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

**Abbreviations and definitions:**

AIC, Akaike Information Criterion

APO, apolipoprotein

AUC, area under the curve

BMI, body mass index

CAF, caffeine

CAT, catechins

ELISA, enzyme linked immunosorbent assay

HDL-c, high-density lipoprotein cholesterol

iAUC, incremental area under the curve

K3 EDTA, andtripotassium ethylene diamine tetraacetic acid

LDL-c, low-density lipoprotein cholesterol

LMM, linear mixed model

NaCL, sodium chloride

NEFA, non-esterified fatty acids

PP, polymerized polyphenols from oolong tea

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

SGLT1, sodium-dependent glucose transporter

TAG, triacylglycerol

VLDL-c, very low-density lipoprotein cholesterol

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

(NCT03324191)

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

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  $kg/m^2$; 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*

**Introduction**

Excessive postprandial elevations in triacylglycerol concentrations and other aspects of lipid

metabolism play a major role in cardiovascular disease risk. Indeed, the results of studies

using Mendelian randomization approaches support a causal role for plasma triacylglycerol

concentrations in coronary heart disease (1). Moreover, the postprandial state is particularly

important, since this scenario captures the total amount of atherogenic lipoproteins in plasma,

and also reflects the metabolic state in which people in middle-to-high income countries

spend most of their time (2). The most recent National Nutrition and Diet Survey (3)

highlights that the typical UK diet is particularly high in fat, which is the key driver in

postprandial triacylglycerol concentration. Therefore, strategies that reduce postprandial

triacylglycerol concentrations may have potential to reduce the incidence of cardiovascular

disease.

Some of the most effective strategies for reducing postprandial triacylglycerol concentrations

include medications that inhibit gastrointestinal lipase activity, such as Orlistat. The addition

of Orlistat to a high-fat meal can reduce the postprandial triacylglycerol area under the curve

by ~40%, in addition to altering various other components of lipid metabolism, such as low

density lipoprotein (LDL-c) cholesterol concentrations, very low-density lipoprotein (VLDL-

c) subclass concentration, mean VLDL size, and small high density lipoprotein (HDL-c)

particle concentrations (4, 5). However, Orlistat is a relatively aggressive pharmacological

means of inhibiting lipase activity and thus carries side effects such as diarrhea (6), whereas

naturally occurring nutrient compounds may provide more modest lipase inhibition but

without the severity of side-effects seen with pharmacological interventions.

One such example is a class of high-molecular-weight polymerized polyphenols (PP) that are

particularly abundant in oolong tea. These polyphenols have been found to markedly inhibit

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 $kg/m^2$; 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 (±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 [1,1,1-13C3] 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 (Mercodia, 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 mg·180min·dL$^{-1}$, compared to 8200 mg·180min·dL$^{-1}$ with placebo (within participant

248 standard deviation, calculated as the root mean squared error, of 424 mg·min·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 mg·180min·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) kg/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 pmol·L$^{-1}$, and for APO CII, 12.0% of data points were below

269   the lowest detectable value of 1.1 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 ± 24, 100 mg PP: 31 ± 24, 150 mg PP: 37 ± 27, 100 mg PP+CC 32 ±

274   21 mmol·L$^{-1}$*180 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 to 33] mmol·L$^{-1}$ higher than

278   the placebo condition, 7 [-15 to 29] mmol·L$^{-1}$ higher than the 150 mg PP condition, and 8 [-14

279   to 30] mmol·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 to 0.14] mmol·L$^{-1}$ higher than in

282   the placebo condition, and 0.05 [-0.02 to 0.12] mmol·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 to 0.01] mmol·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 to 0.3] mmol·L$^{-1}$ lower than the placebo and 150 mg PP conditions, and 0.2 [0.0 to 0.4]

290   mmol·L$^{-1}$ lower than the 100 mg PP condition. Peak insulin concentration was 9 [0 to 18]

291   pmol·L$^{-1}$ lower than the placebo condition, 16 [6 to 26] pmol·L$^{-1}$ lower than the 100 mg PP

292   condition and 11 [1 to 21] pmol·L$^{-1}$ lower than the 150 mg PP condition. Peak C-peptide

293 concentration was 49 [-10 to 108] pmol·L$^{-1}$ lower than the placebo condition, 75 [14 to 136]

294 pmol·L$^{-1}$ lower than the 100 mg PP condition and 55 [-8 to 118] pmol·L$^{-1}$ 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$^{-1}$, 42

304 $\pm 18$ pmol·L$^{-1}$, and $507 \pm 143$ pmol·L$^{-1}$ respectively) than in the placebo ($5.52 \pm 0.46$ mmol·L$^{-}$

305 $^1$ ($p < 0.001$), $55 \pm 28$ pmol·L$^{-1}$ (p = 0.004), and $580 \pm 163$ pmol·L$^{-1}$ ($p < 0.001$) respectively),

306 the 100 mg PP ($5.5 \pm 0.59$ mmol·L$^{-1}$ ($p < 0.001$), $62 \pm 31$ pmol·L$^{-1}$ (p < 0.001), and $610 \pm 173$

307 pmol·L$^{-1}$ (p < 0.001) respectively), and the 150 mg PP conditions ($5.49 \pm 0.46$ mmol·L$^{-1}$ ($p <$

308 0.001), $58 \pm 30$ pmol·L$^{-1}$ (p < 0.001), and $587 \pm 187$ pmol·L$^{-1}$ (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$^{-1}$ vs $550 \pm 160$ pmol·L$^{-1}$ (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$^{-1}$ ($p = 0.009$)

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$^{-1}$ ($p = 0.004$) 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$^{-1}$

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 ± 142 vs 466 ± 142 pmol·L$^{-1}$, ($p$

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$^{-1}$

372    $^{1}$ 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$^{-1}$*180 min higher, and nadir less than

375    0.1 mmol·L$^{-1}$ 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

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 ± SD, and differences analyzed with a single factor (condition, 4 levels) repeated measures linear mixed model.

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

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| APO AI AUC (g·L⁻¹*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⁻¹) | 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⁻¹*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⁻¹) | 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⁻¹*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⁻¹) | 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⁻¹*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⁻¹) | 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⁻¹*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⁻¹) | 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⁻¹*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⁻¹) | 2.7 ± 0.8 | 2.7 ± 0.8 | *0.0 [-0.3 to 0.3]* | 2.7 ± 0.9 | *0.0 [-0.3 to 04]* | 2.7 ± 0.9 | *0.0 [-0.4 to 0.3]* | 0.92 |

[a]$p \leq 0.05$ *versus* PLACEBO; [b]$p \leq 0.05$ *versus* 100mgPP; [c]$p \leq 0.05$ *versus* 150mgPP; [d]$p \leq 0.05$ *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 ± SD. Condition differences for serum TAG 3h-iAUC was compared with a single factor (condition, 4 levels) repeated measures liner 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 ± 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 ± 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, $p < 0.001$. [a]$p \leq 0.05$ 100 mg PP+CC *versus* PLACEBO; [b]$p \leq 0.05$ 100 mg PP+CC *versus* 100 mg PP; [c]$p \leq 0.05$ 100 mg PP+CC *versus* 150 mg PP, [d]$p \leq 0.05$ placebo *versus* 100 mg PP, [e]$p \leq 0.05$ 100 mg PP *versus* 150 mg PP.