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The effects of acute interval exercise and strawberry intake on postprandial lipaemia

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Abstract

Purpose: Raised postprandial triglycerides (TAG) and related oxidative stresses are strongly associated with increased cardiovascular disease (CVD) risk. Acute exercise and strawberry ingestion independently ameliorate postprandial lipid excursions and oxidative stress. However, the combined effects of these lifestyle interventions is unknown. We investigated whether acute exercise and strawberry consumption improved postprandial responses to an oral fat tolerance test (OFTT) in overweight/obese males.

Methods: Overweight/obese adult males underwent four separate OFTT (73 g fat, 33 g carbohydrate) with blood sampled at baseline and hourly for 4 h after OFTT. Two OFTT contained 25 g freeze-dried strawberries and two contained strawberry flavouring (placebo). Participants performed 40 min of submaximal high intensity interval cycling exercise (HIIE)

16 h before one strawberry and one placebo OFTT, and rested before the remaining two OFTT. Serum TAG was analysed and TAG area under curve (AUC) and incremental AUC (iAUC) were calculated. Oxidative stress markers were measured at baseline and 4 h. Differences between conditions (strawberry/placebo and exercise/rest) were assessed using repeated measures ANOVA.

Results: Ten males (Age, 31.5 IQR 17.8 years; BMI, $29.9 \pm 1.8 \text{ kg}\cdot\text{m}^{-2}$) completed the study. TAG AUC was $1.5 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ lower for the exercise conditions compared to the rest conditions (95 % confidence interval [CI]= -2.3 to -0.8 $\text{mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p= 0.001$). TAG AUC was not different between the strawberry and placebo conditions (CI= -1.3 to 0.6 $\text{mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p= 0.475$). TAG iAUC was $0.5 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ greater for the strawberry compared to the placebo conditions (CI= 0.1 to 1.0 $\text{mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p= 0.021$). There were no changes in markers of lipid related oxidative stress ($P> 0.05$).

Conclusion: Acute submaximal HIIE appears effective in reducing postprandial lipaemia in overweight/obese adult males. However, strawberry ingestion did not improve postprandial TAG.

Key words: OFTT, polyphenols, HIIE, Triglycerides, lipids

Introduction

Impaired lipid handling after oral fat ingestion results in increased circulating lipids and associated metabolic stress for prolonged time periods. This postprandial characteristic is often reported in physical inactivity, obesity and type 2 diabetes and is strongly associated with atherosclerosis (32). Acute endothelial dysfunction, increased inflammation and oxidative stress occur during postprandial lipaemia and may contribute to an atherogenic environment

(9, 37). Furthermore, elevated circulating postprandial lipids likely increase the propensity for oxidation of lipids, such as LDL, which are key protagonists of atherosclerosis (17). Attenuation of the postprandial triglyceride (TAG) response, total and oxidised LDL (oxLDL), is therefore likely to be beneficial for optimising long-term cardiovascular and metabolic health, particularly in overweight or obese individuals.

Exercise performed acutely before a high fat meal (typically 4-24 h prior to meal ingestion) reduces postprandial TAG (for a recent review see; (13)). Many studies have investigated the effects of continuous moderate intensity exercise, with most showing favourable postprandial responses after exercise. These studies have been reviewed in detail elsewhere (13). Interval exercise involving several bursts of high intensity exercise (lasting 6 to 240 s) interspersed with light exercise is also an effective strategy to reduce postprandial lipaemia but few studies have been conducted (for a recent review see; (3)). Burns and colleagues (2015) identified that most studies reported significant reductions in postprandial TAG for both submaximal and supramaximal high intensity interval exercise modes (defined relative to $\dot{V}O_2\text{max}$) compared to no exercise conditions (3). When compared to moderate intensity continuous exercise, submaximal high intensity interval exercise has been shown to be similar (12), or more effective (36), at reducing postprandial TAG. Supramaximal high intensity exercise has the added benefit of reducing the time required to complete a fixed amount of work compared to exercise of lower intensities (24). Although this is appealing, because lack of time to exercise is a common reason for people not performing exercise (3, 24), the practicality (24) and safety (11) of supramaximal exercise is not fully understood in sedentary populations. As such, the use of submaximal high intensity interval exercise to lower postprandial lipaemia may be warranted. However, few studies have investigated this mode of exercise on modifying postprandial lipaemia within adults at higher metabolic risk (3).

Having a healthy diet is inversely related to cardiovascular disease and all-cause mortality (40). Consuming sufficient portions of fruit and vegetables each day is an important component of a healthy diet, according to international guidelines (21). In addition to being rich in dietary fibre and essential nutrients, many fruits and vegetables are functional foods; those that provide health benefits in addition to basic nutrition (1). The strawberry is considered to be a functional food due to its antioxidant, anti-inflammatory, antihypertensive and lipid lowering effects (for a recent review see; (1)). The high content of phenols (which include; anthocyanins, catechins, ellagitannins, perlargonidins and quercetin) within strawberries are proposed to be important for modifying circulating lipids and lipid oxidation in the postprandial period (4). Consumption of 10g freeze dried strawberries (equivalent to 110 g fresh weight strawberries) with a moderate fat (31 g) high carbohydrate (135 g) meal compared to a placebo acutely reduced postprandial TAG, oxLDL, and markers of inflammation (C-reactive protein, Interleukin-6) in overweight men and women (4, 10). However, the acute effects of strawberries on the postprandial responses to a high-fat, low-carbohydrate meal has, to our knowledge, not been investigated. This is important in order to understand more comprehensively, the potential use of strawberry intake in reducing postprandial cardio-metabolic stresses associated with fat ingestion.

Prior submaximal high intensity interval exercise and strawberry consumption appear to be independently beneficial in acutely reducing lipid-induced metabolic dysregulation after moderate or high fat meal ingestion. However, the combined effect of these lifestyle interventions has not been investigated to date. We aimed to investigate the separate and combined effects of prior acute exercise and strawberry consumption on reducing postprandial TAG responses and oxidative stress after an oral fat tolerance test (OFTT) in inactive overweight and obese adult males. We hypothesised that exercise and strawberry interventions would independently reduce postprandial TAG and that we would observe an interaction effect for strawberry and exercise in reducing postprandial TAG.

98 **Methods**

99 *Participants*

100 Overweight and obese adult males [Body Mass Index (BMI) $>25\text{ kg.m}^{-2}$, waist circumference
101 $>94\text{ cm}$] with no known cardio-metabolic disorders were recruited. Participants were excluded
102 if they smoked, had known cardio-metabolic disease, were taking lipid lowering medication,
103 had poorly controlled blood pressure, or had abnormalities identified by the cardiopulmonary
104 exercise test during the screening visit that would increase the risk of performing the
105 subsequent exercise trials. This study was conducted according to the declaration of Helsinki
106 and approved by the Department of Sport, Health and Exercise Science Ethics Committee,
107 University of Hull. Written informed consent was given by all participants before study
108 commencement.

109 *Study design*

110 This prospective randomised, single blinded, crossover study investigated the separate and
111 combined effects of acute prior exercise and acute strawberry consumption on postprandial
112 lipaemic responses (serum TAG concentrations) and oxidative stress responses (serum oxLDL
113 and lipid hydroperoxides). There were four experimental conditions which included either an
114 abbreviated OFTT meal containing; whole milk (257.5 g, Tesco, UK), double cream (117.5 g,
115 Tesco, UK) and either strawberry milkshake mix [(placebo), 20 g, Tesco, UK] or freeze dried
116 strawberries [(intervention), 25 g, European Freeze Dry Ltd, Preston, UK] (detailed below).
117 The OFTT meals were preceded by either rest or submaximal high intensity interval exercise
118 (detailed below) conducted on the day before OFTT. Each participant completed all
119 experimental conditions, these were; 1. Placebo OFTT rest condition (R-P), 2. Strawberry
120 OFTT rest condition (R-S), 3. Placebo OFTT exercise condition (Ex-P), 4. Strawberry OFTT
121 exercise condition (Ex-S). Participants attended the research laboratory before 10:00 am on

four separate occasions, separated by at least 72 h. During the acute exercise conditions, participants attended the laboratory after 3:30 pm, 16 to 18 h before the scheduled OFTT. The order in which the trial conditions were performed was randomised a priori for each participant using Research Randomizer software (39). Participants refrained from alcohol and exercise (other than that prescribed within the experimental protocol) for 24 h before each OFTT visit and attended the research laboratory having fasted overnight. All tests were completed within 8 weeks of the screening visit.

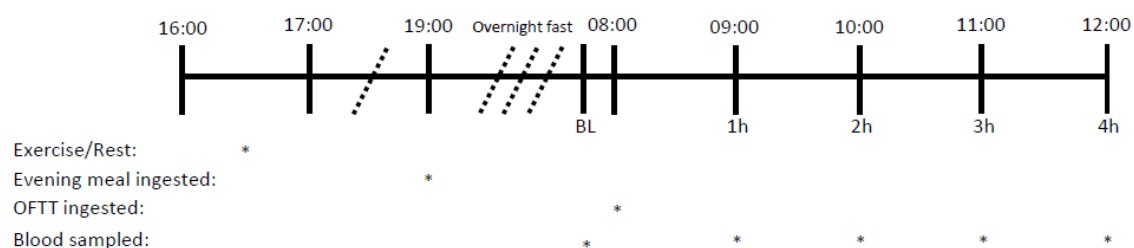


Figure 1. A schematic diagram of the study design. Dotted lines indicate lapses in time periods; * denotes the time point that each corresponding activity was performed or sample was taken.

Screening visit

Participants fasted for 2 h before the screening visit. After providing their written informed consent, baseline stature (Harpender Stadiometer, Holtain Limited, Crymych Pembrokeshire), body mass (Seca Balance Scales, Seca, Hamburg, Germany), waist and hip circumferences (Seca 201 ergonomic circumference measuring tape, Hamburg, Germany) were measured in line with ACSM's Guidelines for Exercise Testing and Prescription (31). Body fat content (percentage) was estimated using bioimpedance (BF900 Maltron Body Composition Analyser, Essex, UK). Blood pressure (Omron M6, Omron Healthcare LTD, Milton Keynes, UK) and resting ECG measurements (GE CASE system, GE Healthcare, Freiburg, Germany) were taken and this was followed by a symptom-limited maximal cardiopulmonary exercise test (CPET) to volitional exhaustion (detailed below).

Visits 1-4

Participants randomised to the exercise condition attended the laboratory the afternoon before the OFTT having refrained from exercise that day. Participants randomised to the rest condition refrained from exercise 24 h before OFTT and did not attend the laboratory. All participants were provided with a commercial “ready meal” (detailed below) to consume as their only nutritional intake that evening and were asked to consume the same meal at a similar time before every OFTT study visit. Participants attended the laboratory before 10 am the following morning having fasted overnight (>10 h). After 10 min of rest, three blood pressure measurements were taken over a period of 10 min. A cannula was inserted in to a vein in the antecubital fossa and a blood sample was drawn. Once the participant was provided with an OFTT meal, they were invited to consume it within 5 min. The OFTT meal either contained freeze dried strawberries (intervention) or strawberry flavouring (placebo). A blood sample was drawn on the hour for 4 h after OFTT meal ingestion.

Oral fat tolerance test (OFTT)

The 4 h abbreviated OFTT has been validated against the standard 8 h test (41) and we have demonstrated the repeatability of this test within our laboratory (28). The OFTT meal (Table 1) was designed specifically for this investigation and was made primarily with dairy products and flavoured with 20 g commercially available strawberry milkshake powder (placebo) or 25 g freeze dried strawberries (European Freeze Dry Ltd, Preston, UK). The high fat meal was designed for participant palatability and in accordance with OFTT expert statement guidelines which recommended 75 g fat, 25 g carbohydrates, 10 g protein (23).

Table 1. Oral Fat Tolerance Test meal composition

| | Placebo OFTT | Strawberry OFTT |
|-------------------------|---------------------|------------------------|
| Energy (kcal) | 832 | 831 |
| Fat (g) | 73.3 | 73.5 |
| Saturated (g) | 45.7 | 45.7 |
| Carbohydrate (g) | 33.3 | 32.4 |
| Fructose (g) | 0.0 | 6.0 |
| Protein (g) | 10.5 | 12.3 |
| Fibre (g) | 0.1 | 2.8 |

Cardiopulmonary exercise test (CPET)

Participants performed an incremental ramp-based CPET to volitional exhaustion on an electronically braked cycle ergometer (eBike ergometer, GE Healthcare, Freiburg, Germany) with on-line breath-by-breath expired gas analysis (Cortex Metalyzer 3B, Leipzig, Germany), and 12 lead ECG (GE CASE system, GE Healthcare, Freiburg, Germany) recorded throughout. CPET was performed and analysed for Peak oxygen consumption ($\dot{V}O_{2\text{peak}}$, ml.kg⁻¹.min⁻¹) and oxygen consumption at the anaerobic threshold (AT, ml.kg⁻¹.min⁻¹) in accordance with our previously described methods (28).

Submaximal high intensity interval exercise

Submaximal high intensity interval exercise was performed on a cycle ergometer (eBike ergometer, GE Healthcare, Freiburg, Germany) using individualised protocols during each of the two exercise sessions. Before interval exercise, there was 6 min of exercise at 20 W immediately followed by 6 min of exercise at a work rate selected at 90% of the oxygen consumption at the AT, performed as a warm-up. The low intensity interval exercise was set at 50% of the work rate at the AT. The high intensity interval exercise was set at 50% of the difference between work rates at AT and $\dot{V}O_{2\text{peak}}$. The high to low intensity exercise ratio was 1 min high intensity exercise to 1 min low intensity exercise for 40 min. Work rates were

calculated from CPET with adjustment for oxygen kinetics and ramp rate as described previously (28).

Evening meal

The nutritional composition of the meal consumed on the evening before OFTT influences the postprandial response to OFTT (34). To control for this, participants were provided with a standardised commercial meal. Participants chose one of two meals and the same meal was consumed by the participant on the evening before all OFTTs. The mean (SD) nutritional contents of the meals were: calories, 755.5 (13.4) kcal; protein, 34.7 (1.1) g; carbohydrates, 77.9 (5.0) g; fat, 32.5 (0.3) g; saturated fat, 14.8 (4.0) g.

Blood sampling and analysis

Blood samples were drawn from a 20-gauge peripheral venous cannula (Braun Introcan Safety 20 G Closed Catheter, Pennsylvania, USA) inserted in to a vein in the antecubital fossa. The cannula was kept patent between blood draws with a mandarin stylet (Braun Vasofix Stylet, Pennsylvania, USA). Up to 25 ml of blood was drawn at each time point. Fluoride/oxalate blood collection tubes were spun immediately at 2383 g for 15 min at 4°C. SST II blood collection tubes were stored at room temperature for 30 min to allow blood to clot and then spun at 1992 g for 10 min at 4 °C. Serum and plasma samples were aliquoted and stored at -80 °C until analyses.

The ABX Pentra 400 biochemistry autoanalyser (Horiba, Montpellier, France) was used to analyse serum TAG, total cholesterol, high density lipoprotein cholesterol (HDL-c), and plasma glucose. Calibration and quality controls were performed prior to use in accordance with manufacturer's guidelines and samples were measured in duplicate. Low Density

Lipoprotein (LDL-c) was estimated from the Friedewald equation (14). Serum oxLDL was determined by using an enzyme-linked immunosorbent assay (ELISA) performed in accordance with the manufacturer's guidelines (Mercodia Inc, Upsala, Sweden), each sample was measured in duplicate. Serum lipid peroxidation was estimated by using the ferrous oxidation in xylenol orange (FOX1) assay in line with established methods (42).

Antioxidant capacity of strawberry product

The Folin-Ciocalteu assay was performed on the freeze dried strawberry product and on the placebo product in keeping with established methods but using epicatechin equivalents in place of gallic acid equivalents (35). Briefly, the strawberry/placebo product was mixed with 100 % dimethyl sulfoxide to make a 50 mg·mL⁻¹ sample concentration. Then 15 µL of this sample, 170 µL double-distilled water, 12 µL Folin-Ciocalteu reagent and 30 µL sodium carbonate solution (concentration 200 g·L⁻¹) was added to each well of a 96 well plate. This was incubated in the dark for 1 h at 21 °C and then 73 µL double-distilled water was added to each well. Absorbance was then measured at 765 nm.

Outcome measures

The primary outcome was TAG AUC during OFTT. Secondary outcome measures were TAG iAUC, oxLDL and lipid peroxidation (FOX1 assay).

Statistical Analyses

Normal (Gaussian) distribution of data was verified using the Shapiro-Wilk test, tests for skewness and kurtosis of distributions and visual inspection of histogram charts was conducted.

Data are presented as mean and standard deviation (SD) for normal data, and non-normally distributed data (age, VO₂max, VO₂AT, fasting glucose, glucose AUC) are presented as median and quartiles 1 and 3 (Q1, Q3). Differences between peak heart rate responses for each exercise session were compared using paired t-tests and Cohen's d was used to demonstrate effect size. Total area under the curve (AUC) and incremental AUC (iAUC) for TAG, cholesterol, HDL-c and glucose was determined by the trapezoidal method (25). oxLDL and lipid hydroperoxides were measured at baseline and at 4 h and the difference between baseline and 4 h was calculated. To assess the differences between outcome measures for each trial condition, 2x2 repeated measures analysis of variance (ANOVA) was used for normally distributed (TAG, cholesterol, HDL-c, LDL-c, oxLDL, lipid hydroperoxides) and non-normally distributed (glucose) AUC and oxidative stress data. Specifically, activity (exercise/ no exercise) was treated as a study condition and nutritional content (strawberry/ no strawberry) was treated as a study condition. Each activity/nutritional intervention and placebo appeared twice across the study trials therefore the 2x2 repeated measures ANOVA enabled the exercise and strawberry interventions to be assessed independently across the study and the interaction revealed whether a combination of the study conditions influenced postprandial responses. Mean difference with 95% confidence intervals (CI), p values and effect sizes using partial eta squared (η^2) are reported. The alpha level was set at 0.05, and η^2 was used to determine the effect size with small, medium and large effects set at 0.01, 0.06 and 0.14, respectively (8). Where significance was reached, post hoc pairwise comparisons were made with Bonferroni adjustment and reported as mean difference, CI, p values and η^2 . Microsoft Excel (2013) and SPSS (Version 22) (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

The complexity of the 2x2 repeated measures ANOVA with two within factors makes sample size estimation for this design challenging (33). As such, we estimated the sample size required to detect differences between the main effects for the diet condition and the exercise condition

using a one way repeated measures ANOVA design with two measures for each condition. Based on previous data (41) we expected that the repeatability of our primary outcome, TAG AUC, would be high (ICC=0.83). Using a more conservative estimate of $\rho=0.7$, an effect size of 0.7, an alpha value of 0.05 and 80 % power we obtained a sample size of 10 participants.

Results

Ten of eleven males (median age, 31.5 Q1, 28.5 Q3, 46.3 years; mean \pm SD BMI, 29.9 ± 1.8 $\text{kg}\cdot\text{m}^{-2}$; waist circumference: 1.05 ± 0.05 m) completed all study visits. Demographics for these participants are reported in table 2. One participant dropped out of the study after the screening visit for personal reasons. Six participants were overweight (BMI $25 \text{ kg}\cdot\text{m}^{-2}$ to $30 \text{ kg}\cdot\text{m}^{-2}$), four were obese (BMI $>30 \text{ kg}\cdot\text{m}^{-2}$) and all were inactive (defined by self-reported exercise <150 min per week). All participants completed the two submaximal high intensity interval exercise protocols which lasted 1 h in total. The peak heart rates achieved during exercise were $93 \pm 4\%$ of peak heart rates measured in CPET. There were no differences in peak heart rates between the two interventions, exercise session 1: 154 ± 14 $\text{beats}\cdot\text{min}^{-1}$; exercise session 2: 153 ± 11 $\text{beats}\cdot\text{min}^{-1}$ (CI: -2 to 5 $\text{beats}\cdot\text{min}^{-1}$; $p=0.504$; Cohen's $d=0.09$). The mean (SD) work rate (W) for the low and high intensity intervals were 48 ± 16 W and 181 ± 49 W, respectively. Participants performed the same pre-programmed tailored exercise protocol for both exercise sessions on an electromagnetically braked cycle ergometer. The Folin-Ciocalteu assay identified that freeze dried strawberry had 4.5 fold greater phenolic capacity compared to the placebo (895 mg vs. 194 mg). There were no adverse effects during or following the exercise interventions or high fat meal ingestion.

283 **Table 1.** Mean (SD) Baseline Demographics

| Baseline measures | |
|--|-------------------|
| Age (years)* | 31.5 (28.5, 46.3) |
| Body mass (kg) | 91.9 (6.8) |
| BMI (Kg·m ⁻²) | 29.9 (1.8) |
| Waist circumference (m) | 1.05 (0.05) |
| Waist to hip ratio | 0.97 (0.05) |
| Body fat (%) | 25.5 (5.2) |
| Blood Pressure (mmHg) | 129 (7)/ 80 (9) |
| $\dot{V}O_2$ peak (ml·kg ⁻¹ ·min ⁻¹)* | 33.2 (26.7, 36.5) |
| $\dot{V}O_2$ AT (ml·kg ⁻¹ ·min ⁻¹)* | 18.1 (15.8, 19.3) |
| Triglycerides (mmol·l ⁻¹) | 1.3 (0.4) |
| HDL (mmol·l ⁻¹) | 1.2 (0.1) |
| Cholesterol (mmol·l ⁻¹) | 5.3 (1.1) |
| Glucose (mmol·l ⁻¹)* | 5.2 (4.9, 5.4) |

*Reported as median (IQ1, IQ3)

Serum TAG responses to OFTT

Mean (SD) TAG responses at each time point for each condition are presented in figure 2. TAG increased from baseline in all conditions and peaked at 3-4 h.

Total AUC

TAG AUC was 1.5 mmol·4h⁻¹·L⁻¹ lower (95% confidence interval [CI]= -2.3 to -0.8 mmol·4h⁻¹·L⁻¹, p= 0.001, η^2 = 0.71) for the two exercise conditions compared to the two resting conditions. Post hoc pairwise comparisons with Bonferroni adjustment identified that TAG AUC was 1.6 mmol·4h⁻¹·L⁻¹ lower in the exercise condition compared to rest condition for the placebo OFTT (CI= -2.5 to -0.5 mmol·4h⁻¹·L⁻¹, p= 0.009, η^2 = 0.55) and by 1.5 mmol·4h⁻¹·L⁻¹ for the strawberry OFTT (CI= -2.9 to -0.2 mmol·4h⁻¹·L⁻¹, p= 0.033, η^2 = 0.41). There were no differences in TAG AUC between the strawberry OFTT and placebo OFTT (Mean difference= -0.3 mmol·4h⁻¹·L⁻¹ CI= -1.3 to 0.7 mmol·4h⁻¹·L⁻¹, p= 0.475, η^2 = 0.06). There was no exercise and strawberry interaction (p= 0.970, η^2 < 0.001).

Incremental AUC

There was a large effect size for lower TAG iAUC (Mean difference= $-0.4 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, CI = -1.1 to $0.2 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, $p= 0.175$, $\eta p^2= 0.19$) in the exercise conditions compared to the resting conditions. TAG iAUC was $0.5 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$ lower in the placebo conditions than the strawberry conditions (CI= -1.0 to $-0.1 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, $p= 0.021$, $\eta p^2= 0.47$). Post hoc analyses identified that TAG iAUC was $0.7 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$ lower for the placebo condition compared to strawberry condition with exercise (CI= -1.1 to $-0.3 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, $p= 0.005$, $\eta p^2= 0.61$) but not with rest (mean difference= $-0.4 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, CI= -1.2 to $0.5 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$, $p= 0.331$, $\eta p^2= 0.11$). There was no interaction between conditions ($p= 0.516$, $\eta p^2= 0.05$).

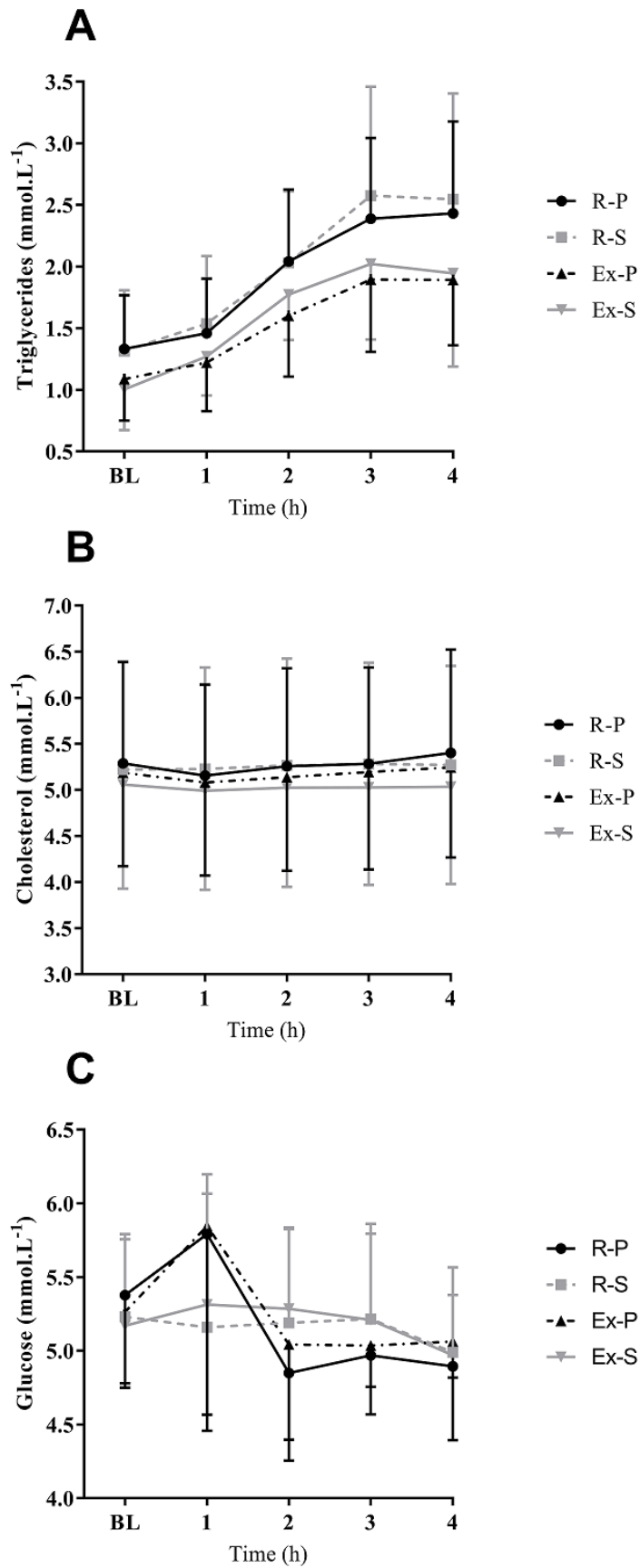
Baseline

Baseline TAG was $0.3 \text{ mmol}\cdot\text{L}^{-1}$ lower (CI= -0.4 to $-0.2 \text{ mmol}\cdot\text{L}^{-1}$, $p= 0.001$, $\eta p^2= 0.74$) in the exercise conditions compared to the resting conditions. Post hoc analyses identified that baseline TAG was $0.2 \text{ mmol}\cdot\text{L}^{-1}$ lower with exercise compared to rest condition with the placebo (CI= -0.4 to $-0.1 \text{ mmol}\cdot\text{L}^{-1}$, $p= 0.011$, $\eta p^2= 0.53$) and $0.3 \text{ mmol}\cdot\text{L}^{-1}$ lower with the strawberry condition (CI= -0.5 to $-0.1 \text{ mmol}\cdot\text{L}^{-1}$, $p=0.014$, $\eta p^2= 0.50$). There were no differences in baseline TAG in the strawberry conditions compared to the placebo conditions (Mean difference= $0.1 \text{ mmol}\cdot\text{L}^{-1}$, CI= -0.1 to $0.2 \text{ mmol}\cdot\text{L}^{-1}$, $p= 0.484$, $\eta p^2= 0.06$). There was no interaction effect between conditions ($p= 0.660$, $\eta p^2= 0.02$).

319 *Table 3. Postprandial responses for each study condition expressed as mean (SD)*

| | Rest & Placebo | Rest & Strawberry | Exercise & Placebo | Exercise & Strawberry |
|--|-------------------------------|----------------------------------|-----------------------------------|--------------------------------------|
| TG AUC (mmol·4h·l⁻¹) | 7.8 (2.2) | 8.1 (2.6) | 6.2 (1.8)* | 6.5 (1.7)* |
| TG iAUC (mmol·4h·l⁻¹) | 2.4 (0.8)** | 2.8 (1.2) | 1.9 (0.8)** | 2.5 (0.6) |
| TG baseline (mmol·l⁻¹) | 1.3 (0.4) | 1.3 (0.5) | 1.1 (0.3)* | 1.0 (0.3)* |
| Δ oxLDL (mU·l⁻¹) | -10.7 (15.7) | 0.4 (14.2) | 1.1 (9.4) | -4.0 (10.6) |
| Δ Lipid hydroperoxides (μmol·l⁻¹) | 11.6 (10.1) | 8.0 (11.7) | 4.4 (11.6) | 13.6 (16.6) |
| Cholesterol AUC (mmol·l⁻¹) | 21.0 (4.2) | 21.0 (4.5) | 20.6 (4.1)* | 20.1 (4.3)* |
| HDL AUC (mmol·l⁻¹) | 4.7 (0.6) | 4.6 (0.4) | 4.6 (0.5) | 4.6 (0.4) |
| LDL AUC (mmol·l⁻¹) | 12.8 (3.4) | 12.7 (4.4) | 13.1 (3.6) | 12.5 (3.8) |
| Glucose AUC (mmol·l⁻¹)[#] | 20.0 (4.3) | 19.8 (1.8) | 20.6 (2.5) | 20.2 (1.5) |

320 **lower for the exercise conditions (p<0.05) **lower for the placebo conditions (p<0.05) [#]Glucose is*
321 *expressed as median (interquartile range)*



322

323 **Figure 2.** TAG (Panel A), cholesterol (Panel B) and glucose (Panel C) responses to OFTT

324

325 *Oxidative stress responses to OFTT*

326 Mean (SD) change (Δ) in oxLDL and lipid hydroperoxides from baseline to 4 h are reported in
327 table 3. There were no differences in oxLDL for the exercise (Mean difference= $-3.6 \text{ mU}\cdot\text{L}^{-1}$,
328 CI= -14.3 to $7.0 \text{ mU}\cdot\text{L}^{-1}$, $p=0.45$, $\eta^2=0.06$) or strawberry (Mean difference= $-2.9 \text{ mU}\cdot\text{L}^{-1}$, CI=
329 -9.6 to $3.7 \text{ mU}\cdot\text{L}^{-1}$, $p=0.34$, $\eta^2=0.10$) conditions. However, there was a large interaction effect
330 size between conditions ($p=0.16$, $\eta^2=0.21$). There were no differences in lipid
331 hydroperoxides for the exercise (Mean difference= $0.8 \mu\text{mol}\cdot\text{L}^{-1}$, CI= -8.0 to $9.6 \mu\text{mol}\cdot\text{L}^{-1}$, $p=$
332 0.84 , $\eta^2=0.01$) or strawberry (Mean difference= $-2.8 \mu\text{mol}\cdot\text{L}^{-1}$, CI= -11.1 to $5.6 \mu\text{mol}\cdot\text{L}^{-1}$, $p=$
333 $0.47 \mu\text{mol}\cdot\text{L}^{-1}$, $\eta^2=0.06$) conditions. However, there was a large interaction effect size
334 between the conditions ($p=0.13$, $\eta^2=0.24$).

335

336 *Cholesterol, HDL, LDL and glucose responses to OFTT*

337 The cholesterol, HDL, LDL and glucose AUC in response to OFTT are presented in Table 3.
338 Cholesterol AUC was $0.7 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ lower in the exercise conditions compared to the rest
339 conditions (CI= -1.1 to $-0.2 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.01$, $\eta^2=0.58$). There was no effect for exercise
340 (Mean difference= $0.01 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -0.13 to $0.14 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.94$, $\eta^2=0.001$)
341 or strawberry (Mean difference= $0.03 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -0.06 to $0.14 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.43$,
342 $\eta^2=0.07$) conditions on HDL responses to OFTT. There was no effect for exercise (Mean
343 difference= $-0.05 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -0.58 to $0.49 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.85$, $\eta^2=0.004$) or
344 strawberry (Mean difference= $0.39 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -0.74 to $1.52 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.46$,
345 $\eta^2=0.06$) conditions on LDL responses to OFTT. There was no effect for exercise (Mean
346 difference= $0.29 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -1.04 to $0.43 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.387$, $\eta^2=0.08$) or
347 strawberry (Mean difference = $0.14 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, CI= -0.55 to $0.83 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$, $p=0.655$,
348 $\eta^2=0.02$) on glucose responses to OFTT.

349

350 **Discussion**

351 We investigated the separate and combined effects of acute submaximal high intensity interval
352 exercise and strawberry consumption on postprandial responses to OFTT among overweight
353 and obese adult males. We have demonstrated that acute submaximal high intensity interval
354 exercise was effective in reducing TAG AUC after OFTT. This significant effect of acute
355 exercise in lowering postprandial TAG was evident both with and without strawberry
356 consumption. However, contrary to our hypotheses, strawberry consumption with OFTT did
357 not alter TAG AUC and there was no interaction between strawberry consumption and
358 submaximal high intensity interval exercise. Our secondary findings indicate that there was a
359 large effect size observed for acute submaximal high intensity interval exercise reducing TAG
360 iAUC. Whereas, TAG iAUC was increased with strawberry consumption. There were no
361 significant changes in lipid related oxidative stress responses between conditions.

362

363 *Exercise and postprandial TAG*

364 We observed a reduction in TAG AUC in response to the OFTT by approximately 20 % in the
365 submaximal high intensity interval exercise conditions compared to the control conditions.
366 Acute prior exercise significantly lowered baseline TAG and there was a large effect size for
367 lower TAG iAUC which contributed to the reduction in total AUC. Reductions in TAG AUC
368 of a similar magnitude have been reported in response to moderate continuous exercise (13)
369 and high intensity interval exercise (12, 36). We selected an individualised submaximal high
370 intensity interval exercise protocol consistent with exercise intensity domains identified by
371 analysis of expired ventilatory gasses measured during a CPET (29). Other submaximal high
372 intensity interval exercise interventions that have successfully reduced postprandial lipaemia
373 lasted approximately 40 min and were stopped when participants had expended 500 kcal (12)

374 or 660 kcal (36). We recruited an older, more overweight, and less active population with
375 higher mean fasting TAG concentrations compared to these studies. For practical reasons (this
376 is, to avoid unrealistic length of exercise sessions) and real life application, we predefined the
377 40 min duration of high intensity interval exercise (rather than a target energy expenditure) and
378 investigated the effects of individualised interventions at clearly defined exercise intensities.
379 We believe this to be important because participants with lower levels of cardiorespiratory
380 fitness, exercising at the same relative intensity, will need to exercise for longer than a fitter
381 individual to attain the same overall energy expenditure. A high total energy expenditure (>500
382 kcal) would typically require exercise sessions in excess of 1 h for a less fit individual. Such
383 study designs may not be ecologically valid because >1 h of exercise is above recommended
384 target exercise guidelines, which are seldom met (38). Furthermore, standard equations used
385 for calculating energy expenditure from expired oxygen and carbon dioxide are inaccurate
386 during interval exercise that involves exercise intensities above the anaerobic threshold.
387 Carbon dioxide ($\dot{V}CO_2$) is produced from non-oxidative processes and oxidative metabolism
388 during exercise at an intensity above the anaerobic threshold (2). The respiratory exchange
389 ratio ($\dot{V}CO_2/\dot{V}O_2$), used to infer substrate specific oxidative metabolism, is increased by non-
390 oxidative carbon dioxide production from anaerobic metabolism (2). This overestimates
391 oxidative glucose and underestimates oxidative fat metabolism and will therefore reduce the
392 accuracy calculations to estimate energy expended (19). Furthermore, in our protocol, exercise
393 intervals at an intensity above the anaerobic threshold lasted 1 min. Oxygen kinetics during
394 exercise take longer than 1 min to reach a steady state (and may never reach a steady state at
395 higher intensities). Attaining steady state is a requirement of energy expenditure estimation
396 from expired gasses. Therefore, the validity of high intensity interval exercise interventions
397 that use predefined estimated energy expenditure targets based on expired oxygen and carbon
398 dioxide calculations could be questioned. Finally, as considered later, total energy expenditure

may not be the key mechanism involved in reducing postprandial TAG with high intensity interval exercise (3).

Interval exercise has the advantage of enabling a greater volume of work to be completed within a period of time (24, 36), as well as varying the physiological challenge on the body when compared to continuous moderate intensity exercise. High intensity interval exercise has superior levels of enjoyment (18), lower perceived work (22) and increased likelihood of continuing regular exercise (18, 22) in addition to the numerous cardio-metabolic benefits (16, 24) compared to moderate intensity continuous exercise. Furthermore, the activity of lipoprotein lipase (LPL; a key enzyme involved with the removal of TAG) appears to be increased following high intensity interval exercise training (3, 36). This is important because TAG clearance appears to be the primary mechanism of reducing postprandial TAG after high intensity interval exercise (3).

One mechanism by which high intensity interval exercise elicits greater reductions in postprandial TAG compared to moderate intensity continuous exercise could be explained by the regulation of LPL and its specificity to type 2 muscle fibres (3). A greater number of type 2 muscle fibres will likely be recruited during high intensity interval exercise and subsequently type 2 muscle fibre specific LPL activity may be increased (36). Reductions in postprandial TAG with moderate intensity continuous exercise may also occur via this mechanism because type 2 muscle fibre recruitment increases with prolonged moderate intensity exercise. In addition to increased LPL activity, moderate intensity exercise increases the affinity of VLDL1 for LPL which will likely facilitate systemic removal of lipids (15). However, the effects of high intensity interval exercise on altering VLDL1 affinity for LPL is unknown. Further mechanistic investigations in to the effects of acute high intensity interval exercise induced attenuation in postprandial TAG excursions, fibre specific LPL activity and VLDL1 affinity

for LPL would help to identify optimal exercise interventions for those at risk of cardiovascular metabolic disease.

Our data support the use of submaximal high intensity interval exercise as a training modality to reduce postprandial TAG which may favourably modify lipid-related cardiovascular risk in overweight and obese men.

Exercise and postprandial oxidative stress

We did not observe improvements in markers of oxidative stress with exercise in the present study. This could be due to the small sample size within our study and the variability within these markers. These were also secondary outcome measures and therefore the study was not adequately powered to detect differences between interventions for these markers.

There were no changes in postprandial oxLDL concentrations or lipid hydroperoxides with prior acute submaximal high intensity interval exercise. Reduced oxLDL with endurance cycling exercise (70 % $\dot{V}O_2$ max for approximately 47 min) performed 16 h before high fat meal ingestion has been previously reported (20). Compared to the present study, the high fat meal utilised in the study by Jenkins and colleagues (20) contained approximately 50 g more fat. The higher fat intake is likely to have contributed to a larger and prolonged lipaemic response. Higher circulating lipids provides a greater capacity for postprandial LDL oxidation (17) and therefore there may have been a greater capacity for reduction in oxLDL with exercise compared to the present study.

A reduction in lipid hydroperoxides with the exercise session performed either immediately before OFTT, or 1 h after OFTT has been demonstrated previously (7, 26). However, to our knowledge the effects of exercise performed 16 h before OFTT on lipid hydroperoxides, as in our protocol, has not been investigated. Of the studies that have investigated the effects of exercise in reducing postprandial oxidative stress, all employed continuous endurance exercise

lasting 47 (20) or 60 (7, 26) min at an intensity of 70 % VO₂max (20), 60 % predicted maximum heart rate (7) or 60 % maximum heart rate (26). The timing of exercise and perhaps the mode of exercise required to reduce oxidative stress may therefore be important.

Strawberry consumption and postprandial TAG

In contrast to previous research (4), strawberry consumption had no effect on TAG AUC. Interestingly, TAG iAUC was higher with strawberry consumption than with the placebo. In contrast to the beneficial effects of strawberry consumption on postprandial TAG that have been reported previously (4) the present findings suggest that strawberry consumption had no effect on postprandial TAG.

Our OFTT had a higher fat content (73 g versus 31 g) and our carbohydrate content was considerably lower (33 g versus 135 g) compared to a previous study which demonstrated reduced TAG after OFTT with strawberry consumption (4). Additionally, our OFTT was composed of milk and cream as opposed to typical American breakfast foods. We propose that the differences in carbohydrate quantities of the OFTT and the amount of fructose relative to the total carbohydrate content may explain these findings. Approximately 20 % of the carbohydrate content of our strawberry OFTT was fructose, with glucose the predominant carbohydrate source in the placebo high fat meal (which did not contain fructose). It has been demonstrated previously that an OFTT containing fructose resulted in a higher postprandial TAG response compared to the same OFTT when the carbohydrate content was glucose (6). It was proposed by Chong and colleagues (2007) (6) that the lower insulin response to fructose compared to glucose may explain the greater postprandial TAG response. The fructose content in our strawberry OFTT may therefore have contributed to the greater incremental increase in postprandial TAG in our study compared to placebo. Given the relatively small fructose contribution to the high total carbohydrate in the test meals of Burton-Freeman and colleagues

(4), the overall effect of fructose on the insulin response was likely minimal in this study. Further, strawberry polyphenols promote increased insulin sensitivity (10). This could potentially stimulate enhanced insulin mediated TAG storage in adipose tissue and thus increased TAG clearance from the circulation, when carbohydrate is high as was the case in the study by Burton-Freeman and colleagues (4).

Strawberry consumption and postprandial oxidative stress

There were no changes in oxLDL or lipid hydroperoxides between groups. Previous studies have demonstrated the benefits of strawberries on reducing postprandial oxLDL after lipid ingestion (4, 30). We gave a dose of strawberries (25 g Freeze dried strawberries) which is similar to the optimal dose (20 g) for lowering postprandial TAG identified by Park and colleagues (2016) (30). We used a higher fat content and specifically a higher dairy fat content in our OFTT meal compared to that of other studies (4, 30). Dairy products within our high fat meal may have reduced circulating bioavailability of the strawberry polyphenols because milk proteins and fat may reduce bioavailability of berry polyphenols (5, 43). However, despite the bioavailability of berry polyphenols being lower when combined with milk, this may not necessarily reduce the intestinal-blood transfer of berry polyphenols according to in vitro experiments (5). Notably, reduced circulating oxLDL and increased circulating strawberry polyphenols have been observed after consumption of a strawberry drink containing milk in humans (30). It is therefore unclear whether dairy products reduced the bioavailability of strawberry polyphenols and therefore capacity to reduce oxLDL in the present study. Lipid hydroperoxides, which increase during postprandial lipaemia (7, 26, 27) are reduced after anthocyanin intake from grapes (27). However, we did not observe this reduction in the present study involving assumed strawberry anthocyanin intake. As discussed, the potential for reduced bioavailability with dairy products may explain our findings. Differences in the

agricultural and preparation processes of the strawberry products could also contribute to the discrepancies between the present study and previous studies (1).

Limitations

We have eluded to some of the limitations that exist within the present study in the discussion. A further limitation is that only the evening meal on the day preceding the OFTT was standardised. Therefore, we cannot completely exclude the influence of food intake 24 h before OFTT. We gave strict instructions to participants to continue with their habitual diet and abstain from alcohol and caffeine. We trusted their adherence to our instructions as we did in our previous repeatability study (28). This was the same for restricting physical activity beyond their habitual levels (which were self-reported to be below standard guidelines), however, previous studies have attempted to measure activity levels during this period (36). Participants did not attend the laboratory on the day before OFTT for the resting conditions at the equivalent time to when they reported to the laboratory to perform exercise during the exercise conditions, this could have influenced the metabolic responses measured. The abbreviated 4 h OFTT has been shown to be predictive of the 8 h time period (41) and is a repeatable test (28), however, it does not allow assessment of clearance of postprandial TAG (this is, chylomicrons and their remnants), which may have been beneficial to evaluate. Finally, the amount of fat in OFTT is not representative of typical western diets and therefore although these findings are important, investigating this exercise protocol using ecologically valid meals is of interest.

Conclusions

Our findings support the use of acute submaximal high intensity interval exercise as an effective intervention to reduce lipoprotein-related cardiovascular risk factors in overweight and obese adult men. This mode of structured exercise could be incorporated in to lifestyle

management of overweight and obese adult males to reduce cardiovascular risk. However, freeze-dried strawberry supplementation within an OFTT containing dairy products did not improve postprandial TAG response which may be related to the fructose and total carbohydrate content of meal. Nevertheless, this is an interesting finding that merits further investigation. We recommend that future studies: 1. Investigate the role of carbohydrate and polyphenols in reducing postprandial lipaemia and 2. Evaluate the effects of acute submaximal high intensity exercise on reducing postprandial lipaemia in dyslipidaemic males and females.

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Conflict of interest

The authors have no personal or financial conflicts of interest to declare with regards to the present study. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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