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Human Kinetics
ORIGINAL RESEARCH

Cold Ambient Temperature Does Not Alter Subcutaneous Abdominal Adipose Tissue Lipolysis and Blood Flow in Endurance-Trained Cyclists

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This study sought to investigate the effect of cold ambient temperature on subcutaneous abdominal adipose tissue (SCAAT) lipolysis and blood flow during steady-state endurance exercise in endurance-trained cyclists. Ten males (age: 23 ± 3 years; peak oxygen consumption: 60.60 ± 4.84 ml·kg^{-1}·min^{-1}; body fat: 18.4% ± 3.5%) participated in baseline lactate threshold (LT) and peak oxygen consumption testing, two familiarization trials, and two experimental trials. Experimental trials consisted of cycling in COLD (3 °C; 42% relative humidity) and neutral (NEU; 19 °C; 39% relative humidity) temperatures. Exercise consisted of 25 min cycling at 70% LT and 25 min at 90% LT. In situ SCAAT lipolysis and blood flow were measured via microdialysis. Heart rate, core temperature, carbohydrate and fat oxidation, blood glucose, and blood lactate were also measured. Heart rate, core temperature, oxygen consumption, and blood lactate increased with exercise but were not different between COLD and NEU. SCAAT blood flow did not change from rest to exercise or between COLD and NEU. Interstitial glycerol increased during exercise ($p < .001$) with no difference between COLD and NEU. Fat oxidation increased ($p < .001$) at the onset of exercise and remained elevated thereafter with no difference between COLD and NEU. Carbohydrate oxidation increased with increasing exercise intensity and was greater at 70% LT in COLD compared to NEU ($p = .030$). No differences were observed between conditions for any other variable. Cycling exercise increased SCAAT lipolysis but not blood flow. Ambient temperature did not alter SCAAT metabolism, SCAAT blood flow, or fat oxidation in well-trained cyclists, though cold exposure increased whole-body carbohydrate oxidation at lower exercise intensities.

Keywords: environmental physiology, thermoregulation, microdialysis

At rest and during exercise, free fatty acids (FFAs) are liberated from adipocytes (i.e., lipolysis). These FFAs may then be transported to muscle tissue for oxidation, a process potentially limited by adipose tissue blood flow (Frayn & Karpe, 2014). Subcutaneous abdominal adipose tissue (SCAAT) is the predominate supplier of FFAs to the circulation during exercise (Horowitz, 2003). Thus, the lipolytic rate of SCAAT may influence whole-body fat oxidation and metabolic rate. Previous research reported increased SCAAT lipolysis and blood flow during endurance exercise (Baur et al., 2018). While the metabolic responses to exercise have been well studied, less is known about how environmental conditions alter these metabolic responses.

Military personnel and athletes are often exposed to challenging environments during training, athletic competition, and military operations (Pasiakos, 2020). These environmental conditions pose safety risks to the athlete or warfighter, alter physiological response and nutritional requirements, and can impair exercise performance (Pasiakos, 2020; Périard et al., 2021). Increased thermal strain of the environment (i.e., greater ambient temperature and humidity) is known to reduce exercise performance (Périard et al., 2021) and to result in greater muscle glycogenolysis, increased carbohydrate (CHO) oxidation, and increased blood lactate (Fink et al., 1975; Hargreaves et al., 1996).

In contrast, cold ambient temperatures may induce greater reliance on fat as a fuel source during exercise (Gagnon et al., 2013, 2020). However, the contribution of various fat depots during exercise in the cold has not been elucidated. Cold exposure may induce vasoconstriction to peripheral adipose tissue, reduce blood flow, and potentially impair lipid mobilization (Doubt, 1991; Layden et al., 2002). Though SCAAT represents a major adipose depot, no studies have examined the effect of cold ambient temperature on SCAAT lipolysis or SCAAT blood flow during endurance exercise. As cold ambient temperatures are common among athletes and warfighters, determining the metabolic response and the contributions of specific metabolic sites (e.g., SCAAT) to cold ambient temperatures during exercise may impact safety, nutritional and training recommendations, and performance optimization. Trained individuals have altered substrate metabolism compared to untrained individuals, making it essential to examine these responses in athletes (Phillips et al., 1996). Therefore, this study investigated the effect of cold ambient temperature during cycling exercise on SCAAT lipolysis and SCAAT blood flow in endurance-trained cyclists. We hypothesized that SCAAT lipolysis would increase and SCAAT blood flow would decrease in COLD compared to neutral (NEU) ambient temperatures.

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Materials and Methods

Participants

Ten healthy, endurance-trained male cyclists participated in this study. Subjects were required to meet the following inclusion criteria: (a) ages 18–45 years, (b) cycling peak oxygen consumption ($\dot{V}O_2$peak) ≥55 ml·kg^{-1}·min^{-1} or peak power output ≥350 W, and (c) ≥2 years cycling experience and cycled ≥3 days per week and ≥7 hr per week for the preceding 2 months while regularly competing in races. Participants were excluded if they had: (a) existing diseases or musculoskeletal disorders or injuries that could be exacerbated by cycling, (b) currently smoked or used any medications known to affect substrate metabolism, or (c) used anti-inflammatory drugs. After having the study explained, each participant provided their oral and written informed consent. The Florida State University Institutional Review Board approved all procedures (HSC 2015.15952).

Study Design

This was a randomized, crossover study consisting of five visits to the laboratory (separated by four to nine days). Lactate threshold (LT) and $\dot{V}O_2$peak were determined at baseline. Familiarization trials mimicked experimental procedures in a thermoneutral environment. Experimental trials were conducted in randomized order consisting of COLD (3.1 ± 1.8 °C; 41.6% ± 5.6% relative humidity) and NEU (19.4 ± 1.0 °C; 39.0% ± 2.2% relative humidity) temperatures. Before experimental trials, exercise and diet were standardized with participants replicating their diets via matching 48-hr dietary logs. Participants were asked to abstain from exercise, alcohol, and caffeine for 24 hr preceding each trial. Participants consuming dietary or ergogenic supplements were instructed to stop consumption and complete a washout period before participation. All trials took place between 0500 and 0900 hr to account for any alterations due to the circadian cycle.

Baseline Testing

Height and weight were assessed via wall-mounted stadiometer and digital scale, respectively (Seca Corporation). Body composition was assessed using dual-energy X-ray absorptiometry (Hologic Discovery W) by a certified technician according to manufacturer's specifications. Participants were then fitted to an electronically braked cycle ergometer (Velotron). Participants wore their personal cycling shoes and kit during testing. A chest strap heart rate (HR) monitor (Polar Electro Inc.) was fitted prior to performing a 5-min self-selected warm-up for a graded exercise test. Expired gases were continuously measured using a face mask (Cosmed V2 mask, COSMED) and TrueOne 2400 metabolic cart (ParvoMedics). Following the warm-up, participants cycled at a workload which was "comfortable, but not an easy pace for a 1-hr ride." Workload increased 20 W every 3 min until LT was achieved. Fingerstick blood samples were taken and analyzed immediately after each stage to determine power output at LT (blood lactate ≥4.0 mmol/L). Once LT was achieved, workload increased 30 W and stages were shortened to 2 min to determine $\dot{V}O_2$peak. Testing continued until participants were unable to maintain a cadence of >50 rpm or volitional exhaustion. To determine that $\dot{V}O_2$peak was achieved, the following criteria were used (Klein et al., 1996): (a) respiratory exchange ratio >1.15, (b) $\dot{V}O_2$ and HR leveling off despite increasing workload, and (c) attained predicted maximal HR (220–age). $\dot{V}O_2$peak was assessed as the highest 5-s average for oxygen consumption.

Experimental Trials

To assess core temperature (CT), each participant consumed a CorTemp radio-frequency telemetered thermometer pill (HQ, Inc.) 8–10 hr prior to arriving at the laboratory. Upon arrival, each participant immediately lay supine for insertion of the microdialysis probe using techniques previously described (Hickner, 2000). After probe insertion, 60 min was allowed for equilibration while the participant lay supine. During the final 10 min of the equilibration period, venous blood, urine-specific gravity (Atago PAL-10S; Atago USA, Inc.), and nude body weight measurements were taken. A resting microdialysis sample was taken for 5 min while seated following equilibration. Energy expenditure (5-min collection) via breath analysis was also measured using indirect calorimetry, and a resting fingerstick blood sample was obtained. Total CHO and fat oxidation were calculated via stoichiochemical equations described elsewhere (Jeukendrup & Wallis, 2005).

Participants then entered the environmental chamber and began the cycling protocol. No food, water, outside encouragement, or music was permitted. Cycling began at a wattage that elicited 70% LT for 25 min. The cycling intensity increased to a wattage that elicited 90% LT for an additional 25 min.

HR, CT, and dialysate measurements were taken at rest and every 5 min during the exercise protocol. Dialysate samples were stored on ice until completion of the testing bout, then at 4 °C, and analyzed for ethanol within 24 hr as described below. Subsequently, dialysate samples were stored at −80 °C for later analysis of interstitial glycerol concentration. Metabolic measurements via gas exchange were measured for 5 min at rest and for 1.5 min during exercise (rest: −5 to 0 min; 70% LT: 3.5 to 5 min, 23.5–25 min; 90% LT: 28.5–30 min, and 48.5–50 min). Fingerstick blood samples were also taken (rest: 0 min; 70% LT: 5 min, 25 min; 90% LT: 30 min, 50 min) to analyze blood glucose and lactate concentrations, then stored on ice until the end of data collection and analyzed via a glucose and lactate analyzer (YSI 2300 Stat, Yellow Springs Instruments).

Dialysate Analysis

Dialysate glycerol was measured according to manufacturer's instructions using an automated microdialysis analyzer (CMA 600 analyzer, CMA Microdialysis). To calculate interstitial glycerol concentrations, a separate in vitro experiment was conducted using the same probes and flow rates as in the in vivo conditions of this study (i.e., 5.0 µl/min). The probes were placed in a beaker solution (Dulbecco's phosphate-buffered saline, 0.1% bovine serum albumin, 5 mM glucose, 0.2 mM glycerol, 5 mM ethanol, and 2 mM of lactate), similar to conditions previously described (Pierce et al., 2015). Using the known concentrations of the beaker solution, in vitro recovery rates for a perfusion rate of 5.0 µl/min were determined by sampling both the beaker solution and the dialysate every 30 min over the course of 2 hr. In vitro recovery for glycerol at a perfusion rate of 5.0 µl/min (47.39%) was used as a reference point in the calculation to determine interstitial concentration for each condition. Interstitial concentrations at 5.0 µl/min were calculated using the following equation:

$$[\text{Dialysate glycerol}_{\text{flow rate}} / \text{Recovery}_{\text{in vitro}}] \times 100.$$

Intra- and interassay CV were recorded for glycerol (4.1% and 2.9%, respectively).

The ethanol technique (Hickner et al., 1992; Pierce et al., 2015) was used to estimate blood flow in the area surrounding the probes. As ethanol is not metabolized to any significant degree

in adipose tissue, its local removal is related to blood flow surrounding the probe (Hickner et al., 1992). A multimode microplate reader (SpectraMax M5, Molecular Devices) was used to measure ethanol concentrations within 24 hr of dialysate collection. Dialysate sample (2 µl) was added to a mixture containing an ethanol buffer (pH 8.9; 74 mM sodium pyrophosphate, 60 mM hydrazine sulfate, and 22 mM glycine) and a nicotinamide adenine dinucleotide solution (NADH, 100 mM) in black 96-well microplates (ThermoScientific Immuno Plates). Alcohol dehydrogenase (10 µl) was added to each well and incubated for 1 hr at room temperature in the dark. Samples were read with fluorescence at an excitation and emission of 360 and 415 nm, respectively. The fluorescent NADH product is directly proportional to ethanol in the sample (Hickner et al., 1992; Pierce et al., 2015). The intraassay CV for ethanol was 7.1%.

Statistical Analysis

Data were analyzed using SPSS (version 27) Statistics software package (IBM) with significance set at $p < .05$. Mixed repeated-measures analysis of variance (ANOVA) was used to identify differences between conditions for: CT, HR, SCAAT glycerol, blood glucose and lactate, CHO and fat oxidation rates, $\dot{V}O_2$, and blood flow. Using an α level of .05 and β of 0.8, it was estimated that nine participants would be sufficient to detect moderate to large differences (effect size = 0.32; G*Power, version 3.1) based on previous literature examining temperature effects on lipid metabolism during exercise (Gagnon et al., 2013, 2020; Sink et al., 1989). To determine the effect of exercise intensity, a secondary analysis was performed using mixed repeated-measures ANOVA for 70% LT (0–25 min) and 90% LT (25–50 min) separately. Greenhouse–Geisser corrections were used if sphericity was violated. If present, outliers were included in final analyses. If a significant Condition × Time interaction was observed, one-way ANOVA was used to determine significance between conditions at individual time points. All Condition × Time interactions are reported as ($F_{\text{dftreatment, dferror}}$) = F statistic, p value, partial eta squared (η_p^2). Effect sizes were qualified as follows: small = 0–0.02, medium = 0.02–0.13, and large = >0.13. All values are reported as mean ± SD, unless otherwise noted.

Results

Descriptive Characteristics

Ten volunteers were recruited from the local community. Table 1 summarizes the participant characteristics at baseline.

Environmental Conditions

Chamber temperatures (COLD: 3.1 ± 1.8 °C, NEU: 19.4 ± 1.0 °C; $p < .001$) but not humidity (COLD: 41.6% ± 5.6%, NEU: 39.0% ± 2.2%; $p = .202$) were significantly different between conditions.

Central Response

HR and CT (Figure 1) were not different at baseline ($p = .964$ and $p = .324$, respectively). HR ($p < .001$) and CT ($p < .001$) increased over time. No Condition × Time ($F_{3.646,\ 61.977} = 2.011$, $p = .110$, $\eta_p^2 = 0.106$) or main ($p = .166$) effects were observed for HR. Similarly, no Condition × Time ($F_{3.582,\ 64.483} = 2.153$, $p = .091$, $\eta_p^2 = 0.107$) or main ($p = .788$) effects were observed for CT.

Table 1 Baseline Descriptive Variables

	n	Baseline
Age (years)	10	23 ± 3
Height (cm)	10	178.6 ± 5.9
Body mass (kg)	10	74.0 ± 11.4
Body fat (%)	10	18.4 ± 3.5
$\dot{V}O_2$peak (ml·kg^{-1}·min^{-1})	10	60.60 ± 4.84
Power output at LT (W)	10	234 ± 35
Urine-specific gravity (g/ml)	9	1.015 ± 0.008

Note. Data are expressed as mean ± SD. LT = lactate threshold; $\dot{V}O_2$peak = peak oxygen consumption.

Metabolic Response: All Data

There were no differences at baseline for any measure. When all time points were analyzed (0–50 min), interstitial glycerol significantly increased over time ($p < .001$) but no Condition × Time ($F_{1.316,\ 18.428} = 0.354$, $p = .618$, $\eta_p^2 = 0.025$) or main ($p = .582$) effects were observed (Figure 2). CHO oxidation increased at the onset of exercise and as exercise intensity increased from 70% to 90% LT ($p < .001$); however, no Condition × Time ($F_{1.720,\ 18.920} = 1.266$, $p = .300$, $\eta_p^2 = 0.103$) or main ($p = .178$) effects were observed (Figure 3). Fat oxidation increased at the onset of exercise ($p < .001$) and remained elevated thereafter, but no Condition × Time ($F_{1.474,\ 16.209} = 1.528$, $p = .243$, $\eta_p^2 = 0.122$) or main ($p = .260$) effects were observed (Figure 3). There was a significant time ($p = .020$) but not Condition × Time ($F_{1.470,\ 23.528} = 0.996$, $p = .361$, $\eta_p^2 = 0.059$) or main ($p = .745$) effect for blood glucose. Blood lactate increased over time ($p < .001$), and a trend for a significant Condition × Time effect ($F_{1.533,\ 24.520} = 3.657$, $p = .051$, $\eta_p^2 = 0.186$) was observed (Figure 4). However, one-way ANOVA revealed no differences between conditions at any time point. $\dot{V}O_2$ continuously increased ($p < .001$), but no Condition × Time ($F_{1.721,\ 18.933} = 0.390$, $p = .652$, $\eta_p^2 = 0.034$) or main ($p = .398$) effects were observed (Figure 5).

Metabolic Response: 70% LT

When analyzing only the 70% LT portion of the exercise bout (0–25 min), interstitial glycerol increased over time ($p < .001$) but no Condition × Time ($F_{1.780,\ 30.255} = 1.533$, $p = .233$, $\eta_p^2 = 0.083$) or main ($p = .561$) effects were observed. Fat oxidation increased ($p < .001$) at the onset of exercise and remained elevated thereafter. No Condition × Time ($F_{1.412,\ 19.771} = 0.520$, $p = .119$, $\eta_p^2 = 0.153$) or main ($p = .146$) effects were observed. At the lower exercise intensity, CHO oxidation increased over time ($p < .001$), but no Condition × Time ($F_{1.188,\ 16.626} = 3.057$, $p = .094$, $\eta_p^2 = 0.179$) effect was observed. Main effects indicated significantly greater CHO oxidation in COLD compared to NEU ($p = .030$, $\eta_p^2 = 0.295$). Blood glucose did not change with time ($p = .374$), and no Condition × Time ($F_{2,\ 34} = 0.508$, $p = .606$, $\eta_p^2 = 0.029$) or main ($p = .963$) effects were observed. Blood lactate increased ($p < .001$) with no Condition × Time ($F_{2,\ 34} = 1.037$, $p = .366$, $\eta_p^2 = 0.057$) or main ($p = .531$) effects. $\dot{V}O_2$ increased ($p < .001$), but no Condition × Time ($F_{2,\ 28} = 0.838$, $p = .443$, $\eta_p^2 = 0.056$) or main ($p = .346$) effects were observed.

Metabolic Response: 90% LT

When analyzing only the 90% LT portion of the exercise bout (25–50 min), interstitial glycerol continued to increase ($p < .001$) with

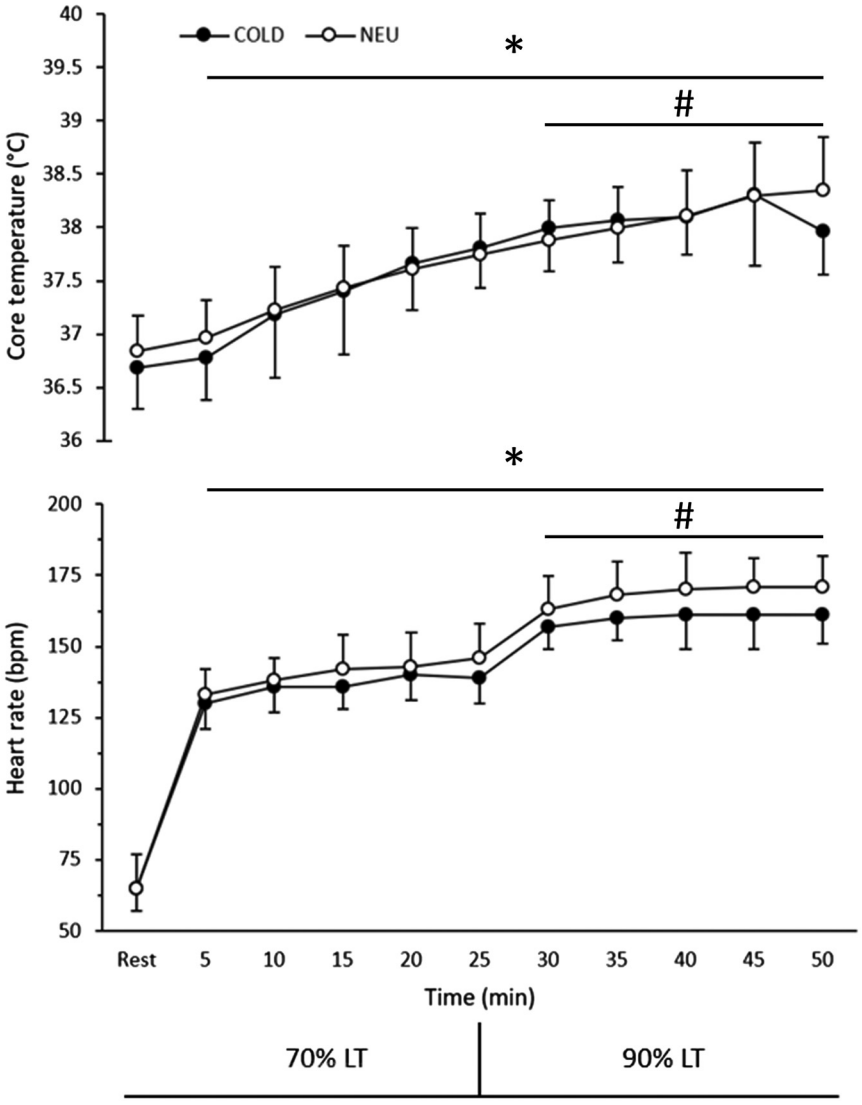

Figure 1 — Central response during cycling exercise. Core temperature (top; $n = 10$) and heart rate (bottom; COLD: $n = 10$, NEU: $n = 9$) are presented as mean ± *SD*. There were no differences between temperature conditions. *Significantly greater compared with rest ($p < .01$). #Significantly greater from 70% LT ($p < .01$). NEU = neutral; LT = lactate threshold.

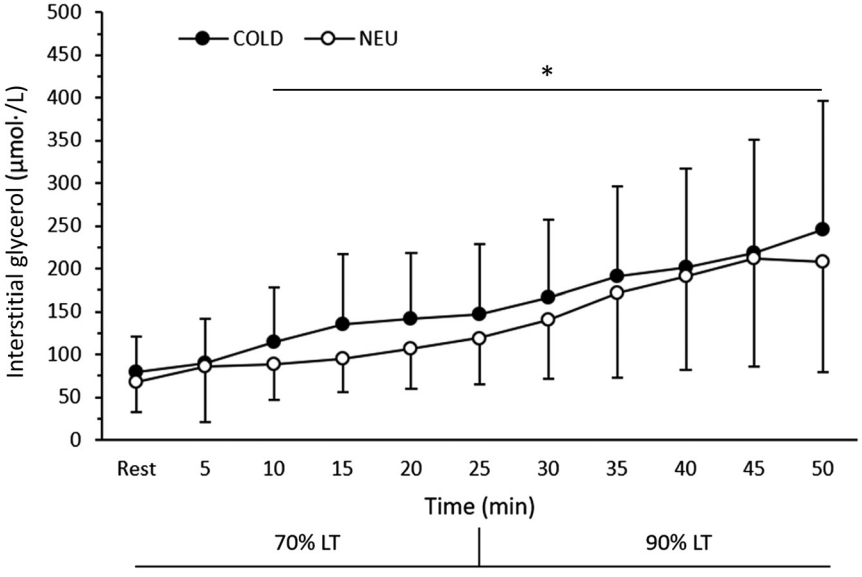

Figure 2 — Interstitial glycerol concentration during cycling exercise (due to missed samples, $n = 8$; mean ± *SD*). There were no significant differences between temperature conditions. *Significantly greater compared with rest ($p < .01$). NEU = neutral; LT = lactate threshold.

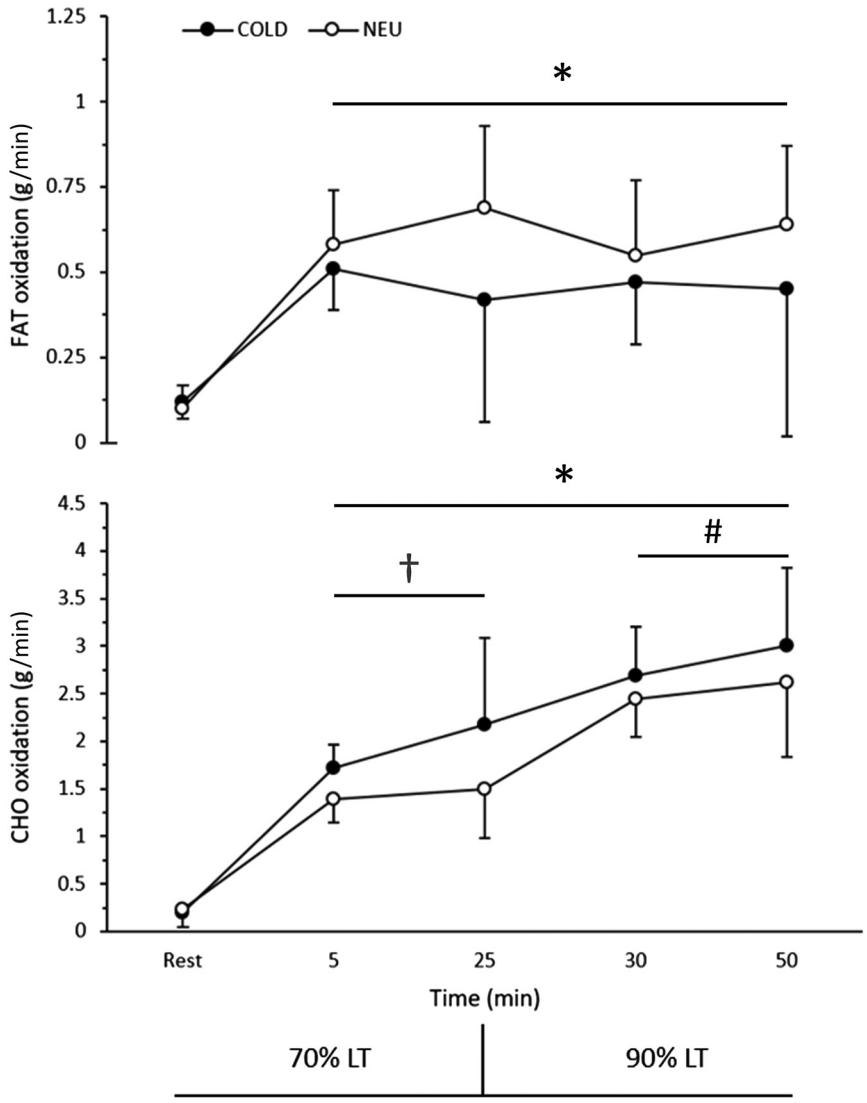

Figure 3 — Whole-body substrate utilization during cycling exercise for fat oxidation (top) and CHO oxidation (bottom; due to missed samples, COLD: $n = 6$; NEU: $n = 7$; mean ± *SD*). *Significantly greater compared with rest ($p < .001$). #Significantly greater from 70% LT ($p < .01$). †CHO oxidation was significantly greater in COLD at 70% LT ($p < .05$). NEU = neutral; LT = lactate threshold; CHO = carbohydrate.

no Condition × Time ($F_{1.311,\ 18.357} = 0.477$, $p = .549$, $\eta_p^2 = 0.033$) or main ($p = .692$) effects. No time ($p = .723$), Condition × Time ($F_{1.294,\ 16.825} = 0.814$, $p = .410$, $\eta_p^2 = 0.059$), or main ($p = .273$) effects were observed for fat oxidation. CHO oxidation increased ($p < .001$) at the onset of the greater exercise intensity and remained elevated thereafter, but no Condition × Time ($F_{2,\ 26} = 1.127$, $p = .339$, $\eta_p^2 = 0.080$) or main ($p = .468$) effects were observed. There was a significant time effect ($p = .024$) such that blood glucose was increased at 50 min compared to 25 and 30 min, but no Condition × Time ($F_{1.141,\ 18.263} = 0.987$, $p = .345$, $\eta_p^2 = 0.058$) or main ($p = .573$) effects were observed. Blood lactate increased at the onset of the greater exercise intensity and remained thereafter ($p < .001$). Significant Condition × Time ($F_{1.337,\ 21.400} = 4.079$, $p = .046$, $\eta_p^2 = 0.203$) effects were observed. However, one-way ANOVA revealed no differences between conditions at any time point. $\dot{V}O_2$ continued to increase ($p < .001$) but no Condition × Time ($F_{1.352,\ 17.581} = 0.761$, $p = .433$, $\eta_p^2 = 0.055$) or main ($p = .967$) effects were observed.

Blood Flow

SCAAT blood flow did not change over time ($p = .320$) and was not different between conditions at rest ($p = .144$) or during exercise ($p = .308$). No main effects were observed ($p = .816$). Therefore, no blood flow adjustments were necessary.

Discussion

Exercise increases lipid mobilization from SCAAT (Baur et al., 2018; Ormsbee et al., 2007, 2009); however, there are no data demonstrating the effect of cold exposure during exercise on glycerol mobilization from SCAAT using microdialysis. Using microdialysis, we were able to determine dialysate glycerol concentration in SCAAT before and during exercise. The novel finding from the present study is that COLD did not influence SCAAT lipolysis, SCAAT blood flow, or fat oxidation during steady-state exercise below the LT (70% and 90% LT) compared to NEU.

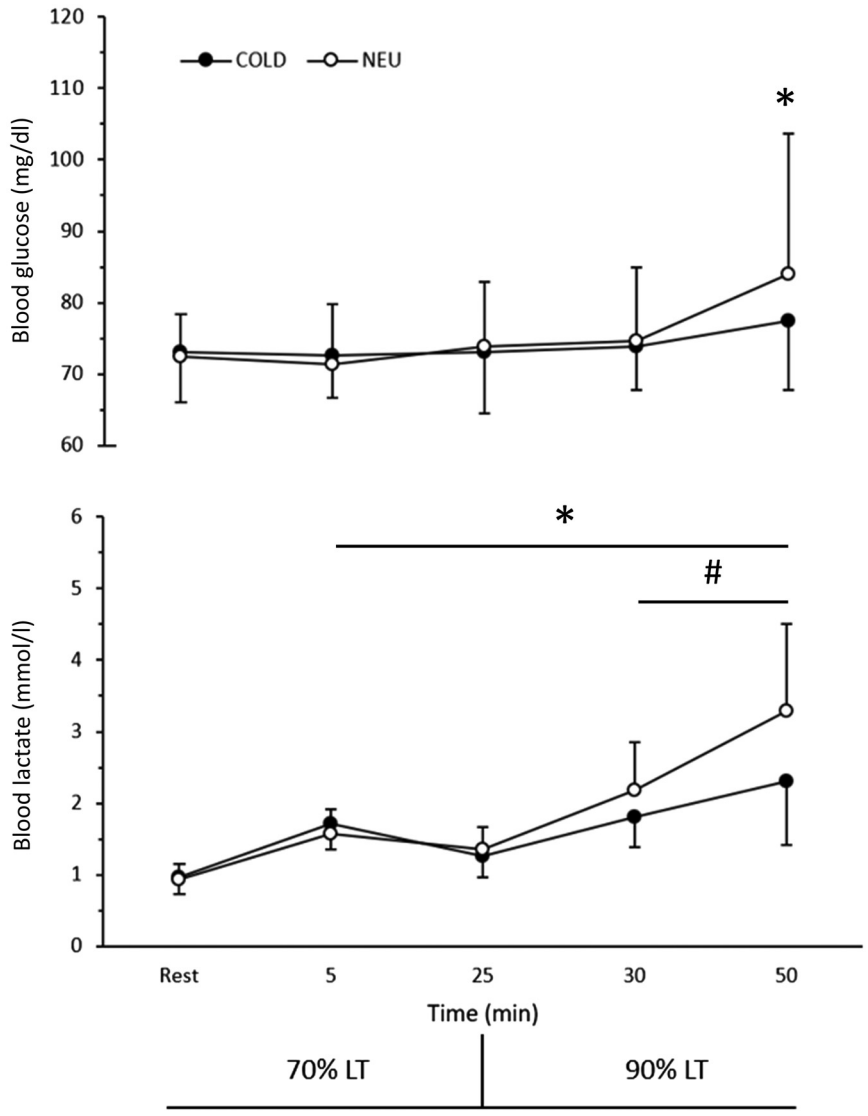

Figure 4 — Blood glucose (top) and blood lactate (bottom) response during cycling exercise ($n = 9$; mean ± *SD*). There were no differences between temperature conditions. *Significantly greater compared with rest ($p < .05$). #Significantly greater from 70% LT ($p < .05$). LT = lactate threshold; NEU = neutral.

As no studies have examined the effects of cold ambient temperature on SCAAT lipolysis with microdialysis, it is not possible to make direct comparisons. However, since previous research has shown that circulating plasma glycerol and local SCAAT glycerol concentrations respond to stimuli in a similar manner (Hickner et al., 1999), and as we observed no alteration in SCAAT blood flow which suggests fuel mobilization impairment was unlikely, inferences can be made based on currently available literature. Aerobic exercise increases SCAAT lipolysis (Baur et al., 2018), and the present study validates those findings. Previous work investigating cold air exposure, alone, on lipolysis has reported increased plasma glycerol concentrations in 5 °C compared to 29 °C (Vallerand et al., 1999). Yet, when aerobic exercise is combined with cold exposure, it does not appear that plasma glycerol concentrations are altered (Gagnon et al., 2013; Galloway & Maughan, 1997). No differences in plasma glycerol during walking or running exercise in 0 or 22 °C were observed despite increased fat oxidation in the cold (Gagnon et al., 2013). To explain the increased fat oxidation despite no change in blood parameters, these authors speculated an increased reliance on intramuscular triglyceride (IMTG) stores in cold conditions, though this was not measured. While SCAAT represents the largest contribution of fatty acids during exercise (Horowitz, 2003), IMTGs are also utilized to a significant degree, contributing between 10% and 50% of total fat oxidation during moderate-intensity exercise (Horowitz, 2003; van Loon et al., 2003) with endurance-trained individuals having an increased capacity to utilize IMTGs compared to sedentary (Phillips et al., 1996). Given the highly trained status of our participants, it is possible that no differences were observed between conditions due to partitioning of other adipose tissue depots (e.g., IMTGs). Therefore, multiple sites of lipolytic activity during exercise in cold ambient temperatures should be investigated to quantify the contribution of each depot. However, as we observed no differences in fat oxidation between COLD and NEU, it seems unlikely that IMTG utilization altered our findings.

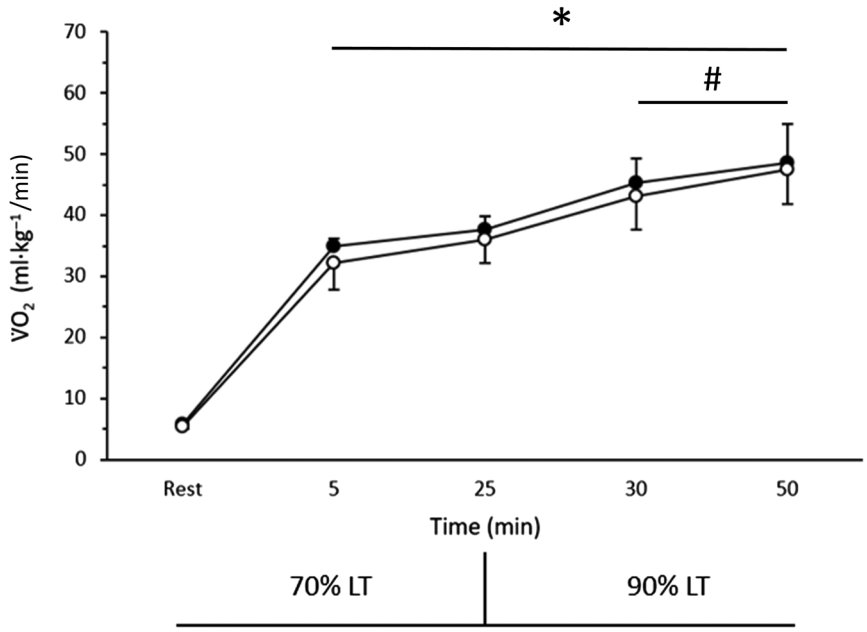

Figure 5 — $\dot{V}O_2$ response during cycling exercise (due to missed samples, COLD: $n = 6$; NEU: $n = 7$; mean ± *SD*). *Significantly greater compared with rest ($p < .01$). #Significantly greater from 70% LT ($p < .01$). LT = lactate threshold; NEU = neutral; $\dot{V}O_2$ = oxygen consumption.

No differences in blood glycerol concentrations or total fat oxidation rates were observed between 4°C and 21 °C during time to exhaustion cycling at 70% $\dot{V}O_{2\,max}$ despite reduced CT in the cold (Galloway & Maughan, 1997). Exercise modality differences could explain the incongruent results. In support of this, recent work demonstrated that exercise modality alters energy substrate metabolism (Gagnon et al., 2020). These authors reported greater maximal fat oxidation in cold (4.6 °C) versus warm (34.1 °C) conditions during treadmill but not cycling exercise. Our findings corroborate that fat oxidation is not altered in cold conditions during cycling exercise. Therefore, the most likely explanation for not observing differences in SCAAT lipolysis or fat oxidation in the COLD condition is the cycling exercise utilized in the present study.

It has been suggested that results not showing an augmented, cold-induced lipolytic response are due, at least partially, to peripheral perfusion limitations to adipose during exercise, as cold exposure has been speculated to decrease adipose blood flow via potentiation of adrenergic vasoconstriction (Hjemdahl & Sollevi, 1978). Thus, diminished peripheral blood flow may limit the liberation of FFA and glycerol from adipose into the blood (Doubt, 1991; Hjemdahl & Sollevi, 1978; Layden et al., 2002), although this has not been verified. Therefore, we measured the microcirculatory blood flow in SCAAT to ensure that any alterations in interstitial markers were a direct result of environmental temperature and not temperature-induced alterations in blood flow. In contrast, we observed no reduction of SCAAT blood flow in COLD. Our data demonstrated that neither cycling exercise nor the environmental conditions alter in situ SCAAT blood flow. To our knowledge, this is the first study to address the environmental influence on SCAAT blood flow during endurance exercise. Others reported increased SCAAT lipolysis and no change in SCAAT blood flow with moderate-intensity cycling, alone (de Glisezinski et al., 1998). In contrast, treadmill running increased SCAAT blood flow (Baur et al., 2018). The disparity in exercise modality likely explains incongruent findings.

We observed increased CHO oxidation in COLD compared to NEU at 70% LT. In agreement, increased CHO oxidation was reported during cycling at 0 °C and −10 °C compared to 10 °C and 20 °C (Layden et al., 2002). These authors speculated increased CHO utilization could be due to reduced lipolysis, mobilization of FFA, uptake of FFA into muscle, or oxidative capacity of the muscle. As we observed no differences in SCAAT lipolysis, this explanation appears unlikely. While we did not observe alterations in SCAAT blood flow, we cannot rule out impairments to FFA mobilization as blood glycerol was not measured. Similarly, FFA uptake into the muscle was not directly measured and, therefore, cannot be ruled out. Others reported increased CHO oxidation in 4 °C compared to 11 °C and 21 °C (Galloway & Maughan, 1997). These authors suggested reduced mechanical efficiency during cold exposure may explain their findings. We observed increased CHO utilization at lower exercise intensities but not higher exercise intensities where greater metabolic heat production would occur. Therefore, it is possible that we observed increased CHO reliance at 70% LT in COLD due to reduced mechanical efficiency.

We observed no differences in CT between conditions, suggesting the cold stimuli may have been insufficient to induce physiological alterations. Lind (1963) described the "prescriptive zone," whereby CT is influenced by metabolic rate during treadmill walking rather than ambient temperature within a certain range of environmental conditions (Lind, 1963). This work investigated temperatures as low as 10 °C; however, it is possible that the ambient temperature utilized in the present study falls within this prescriptive zone. Previous reports have shown reduced CT during cycling exercise at 4 °C (Galloway & Maughan, 1997), while we, and others, did not observe differences in CT during cycling (Layden et al., 2002) or running (Gagnon et al., 2013). This would suggest that the present study likely does fall within the prescriptive zone. Nonetheless, previous reports have shown alterations in glycerol concentrations with no change in CT (Layden et al., 2002) and no alterations in glycerol concentration despite CT differences (Galloway & Maughan, 1997). Thus, the impact of

internal temperature on lipolysis remains unclear. Others have suggested fat metabolism is altered only when temperatures are ≤0 °C (Layden et al., 2002, 2004). Our findings seemingly support this as the COLD condition in the current study was 3.1 ± 1.8 °C, and we observed no differences in fat metabolism. Therefore, the cold stimulus in the present study was perhaps insufficient to induce fat-related metabolic alterations. Future work should investigate the effect of temperatures at or below that threshold on SCAAT lipolysis during endurance exercise.

Neither skin nor muscle temperature was measured in the present study, limiting our ability to quantify the magnitude of cold exposure experienced by our participants. However, as cold ambient air has been suggested to result in little or no change in muscle temperature (Castellani et al., 2021), this is unlikely to have influenced our results. Further, studies utilizing similar environmental conditions have consistently shown reduced skin temperature with mixed results on metabolic alterations (Gagnon et al., 2013; Galloway & Maughan, 1997; Layden et al., 2002; Sink et al., 1989), suggesting that, though not directly measured, skin temperature would have been reduced. Though ice storage preserves samples compared with no ice (Higgins et al., 2023), it is possible that our results, particularly lactate, were altered compared with immediate analysis. LT was determined in thermoneutral conditions. Due to the fixed-rate protocol (70% and 90% LT), it is possible that temperature-mediated alterations in work rate ensued. As females were not included in this study, results may not translate to females. Finally, our observed power for metabolic variables was low. While our initial sample size was in line with other studies investigating cold-induced metabolic alterations during exercise (Gagnon et al., 2013, 2020; Sink et al., 1989), the present study is likely underpowered to detect smaller differences which may exist. With a larger sample size, differences could have emerged between temperature conditions. Despite these limitations, we believe the present study has practical implications as athletes and military personnel are likely to experience similar environmental conditions during training, competition, or military operations. These findings suggest that training, nutrition, and supplementation strategies need not be altered to accommodate cold, but above freezing, ambient temperatures.

In conclusion, by using microdialysis, we observed an increase in SCAAT lipolysis during cycling exercise. However, cold ambient temperature did not influence in situ SCAAT lipolysis or SCAAT blood flow, nor did it alter whole-body fat metabolism below the LT.

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