie A ($r=0,40$, $p=0,01$), fenotypie B ($r=0,47$, $p=0,03$) oraz fenotypie C ($r=0,66$, $p<0,01$), natomiast w fenotypie D nie stwierdzono istotnego związku ($r=0,36$, $p=0,18$).

4.2.2.4 Zależność pomiędzy stężeniem androgenów w surowicy a VAT oraz wskaźnikiem A/G oszacowanymi za pomocą DXA w badanych grupach

We wszystkich fenotypach PCOS obserwowano korelacje FAI ze wskaźnikiem A/G (wszystkie $p<0,05$). W fenotypie C wykazano związek pomiędzy stężeniem DHEA-S i androstendionu w surowicy a wskaźnikiem A/G (odpowiednio $r=0,46$, $p=0,03$; $r=0,53$, $p=0,01$). Jedynie w fenotypie A stwierdzono dodatnią korelację FAI z VAT ($r=0,58$, $p<0,01$).

4.2.3 Wnioski

1. Pacjentki z fenotypem A PCOS, ze względu na większą masę trzewnej tkanki tłuszczowej mają większe ryzyko rozwoju zaburzeń metabolicznych w porównaniu z grupą kontrolną.

2. Masa trzewnej tkanki tłuszczowej wpływa na rozwój insulinooporności oraz stężenie androgenów w surowicy zarówno w fenotypach normoandrogennych, jak i hiperandrogennych PCOS.

4.3 Praca pt. „The Association of Serum Levels of Leptin and Ghrelin with the Dietary Fat Content in Non-Obese Women with Polycystic Ovary Syndrome”

Celem pracy była ocena związku pomiędzy stężeniem leptyny i greliny w osoczu a składnikami diety u kobiet z PCOS.

Szczegółowe informacje dotyczące celu pracy, materiałów i metod, wyników oraz wniosków zostały zaprezentowane w pracy oryginalnej wchodzącej w skład rozprawy doktorskiej:

Aleksandra Maria Polak, Anna Krentowska, Agnieszka Łebkowska, Angelika Buczyńska, Marcin Adamski, Edyta Adamska-Patruno, Joanna Fiedorczuk, Adam Jacek Krętowski, Irina Kowalska, Agnieszka Adamska: *The Association of Serum Levels of Leptin and Ghrelin with the Dietary Fat Content in Non-Obese Women with Polycystic Ovary Syndrome.* Nutrients, 2020. 12(9), 2753. Doi: 10.3390/nu12092753

4.3.1 Materiał i metody

4.3.1.1 Grupa badana

Grupa badana składała się z 73 kobiet: 39 kobiet z PCOS i 34 kobiet stanowiących grupę kontrolną dobraną pod względem BMI. Kobiety z PCOS były pacjentkami leczonymi w Klinice Endokrynologii, Diabetologii i Chorób Wewnętrznych oraz Klinice Chorób Wewnętrznych i Chorób Metabolicznych Uniwersytetu Medycznego w Białymstoku. Grupę kontrolną rekrutowano spośród studentek Uniwersytetu Medycznego w Białymstoku. Kryteria włączenia do badania pacjentek z PCOS i grupy kontrolnej były takie same jak w poprzedniej pracy, gdzie zostały one szczegółowo opisane.

Zgoda na przeprowadzenie badań w ramach przygotowania niniejszej pracy została wydana przez Komisję Bioetyczną Uniwersytetu Medycznego w Białymstoku (numer zgody: APK.002.171.2020).

4.3.1.2 Protokół badania

U wszystkich pacjentek przeprowadzono 3-dniowy wystandaryzowany kwestionariusz oceny spożycia składników pokarmowych, na podstawie którego wyliczono spożywane składniki pokarmowe w g/dzień. Badane zostały poinformowane o konieczności utrzymania dotychczasowego stylu życia (w tym zachowań żywieniowych). Dzienne spożycie składników odżywczych wyliczono przy użyciu programu Dieta 5.0 opracowanego przez Instytut Żywności i Żywienia. Przeprowadzony kwestionariusz żywieniowy pozwolił na uzyskanie informacji dotyczącej dziennej zawartości energii, białka, węglowodanów, tłuszczu całkowitego, nasyconych kwasów tłuszczowych (saturated fatty acids, SFA), jednonienasyconych kwasów tłuszczowych (monounsaturated fatty acids, MUFA), wielonienasyconych kwasów tłuszczowych (polyunsaturated fatty acids, PUFA), długołańcuchowych wielonienasyconych kwasów tłuszczowych (long-chain polyunsaturated fatty acids, LC-PUFA), cholesterolu całkowitego, błonnika pokarmowego, witamin i pierwiastków śladowych. Oceniony został także procent energii pochodzącej ze spożycia białka, węglowodanów i tłuszczów.

4.3.1.3 Pomiary antropometryczne

U wszystkich badanych zostało wykonane pełne badanie lekarskie oraz przeprowadzono pomiary antropometryczne. Wskaźnik masy ciała BMI obliczono jako masę ciała wyrażoną w kilogramach podzieloną przez kwadrat wzrostu wyrażonego w metrach (kg/m2). Współczynnik

obwodu talii do obwodu bioder (waist-hip ratio, WHR) obliczono w pozycji stojącej jako stosunek obwodu talii (najmniejszy obwód pomiędzy klatką piersiową a grzebieniem biodrowym) do obwodu bioder (maksymalny obwód na wysokości krętarzy kości udowych).

4.3.1.4 Analizy biochemiczne

Próbki krwi do badania pobierano na czczo pomiędzy 3. a 6. dniem cyklu bądź niezależnie od fazy cyklu w przypadku braku miesiączki dłuższego niż 3 miesiące. Stężenia leptyny w osoczu krwi badanych oznaczono metodą immunoenzymatyczną (Human Leptin ELISA, BioVendor, Brno, Republika Czeska). Stężenia całkowitej i aktywnej greliny mierzono metodą radioimmunometryczną przy użyciu specyficznych przeciwciał odpowiednio dla całkowitej i aktywnej postaci greliny. Stężenia całkowitej greliny oznaczano przy użyciu zestawu dla greliny całkowitej (GHRT-89HK, RIA, Millipore, USA), natomiast stężenia aktywnej postaci greliny mierzono zestawem dla greliny aktywnej (GHRA-88HK, RIA, Millipore, Burlingtone, MA, USA). Ponadto wyliczono wskaźnik leptyny do greliny.

U wszystkich badanych we krwi oznaczono stężenie cholesterolu całkowitego, HDL-cholesterolu (high-density lipoprotein, HDL), LDL-cholesterolu (low-density lipoprotein, LDL), trójglicerydów oraz stężenia LH, FSH, hormonu tyreotropowego (thyroid stimulating hormone, TSH), estradiolu, testosteronu całkowitego i SHBG, a także obliczono współczynnik FAI. Ponadto oznaczono stężenie glukozy i insuliny na czczo oraz 2 godziny po doustnym obciążeniu 75g glukozy oraz obliczono wskaźnik HOMA-IR.

4.3.1.5 Analiza statystyczna

Analiza statystyczna została przeprowadzona przy użyciu oprogramowania pakietu Statistica (Statistica 13.3, Statsoft, Kraków). Zmienne przetestowano pod kątem normalności rozkładu za pomocą testu Shapiro–Wilka. W związku z tym, że dane nie wykazywały rozkładu normalnego, zastosowano testy nieparametryczne, a wszystkie wartości wyrażono jako medianę i przedział międzykwartylowy. Porównanie grupy PCOS i grupy kontrolnej przeprowadzono testem Manna-Whitneya. Do analizy korelacji zastosowano test Spearmana. Wartość $p<0,05$ uznano za istotną statystycznie.

4.3.2 Wyniki

4.3.2.1 Charakterystyka grupy badanej

Badane grupy nie różniły się istotnie pod względem BMI i WHR (wszystkie $p>0,05$).

Nie wykazano istotnych różnic pomiędzy badanymi grupami w stężeniach leptyny, greliny całkowitej i greliny aktywnej w osoczu oraz wartości wskaźnika leptyny do greliny (wszystkie $p>0,05$). Ponadto grupy nie różniły się istotnie pod względem spożycia makroskładników (białek, tłuszczów i węglowodanów), SFA, MUFA, PUFA, LC-PUFA oraz mikroskładników (sód, potas, wapń, fosfor, magnez, żelazo, cynk, wiatamina A, witamina E, witamina D, witamina C, witamina B3, witamina B6, witamina B12 oraz jod) (wszystkie $p>0,05$). Nie zanotowano również różnic pomiędzy badanymi grupami w dziennej ilości przyjmowanej energii ($p=0,51$) oraz w procentowej ilości energii dostarczanej ze spożycia białek, węglowodanów i tłuszczów (wszystkie $p>0,05$). W grupie pacjentek z PCOS stężenie całkowitego testosteronu w surowicy oraz FAI były istotnie wyższe, podczas gdy stężenie SHBG

w surowicy było istotnie niższe w porównaniu do grupy kontrolnej (wszystkie p <0,05).

4.3.2.2 Zależność pomiędzy stężeniem leptyny i greliny w osoczu oraz wskaźnikiem leptyna/ grelina a spożyciem makroskładników odżywczych

W grupie kobiet z PCOS stężenie leptyny w osoczu korelowało dodatnio ze spożyciem tłuszczów (r=0,36, p=0,02), SFA (r=0,43, p<0,01) i MUFA (r=0,37, p=0,02), natomiast stężenie greliny w osoczu korelowało ujemne ze spożyciem tłuszczów (r=-0,37, p=0,02), cholesterolu całkowitego (r=-0,36, p=0,02), MUFA (r=-0,37, p=0,02), PUFA (r=-0,34, p=0,03) i LC-PUFA (r=-0,38, p=0,02). Ponadto w grupie PCOS wykazano związek pomiędzy wskaźnikiem leptyna/grelina a spożyciem tłuszczu całkowitego (r=0,45, p<0,01), SFA (r=0,49, p<0,01), MUFA (r=0,45, p<0,01) i PUFA (r=0,34, p=0,04). W tej grupie stwierdzono również ujemną korelację pomiędzy stężeniem greliny acylowanej (aktywnej) a zawartością białka w diecie (r=−0,35, p=0,03).

4.3.2.3 Zależność pomiędzy stężeniem hormonów we krwi oraz HOMA-IR a spożyciem makroskładników w diecie

W grupie kobiet z PCOS stwierdzono ujemny związek HOMA-IR ze stężeniem greliny w osoczu (r=-0,4, p=0,03) oraz dodatni ze stężeniem leptyny w osoczu (r=0,5, p<0,01), a także korelację pomiędzy HOMA-IR a zawartością tłuszczów (r=0,38, p=0,03) i MUFA w diecie (r=0,35, p =0,04).

U pacjentek z PCOS wykazana została również zależność pomiędzy stężeniem leptyny w osoczu a FAI (r=0,38, p=0,01) oraz stężeniem SHBG w surowicy (r=−0,4, p<0,01). Ponadto w grupie PCOS zaobserwowano zależność pomiędzy FAI a spożyciem SFA (r=0,34, p=0,04) oraz ujemny związek pomiędzy stężeniem SHBG w surowicy

a spożyciem w diecie tłuszczu całkowitego (r=−0,38, p=0,02), SFA (r=−0,51, p<0,01) i MUFA (r=−0,35, p=0,03).

4.3.3 Wnioski

Dieta bogatotłuszczowa u pacjentek z PCOS wiąże się z rozwojem insulinooporności, zaburzeniami w równowadze pomiędzy stężeniem leptyny i greliny w osoczu prowadząc do rozwoju otyłości.

5. Kopie publikacji wchodzących w skład rozprawy doktorskiej

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Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes

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Abstract: Insulin resistance and hyperandrogenemia observed in polycystic ovary syndrome (PCOS) are associated with metabolic disturbances and could be connected with body composition pattern. To date, several studies defining the parameters of body composition using dual energy X-ray absorptiometry (DXA) method in the group of PCOS patients have been published, however, without the analysis in different phenotypes. The aim of the present study was to investigate the relationships between serum androgens concentration, insulin resistance and distribution of fat mass using DXA method in various PCOS phenotypes according to the Rotterdam criteria. We examined 146 women: 34 (38%) had PCOS phenotype A, 20 (23%) phenotype B, 20 (23%) phenotype C and 15 (16%) phenotype D (with mean age of each phenotype 25 years), and 57 control subjects (mean age of 25.5 years). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Serum concentrations of testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEA-S) were assessed and free androgen index (FAI) was calculated. In phenotypes A, B and C, we observed higher FAI in comparison to the control group (all $p < 0.01$). Serum concentrations of androstenedione and DHEA-S were higher in phenotypes A and C in comparison to the control group (all $p < 0.01$). However, only in phenotype A we found higher visceral adipose tissue (VAT) mass and android/gynoid ratio (A/G ratio) in comparison to the control group (all $p < 0.01$). In phenotype A, we observed connection of VAT with FAI ($r = 0.58$, $p < 0.01$). Accordingly, A/G ratio was related with FAI in all phenotypes (all $p < 0.05$). Additionally, in phenotype C, A/G ratio was related to serum concentrations of DHEA-S and androstenedione ($r = 0.46$, $p = 0.03$; $r = 0.53$, $p = 0.01$, respectively). We also found connections of HOMA-IR with VAT and A/G ratio in all phenotypes (all $p < 0.05$). Women with phenotype A had higher amount of VAT and A/G ratio in comparison to the control group. Serum concentration of androgens and insulin resistance are connected with VAT and A/G ratio in normoandrogenic and hyperandrogenic PCOS phenotypes.

Keywords: body composition; insulin resistance; androgens; PCOS phenotypes

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women of reproductive age, with a prevalence of 6–20% according to the criteria used [1]. Most women with PCOS are also

characterized by metabolic abnormalities like abdominal obesity or insulin resistance, which form the risk factors for metabolic syndrome [2]. A number of studies have indicated that insulin resistance plays a crucial role in the pathogenesis of polycystic ovary syndrome [3]. Insulin acts synergistically with luteinizing hormone (LH), leading to increased production of androgens in the ovarian theca cells [4]. Hyperandrogenemia includes elevated serum concentrations of total and free testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEA-S). Previous data have shown that hyperandrogenemia may affect the distribution of adipose tissue in PCOS patients [5]. Additionally, it has been reported that increased serum testosterone levels in PCOS women are associated with excess of visceral fat amount [5], as well as with insulin resistance and more frequent occurrence of impaired glucose tolerance [3].

In the Rotterdam Consensus, it was defined that in order to diagnose PCOS, at least two of the following criteria have to be fulfilled: oligoovulation and/or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology in transvaginal ultrasound [6]. The Rotterdam criteria for PCOS recognize four clinical phenotypes of the syndrome. The most prevalent phenotype is the classic form [7], which meets all three current criteria for PCOS: clinical and/or biochemical hyperandrogenism (HA), menstrual dysfunction (oligo/amenorrhea) (Oligo) and polycystic ovarian morphology (PCOM)-phenotype A (Oligo + HA + PCOM). Phenotype B (HA + Oligo) and phenotype C (HA + PCOM) are less frequent. The Rotterdam criteria also recognize a fourth phenotype, D, which is defined by oligomenorrhea, polycystic ovarian morphology in ultrasound and normal androgen levels (Oligo + PCOM) [8]. An increased incidence of metabolic disorders is observed among women with phenotypes A, B and C [9], whereas phenotype D is probably characterized by fewer metabolic abnormalities [1]. However, not all published data confirm this hypothesis [1].

To date, several studies defining the parameters of body composition using dual energy X-ray absorptiometry (DXA) method in the group of PCOS patients have been published [10–12], however, without the division into phenotypes. Magnetic resonance imaging is considered the gold standard in the assessment of fat distribution (visceral and subcutaneous adipose tissue). However, this technique requires advanced equipment and highly qualified staff. It has been shown that visceral obesity might be detected at an early stage by DXA. Moreover, due to high reproducibility of this method, repeated measurements might be performed in the same patient to monitor changes in body composition over time [13].

As it was mentioned previously, hyperandrogenemia is connected with adverse metabolic parameters, therefore, we hypothesized that women with phenotypes characterized by elevated serum concentration of androgens (phenotypes A, B and C) presented insulin resistance and adverse fat distribution compared with those with normal serum level of androgens (phenotype D). Therefore, the aim of the present study was to investigate the relationships between serum androgen concentrations, insulin resistance and distribution of fat mass using the DXA method in various PCOS phenotypes.

2. Materials and Methods

2.1. Subjects

A prospective, cross-sectional study was conducted between March 2018 and June 2019. The study group consisted of 146 women: 89 patients with PCOS divided into four phenotypes (phenotype A, B, C, D with mean age of 25 years), and 57 control women (mean age of 25.5 years). PCOS women were patients treated in the Department of Endocrinology, Diabetology and Internal Medicine and the Department of Internal Medicine and Metabolic Diseases, Medical University of Białystok. The control group was recruited from students who met exclusion criteria and met the following criteria: they were normoandrogenic, without hirsutism, had a history of regular, ovulatory menstrual cycles and morphologically normal ovaries on ultrasound. The diagnosis of PCOS was made according to the 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group diagnostic criteria. We defined PCOS by the presence of at least two out of three criteria: clinical and/or biochemical hyperandrogenism,

oligo/anovulation, and polycystic ovaries in ultrasound (>12 follicles measuring 2–9 mm in diameter or ovarian volume >10 mL in at least one ovary) [6]. The phenotypes of PCOS (A, B, C, D) were classified according to Rotterdam criteria described in the Introduction section [6]. Exclusion criteria included: other conditions causing menstrual irregularity and/or hyperandrogenism (i.e., hyperprolactinemia, Cushing's syndrome (based on history taking and physical examination), late-onset congenital adrenal hyperplasia (for this purpose, we determined the serum levels of 17-hydroxyprogesterone), hypothyroidism and hyperthyroidism, pregnancy (appropriate test was performed) and breastfeeding, type 1 or type 2 diabetes, chronic or acute infection (within the previous 30 days), any other serious medical problem, hormonal contraception and/or anti-androgen therapy (within the previous 6 months), and the use of medications for obesity, hyperglycemia, dyslipidemia or hypertension. All the patients participating in the study were Caucasians. The study was approved by the Institutional Review Board (Ethics Committee of the Medical University of Białystok, Białystok, Poland; approval no. R-I-002/127/2018) and was concordant with the Declaration of Helsinki. All the procedures were performed in accordance with the relevant guidelines and regulations. All women participated in the study voluntarily and gave their written informed consent for inclusion. All the procedures were explained to the participants in detail before the beginning of the study.

2.2. Study Protocol

All women underwent physical examination. Clinical hyperandrogenism-hirsutism (defined as more than eight points in the modified Ferriman-Gallwey score) [14] and presence of acne were evaluated. Oligo/amenorrhea and anovulation were defined as fewer than six menses during the previous year.

BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured in the standing position, at the smallest circumference between the rib cage and the iliac crest. Systolic and diastolic blood pressure was recorded. Transvaginal ultrasound was performed in all women by the same gynecologist with a 5–9 MHz transvaginal transducer (Voluson 730 Expert GE Healthcare) in the early follicular phase. Ovarian volume was calculated using the simplified formula for a prolate ellipsoid [15].

In the morning, blood samples were obtained between the 3rd and 6th day of the cycle or independently of cycle phase in the presence of amenorrhea, at least 3 months from the last menses. Oral glucose tolerance test with 75 g of glucose was performed in all subjects to exclude diabetes.

2.3. Biochemical Analyses

Fasting plasma glucose and serum insulin concentrations, as well as plasma concentrations of glucose and serum levels of insulin two hours after the ingestion of 75 g of glucose were determined. Plasma glucose concentrations were assessed by the hexokinase method, and plasma lipid concentrations (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG)) were measured by enzymatic colorimetric method (Cobas c111, Roche Diagnostic Ltd., Switzerland). Plasma low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald's formula. Serum insulin concentrations were assessed with the immunoradiometric method (DIAsource ImmunoAssays S.A., Belgium) (minimum detectable concentration (MDC)—1 µIU/mL; intra-assay coefficient of variation (CV)—below 2.2%, inter-assay CV—below 6.5%,). There is no cross-reaction between human and animal proinsulins in this method.

Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined with immunoradiometric method (DIAsource ImmunoAssays S.A., Belgium) (LH: intra-assay CV—below 3.9%, inter-assay CV—below 8%; FSH: intra-assay CV—below 2%, inter-assay CV—below 4.4%). Concentrations of total testosterone were measured by radioimmunoassay (DIAsource ImmunoAssays S.A., Belgium) (MDC—0.05 ng/mL, intra-assay CV—3.3%, inter-assay CV—4.8%). Serum sex hormone–binding globulin (SHBG) concentrations were assessed with immunoradiometric method (ZenTech, Angleur, Belgium) (intra-assay CV—below 5.2%, inter-assay CV—below 5.8%).

Serum concentrations of DHEA-S and androstenedione were measured with radioimmunoassay (DIAsourceImmunoAssays S.A., Belgium) (MDC for DHEA-S—1.23 µg/dL, for androstenedione—0.03 ng/mL; intra-assay and inter-assay CV for DHEA-S—3.6% and 6.5%, for androstenedione—3.2% and 5.9%).

2.4. Calculations

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (µIU/mL) x fasting plasma glucose (mmol/L)/22.5 [16]. Free androgen index (FAI) was calculated according to the formula: serum total testosterone (nmol/L) × 100/SHBG (nmol/L) [14].

2.5. Body Composition Analysis

Body composition analysis was conducted using DXA (GE Healthcare, Chicago, IL, USA, Lunar iDXA) by qualified physicians at the Clinical Research Centre, Medical University of Białystok. The equipment was calibrated before every examination. The patients were positioned on the examination table in a supine position, with their feet secured together with an adjustable strap and hands lying flat adjacent to the sides of the body. Each examination took approximately 8 min. On the basis of the scans, CoreScan software estimated mass of visceral adipose tissue (VAT) within the android region. Additionally, android/gynoid ratio (A/G ratio) was calculated. DXA assessed fat mass with the precision (coefficient of variation) of 2.0% and 8.0%, respectively.

2.6. Statistical Analysis

The statistical analysis for the present study was performed with the Statistica package (Statistica 13.3, Statsoft, Cracow, Poland). All analyzed variables were tested for normality of distribution with the Shapiro–Wilk test. Due to non-normal distribution, all values were expressed as median (interquartile range). Differences between the studied groups were assessed with non-parametric Kruskal–Wallis test with post-hoc multiple comparisons of mean ranks of all pairs of groups. Correlation analysis was performed using the Spearman test. Afterwards, multivariate regression analysis was performed to investigate independent relationships. A p-value < 0.05 was considered statistically significant.

3. Results

The clinical characteristics of the studied groups are presented in Table 1. In PCOS group, 34 (38%) women had phenotype A, 20 (23%) women had phenotype B, 20 (23%) women presented phenotype C and 15 (16%) were diagnosed with phenotype D. The groups were similar in terms of age and BMI (all $p > 0.05$) (Table 1).

Serum concentrations of total testosterone were significantly higher in phenotype A ($p < 0.01$ in post-hoc analysis) and C ($p < 0.01$ in post-hoc analysis) in comparison to the control group. Similarly, a higher level of total testosterone was observed in phenotypes A, B and C in comparison to phenotype D (in post-hoc analysis $p < 0.01$; $p = 0.01$; $p < 0.01$; respectively). We noticed lower serum concentration of SHBG in phenotype A and B in comparison to the controls (in post-hoc analysis both $p < 0.01$). In phenotypes A, B and C, FAI was higher in comparison to the controls (in post-hoc analysis all $p < 0.01$). Accordingly, we observed higher FAI in phenotype A in comparison to phenotype D ($p < 0.01$). Serum concentrations of DHEA-S were higher in phenotype A and C in comparison to the healthy women (in post-hoc analysis both $p < 0.01$). Similarly, we noticed higher serum concentrations of androstenedione in phenotype A and C in comparison to the control group (in post-hoc analysis both $p < 0.01$). We did not observe differences in HOMA-IR between the studied groups ($p = 0.25$), however, fasting glucose was higher in phenotype A vs. C (in post-hoc analysis $p = 0.04$) (Table 1).

Table 1. Clinical and biochemical characteristics of the studied groups.

	Control Group (n = 57)	Phenotype A (n = 34)	Phenotype B (n = 20)	Phenotype C (n = 20)	Phenotype D (n = 15)	p Value
Age (years)	25 (23–28)	24 (22–27)	24 (23–27.5)	24 (21.5–27.5)	26 (22–28)	0.60
BMI (kg/m^2)	22.4 (21.7–24.3)	23.7 (21.1–29.4)	24.9 (22.2–29.5)	23.1 (21.6–25.2)	23.4 (20.5–27.1)	0.60
WC (cm)	80 (74–84)	80 (73–96)	81.5 (77.5–97)	78.5 (73.5–89)	82 (69–91)	0.72
Ferriman-Gallwey score	3 (2–5)	9 (4–12) [1,2]	11 (9–15) [3,6]	9 (3–11) [4,5]	1 (1–5) [2,3,5]	<0.01
FSH (IU/L)	5.40 (4.4–6.3)	5.61 (3.62–6.39)	4.76 (3.91–6.02)	5.67 (5.14–6.57)	5.19 (4.34–6.6)	0.41
LH (IU/L)	3.70 (2.7–4.7)	4.66 (3.1–7.2)	3.66 (2.57–4.56)	3.71 (2.95–4.91)	4.42 (3.58–5.95)	0.07
TT (ng/mL)	0.56 (0.42–0.69)	0.72 (0.63–0.94) [1,2]	0.78 (0.59–0.88) [3]	0.80 (0.65–0.89) [4,5]	0.51 (0.41–0.59)	<0.01
SHBG (nmol/L)	66.9 (54.6–92.5)	43.2 (27.2–51.8) [1]	34.7 (25.8–86.6) [6]	56.3 (36.7–73.4)	57.5 (51.5–79.7)	<0.01
FAI	2.70 (1.7–3.8)	6.18 (4.34–9.73) [1,2]	5.38 (2.69–8.94) [4]	4.65 (3.19–6.59) [6]	2.58 (2.04–3.5)	<0.01
Androstenedione (ng/mL)	3.10 (2.49–4.04)	4.60 (3.3–5.1)[1]	3.73 (3.2–4.62)	4.68 (3.26–5.85) [4]	3.43 (2.9–4.95)	<0.01
DHEA-S (ug/dL)	230.1 (185.6–338)	300.3 (256.9–368.6) [1]	287.1 (222.8–400.6)	358.6 (241.9–441.6) [4]	238 (201.5–301.4)	<0.01
Glucose 0′ OGTT (mg/dL)	92 (88–97)	95 (90–100) [7]	96.5 (91–100)	90 (84–92)	90 (87–94)	0.04
Glucose 120′ OGTT (mg/dL)	91 (75–101)	98 (86–121)	96.5 (85.5–111)	85 (78–98)	83 (77–95)	0.03
Insulin 0′ OGTT (uIU/mL)	8.80 (7.2–11.6)	10.60 (7.5–14.8)	9.81 (6.6–14.2)	8.36 (7.1–10.0)	8.20 (6.8–13.5)	0.38
Insulin 120′ OGTT (uIU/mL)	27.1 (18.7–38)	41 (25.5–67.9)	29.6 (25.8–46.6)	23.8 (17.2–40.3)	29.2 (19.2–57.1)	0.04
HOMA-IR	2.06 (1.64–2.9)	2.60 (1.91–3.49)	2.35 (1.45–3.61)	1.85 (1.46–2.3)	1.92 (1.46–2.73)	0.25
Total cholesterol (mg/dL)	171 (149–195)	172 (157–199)	169.5 (160–182)	168 (157–179.5)	174 (140–193)	0.80
HDL-cholesterol (mg/dL)	63 (57–75)	67 (49–75)	60.5 (50.5–69.5)	69.5 (59–77.5)	69 (51–79)	0.38
LDL-cholesterol (mg/dL)	90 (76–106)	96.6 (81.2–110.6)	91.3 (83.6–103)	86.6 (68.8–91.6)	90.8 (72–104)	0.28
TG (mg/dL)	59 (42–81)	67 (49–92)	68 (51.5–102)	60 (47.5–83.5)	60 (50–76)	0.30
VAT mass (g)	168 (68–336)	242 (125–897) [1]	220 (88–667)	157 (57–356)	219 (118–420)	0.01
A/G ratio	0.79 (0.67–0.89)	0.94 (0.74–1.09) [1]	0.88 (0.78–1.13)	0.82 (0.70–0.93)	0.76 (0.69–0.93)	0.005

Values are expressed as median (interquartile range): [1] $p < 0.05$ phenotype A vs. control; [2] $p < 0.05$ phenotype A vs. phenotype D; [3] $p < 0.05$ phenotype B vs. phenotype D; [4] $p < 0.05$ phenotype C vs. control; [5] $p < 0.05$ phenotype C vs. phenotype D; [6] $p < 0.05$ phenotype B vs. control; [7] $p < 0.05$ phenotype A vs. phenotype C. BMI: body mass index; WC: waist circumference; TT: total testosterone; DHEA-S: dehydroepiandrosterone sulfate; TG: triglycerides; OGTT: oral glucose tolerance test; FSH: follicle-stimulating hormone; LH: luteinizing hormone; FAI: free androgen index; SHBG: sex hormone binding globulin; HOMA-IR: homeostasis model assessment of insulin resistance; TSH: thyroid-stimulating hormone; VAT: visceral adipose tissue; A/G ratio: android/gynoid ratio.

DXA analysis revealed higher VAT mass (in post-hoc analysis $p = 0.04$) and A/G ratio (in post- hoc analysis $p = 0.01$) in phenotype A than in the control group (Figure 1).

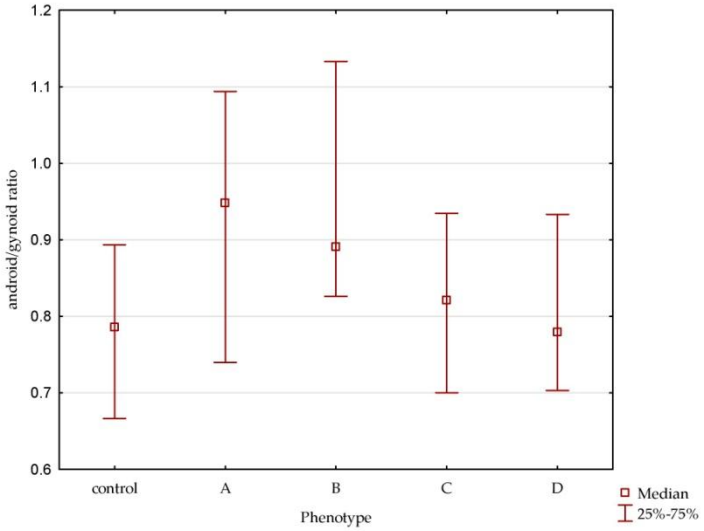

Figure 1. Android/gynoid ratio in different PCOS phenotypes and the control group.

In phenotype A, we observed relationships between FAI and VAT ($r = 0.58$, $p < 0.01$). We also found connections of HOMA-IR with VAT in phenotypes A, B, C and D (all $p < 0.05$). We did not find significant relationships of serum concentrations of DHEA-S and androstenedione with VAT estimated with DXA in phenotypes A, B, C and D (all $p > 0.05$) (Table 2).

Table 2. Relationship of HOMA-IR and serum concentration of androgens with VAT estimated with DXA method in the studied groups.

	Control Group (*n* = 57)	**Phenotype A (*n* = 34)**	**Phenotype B (*n* = 20)**	**Phenotype C (*n* = 20)**	**Phenotype D (*n* = 15)**
HOMA-IR	$r = 0.12$, $p = 0.37$	$r = 0.61$, $p < 0.01$ *	$r = 0.70$, $p < 0.01$ *	$r = 0.51$, $p = 0.02$ *	$r = 0.57$, $p = 0.03$ *
TT (ng/mL)	$r = 0.14$, $p = 0.27$	$r = 0.20$, $p = 0.86$	$r = 0.01$, $p = 0.94$	$r = 0.13$, $p = 0.56$	$r = 0.05$, $p = 0.85$
FAI	$r = 0.22$, $p = 0.08$	$r = 0.58$, $p < 0.01$ *	$r = 0.38$, $p = 0.10$	$r = 0.44$, $p = 0.05$	$r = 0.50$, $p = 0.06$
Androstenedione (ng/mL)	$r = 0.18$, $p = 0.18$	$r = 0.09$, $p = 0.58$	$r = 0.11$, $p = 0.63$	$r = 0.22$, $p = 0.43$	$r = 0.11$, $p = 0.63$
DHEA-S (ug/dl)	$r = 0.09$, $p = 0.50$	$r = 0.12$, $p = 0.48$	$r = 0.05$, $p = 0.84$	$r = 0.21$, $p = 0.35$	$r = 0.27$, $p = 0.34$

Data are derived from Spearman correlation coefficient. The level of significance was accepted at * $p < 0.05$. VAT: visceral adipose tissue; DXA: dual energy X-ray absorptiometry; HOMA-IR: homeostasis model assessment of insulin resistance; TT: total testosterone; FAI: free androgen index; DHEA-S: dehydroepiandrosterone sulfate.

In phenotypes A, B, C and D, we observed relationships between FAI and A/G ratio (all $p < 0.01$). We also found connections of HOMA-IR with A/G ratio in phenotypes A, B, C and D (all $p < 0.05$). However, only in phenotype C, serum concentrations of DHEA-S and androstenedione were connected with A/G ratio ($r = 0.46$, $p = 0.03$; $r = 0.53$, $p = 0.01$, respectively). We found no correlation of serum concentration of androstenedione and DHEA-S with A/G ratio estimated with DXA in phenotype A, B and D (all $p > 0.05$) (Table 3).

Table 3. Relationship of HOMA-IR and serum concentration of androgens with A/G ratio estimated with DXA in the studied groups.

	Control Group ($n = 57$)	Phenotype A ($n = 34$)	Phenotype B ($n = 20$)	Phenotype C ($n = 20$)	Phenotype D ($n = 15$)
HOMA-IR	$r = 0.12$, $p = 0.36$	$r = 0.53$, $p < 0.01$ *	$r = 0.53$, $p = 0.01$ *	$r = 0.50$, $p = 0.02$ *	$r = 0.58$, $p = 0.02$ *
TT (ng/mL)	$r = 0.05$, $p = 0.67$	$r = 0.16$, $p = 0.33$	$r = 0.08$, $p = 0.74$	$r = 0.51$, $p = 0.01$	$r = 0.06$, $p = 0.82$
FAI	$r = 0.26$, $p = 0.04$ *	$r = 0.53$, $p < 0.01$ *	$r = 0.50$, $p = 0.02$ *	$r = 0.61$, $p = 0.003$ *	$r = 0.52$, $p = 0.04$ *
Androstendione (ng/mL)	$r = 0.09$, $p = 0.48$	$r = 0.02$, $p = 0.89$	$r = -0.05$, $p = 0.83$	$r = 0.53$, $p = 0.01$ *	$r = -0.05$, $p = 0.83$
DHEA-S (ug/dL)	$r = 0.08$, $p = 0.55$	$r = 0.002$, $p = 0.48$	$r = 0.24$, $p = 0.30$	$r = 0.46$, $p = 0.03$ *	$r = 0.48$, $p = 0.06$

Data are derived from Spearman correlation coefficient. The level of significance was accepted at * $p < 0.05$. A/G: android/gynoid ratio; HOMA-IR: homeostasis model assessment of insulin resistance; TT: total testosterone; FAI: free androgen index; DXA: dual energy x-ray absorptiometry; DHEA-S: dehydroepiandrosterone sulfate.

We found relationships between FAI and HOMA-IR in phenotype A ($r = 0.40$, $p = 0.01$), phenotype B ($r = 0.47$, $p = 0.03$) and phenotype C ($r = 0.66$, $p = 0.001$), but not in phenotype D ($r = 0.36$, $p = 0.18$).

In the entire group, multiple regression analysis showed that FAI ($\beta = 0.33$, $p < 0.01$) and HOMA-IR ($\beta = 0.36$, $p < 0.01$) were significantly associated with A/G ratio and there was no significant interaction with phenotypes. Additionally, in the entire group, multiple regression analysis showed that FAI ($\beta = 0.37$, $p < 0.01$) and HOMA-IR ($\beta = 0.52$, $p < 0.01$) were significantly associated with VAT mass and there was no significant interaction with phenotypes.

In the control group, we found no correlation between HOMA-IR, serum concentrations of androstenedione, DHEA-S and VAT estimated with DXA (all $p > 0.05$) (Table 2). We observed relationships between A/G ratio and FAI ($r = 0.26$, $p = 0.04$) in the control group (Table 3).

4. Discussion

In our study, we demonstrated the relationships of serum concentrations of different androgens and HOMA-IR with body composition estimated with DXA in different phenotypes of PCOS. In phenotype A, we observed higher VAT amount, as well as A/G ratio and FAI in comparison to the control group, and a connection between FAI and VAT and A/G ratio in this phenotype. Previous studies have shown contrasting results of fat content in PCOS women [12,17,18]. In some studies, increased abdominal fat was observed in overweight and lean PCOS women in comparison to controls [18], whereas in others, fat mass in trunk and arms were significantly higher in patients with PCOS vs. control [12]. In the cited study, FAI positively correlated only with fat mass in arms in women with PCOS [12]. However, they did not examine various PCOS phenotypes, as we did. In one study, there were no differences in fat distribution in DXA method between phenotypes. However, the authors found that A/G ratio was connected positively with HOMA-IR and negatively with insulin sensitivity index [19]. Previous studies have shown that VAT is metabolically more active than subcutaneous adipose tissue, and that the increased amount of VAT is associated with higher risk of metabolic disturbances, e.g., hypertension, dyslipidemia, insulin resistance and type 2 diabetes [20]. It has also been observed that fat distribution

is altered in PCOS patients and that this group presents greater tendency to increased VAT accumulation in comparison to the general population [17]. Moreover, it has been shown that VAT is associated with insulin resistance and increased metabolic risk in PCOS women [21], and that increased concentrations of androgens are connected with abdominal fat deposition [22]. Therefore, our results confirmed that phenotype A could be considered a phenotype with increased risk of obesity, type 2 diabetes, coronary heart disease and other metabolic disorders [23], and it may be related to significantly higher FAI in this group of patients. However, prospective studies are needed to confirm this hypothesis.

It is unclear whether hyperandrogenic PCOS phenotypes are at an increased cardiovascular risk in comparison to normoandrogenic phenotype. In the present study, relationships between serum concentration of androgens and body distribution in various PCOS phenotypes were observed. We revealed relationships between FAI and A/G ratio in phenotypes with hyperandrogenism and normoandrogenic phenotype. We also observed that serum concentrations of DHEA-S and androstenedione were connected with A/G ratio in phenotype C. Furthermore, we demonstrated the association of HOMA-IR and FAI with A/G ratio in all phenotypes. Interestingly, in phenotypes C and D, the relationship between FAI and VAT almost reached statistical significance. Therefore, we could not exclude connections between fat distribution and FAI in those phenotypes. Therefore, it seems that women without elevated serum androgens concentration are not protected from metabolic disturbances. On the contrary, Carmina et al. [18] did not find any correlation between fat parameters and serum testosterone levels in PCOS patients. However, they did not study various PCOS phenotypes. Our observation can be supported by the fact that insulin resistance in PCOS women is related to excessive serine phosphorylation of the insulin receptor 1 (IRS-1) [24], and serine phosphorylation modulates the activity of the key regulatory enzyme of androgen biosynthesis, P450c17 [25].

In our study, we reported a significant difference among the four studied phenotypes in terms of total testosterone levels. However, we found that in phenotype D, serum concentration of total testosterone was similar to control group and lower in comparison to other phenotypes. Our findings are in accordance with Jamil et al. [26], who also reported higher total testosterone levels in phenotype A, B and C than in phenotype D. Additionally, Yilmaz et al. reported that phenotype D was more similar to the control group than the other PCOS phenotypes [27]. Those results suggest that PCOS patients are not a homogenous group in relation to androgen excess. Previous studies [2,26,28] confirmed that metabolic abnormalities are less severe in normoandrogenic women with PCOS in comparison to phenotypes with hyperandrogenism. However, based on our data, we could not confirm that phenotype D is characterized by milder endocrine and metabolic abnormalities than other PCOS phenotypes.

The limitation of the present study is a relatively small number of participants representing different PCOS phenotypes, however, they are very well characterized. Another limitation is the use of HOMA-IR to estimate insulin resistance. The gold standard in the assessment of whole-body insulin sensitivity is hyperinsulinemic euglycemic clamp, however, it is time-consuming and difficult to perform [29]. Other methods of insulin sensitivity assessment could be minimal model S_I and indirect indices calculated from OGTT [30]. HOMA-IR takes into account fasting glucose and insulin levels and only reflects hepatic insulin sensitivity [31]. Therefore, the correlation between HOMA-IR and M index derived from the clamp is only moderate [29]. It should also be emphasized that concentrations of total testosterone were measured by RIA. The currently recommended method, considered a gold standard in the assessment of testosterone concentrations, is liquid chromatography-tandem mass spectrometry (LC-MS), however, its use is limited by high costs of the technique.

5. Conclusions

In conclusion, women with phenotype A have a higher amount of VAT and A/G ratio in comparison to the control group, therefore, metabolic disturbances could be more pronounced in this phenotype. Serum concentration of androgens and insulin resistance are connected with VAT and A/G ratio in normoandrogenic and hyperandrogenic PCOS phenotypes.

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The Association of Serum Levels of Leptin and Ghrelin with the Dietary Fat Content in Non-Obese Women with Polycystic Ovary Syndrome

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Abstract: Women with polycystic ovary syndrome (PCOS) are at an increased risk of developing insulin resistance and abdominal obesity in the state of an improper diet balance. Leptin is a peptide considered to be a satiety hormone that plays an important role in the long-term energy balance, whereas ghrelin is a hormone that controls short-term appetite regulation and is considered a hunger hormone. The aim of the present study was to assess the relationship between serum leptin and ghrelin concentrations and the dietary macronutrient content in PCOS women. We examined 73 subjects: 39 women diagnosed with PCOS by the Rotterdam criteria and 34 healthy controls, matched by the body mass index. The subjects completed a consecutive three-day dietary diary to identify the macronutrient and micronutrient intake. Serum concentrations of leptin and total ghrelin were measured and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. The studied groups did not differ significantly in terms of the intake of macronutrients (proteins, fats, and carbohydrates) and serum concentrations of ghrelin and leptin (all $p > 0.05$). In the PCOS group, the serum leptin concentration positively correlated with the intake of total fat ($r = 0.36$, $p = 0.02$), total cholesterol ($r = -0.36$, $p = 0.02$), saturated fatty acids ($r = 0.43$, $p < 0.01$), and monounsaturated fatty acids (MUFA) ($r = 0.37$, $p = 0.02$), whereas the serum ghrelin concentration correlated in an inverse manner with the intake of total fat ($r = -0.37$, $p = 0.02$), MUFA ($r = -0.37$, $p = 0.02$), polyunsaturated fatty acids ($r = -0.34$, $p = 0.03$), and long chain polyunsaturated fatty acids ($r = -0.38$, $p = 0.02$). In this group, we also found a negative association of HOMA-IR with serum ghrelin levels ($r = -0.4$, $p = 0.03$) and a positive relationship with the serum leptin concentration ($r = 0.5$, $p < 0.01$) and relationships between HOMA-IR and total dietary fat ($r = 0.38$, $p = 0.03$) and MUFA ($r = 0.35$, $p = 0.04$) intake. In PCOS women, dietary components such as the total fat and type of dietary fat and HOMA-IR are positively connected to serum leptin concentrations and negatively connected to serum ghrelin concentrations, which may influence the energy balance.

Keywords: leptin; ghrelin; macronutrients; PCOS

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting reproductive-age women, with a prevalence reaching 6–20%, depending on the criteria used [1]. Polycystic ovary syndrome is diagnosed by the presence of at least two of the following criteria: oligoovulation and/or anovulation, clinical and/or biochemical hyperandrogenism, and a polycystic ovarian morphology in a transvaginal ultrasound [2]. Insulin resistance and hyperinsulinemia play a crucial role in the pathogenesis of PCOS, which is associated with a higher risk of developing abdominal obesity, metabolic syndrome, pre-diabetes, and, as a consequence, type 2 diabetes, in comparison to the general population [3]. It has been shown that more than 50% of women with PCOS are overweight or obese, which is largely related to dietary patterns [4]. The three principal dietary components (macronutrients) are fats, proteins, and carbohydrates. Fats are classified into subgroups on the basis of the carbon chain length and the degree of saturation [5]. Saturated fatty acids (SFA) have been deemed the predisposing factors of cardiovascular disease [6], while monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have been considered to be protective factors [7,8]. There are conflicting data on which macronutrient in the diet is connected the most with human obesity [9–14]. Some studies have shown an association of an increased consumption of total fat and saturated fatty acids with weight gain and obesity [9,11]. It has been established that fat provides more energy per gram than carbohydrates [12]. Therefore, a higher proportion of fat in the diet can lead to weight gain through an excess energy intake [13]. On the other hand, it has been observed that the percentage of fat in the diet is not connected with excess body fat in Western countries [15]. On the contrary, other authors have demonstrated weight gain with an increased carbohydrate intake [10] and with an increased calorie intake [16]. These conflicting data may result from several methodological issues that make findings from studies on diet and body weight difficult to interpret. Moreover, there are indications that the intake of carbohydrates and fat is more subject to underreporting than the intake of protein, which can affect the results of studies on the macronutrient composition and body weight [12,14] and therefore may confound the association between dietary intake and body weight.

Adipose tissue is considered the primary site able to store energy excess, but also an organ of endocrine secretion. Adipocytes synthesize and secrete biologically active substances, including leptin and ghrelin [17,18]. It has been shown that leptin, which is a product of the leptin gene, is secreted proportional to the amount of adipose tissue [19]. This hormone affects the energy balance, resulting in a decrease in food intake and an increase in energy expenditure [20]. Accordingly, leptin participates in the regulation of metabolism of energy substrates—lipids and carbohydrates [21]. It has been shown that obesity is associated with increased serum leptin concentrations [22,23]. However, in obesity, despite elevated leptin concentrations, the effect of leptin is reduced due to leptin resistance [24,25]. To date, several studies have been published to determine the relationship between dietary components and serum leptin levels, although the existing reports are contradictory. It has been found that high-carbohydrate meals increase serum leptin concentrations in subjects with a normal weight, while obese participants have both fasting and postprandial leptin concentrations higher than those with a normal weight [23]. Pourghassem et al., in a study on PCOS women, showed that high-fat meals reduced the concentrations of circulating leptin [26]. Similar results were obtained by Kong et al. in a study carried out on a group of obese and overweight postmenopausal women. Additionally, they observed an inverse relationship between the serum leptin concentration and the percentage of carbohydrate energy [27]. In contrast to the results of the studies cited above, Yannakoulia et al. showed a positive correlation between the serum leptin concentration and dietary fat intake [28]. In turn, other studies failed to show any association of dietary fat with the serum leptin concentration [29].

Another peptide hormone that has a significant impact on the energy balance, food intake, and regulation of body mass is ghrelin [30]. Ghrelin is secreted by cells in the stomach, pancreas, kidneys, and gonads, and, as mentioned above, by adipose tissue [31]. It plays an important role in the short-term regulation of appetite by stimulating the food intake, and its concentration in blood rises before a meal and decreases after food ingestion [30]. Low serum ghrelin levels were found in

conditions of a positive energy balance, such as obesity, and therefore are associated, in an inverse manner, with insulin resistance and type 2 diabetes [32]. Previous studies have shown lower serum ghrelin concentrations in a group of women with PCOS in relation to a control group with a comparable body mass index (BMI) [33]. Studies on the relationship between dietary nutrients and serum ghrelin levels in PCOS subjects are limited and present conflicting results. Pourghassem et al. did not show an association between the serum ghrelin concentration and dietary macronutrient intake in PCOS patients and control subjects [26]. In turn, Barber et al. found that an intake of oral glucose reduced ghrelin secretion in women with PCOS [34].

Some studies have suggested a link between weight gain and imbalance in leptin and ghrelin concentrations in women with PCOS [35,36]; therefore, it seems to be important to determine macronutrients altering leptin and ghrelin concentrations. Accordingly, few studies so far have addressed the relation between dietary macronutrients and serum leptin and ghrelin concentrations in PCOS. Considering the insufficient data and contradictory results of studies conducted in women with PCOS, the purpose of this study was to assess the relationship between serum leptin and ghrelin concentrations and the dietary macronutrient intake in women with PCOS.

2. Materials and Methods

2.1. Study Participants

The study group consisted of 73 women: 39 subjects with PCOS and 34 healthy women, matched for BMI. Women were recruited from the Department of Endocrinology, Diabetology and Internal Medicine, as well as from the Department of Internal Medicine and Metabolic Diseases, Medical University of Bialystok, Poland. The control group consisted of healthy women recruited from students and staff who met the exclusion criteria and met the following criteria: they did not present hyperandrogenemia or hirsutism; they had a history of regular, ovulatory menstrual cycles; and they had morphologically normal ovaries, assessed by a transvaginal ultrasound. The diagnosis of PCOS was made according to the 2003 Rotterdam European Society of Human Reproduction and Embryology/American Society of Reproductive Medicine (ESHRE/ASRM) PCOS Consensus Workshop Group diagnostic criteria, i.e., the presence of at least two out of three of the following criteria: clinical and/or biochemical hyperandrogenism, oligo/anovulation, and polycystic ovaries on an ultrasound (≥12 follicles measuring 2–9 mm in diameter or an ovarian volume >10 mL in at least one ovary) [2]. The exclusion criteria for all subjects included other causes of irregular menstrual cycles and/or androgen excess, i.e., hyperprolactinemia; Cushing's syndrome (excluded on the basis of history taking and a physical examination); late-onset congenital adrenal hyperplasia (excluded on the basis of serum levels of 17-hydroxyprogesterone); hypothyroidism or hyperthyroidism; pregnancy (excluded on the basis of an appropriate test) and breastfeeding; type 1 or type 2 diabetes; chronic or acute infection (within the previous 30 days); any other serious medical condition; hormonal contraception and/or anti-androgen therapy (within the previous 6 months); and the use of medications for obesity, hyperglycemia, dyslipidemia, or hypertension.

The study protocol was approved by the Ethics Committee of the Medical University of Białystok, Poland (approval no. APK.002.171.2020) and was concordant with the Declaration of Helsinki. After being fully informed on the purpose and procedures of the study, all subjects signed an informed consent form.

2.2. Dietary Intake

Subjects completed a consecutive three-day dietary diary to identify the macronutrient and micronutrient intake. Dietary intake was assessed on the basis of a completed questionnaire regarding the type and amount of products consumed on the previous three days. The subjects were instructed to maintain their lifestyle (including eating behaviors) before the blood collection, and they were asked to record the food intake during the three days preceding the blood collection. The reported amount of

studied food items was converted to grams using household measures. For mixed meals, nutrients were calculated based on their components. Food intake data obtained from the participants were analyzed for energy, protein, carbohydrates, total fat, SFA, MUFA, PUFA, long chain polyunsaturated fatty acids (LC-PUFA), total cholesterol, dietary fiber, vitamins, and trace elements, as well as the percentage of energy from protein, carbohydrate, fat, and alcohol. The content of daily nutrient intake was calculated by the Diet 5.0 program developed by the Institute of Food and Nutrition, Poland.

2.3. Anthropometric Measurements

All women underwent a physical examination. The body mass index was calculated as the body weight in kilograms divided by the height in meters squared (kg/m^2). The waist-hip ratio (WHR) was calculated from the waist circumference (the smallest circumference between the rib cage and the iliac crest) and hip circumference (the maximum circumference at the level of the femoral trochanters), measured in the standing position.

2.4. Biochemical Analysis

Blood was sampled in the morning between the 3rd and 6th day of the menstrual cycle, or, if the woman suffered from amenorrhea, in any phase of the cycle at least 3 months from the last spontaneous menses. Fasting concentrations of plasma glucose and serum insulin, as well as plasma concentrations of glucose and serum levels of insulin two hours after the ingestion of 75 g of glucose, were assessed. Plasma glucose concentrations were determined with the hexokinase method and serum insulin levels were determined by the immunoradiometric method (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) (minimum detectable concentration (MDC)—1 µIU/mL; intra-assay coefficient of variation (CV)—below 2.2%, inter-assay CV—below 6.5%). Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were assessed by the enzymatic colorimetric method (Cobas c111, Roche Diagnostic Ltd., Rotkreuz, Switzerland). The plasma low-density lipoprotein cholesterol (LDL-C) concentration was calculated with Friedewald's formula.

Serum leptin concentrations were measured using an immunoenzymatic method (Human Leptin ELISA, BioVendor, Brno, Czech Republic) (MDC—0.2 ng/mL; intra-assay CV—5.9%, inter-assay CV—5.6%). Total and active ghrelin concentrations were measured by the radioimmunometric method, using specific antibodies for the total and active ghrelin form, respectively. Total ghrelin concentrations were assayed using the commercial kit for ghrelin (total) (GHRT-89HK, RIA, Millipore, USA, with MDC—100 pg/mL, intra-assay CV—below 10.0%, inter-assay CV—below 17.8%). The level of the active form of ghrelin was measured by a ghrelin (active) kit (GHRA-88HK, RIA, Millipore, Burlingtone, MA, USA, with MDC—10 pg/mL, intra-assay CV—below 9.5%, inter-assay CV—below 16.2%). Accordingly, the leptin/ghrelin ratio was calculated.

The levels of serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were assessed with the immunoradiometric method (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) (LH: intra-assay CV—below 3.9%, inter-assay CV—below 8%, FSH: intra-assay CV—below 2%, inter-assay CV—below 4.4%). The serum concentration of estradiol was determined by a radioimmunoassay (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) (MDC—2.7 pg/mL, intra-assay and inter-assay CV—4.7% and 10.4%, respectively). The measurement of total testosterone was performed using a radioimmunoassay (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) (MDC—0.05 ng/mL, intra-assay CV—3.3%, inter-assay CV—4.8%). Serum sex hormone–binding globulin (SHBG) was measured by an immunoradiometric assay (ZenTech, Angleur, Belgium) (intra-assay CV—below 5.2%, inter-assay CV—below 5.8%). The free androgen index (FAI) was calculated as the serum total testosterone (nmol/L) × 100/SHBG (nmol/L) ratio [37]. The serum TSH concentration was measured with the immunoradiometric method (DIAsource ImmunoAssays S.A., Louvain-La-Neuve, Belgium) (sensitivity 0.025 µIU/mL; intra-assay CV—0.6%; inter-assay CV—2.1%).

Insulin resistance was estimated by using the homeostasis model assessment index (HOMA-IR), which was calculated according to the following formula: (fasting insulin [μU/mL] × fasting glucose [mmol/L])/22.5 [38].

2.5. Statistical Analysis

Statistical analyses were performed using the Statistica 13.3 package (Statsoft, Cracow, Poland). The variables were tested for a normal distribution using the Shapiro–Wilk test. Due to a non-normal distribution of the data, non-parametric tests were applied and all values were expressed as the median and interquartile range. Comparisons of the PCOS and control group were performed by the Mann–Whitney U test. The Spearman test was used for correlation analysis. A p-value < 0.05 was considered statistically significant. We did not correct for multiple correlation analyses employing the same subjects and involving many parameters.

3. Results

The clinical and biochemical characteristics of the studied groups are presented in Table 1. The studied groups did not differ significantly in terms of BMI and WHR (all $p > 0.05$), although women with PCOS were younger than controls. In the PCOS group, the serum level of total testosterone and FAI were significantly higher in comparison to the control group, whereas the serum SHBG concentration was found to be lower in the PCOS group in comparison to the control group (all $p < 0.05$) (Table 1).

Table 1. Clinical and biochemical characteristics of the studied groups.

	Control Group (n = 34)	PCOS (n = 39)	p Value
Age (years)	26 (24.0–28.0)	23 (21–27)	<0.01 *
BMI (kg/m^2)	22.92 (20.64–24.9)	23.54 (21.47–25.93)	0.24
WHR	0.8 (0.77–0.85)	0.83 (0.79–0.87)	0.12
FSH (IU/L)	5.64 (4.43–6.56)	5.69 (4.39–7.0)	0.62
LH (IU/L)	3.79 (2.93–6.0)	4.05 (2.94–5.56)	0.62
Estradiol (ng/L)	66.68 (48.46–77.96)	58.26 (49.17–76.46)	0.72
TT (ng/mL)	0.63 (0.49–0.79)	0.78 (0.61–0.89)	0.01 *
SHBG (nmol/L)	72.23 (56.51–91.36)	47.02 (31.56–64.68)	<0.01 *
FAI	2.79 (2.01–4.01)	5.03 (2.86–8.5)	<0.01 *
TSH (mIU/L)	1.69 (1.36–2.33)	2.17 (1.34–2.8)	0.25
Glucose 0′ OGTT (mg/dL)	92 (88–99)	93 (89–98)	0.81
Glucose 120′ OGTT (mg/dL)	94 (86–104)	96 (80–106)	0.97
Insulin 0′ OGTT (uIU/mL)	7.68 (6.78–10.16)	9.01 (6.36–11.67)	0.42
Insulin 120′ OGTT (uIU/mL)	34.05 (22.3–45.21)	38.37 (25.3–62.91)	0.15
HOMA-IR	1.81 (1.52–2.31)	2.19 (1.42–2.86)	0.47
Total cholesterol (mg/dL)	170.5 (149–195)	172 (155–180)	0.62
HDL-cholesterol (mg/dL)	73 (60–81)	68 (57–75)	0.11
LDL-cholesterol (mg/dL)	88.9 (72–105)	90.8 (81–103)	0.76
TG (mg/dL)	50.5 (40–70)	57 (47–79)	0.14
Ghrelin (total) (pg/mL)	1017.60 (823.06–1124.05)	869.39 (702.34–1101.45)	0.08
Ghrelin (active) (pg/mL)	39.62 (33.58–51.12)	41.95 (32.53–55.7)	0.78
Leptin (ng/mL)	9.94 (5.59–14.94)	12.84 (5.68–19.75)	0.46
Leptin/Ghrelin ratio	0.01 (0.01–0.02)	0.01 (0.01–0.03)	0.28

Values are expressed as the median (interquartile range): * $p < 0.05$. Abbreviations: BMI: body mass index; WHR: waist-hip ratio; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; SHBG: sex hormone-binding globulin; FAI: free androgen index; TSH: thyroid-stimulating hormone; OGTT: oral glucose tolerance test; HOMA-IR: homeostasis model assessment of insulin resistance; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PCOS: polycystic ovary syndrome.

We did not observe significant differences in total ghrelin, active ghrelin, and leptin serum concentrations or the leptin/ghrelin ratio between the groups (all $p > 0.05$) (Table 1).

The studied groups did not differ in the amount of daily macronutrient (proteins, fats, and carbohydrates), as well as SFA, MUFA, PUFA, LC-PUFA, and micronutrient intake (sodium, potassium, calcium, phosphor, magnesium, iron, zinc, Vitamin A, Vitamin E, Vitamin D, Vitamin C, Vitamin B3, Vitamin B6, Vitamin B12, and iodine) (all $p > 0.05$). Moreover, no differences in the total energy intake during the day were observed between the studied groups ($p = 0.51$). We did not observe differences between the percentage of energy from fat, proteins, and carbohydrates between PCOS women and the control group (all $p > 0.05$) (Table 2).

Table 2. Macronutrient and micronutrient intake in the studied groups.

	Control Group (n = 34)	PCOS (n = 39)	p Value
Carbohydrate (g)	224.74 (167.37–278.24)	201.01 (166.21–227.37)	0.28
Protein (g)	64.58 (58.22–75.3)	66.34 (59.67–78.49)	0.4
Total fat (g)	51.58 (42.52–63.79)	52.35 (36.91–68.46)	1
SFA (g)	18.40 (15.21–22.59)	18.72 (13.56–23.05)	0.74
MUFA (g)	20.9 (16.2–25.76)	21.89 (14.5–28.59)	0.95
PUFA (g)	7.83 (5.47–10.46)	7.24 (5.52–10.52)	0.8
LC-PUFA (g)	0.06 (0.02)	0.07 (0.03–0.25)	0.55
Total dietary cholesterol (mg)	190.11 (154.66–290.59)	239.19 (154.37–307.62)	0.53
Total dietary fiber (g)	17.96 (12.89–26.33)	16.02 (12.21–22.16)	0.32
Total energy intake (kcal)	1575.20 (1240.07–1935.8)	1556.85 (1217.86–1791.39)	0.51
Percentage of energy from carbohydrate (%)	52.0 (43.51–56.27)	49.55 (43.16–52.52)	0.27
Percentage of energy from protein (%)	16.45 (14.56–19.41)	18.39 (15.28–21.39)	0.13
Percentage of energy from fat (%)	28.26 (24.25–36.04)	30.58 (26.57–35.05)	0.69
Sodium (mg)	2507.58 (2240.78–3177.19)	2454.1 (2161.57–3307.55)	1
Potassium (mg)	2864.78 (2205.7–4234.38)	2725.26 (2304.4–3706.78)	0.47
Calcium (mg)	643.95 (458.71–807.84)	603.75 (418.67–773.5)	0.33
Phosphor (mg)	1120.78 (902.5–1449.26)	1138.16 (958.67–1403.96)	0.87
Magnesium (mg)	274.67 (232.25–330.97)	287.56 (206.36–351.9)	0.85
Iron (mg)	9.23 (7.52–15.68)	9.21 (8.08–12.11)	0.65
Zinc (mg)	8.3 (6.87–9.8)	8.43 (6.89–9.72)	0.89
Vitamin A (µg)	792.2 (614.91–1128.55)	839.89 (537.03–1196.49)	0.63
Vitamin E (mg)	7.43 (4.96–11.42)	7.7 (5.35–9.85)	0.71
Vitamin B3 (mg)	18.57 (12.45–23.04)	17.27 (12.68–22.5)	0.93
Vitamin B6 (mg)	1.62 (1.34–2.26)	1.69 (1.3–2.18)	0.82
Vitamin B12 (mg)	2.44 (2.02–3.93)	2.56 (1.8–4.13)	0.93
Vitamin C (mg)	70.59 (44.93–111.29)	81.3 (60.8–112.08)	0.5
Vitamin D (µg)	2.14 (1.18–3.35)	1.66 (1.32–3.23)	0.87
Iodine (µg)	95.53 (62.49–128.72)	95.99 (69.72–131.17)	0.57

Values are expressed as the median (interquartile range): The level of significance was accepted at $p < 0.05$. Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids; PCOS: polycystic ovary syndrome.

We observed relationships between the BMI and serum concentration of leptin ($r = 0.61$, $p < 0.01$) and ghrelin ($r = -0.39$, $p = 0.01$) in PCOS women. We also noticed a correlation between the BMI and serum concentration of leptin ($r = 0.69$, $p < 0.01$), but no relationship between the BMI and serum level of ghrelin ($r = -0.1$, $p = 0.56$) in the control group.

In the PCOS group, the serum leptin concentration correlated with the total fat ($r = 0.36$, $p = 0.02$), SFA ($r = 0.43$, $p < 0.01$), and MUFA ($r = 0.37$, $p = 0.02$) contained in the diet, whereas serum ghrelin

concentrations correlated, in an inverse manner, with the total fat (r = −0.37, $p = 0.02$), total cholesterol (r = −0.36, $p = 0.02$), MUFA (r = −0.37, $p = 0.02$), PUFA (r = −0.34, $p = 0.03$), and LC-PUFA (r = −0.38, $p = 0.02$) contained in the diet. Additionally, in the group of PCOS women, we found a negative correlation between the acylated ghrelin concentration and proteins contained in the diet (r = −0.35, $p = 0.03$). Furthermore, in the PCOS group, we observed a positive association between the leptin/ghrelin ratio and total fat (r = 0.45, $p < 0.01$), SFA (r = 0.49, $p < 0.01$), MUFA (r = 0.45, $p < 0.01$), and PUFA (r = 0.34, $p = 0.04$) contained in the diet. There was also a correlation of borderline significance between the leptin/ghrelin ratio and the percentage of energy from fats (r = 0.32, $p = 0.05$) (Table 3).

Table 3. Correlations of leptin and ghrelin concentrations and the leptin/ghrelin ratio with the dietary intake of macronutrients.

	Control Group (n = 34)			PCOS (n = 39)		
	Leptin	**Ghrelin**	**Leptin/Ghrelin**	**Leptin**	**Ghrelin**	**Leptin/Ghrelin**
Total energy intake (kcal)	r = −0.14 $p = 0.45$	r = 0.23 $p = 0.19$	r = −0.18 $p = 0.32$	r = 0.19 $p = 0.26$	r = −0.2 $p = 0.23$	r = 0.25 $p = 0.13$
Carbohydrate (g)	r = −0.05 $p = 0.79$	r = 0.34 $p = 0.05$	r = −0.13 $p = 0.48$	r = 0.06 $p = 0.7$	r = −0.01 $p = 0.94$	r = 0.11 $p = 0.52$
Protein (g)	r = −0.45 $p < 0.01$ *	r = 0.07 $p = 0.7$	r = −0.44 $p < 0.01$ *	r = 0.21 $p = 0.19$	r = −0.19 $p = 0.24$	r = 0.2 $p = 0.23$
Total fat (g)	r = −0.02 $p = 0.9$	r = 0.01 $p = 0.94$	r = −0.01 $p = 0.96$	r = 0.36 $p = 0.02$ *	r = −0.37 $p = 0.02$ *	r = 0.45 $p < 0.01$ *
SFA (g)	r = −0.12 $p = 0.5$	r = −0.02 $p = 0.92$	r = −0.09 $p = 0.63$	r = 0.43 $p < 0.01$ *	r = −0.24 $p = 0.13$	r = 0.49 $p < 0.01$ *
MUFA (g)	r = 0.04 $p = 0.83$	r = −0.02 $p = 0.9$	r = 0.05 $p = 0.79$	r = 0.37 $p = 0.02$ *	r = −0.37 $p = 0.02$ *	r = 0.45 $p < 0.01$ *
PUFA (g)	r = −0.07 $p = 0.69$	r = −0.07 $p = 0.71$	r = −0.02 $p = 0.89$	r = 0.25 $p = 0.12$	r = −0.34 $p = 0.03$ *	r = 0.34 $p = 0.04$ *
LC-PUFA (g)	r = −0.06 $p = 0.75$	r = 0.09 $p = 0.59$	r = −0.12 $p = 0.5$	r = 0.1 $p = 0.56$	r = −0.38 $p = 0.02$ *	r = 0.18 $p = 0.27$
Total dietary cholesterol (mg)	r = −0.09 $p = 0.62$	r = 0.04 $p = 0.82$	r = −0.1 $p = 0.56$	r = 0.13 $p = 0.45$	r = −0.36 $p = 0.02$ *	r = 0.2 $p = 0.23$
Percentage of energy from carbohydrate (%)	r = 0.12 $p = 0.48$	r = 0.32 $p = 0.07$	r = 0.02 $p = 0.91$	r = −0.11 $p = 0.52$	r = 0.22 $p = 0.17$	r = −0.12 $p = 0.46$
Percentage of energy from protein (%)	r = −0.35 $p = 0.04$ *	r = −0.24 $p = 0.16$	r = −0.27 $p = 0.13$	r = −0.05 $p = 0.78$	r = −0.1 $p = 0.53$	r = −0.1 $p = 0.56$
Percentage of energy from fat (%)	r = −0.01 $p = 0.97$	r = −0.24 $p = 0.18$	r = 0.06 $p = 0.72$	r = 0.25 $p = 0.12$	r = −0.28 $p = 0.09$	r = 0.32 $p = 0.05$

Data are derived from the Spearman correlation coefficient. The level of significance was accepted at * $p < 0.05$. Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids; PCOS: polycystic ovary syndrome.

In the control group, we did not observe relationships between serum ghrelin concentrations and dietary macronutrients (all $p > 0.05$). We found a correlation between the serum leptin concentration and protein intake in the diet (r = −0.45, $p < 0.01$) and between the leptin/ghrelin ratio and proteins in the diet (r = −0.44, $p < 0.01$) (Table 3).

In the PCOS group, we observed a negative association of HOMA-IR with serum ghrelin levels (r = −0.4, $p = 0.03$) and a positive relationship with the serum leptin concentration (r = 0.5, $p < 0.01$). We also found an association between HOMA-IR and the dietary intake of total fat (r = 0.38, $p = 0.03$) and MUFA (r = 0.35, $p = 0.04$) in this group (Table 4).

Table 4. Correlations between hormones, HOMA-IR, and the dietary intake of macronutrients in polycystic ovary syndrome (PCOS) women.

PCOS (n = 39)			
	HOMA-IR	**SHBG**	**FAI**
Leptin (ng/mL)	r = 0.5 $p < 0.01$ *	r = −0.4 $p < 0.01$ *	r = 0.38 $p = 0.01$
Total ghrelin (pg/mL)	r = −0.4 $p = 0.03$ *	r = 0.2 $p = 0.1$	r = 0.2 $p = 0.1$
Total energy intake (kcal)	r = 0.3 $p = 0.08$	r = −0.31 $p = 0.06$	r = 0.16 $p = 0.35$
Carbohydrate (g)	r = 0.26 $p = 0.14$	r = −0.26 $p = 0.11$	r = 0.18 $p = 0.28$
Protein (g)	r = 0.41 $p = 0.02$ *	r = −0.32 $p = 0.04$	r = 0.19 $p = 0.25$
Total fat (g)	r = 0.38 $p = 0.03$ *	r = −0.38 $p = 0.02$ *	r = 0.22 $p = 0.18$
SFA (g)	r = 0.26 $p = 0.14$	r = −0.51 $p < 0.01$ *	r = 0.34 $p = 0.04$ *
MUFA (g)	r = 0.35 $p = 0.04$ *	r = −0.35 $p = 0.03$ *	r = 0.19 $p = 0.25$
PUFA (g)	r = 0.34 $p = 0.05$	r = −0.28 $p = 0.08$	r = 0.14 $p = 0.38$
LC−PUFA (g)	r = 0.12 $p = 0.49$	r = −0.12 $p = 0.46$	r = 0.11 $p = 0.52$
Total dietary cholesterol (mg)	r = 0.15 $p = 0.4$	r = −0.13 $p = 0.43$	r = 0.06 $p = 0.71$
Percentage of energy from carbohydrate (%)	r = −0.04 $p = 0.83$	r = 0.05 $p = 0.78$	r = 0.05 $p = 0.75$
Percentage of energy from protein (%)	r = 0.01 $p = 0.94$	r = 0.16 $p = 0.33$	r = −0.18 $p = 0.28$
Percentage of energy from fat (%)	r = 0.22 $p = 0.2$	r = −0.24 $p = 0.14$	r = 0.11 $p = 0.49$

Values are expressed as the median (interquartile range): * $p < 0.05$. Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids; PCOS: polycystic ovary syndrome; HOMA-IR: homeostasis model assessment of insulin resistance; SHBG: Serum sex hormone–binding globulin; FAI: free androgen index.

We observed a positive relationship between FAI and the serum levels of leptin (r = 0.38, $p = 0.01$) and a negative association between the serum concentration of SHBG and serum levels of leptin (r = −0.4, $p < 0.01$) in PCOS women (Table 4). In the control group, we did not observe relationships of total testosterone and FAI with serum levels of leptin and ghrelin (all $p > 0.05$).

Furthermore, in the PCOS group, we observed a positive association between FAI and SFA intake (r = 0.34, $p = 0.04$) and a negative association between SHBG concentrations and the dietary intake of total fat (r = −0.38, $p = 0.02$), SFA (r = −0.51, $p < 0.01$), and MUFA (r = −0.35, $p = 0.03$) (Table 4). In the control group, we did not observe relationships of FAI and SHBG with fat or subgroups of fatty acids (all $p > 0.05$).

4. Discussion

In our study, we did not observe significant differences in diet and serum leptin and ghrelin concentrations between PCOS patients and control subjects. However, we found a positive association of the dietary total fat, SFA, and MUFA intake with serum leptin concentrations exclusively in non-obese PCOS women; whereas, in the control group, we did not notice the above associations. Additionally, we observed a negative relationship between serum levels of leptin and HOMA-IR and a positive association between the dietary fat intake and HOMA-IR. According to the available evidence, dietary SFA increase the risk of obesity [9], which may result in changes in leptin concentrations. Some studies have reported that a high-fat diet is associated with an increased leptin concentration [39–41],

while other studies have shown that dietary fat reduced the serum leptin concentration [26,42,43]. In turn, some researchers did not show the influence of dietary fat on serum leptin concentrations [44]. The above conflicting data may reflect the lack of adjustment for gender, the total energy intake, and BMI. Yannakoulia et al. [28] found that the free leptin index is negatively associated with the energy intake from carbohydrates and positively associated with the energy intake from dietary fat. Moreover, they observed that the serum leptin concentration reflects the amount of body fat and is higher in women compared with men. Sexual dimorphism of the body fat distribution or differences in sex steroid hormones between genders have been proposed to be responsible for the observed differences in leptin concentrations [28]. It has been found that the intake of SFA increases serum leptin concentrations [45]. This relationship can be explained by the induction of insulin resistance by dietary fat and SFA [46]. The association between dietary fat intake and an increase in the serum leptin concentration could be related to changes in serum insulin concentrations. As mentioned previously, we found a positive association between the dietary fat intake and HOMA-IR and a positive relationship between serum levels of leptin and HOMA-IR. It has been previously reported that serum leptin concentrations are positively associated with insulin resistance in obese women [47], and that an elevated serum insulin concentration that follows insulin resistance can stimulate leptin mRNA expression in adipocytes and increase circulating leptin concentrations [48,49]. Moreover, the available data have shown that the ingestion of a high-fat diet induces a state of leptin resistance in the absence of an increasing serum leptin concentration and body fat mass in rodents [50]. These findings suggest that certain macronutrients may be involved in the induction of leptin resistance prior to an increase in the leptin concentration and body weight and may play an important role in the development of obesity.

In our study, we did not observe an association between the dietary fiber intake and serum leptin concentration; however, a study carried out on young Japanese women showed that a higher intake of dietary fiber was associated with a lower serum leptin concentration [29]. Additionally, the protein and PUFA intake was inversely related to the serum leptin concentration, although this association was dependent on the intake of other nutrients. The suggested explanation of this pattern is that the dietary fiber intake may decrease the serum leptin concentration directly through a decrease in leptin production or indirectly through an increase in leptin sensitivity, which in turn leads to a decrease in leptin production through feedback mechanisms [29].

In our study, we did not observe differences in serum levels of ghrelin between women with PCOS and control subjects, although our results indicate that ghrelin serum concentrations are connected, in an inverse manner, to the dietary intake of total fat, MUFA, PUFA, LC-PUFA, and total cholesterol in the PCOS group. The findings of previously conducted studies suggest that, besides leptin disturbances, an imbalance in the ghrelin concentration may also be associated with weight gain in women with PCOS [35,36,51,52]. Our results contrast with those of some other studies, which showed that an increased intake of fat and carbohydrates is associated with higher ghrelin concentrations [27] and those which showed no relationship between dietary macronutrients and serum ghrelin concentrations [26,53]. The above conflicting data may reflect the differences in ethnic groups; lack of adjustment for potential confounding factors, such as the BMI of the studied group; or different methods of diet analysis. An inverse association between ghrelin concentrations and the dietary intake of fat and fatty acids observed in our study can be explained by the involvement of fat and fatty acids in the induction of insulin resistance, which is suggested by the positive relationship of total fat and MUFA in the diet with HOMA-IR. Moreover, it has been previously shown that an increased insulin concentration leads to a decrease in the serum ghrelin concentration [54,55].

It has been proposed that in the analysis of the hormonal response to meal intake, leptin and ghrelin effects should be combined [56], as the energy balance and the final clinical effect depend on the interplay between both hormones [57]. Therefore, in our study, we also analyzed the ratio of leptin and ghrelin. In the PCOS group, we found a positive correlation between the leptin/ghrelin ratio and total fat, SFA, MUFA, and PUFA contained in the diet. Previous studies noted that a higher leptin/ghrelin ratio was associated with a lower resting metabolic rate [58]. It has been found that overweight and

obese subjects present a higher leptin/ghrelin ratio [56]. Therefore, we can suspect that a higher intake of total fat, SFA, MUFA, and PUFA in PCOS individuals may increase the leptin/ghrelin ratio and contribute to the development of obesity.

As previously mentioned, in our study, we did not find any significant differences in leptin and ghrelin concentrations between the PCOS patients and the control group. These results support the previous findings of other studies, which presented similar leptin and ghrelin levels in both PCOS patients and control group [59]. Contradictory results have been reported by Pekhlivanov et al. [60] and Jalilian et al. [61], who noted increased leptin concentrations in PCOS patients. In addition, it has been found, similarly to our study, that the serum leptin concentration is closely related to BMI [61]. Therefore, these controversial results and a lack of differences in the serum leptin concentration shown in our study may result from the fact that the studied groups did not differ in BMI and our groups were non-obese. This statement is based on the fact that leptin is predominantly produced by adipocytes, and therefore, patients with a higher BMI, which reflects the amount of adipose tissue, may present higher serum leptin concentrations [61]. Interestingly, it has been proposed that, in part by increased intra-follicular levels of leptin, obesity directly affects ovarian functions in PCOS and may induce a relative resistance to gonadotropins [62]. In addition, the small size of the group may affect the obtained results, which was indicated in the limitations of this study. Another explanation of the obtained results could be connected to the fact that we only studied Caucasian women, in contrast to other studies.

In our study, we also observed a negative correlation between the serum acylated ghrelin concentration and diet protein intake in the group of PCOS women. Proteins have been shown to reduce the appetite more than equivalent calories from carbohydrates or lipids [63–65]. The higher satiety associated with the protein intake seems to be related to ghrelin suppression [64]. Studies have shown that postprandial ghrelin suppression is greatest after protein ingestion [66,67]. Moreover, protein intake suppresses ghrelin longer than other types of macronutrients [64,68]. The prolonged suppression of ghrelin after the intake of protein might be associated with a prolonged emptying of proteins from the stomach [66]. Moreover, proteins are able to stimulate the secretion of specific gastrointestinal peptides (cholecystokinin, glucagon-like peptide-1, and gastric inhibitory polypeptide), which delay gastric emptying [64,69]. Additionally, after protein intake, the concentration of circulating amino acids increases, which stimulates hepatic gluconeogenesis, preventing hypoglycemia and thus causing satiety [69]. Therefore, based on the inverse relationship between the serum acylated ghrelin concentration and diet protein intake in the PCOS group, we can speculate that the mechanisms described above are sufficient in these women. However, this association requires further research.

We also noticed that PCOS women had higher concentrations of serum testosterone and FAI, whereas SHBG was lowered compared to healthy women. Additionally, in our study, we only observed a relationship between FAI and serum levels of leptin in PCOS women. In the literature, the relationship between the serum concentration of leptin and androgens in PCOS is still controversial, and the interactions between gonadotropins, insulin, and leptin are very complex [35]. Leptin inhibits the insulin-mediated promotion of gonadotropin-stimulated ovarian steroidogenesis [35]. However, in our study, we did not observe differences in the serum concentration of leptin and ghrelin between PCOS women and healthy controls, despite the fact that a high proportion of women with PCOS were hyperandrogenic. In the aforementioned study, it has been shown that an elevated serum concentration of leptin is associated with elevated levels of testosterone in PCOS women. Therefore, it has been postulated that there is as yet an undefined mechanism, probably mediated by insulin, that would explain these relationships [35]. Elevated levels of androgens in PCOS are due to an excessive production of androgens by the ovaries [70] in the state of insulin resistance [71]. Dietary components seem to play a role in the regulation of androgens and SHBG concentrations in PCOS subjects [72,73], and it is possible that insulin resistance is involved in this process. It has been found that diets containing higher amounts of SFA are able to reduce insulin sensitivity more than diets consisting of other types of fatty acids [74]. In our study, we found that SFA correlate positively with FAI and negatively with SHBG.

Moreover, the dietary total fat intake was negatively associated with SHBG concentrations. Given the important role of insulin resistance in the development of hyperandrogenemia, it is possible that fat and saturated fatty acids induce insulin resistance and compensatory hyperinsulinemia, which affect the changes in the androgen level.

The major limitation of the present study is that women in the PCOS group were younger than the control individuals, what could have affected the results when comparing the differences in parameters which are partially dependent on age. Another limitation of our study is the relatively small sample size; however, the participants were very well-characterized. Moreover, we only measured leptin and ghrelin concentrations in the fasting state, without an assessment of the postprandial state or hormone response to given macronutrient test meals. The use of a self-reported dietary intake may also be considered a limitation of the present study because it may not accurately reflect the real amount and type of food consumed. In addition, the results indicate a relationship of leptin and ghrelin with dietary macronutrients; however, they do not explain the causal relationships, but only the associations. Considering the results of our study, which suggest an association of dietary macronutrients with serum leptin and ghrelin concentrations, it seems to be important to find the causal relationship arising from these findings. Therefore, future research is needed. In order to reach a full understanding of the mechanism and effect of dietary macronutrients, as well as the possible interactions between dietary components and the energy balance, the omics approach may be considered. This approach would provide more accurate information on mechanisms involved in the response to the consumption of different dietary macronutrients [75]. Omics-based nutrition research can identify those individuals who are likely to respond maximally and will provide personalized dietary recommendations [76,77] for women with PCOS. This is very important for this group of patients, as they have an increased risk of obesity, metabolic syndrome, and type 2 diabetes in relation to the general population [3]. However, this approach requires advanced skills for data analysis and is relatively expensive; therefore, the availability of sufficient funding is the main limiting factor for performing such studies [78]. Additionally, in our study, we included a unique, non-obese group of women with PCOS, compared to their control counterparts. In our study, PCOS women differed from the control group in terms of the serum levels of total testosterone, SHBG, and FAI, whereas we did not observe differences between the studied groups in terms of the serum concentration of glucose and insulin during OGTT, as well as HOMA-IR. However, we used HOMA-IR instead of a euglycemic hyperinsulinemic clamp to estimate insulin resistance and we therefore cannot exclude insulin resistance in the non-obese PCOS group. On the other hand, this group differs profoundly from obese PCOS women. Therefore, it is an advantage of our study that we examined non-obese subjects, because the impact of obesity on the studied parameters was removed. It is important to study non-obese PCOS women, because they could present a tendency for weight gain and obesity [3] in the future. Moreover, it has been shown that women with PCOS are characterized by atherogenic dyslipidemia [79]. In our study, we did not observe differences in serum levels of lipids between non-obese PCOS women and the control group, but we found relationships between fat in the diet and the leptin and ghrelin serum concentration. Therefore, dietary fat could impact the physiological function in PCOS women, and it could be indicative of an abnormal lipid function in PCOS per se. Therefore, it seems to be important to assess whether the non-obese PCOS group has any metabolic abnormalities and what kind of abnormalities they present before the onset of obesity, which could be accompanied by leptin resistance [24]. Based on our findings, we can speculate that limiting food products high in fat (especially SFA) would be beneficial for the appropriate control and management of the metabolic status of PCOS patients. However, to confirm this hypothesis, a prospective study should be performed.

5. Conclusions

In PCOS women, dietary components, especially the total fat intake and the types of dietary fat, as well as HOMA-IR, correlate positively with serum leptin concentrations and negatively with serum ghrelin concentrations, which may influence the energy balance.

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6. Streszczenie w języku polskim

Wstęp

Zespół policystycznych jajników (Polycystic Ovary Syndrome, PCOS) jest najczęstszą endokrynopatią u kobiet w wieku rozrodczym. Według kryteriów rotterdamskich wyodrębniono cztery kliniczne fenotypy zespołu policystycznych jajników: fenotyp A spełniający wszystkie trzy kryteria PCOS: kliniczny/biochemiczny hiperandrogenizm, zaburzenia miesiączkowania i policystyczna morfologia jajników (PCOM), fenotyp B, w którym występuje kliniczny/biochemiczny hiperandrogenizm i zaburzenia miesiączkowania, fenotyp C, charakteryzujący się klinicznym/biochemicznym hiperandrogenizmem i PCOM, oraz fenotyp D, w którym obserwuje się zaburzenia miesiączkowania i PCOM. W PCOS obok zaburzeń funkcji jajnika występują zaburzenia metaboliczne, takie jak otyłość brzuszna i insulinooporność. W insulinooporności, której towarzyszy hiperinsulinemia, insulina działa na komórki tekalne jajnika, powodując wzmożoną produkcję androgenów. Badania wykazały, że zwiększone stężenia całkowitego testosteronu w surowicy u kobiet z PCOS są związane z nadmiarem tkanki tłuszczowej trzewnej, co może prowadzić do rozwoju insulinooporności i częstszego występowania zaburzeń tolerancji glukozy.

Nieprawidłowo zbilansowana dieta pacjentek z PCOS prowadzi do otyłości brzusznej oraz insulinooporności, jednak nie stwierdzono dotychczas, który makroskładnik odżywczy zawarty w diecie (białka, tłuszcze, węglowodany) jest najbardziej powiązany z rozwojem otyłości. Wcześniejsze badania wykazały, że do otyłości prowadzi zachwianie równowagi pomiędzy stężeniem leptyny i greliny w osoczu. Leptyna powoduje zmniejszenie łaknienia i wzrost wydatku energetycznego, natomiast grelina zwiększa apetyt.

Celem pracy była ocena stężeń androgenów, leptyny i greliny we krwi pacjentek z zespołem policystycznych jajników w korelacji z parametrami antropometrycznymi i dietą.

Materiał i Metody:

Pierwszą badaną grupę stanowiło 89 pacjentek z PCOS przydzielonych do jednego z czterech fenotypów sklasyfikowanych zgodnie z kryteriami rotterdamskimi (fenotyp A, B, C, D) oraz 57 kobiet z grupy kontrolnej. W grupie tej oznaczono w surowicy stężenia

testosteronu całkowitego, androstendionu i DHEA-S metodą radioimmunologiczną, natomiast stężenia w surowicy globuliny wiążącej hormony płciowe oznaczono metodą radioimmunometryczną. U wszystkich kobiet wyliczono wskaźnik insulinooporności (Homeostasis Model Assessment of Insulin Resistance, HOMA-IR) oraz obliczono współczynnik wolnych androgenów (free androgen index, FAI) oraz wykonano badanie dwuwiązkowej absorpcjometrii promieniowania rentgenowskiego (dual-energy X-ray absorptiometry, DXA) szacując masę trzewnej tkanki tłuszczowej (visceral adipose tissue, VAT) oraz gynoidalnej tkanki tłuszczowej, a następnie obliczono wskaźnik tkanki tłuszczowej androidalnej (A) do gynoidalnej (G) (wskaźnik A/G).

Drugą grupę badaną stanowiły 73 kobiety: 39 kobiet z PCOS i 34 kobiety z grupy kontrolnej. U wszystkich badanych przeprowadzono wystandaryzowany 3-dniowy kwestionariusz spożycia składników pokarmowych. Następnie za pomocą programu Dieta 5.0 wyliczono spożywane składniki pokarmowe w g/dzień (białka, tłuszcze, węglowodany), a także w osoczu krwi stężenie leptyny oznaczono metodą immunoenzymatyczną natomiast stężenie całkowitej greliny oznaczono metodą radioimmunometryczną.

Wyniki

W fenotypach A, B i C FAI był istotnie wyższy w porównaniu z grupą kontrolną (wszystkie $p<0,01$), ponadto w fenotypie A był istotnie wyższy w porównaniu z fenotypem D ($p<0,01$). Jedynie w fenotypie A zaobserwowano większą masę VAT oraz większy wskaźnik A/G (wszystkie $p<0,01$) w odniesieniu do grupy kontrolnej. We wszystkich fenotypach PCOS wykazano związek HOMA-IR z VAT oraz ze wskaźnikiem A/G (wszystkie $p<0,05$), ponadto stwierdzono korelację pomiędzy FAI i HOMA-IR w fenotypie A ($r=0,40$, $p=0,01$), fenotypie B ($r=0,47$, $p=0,03$) oraz fenotypie C ($r=0,66$, $p<0,01$). We wszystkich fenotypach PCOS obserwowano dodatnie korelacje FAI ze wskaźnikiem A/G (wszystkie $p<0,05$).

W grupie PCOS stężenie leptyny w osoczu korelowało dodatnio a greliny ujemnie z zawartością w diecie tłuszczów ($r=0,36$, $p=0,02$; $r=-0,37$, $p=0,02$). Jednocześnie w grupie PCOS wykazano zależność pomiędzy HOMA-IR a stężeniem leptyny w osoczu ($r=0,5$, $p<0,01$), orazpomiędzy HOMA-IR a stężeniem greliny w osoczu ($r=-0,4$, $p=0,03$). Ponadto wykazano dodatni związek pomiędzy HOMA-IR a zawartością w diecie tłuszczów ($r=0,38$, $p=0,03$).

Wnioski:

1. Pacjentki z fenotypem A PCOS, ze względu na większą masę trzewnej tkanki tłuszczowej mają większe ryzyko rozwoju zaburzeń metabolicznych w porównaniu z grupą kontrolną.
2. Masa trzewnej tkani tłuszczowej wpływa na rozwój insulinooporności oraz stężenie androgenów w surowicy zarówno w fenotypach normoandrogennych, jak i hiperandrogennych PCOS.
3. Uzyskane wyniki potwierdzają, że dieta bogatotłuszczowa u pacjentek z PCOS wiąże się z rozwojem insulinooporności, zaburzeniami w równowadze pomiędzy stężeniem leptyny i greliny w osoczu prowadząc do rozwoju otyłości.

7. Streszczenie w języku angielskim

Polycystic ovary syndrome (PCOS) is the common endocrinopathy in women of reproductive age. The Rotterdam criteria for PCOS recognize four clinical phenotypes of the syndrome. Phenotype A, which meets all three current criteria for PCOS: clinical/biochemical hyperandrogenism, menstrual dysfunction and polycystic ovarian morphology (PCOM), phenotype B involving clinical/biochemical hyperandrogenism and menstrual dysfunction, phenotype C characterized by clinical/biochemical hyperandrogenism and PCOM, phenotype D in which menstrual dysfunction and PCOM are observed. In PCOS, apart from ovarian dysfunction, we can observe metabolic disorders such as abdominal obesity and insulin resistance. In state of insulin resistance, accompanied by hyperinsulinemia, insulin acts on the ovarian theca cells, leading to increased production of androgens. It has been reported that increased serum testosterone levels in PCOS women are associated with excess of visceral fat amount, which may lead to development of insulin resistance and more frequent occurrence of impaired glucose tolerance. Improper diet balance of patients with PCOS leads to abdominal obesity and insulin resistance, however there are conflicting data on which macronutrient in the diet (proteins, fats, carbohydrates) is connected the most with human obesity. Previous studies have suggested a link between weight gain and imbalance in leptin and ghrelin concentrations in women with PCOS. Leptin reduces appetite and increases energy expanditure, while ghrelin increases appetite.

The aim of the study was to assess the concentrations of androgens, leptin and ghrelin in the blood of patients with polycystic ovary syndrome in correlation with anthropometric parameters and diet.

Material and Methods:

The first study group consisted of 146 women: 89 patients with PCOS divided into four phenotypes according to Rotterdam criteria (phenotype A, B, C, D) and 57 control women. In this group serum concentrations of total testosterone androstenedione and DHEA-S were measured with radioimmunoassay (DIAsource ImmunoAssays S.A., Belgium), whereas serum concentrations of serum sex hormone–binding globulin (SHBG) were assessed with immunoradiometric method (ZenTech, Angleur, Belgium). In all women the insulin resistance index (Homeostasis Model Assessment of Insulin Resistance, HOMA-IR) was calculated, the free androgen index (FAI) was calculated and the dual-energy X-ray absorptiometry (DXA) analysis was performed, estimating the weight visceral adipose tissue (VAT) and gynoid adipose tissue, and then the android (A) to gynoid (G) ratio (A/G ratio) was calculated.

In the second group of 73 women: 39 subjects with PCOS and 34 healthy women completed a consecutive three-day dietary diary to identify the macronutrient and micronutrient intake. Subsequently, using Diet 5.0 program the content of daily nutrients intake in g/day (proteins, fats, carbohydrates) were calculated and the concentration of leptin in the blood plasma was determined by the immunoenzymatic method (Human Leptin ELISA, BioVendor, Brno, Czech Republic), while the concentration of total ghrelin was determined by the radioimmunometric method (GHRT-89HK, RIA, Millipore, USA).

Results:

In phenotypes A, B and C, we observed higher FAI in comparison to the control group (all $p < 0.01$), as well as in phenotype A in comparison to phenotype D ($p < 0.01$). Only in phenotype A we found higher visceral adipose tissue (VAT) mass and android/gynoid ratio (A/G ratio) in comparison to the control group (all $p < 0.01$). We found

a positive correlations of HOMA-IR with VAT and A/G ratio in all phenotypes (all $p < 0.05$), moreover we found relationships between FAI and HOMA-IR in phenotype A ($r = 0.40$, $p = 0.01$), phenotype B ($r = 0.47$, $p = 0.03$) and phenotype C ($r = 0.66$, $p < 0.01$). Accordingly, A/G ratio was related with FAI in all phenotypes (all $p < 0.05$).

In the PCOS group, the plasma leptin concentration correlated positively, and ghrelin plsma concentration correlated negatively with the dietary fat content (r = 0.36, p = 0.02; r = -0.37, p = 0.02). Accordingly, in the PCOS group we found a positive association of HOMA-IR with serum leptin levels (r = 0.5, $p < 0.01$) and a negative relationship with the serum ghrelin concentration (r = −0.4, $p = 0.03$). Moreover, we found a positive relationship between HOMA-IR and total dietary fat (r = 0.38, $p = 0.03$).

Conclusions:

1. Due to the greater amount of visceral adipose tissue in the A PCOS phenotype, patients presenting this phenotype have a greater risk of developing metabolic disorders compared to the control group.
2. The amount of visceral adipose tissue is associated with insulin resistance and serum concentration of androgens in both the normoandrogenic and hyperandrogenic phenotypes of PCOS.
3. A high-fat diet in patients with PCOS is associated with the development of insulin resistance, disturbances in the balance between the concentration of leptin and ghrelin in the plasma, leading to the development of obesity.

8. Piśmiennictwo

Piśmiennictwo

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9. Oświadczenie współautorów

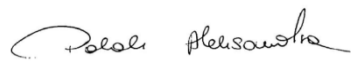
9.1. Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu (praca oryginalna)

Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: ***Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes.*** Journal of Clinical Medicine, 2020. 9(3), 732. doi: 10.3390/jcm9030732

Imię i nazwisko współautora	*Charakter udziału*	*Procentowy wkład*
doktorant – lek. Aleksandra Polak	Udział w planowaniu eksperymentów, przeprowadzanie eksperymentów prezentowanych w pracy, opracowanie i analiza wyników, przygotowanie manuskryptu, przygotowanie tabel wchodzących w skład manuskryptu	62%
Dr hab. n. med. Agnieszka Adamska	Stworzenie koncepcji pracy, udział w planowaniu eksperymentów, pomoc przy przygotowaniu manuskryptu, konsultacja merytoryczna	18%
Lek. Anna Krentowska	Pomoc przy rekrutowaniu pacjentów do badania, tworzeniu bazy danych, korekta językowa	7%
Dr n. med. Agnieszka Łebkowska	Pomoc przy rekrutowaniu pacjentów i tworzeniu bazy danych	1%
Mgr Justyna Hryniewicka	Współuczestnictwo w przeprowadzaniu eksperymentów prezentowanych w pracy	1%
Dr inż. Marcin Adamski	Pomoc w przeprowadzeniu analizy statystycznej	1%
Prof. dr hab. n. med. Irina Kowalska	Stworzenie koncepcji pracy, konsultacja merytoryczna	10%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Aleksandry Marii Polak

Podpis doktoranta



Białystok, 10.10.2020r.

Dr. hab. n. med. Agnieszka Adamska

Klinika Endokrynologii, Diabetologii

i Chorób Wewnętrznych

Uniwersytetu Medycznego w Białymstoku

ul. M. Skłodowskiej-Curie 24 A

15-276 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w publikacji:

Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: Body composition, serum concentrations of androgens and insulin resistance in different polycystic ovary syndrome phenotypes. Journal of Clinical Medicine, 2020; 9(3): 732. doi: 10.3390/jcm9030732, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 18% polegał na stworzeniu koncepcji pracy, planowaniu eksperymentów, pomocy przy przygotowaniu manuskryptu i konsultacji merytorycznej.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Białystok, 10.10.2020r.

Lek. Anna Krentowska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

Uniwersytetu Medycznego w Białymstoku

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15-276 Białystok

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Oświadczam, iż mój udział w publikacji:

Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: Body composition, serum concentrations of androgens and insulin resistance in different polycystic ovary syndrome phenotypes. Journal of Clinical Medicine, 2020; 9(3): 732. doi: 10.3390/jcm9030732, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 7% polegał na pomocy przy rekrutowaniu pacjentów, tworzeniu bazy danych oraz korekcie językowej.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Anna Krentowska

Białystok, 10.10.2020r.

Dr n. med. Agnieszka Łebkowska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

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15-276 Białystok

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Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: Body composition, serum concentrations of androgens and insulin resistance in different polycystic ovary syndrome phenotypes. Journal of Clinical Medicine, 2020; 9(3): 732. doi: 10.3390/jcm9030732, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 1% polegał na pomocy przy rekrutowaniu pacjentów i tworzeniu bazy danych.

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Dr n. med. Agnieszka Łebkowska
specjalista chorób wewnętrznych
2342201

Białystok, 10.10.2020r.

Dr Justyna Hryniewicka

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i Chorób Wewnętrznych

Uniwersytetu Medycznego w Białymstoku

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Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: Body composition, serum concentrations of androgens and insulin resistance in different polycystic ovary syndrome phenotypes. Journal of Clinical Medicine, 2020; 9(3): 732. doi: 10.3390/jcm9030732, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 1% polegał na pomocy w przeprowadzaniu eksperymentów prezentowanych w pracy.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Justyna Hryniewicka

Białystok, 10.10.2020r.

Dr inż. Marcin Adamski

Wydział Informatyki

Politechniki Białostockiej

ul. Wiejska 45A

15-351 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w publikacji:

Aleksandra Maria Polak, Agnieszka Adamska, Anna Krentowska, Agnieszka Łebkowska, Justyna Hryniewicka, Marcin Adamski, Irina Kowalska: Body composition, serum concentrations of androgens and insulin resistance in different polycystic ovary syndrome phenotypes. Journal of Clinical Medicine, 2020; 9(3): 732. doi: 10.3390/jcm9030732, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 1% polegał na pomocy w przeprowadzeniu analizy statystycznej.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Białystok, 10.10.2020r.

Prof. dr hab. n. med. Irina Kowalska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

Uniwersytetu Medycznego w Białymstoku

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

I. Kowalska

9.2. Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu (praca oryginalna)

Aleksandra Maria Polak, Anna Krentowska, Agnieszka Łebkowska, Angelika Buczyńska, Marcin Adamski, Edyta Adamska-Patruno, Joanna Fiedorczuk, Adam Jacek Krętowski, Irina Kowalska, Agnieszka Adamska: *The Association of Serum Levels of Leptin and Ghrelin with the Dietary Fat Content in Non-Obese Women with Polycystic Ovary Syndrome.* Nutrients, 2020. 12(9), 2753. Doi: 10.3390/nu12092753

Imię i nazwisko współautora	*Charakter udziału*	*Procentowy wkład*
doktorant – lek. Aleksandra Polak	Udział w planowaniu eksperymentów, przeprowadzanie eksperymentów prezentowanych w pracy, opracowanie i analiza wyników, przygotowanie manuskryptu, przygotowanie tabel wchodzących w skład manuskryptu	60%
Lek. Anna Krentowska	Pomoc przy rekrutowaniu pacjentów do badania, tworzeniu bazy danych, korekta językowa	5%
Dr n. med. Agnieszka Łebkowska	Pomoc przy rekrutowaniu pacjentów i tworzeniu bazy danych	1%
Mgr Angelika Buczyńska	Pomoc w przeprowadzaniu eksperymentów prezentowanych w pracy	1%
Dr inż. Marcin Adamski	Pomoc w przeprowadzeniu analizy statystycznej	1%
Dr Edyta Adamska-Patruno	Konsultacja merytoryczna	2%
Mgr Joanna Fiedorczuk	Pomoc przy tworzeniu bazy danych	1%
Prof. dr hab. n. med. Adam Jacek Krętowski	Konsultacja merytoryczna	1%
Prof. dr hab. n. med. Irina Kowalska	Stworzenie koncepcji pracy, konsultacja merytoryczna	10%
Dr hab. n. med. Agnieszka Adamska	Stworzenie koncepcji pracy, udział w planowaniu eksperymentów, pomoc przy przygotowaniu manuskryptu, konsultacja merytoryczna	18%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Aleksandry Marii Polak

Podpis doktoranta

Białystok, 10.10.2020r.

Lek. Anna Krentowska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

Uniwersytetu Medycznego w Białymstoku

ul. M. Skłodowskiej-Curie 24A

15-276 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w publikacji:

Aleksandra Maria Polak, Anna Krentowska, Agnieszka Łebkowska, Angelika Buczyńska, Marcin Adamski, Edyta Adamska-Patruno, Joanna Fiedorczuk, Adam Jacek Krętowski, Irina Kowalska, Agnieszka Adamska: The association of serum levels of leptin and ghrelin with the dietary fat content in non-obese women with polycystic ovary syndrome. Nutrients, 2020; 12(9): 2753. doi: 10.3390/nu12092753, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 5% polegał na pomocy przy rekrutowaniu pacjentów, tworzeniu bazy danych oraz korekcie językowej.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Anna Krentowska

Białystok, 10.10.2020r.

Dr n. med. Agnieszka Łebkowska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

Uniwersytetu Medycznego w Białymstoku

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Aleksandra Maria Polak, Anna Krentowska, Agnieszka Łebkowska, Angelika Buczyńska, Marcin Adamski, Edyta Adamska-Patruno, Joanna Fiedorczuk, Adam Jacek Krętowski, Irina Kowalska, Agnieszka Adamska: The association of serum levels of leptin and ghrelin with the dietary fat content in non-obese women with polycystic ovary syndrome. Nutrients, 2020; 12(9): 2753. doi: 10.3390/nu12092753, wchodzącej w skład rozprawy doktorskiej lek. Aleksandry Marii Polak, wynoszący 1% polegał na pomocy przy rekrutowaniu pacjentów i tworzeniu bazy danych.

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Dr n. med. Agnieszka Łebkowska
specjalista chorób wewnętrznych
2342301

Białystok, 10.10.2020r.

Mgr Angelika Buczyńska

Klinika Endokrynologii, Diabetologii

i Chorób Wewnętrznych

Uniwersytetu Medycznego w Białymstoku

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Angelika Buczyńska

Białystok, 10.10.2020r.

Dr inż. Marcin Adamski

Wydział Informatyki

Politechniki Białostockiej

ul. Wiejska 45A

15-351 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w publikacji:

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Białystok, 10.10.2020r.

Dr Edyta Adamska-Patruno

Centrum Badań Klinicznych

Uniwersytetu Medycznego w Białymstoku

ul. M. Skłodowskiej-Curie 24 A

15-276 Białystok

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Edyta Adamska-Patruno

Białystok, 10.10.2020r.

Mgr Joanna Fiedorczuk

Centrum Badań Klinicznych

Uniwersytetu Medycznego w Białymstoku

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Joanna Fiedorczuk

Białystok, 10.10.2020r.

Prof. dr hab. n. med. Adam Jacek Krętowski

Klinika Endokrynologii, Diabetologii

i Chorób Wewnętrznych

Uniwersytetu Medycznego w Białymstoku

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15-276 Białystok

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Aleksandrę Marię Polak powyższej publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

KIEROWNIK
Kliniki Endokrynologii, Diabetologii
i Chorób Wewnętrznych
prof. dr hab. n. med. Adam Krętowski

Białystok, 10.10.2020r.

Prof. dr hab. n. med. Irina Kowalska

Klinika Chorób Wewnętrznych

i Chorób Metabolicznych

Uniwersytetu Medycznego w Białymstoku

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15-276 Białystok

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I. Kowalska

Białystok, 10.10.2020r.

Dr. hab. n. med. Agnieszka Adamska

Klinika Endokrynologii, Diabetologii

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Uniwersytetu Medycznego w Białymstoku

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