Metabolic flexibility is unimpaired during exercise in the cold following acute glucose ingestion in young healthy adults

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Abstract

Purpose: Metabolic flexibility is compromised in individuals suffering from metabolic diseases, lipo- and glucotoxicity, and mitochondrial dysfunctions. Exercise studies performed in cold environments have demonstrated an increase in lipid utilization, which could lead to a compromised substrate competition, glycotoxic-lipotoxic state, or metabolic inflexibility. Whether metabolic flexibility is altered during incremental maximal exercise to volitional fatigue in a cold environment remains unclear.

Methods: Ten young healthy participants performed four maximal incremental treadmill tests to volitional fatigue, in a fasted state, in a cold (0°C) or a thermoneutral (22.0°C) environment, with and without a pre-exercise ingestion of a 75-g glucose solution. Metabolic flexibility was assessed via indirect calorimetry using the change in respiratory exchange ratio (ΔRER), maximal fat oxidation (ΔMFO), and where MFO occurred along the exercise intensity spectrum (ΔFat_{max}), while circulating lactate and glucose levels were measured pre and post exercise.

Results: Multiple linear mixed-effects regressions revealed an increase in glucose oxidation from glucose ingestion and an increase in lipid oxidation from the cold during exercise ($p<0.001$). No differences were observed in metabolic flexibility as assessed via ΔRER (0.05 ± 0.03 *vs.* 0.05 ± 0.03; p=0.734), ΔMFO (0.21 ± 0.18 *vs.* 0.16 ± 0.13 g•min^{-1}; p=0.133) and ΔFat_{max} (13.3 ± 19.0 *vs.* 0.6 ± 21.3 %$\dot{V}O_{2peak}$; p=0.266) in cold and thermoneutral, respectively.

Conclusions: Following glucose loading, metabolic flexibility was unaffected during exercise to volitional fatigue in a cold environment, inducing an increase in lipid oxidation. These results suggest that competing pathways responsible for the regulation of fuel selection during exercise and cold exposure may potentially be mechanistically independent. Whether long-term metabolic

influences of high-fat diets and acute lipid overload in cold and warm environments would impact metabolic flexibility remain unclear.

Keywords: Exercise, thermal stress, indirect calorimetry, metabolism, health

Abbreviations:

CHO – carbohydrate
Fat_{max} – maximal fat oxidation (%VO_{2peak})
HR – heart rate
MFO – maximal fat oxidation
NEFA – non-esterified fatty acid
RER – respiratory exchange ratio
$\bar{T}_{sk}$ – mean skin temperature
$\dot{V}CO_2$ – volume carbon dioxide produced
$\dot{V}O_2$ – volume oxygen consumption
$\dot{V}_E$ – minute ventilation BTPS
$\dot{V}_t$ – tidal volume BTPS

Conditions:
CO-CON – Cold Control
CO-GLU – Cold Glucose
TN- CON – Thermoneutral Control
TN-GLU – Thermoneutral Glucose

1. Introduction

Metabolic flexibility is the ability of the organism to adjust fuel utilization according to a multi-factorial network that includes substrate sensing, trafficking, storage, availability, and demand (Smith et al., 2018), and is a key indicator of mitochondrial dysfunction via its link to type 2 diabetes mellitus (Galgoni, et al., 2008), metabolic syndrome (Fonseca, 2005), insulin resistance (Kelley et al., 1999; Kelley and Mandarino, 2000), obesity (Kelley et al., 1999) and health (Duchan, 2004; Nicolson, 2007; Galgani et al., 2008; Galgani, et al., 2008). Metabolic flexibility is known to be heavily influenced by lifestyle changes, including diet, weight loss, and increased physical activity (Achten & Jeukendrup, 2004; Battaglia et al., 2012; Corpeleijn et al., 2009).

Metabolic inflexibility is classified as a "nutrient overload" or a "heightened substrate competition" (Muoio, 2014), resulting in a lack plasticity during metabolic adjustments. Inflexibility has been importantly associated to liver and skeletal muscle insulin resistance (Flier et al, 1979; Holland & Summers, 2008; Shulman, 2004). In a healthy model, the flux:ratio of pyruvate dehydrogenase (PDH) / β oxidation at the mitochondrial level seem to be the main regulator of substrate selection. Glycolysis increases malonyl-CoA activity, inhibiting carnitine palmitoyl-transferase I (CPT-1), responsible for transporting long-chain fatty acid from the cytosol to the mitochondria. Under fat oxidation dominance, not only CPT-1 activity is increased, but PDK is activated, resulting in the inactivation of PDH and lower acetyl-CoA production from glycolysis. This flux:ratio tends to disappear under chronic metabolic challenges, such as following obesogenic diets with high-fat or high-sugar content (Kuzmiak-Glancy and Willis, 2014; Jorgensen et al., 2017).

Exercise and acute substrate loading elevate the body from its minimal homeostatic oxidation rate and provide a wider scope of mitochondrial flexibility and fuel selection (Romjin et

al., 1993; Brooks and Mercier, 1994; Smith, 2018). An increase in exercise intensity displays metabolic fluxes between lipids and CHO oxidation (van Loon et al, 2001; Brooks, 1997) where peak fat oxidation occurs at approximately 50% of maximal oxygen consumption ($\dot{V}O_{2max}$) whereas, CHO subsequently becomes the main source of energy and peaks at maximal exercise intensities (Romijn et al., 1993; Brooks & Mercier, 1994; Achten et al. 2002; Achten & Jeukendrup, 2004; Storlien et al., 2004; Venables et al., 2005). As exercise intensity increases, lactate accumulates and muscle pH decreases, where the increase in H^+ ions results from the high energy demand and the lack of oxygen supply to the muscles (Sahlin, 1986). Moreover, carnitine, and CPT-1, regulate the production of acetyl-CoA and NADH during exercise (Arenas, 1991; Sahlin, 1990). As exercise intensity increases, there is a decrease in free carnitine, limiting lipid oxidation as less carnitine can be converted to acetyl-CoA to allow for fatty acid oxidation to occur (Sahlin, 1990), thereby causing a progressive alteration toward CHO reliance. During acute substrate loading, carbohydrate ingestion leads to greater circulating glucose and insulin concentrations, which increase glucose uptake in tissue, glycolysis, pyruvate oxidation through PDH activity, and suppress fatty acids oxidation via the increase in malonyl-CoA and decrease in CPT-1 activity (Smith, 2018). Independently, or combined, exercise and acute substrate loading can challenge energy homeostasis and give a window on metabolic flexibility state.

Exercising in the cold have demonstrated increases in fat oxidation compared to thermoneutral conditions (Hurley & Haymes, 1982; Timmons et al., 1985; Shephard, 1992). Gagnon et al. (2013, 2019) suggested a greater reliance on intra-muscular triglycerides oxidation and a metabolically-independent increase in CPT-1 activity to explain this increase in fat oxidation. Moreover, cold exposure and its associated vasoconstriction on peripheral tissues, could regulate a greater affinity of working muscles to extract non-esterified fatty acids (NEFA) due to a longer

mean capillary transit time (Kiens et al. 1993). Together, the observed shift in energy metabolism towards lipid metabolism combined with the possibility of improved regulation of NEFA influx in skeletal muscles could influence metabolic substrate flux and mitochondrial flexibility. Since the increase in glycolysis and malonyl-CoA during exercise could be impaired by a potential rise in CPT-1 activity in the cold, investigating metabolic flexibility in the cold could give us some insights on the interaction of competing pathways.

In participants with diabetes mellitus, metabolic syndrome, and obesity, metabolic flexibility is compromised, as there is an inability to switch between available substrates, leading to lipo- and gluco-toxicity (Boden & Shulman, 2002). Whether or not a cold-induced increase in lipid oxidation during exercise compromises metabolic flexibility, via the theoretically confounding increase in malonyl-CoA and the deactivation of CPT-1 from acute glucose loading, is unknown. Thereby, this study investigated the effects of a cold environment and acute glucose loading on metabolic flexibility during incremental exercise across a wide range of intensities.

2. Methods

2.1 Participants

Ten young, healthy participants (6M-4W) completed the study. Participants' anthropometric and fitness characteristics are presented in Table 1. Data collection was conducted during the months of January and February. Each participant provided informed consent and completed a physical activity readiness questionnaire and a health-screening form for cardiovascular, respiratory, and metabolic conditions that could be aggravated by exercise or cold. None of the participants were on medication. All female participants reported to use oral contraceptives. This study was approved by the Laurentian University Research Ethics Board, and performed according to the declaration of Helsinki.

2.2 Experimental Design

All participants completed four experimental sessions, dressed in shorts and t-shirt. Each session was separated by a minimum of 1 week, followed a balanced design, and occurred at the same designated time of day to control for circadian influences. Participants completed the experimental sessions following a 10-12 hour overnight fast. They were also requested to avoid alcohol consumption, strenuous exercise, caffeine and tobacco 24 hours prior to each experimental exercise session. Female menstrual cycles were not recorded but the experimental design and distribution of exercise conditions over a period of four weeks likely washed out any menstrual effects of metabolic variables. To control for dietary influences on metabolism during exercise, participants maintained the same diet each day prior to their exercise sessions, with the built-in assumption that this energy standardization method yields significant variability in food intake between participants (Jeacocke & Burke 2010). Arrival to the laboratory was between 0700 to 1000 h, preceded by an overnight fast. Water was available at request before all sessions. Participants took part in four maximum incremental treadmill tests within a thermal chamber at two different environmental temperatures (thermoneutral (TN): 21.9 ± 1.1ºC, cold (CO): 0.2 ± 2.0°C), each with (GLU) and without (CON) an acute ingestion of a glucose solution.

2.3 Preliminary Session

Prior to the initial exercise test, anthropometric data was collected, including height, body mass, and percent body fat. Percentage body fat was estimated via skin folds measured at four sites (biceps, triceps, subscapular, and supraliac) (Jackson & Pollock, 1978; Jackson & Pollock, 1980). Body surface area was calculated using height and weight (Dubois & Dubois, 1916).

2.4 Experimental Protocol

Upon arrival at the laboratory, fingertip blood lactate, and blood glucose were measured. Then, participant either ingested a glucose solution (300mL solution, deoxygenated water and 75g dissolved Lantic Sugar) (Mähler et al., 2012; Oberbach et al., 2008; Afman et al., 2014), or water only, followed by 30-minutes seated rest (Engeroff et al., 2017; Virkkunen et al., 1994). Following the seated rest, a second fingertip blood glucose measure was collected, and participants proceeded to instrumentation outside of the thermal chamber. The participants entered the thermal chamber and immediately performed an incremental maximal treadmill protocol (Gagnon et al., 2019). Briefly, each stage of the protocol was 3 min, and began at 2.2 mph at a 1% incline. For the next four stages, speed was increased to 2.7 mph, 3.4 mph, 4.0 mph, and 4.7 mph. Following these stages, the speed was maintained at 4.7 mph and the grade increased by 2% at every stage. The participant continued until their respiratory exchange ratio (RER) reached 1.0 (point from which no fat contributes to energy expenditure), then, speed was increased to 6.0 mph with the grade maintained, and the speed increased 1.0 mph every minute until volitional fatigue. The continuation of the protocol after RER reached 1.0 was performed to obtain a measure for maximum $\dot{V}O_2$ for relative comparisons between conditions in a short period of time. Immediately following the exercise protocol, a fingertip blood sample was collected for lactate and glucose concentrations.

2.5 Instrumentation

The participants were instrumented outside of the thermal chamber while standing in anatomical position. Skin temperature was measured via Thermochron temperature data loggers

(iButton DS1922L, iButtonLink, Technology, WI, USA) placed at six locations on the body (forehead, chest, lateral forearm, hand, lateral thigh, and lower back). The temperature readings from each location were used to calculate mean skin temperature ($\overline{T}_{sk}$) (Palmes & Park, 1947).

A polar heart rate monitor (Polar Electro Oy, V800, Kempele, Finland) was secured around the participant's chest, and recorded heart rate (HR) continuously throughout each session. Data for $\dot{V}O_2$, RER, and volume of carbon dioxide produced ($\dot{V}CO_2$) were collected using an open-circuit ergospirometer in breath-by-breath mode (Ultima CPX, MGC Diagnostics, St. Paul, MN, USA). Before each trial, the gas analyzers were calibrated with air tanks containing 15% O_2 and 5% CO_2, respectively, in the pre-determined environmental condition. This was performed volume calibration, while the bidirectional flow sensor was calibrated with a 3-L syringe. The sensor, connected to a flexible properly-adjusted face mask, was used in all experimental sessions to measure the flow of gases.

2.6 Blood Sampling

A standard finger prick method, using a disposable lancet, was used to measure lactate and glucose levels. A blood lactate meter (Lactate Pro, Arkray, Shiga, Japan) was used to collect the participant's blood lactate before and immediately following each treadmill protocol (Goodwin, Harris, Hernández, & Gladden, 2007). Also, a blood glucose monitoring system (FreeStyle, Precision Neo, ON, CA) was used to measure blood glucose levels at three designated times: before the glucose treatment ingestion, 30-minutes following glucose ingestion, and directly following exercise. For control conditions, the 30-minutes following glucose ingestion time point was omitted.

2.7 Data Analyses

$\dot{V}O_2$, $\dot{V}CO_2$, RER, minute ventilation ($\dot{V}_E$), tidal volume ($\dot{V}_t$), HR, and skin temperature were all measured continuously during exercise. For each measure, the last minute of every stage was averaged for analysis. For peak value variables (maximal fat oxidation (MFO), maximal intensity of fat oxidation (Fat_{max}), $\dot{V}O_{2max}$, HR_{max}) the highest mean value was selected. $\overline{T}_{sk}$ was calculated using the following equation:

(1) $\overline{T}_{sk} = 0.14(T_{face}) + 0.19(T_{chest}) + 0.11(T_{forarm}) + 0.05(T_{hand}) + 0.32(T_{lat\ thigh}) + 0.19(T_{back})$

CHO utilization and fat utilization were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values using stoichiometric equations (Jeukendrup & Wallis, 2005).

For exercise stages working below or at 50% of each participant's $\dot{V}O_{2max}$:

(2) $Fat = 1.695 \bullet \dot{V}O_2 - 1.701 \bullet \dot{V}CO_2 - 1.77 \bullet n$

(3) $CHO = 4.344 \bullet \dot{V}CO_2 - 3.061 \bullet \dot{V}O_2 - 0.4 \bullet n$

For exercise stages working above 50% of each participant's $\dot{V}O_{2max}$:

(2) $Fat = 1.695 \bullet \dot{V}O_2 - 1.701 \bullet \dot{V}CO_2 - 1.77 \bullet n$

(4) $CHO = 4.21 \bullet \dot{V}CO_2 - 2.962 \bullet \dot{V}O_2 - 0.4 \bullet n$

CHO and fat oxidation are in $g \bullet min^{-1}$, while n is the nitrogen excretion from any protein oxidation that has taken place ($n = 135\ \mu g \bullet kg^{-1} \bullet min^{-1}$) (Jeukendrup & Wallis, 2005).

2.8 Statistical Analyses

A paired two-tailed t-test with Cohen's d effect size with lower and upper limit confidence intervals was conducted for primary outcomes measures; ΔMFO, ΔFat_{max}, ΔCrossover, ΔRER. Three-way repeated measures ANOVA (temperature; CO and TN: glucose; CON and GLU:

exercise stage) was used to identify differences for %fat oxidation, %CHO oxidation, and RER, as well as blood lactate and glucose (temperature; CO and TN: glucose; CON and GLU: time: pre and post). To assess if there were differences across substrate oxidation curves, multiple linear mixed-effects regression analyses were used to examine the differences for absolute fat and CHO oxidation curves with temperature, glucose treatment, and $\%\dot{V}O_{2peak}$ as variables. To account for the quadratic and exponential natures of the two curves, $\%\dot{V}O_{2peak}$ squared was added within the model. It was estimated that a sample of ten participants would be sufficient for detection of large effect sizes, assuming standard deviations similar to those previously observed (Layden et al., 2002; Achten et al., 2003b; Gagnon et al., 2013, 2019) with a statistical power (1-β err. prob.) of 80% (G*power, Düsseldorf, Germany). Results were analyzed using STATA 15. Data are presented as mean ± SD.

3. Results

3.1 Thermal and Cardiorespiratory Variables

Mean $\dot{V}O_{2peak}$ was not significantly different between temperatures (p=0.257) or glucose treatment (p=0.554, CO-CON: 44.9 ± 6.72 ml • min^{-1}• kg^{-1}, TN-CON: 42.1 ± 6.42 ml • min^{-1}• kg^{-1}, CO-GLU: 42.7 ± 5.29 ml • min^{-1} • kg^{-1} •, TN-GLU: 43.7 ± 4.93 ml • min^{-1} • kg^{-1}). HR_{max} was also not significantly different between temperature conditions (p=0.188) or glucose treatment (p=0.309, CO-CON: 176 ± 15 bpm, TN-CON: 179 ± 15 bmp, CO-GLU: 171 ± 25 bpm, TN-GLU: 187 ± 13 bpm). $\bar{T}_{sk}$ was lower during in CO (p<0.001, 24.3 ± 2.50ºC) compared to TN condition (30.8 ± 1.03ºC), but was not influenced by glucose treatment (p=0.174). Other cardiorespiratory variables throughout the intensities of each trial are shown in supplementary data files.

3.2 Metabolic Flexibility Variables

Figure 1 shows mean (±SD) for (A) MFO (B) Fat_{max}, and (C) Crossover Point. MFO was greater in the CO *vs.* TN (p=0.025; CO: 0.63 ± 0.26 *vs.* TN: 0.53 ± 0.21 g • min^{-1}), as well as in CON *vs.* GLU (p=0.003; CON: 0.67 ± 0.27 *vs.* GLU: 0.79 ± 0.24). However, there were no interactions between temperature and treatment (p=0.133). The point at which CHO usage and fat utilization intersected (crossover point) was significantly higher in CO (p=0.019, CO: 30.6 ± 22.9 $\%VO_{2peak}$ *vs.* TN: 18.9 ± 21.9 $\%VO_{2peak}$), and in CON (p=0.002, CON: 38.4 ± 17.9 $\%VO_{2peak}$ *vs.* GLU: 11.1 ± 18.8 $\%VO_{2peak}$). There were also no interactions between temperature and glucose on the crossover point (p=0.728). Moreover, no differences were observed across temperature (p=0.162) or glucose (p=0.072) for the intensity at which maximal fat oxidation occurred (Fat_{max}). Importantly, Table 2 presents metabolic flexibility variables, namely, ΔMFO, ΔFat_{max}, ΔCrossover, and ΔRER. There were no differences in any of the aforementioned variables.

Figure 2 presents the energy contributions of both CHO and lipids during each stage of exercise in a cold and thermoneutral environment. Percentage of CHO oxidation was significantly lower in CO (p=0.005, CO: 65.89 ± 23.36%, TN: 69.19 ± 23.30%), increased by glucose treatment (p<0.001, GLU: 76.07 ± 16.75%, CON: 59.13 ± 25.85%), and as exercise stages progressed (p< 0.001). Evidently, percentage of fat oxidation responded oppositely and was significantly higher in the cold (p=0.005, COL: 34.11 ± 23.36%, TN: 30.81 ± 23.30%), decreased by glucose treatment (p<0.001, GLU: 23.93 ± 16.75, CON: 40.87 ± 25.85), and exercise stage (p<0.001). RER was also significantly decreased by cold temperature (p=0.004, CO: 0.85 ± 0.07, TN: 0.86 ± 0.07), increased by glucose treatment (p<0.001, GLU: 0.88 ± 0.06, CON: 0.83 ± 0.07), and exercise stage (p<0.001). There was no significant interaction between temperature, glucose treatment and exercise stage for either %CHO oxidation, %fat oxidation, or RER (p>0.05).

Figures 3 presents second order polynomial regression models for absolute rates of lipid and CHO oxidation over increasing exercise intensities to peak $\dot{V}O_2$. The curves derived from absolute rates of both lipid and CHO oxidation were significantly influenced by both temperature (p<0.001) and glucose treatment (p<0.001) (Table 3).

3.3 Blood Analyses

Blood lactate concentration levels demonstrated a consistent increase post exercise in every condition (p<0.001), as shown in Figure 4. Further, there was a main effect of temperature on lactate levels where CO was lower compared to TN (p=0.023, CO: 5.4 ± 1.9 mM • L^{-1} and TN: 6.3 ± 2.6 mM • L^{-1}). Lactate concentration was also lower in CON *vs.* GLU (p=0.006, CON: 5.1 ± 2.4 mM•L^{-1} and GLU: 6.7 ± 2.2 mM • L^{-1}). Blood glucose concentration levels (Fig 5B) were elevated in all participants after the ingestion of glucose (CO-GLU: +3.43 ± 1.763 mM • L^{-1} and TN-GLU: +2.88 ± 1.287 mM • L^{-1}), but were not different post exercise (p=0.991). Glucose concentration was unaffected by temperature (p=0.963).

4. Discussion

Previous studies have observed an increase of fat oxidation in cold environments (Hurley & Haymes, 1982; Galloway & Maughan, 1997; Gagnon et al., 2013) and an increase in CHO oxidation under CHO loading (Muoio, 2014; Spriet, 2014; Jorgensen et al., 2017). However, this is the first study to examine if the cold-induced increase in fat oxidation compromises metabolic flexibility during incremental exercise following glucose loading. Our main finding is that exercising in a cold environment, with its concurrent drive in lipid oxidation, does not inhibit metabolic flexibility in healthy participants as acute glucose ingestion modulated the oxidation of both CHO (increase) and lipid (decrease) similarly in both the cold and thermoneutral

environmental conditions. This suggests that pathways responsible for the regulation of fuel selection during exercise and cold exposure may be mechanistically independent. This conclusion is supported via similar changes observed in both thermal conditions on ΔMFO, ΔFat_{max}, ΔRER, and the ΔCrossover Point, with and without pre-exercise glucose ingestion. As individuals with metabolic conditions have a reduced ability to switch between fuels and may suffer from lipo- or gluco-toxicity (Galgani et al., 2008; Kelley, 2002; Schrauwen et al., 2010), it is essential to assess whether changes in the activation of metabolic pathways responsible for the increase in fat oxidation in the cold could induce a state of inflexibility.

Skin temperature was lower in cold ambient temperature. Short-term exercise under mild cold stress does not have a tendency to modulate core temperature (Gagnon et al., 2013, 2019), nor seem to influence the previously established increase in fat oxidation in the cold (Gagnon et al. 2013, 2014), and was thereby not assessed. Our results further demonstrated a linear / curvi-linear increase in CHO oxidation, whilst a parabolic response for fat oxidation, similar to work examining oxidation curves during incremental exercise (Achten et al., 2002; Romijn et al., 1993; Venables et al., 2005; Gagnon et al., 2019). Importantly, exercising in the cold lowered CHO and increased fat contribution to energy expenditure, increased MFO and the Crossover Point, and altered oxidation curves. Acute glucose ingestion, however, increased blood glucose concentrations, increased RER, lowered MFO and the Crossover Point, and modulated both the CHO and fat oxidation curves. Gagnon et al. (2019) demonstrated a greater MFO during exercise in the cold. Previous studies using acute glucose ingestion as a model to assess metabolic flexibility have also observed increases in RER and CHO oxidation during exercise (Mähler et al., 2012; Oberbach et al., 2008; Afman et al., 2014).

MFO was influenced by both temperature and glucose treatment. Glucose treatments have shown similar responses in thermoneutral conditions (Achten & Jeukendrup, 2003a). An increase in MFO is also in line with the studies demonstrating an increase in fat oxidation in cold conditions compared to thermoneutral conditions (Hurley & Haymes, 1982; Timmons et al., 1985; Shephard, 1992). As shown in Figure 1. A, despite shifting the metabolic responses toward fat usage in the cold, the CO-GLU trial adequately returned the oxidation point toward heightened CHO oxidation. This supports that exercise in the cold does not impair metabolic flexibility, as adjustments to available substrates still occurs.

Fat_{max}, originally presented by Jeukendrup and Achten (2001), is the percentage of exercise intensity where maximal fat oxidation occurs. The maximal ability to oxidize fat has been extensively used in exercise studies to quantify skeletal muscles metabolic status (Rosenkilde et al., 2010; Robinson et al., 2015). There is a trend toward differences between cold and thermoneutral trials, however they remain insignificant (Figure 1B). Studies testing the presence of a glucose treatment have shown significant differences in Fat_{max} when using a placebo (Achten & Jeukendrup, 2003a). Discrepancies in the variable may be due to the intra-individual variability linked with moderately fit individuals in repeated trials (Jeukendrup & Wallis, 2005; Croci et al., 2014). Croci et al. (2014) assessed the reproducibility of both Fat_{max} and MFO and determined that due to the variability within each subject, Fat_{max} may be more consistent with larger sample sizes.

The crossover point, where the contribution of lipids and CHO to total energy expenditure is equal, identifies endurance training outcomes (Brooks & Mercier, 1994), and also serves as a marker metabolic flexibility. As expected, the crossover point was significantly higher in the cold, which is indicative of an increase in fat utilization. Compared with the control conditions, glucose treatment demonstrated a significant decrease in the crossover point even within the cold condition.

This result demonstrates that although cold induces higher levels of fat oxidation, metabolic flexibility remains unimpaired in relation to the crossover point, as a shifts occurs in favor of CHO utilization upon the enhancement of glucose presence within the system.

4.1 Potential Mechanisms

As shown in Figure 4, blood lactate concentrations demonstrated a consistent increase from pre and to post maximal intensity exercise. Higher concentrations of blood lactate were further observed after exercise in TN vs. CO, which may partly be responsible for the increase in fat oxidation in cold conditions. In a thermoneutral environment, an increase in lactate and consequently a decrease in blood pH, a window of muscle pH, has been described as a key factor regulating fat and CHO oxidation curves during exercise (van Loon et al., 2001). As adenosine triphosphate (ATP) hydrolysis increases during exercise, its release of hydrogen ions (H^+) lowers muscle pH and inhibits transport of long chain fatty acids (LCFA) into the mitochondria, shifting fuel dominance towards CHO. The regulation of LCFA into the mitochondria may further be regulated by CD36 and CPT-1 activity, both transporting LCFA across the cytosol and mitochondrial membrane, respectively. Cold exposure has been linked to increases in CD36 and CPT-1 protein expression (Putri et al., 2015; Townsend et al., 2013; van den Beukel et al., 2015), facilitating LCFA transport for oxidation at rest. While these findings we primarily observed in adipocytes under resting conditions, a recent report further confirmed the role of CPT-1 in the regulation of fatty acids oxidation in skeletal muscles during exercise (Patrick and Holloway 2019). Further investigations will be essential to determine whether the CPT-1 metabolic axis is regulated by cold exposure during exercise.

Moreover, glucose levels in the CO-CON and TN-CON trials demonstrated increased glucose levels post exercise (4.9 to 6.0 and 4.8 to 6.6 $mM \cdot L^{-1}$ respectively). Within the glucose loading condition, glucose concentrations were elevated before exercise and subsequently decreased post exercise. The pre exercise acute glucose loading effectively elevated blood glucose levels, which we know increases insulin secretion and glucose transporters (GLUT) in cellular membrane, increasing glucose uptake for storage or energy production. During exercise, the increase of glucose uptake in skeletal muscle tissues, raises glycolytic flux and pyruvate oxidation, suppressing fatty acids oxidation via the inhibition of CPT-1 activity (Smith, 2018). It is interesting that despite an increase in fat oxidation from cold exposure, the metabolically known pathway for glucose oxidation with acute glucose loading or CHO-rich meal also regulated CHO oxidation during our experimental protocol. The mechanisms of reciprocal inhibition between glucose and fatty acid oxidation pathways have been previous well defined (Smith et al., 2018). Importantly, neither the effects of increased CHO oxidation and malonyl-CoA on reduced CPT-1 activity, nor the increased in fat oxidation along with the effects of increased levels of acetyl-CoA and NADH on pyruvate dehydrogenase function, key mechanisms inhibiting substrate oxidation, may have been compromised by exercising in a cold environment. This suggest that individuals performing physical work in a cold environment may increase the contribution from fat to total energy expenditure without compromising metabolic flexibility.

4.2 Limitations

Both males and females took part in this study. Woman oxidize more lipids at rest and during exercise along with higher MFO and Fat_{max} (Venables et al., 2005). Although absolute oxidation rates, and the magnitude of responses do differ between sexes, the metabolic changes

observed during exercise and under cold stress was expected to remain similar for all individuals of both sexes (Gagnon et al., 2018, 2019). Furthermore, metabolic results are based on indirect calorimetry. Although we can speculate that metabolic pathways likely remained unimpaired during metabolic flexibility testing for the lack of differences across primary metabolic variables (RER, MFO, Fat_{max}, Crossover), an in-depth analysis of potential alterations in metabolic pathways would have required additional biological samples. Our experimental design also used a glucose loading protocol, which does not include certain metabolic challenges that lipid loading would induce. It would be reasonable to assume that an increase in lipid content, modulating malonyl-CoA, GLUT4, CPT-1, and possibly other regulators of metabolic flexibility, would have further supported the current results. Moreover, our statistical design was built on the assumptions that large effect sizes would be detected, but that medium and small effect sizes could be missed. Consequently, we cannot conclusively state that no differences emerged between our conditions but that if any potential metabolic responses were induced by our experimental model, it would likely be limited. We nonetheless observed sufficient statistical power to assess the effects of both acute glucose ingestion and temperature on metabolism, further suggesting that if potential metabolic flexibility impairment were present but missed, they were likely small. Moreover, respiratory exchange ratio and stoichiometric equations are well established methods to assess changes in metabolism in response to exercise but carry their limitations. Exercise intensities below 25% and above 75 of $\dot{V}O_{2max}$ are known to become less reliable due to CO_2 production from lower blood pH (Gauthier et al. 1996; Péronnet and Anguilaniu 2006). Analyses were conducted with and without outside of those parameters and resulted in similar outcomes. Finally, the study population was healthy without metabolic dysfunctions. Whether the current results apply to individuals suffering from metabolic diseases remains to be investigated.

5. Conclusion

The present study investigated whether metabolic flexibility is compromised by the cold-induced increase fat oxidation during exercise, in an attempt to gain insights of potentially interacting competitive pathways regulating fuel selection. Although both temperature and glucose ingestion modulated energy metabolism, metabolic flexibility remained unimpaired during incremental treadmill exercise. The present results suggest that in healthy participants, competing pathways responsible for the regulation of fuel selection during exercise and cold exposure may be mechanistically independent. Further studies should investigate metabolic flexibility under acute lipid loading and heat stress

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Disclosure of Interest

The authors report no conflict of interest.

Author Contribution Statement

AM and DDG conceptualized the work, conducted experiments, analyzed and interpreted the data, and drafted the manuscript. SM and KHH analyzed and interpreted the data, and critically revised the manuscript. All authors read and approved the manuscript.

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REFERENCES

1. Achten, J., Gleeson, M., & Jeukendrup, A. E. (2002). Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc, 34*(1), 92-97.
2. Achten, J., & Jeukendrup, A.E. (2003a). The effect of pre-exercise carbohydrate feedings on the intensity that elicits maximal fat oxidation. *Journal of Sports Sciences,* 21, 1017-1024. doi: 10.1080/02640410310001641403
3. Achten, J., Venables, M. C., & Jeukendrup, A. E. (2003b). Fat oxidation rates are higher during running compared to cycling over a wide range of intensities. *Metabolism*, 52(6), 747-752. doi.org/10.1016/S0026-0495(03)00068-4
4. Achten, J., & Jeukendrup, A. E. (2004). Optimizing fat oxidation through exercise and diet. *Nutrition*, *20*(7–8), 716–727. doi.org/10.1016/j.nut.2004.04.005
5. Afman, G., Garside, R. M., Dinan, N., Gant, N., Betts, J. A., & Williams, C. (2014). Effect of carbohydrate or sodium bicarbonate ingestion on performance during a validated basketball simulation test. *International Journal of Sport Nutrition and Exercise Metabolism, 24*(6), 632–644. doi.org/10.1123/ijsnem.2013-0168
6. Battaglia, G.M., Zheng, D., Hickner, R.C., Houmard, J.A. 2012. Effect of exercise training in metabolic flexibility in response to a high-fat diet in obese individuals. AJP Endocrinol. Metab. 303: E1440-E1445
7. Brooks, G. A. (1997). Importance of the "crossover" concept in exercise metabolism. *Clinical and Experimental Pharmacology and Physiology*, *24*(11), 889–895.
8. Corpeleijn, E., Saris, W. H. M., & Blaak, E. E. (2009). Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. *Obesity Reviews, 10*(2), 178–193. doi.org/10.1111/j.1467-789X.2008.00544.x

9. Croci, I., Borrani, F., Byrne, N. M., Wood, R. E., Hickman, I. J., Chenevière, X., & Malatesta, D. (2014). Reproducibility of Fat_{max} and fat oxidation rates during exercise in recreationally trained males. *PloS one*, *9*(6), e97930. doi:10.1371/journal.pone.0097930
10. Du Bois, D. & Du Bois, E. F. (1916). A formula to estimate the approximate surface area if height and weight be known. *Nutrition.* 1989(5), 303-11.
11. Engeroff, T., Fleckenstein, J., & Banzer, W. (2017). Glucose metabolism from mouth to muscle: a student experiment to teach glucose metabolism during exercise and rest. *Advances in Physiology Education*, *41*(1), 82–88. doi.org/10.1152/advan.00124.2016
12. Flier, J. S., Kahn, C. R., and Roth, J. (1979). Receptors, antireceptor antibodies and mechanisms of insulin resistance. *N. Engl. J. Med.* 300, 413–419.
13. Gagnon, D. D., Gagnon, S. S., Rintamäki, H., Törmäkangas, T., Puukka, K., Herzig, K.-H., & Kyröläinen, H. (2014). The effects of cold exposure on leukocytes, hormones and cytokines during acute exercise in humans. *PLoS ONE*, *9*(10), e110774. doi.org/10.1371/journal.pone.0110774
14. Gagnon, D. D., Rintamäki, H., Gagnon, S. S., Cheung, S. S., Herzig, K.-H., Porvari, K., & Kyröläinen, H. (2013). Cold exposure enhances fat utilization but not non-esterified fatty acids, glycerol or catecholamines availability during submaximal walking and running. *Frontiers in Physiology*, *4*. doi.org/10.3389/fphys.2013.00099
15. Gagnon, D. D., Rintamäki, H., Gagnon, S. S., Oksa, J., Porvari, K., Cheung, S. S., Herzig, K.H., Kyröläinen, H. (2014). Fuel selection during short-term submaximal treadmill exercise in the cold is not affected by pre-exercise low-intensity shivering. *Applied Physiology, Nutrition, and Metabolism*, *39*(3), 282–291. doi.org/10.1139/apnm-2013-0061

16. Gagnon D.D., McCue A., Dorman S.C., Serresse O. (2018). Influences of a Cold and Warm Environment on Substrate Metabolism in Women during Running and Cycling. *FASEB,* 32 (1 suppl), 589-1.
17. Gagnon, D. D., Perrier, L., Dorman, S. C., Oddson, B., Larivière, C., & Serresse, O. (2020). Ambient temperature influences metabolic substrate oxidation curves during running and cycling in healthy men. *European Journal of Sport Science, 20*(1), 90-99.
18. Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., et al., and the Look AHEAD Adipose Research Group. (2008). Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes, 57*(4), 841–845. doi.org/10.2337/db08-0043
19. Galgani, J. E., Moro, C., & Ravussin, E. (2008). Metabolic flexibility and insulin resistance. *AJP: Endocrinology and Metabolism*, *295*(5), E1009–E1017. doi.org/10.1152/ajpendo.90558.2008
20. Galloway, S. D. & Maughan, R. J., (1997). Effects of ambient temperature on the capacity to perform prolonged cycling exercise in men. *Medicine and Science in Sports and Exercise.* 29(9), 1240-9
21. Gauthier, J.F., Pirnay, F., Lacroix, M., Mosora, F., Sciieen, A.J., Cathelineau, G., & Lefèbvre, P.J. (1996). Changes in breath $^{13}CO_2/^{12}CO_2$ during exercise of different intensities. *J. Appl. Physiol.* 81(3): 1096-1102.
22. Ghanassia, E., Brun, J.-F., Mercier, J., & Raynaud, E. (2007). Oxidative mechanisms at rest and during exercise. *Clinica Chimica Acta, 383*(1–2), 1–20. doi.org/10.1016/j.cca.2007.04.006

23. Hoeks, J., van Herpen, N.A., Mensink, M., Moonen-Kornips, E., van Beurden, D., Hesselink, M.K., and Schrauwen, P. (2010). Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes* 59, 2117–2125.
24. Holland, W.L., and Summers, S.A. (2008). Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. *Endocr. Rev.* 29, 381–402.
25. Hurley, B., Haymes E. M. (1982). The effects of rest and exercise in the cold on substrate mobilization and utilization. *Aviat. Space Environ. Med*. 53, 1193–1197.
26. Jackson, A. S. & Pollock, M. L., (1978). Generalized equations for predicting body density of men. *British Journal of Nutrition.* 40(3), 497-504
27. Jackson, A. S. & Pollock, M. L., (1980). Generalized equations for predicting body density of women. *Medicine and Science in Sports and Exercise.* 12(3), 175-182
28. Jeacocke, N. A., & Burke, L. M. (2010). Methods to standardize dietary intake before performance testing. International Journal of Sport Nutrition and Exercise Metabolism, 20, 87–103.
29. Jeukendrup, A. E., Achten, J., (2001). Fatmax: A new concept to optimize fat oxidation during exercise? *European Journal of Sport Science,* 1(5). doi: 10.1080/17461390100071507
30. Jeukendrup, A. E., Wallis, G. A., (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med,* 26(1), S28-S37 doi: 10.1055/s-2004-830512

31. Jorgensen, W., Rud, K.A., Mortensen, O.H., Frandsen, L., Grunnet, N., and Quistorff, B. 2017. Your mitochondria are what you eat: a high-fat or a high-sucrose diet eliminates metabolic flexibility in isolated mitochondria from rat skeletal muscle. Physiol. Rep. 5(6): e13207. doi:10.14814/phy2.13207
32. Kiens, B., Éssen-Gustavsson, B., Christensen, N. J., and Saltin, B. (1993). Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training. *J. Physiol*. 469, 459–478.
33. Kuzmiak-Glancy, S., and Willis, W.T. 2014. Skeletal muscle fuel selection occurs at the mitochondrial level. J. Exp. Biol. doi : 10.1242/jeb.098863
34. Layden, J. D., Malkova, D., & Nimmo, M. A. (2004a). During exercise in the cold increased availability of plasma nonesterified fatty acids does not affect the pattern of substrate oxidation. *Metabolism*, *53*(2), 203–208. doi.org/10.1016/j.metabol.2003.09.018
35. Layden, J. D., Malkova, D., & Nimmo, M. A. (2004b). Fat oxidation after acipimox-induced reduction in plasma nonesterified fatty acids during exercise at 0°C and 20°C. *Metabolism*, *53*(9), 1131–1135. doi.org/10.1016/j.metabol.2004.03.016
36. Mähler, A., Steiniger, J., Bock, M., Brandt, A. U., Haas, V., Boschmann, M., & Paul, F. (2012). Is metabolic flexibility altered in multiple sclerosis patients? *PLoS ONE*, *7*(8), e43675. doi.org/10.1371/journal.pone.0043675
37. Muoio, D. M., (2014). Metabolic inflexibility: When mitochondrial indecision leads to metabolic gridlock. *Cel.* 159(6), 1253-1262. doi: 10.1016/j.cell.2014.11.034
38. Nicolson, G. L. (2007). Metabolic syndrome and mitochondrial function: Molecular replacement and antioxidant supplements to prevent membrane peroxidation and restore

mitochondrial function. *Journal of Cellular Biochemistry*, 100(6), 1352–1369. doi.org/10.1002/jcb.21247

39. Oberbach, A., Lehmann, S., Kirsch, K., Krist, J., Sonnabend, M., Linke, A., … Kovacs, P. (2008). Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the -174G/C variant in IL-6 gene. *European Journal of Endocrinology*, 159(2), 129–136. doi.org/10.1530/EJE-08-0220
40. Palmes, E. D., & Park, C. R. (1948). An improved mounting for thermocouples for the measurement of the surface temperature of the body. The *Journal of Laboratory and Clinical Medicine*, 33(8), 1044-6.
41. Péronnet, F., & Anguilaniu, B. (2006). Lactic acid buffering, nonmetbaolic CO_2 and exercise hyperventilation: A critical reappraisal. *Resp. Physiol. Neurobiol*. 150(1): 4-18.
42. Putri, M., Syamsunarno, M.R.A.A., Iso, T., Yamaguchi, A., Hanaoka, H., et al. (2015) CD36 is indispensable for thermogenesis under conditions of fasting and cold stress. *Biochemical and Biophysical Research Communications*, 457 (4), 520-525.
43. Robinson, S.L., Hattersley, J., Frost, G.S., Chambers, E.S., and Wallis, G. (2015) Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. J. Appl. Physiol. 118(11): 1415-1422
44. Sahlin, K. (1986). Muscle fatigue and lactic acid accumulation. *Acta Physiol Scand Suppl.*, 556, 83-91. PMID: 3471061
45. Sahlin, K. (1990). Muscle carnitine metabolism during incremental dynamic exercise in humans. *Acta Pbysiol Scand*. 138, 259-262
46. Schrauwen, P., Schrauwen-Hinderling, V., Hoeks, J., & Hesselink, M. K. C. (2010). Mitochondrial disfunction and lipotoxicity. *Biochimica et Biophysica Acta.* 1801, 266-271

47. Shephard, R. J. (1992). Fat metabolism, exercise, and the cold. *Can J Sport Sci., 17*(2), 83-90. PMID: 1324116
48. Shulman, G. I. (2004). Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology (Bethesda)* 19, 183–190.
49. Spriet, L.L. (2014). New insights into the interaction of carbohydrate and fat metabolism during exercise. Sports Med. 44 (suppl. 1), S87-96
50. Storlien, L., Oakes, N. D., & Kelley, D. E. (2004). Metabolic flexibility. *Proceedings of the Nutrition Society*, *63*(2), 363–368. doi.org/10.1079/PNS2004349
51. Smith, R.L., Soeters, M.R., Wust, R.C.I., and Houtkooper, R.H. 2018. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. Endocrine Rev. doi: 10.1210/er.2017-00211
52. Timmons, B. A., Araujo, J., & Thomas, T. R. (1985). Fat utilization enhanced by exercise in a cold environment. *Med. Sci. Sports Exerc.* 17, 673–678.
53. Vallerand, A. L., & Jacobs, I. (1989). Rates of energy substrates utilization during human cold exposure. *European Journal of Applied Physiology and Occupational Physiology*, *58*(8), 873–878.
54. van den Beukel, J.C, Boon, M.R., Steenbergen, J., Rensen, P.C.N., Meijer, O.C., Themmen, A.P.N., Grefhorst, A. (2015) Cold Exposure Partially Corrects Disturbances in Lipid Metabolism in a Male Mouse Model of Glucocorticoid Excess, *Endocrinology*, 156 (11), 4115–4128, https://doi.org/10.1210/en.2015-1092
55. Venables, M. C., Achten, J., & Jeukendrup A. E. (2005). Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *Journal of Applied Physiology*, 98(1), 160-167.

56. Virkkunen, M., Rawlings, R., Tokola, R., Poland, R. E., Guidotti, A., Nemeroff, C., Bisette, G., Kalogeras, K., Karonen, S., & Linnoila, M. (1994). CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. *Archives of General Psychiatry*, *51*(1), 20-7.

57. Womersley, J., Durnin, J. V. G. A., Armstrong, W. H., & Fiskey, M. (1973). An experimental study on variability of measurements of skinfold thickness on young adults. *Human Biology*, *45*(2), 281-292.

Table 1. Participant's anthropometric and fitness characteristics (Mean ± SD)

N	10 (6M and 4W)
Age (year)	22.3 ± 1.49
Height (cm)	169.7 ± 4.89
Body Mass (kg)	68.1 ± 7.83
[1]Body Fat (%)	21.1 ± 9.70
[2]BSA (m^2)	1.78 ± 0.11
W-H Ratio	0.40 ± 0.04
HR_{peak} (bpm)	189.7 ± 13.0
[3]$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	46.4 ± 5.67
$\dot{V}_{Epeak}$ ($L \cdot min^{-1}$)	86.8 ± 13.45
$\dot{V}_{tpeak}$ (mL)	2220.7 ± 340.63
$Lactate_{peak}$ (mM)	12.2 ± 4.54

BSA, body surface area; W-H ratio, waist-to-hip ratio; HR_{peak}, peak heart rate; $\dot{V}O_{2peak}$, peak oxygen consumption; $\dot{V}_{Epeak}$, peak minute ventilation; $\dot{V}_{tpeak}$, peak tidal volume.[1]Estimated from skinfolds (Durnin and Womersley, 1974). [2]Calculated from height (H) and weight (W) as follows: A = Weight $(kg)^{0.425}$ x Height$(cm)^{0.725}$ x 0.007184 (Dubois and Dubois, 1916). [3]Highest value from all conditions during maximal incremental treadmill test

Table 2. Metabolic flexibility variables

							95% CI Cohen's d	
	COC - COG	***TNC - TNG***	***t***	***df***	***p value***	***Cohen's d***	***Lo. limit***	***Up. Limit***
ΔMFO ($g \cdot min^{-1}$)	0.2154 ± 0.18	0.1575 ± 0.13	1.649	18	0.133	0.52	0.20	0.84
ΔFat_{max} (%)	13.33 ± 18.98	0.55 ± 21.31	1.186	18	0.266	0.36	0.14	0.61
ΔCrossover (%)	25.95 ± 25.15	28.77 ± 23.17	-0.358	18	0.728	-0.11	-0.04	-0.18
ΔRER	-0.050 ± 0.029	-0.054 ± 0.027	0.351	18	0.734	0.11	0.04	0.18

ΔMFO, Change in maximal fat oxidation; ΔFat_{max}, change of the exercise intensity where MFO occurred; ΔCrossover, change of the crossover point; ΔRER, change in respiratory exchange ratio.

Table 3. Multiple linear mixed-effects regression analysis for absolute fat and CHO oxidation rates.

FAT	Coefficient (β)	Std. Err.	z	P value	95% Conf. Interval	
Temperature	.0751297	.0153902	4.88	<0.001	.0449655	.1052939
Glucose	-.1714098	.0153023	-11.20	<0.001	-.2014017	-.1414179
Fat_{max}	1.30626	.1988997	6.57	<0.001	.9164242	1.696097
Fat_{max2}	-.9573326	.1741409	-5.50	<0.001	-1.298642	-.6160228
Constant	.0816893	.0679609	1.20	=0.229	-.0515116	.2148902
CHO						
Temperature	-.2026358	.0387223	-5.23	<0.001	-.2785301	- .1267414
Glucose	.3987507	.038519	10.36	<0.001	.3232885	.47423
Fat_{max}	1.441568	.5004183	2.88	=0.004	.4607665	2.42237
Fat_{max2}	2.075858	.4381056	4.74	<0.001	1.217186	2.934529
Constant	-.266836	.1602862	-1.66	=0.096	-.5809912	.0473192

FIGURE LEGEND

Figure 1. Mean values (± SD) for MFO (A), Fat_{max} (B), and the Crossover Point (%) where fat and CHO usage intersect (C). *Significant differences between thermoneutral and cold ($p < 0.05$). ‡Significant differences between control and glucose ($p < 0.05$).

Figure 2. Relative energy contribution (%) between fat (white) and CHO (black) to total energy expenditure for CO-CON, TN-CON, CO-GLU, and TN-GLU.

Figure 3. Scatter plots of fat oxidation and CHO oxidation over $\dot{V}O_{2peak}$ (%) in cold and thermoneutral environment, in the control and glucose condition. The trend line represents a non-linear 2nd order polynomial regression, displaying 95% confidence intervals.

Figure 4. Individual and mean values of circulating venous blood lactate and glucose concentrations in CO-CON, CO-GLU, TN-CON, and TN-GLU. *Significant differences between thermoneutral and cold ($p < 0.05$). ‡Significant differences between control and glucose ($p < 0.05$). # Significant differences between pre and post ($p < 0.05$).