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1 **Regional cerebral activation accompanies sympathoexcitation in women with polycystic**
2 **ovary syndrome**

3

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5

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12 **Abbreviated title:** Sympathetic neural activation in PCOS

13 **Keywords:** Polycystic ovary syndrome; insulin resistance; sympathetic nervous system;

14 orbitofrontal cortex; fMRI

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18

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20

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22

23 **Abstract**

24 **Context:** Polycystic ovary syndrome (PCOS) is associated with increased sympathetic nervous
25 system (SNS) activation but the cerebral pathways involved are unclear.

26 **Objective:** To compare cerebral (blood oxygen level-dependent [BOLD] fMRI), pressor
27 (blood pressure [BP], heart rate [HR]) and muscle sympathetic nerve activity (MSNA)
28 responses to isometric forearm contraction (IFC) in women with PCOS and matched controls.

29 **Design:** Case-control study

30 **Setting:** Referral center

31 **Participants:** 20 subjects with PCOS (age 29.8 ± 4.8 yrs, BMI 26.1 ± 4.9 kg/ m²) and 20
32 age/BMI-matched controls (age 29.7 ± 5.0 yrs, BMI 26.1 ± 4.8 kg/ m²)

33 **Main outcome measures:** BP, HR, catecholamine and MSNA responses to 30% IFC. BOLD
34 signal change modelled for blood pressure response to 30% IFC.

35 **Results:** Whilst HR and BP increased to a similar extent in both groups following IFC, MSNA
36 burst frequency increased by 68% in the PCOS group (n=7) compared to 11.9% in controls
37 (n=7) (p=0.002). Brain activation indexed by the BOLD signal in response to IFC was
38 significantly greater in the PCOS group (n=15) compared to controls (n=15) in the right
39 orbitofrontal cortex (p<0.0001). Adjustment for insulin sensitivity, but not hyperandrogenism,
40 abolished these between-group differences.

41 **Conclusions:** Our study confirms enhanced sympathoexcitation in women with PCOS and
42 demonstrates increased regional brain activation in response to IFC. The right orbitofrontal
43 cortex BOLD signal change in women with PCOS is associated with insulin sensitivity. Further
44 studies are warranted to clarify whether this may offer a novel target for cardiovascular risk
45 reduction.

46

47 **Précis**

48 In women with PCOS, enhanced sympathoexcitation is accompanied by cerebral activation in
49 the right orbitofrontal cortex that is influenced by insulin sensitivity.

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70 **Introduction**

71 Polycystic ovary syndrome (PCOS) is a common metabolic disorder characterized by defects
72 in insulin secretion and action. This leads to an increased risk of metabolic syndrome and
73 disorders of glucose tolerance, including type 2 diabetes [1]. Women with PCOS also display
74 a higher prevalence of cardiovascular risk markers, including dyslipidemia [2], hypertension
75 [3] and endothelial dysfunction [4], although studies are yet to confirm if this leads to increased
76 cardiovascular morbidity and mortality.

77

78 Sympathetic nervous system (SNS) activation may also contribute to this enhanced
79 cardiometabolic risk [5], since conditions associated with chronic sympathoexcitation, such as
80 obesity, hyperinsulinemia and obstructive sleep apnoea (OSA), are common in women with
81 PCOS. In support of this, heart rate variability is altered [6-8] and heart rate and blood pressure
82 recovery after exercise is delayed [9-10] in women with PCOS compared to matched controls,
83 consistent with enhanced sympathetic stimulation and increased peripheral arterial resistance.
84 Direct measurement of muscle sympathetic nerve activity (MSNA) by microneurography has
85 also confirmed enhanced sympathetic outflow in women with PCOS compared with age- and
86 BMI-matched controls [11-12].

87

88 The mechanisms by which this enhanced sympathetic activation occurs are not entirely clear,
89 although both hyperinsulinemia [12] and hyperandrogenism [11] have been implicated. The
90 origins of this activation are also uncertain, although the hypothalamus [13], brainstem [14]
91 and higher brain centers [15] appear to be involved in regulating sympathetic tone in rodents.
92 Contemporary imaging techniques, such as positron emission tomography [16-17] and blood
93 oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) [18-20],
94 facilitate neuroanatomical localization of these responses in humans, and have identified a

95 number of cortical and brainstem regions involved in this process. To our knowledge, similar
96 studies have not been undertaken in metabolic disorders characterized by insulin resistance,
97 including PCOS, in which compensatory hyperinsulinemia might be anticipated to amplify the
98 cerebral responses to sympathoexcitation.

99

100 We hypothesized that women with PCOS would have evidence of sympathoexcitation
101 accompanied by functional differences in higher brain centres. We therefore set out to compare
102 cerebral (BOLD fMRI), pressor (blood pressure and heart rate) and MSNA responses to an
103 isometric forearm contraction model of sympathoexcitation in women with PCOS and matched
104 controls.

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108

109 **Materials and Methods**

110 **Participants**

111 Patients with PCOS (n=20) were recruited from the endocrine clinic at the University Hospital
112 of Wales, the endocrine clinic at Morriston Hospital, Swansea, and Morlais Medical Practice,
113 Merthyr Tydfil. Diagnosis was made according to the Rotterdam criteria [21]. Congenital
114 adrenal hyperplasia, Cushing's syndrome, androgen-secreting neoplasms, hyperprolactinemia
115 and thyroid disease were excluded by biochemical testing. Patients were aged between 18 and
116 45 years. Exclusion criteria were: pregnancy and breastfeeding, hyperlipidemia or use of lipid-
117 lowering agents, hypertension or use of anti-hypertensives, use of glucocorticoids or anti-
118 obesity drugs, diabetes or use of antidiabetic drugs within 3 months. Patients with any
119 contraindication to MRI were also excluded. Of the 20 women, 12 had polycystic ovaries
120 (PCO), hyperandrogenism and anovulation, 5 had hyperandrogenism and anovulation, 2 had
121 PCO and hyperandrogenism, and 1 had PCO and anovulation.

122

123 Healthy volunteers (n=20) were recruited as controls. For each individual patient, a control was
124 identified matched for age (within 2 yrs) and BMI (within 2 kg/m²). Controls needed to have
125 regular menstrual cycles (menses every 27–32 days). Their healthy state was determined by
126 history, examination and hormonal evaluation (testosterone, androstenedione, thyroid function,
127 prolactin). Control subjects with signs of hirsutism or with a personal history of diabetes or
128 hypertension, or a family history of PCOS, or current pregnancy were excluded. Those with
129 any contraindication to MRI were also excluded. Healthy volunteers were recruited by
130 advertisement among staff and students at the University Hospital of Wales, Cardiff University
131 and in the local press. The study was approved by Cardiff University (study sponsors), Cardiff

132 and Vale University Health Board and the South East Wales Research Ethics Committee
133 (reference 12/WA/0239). All subjects gave written, informed consent.

134

135 **Anthropometric and biochemical measurements**

136 Height, weight, waist and hip circumference were measured according to our previously
137 published protocol [22]. Blood samples were collected after an overnight fast. Serum total
138 cholesterol and triglycerides were assayed using an Aeroset analyzer (Abbott Diagnostics).
139 Insulin was measured using an immunometric assay specific for human insulin (Invitron), and
140 glucose was measured using the Aeroset chemistry system (Abbott Diagnostics). Total
141 testosterone was measured by liquid chromatography-tandem mass spectrometry (Quattro™
142 Premier XE triple quadrupole tandem mass spectrometer; Waters Ltd). Androstenedione was
143 measured by tandem mass spectrometry using an in-house method. Thyroid function tests were
144 assayed using the Abbott Architect platform (Abbott Laboratories). HbA1c was determined
145 using a high-performance liquid chromatography (HPLC) assay (Tosoh HLC-723G8, Tosoh
146 Corporation). The intra- and inter-assay coefficients of variation were all <9%.

147

148 A standard 75-g oral glucose tolerance test was performed in all participants to determine post-
149 prandial insulin sensitivity. Glucose and insulin were measured at 0, 30, 60, 90, and 120
150 minutes. The areas under the curve (AUCs) for insulin and glucose were calculated using the
151 trapezoid method. The homeostatic model assessment (HOMA) method was also used to
152 estimate fasting insulin resistance (HOMA-IR) according to the formula (fasting insulin
153 (mU/L) x fasting glucose (mg/dL)/405) [23].

154

155 **Isometric forearm contraction (IFC) protocol**

156 Isometric forearm contraction (IFC) at 30% maximum voluntary contraction was used to
157 generate a peripheral haemodynamic and SNS response. Maximum grip strength was
158 determined by asking the volunteer to squeeze an electronic hand dynamometer (90kg capacity
159 range) (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) with their dominant hand to
160 maximum effort on three separate attempts, with a 60 second period of rest between each
161 squeeze, as previously recommended [24]. The mean maximum grip strength was determined
162 and 30% IFC subsequently calculated. This was then applied in a protocol which followed a
163 block design of 12 minutes in total, comprising 1 minute rest, 3 minutes squeeze, 2.5 minutes
164 rest, 3 minutes squeeze and 2.5 minutes rest. The subjects were cued for the rest and squeeze
165 periods, and targeted to sustain 30% IFC during the squeeze periods (figure 1).

166

167 **Sympathetic activity measurements**

168 *Blood pressure and heart rate.* Resting blood pressure (mmHg) and heart rate (beats/min) were
169 measured at baseline using an Omron HEM-907 blood pressure monitoring device (Omron
170 Healthcare UK Ltd) on the non-dominant arm and every 30 seconds throughout the 12 minute
171 IFC protocol. Mean arterial blood pressure (MAP) was calculated. The mean of the values at
172 rest were calculated as a pre-IFC blood pressure and heart rate, and the mean of values at the
173 end of each 3 minute squeeze to give a post-IFC blood pressure and heart rate.

174

175 *Plasma catecholamines.* Blood was drawn from the non-dominant arm of the subject in a
176 supine position after a 10 minute rest period (pre-IFC catecholamines). Following 3 minutes
177 of IFC at 30% maximum handgrip strength, further blood was drawn for post-IFC

178 catecholamines. Samples were centrifuged at 2000rpm at 4°C within 10 minutes of collection
179 and aliquots stored at -80°C until analysis. Catecholamines were measured using an
180 Epinephrine ELISA Kit (Abnova, Taoyuan County, Taiwan) and Norepinephrine ELISA Kit
181 (Abnova, Taoyuan County, Taiwan). The intra- and inter-assay coefficients of variation were
182 <15.4% and <16.1% respectively.

183

184 *Microneurography*. A subset of patients (n=7, age 29.6 ± 6.4 yrs, BMI 27.3 ± 4.9 kg/m²) and
185 controls (n=7, age 30.1 ± 6.2 yrs, BMI 27.1 ± 6.2 kg/m²) agreed to undergo microneurography.

186 Studies were conducted on a separate day between 0830 and 1530 hours in a quiet physiological

187 lab maintained at 20°C and performed by a single observer blind to subject status (YS). Direct

188 recordings of multiunit efferent postganglionic muscle sympathetic nerve activity (MSNA)

189 were obtained with a tungsten microelectrode with a tip diameter of a few micrometers inserted

190 into a muscle fascicle of the peroneal nerve, posterior to the fibular head. A low-impedance

191 reference electrode was inserted subcutaneously a few centimeters from the fibular head. When

192 a muscle nerve fascicle was identified, small electrode adjustments were made until a site was

193 found in which spontaneous, pulse-synchronous bursts of neural activity could be recorded.

194 Details of the nerve recording technique and criteria for MSNA have been reported previously

195 [25]. Bursts identified by inspection of the mean voltage neurogram were expressed as burst

196 frequency (number of pulse synchronic sympathetic bursts per minute) [bursts/min (BF)] and

197 burst incidence (number of pulse synchronic sympathetic bursts per 100 heart beats)

198 [bursts/100 heartbeats (BI)]. Total MSNA activity was measured to take into account both the

199 frequency and size of a sympathetic burst (the product of burst per minute and mean burst
200 amplitude), expressed in arbitrary units. The total MSNA during the last 60 seconds of a rest
201 period was used as a baseline to establish the percentage change in MSNA during the last 60
202 seconds of the 30% IFC.

203

204

205 **MRI data acquisition**

206 MRI was performed on a 3T GE HDx MRI system (General Electric). The head was held
207 immobile in an eight-channel receive only head coil by foam pads. A continuous series of 232
208 fMRI image volumes (echo-planar images using BOLD contrast, scan time = 12 mins, TR =
209 3.1s, TE = 25ms) were collected for each run. In-plane voxel size was $1.5 \times 1.5 \text{ mm}^2$, matrix
210 $128 \times 128 \times 40$ and Field-of-view (FOV) $192 \times 192 \text{ mm}^2$ in plane. The slice thickness was 2.2mm
211 and slice gap 0.8mm. Each volume covered the entire brain and brainstem. Slices were tilted
212 10° - 15° from the axial to the coronal plane to reduce signal loss due to dephasing in the
213 brainstem resulting from through-slice susceptibility-induced gradients [26]. Structural images
214 were collected using a T1-weighted sequence in order to facilitate visualization.

215

216 **Blood oxygen level-dependent (BOLD) fMRI scan protocol**

217 The scan protocol aimed to reveal BOLD signal correlates with the IFC task, using a block
218 design. Subjects were fitted with a nasal cannula to measure end tidal CO_2 . Respiration pattern
219 was determined by a strain-gauge band around the chest. Heart rate was measured from a pulse
220 oximeter on the left hand (MedRad, USA). Physiological data were collected with a computer-

221 based data acquisition and analysis system (CED 1401, Cambridge, UK). An in-house MRI-
222 compatible handgrip device was positioned in the dominant hand and connected to a pressure
223 transducer. The pressure signal was collected with a computer-based data acquisition and
224 analysis system (CED 1401, Cambridge, UK) and displayed on a screen located inside the
225 scanner. Subjects followed visual instructions presented on the screen as to the rest and squeeze
226 periods, with a target bar showing when 30% squeeze had been achieved. PsychoPy version
227 1.78 [27] was used to run the visual stimulus. Subjects performed the previously described
228 block paradigm twice with time to rest between the runs.

229

230 **Image and statistical analyses**

231 Analysis of the scans was by FEAT (fMRI Expert Analysis Tool, version 6.00) software
232 (available on-line at www.fmrib.ox.ac.uk/fsl). Each T1 scan was registered to the MNI152, an
233 average T1 brain image constructed from 152 normal subjects at the Montreal Neurological
234 Institute (MNI), Montreal, QC, Canada, using linear registration (FLIRT within the FMRIB
235 Software Library (FSL)) [28-29]. The functional BOLD scans were then registered to each
236 individual's T1 structural image. fMRI images were un-warped, motion corrected and spatially
237 smoothed. Physiological noise from cardiac and respiratory signals was retrospectively
238 regressed out from the images. FSL contains the software FLIRT (FMRIB's Linear Image
239 Registration Tool) that allowed the linear transformation of imaging data [28, 30]. A high-pass
240 filter of 330 seconds was used. To generate contrast images, task-related BOLD activation was
241 estimated with a design matrix specifying a general linear model (GLM) that included a
242 waveform based on each person's IFC recording obtained during the scan protocol from the
243 hand grip device. The visual stimulus shown in the scan session was also included in this
244 analysis. BOLD signal changes for blood pressure condition were modelled with a waveform
245 derived from the blood pressure recordings made out of scanner during the 12-minute

246 paradigm. Z statistic images were thresholded using clusters determined by $z > 2.3$ and a cluster
247 significance threshold of $P = 0.05$ [31]. Significant BOLD signal intensity changes were color
248 coded and rendered onto an individual's T1-weighted anatomic image set. The resulting
249 statistical parametric maps were used in higher level analysis to determine differences between
250 PCOS and control groups. As the paradigm was run twice, an intermediate level FEAT analysis
251 was run for each subject by combining their two lower-level FEAT outputs, to produce an
252 average for each subject. These were then used in the higher-level FEAT analysis that could
253 be used in the group analyses to examine BOLD activation in the PCOS and control groups
254 and the differences in activation between groups ($z > 2.3$, $p = 0.05$).

255

256 For the pressor, MSNA and catecholamine responses, statistical analysis was performed using
257 SPSS version 20.0 (IBM, New York). An independent-samples t-test was used to compare the
258 difference between the PCOS and control group means. A p-value of < 0.05 was considered
259 statistically significant.

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272 **Results**

273 **Baseline characteristics**

274 Table 1 shows the clinical, anthropometric and metabolic characteristics of the two groups. The
275 groups were closely matched for age, BMI, resting heart rate and blood pressure. Testosterone
276 and androstenedione levels were non-significantly higher in PCOS subjects than controls.
277 Similarly, the insulin response to oral glucose challenge (insulin AUC) and HOMA-IR values
278 were higher in PCOS subjects but fell just short of statistical significance. Triglyceride levels
279 in the PCOS group were higher than in controls.

280

281 **Sympathetic activity measurements**

282 *Pressor response*

283 19 PCOS and 19 controls had heart rate (HR) and blood pressure (BP) measured in response
284 to the IFC paradigm (table 2). As anticipated, IFC induced a significant rise in HR and BP in
285 both groups. However, there were no between-group differences in the HR or BP increase from
286 baseline in response to IFC.

287

288 *Catecholamines*

289 The plasma catecholamine response to IFC was assessed in 39 subjects (20 PCOS, 19 controls)
290 (table 2). Mean resting catecholamine concentrations were not different between groups.
291 Following IFC, norepinephrine levels did not change but epinephrine concentrations increased
292 significantly in the PCOS group ($p < 0.001$). However, differences between groups in
293 epinephrine response to IFC were not apparent.

294

295 *MSNA*

296 Resting data were obtained from 16 subjects (8 PCOS, 8 controls). Only 14 of these (7 PCOS,
297 7 controls) were able to proceed with full MSNA recordings post-IFC due to technical
298 difficulties, including inability to locate the peroneal nerve for recordings (n=1) and a
299 participant who was unable to keep their leg in position (n=1).

300

301 Resting burst frequency (BF), burst incidence (BI) and total MSNA was not different between
302 groups (table 2). The increase in BF was significantly greater (68%) in the PCOS group
303 compared to controls (11.9%; $p=0.002$). The increases in BI (PCOS: 55.4%, controls: 20.5%)
304 and total MSNA (PCOS: 124.1%, controls: 86.4%) were not significantly different between
305 groups.

306

307 **fMRI BOLD signal activation**

308 30 participants (15 PCOS, 15 controls) underwent fMRI scanning with out-of-scanner HR and
309 BP changes recorded every 30 seconds in response to the IFC paradigm. There were no
310 significant differences in the age, BMI, testosterone, HOMA-IR, resting HR or resting BP
311 between groups. The change in BOLD signal intensity that fitted the modelled blood pressure
312 response showed activation in the PCOS group in the right cerebral cortex, right pallidum, right
313 thalamus and right parietal operculum cortex ($p<0.0001$) and control group in the intracalcarine
314 cortex and lingual gyrus ($p=0.003$). BOLD signal activation was significantly greater in the
315 PCOS group compared to controls in the right orbitofrontal cortex ($p<0.0001$), and less so in
316 the left angular gyrus and lateral occipital cortex ($p=0.04$) (figures 2(a) and 2(b)). No
317 differences were observed in the brainstem.

318

319 **Metabolic influences on fMRI BOLD signal change**

320 When the BOLD signal change modelled for hemodynamic response was adjusted for variance
321 associated with testosterone, using testosterone as a covariate at the group level, BOLD
322 activation in the right orbitofrontal cortex was still greater in the PCOS group compared to
323 controls ($p < 0.0001$). However, when the BOLD signal was separately adjusted for insulin
324 sensitivity (HOMA-IR), the BOLD signal differences between groups in the right orbitofrontal
325 cortex were no longer significant. When corrected for HOMA-IR, the BOLD signal in the left
326 angular gyrus and lateral occipital cortex remained significant.

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343 **Discussion**

344 Our study demonstrates that women with PCOS have evidence of enhanced
345 sympathoexcitation in response to IFC compared to age- and BMI-matched controls, and that
346 this is accompanied by a difference in BOLD signal change that localizes to the right
347 orbitofrontal cortex. This finding is consistent with previous studies implicating this region in
348 the neural control of blood pressure [17, 32, 33], but to our knowledge is the first to confirm
349 enhanced activation in this region in young women with insulin resistance. These observations
350 may extend our understanding of the mechanisms involved in neurogenic hypertension in
351 young ‘at risk’ subjects.

352

353 In common with many previous studies, we used IFC at 30% of maximum grip as our stimulus
354 to induce a blood pressure rise. In young adult volunteers this has been shown not to increase
355 nociception [18]. The pressor response we observed was of a similar magnitude to other studies
356 [18, 34-35] and did not differ between women with PCOS and controls. This is in keeping with
357 observations in patients with type 2 diabetes whereby systolic and diastolic blood pressure rose
358 in parallel to controls in response to IFC, despite differences in resting blood pressure between
359 groups [36].

360

361 We did not observe any rise in concentrations of the sympathetic neurotransmitter
362 norepinephrine in either group but plasma measurement offers limited sensitivity and
363 reproducibility, unlike radiolabelled techniques which may be used reliably to measure
364 regional sympathetic activity in individual organs. Furthermore, plasma norepinephrine
365 measurement cannot distinguish between increased central catecholamine production and

366 reduced clearance [37]. For these reasons, the significance of the greater rise in plasma
367 epinephrine concentrations in the PCOS group following IFC is uncertain.

368

369 In contrast to plasma catecholamines, microneurography represents a more direct measurement
370 of sympathetic neural output. In common with many studies, we chose the common peroneal
371 nerve, in view of its easy accessibility, to measure efferent MSNA. Importantly, MSNA
372 correlates well with autonomic effector (including blood pressure and heart rate) responses
373 [25], and provides immediate data on sympathetic output. However, it is invasive, hence we
374 were only able to recruit a proportion of our total group to this sub-study. Nevertheless, women
375 with PCOS showed a greater rise in burst frequency in response to IFC than controls, although
376 resting measures were not different between groups. This contrasts with previous studies,
377 where higher resting MSNA values were observed in women with PCOS [11-12]. However,
378 it is noticeable that the resting burst frequency and burst incidence values in our control group
379 were significantly greater than those reported in these previous studies, and this may go some
380 way to explain the absence of differences in MSNA between our two groups at baseline.

381

382 This study identified several cortical areas whose BOLD signal change correlated with the
383 modelled BP response to static exercise. Of these, between-group differences were most
384 apparent in the right orbitofrontal cortex. This cerebral region has previously been shown to
385 associate with a pressor response in humans. In a positron emission tomography study,
386 Critchley and colleagues identified the right orbitofrontal cortex as one of several brain regions
387 implicated in the cardiovascular response to isometric exercise and mental stress [17]. Harper
388 *et al.* used functional MRI to demonstrate increased activity in the right orbitofrontal cortex
389 during hypertension induced by cold pressor and Valsalva stimuli [33], whilst Gianaros *et al.*
390 showed that the orbitofrontal cortex was similarly activated in response to a behavioral stressor

391 [32]. More recently, Macefield and Henderson contemporaneously captured skin sympathetic
392 nerve activity (SSNA) directly during BOLD fMRI of the brain [38], showing correlation of
393 spontaneous SSNA with BOLD signal intensity in the right orbitofrontal cortex. Furthermore,
394 in animal studies, the orbitofrontal cortex has been shown to connect to the insular cortex, a
395 key regulator in the pressor response [39]. Our data therefore support the prevailing view that
396 a cortical and sub-cortical network exists in humans to control cardiovascular responses.
397 Studies in patients with intractable epilepsy undergoing intracranial electrode implantation and
398 deep brain stimulation appear to confirm this, whereby stimulation of the subcallosal
399 neocortex, which lies adjacent to the orbitofrontal cortex, elicited marked systolic hypotensive
400 changes likely as a result of reduced sympathetic drive [40].

401

402 In an attempt to understand the potential metabolic drivers of the altered BOLD signal
403 response, we extended our analyses to sequentially adjust for hyperandrogenism and insulin
404 resistance, observing that adjustment for HOMA-IR, but not testosterone, abolished the
405 between-group differences in BOLD signal intensity in the right orbitofrontal cortex. This
406 implies that differences in insulin sensitivity, and compensatory hyperinsulinemia, might
407 account for the differences we observed in the BOLD signal response in this area in response
408 to IFC. Our findings may thus have relevance for other metabolic disorders characterized by
409 insulin resistance, such as metabolic syndrome and type 2 diabetes, which we speculate might
410 similarly be affected by altered BOLD signal in this cerebral region. Although little insulin is
411 produced in the brain, insulin receptors are widely distributed in the brain and peripherally-
412 made insulin can cross the blood-brain barrier [41]. Furthermore, intracerebroventricular
413 injection of insulin in rodents induces sympathoexcitation via the arcuate nucleus [13, 42]. In
414 humans, hyperinsulinemia increases MSNA and modifies baroreflex control of sympathetic
415 activity [43-44] although these effects of insulin on sympathetic outflow may be blunted in

416 insulin-resistant states such as obesity and the metabolic syndrome [45-46]. We therefore
417 speculate that the enhanced activation observed in the right orbitofrontal cortex in women with
418 PCOS may reflect preserved insulin sensitivity in this cerebral region. This raises the
419 possibility that insulin sensitization might have therapeutic benefit in reducing sympathetic
420 output in PCOS and consequently improving cardiometabolic outcomes. Indeed, metformin
421 caused a dose-dependent reduction in heart rate, blood pressure and renal sympathetic nerve
422 activity in spontaneously hypertensive rats [49], but similar benefits were not observed short-
423 term in obese hypertensive men [50]. In contrast, both rosiglitazone and pioglitazone have been
424 shown to reduce sympathetic nerve activity in subjects with type 2 diabetes [51-52].

425

426 In contrast to other studies [18], we did not find any change in BOLD signal in the brainstem
427 following IFC, a region that we hypothesized at the outset might be activated in response to
428 this paradigm. In particular, medullary structures are implicated in autonomic control of
429 cardiovascular responses. Reasons for this might include physiological noise due to cardiac
430 and respiratory motion, and the presence of magnetic field inhomogeneity caused by the nearby
431 sphenoid sinus. Furthermore, the small size of brainstem nuclei in humans [53] makes
432 localization challenging even when using MRI scanners (3T) that image with greater resolution
433 than conventional systems. In this regard, the enhanced signal and spatial resolution offered by
434 7T systems may offer an important advance.

435

436 Our study has some limitations. Firstly, we chose to define our subjects with PCOS by the
437 Rotterdam criteria since this embraces a ‘milder’ metabolic phenotype characterized by lesser
438 degrees of hyperandrogenism and insulin resistance than other definitions such as the NIH
439 criteria [54]. Whilst this allowed us to explore the effects of relatively mild insulin resistance
440 on cerebral and pressor responses to IFC, the study group was heterogeneous and it is difficult

441 to be certain if our findings extend to all sub-phenotypes of the syndrome; further studies are
442 needed in this regard. Since patients with hyperandrogenic PCOS carry a worse
443 cardiometabolic risk profile, we speculate that inclusion of patients with more severe
444 hyperandrogenism may have exaggerated the differences we observed in orbitofrontal cortex
445 activation and/or unmasked other cerebral regions implicated in the neurogenic regulation of
446 blood pressure. Inclusion of a young population nevertheless avoids the potentially
447 confounding influences of vascular pathology (from e.g. diabetes and hypertension) on blood
448 flow and therefore BOLD signal. Secondly, MSNA and pressor recordings were undertaken
449 out-of-scanner; it would have been preferable to do so during scanning, as demonstrated
450 recently by others [20, 38] but this is beyond our current technical ability. Thirdly, our study
451 used static hand grip to induce a pressor response, which is a motor task cued by a visual
452 stimulus. Although the potential confounding influence of this model was reduced by factoring
453 the motor and visual tasks into the FEAT analysis, we nevertheless observed a change in BOLD
454 signal intensity in the intracalcarine cortex and lingual gyrus in controls, in the parietal
455 operculum in subjects with PCOS, and between-group differences in the lateral occipital cortex
456 and left angular gyrus, which are likely to relate to remaining confounding effects of the visual
457 stimulus. Similarly, the signal change in the right thalamus, pallidum and cerebral cortex in the
458 PCOS group may reflect residual confounding by the motor component of the hand grip task.
459 However, imaging studies have also suggested that areas of the thalamus may be implicated in
460 blood pressure control, potentially via increasing vagal tone and reducing sympathoexcitation
461 [55].

462

463 In conclusion, our study supports previous observations of enhanced sympathetic output in
464 women with PCOS but demonstrates for the first time that this is accompanied by regional
465 differences in cerebral activation that are most marked in the right orbitofrontal cortex. This

466 differential activation appears to relate to altered insulin sensitivity, and suggests that
467 treatments targeted at reducing hyperinsulinemia in young women with PCOS may have
468 benefits in reducing sympathetic output and improving cardiovascular health.

469

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674 **Tables and figures.**675 **Table 1.** Anthropometric and metabolic characteristics of the study population

	PCOS (n=20)* Mean ± SD	Control (n=20) Mean ± SD	p- value
Age (yrs)	29.80 ± 4.78	29.65 ± 4.96	0.92
BMI (Kg/m ²)	26.05 ± 4.90	26.11 ± 4.83	0.97
WHR	0.88 ± 0.07	0.84 ± 0.04	0.04
Waist circumference (cm)	85.9 ± 13.7	85.1 ± 11.1	0.86
Hip circumference (cm)	97.2 ± 10.4	101.4 ± 11.8	0.24
Testosterone (nmol/L)	1.41 ± 0.77	1.03 ± 0.53	0.09
Androstenedione (nmol/L)	4.51 ± 2.99	3.64 ± 1.28	0.25
HbA1c (mmol/mol)	34.15 ± 2.76	34.21 ± 2.64	0.95
Total cholesterol (mmol/L)	5.22 ± 1.05	4.79 ± 0.55	0.12
Triglycerides (mmol/L)	1.34 ± 0.68	0.90 ± 0.36	0.02
Insulin AUC (pmol min/L)	55519.50 ± 41547.67	35320.26 ± 21008.31	0.07
Glucose AUC (mmol min/L)	764.85 ± 239.02	661.89 ± 219.03	0.17
HOMA-IR	1.41 ± 1.10	0.88 ± 0.65	0.08
Resting HR (beats/min)	71.05 ± 8.59	71.26 ± 7.65	0.94
Resting SBP (mmHg)	114.53 ± 9.33	117.58 ± 12.62	0.40
Resting DBP (mmHg)	65.16 ± 13.33	65.47 ± 14.31	0.94
Resting MAP (mmHg)	81.63 ± 11.26	83.84 ± 10.54	0.54

676 BMI, body mass index; AUC, area under the curve during oral glucose tolerance test;

677 HOMA-IR, homeostatic model assessment of insulin resistance. *19 controls underwent an

678 oral glucose tolerance test

679

Table 2. Pressor, catecholamine and MSNA responses to IFC in PCOS and control groups

	PCOS Mean \pm SD			Controls Mean \pm SD			p-value PCOS vs controls
	Pre-IFC	Post-IFC	p-value	Pre-IFC	Post-IFC	p-value	
Pressor response	n=19			n=19			
HR (beats/min)	71.05 \pm 8.59	76.68 \pm 8.04	<0.001	71.26 \pm 7.65	75.11 \pm 8.43	<0.001	0.155
SBP (mmHg)	114.53 \pm 9.33	127.11 \pm 13.69	<0.001	117.58 \pm 12.62	125.84 \pm 11.21	<0.001	0.090
DBP (mmHg)	65.16 \pm 13.33	74.84 \pm 15.79	<0.001	65.47 \pm 14.31	74.21 \pm 10.68	<0.001	0.157
MAP (mmHg)	81.63 \pm 11.26	92.37 \pm 13.97	<0.001	83.84 \pm 10.54	91.32 \pm 9.27	<0.001	0.058
Catecholamines	n=20			n=19			
Epinephrine concentration (ng/mL)	0.68 \pm 0.53	1.23 \pm 0.71	<0.001	0.77 \pm 0.59	0.99 \pm 0.61	0.14	0.32
Norepinephrine concentration (ng/mL)	18.11 \pm 11.18	16.77 \pm 10.01	0.38	22.99 \pm 13.33	20.99 \pm 12.12	0.25	0.42
MSNA	n=7			n=7			
BF (bursts/min)	25.9 \pm 4.4	42.9 \pm 8.2	0.001	29.6 \pm 7.1	34.9 \pm 4.5	0.149	0.002
BI (bursts/100 heartbeats)	36.3 \pm 9.9	54.4 \pm 12.1	0.004	42.0 \pm 10.3	47.9 \pm 7.1	0.199	0.133
Total MSNA	2.4 \pm 1.3	5.5 \pm 3.1	0.004	2.6 \pm 0.7	4.4 \pm 1.7	0.048	0.420

682 HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve
683 activity; BF, burst frequency; BI, burst incidence.

684

685 **Legends for figures**

686 **Figure 1.** 12 minute IFC paradigm comprising 1 minute rest, 3 minutes 30% IFC, 2.5 minutes rest, 3 minutes 30% IFC and 2.5 minutes rest. The
687 timings of MSNA, catecholamine, heart rate and blood pressure measurements are indicated.

688

689 **Figure 2.** BOLD signal activation (modelled for blood pressure) differences between PCOS and controls in the right orbitofrontal cortex (a) and
690 between PCOS and controls in the left angular gyrus and lateral occipital cortex (b). The significant region is displayed with a threshold of
691 $Z > 2.3$, with a cluster probability threshold of $p < 0.05$.

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