Brown adipose tissue activity is reduced in women with polycystic ovary syndrome

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Abstract

Objective: To evaluate whether brown adipose tissue (BAT) activity is altered in women with polycystic ovary syndrome (PCOS), and whether BAT activity correlates with plasma levels of irisin, a myokine that can induce BAT formation.

Design: We performed a cross-sectional study including women with PCOS ($n = 45$) and a healthy control group ($n = 25$) matched by age and body mass index (BMI).

Methods: BAT activity was measured using ^{18}F -FDG positron emission tomography-computed tomography (PET-CT) and plasma irisin levels were measured by a validated enzyme immunoassay.

Results: Total BAT activity was significantly reduced in women with PCOS (maximal standardized uptake value (SUV_{max}): median 7.4 g/mL, interquartile range 0.9–15.4) compared to controls (median 13.0 g/mL, interquartile range 4.7–18.4, $P = 0.047$). However, this difference was no longer significant after adjustment for waist circumference, a surrogate marker of central adiposity. In the PCOS group, BAT activity correlated negatively with BMI (Spearman's $r = -0.630$, $P = 0.000$) and waist circumference ($r = -0.592$, $P = 0.000$) but not with plasma irisin levels.

Conclusions: BAT activity was reduced in women with PCOS possibly due to increased central adiposity. In PCOS women, BAT activity did not correlate with plasma irisin levels.

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Introduction

Brown adipose tissue (BAT) has been long known in small mammals and newborns to maintain body temperature through thermogenesis (1). In contrast to the energy-storing function of white adipose tissue, BAT is a highly energetic tissue with a unique mitochondrial expression of uncoupling protein-1 (UCP-1) that converts the cell respiration electrochemical gradient into heat, using glucose and fatty acids as fuels (2, 3, 4).

Although some indirect evidence had led a few authors to postulate the presence of BAT in adult humans (5), it could be demonstrated only with the availability of positron emission tomography (PET) with ^{18}F -fluorodeoxyglucose (^{18}F -FDG), a functional imaging method that evaluates areas of high metabolic activity

(4, 6, 7, 8). BAT cells are found in adult humans mainly in the supraclavicular, cervical and paraesternal regions (9). This discovery attracted great interest and led to the hypothesis that BAT distribution and function may be altered in metabolic diseases (4, 6, 9, 10).

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5–15% of women of reproductive age. It is characterized by clinical and/or biochemical hyperandrogenism, ovulation failure and the presence of enlarged and/or polycystic ovaries in ultrasound images (11). Functional abnormalities of the adipose tissue have been reported in women with PCOS, primarily linked to insulin resistance, even in the absence of obesity (12). Yuan *et al.* demonstrated that BAT activity

was dramatically decreased in a rat model of PCOS and that BAT transplantation reversed PCOS-like features such as estrous cycle irregularity and insulin resistance (13).

During the past decade, skeletal muscle has also been identified as an endocrine organ that releases cytokines and other peptides collectively named myokines. Irisin is an exercise-induced myokine that has been proposed to mediate the beneficial effects of physical exercise on metabolism (14, 15, 16). The active irisin molecule derives from proteolytic cleavage of fibronectin type III domain-containing 5 (FNDC5) and induces 'browning' of white adipocytes through the activation of UCP-1, leading to increased cell respiration and energy expenditure (15, 16, 17, 18). Irisin levels are positively associated with BMI and muscle mass (19). However, patients with type 2 diabetes have paradoxically lower irisin levels despite the association of this condition with obesity (17); therefore, the relationship between irisin and insulin resistance is complex and not fully understood (17, 20).

In women with PCOS, the evidence about irisin regulation remains largely conflicting (21, 22, 23). A study found high serum irisin levels when hyperandrogenism was part of the PCOS phenotype (21), but a recent meta-analysis showed that, with adjustment for BMI, circulating irisin levels are similar in PCOS patients and healthy controls (22). In addition, BAT activity had not been previously assessed in women with PCOS, and we hypothesized that BAT activity and its relation with irisin might be a novel metabolic pathway disrupted in PCOS, with implications for the development of future treatments. Therefore, the aim of this study was to evaluate whether BAT activity is altered in women with PCOS and if it correlates with body measures or with plasma irisin levels.

Subjects and methods

Study participants and ethical approval

This was a cross-sectional study including 70 women aged 18–45 years divided into a group with PCOS ($n=45$) and a healthy control group ($n=25$). The groups were matched by age and BMI based on group means without individual pairing (24), and we stopped enrolment when both groups had reached the minimum sample size with similar age and BMI. Participants were enrolled from April 2015 to September 2017 at the outpatient facilities of two teaching hospitals in Belo Horizonte, Brazil. The study was approved by the Research Ethics Committee of Universidade Federal

de Minas Gerais (Protocol ID 17127713.2.0000.5149) and all patients signed an informed consent.

The diagnosis of PCOS was made according to the 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group diagnostic criteria (25). As per the Rotterdam criteria, we excluded patients with hypo or hyperthyroidism, hyperprolactinemia, 21-hydroxylase deficiency, Cushing's syndrome and androgen-producing tumors. To reduce potential confounders, we also excluded patients who had used antiandrogen drugs in the last six months or any hormones or metformin in the last 2 months. Finally, for safety concerns about the use of intravenous ^{18}F -FDG, we excluded pregnant or puerperal women, women who were breastfeeding, and patients with diabetes or oral glucose intolerance. The control group consisted of women with regular menstrual cycles and without any clinical or biochemical hyperandrogenism or polycystic ovarian morphology and with no use of hormonal therapy or metformin in the past 2 months.

Antropometric measures, lifestyle and laboratory assessment

Body weight, height and waist circumference were measured by the same professional with the participants wearing lightweight clothing without shoes and BMI was calculated according to the standard formula (weight in kilograms divided by squared height in centimeters).

Physical activity was assessed through the International Physical Activity Questionnaire, quantified in min/week and classified into very active, active or sedentary as detailed elsewhere (26, 27). Women were classified as alcohol drinkers if they reported any quantity of alcohol consumption in the past month and as smokers if they smoked at least one cigarette per day (28, 29).

Blood samples were collected at 9:00 h after an overnight fast between the 3rd and 5th days of menstrual cycle. For serum collection, blood was withdrawn in a plain tube, allowed to clot and centrifuged for 10 min at 1500g at room temperature. For plasma collection, blood was withdrawn in an EDTA-coated tube and centrifuged for 10 min at 2500g at 4°C. The serum and plasma aliquots were stored at -80°C until assayed.

Serum levels of follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), prolactin, glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides were measured using commercial assays (30). Total testosterone was assayed by a competitive

immunoassay (Vitros® system, Ortho Clinical Diagnostics, Raritan, NJ, USA). Lipid accumulation product (LAP) index was calculated as abdominal circumference (cm) – $58 \times \text{triglycerides (millimol/liter)}$ (31).

Plasma irisin assay

Plasma irisin levels were assayed using a commercial ELISA kit (CUSABIO®, catalog number CSB-EQ027943HU), following strictly the manufacturer's instructions, in duplicate and with the operator blinded to the patient group. The intra-assay and inter-assay coefficients of variation, using three samples of known concentration, were <8% and <10%, respectively. The assay sensitivity was 0.78 ng/mL.

Brown adipose tissue activity measurement

BAT activity was measured using ^{18}F -FDG PET-CT with cold activation in the morning after 9–12h fasting. The method of cold activation has been previously described and validated (32). In brief, the participants remained in a room at 19°C for 2h wearing only underwear and a thin, sleeveless short gown with an insulation effect of 0.18 clo (33), which corresponds to 18% of the insulating value of clothing needed to maintain a person in comfort sitting at rest in a room at 21°C with air movement of 0.1 m/s and humidity less than 50% (34). During the cold exposure no shivering activity was self-reported by the participants or noted by the researchers. After 1h resting at 19°C, ^{18}F -FDG (0.1 mCi/kg) was administered intravenously and the subjects remained in the same cold conditions for another hour. Afterward, a whole-body PET-CT scan was performed at 20°C using a dedicated PET-CT system (Discovery PET-CT 600, GE Healthcare). Low-dose CT without contrast enhancement was performed first and used for attenuation correction and anatomic image fusion (90 kV, 10–120 mAs (smart mA) with 3.75 mm section thickness). The PET images were reconstructed in a 192×192 matrix, using an Ordered Subsets Expectation Maximization (OSEM)-like algorithm, with 2 iterations and 24 subsets.

^{18}F -FDG uptake in fat areas identified by CT was quantified using a dedicated workstation (Xeleris, GE Healthcare) as described elsewhere (35). BAT activity was expressed in g/mL as the maximum standardized uptake value (SUV_{max}), defined as the tissue radioactivity concentration (KBq/mL) divided by the injection dose per lean body mass (KBq/g). In each one of four body regions (cervico-thoracic, axillary, thoracic, and abdominal) a

region of interest (ROI) was delimited around the most dense uptake zone and used to calculate the SUV_{max} . The highest BAT uptake value was used as the total BAT activity. For categorical analysis, the presence of active BAT was defined as a SUV_{max} of at least 2.0 g/mL, as previously described (6). All images were evaluated by two independent readers (experienced nuclear physician and radiologist), blinded to the clinical findings and laboratorial results.

Statistical analysis

Sample size calculation indicated that 24 participants per group would be sufficient to detect a minimum difference of 5 g/mL (0.8 standard deviation) in BAT activity with 95% confidence and 80% statistical power.

Continuous variables were tested for normal distribution by Shapiro–Wilk test. Normally distributed variables were expressed as means $\pm$ standard deviation (s.d.) and compared between groups using unpaired Student's *t* test, whereas variables that departed from normality were expressed as medians and quartiles and compared by the non-parametric Mann–Whitney *U* test. Differences between groups were adjusted for potential confounders by analysis of covariance (ANCOVA) (36). Categorical variables were submitted to chi-square test with continuity (Yates) correction or Fisher's exact test, as appropriate. Linear correlations were evaluated by Spearman's rank correlation coefficient. We used Prism GraphPad version 6 and SPSS, version 22 software packages. All tests were two-tailed and exact *P* values are reported with 3 decimal places (37).

Results

Baseline characteristics

Table 1 summarizes the characteristics of the two study groups. The PCOS and control groups had similar age, BMI, blood pressure, smoking and alcohol habits, physical activity, serum cholesterol, glucose and TSH levels. The only anthropometric variable that differed between groups was waist circumference, which was larger in PCOS (mean $\pm$ s.d.: 91.0 ± 12.3 cm) than in controls (82.4 ± 10.9 cm, $P=0.006$). Among the PCOS patients, 28 were hyperandrogenic (of whom 26 had all three diagnostic criteria and 2 had hyperandrogenism with polycystic ovarian morphology but not menstrual irregularity) and 17 were normoandrogenic.

Table 1 Characteristics of the study participants.

	PCOS (n = 45)	Control (n = 25)	Mean difference (95% CI)	P value
Age (years)	30.6 ± 5.5	33.2 ± 6.7	−2.6 (−5.6 to 0.3)	0.083*
Weight (kg)	73.4 ± 13.1	69.2 ± 12.1	4.2 (−2.2 to 10.5)	0.195*
Height (cm)	161.1 ± 6.2	160.4 ± 6.3	0.8 (−2.3 to 3.9)	0.613*
Body mass index (kg/m ²)	28.2 ± 4.6	26.9 ± 4.6	1.2 (−1.1 to 3.5)	0.288*
Waist circumference (cm)	91.0 ± 12.3	82.4 ± 10.9	8.5 (2.6–14.5)	0.006*
Blood pressure (mmHg)				
Systolic	110 (110–120)	110 (100–120)		0.557 [†]
Diastolic	70 (70–80)	75 (68–80)		0.825 [†]
Smoking (n, %)	6 (13%)	0 (0%)		0.062 [‡]
Alcohol drinking (n, %)	10 (22%)	3 (12%)		0.235 [‡]
Physical activity duration (min/week)	400 (180–635)	420 (275–930)		0.398 [‡]
Physical activity classification (n, %)	8 (18%)	4 (16%)		0.822 [§]
Very active	27 (60%)	15 (60%)		
Active Sedentary	10 (22%)	6 (24%)		
Current hypocaloric diet (n, %)	7 (17%)	4 (16%)		0.613 [‡]
Serum cholesterol (mg/dL)				
Total	188.4 ± 38.7	184.3 ± 16.2	4.1 (−26.1 to 34.3)	0.786*
HDL-c	47.5 ± 12.4	54.1 ± 11.4	−6.7 (−16.8 to 3.5)	0.192*
Fasting Glucose (mg/dL)	88.3 ± 7.1	92.4 ± 7.8	−3.1 (−6.6 to 0.5)	0.088*
Serum TSH (μIU/mL)	2.54 ± 2.45	2.95 ± 0.79	−0.4 (−2.5 to 1.6)	0.691*

Data are expressed as means ± s.d. or medians (interquartile ranges). P values refer to *Unpaired t test, [†]Mann-Whitney test, [‡]Fisher's exact test or [§]chi-square test, as appropriate.

BAT distribution and correlations

Fig. 1 illustrates typical ¹⁸F-FDG PET-CT images for detection of BAT in women with PCOS and healthy controls. Specific BAT activity was detected in all body regions evaluated and was present in 46/70 (65.7%) of the participants.

BAT activity was significantly reduced in women with PCOS (median SUV_{max}: 7.4, interquartile range: 0.9–15.4) compared to controls (median SUV_{max}: 13.0, interquartile range: 4.7–18.4, *P* = 0.047, Mann-Whitney test, Fig. 2). When considering each anatomic region evaluated, the BAT activity level measured at region 3 (thorax) was

Figure 1

Typical localization of brown fat activity with ¹⁸F-FDG PET-CT. (A) Maximal intensity projection showing diffuse uptake of ¹⁸F-FDG at different sites of brown fat. (B, C, D, E, F, G, H and I) Representative axial slices throughout a patient's body showing sites of brown fat (red arrows). (B) Bilateral cervical regions; (C) bilateral cervico-thoracic transition; (D) bilateral cervico-thoracic transition, bilateral axillary regions; (E) bilateral axillary regions and anterior thoracic region; (F) mediastinal, bilateral axillary and bilateral intercostal regions; (G) thoracic-abdominal transition (close to esophagus); (H) right perirenal space; (I) intraabdominal region. A full colour version of this figure is available at <https://doi.org/10.1530/EJE-19-0505>.

significantly reduced in women with PCOS (median SUV_{max} : 1.0, interquartile range: 0.9–7.0) compared to controls (SUV_{max} : 6.4, interquartile range: 1.0–10.8, $P=0.010$, Mann–Whitney test, Fig. 2). However, these differences were no longer significant when adjusted for waist circumference ($P=0.885$ and 0.205 for total BAT and region 3, respectively).

Correlation analyses pooling PCOS women and controls showed that BAT activity was negatively correlated with BMI (Spearman's $r=-0.547$, $P=0.000$) and waist circumference ($r=-0.562$, $P=0.000$). In addition, BAT was weakly and negatively correlated with plasma irisin levels ($r=-0.266$, $P=0.026$, Fig. 2).

When considering only women with PCOS, BAT activity correlated inversely with BMI, waist circumference and LAP index in total and in most anatomical regions analyzed. Remarkably, in women with PCOS total BAT activity showed no correlation with age, serum testosterone, serum insulin or plasma irisin levels (Table 2). Secondary analysis did not show any significant difference in total BAT activity between hyperandrogenic

and normoandrogenic women with PCOS (adjusted $P=0.165$).

Plasma irisin levels

Fig. 3 shows that plasma irisin levels were similar in women with PCOS and controls, but there was a positive correlation between irisin and BMI in the whole study sample ($r=0.581$, $P=0.000$), as well as in each study group (PCOS, $r=0.542$, $P=0.000$; control, $r=0.662$, $P=0.000$).

Discussion

The main finding of our study was that patients with PCOS had a lower BAT activity than control patients. This finding was due to the decreased BAT activity in the thoracic region. We also found that BAT activity is inversely correlated with BMI and waist circumference but not with plasma irisin levels in PCOS women.

To the best of our knowledge, this is the first study that evaluated BAT activity and anatomical distribution

Figure 2

Quantification of brown adipose tissue activity by ^{18}F -FDG uptake in control ($n = 25$) and PCOS ($n = 45$) groups. The boxplots represent the medians and quartiles, with error bars indicating the 10th and 90th percentiles. The scatter plots in the upper panel show negative correlations between total BAT and BMI, waist circumference and plasma irisin. The lower plots compare BAT activity in the two groups at Region 1 (cervico-thoracic), Region 2 (axillary), Region 3 (thoracic), and Region 4 (abdominal). P values refer to Mann–Whitney test.

Table 2 Linear correlation between brown adipose tissue activity at different body regions and some anthropometric and metabolic variables in PCOS women ($n = 45$).

	Total	Region 1 cervico-thoracic	Region 2 axillary	Region 3 thoracic	Region 4 abdominal
Age	-0.241 (0.111)	-0.134 (0.380)	-0.148 (0.331)	-0.136 (0.374)	-0.007 (0.961)
Body mass index	-0.630 (0.000)	-0.602 (0.000)	-0.675 (0.000)	-0.534 (0.000)	-0.498 (0.001)
Waist circumference	-0.592 (0.000)	-0.503 (0.001)	-0.628 (0.000)	-0.590 (0.000)	-0.437 (0.003)
LAP index	-0.392 (0.022)	-0.440 (0.009)	-0.549 (0.001)	-0.307 (0.077)	-0.359 (0.037)
Serum total testosterone	0.162 (0.383)	0.151 (0.419)	0.112 (0.549)	0.303 (0.097)	0.149 (0.423)
Serum insulin	-0.109 (0.666)	-0.083 (0.745)	-0.265 (0.289)	-0.176 (0.486)	-0.075 (0.767)
Plasma irisin	-0.250 (0.098)	-0.263 (0.081)	-0.288 (0.055)	-0.257 (0.089)	-0.251 (0.097)

Data are presented as Spearman's rank correlation coefficient and P values are shown in parentheses.

in women with PCOS using ^{18}F -FDG PET-CT. However, a recent study detected lower skin temperature at supraclavicular region in women with PCOS, which also suggests lower BAT activity compared to controls (38). Based on studies involving other metabolic diseases, we hypothesized that BAT activity might be lower in women with PCOS. In patients with cardiovascular disease (39) or pheochromocytoma (40) the presence of BAT has been negatively associated with central obesity and metabolic dysfunction. Other studies have shown that BAT activity is negatively correlated with visceral fat area (8) and proportion of fat body mass (4) in healthy volunteers.

Here we have expanded this observation to women with PCOS, whose BAT activity was decreased in comparison to controls and was inversely correlated with waist circumference, a surrogate for central adiposity. By using axial magnetic resonance imaging at selected anatomical sites, Barber *et al.* found the same amount of visceral fat in women with and without PCOS (41). However, their groups were matched for fat mass and had equal mean waist circumference, whereas ours were balanced by age and BMI but differed in waist circumference, which was larger in the PCOS group. Thus, it is uncertain whether the lower BAT activity is directly related to PCOS or whether it is associated with visceral adiposity, since the difference between groups does not hold when the results are adjusted for waist circumference. Furthermore,

the lack of correlation between BAT activity and plasma irisin levels in the PCOS group suggests that the lower BAT activity is not explainable by low irisin levels.

The lower activity of brown fat identified in PCOS patients when compared to healthy women was due to differences in the thoracic region. We hypothesize that this might be due to some topographic variation of hormonal mechanisms affecting human BAT metabolism, which however remains to be investigated. Furthermore, there was an inverse correlation between BAT and LAP index, an insulin resistance and cardiovascular risk marker (31). Surprisingly there was no relationship between serum testosterone and BAT activity in any of the studied regions, suggesting that the decrease in brown fat is related to a metabolic issue independent of changes in the hypothalamic-pituitary-ovary axis, and that the reproductive changes of PCOS may not affect the regulation of the browning pathway of white adipose tissue. Accordingly, supraclavicular skin temperature, which indirectly measures BAT activity, was found weakly associated with testosterone levels in women with PCOS (38). Nevertheless, a link between androgens and BAT activity cannot be ruled out since we measured serum testosterone with an immunoassay, which is not as accurate as the gold standard chromatography-mass spectrometry, and we did not assess androgen precursors such as androstenedione and dehydroepiandrosterone sulfate.

Studies suggested that, compared to white fat, BAT depots are less susceptible to developing local inflammation in response to obesity. However, strong obesogenic insults ultimately induce a local pro-inflammatory environment in brown fat. In mice, this condition directly alters the thermogenic activity of BAT by impairing its energy expenditure mechanism and reducing glucose uptake for use as a fuel substrate (42). This mechanism might justify the lower BAT activity in PCOS patients as the syndrome is often associated with chronic low-grade inflammation (43).

There is growing interest in exploring circulating irisin levels in PCOS patients, as many studies demonstrated

Figure 3

Plasma irisin levels in control ($n = 25$) and PCOS ($n = 45$) groups and their correlation with BMI.

that irisin can be involved with PCOS pathophysiology (22). In the current study, PCOS women had similar plasma irisin concentrations in comparison to the control group. In the literature, the characterization of irisin in PCOS is quite controversial since normal (44), increased (18, 21, 23) and decreased (16) irisin concentrations have been described in these patients. This inconsistency may be partly due to differences in the assay methods, but it may also be due to the interference of confounding variables associated with PCOS. Recently, two meta-analyses have summarized irisin levels in women with PCOS. The first meta-analysis included eight studies involving 1918 PCOS patients and 528 controls. The pooled data indicated that the levels of irisin were higher in patients with PCOS and were unrelated to insulin resistance (45). The other meta-analysis included eight studies with 918 PCOS patients and 528 healthy controls and demonstrated that, when adjusted for BMI, circulating irisin in PCOS patients seems comparable to healthy controls (22).

One strength of the present study is that the PCOS and control groups were matched by age and BMI and were similar except by abdominal circumference, which is often increased in PCOS (31, 46). In addition, PET-CT examinations were performed by the same professionals unaware of the participant's group, while plasma irisin assays were performed blindly and by a widely validated technique. Our study also managed to recruit the number of participants calculated to achieve statistical power in the comparisons between PCOS and control groups. One limitation of this study is the cross-sectional design that does not allow us to define whether the lower BAT activity was present before the onset of PCOS symptoms. Another limitation is that androgen levels were assessed only by total testosterone immunoassay.

In conclusion, BAT activity was reduced in women with PCOS possibly due to increased central adiposity. In PCOS women, BAT activity did not correlate with plasma irisin levels.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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References

- Nicholls DG, Bernson VS & Heaton GM. The identification of the component in the inner membrane of brown adipose tissue mitochondria responsible for regulating energy dissipation. *Experientia. Supplementum* 1978 **32** 89–93. (https://doi.org/10.1007/978-3-0348-5559-4_9)
- Rothwell NJ & Stock MJ. Effects of age on diet-induced thermogenesis and brown adipose tissue metabolism in the rat. *International Journal of Obesity* 1983 **7** 583–589.
- Gesta S, Tseng YH & Kahn CR. Developmental origin of fat: tracking obesity to its source. *Cell* 2007 **131** 242–256. (<https://doi.org/10.1016/j.cell.2007.10.004>)
- van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P & Teule GJ. Cold-activated brown adipose tissue in healthy men. *New England Journal of Medicine* 2009 **360** 1500–1508. (<https://doi.org/10.1056/NEJMoa0808718>)
- Jung RT, Leslie P, Nicholls DG, Cunningham S & Isles TE. Energy expenditure in normal and diabetic man: the role of brown adipose tissue. *Health Bulletin* 1988 **46** 55–62.
- Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A *et al.* Identification and importance of brown adipose tissue in adult humans. *New England Journal of Medicine* 2009 **360** 1509–1517. (<https://doi.org/10.1056/NEJMoa0810780>)
- Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S *et al.* Functional brown adipose tissue in healthy adults. *New England Journal of Medicine* 2009 **360** 1518–1525. (<https://doi.org/10.1056/NEJMoa0808949>)
- Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K *et al.* High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 2009 **58** 1526–1531. (<https://doi.org/10.2337/db09-0530>)
- Nedergaard J, Bengtsson T & Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology: Endocrinology and Metabolism* 2007 **293** E444–E452. (<https://doi.org/10.1152/ajpendo.00691.2006>)
- Hany TE, Gharehpapagh E, Kamel EM, Buck A, Himms-Hagen J & von Schulthess GK. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *European Journal of Nuclear Medicine and Molecular Imaging* 2002 **29** 1393–1398. (<https://doi.org/10.1007/s00259-002-0902-6>)
- Rosenfield RL & Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocrine Reviews* 2016 **37** 467–520. (<https://doi.org/10.1210/er.2015-1104>)
- Villa J & Pratley RE. Adipose tissue dysfunction in polycystic ovary syndrome. *Current Diabetes Reports* 2011 **11** 179–184. (<https://doi.org/10.1007/s11892-011-0189-8>)
- Yuan X, Hu T, Zhao H, Huang Y, Ye R, Lin J, Zhang C, Zhang H, Wei G, Zhou H *et al.* Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *PNAS* 2016 **113** 2708–2713. (<https://doi.org/10.1073/pnas.1523236113>)
- Pedersen BK & Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature Reviews Endocrinology* 2012 **8** 457–465. (<https://doi.org/10.1038/nrendo.2012.49>)
- Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ *et al.* A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012 **481** 463–468. (<https://doi.org/10.1038/nature10777>)
- Abali R, Temel Yuksel I, Yuksel MA, Bulut B, Imamoglu M, Emirdar V, Unal F, Guzel S & Celik C. Implications of circulating irisin and Fabp4 levels in patients with polycystic ovary syndrome. *Journal of*

- Obstetrics and Gynaecology* 2016 **36** 897–901. (<https://doi.org/10.3109/01443615.2016.1174200>)
- 17 Polyzos SA, Anastasilakis AD, Efstathiadou ZA, Makras P, Perakakis N, Kountouras J & Mantzoros CS. Irisin in metabolic diseases. *Endocrine* 2018 **59** 260–274. (<https://doi.org/10.1007/s12020-017-1476-1>)
 - 18 Li M, Yang M, Zhou X, Fang X, Hu W, Zhu W, Wang C, Liu D, Li S, Liu H *et al.* Elevated circulating levels of irisin and the effect of metformin treatment in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1485–1493. (<https://doi.org/10.1210/jc.2014-2544>)
 - 19 Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE & Mantzoros CS. FND5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism: Clinical and Experimental* 2012 **61** 1725–1738. (<https://doi.org/10.1016/j.metabol.2012.09.002>)
 - 20 Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, Ricart W & Fernandez-Real JM. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E769–E778. (<https://doi.org/10.1210/jc.2012-2749>)
 - 21 Zhang L, Fang X, Li L, Liu R, Zhang C, Liu H, Tan M & Yang G. The association between circulating irisin levels and different phenotypes of polycystic ovary syndrome. *Journal of Endocrinological Investigation* 2018 **41** 1401–1407. (<https://doi.org/10.1007/s40618-018-0902-4>)
 - 22 Cai X, Qiu S, Li L, Zugel M, Steinacker JM & Schumann U. Circulating irisin in patients with polycystic ovary syndrome: a meta-analysis. *Reproductive Biomedicine Online* 2018 **36** 172–180. (<https://doi.org/10.1016/j.rbmo.2017.10.114>)
 - 23 Chang CL, Huang SY, Soong YK, Cheng PJ, Wang CJ & Liang IT. Circulating irisin and glucose-dependent insulinotropic peptide are associated with the development of polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E2539–E2548. (<https://doi.org/10.1210/jc.2014-1180>)
 - 24 Costanza MC. Matching. *Preventive Medicine* 1995 **24** 425–433. (<https://doi.org/10.1006/pmed.1995.1069>)
 - 25 Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and Sterility* 2004 **81** 19–25.
 - 26 Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF *et al.* International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise* 2003 **35** 1381–1395. (<https://doi.org/10.1249/01.MSS.0000078924.61453.FB>)
 - 27 Macfarlane DJ, Lee CC, Ho EY, Chan KL & Chan D. Convergent validity of six methods to assess physical activity in daily life. *Journal of Applied Physiology* 2006 **101** 1328–1334. (<https://doi.org/10.1152/jappphysiol.00336.2006>)
 - 28 Colpani V, Oppermann K & Spritzer PM. Causes of death and associated risk factors among climacteric women from Southern Brazil: a population based-study. *BMC Public Health* 2014 **14** 194. (<https://doi.org/10.1186/1471-2458-14-194>)
 - 29 Bastos CA, Oppermann K, Fuchs SC, Donato GB & Spritzer PM. Determinants of ovarian volume in pre-, menopausal transition, and post-menopausal women: a population-based study. *Maturitas* 2006 **53** 405–412. (<https://doi.org/10.1016/j.maturitas.2005.07.002>)
 - 30 Lauria PB, Del Puerto HL, Reis AM, Candido AL & Reis FM. Low plasma atrial natriuretic peptide: a new piece in the puzzle of polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4882–4889. (<https://doi.org/10.1210/jc.2013-2141>)
 - 31 Wiltgen D, Benedetto IG, Mastella LS & Spritzer PM. Lipid accumulation product index: a reliable marker of cardiovascular risk in polycystic ovary syndrome. *Human Reproduction* 2009 **24** 1726–1731. (<https://doi.org/10.1093/humrep/dep072>)
 - 32 Martinez-Tellez B, Sanchez-Delgado G, Garcia-Rivero Y, Alcantara JMA, Martinez-Avila WD, Munoz-Hernandez MV, Olza J, Boon MR, Rensen PCN, Llamas-Elvira JM *et al.* A new personalized cooling protocol to activate brown adipose tissue in young adults. *Frontiers in Physiology* 2017 **8** 863.
 - 33 Engineers ASoHRAA-C. 2009 ASHRAE Handbook - Fundamentals. ASHRAE, 2009.
 - 34 ToolBox E. Clo - Clothing and Thermal Insulation, 2004.
 - 35 Sugawara Y, Zasadny KR, Neuheff AW & Wahl RL. Reevaluation of the standardized uptake value for FDG: variations with body weight and methods for correction. *Radiology* 1999 **213** 521–525. (<https://doi.org/10.1148/radiology.213.2.r99nv37521>)
 - 36 Pourhoseingholi MA, Baghestani AR & Vahedi M. How to control confounding effects by statistical analysis. *Gastroenterology and Hepatology from Bed to Bench* 2012 **5** 79–83.
 - 37 Dekkers OM. Why not to (over)emphasize statistical significance. *European Journal of Endocrinology* 2019 **181** E1–E2. (<https://doi.org/10.1530/EJE-19-0531>)
 - 38 Shorakae S, Jona E, de Courten B, Lambert GW, Lambert EA, Phillips SE, Clarke IJ, Teede HJ & Henry BA. Brown adipose tissue thermogenesis in polycystic ovary syndrome. *Clinical Endocrinology* 2019 **90** 425–432. (<https://doi.org/10.1111/cen.13913>)
 - 39 Franssens BT, Hoogduin H, Leiner T, van der Graaf Y & Visseren FLJ. Relation between brown adipose tissue and measures of obesity and metabolic dysfunction in patients with cardiovascular disease. *Journal of Magnetic Resonance Imaging* 2017 **46** 497–504. (<https://doi.org/10.1002/jmri.25594>)
 - 40 Wang Q, Zhang M, Ning G, Gu W, Su T, Xu M, Li B & Wang W. Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PLoS ONE* 2011 **6** e21006. (<https://doi.org/10.1371/journal.pone.0021006>)
 - 41 Barber TM, Golding SJ, Alvey C, Wass JA, Karpe F, Franks S & McCarthy MI. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 999–1004. (<https://doi.org/10.1210/jc.2007-2117>)
 - 42 Villarroya F, Cereijo R, Gavalda-Navarro A, Villarroya J & Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. *Journal of Internal Medicine* 2018 **284** 492–504. (<https://doi.org/10.1111/joim.12803>)
 - 43 Spritzer PM, Lecke SB, Satler F & Morsch DM. Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome. *Reproduction* 2015 **149** R219–R227. (<https://doi.org/10.1530/REP-14-0435>)
 - 44 Pukajlo K, Laczmannski Ł, Kolackov K, Kuliczowska-Plaksej J, Bolanowski M, Milewicz A & Daroszewski J. Irisin plasma concentration in PCOS and healthy subjects is related to body fat content and android fat distribution. *Gynecological Endocrinology* 2015 **31** 907–911. (<https://doi.org/10.3109/09513590.2015.1065482>)
 - 45 Wang C, Zhang XY, Sun Y, Hou XG & Chen L. Higher circulating irisin levels in patients with polycystic ovary syndrome: a meta-analysis. *Gynecological Endocrinology* 2018 **34** 290–293. (<https://doi.org/10.1080/09513590.2017.1393065>)
 - 46 Couto Alves A, Valcarcel B, Makinen VP, Morin-Papunen L, Sebert S, Kangas AJ, Soininen P, Das S, De Iorio M, Coin L *et al.* Metabolic profiling of polycystic ovary syndrome reveals interactions with abdominal obesity. *International Journal of Obesity* 2017 **41** 1331–1340. (<https://doi.org/10.1038/ijo.2017.126>)

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