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Title: Brown adipose tissue thermogenesis in polycystic ovary syndrome

Running title: BAT thermogenesis in PCOS

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Summary

Objective. Polycystic ovary syndrome (PCOS) is associated with increased obesity with a greater propensity to weight gain and a lack of sustainable lifestyle interventions. Altered brown adipose tissue (BAT) thermogenesis is a potential contributor to obesity in PCOS.

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BAT activity and modulation has not been studied in PCOS. This observational study explored BAT thermogenesis and its associations in women with and without PCOS.

Participants and methods. Cutaneous temperature was recorded from supraclavicular (indicator of BAT activity) and upper arm regions using Dataloggers (SubCue, Calgary, Canada) in a cross sectional sub-study, nested within a randomized control trial, of community recruited pre-menopausal women with (n=47, Rotterdam diagnostic criteria) and without (n=11) PCOS.

Results. Complete temperature data were available in 44 PCOS (mean age: 30.0±6.2, mean BMI: 29.3±5.5) and 11 non-PCOS (mean age: 33.0±7.0, mean BMI: 25±3) women. Women with PCOS had lower supraclavicular skin temperature compared to controls overall (33.9±0.7 vs 34.5±1, p < 0.05) and during sleep (34.5±0.6 vs 35.2±0.9, p < 0.001). In the PCOS group, supraclavicular skin temperature overall and over sleep and waking hours correlated inversely with testosterone (r= - 0.41 p <0.05, r= -0.485 p <0.01 and r= -0.450 p <0.01 respectively). Testosterone levels explained approximately 15%, 30% and 20% of the variability in supraclavicular skin temperature overall and over sleep and waking hours in women with PCOS, respectively.

Conclusion. Women with PCOS have lower BAT activity compared to controls. BAT thermogenesis is negatively associated with androgen levels in PCOS.

Keywords: Obesity, sympathetic nervous system, hyperandrogenism, brown adipose tissue

Introduction

Polycystic ovary syndrome is a complex, common endocrinopathy affecting 8-13% of reproductive age women¹ and is associated with increased cardiometabolic risk factors including visceral obesity, dyslipidemia, impaired glucose homeostasis, and potentially cardiovascular disease^{2,3}. PCOS is characterised by reproductive, metabolic and psychological features with androgen excess and insulin resistance being the key pathophysiological mechanisms underpinning the disease³.

Overweight (body mass index (BMI) 25–29.9 kg/m²) and obesity (BMI ≥ 30 kg/m²) are major global health concerns with rising prevalence, and health burden. Excess body fat is closely associated with morbidity and mortality, with central or visceral adiposity inducing greater insulin resistance and cardiometabolic complications⁴. Women with PCOS have higher prevalence of obesity, particularly central and visceral obesity⁵ and obesity, independently, exacerbates reproductive, metabolic and psychological features in PCOS⁶. Weight loss

through diet and lifestyle interventions is the first line treatment in PCOS⁶, yet sustainability and long term efficacy of dietary interventions is limited⁷. Metabolically active brown adipose tissue (BAT), contributing to energy balance via heat dissipation, has been recognised in humans⁸. Brown adipocyte mitochondria have a unique inner membrane protein, Uncoupling Protein 1 (UCP-1), which facilitates heat production via uncoupling aerobic respiration⁹. This potential capacity of stimulated BAT renders it an appealing target for prevention and treatment of obesity¹⁰. BAT thermogenesis is regulated by environmental temperature, physical activity, the sympathetic nervous system (SNS), inflammation, thyroid hormones, sex steroids, metabolic factors, age and nutritional status¹¹⁻¹³. Recent studies in rat models of PCOS suggest significant reduction in BAT activity when compared to normal rats and BAT transplantation alleviated the metabolic and reproductive features in PCOS animals¹⁴. Given the association of PCOS with SNS dysfunction, chronic low-grade inflammation and hyperandrogenism^{15,16}, altered BAT thermogenesis is a potential contributor to propensity for obesity in PCOS. Therefore, understanding BAT thermogenesis and its regulation in PCOS, may shed some light on the mechanisms underpinning obesity in this condition. The current gold standard method for detection of BAT and quantifying its activity is ¹⁸F-FDG uptake assessed by PET-CT, but this has limitations due to high costs and the level of radiation exposure¹⁷. The use of PET-CT provides a single snapshot in time and in the vast majority of subjects, active BAT is only detectable in response to cold exposure. Previous work has validated the measurement of cutaneous temperature recordings of the supraclavicular region as a qualitative measurement of BAT activity¹⁸⁻²¹. Here, we aimed to quantify BAT activity utilising this technique (primary outcome) with the aim to extend animal data with a novel study exploring BAT thermogenesis and its associations (secondary outcomes), in women with and without PCOS.

Methods

Study design and participants

This observational study is a sub-study using baseline data from a double blind randomised controlled trial (NCT01504321) in PCOS, which was approved by Local Ethics committees at the Alfred Hospital and Monash Health. All subjects gave written informed consent before participation. In addition, a group of participants without PCOS (control group) were recruited for the purpose of this study of brown adipose tissue thermogenesis.

Pre-menopausal women with and without PCOS were recruited by advertisement from the local community between January 2013 and March 2018. PCOS was diagnosed according to Rotterdam criteria with presence of the two of the following three, irregular menstruation (cycle length greater than 35 days), clinical (hirsutism) or biochemical hyperandrogenism

and polycystic ovarian morphology on ultrasound (presence of 12 or more follicles measuring 2-9 mm in each or both ovaries)²². Hirsutism was evaluated using a modified Ferriman-Gallwey scoring (m-FG score) system²³. Clinical hyperandrogenism was defined as an m-FG score above 8 in Caucasian and above 6 in Asian women.

Exclusion criteria included pregnancy, diabetes, any medication that could interfere with activity of the sympathetic nervous system and insulin resistance within 3 months of recruitment, a history of secondary hypertension, cardiovascular, cerebrovascular, renal, liver, thyroid or lung disease and severe mental illness. Women who were taking oral contraceptive pills (OCP) or metformin were asked to stop the medication, use barrier contraception if desired and if no contraindications and went through a 3 month wash out period for OCP and 1 month wash out period for metformin prior to recruitment.

Fasting venous blood samples were drawn from participants. Plasma was collected and stored at -80 °C for measurement of progesterone, total testosterone, sex hormone binding globulin (SHBG), thyroid stimulating hormone (TSH), glucose, insulin, total cholesterol, and triglyceride (Tg) concentrations.

Anthropometric measurements

Body weight was measured in underclothes without shoes, using a digital scale. BMI was calculated as weight (kg)/height squared (m²). Waist circumference was measured at the midpoint between iliac crest and the lowest rib.

Biochemical and hormone assays

Insulin resistance was determined using the Homeostatic Model Assessment for insulin resistance (HOMA-IR), calculated as (fasting insulin x fasting glucose/22.5). The Access SHBG assay was performed using a sequential two-step immunoenzymatic ('sandwich') assay carried out on a Beckman Coulter Unicel DXI 800 (Beckman Coulter, Lane Cove, NSW, Australia). Testosterone assay was performed by high performance liquid chromatography–mass spectrometry (HPLCMS/MS) method using a liquid sample extraction (AB Sciex Triple Quad 5500 LC/MS/MS system; Mt Waverley, VIC, Australia). Free Androgen Index (FAI) was calculated as (total testosterone x 100)/SHBG. Plasma progesterone levels were measured by radioimmunoassay using the double antibody ImmuChem kit (MP Biomedicals). Across two assays the average sensitivity was 0.03 ng/ml and the intra-assay co-efficient of variation was 5 %.

Sympathetic Nerve activity

Muscle sympathetic nerve activity (MSNA), expressed as burst frequency and burst incidence, was measured only in the PCOS group as described previously²⁴.

Cutaneous Temperature Measurement

Wireless cutaneous temperature monitors (Dataloggers; 2cm diameter, 0.5 depth) obtained from SubCue (Calgary, Canada) were attached to the supraclavicular and upper arm skin

using adhesive tape for 4 consecutive days. Temperature was measured continuously at 15 to 30 minute intervals whilst participants underwent routine daily activities. The dataloggers have been used extensively in large animal models to monitor temperature over extended times and fixed intervals²⁵. Participants were taught how to replace the monitors on the skin on the supraclavicular area and outer/upper arm in case the probes required replacement. Participants were asked to fill in a simple food and activity diary while wearing temperature monitors. Data were downloaded from each temperature monitor following completion of the recording period, by transfer to a computer with software designed for these devices (SubCue). Data obtained across the 48 h period in the middle of the 4 day recording period was used for analyses. Recording periods that coincided with physical activity i.e. sex and exercise were excluded, and the remaining data was used to calculate the average skin temperature from supraclavicular ($Temp_{sc}$) and upper arm ($Temp_{arm}$) regions for each participant. Subsequently average skin temperature was calculated over sleep ($Temp_{arm/sleep}$, $Temp_{sc/sleep}$) and waking ($Temp_{arm/awake}$, $Temp_{sc/awake}$) hours for each region, considering recordings during sleep hours to be more reflective of pure BAT thermogenesis and not being affected by physical activity or food consumption.

Statistical Analysis

Data were analysed using SPSS Statistics, version 22 (Armonk, NY, USA). Data are presented as mean $\pm$ SD or median (IQR) if data were skewed. Histograms and the Komolov-Smirnov test were used to determine data distribution and skewed data were log transformed if appropriate.

A sample size of 25 (PCOS to non-PCOS ratio: 4) was needed to demonstrate a difference in supraclavicular temperature of 1.3 ± 0.6 with 90% power ($\alpha = 0.05$). This was based on data on mean supraclavicular temperature in healthy subjects^{18,19} and our previous finding from the PCOS cohort of this study (recruited through the double blind randomised controlled trial as explained in the study design).

For descriptive analysis, participants with PCOS were grouped according to BMI categories (lean: BMI < 25, overweight: $25 \leq BMI < 30$, obese: BMI ≥ 30) and metabolic syndrome defined according to International Diabetes Federation (IDF) guidelines²⁶. Student's t-tests were used to compare mean supraclavicular and arm temperatures, overall and during sleeping and waking hours between the two groups. Correlations were determined using Pearson's correlation coefficient and variables were entered into a linear regression model where an individual correlation achieved statistical significance (p-value cut off 0.1) and/or if clinically indicated. Regression models were tested for collinearity.

All analyses were adjusted for season of study (warm season: September – February, cold season: March – August) to account for the effect of environmental temperature on

thermogenesis. Methods were confirmed with an experienced statistician. Statistical significance was defined as a p-value less than 0.05.

Results

Characteristics of participants

Forty-seven women with PCOS and 11 control women without PCOS were recruited. Recorded temperature data were available in 44 PCOS (mean age: 30.0 ± 6.2 , mean BMI: 29 ± 6) and 11 non-PCOS women (mean age: 33.0 ± 7.0 , mean BMI: 25 ± 3). In the PCOS group, 36 women reported menstrual irregularity and 41 were classified as hyperandrogenic (39 had clinical hyperandrogenism and 15 had biochemical hyperandrogenism). Women with PCOS had higher BMI, WC, fasting insulin, HOMA-IR, testosterone, FAI and m-FG score and lower heart rate compared to controls. In the PCOS group, 11 women (25%) were lean, 12 (27%) were overweight and 21 (48%) were obese. Nine women (21%) met the criteria for metabolic syndrome. Other participant characteristics are summarised in Table 1.

Cutaneous temperature

Supraclavicular skin temperature was higher than arm skin temperature overall and during waking and sleep hours in both groups (Table 2). Supraclavicular skin temperature was higher during sleep hours compared to waking hours in both groups (Table 2). Women with PCOS had significantly lower supraclavicular skin temperature compared to the control group overall (33.9 ± 0.7 vs 34.5 ± 1 , $p < 0.05$) and during sleep hours (34.5 ± 0.6 vs 35.2 ± 0.9 , $p < 0.001$) (Figure 1). The difference in supraclavicular temperature overall and over sleep hours remained significant ($p < 0.05$ and $p < 0.01$ respectively) after adjustment for BMI and season alone or together. The difference in arm temperature was not significant between the two groups.

Correlations

The univariate correlation of supraclavicular skin temperature with anthropometric, haemodynamic, metabolic, sympathetic (only available in the PCOS group) and reproductive parameters was explored in each group.

In the PCOS group, supraclavicular skin temperature overall ($Temp_{sc}$) and over sleep hours ($Temp_{sc/sleep}$) and waking hours ($Temp_{sc/awake}$) correlated inversely with testosterone ($r = -0.41$ $p < 0.05$, $r = -0.485$ $p < 0.01$ and $r = -0.450$ $p < 0.01$ respectively) (Figure 2). Arm skin temperature ($Temp_{arm}$) did not correlate with testosterone levels in the PCOS group. In the control group, supraclavicular skin temperature did not correlate with testosterone levels (Figure 2).

Body mass index, age, season, TSH and Testosterone were included in a regression model to explore relationships with supraclavicular skin temperature ($Temp_{sc}$, $Temp_{sc/sleep}$ and $Temp_{sc/awake}$) in women with PCOS. In the final model, testosterone levels explained

approximately 15%, 30% and 20% of the variability in supraclavicular skin temperature overall ($p < 0.05$), over sleep hours ($p < 0.01$) and waking hours ($p < 0.01$), respectively (Table 3).

Discussion

In this novel study, we investigated BAT activity (on skin temperature at two sites using cutaneous temperature probes) in women with and without PCOS, a known metabolic condition with a high prevalence of obesity. Supraclavicular skin temperature was lower in women with PCOS and correlated inversely with testosterone levels. In women with PCOS, testosterone levels explained approximately 15%, 30% and 20% of the variability in supraclavicular skin temperature overall, over sleep hours and waking hours respectively.

There is an increased prevalence of overweight and obesity in PCOS (40% to 60%) with these women having greater abdominal or visceral adiposity compared to their weight-matched controls^{6,27}. While influenced by the worldwide increase in prevalence of obesity, specific drivers of weight gain in PCOS are poorly understood, with evidence suggesting that diet and physical activity alone, do not sufficiently explain excess weight gain in this condition^{6,28}. In this study, women with PCOS had lower supraclavicular temperature compared to control women by 0.6-0.7 °C. While this is the first study to compare supraclavicular temperature in PCOS versus controls, it is important to note that a temperature difference of 0.3 °C in supraclavicular area has been considered clinically significant by previous literature in the context of assessing BAT thermogenesis¹⁹. This lower supraclavicular temperature, suggests that altered BAT thermogenesis could potentially contribute to propensity for obesity in women with PCOS. In this study women with PCOS were more obese than control women, however the p-value for difference in mean supraclavicular temperature between the two groups remained significant after adjusting the analysis for BMI. Moreover regressing supraclavicular temperature for group and BMI indicated a more significant effect of group (B coefficient= -0.6, $p < 0.05$) compared to BMI (B coefficient= - 0.005, $p > 0.1$).

There is no single method to comprehensively characterize BAT activity, volume and morphology in humans²⁹. A safe, reliable and easily accessible technique is desirable to expedite the quantification of BAT activity in human studies. Animal studies have used surgically implanted, biocompatible temperature probes to directly measure heat production within discrete tissue beds including BAT, white adipose tissue and skeletal muscle³⁰⁻³². The invasive nature of such procedures makes this technique inappropriate for temperature measurement in humans. Ingestible telemetric capsules or wireless cutaneous temperature monitors have also been validated in human studies of BAT thermogenesis^{18,19}. However, these provide an index of core body temperature and are not a direct measure of BAT

activity. Previous work has validated the measurement of cutaneous temperature recordings of the supraclavicular region as a qualitative measurement of BAT activity in human¹⁸⁻²¹. Boon et al. reported supraclavicular skin temperature positively correlated with both total (R²=0.28, P=0.010) and clavicular BAT volume (R²=0.20, P=0.030) and the clavicular maximum standardised uptake volume (SUV max) (R²=0.27, P=0.010) measured by 18F-FDG PET/CT. Van der Lans et al. also reported a significant positive correlation between the change in supraclavicular skin temperature and BAT activity (R²=0.23, P<0.01), and the change in supraclavicular skin temperature and non-shivering thermogenesis (R²=0.18, P<0.01) measured by 18F-FDG PET. Here we applied cutaneous temperature measurement of the supraclavicular area, where most human BAT is concentrated and showed higher temperatures than that of the upper arm skin (overlying muscle) by approximately 1.5°C. This temperature difference remained consistent during sleep and waking hours, highlighting usefulness for cutaneous temperature recordings for the estimation of BAT activity. The thermogenic activity of BAT is predominantly regulated by the hypothalamus, a primary locus for coordinating and integrating metabolic and thermoregulatory information. This information is then relayed to the periphery via sympathetic efferent nerves, which innervate brown adipose depots¹¹. Rodent studies have proven the stimulatory effect of noradrenaline on proliferation, differentiation and activity of BAT via activation of β -adrenergic receptors. Indeed, deletion of all three β -adrenergic receptors in BAT of mice abolishes thermogenesis and leads to severe obesity³³. In humans, similar evidence in pheochromocytoma shows a higher abundance of BAT occurs in peri-renal, peri-adrenal and omental fat biopsies³⁴ and a higher FDG uptake in BAT depots correlates with circulating catecholamine levels³⁵. However, it is important to note that sympathetic regulation of BAT thermogenesis in humans is complex, involving specific sympathetic pathways and is influenced by BMI³⁶⁻³⁹. In the present study, muscle sympathetic activity did not correlate with supraclavicular skin temperature in the PCOS group. Keeping in mind that sympathetic activity and responses to stimuli display regional specificity the lack of association between MSNA and skin temperature indicates that, at least in this context, the muscle vasoconstrictor outflow is not associated with thermoregulation. While women with PCOS are known to have increased sympathetic activity compared to controls²⁴, in this study women with PCOS had lower heart rate than controls, which despite a significant p-value was not clinically significant. Moreover the heart rate is not a reliable marker of sympathetic activity⁴⁰ as there are other determinants of heart rate in addition to the sympathetic nervous system.

As evident from animal studies, sex steroids exert gender dependent effects on thermogenesis via different mechanisms including modulating adrenergic control of BAT activity and regulating the UCP1 transcription process⁴¹⁻⁴⁴. Testosterone inhibits

thermogenesis via downregulation of lipolysis and UCP1 mRNA expression and protein transcription and modulation of SNS activity in cultured brown adipocytes ⁴¹. Nahora et al. also reported leptin failure to activate BAT UCP1 gene expression subsequent to chronic androgen exposure in adult female mice ⁴⁵. Here, we demonstrate a significant negative correlation of supraclavicular skin temperature with serum free testosterone, which remained consistent over sleeping and waking hours. While the existing literature on the gender dependent effects of sex hormones on BAT activity is controversial, our findings provide insights into potential links between androgens, obesity and metabolic features in PCOS.

Core body temperature follows a circadian rhythm regulated by the suprachiasmatic nuclei (SCN) in the hypothalamus and peripheral tissue clocks ⁴⁶, and BAT activity is found to coincide with this rhythm, rising prior to the active period (day time) and falling at the start of the rest period (night time) ⁴⁷. In this study, both supraclavicular and arm temperature were higher during sleep hours compared to waking hours in women with and without PCOS and this remained consistent regardless of season. This is despite general falls in oxygen consumption and energy expenditure during sleep ⁴⁸. It is important to note, however, that we did not control for other factors affecting recorded temperatures including room temperature, clothing and bed covering during sleep.

Conclusion

In conclusion, we contribute novel data showing lower BAT activity in women with PCOS compared to controls with the negative association of BAT thermogenesis with androgen levels in women with PCOS. We also confirm applying cutaneous temperature recordings is a safe and feasible method of estimating BAT activity in humans. While this novel finding provides insights into potential negative impacts of hyperandrogenism on BAT thermogenesis in PCOS, further research including intervention studies to modulate androgens and BAT activity is warranted to explore the nature of these relationships and advance understanding of drivers of obesity and other metabolic abnormalities in PCOS.

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Table 1. Participant characteristics

Variable	Control (n=11)	PCOS (n=44)	p-value
Age (years)	33±7	30±6	
BMI (Kg/m ²)	25±3	29±6	<0.05
WC (cm)	77.8±3.8	101.6±14.5	<0.01
SBP (mmHg)	104±8	111±12	
DBP (mmHg)	72±5	70±10	
Heart rate (per minute)	74±9	65±9	<0.05
Fasting Glucose (mmol/L)	4.8±0.3	4.7±0.5	
Fasting Insulin (uU/ml)	4.1(1.5)	17.9(13.3)	<0.01
HOMA-IR	0.9(0.4)	3.6(3.2)	<0.01
TC (mmol/L)	4.7±0.9	4.8±0.8	
Tg (mmol/L)	0.8(0.4)	0.9(0.6)	
Testosterone (nmol/L)	0.9±0.5	1.4±0.6	0.05
SHBG (nmol/L)	61±19	42±19	<0.05
FAI	1.6(0.9)	3.5(2.9)	<0.01
m-FG score	3±2	13±6	<0.01
Progesterone (ng/ml)	0.5(11)	0.8(11)	

Note: Data expressed as mean±SD or median(IQR)

body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), Homeostatic Model Assessment for insulin resistance (HOMA-IR), total cholesterol (TC), triglyceride (Tg), sex hormone binding globulin (SHBG), free androgen index (FAI), modified Ferriman-Gallwey score (m-FG score),

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Table 2. Supraclavicular and arm temperature overall and over waking and sleep hours in PCOS and control groups

Temperature	Temp _{sc}	Temp _{arm}	P-value
Control group			
Overall	34.5±1	32.8±1.4	< 0.01
Waking hours	34±1.2	32.2±1.7	< 0.01
Sleep hours	35.5±0.9 [*]	33.7±1.2	< 0.01

PCOS group			
Overall	33.9±0.7	32.3±0.8	< 0.001
Waking hours	33.7±0.9	31.9±0.8	< 0.001
Sleep hours	34.1±0.6 *	33.1±1	< 0.001

Supraclavicular skin temperature (Temp_{sc}), Arm skin temperature (Temp_{arm})

* p < 0.05, supraclavicular temperature over waking hours vs sleep hours

Table 3. Regression analysis of supraclavicular skin temperature (Temp_{sc}, Temp_{sc/sleep} and Temp_{sc/awake}) in women with PCOS

Variable		Temp _{sc}	Temp _{sc/sleep}	Temp _{sc/awake}
Testosterone	B coefficient	-0.407	-0.446	-0.606
	P-value	< 0.05	<0.01	< 0.01
BMI	B coefficient		0.03	
	P-value		<0.05	
Age	B coefficient			
	P-value			
TSH	B coefficient			
	P-value			
Season	B coefficient			
	P-value			
Final model adjusted R²		0.15	0.30	0.18
Final model p-value		<0.05	<0.01	<0.01

Supraclavicular skin temperature overall (Temp_{sc}), over sleep hours (Temp_{sc/sleep}) and waking hours (Temp_{sc/awake})

Legends to figures

Figure 1. Mean supraclavicular skin temperature overall ($Temp_{sc}$) and over waking ($Temp_{sc/awake}$) and sleeping ($Temp_{sc/sleep}$) in women with and without PCOS

Figure 2. Correlation of testosterone level with supraclavicular skin temperature overall ($Temp_{sc}$) and over waking ($Temp_{sc/awake}$) and sleeping ($Temp_{sc/sleep}$) in women with and without PCOS

Figure 1.

Note: * $p < 0.0001$ $Temp_{sc}$ PCOS vs Control, ** $p < 0.001$ $Temp_{sc/sleep}$ PCOS vs Control

Figure 2.

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