Perkin, O, Chen, Y-C, Johnson, D, Thomas, J, Atkinson, G, Betts, J and Gonzalez, J

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

Title Page

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

Author names: Oliver J. Perkin[1,2], Yung-Chih Chen[1,2,3], Drusus A. Johnson[1], Joel E. Thomas[1], Greg Atkinson[4], James A. Betts[1,2], Javier T. Gonzalez[1,2]

Author affiliations: [1]Department for Heath, University of Bath, Bath, UK

[2]Centre for Nutrition, Exercise and Metabolism, University of Bath, Bath, UK

[3]Department of Physical Education and Sport Sciences, National Taiwan Normal University, Taipei, Taiwan

[4]School of Sport and Exercise Sciences, Liverpool John Moore's University, Liverpool, UK

Disclaimers: J.T.G has received funding from the British Heart Foundation, Medical Research Council, Biotechnology and Biological Sciences Research Council, Arla Foods Ingredients, Kenniscentrum Suiker and Voeding and Lucozade Ribena Suntory. J.A.B has received funding from the BBSRC, GlaxoSmithKline, Lucozade Ribena Suntory, Kellogg's, Nestlé and PepsiCo.

Corresponding author: James A. Betts, Ph.D., F.A.C.S.M., Department for Health, University of Bath, Bath, BA2 7AY, United Kingdom; Telephone: +44 1225 383 448 E-mail: J.Betts@bath.ac.uk

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

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].

Abstract

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

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

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

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

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

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

Participants and Methods

Study design overview

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

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

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

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

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

Participants

Fifty participants completed the study (mean (SD) age: 26 (7) years; weight: 75.2 (11.9) kg; BMI: 24.0 (2.7) kg/m^2; females n=16 (32%)). Exclusion criteria were body mass index (BMI) <18 or >35 kg/m^2; pregnancy; current breast-feeding; allergy or intolerance to study materials; blood donation of more than 400 mL within three months prior to participation; body weight shift >3 kg within six months prior to participation. Participants were fully informed as to the nature and potential risks of participation before written informed consent was obtained. The study was approved by the University of Bath, Research Ethics Approval Committee for Health (Ref: EP17/18 005) and undertaken in accordance with the Helsinki Declaration of 1975 as revised in 1983. All data collection was completed from October 2017 to March 2018.

Experimental procedures

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

Participants arrived at the laboratory following a >5 hour fast, having ingested 0.568 L (one pint) of water before arrival to facilitate consistent hydration between visits, and having refrained from alcohol and caffeine consumption for the >12 hours prior. Once diet and physical activity replication had been confirmed, a cannula was inserted into a forearm antecubital vein, and a baseline 10 mL blood sample drawn. Immediately thereafter, participants consumed a high fat liquid meal challenge, and whichever test drink has been allocated for that visit. The fat meal challenge drink provided 40 g dietary fat, <2 g carbohydrate, and 1.2 g protein, comprising of 86 mL of fresh cream (Tesco Fresh Double Cream), made up to 150 mL with cold water, with 0.5 mL vanilla flavor droplets added (My Protein Flavdrops, Northwich, Cheshire, UK). Test drinks were provided to participants as a 350 mL bolus at room temperature. Both drinks were consumed within 15 minutes, with participants asked to consume ~50% of the meal challenge within the first five minutes, followed by ~50% of the test drink in the next two and a half minutes, with this process repeated for the remaining drink over the subsequent seven and a half minutes. The meal challenge vessel was swilled with 50 mL of room temperature water, which the participant consumed. During the final visit, participants completed an exit questionnaire to verify successful blinding. Forty-six participants reported that they could identify a difference between the test drinks consumed across the four visits. Fourteen of these participants believed that they could identify at least one of the test drinks consumed, with 12 participants correctly identified the 100mgPP+CC test beverage. No participants successfully identified other test drinks.

Thirty minutes after participants started consuming the test drink, serial 10 mL blood samples were collected every 30 minutes until two hours, with a final 10 mL blood sample at three hours. Cannulae were flushed with 5-10 mL 0.9% NaCL after each sample to maintain patency. Blood samples were drawn into a syringe, and immediately dispensed into untreated

serum tubes with silicate clotting activator, and tripotassium ethylenediaminetetraacetic acid (K3 EDTA) treated tubes (both Sarstedt, Nümbrecht, Germany) for serum and plasma separation respectively. Before centrifugation, blood samples for serum separation were allowed to clot at room temperature for 20 minutes. Samples were centrifuged at 1300 g for 15 minutes at 4°C, then supernatant was immediately aliquoted, frozen on dry ice, and stored at -80°C for later analysis.

A randomly selected and blinded sub-group of 15 participants were assigned to also receive a 300 mg dose of [1,1,1-13C3] labelled tripalmitin in the meal challenge drink to trace incorporation of dietary lipid into plasma fatty acids. However, due to analytical issues, the tracer enrichment data could not be obtained, and this sub-group analysis was abandoned.

Sample analysis

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

Statistical analysis

In line with our primary hypothesis of sustained (3 h) postprandial differences between conditions, 3-h iAUC was calculated for serum TAG, and plasma TAG, glucose, insulin, and C-peptide, concentrations using the trapezoid method (13), ignoring values below the baseline (14). The total AUC was calculated for NEFA, glycerol, HDL-c and LDL-c, and APO AI, AII, B, CII, CIII, and E, concentrations, since these were suppressed below baseline following ingestion of the test drink. Condition differences in iAUC, total AUC, and differences

between peak/nadir metabolite concentrations were analyzed with a single factor (condition, 4 levels) repeated measures (within-subjects) linear mixed model (LMM) (15). Various time-point correlation and variance structures were explored with our statistical models, and the structure that consistency provided the lowest Akaike Information Criterion (AIC) was the compound symmetry structure (16).

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

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

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

Sample size

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

Results

Model residuals associated with each measured variable were reasonably Normally distributed, apart from the residuals for glycerol and HDL-c, both of which were skewed and subsequently transformed with log to base E before analysis. The histograms for the model residuals resulting from analysis of the primary variables of iAUC/AUC and time course data are shown in Supplementary File 2. Fifty-three participants were screened into the study but three did not complete the study (see **Figure 1** for study CONSORT diagram). One participant could not schedule study visits, and two others consumed each other's test drinks during a study visit so were excluded). For insulin, 2.8% of data points were below the lowest detectable value of 6.0 $pmol \cdot L^{-1}$, and for APO CII, 12.0% of data points were below the lowest detectable value of 1.1 mg/dL.

Following ingestion of the fat meal challenge and test drinks, serum TAG concentrations increased in a sustained manner over the duration of the three-hour postprandial period, however no differences between conditions were found for serum TAG

3h-iAUC (Placebo: 29 ± 24, 100 mg PP: 31 ± 24, 150 mg PP: 37 ± 27, 100 mg PP+CC 32 ± 21 mmol·L^{-1}*180 min; p = 0.12; **Figure 2A**). Similarly, there were no effects of condition on any other marker of lipid metabolism 3h-iAUC or AUC, apart from HDL-c for which the AUC was significantly greater in the 100 mg PP condition compared to all other conditions (*p* = 0.02). In the 100 mg PP condition HDL-c 3h-iAUC was 9 [23] mmol·L^{-1} higher than the placebo condition, 7 [22] mmol·L^{-1} higher than the 150 mg PP condition, and 8 [22] mmol·L^{-1} higher than the 100 mg PP+CC condition. There were no differences in peak or nadir concentrations of lipid metabolism markers apart from NEFA and HDL-c. For NEFA, the nadir was for the 100 mg PP+CC was 0.07 [0.07] mmol·L^{-1} higher than in the placebo condition, and 0.05 [0.07] mmol·L^{-1} higher compared to the other conditions (**Table 1**). For HDL-c the nadir was significantly lower than all other conditions (*p* = 0.02) (**Table 1**), albeit the mean difference was 0.0 [0.01] mmol·L^{-1} compared to all other conditions when reporting to an appropriate degree of accuracy for the measurement.

There were no differences in 3h-iAUC for glucose metabolism markers. For 100 mg PP+CC, peak glucose, insulin, and C-peptide concentrations were significantly lower than all other conditions (all *p* <0.001) (**Table 1**). Specifically, peak glucose concentration was 0.1 [0.2] mmol·L^{-1} lower than the placebo and 150 mg PP conditions, and 0.2 [0.2] mmol·L^{-1} lower than the 100 mg PP condition. Peak insulin concentration was 9 [9] pmol·L^{-1} lower than the placebo condition, 16 [10] pmol·L^{-1} lower than the 100 mg PP condition and 11 [10] pmol·L^{-1} lower than the 150 mg PP condition. Peak C-peptide concentration was 49 [59] pmol·L^{-1} lower than the placebo condition, 75 [61] pmol·L^{-1} lower than the 100 mg PP condition and 55 [63] pmol·L^{-1} lower150 mg PP condition.

In terms of exploring differences between condition in terms of time course responses, no condition x time interaction effects were observed for any of the lipid metabolism markers. Time course of responses for serum TAG, and plasma NEFA and glycerol are shown in

Figures 2B, 2C and **2D** respectively, with time course responses to all other lipid metabolism markers in **Figure 3**.

Significant condition x time interactions were observed for glucose ($p < 0.001$), insulin ($p < 0.001$), and C-peptide ($p < 0.001$) (**Figure 4**). Of note, glucose, insulin, and C-Peptide at 30 minutes were lower in the 100 mg PP+CC condition (5.34 ± 0.42 mmol·L^{-1}, 42 ± 18 pmol·L^{-1}, and 507 ± 143 pmol·L^{-1} respectively) than in the placebo (5.52 ± 0.46 mmol·L^{-1} ($p < 0.001$), 55 ± 28 pmol·L^{-1} (p = 0.004), and 580 ± 163 pmol·L^{-1} (p < 0.001) respectively), the 100 mg PP (5.5 ± 0.59 mmol·L^{-1} ($p < 0.001$), 62 ± 31 pmol·L^{-1} (p < 0.001), and 610 ± 173 pmol·L^{-1} (p < 0.001) respectively), and the 150 mg PP conditions (5.49 ± 0.46 mmol·L^{-1} ($p < 0.001$), 58 ± 30 pmol·L^{-1} (p < 0.001), and 587 ± 187 pmol·L^{-1} (p < 0.001 respectively). At 60 minutes, C-peptide remained lower in the 100 mg PP+CC condition than in the 100 mg PP condition (498 ± 142 pmol·L^{-1} vs 550 ± 160 pmol·L^{-1} (p = 0.003)). In the 100 mg PP+CC condition, glucose was elevated at 60 minutes compared to the placebo and 100 mg PP conditions (5.11 ± 0.41, vs 4.97 ± 0.46 ($p = 0.003$), and 4.96 ± 0.49 mmol·L^{-1} ($p = 0.009$) respectively) and was still elevated at 90 minutes (5.15 ± 0.30, vs 5.04 ± 0.37 ($p = 0.012$), and 4.99 ± 0.46 mmol·L^{-1} ($p = 0.004$) respectively). At 30 minutes, insulin concentration in the placebo condition was lower than in the 100 mg PP condition (55 ± 28, vs 62 ± 31 pmol·L^{-1} ($p < 0.001$)), and at 120 minutes, C-peptide concentration was significantly lower in the 100 mg PP condition compared to the 150 mg PP condition (454 ± 142 vs 466 ± 142 pmol·L^{-1}, ($p = 0.029$)).

Discussion

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

Postprandial triacylglycerol concentrations are a marker of cardiovascular disease risk, and prior evidence indicates that gastrointestinal lipase inhibition may lower postprandial triacylglycerol concentrations (1, 4). Furthermore, since polymerized polyphenols from oolong tea have been shown to display lipase inhibitory activity *in vitro* (7), and suggested to reduce postprandial serum triacylglycerol concentrations in Japanese and Thai adults (10, 11), it was hypothesized that ingesting polymerized polyphenols from oolong tea alongside a high-fat beverage would lower postprandial triacylglycerol concentrations. The data in the present study did not replicate those findings and indicate that polymerized polyphenols from oolong tea ingested in either doses typically found in commercially available oolong tea drinks (21) (100 mg), or in markedly higher doses (150 mg) do not alter postprandial lipemia in healthy, non-obese males or females. Prior work indicated that 70 mg PP ingested with 40 g fat lowers postprandial serum triacylglycerol concentrations within 3 hours following ingestion (10, 11). There are no data to suggest that the inhibition of pancreatic lipase by polymerized polyphenols from oolong tea is specific to people with a particular ethnic

heritage (e.g. Japan or Thai vs UK), however differences in participant characteristics or methodology between the present study and the aforementioned studies are noteworthy. For example, participant age (26 ± 7 years, versus 50 ± 9 and 36 ±11 years in (10, 11) respectively) and fasting serum TAG (75 ± 34 mg/dL versus 145 ± 54 and 151 ± 52 mg/dL in (10, 11) respectively) were markedly different. In addition, increase of postprandial TAG in the present study was dramatically lower than observed in the aforementioned studies. This may reflect habitual dietary fat intake, which was not controlled for in the present study. The fat meal challenge was also different between studies, in the present study was a dairy based milkshake whereas the previously mentioned studies administered a corn-based soup. In any case, it is unclear why polymerized polyphenols from oolong tea (at even higher doses of 100 and 150 mg) did not alter serum or plasma triacylglycerol concentrations, nor meaningfully alter any other aspect of lipid metabolism within the present investigation.

Pharmacological lipase inhibition (Orlistat) has been shown to potently lower postprandial triacylglycerol concentrations, and also alter the postprandial responses of other components of lipid metabolism such as LDL-cholesterol concentrations (4, 5). It might be expected that polymerized polyphenols from oolong tea would display more subtle effects on triacylglycerol responses than pharmacological inhibitors of lipase activity such as Orlistat. Therefore, as exploratory outcomes, a variety of other components of lipid metabolism were determined in an attempt to detect more subtle effects on postprandial lipemia. These included markers of lipolysis (non-esterified fatty acid and glycerol concentrations), components of forward cholesterol transport (LDL-cholesterol concentrations, apolipoprotein B, apolipoprotein E) reverse cholesterol transport (HDL-cholesterol, apolipoprotein AI and apolipoprotein AII concentrations), and key activators (apolipoprotein CII) and inhibitors (apolipoprotein CIII) of lipoprotein lipase, which catalyzes triacylglycerol hydrolysis in the periphery; the rate-limiting step for triacylglycerol clearance. No meaningful differences were

detected between conditions with any of these components of lipid metabolism. The nadir of non-esterified fatty acid concentrations was 0.05 [0.07] to 0.07 [0.07] mmol·L^{-1} higher in the condition where caffeine and catechins were ingested alongside 100 mg PP compared to the other conditions. Likewise, the total AUC of high-density lipoprotein cholesterol was 7 [22] to 9 [23] mmol·L^{-1}*180 min higher, and nadir less than 0.1 mmol·L^{-1} higher in the 100 mg PP condition than the other conditions (**Table 1).** Neither of the statistically significant differences represent physiologically meaningful changes in lipid metabolism. Therefore, it is unlikely that polymerized polyphenols from oolong tea affect digestion, absorption, or postprandial lipid metabolism at doses of up to 150 mg.

In addition to polyphenols, tea often contains caffeine and catechins. Since caffeine and catechins may alter lipid metabolism (22, 23), an additional aim of the present study was to assess the metabolic responses to polymerized polyphenols from oolong tea ingested alongside caffeine and catechins. Whilst caffeine and catechins added to polymerized polyphenols from oolong tea did not alter any of the measured components of lipid metabolism, there was evidence of effects on carbohydrate metabolism. Caffeine and catechins ingested alongside polymerized polyphenols from oolong tea lowered glucose, insulin and C-peptide responses. As high-dose (~300 mg) caffeine ingestion has been shown to increase glucose and insulin responses to carbohydrate ingestion (24), it seems unlikely that the ~50 mg caffeine provided in the present study was responsible for the reduced glucose, insulin and C-peptide responses observed. Notwithstanding the use of a relatively small caffeine dose in the present study, it is more likely that catechins were responsible for this effect. In turn, the lower insulinemia likely explains the slightly higher nadir for non-esterified fatty acid concentrations during this condition *versus* placebo. Glucose is primarily absorbed across the intestinal membrane via the sodium-dependent glucose transporter, SGLT1 (25), and tea catechins have been shown to inhibit SGLT-1 activity *in vitro* (26).

Therefore, it is possible that the lower glucose response is due to catechin-inhibition of SGLT1 activity and thus intestinal glucose absorption. Consequently, slower intestinal glucose absorption rates would provide less of a stimulus for insulin secretion, thereby attenuating the rise in insulin concentrations.

Some potential limitations with the present investigation include the three-hour postprandial period and the lack of measurement of lipase activity as the hypothesized mechanism. Nevertheless, the three-hour triacylglycerol iAUC seems to be essentially identical to the five-hour iAUC based on prior work using oral fat tolerance tests (12). Furthermore, abbreviated oral fat tolerance tests (e.g., four-hour) have been shown to be valid and reliable compared to six-hour tests, even when consuming a large bolus of fat (80 g) (27, 28). Finally, prior work examining the effect of polymerized polyphenols from oolong tea on postprandial lipemia demonstrated differences in triacylglycerol concentrations at the three-hour timepoint. Therefore, the three-hour postprandial period in the present study is likely to have been a sufficient representation of postprandial lipid metabolism, especially considering the hypothesized mechanism relates to lipid absorption and systemic appearance (rather than peripheral clearance). Of note, 46 participants identified a difference between test drinks, with 12 correctly identifying one of the test drinks. It is likely that this was because the test drink containing caffeine and catechins was a formula that may have been familiar to participants who habitually consume oolong tea beverages. Whilst it seems unlikely that this would impact physiological/metabolic responses (i.e., that are not under conscious control), improving the flavor matching of drinks is a consideration for future research in this area. It should also be noted that three participants withdrew from the study, two due to a protocol violation and one due to scheduling commitments. However, a strength of the study was that only 56 data points were missed out of a possible 18,000 (50 participants * 4 conditions * 6 timepoints * 15 analytes), and as such confidence can be taken in the sample size providing

sufficient statistical power to have detected effects of the interventions. Furthermore, the counterbalanced nature of the condition order through a 4x4 Williams Latin square design with 50 participants, and the use of linear mixed model with the baseline (pre-ingestion) timepoint as a time varying covariate considers the possibility of carry over effects. Future work should also aim to assess gastrointestinal lipase inhibition in humans *in vivo*, potentially via gastric and intestinal sampling (29)

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

Acknowledgements

We thank the participants for their time and willingness to participate in this study.

Conflicts of interest: J.T.G. has received funding from the British Heart Foundation, Medical Research Council, Biotechnology and Biological Sciences Research Council, Arla Foods Ingredients, Kenniscentrum Suiker and Voeding and Lucozade Ribena Suntory. J.A.B. has received funding from the BBSRC, GlaxoSmithKline, Lucozade Ribena Suntory, Kellogg's, Nestlé and PepsiCo. O.J.P., Y.C.C., D.A.J., and J.E.T. declare no COI's.

Author contributions: J.T.G and J.A.B designed the research. O.J.P., Y.C.C., D.A.J., J.E.T., 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. consulting on statistical analysis. O.J.P., J.T.G, and J.A.B. wrote the manuscript. All authors have read and approved the final version.

References

1. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, Dale CE, Padmanabhan S, Finan C, Swerdlow DI, et al. Mendelian randomization of blood lipids for coronary heart disease. Eur Heart J 2015;36(9):539-50. doi: 10.1093/eurheartj/eht571.
2. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama 2007;298(3):299-308. doi: 10.1001/jama.298.3.299.
3. Bates B, Collins D, Jones K, Page P, Roberts C, Steer T, Swan G. National Diet and Nutrition Survey Rolling Programme Years 9 to 11 (2016/2017 to 2018/2019), 2020.
4. Suter PM, Marmier G, Veya-Linder C, Hänseler E, Lentz J, Vetter W, Otvos J. Effect of orlistat on postprandial lipemia, NMR lipoprotein subclass profiles and particle size. Atherosclerosis 2005;180(1):127-35. doi: 10.1016/j.atherosclerosis.2004.11.023.
5. Gabriel FS, Samson CE, Abejuela ZR, Sicat-Gabriel PR, Sumpio JP, Zacarias MB, Mercado-Asis LB. Postprandial effect of orlistat on the peaking of lipid level after sequential high fat meals. Int J Endocrinol Metab 2012;10(2):458-63. doi: 10.5812/ijem.2920.
6. Ballinger A. Orlistat in the treatment of obesity. Expert Opinion on Pharmacotherapy 2000;1(4):841-7. doi: 10.1517/14656566.1.4.841.
7. Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, Mitsunaga T, Hashimoto F, Kiso Y. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. J Agric Food Chem 2005;53(11):4593-8. doi: 10.1021/jf047814+.
8. Yi DQ, Tan XR, Zhao ZG, Cai YM, Li YM, Lin XY, Lu SL, Chen YS, Zhang QY. Reduced risk of dyslipidaemia with oolong tea consumption: a population-based study in southern China. British Journal of Nutrition 2014;111(8):1421-9. doi: 10.1017/s0007114513003644.
9. Hsu TF, Kusumoto A, Abe K, Hosoda K, Kiso Y, Wang MF, Yamamoto S. Polyphenol-enriched oolong tea increases fecal lipid excretion. European journal of clinical nutrition 2006;60(11):1330-6. doi: 10.1038/sj.ejcn.1602464.
10. Hara Y, Moriguchi S, Kusumoto A, Nakai M, Ono Y, Abe K, Ohta H, Shibata H, Kiso Y, Egawa K. Suppressive effect of Oolong tea polymerized polyphenols-enriched oolong tea on Postprandial serum triglyceride elevation. Japanese Pharmacology and Therapeutics 2004;32:335-42.
11. Bumrungpert A, Chongsuwat R. The Effect of Oolong Tea Consumption on Postprandial Triglyceride Levels: A Randomized, Double-blind, Placebo-controlled Crossover Study. Journal of Public Health 2015;45(1):6-17.
12. Watkins J, Simpson A, Betts JA, Thompson D, Holliday A, Deighton K, Gonzalez JT. Galactose Ingested with a High-Fat Beverage Increases Postprandial Lipemia Compared with Glucose but Not Fructose Ingestion in Healthy Men. J Nutr 2020;150(7):1765-72. doi: 10.1093/jn/nxaa105.
13. Narang BJ, Atkinson G, Gonzalez JT, Betts JA. A Tool to Explore Discrete-Time Data: The Time Series Response Analyser. Int J Sport Nutr Exerc Metab 2020;30(5):374-81. doi: 10.1123/ijsnem.2020-0150.
14. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, Wolever TM. Glycaemic index methodology. Nutrition research reviews 2005;18(1):145-71. doi: 10.1079/nrr2005100.

15. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Stat Med 1997;16(20):2349-80. doi: 10.1002/(sici)1097-0258(19971030)16:20<2349::aid-sim667>3.0.co;2-e.
16. Ferron J, Dailey R, Yi Q. Effects of Misspecifying the First-Level Error Structure in Two-Level Models of Change. Multivariate Behav Res 2002;37(3):379-403. doi: 10.1207/s15327906mbr3703_4.
17. Dziura JD, Post LA, Zhao Q, Fu Z, Peduzzi P. Strategies for dealing with missing data in clinical trials: from design to analysis. Yale J Biol Med 2013;86(3):343-58.
18. Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. BMJ 2001;323(7321):1123-4. doi: 10.1136/bmj.323.7321.1123.
19. Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998;316(7139):1236-8. doi: 10.1136/bmj.316.7139.1236.
20. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310(6973):170. doi: 10.1136/bmj.310.6973.170.
21. Sajilata MG, Bajaj PR, Singhal RS. Tea Polyphenols as Nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 2008;7(3):229-54. doi: doi.org/10.1111/j.1541-4337.2008.00043.x.
22. Hursel R, Viechtbauer W, Dulloo AG, Tremblay A, Tappy L, Rumpler W, Westerterp-Plantenga MS. The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 2011;12(7):e573-81. doi: 10.1111/j.1467-789X.2011.00862.x.
23. Gonzalez JT, Stevenson EJ. New perspectives on nutritional interventions to augment lipid utilisation during exercise. Br J Nutr 2012;107(3):339-49. doi: 10.1017/S0007114511006684.
24. Smith HA, Hengist A, Thomas J, Walhin JP, Heath P, Perkin O, Chen YC, Gonzalez JT, Betts JA. Glucose control upon waking is unaffected by hourly sleep fragmentation during the night, but is impaired by morning caffeinated coffee. Br J Nutr 2020;124(10):1114-20. doi: 10.1017/S0007114520001865.
25. Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJ. Glucose Plus Fructose Ingestion for Post-Exercise Recovery-Greater than the Sum of Its Parts? Nutrients 2017;9(4). doi: 10.3390/nu9040344.
26. Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y. Regulation of intestinal glucose transport by tea catechins. Biofactors 2000;13(1-4):61-5. doi: 10.1002/biof.5520130111.
27. Maraki M, Aggelopoulou N, Christodoulou N, Katsarou C, Anapliotis P, Kavouras SA, Panagiotakos D, Sidossis LS. Validity of abbreviated oral fat tolerance tests for assessing postprandial lipemia. Clin Nutr 2011;30(6):852-7. doi: 10.1016/j.clnu.2011.05.003.
28. Weiss EP, Fields DA, Mittendorfer B, Haverkort MA, Klein S. Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test. Metabolism 2008;57(10):1479-85. doi: 10.1016/j.metabol.2008.05.020.
29. Sternby B, Hartmann D, Borgström B, Nilsson A. Degree of in vivo inhibition of human gastric and pancreatic lipases by Orlistat (Tetrahydrolipstatin, THL) in the stomach and small intestine. Clin Nutr 2002;21(5):395-402. doi: 10.1054/clnu.2002.0565.

Tables

Table 1. Plasma metabolite and hormone responses 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 liner mixed model.

Plasma variable	PLACEBO	100mgPP	150mgPP	100mgPP+CC	LLM p value
TAG iAUC (mmol·L^{-1}*180 min)	28 ± 21	30 ± 21	34 ± 24	30 ± 19	0.11
TAG peak (mmol·L)	1.15 ± 0.53	1.16 ± 0.48	1.23 ± 0.60	1.13 ± 0.50	0.25
NEFA AUC (mmol·L^{-1}*180 min)	100 ± 43	105 ± 48	102 ± 39	110 ± 38	0.14
NEFA nadir (mmol·L^{-1})	0.34 ± 0.18	0.37 ± 0.18	0.36 ± 0.17	0.41 ± 0.20^{a,b,c}	**0.02**
Glycerol AUC (mmol·L^{-1}*180 min)	74 ± 41	77 ± 40	76 ± 32	81 ± 34	0.24
Glycerol nadir (mmol·L^{-1})	0.27 ± 16	0.28 ± 17	0.27 ± 13	30 ± 15	0.26
Glucose iAUC (mmol·L^{-1}*180 min)	9 ± 18	13 ± 35	9 ± 15	7 ± 12	0.80
Glucose peak (mmol·L^{-1})	5.6 ± 0.4	5.7 ± 0.5	5.6 ± 0.4	5.5 ± 0.4^{a,b,c}	**0.003**
Insulin iAUC (pmol·dL^{-1}*180 min)	144 ± 111	168 ± 169	174 ± 158	131 ± 116	0.19
Insulin peak (pmol·L^{-1})	58 ± 28	64 ± 30	59 ± 30	47 ± 17^{a,b,c,}	**<0.001**
C-peptide iAUC (mmol·L^{-1}*180 min)	15 ± 8	14 ± 10	15 ± 9	13 ± 10	0.14
C-peptide peak (pmol·L^{-1})	596 ± 165	622 ± 173	602 ± 183	547 ± 134^{a,b,c}	**<0.001**
LDL-c AUC (mmol·L^{-1}*180 min)	411 ± 134	417 ± 139	417 ± 134	412 ± 137	0.79
LDL-c nadir (mmol·L^{-1})	2.2 ± 0.7	2.2 ± 0.8	2.3 ± 0.7	2.2 ± 0.7	0.83
HDL-c AUC (mmol·L^{-1}*180 min)	227 ± 60	237 ± 59^{a,c,d}	230 ± 54	228 ± 59	**0.02**
HDL-c nadir (mmol·L^{-1})	1.2 ± 0.3	1.3 ± 0.3^{a,c,d}	1.2 ± 0.3	1.2 ± 0.3	**0.02**
APO AI AUC (g·L^{-1}*180 min)	2.4 ± 0.5	2.5 ± 0.4	2.4 ± 0.4	2.4 ± 0.5	0.46
APO AI nadir (mg·dL^{-1})	130 ± 26	132 ± 23	131 ± 23	130 ± 28	0.71
APO AII AUC (g·dL^{-1}*180 min)	5.0 ± 0.8	5.1 ± 0.9	5.0 ± 0.8	5.0 ± 0.9	0.15
APO AII nadir (mg·dL^{-1})	27 ± 5	27 ± 5	27 ± 5	27 ± 5	0.33
APO B AUC (g·L^{-1}*180 min)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.4	1.2 ± 0.4	0.63
APO B nadir (mg·dL^{-1})	63 ± 16	64 ± 18	64 ± 20	63 ± 19	0.80
APO CII AUC (mg·dL^{-1}*180 min)	478 ± 225	509 ± 244	479 ± 209	499 ± 229	0.37
APO CII nadir (mg·dL^{-1})	2.4 ± 1.3	2.6 ± 1.3	2.5 ± 1.1	2.6 ± 1.2	0.43
APO CIII AUC (g·dL^{-1}*180 min)	1.3 ± 0.4	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	0.84
APO CIII AUC (mg·dL^{-1})	6.7 ± 2.3	6.8 ± 2.6	6.9 ± 2.8	6.8 ± 2.7	0.80
APO E AUC (mg·dL^{-1}*180 min)	512 ± 165	512 ± 152	511 ± 162	510 ± 164	0.99
APO E nadir (mg·dL^{-1})	2.7 ± 0.8	2.7 ± 0.8	2.7 ± 0.9	2.7 ± 0.9	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 TAG, triacylglycerol; NEFA, non-esterified fatty acids; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; APO, apolipoprotein; iAUC, incremental area under the curve; AUC, area under the curve; PP, polymerized polyphenols from oolong tea; CC, caffeine and catechins; LLM, Linear mixed model.

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.