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Comparison of regional fat mass measurement by whole body DXA scans and anthropometric measures to predict insulin resistance in women with polycystic ovary syndrome and controls

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Key words
Dual-energy X-ray absorptiometry scan, regional fat mass, body mass index, waist, visceral adiposity index, lipid accumulation product, lean body mass

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Conflict of interest
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Abstract

Introduction. Polycystic ovary syndrome (PCOS) is characterized by obesity and insulin resistance. Measures of regional obesity may be used to predict insulin resistance. In the present study we compared fat distribution in patients with PCOS vs. controls and established the best measure of fat mass to predict insulin resistance in patients with PCOS. *Material and methods.* The study was cross-sectional in an academic tertiary-care medical center with 167 pre-menopausal women with PCOS and 110 controls matched for ethnicity, BMI and age. Total and regional fat and lean body mass were assessed by whole body dual-energy X-ray absorptiometry (DXA) scans. Anthropometric measures (BMI, waist) and fasting metabolic analyses [insulin, glucose, lipids, Homeostasis model assessment (HOMA-IR), lipid accumulation product, and visceral adiposity index] were determined. Trial registration numbers: NCT00451568, NCT00145340. *Results.* Women with PCOS had higher central fat mass (waist, waist–hip ratio, and upper/lower fat ratio) compared with controls. In bivariate associations, the strongest associations were found between HOMA-IR and the fat mass measures trunk fat ($r = 0.59$), waist ($r = 0.57$) and BMI ($r = 0.56$), all $p < 0.001$. During multiple regression analyses, trunk fat, waist and BMI were the best predictors of HOMA-IR ($R^2 = 0.48$, 0.49, and 0.47, respectively). *Conclusions.* Women with PCOS were characterized by central obesity. Trunk fat, waist and BMI were the best predictors of HOMA-IR in PCOS, but only limited information regarding insulin resistance was gained by whole body DXA scan.

Abbreviations: BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; DXA, dual-energy X-ray absorptiometry; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment; LAP, lipid accumulation product; PCOS, polycystic ovary syndrome; SHBG, sex-hormone binding globulin; TG, triglyceride; VAI, visceral adiposity index; WHR, waist–hip ratio.

Introduction

Polycystic ovary syndrome (PCOS) is a lifelong endocrine condition with a prevalence ranging from 6 to 20% in reproductive-aged women (1,2). PCOS is characterized by oligo/anovulation, hyperandrogenism, and polycystic ovaries (1). The majority of women with PCOS are insulin-resistant (3). Hyperinsulinemia is a consequence of insulin resistance and high levels of insulin stimulate ovarian androgen production and suppress hepatic production of sex-hormone binding globulin (SHBG),

thereby causing elevated circulating levels of free testosterone (3). Insulin resistance in PCOS is often established by the measurement of increased levels of fasting insulin or the homeostasis model assessment (HOMA-IR) (1).

It is currently estimated that approximately 75% of women with PCOS are overweight or obese, but the prevalence of overweight varies among different study populations (4,5). Increasing body mass index (BMI) is closely associated with PCOS (4). Weight gain may induce the PCOS phenotype in predisposed women, and medical intervention or a change in lifestyle resulting in weight loss may fully resolve PCOS and restore fertility (3,6). In prospective studies in PCOS, BMI was the most important predictive factor for the development of type 2 diabetes (7). In obesity, the number of adipose tissue-resident macrophages is increased and the circulating mononuclear cells show more inflammatory activity (8). The distribution of fat is important for the metabolic risk, as central obesity is associated with insulin resistance and predicts the development of type 2 diabetes and cardiovascular disease (9). Patients with PCOS have decreased levels of adiponectin and increased secretion of other adipokines and inflammatory markers, which may link central obesity, inflammation and insulin resistance in PCOS (10). Central obesity is both present in normal weight and overweight patients with PCOS (4). Furthermore, the metabolic disturbances in PCOS are more pronounced in patients with hyperandrogenemia than in patients with normal testosterone levels (11). Therefore, the pathogenesis of PCOS may be described as a vicious cycle involving hyperandrogenemia, central obesity, and insulin resistance/hyperinsulinemia (10).

The importance of central obesity for the metabolic risk in patients with PCOS makes individual metabolic risk assessment important in the daily clinic. Position and consensus statements recommend that all patients with PCOS are routinely screened for the metabolic syndrome and cardiovascular risk factors (10,12). The definition of the metabolic syndrome in PCOS includes a waist circumference of more than 88 cm (1). The cut-off limit for waist circumference is based on criteria for the metabolic syndrome in non-PCOS-populations (12). Whole body dual-energy X-ray absorptiometry (DXA) scans offer the opportunity to estimate total, abdominal and extremity fat mass (13). Previous studies found increased trunk fat in patients with PCOS than in controls (14–16), but whether trunk fat is superior to BMI and waist as a metabolic risk marker in PCOS remains to be established. The lipid accumulation product (LAP), visceral adiposity index (VAI), and fat/lean body mass ratio were applied as markers of visceral adiposity and insulin resistance in recent studies (17–20) and one of these markers could be superior to the measurement of BMI or waist circumference as predictor of metabolic risk in PCOS.

The aim of the present study was to compare the usefulness of regional fat mass, waist circumference and BMI to predict insulin resistance in PCOS. A group of healthy controls was included to compare body composition in PCOS vs. controls.

Material and methods

The study population consisted of 187 White women of reproductive age diagnosed with PCOS according to the Rotterdam criteria, who were participating in previous clinical studies and one ongoing clinical study at the Department of Endocrinology, Odense University Hospital (21–23). All included patients fulfilled two of the criteria: (1) irregular periods for more than 1 year in combination with a cycle length >35 days; (2) total or free testosterone levels above reference interval (upper limits: total testosterone >1.8 nmol/l, free testosterone >0.035 nmol/l) or hirsutism; and (3) transvaginal ultrasound with at least one polycystic ovary. Hirsutism was scored according to the generally used Ferriman–Gallwey score (FG score) (1). Serious endocrine diseases were excluded as previously reported (24) and included routine measurement of prolactin, thyroid stimulating hormone, and 17-hydroxyprogesterone. Patients with diabetes were excluded and therefore all included patients had HbA1c <6.5% (25). Patients suspended taking oral contraceptives and metformin for at least 3 months prior to examination.

Controls

Data regarding 117 healthy women were already available from previous studies (21–23). The controls were recruited by advertising in local newspapers, the local university, nursing school, and at Odense University Hospital. All controls had regular menstrual cycles, testosterone levels within reference interval, and did not suffer from hirsutism. Controls paused oral contraceptives for at least 3 months before evaluation.

Key Message

Individual metabolic risk assessment is important in women with PCOS. The findings of the present study support the use of waist measurement and BMI in the outpatient clinic as markers of insulin resistance in women with PCOS.

Methods

Evaluation included medical history, clinical examination, transvaginal ultrasound, and fasting blood samples. Waist circumference was measured to the nearest cm in a standing position midway between the lower costal margin and the iliac crest. Transvaginal ultrasound was performed at the Department of Gynecology, Odense University Hospital. Fasting blood samples were drawn in follicular phase (cycle days 2–8) in patients with a cycle length shorter than 3 months. Patients with cycle length >3 months had the blood samples drawn on a random cycle day. Blood tests included androgens [total testosterone, free-testosterone, SHBG, dehydroepiandrosterone sulfate (DHEAS)], fasting insulin, glucose and fasting lipid profile.

Assays

Insulin was analyzed by a time-resolved fluoroimmunoassay using a commercial kit (AutoDELFIA, Wallac Oy, Turku, Finland) with an intra-assay coefficient of variation 2.1–3.7% and an inter-assay coefficient of variation 3.4–4.0%. Blood glucose was measured on capillary ear blood using Hemo Cue. We calculated HOMA-IR = fasting insulin * fasting glucose/22.5. Plasma total cholesterol, high-density lipoprotein (HDL), cholesterol and triglyceride (TG) were analyzed by enzymatic colorimetric reactions (Modular P, Roche), and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Serum total testosterone and SHBG were analyzed using an in-house method based on the extraction of steroids from serum by ether, separation of extracted steroids by liquid chromatography and quantification by radioimmunoassay as previously described (26). In this method, testosterone, dihydrotestosterone and androstendione are extracted before applying radioimmunoassay, avoiding overestimation of testosterone levels (26,27). The method shows a close correlation with the determination of testosterone levels using mass spectrometry (26,27). In a subset of participants ($n = 56$), total testosterone was measured by mass spectrometry. The intra-assay coefficient of variation for total testosterone was 8.2% and for SHBG it was 5.2%. The inter-assay coefficient of variation for total testosterone was 13.8% and for SHBG 7.5%. Double measurements were performed to ensure that the two methods were comparable (Statens Serum Institut, Denmark, personal communication). Free testosterone levels were calculated from measurements of total testosterone and SHBG. DHEAS was measured by radioimmunoassay on diluted serum using specific antibodies. The inter-assay variation for DHEAS was 10.7%. The LAP was calculated as (waist – 58)*TG (28) and the VAI was calculated as waist/[36.58 + (1.89*BMI)]*(TG/0.81)*(1.52/HDL) (29).

DXA scans

Dual-energy X-ray absorptiometry in whole body array mode (DXA, Hologic QDR-4500) was used to measure whole body fat mass and to differentiate between peripheral (legs and arms) and central fat mass. Default software readings were used to divide the body into six compartments: head, trunk, arms and legs. The trunk was defined by a horizontal line below the chin and vertical lines passing through colli femori. The arm regions were separated from the trunk at the levels of the shoulder joint. Fat mass was determined for each region. Whole body fat mass was calculated by subtracting head fat mass from total body fat mass. Technical performance was monitored by daily calibration scans using an anthropomorphic Hologic phantom. The coefficient of variation for replicate scans of the same individual was 0.8% for fat mass and 0.6% for lean body mass. The coefficient of variation was determined by repeated scanning in 10 patients with PCOS (22).

Statistical analysis

Parameters were not normally distributed and were therefore described using medians and quartiles. Spearman bivariate associations were performed to evaluate associations between individual markers of fat mass and free testosterone and HOMA-IR.

We performed multiple regression analyses to investigate the association between individual markers of fat mass, VAI, and LAP and insulin resistance (HOMA-IR) after correcting for patient status (control = 0, PCOS = 1), age (years) and free testosterone. Linear regression analyses were performed on log-transformed data with all variables entered at one time. In these regression analyses, log (HOMA-IR) was entered as the dependent variable and patient status, log (free testosterone) and log (age) as the explanatory variables along with one of the metabolic risk markers: log (BMI, waist, waist–hip ratio, trunk fat, total body fat, fat ratio, lean body mass, total body fat/total lean mass, LAP or VAI). The R^2 for the individual regression models and B-levels, and p-values for the relevant explanatory variable are presented in tables. We used SPSS version 17 (SPSS, Chicago, IL, USA) in our calculations. The p-values <0.05 were considered significant.

The trials were registered at www.clinicaltrials.gov (registration numbers NCT00145340, NCT00451568). The local ethics committee approved all studies and all women included gave written informed consent. The study furthermore complied with the Declaration of

Helsinki. ID numbers, date of acceptance by the Ethics committee Region of Southern Denmark: S-20020010, 24 January 2002 (21), S-20010046, 12 June 2001 (22), S-20070020, 1 March 2007 (23), and S-20130666, 10 July 2013 (previously unpublished data).

Results

Women with PCOS were significantly younger than controls [median (interquartile range) 29 (24–33) years vs. 30 (25–36) years, $p = 0.006$] and had significantly higher BMI [29.3 (24.1–32.8) kg/m^2 vs. 27.1 (23.2–31.1) kg/m^2]. The difference in age between patients and controls could be explained partly by a different age range in women with PCOS (18–44 years) than controls (18–54 years) and, accordingly, seven controls aged >44 years were excluded from the study population (comparison of age in patients vs. controls, $p = 0.07$). Thereafter, patients and controls were divided in five BMI groups (BMI <20 kg/m^2, BMI 20–24.9 kg/m^2, BMI 25–29.9 kg/m^2, BMI 30–34.9 kg/m^2 and BMI ≥ 35 kg/m^2). The distribution of patients and controls according to BMI group differed significantly (Chi-square test, $p = 0.025$). The 15 youngest patients with BMI 30–34.9 kg/m^2 and the five youngest patients with BMI ≥35 kg/m^2 were then excluded to ensure equal distribution in BMI groups (Chi-square test, $p = 0.37$, data not shown) and comparable age between patients and controls. Data of the remaining 167 patients and 110 controls are presented in Table 1. A total of 111/167 (66.5%) patients vs. 69/110 (62.7%) controls had BMI ≥25 kg/m^2, Chi-square test $p = 0.52$.

In all, 125/167 (74.9%) patients fulfilled all three Rotterdam criteria, 22 (13.1%) patients had hyperandrogenism (HA) and polycystic ovaries (PCOM), 15 (8.9%) patients had HA and oligo/amenorrhea (OA), and 5

Table 1. Clinical and biochemical data in patients with polycystic ovary syndrome (PCOS) and controls.

	PCOS *n* = 167	Controls *n* = 110	*p*-level
Age (years)	30 (25–33)	30 (25–36)	0.16
Weight (kg)	80.6 (69.1–90.7)	77.9 (66.7–89.6)	0.35
BMI (kg/m^2)	28.6 (23.8–32.2)	26.7 (22.9–30.4)	0.11
Waist (cm)	91 (78–104)	82 (74–95)	<0.001
Hip (cm)	109 (101–116)	110 (102–117)	0.25
WHR	0.83 (0.78–0.90)	0.75 (0.72–0.81)	<0.001
Trunk fat (kg)	14.1 (9.1–18.2)	11.7 (8.1–16.2)	0.07
Fat legs (kg)	10.3 (8.2–13.2)	10.5 (8.0–13.9)	0.45
Total body fat (kg)	27.3 (19.5–35.5)	26.2 (18.9–33.9)	0.36
Total body fat (%)	37.8 (32.5–42.0)	36.2 (30.2–43.0)	0.25
Fat ratio (upper/lower)	1.7 (1.3–1.9)	1.4 (1.2–1.7)	<0.001
Lean body mass (kg)	44.1 (40.9–47.9)	44.3 (40.0–48.3)	0.75
Total body fat/lean mass	0.63 (0.50–0.76)	0.59 (0.45–0.78)	0.29
Fasting insulin (pmol/l)	52 (38–93)	37 (24–50)	<0.001
Glucose (mmol/l)	5.3 (5.0–5.6)	5.3 (5.0–5.7)	0.36
HOMA-IR (pmol mmol l^{-2})	12.7 (8.5–22.3)	9.1 (5.4–12.3)	<0.001
Cholesterol (mmol/l)	4.7 (4.1–5.3)	4.6 (4.1–5.1)	0.58
TG (mmol/l)	1.0 (0.7–1.4)	0.9 (0.6–1.1)	0.01
LDL (mmol/l)	2.7 (2.3–3.3)	2.5 (1.9–3.0)	0.02
HDL (mmol/l)	1.3 (1.2–1.6)	1.5 (1.3–1.9)	<0.001
LAP	30.4 (17.0–60.4)	22.9 (13.6–38.6)	0.02
VAI	1.5 (0.8–2.4)	0.9 (0.7–1.5)	<0.001
FG score	8 (3–13)	0 (0–1)	<0.001
Total testosterone (nmol/l)	1.90 (1.34–2.42)	1.37 (1.04–1.56)	<0.001
Free testosterone (nmol/l)	0.036 (0.023–0.053)	0.023 (0.017–0.027)	<0.001
SHBG (nmol/l)	41 (29–61)	54 (37–78)	0.002
DHEAS (10^3 μmol/l)	5.1 (3.7–7.5)	4.6 (3.6–5.9)	0.51
PCO (yes)	152 (91%)		
Hyperandrogenism (yes)	161 (96%)		
Oligomenorrhea (yes)	145 (87%)		

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; FG, Ferriman-Gallwey; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LAP, lipid accumulation product; LDL, low density lipoprotein; PCO, polycystic ovary; SHBG, sex hormone binding globulin; TG, triglyceride; VAI, visceral adiposity index; WHR, waist-hip ratio.
Data presented as median (quartiles) or *n* (%).

(3.0%) patients had PCO and OA (Table 1). Patients with PCOS had higher central fat mass (waist, waist–hip ratio and fat ratio) compared with controls. The metabolic risk markers fasting insulin, HOMA-IR, TG, LDL, LAP and VAI were significantly higher and HDL and SHBG levels were significantly lower in patients than in controls.

Table 2 shows bivariate associations between HOMA-IR, free testosterone, measures of fat and lean mass, LAP and VAI in patients with PCOS. The metabolic risk markers trunk fat, BMI, waist and LAP were most closely associated with HOMA-IR and free testosterone. Age was positively associated with all fat mass measures and LAP (r-values from 0.15 to 0.25, all p-values <0.05).

Table 3 shows bivariate associations between individual measures of fat mass in patients with PCOS. The closest associations were found between trunk fat and BMI ($r = 0.94$) and between trunk fat and waist circumference ($r = 0.90$).

Multiple regression analyses were performed to evaluate the independent effect of measures of fat and lean mass, LAP and VAI on HOMA-IR after adjusting for patient group (patient or control), free testosterone and age (Table 4). Trunk fat, BMI and waist were the best predictors of HOMA-IR. The diagnostic group (control/patient) had a significant independent effect on HOMA-IR. Free testosterone had a significant independent effect on HOMA-IR in models that included total body fat, fat ratio (upper/lower), lean mass, total body fat/lean mass and VAI.

Table 2. Bivariate associations between homeostasis model assessment (HOMA-IR), free testosterone, measures of fat and lean mass, lipid accumulation product, and visceral adiposity index in patients with polycystic ovary syndrome (PCOS).

	PCOS $n = 167$			
	HOMA-IR		Free-T	
	r-value	*p*-level	*r*-value	*p*-level
BMI (kg/m^2)	0.56	<0.001	0.30	<0.001
Waist (cm)	0.57	<0.001	0.26	<0.001
Hip (cm)	0.43	<0.001	0.18	0.02
WHR	0.46	<0.001	0.20	0.009
Trunk fat (kg)	0.59	<0.001	0.30	<0.001
Fat legs (kg)	0.38	<0.001	0.18	0.02
Total body fat (kg)	0.54	<0.001	0.23	0.002
Total body fat (%)	0.52	<0.001	0.21	0.006
Fat ratio (upper/lower)	0.43	<0.001	0.24	0.002
Total lean mass (kg)	0.41	<0.001	0.21	0.004
Total body fat/lean mass	0.50	<0.001	0.19	0.01
LAP	0.58	<0.001	0.25	0.001
VAI	0.49	<0.001	0.17	0.03

BMI, body mass index; WHR, waist-hip ratio.
Data presented as *r*-value and *p*-level.
Spearman's rank correlations between HOMA-r, free testosterone (free-T) and individual measures of fat and lean mass, lipid accumulation product (LAP), and visceral adiposity index (VAI).

Discussion

In the present study, trunk fat established by whole body DXA scan was the best predictor of insulin resistance in patients with PCOS. However, trunk fat was closely associated with waist and BMI, and during multiple regression analyses, trunk fat, waist and BMI were equally good predictors of insulin resistance. Free testosterone was positively associated with all fat mass measures during bivariate associations; in multiple regression analyses, free testosterone and PCOS status were independent predictors of HOMA-IR. Our study therefore supports that trunk fat, waist and BMI may be used to predict insulin resistance in the outpatient clinic, but only limited information was gained by the use of a DXA scan. Furthermore, hyperandrogenemia and the presence of PCOS were independent predictors of insulin resistance.

Our findings of adverse effects of abdominal fat mass on insulin sensitivity are in accordance with previous studies in PCOS (14,30–34). Those studies included various numbers of patients with PCOS [$n = 35$ (30), $n = 20$ (31), $n = 110$ (32), $n = 30$ (33), $n = 74$ (34), $n = 116$ (14)] and reported an inverse relation between insulin sensitivity established by HOMA-IR (31), QUICKY (32) or the euglycemic clamp (14,30,34) and central fat mass established by whole body DXA scans (30–32), ultrasound (33) or magnetic resonance imaging (34). The power of many of these studies was not sufficient to perform multiple regresson analyses (30–33). However, the study by Manneras-Holm reported that adipocyte size, adiponectin and waist circumference were the factors most strongly associated with insulin sensitivity established by the euglycemic clamp, therefore supporting our findings of waist circumrefence as an important predictor of insulin resistance in PCOS (34). Recently, Tosi et al. (14) found that total adiposity and central fat mass were independent predictors of insulin sensitivity established by the euglycemic clamp after correcting for age and free testosterone using a similar regression analysis as in the present study.

The isolated effect of hyperandrogenism on fat mass in PCOS is debated. Previous studies supported our findings of positive associations between testosterone and measures of fat mass (14,35). A recent meta-analysis included studies assessing adiposity by BMI and waist circumference or waist–hip ratio and concluded that fat excess was associated with hyperandrogenemia (9). Tosi et al. found a positive association between free testosterone and body fat and regional fat, but during multiple regression

Table 3. Bivariate associations between metabolic risk markers in patients with polycystic ovary syndrome (n = 167).

	BMI		Waist		LAP		VAI	
	r-value	*p*-level	*r*-value	*p*-level	*r*-value	*p*-level	*r*-value	*p*-level
DXA scan measure								
Trunk fat (kg)	0.95	<0.001	0.91	<0.001	0.80	<0.001	0.54	<0.001
Total body fat (kg)	0.94	<0.001	0.87	<0.001	0.74	<0.001	0.46	<0.001
Total body fat (%)	0.87	<0.001	0.78	<0.001	0.65	<0.001	0.38	<0.001
Fat ratio (upper/lower)	0.50	<0.001	0.58	<0.001	0.62	<0.001	0.56	<0.001
Total lean mass (kg)	0.71	<0.001	0.74	<0.001	0.66	<0.001	0.45	<0.001
Total fat/lean mass	0.86	<0.001	0.77	<0.001	0.64	<0.001	0.38	<0.001

BMI, body mass index; DXA, dual-energy X-ray absorptiometry; LAP, lipid accumulation product; VAI, visceral adiposity index.
Data are presented as *r*-values and *p*-levels.
Spearman's rank correlations.

Table 4. Individual body composition measures, lipid accumulation product (LAP) and visceral adiposity index (VAI) as predictors of homeostasis model assessment of insulin resistance (HOMA-IR) in all included subjects (n = 277).

Dependent variable HOMA-IR

Independent variables

Metabolic risk marker		Control/PCOS	Free-T	Age	R^2 model
1. BMI (kg/m^2)	2.1 (<0.001)	0.17 (<0.001)	0.12 (0.12)	−0.34 (0.08)	0.47 (<0.001)
2. Waist (cm)	2.7 (<0.001)	0.17 (<0.001)	0.07 (0.39)	−0.47 (0.02)	0.49 (<0.001)
3. WHR	3.3 (<0.001)	0.12 (0.008)	0.13 (0.14)	−0.22 (0.29)	0.38 (<0.001)
4. Trunk fat (kg)	0.9 (<0.001)	0.16 (<0.001)	0.10 (0.19)	−0.33 (0.09)	0.48 (<0.001)
5. body fat (kg)	0.9 (<0.001)	0.15 (<0.001)	0.28 (<0.001)	−0.14 (0.47)	0.45 (<0.001)
6. Fat ratio	1.1 (<0.001)	0.14 (0.002)	0.21 (0.02)	−0.08 (0.72)	0.33 (<0.001)
7. Lean mass (kg)	2.3 (<0.001)	0.19 (<0.001)	0.20 (0.02)	−0.08 (0.91)	0.36 (<0.001)
8. Fat/lean mass	1.0 (<0.001)	0.14 (<0.001)	0.35 (<0.001)	−0.02 (0.90)	0.39 (<0.001)
9. LAP	0.5 (<0.001)	0.15 (<0.001)	0.11 (0.16)	−0.32 (0.10)	0.46 (<0.001)
10. VAI	0.4 (<0.001)	0.13 (0.004)	0.25 (0.003)	0.09 (0.67)	0.34 (<0.001)

PCOS, polycystic ovary syndrome; T, testosterone.
Multiple regression analyses were performed with log (HOMA-r) as the dependent variable and one individual metabolic risk marker log [body mass index (BMI), waist, waist–hip ratio (WHR), trunk fat, total body fat, fat ratio (upper/lower), lean mass, total body fat/lean mass, lipid accumulation product (LAP) or visceral adiposity index (VAI)] was entered as the explanatory variable along with patient group (control = 0, PCOS = 1), log (free-T) and log (age).
Model 1: Entered explanatory variables: log (BMI), patient group, log (free-T), log (age). Model 2: entered explanatory variables: log (waist), patient group, log (free-T), log (age). Model 3: entered explanatory variables: log (WHR), patient group, log (free-T), log (age), etc.
Data presented as B-value (*p*-level).

analyses the association between body fat and hyperandrogenemia could be explained by insulin resistance (9). The association between body fat and hyperandrogenemia was therefore at least in part mediated by insulin resistance (9). The positive association between hyperandrogenemia and central fat mass in PCOS may be further investigated during long-term androgen suppression. We recently reported unchanged regional fat distribution despite a median weight gain of 1.2 kg during 12 months of oral contraceptive treatment in PCOS and changes in testosterone levels were not associated with changes in fat distribution (23). At present, limited data are available from prospective studies with hard endpoints including type 2 diabetes mellitus and cardiovascular disease, and we are not aware of prospective studies using more sophisticated measures than BMI and waist to determine regional fat mass in PCOS. A recent prospective study in 410 patients with PCOS found that BMI >25 kg/m^2, waist ≥80 cm, and a family history of diabetes were the strongest predictors of metabolic risk along with the hyperandrogenic PCOS phenotype (36). Therefore, even if hyperandrogenism in PCOS were not an independent predictor of insulin resistance and central obesity in some clinical studies, long-term androgen stimulation for example starting prenatally or during puberty could affect metabolic risk in PCOS. Future prospective studies need

to confirm whether high trunk fat is superior to high BMI and waist as a metabolic risk marker.

LAP and VAI were elevated in PCOS and were associated with HOMA-IR and regional fat mass, but especially VAI was inferior to central fat mass, BMI and waist as predictors of HOMA-IR. The formulas for the calculation of LAP and VAI include waist and TG and it was hypothesized that a hypertriglyceridemic waist phenotype could reflect visceral adiposity and therefore would be associated with metabolic risk (17). Several recent studies reported that high LAP and high VAI levels could be used as metabolic risk markers in PCOS (17,18). Differences in the included study populations regarding ethnicity, and metabolic risk and differences in the applied methods regarding measurement of body composition and insulin resistance, could explain the various findings of possible markers for metabolic risk in these studies (17,18). Prospective studies in well described study populations with PCOS are needed to further clarify the benefit of LAP and VAI.

The negative effect of fat mass on metabolic risk could be counterbalanced by increased lean body mass and the net effect could be estimated by calculating fat/lean body mass ratio (19). However, in the present study, lean body mass and fat/lean mass were comparable in PCOS and controls; during multiple regression, central fat mass, BMI and waist were better predictors of insulin resistance than fat/lean mass. We found a positive association between lean body mass and HOMA-IR, which did not support that lean body mass protects against insulin resistance. These results contrasted the results of Ezeh et al. (19), who reported higher fat/lean mass in patients with PCOS than controls, and that high fat/lean mass predicted increasing HOMA-IR (19). The study populations of the present study and Ezeh et al. were comparable regarding age and BMI, whereas patients of mixed ethnicity were included by Ezeh, and patients with PCOS tended to be younger than controls (19). In the present study, age and central fat mass were positively associated and recent studies supported that increasing age in PCOS is associated with more metabolic risk factors, whereas hyperandrogenemia decreases with increasing age (37). The use of bioelectrical impedance to establish fat and lean body mass may also have affected study results (38). Our findings are supported by a study by Comerford et al. (39). They hypothesized that additional lean mass could exaggerate the effects of visceral fat mass on insulin resistance in PCOS and that hyperinsulinemia in PCOS could affect muscle quality (39). More studies are needed to test this hypothesis.

Strengths and limitations of the present study need to be discussed. The study population included a large well-characterized group of patients and we are not aware of previous studies that performed DXA scans in such a large study population of women with PCOS. The number of study controls was relatively limited in the present study. However, patients and controls were matched for ethnicity, age and BMI. Ethnicity may have important effects on body composition and metabolic risk factors (40) in PCOS and we therefore consider the inclusion of a study group with uniform ethnicity a study strength. However, our findings may not apply in populations with other ethnic backgrounds. Furthermore, the majority of included patients had hyperandrogenism, which could have affected study results. We applied HOMA-IR as a measure of insulin resistance. The gold standard for measurement of insulin resistance is the euglycemic clamp, but HOMA-IR correlates well with clamp-established measures of insulin sensitivity (41). Furthermore, all the women had HbA1c levels within reference interval and normal fasting glucose levels. We therefore find it unlikely that high glucose levels affected our study results. Fasting insulin and glucose are subject to day-to-day variation but this was partly accounted for by the inclusion of a large group of women. DXA scans offer the opportunity to estimate total, abdominal and extremity fat mass (13), whereas magnetic resonance imaging is the best method for discriminating between intra-abdominal and subcutaneous fat mass. However, several studies found similar adipose tissue volume and distribution during magnetic resonance imaging in patients with PCOS and controls (34,42) despite a higher waist circumference in patients with PCOS (34). Recent studies furthermore suggested that subcutaneous abdominal and intra-abdominal fat have similar adverse effects on insulin resistance (43).

In conclusion, waist and BMI could be used to predict insulin resistance in patients with PCOS, whereas limited information was gained by measures of central fat mass established from DXA scans.

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