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Original Article

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Resting energy expenditure in women with polycystic ovary syndrome

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ABSTRACT

STUDY QUESTION: Is resting energy expenditure (REE) altered in women with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: Women with PCOS have a reduction in REE, when corrected for fat-free mass, independent of PCOS clinical phenotypes and BMI categories.

WHAT IS KNOWN ALREADY: Obesity is an important issue in women with PCOS, in terms of frequency and pathophysiological implications. It has been hypothesized that obesity may be favoured by alterations in REE, but the studies have been limited and conflicting.

STUDY DESIGN, SIZE, DURATION: This case–control study was a comparison of 266 women with PCOS and 51 healthy controls, recruited in the Verona 3P study from 2010 to 2021.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women with PCOS diagnosed by the Rotterdam criteria, with normal thyroid function and no interfering medications, were referred to the outpatient clinic of a tertiary care centre of endocrinology and metabolism for a measurement of REE. Healthy controls were recruited in the same period and submitted to the same procedure. In all subjects, REE was measured by indirect calorimetry and serum androgens were measured by LC-MS/MS. In women with PCOS, insulin sensitivity was assessed using the hyperinsulinemic–euglycemic clamp.

MAIN RESULTS AND THE ROLE OF CHANCE: REE was similar in women with PCOS and controls. However, REE corrected for fat-free mass (REE/FFM) was significantly lower in women with PCOS than in controls (31.8 ± 4.0 vs 35.4 ± 3.9 kcal/kgFFM·day, $P < 0.001$). REE/FFM did not differ between normal-weight, overweight, or obese women with PCOS, and each of these subgroups showed lower REE/FFM values than controls. Reduced REE/FFM values were found in each phenotype of the syndrome. In multiple regression analysis, REE/FFM was independently associated with age and PCOS status, but not with fat mass. In PCOS women, REE/FFM was independently and directly associated with ovarian follicle number.

LIMITATIONS, REASONS FOR CAUTION: Limitations of the study are the cross-sectional design, which limits the causal inference of the results, and the unavailability of precise information about lifestyle factors, which may be potential confounders. Further prospective studies are needed to establish the importance of this phenomenon in contributing to the weight excess of PCOS.

WIDER IMPLICATIONS OF THE FINDINGS: A reduction of REE could potentially favour weight gain in women with PCOS and possibly contribute to the altered metabolic profile typical of this condition, even counteracting the therapeutic strategies aimed to reduce excess body fat in these women. Nevertheless, the presence of this abnormality in both obese/overweight and normal-weight patients suggests that other factors must play a role in this phenomenon.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous condition characterized by endocrine alterations and reproductive dysfunction, often associated with metabolic abnormalities.

Obesity is a frequent finding in these women, affecting up to 70% of people with PCOS (Lim *et al.*, 2012). Notably, weight excess markedly exacerbates the endocrine and reproductive alterations of PCOS and the metabolic consequences of the syndrome. Therefore, understanding and targeting obesity in PCOS is an important issue.

Despite the large epidemiological dimension and the key pathophysiological implications of weight excess, studies conducted to explain this clinical problem in PCOS are limited and inconclusive.

Obesity is the result of increased energy intake, reduced energy expenditure, or their combinations. In particular, the daily energy expenditure is determined by three components: resting energy expenditure (REE), the thermic effect of food (post-prandial thermogenesis), and energy expenditure related to physical activity (Westerterp, 2017). REE, which represents the metabolic rate required to maintain vital physiological functions of an individual who is at rest, awake, in a fasted state and in a thermoneutral environment (Westerterp, 2017), is generally the major component, accounting for ~65% of the total daily energy expenditure.

The hypothesis that a reduced REE may be a predisposing factor for obesity in women with PCOS has been explored by few studies. Cosar *et al.* (2008) and Larsson *et al.* (2016), using indirect calorimetry to measure REE, did not find significant differences of this parameter between women with PCOS and controls, even after adjusting for age and BMI (Larsson *et al.*, 2016). Similarly, Romualdi *et al.* (2019), using a SenseWear Armband to estimate REE, did not find differences between overweight PCOS women and BMI-matched healthy controls. Conversely, Georgopoulos *et al.* (2009), who compared adjusted REE (REEadj), using a formula that takes into account fat mass, fat-free mass, and age, in 91 women with PCOS and 48 controls, reported that this value was significantly lower in PCOS women than in controls. Furthermore, REEadj was significantly lower in insulin-resistant than in insulin-sensitive women with PCOS, subdivided by the HOMA-IR index (Georgopoulos *et al.*, 2009). On the whole, most of the available evidence did not adequately take into account the differences in body composition between women with PCOS and controls, whereas the only study that corrected data for free fat mass used an imprecise surrogate index of insulin sensitivity to estimate the potential association between REE and insulin resistance. In addition, no previous studies used a reliable measure of serum androgens, which may potentially be another factor responsible for energy expenditure differences between groups, and none of them distinguished between the clinical phenotypes of PCOS. This distinction, which derives from the various possible combinations of the diagnostic elements of PCOS observed in each individual subject, may also be important, as it has several implications, such as striking differences in metabolic abnormalities (Moghetti *et al.*, 2013).

The aims of this study were: (i) to compare REE in women with PCOS and healthy controls, taking into account the differences in body composition; (ii) to evaluate the relationships between this parameter and the main clinical, endocrine, and metabolic characteristics of PCOS, also assessing if there may be differences in REE between the clinical phenotypes of PCOS; and (iii) to identify the independent predictors of REE in women with PCOS.

Materials and methods

Subjects

A total of 266 women with PCOS were selected from 378 women referred to the outpatient clinic of the Division of Endocrinology, Diabetes and Metabolism of Verona City Hospital from 2010 to 2021, and included in the Verona 3P Study cohort (Moghetti *et al.*, 2013). The subjects selected were those with a measurement of REE by indirect calorimetry. Of the non-selected women, REE could not be measured in 70 women because the calorimeter was not available at that time, 5 women refused their consent to the evaluation of REE by indirect calorimetry, mainly due to claustrophobia, and 37 tests were not conclusive for technical problems. The study also included 51 healthy women, recruited in the same period, and submitted to the same procedure, as controls.

Women with PCOS had been referred to the outpatient clinic of the Division of Endocrinology, Diabetes and Metabolism of Verona City Hospital (Italy) for clinical hyperandrogenism and/or menstrual dysfunction. In this cohort, PCOS was diagnosed according to the Rotterdam workshop criteria, i.e. presence of at least two among the three following features: clinical and/or biochemical hyperandrogenism, chronic oligo-anovulation, and polycystic ovary morphology (PCOm), after exclusion of secondary causes (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Clinical hyperandrogenism was defined by the presence of hirsutism (modified Ferriman–Gallwey score ≥8), and biochemical hyperandrogenism by increased free testosterone (FT) levels, which is the most sensitive measure of hyperandrogenemia according to international guidelines (Teede *et al.*, 2023). The cut-off in our lab to define increased FT was >0.46 ng/dl (Tosi *et al.*, 2016). The presence of irregular cycles and/or ovulatory dysfunction was diagnosed by the presence of either oligoamenorrhea (≤8 cycles/year) or luteal phase serum progesterone <12 nmol/l in two consecutive menstrual cycles, according to both the original Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) and current international guidelines (Teede *et al.*, 2023). Our cohort of women with PCOS was studied over a long time frame, from 2010 to 2021. Over the course of this period, the improvement in ultrasonographic devices resulted in a change in ultrasonographic criteria, in particular regarding the number of follicles (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004; Teede *et al.*, 2023). For diagnosis of PCOm, we used thresholds in the number of follicles, which were appropriate for the period and the devices used, and/or an ovarian volume ≥10 ml, which remained unchanged over time. We have subsequently verified that, in women with PCOm in whom diagnosis was based on follicle count, the follicle number per section, which was available in all women, was ≥10, as defined by previous studies and confirmed by current 2023 international guidelines (Teede *et al.*, 2023). A transvaginal approach was used whenever possible, and this occurred in more than 95% of subjects.

In order to rule out potential secondary causes, serum TSH, prolactin, and 17-hydroxyprogesterone were assessed in all subjects, whereas other investigations were carried out when indicated on clinical grounds.

According to the combinations of diagnostic features, the clinical phenotypes of women with PCOS were as follows: 183 (68.8%) with the complete phenotype (hyperandrogenism, oligoanovulation, and PCOm), 15 (5.6%) with the classic phenotype (hyperandrogenism and oligoanovulation), 40 (15.0%) with the ovulatory phenotype (hyperandrogenism and PCOm), and 28 (10.6%) with the normoandrogenic phenotype (oligoanovulation and PCOm).

No patients were suffering from other diseases. In particular, none of them had thyroid dysfunction or were taking medications which could potentially interfere with the evaluations carried out in the study (oral contraceptives, insulin-sensitizing agents, antiandrogens, or glucocorticoids).

Control subjects were normal-weight healthy women with regular and ovulatory menstrual cycles and no clinical signs of hyperandrogenism nor micropolycystic ovarian morphology.

All subjects gave their written informed consent before the study, which was approved by our institutional Ethical Committee (Comitato Etico per la Sperimentazione, Azienda Ospedaliera Universitaria Integrata Verona).

Protocol

All the participants underwent a complete medical examination, including measurement of body weight, height, waist and hip circumferences, fat and fat-free mass by bioimpedance (BIA 103, Akern, Florence, Italy) (Gray *et al.*, 1989), and blood pressure. Hirsutism was quantified by the modified Ferriman–Gallwey score (Escobar-Morreale *et al.*, 2012).

In the early follicular phase of a spontaneous menstrual cycle, or, in women with severe menstrual dysfunction, after at least three months of amenorrhea, a venous blood sample was drawn in the morning, after 10–12 h of fasting, for hormonal and metabolic assessment.

The hormonal assessment included FSH, LH, AMH, sex hormone-binding globulin (SHBG), total and FT, androstenedione, and dehydroepiandrosterone sulphate (DHEAS).

The metabolic evaluation comprised fasting plasma glucose and insulin, lipid profile (total and HDL-cholesterol, triglycerides), and uric acid. In addition, an oral glucose tolerance test was carried out in 256 PCOS subjects, according to the WHO procedure (World Health Organization Study Group, 1985). Diagnosis of metabolic syndrome was carried out using the 2009 joint criteria by the IDF and other Societies (Alberti *et al.*, 2009). According to these criteria, at least three of the following are needed for diagnosis in female subjects: (i) waist circumference ≥80 cm, (ii) fasting glucose ≥100 mg/dl or altered glucose tolerance, (iii) HDL-cholesterol <50 mg/dl, (iv) triglycerides ≥150 mg/dl, and (v) systolic blood pressure ≥130 and/or diastolic blood pressure ≥85 mmHg.

In 240 women, among those with PCOS, insulin sensitivity was measured by the hyperinsulinemic–euglycemic clamp technique, as previously described (DeFronzo *et al.*, 1979). This test was not performed in 26 women, because they did not give their consent to the procedure (n = 3) or because technical problems made it impossible to maintain venous access throughout the test (n = 23). Briefly, after overnight fasting, a continuous insulin infusion (Humulin R, Lilly, Indianapolis, IN, USA) was started and maintained at a constant rate of 80 mU/m^2 per min for 120 min. Euglycemia was maintained throughout the test by a variable infusion of 20% dextrose, adjusted by monitoring plasma glucose levels in arterialized venous blood every 5–10 min. Since, in non-diabetic hyperandrogenic women and controls, endogenous glucose production is negligible at this insulin infusion rate (Moghetti *et al.*, 1996), the amount of glucose infused into each subject can be considered equivalent to the whole-body insulin-mediated glucose uptake. Because muscle is responsible for most insulin-induced glucose metabolism (DeFronzo, 1988), glucose disposal data (M-clamp) were expressed per fat-free mass (mg/kgFFM·min^{-1}).

In all subjects, REE was evaluated fasting in the morning, in a warm room, by indirect calorimetry (Ferrannini, 1988). Gas exchanges, O_2 consumption and CO_2 production, were measured over 30 min, using a Quark RMR instrument (Cosmed, Cernusco sul Naviglio, Italy) equipped with a ventilated hood.

Assays

Plasma glucose was measured by the glucose-oxidase method, using a glucose analyzer (YSI-2300 Stat Plus; YSI Inc, Yellow Springs, OH, USA). Total and HDL-cholesterol, triglycerides, and uric acid were assayed by an automated analyzer, according to standard laboratory procedures (Dimension Vista 1500, Siemens, Milan, Italy).

Liquid chromatography-mass spectrometry (LC-MS/MS) measures of total testosterone and androstenedione were obtained by using a Micromass Quattro Premier XE Mass Spectrometer from Waters Corporation (Milford, MA, USA) as previously described (Tosi *et al.*, 2016). For total testosterone, the intra-assay CV was 9.1% at a concentration 14 ng/dl and inter-assay CV 9.3% at 26 ng/dl. For androstenedione, the intra-assay CV was 3% at 64 ng/dl, and the inter-assay CV 4.6% at 58 ng/dl. FT was calculated by the Vermeulen formula (Vermeulen *et al.*, 1999), using the ISSAM online calculator (http://www.issam.ch/freetesto.htm), and including a default value of albumin of 4.3 g/l.

DHEAS was measured by a direct automated chemiluminescent (CLIA) method (Immulite 2000, Siemens, Erlangen, Germany). SHBG and insulin were assayed by immunoradiometric methods (Orion Diagnostica, Espoo, Finland; and Biosource, Fleurus, Belgium, respectively). LH and FSH were assayed by an automated CLIA method (Advia Centaur XP, Siemens, Tarrytown, NY, USA). TSH was measured by an electrochemiluminescence analyzer, Modular Analytics E170, and Elecsys Cobas Reagents (Roche Diagnostics, Milan, Italy). AMH was assayed by the ELISA AMH Gen II Kit (Beckman Coulter, Inc., Webster, TX, USA), using a Triturus Analyzer (Grifols, Barcelona, Spain). For all these assays, the intra- and inter-assay CVs were <10% and <16%, respectively.

Calculations

BMI was calculated as weight (kg)/height (m)^2 and WHR as waist circumference (cm)/hip circumference (cm).

Glucose disposal rate during the steady-state period of the clamp (M-clamp) was calculated with standard formula (DeFronzo *et al.*, 1979).

REE corrected for fat-free mass was calculated as REE (kcal/day)/FFM (kg) measured by bioimpedance.

Ovarian volume (ml) was calculated using the ellipsoid formula: $\pi/6$ [length (cm) × height (cm) × width (cm)].

Power calculations

A *post hoc* power calculation was performed. Setting the significance level alpha to 0.05, with a sample size of 266 for the PCOS and 51 for controls, the power to detect a difference in REE/FFM between 31.8 kcal/kgFFM·day (SD = 4.0) and 35.4 kcal/kgFFM·day (SD = 3.9) in PCOS and controls, respectively, was equal to 100%.

Statistical analysis

Continuous variables were described as mean ± SD and categorical variables were summarized by percentages. Comparisons of continuous variables between women with PCOS and healthy controls, as well as between subgroups of patients, were made by Student's *t*-test for unpaired data and ANOVA. Non-normally distributed variables were log or square-root transformed before analysis.

Pearson's correlations were performed to compare REE or REE/FFM values with clinical, metabolic, and hormonal variables.

Subsequently, multiple regression analysis was performed to identify the independent predictors of REE or REE/FFM. In this analysis, independent variables were chosen on the basis of bivariate correlations and/or biological plausibility.

P values <0.05 were considered statistically significant. Analyses were performed using STATA version 10.1 (Stata-Corp, College Station, TX, USA).

Results

The main characteristics of women with PCOS and controls are shown in Table 1.

Age was significantly lower in women with PCOS than in controls. However, the difference was small and with unlikely biological significance in the considered age range. Indexes of adiposity (BMI, waist circumference, WHR, fat mass) and fat-free mass were all significantly higher in women with PCOS than in controls. In particular, among the subjects with PCOS, 60 women (22.6%) were overweight (BMI ≥ 25 and <30 kg/m^2) and 101 (38.0%) were obese (BMI ≥ 30 kg/m^2). As regards the metabolic profile, fasting insulin, triglycerides, uric acid, and diastolic blood pressure were higher, while HDL-cholesterol was lower, in PCOS patients than in controls. Mean insulin sensitivity (M-clamp value), evaluated in PCOS women only, was lower than the reference cut-off established in our lab (9.8 ± 3.7 mg/kgFFM·min^{-1}, reference cut-off >11.76). In particular, 174 of these women with PCOS (72.5%) were insulin resistant. Three women with PCOS had mild type 2 diabetes mellitus, while 33 of them had prediabetes (impaired fasting glucose and/or impaired glucose tolerance).

Table 1. Main characteristics of women with PCOS and healthy controls included in the study (mean ± SD).

	PCOS n = 266	Controls n = 51	P[a]
Age (years)	23.3 ± 5.2	25.2 ± 3.6	**0.014**
BMI (kg/m^2)	28.3 ± 7.4	20.5 ± 2.0	**<0.001**
Waist circumference (cm)	90.1 ± 18.0	71.2 ± 5.3	**<0.001**
WHR	0.83 ± 0.01	0.77 ± 0.01	**<0.001**
Fat mass (kg)	27.1 ± 14.4	13.6 ± 5.4	**<0.001**
Fat-free mass (kg)	49.0 ± 7.7	42.8 ± 4.6	**<0.001**
Systolic blood pressure (mmHg)	118 ± 13	115 ± 10	**0.148**
Diastolic blood pressure (mmHg)	75.4 ± 10.2	70.5 ± 9.8	**0.004**
Fasting glucose (mg/dl)	85.3 ± 9.5	83.4 ± 5.9	0.205
Fasting insulin (mU/l)	16.2 ± 12.6	7.0 ± 5.0	**<0.001**
M-clamp (mg/kgFFM × min^{-1})[b]	9.8 ± 3.7	–	
Total cholesterol (mg/dl)	162 ± 32	162 ± 27	0.755
HDL-cholesterol (mg/dl)	52.4 ± 14.3	63.0 ± 10.4	**<0.001**
Triglycerides (mg/dl)	83.9 ± 53.6	53.4 ± 21.8	**0.001**
Uric acid (mg/dl)	4.4 ± 1.1	3.7 ± 0.7	**0.001**
Metabolic syndrome (%)	27.4	0	**<0.001**
Ferriman–Gallwey score	9.4 ± 6.9	1.4 ± 0.6	**<0.001**
LH/FSH ratio	1.8 ± 1.1	0.7 ± 0.3	**0.001**
SHBG (nmol/l)	35.8 ± 20.8	68.2 ± 20.9	**<0.001**
Total testosterone (ng/dl)	39.7 ± 16.2	25.8 ± 9.7	**<0.001**
Free testosterone (ng/dl)[c]	0.7 ± 0.3	0.3 ± 0.1	**<0.001**
Androstenedione (ng/dl)	171 ± 64	110 ± 45	**<0.001**
DHEAS (μmol/l)	6.5 ± 2.8	4.9 ± 1.9	**0.017**
Follicle number	14.6 ± 4.2	4.1 ± 1.8	**<0.001**
Ovarian volume (ml)	12.6 ± 5.2	7.0 ± 2.0	**0.001**
AMH (μg/l)	8.9 ± 6.3	4.4 ± 3.5	**<0.001**

[a] Significant differences are in bold type.
[b] Insulin-stimulated glucose uptake adjusted for FFM (available in 240 women with PCOS).
[c] Calculated by the Vermeulen formula (Vermeulen *et al.*, 1999).
BMI, body mass index; WHR, waist circumference:hip circumference ratio; M-clamp, glucose disposal during the clamp; HDL, high density lipoproteins; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulphate.

Metabolic syndrome was diagnosed in 73 (27.4%) PCOS patients, whereas no control women had this metabolic abnormality.

As regards hormonal parameters, as expected, serum androgens, AMH and LH/FSH ratio were higher and SHBG levels were lower in women with PCOS than in controls. Moreover, as expected on the basis of diagnostic criteria, the number of follicles and the ovarian volume were higher in subjects with PCOS than in controls.

In accordance with selection criteria, serum concentrations of TSH were in the normal range in all subjects and similar between groups.

Energy expenditure in women with PCOS and healthy controls

Figure 1A shows the box-plots of REE in women with PCOS and healthy controls. This parameter was similar in the two groups (1509 ± 201 vs 1554 ± 277 kcal/day, $P = 0.273$). However, REE corrected for fat-free mass (REE/FFM) was lower in women with PCOS than in controls (31.8 ± 4.0 vs 35.4 ± 3.9 kcal/kgFFM·day, $P < 0.001$, 95% CI of the mean difference −3.6 kcal/kgFFM·day equal to −4.8, −2.4 kcal/kgFFM·day, and Cohen's *d* effect size equal to 0.86) (Fig. 1B).

When women with PCOS were subdivided according to BMI categories, REE progressively increased from normal to overweight and to obese subjects. Compared with control women, who were all normal-weight, REE was significantly higher in obese PCOS women but significantly lower in normal-weight PCOS women than in controls, whereas the values were similar in overweight PCOS patients and lean controls (Fig. 1C).

Conversely, REE/FFM did not significantly differ between normal, overweight, and obese PCOS patients, whereas each of these three subgroups had REE/FFM values significantly lower than controls (Fig. 1D).

The findings were unchanged after exclusion of subjects with altered glucose tolerance (data not shown).

Resting energy expenditure and features of PCOS

To avoid redundant information, findings on the relationships between energy expenditure and PCOS features are shown considering the REE/FFM data only (Fig. 2). Moreover, in comparing REE/FFM values in the different phenotypes of PCOS, the complete and the classic phenotype subgroups were pooled, due to the small number of subjects without PCOm.

Each of the clinical phenotypes of PCOS showed a significantly lower REE/FFM value than controls. Moreover, the complete/classic phenotype showed a marginally lower REE/FFM than the ovulatory phenotype (Fig. 2A).

Considering each diagnostic feature contributing to PCOS diagnosis separately, REE/FFM did not differ between hirsute and non-hirsute (Fig. 2B) nor between hyperandrogenemic and normoandrogenemic women (Fig. 2C). Also, the difference in REE/FFM between anovulatory and ovulatory women did not reach the conventional statistical significance ($P = 0.054$, Fig. 2D). Conversely, REE/FFM was higher in PCOS women with, versus those without, PCOm ($P = 0.014$) (Fig. 2E).

Finally, REE/FFM was similar in insulin-resistant versus insulin-sensitive women with PCOS, as well as in those with versus those without metabolic syndrome (Supplementary Fig. S1). REE/FFM was also similar in subjects with normal versus altered glucose tolerance (data not shown).

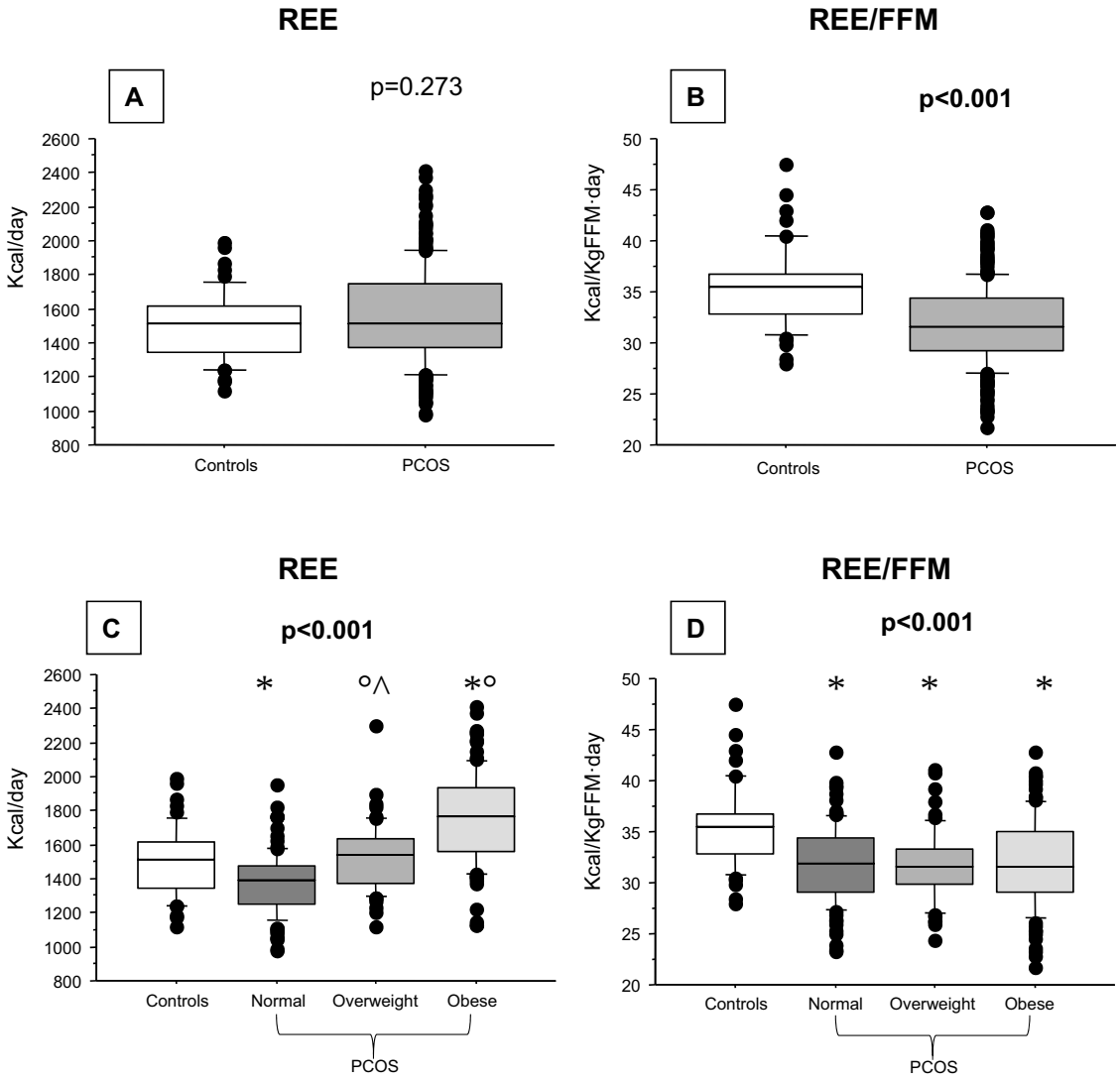

Figure 1. Box-plots of resting energy expenditure (REE) and resting energy expenditure corrected for fat-free mass (REE/FFM). Data are compared in women with PCOS versus healthy controls (panels **A** and **B**) and in women with PCOS subdivided according to BMI category versus healthy controls (panels **C** and **D**). *P<0.001 versus Controls; °P < 0.001 versus Normal-weight; ^P < 0.001 versus Obese.

Relationships between energy expenditure and anthropometric, metabolic, and hormonal parameters

Table 2 shows the bivariate relationships between either REE or REE/FFM and the main characteristics of women with PCOS. REE was associated with several anthropometric and metabolic parameters. In particular, a direct association was found with indexes of adiposity, fat-free mass, blood pressure, fasting glucose and insulin, triglycerides, and uric acid, whereas an inverse association was found with HDL-cholesterol and M-clamp values (Table 2). However, when REE was corrected for FFM, many of these relationships were lost. In particular, REE/FFM showed a direct association with fasting glucose ($r = 0.134$, $P = 0.029$) and an inverse association with total cholesterol only ($r = -0.128$, $P = 0.038$) (Table 2).

As regards the endocrine and ultrasonographic features, direct relationships were observed between REE and measures of hyperandrogenism (hirsutism score and serum FT), whereas inverse relationships were observed between REE and LH/FSH ratio, SHBG and AMH (Table 2). However, when REE/FFM was considered, none of these relationships remained statistically significant. REE/FFM was, however, directly associated with the number of ovarian follicles ($r = 0.232$, $P = 0.001$) (Table 2).

In the multiple regression model to identify the independent predictors of REE, on the entire sample, age, fat mass, fat-free mass, and the presence or absence of PCOS status were included as independent variables. In this analysis, fat mass and fat-free mass, directly, and PCOS status and age, inversely, were independent predictors of REE (Table 3). In a similar model in which REE/FFM was the dependent variable, age (b coefficient=−0.106, 95% CI=−0.193; −0.019, $P = 0.018$) and PCOS status (b coefficient=−3.896, 95% CI=−5.165; −2.626, $P < 0.001$) were still negative independent predictors of REE/FFM, whereas fat mass was no longer an independent predictor (Table 3). In both models, the inclusion of FT as an additional independent variable did not change the results (data not shown).

Finally, to identify the potential predictors of REE/FFM in women with PCOS, the following variables, chosen according to biological plausibility or the results of bivariate regression analysis, were included as independent variables: age, fat mass, FT, follicle number, fasting glucose, total cholesterol, and M-clamp value. In this model, the number of ovarian follicles was a direct independent predictor of REE/FFM (b coefficient = 0.269, 95% CI = 0.118–0.420, $P = 0.001$), whereas fasting glucose showed borderline statistical significance ($P = 0.063$) (Supplementary Table S1).

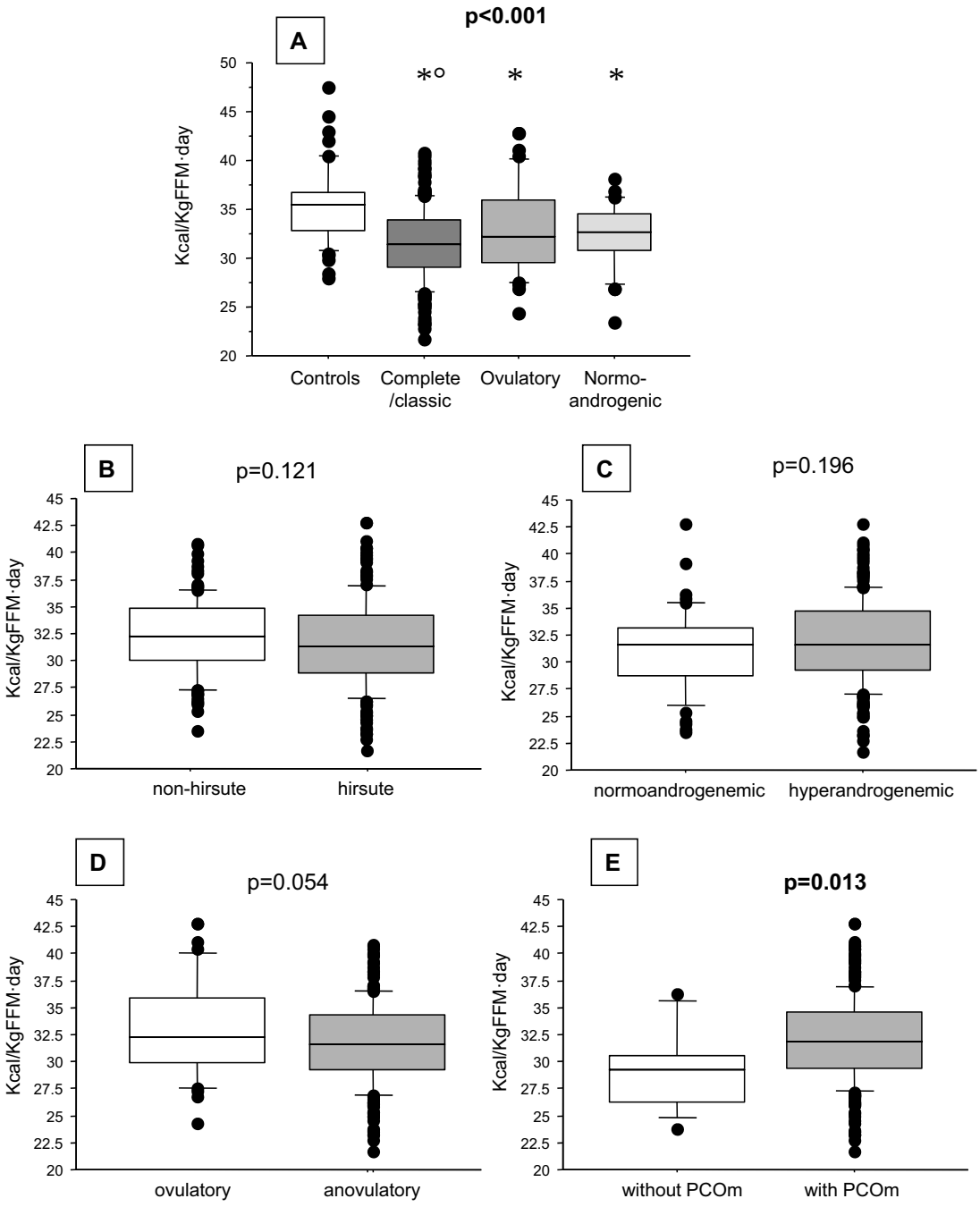

Figure 2. Box-plots of resting energy expenditure corrected for fat-free mass (REE/FFM). Data are compared in healthy controls versus women with PCOS subdivided according to the clinical phenotypes (panel **A**) and in women with PCOS subdivided according to the presence or absence of hirsutism (panel **B**), hyperandrogenemia (panel **C**), oligoanovulation (panel **D**), or polycystic ovary morphology (PCOm) (panel **E**). *$P < 0.005$ versus Controls; °$P = 0.025$ versus Ovulatory phenotype.

Discussion

This study investigated REE, measured by indirect calorimetry, in a large cohort of women with PCOS and healthy controls. This parameter did not differ between the two groups. However, women with PCOS and controls differed in terms of body composition. In particular, both fat mass and fat-free mass were higher in PCOS women than in controls. Importantly, lean mass is metabolically the most active tissue and therefore, it is the main determinant of REE (Westerterp, 2017; Heymsfield *et al.*, 2021). Interestingly, when REE was corrected for fat-free mass (REE/FFM), this value was significantly lower in women with PCOS than in controls. Moreover, each BMI category of PCOS patients (normal, overweight, or obese), as well as each clinical phenotype of the syndrome (classic/complete, ovulatory, or normoandrogenic) showed a lower REE/FFM when compared with healthy controls. In the entire population, age and PCOS status were negative independent predictors of REE/FFM. These data confirm that REE, corrected for body composition, declines with age (Karppinen *et al.*, 2023), and suggest that it is also affected by PCOS status *per se*. However, the mechanisms underlying this effect of PCOS remain unclear. Among the several metabolic and endocrine parameters examined in the study, the number of ovarian follicles was the only independent predictor of REE/FFM in women with PCOS.

Interestingly, a reduced REE may be a predisposing factor for obesity, which is a frequent finding in women with PCOS, with relevant pathophysiological implications. This phenomenon can also be relevant in the management of these women, as it may counteract weight reduction strategies in overweight patients. Moreover, it can be hypothesized that it may even play a pathogenic role in the syndrome, as Mendelian randomization studies have shown that obesity has a causal role in PCOS (Zhu and Goodarzi, 2022).

Noteworthy, REE is the major component of the daily energy expenditure in humans. It represents the energy required for the conservation of normal functions while the body activity is reduced as much as possible. Unlike the other two components of the daily energy expenditure, post-prandial thermogenesis and energy expenditure linked to physical activity, which change in relation to the lifestyle, the intra-individual variability of REE, over days or months, is relatively low. Environmental factors, such as the ambient temperature, as well as the body size and composition are the major determinants of REE. For this reason, indirect calorimetry must be performed in a thermo-neutral ambient, and REE of each subject should be corrected for the amount of fat-free mass (Westerterp, 2017; Heymsfield et al., 2021).

A very limited number of studies have previously evaluated REE in women with PCOS. In general, they have examined small samples of subjects and used different methods (Cosar et al., 2008; Georgopoulos et al., 2009; Larsson et al., 2016; Romualdi et al., 2019). Most of these studies did not find statistically significant differences of REE between women with PCOS and controls. Cosar et al. (2008) measured REE by indirect calorimetry in 31 women with PCOS and 29 age- and BMI-matched controls, with different body fat distribution as assessed by WHR. In this study, REE was similar between the two groups. Data were not corrected for fat-free mass. However, these findings suggest that central adiposity is not a predictor of REE in women with PCOS.

Larsson et al. (2016) tested the hypothesis that women with PCOS may display altered dietary intakes and eating behaviours compared to controls. In this study, 72 women with PCOS and 30 controls, with different mean BMI (28 vs 24 kg/m^2), completed questionnaires regarding eating behaviour and underwent indirect calorimetry. Women with PCOS showed greater concerns about their weight and dieting. The difference in REE between the two groups did not reach statistical significance, even after adjusting for age and BMI. The authors concluded that energy expenditure does not have a significant role in the tendency toward weight excess often observed in women with PCOS.

Finally, in the study by Georgopoulos et al. (2009), 91 women with PCOS and 48 controls were investigated, in order to assess the relationship between REE, measured by indirect calorimetry, and insulin sensitivity, estimated by several surrogate indexes (HOMA-IR, QUICKI, serum insulin, glucose/insulin ratio). In this study, the individual REE of each subject was corrected (REEadjusted) using the Hume formula, which takes into account lean mass, fat mass, and age. PCOS patients had significantly lower adjusted REE values than controls. Moreover, adjusted REE was significantly lower in insulin-resistant than in insulin-sensitive women with PCOS.

The results of this latter study are, at least in part, in agreement with the results of our study. In fact, after adjustment of REE for body composition (fat-free mass and fat mass in the study by Georgopoulos et al. (2009) and fat-free mass in our study), a significant difference in energy expenditure emerged in the comparison of women with PCOS versus controls. In both studies, energy expenditure per unit of fat-free mass was lower in PCOS patients than in healthy controls.

In our study, we did not identify a specific characteristic of PCOS associated with the impaired REE. In these women, REE/

Table 2. Simple correlations between resting energy expenditure (REE) or resting energy expenditure corrected for fat-free mass (REE/FFM) and the main parameters evaluated in women with PCOS.

	REE		REE/FFM	
	r	*P*[a]	*r*	*P*[a]
Age	−0.055	0.372	−0.094	0.128
BMI	0.674	**<0.001**	−0.038	0.534
Waist circumference	0.645	**<0.001**	−0.028	0.655
WHR	0.336	**<0.001**	−0.035	0.575
Fat mass	0.726	**<0.001**	0.030	0.630
Fat-free mass	0.718	**<0.001**	–	–
Systolic blood pressure	0.385	**<0.001**	0.075	0.228
Diastolic blood pressure	0.367	**<0.001**	0.071	0.249
Fasting glucose	0.401	**<0.001**	0.134	**0.029**
Fasting insulin	0.471	**<0.001**	0.074	0.229
M-clamp[b]	−0.347	**<0.001**	−0.052	0.425
Total cholesterol	0.040	0.523	−0.128	**0.038**
HDL-cholesterol	−0.381	**<0.001**	−0.090	0.146
Triglycerides	0.378	**<0.001**	0.075	0.221
Uric acid	0.415	**<0.001**	0.044	0.501
Ferriman–Gallwey score	0.181	**0.004**	−0.064	0.310
LH/FSH ratio	−0.152	**0.017**	0.039	0.543
SHBG	−0.366	**<0.001**	−0.073	0.239
Total testosterone	0.040	0.517	0.058	0.351
Free testosterone[c]	0.312	**<0.001**	0.086	0.164
Androstenedione	−0.038	0.538	0.076	0.223
DHEAS	0.075	0.236	0.105	0.097
Follicle number	−0.025	0.733	0.232	**0.001**
Ovarian volume	0.035	0.605	0.049	0.468
AMH	−0.213	**0.003**	−0.073	0.318

[a] Significant differences are in bold type.
[b] Insulin-stimulated glucose uptake adjusted for FFM (available in 240 women with PCOS).
[c] Calculated by the Vermeulen formula (Vermeulen et al., 1999).
BMI, body mass index; WHR, waist circumference:hip circumference ratio; M-clamp, glucose disposal during the clamp; HDL, high density lipoproteins; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulphate.

Table 3. Predictors of resting energy expenditure (REE) or resting energy expenditure corrected for fat-free mass (REE/FFM) in multivariable regression analysis conducted in the entire population.

	REE (explained variance 56.1%)			REE/FFM (explained variance 11.7%)		
Independent variables:	**b coefficient**	**95% CI**	***P*[a]**	**b coefficient**	**95% CI**	***P*[a]**
Age (years)	−5.56	−9.53, −1.60	**0.006**	−0.106	−0.193, −0.019	**0.018**
Fat mass (kg)	7.30	4.80, 9.80	**<0.001**	0.010	−0.023, 0.042	0.562
Fat-free mass (kg)	15.14	10.54, 19.73	**<0.001**			
Group (PCOS)	−159.1	−216.7, −101.5	**<0.001**	−3.896	−5.165, −2.626	**<0.001**

[a] Significant relationships are in bold type.

FFM values were similar in patients with versus those without hyperandrogenism, in insulin-resistant versus insulin-sensitive patients, in those with or without oligoanovulation, as well as in each clinical phenotype of the syndrome. Only the presence or absence of PCOm was associated with differences in the REE/FFM values. However, a reduction in energy expenditure was found in women with a lower follicle count, which is not a characteristic of PCOS. This finding is intriguing and cannot easily be explained.

Overall, our findings may suggest the existence of a common defect in the mechanism(s) leading to resting energy production that involves all women with PCOS, although this defect remains undefined. Some studies have suggested that PCOS may be associated with thyroid dysfunction (Gaberšček *et al.*, 2015) which could potentially be responsible for an altered REE. However, in our study, all women had normal thyroid function, as assessed by serum TSH, and no relationships were found between TSH and either REE or REE/FFM.

In human cells, the majority of metabolizable energy is processed via the Krebs cycle in mitochondria. Krebs cycle enzymes, which are located on the inner mitochondrial membrane, generate ATP via oxidative phosphorylation. During this process, protons create an electrochemical gradient that powers the conversion of chemical energy in glucose to ATP. This process is tightly coupled, although partial uncoupling of substrate oxidation to ATP generation releases the heat that largely accounts for REE (Rolfe and Brown, 1997; Heymsfield *et al.*, 2021). In this regard, two components of mitochondrial heat generation are recognized: a basal proton leak, that accounts for up to 20–30% of oxygen consumption and likely affects whole-body energy utilization, and inducible proton leaks, that are involved in the heat generated by brown adipose tissue (Rolfe and Brown, 1997). Therefore, mitochondrial dysfunction may potentially account for the impaired energy expenditure in women with PCOS. However, the available information on this issue is inconsistent.

Using 31P-MR spectroscopy, Cree-Green *et al.* (2015) reported that mitochondrial oxidative phosphorylation was lower in hyperandrogenic obese adolescents than in age- and BMI-matched controls. Moreover, the expression of key genes for oxidative phosphorylation was reported to be reduced in the skeletal muscle of women with PCOS (Skov *et al.*, 2007). Studies of oxygen consumption in blood mononuclear cells have indicated that mitochondrial complex I respiration is reduced in women with PCOS, as compared with age- and BMI-matched controls (Victor *et al.*, 2009). These data support the hypothesis of a mitochondrial dysfunction in women with PCOS. However, other authors have not found any difference in skeletal muscle mitochondrial respiration in women with PCOS versus healthy controls, nor in mitochondrial content between the two groups (Rabøl *et al.*, 2011).

It is important to bear in mind that other tissues, apart from the skeletal muscle, contribute to determining resting energy production. In particular, the brain and the liver are important contributors in this process (Heymsfield *et al.*, 2021). Whether this may account for the impaired energy expenditure of women with PCOS is unknown. In particular, the liver could be an interesting actor in this phenomenon. However, to the best of our knowledge, no information is available on this issue.

Looking for the potential factors that could influence REE in women with PCOS, we hypothesized a role for androgens. In this regard, no previous studies have investigated this hypothesis. However, interestingly, Lerner *et al.* (2021), showed that *in vitro* exposure to the non-aromatizable androgen DHT influenced the expression of genes involved in thermogenesis and inhibited the mitochondrial respiration in brown adipose tissue of rats, suggesting that androgens may impair post-prandial thermogenesis. The extrapolation of these results to humans requires caution. Moreover, in our study, no association was found between serum androgens and REE corrected for fat-free mass.

In the previously mentioned study by Georgopoulos *et al.* (2009), in which REE was corrected for lean and fat body mass, this parameter was higher in insulin-sensitive than in insulin-resistant women with PCOS and both these subgroups of patients had lower values than controls. This observation suggested a potential association between REE and insulin sensitivity. However, in this study, the presence versus absence of insulin resistance was defined using surrogate indexes, which show a poor performance in recognizing insulin-resistant women (Tosi *et al.*, 2017). In our study, we did not find any relationship between REE/FFM and M-clamp values, a direct measure of insulin sensitivity obtained by the gold standard hyperinsulinemic–euglycemic clamp methodology (DeFronzo *et al.*, 1979).

The main strengths of the present study are the large cohort of women with PCOS investigated and the use of gold standard methodologies to measure some parameters of potential pathophysiological relevance, such as insulin sensitivity and serum androgens concentrations. A limitation of the study is its cross-sectional design, which did not permit us to establish cause-effect relationships. Moreover, it was not possible to measure lean mass by DXA in these women. However, a good correlation was previously reported between FFM measures obtained by BIA and either DXA (Yang *et al.*, 2018) or the gold standard underwater weighing (Gray *et al.*, 1989). Another limitation is the lack of information on some possible confounders in the study, in particular as regards previous weight loss efforts, diet, and physical activity. The personal history of women with PCOS included weight changes over time. However, this information was not easily available in all subjects. It should be noted that we were careful to avoid including subjects with weight changes in recent months, and the vast majority of subjects were sedentary. Moreover, the findings were totally independent of body weight. Studies aiming to assess the effect of diet and/or physical exercise intervention on REE in women with PCOS are extremely limited and do not suggest changes in this parameter during the weight maintenance phase after hypocaloric diet, or differences depending on diet composition (Moran *et al.*, 2006). No comparison with control women was carried out in these studies. Further prospective studies with an adequate sample size are required to clarify these aspects.

In conclusion, women with PCOS have a reduction in REE, when corrected for fat-free mass, which is independent of the phenotypes of the syndrome and the BMI category of subjects. These results suggest that these women have an intrinsic alteration in REE, which might potentially favour weight gain in many of them and possibly contribute to the altered metabolic profile typical of this condition. This phenomenon could also oppose the therapeutic strategies aimed to reduce excess body fat in these women. However, the mechanisms underlying this phenomenon remain undefined. Further prospective studies are needed to understand these mechanisms, and the contribution of this phenomenon to obesity associated with PCOS. Nevertheless, the presence of this abnormality in both obese/overweight and normal-weight patients suggests that other factors also play a role in this process.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

F.T. conceived the study, analysed the data, and wrote the draft manuscript; F.R., V.G., and F.L. contributed to the acquisition of the glucose clamp and indirect calorimetry data and critically revised the manuscript; M.Z. contributed to analysis of the data and critically revised the manuscript; M.E.Z. contributed to statistical analysis of the data and critically revised the manuscript; T.F. and J.-M.K. performed the androgen assays and critically revised the manuscript; P.M. contributed to designing the study, interpreting the data, and writing the manuscript. All authors approved the final version of the manuscript, and agreed to be accountable for all aspects of the work in ensuring the accuracy and integrity of any part of it.

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Conflict of interest

The authors have nothing to disclose.

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