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## Influence of acute exercise with and without carbohydrate replacement on postprandial lipid metabolism

Michael Harrison,<sup>1</sup> Donal J. O’Gorman,<sup>2</sup> Noel McCaffrey,<sup>2</sup> Marc T. Hamilton,<sup>3</sup> Theodore W. Zderic,<sup>3</sup> Brian P. Carson,<sup>2</sup> and Niall M. Moyna<sup>2</sup>

<sup>1</sup>Department of Health, Sport and Exercise Science, Waterford Institute of Technology, Waterford, Ireland; <sup>2</sup>School of Health and Human Performance, Dublin City University, Dublin, Ireland; and <sup>3</sup>Department of Biomedical Sciences, University of Missouri, Columbia, Missouri

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**Harrison M, O’Gorman DJ, McCaffrey N, Hamilton MT, Zderic TW, Carson BP, Moyna NM.** Influence of acute exercise with and without carbohydrate replacement on postprandial lipid metabolism. *J Appl Physiol* 106: 943–949, 2009. First published December 26, 2008; doi:10.1152/jappphysiol.91367.2008.—Acute exercise, undertaken on the day before an oral fat tolerance test (OFTT), typically reduces postprandial triglycerides (TG) and increases high-density lipoprotein-cholesterol (HDL-C). However, the benefits of acute exercise may be overstated when studies do not account for compensatory changes in dietary intake. The objective of this study was to determine the influence of acute exercise, with and without carbohydrate (CHO) replacement, on postprandial lipid metabolism. Eight recreationally active young men underwent an OFTT on the morning after three experimental conditions: no exercise [control (Con)], prolonged exercise without CHO replacement (Ex-Def) and prolonged exercise with CHO replacement to restore CHO and energy balance (Ex-Bal). The exercise session in Ex-Def and Ex-Bal consisted of 90 min cycle ergometry at 70% peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) followed by 10 maximal 1-min sprints. CHO replacement was achieved using glucose solutions consumed at 0, 2, and 4 h postexercise. Muscle glycogen was  $40 \pm 4\%$  ( $P < 0.05$ ) and  $94 \pm 3\%$  ( $P = 0.24$ ) of Con values on the morning of the Ex-Def and Ex-Bal OFTT, respectively. Postprandial TG were  $40 \pm 14\%$  lower and postprandial HDL-C, free fatty acids, and 3-hydroxybutyrate were higher in Ex-Def compared with Con ( $P < 0.05$ ). Most importantly, these exercise effects were not evident in Ex-Bal. Postprandial insulin and glucose and the homeostatic model assessment of insulin resistance (HOMA<sub>IR</sub>) were not significantly different across trials. There was no relation between the changes in postprandial TG and muscle glycogen across trials. In conclusion, the influence of acute exhaustive exercise on postprandial lipid metabolism is largely dependent on the associated CHO and energy deficit.

postprandial lipemia; high-density lipoprotein-cholesterol; energy deficit; glycogen

EPIDEMIOLOGICAL EVIDENCE links high postprandial triglycerides (TG) and low levels of high-density lipoprotein-cholesterol (HDL-C) (15) to the development of atherosclerotic cardiovascular disease. Evidence exists from animal and human studies that the remnants of TG-rich lipoproteins (TGRL) are atherogenic (34). In addition, a postprandial TG-rich environment of prolonged duration can exert an atherogenic influence on other lipoproteins, including HDL particles (30). As humans spend the majority of each 24-h period in a postprandial state,

strategies are required to reduce postprandial lipemia and increase HDL-C.

Acute exercise attenuates the magnitude and duration of postprandial lipemia (13, 36) and increases HDL-C (8). It has been suggested that much of the influence of regular exercise on TG metabolism and HDL-C is acute in nature and likely to be related to factors associated with exercise energy expenditure (35). The exercise effect on postprandial lipemia is independent of exercise intensity, with isocaloric sessions of different intensity resulting in similar reductions (36). Postprandial lipemia decreases (13) and HDL-C increases (8) in a dose-dependent manner with increasing exercise energy expenditure. It does not necessarily follow, however, that these exercise effects are mediated by an energy or substrate deficit per se. The effect of acute exercise on postprandial lipemia is considerably greater than a similar dietary-induced energy deficit (12). It is possible that exercise energy expenditure is a surrogate for other perturbations associated with the increasing duration of physical activity in these studies. Clarity is needed as to whether the exercise effects on postprandial lipemia and HDL-C are dependent on, or independent of, energy and substrate deficits.

Studies of acute exercise may not be representative of real-life conditions as they often fail to address the issue of compensatory increases in energy intake. There is evidence of increased appetite during the later stages of recovery following acute exercise (25). The internal validity of exercise studies that do not control energy status has recently been questioned, as energy deficits can have a profound effect on metabolism (4). Studies of postprandial lipemia typically examine the effects of a single exercise session undertaken on the day before an oral fat tolerance test (OFTT), but without dietary adjustment to take account of energy expenditure during exercise. In one recent study (5), however, energy replacement achieved with additional carbohydrate (CHO), fat, and protein attenuated the effects of prior walking on postprandial lipemia. As that exercise and diet combination created and largely replenished both a CHO and fat deficit, the relative importance of each is unclear.

In this study the influence of acute exercise on postprandial lipid metabolism was determined with and without postexercise CHO replacement to restore CHO and energy balance. The vigorous exercise protocol, culminating with 10 maximal sprints, aimed to maximize CHO oxidation. Consequently, we

Address for reprint requests and other correspondence: M. Harrison, Dept. of Health, Sport and Exercise Science, Waterford Institute of Technology, Waterford, Ireland (e-mail: mharrison@wit.ie).

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felt justified in refeeding with glucose. We hypothesized that any protective effects of exercise on postprandial lipemia and HDL-C would be attenuated by restoring CHO and energy balance in advance of the OFTT.

## METHODS

**Subjects.** Eight recreationally active men [mean  $\pm$  SD: age 26.9  $\pm$  4.1 yr, peak oxygen uptake ( $\dot{V}O_{2peak}$ ) 46.8  $\pm$  4.9 ml $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ , body mass 83.5  $\pm$  13.7 kg, body mass index (BMI) 26.0  $\pm$  3.6 kg/m, %body fat 15.2  $\pm$  5.0%] volunteered for this study. Subjects were nonsmokers, normolipidemic, free from cardiovascular disease and diabetes, and not taking medication known to influence carbohydrate or lipid metabolism. Ethical approval was granted by the Dublin City University Research Ethics Committee and the University of Missouri Institutional Review Board. Written informed consent was also obtained.

**Experimental design.** Subjects underwent three 6-h oral fat tolerance tests (OFTT) in random order, separated by 7 days approximately (Fig. 1). As the effects of exercise training on postprandial lipemia are lost within 60 h of the last training session (18), a 7-day washout period between trials was considered sufficient. On the evening before one OFTT, subjects rested quietly at home [control (Con)]. On the evening before two OFTTs, subjects exercised in the laboratory. Following one of these exercise sessions, glucose was consumed to restore CHO and energy balance (Ex-Bal). Following the other exercise session (Ex-Def), only water was permitted, thus maintaining a CHO and energy deficit.

**Preliminary measurements.** During a preliminary visit, subjects underwent a physical examination, had their body composition assessed, and  $\dot{V}O_{2peak}$  determined.  $\dot{V}O_{2peak}$  was determined via a breath-by-breath metabolic system (Vmax 229, SensorMedics, Yorba Linda, CA) during an incremental exercise test with 2-min stages on a cycle ergometer (Monark, Vansbro, Sweden). The initial workload and increments were individualized so that the test was 8–12 min in duration. The workload corresponding to 70%  $\dot{V}O_{2peak}$  was determined during a subsequent submaximal test from the relation between oxygen uptake and workload. Percentage body fat was estimated from the sum of seven skinfolds using the Jackson and Pollock equation (20).

**Exercise session and energy expenditure.** The experimental exercise sessions were conducted between 1700 and 1900 on the evening before the OFTT. Subjects cycled for 90 min at a load equaling 70%  $\dot{V}O_{2peak}$ , followed by ten 1-min full-effort sprints interspersed with 1 min of resting recovery. Flywheel resistance was increased by 25% for the sprints, and the flywheel revolutions were recorded every 20 s to estimate energy expenditure. Expired air was collected continuously during the continuous cycling. Energy expenditure and substrate oxidation were estimated using indirect calorimetry (32). Energy

expenditure during the sprints was estimated from an American College of Sports Medicine metabolic equation (1) based on flywheel resistance and revolutions. Heart rate was recorded continuously using short-range telemetry (Polar Electro Oy).

**CHO replacement.** Following one of the exercise sessions (Ex-Bal), CHO was provided. Subjects consumed 105% of the CHO oxidized during exercise (4.4  $\pm$  0.2 g/kg body mass) to restore both CHO and energy balance (Table 1). High-glycemic index CHO was delivered in the form of an 18% CHO drink and 85% glucose confectionary. The CHO was divided into three equal boluses and consumed immediately and 2 h and 4 h postexercise. Thus CHO replacement was complete 4 h postexercise and at least 10 h before the subsequent OFTT. Water was consumed at equivalent time points in Con and Ex-Def.

**Dietary control.** Subjects were required to abstain from alcohol and not to engage in exercise or heavy physical work for 3 days before each OFTT. On the day before each OFTT, diet was strictly controlled with subjects consuming three meals provided by the laboratory. The meals consisted of breakfast cereal, milk, toast, butter, chocolate biscuits, and ham and cheese sandwiches. This pre-OFTT diet was individualized to provide an energy content equal to 1.4 times basal metabolic rate (BMR), with 56% as CHO, 30% as fat, and 30% as protein. BMR was estimated from the Harris-Benedict equation (16). On the 2 days before this, subjects consumed their normal diet. Food items consumed along with portion size were recorded on sheets provided, before the first OFTT. This diet was then replicated in advance of subsequent OFTTs. Subjects were reminded of these diet and activity requirements during phone contact in advance of each OFTT. Compliance was checked verbally on the morning of each OFTT.

**OFTTs.** Subjects traveled to the laboratory on the morning of each OFTT by motorized transport. They had been fasting for at least 10 h. An intravenous catheter was inserted into a forearm vein and a fasting blood sample obtained (0 h). This catheter was kept patent during the 6 h postprandial follow-up period by flushing regularly with a 0.9% saline solution. The test meal consisted of croissants, butter, high-fat ice cream, chocolate, and potato crisps with a macronutrient composition per 2 m $^2$  body surface area of 97 g fat, 124 g CHO, and 1,450 kcal. The high-fat mixed meal had a considerable CHO content to ensure an insulin response, as insulin plays a crucial role in regulating postprandial TG metabolism. The meal was well tolerated. Water intake was ad libitum during the first trial. This pattern of water intake was recorded and repeated during subsequent trials. Subjects rested quietly in the laboratory during the observation period with blood sampled at 0.5, 1, 2, 4, and 6 h postprandially.

**Muscle biopsy and muscle glycogen.** A muscle biopsy was obtained from the midway point of the vastus lateralis after the venous cannula was inserted and immediately before each OFTT. Biopsies were obtained from alternate legs for different trials after local anesthesia

Fig. 1. Experimental design. Con, control trial (no exercise); Ex-Def, exercise deficit trial (prolonged exercise without carbohydrate replacement); Ex-Bal, exercise balance trial (prolonged exercise with postexercise carbohydrate replacement).

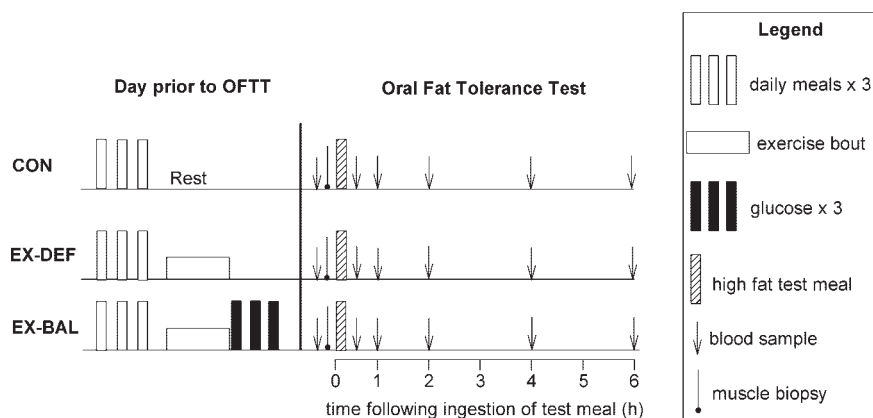


Table 1. Energy and carbohydrate oxidized during exercise and replaced in the hours postexercise in Ex-Bal

	Oxidized	Refed
Energy, kcal	1,507±75	1,420±69
Carbohydrate, g	349±17	367±18
Fat, g	16±2	0

Values are means ± SE; *n* = 8. Ex-Bal, prolonged exercise with carbohydrate replacement.

with 2% lidocaine. Samples were snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis. Frozen muscle samples were freeze-dried and dissected free of connective tissue. Glycogen concentrations were determined at a later stage in duplicate by a standard enzymatic technique with fluorimetric detection (29). Briefly, 2 mg freeze-dried muscle was allowed to thaw to  $-15^{\circ}\text{C}$ . Muscle was incubated in 0.5 ml of 2 N hydrochloric acid for 2 h at  $100^{\circ}\text{C}$ . Samples were then reconstituted to original weight with distilled  $\text{H}_2\text{O}$  before being neutralized with 1.5 ml of 0.67 N NaOH. One milliliter of reagent mix containing Tris base, HCl,  $\text{MgCl}_2$ , DTT, ATP, NADP, hexokinase, and glucose-6-phosphate dehydrogenase was added to samples and glycogen content determined by fluorimeter. The coefficient of variation for this assay was 3.6%.

**Serum analyses.** Serum was centrifuged at 1,600 g for 15 min at  $4^{\circ}\text{C}$ . Serum TG, HDL-C, 3-hydroxybutyrate (3-HB), free fatty acids (FFA), and glucose were determined using spectrophotometric assays. The spectrophotometric analyses were performed on an automated bench-top clinical chemistry system (ACE, Alfa Wassermann) using appropriate reagents, calibrators, and controls (Randox Laboratories). Serum insulin was determined by fluoroimmunoassay using a commercially available kit (Perkin-Elmer, Wellesley, MA). Hematocrit values and hemoglobin concentrations were determined from an EDTA whole blood sample using an automated hematology analyzer (AcTdiff2, Beckman Coulter, Fullerton, CA). The intra-assay coefficients of variations were <3% for the spectrophotometric assays and the fluoroimmunoassay.

**Data analysis.** Postprandial responses to the test meal are summarized as time-averaged postprandial values. Time-averaged postprandial values represent the total area under the concentration vs. time curve (AUC), calculated using the trapezoidal rule, divided by the length of the postprandial period (6 h). The time-averaged TG increment represents the AUC above fasting values, divided by the length of the postprandial period. Insulin resistance was estimated from fasting insulin and glucose values using the homeostatic model assessment ( $\text{HOMA}_{\text{IR}}$ ) method (26).

The study data including the TG data were normally distributed. The significance of time-averaged postprandial differences was examined using a one-way (trial) repeated-measures ANOVA. Changes over the postprandial period were examined using a two-way (trial × time) repeated-measures ANOVA with between-trial differences at individual time points examined post hoc. The Fisher least significant difference post hoc test was used to identify where differences lay if the *F* ratio was significant. Associations between selected variables were determined using Pearson correlations. Data are reported as means ± SE. Significance was set at  $P < 0.05$ .

## RESULTS

The work rate ( $197 \pm 9$  vs.  $197 \pm 9$  W),  $\dot{V}\text{O}_2$  ( $33.4 \pm 1.1$  vs.  $33.2 \pm 1.0$   $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), respiratory exchange ratio ( $0.96 \pm 0.005$  vs.  $0.96 \pm 0.004$ ), and heart rate ( $151 \pm 3$  vs.  $150 \pm 4$  beats/min) during the 90 min of continuous cycling did not differ in Ex-Def and Ex-Bal, respectively. The work rate during the Ex-Def and Ex-Bal sprints was  $282 \pm 12$  and  $290 \pm 17$  W, respectively, equivalent to  $\sim 93 \pm 3\%$   $\dot{V}\text{O}_{2\text{peak}}$ .

Estimates of CHO oxidation ( $352 \pm 21$  vs.  $349 \pm 17$  g), fat oxidation ( $15 \pm 2$  vs.  $16 \pm 2$  g), and energy expenditure ( $1,509 \pm 78$  vs.  $1,507 \pm 75$  kcal) in Ex-Def and Ex-Bal exercise sessions (continuous cycling and sprints) were also similar. As mean hemoglobin ( $P = 0.92$ ) and hematocrit ( $P = 0.69$ ) were similar in Con ( $13.9 \pm 0.4$  g/dl and  $41.8 \pm 1.0\%$ ), Ex-Def ( $14.0 \pm 0.3$  g/dl and  $41.2 \pm 1.1\%$ ) and Ex-Bal ( $14.0 \pm 0.2$  g/dl and  $41.5 \pm 0.7\%$ ), serum concentrations were not adjusted for changes in plasma volume. Muscle glycogen was  $40 \pm 4\%$  ( $P < 0.05$ ) and  $94 \pm 3\%$  ( $P = 0.24$ ) of the Con value on the morning of the Ex-Def and Ex-Bal OFTT, respectively (Fig. 2).

The influence of the test meal on serum TG in Con, Ex-Def, and Ex-Bal is shown in Fig. 3A. Postprandial TG were lower ( $P < 0.05$ ) in Ex-Def (Cohen's  $d = -0.92$ ) compared with Con and Ex-Bal (Fig. 4). Values were not different between Con and Ex-Bal ( $P = 0.29$ ) (Fig. 4). Postprandial TG increment was lower in Ex-Def compared with Con. In Ex-Bal, postprandial TG increment did not differ significantly from Ex-Def or Con values (Table 2). Fasting TG were closely related to postprandial TG in Con ( $r = 0.92$ ,  $P < 0.05$ ), Ex-Def ( $r = 0.98$ ,  $P < 0.05$ ), and Ex-Bal ( $r = 0.76$ ,  $P < 0.05$ ). There was no relation between the change in muscle glycogen and the change in any index of lipemia (fasting TG, postprandial TG, or postprandial TG increment) across trials. HDL-C did not change during the postprandial period. Postprandial HDL-C was similar in Con and Ex-Bal with values higher ( $d = 0.77$ ) ( $P < 0.05$ ) in Ex-Def (Fig. 4). There was no relation between the change in HDL-C and the change in any index of lipemia across trials.

The influence of the test meal on serum glucose, insulin, FFA, and 3-HB in Con, Ex-Def, and Ex-Bal is shown in Fig. 3, B–E. Postprandial insulin, postprandial glucose, and  $\text{HOMA}_{\text{IR}}$  were not significantly different across trials (Table 2). Fasting glucose was similar in Con and Ex-Bal but lower ( $P < 0.05$ ) in Ex-Def. Postprandial FFA and 3-HB were higher ( $P < 0.05$ ) in Ex-Def than in Con and Ex-Bal (Table 2). There was no relation between the change in 3-HB and the change in any index of lipemia across trials.

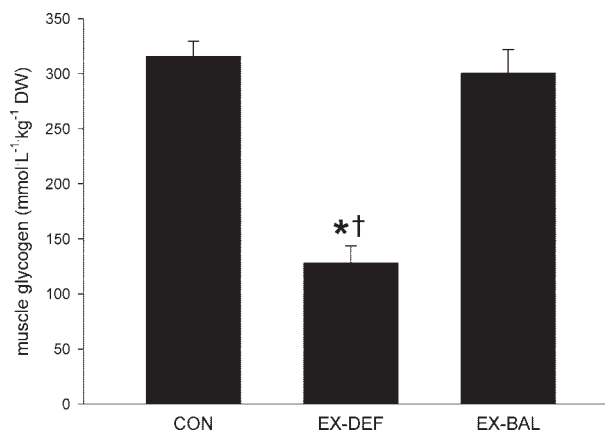


Fig. 2. Values are means ± SE; *n* = 8. Muscle glycogen concentrations on the morning following no exercise (Con), prolonged exercise without carbohydrate replacement (Ex-Def), and prolonged exercise with carbohydrate replacement (Ex-Bal). DW, dry weight. Differences assessed using 1-way repeated-measures ANOVA followed by least significant difference (LSD) post hoc test. \*Con vs. Ex-Def,  $P < 0.05$ . †Ex-Def vs. Ex-Bal,  $P < 0.05$ . Con vs. Ex-Bal,  $P =$  nonsignificant (NS).

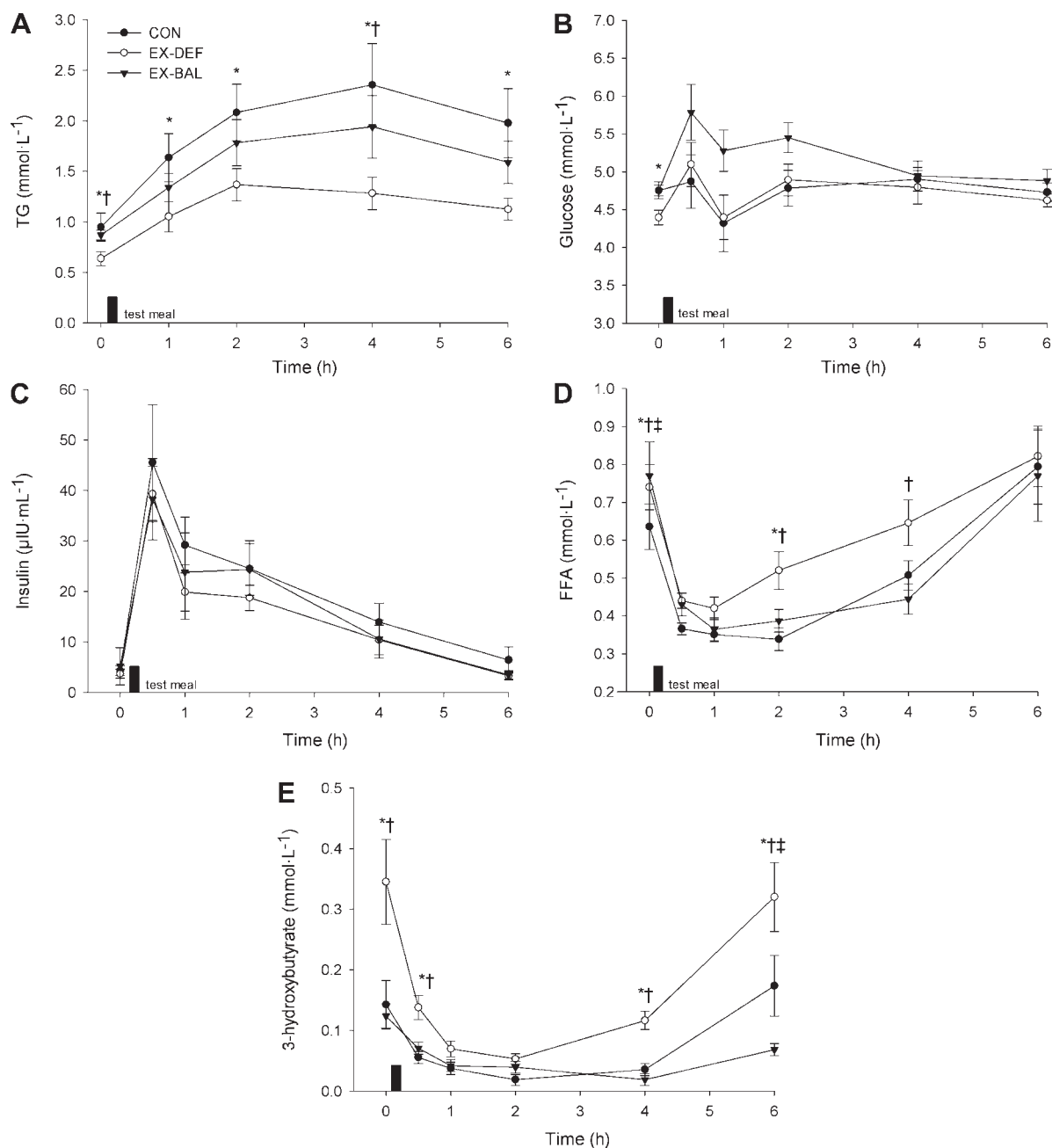


Fig. 3. Values are means  $\pm$  SE;  $n = 8$ . Triglyceride (TG) (A), glucose (B), insulin (C), free fatty acid (FFA) (D), and 3-hydroxybutyrate (E) response to a high-fat meal on the morning following no exercise (Con), prolonged exercise without carbohydrate replacement (Ex-Def), and prolonged exercise with carbohydrate replacement (Ex-Bal). Differences assessed using 2-way repeated-measures ANOVA followed by LSD post-hoc. \*Con vs. Ex-Def,  $P < 0.05$ . †Ex-Def vs. Ex-Bal,  $P < 0.05$ . ‡Con vs. Ex-Bal,  $P < 0.05$ .

## DISCUSSION

The major finding of this study is that the effects of acute exercise on fasting and postprandial lipids are dependent, at least in part, on the associated CHO and energy deficit. No exercise effect on fasting TG or HDL-C was evident when CHO replacement occurred. The effect on postprandial TG was significantly attenuated by CHO replacement, although the possibility still exists that a minor portion of this exercise effect is independent of the associated CHO and energy deficit. No exercise effect on postprandial FFA or 3-HB was evident when CHO replacement occurred. There were no exercise effects on

postprandial insulin or glucose in this study, with or without CHO replacement. In contrast to the study of Burton et al. (5) in which subjects walked for  $\sim 90$  min, the exercise session employed in this study was vigorous and exhausting. Thus the absence of independent exercise effects on postprandial lipemia and HDL-C cannot be attributed to a suboptimal exercise stimulus.

The relative importance of an energy, CHO, and fat deficit to the acute exercise effect on postprandial lipemia is open to debate. The reduction in postprandial lipemia observed in this study (%change and effect size) is one of the largest docu-

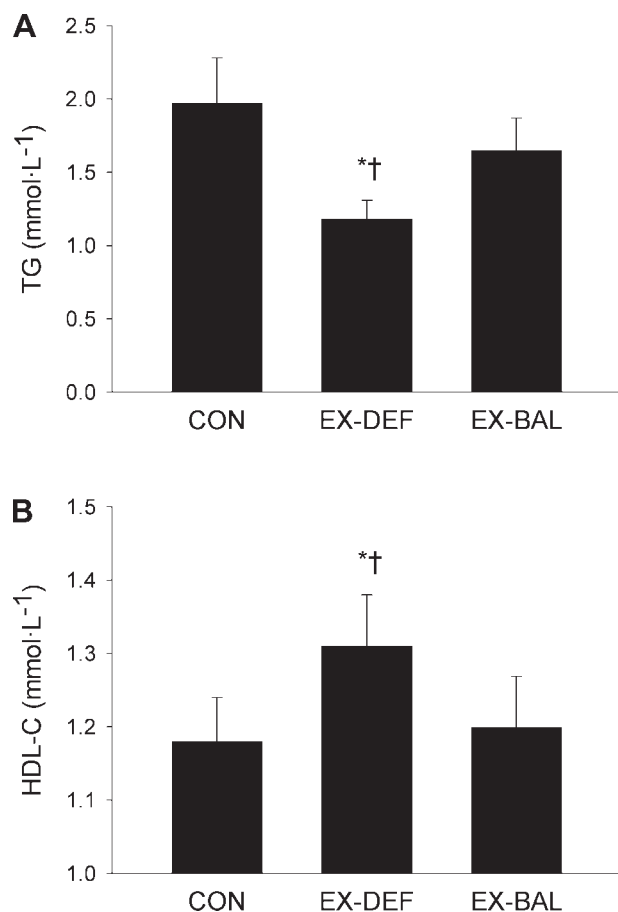


Fig. 4. Values are means  $\pm$  SE;  $n = 8$ . Time-averaged postprandial TG (A) and high-density lipoprotein-cholesterol (HDL-C) (B) on the morning following no exercise (Con), prolonged exercise without carbohydrate replacement (Ex-Def), and prolonged exercise with carbohydrate replacement (Ex-Bal). Differences assessed using 1-way repeated-measures ANOVA followed by LSD post hoc test. \*Con vs. Ex-Def,  $P < 0.05$ . †Ex-Def vs. Ex-Bal,  $P < 0.05$ .

mented in reviews examining the influence of previous day exercise on postprandial lipemia (14, 33). This reduction was achieved with one of the lowest contributions of fat oxidation to total energy expenditure and reversed with glucose refeeding. Consequently our results may indicate a particular importance for a CHO deficit over a fat deficit in mediating the exercise effects on postprandial metabolism. Koutsari et al. (22) documented the importance of short-term changes in CHO balance in a study examining the influence of isocaloric low-CHO and high-CHO diets on postprandial lipemia, with and without exercise (22). In that study, postprandial lipemia was highest on a high-CHO diet but similarly reduced on a low-CHO diet without exercise and a high-CHO diet with accompanying exercise. Taken together these studies suggest that exercise can oppose the hypertriglyceridemic effects of a high CHO intake, and restoration of CHO balance can oppose the hypotriglyceridemic effects of exercise. Although less likely, the primacy of the total energy deficit over a specific CHO or fat deficit also deserves consideration. Substrate utilization during exercise has been manipulated by administering acipimox to inhibit lipolysis while holding total energy expenditure constant (24). The exercise attenuation of postprandial lipemia was similar regardless of substrate utilization. However, the

effect of this manipulation on CHO oxidation during exercise (%difference between trials) was relatively small. The importance of the total energy deficit in mediating these acute exercise effects remains to be investigated in future studies by maximizing the contribution of one substrate to energy expenditure during exercise while adjusting the diet with another.

Dietary-induced carbohydrate deficits, particularly following days of CHO restriction, can also exert a considerable influence on lipid metabolism. The exercise effects on TG, HDL-C, FFA, and 3-HB documented in the present study have all been reported previously when individuals switch to a low-CHO diet (9, 17, 22, 31, 37). However, the relative importance of tissue-specific CHO deficits vs. a whole body CHO deficit is unclear (37). Fasting glucose was reduced during Ex-Def and restored during Ex-Bal in the present study. However, postprandial glucose was similar across trials. As acute exercise frequently reduces postprandial lipemia in the absence of changes in glucose (5, 12, 13, 22, 24, 27, 36), we do not believe that the Ex-Def decrease in fasting glucose was of primary importance in explaining the observed changes in TG, HDL-C, FFA, and 3-HB. Muscle glycogen was also reduced in Ex-Def and restored in Ex-Bal without supercompensation. There is evidence of a regulatory role for muscle glycogen content in substrate metabolism (37). Low muscle glycogen, manipulated through exercise and diet, results in higher FFA and norepinephrine concentrations. In addition, reductions in postprandial lipemia can be achieved with short sessions of moderate-intensity exercise that are unlikely to deplete liver glycogen (27). However, the changes in muscle glycogen across trials were not correlated with the changes in postprandial lipemia or FFA in the present study. Data from Casey et al. (6) suggest that liver glycogen may have been similarly depleted during strenuous exercise and restored with the CHO replacement protocol. There is some evidence of a regulatory role for liver glycogen in lipid metabolism (17). Changes in TG, insulin, FFA, and 3-HB have been documented during a

Table 2. Fasting and time-averaged postprandial values on the morning following no exercise (Con), prolonged exercise without carbohydrate replacement (Ex-Def), and prolonged exercise with carbohydrate replacement (Ex-Bal)

	Con	Ex-Def	Ex-Bal
Fasting TG, mmol/l	0.95 $\pm$ 0.13	0.63 $\pm$ 0.07*	0.87 $\pm$ 0.05
Postprandial TG, mmol/l	1.97 $\pm$ 0.31	1.17 $\pm$ 0.13*	1.65 $\pm$ 0.22
Postprandial TG increment, mmol/l	1.02 $\pm$ 0.18	0.54 $\pm$ 0.06†	0.78 $\pm$ 0.18
Fasting HDL-C, mmol/l	1.20 $\pm$ 0.07	1.30 $\pm$ 0.08*	1.20 $\pm$ 0.07
Postprandial HDL-C, mmol/l	1.18 $\pm$ 0.06	1.31 $\pm$ 0.07*	1.20 $\pm$ 0.07
Fasting insulin, mmol/l	4.30 $\pm$ 0.96	3.70 $\pm$ 0.90	5.19 $\pm$ 1.30
Postprandial insulin, mmol/l	19.5 $\pm$ 4.2	14.6 $\pm$ 1.9	18.4 $\pm$ 3.4
Fasting glucose, mmol/l	4.75 $\pm$ 0.07	4.40 $\pm$ 0.09*	4.75 $\pm$ 0.11
Postprandial glucose, mmol/l	4.76 $\pm$ 0.12	4.72 $\pm$ 0.08	5.11 $\pm$ 0.12
HOMA <sub>IR</sub>	0.92 $\pm$ 0.21	0.74 $\pm$ 0.20	1.10 $\pm$ 0.31
Postprandial FFA, mmol/l	0.49 $\pm$ 0.03	0.60 $\pm$ 0.04*	0.49 $\pm$ 0.04
Postprandial 3-hydroxybutyrate, mmol/l	0.093 $\pm$ 0.03	0.209 $\pm$ 0.03*	0.063 $\pm$ 0.01

Values are means  $\pm$  SE;  $n = 8$ . TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; HOMA<sub>IR</sub>, homeostatic model assessment of insulin resistance; FFA, free fatty acids; Ex-Def, prolonged exercise without carbohydrate replacement. Differences assessed using 1-way repeated-measures ANOVA followed by least significant difference post hoc test. \* $P < 0.05$  compared with Con and Ex-Bal. † $P < 0.05$  compared with Con.

short period of CHO restriction, in the absence of changes in fasting glucose and with only minimal decreases in muscle glycogen. Despite these observations, it is difficult to differentiate the effects of low muscle and liver glycogen. Presently, we cannot be certain of the primacy of either store when accounting for the influence of CHO deficits on lipid metabolism. Although CHO oxidation accounted for ~90% of total energy expenditure in the present study, it is possible that intramuscular triglyceride (IMTG) stores were depleted between the end of the exercise session (Ex-Def) and the start of the OFTT. There is evidence (21) that IMTG are not reduced during prolonged acute exercise but decrease during the subsequent 18-h postexercise period to fuel muscle metabolism, while dietary CHO are directed toward storage. Thus a role for IMTG in explaining the effect of acute exercise on postprandial lipemia cannot be excluded.

Dietary-induced CHO deficits are typically accompanied by a decrease in insulin (9, 17) and increases in glucagon (9), norepinephrine (31, 37), growth hormone (9), and possibly epinephrine (37). Less data is available, however, linking exercise-induced changes in lipid metabolism during the 12- to 24-h postexercise period to hormonal changes. Exercise did not reduce postprandial insulin in either Ex-Def or Ex-Bal in the present study. Although exercise typically reduces postprandial lipemia, the effect on postprandial insulin is less consistent, particularly in young, lean individuals (11, 19, 23). In addition, changes in postprandial lipemia and insulin are not correlated (14). Acute exercise has also been shown to reduce postprandial lipemia in the absence of changes in glucagon (12). In the present study, the severity of the exercise bout, the magnitude of the CHO deficit, and the increase in FFA may have offset any insulin-sensitizing effects of exercise on muscle. Whereas energy restriction can enhance muscle insulin action (10), prolonged fasting can cause insulin resistance particularly in lean subjects (2). We are not aware of data that link exercise-induced changes in lipid metabolism to changes in catecholamines. However, epinephrine can still be elevated 12 h after a prolonged bout of cycling (28). Investigations are clearly warranted to clarify the role of hormonal changes in mediating the effects of exercise-induced CHO deficits on lipid metabolism.

The results of this study underline the importance of controlling energy balance in studies of acute exercise, to distinguish the effects of an energy deficit from the effects of exercise (4). The exercise effects on postprandial TG, HDL-C, FFA, and 3-HB observed in Ex-Def are typical of those observed elsewhere (5, 12, 13, 22, 36) but none of these effects were evident in Ex-Bal, despite the exhaustive nature of the exercise session. The beneficial effects of exercise on insulin action (3) and glucose control (7) may also be attenuated when energy intake is adjusted in line with energy expenditure. Consequently, the magnitude and duration of these acute exercise effects are likely to be overstated in the literature. However, transient exercise-induced energy and substrate deficits may still persist through one or more postprandial periods under real-life conditions, in the absence of deliberate attempts postexercise to restore balance. Indeed postexercise CHO loading strategies may not be necessary or appropriate for a general population that does not engage in glycogen-depleting exercise on a daily basis. Novel study designs are required to mimic free living conditions in the laboratory setting and elucidate the

effects of repeated postexercise energy and substrate deficits on energy intake, substrate utilization, and postprandial metabolism.

In summary, the effects of acute exercise on subsequent postprandial lipid metabolism appear to be at least in part dependent on the resultant CHO and energy deficit. Further work is needed to identify the relative importance of specific substrate stores in mediating these exercise effects. Acute exercise studies that do not take account of the resultant CHO and energy deficits are not ecologically valid and may be overstating the benefits of regular exercise.

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