

1 **Title: Effects of empagliflozin on metabolic parameters in polycystic ovary syndrome:**  
2 **a randomised controlled study**

3  
4 Zeeshan Javed<sup>1, 2</sup>, Maria Papageorgiou<sup>1, 3</sup>, Harshal Deshmukh<sup>1</sup>, Alan S. Rigby<sup>4</sup> Unaiza Qamar<sup>5</sup>,  
5 Jehangir Abbas<sup>2</sup>, Amer Y. Khan<sup>6</sup>, Eric S. Kilpatrick<sup>7</sup>, Stephen L. Atkin<sup>7</sup> and Thozhukat  
6 Sathyapalan<sup>1,\*</sup>

7  
8 <sup>1</sup>Department of Academic Diabetes, Endocrinology and Metabolism, Hull York Medical  
9 School, University of Hull, Hull, United Kingdom

10 <sup>2</sup>Department of Endocrinology and Diabetes, Pakistan Kidney & Liver Institute and  
11 Research Centre, DHA Phase 6, Knowledge City, Lahore, Pakistan

12 <sup>3</sup>Department of Pathophysiology and Allergy Research, Center of Pathophysiology,  
13 Infectiology, and Immunology, Medical University of Vienna, Vienna, Austria.

14 <sup>4</sup> Hull York Medical School, University of Hull, Hull, United Kingdom

15 <sup>5</sup>Department of Pathology, Pakistan Kidney & Liver Institute and Research Centre, DHA  
16 Phase 6, Knowledge City, Lahore, Pakistan

17 <sup>6</sup>Department of Medicine, Pakistan Kidney & Liver Institute and Research Centre, DHA  
18 Phase 6, Knowledge City, Lahore, Pakistan

19 <sup>7</sup>Department of Pathology, Sidra Medical and Research Center, Doha, Qatar

20 <sup>8</sup>Weill Cornell Medical College Qatar, Education City, PO Box 24144, Doha, Qatar

21  
22 **\*Corresponding author and person to who reprint request should be addressed:**

23 Email: [Thozhukat.Sathyapalan@hyms.ac.uk](mailto:Thozhukat.Sathyapalan@hyms.ac.uk).

24 **Abstract**

25 **Background:** Empagliflozin is a sodium-glucose-cotransporter-2 inhibitor that improves  
26 cardiovascular risk and promotes weight loss in patients with type 2 diabetes. Polycystic ovary  
27 syndrome (PCOS) is associated with obesity and increased cardiovascular risk; therefore,  
28 empagliflozin may be of benefit for these women. The aim of this study was to compare the effects of  
29 empagliflozin vs. metformin on anthropometric and body composition, hormonal and metabolic  
30 parameters in women with PCOS.

31 **Materials and methods:** A randomized open-label study was conducted in women with PCOS who  
32 were randomised to either empagliflozin 25mg (n=19) or metformin 1500mg (n=20) daily for 12  
33 weeks. The main outcomes assessed were changes in anthropometric and body composition,  
34 hormonal and metabolic parameters.

35 **Results:** Univariate analysis showed significant differences in weight (empagliflozin:  $-1.4\pm 3.2\%$  vs.  
36 metformin:  $1.2\pm 2.3\%$ ;  $p=0.006$ ), body mass index (empagliflozin:  $-1.4\pm 3.2\%$  vs. metformin:  
37  $1.1\pm 2.2\%$ ;  $p=0.006$ ), waist circumference (empagliflozin:  $-1.6\pm 2.8\%$  vs. metformin:  $0.2\pm 2.1\%$ ;  
38  $p=0.029$ ) and hip circumference (empagliflozin:  $-2.0\pm 3.0\%$  vs. metformin:  $1.1\pm 1.9\%$ ;  $p=0.001$ ), basal  
39 metabolic rate (empagliflozin:  $-1.8\pm 2.9\%$  vs. metformin:  $0.1\pm 1.9\%$ ,  $p=0.024$ ) and fat mass  
40 (empagliflozin:  $-0.7\pm 4.9\%$  vs. metformin,  $3.2\pm 5.0\%$ ;  $p=0.023$ ) between the empagliflozin and the  
41 metformin groups. These differences were confirmed in linear regression analysis after adjustment for  
42 relevant covariates. There were no significant changes in hormonal or metabolic parameters between  
43 both groups.

44 **Conclusion:** There was a significant improvement in anthropometric parameters and body  
45 composition, in overweight and obese women with PCOS after 12 weeks of treatment with  
46 empagliflozin compared to metformin, although no changes were seen in hormonal or  
47 metabolic parameters.

48

49 **Key words:** Polycystic ovary syndrome, SGLT2 inhibitors, empagliflozin, body  
50 composition, hormones, metabolic parameters

## 51 **Introduction**

52 Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder featured by  
53 hyperandrogenism, menstrual irregularities and polycystic ovaries that affects women of  
54 reproductive age.<sup>1</sup> PCOS is also associated with infertility, obesity, metabolic disturbances  
55 and increased cardiovascular risk.<sup>1,2</sup>

56 Accordingly, the treatment of PCOS is commonly symptom-based, while the ideal  
57 treatment would address both the reproductive and metabolic abnormalities related to  
58 PCOS.<sup>1, 3</sup> Hormonal contraceptives alone or combined with anti-androgens have been the  
59 cornerstone for managing menstrual disturbances and clinical or biochemical  
60 hyperandrogenaemia;<sup>4</sup> however, some hormonal contraceptives may unfavourably affect  
61 the lipid profile in PCOS<sup>5</sup> and increase the risk of thrombosis and cardiovascular events in  
62 the general population.<sup>6</sup> Glucose lowering agents including metformin and  
63 thiazolidinedione have been shown to be effective in managing the metabolic abnormalities  
64 (*i.e.*, insulin resistance, hyperinsulinaemia, and diabetes mellitus) and chronic anovulation;  
65 however, their use has been inconsistently associated with improvements in weight loss and  
66 body composition, menstrual irregularity or clinical symptoms of excess androgens.<sup>7,8</sup>

67 Empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, is a new treatment  
68 option for adults with type 2 diabetes,<sup>9</sup> however, its effects in PCOS have not been  
69 previously explored. Its principal action involves inhibition of glucose reabsorption by the  
70 kidney and therefore, glucose excretion via urine. Notably, this action mechanism is  
71 insulin-independent; as such it does not increase the risk of hypoglycaemia, making it  
72 attractive for use in normoglycaemic individuals.<sup>10, 11</sup> Recent trials have demonstrated that  
73 treatment with empagliflozin promotes weight loss,<sup>12</sup> exerts positive effects on arterial  
74 stiffness, vascular resistance and blood pressure and decreases the relative risk for  
75 cardiovascular and all-cause mortality in patients with type 2 diabetes.<sup>10, 13</sup> Evidence from  
76 preclinical studies suggests that these cardio-protective effects may be due to the reduction  
77 in oxidative stress and suppressed markers of inflammation and fibrosis.<sup>14, 15</sup> In humans, the  
78 cardiovascular benefits may also be mediated by reductions in HbA1c, insulin resistance,

79 plasma volume, weight/fat mass and inflammation.<sup>16, 17</sup> Given that these pathological  
80 features are also common in PCOS;<sup>1, 2</sup> empagliflozin may be of potential benefit for this  
81 population.

82 Therefore, the aim of this study was to explore and compare the effects of empagliflozin  
83 vs. metformin on anthropometric, body composition, hormonal and metabolic parameters in  
84 women with PCOS.

85

## 86 **Materials and Methods**

87 An open-label, randomised, comparative study in women with PCOS was performed in the  
88 Academic Diabetes, Endocrinology and Metabolism research centre at Hull Royal Infirmary. All  
89 participants were women, aged between 18 and 45 years, had a body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>,  
90 were diagnosed with PCOS based on the Rotterdam criteria [biochemical hyperandrogenism, as  
91 indicated by a free androgen index (FAI)  $>4$ , and self-reported oligomenorrhea (cycle length  $>35$  days  
92 and 9 or fewer periods per year) or amenorrhea (absence of menses for a period  $\geq 3$  months)].<sup>18</sup>  
93 Women with differential diagnoses of non-classical 21-hydroxylase deficiency, hyperprolactinaemia,  
94 Cushing's disease and androgen-secreting tumours were excluded from participation. Additional  
95 exclusion criteria included pregnancy or intention to become pregnant, breastfeeding, documented use  
96 of oral hormonal contraceptives and hormone-releasing implants, metformin or other insulin-  
97 sensitizing medications, clomiphene citrate or estrogen modulators, gonadotropin-releasing hormone  
98 (GnRH) modulators and Minoxidil, diagnosis of diabetes, history or presence of malignant neoplasms  
99 within the last 5 years, pancreatitis (acute or chronic), recurrent urinary tract infections or  
100 gastrointestinal tract surgery, ongoing, inadequately controlled thyroid disorder and known  
101 hypersensitivity to the investigational medicinal products or any of their excipients. All participants  
102 provided their written informed consent. This study was approved by the Medicines and Healthcare  
103 Products Regulatory Authority (MHRA) (Ref: 21411/0254/001-0001), the Yorkshire & Humber  
104 Health Research Authority and Leeds East Research Ethics Committee (REC reference: 17/YH/0118),  
105 registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT03008551) and conducted in accordance with the  
106 Declaration of Helsinki and local regulations.

107 Women with PCOS were randomized on a 1:1 ratio using an online web-based randomisation  
108 service (<https://www.sealedenvelope.com>) to receive either empagliflozin 25mg (Jardiance) or  
109 metformin SR (slow release) 1500mg (Bolamyn) daily for 12 weeks. The dosage of empagliflozin (25  
110 mg) was chosen to get the maximum metabolic response with comparable duration to metformin  
111 treatment group. Metformin group received Metformin SR 1500mg, which is the standard dose  
112 commonly used in patients with PCOS in clinical practice.<sup>19</sup> Metformin SR was preferred over  
113 immediate release metformin in view of better gastrointestinal tolerability.<sup>20</sup> All participants were  
114 advised to maintain their usual dietary and lifestyle habits during the study.

115 Participants attended three visits (visits 1-3). During Visit 1, participants were screened against  
116 inclusion and exclusion criteria by medical history and clinical examination, routine blood tests (i.e.,  
117 full blood count, liver function tests, urea and electrolytes, clotting screen and a pregnancy test), urine  
118 pregnancy test and anthropometric measurements. During Visit 2 (baseline) and Visit 3 (12-week  
119 follow-up), participants underwent anthropometric [weight, BMI, waist circumference (WC) and hip  
120 circumference (HC)] and body composition assessments and an endothelial function measurement.  
121 Blood samples were collected at these time points and analysed for reproductive hormones and  
122 cardio-metabolic parameters [fasting glucose, fasting insulin, HOMA-IR, total cholesterol, LDL-C,  
123 HDL-C, triglycerides (TG), and hs-CRP].

124

## 125 **Procedures**

126 Height and weight were recorded with participants wearing light clothing and no shoes using a  
127 weighing scale with attached stadiometer (MS-4202L, Marsden Weighing Machine Group Limited,  
128 Rotherham, UK) and BMI was calculated as weight (kg) divided by the square of height (m<sup>2</sup>). Blood  
129 pressure readings were recorded using an automated device (NPB-3900; Nellcor Puritan Bennett,  
130 Pleasanton, CA). Three readings were taken at least two minutes apart, and then the average of the  
131 readings was obtained. Waist circumference and hip circumference were measured using a tape  
132 measure by wrapping it around the patient's waist at the midway point between the top of iliac crest  
133 and the bottom of the ribs. Basal metabolic rate, total body fat percentage, fat mass, fat free mass,  
134 total body impedance and total body water were measured by using a body composition analyser (BC

135 418 MA, Tanita Corporation Itabashi-ku, Tokyo, Japan). All these measurements were performed at  
136 baseline and 12 weeks after the empagliflozin and metformin treatments.

137 Endothelial function was assessed using a plethysmographic device Endo-PAT 2000 (Itamar  
138 Medical Ltd, Caesarea, Israel). Participants relaxed for at least 15 minutes in a quiet, controlled  
139 temperature (22 – 24 °C) room. Endo-PAT biosensors were placed on the index fingers of both hands.  
140 During the measurement, participants were instructed to relax and refrain from talking or making any  
141 sudden movements. The probes were inflated, and the signals were recorded on the computer  
142 according to manufacturer's instructions. This measurement consisted of 5 minutes of baseline  
143 recording, followed by blood pressure cuff inflation to a supra-systolic level (at least 60 mmHg above  
144 systolic pressure and no less than 200 mmHg) sustained for 5 minutes and subsequent blood pressure  
145 cuff deflation and recording of Endo-PAT readings over a further 5-minute period. Output variables  
146 namely Reactive Hyperaemia Index (RHI), a measure for endothelial function, and Augmentation  
147 Index (AI), a measure for arterial stiffness, were assessed using an automated computer software  
148 (EndoPAT2000 version 3.3.2, Itamar Medical Ltd). Compliance with the treatments was calculated by  
149 counting the returned medications at the end of the 12-week period.

150

### 151 **Blood sampling and biochemical analyses**

152 Following an overnight fast, blood samples were collected both at the baseline and final visit (end  
153 of the 12-week period). The fasting venous blood was collected into fluoride oxalate and serum gel  
154 tubes. Blood samples were separated by centrifugation at 3,500 x G for 15 minutes at 5°C, and the  
155 aliquots were stored at -80°C within 1 hour of collection.

156 Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the  
157 manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK), with a coefficient of  
158 variation (CV) was 6%, and no stated cross-reactivity with proinsulin. The plasma glucose was  
159 measured using a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, United Kingdom)  
160 and according to the manufacturer's recommended protocol. Total cholesterol, high-density  
161 lipoprotein cholesterol (HDL-C), triglycerides (TG), alanine aminotransferase (ALT), aspartate  
162 aminotransferase (AST) and high sensitivity C-reactive protein (*hs*-CRP) levels were measured

163 enzymatically using a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, UK) with  
164 CVs of <4.9, 0.9, 1.6 and 8.4 %. Low-density lipoprotein cholesterol (LDL-C) was calculated using  
165 the Friedewald equation. Serum testosterone and androstenedione were quantified using isotope-  
166 dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). Sex hormone-binding  
167 globulin (SHBG), estrogen and dehydroepiandrosterone (DHEAS) were measured using a  
168 chemiluminescent immunoassay on the Beckman-Coulter UniCel DxI 800 analyser, applying the  
169 manufacturer's recommended protocol. The free androgen index (FAI) was calculated as: (total  
170 testosterone/SHBG) x 100.

171

## 172 **Statistical analysis**

173 There are no previous studies on the effect of empagliflozin or any other SGLT-2  
174 inhibitors on women with PCOS. Sample size was based on an independent t-test with an  
175 arbitrary level of 5% significance (2-tailed) and power of 80%. Assuming a common  
176 standard deviation of 16% for radial augmentation index and a 4% reduction as a significant  
177 change,<sup>21</sup> a sample size of 16 patients per group allowed us to detect a between-group mean  
178 difference of 4%. To allow for loss-to-follow-up we aimed to recruit 20 patients per group.

179 All data were checked for normality according to the Shapiro-Wilk test. Mean  
180 differences for all parameters expressed as % change from baseline between women with  
181 PCOS in the empagliflozin group and the metformin group were analysed with independent  
182 t test or Mann-Whitney U-test for normally and non-normally distributed data, respectively.  
183 Mean differences between baseline and 12-week follow-up within each treatment group  
184 were analysed with a paired t-test or a signed-rank test for normally and non-normally  
185 distributed data, respectively. Values are presented as mean SD, if the variables were  
186 normally distributed, or median and interquartile range, if the variables were skewed. All  
187 statistical analyses were performed using IBM-SPSS version 24.0 (Chicago, IL, USA) with  
188 p-values  $\leq 0.05$  considered to be significant. Linear regression analysis using function  $\ln R$   
189 was used to confirm the findings of univariate analysis after adjustments for confounders.

190 Since this was a randomised controlled trial, we only adjusted for age and age + BMI,  
191 where relevant.

192

## 193 **Results**

194 Forty-two participants with PCOS were screened; two participants were excluded from  
195 participation because they did not meet inclusion criteria, as such 40 participants were  
196 randomised to the two treatment arms (empagliflozin, n=20 and metformin, n=20) (Figure  
197 1). During the intervention phase of the study, one participant in the empagliflozin group  
198 was lost to follow-up. Finally, 19 participants in the empagliflozin group [age: 26.0 (8.0)  
199 years, BMI: 37.1±6.2 kg/m<sup>2</sup>] and 20 participants in the metformin group [age: 31.5 (20.0)  
200 years, BMI: 38.7±7.8 kg/m<sup>2</sup>] completed the trial and their data were included in the final  
201 analysis (Figure 1). The baseline characteristics of both groups are presented in Table and  
202 Supplementary Table 1. Compliance was over 90% in both groups. There were no adverse  
203 events or serious adverse events in the metformin group. In the empagliflozin group, two  
204 patients reported adverse events (headache and dizziness, n=1; mild rash, n=1), which were,  
205 however, unrelated to the study drug.

215

### 216 **Anthropometric and body composition parameters**

217 In the empagliflozin group, waist circumference ( $p=0.024$ ), hip circumference  
218 ( $p=0.013$ ), BMR ( $p=0.016$ ), fat free mass (FFM) ( $p=0.013$ ) and total body water ( $p=0.014$ )  
219 at 12 weeks decreased significantly compared to baseline, but no changes were seen in total  
220 mass ( $p=0.079$ ) or BMI ( $p=0.069$ ) (Table 1). In the metformin group, body mass ( $p=0.019$ ),  
221 BMI ( $p=0.024$ ) and hip circumference ( $p=0.031$ ), total fat percentage ( $p=0.015$ ) or fat mass  
222 ( $p=0.005$ ) significantly increased after 12 weeks of treatment (Table 1).

223 When data were expressed as percentage change from baseline in each group, significant  
224 differences in weight (empagliflozin:  $-1.4\pm 3.2\%$  vs. metformin:  $1.2\pm 2.3\%$ ;  $p=0.006$ ), BMI  
225 (empagliflozin:  $-1.4\pm 3.2\%$  vs. metformin:  $1.1\pm 2.2\%$ ;  $p=0.007$ ), waist (empagliflozin:  $-$   
226  $1.6\pm 2.8\%$  vs. metformin:  $0.2\pm 2.1\%$ ;  $p=0.029$ ) and hip circumference (empagliflozin:  $-$

227 2.0±3.0% vs. metformin: 1.1±1.9%;  $p=0.001$ ) were seen between the treatment groups.  
228 Similarly, the percentage changes from baseline in BMR (empagliflozin: -1.8±2.9% vs.  
229 metformin: 0.1±1.9%,  $p=0.024$ ) and fat mass (empagliflozin: -0.7±4.9% vs. metformin,  
230 3.2±5.0%;  $p=0.023$ ) were significantly different between the empagliflozin and metformin  
231 groups (Table 1, Figure 2). The proportion of women with PCOS who experienced (a) a  
232 decrease >5%, (b) an increase >5%, or (c) a change ≤5% from baseline in anthropometric  
233 and body composition parameters are presented in Supplementary Table 2.

234

### 235 **Hormonal and metabolic parameters**

236 In the empagliflozin group, significant increases in SHBG ( $p=0.049$ ) and oestradiol levels  
237 ( $p=0.032$ ) were seen after 12 weeks of treatment (Table 1). There were no other hormonal changes for  
238 either group (Table 1). No differences were seen in percentage change from baseline for any of the  
239 hormone parameters between groups (Table 1, Figure 2).

240 There were no changes following 12 weeks of treatment in blood pressure, endothelial function  
241 (RHI, AI), insulin sensitivity (insulin, fasting glucose, HOMA-IR), fasting lipid profile or *hs*-CRP in  
242 either treatment arm (Table 1). Between groups comparisons did not reveal any differences in  
243 percentage changes from baseline for any of these metabolic parameters (Table 1, Figure 2).

244

### 245 **Regression analysis modelling**

246 Table 2 shows the results of the linear regression analysis modelling percentage changes from  
247 baseline in anthropometric characteristics, hormonal and metabolic parameters as function of  
248 metformin or empagliflozin treatment. The results confirm statistically significant reduction in weight,  
249 BMI, WC, HC, fat mass and BMR in those randomized to empagliflozin group as compared to those  
250 in metformin group.

251

### 252 **Discussion**

253 In this first study on the comparative effects of the SGLT2 inhibitor, empagliflozin, and metformin  
254 in overweight and obese women with PCOS, we demonstrated that treatment with empagliflozin over

255 a 12-week period, resulted in significant reductions in weight, BMI, waist and hip circumference, total  
256 body fat mass and BMR, compared to treatment with metformin, but did not differentially affect  
257 hormonal or metabolic parameters.

258 Obesity is prevalent among women with PCOS and has been associated directly and/or indirectly  
259 with negative metabolic, cardiovascular, endocrine, reproductive and mental health outcomes.<sup>1, 2</sup>  
260 Weight reduction exerts positive effects on PCOS-related outcomes; therefore, it is a primary goal of  
261 the management of the condition.<sup>3</sup> In the present study, women with PCOS in the empagliflozin  
262 group had a mean weight loss of 1.5 kg, which is similar to the weight loss reported in previous  
263 short-term trials.<sup>12</sup> Mechanistically, initial weight loss in empagliflozin studies is attributed to the  
264 calorie loss (approximately 200-300 kcal/day) associated with glucose excretion, but also to the mild  
265 diuretic effects of the drug.<sup>9, 12</sup> Conversely, the steady-state weight loss associated with SGLT2  
266 inhibitors treatment may result from fat loss.<sup>22, 23</sup> In animal models, SGLT2 inhibitors have been  
267 shown to cause reduction in body weight and fat mass by enhancing lipolysis, fatty acid oxidation  
268 and adipose tissue browning.<sup>14, 24</sup> These findings coincide with the alterations seen in substrate  
269 utilisation from carbohydrates to lipids and potentially, ketone bodies.<sup>9, 25</sup> Reductions in other  
270 measures of adiposity including waist and hip circumference, visceral and subcutaneous fat depots or  
271 indices, which may better reflect risk for metabolic disturbances and cardiovascular disease, have  
272 also been demonstrated in patients with type 2 diabetes following treatment with SGLT2 inhibitors,  
273 <sup>22, 23</sup> with these findings being in agreement with the improvements in waist and hip circumference  
274 seen in our women with PCOS assigned to receive empagliflozin.

275 In the present study, the metformin group experienced modest increases in body weight. Studies  
276 on the effect of metformin on body weight in women with PCOS have yielded mixed results.<sup>8, 26, 27</sup>  
277 While some studies have suggested that metformin therapy may result in weight reduction, some  
278 randomized controlled trials have failed to confirm this. For example, a large, randomised, double-  
279 blind, placebo-controlled trial evaluated the combined effects of lifestyle modification and  
280 metformin (850 mg twice daily), by studying 143 anovulatory women in the UK with a mean BMI  
281 of 38 kg/m<sup>2</sup> and showed that it is no different than placebo in terms of weight reduction.<sup>26</sup> However,  
282 women in this study were not required to have clinical and biochemical evidence of

283 hyperandrogenemia- an essential component of the diagnosis of PCOS. Conversely, a Finnish  
284 multicentre randomized study<sup>27</sup> compared metformin with placebo in 320 women with PCOS and  
285 the authors reported significantly higher live birth rates in the metformin group (41.9% versus  
286 28.8%;  $p=0.014$ ) and maximal effect was seen in obese women with PCOS. A recent Cochrane  
287 review explored the effect of metformin on PCOS (40 studies, total  $n= 3848$  women) failed to  
288 provide any conclusive evidence against or for metformin in women with PCOS.<sup>8</sup> These mixed  
289 results in the literature with regards to metformin treatment indicate that there are subtypes of PCOS  
290 which might respond beneficially to PCOS. Our study is not powered to assess the phenotypic  
291 heterogeneity in PCOS with regards to response to metformin- and highlights the need to study this  
292 with large scale studies looking at the effect of metformin on PCOS subtypes.

293 Women with PCOS experience higher prevalence of insulin resistance, type 2 diabetes mellitus,  
294 dyslipidaemia, endothelial dysfunction and atherosclerosis compared to age-matched women without  
295 PCOS.<sup>2</sup> Such metabolic disturbances are characterised by chronic low-grade inflammation and  
296 vascular impairments which increase cardiovascular risk.<sup>2</sup> The effects of SGLT-2 inhibitors on  
297 glycaemic control have been evaluated as the primary outcome in the majority of the studies  
298 investigating this new class of glucose lowering agents in type 2 diabetes. A meta-analysis of 13  
299 randomized trials on the efficacy of SGLT-2 inhibitors compared to placebo demonstrated  
300 improvements in glycaemic control in type 2 diabetes patients, as evidenced by reductions in HbA1c  
301 (-0.49% and -0.50% after one and two years of treatments) and fasting plasma glucose levels (-0.81  
302 mmol/L and -0.76 mmol/L after one and two years of treatment).<sup>28</sup> Similar results were shown in a  
303 meta-analysis (10 studies, 6203 participants) on the efficacy and safety of empagliflozin only.<sup>29</sup> In  
304 addition to glycaemic control, use of SGLT-2 inhibitors results in a reduction in TG levels and  
305 increases in HDL-cholesterol levels, but also LDL-cholesterol levels, possibly due to the shifted  
306 metabolism favouring lipid utilisation.<sup>30, 31</sup> In contrast to these beneficial effects on glycaemic  
307 control and less pronounced lipids effects of SGLT-2 inhibitors reported in type 2 diabetes, we did  
308 not observe significant changes in fasting glucose, insulin fasting lipids or *hs*-CRP at 3 months after  
309 empagliflozin treatment compared to baseline or any differences between our treatment groups.

310 These results may be related to the short duration of the study or to the baseline characteristics of our  
311 participants with PCOS, who were young and did not have diabetes.

312 Further evidence from studies in patients with type 2 diabetes suggest that empagliflozin, and  
313 other SGLT2 inhibitors such as canagliflozin and dapagliflozin cause reductions in blood pressure,  
314 as a result of their natriuretic effects or due to the intensification of anti-hypertensive therapy.<sup>32, 33</sup>  
315 No such blood pressure changes were demonstrated in our women with PCOS, though these subjects  
316 were normotensive and changes may not have been expected. Similarly, measures of endothelial  
317 function (RHI) or arterial stiffness (AI) were not altered compared to baseline in either treatment  
318 groups. Empagliflozin has been shown to improve endothelial dysfunction in preclinical studies in  
319 diabetic rat models,<sup>34, 35</sup> but human data are scarce. A recent 16-week study demonstrated that  
320 dapagliflozin add-on therapy to metformin improved endothelial function, as evaluated by flow-  
321 mediated dilation, in patients with inadequately controlled early-stage type 2 diabetes mellitus.<sup>36</sup>  
322 Although there are no comparative data from studies that have investigated the effects of SGLT-2  
323 inhibitors in women with PCOS, the results of our 12-week intervention contrast those of a longer  
324 study which demonstrated that metformin treatment for 6 months improved or even normalised  
325 abnormal flow-mediated dilatation (FMD) on the brachial artery and improved plasma endothelin-1  
326 (ET-1) levels in women with PCOS.<sup>37</sup> The discrepancies in these results may be at least partially  
327 explained by differences in study duration and the use of different endothelial function measures.

328 There were significant increases in the SHBG and oestradiol levels in the empagliflozin group,  
329 but no significant reductions were seen in FAI and serum total testosterone levels. The % changes  
330 from baseline in hormonal levels did not differ to metformin. Metformin use in women with PCOS  
331 has been associated with improvements in hormonal levels. A recent meta-analysis demonstrated  
332 that metformin treatment resulted in small improvements in serum testosterone, but no changes in  
333 free testosterone, FAI, SHBG, DHEAS LH, FSH, LH/FSH ratio, oestradiol or progesterone  
334 compared to placebo in women with PCOS.<sup>38</sup> Metformin may also have some beneficial effects on  
335 ovulation and menstrual frequency.<sup>8</sup> Given the short follow-up of the present study, we did not  
336 assess these parameters, which is a limitation of the present study.

337

338 **Conclusions**

339 Empagliflozin treatment over a 12-week period had beneficial effects on weight, BMI, waist and  
340 hip circumference and total body fat in overweight and obese women with PCOS compared to  
341 metformin, but no differences were seen in hormonal and metabolic parameters including insulin  
342 resistance and androgen levels. Placebo-controlled and comparative treatment RCTs of longer-term  
343 duration are needed to confirm these findings and provide further insights into the effects of  
344 empagliflozin on PCOS-related outcomes in women with PCOS with different PCOS phenotypes and  
345 PCOS-related complications (e.g., with/ without diabetes), before empagliflozin gains a therapeutic  
346 place in PCOS. Lifestyle interventions (preferably multicomponent including diet, exercise and  
347 behavioural strategies) should still be considered the first line of treatment for overweight/obese  
348 women with PCOS for reductions in body weight, central obesity and insulin resistance.<sup>3</sup>

349

350 **Acknowledgements**

351 This study was supported internally by the Academic Diabetes Endocrinology and Metabolism Unit at  
352 University of Hull. We gratefully acknowledge the study participants who contributed to the study

353

354 **Disclosure Statement**

355 The authors report no conflict of interest in this work.

356

357 **Data availability statement**

358 The data that support the findings of this study are available from the corresponding author upon  
359 reasonable request.

360

361 **Contributions**

362 ZJ, ESK, SLA, TS participated in study conception and design. ZJ, TS performed the acquisition of  
363 data. ZJ, MP, HD, UQ, JA, AYK, ASR, ESK, SLA and TS participated in analysis and/or

364 interpretation of data. ZJ and MP drafted the paper; all authors reviewed and approved the final  
365 manuscript. TS is the guarantor of the study.

## References

1. Legro RS, Arslanian SA, Ehrmann DA et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2013; 98:4565-4592.
2. Moran LJ, Norman RJ, Teede HJ. Metabolic risk in PCOS: phenotype and adiposity impact. *Trends Endocrin Met.* 2015; 26:136-143.
3. Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf.* 2018, 89:251-268.
4. Yildiz BO. Approach to the patient: contraception in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2015; 100:794-802 (2015).
5. Amiri M, Ramezani Tehrani F, Nahidi F, Kabir A, Azizi F, Carmina E. Effects of oral contraceptives on metabolic profile in women with polycystic ovary syndrome: A meta-analysis comparing products containing cyproterone acetate with third generation progestins. *Metabolism.* 2017; 73:22-35.
6. de Bastos M, Stegeman BH, Rosendaal FR, et al. Combined oral contraceptives: venous thrombosis. *Cochrane Database Syst Rev.* 2014; 3:CD010813.
7. Sam S, Ehrmann DA. Metformin therapy for the reproductive and metabolic consequences of polycystic ovary syndrome. *Diabetologia.* 2017; 60:1656-1661.
8. Morley LC, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev.* 2017; 11:CD003053.
9. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. *Diabetologia.* 2017; 60:215-225.
10. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* 2015; 373:2117-2128.
11. Scheen AJ. Pharmacokinetic and pharmacodynamic profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor. *Clin Pharmacokinet.* 2014; 53:213-225.
12. Vasilakou D, Karagiannis T, Athanasiadou E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med.* 2013; 159:262-274.
13. Fitchett D, Zinman B, Wanner C, et al. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME (R) trial. *Eur Heart J.* 2016, 37:1526-1534.
14. Xu L, Nagata N, Nagashimada M, et al. SGLT2 Inhibition by Empagliflozin Promotes Fat Utilization and Browning and Attenuates Inflammation and Insulin Resistance by Polarizing M2 Macrophages in Diet-induced Obese Mice. *Ebiomedicine.* 2017; 20: 137-149.
15. Gallo LA, Ward MS, Fotheringham AK, et al. Once daily administration of the SGLT2 inhibitor, empagliflozin, attenuates markers of renal fibrosis without improving albuminuria in diabetic db/db mice *Sci Rep.* 2016; 6: 28124.

16. Inzucchi SE, Zinman B, Fitchett D, et al. How does empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. *Diabetes Care*. 2018; 41: 356-363.
17. Staels B. Cardiovascular protection by sodium glucose cotransporter 2 inhibitors: potential mechanisms. *Am J Cardiol*. 2017; 120: S28-S36.
18. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004; 19: 41-47.
19. Lashen H. Role of metformin in the management of polycystic ovary syndrome. *Ther Adv Endocrinol Metab*. 2010; 1:117-128.
20. Blonde L, Dailey GE, Jabbour SA, Reasner CA, Mills DJ. Gastrointestinal tolerability of extended-release metformin tablets compared to immediate-release metformin tablets: results of a retrospective cohort study. *Curr Med Res Opin*. 2004; 20:565-572.
21. Cherney DZ, Perkins BA, Soleymanlou N, et al. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. *Cardiovasc Diabetol*. 2014; 13:28.
22. Bolinder J, Ljunggren Ö, Johansson L, et al. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. *Diabetes Obes Metab*. 2014; 16:159-169.
23. Neeland IJ, McGuire DK, Chilton R, et al. Empagliflozin reduces body weight and indices of adipose distribution in patients with type 2 diabetes mellitus. *Diabetes Vasc Dis Res*. 2016; 13: 119-126.
24. Yokono M, Takasu T, Hayashizaki Y, et al. SGLT2 selective inhibitor ipragliflozin reduces body fat mass by increasing fatty acid oxidation in high-fat diet-induced obese rats. *Eur J Pharmacol*. 2014; 727: 66-74 (2014).
25. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest*. 2014; 124:499-508.
26. Tang T, Glanville J, Hayden CJ, White D, Barth JH, Balen AH. Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multicentre study. *Hum Reprod*. 2006; 21: 80-89.
27. Morin-Papunen L, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. *J Clin Endocrinol Metab* 2012; 97: 1492-1500.
28. Liu XY, Zhang N, Chen R, Zhao JG, Yu P. Efficacy and safety of sodium-glucose cotransporter 2 inhibitors in type 2 diabetes: a meta-analysis of randomized controlled trials for 1 to 2 years. *J Diabetes Complicat*. 2015; 29:1295-1303.
29. Liakos A, Karagiannis T, Athanasiadou E, et al. Efficacy and safety of empagliflozin for type 2 diabetes: a systematic review and meta-analysis. *Diabetes Obes Metab*. 2014; 16:984-993.

30. Briand F, Mayoux E, Brousseau E, *et al.* Empagliflozin, via Switching Metabolism Toward Lipid Utilization, Moderately Increases LDL Cholesterol Levels Through Reduced LDL Catabolism. *Diabetes*. 2016; 65: 2032-2038.
31. Inzucchi SE, Zinman B, Wanner C, *et al.* SGLT-2 inhibitors and cardiovascular risk: Proposed pathways and review of ongoing outcome trials. *Diabetes Vasc Dis Re*. 2015; 12: 90-100.
32. Mancia G, Cannon CP, Tikkanen I, *et al.* Impact of empagliflozin on blood pressure in patients with type 2 diabetes mellitus and hypertension by background antihypertensive medication. *Hypertension*. 2016; 68:1355-1364.
33. Mearns ES, Sobieraj DM, White CM, *et al.* Comparative Efficacy and Safety of Antidiabetic Drug Regimens Added to Metformin Monotherapy in Patients with Type 2 Diabetes: A Network Meta-Analysis. *Plos One*. 2015; 10: :e0125879.
34. Oelze M, Kröller-Schön S, Welschof P, *et al.* The Sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. *Plos One*. 2014; 9:e112394.
35. Steven S, Oelze M, Hanf A, *et al.* The SGLT2 inhibitor empagliflozin improves the primary diabetic complications in ZDF rats. *Redox Biol*. 2017; 13: 370-385.
36. Shigiyama F, Kumashiro N, Miyagi M, *et al.* Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. *Cardiovasc Diabetol*. 2017; 16:84.
37. Diamanti-Kandarakis E, Alexandraki K, Protogerou A, *et al.* Metformin administration improves endothelial function in women with polycystic ovary syndrome. *Eur J Endocrinol*. 2005; 152: 749-756.
38. Patel R, Shah G. Effect of metformin on clinical, metabolic and endocrine outcomes in women with polycystic ovary syndrome: a meta-analysis of randomized controlled trials. *Curr Med Res Opin*. 2017; 33: 1545-1557.

**Table 1:** Changes in anthropometric, body composition, hormonal and metabolic parameters following 12 weeks of empagliflozin and metformin treatment.

Parameter	Empagliflozin (n = 19)			Metformin (n=20)		
	Baseline	12 weeks	% baseline change	Baseline	12 weeks	% baseline change
Weight (kg)	102.3±16.6	101.5±16.3	-1.4±3.2**	108.8±25.3	110.1±25.7*	1.2±2.3
BMI (kg/m <sup>2</sup> )	37.1±6.2	36.6±6.0	-1.4±3.2**	38.7±7.8	39.2±7.9*	1.1±2.2
Waist circumference (cm)	101.2±9.7	99.6±9.5*	-1.6±2.8**	106.2±15.7	106.3±15.4	0.2±2.1
Hip circumference (cm)	121.6±11.5	119.2±11.4*	-2.0±3.2**	124.1±17.4	125.3±16.7*	1.1±1.9
BMR (kcal) <sup>1</sup>	1761±205	1728±200*	-1.8±2.9**	1783 (304)	1797 (305)	0.1±1.9
Body fat (%) <sup>1</sup>	46.7±3.5	47.1±3.4	0.6 (3.2)	46.8±6.2	47.6±5.9*	1.1 (3.8)
Fat mass (kg) <sup>1</sup>	48.9±11.0	48.6±11.0	-0.7±4.9**	52.3±10.9	53.7±18.3*	3.2±5.0
FFM (kg) <sup>1</sup>	54.8±5.9	53.7±5.8*	-2.0±3.2	56.7±7.9	56.5±7.9	-0.3±2.2
TBW (kg) <sup>1</sup>	40.1±4.3	39.3±4.3*	-2.0±3.2	41.5±5.8	41.4±5.8	-0.3±2.2
FAI	10.3±3.0	9.4±3.6	-7.0±31.4	7.5 (6.4)	8.0 (6.4)	-9.7 ±34.0
Testosterone (nmol/L)	1.6±0.4	1.6±0.6	2.6 (37.0)	1.7 (1.2)	1.5 (1.2)	-14.0 (33.6)
SHBG (nmol/L)	17.3±6.4	19.2±8.5*	9.9±22.6	19.5(13.5)	19.5(14.5)	6.4±25.5
Androstenedione (nmol/L)	5.7±1.4	5.7±1.9	-2.2 (24.4)	4.3 (4.4)	5.0 (2.8)	5.6 (59.8)
DHEAS (µmol/L)	6.1±1.6	6.2 ± 2.1	1.0±20.1	5.5 ± 3.3	5.8 ± 3.0	8.1±15.0
Oestradiol (pmol/L)	200 (80)	280 (340)*	39.1 (121)	240 (140)	210 (190)	-8.7 (113.1)
SBP (mmHg)	118.1±11.7	117.5±14.2	-0.8 (5.9)	124.4±15.5	125.9±15.8	1.1 (6.8)
DBP (mmHg)	74.0 (10.0)	73.0 (8.0)	-3.1±9.0	80.3 ± 10.7	80.7 ± 9.8	0.8±7.1
RHI	1.6 (0.5)	1.5 (0.7)	2.6 (48.1)	1.7 (0.5)	1.6 (0.7)	-1.9 (53.7)
AI	-3.3 ± 12.0	-3.4± 13.3	-57.0±170	0.6 ± 8.1	2.3 ± 10.4	-47.5±146
Fasting glucose (mmol/L) <sup>1</sup>	4.5 (0.6)	4.5 (0.6)	-0.8±5.8	4.7 (0.5)	4.4 (0.6)	-2.3±8.0
Fasting insulin (µIU/ml)	12.6 (11.6)	12.7 (14.4)	-21.5(80.1)	16.6 (11.4)	14.0 (22.7)	-14.1 (52.5)
HOMA-IR <sup>1</sup>	2.6 (2.1)	2.4 (2.7)	-20.5 (84.6)	3.7 (2.4)	3.2 (4.9)	-18.9 (53.5)
TC (mmol/L)	4.8 ± 1.0	4.7±1.1	-1.6 ± 13.7	4.7 ± 0.9	4.5 ± 0.9	-2.2 ± 8.5
LDL-C (mmol/L)	2.8 ± 1.0	2.7 ± 1.1	2.7 (30.2)	2.8 (0.6)	2.8 (0.9)	-3.4 (9.6)
HDL-C (mmol/L)	1.1 ± 0.2	1.1 ± 0.2	-0.6 ± 9.2	1.2 ± 0.2	1.1 ± 1.9	-3.4 ± 9.6
TG (mmol/L)	1.5 (1.3)	1.4 (0.9)	-6.7 (35.8)	1.1 (0.9)	1.2 (0.7)	-9.0 (49.8)
hs-CRP (mg/L)	5.4 (6.6)	3.3 (5.9)	9.8±59.1	6.1 (9.7)	5.1 (10.9)	8.4±34.0

Data are presented as mean ± SD if normally distributed or as median (interquartile range), if skewed. \*,  $p < 0.05$ , significant difference from baseline within treatment group; \*\*,  $p < 0.05$ , significant difference from metformin group; AI, Augmentation index; BMI, body mass index; BMR, Basal metabolic rate; DBP, diastolic blood pressure; DHEAS, Dehydroepiandrosterone sulphate; FAI, Free androgen index; FFM, Fat free mass; HDL, high density lipoproteins; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high sensitivity-C-reactive protein; LDL, low density lipoproteins; RHI, Reactive hyperaemic index; SBP, systolic blood pressure in; SHBG, Sex hormone binding globulin; TBW, Total body water; TC, total cholesterol; TG, triglycerides. <sup>1</sup>Data available for 18 participants in the empagliflozin group.

**Table 2:** Linear regression analysis modelling percentage (%) changes in key anthropometric characteristics, hormonal and metabolic parameters as function of metformin or empagliflozin

	Beta	SE	P-value
% change in BMI*	-2.27	0.95	0.02
% change in weight*	-2.28	0.96	0.02
% change in fat mass*	-3.43	1.74	0.05
% change in WC*	-1.76	0.85	0.04
% change in HC*	-2.87	0.88	0.002
% change in BMR*	-1.73	0.86	0.05
% change in FAI**	5.42	11.97	0.65
% change in SHBG**	5.89	8.69	0.5
% change in TC*	2.78	3.85	0.47
% change in fasting glucose*	1.12	2.48	0.65

BMI, body mass index; BMR, Basal metabolic rate; WC, waist circumference; HC, HIP circumference; FAI, Free androgen index; FFM, Fat free mass; SHBH, sex hormone binding globulin; TC, total cholesterol.

Metformin group used as reference group

\*adjusted for age

\*\*adjusted for age and BMI

## Legends of figures

**Figure 1:** Flow diagram of the study.

**Figure 2:** Percentage changes from baseline in anthropometric and body composition parameters after 12 weeks with empagliflozin and metformin treatment.

\*,  $p < 0.05$ ; BMI, body mass index; WC, waist circumference; HC, hip circumference; BMR, Basic metabolic rate; FFM, Fat free mass; TBW, Total body water. Body composition data (BMR, body fat %, fat mass, FFM and TBW) are presented for 18 participants in the empagliflozin group with available data.

