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Exercise prior to fat ingestion lowers fasting and postprandial VLDL and decreases adipose tissue IL-6 and GIP receptor mRNA in hypertriacylglycerolemic men

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Abstract

Fasting and postprandial triacylglycerol (TAG) concentrations are risk factors for cardiovascular disease. This study evaluated whether interleukin-6 (IL-6) and incretin hormones [gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1) (active)] were associated with fasting and postprandial TAG in response to an oral lipid load, including very-low-density lipoprotein (VLDL) and chylomicron (CM) TAG, following one bout of exercise in nine men (age, 59±2 years; body mass index, 34±2 kg/m^2; waist circumference, 113±3 cm) with high fasting TAG (2.9±0.2 mmol/L). Subjects completed two oral fat tolerance tests (OFTTs), randomized 1 week apart, that consisted of 1g fat/kg body weight emulsified lipids in the absence of carbohydrate and protein. Approximately 16 h prior to one OFTT, subjects completed 60 min of treadmill walking (estimated 55% VO_2 peak; heart rate, 122±4 beats/min). No exercise was performed on the day before the other OFTT. Fasted (0 h) and postprandial (1, 2, 3, 4, 5 and 6 h) blood samples were taken for analysis of TAG, IL-6 and incretins. Subcutaneous adipose tissue biopsies were taken at 0 and 6 h after OFTT ingestion for IL-6 and GIP receptor (GIPr) mRNA quantification. Exercise lowered fasting and postprandial TAG (P<.05) and VLDL TAG (P<.05), while postprandial CM TAG were similar in both OFTT trials (P>.05). Fasting and postprandial plasma IL-6, GIP and GLP-1 did not differ between rest and exercise OFTT trials (P>.05). Exercise reduced IL-6 and GIPr mRNA (P<.05) in adipose tissue. Our results suggest that the reduction in VLDL TAG following an acute bout of exercise is not associated with circulating IL-6 or incretin concentrations, despite reductions in the adipose tissue expression of IL-6 and GIPr.

Keywords: Exercise; Postprandial; Oral fat tolerance test; VLDL; Incretins; Inflammation; Adipose tissue biopsy

1. Introduction

Elevated postprandial triacylglycerol (TAG) concentration is an independent risk factor for cardiovascular disease (CVD) in men and women [1,2]. Furthermore, an exaggerated postprandial TAG response to an oral fat load occurs in individuals with high fasting TAG compared to well-matched individuals with low fasting TAG [3,4]. Fortunately, blood TAG levels may favorably respond to lifestyle interventions such as diet and exercise. Several studies have demonstrated that an acute bout of moderate exercise can reduce fasting and postprandial TAG concentrations. Past investigations have primarily focused on young [5–7] and older [8,9] healthy populations. Gill et al. [10] found that acute moderate exercise in lean and centrally obese middle-aged men resulted in ~25% decreases in both fasting and postprandial TAG. Despite strong evidence that a reduction in very-low-density lipoprotein (VLDL) is responsible for the TAG-lowering phenomenon, supportive studies have used either 1.5 h of walking [7,8] or 2 h of running between 50% and 60% VO_2 peak [11] and have not examined exercise in overweight/obese men with hypertriacylglycerolemia. Individuals with elevated fasting TAG also exhibit the hypotriacylglycerolemic effect of exercise [12]. However, the mechanisms behind the TAG-lowering effect of a single bout of exercise have not been fully explained.

Multiple tissues and systems, such as the liver, gastrointestinal system and adipose tissue, are involved in the regulation of TAG metabolism [13]. In addition to well-recognized factors associated with TAG-rich lipoprotein (TRL) metabolism such as lipoprotein lipase, other factors such as adipokines and incretin hormones are secreted from these tissues and can have metabolic consequences. Interleukin-6 (IL-6) is produced by adipose tissue [14] and exercising muscle [15], and while it acutely stimulates lipolysis and fat oxidation [16], it is also a known CVD risk factor [17,18]. We have previously demonstrated that men with high fasting TAG also exhibit an elevated postprandial IL-6 concentration [4]. In rats, an IL-6 infusion significantly increased fasting TAG, further illustrating a relationship between these CVD factors [19]. The incretin hormone gastric inhibitory peptide (GIP) is also elevated in men with high fasting TAG [20]. On the other hand, GIP administration decreases chylomicron (CM) TAG in dogs undergoing a postprandial challenge [21]. Both GIP and glucagon-like peptide-1 (GLP-1) are responsive to fat

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ingestion [22,23], and intravenous administration of GLP-1 completely abolished the postprandial rise in TAG following a high-fat meal [24]. Furthermore, adipose tissue expresses a GIP receptor (GIPr) [25], but the impact of dietary fat and exercise on expression is unknown. While exercise may modulate circulating incretin concentrations [26], it is unknown whether these gut-derived factors are involved in the TAG-lowering effect of exercise.

The purpose of this study was to evaluate the postprandial response to an oral fat load consumed 16 h after 60 min of moderate walking exercise in men with elevated (>2.0 mmol/L) fasting TAG concentrations to determine whether IL-6 and/or incretin hormones are also changed and thus may contribute to the TAG-lowering phenomenon. A previously developed oral fat tolerance test (OFTT) [27] that delivers a fat load consisting solely of emulsified lipids was used as fat challenge. This pure lipid challenge allows for a clear investigation of postprandial response to fat alone. We hypothesized that: (a) the exercise-induced reduction in postprandial TAG will be associated with a decrease in VLDL and CM; however, the proportional decrease will be such that VLDL decreases in a greater manner following exercise; (b) exercise will decrease fasting and postprandial IL-6 and will alter incretin hormones (decrease in GIP and increase in GLP-1); and (c) exercise will decrease the expression of IL-6 and GIPr in subcutaneous adipose tissue.

2. Materials and methods

2.1. Materials

Palm stearin, soybean oil and bleaching clay (Engelhard F105) were generously provided by Bunge Canada (Toronto, ON). Sodium methoxide (Sigma Aldrich) and citric acid (VWR International) were used in lipid interesterification. Myverol 18-99K and Tween80 (both from Nealanders International, Oakville, ON) were used to prepare the emulsified OFTT beverages, which included a commercial food flavoring agent (David Michaels and Co., Philadelphia, PA) and a noncarbohydrate sweetener (SugarTwin Liquid Sweetener; Alberto-Culver, Melrose Park, IL).

2.2. OFTT preparation

To study postprandial metabolic responses, we produced an emulsified, artificially flavored and sweetened beverage that was devoid of protein or carbohydrate, as previously described in detail [27]. Briefly, prior to the experimental trials, a fat blend of palm stearin (63% palmitic acid; 16:0) and soybean oil (53% linoleic acid; 18:2$n-6$) was prepared to achieve the desired P/S ratio of 1.0. The blend was chemically interesterified in order to achieve a completely random distribution of the fatty acids present [27]. The fatty acid composition of the OFTT was confirmed (Table 1) by gas chromatography, as previously described [27].

Table 1
Fatty acid composition of the OFTT beverage consumed by the subjects

Fatty acid[a]	% wt/wt total fatty acids
12:0	0.7
14:0	0.7
16:0	32.0
18:0	4.4
18:1 c9	22.1
18:1 t9	0.6
18:1 c11	0.5
18:2 $n-6$	32.2
18:3 $n-6$	0.3
18:3 $n-3$	4.6
20:0	0.4
22:0	0.3
Total saturated fatty acid	38.5
Total monounsaturated fatty acid	23.1
Total polyunsaturated fatty acid	37.1
P/S ratio[b]	1.0

[a] Only major fatty acids (i.e., >0.2) are presented.
[b] P/S ratio was determined by dividing the total polyunsaturated fatty acid by the total saturated fatty acid.

2.3. Subjects and preliminary screening

This study was approved by the University of Guelph Research Ethics Board. Ten males were recruited to participate in this study through a combination of newspaper, poster and website advertisements. Subjects were required to be between 40 and 70 years of age and to be nonsmokers. In addition, a body mass index (BMI) greater than 27 kg/m^2 was required for inclusion in the study. Individuals taking medications for control of blood lipids and/or inflammation were excluded from participation in this study. Subjects were asked to read and to complete an informed consent form identifying potential risks associated with participation in the study. Subjects were required to attend one pretrial session to complete a subject screening questionnaire and blood screening, and to have weight and height measured. During the preliminary screening visit, subjects arrived in the laboratory following a 12-h fast and following instruction to refrain from exercise for 48 h prior to the visit to the laboratory. Plasma sample was obtained by a medically trained technician via venous puncture for immediate analysis of TAG using Cholestech LDX lipid cassettes (Cholestech Corp., Hayward, CA). To be considered for the study, all subjects were required to have a fasting plasma TAG concentration of ≥2.0 mmol/L. All subjects who met the TAG requirement were required to undergo a 2-h screening with 75 g of oral glucose tolerance test for assessment of glucose tolerance. Any person meeting the criteria for type 2 diabetes [28] was excluded from the study and instructed to consult a physician. Subjects who met all inclusion criteria had their waist circumference measured and body composition determined using bioelectrical impedance analysis (Bodystat, Tampa, FL).

2.3.1. Exercise

All participants were required to attend a preliminary submaximal incremental treadmill exercise session approximately 1 week prior to the OFTTs to determine the speed and gradient required to perform exercise at an estimated 55% VO_2 peak using the heart rate reserve method in accordance with the *ACSM's Guidelines for Exercise Testing and Prescription* [29]. Heart rate was recorded by short-range telemetry (Polar Electro, Lake Success, NY). Subjects then completed a randomized cross-over study consisting of one exercise OFTT and one rest OFTT separated by at least 1 week. On the afternoon (approximately 16 h) before the exercise OFTT, all participants completed 60 min of treadmill walking, maintaining the predetermined speed and gradient. No exercise was performed prior to the rest OFTT.

2.3.2. Pretrial requirements

All subjects were instructed to record their food intake for the 3 days prior to each of the OFTT trials (i.e., a total of 6 days for the entire study). In addition, subjects consumed a standardized meal on the evening prior to each OFTT. Subjects were also required to abstain from alcohol and additional exercise for 48 h prior to each OFTT trial and to arrive at the laboratory following a 12-h fast.

2.4. Study design

2.4.1. Beverage preparation

On each trial day, 1 g lipids/kg subject body weight of the 1.0 OFTT blend was measured into a glass mug. The fat was warmed to approximately 50°C and subsequently emulsified in water (73% wt/wt). To maintain the emulsified pure fat challenge, we added the monoglyceride Myverol (2% wt/wt) and the polysorbate Tween 80 (0.15% wt/wt) using a handheld homogenizer. Each OFTT was sweetened with nonnutritive liquid sweetener (SugarTwin Liquid Sweetener) and flavored with a commercially designed flavor (David Michaels and Co.).

2.5. Experimental trials

Upon the subjects' arrival at the laboratory, a catheter was inserted into a forearm vein for the withdrawal of blood samples. Saline was administered to maintain the catheter for repeated blood sampling. On each day, following an initial fasting blood sample (0 h), subjects ingested the OFTT. Blood was taken at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h following the OFTT. A subcutaneous adipose tissue sample (~200–300 mg) was obtained by a physician using the percutaneous needle biopsy technique with suction under local anesthetic prior to the start of each OFTT trial (0 h) and at 6 h postingestion of the OFTT. Adipose tissue samples were immediately flash frozen in liquid nitrogen and remained frozen at −80°C until analysis.

2.6. Blood glucose and plasma TAG measurements

Blood glucose was analyzed in duplicate by a glucose oxidase method (YSI 2300 Stat Plus Glucose Analyzer; Stat Plus, Yellow Springs, OH). At three designated time points (0, 3 and 6 h), 9 ml of blood was drawn into EDTA-treated tubes for plasma TAG analysis. Blood collection tubes were spun immediately at 1000×*g* for 15 min at 4°C. The plasma was collected, pooled for each subject and time point, packaged on ice and shipped overnight to the University of Ottawa for CM and VLDL TAG and cholesterol analysis. Upon receipt, samples were ultracentrifuged in a 50Ti fixed-angle rotor (Beckman Coulter, Inc., Mississauga, ON) at 40,000 rpm for 18 h at 10°C. The lipoprotein fraction VLDL (d<1.006 g/ml) and the sum of intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL)