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RESEARCH ARTICLE

High-intensity interval exercise in the cold regulates acute and postprandial metabolism

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Abstract

High-intensity interval exercise (HIIE) has been shown to be more effective than moderate-intensity exercise for increasing acute lipid oxidation and lowering blood lipids during exercise and postprandially. Exercise in cold environments is also known to enhance lipid oxidation; however, the immediate and long-term effects of HIIE exercise in cold are unknown. The purpose of this study was to examine the effects cold stress during HIIE on acute exercise metabolism and postprandial metabolism. Eleven recreationally active individuals (age: 23 ± 3 yr, weight: 80 ± 9.7 kg, $\dot{V}O_{2peak}$: 39.2 ± 5.73 mL·kg^{-1}·min^{-1}) performed evening HIIE sessions (10 × 60 s cycling, 90% $\dot{V}O_{2peak}$ interspersed with 90 s active recovery, 30% $\dot{V}O_{2peak}$) in thermoneutral (HIIE-TN, control; 21°C) and cold environment (HIIE-CO; 0°C), following a balanced crossover design. The following morning, participants consumed a high-fat meal. Indirect calorimetry was used to assess substrate oxidation, and venous blood samples were obtained to assess changes in noncellular metabolites. During acute exercise, lipid oxidation was higher in HIIE-CO (P = 0.002) without differences in $\dot{V}O_2$ and energy expenditure ($P \geq 0.162$) between conditions. Postprandial $\dot{V}O_2$, lipid and CHO oxidation, plasma insulin, and triglyceride concentrations were not different between conditions ($P > 0.05$). Postprandial blood LDL-C levels were higher in HIIE-CO 2 h after the meal (P = 0.003). Postprandial glucose area under curve was 49% higher in HIIE-CO versus HIIE-TN (P = 0.034). Under matched energy expenditure conditions, HIIE demonstrated higher lipid oxidation rates during exercise in the cold; but only marginally influenced postprandial lipid metabolism the following morning. In conclusion, HIIE in the cold seemed to be less favorable for postprandial lipid and glycemic responses.

NEW & NOTEWORTHY This is the first known study to investigate the effects of cold ambient temperatures on acute metabolism during high-intensity interval exercise, as well as postprandial metabolism the next day. We observed that high-intensity interval exercise in a cold environment does change acute metabolism compared to a thermoneutral environment; however, the addition of a cold stimulus was less favorable for postprandial metabolic responses the following day.

high-fat meal; high-intensity interval exercise; lipid oxidation; postprandial lipemia

INTRODUCTION

Dysfunctional postprandial metabolism (the way the body metabolizes macronutrients after a meal) and increased postprandial lipemia (PPL), the rise in blood lipids after ingestion of food, are both predictors of the development of type 2 diabetes (1), liver disease (2), and cardiovascular diseases (3). Reducing vascular inflammation associated with postprandial metabolism and lowering postprandial lipemia are both strategies to lower the risk of developing these common diseases. Multiple factors may impact postprandial metabolism and lipemia including intestinal adsorptive functioning, the meal components on postprandial vasculature function, and physical activity (4). Regular physical activity (PA) is associated with lower PPL and lower postprandial triglyceride levels (5). Interestingly, the impact of PA on PPL can be influenced by exercise modality (continuous exercise vs. interval exercise) and exercise intensity (moderate- vs. high-intensity). Short, high-intensity interval exercise reduces PPL to a greater extent than long, moderate-intensity continuous exercise (6, 7). Beyond modality and intensity, many other factors also influence both metabolism during exercise and postprandially (e.g., sex, age, fitness level). Exposure to cold temperature environments also influences the metabolism of lipids both during rest and exercise (8, 9). However, it is unknown whether such environmental manipulations, in combination with exercise, would alter postprandial metabolism and/or PPL.

There has been renewed interest in training methods that involve short, high-intensity exercise bouts (e.g., high-intensity interval exercise, HIIE) as a time-effective protocol for aerobic energy metabolism and reducing body fat. Low

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volumes of HIIE training can induce the same metabolic adaptations compared to high volumes of moderate-intensity continuous exercise (MICE) training, but in a shorter amount of time (10). Adaptations following HIIE training (2–6 wks) include increased aerobic capacity, muscle oxidative capacity, and lipid oxidation; while reducing muscle glycogenolysis and phosphocreatine utilization (11). Interestingly, a single bout of high-intensity exercise has been shown to lower the postexercise respiratory exchange ratio (RER) and increase circulating plasma catecholamines compared to endurance exercise (12, 13). However, these responses did not produce differences in postexercise lipid oxidation between exercise intensity (13).

Quantifying postexercise metabolic adaptations can also be conducted via the assessment of postprandial metabolism. Previous research examining the effect of prior exercise on postprandial metabolism has found that the type, and mode, of exercise can influence the postprandial lipid and glycemic responses. The effect of HIIE versus MICE on postprandial metabolism has been examined in multiple studies (6, 7, 14). Lee et al. demonstrated that individuals that performed HIIE have greater postprandial lipid oxidation after ingestion of a high-fat meal (HFM) compared to those who performed MICE. Similarly, both Freese et al. and Trombold et al. found that postprandial lipid oxidation was significantly higher, and consequently, PPL was lower, following HIIE compared to both MICE and control groups. All studies noted that exercise intensity played a significant role in PPL regulation, that is, the higher the intensity, the lower the PPL. However, the mechanisms responsible for increased postprandial metabolism following higher intensity exercise remain unclear.

Environmental temperature is known to impact metabolic and molecular changes during exercise in human and animal models. A recent study by Raun et al. (15) highlighted the importance of ambient temperature during exercise by demonstrating that multiple training adaptations were improved when mice exercised under mild cold stress, including increased glucose tolerance, improved insulin-stimulated glucose uptake, increased metabolic rate, and upregulation of pyruvate dehydrogenase (PDH) and subunits of the electron transport chain. Cold exposure is also known to regulate the metabolism of lipids and carbohydrates (CHO) differently than in a thermoneutral environment. While shivering and nonshivering thermogenesis at rest, in the cold, elicits greater CHO metabolism, the metabolism of lipids is favored during exercise in the cold (8, 16, 17). Increased lipid oxidation during exercise in the cold is suggested to originate from an increase in intramuscular triglyceride utilization, a greater lactate clearance, and a greater CPT-1 activity, regulating long-chain fatty acid (LCFA) transport across the mitochondrial outer membrane (8, 16, 17). Cold exposure also increases the secretion of catecholamines, thereby increasing whole-body lipolysis (18). To the best of our knowledge, the effects of exercise in the cold on postprandial metabolism have yet to be investigated.

Continuous exercise in the cold has consistently demonstrated increased lipid oxidation; however, it is unknown whether those metabolic responses are similar during HIIE in the cold. Additionally, HIIE has been shown to be more efficient in upregulating postprandial lipid metabolism compared to MICE. Whether combining HIIE with cold exposure would further increase postprandial lipid metabolism and its effects on postprandial CHO metabolism is unknown. Therefore, the aims of this study were: *i*) to examine whether HIIE, under cold stress would increase lipid oxidation during exercise and *ii*) to examine whether performing HIIE under cold stress would upregulate postprandial lipid metabolism and/or alter carbohydrate metabolism, the next morning. It was hypothesized that performing HIIE in a cold environment would increase lipid oxidation, decrease carbohydrate metabolism, and would increase postprandial lipid metabolism more effectively than a thermoneutral environment.

METHODS

Participants

Eleven healthy, recreationally active individuals (male: n = 7, female: n = 4) participated in this study. Participant characteristics are presented in Table 1. The inclusion criteria were an age between 18 and 30 yrs old and a BMI between 25 and 30 $kg \cdot m^{-2}$. Participants were required to be moderately fit ($\dot{V}O_{2peak}$ within the "Fair" or "Good" zones; 41.6–50.5 $mL \cdot kg^{-1} \cdot min^{-1}$ for males and 35.0–41.9 $mL \cdot kg^{-1} \cdot min^{-1}$ for females), but not formally trained for varsity or professional sports, to participate in the study. Female menstrual cycles were dispersed over the follicular, ovulation, and luteal phases for the thermoneutral condition, and the ovulation, luteal, and menstrual phases for cold condition. The distribution likely washed out any menstrual cycle effects. The exclusion criteria included a history of CVDs and/or patho-metabolic conditions, pregnant and/or nursing women, varsity, and professional athletes. Informed, written consent was provided before testing. Participants were instructed to refrain from physical activity, caffeine, and alcohol consumption for 24 h before each visit to the laboratory. Participants were screened with a Get Active Questionnaire and a health screening form for health conditions or diseases that could be aggravated by cold or exercise. All procedures were in accordance with the Declaration of Helsinki and approved by the university's Research Ethics Board.

Table 1. *Participant physical characteristics and fasting blood parameters*

Characteristic	Means ± SD
Height, cm	173 ± 7.47
Weight, kg	80 ± 9.7
Age, yr	23 ± 3
Body mass index (BMI), $kg \cdot m^{-2}$	26.4 ± 1.89
Body fat, %	23.6 ± 7.25
Fat-free mass, kg	61 ± 10.1
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	39.2 ± 5.73
Fasting blood glucose, $mg \cdot dL^{-1}$	87.3 ± 7.68
Fasting insulin, $mU \cdot L^{-1}$	6.5 ± 1.71
Fasting triglycerides, $mg \cdot dL^{-1}$	36.9 ± 15.25
Fasting total cholesterol, $mmol \cdot L^{-1}$	5.0 ± 1.22
Fasting LDL-C, $mmol \cdot L^{-1}$	2.05 ± 0.342
Fasting HDL-C, $mmol \cdot L^{-1}$	0.81 ± 0.199
HOMA-IR	1.4 ± 0.42

HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol.

Experimental Protocol

Participants completed one initial assessment and two, 2-day experimental visits. Experimental visits consisted of an evening exercise session under controlled environmental conditions followed by a postprandial session the next morning at room temperature (Fig. 1). Experimental visits were conducted on the same day of the week, at least 1 wk apart (9 ± 3 days), in a balanced design for the following conditions: *1*) thermoneutral environment (HIIE-TN; 21°C) and *2*) cold environment (HIIE-CO; 0°C).

At least 1 wk before the experimental visit, participants visited the laboratory for initial assessment and determination of peak oxygen consumption uptake ($\dot{V}O_{2peak}$). Participants arrived at the laboratory between 07:30 and 10:30 in a fasted state. Once approved for exercise, a fasting blood sample was collected. Subsequently, anthropometric data were collected and percent body fat was estimated by hydrostatic underwater weighing (19). Thereafter, participants completed an incremental exercise test on a cycle ergometer in order to determine peak power (W_{max}) and $\dot{V}O_{2peak}$. The incremental exercise protocol began at 50 W, increasing by 25 W every minute until volitional fatigue.

On day one of the experimental visits, participants reported to the laboratory at 1800. Upon arrival, participants were instructed to change into shorts and T-shirt for both conditions and were instrumented (see Instrumentation and Analyses section). Then, participants entered a climate-controlled chamber set at either thermoneutral (21°C) or cold (0°C) and sat quietly in a chair for 15 min before starting exercise. At 1900, participants performed a 5-min warm-up on a cycle ergometer at 40% $\dot{V}O_{2peak}$. Exactly 1 min following the warm-up, participants began the exercise protocol. The exercise protocol consisted of ten 60-s cycling sprints at a fixed resistance of 90% $\dot{V}O_{2peak}$, interposed with 90 s of active recovery at 30% $\dot{V}O_{2peak}$ (6). Participants were instructed to pedal as fast as possible during the sprints. Following the completion of all 10 intervals, participants were then allowed to cool down on the bike or by walking in the laboratory and then were deinstrumented. They were also instructed to go to sleep at 2200 that evening (6).

Similar to Lee et al., on the second day (i.e., the following morning), participants arrived at the laboratory by 07:45. Upon arrival, a fasting blood sample was obtained, and participants resting on the hospital bed for 10 min for collection of fasting, resting metabolic data. Following this, they ingested a high-fat meal (HFM) at approximately 08:15. Thereafter, the participants sat on the hospital bed for the next three and a half hours. During this time, resting energy expenditure data were obtained from indirect calorimetry, and blood samples were collected every hour (6). No other food was ingested during this time; only limited water was permitted.

Dietary Control and Test Meal

Participants were instructed to follow their typical diet the day of the first experimental exercise session but were instructed to eat dinner at 1600 that day, therefore providing 3 h to digest consumed food before exercise. Participants were provided with a dietary log sheet and instructed to record all food intake (food type and quantity) during the day. They were then instructed to repeat the same diet on the day of the second experimental session. The average macronutrient content of the meals consumed during the day of each experimental session was 50.8 ± 13.25% CHO, 32.7 ± 9.48% fat, and 18.3 ± 5.41% protein. This equated to an average of 1591.8 ± 245.58 kcal consumed.

After each evening exercise session, participants were given a nutrition bar (45 g of CHO, 5 g of fat, and 9 g of protein) to eat at 2000 and were instructed to avoid any other food intake throughout the night. Participants were permitted to drink water as necessary.

The HFM breakfast on the second day comprised whole grain bread, unsalted butter, avocado, banana, egg, and cheddar cheese. The exact amount of each item and the caloric composition of the HFM was determined by the participant's body mass. This was equal to 1.1 g of fat (66% kcal), 1.0 g of carbohydrate (26% kcal), and 0.3 g of protein (8% kcal); per kilogram of body mass (6) (1201 ± 140 total kcal), similar to that of Freese et al. (14). Following the ingestion of the HFM, no other meals or calorie-containing beverages were consumed for 4 h, but water was allowed.

Instrumentation and Analyses

Skin temperature was measured via wireless surface skin temperature probes (Thermochron iButton, Maxim Integrated, San Jose) at six sites: forehead, chest, hand, lateral forearm,

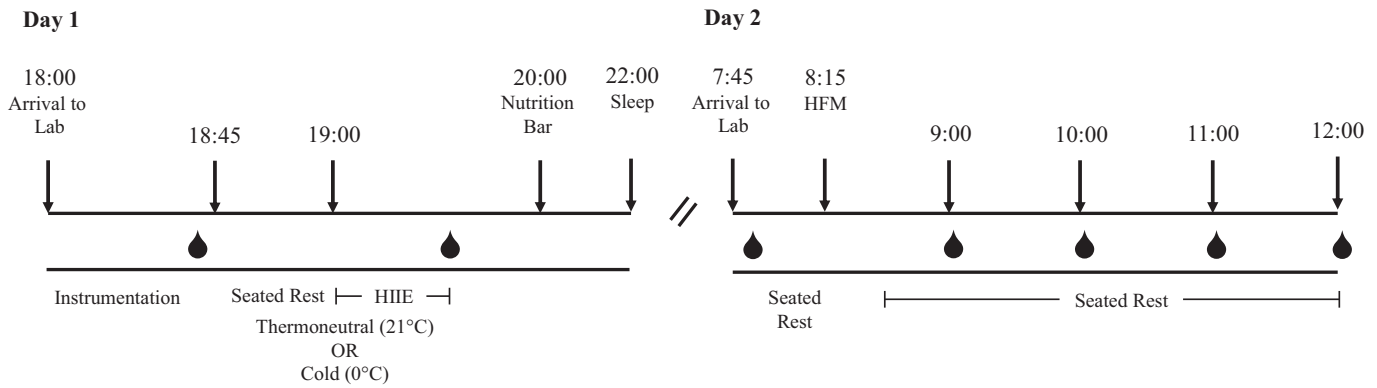

Figure 1. Schematic diagram of the experimental protocol. Blood drops represent blood samples. All participants (n = 11) completed both thermoneutral exercise (HIIE-TN) and cold exercise (HIIE-CO) sessions. HIIE, high-intensity interval exercise; HFM, high-fat meal.

lower back, and lateral thigh. Mean skin temperature ($\bar{T}_{sk}$) was calculated using the following equation (20):

$$\bar{T}_{sk} = 0.14\,(T_{face}) + 0.19\,(T_{chest}) + 0.11\,(T_{forearm}) + 0.05\,(T_{hand}) + 0.32\,(T_{thigh}) + 0.19\,(T_{back})$$

Core temperature (T_c) was measured through a rectal thermistor (Physitemp, New Jersey) self-inserted 10 cm beyond the anal sphincter. A thoracic electrical bioimpedance device (Enduro, Physioflow, France) was used to measure cardiac function in real-time including heart rate (HR), stroke volume (SV), and cardiac output ($\dot{Q}$) via six electrodes (Skintact, Austria) on the thoracic area.

Muscle oxygenation of the vastus lateralis (VL) was assessed via near-infrared spectroscopy (NIRS). NIRS signals were obtained using a portable continuous-wave NIRS device (PortaMon MK II, Artinis Medical Systems) positioned on the medial axis on the VL, ~10 cm above the knee joint. The NIRS device simultaneously uses the modified Lambert–Beer law and spatially resolved spectroscopy for the observation of relative changes in tissue oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), and total hemoglobin (tHb) and provides a measure of tissue saturation index (TSI) (21). Variables were measured via differences in absorption characteristics of three light sources (wavelengths: 760 nm and 850 nm). The difference between O_2Hb and HHb was calculated (HbDiff). Due to the path length of the photons through the tissue being unknown while using continuous-wave spectroscopy, the differential path length factor was set to 4.0 (21). Data from the NIRS device were collected at 1 Hz.

Breath-by-breath measurements were measured continuously at the mouth during exercise and for the 4 h following the HFM. During exercise, an open circuit ergospirometer in breath-by-breath, exercise capacity mode (CPX, MGC Diagnostics, Saint Paul, MN) was used to obtain measures of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER). During the postprandial rest period, the cart gas exchange settings were changed to nutritional assessment mode for resting metabolic rate and resting energy expenditure. All calibration procedures were performed to the manufacturer's specifications immediately before each testing session. Gas flow was measured through a bidirectional pitot tube flow sensor attached to the face-fitting mask worn by all participants during the exercise and postprandial testing sessions.

From the breath-by-breath metabolic measurements, calculations of lipid and carbohydrate oxidation were completed using the following stoichiometric equations (22):

$$\left(<50\%\,\dot{V}O_{2peak}\right)\ CHO\ (g\cdot min^{-1}) = 4.344\cdot\dot{V}CO_2 - 3.061\cdot\dot{V}O_2 - 0.40\cdot n$$

$$\left(>50\%\,\dot{V}O_{2peak}\right)\ CHO\ (g\cdot min^{-1}) = 4.210\cdot\dot{V}CO_2 - 2.962\cdot\dot{V}O_2 - 0.40\cdot n$$

$$Lipid\,(g\cdot min^{-1}) = 1.695\cdot\dot{V}CO_2 - 1.701\cdot\dot{V}CO_2 - 1.77\cdot n$$

where values of $\dot{V}O_2$ and $\dot{V}CO_2$ ($L\cdot min^{-1}$) were collected during exercise and during the postprandial rest period, and n represents nitrogen excretion from the oxidation of proteins. Nitrogen excretion is negligible in most exercise conditions (22) and was not accounted for in these equations. An average value for $\dot{V}O_2$ and $\dot{V}CO_2$ was calculated over all 10 HIIE exercise bouts, as well as during the last 15 min of every postprandial hour. Absolute oxidation values, as well as relative contributions of the oxidation of CHO, and lipid were calculated for the entire duration of each exercise session and for the last 15 min of every hour of the postprandial period. Relative contributions were calculated according to the predetermined energy obtained from 1 g of each substrate (CHO < 50% $\dot{V}O_{2peak}$ 3.95 kcal, CHO > 50% $\dot{V}O_{2peak}$ 4.07 kcal, lipid 9.75 kcal) (22).

Resting energy expenditure (REE) was calculated using values of $\dot{V}O_2$ and $\dot{V}CO_2$ ($L\cdot min^{-1}$) during postprandial seated rest (23):

$$REE\ (kcal\cdot min^{-1}) = (3.716\cdot\dot{V}O_2) + (1.332\cdot\dot{V}CO_2)$$

Exercise energy expenditure (EE) was calculated using values of $\dot{V}O_2$ and $\dot{V}CO_2$ ($L\cdot min^{-1}$) during the exercise protocol (23):

$$EE\ (kcal\cdot min^{-1}) = [(3.716\cdot\dot{V}O_2) + (1.332\cdot\dot{V}CO_2)] - REE$$

Blood Sampling and Analyses

During the evening exercise sessions, venous blood samples from an antecubital vein were collected via independent venipuncture before participants entering the climate-controlled chamber and immediately following the last (10th) HIIT bout. The following morning, a venous catheter (20 g, BD Shielded I.V. Catheter) was inserted into an antecubital vein. Blood samples were collected via the venous catheter before the meal (fasting), and at 1, 2, 3, and 4 h following the meal. A 5-mL blood sample was collected in a tube containing a serum clot activator and gel for serum separation (SST BD Vacutainer, BD Worldwide, Franklin Lakes) and a 6-mL blood sample was collected in a tube containing a clot inhibitor for plasma preparation (K2 EDTA BD Vacutainer, BD Worldwide, Franklin Lakes). Each collection was immediately centrifuged room temperature (~21°C) for 10 min at a rotational centrifugal force of ~4,000 Gs. Samples were separated into 2-mL microcentrifuge tubes and stored frozen at −80°C. Blood plasma or serum was analyzed for plasma glucose (Cayman Chemical), insulin (Mercodia, Sweden), triglycerides (Cayman Chemical), total cholesterol (TC, Cayman Chemical), and low/high-density lipoprotein cholesterol (LDL-C/HDL-C, Cell Biolabs) following manufacturer's guidelines. Briefly, glucose was analyzed using 100 μL of 1:5 diluted plasma, insulin was examined with 25 μL of undiluted plasma, triglycerides via 10 μl of undiluted plasma, total cholesterol with 50 μL of 1:400 diluted serum, and HDL-C and LDL-C were analyzed using 50 μL of 1:25 diluted serum.

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used as a proxy to estimate insulin resistance to ensure none of our participants were classified as insulin resistant (noninsulin resistant: ≤1.9, insulin resistant: >2.0). HOMA-IR was calculated using fasting venous insulin

and glucose concentrations collected during the initial assessment visit and during Day 2 of each experimental session using the following equation (24):

$$\text{HOMA-IR} = (\text{insulin} \cdot \text{glucose})/405$$

where fasting levels of insulin are expressed in $\text{mU}\cdot\text{L}^{-1}$ and glucose levels in $\text{mg}\cdot\text{dL}^{-1}$.

Postprandial responses for glucose, insulin, and triglycerides were quantified by summing 4 h area under curve (AUC) for plasma/serum concentrations versus time using the trapezoidal method to obtain total AUC responses (tAUC). Net incremental area under the curve (iAUC) was calculated by subtracting the fasting value from the postprandial value before calculating AUC (25).

Statistical Analysis

All data are presented as means ± SD. The normality of the data was tested using the Shapiro–Wilk test. Two-way analysis of variance with repeated measures was used to analyze the differences between HIIE temperature conditions (HIIE-TN vs. HIIE-CO) during each interval of acute HIIE for the following variables: $\bar{T}_{sk}$, T_c, HR, SV, $\dot{Q}$, $\dot{V}O_2$, RER, EE, substrate oxidation, and blood variables collected. Additionally, one-way analysis of variance with repeated measures was used to compare fasting pre-HFM blood samples, tAUC, and iAUC between conditions. Two-way analysis of variance with repeated measures was used to analyze difference between HIIE temperature conditions (HIIE-TN vs. HIIE-CO) over time (1, 2, 3, and 4 h) during the postprandial period for metabolic (resting $\dot{V}O_2$, resting RER, REE, resting substrate oxidation rates, and percent substrate contributions) and blood variables collected. Additionally, one-way analysis of covariance [covariates: fat-free mass (FFM), $\dot{V}O_{2peak}$], where the covariates were centered across all participants, was run to determine whether the linear relationship between the covariate and the dependent variable differed between temperature conditions (i.e., covariate–dependent variable interaction) (26). When a significant F ratio was observed, all pairwise multiple comparison procedures with Bonferroni corrections were conducted. All effects were tested at a 95% confidence interval ($P \leq 0.05$). It was estimated that a sample of nine participants would be sufficient for detection of large effect sizes, assuming standard deviations similar to those previously observed (6) with a statistical power (1 − β err. prob.) of 80% (G*Power, Düsseldorf, Germany). All statistical analyses were performed using Sigma Plot, version 14.0.

RESULTS

Thermal and Cardiovascular Measures

Mean $\bar{T}_{sk}$ and T_c were significantly lower during HIIE-CO exercise compared to HIIE-TN ($P < 0.001$, 36.91 ± 0.311 °C) versus HIIE-TN (37.47 ± 0.306 °C) (Fig. 2, *A* and *B*). However, ΔT_c was not significantly different between conditions (P = 0.107). Mean HR and $\dot{Q}$ were significantly lower during HIIE-CO (HR: 166.5 ± 12.45 bpm, $\dot{Q}$: 20.68 ± 3.53 $\text{L}\cdot\text{min}^{-1}$) exercise compared to HIIE-TN ($P \leq 0.028$, HR: 171.5 ± 11.59 bpm, $\dot{Q}$: 22.25 ± 3.481 $\text{L}\cdot\text{min}^{-1}$). SV was not different between temperature conditions (P = 0.139, HIIE-TN: 130.35 ± 21.915 mL, HIIE-CO: 125.92 ± 20.079 mL). There were no covariate–dependent variable interactions ($P \geq 0.094$).

Metabolic Rate, Substrate Utilization, and Skeletal Muscle Oxidation

During acute exercise, mean $\dot{V}O_2$ and EE were not significantly different between HIIE-CO and HIIE-TN ($\dot{V}O_2$: P = 0.162; EE: P = 0.476), and both $\dot{V}O_2$ and EE were lower at interval one versus all other HIIE intervals ($P \leq 0.008$) (Fig. 2*C*, 2*D*). Muscle oxidative measures also were not different between temperature conditions (TSI: P = 0.207, O_2Hb: P = 0.740, HHb: P = 0.069, tHb: P = 0.351, HbDiff: P = 0.322). However, RER was significantly lower during HIIE-CO (P = 0.019, HIIE-TN: 0.99 ± 0.029, HIIE-CO: 0.94 ± 0.051). Accordingly, lipid oxidation rate was higher (P = 0.008) during HIIE-CO exercise compared to HIIE-TN, as well as during intervals 6–10 versus 1–4 ($P \leq 0.048$) (Fig. 2*E*). CHO oxidation rate was not different between conditions (P = 0.130) (Fig. 2*F*). There were no covariate–dependent variable interactions ($P \geq 0.276$).

During the postprandial period, the mean $\dot{V}O_2$ or REE was not significantly different between conditions ($\dot{V}O_2$: P = 0.439, HIIE-TN: 350.34 ± 47.137 $\text{mL}\cdot\text{min}^{-1}$, HIIE-CO: 342.75 ± 58.603 $\text{mL}\cdot\text{min}^{-1}$; REE: P = 0.621, HIIE-TN: 1.67 ± 0.228 $\text{kcal}\cdot\text{min}^{-1}$, HIIE-CO: 1.64 ± 0.290 $\text{kcal}\cdot\text{min}^{-1}$) but was lower at fasting baseline compared to hours 2, 3, and 4, regardless of condition ($\dot{V}O_2$: P = 0.036; REE: P = 0.027). Mean $\dot{V}CO_2$ was not different between conditions (P = 0.422, HIIE-TN: 286.70 ± 42.247 $\text{mL}\cdot\text{min}^{-1}$, HIIE-CO: 281.36 ± 51.714 $\text{mL}\cdot\text{min}^{-1}$) but was higher at hour 2 (294.09 ± 48.371 $\text{mL}\cdot\text{min}^{-1}$) compared to hour 4 ($P$ = 0.004, 272.30 ± 41.485 $\text{mL}\cdot\text{min}^{-1}$). Mean RER was not different the following morning between either HIIE-TN (0.82 ± 0.035) or HIIE-CO (0.82 ± 0.044) condition (P = 0.952) but was significantly lower at hour 4 (0.80 ± 0.024) compared to hour 1 (P = 0.004, 0.84 ± 0.045) and hour 2 (P = 0.013, 0.83 ± 0.046). Neither lipid or CHO oxidation rate, nor percent lipid or CHO contribution, had a significant main effect of temperature condition ($P \geq 0.711$). However, there was a main effect of time ($P \leq 0.017$), where lipid oxidation rate was higher at hour 4 compared to fasting (P = 0.001) and hour 1 (P = 0.017), and CHO oxidation rate was lower at hour 4 compared to fasting (P = 0.034), hour 1 (P = 0.002) and hour 2 (P = 0.013) (Fig. 3, *A* and *B*). Accordingly, percent lipid contribution was higher, and percent CHO contribution lower, at hour 4 compared to fasting (P = 0.007), and hours 1 (P = 0.036) and 2 (P = 0.039) (Fig. 3*C*). There were no covariate–dependent variable interactions ($P \geq 0.195$).

Blood Parameters

During the acute exercise sessions, blood insulin, glucose, and triglyceride concentrations were not different between conditions ($P \geq 0.334$) (Fig. 4, *A–C*). Additionally, total cholesterol, LDL-C, and HDL-C concentrations were not significantly different between conditions postexercise ($P \geq 0.346$) (Fig. 4, *D–F*).

The following morning, pre-HFM fasting blood samples were not different between temperature conditions for any parameter ($P \geq 0.086$). Fasting insulin resistance (HOMA-IR)

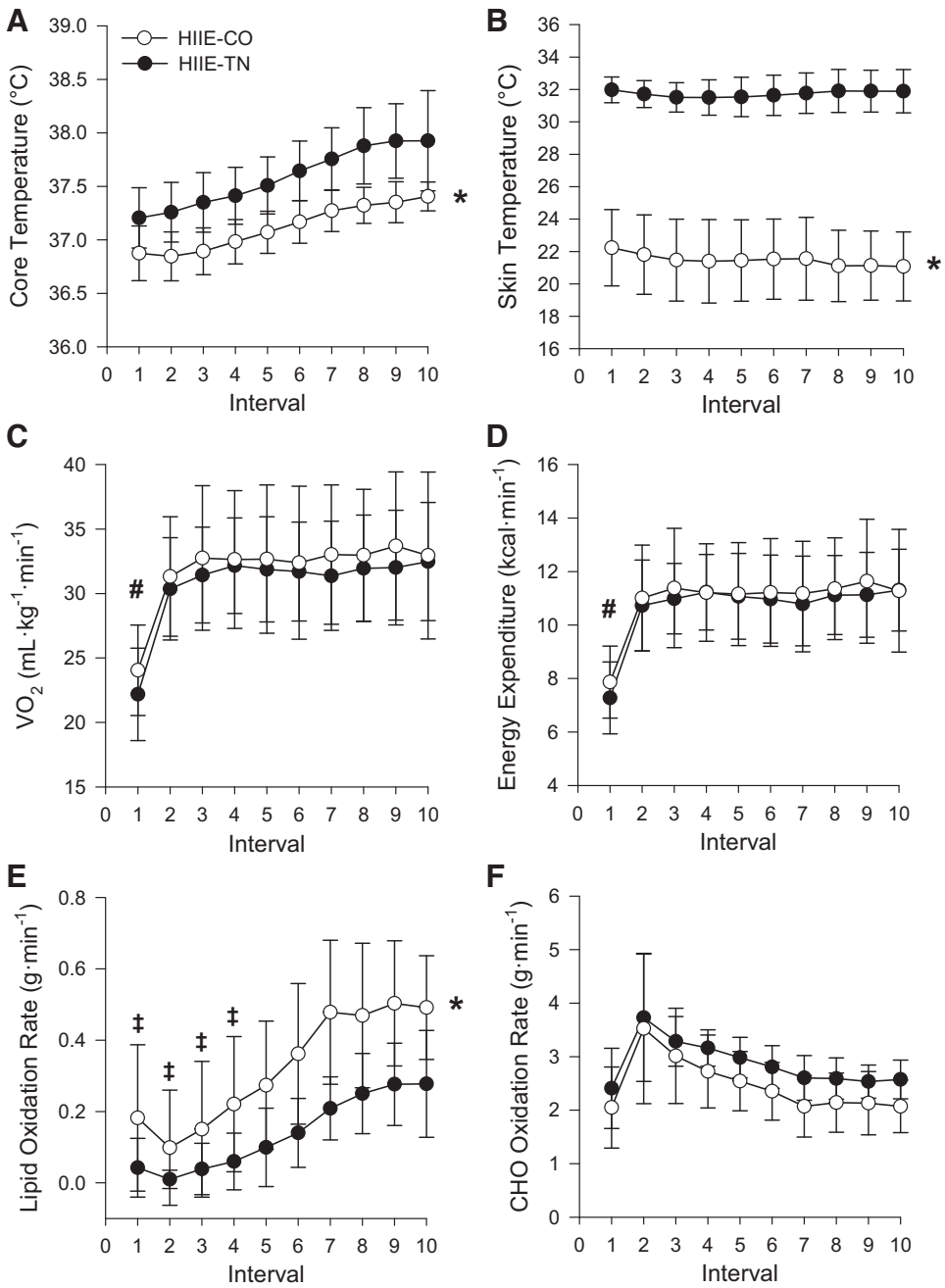

Figure 2. Means ± SD of core temperature (*A*), skin temperature (*B*), oxygen consumption (*C*), energy expenditure (*D*), lipid oxidation rate (*E*), and carbohydrate oxidation rate (*F*), at each high-intensity interval during each exercise condition. *Significantly different from HIIE-TN ($P < 0.05$), #significantly different versus all other intervals ($P < 0.05$), ‡significantly different from intervals 6–10 ($P < 0.05$). HIIE-TN, high-intensity interval exercise-thermoneutral exercise.

was also not different between conditions ($P = 0.091$, HIIE-TN: 1.39 ± 0.634, HIIE-CO: 1.01 ± 0.363). During the postprandial period following the HFM, significant differences were observed over time for blood insulin ($P < 0.001$) (Fig. 5*A*), glucose ($P = 0.015$) (Fig. 5*B*), triglycerides ($P < 0.001$) (Fig. 5*C*), and TC ($P = 0.024$) (Fig. 5*D*). Across both conditions, insulin was higher, whereas triglycerides were lower, at hour 1 compared to hours 2, 3, and 4. Glucose levels were higher at hour 1 compared to hour 2. TC was higher at hour 4 compared to hour 1 ($P = 0.018$). There was a significant condition–time interaction observed for LDL-C ($P = 0.009$) (Fig. 5*E*). At hour 2, LDL-C was higher in HIIE-CO versus HIIE-TN ($P = 0.003$). Further, within HIIE-TN, LDL-C was lower at hours 1 and 2 compared to hours 3 and 4 ($P \leq 0.016$). There were no differences in HDL-C levels between conditions or over time ($P \geq 0.118$, Fig. 5*F*).

AUC data are presented in Table 2. During the postprandial period, glucose iAUC was significantly higher following HIIE-CO versus HIIE-TN ($P = 0.034$). However, insulin iAUC and triglyceride iAUC were not different between conditions ($P \geq 0.077$). tAUC was not different for any blood variable between conditions ($P \geq 0.561$).

DISCUSSION

Previous studies have shown that a single bout of HIIE can enhance lipid oxidation postprandial the morning following exercise. Previous studies have also shown that exercise in the

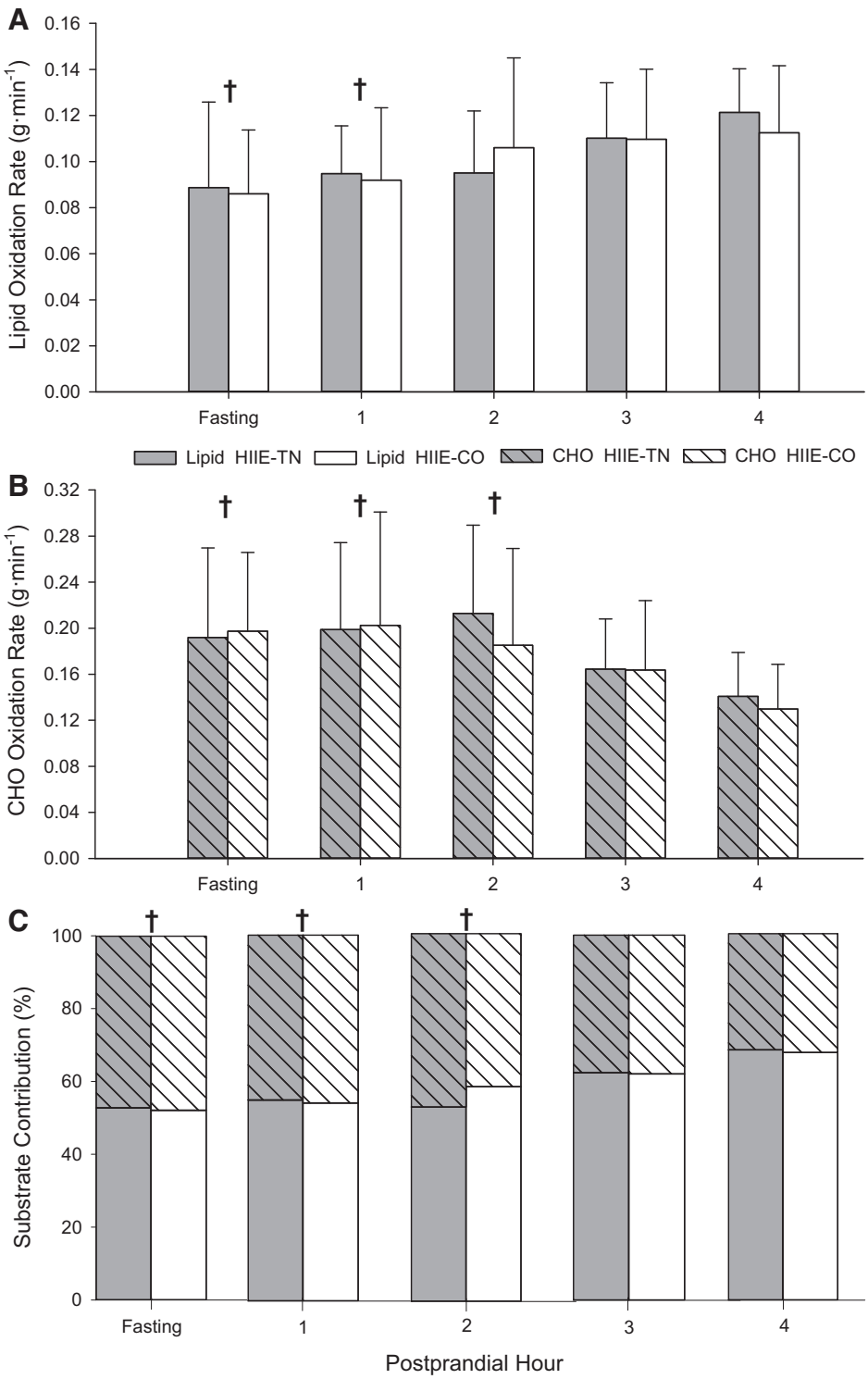

Figure 3. Means ± SD values of lipid oxidation rate (*A*), carbohydrate oxidation rate (*B*), and percent substrate concentration (*C*), at fasting pre-HFM and each hour postprandial for each condition. †Significantly different from hour 4 ($P < 0.05$). HFM, high-fat meal.

cold can enhance lipid oxidation during acute exercise. However, to date, no one has examined whether HIIE in the cold can enhance lipid oxidation or improve the postprandial metabolic state the following day. Therefore, the present study compared substrate oxidation between a thermoneutral and a cold environment: *1*) during high-intensity interval exercise and *2*) the postprandial metabolic state the next morning. The main findings of this study were *1*) lipid oxidation was higher in the cold during high-intensity interval exercise, *2*) lipid metabolism was marginally influenced in the postprandial state with greater LDL-C concentrations in the HIIE-CO condition at 2 h, and *2*) the postprandial glycemic response was flattened following the high-fat meal in the HIIE-TN condition.

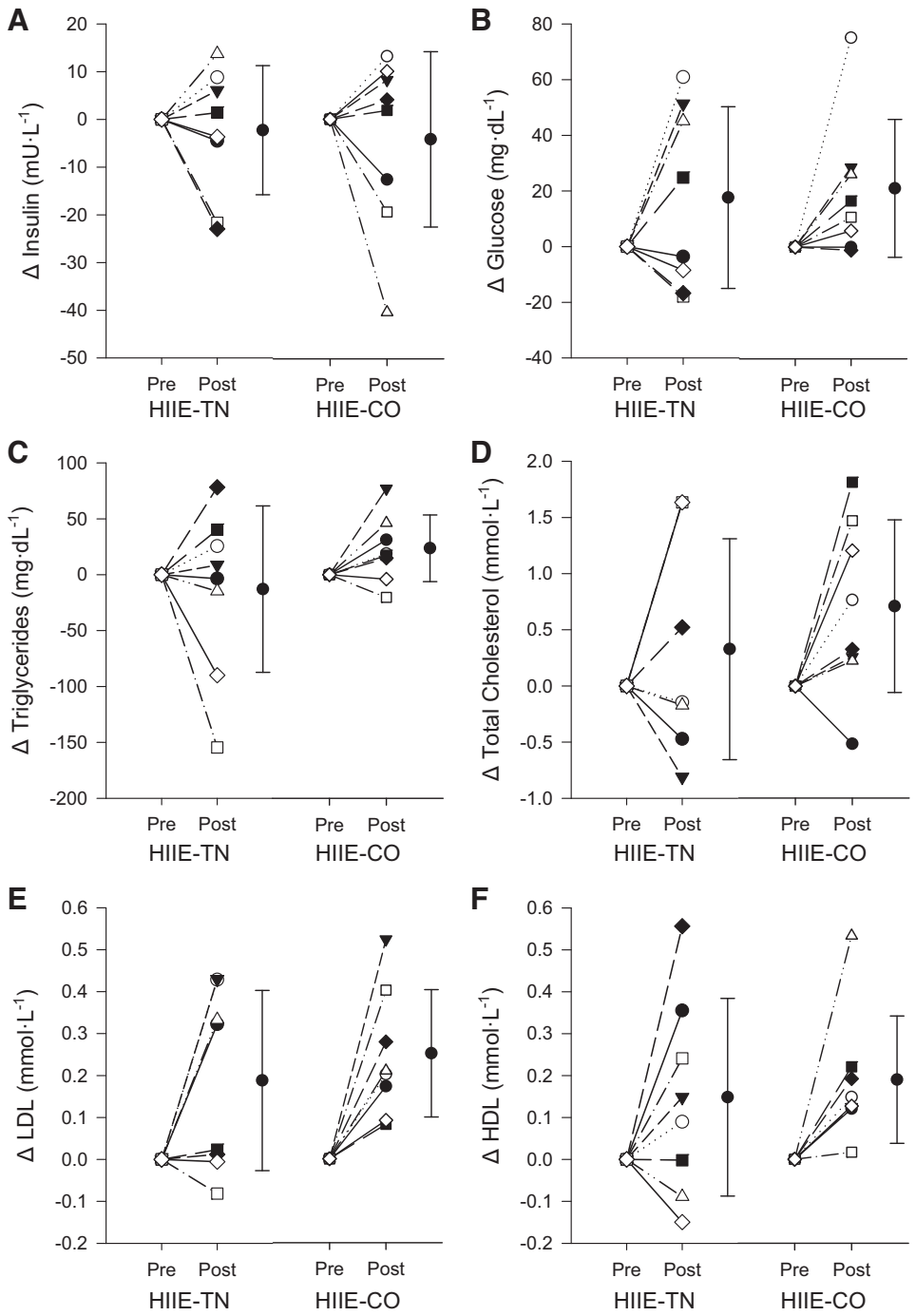

Figure 4. Individual participant values and overall mean change ± SD from preexercise values of insulin concentration (*A*), glucose concentration (*B*), triglyceride concentration (*C*), total cholesterol (*D*), low-density lipoprotein concentration (*E*), and high-density lipoprotein concentration (*F*), during each exercise condition.

To the best of our knowledge, there is currently no research focusing on postprandial metabolism while comparing high-intensity interval exercise conducted under thermal stress, which is known to acutely modulate metabolism (8, 17). Previous research that examined postprandial metabolism following exercise has identified multiple factors that may regulate the postprandial response, including exercise intensity, energy expenditure, and meal timing (6, 7, 14). Studies concentrating on differences in exercise type (endurance exercise vs. interval exercise) have indicated that exercise intensity likely plays a significant role in postprandial lipemia regulation (6, 7, 27). Higher intensity exercise has been shown to produce greater lipid oxidation and oxygen consumption than low-intensity exercise, even when EE is equivalent (7, 27). Further, both Freese et al. and Lee et al. demonstrated greater lipid oxidation, thus reduced PPL, 12–18 h following HIIE versus MICE, even when the protocols were not isocaloric. Since the intensity of exercise is a driving factor behind changes in postprandial metabolism, and given that the intensity between the HIIE conditions were identical, and no significant differences were detected in $\dot{V}O_2$ and EE, metabolic differences observed in the present study were likely the result of the cold environment.

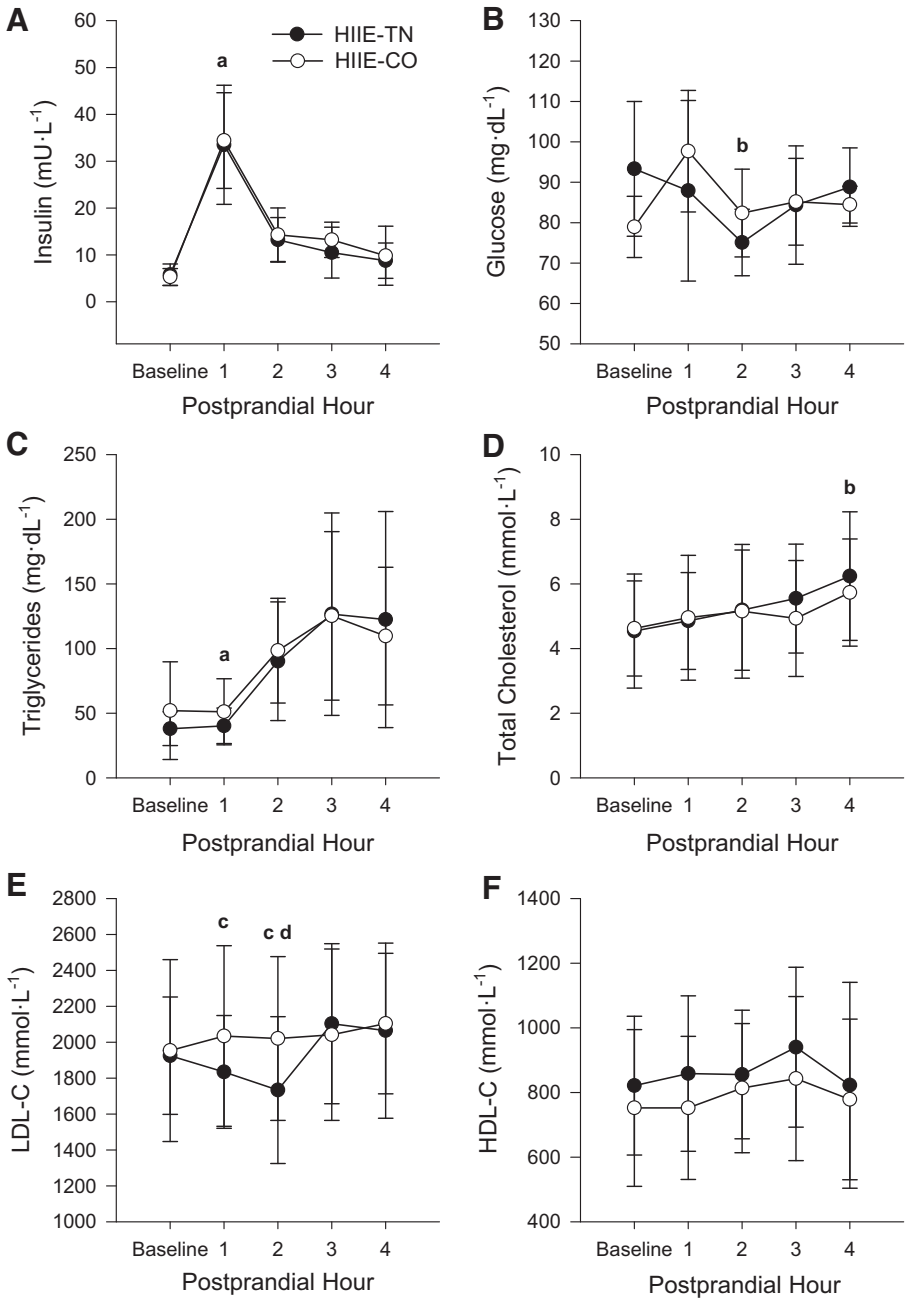

Figure 5. Means ± SD of insulin concentration (*A*), glucose concentration (*B*), triglyceride concentration (*C*), total cholesterol (*D*), low-density lipoprotein concentration (*E*), and high-density lipoprotein concentration (*F*), at each hour postprandial for each condition. [a]Significantly different from hours 2, 3, and 4 ($P < 0.05$), [b]significantly different from hour 1 ($P < 0.05$), [c]significantly different from hours 3 and 4 ($P < 0.05$), [d]significantly different between HIIE-TN and HIIE-CO at hour 2 postprandial ($P < 0.01$). HIIE-TN, high-intensity interval exercise-thermoneutral exercise; HIIE-CO, high-intensity interval exercise-cold exercise.

Thermal Effects during Acute Exercise

The present study found that high-intensity exercise in the cold increased lipid oxidation by 358% during the exercise bout in comparison to high-intensity exercise in a thermoneutral environment. Other studies examining substrate utilization during continuous exercise at low- and moderate-intensity exercise in the cold have found similar results (8, 16, 17). However, even though lipid metabolism was increased during HIIE-CO, there were no differences in post-exercise triglyceride blood levels between conditions. Vallerand and Jacobs (28), and Gagnon et al. (8) made similar observations both during cold exposure and following cold exercise, respectively. It was suggested that intramuscular triglycerides may be the preferred fuel sources for oxidation, rather than plasma triglycerides, even when triglyceride blood concentrations are elevated. Cold exposure can also be a strong stimulus of the sympathetic nervous system, increasing β-adrenergic receptor activity and causing an increase in the initiation of lipolysis and mobilization of FFAs (18, 29). It has also been noted that the increase in lipid oxidation after HIIE might be related to an increase of β-adrenergic receptor stimulation, as elevations in plasma epinephrine and norepinephrine immediately following HIIE have been observed (13). While we did not directly measure FFA concentrations, previous literature has shown that plasma FFA concentrations are similar following exercise in cold and thermoneutral environments (8). Activation of

Table 2. *Area under the curve responses of glucose, insulin, and triglycerides 4 h postprandial*

	HIIE-TN	HIIT-CO
Glucose		
tAUC, mg·dL^{-1}·4 h^{-1}	345.15 ± 43.241	343.54 ± 36.335
iAUC, mg·dL^{-1}·4 h^{-1}	−36.95 ± 51.417	22.48 ± 13.395*
Insulin		
tAUC, mU·L^{-1}·4 h^{-1}	65.88 ± 25.623	70.00 ± 19.300
iAUC, mU·L^{-1}·4 h^{-1}	25.45 ± 12.048	33.94 ± 8.235
Triglycerides		
tAUC, mg·dL^{-1}·4 h^{-1}	358.55 ± 232.579	370.80 ± 222.156
iAUC, mg·dL^{-1}·4 h^{-1}	165.00 ± 140.968	136.776 ± 132.872

*Significantly different from HIIT-TN ($P < 0.05$). HIIE-TN, high-intensity interval exercise-thermoneutral exercise; HIIE-CO, high-intensity interval exercise-cold exercise; iAUC, incremental area under the curve; tAUC, total area under the curve.

α-adrenergic receptors elicits peripheral vasoconstriction during cold exposure, particularly when the skin is cooled (8, 30). Peripheral vasoconstriction may reduce blood flow in subcutaneous adipose tissue, potentially limiting the mobilization of FFA and lipolytic effects of cold exposure (8, 31). Additionally, FFAs can be reesterified to form triacylglycerol in the cytoplasm of the cell (32). Therefore, plasma FFA may not render an accurate measurement of whole-body lipolysis. Increased central blood volume, due to peripheral vasoconstriction, in combination with vasoconstrictor activated baroreceptor reflexes (8) likely led to the reduced HR observed during HIIE-CO. While we did not measure changes in catecholamines, a higher lipid oxidation rate during HIIE in the cold (13, 18) is likely due to a complex integration of α- and β-adrenergic receptor activity. The shift toward lipid metabolism during HIIE-CO may be responsible for the elevation of postprandial circulating blood glucose concentrations the following morning compared to HIIE-TN.

Increased exercise intensity may also be related to the expression of muscle lipoprotein lipase (LPL), which is responsible for increasing the hydrolysis of triglycerides. Grewiwe, Holloszy, and Semenkovich (2000) found that a single bout of exercise increases the expression of skeletal muscle LPL (19). Herd et al. (34) further found an inverse relationship between muscle LPL activity and postprandial lipemia. While catecholamines are known to be involved in the expression of LPL, interactions between catecholamines and adrenergic receptors are not required to produce an exercise induction of LPL (33). The exact mechanism by which exercise-induced LPL induction occurs is still unknown. Short-term cold exposure has been shown to elevate LPL activity in brown adipose tissue in humans (35). Increased LPL activity in the cold may mediate greater concentrations of LDL-C particles via the slow lipolysis of VLDL (36) or increased cholesteryl ester transfer protein breakdown of VLDL-TG, followed by subsequent LPL hydrolysis (35). Taken together, increased circulating catecholamines, stimulated by both high-intensity exercise and cold exposure, may have led to greater LPL activity, and subsequently, greater lipolysis during HIIE-CO compared to HIIT-TN.

An increase in substrate availability does not result in increased utilization during exercise in the cold (8, 9, 31, 37), and intramuscular mechanisms have been proposed to explain some of the discrepancies. The oxidation of intramuscular triglycerides is regulated via carnitine palmitoyltransferase-1 (CPT-1). CPT-1 is located within the outer mitochondrial membrane and is responsible for the transport of long-chain fatty acids (LCFAs) into the mitochondria for β-oxidation (54). Acute cold exposure may have a muscle-specific influence on the interaction of CPT-1 and malonyl-CoA, a CPT-1 inhibitor, favoring lipid oxidation (38). Cold exposure has also been shown to increase the transcription of proteins involved in the transport of acyl-CoA, such as CPT-1, in rat brown adipose tissue (39) and liver (55). However, the influence of cold on CPT-1 regulation may only occur after a specific amount of cold exposure (≥4 h) and/or with shivering thermogenesis (38). Further investigation of this mechanism is needed, particularly in human muscle tissue.

Thermal Effects on Postprandial Metabolism

Our results indicate that the addition of an acute cold stimulus during high-intensity interval exercise did not alter triglyceride levels nor further increase lipid oxidation rates during the postprandial period compared to thermoneutral exercise. Greater lipid oxidation rates during acute exercise in HIIE-CO the evening prior may have spared glucose, leading to higher CHO oxidation and lower lipid oxidation the following morning. The lack of greater lipid oxidation during the postprandial period following HIIE-CO may indicate that an energy turnover occurred, utilizing the substrate that was not prioritized the evening prior (i.e., CHO). Another factor commonly considered in the regulation of postprandial metabolism is the presence of an exercise-induced energy deficit. Energy expenditure during the exercise sessions in the current study was similar between conditions. Freese et al. (14) found that PPL was halved when the energy deficit created by exercise was replaced with a meal bar, compared to when the deficit was not replaced. If maintenance of an energy deficit is a critical factor in the reduction of PPL, the nutrition bar given to participants following each exercise session may have minimized potential differences in the postprandial response between conditions.

Additionally, participants were only precooled for 15-min before starting exercise, and while skin temperature was affected, a significant drop in core temperature did not occur during this period. Though T_c was slightly lower at the start of HIIE-CO (36.82 °C) versus HIIE-TN (37.19 °C), the acute effect of the cold environment acted to attenuate the exercise-induced rise in core temperature (Δ0.60 °C) compared to HIIE-TN (Δ0.81 °C) (8, 21). Attenuation of the rise in core temperature may be associated with attenuation of sympatho-adrenal responses (40), as discussed above. In this case, while the cold environment influenced

acute substrate metabolism, there may have not been a strong enough cold stimulus to produce a continued increase in lipid metabolism the following morning compared to thermoneutral exercise.

Even though there were no differences in postprandial triglyceride or insulin concentrations between conditions, the glucose response was quite diverse. Glucose AUC was significantly lower following HIIE-TN and seemingly lacked a glucose spike following the meal. Physical activity plays a significant role in improving glucose uptake (41), with the effects lasting 48–72 h after exercise (42). As exercise intensity increases, there is a greater reliance on muscle glycogen and when these stores are depleted, insulin stimulates glucose uptake through an increase of GLUT-4 translocation. A single bout of exercise has also been shown to prevent excessive increases in postprandial blood glucose in healthy individuals (42, 43). In the current study, higher glucose utilization during HIIE-TN exercise may have reduced muscle glycogen stores, and subsequently, increased glucose uptake the next morning following the consumption of the meal. Glycemic responses have also been shown to be lowered, or flattened, following the consumption of a high-fat meal, as indicated by a lower iAUC and glycemic peak (44, 45). Therefore, the absence of a glucose peak, and a lower glucose AUC following HIIE-TN may be due to a combination of greater CHO reliance during exercise and the high-fat content of the meal.

In addition to different glucose concentrations between conditions, plasma LDL-C concentrations remained constant for the first 2 h postprandial following HIIE-CO, whereas postprandial concentrations decreased following HIIE-TN. LDL-C concentrations are an indicator of lipolysis, and when present after a meal, may indicate risk of dyslipidemia and development of cardiovascular diseases. Previous research has shown that LDL-C concentrations decrease after the consumption of a meal in individuals both with and without abdominal adiposity (46). Yet, elevated LDL-C levels have been observed following cold exposure in humans (47, 48) and mice (47). Elevated LDL-C could be due to increased LPL activity in the cold, as previously discussed. It may also be indicative of elevated synthesis of LDL-C via the upregulation of enzymes and transcription factors following cold exposure (47). Further research is needed to confirm the mechanisms behind this response.

Limitations

A strength of this study is the inclusion of healthy overweight individuals, as opposed to lean individuals. Individuals under this classification have a worse triglyceride response to high-fat meals, and therefore, the benefits of exercise reducing postprandial triglyceride responses in this population is important (49). There is also some evidence to suggest that overweight/obese individuals may have increased lipid oxidation rates during exercise, related to an integration of increased, or maintained, plasma free fatty acid (FFA) availability, uptake, and oxidation, with elevated availability and oxidation of nonplasma lipids (e.g., intramuscular triglycerides) (50, 51). It should be acknowledged that lean individuals may have a different postprandial response following exercise under cold stress.

During the postprandial period, blood samples were collected in 1-h intervals. More frequent samples, particularly during the first hour, may have captured time-sensitive differences in blood variables. Participants were also instructed to stay still and silent during the postprandial period. On a few occasions, they had to voice technical or comfort issues, which could have had a minimal effect on respiratory data. Further, while we evaluated substrate oxidation via indirect calorimetry using equations proposed for high-intensity exercise (22), when intensities rise above an individual's lactate steady state, a shift in acid–base balance causes additional CO_2 excretion via hyperpnoea. The potential elevation of $\dot{V}CO_2$ may have overestimated CHO oxidation, while underestimating lipid oxidation (52). A nutrition bar was given to participants postexercise to reduce the likelihood of participant consuming food that night; however, it was not portioned per body mass, as per the HFM. Therefore, both the inclusion and the size of the nutrition bar may have reduced lipid oxidation the following morning by replacing all, or more than, the energy deficit induced by the HIIE session (14). Additionally, sex has been identified as a significant moderator on the effect of exercise on PPL (53). Freese et al. noted that women typically have a larger reduction in the total triglyceride response following exercise compared to men, and Gonzalez-Rodriguez et al. found sex differences in postprandial glycemic responses, which were linked to body-fat distribution and hormonal responses (44, 53). Due to the small sample size in the current study (n = 11), we were unable to evaluate potential sex differences.

Conclusions

Compared to a thermoneutral environment, performing acute high-intensity interval exercise under cold stress increases lipid oxidation and reduces CHO oxidation. However, during the postprandial period the following morning, after a high-fat meal, postprandial lipid metabolism was only marginally influenced by the cold exercise, whereas the glycemic response was lowered, or flattened, following the thermoneutral exercise. Therefore, while acute benefits seem to be present during acute HIIE in the cold, postprandial metabolic responses are less favorable when high-intensity interval exercise is performed with acute cold exposure.

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DISCLAIMERS

The authors declare that the results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.M., S.C.D., and D.D.G. conceived and designed research; S.M., L.M., and D.D.G. performed experiments; S.M., L.M., J.G., and A.M. analyzed data; S.M., J.G., and D.D.G. interpreted results of experiments; S.M. and D.D.G. prepared figures; S.M. and D.D.G. drafted manuscript; S.M., J.G., S.C.D., A.M., and D.D.G. edited and revised manuscript; S.M., L.M., J.G., S.C.D., A.M., and D.D.G. approved final version of manuscript.

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