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1 **Title Page**

2 **Title:** Postprandial metabolic responses to high-fat feeding in healthy adults following  
3 ingestion of oolong tea-derived polymerized polyphenols: a randomized, double-blinded,  
4 placebo-controlled crossover study

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21 **Sources of support:** This study was funded by Lucozade Ribena Suntory.

22 **Short running head:** Polyphenols and postprandial lipemia.

23 **Abbreviations and definitions:**

24 AIC, Akaike Information Criterion

25 APO, apolipoprotein

26 AUC, area under the curve

27 BMI, body mass index

28 CAF, caffeine

29 CAT, catechins

30 ELISA, enzyme linked immunosorbent assay

31 HDL-c, high-density lipoprotein cholesterol

32 iAUC, incremental area under the curve

33 K3 EDTA, andtripotassium ethylene diamine tetraacetic acid

34 LDL-c, low-density lipoprotein cholesterol

35 LMM, linear mixed model

36 NaCL, sodium chloride

37 NEFA, non-esterified fatty acids

38 PP, polymerized polyphenols from oolong tea

39 PP+CC, polymerized polyphenols from oolong tea plus caffeine and catechins

40 SGLT1, sodium-dependent glucose transporter

41 TAG, triacylglycerol

42 VLDL-c, very low-density lipoprotein cholesterol

43

44 **Clinical Trial Registration:** <https://clinicaltrials.gov/ct2/show/NCT03324191>

45 (NCT03324191)

46 Data described in the manuscript, code book, and analytic code will be made publicly and freely

47 available without restriction at [URL to be generated upon publication].

48 **Abstract**

49 BACKGROUND: Polymerized polyphenols (PP) found in oolong tea can inhibit pancreatic  
50 lipase activity *in vitro* and pilot work indicates this may reduce postprandial lipemia. Since  
51 tea contains caffeine and catechins, the interactions between these ingredients and PP  
52 warrants investigation.

53 OBJECTIVE: Assess whether PP ingested alone or with caffeine and catechins lowers  
54 postprandial lipemia.

55 DESIGN: Fifty healthy adults (mean (standard deviation) age: 26 (7) years; BMI: 24.0 (2.7)  
56 kg/m<sup>2</sup>; female n=16) completed 4 oral lipid tolerance tests in a placebo-controlled  
57 randomized, crossover design. Participants ingested 40 g fat with either: 1) placebo; 2) 100  
58 mg PP; 3) 150 mg PP; or 4) 100 mg PP plus 50 mg caffeine and 63 mg catechins (PP+CC).  
59 Blood was sampled for 3 hours postprandially to assess concentrations of serum and plasma  
60 triacylglycerol and plasma markers of lipid (non-esterified fatty acid (NEFA), glycerol, low-  
61 and high-density lipoprotein-cholesterol (LDL-c & HDL-c), and apolipoprotein-AI, -AII, -B, -  
62 CII, -CIII and -E) and glucose metabolism (glucose, insulin, and C-peptide).

63 RESULTS: Serum and plasma triacylglycerol concentrations and lipid metabolism variables  
64 generally increased following any test drink ingestion (main effect of time,  $p < 0.001$ ).  
65 Nevertheless, for the lipid metabolism responses, there were no statistically significant  
66 condition x time interactions and no statistically significant differences in incremental or total  
67 area under the curve between conditions, apart from HDL-c ( $p = 0.021$ ). Ingesting 100 mg  
68 PP+CC lowered peak plasma glucose, insulin, and C-peptide concentrations versus all other  
69 conditions 30 minutes post-ingestion ( $p < 0.001$ ), with persistent alterations in glucose  
70 concentrations observed for 90 minutes compared with placebo and 100 mg PP conditions.

71 CONCLUSIONS: PP ingested at doses up to 150 mg do not clearly alter early-phase  
72 postprandial triacylglycerol concentrations in healthy adults, irrespective of the presence or  
73 absence of caffeine and catechins. Nevertheless, caffeine and catechins added to PP lowered  
74 postprandial glucose and insulin concentrations.

75 Keywords: *Lipid Metabolism, Blood lipids, Tea extract, Triglycerides, Energy metabolism,*  
76 *Glucose control*

## 77 **Introduction**

78 Excessive postprandial elevations in triacylglycerol concentrations and other aspects of lipid  
79 metabolism play a major role in cardiovascular disease risk. Indeed, the results of studies  
80 using Mendelian randomization approaches support a causal role for plasma triacylglycerol  
81 concentrations in coronary heart disease (1). Moreover, the postprandial state is particularly  
82 important, since this scenario captures the total amount of atherogenic lipoproteins in plasma,  
83 and also reflects the metabolic state in which people in middle-to-high income countries  
84 spend most of their time (2). The most recent National Nutrition and Diet Survey (3)  
85 highlights that the typical UK diet is particularly high in fat, which is the key driver in  
86 postprandial triacylglycerol concentration. Therefore, strategies that reduce postprandial  
87 triacylglycerol concentrations may have potential to reduce the incidence of cardiovascular  
88 disease.

89 Some of the most effective strategies for reducing postprandial triacylglycerol concentrations  
90 include medications that inhibit gastrointestinal lipase activity, such as Orlistat. The addition  
91 of Orlistat to a high-fat meal can reduce the postprandial triacylglycerol area under the curve  
92 by ~40%, in addition to altering various other components of lipid metabolism, such as low  
93 density lipoprotein (LDL-c) cholesterol concentrations, very low-density lipoprotein (VLDL-  
94 c) subclass concentration, mean VLDL size, and small high density lipoprotein (HDL-c)  
95 particle concentrations (4, 5). However, Orlistat is a relatively aggressive pharmacological  
96 means of inhibiting lipase activity and thus carries side effects such as diarrhea (6), whereas  
97 naturally occurring nutrient compounds may provide more modest lipase inhibition but  
98 without the severity of side-effects seen with pharmacological interventions.

99 One such example is a class of high-molecular-weight polymerized polyphenols (PP) that are  
100 particularly abundant in oolong tea. These polyphenols have been found to markedly inhibit  
101 pancreatic lipase activity *in vitro* (7). Moreover, cross-sectional data indicate that long-term

102 consumption of oolong tea may be associated with improved blood lipid profile (8) and  
103 experimental evidence indicates increased fecal fat content following 10 days of PP  
104 supplementation (9). A randomized controlled trial in 22 Japanese adults suggests that 70 mg  
105 PP may reduce postprandial triacylglycerol concentrations in response to feeding of a high fat  
106 meal (40g of dietary fat) by up to 18% *versus* placebo (10). Similar suppression of the  
107 postprandial increase in serum triacylglycerol following a high fat meal has been report in  
108 adults in Thailand (11).

109 The aim of this study was to assess the effect of polymerized polyphenols from oolong tea on  
110 postprandial triacylglycerol concentrations and additional components of lipid metabolism.  
111 Since tea products typically also contain caffeine and catechins, an additional aim was to  
112 examine any interactions between these added ingredients with polymerized polyphenols. It  
113 was hypothesized that oolong tea polymerized polyphenols would reduce postprandial serum  
114 triacylglycerol responses to a high-fat drink independent of the other ingredients, and in a  
115 sustained manner over the 3-h postprandial study period.

116

## 117 **Participants and Methods**

### 118 **Study design overview**

119 This study was a double-blinded, single center, randomized controlled trial using a repeated-  
120 measures crossover experimental design, with four experimental conditions. The effect of test  
121 drink ingestion on three-hour blood lipid response to a high fat meal challenge was assessed.  
122 Whilst five or six hours is commonly used for oral fat tolerance tests, re-examination of our  
123 previous work has indicated that the three-hour incremental area under the curve (3h-iAUC)  
124 for triacylglycerol provides a valid representation of the five-hour iAUC ( $r = 0.91$ ,  $p < 0.01$ ;  
125 Supplementary Figure 1, and (12)). Whilst these two parameters of course share the same first

126 three hours and so the correlation is subject to mathematical coupling, the strength of this  
127 correlation at least demonstrates that the iAUC over 5 hours does not typically show an  
128 opposite response to that observed over 3 hours. Moreover, prior work from others  
129 demonstrated a lowering of serum triacylglycerol concentrations three hours following  
130 ingestion (10). Therefore, to replicate that previous work and minimize participant burden, a  
131 three-hour postprandial period was chosen for the present study. The four test drink  
132 conditions were as follows;

- 133 1) a placebo drink, containing <1 mg of isolated polymerized polyphenols from oolong  
134 tea (PLACEBO)
- 135 2) a drink containing a moderate amount (approx. 100 mg) of isolated polymerized  
136 polyphenols from oolong tea (100mgPP)
- 137 3) a drink containing a large amount (approx. 150 mg) of isolated polymerized  
138 polyphenols from oolong tea (150mgPP)
- 139 4) a drink containing a moderate amount (approx. 100 mg) of isolated polymerized  
140 polyphenols from oolong tea plus 50 mg caffeine and 63 mg catechins (100mgPP+CC)

141 The 350 mL test drinks were flavor- and color-matched, and provided by Lucozade Ribena  
142 Suntory Ltd in sealed pre-labelled containers identified by participant identification number  
143 and visit number only. Therefore, none of the participants or research team was aware of  
144 treatment allocation. The randomization schedule was generated using a single 4-by-4  
145 Williams Latin square. For every block of 4 participants, the rows of this reference 4-by-4  
146 square were randomly permuted. The net result was a randomization schedule with 4 unique  
147 treatment sequences and each treatment followed every other treatment an equal number of  
148 times. It was generated in SAS version 9.4 (SAS Institute., Cary, NC, USA).



149 The primary outcome measure was serum triacylglycerol (TAG) 3h-iAUC, for direct  
150 comparison with findings from previous research (10). Secondary outcome measures were 3h-  
151 iAUC for plasma TAG, insulin, C-peptide, and total area under the curve (AUC) for non-  
152 esterified fatty acids (NEFA), glycerol, high-density lipoprotein cholesterol, low-density  
153 lipoprotein cholesterol, glucose, and apolipoproteins (APO) AI, AII, B, CII, CIII, and E. The  
154 time course of response for all of the aforementioned markers, and their respective peak or  
155 nadir concentrations were also examined.

### 156 **Participants**

157 Exclusion criteria were body mass index (BMI)  $<18$  or  $>35$  kg/m<sup>2</sup>; pregnancy; current breast-  
158 feeding; allergy or intolerance to study materials; blood donation of more than 400 mL within  
159 three months prior to participation; body weight shift  $>3$  kg within six months prior to  
160 participation. Participants were fully informed as to the nature and potential risks of  
161 participation before written informed consent was obtained. The study was approved by the  
162 University of Bath, Research Ethics Approval Committee for Health (Ref: EP17/18 005) and  
163 undertaken in accordance with the Helsinki Declaration of 1975 as revised in 1983.

### 164 **Experimental procedures**

165 Prior to their first testing day, each participant recorded habitual activity and diet for two  
166 days, to facilitate diet and physical activity replication for two days ahead of subsequent  
167 visits. As such, a minimum wash-out period of two days between trials was observed, and  
168 each participant was tested at the same time of day for all visits ( $\pm 1$  hour). Female participants  
169 with regular menstrual cycles were tested within a week of the same day of their menstrual  
170 cycle on all occasions.

171 Participants arrived at the laboratory following a  $>5$  hour fast, having ingested 0.568 L (one  
172 pint) of water before arrival to facilitate consistent hydration between visits, and having

173 refrained from alcohol and caffeine consumption for the >12 hours prior. Once diet and  
174 physical activity replication had been confirmed, a cannula was inserted into a forearm  
175 antecubital vein, and a baseline 10 mL blood sample drawn. Immediately thereafter,  
176 participants consumed a high fat liquid meal challenge, and whichever test drink has been  
177 allocated for that visit. The fat meal challenge drink provided 40 g dietary fat, <2 g  
178 carbohydrate, and 1.2 g protein, comprising of 86 mL of fresh cream (Tesco Fresh Double  
179 Cream), made up to 150 mL with cold water, with 0.5 mL vanilla flavor droplets added (My  
180 Protein Flavdrops, Northwich, Cheshire, UK). Test drinks were provided to participants as a  
181 350 mL bolus at room temperature. Both drinks were consumed within 15 minutes, with  
182 participants asked to consume ~50% of the meal challenge within the first five minutes,  
183 followed by ~50% of the test drink in the next two and a half minutes, with this process  
184 repeated for the remaining drink over the subsequent seven and a half minutes. The meal  
185 challenge vessel was swilled with 50 mL of room temperature water, which the participant  
186 consumed. During the final visit, participants completed an exit questionnaire to verify  
187 successful blinding. Forty-six participants reported that they could identify a difference  
188 between the test drinks consumed across the four visits. Fourteen of these participants  
189 believed that they could identify at least one of the test drinks consumed, with 12 participants  
190 correctly identified the 100mgPP+CC test beverage. No participants successfully identified  
191 other test drinks.

192 Thirty minutes after participants started consuming the test drink, serial 10 mL blood samples  
193 were collected every 30 minutes until two hours, with a final 10 mL blood sample at three  
194 hours. Cannulae were flushed with 5-10 mL 0.9% NaCL after each sample to maintain  
195 patency. Blood samples were drawn into a syringe, and immediately dispensed into untreated  
196 serum tubes with silicate clotting activator, and tripotassium ethylenediaminetetraacetic acid  
197 (K3 EDTA) treated tubes (both Sarstedt, Nümbrecht, Germany) for serum and plasma

198 separation respectively. Before centrifugation, blood samples for serum separation were  
199 allowed to clot at room temperature for 20 minutes. Samples were centrifuged at 1300 g for  
200 15 minutes at 4°C, then supernatant was immediately aliquoted, frozen on dry ice, and stored  
201 at -80°C for later analysis.

202 A randomly selected and blinded sub-group of 15 participants were assigned to also receive a  
203 300 mg dose of [1,1,1-<sup>13</sup>C<sub>3</sub>] labelled tripalmitin in the meal challenge drink to trace  
204 incorporation of dietary lipid into plasma fatty acids. However, due to analytical issues, the  
205 tracer enrichment data could not be obtained, and this sub-group analysis was abandoned.

### 206 **Sample analysis**

207 Serum TAG, and plasma TAG, NEFA, glycerol, glucose, HDL-c, LDL-c, and APOs AI, AII,  
208 B, CII, CIII, and E, were measured with commercially available spectrophotometric assays  
209 (Daytona Rx, Randox, Crumlin, UK) as per the manufacturer's instructions. Commercially  
210 available enzyme linked immunosorbent assay (ELISA) was used to determine concentrations  
211 of plasma insulin (Merckodia, Uppsala, Sweden) and C-peptide (MilliporeSigma,  
212 Massachusetts, USA).

### 213 **Statistical analysis**

214 In line with our primary hypothesis of sustained (3 h) postprandial differences between  
215 conditions, 3-h iAUC was calculated for serum TAG, and plasma TAG, glucose, insulin, and  
216 C-peptide, concentrations using the trapezoid method (13), ignoring values below the baseline  
217 (14). The total AUC was calculated for NEFA, glycerol, HDL-c and LDL-c, and APO AI,  
218 AII, B, CII, CIII, and E, concentrations, since these were suppressed below baseline following  
219 ingestion of the test drink. Condition differences in iAUC, total AUC, and differences  
220 between peak/nadir metabolite concentrations were analyzed with a single factor (condition, 4  
221 levels) repeated measures (within-subjects) linear mixed model (LMM) (15). Various time-

222 point correlation and variance structures were explored with the statistical models (16). See  
223 supplementary file 2 for Akaike Information Criterion (AIC) comparisons between various  
224 structures for primary outcome and condition comparison (AUC of serum TAG).

225 Of a possible 18,000 data points, 56 data points (0.3%) were missing due to insufficient  
226 plasma or serum for analysis. When this was the case and adjacent samples were available, for  
227 analysis the mean of samples on each side of this timepoint were taken (e.g. for a missing 90-  
228 minute sample, the mean of 60-minute and 120-minute samples were used), or for missing  
229 baseline samples, the mean of the three other baseline samples was taken (17). When  
230 concentrations were below the detectable limit of the assay, the lowest detectable value was  
231 assumed.

232 As an exploratory secondary analysis, data were also analyzed with a condition (4 levels) x  
233 time (5 levels) repeated measures (within-subjects) LMM with the baseline time point  
234 included as a time varying covariate (18) to identify any condition x time interactions and,  
235 subsequently, the location during the postprandial period of any statistically significant  
236 differences in time course of responses between conditions using the Least Significant  
237 Difference approach .

238 The residuals from each linear mixed model were explored for parity with a Normal  
239 distribution using a histogram, with appropriate transformation (generally log (base E)  
240 transformation) of data employed if required. Descriptive data including participant  
241 characteristics are reported as mean  $\pm$  SD, and mean differences are reported with 95%  
242 confidence intervals [CI]. Statistical significance was accepted at  $p \leq 0.05$ . Data was analyzed  
243 using SPSS v26 and v.28.0 (SPSS Inc., Chicago, IL).

## 244 **Sample size**

245 Sample size estimation was based on a previous study using a similar design (10), in which  
246 serum TAG iAUC (0-3 hours) mean response to an PP and high fat meal was 7000  
247  $\text{mg}\cdot 180\text{min}\cdot \text{dL}^{-1}$ , compared to 8200  $\text{mg}\cdot 180\text{min}\cdot \text{dL}^{-1}$  with placebo (within participant  
248 standard deviation, calculated as the root mean squared error, of 424  $\text{mg}\cdot \text{min}\cdot \text{dL}^{-1}$ ). This  
249 represented a reduction compared to placebo of approximately 15% and was considered to be  
250 a clinically meaningful effect. Assuming the true effectiveness of the PP to be similar as that  
251 previously reported (10), a sample size of 50 participants completing the study was estimated  
252 to give >90% power to detect a difference of 1200  $\text{mg}\cdot 180\text{min}\cdot \text{dL}^{-1}$  between PP and placebo  
253 with alpha set to 0.05.

254

## 255 **Results**

256 Fifty participants completed the study (mean (SD) age: 26 (7) years; weight: 75.2 (11.9) kg;  
257 BMI: 24.0 (2.7)  $\text{kg}/\text{m}^2$ ; females n=16 (32%)). All data collection was completed from October  
258 2017 to March 2018.

259 The structure that consistently provided the lowest AIC was the compound symmetry  
260 structure (16). Model residuals associated with each measured variable were reasonably  
261 Normally distributed, apart from the residuals for glycerol and HDL-c, both of which were  
262 skewed and subsequently transformed with log to base E before analysis. The histograms for  
263 the model residuals resulting from analysis of the primary variables of iAUC/AUC and time  
264 course data are shown in Supplementary File 2. Fifty-three participants were screened into  
265 the study but three did not complete the study (see **Figure 1** for study CONSORT diagram).  
266 One participant could not schedule study visits, and two others consumed each other's test  
267 drinks during a study visit so were excluded). For insulin, 2.8% of data points were below the

268 lowest detectable value of  $6.0 \text{ pmol}\cdot\text{L}^{-1}$ , and for APO CII, 12.0% of data points were below  
269 the lowest detectable value of  $1.1 \text{ mg/dL}$ .

270           Following ingestion of the fat meal challenge and test drinks, serum TAG  
271 concentrations increased in a sustained manner over the duration of the three-hour  
272 postprandial period, however no differences between conditions were found for serum TAG  
273 3h-iAUC (Placebo:  $29 \pm 24$ , 100 mg PP:  $31 \pm 24$ , 150 mg PP:  $37 \pm 27$ , 100 mg PP+CC  $32 \pm$   
274  $21 \text{ mmol}\cdot\text{L}^{-1}\cdot 180 \text{ min}$ ;  $p = 0.12$ ; **Figure 2A**). Similarly, there were no effects of condition on  
275 any other marker of lipid metabolism 3h-iAUC or AUC, apart from HDL-c for which the  
276 AUC was significantly greater in the 100 mg PP condition compared to all other conditions ( $p$   
277  $= 0.02$ ). In the 100 mg PP condition HDL-c 3h-iAUC was  $9 [-9 \text{ to } 33] \text{ mmol}\cdot\text{L}^{-1}$  higher than  
278 the placebo condition,  $7 [-15 \text{ to } 29] \text{ mmol}\cdot\text{L}^{-1}$  higher than the 150 mg PP condition, and  $8 [-14$   
279  $\text{ to } 30] \text{ mmol}\cdot\text{L}^{-1}$  higher than the 100 mg PP+CC condition. There were no differences in peak  
280 or nadir concentrations of lipid metabolism markers apart from NEFA and HDL-c. For  
281 NEFA, the nadir was for the 100 mg PP+CC was  $0.07 [0.00 \text{ to } 0.14] \text{ mmol}\cdot\text{L}^{-1}$  higher than in  
282 the placebo condition, and  $0.05 [-0.02 \text{ to } 0.12] \text{ mmol}\cdot\text{L}^{-1}$  higher compared to the other  
283 conditions (**Table 1**). For HDL-c the nadir was significantly lower than all other conditions ( $p$   
284  $= 0.02$ ) (**Table 1**), albeit the mean difference was  $0.0 [-0.01 \text{ to } 0.01] \text{ mmol}\cdot\text{L}^{-1}$  compared to all  
285 other conditions when reporting to an appropriate degree of accuracy for the measurement.

286           There were no differences in 3h-iAUC for glucose metabolism markers. For 100 mg  
287 PP+CC, peak glucose, insulin, and C-peptide concentrations were significantly lower than all  
288 other conditions (all  $p < 0.001$ ) (**Table 1**). Specifically, peak glucose concentration was  $0.1 [-$   
289  $0.1 \text{ to } 0.3] \text{ mmol}\cdot\text{L}^{-1}$  lower than the placebo and 150 mg PP conditions, and  $0.2 [0.0 \text{ to } 0.4]$   
290  $\text{ mmol}\cdot\text{L}^{-1}$  lower than the 100 mg PP condition. Peak insulin concentration was  $9 [0 \text{ to } 18]$   
291  $\text{ pmol}\cdot\text{L}^{-1}$  lower than the placebo condition,  $16 [6 \text{ to } 26] \text{ pmol}\cdot\text{L}^{-1}$  lower than the 100 mg PP  
292 condition and  $11 [1 \text{ to } 21] \text{ pmol}\cdot\text{L}^{-1}$  lower than the 150 mg PP condition. Peak C-peptide

293 concentration was 49 [-10 to 108] pmol·L<sup>-1</sup> lower than the placebo condition, 75 [14 to 136]  
294 pmol·L<sup>-1</sup> lower than the 100 mg PP condition and 55 [-8 to 118] pmol·L<sup>-1</sup> lower than the 150  
295 mg PP condition.

296 In terms of exploring differences between condition in terms of time course responses,  
297 no condition x time interaction effects were observed for any of the lipid metabolism markers.  
298 Time course of responses for serum TAG, and plasma NEFA and glycerol are shown in  
299 **Figures 2B, 2C and 2D** respectively, with time course responses to all other lipid metabolism  
300 markers in **Figure 3**.

301 Significant condition x time interactions were observed for glucose ( $p < 0.001$ ),  
302 insulin ( $p < 0.001$ ), and C-peptide ( $p < 0.001$ ) (**Figure 4**). Of note, glucose, insulin, and C-  
303 Peptide at 30 minutes were lower in the 100 mg PP+CC condition ( $5.34 \pm 0.42$  mmol·L<sup>-1</sup>,  $42$   
304  $\pm 18$  pmol·L<sup>-1</sup>, and  $507 \pm 143$  pmol·L<sup>-1</sup> respectively) than in the placebo ( $5.52 \pm 0.46$  mmol·L<sup>-1</sup>  
305  $^1$  ( $p < 0.001$ ),  $55 \pm 28$  pmol·L<sup>-1</sup> ( $p = 0.004$ ), and  $580 \pm 163$  pmol·L<sup>-1</sup> ( $p < 0.001$ ) respectively),  
306 the 100 mg PP ( $5.5 \pm 0.59$  mmol·L<sup>-1</sup> ( $p < 0.001$ ),  $62 \pm 31$  pmol·L<sup>-1</sup> ( $p < 0.001$ ), and  $610 \pm 173$   
307 pmol·L<sup>-1</sup> ( $p < 0.001$ ) respectively), and the 150 mg PP conditions ( $5.49 \pm 0.46$  mmol·L<sup>-1</sup> ( $p <$   
308  $0.001$ ),  $58 \pm 30$  pmol·L<sup>-1</sup> ( $p < 0.001$ ), and  $587 \pm 187$  pmol·L<sup>-1</sup> ( $p < 0.001$  respectively). At 60  
309 minutes, C-peptide remained lower in the 100 mg PP+CC condition than in the 100 mg PP  
310 condition ( $498 \pm 142$  pmol·L<sup>-1</sup> vs  $550 \pm 160$  pmol·L<sup>-1</sup> ( $p = 0.003$ )). In the 100 mg PP+CC  
311 condition, glucose was elevated at 60 minutes compared to the placebo and 100 mg PP  
312 conditions ( $5.11 \pm 0.41$ , vs  $4.97 \pm 0.46$  ( $p = 0.003$ ), and  $4.96 \pm 0.49$  mmol·L<sup>-1</sup> ( $p = 0.009$ )  
313 respectively) and was still elevated at 90 minutes ( $5.15 \pm 0.30$ , vs  $5.04 \pm 0.37$  ( $p = 0.012$ ), and  
314  $4.99 \pm 0.46$  mmol·L<sup>-1</sup> ( $p = 0.004$ ) respectively). At 30 minutes, insulin concentration in the  
315 placebo condition was lower than in the 100 mg PP condition ( $55 \pm 28$ , vs  $62 \pm 31$  pmol·L<sup>-1</sup>  
316 ( $p < 0.001$ )), and at 120 minutes, C-peptide concentration was significantly lower in the 100

317 mg PP condition compared to the 150 mg PP condition ( $454 \pm 142$  vs  $466 \pm 142$  pmol·L<sup>-1</sup>, (*p*  
318 = 0.029)).

319

## 320 **Discussion**

321 These data demonstrate that neither 100 mg nor 150 mg of polymerized polyphenols from  
322 oolong tea, alter postprandial triacylglycerol concentrations following ingestion of 40 g of fat,  
323 irrespective of the presence or absence of caffeine and catechins in healthy adults. Moreover,  
324 lipid metabolism measured in the present study was generally unaltered by the ingestion of  
325 polymerized polyphenols from oolong tea with or without caffeine and catechins (e.g. non-  
326 esterified fatty acid, glycerol, LDL-cholesterol, apolipoproteins AI, AII, B, CII, CIII or E  
327 concentrations), albeit HDL-cholesterol appeared to be have been elevated with ingestion of  
328 100 mg polymerized polyphenols compared to other conditions in the postprandial state.  
329 Caffeine and catechins ingested alongside polymerized polyphenols from oolong tea lowered  
330 postprandial glucose, insulin and C-peptide concentrations 30-minutes post ingestion.

331 Postprandial triacylglycerol concentrations are a marker of cardiovascular disease risk, and  
332 prior evidence indicates that gastrointestinal lipase inhibition may lower postprandial  
333 triacylglycerol concentrations (1, 4). Furthermore, since polymerized polyphenols from  
334 oolong tea have been shown to display lipase inhibitory activity *in vitro* (7), and suggested to  
335 reduce postprandial serum triacylglycerol concentrations in Japanese and Thai adults (10,  
336 11), it was hypothesized that ingesting polymerized polyphenols from oolong tea alongside a  
337 high-fat beverage would lower postprandial triacylglycerol concentrations. The data in the  
338 present study did not replicate those findings and indicate that polymerized polyphenols from  
339 oolong tea ingested in either doses typically found in commercially available oolong tea  
340 drinks (19) (100 mg), or in markedly higher doses (150 mg) do not alter postprandial lipemia



341 in healthy, non-obese males or females. Prior work indicated that 70 mg PP ingested with 40  
342 g fat lowers postprandial serum triacylglycerol concentrations within 3 hours following  
343 ingestion (10, 11). There are no data to suggest that the inhibition of pancreatic lipase by  
344 polymerized polyphenols from oolong tea is specific to people with a particular ethnic  
345 heritage (e.g. Japan or Thai vs UK), however differences in participant characteristics or  
346 methodology between the present study and the aforementioned studies are noteworthy. For  
347 example, participant age ( $26 \pm 7$  years, versus  $50 \pm 9$  and  $36 \pm 11$  years in (10, 11)  
348 respectively) and fasting serum TAG ( $75 \pm 34$  mg/dL versus  $145 \pm 54$  and  $151 \pm 52$  mg/dL in  
349 (10, 11) respectively) were markedly different. In addition, increase of postprandial TAG in  
350 the present study was dramatically lower than observed in the aforementioned studies. This  
351 may reflect habitual dietary fat intake, which was not controlled for in the present study. The  
352 fat meal challenge was also different between studies, in the present study was a dairy based  
353 milkshake whereas the previously mentioned studies administered a corn-based soup. In any  
354 case, it is unclear why polymerized polyphenols from oolong tea (at even higher doses of 100  
355 and 150 mg) did not alter serum or plasma triacylglycerol concentrations, nor meaningfully  
356 alter any other aspect of lipid metabolism within the present investigation.

357 Pharmacological lipase inhibition (Orlistat) has been shown to potently lower postprandial  
358 triacylglycerol concentrations, and also alter the postprandial responses of other components  
359 of lipid metabolism such as LDL-cholesterol concentrations (4, 5). It might be expected that  
360 polymerized polyphenols from oolong tea would display more subtle effects on  
361 triacylglycerol responses than pharmacological inhibitors of lipase activity such as Orlistat.  
362 Therefore, as exploratory outcomes, a variety of other components of lipid metabolism were  
363 determined in an attempt to detect more subtle effects on postprandial lipemia. These included  
364 markers of lipolysis (non-esterified fatty acid and glycerol concentrations), components of  
365 forward cholesterol transport (LDL-cholesterol concentrations, apolipoprotein B,

366 apolipoprotein E) reverse cholesterol transport (HDL-cholesterol, apolipoprotein AI and  
367 apolipoprotein AII concentrations), and key activators (apolipoprotein CII) and inhibitors  
368 (apolipoprotein CIII) of lipoprotein lipase, which catalyzes triacylglycerol hydrolysis in the  
369 periphery; the rate-limiting step for triacylglycerol clearance. No meaningful differences were  
370 detected between conditions with any of these components of lipid metabolism. The nadir of  
371 non-esterified fatty acid concentrations was 0.05 [0.02 to 0.13] to 0.07 [0.00 to 0.14] mmol·L<sup>-1</sup>  
372 <sup>1</sup> higher in the condition where caffeine and catechins were ingested alongside 100 mg PP  
373 compared to the other conditions. Likewise, the total AUC of high-density lipoprotein  
374 cholesterol was 7 [-15 to 29] to 9 [-14 to 32] mmol·L<sup>-1</sup>\*180 min higher, and nadir less than  
375 0.1 mmol·L<sup>-1</sup> higher in the 100 mg PP condition than the other conditions (**Table 1**). Neither  
376 of the statistically significant differences represent physiologically meaningful changes in  
377 lipid metabolism. Therefore, it is unlikely that polymerized polyphenols from oolong tea  
378 affect digestion, absorption, or postprandial lipid metabolism at doses of up to 150 mg.

379 In addition to polyphenols, tea often contains caffeine and catechins. Since caffeine and  
380 catechins may alter lipid metabolism (20, 21), an additional aim of the present study was to  
381 assess the metabolic responses to polymerized polyphenols from oolong tea ingested  
382 alongside caffeine and catechins. Whilst caffeine and catechins added to polymerized  
383 polyphenols from oolong tea did not alter any of the measured components of lipid  
384 metabolism, there was evidence of effects on carbohydrate metabolism. Caffeine and  
385 catechins ingested alongside polymerized polyphenols from oolong tea lowered glucose,  
386 insulin and C-peptide responses. As high-dose (~300 mg) caffeine ingestion has been shown  
387 to increase glucose and insulin responses to carbohydrate ingestion (22), it seems unlikely that  
388 the ~50 mg caffeine provided in the present study was responsible for the reduced glucose,  
389 insulin and C-peptide responses observed. Notwithstanding the use of a relatively small  
390 caffeine dose in the present study, it is more likely that catechins were responsible for this

391 effect. In turn, the lower insulinemia likely explains the slightly higher nadir for non-  
392 esterified fatty acid concentrations during this condition *versus* placebo. Glucose is primarily  
393 absorbed across the intestinal membrane via the sodium-dependent glucose transporter,  
394 SGLT1 (23), and tea catechins have been shown to inhibit SGLT-1 activity *in vitro* (24).  
395 Therefore, it is possible that the lower glucose response is due to catechin-inhibition of  
396 SGLT1 activity and thus intestinal glucose absorption. Consequently, slower intestinal  
397 glucose absorption rates would provide less of a stimulus for insulin secretion, thereby  
398 attenuating the rise in insulin concentrations.

399 Some potential limitations with the present investigation include the three-hour postprandial  
400 period and the lack of measurement of lipase activity as the hypothesized mechanism.  
401 Nevertheless, the three-hour triacylglycerol iAUC seems to be essentially identical to the five-  
402 hour iAUC based on prior work using oral fat tolerance tests (12). Furthermore, abbreviated  
403 oral fat tolerance tests (e.g., four-hour) have been shown to be valid and reliable compared to  
404 six-hour tests, even when consuming a large bolus of fat (80 g) (25, 26). Finally, prior work  
405 examining the effect of polymerized polyphenols from oolong tea on postprandial lipemia  
406 demonstrated differences in triacylglycerol concentrations at the three-hour timepoint.  
407 Therefore, the three-hour postprandial period in the present study is likely to have been a  
408 sufficient representation of postprandial lipid metabolism, especially considering the  
409 hypothesized mechanism relates to lipid absorption and systemic appearance (rather than  
410 peripheral clearance). Of note, 46 participants identified a difference between test drinks, with  
411 12 correctly identifying one of the test drinks. It is likely that this was because the test drink  
412 containing caffeine and catechins was a formula that may have been familiar to participants  
413 who habitually consume oolong tea beverages. Whilst it seems unlikely that this would  
414 impact physiological/metabolic responses (i.e., that are not under conscious control),  
415 improving the flavor matching of drinks is a consideration for future research in this area. It

416 should also be noted that three participants withdrew from the study, two due to a protocol  
417 violation and one due to scheduling commitments. However, a strength of the study was that  
418 only 56 data points were missed out of a possible 18,000 (50 participants \* 4 conditions \* 6  
419 timepoints \* 15 analytes), and as such confidence can be taken in the sample size providing  
420 sufficient statistical power to have detected effects of the interventions. Furthermore, the  
421 counterbalanced nature of the condition order through a 4x4 Williams Latin square design  
422 with 50 participants, and the use of linear mixed model with the baseline (pre-ingestion)  
423 timepoint as a time varying covariate considers the possibility of carry over effects. Future  
424 work should also aim to assess gastrointestinal lipase inhibition in humans *in vivo*, potentially  
425 via gastric and intestinal sampling (27)

426 In summary, neither moderate nor large doses of polymerized polyphenols from oolong tea,  
427 consumed with and without caffeine and additional catechins, altered postprandial  
428 triacylglycerol concentrations in healthy adults. Furthermore, no other aspects of lipid  
429 metabolism measured were affected (non-esterified fatty acid, glycerol, LDL-cholesterol,  
430 HDL-cholesterol, or any of the major apolipoprotein concentrations).

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433

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439

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441 J.T.G. and J.A.B. conducted the research. O.J.P., J.T.G and J.A.B., analyzed data, with G.A.

442 consulting on statistical analysis. O.J.P., J.T.G, and J.A.B. wrote the manuscript. All authors

443 have read and approved the final version.

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## Tables

**Table 1.** Plasma metabolite and hormone responses (iAUC or AUC) to 40 g fat ingested with either placebo, 100 mg or 150 mg polymerized polyphenols from oolong tea, or 100 mg polymerized polyphenols from oolong tea plus caffeine and catechins in healthy adults ( $n = 50$ ). Data presented as means  $\pm$  SD, and differences analyzed with a single factor (condition, 4 levels) repeated measures linear mixed model.

Plasma variable	PLACEBO	100mgPP	150mgPP	100mgPP+CC	LMM Condition effect <i>p</i> value			
	Mean $\pm$ SD	Mean $\pm$ SD	Mean <i>Mean</i> $\pm$ SD <i>difference</i> [95% CI] vs PLACEBO	Mean <i>Mean</i> $\pm$ SD <i>difference</i> [95% CI] vs PLACEBO				
TAG iAUC (mmol·L <sup>-1</sup> *180 min)	28 $\pm$ 21	30 $\pm$ 21	3 [-6 to 11]	34 $\pm$ 24	7 [-2 to 16]	30 $\pm$ 19	2 [-6 to 10]	0.11
TAG peak (mmol·L <sup>-1</sup> )	1.15 $\pm$ 0.53	1.16 $\pm$ 0.48	0.01 [-0.19 to 0.21]	1.23 $\pm$ 0.60	0.09 [-0.14 to 0.31]	1.13 $\pm$ 0.50	-0.02 [-0.22 to 0.18]	0.25
NEFA AUC (mmol·L <sup>-1</sup> *180 min)	100 $\pm$ 43	105 $\pm$ 48	5 [-13 to 23]	102 $\pm$ 39	3 [-14 to 19]	110 $\pm$ 38	11 [-5 to 27]	0.14
NEFA nadir (mmol·L <sup>-1</sup> )	0.34 $\pm$ 0.18	0.37 $\pm$ 0.18	0.02 [-0.05 to 0.10]	0.36 $\pm$ 0.17	0.02 [-0.05 to 0.09]	0.41 $\pm$ 0.20 <sup>a,b,c</sup>	0.07 [-0.01 to 0.14]	<b>0.02</b>
Glycerol AUC (mmol·L <sup>-1</sup> *180 min)	74 $\pm$ 41	77 $\pm$ 40	4 [-12 to 20]	76 $\pm$ 32	2 [-12 to 17]	81 $\pm$ 34	7 [-7 to 22]	0.24
Glycerol nadir (mmol·L <sup>-1</sup> )	0.27 $\pm$ 0.16	0.28 $\pm$ 0.17	0.01 [-0.05 to 0.08]	0.27 $\pm$ 0.13	0.00 [-0.06 to 0.05]	30 $\pm$ 15	0.02 [-0.04 to 0.09]	0.26
Glucose iAUC (mmol·L <sup>-1</sup> *180 min)	9 $\pm$ 18	13 $\pm$ 35	4 [-7 to 15]	9 $\pm$ 15	0 [-6 to 6]	7 $\pm$ 12	-2 [-8 to 3]	0.80
Glucose peak (mmol·L <sup>-1</sup> )	5.6 $\pm$ 0.4	5.7 $\pm$ 0.5	0.1 [-0.1 to 0.2]	5.6 $\pm$ 0.4	0.0 [-0.1 to 0.2]	5.5 $\pm$ 0.4 <sup>a,b,c</sup>	-0.1 [-0.3 to 0.0]	<b>0.003</b>
Insulin iAUC (pmol·dL <sup>-1</sup> *180 min)	144 $\pm$ 111	168 $\pm$ 169	24 [-32 to 80]	174 $\pm$ 158	30 [-25 to 82]	131 $\pm$ 116	-13 [-59 to 30]	0.19
Insulin peak (pmol·L <sup>-1</sup> )	58 $\pm$ 28	64 $\pm$ 30	6 [-5 to 18]	59 $\pm$ 30	1 [-10 to 13]	47 $\pm$ 17 <sup>a,b,c</sup>	-9 [-19 to 0]	<b>&lt;0.001</b>
C-peptide iAUC (mmol·L <sup>-1</sup> *180 min)	15 $\pm$ 8	14 $\pm$ 10	-1 [-4 to 3]	15 $\pm$ 9	0 [-3 to 4]	13 $\pm$ 10	-3 [-6 to 1]	0.14
C-peptide peak (pmol·L <sup>-1</sup> )	596 $\pm$ 165	622 $\pm$ 173	26 [-41 to 32]	602 $\pm$ 183	6 [-63 to 74]	547 $\pm$ 134 <sup>a,b,c</sup>	-49 [-108 to 10]	<b>&lt;0.001</b>
LDL-c AUC (mmol·L <sup>-1</sup> *180 min)	411 $\pm$ 134	417 $\pm$ 139	6 [-47 to 60]	417 $\pm$ 134	6 [-46 to 58]	412 $\pm$ 137	1 [-52 to 54]	0.79
LDL-c nadir (mmol·L <sup>-1</sup> )	2.2 $\pm$ 0.7	2.2 $\pm$ 0.8	0.0 [-0.3 to 0.3]	2.3 $\pm$ 0.7	0.0 [-0.3 to 0.3]	2.2 $\pm$ 0.7	0.0 [-0.3 to 0.3]	0.83
HDL-c AUC (mmol·L <sup>-1</sup> *180 min)	227 $\pm$ 60	237 $\pm$ 59 <sup>a,c,d</sup>	9 [-14 to 32]	230 $\pm$ 54	2 [-20 to 25]	228 $\pm$ 59	1 [-23 to 24]	<b>0.02</b>
HDL-c nadir (mmol·L <sup>-1</sup> )	1.2 $\pm$ 0.3	1.3 $\pm$ 0.3 <sup>a,c,d</sup>	0.1 [-0.1 to 0.2]	1.2 $\pm$ 0.3	0.0 [-0.1 to 0.1]	1.2 $\pm$ 0.3	0.0 [-0.1 to 0.1]	<b>0.02</b>



APO AI AUC (g·L <sup>-1</sup> *180 min)	2.4 ± 0.5	2.5 ± 0.4	0.0 [-0.1 to 0.2]	2.4 ± 0.4	0.1 [-0.1 to 0.2]	2.4 ± 0.5	0.0 [-0.2 to 0.2]	0.46
APO AI nadir (mg·dL <sup>-1</sup> )	130 ± 26	132 ± 23	2 [-8 to 12]	131 ± 23	1 [-8 to 11]	130 ± 28	0 [-11 to 11]	0.71
APO AII AUC (g·dL <sup>-1</sup> *180 min)	5.0 ± 0.8	5.1 ± 0.9	0.1 [-0.2 to 0.5]	5.0 ± 0.8	0.0 [-0.3 to 0.4]	5.0 ± 0.9	0.1 [-0.3 to 0.4]	0.15
APO AII nadir (mg·dL <sup>-1</sup> )	27 ± 5	27 ± 5	0 [-1 to 2]	27 ± 5	0 [-2 to 2]	27 ± 5	0 [-2 to 2]	0.33
APO B AUC (g·L <sup>-1</sup> *180 min)	1.2 ± 0.3	1.2 ± 0.3	0.0 [-0.1 to 0.1]	1.2 ± 0.4	0.0 [-0.1 to 0.1]	1.2 ± 0.4	0.0 [-0.1 to 0.1]	0.63
APO B nadir (mg·dL <sup>-1</sup> )	63 ± 16	64 ± 18	1 [-6 to 8]	64 ± 20	1 [-6 to 8]	63 ± 19	0 [-7 to 7]	0.80
APO CII AUC (mg·dL <sup>-1</sup> *180 min)	478 ± 225	509 ± 244	31 [-62 to 123]	479 ± 209	1 [-84 to 86]	499 ± 229	21 [-68 to 110]	0.37
APO CII nadir (mg·dL <sup>-1</sup> )	2.4 ± 1.3	2.6 ± 1.3	0.1 [-0.4 to 0.6]	2.5 ± 1.1	0.0 [-0.4 to 0.5]	2.6 ± 1.2	0.1 [-0.3 to 0.6]	0.43
APO CIII AUC (g·dL <sup>-1</sup> *180 min)	1.3 ± 0.4	1.3 ± 0.5	0.0 [-0.1 to 0.2]	1.3 ± 0.5	0.0 [-0.1 to 0.2]	1.3 ± 0.5	0.0 [-0.1 to 0.2]	0.84
APO CIII AUC (mg·dL <sup>-1</sup> )	6.7 ± 2.3	6.8 ± 2.6	0.1 [-0.8 to 1.1]	6.9 ± 2.8	0.2 [-0.8 to 1.2]	6.8 ± 2.7	0.1 [-0.8 to 1.1]	0.80
APO E AUC (mg·dL <sup>-1</sup> *180 min)	512 ± 165	512 ± 152	0 [-62 to 62]	511 ± 162	-1 [-65 to 63]	510 ± 164	-2 [-66 to 63]	0.99
APO E nadir (mg·dL <sup>-1</sup> )	2.7 ± 0.8	2.7 ± 0.8	0.0 [-0.3 to 0.3]	2.7 ± 0.9	0.0 [-0.3 to 0.4]	2.7 ± 0.9	0.0 [-0.4 to 0.3]	0.92

<sup>a</sup> $p \leq 0.05$  versus PLACEBO; <sup>b</sup> $p \leq 0.05$  versus 100mgPP; <sup>c</sup> $p \leq 0.05$  versus 150mgPP; <sup>d</sup> $p \leq 0.05$  versus 100mgPP+CC APO, apolipoprotein; AUC, area under the curve; CC, caffeine and catechins; HDL-c, high-density lipoprotein cholesterol; iAUC, incremental area under the curve; LDL-c, low-density lipoprotein cholesterol; LMM, Linear mixed model; NEFA, non-esterified fatty acids; PP, polymerized polyphenols from oolong tea; TAG, triacylglycerol.

## Legends for figures

**Figure 1.** CONSORT diagram.

**Figure 2.** Serum triacylglycerol three-hour incremental area under the curve (**A**), concentrations (**B**), and plasma non-esterified fatty acid, (**C**) and glycerol (**D**) concentrations following ingestion of 40 g fat with either placebo, 100 mg, 150 mg polymerized polyphenols from oolong tea, or 100 mg polymerized polyphenols from oolong tea plus caffeine and catechins in healthy adults ( $n = 50$ ). Data are presented as means  $\pm$  SD. Condition differences for serum TAG 3h-iAUC was compared with a single factor (condition, 4 levels) repeated measures linear mixed model. Time course of responses (B, C, D above) were compared with a condition (4 levels) x time (5 levels) repeated measures linear mixed model. Significance accepted at  $p \leq 0.05$ . CC, caffeine and catechins; NEFA, non-esterified fatty acids; PP, polymerized polyphenols from oolong tea; TAG, triacylglycerol. The condition interaction for serum TAG 3h-iAUC was  $F(3, 147) = 1.970$ ,  $p = 0.12$ ). The conditions x time interactions were: serum TAG;  $F(12, 925.673) = 1.050$ ,  $p = 0.40$ , NEFA;  $F(12, 927.276) = 0.363$ ,  $p = 0.976$ , Glycerol;  $F(12, 926.875) = 0.880$ ,  $p = 0.567$ .

**Figure 3.** Plasma LDL-c (**A**), HDL-c (**B**), APO AI (**C**), AII (**D**), B (**E**), CII (**F**), CIII (**G**) and E (**H**) concentrations following ingestion of 40 g fat with either placebo, 100 mg, 150 mg polymerized polyphenols from oolong tea, or 100 mg polymerized polyphenols from oolong tea plus caffeine and catechins in healthy adults ( $n = 50$ ). Data are presented as means  $\pm$  SD and compared with a condition (4 levels) x time (5 levels) repeated measures linear mixed model, with significance accepted at  $p \leq 0.05$ . APO, apolipoprotein; CC, caffeine and catechins; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein

cholesterol; PP, polymerized polyphenols from oolong tea. The conditions x time interactions were: LDL-c;  $F(12, 924.981) = 0.353, p = 0.979$ , HDL-c;  $F(12, 910.981) = 0.349, p = 0.979$ , APO AI;  $F(12, 926.255) = 0.385, p = 0.969$ , APO AII;  $F(12, 897.232) = 1.102, p = 0.355$ , APO B;  $F(12, 890.554) = 0.301, p = 0.989$ , APO CII;  $F(12, 924.441) = 0.628, p = 0.820$ , APO CIII;  $F(12, 928.687) = 0.317, p = 0.987$ , APO E;  $F(12, 918.277) = 0.503, p = 0.503$ .

**Figure 4.** Plasma glucose (A), insulin (B), and C-peptide (C) concentrations following ingestion of 40 g fat with either placebo, 100 mg, 150 mg polymerized polyphenols from oolong tea, or 100 mg polymerized polyphenols from oolong tea plus caffeine and catechins in healthy adults ( $n = 50$ ). Data are presented as means  $\pm$  SD and compared with a condition (4 levels) x time (5 levels) repeated measures linear mixed model, with significance accepted at  $p \leq 0.05$ . CC, caffeine and catechins; PP, polymerized polyphenols from oolong tea. The conditions x time interactions were: glucose;  $F(12, 928.191) = 2.936, p < 0.001$ , insulin;  $F(12, 925.175) = 5.396, p < 0.001$ , and C-peptide;  $F(12, 924.633) = 4.278, p < 0.001$ . <sup>a</sup> $p \leq 0.05$  100 mg PP+CC versus PLACEBO; <sup>b</sup> $p \leq 0.05$  100 mg PP+CC versus 100 mg PP; <sup>c</sup> $p \leq 0.05$  100 mg PP+CC versus 150 mg PP, <sup>d</sup> $p \leq 0.05$  placebo versus 100 mg PP, <sup>e</sup> $p \leq 0.05$  100 mg PP versus 150 mg PP.