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

O'Doherty, Alasdair F.[1,2]; Jones, Huw S.[2]; Sathyapalan, Thozhukat[3]; Ingle, Lee[2]; Carroll, Sean[2]

1. Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University, Newcastle-Upon-Tyne, UK; 2. Sport, Health & Exercise Science, School of Life Sciences, University of Hull, Hull, UK; 3. Academic Diabetes, Endocrinology and Metabolism, Hull York Medical School, University of Hull, Hull, UK

Corresponding Author: Alasdair Fraser O'Doherty

Mailing address: Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University, Newcastle-Upon-Tyne, UK; Telephone: +447793885801; email: alasdair.odoherty@northumbria.ac.uk

## 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 (73g fat, 33g carbohydrate) with blood sampled at baseline and hourly for 4 h after OFTT. Two OFTT contained 25g freeze-dried strawberries and two contained strawberry flavouring (placebo). Participants performed 40 minutes 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 ±1.8 $kg \cdot m^{-2}$) completed the study. TAG AUC was 1.5 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower for the exercise conditions compared to the rest conditions (95% confidence interval [CI]= -2.3 to 0.8, p= 0.001). TAG AUC was not different between the strawberry and placebo conditions (CI= -1.3 to 0.6, p= 0.475). TAG iAUC was 0.5 $mmol \cdot 4h^{-1} \cdot L^{-1}$ greater for the strawberry compared to the placebo conditions (CI= 0.1 to 1.0, 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 (29). Acute endothelial dysfunction, increased inflammation and oxidative stress occur during postprandial lipaemia and may contribute to an atherogenic

environment (8, 34). Furthermore, elevated circulating postprandial lipids likely increase the propensity for oxidation of lipids, such as LDL, which are key protagonists of atherosclerosis (15). 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; (12)). 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 (12). 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; (2)). 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 $\dot{V}O_2max$) compared to no exercise conditions (2). When compared to moderate intensity continuous exercise, submaximal high intensity interval exercise has been shown to be similar (11), or more effective (33), 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 (21). Although this is appealing, because lack of time to exercise is a common reason for people not performing exercise (2, 21), the practicality (21) and safety (10) of supramaximal exercise is not fully understood in sedentary populations. As such, the use of submaximal high intensity interval exercise to lower PPL may be warranted. However, few studies have investigated this mode of exercise on modifying postprandial lipaemia within adults at higher metabolic risk (2).

Having a healthy diet is inversely related to cardiovascular disease and all-cause mortality (37). Consuming sufficient portions of fruit and vegetables each day is an important component of a healthy diet, according to international guidelines (18). 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 (3). Consumption of 10g freeze dried strawberries (equivalent to 110g fresh weight strawberries) with a moderate fat (31g) high carbohydrate (135g) meal compared to a placebo acutely reduced postprandial TAG, oxLDL, and markers of inflammation (C-reactive protein, Interleukin-6) in overweight men and women (3, 9). 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 to help fully understand 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 triglycerides and that we would

observe an interaction effect for strawberry and exercise in reducing postprandial triglycerides.

## Methods

### *Participants*

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

### *Study Design*

This prospective randomised, single blinded, crossover study investigated the separate and combined effects of acute prior exercise and acute strawberry consumption on postprandial lipaemic responses (serum TAG concentrations) and oxidative stress responses (serum oxidised LDL and lipid hydroperoxides). There were four experimental conditions which included either an abbreviated OFTT meal containing; whole milk (257.5 g, Tesco, UK), double cream (117.5 g, Tesco, UK) and either strawberry milkshake mix [(placebo), 20 g, Tesco, UK] or freeze dried strawberries [(intervention), 25 g, European Freeze Dry Ltd] (detailed below). The OFTT meals were preceded by either rest or submaximal high intensity interval exercise (detailed below) conducted on the day before OFTT. Each participant completed all experimental conditions, these were; 1. Placebo OFTT rest condition (R-P), 2.

Strawberry OFTT rest condition (R-S), 3. Placebo OFTT exercise condition (Ex-P), 4. Strawberry OFTT 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 3:30pm, 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 (36). 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 height (Harpenden 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 (28). Body fat content (percentage) was estimated using 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).

147 *Visits 1-4*

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

160

161 *Oral Fat Tolerance Test*

162 The 4-hour abbreviated OFTT has been validated against the standard 8 hour test (38) and we
163 have demonstrated the repeatability of this test within our laboratory (25). The OFTT meal
164 (Table 1) was designed specifically for this investigation and was made primarily with dairy
165 products and flavoured with 20g commercially available strawberry milkshake powder
166 (placebo) or 25g freeze dried strawberries (European Freeze Dry Ltd, Preston). The high fat
167 meal was designed for participant palatability and in accordance with OFTT expert statement
168 guidelines which recommended 75g fat, 25g carbohydrates, 10g protein (20).

169

170 ***Table 1.*** *Oral Fat Tolerance Test meal composition*

*Cardiopulmonary Exercise Test*

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 ($\dot{V}O_2$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 (25).

*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 20W 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 $\dot{V}O_2$peak. The high to low intensity ratio was 1 minute high intensity to 1 minute low intensity for 40 min. Work rates were calculated from CPET with adjustment for oxygen kinetics and ramp rate as described previously (25).

*Evening meal*

The nutritional composition of the meal consumed on the evening before OFTT influences the postprandial response to OFTT (31). 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 20G 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 25ml of blood was drawn at each time point. Fluoride/oxalate blood collection tubes were spun immediately at 2383g 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 1992g 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 (13). Serum oxidised LDL 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 (39).

*Antioxidant capacity of strawberry product*

The Folin-Ciocalteau 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 (32). Briefly, the strawberry/placebo product was mixed with 100% dimethyl sulfoxide to make a 50 $mg \cdot mL^{-1}$ sample concentration. Then 15 µL of this sample, 170 µL double-distilled water, 12 µL Folin-Ciocalteau reagent and 30 µL sodium carbonate solution (concentration 200 $g \cdot L^{-1}$) was added to each well of a 96 well plate. This was incubated in the dark for 1 hour 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. Non-normally distributed data were analysed using non-parametric analyses. Data are presented as mean and standard deviation (SD) for normal data, and non- normally distributed data are presented as median and quartiles 1 and 3 (Q1, Q3). Total area under the curve (AUC) and incremental AUC (iAUC) for triglyceride, cholesterol, HDL-c and glucose was determined by the trapezoidal method (22). Oxidised LDL 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. 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 influence of exercise and strawberry to be assessed independently across the study and the interaction revealed whether a combination of the study conditions influenced postprandial TAG. Mean difference with 95% confidence intervals (CI), p values and effect sizes using partial eta squared ($\eta p^2$) are reported. The alpha level was set at 0.05, and $\eta p^2$ was used to determine the effect size with small, medium and large effects set at 0.01, 0.06 and 0.14, respectively (7). Where significance was reached, post hoc pairwise comparisons were made with Bonferroni adjustment and reported as mean difference, CI, p values and $\eta p^2$. 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 (30). 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 (38) 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 ±SD BMI, 29.9 ±1.8 $kg \cdot m^{-2}$; 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 $kg \cdot m^{-2}$ to 30 $kg \cdot m^{-2}$), four were obese (BMI >30 $kg \cdot m^{-2}$) 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 one hour in total. The peak heart rates achieved during exercise were 93 ±4% of peak heart rates measured in CPET and there were no differences in peak heart rates between the two interventions (p= 0.504). The mean (SD) work rate (W) for the low and high intensity intervals were 48 ±16 W and 181 ±49 W, respectively. The Folin-Ciocalteau 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.

*Table 2. Mean (SD) Baseline Demographics*

*Serum Triglyceride 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 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower (95% confidence interval [CI]= -2.3 to -0.8, p= 0.001, $\eta p^2$= 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 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower in the exercise condition compared to rest condition for the placebo OFTT (CI= -2.5 to -0.5, p= 0.009, $\eta p^2$= 0.55) and by 1.5 $mmol \cdot 4h^{-1} \cdot L^{-1}$ for the strawberry

OFTT (CI= -2.9 to -0.2, p= 0.033, $\eta p^2$= 0.41). There were no differences in TAG AUC between the strawberry OFTT and placebo OFTT (Mean difference= -0.3 $mmol \cdot 4h^{-1} \cdot L^{-1}$ CI= -1.3 to 0.7, p= 0.475, $\eta p^2$= 0.06). There was no exercise and strawberry interaction (p= 0.970, $\eta p^2 < 0.001$).

*Incremental AUC*

There was a large effect size for lower TAG iAUC (Mean difference= 0.4 $mmol \cdot 4h^{-1} \cdot L^{-1}$, CI = -0.2 to 1.1, p= 0.175, $\eta p^2$= 0.19) in the exercise conditions compared to the resting conditions. TAG iAUC was 0.5 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower in the placebo conditions than the strawberry conditions (CI= -1.0 to -0.1, p= 0.021, $\eta p^2$= 0.47). Post hoc analyses identified that TAG iAUC was 0.7 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower for the placebo condition compared to strawberry condition with exercise (CI= -1.1 to -0.3, p= 0.005, $\eta p^2$= 0.61) but not with rest (mean difference= 0.4 $mmol \cdot 4h^{-1} \cdot L^{-1}$, CI= -1.2 to 0.5, 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 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower (CI= -0.4 to 0.2, 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 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower with exercise compared to rest condition with the placebo (CI= -0.4 to -0.1, p=0.011, $\eta p^2$= 0.53) and 0.3 $mmol \cdot 4h^{-1} \cdot L^{-1}$ lower with the strawberry condition (CI= -0.5 to -0.1, 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 $mmol \cdot 4h^{-1} \cdot L^{-1}$, CI= -0.1 to 0.2, p=0.484, $\eta p^2$=0.06). There was no interaction effect between conditions (p=0.660, $\eta p^2$=0.02).

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

***Figure 2.*** *TAG responses to OFTT*

*Oxidative stress responses to OFTT*

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

*Cholesterol, HDL, LDL and Glucose responses to OFTT*

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

## Discussion

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

### *Exercise and postprandial triglycerides*

We observed a reduction in TAG AUC in response to the OFTT by approximately 20% in the submaximal high intensity interval exercise conditions compared to the control conditions. Acute prior exercise significantly lowered baseline TAG and there was a large effect size for lower TAG iAUC which contributed to the reduction in total AUC. Reductions in TAG AUC of a similar magnitude have been reported in response to moderate continuous exercise (12) and high intensity interval exercise (11, 33). We selected an individualised submaximal high intensity interval exercise protocol consistent with exercise intensity domains identified by analysis of expired ventilatory gasses measured during a CPET (26). Other submaximal high intensity interval exercise interventions that have successfully reduced postprandial lipaemia

lasted approximately 40 min and were stopped when participants had expended 500 kcal (11) or 660 kcal (33). We recruited an older, more overweight, and less active population with higher mean fasting triglyceride concentrations compared to these studies. For practical reasons (this is, to avoid unrealistic length of exercise sessions) and real life application, we predefined the 40 minute duration of high intensity interval exercise (rather than a target energy expenditure) and investigated the effects of individualised interventions at clearly defined exercise intensities. We believe this to be important because cardiorespiratory fitness is inversely related to cardio-metabolic health. Accordingly, participants with lower levels of cardiorespiratory fitness, exercising at the same relative intensity, will need to exercise for longer than a fitter individual to attain the same overall energy expenditure. Given the frequently cited barriers to exercise being time, it is unrealistic to expect an individual with poor cardiorespiratory fitness to exercise to attain a high total energy expenditure (>500 kcal) as this would typically require exercise sessions in excess of one hour. An exercise session duration of greater than one hour is in excess of recommended target guidelines for apparently healthy populations, which are seldom met (35). Therefore investigating the effects of acute exercise by predefining a fixed amount of time may be more ecologically valid. Furthermore, standard equations used for calculating energy expenditure from expired oxygen and carbon dioxide are inaccurate during interval exercise that involves exercise intensities above the anaerobic threshold. Therefore the validity of high intensity interval exercise interventions that use predefined estimated energy expenditure targets could be questioned. Finally, as considered later, total energy expenditure may not be the key mechanism involved in reducing postprandial triglycerides with high intensity interval exercise (2).

Interval exercise has the advantage of enabling a greater volume of work/energy expenditure to be completed within a period of time (21, 33), 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 (16), lower perceived work (19) and increased likelihood of continuing regular exercise (16, 19) in addition to the numerous cardio-metabolic benefits (14, 21) 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 (2, 33). This is important because TAG clearance appears to be the primary mechanism of reducing postprandial TAG after high intensity interval exercise (2). Many early exercise interventions designed to reduce postprandial lipids employed moderate intensity continuous exercise and it became widely accepted that estimated energy expenditure was central to these reductions (12). However, as reviewed by Burns and colleagues, the estimated energy expenditure during high intensity interval exercise interventions that reduce postprandial TAG appear to be lower than that during moderate intensity exercise interventions (2). Additionally, when estimated energy expenditure during exercise is matched, high intensity interval exercise has been shown to have a greater effect on reducing postprandial TAG (33).

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 (2). 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 greatly increased (33). Reductions in postprandial TAG with moderate intensity continuous exercise may still occur via this mechanism because type 2 muscle fibre recruitment increases with prolonged moderate intensity exercise. Exercise duration and energy expenditure for moderate intensity exercise are closely related, it is therefore possible that type 2 muscle fibre recruitment during prolonged moderate continuous exercise increases LPL activity in type 2 fibres. Higher

energy expenditure may reflect greater duration of exercise or exercise at higher intensities and thus increased type 2 muscle fibre recruitment. However, as already discussed, accurate assessment of energy expenditure during high intensity interval exercise is challenging and therefore comparison between moderate intensity exercise and high intensity interval exercise with regards to energy expenditure may be misleading. Further mechanistic investigations in to the effects of acute high intensity exercise induced attenuation in postprandial TAG excursions and fibre specific LPL activity would help to identify optimal exercise interventions for those at risk of cardio-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 oxidised LDL 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 (17). Compared to the present study, the high fat meal utilised in the study by Jenkins and colleagues (17) contained approximately 50g 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

(15) and therefore there may have been a greater capacity for reduction in oxidised LDL with exercise compared to the present study.

A reduction in lipid hydroperoxides with the exercise session performed either immediately before OFTT, or 1 hour after oral fat ingestion has been demonstrated previously (6, 23). 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 (17) or 60 (6, 23) min at an intensity of 70% VO2max (17), 60% predicted maximum heart rate (6) or 60% maximum heart rate (23). The timing of exercise and perhaps the mode of exercise required to reduce oxidative stress may therefore be important.

*Strawberry consumption and postprandial triglycerides*

In contrast to previous research (3), 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 (3) the present findings suggest that strawberry consumption may be detrimental to postprandial TAG.

Our OFTT had a higher fat content (73g versus 31g) and our carbohydrate content was considerably lower (33g versus 135g) compared to a previous study which demonstrated reduced TAG after OFTT with strawberry consumption (3). 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 (5). It was proposed by Chong and colleagues (2007) (5) 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 (3), the overall effect of fructose on the insulin response was likely minimal in this study. Further, strawberry polyphenols promote increased insulin sensitivity (9). This could potentially stimulate enhanced insulin mediated triglyceride storage in adipose tissue and thus increased triglyceride clearance from the circulation, when carbohydrate is high as was the case in the study by Burton-Freeman and colleagues (3).

*Strawberry consumption and postprandial oxidative stress*

There were no changes in oxidised LDL or lipid hydroperoxides between groups. Previous studies have demonstrated the benefits of strawberries on reducing postprandial oxidised LDL after lipid ingestion (3, 27). We gave a dose of strawberries (25g Freeze dried strawberries) which is similar to the optimal dose (20g) for lowering postprandial TAG identified by Park and colleagues (2016) (27). We used a higher fat content and specifically a higher dairy fat content in our OFTT meal compared to that of other studies (3, 27). 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 (4, 40). 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 (4). Notably, reduced circulating oxLDL and increased circulating strawberry polyphenols have been observed after consumption of a strawberry drink containing milk in humans (27). 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 (6, 23, 24) are reduced after anthocyanin intake from grapes (24). 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 hours before OFTT. We gave strict instructions to participants to abstain from alcohol, caffeine and trusted their adherence. This was the same for restricting physical activity beyond their habitual levels (which were self-reported to be below standard guidelines), other studies have attempted to measure activity levels during this period. Additionally, although the abbreviated 4 hour OFTT has been shown to predictive of the 8 hour time period (38) and is a repeatable test (25) it does not allow assessment of clearance of postprandial triglycerides (this is, chylomicrons and their remnants), which may have been useful to evaluate.

**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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