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Effects of Peripheral Cooling on Substrate Selection During Exercise

By

David Elias Lankford

B.S. University of Utah

Salt Lake City, UT 2001

Presented in partial fulfillment for the requirements for the degree of:

Master of Science

The University of Montana

Missoula, MT 2003

Approved By:

Chairperson

Dean, Graduate School

Date

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Effects of Peripheral Cooling on Substrate Selection During Exercise

Director: Brent C. Ruby, Ph.D.

Previous research has demonstrated that exercise in the heat significantly increases the rate of muscle glycogenolysis compared to exercise under normal ambient temperatures. **Purpose:** To examine the effects of peripheral cooling via the RTX cooling device (Avacore Technologies Inc.) on whole body substrate oxidation during submaximal exercise at a moderate ambient temperature (28°C). **Methods:** Subjects included recreationally active college aged males (N=16). Each subject performed an initial cycle ergometer VT/VO₂ max test. Subjects completed two 45-minute cycle ergometer trials at a work rate equivalent to approximately 90% VT, one with cooling from the RTX cooling device (manifold T= 22°C, P=635mmHg) and one without. Rectal temperature was continuously recorded throughout both trials. Trials were performed in a random, crossover design. Dependent variables were analyzed using pre-planned comparisons. Statistical significance was established at p<0.05. **Results:** Both groups maintained constant carbohydrate use during the cooling trial, whereas without cooling carbohydrate use was greater at the beginning of exercise (p<0.05) and decreased with time (p<0.05). Fat use increased with time in both trials for both groups. There was no difference in rectal temperature between trials. **Conclusion:** Despite a similar response in rectal temperature across trials, peripheral cooling with the AVAcORE RTX cooling device altered substrate use during submaximal exercise without any difference in total energy expenditure.

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Chapter One: Introduction

It is widely recognized that an augmented rise in body temperature during submaximal exercise will increase carbohydrate and more specifically glycogen utilization during exercise ^{3,5,7}. Previous research has shown that an attenuated rise in body temperature either by reducing ambient temperatures ^{5,6}, or by cooling ^{13,18} will reduce glycogen flux and ultimately total carbohydrate oxidation.

Heat stress also influences lipid mobilization and oxidation. Plasma free fatty acid levels are unaltered by exercise and heat stress ⁷. However, it has been shown that intramuscular triacylglycerol utilization ⁷ and free fatty uptake ¹¹ are reduced with thermal stress, possibly suggesting that free fatty acid release by adipocytes may be reduced during the combination of heat stress and exercise ². Brooks et al. ¹ research indicates that elevated temperature decreases phosphorylative efficiency in isolated skeletal muscle mitochondria also causing an increased reliance on carbohydrate as the primary substrate. Febbraio ³ suggests that elevated muscle temperature may impair mitochondrial function. Of note, findings by Mills et al. ¹⁵ observed an increase of lipid peroxides, an indicator of oxidative stress, in the plasma of hyperthermic horses exercising to fatigue, possibly supporting the notion that heat stress may cause metabolic dysfunction.

Studies have examined RER ^{6,12}, rate of glucose disposal (glucose Rd) ¹², carbohydrate oxidation ⁹, and muscle glycogen depletion ^{6,18} to point toward the use of carbohydrates during heat stress. It has been shown that heat stress stimulates an increase in hepatic glucose production without an augmented glucose disappearance rate ¹². Febbraio ³ suggests that an increase in body temperature and perceived effort promote a

feed-forward effect resulting in increased blood catecholamines which in return increases glycogen utilization in contracting skeletal muscle. Assuming non-protein use, past research has shown that RER is increased with heat stress^{6, 12, 20}. Augmented glycogen utilization during heat stress was first reported by Fink et al.⁷, while comparing exercise in 40°C with 9°C. Fink also noted a shift in RER and reduced intramuscular triglyceride use indicating increased carbohydrate utilization. Using water-perfused cuffs to cool one thigh and heat the other during submaximal exercise, Starkie et al.¹⁸ found that muscle glycogen levels were lower in the heated leg compared with the cool leg.

Studies have consistently shown that enhanced heat stress increases blood lactate accumulation during exercise^{6, 7, 12, 20}. Hargreaves et al.¹² suggests that an increased blood lactate is associated with increased rate of intramuscular glycogen utilization, since blood glucose uptake is reduced with heat, yet total carbohydrate oxidation is increased.

In regards to body composition and substrate oxidation, previous findings from our lab indicate that high fit (ventilatory threshold >30 ml/kg/min), low fat (10.5±2.3%) individuals are better able to maintain lipid metabolism during increased relative intensities compared with low fit (ventilatory threshold <20 ml/kg/min), high fat mass (27.1±5%) individuals¹⁴. Although explanations for these findings are unknown, the thermal control in low fat individuals may, in part explain the observed increase in lipid oxidation.

For cooling purposes this study will use an AVAcore (AVAcore Technologies Inc.) hand device designed to manipulate core temperature. Built to enhance heat extraction by amplifying blood flow to the hand via negative pressure within the capsule, the device will influence blood temperature in the hand through contact with a metal plate

cooled by 22°C water flow. To our knowledge, no studies have been performed that examine the effects of thermoregulation on substrate utilization, while cooling with a temperature <10°C different from the ambient exercise temperature.

Problem

The purpose of this study will be to examine the effects of peripheral cooling at 28°C ambient temperature on substrate utilization at 90% ventilatory threshold using a hand device developed by AVAcore Technologies. The secondary purpose of this study was to examine the substrate selection response between body compositions to the peripheral cooling.

Hypothesis One

Peripheral cooling with the hand device will attenuate the rise in core body temperature sufficient to attenuate the reliance on carbohydrates and glycogen stores.

Justification of Hypothesis One

Febbraio states that a >.5 degree Celsius difference in body temperature will alter substrate selection³. Young et al.²⁰ and Febbraio et al.⁴ suggest that increased temperature will cause a shift in enzyme action, primarily phosphofructokinase, glycogen phosphorylase, and pyruvate kinase, due to the Q-10 effect thus shifting substrate use towards increased carbohydrate utilization and a reduced lipid oxidation. The cooling device from AVAcore Technology has a negative pressure inside the capsule so when the

hand is placed within it continually circulates superficial blood flow through the hand. Water cooled to 22°C flows through the capsule floor allowing blood to be cooled without stimulating vasoconstriction. Previous findings from our lab found that cooling using the hand device altered substrate selection in submaximal exercise in a 100°F environment.

Hypothesis two

Subjects with greater than eighteen percent fat mass will have a lower fat oxidation at a given time during exercise than those with less than ten percent fat mass.

Justification of Hypothesis Two

Recent findings from our lab ¹⁴ indicate that high fat, low fit individuals had similar fat oxidation at 70% of ventilatory threshold (VT) to those with low fat, high fitness levels. However at 85% and 100% of VT, high fat, low fit individuals had significantly less fat oxidation than those with low fat, high fitness levels.

Significance of the study

Research has shown that attenuated rise in body temperature either by reducing ambient temperature ⁶ or by cooling ^{13, 18} will reduce glycogenolic rate and carbohydrate oxidation. Sparing glycogen stores and increasing lipid metabolism can benefit performance during exercise. Previous research has used extreme temperature difference ⁶, or radical cooling ¹³ and heat temperatures ¹⁸ to alter substrate selection; our study will

use a cooling temperature 6°C lower than ambient to examine if even minute differences in temperature will make significant differences in substrate selection. Our study will also compare differences in substrate use between body compositions.

Rational of the study

Previous attempts to regulate core temperature have been done using methods such as ice packs¹³, lowering ambient temperature^{4,6} or direct cooling of working muscle¹⁸. Our study will examine the effects of peripheral cooling using an AVAcore hand device. We will also examine the efficacy of the AVAcore device to alter substrate use between body compositions. Future use from results of this study may include thermoregulation attempts in athletics to retain glycogen stores or health industry to enhance lipid oxidation duration.

Limitations

i/ Physical capacity of the subjects: The subjects will have differing fitness levels and physical capacities. To correct for this, all subjects will work at a relative intensity.

ii/ Non-randomized samples: The subjects will not be randomly selected. The subjects will be acquired on a volunteer basis.

iii/ Instrumentation: There is inherent error with all instrumentation. Using trained testers and calibrated equipment will minimize this error.

iv/ Body Temperature: Beginning core temperatures cannot be controlled.

Delimitations

i/ Body Composition: Subjects will have either <10% fat mass or >18% fat mass.

ii/ Age of subjects: Only college males will be used in this study. Aging has been shown to influence substrate use during exercise ¹⁶. Therefore, in this study only college-aged subjects will be used.

iii/ Gender: Hormone differences between genders have been shown to alter substrate use ¹⁹. In this study only males will be used to eliminate differences between genders.

iv/ Temperature: Room temperature will be maintained at 28°C.

Definition of Terms

Relative intensity: Intensity relative to a specific level of stress.

Respiratory Exchange Ratio (RER): The ratio of VCO₂ expired to VO₂ consumed. RER is used to indicate the primary substrates being used. Since fat metabolism requires more oxygen, a lower RER indicates increased fat oxidation. Theoretically, an RER of 1.0 is indicative of 100% carbohydrate oxidation and an RER of .71 is indicative of 100% fat oxidation.

Substrate: A selected fuel utilized to produce energy.

VCO₂: The amount of carbon dioxide produced/minute; generally expressed as L/min.

VO₂: The amount of oxygen utilized by the body/min; generally expressed as L/min.

Thermoregulation: Regulation of deep body temperature.

Ventilatory Threshold: An indirect measure of the lactate threshold that is based on non-linear increases in metabolic gases. VT will be defined as the first sustained raise in VE/VO₂ without a rise in VE/VCO₂ concurrent with the first rise in excess CO₂, and the first break above a slope of 1.0 in the v-slope method.

Rate of Perceived Exertion (RPE): Subjective means for measuring an individual's exertion level determined by the Borg scale from 6-20. A higher number indicates in increased perceived exertion.

Chapter Two: Literature Review

Heat and Metabolism by Sequence of Time

Brooks et al. ¹ studied the phosphorylative efficiency of isolated rat muscle mitochondria in vitro by examining the ADP/O ratio over several varying temperatures. Between 25°C and 40°C they observed constant ADP/O ratios. However as muscle temperature increased above 40°C, the ADP/O ratio decreased linearly. These findings suggest that for a given VO_2 the rate of ATP degradation was higher than ADP rephosphorylation.

In a study by Fink et al. ⁷, subjects performed work in heat (41°C) and cold (9°C) to assess the effects of environmental stress on muscle metabolism during exercise. Each trial consisted of work at 70-85% of VO_2 max for 3-bouts of 15-minutes each with a 10-minute rest in between each bout. Findings showed that heart rate, rectal temperature, muscle glycogen utilization and blood lactate were all significantly higher in the heat. Subjects showed reduced muscle triglyceride depletion during exercise in the heat, yet serum triglycerides levels were slightly lower with exercise in the heat. Plasma free fatty acid levels were not different between the trials. In conclusion, Fink noted that blood and muscle lipids indicate a trend toward less lipid metabolism in the heat than in the cold. An enhanced rate of glycolysis during the heat was reflected by a greater lactate production and glycogen utilization.

Young et al. studied the effects of heat acclimation on muscle metabolism during submaximal exercise ²⁰. Subjects were asked to cycle for 30-minutes at 70% maximal

VO₂ in a cool (21°C) and hot (49°C) environment and again after acclimatization. RER and plasma lactate were significantly higher in the heat trials compared with the cold trials. Muscle glycogen utilization did not differ at any point between the heat and cold trials. Acclimation lowered aerobic metabolic rate and plasma lactate in both temperatures. Young concluded that the increased plasma lactate during heat is not resultant upon increased glycogen since no differences in muscle glycogen were found.

Using 11 dogs as subjects, Kozlowski et al.¹³ found that trunk cooling with ice packs improved total running performance. Cooling resulted in a reduced muscle glycogen depletion and blood lactate ($p < 0.05$). Following the exercise to exhaustion, muscle ATP was higher ($p < 0.01$), ADP content was lower ($p < 0.05$) and AMP content was lower ($p < 0.01$) in cooled dogs. Kozlowski concluded that hyperthermia promoted lactate accumulation and more rapid glycogen depletion possibly accounting for local fatigue and decreased performance.

Febbraio et al.⁶ had subjects cycle for 40 minutes at 70% peak VO₂ in ambient temperatures of 20°C and 40°C to examine the effects of heat on metabolism. RER, muscle glycogenolysis, lactate and heart rate were significantly higher in the heat ($p < 0.05$). Rectal temperatures were higher and creatine phosphate stores were lower in the heat trial ($p < 0.05$). There were no differences in post exercise ATP/ADP ratio or IMP concentrations possibly indicating that although ATP demand is increased in the heat, the demand is met by an increase in anaerobic glycolysis and creatine phosphate hydrolysis.

Febbraio et al.⁴ looked at the influence of elevated muscle temperature on metabolism during intense, dynamic exercise. Subjects underwent 2 trials consisting of a 2-minute workload at 115% maximal VO_2 , either without preheating (CT) or after having their thigh wrapped in a heating blanket for 60-minutes (HT). Core temperature was not affected by the heating but muscle temperature within the thigh was significantly different ($p < 0.01$). Muscle glycogen and lactate concentrations were not different before exercise, but lactate concentrations were higher in HT and glycogen concentrations were lower in HT ($p < 0.05$). During exercise ATP degradation was higher in HT than CT ($p < 0.05$). Febbraio suggests that increased adenine nucleotide degradation will result in further allosteric activation of enzymes such as glycogen phosphorylase, PFK, and LDH. He concludes that elevated muscle temperature increases glycogenolysis, glycolysis, and high-energy phosphate degradation during exercise.

In a study done in 20° C and 3°C ambient temperatures, Febbraio et al.⁵ found that exercise in cool ambient temperature attenuated glycogen depletion. Heart rate, plasma catecholamines and rectal temperature were significantly higher ($p < 0.05$) during the heat trial. Lactate and blood glucose levels were not significantly different. However, muscle glycogen levels were significantly higher in the cool trial. Febbraio reasons that findings of a higher RER in the cool trial may be due to involuntary activity in muscles associated with shivering.

Using fit horses, Mills et al.¹⁵ showed that high temperature and humidity exacerbate oxidative stress during exercise. Horses performed prolonged variable-intensity and short-term high intensity exercises under 20°C, 40% relative humidity or 30°C, 80% relative humidity. Horses were unable to complete the exercise protocol in the high heat trial. Correlations ($r=0.85$) were found between the final stages of exercise in the hot/humid trial and lipid hydroperoxides, indicating oxidative stress. Mills concluded that heat and humidity amplified the induced changes in biochemical parameters, which indicate oxidative stress.

Recently, Sial et al.¹⁶ looked at the effects of aging on substrate oxidation. At similar relative intensities, equivalent to 56% VO_2 max in the elderly group, carbohydrate was 40% lower ($p<0.001$) and fat oxidation was 25-35% lower ($p<0.05$) in the elderly group. Sial concluded that the shift in substrate oxidation was caused by age-related changes in skeletal muscle respiratory capacity.

In a study conducted by Hargreaves et al.¹² subjects were studied during 40-minutes of cycling at a workload equivalent to 65% VO_2 max. Trials were randomly performed in ambient temperatures of 20°C (CT) and 40°C (HT). Glucose rate of appearance and disappearance was measured using a primed infusion of a glucose isotope. Following 40-minutes of exercise, heart rate, rectal temperature, RER, and plasma lactate were all higher ($p<0.05$) in HT compared with exercise in CT. Plasma glucose levels were higher following exercise in HT ($p<0.05$) due to an increase in hepatic glucose production. Glucose rate of disappearance and metabolic clearance rate

was not different at any point. Hargreaves concluded that hyperglycemia observed during the heat was caused by an increase in liver glucose without any change in whole body glucose utilization.

Galloway et al.⁹ found that the effects of temperature on exercise capacity follow an inverted U relationship. During trials in temperatures of 3.6°C, 10.5°C, 20.6°C and 30.5°C, exercise time was the greatest during the 11°C trial. RER was the lowest and fat oxidation highest during the 11°C trial. In temperatures higher than 11°C, RER increased although carbohydrate oxidation rates were highest ($p < 0.05$) during the 4°C trial. Galloway concluded that the optimal temperature for prolonged exercise from this study was 11°C.

Starkie et al.¹⁸ used water cuffs to examine the effects of temperature on metabolism during submaximal exercise. 40-minutes prior to exercise, each subject had one thigh warmed with 55°C water (HL) and the other thigh cooled with 0°C (CL) water via water-perfused cuffs and continued wearing them throughout the trial. Subjects performed at 70% VO_2 peak for 20 minutes. Results from muscle biopsy showed that muscle glycogen levels were lower in the HL thigh ($p < 0.05$). Muscle ATP concentrations were not different post exercise when comparing HL to CL. Starkie concluded that the increase in CHO utilization was a direct result of elevated muscle temperature and was not secondary to allosteric activity from enzymes mediated by a reduced ATP content.

A study from Gonzalez-Alonso et al.¹¹ used dehydration-induced reductions in blood flow to examine substrate delivery and utilization. Subjects performed two separate trials at 61% VO_2 max in 35°C heat. The first trial (DE) was done until voluntary exhaustion without any hydration and developing hyperthermia (39.7°C). The second trial (CON) was performed for the same length of time, except fluid ingestion was allowed and core temperatures only reached 38.2°C. Following 20-minutes of exercise, leg blood flow was decreased in DE ($p < 0.05$). DE resulted in a lower ($p < 0.05$) FFA uptake, higher glycogen utilization and higher muscle lactate production. Plasma glucose and FFA levels were not different between trials. Gonzalez-Alonso concluded that hyperthermia was the main factor in fatigue in the heat.

In a review article, Febbraio states that³ if exercise is submaximal in nature and a $> .5^\circ\text{C}$ increase in body temperature is observed, intramuscular carbohydrate utilization is being augmented. He also suggests that when the body is unable to dissipate heat, augmenting the exercise induced rise in core body temperature and perceived effort, a feed forward increase in epinephrine secretion results. This increase in epinephrine further augments glycogen utilization in contracting skeletal muscle. Increased muscle temperature may also impair muscle and metabolic function limiting oxidative phosphorylation in the heat.

In a review article regarding alterations in energy metabolism during heat stress, Febbraio² sites findings by Fink et al.⁷ and Gonzalez-Alonso et al.¹¹ that heat stress does not alter plasma free fatty acid, but reduces contracting free fatty acid uptake.

Febbraio suggests that free fatty acid release by adipocytes may be reduced during exercise in the heat.

Body Composition and Substrate Selection

To find the optimal intensity to oxidize fats, Looper et al.¹⁴ had subjects perform at 70, 85 and 100% ventilatory threshold (VT). Subjects were separated into two groups, those with low fat mass ($10.5 \pm 2.3\%$) and recreational fitness levels (recfit-lowfat) and those with high fat mass ($27.1 \pm 5\%$) and low fitness levels (lowfit-highfat). Fat oxidation was highest at 70% VT for the lowfit-highfat group and decreased with increased intensities. There were no significant differences between intensities for the recfit-lowfat group. Fat oxidation was not different between groups at 70% VT, but was significantly lower at 85% VT and 100% VT in the lowfit-highfat group. Looper concluded that with increased fitness and decreased fat mass, individuals are better able to maintain higher rates of fat oxidation over a range of moderate intensities.

Gender and Substrate Selection

Comparing gender and substrate utilization, Tarnopolsky et al.¹⁹ found that women oxidized significantly more lipid and less protein and carbohydrate compared with the men during exercise at 75% VO_2 -peak. Also, after an attempt to increase muscle glycogen through 4 days of carbohydrate loading, women did not increase muscle glycogen stores whereas men did. Tarnopolsky concluded that his data supports previous observations of greater lipid and lower carbohydrate and protein oxidation by women versus men during submaximal endurance exercise.

Chapter Three: Methodology

Setting

All exercise testing took place at The University of Montana, Human Performance Laboratory.

Subjects

Sixteen apparently healthy college aged men participated in the study. Subjects were divided into two groups based on their body composition. Men with $>18\%$ fat mass ($n=8$) were placed in the high fat group (group 1) and men with $<10\%$ fat mass ($n=8$) were placed in the low fat group (group 2). Prior to participation, each subject completed an IRB approved informed consent form.

Descriptive Data

Prior to testing, the following descriptive data was obtained: age, height (cm) and body weight (kg) using a calibrated digital scale model PS6600T (Belfour Inc., Cedarburg, WI). Residual lung volume was measured using the helium dilution method (Collins Modular Lung Analyzer, Greensboro, NC). Hydrostatic weight was measured with a digital underwater scale (Exertech, Dresbach, MN). Body density was calculated using an average of three underwater weights within 100 grams of each other and corrected for residual volume. Body density was converted to percent fat using the Siri equation ¹⁷.

Exercise Testing

For the initial session subjects completed a graded exercise test on a Monark 824E mechanically braked cycle ergometer (Varberg, Sweden) to determine ventilatory threshold (VT) and VO_2 max. The graded exercise for VT consisted of 1-minute stages until exhaustion. The first stage was performed at approximately 70-watts and each subsequent stage consists of a 15-watt increase. Cadence was maintained at 70 revolutions per minute. Expired gas was collected and analyzed at 15-second intervals using a calibrated Parvo Medics metabolic cart (Parvomedics, Inc., Salt Lake City, UT). VT was determined using a combination of the V-slope method, the ventilatory equivalent method and the excess CO_2 method ¹⁰.

Trials

Each subject was asked to refrain from strenuous exercise, caffeine, alcohol, and tobacco for 24-hours prior to the trial. Subjects were asked to keep a 1-day food diary the day before the test and follow it for each subsequent trial. All trials were performed in the morning following a 10-hour fast. Subjects exercised at a work rate equivalent to 90% of VT for 45-minutes on a cycle ergometer. Exercise chamber temperature was controlled at 28°C for both trials. Subjects received either cooling (WC, 22°C) from the AVAcore hand device or no cooling (NC) in a random repeated measure. Core body temperature was continuously recorded using an Omega data logger and Mon-a-therm (Mallinkrodt Medical, Inc.) rectal probe inserted at least 10 cm. Heart rate was recorded every 10-minutes using a chest strap heart rate monitor (Polar, Port Washington, NY). RPE was recorded every 10-minutes. Blood lactate samples were taken at minute 0, 15,

25, 35 and 45. RER was recorded at minutes 5, 15, 25, 35 and 45. Whole body carbohydrate and fat oxidation were calculated from VO_2 and VCO_2 measures collected at minutes 5, 15, 25, 35 and 45 using the Frayn equation⁸. Following the completion of each trial, a passive recovery took place for 10-minutes.

Statistics

RER, HR, RPE, fat oxidation and CHO oxidation were examined using pre-planned comparison between trials for each group individually during minutes 5, 15, 25, 35, and 45. Pre-planned comparisons examined differences in RER, HR, RPE, fat oxidation and CHO oxidation at minute 5 vs. 25 and 5 vs. 45 within trials for both groups individually. Temperature was analyzed between groups with pre-planned comparisons between trials at minutes 0, 5, 15, 25, 35, 45, and 10-minutes post. Within each trial pre-planned comparisons examined differences between minute 0 vs. 25, 0 vs. 45 and 45 vs. 10-minute post for both groups individually. Blood lactate was examined using pre-planned comparison between trials for each group individually during minutes 0, 15, 25, 35, and 45. Pre-planned comparisons will examine differences in blood lactate at minute 0 vs. 25 and 0 vs. 45 within trials for both groups individually. Significance level was set at $p < 0.05$.

Chapter Four: Results

Descriptive Data

Table 1. Group 1

Subject	Age	Ht. inches	Wt. kg	% body fat	Peak VO ₂ (L/min)	VT (L/min)	% VO ₂ at VT
9	24	71.5	90.2	18.1	3.15	2.0	0.63
10	25	69	94.6	20.6	3.60	2.2	0.61
11	30	70.5	95.0	22.7	4.43	2.8	0.63
12	30	68	88.2	18.1	3.51	2.2	0.61
13	36	72	104.7	26.4	4.13	2.8	0.69
14	37	73	110.3	27	3.87	2.4	0.62
15	19	72	84.35	18.2	2.98	2.7	0.57
16	23	71	144.8	38.7	3.49	1.9	0.56

Table 2. Group 2

Subject	Age	Ht. inches	Wt. kg	% body fat	Peak VO ₂ (L/min)	VT (L/min)	% VO ₂ at VT
1	23	70.7	77.6	4.9	4.24	2.8	0.66
2	32	70.3	68.1	6.2	3.76	2.5	0.66
3	22	73.5	65.9	3.1	4.34	2.8	0.65
4	28	66.5	67.9	6.6	4.44	2.6	0.59
5	20	73	68.8	9.9	4.23	2.9	0.67
6	19	66	61.1	5.2	4.07	3.0	0.74
7	21	71	66.8	5.5	4.87	2.6	0.73
8	19	69	82.9	4.2	3.91	2.2	0.56

CHO Utilization

For group 1, NC showed significantly greater CHO use at minutes 5 and 25. NC showed significant decreases in CHO utilization between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

For group 2, CHO utilization during NC was significantly greater at minutes 5, 15, and 45 compared with WC. During NC, CHO utilization dropped significantly between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

Fat Utilization

For group 1, WC resulted in significantly greater fat utilization at minutes 5 and 25. During both NC and WC, fat utilization increased significantly between minutes 5 and 25, and between minutes 5 and 45 for group 2. All other pre-planned comparisons were not significantly different.

For group 2, fat utilization was significantly greater during WC at minutes 5, 15, 25, and 45. During both NC and WC, fat utilization increased significantly between minutes 5 and 25, and between minutes 5 and 45 for group 1. All other pre-planned comparisons were not significantly different.

RER

For group 1, NC resulted in significantly higher RER values at minutes 5 and 25. Both NC and WC resulted in significant decreases in RER between minutes 5 and 25, and 5 and 45. All other pre-planned comparisons were not significantly different.

For group 2, RER was significantly higher at minutes 5, 15, 25, and 45 during NC. Both NC and WC resulted in significant decreases in RER between minutes 5 and 25, and 5 and 45. All other pre-planned comparisons were not significantly different.

Heart Rate

For group 1, HR was significantly lower at minute 5 during WC. For both NC and WC, HR increased between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

For group 2, there were no significant differences between trials at any point. For both NC and WC, HR increased between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

RPE

For group 1, RPE was significantly lower during WC at minutes 25 and 45 compared with NC. For both NC and WC, RPE increased between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

For group 2, there were no significant differences between trials at any point. For both NC and WC, RPE increased between minutes 5 and 25, and between minutes 5 and 45. All other pre-planned comparisons were not significantly different.

Temperature

For group 1, there were no significant differences between NC and WC at any point. Both NC and WC resulted in increased rectal temperature between minutes 0 and 25, minutes 0 and 45, and decreased rectal temperature between minutes 45 and 10-minute post. All other pre-planned comparisons were not significantly different.

For group 2, there were no significant differences between NC and WC at any point. Both NC and WC resulted in increased rectal temperature between minutes 0 and 25, and 0 and 45. There was no significant difference between minutes 45 and 10-minute post for the NC trial. All other pre-planned comparisons were not significantly different.

Lactate

For group 1, there was a significant difference between NC and WC at minute 45. WC resulted in significant differences between minutes 5 and 25, but non-significant differences between minutes 5 and 45. NC resulted in significant differences between minutes 5 and 25, and between minutes 5 and 45.

For group 2, there were no significant differences between trials. Both WC and NC resulted in significant differences between minutes 5 and 25, and minutes 5 and 45.

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Appendix I Attachments

**HUMAN PERFORMANCE LABORATORY
Dept of Health and Human Performance
UNIVERSITY OF MONTANA
Missoula, Montana**

SUBJECT INFORMATION AND CONSENT FORM

_____ ID # _____ Today's Date: _____ / _____ / _____ Participant
(Print Name) Month Day Year

EFFECTS OF PERIPHERAL COOLING ON MUSCLE FUEL UTILIZATION DURING MODERATE EXERCISE

STUDY DIRECTOR: Brent Ruby, Ph.D. (406) 243-2117 University of Montana

STUDY SPONSOR: AVA core Technologies (650) 321-0824

This consent form may contain words that are new to you. If you read any words that are not clear to you, please ask the person who gave you this form to explain them to you.

PURPOSE OF THE RESEARCH

- You are being asked to take part in a research study to examine the effects of hand/arm cooling on measurements of body temperature and metabolism during two sessions of moderate intensity exercise.
- You have been chosen because you are 18-50 years of age, healthy, and a body fat <10% or with a body fat >18%.
- During this study you will be performing two 45-minute exercise sessions of a submaximal, moderate intensity in a controlled environment of 75° F.

PROCEDURES

On your first visit to the lab we will measure your height and weight and body fat.

Body fat will be determined by using hydrostatic weighing. Body density will be calculated using an average of 3 underwater weights within 100 grams of each other. Body fat will be determined from the average body density. Residual lung volume will be determined using the helium dilution method.

If your percent body fat values are less than 10% or greater than 18%, you are eligible for participation in the study.

On your second visit, you will be asked to perform the following exercise test:

A maximal treadmill test (cycling) to determine your level of aerobic fitness. The performance of a maximal test allows the tester to determine the participant's maximal volume of oxygen breathed in, which is a measure of fitness. This test will also allow us to determine your ventilatory threshold, which will allow us to compute the speed and grade for your two heated exercise sessions. This testing will take approximately 1 hour.

Revised 9/26/02.

2. You will receive information concerning your maximal aerobic capacity and ventilatory threshold, which you may compare with norms for your age, sex and sport. There are no other direct benefits to the participants in the study.

CONFIDENTIALITY

1. Your records will be kept private and will not be released without your consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, your name will not be used.
5. All data, identified only by an anonymous ID #, will be stored in our laboratory.
6. Your signed consent form and information sheet will be stored in a locked office separate from the data.

COMPENSATION FOR INJURY

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms.

"In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University's Claim representative or University Legal Counsel,"

VOLUNTARY PARTICIPATION/WITHDRAWAL

1. You have the right to request that a test be stopped at any time.
2. Your decision to take part in this research study is entirely voluntary.
3. You may refuse to take part in or you may withdraw from the study at any time without penalty or loss of benefits to which you are normally entitled.
4. You may leave the study for any reason.

You may be asked to leave the study for any of the following reasons:

1. Failure to follow the study investigator's instructions.
2. A serious adverse reaction, which may require evaluation.
3. The study director/investigator thinks it is in the best interest of your health and welfare.
4. The study is terminated.

QUESTIONS

- You may wish to discuss this with others before you agree to take part in this study.
- If you have any questions about the research now or during the study contact: Brent Ruby (406) 243-2117.
- If you have any questions regarding your rights as a research subject, you may contact the Chairman, J.A. Rudbach, of the IRB through the Research Office at the University of Montana at 243-6670.

On your third and fourth visit we will measure your responses during a 45 minute exercise trial in a climate controlled room (approximately 75°F). This trial will proceed on a morning after a 12 hour fast. Before the exercise trial, you will place a flexible rectal thermometer so that your core body temperature can be monitored throughout each trial. During the exercise, one arm/hand will be kept cool using a cooling device that creates a low-pressure environment to draw blood into the hand and cools that blood by absorbing heat from the overlying skin. We will also ask you to rate your level of fatigue during the exercise using a 6-20 number scale. Your heart rate will be measured during the exercise using a chest strap heart rate monitor. During the exercise, a small blood sample will be periodically obtained from your finger tip (every 10-minutes) to allow us to measure blood lactate. During the exercise trials, you will not be provided with any water until the exercise period ends as it enhances body cooling. As a result, it is anticipated that you will get thirsty during your participation. Periodically, you will wear a mouthpiece and nose clip so that samples of your exhaled air can be collected and analyzed to determine the amount of oxygen your body is consuming during exercise. You will be asked to repeat the same exercise but without the cooling device. All other measurements listed above will be collected during both of the 45-minute trials. It is expected that these two visits to our lab will take approximately 1 to 1 1/2 hours of your time..

LOCATION AND LENGTH OF TIME REQUIRED

The study will take place at the in the Human Performance Laboratory in McGill Hall (First Floor – enter main doors, go straight through lobby, then enter lab via first door to the right.)

PAYMENT

There is no payment for participation.

RISK/DISCOMFORTS

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Vary rare instances of heart attack have occurred, as with other moderately strenuous exercise activities. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. Equipment and trained personnel are available to deal with unusual situations should they arise. A trained CPR technician will be on hand at all times and the laboratory has standard emergency procedures should any potential need arise.
4. Mild symptoms of dehydration such as headache and general fatigue may result during and after the exercise. To minimize the risk of excessive dehydration, your body temperature will be monitored continuously during exercise. Immediately after the exercise sessions, sports drinks or juices (electrolytes) along with water will be provided to you.
5. You will be informed of any new findings that may affect your decision to remain in the study.
6. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped.

BENEFITS OF PARTICIPATING IN THIS STUDY

1. There is no promise that you will receive any benefit from taking part in this study.

SUBJECT'S STATEMENT OF CONSENT

I have read the above description of this research study. I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. Furthermore, I have been assured that a member of the research team will also answer any future questions I may have. I voluntarily agree to take part. I understand I will receive a copy of this consent form.

Printed (Typed) Name of Subject

Subject's Signature

Date

SUBJECT'S STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

I provide my consent to be photographed during periods of the data collection. I realize that these digital images may be used during presentation of the data at regional and national meetings.

Subject's Signature

Date

Date Approved by IRB: 9/27/02
Approval Expires on: 2/13/03
J. G. Reulbach IRB Chair

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 1994)

PAR - Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES NO

☐	☐	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want—as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active—begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal—this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever—wait until you feel better; or
- if you are or may be pregnant—talk to your doctor before you start becoming more active.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

You are encouraged to copy the PAR-Q but only if you use the entire form

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT _____

WITNESS _____

or GUARDIAN (for participants under the age of majority)

© Canadian Society for Exercise Physiology
Société canadienne de physiologie de l'exercice

Supported by:

Health
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Appendix II: Statistics

Group 1 RER

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	.031	.004				
Trial	1	.003	.003	3.283	.1129	.1129	.1129
Trial * Subject	7	.007	.001				
time	4	.015	.004	15.590	.0001	.0002	.0001
time * Subject	28	.007	2.369E-4				
Trial * time	4	.003	.001	6.469	.0008	.0041	.0008
Trial * time * S...	28	.003	1.052E-4				

Dependent: RER

Means Table

Effect: Trial

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
with	40	.903	.030	.005
without	40	.915	.027	.004

Means Table

Effect: time

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
5 min	16	.933	.034	.008
15 min	16	.912	.026	.006
25 min	16	.908	.023	.006
35 min	16	.898	.023	.006
45 min	16	.894	.026	.006

Means Table

Effect: Trial * time

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	.916	.036	.013
with, 15 min	8	.911	.031	.011
with, 25 min	8	.899	.025	.009
with, 35 min	8	.897	.027	.010
with, 45 min	8	.890	.031	.011
without, 5 min	8	.949	.023	.008
without, 15 min	8	.914	.022	.008
without, 25 min	8	.917	.019	.007
without, 35 min	8	.898	.019	.007
without, 45 min	8	.899	.021	.008

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.004
Mean square	.004
F-Value	41.233
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	3.655E-5
Mean square	3.655E-5
F-Value	.348
P-Value	.5602
G-G	.4793
H-F	.5602

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.001
Mean square	.001
F-Value	11.160
P-Value	.0024
G-G	.0071
H-F	.0024

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	1.896E-6
Mean square	1.896E-6
F-Value	.018
P-Value	.8941
G-G	.8032
H-F	.8941

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	3.228E-4
Mean square	3.228E-4
F-Value	3.070
P-Value	.0907
G-G	.1044
H-F	.0907

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	.001
Mean square	.001
F-Value	10.816
P-Value	.0027
G-G	.0078
H-F	.0027

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	.003
Mean square	.003
F-Value	27.083
P-Value	.0001
G-G	.0002
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	.004
Mean square	.004
F-Value	40.570
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	.010
Mean square	.010
F-Value	97.481
P-Value	.0001
G-G	.0001
H-F	.0001

Group 1 HR

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	12279.934	1754.276				
Trial	1	20.211	20.211	.335	.5810	.5810	.5810
Trial * Subject	7	422.714	60.388				
time	4	4327.352	1081.838	39.892	.0001	.0001	.0001
time * Subject	28	759.332	27.119				
Trial * time	4	41.309	10.327	1.129	.3631	.3518	.3596
Trial * time * S...	28	256.183	9.149				

Dependent: HR

Means Table

Effect: Trial

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
with	40	133.508	15.202	2.404
without	40	134.514	15.254	2.412

Means Table

Effect: time

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
5 min	16	122.572	12.415	3.104
15 min	16	129.618	13.829	3.457
25 min	16	134.564	12.848	3.212
35 min	16	140.368	14.657	3.664
45 min	16	142.933	13.905	3.476

Means Table

Effect: Trial * time

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	120.681	12.265	4.336
with, 15 min	8	129.196	12.984	4.591
with, 25 min	8	134.304	13.424	4.746
with, 35 min	8	140.296	15.308	5.412
with, 45 min	8	143.065	13.671	4.834
without, 5 min	8	124.464	13.102	4.632
without, 15 min	8	130.040	15.518	5.487
without, 25 min	8	134.824	13.168	4.656
without, 35 min	8	140.440	15.032	5.315
without, 45 min	8	142.801	15.080	5.331

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	57.229
Mean square	27.229
F-Value	6.255
P-Value	.0185
G-G	.0410
H-F	.0292

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	2.848
Mean square	2.848
F-Value	.311
P-Value	.58314
G-G	.4387
H-F	.5113

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	1.082
Mean square	1.082
F-Value	.118
P-Value	.7335
G-G	.5573
H-F	.6494

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.083
Mean square	.083
F-Value	.009
P-Value	.9250
G-G	.7693
H-F	.8604

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	.278
Mean square	.278
F-Value	.030
P-Value	.8628
G-G	.6857
H-F	.7838

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	742.290
Mean square	742.290
F-Value	81.130
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	2004.129
Mean square	2004.129
F-Value	219.045
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	429.318
Mean square	429.318
F-Value	46.923
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	1345.056
Mean square	1345.056
F-Value	147.010
P-Value	.0001
G-G	.0001
H-F	.0001

Group 1 Fat utilization

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	82.936	11.848				
Trial	1	4.023	4.023	4.857	.0634	.0634	.0634
Trial * Subject	7	5.798	.828				
time	4	33.785	8.446	20.597	.0001	.0001	.0001
time * Subject	28	11.482	.410				
Trial * time	4	4.265	1.066	4.562	.0058	.0402	.0277
Trial * time * S...	28	6.545	.234				

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

Means Table

Effect: Trial

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with	40	4.058	1.347	.213
without	40	3.609	1.378	.218

Means Table

Effect: time

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
5 min	16	2.724	1.330	.333
15 min	16	3.581	1.156	.289
25 min	16	3.939	1.288	.322
35 min	16	4.376	1.070	.267
45 min	16	4.549	1.327	.332

Means Table

Effect: Trial * time

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	3.329	1.335	.472
with, 15 min	8	3.680	1.412	.499
with, 25 min	8	4.303	1.362	.482
with, 35 min	8	4.335	1.098	.388
with, 45 min	8	4.644	1.397	.494
without, 5 min	8	2.120	1.084	.383
without, 15 min	8	3.481	.921	.326
without, 25 min	8	3.575	1.181	.418
without, 35 min	8	4.416	1.115	.394
without, 45 min	8	4.455	1.342	.474

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	5.844
Mean square	5.844
F-Value	25.004
P-Value	.0001
G-G	.0023
H-F	.0010

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.158
Mean square	.158
F-Value	.676
P-Value	.4179
G-G	.3003
H-F	.3309

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	2.117
Mean square	2.117
F-Value	9.057
P-Value	.0055
G-G	.0260
H-F	.0195

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.026
Mean square	.026
F-Value	.113
P-Value	.4179
G-G	.3003
H-F	.3309

Comparison 5
Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	.143
Mean square	.143
F-Value	.610
P-Value	.4415
G-G	.3135
H-F	.3464

Comparison 6
Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	3.793
Mean square	3.793
F-Value	16.227
P-Value	.0004
G-G	.0075
H-F	.0043

Comparison 7
Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	6.917
Mean square	6.917
F-Value	25.593
P-Value	.0001
G-G	.0013
H-F	.0005

Comparison 8
Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	8.468
Mean square	8.468
F-Value	36.230
P-Value	.0001
G-G	.0007
H-F	.0002

Comparison 9
Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	21.809
Mean square	21.809
F-Value	93.306
P-Value	.0001
G-G	.0001
H-F	.0001

Group 1 CHO

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	71120.364	10160.052				
Trial	1	675.064	675.064	3.835	.0910	.0910	.0910
Trial * Subject	7	1232.048	176.007				
time	4	2344.810	586.203	6.290	.0010	.0150	.0075
time * Subject	28	2609.508	93.197				
Trial * time	4	1168.766	292.192	4.898	.0040	.0197	.0073
Trial * time * S...	28	1670.232	59.651				

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

Means Table

Effect: Trial

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with	40	109.469	32.167	5.086
without	40	115.279	31.943	5.051

Means Table

Effect: time

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
5 min	16	122.226	36.524	9.131
15 min	16	112.317	32.791	8.198
25 min	16	112.409	28.855	7.214
35 min	16	108.224	32.575	8.144
45 min	16	106.693	30.475	7.619

Means Table

Effect: Trial * time

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	112.734	36.565	12.928
with, 15 min	8	110.625	32.484	11.485
with, 25 min	8	108.352	30.029	10.617
with, 35 min	8	110.251	36.643	12.955
with, 45 min	8	105.382	33.063	11.689
without, 5 min	8	131.717	36.271	12.824
without, 15 min	8	114.010	35.247	12.462
without, 25 min	8	116.465	29.065	10.276
without, 35 min	8	106.197	30.361	10.734
without, 45 min	8	108.004	29.885	10.566

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	1
Mean square	1441.531
F-Value	24.166
P-Value	.0001
G-G	.0009
H-F	.0001

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	45.833
Mean square	45.833
F-Value	.768
P-Value	.3882
G-G	.3194
H-F	.3666

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	263.251
Mean square	263.251
F-Value	4.413
P-Value	.0448
G-G	.0676
H-F	.0527

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	65.732
Mean square	65.732
F-Value	1.102
P-Value	.3028
G-G	.2614
H-F	.2907

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	27.484
Mean square	27.484
F-Value	.461
P-Value	.5028
G-G	.3992
H-F	.4695

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	76.781
Mean square	76.781
F-Value	1.287
P-Value	.2662
G-G	.2364
H-F	.2581

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	216.164
Mean square	216.164
F-Value	.3624
P-Value	.0673
G-G	.0887
H-F	.0753

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	930.555
Mean square	930.555
F-Value	15.600
P-Value	.0005
G-G	.0040
H-F	.0011

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	2249.368
Mean square	2249.368
F-Value	37.709
P-Value	.0001
G-G	.0001
H-F	.0001

Group 1 RPE

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	108.972	15.567				
Trial	1	4.753	4.753	2.838	.1359	.1359	.1359
Trial * Subject	7	11.722	1.675				
time	4	97.831	24.458	11.574	.0001	.0083	.0071
time * Subject	28	59.169	2.113				
Trial * time	4	.981	.245	.867	.4956	.4415	.4682
Trial * time * S...	28	7.919	.283				

Dependent: RPE

Means Table

Effect: Trial

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
with	40	12.962	1.766	.279
without	40	13.450	2.056	.325

Means Table

Effect: time

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
5 min	16	11.344	1.207	.302
15 min	16	12.750	1.342	.335
25 min	16	13.344	1.546	.387
35 min	16	14.156	1.767	.442
45 min	16	14.438	2.032	.508

Means Table

Effect: Trial * time

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	11.250	1.309	.463
with, 15 min	8	12.500	1.195	.423
with, 25 min	8	12.938	1.208	.427
with, 35 min	8	14.000	1.690	.598
with, 45 min	8	14.125	1.885	.666
without, 5 min	8	11.438	1.178	.417
without, 15 min	8	13.000	1.512	.535
without, 25 min	8	13.750	1.813	.641
without, 35 min	8	14.312	1.945	.688
without, 45 min	8	14.750	2.252	.796

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.141
Mean square	.141
F-Value	.497
P-Value	.4865
G-G	.3698
H-F	.4253

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	1.000
Mean square	1.000
F-Value	3.536
P-Value	.0805
G-G	.0940
H-F	.0848

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	2.641
Mean square	2.641
F-Value	9.337
P-Value	.0049
G-G	.0194
H-F	.0112

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.391
Mean square	.391
F-Value	1.381
P-Value	.2498
G-G	.2193
H-F	.2362

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	1.562
Mean square	1.562
F-Value	5.525
P-Value	.0260
G-G	.0511
H-F	.0393

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	11.391
Mean square	11.391
F-Value	40.276
P-Value	.0001
G-G	.0002
H-F	.0001

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	33.063
Mean square	33.063
F-Value	116.906
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	21.391
Mean square	21.391
F-Value	75.635
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	43.891
Mean square	43.891
F-Value	155.193
P-Value	.0001
G-G	.0001
H-F	.0001

Group 1 Lactate

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	123.741	17.677				
Trial	1	5.980	5.980	1.974	.2029	.2029	.2029
Trial * Subject	7	21.209	3.030				
time	4	32.022	8.005	6.590	.0007	.0180	.0113
time * Subject	28	34.015	1.215				
Trial * time	4	4.283	1.071	1.594	.2034	.2399	.2292
Trial * time * S...	28	18.804	.672				

Dependent: lactate (mM)

Means Table

Effect: Trial

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
with	40	3.407	1.287	.204
without	40	3.954	2.084	.330

Means Table

Effect: time

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
0 min	16	2.493	1.023	.256
15 min	16	3.852	1.380	.345
25 min	16	3.744	1.310	.327
35 min	16	4.385	1.948	.487
45 min	16	3.929	2.325	.581

Means Table

Effect: Trial * time

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
with, 0 min	8	2.420	.887	.313
with, 15 min	8	3.650	1.145	.405
with, 25 min	8	3.697	1.186	.419
with, 35 min	8	4.015	1.663	.588
with, 45 min	8	3.253	1.111	.393
without, 0 min	8	2.565	1.202	.425
without, 15 min	8	4.054	1.636	.578
without, 25 min	8	3.791	1.505	.532
without, 35 min	8	4.754	2.249	.795
without, 45 min	8	4.605	3.050	1.078

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.084
Mean square	.084
F-Value	.125
P-Value	.7259
G-G	.5268
H-F	.6044

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.655
Mean square	.655
F-Value	.975
P-Value	.3319
G-G	.2645
H-F	.2917

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.035
Mean square	.035
F-Value	.053
P-Value	.8200
G-G	.6120
H-F	.6969

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	2.183
Mean square	2.183
F-Value	3.251
P-Value	.0822
G-G	.1046
H-F	.0988

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	7.305
Mean square	7.305
F-Value	10.877
P-Value	.0027
G-G	.0154
H-F	.0090

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	6.520
Mean square	6.520
F-Value	9.709
P-Value	.0042
G-G	.0196
H-F	.0123

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	2.775
Mean square	2.775
F-Value	4.132
P-Value	.0517
G-G	.0792
H-F	.0705

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	6.040
Mean square	6.010
F-Value	8.949
P-Value	.0057
G-G	.0232
H-F	.0152

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	16.632
Mean square	16.632
F-Value	24.766
P-Value	.0001
G-G	.0016
H-F	.0005

Group 1 Temperature

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	21.218	3.031				
trial	1	.047	.047	.129	.7299	.7299	.7299
trial * Subject	7	2.529	.361				
time	6	11.902	1.984	8.890	.0001	.0108	.0074
time * Subject	42	9.372	.223				
trial * time	6	.093	.015	1.254	.2990	.3152	.3162
trial * time * S...	42	.517	.012				

Dependent: rectal temp

Means Table

Effect: trial

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
with	56	37.549	.634	.085
without	56	37.589	.654	.087

Means Table

Effect: time

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
0	16	37.065	.564	.141
5	16	37.163	.496	.124
15	16	37.479	.425	.106
25	16	37.703	.437	.109
35	16	37.878	.434	.109
45	16	38.001	.457	.114
10 min post	16	37.694	.958	.240

Means Table

Effect: trial * time

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
with, 0	8	37.065	.607	.214
with, 5	8	37.136	.543	.192
with, 15	8	37.444	.458	.162
with, 25	8	37.662	.455	.161
with, 35	8	37.833	.421	.149
with, 45	8	37.965	.479	.169
with, 10 min post	8	37.736	.922	.326
without, 0	8	37.065	.560	.198
without, 5	8	37.189	.481	.170
without, 15	8	37.514	.416	.147
without, 25	8	37.744	.446	.158
without, 35	8	37.923	.472	.167
without, 45	8	38.038	.464	.164
without, 10 min post	8	37.653	1.055	.373

Comparison 1
Effect: Trial * Time

With min 0 vs. Without min 0	
df	1
Sum of squares	-4.44E-16
Mean square	-4.44E-16
F-Value	-3.60E-14
P-Value	1.0000
G-G	*
H-F	*

Comparison 2
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.011
Mean square	.011
F-Value	.912
P-Value	.3450
G-G	.2342
H-F	.2661

Comparison 3
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.020
Mean square	.020
F-Value	1.614
P-Value	.2109
G-G	.1723
H-F	.1877

Comparison 4
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.027
Mean square	.027
F-Value	2.183
P-Value	.1470
G-G	.1406
H-F	.1481

Comparison 5

Effect: Trial * Time

With min 35 vs. Without min35	
df	1
Sum of squares	.032
Mean square	.032
F-Value	2.601
P-Value	.1143
G-G	.1230
H-F	.1264

Comparison 6

Effect: Trial * Time

With min 45 vs. Without min 45	
df	1
Sum of squares	.022
Mean square	.022
F-Value	1.766
P-Value	.1910
G-G	.1627
H-F	.1757

Comparison 7

Effect: Trial * Time

With 10 post vs. Without 10 post	
df	1
Sum of squares	.028
Mean square	.028
F-Value	2.237
P-Value	.1422
G-G	.1381
H-F	.1450

Comparison 8

Effect: Trial * Time

With min 0 vs. With min 25	
df	1
Sum of squares	1.430
Mean square	1.430
F-Value	116.090
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

With min 0 vs. With min 45	
df	1
Sum of squares	3.240
Mean square	3.240
F-Value	263.026
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 10

Effect: Trial * Time

With min 45 vs. With 10 post	
df	1
Sum of squares	.210
Mean square	.210
F-Value	17.041
P-Value	.0002
G-G	.0079
H-F	.0040

Comparison 11
Effect: Trial * Time

Without min 0 vs. Without min 25	
df	1
Sum of squares	1.849
Mean square	1.849
F-Value	150.115
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 12
Effect: Trial * Time

Without min 0 vs. Without min 45	
df	1
Sum of squares	3.793
Mean square	3.793
F-Value	307.899
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 13
Effect: Trial * Time

Without min 45 vs. Without 10 post	
df	1
Sum of squares	.595
Mean square	.595
F-Value	48.341
P-Value	.0001
G-G	.0003
H-F	.0001

Group 2 RER

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	.026	.004				
Trial	1	.005	.005	3.090	.1222	.1222	.1222
Trial * Subject	7	.011	.002				
time	4	.007	.002	3.366	.0227	.0808	.0663
time * Subject	28	.014	.001				
Trial * time	4	.002	4.146E-4	4.830	.0043	.0169	.0048
Trial * time * S...	28	.002	8.583E-5				

Dependent: RER

Means Table

Effect: Trial

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
with	40	.894	.030	.005
without	40	.910	.026	.004

Means Table

Effect: time

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
5 min	16	.918	.045	.011
15 min	16	.905	.021	.005
25 min	16	.897	.022	.006
35 min	16	.900	.020	.005
45 min	16	.890	.025	.006

Means Table

Effect: Trial * time

Dependent: RER

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	.903	.044	.015
with, 15 min	8	.898	.024	.009
with, 25 min	8	.892	.029	.010
with, 35 min	8	.899	.023	.008
with, 45 min	8	.879	.027	.010
without, 5 min	8	.932	.045	.016
without, 15 min	8	.912	.016	.006
without, 25 min	8	.902	.013	.005
without, 35 min	8	.901	.018	.006
without, 45 min	8	.901	.018	.006

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.003
Mean square	.003
F-Value	39.005
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.001
Mean square	.001
F-Value	9.439
P-Value	.0047
G-G	.0138
H-F	.0051

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	3.926E-4
Mean square	3.926E-4
F-Value	4.574
P-Value	.0413
G-G	.0609
H-F	.0427

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	3.008E-5
Mean square	3.008E-5
F-Value	.350
P-Value	.5586
G-G	.4582
H-F	.5520

Comparison 5
Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	.002
Mean square	.002
F-Value	21.968
P-Value	.0001
G-G	.0009
H-F	.0001

Comparison 6
Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	4.893E-4
Mean square	4.893E-4
F-Value	5.701
P-Value	.0239
G-G	.0471
H-F	.0250

Comparison 7
Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	.002
Mean square	.002
F-Value	27.402
P-Value	.0001
G-G	.0003
H-F	.0001

Comparison 8
Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	.004
Mean square	.004
F-Value	42.178
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9
Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	.004
Mean square	.004
F-Value	46.147
P-Value	.0001
G-G	.0001
H-F	.0001

Group 2 HR

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	6017.780	859.683				
Trial	1	3.354	3.354	.007	.9354	.9354	.9354
Trial * Subject	7	3326.857	475.265				
time	4	3756.457	939.114	46.169	.0001	.0001	.0001
time * Subject	28	569.541	20.341				
Trial * time	4	20.426	5.106	.547	.7025	.6418	.7025
Trial * time * S...	28	261.277	9.331				

Dependent: HR

Means Table

Effect: Trial

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
with	40	155.337	13.517	2.137
without	40	155.747	13.230	2.092

Means Table

Effect: time

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
5 min	16	143.413	14.505	3.626
15 min	16	153.516	11.790	2.947
25 min	16	157.170	11.017	2.754
35 min	16	160.703	9.938	2.484
45 min	16	162.909	10.508	2.627

Means Table

Effect: Trial * time

Dependent: HR

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	142.289	14.727	5.207
with, 15 min	8	153.229	12.811	4.529
with, 25 min	8	157.065	11.083	3.919
with, 35 min	8	161.014	9.722	3.437
with, 45 min	8	163.090	10.535	3.725
without, 5 min	8	144.536	15.202	5.375
without, 15 min	8	153.804	11.556	4.086
without, 25 min	8	157.275	11.715	4.142
without, 35 min	8	160.391	10.811	3.822
without, 45 min	8	162.728	11.204	3.961

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	20.205
Mean square	20.205
F-Value	2.165
P-Value	.1523
G-G	.1569
H-F	.1523

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	1.323
Mean square	1.323
F-Value	.142
P-Value	.7094
G-G	.6135
H-F	.7094

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.176
Mean square	.176
F-Value	.019
P-Value	.8916
G-G	.8048
H-F	.8916

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	1.550
Mean square	1.550
F-Value	.166
P-Value	.6867
G-G	.5926
H-F	.6867

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	.526
Mean square	.526
F-Value	.056
P-Value	.8141
G-G	.7166
H-F	.8141

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	873.350
Mean square	873.350
F-Value	93.593
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	1730.768
Mean square	1730.768
F-Value	185.479
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	649.103
Mean square	649.103
F-Value	69.562
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	1323.686
Mean square	1323.686
F-Value	141.854
P-Value	.0001
G-G	.0001
H-F	.0001

Group 2 Fat utilization

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	393.254	56.179				
Trial	1	31.088	31.088	4.591	.0694	.0694	.0694
Trial * Subject	7	47.403	6.772				
time	4	38.647	9.662	3.235	.0266	.0927	.0810
time * Subject	28	83.627	2.987				
Trial * time	4	6.611	1.653	3.471	.0201	.0443	.0201
Trial * time * S...	28	13.334	.476				

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

Means Table

Effect: Trial

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with	40	7.813	2.860	.452
without	40	6.566	2.601	.411

Means Table

Effect: time

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
5 min	16	6.018	4.091	1.023
15 min	16	6.948	2.580	.645
25 min	16	7.485	2.461	.615
35 min	16	7.381	2.147	.537
45 min	16	8.118	2.074	.518

Means Table

Effect: Trial * time

Dependent: Fat oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	7.079	4.152	1.468
with, 15 min	8	7.608	2.989	1.057
with, 25 min	8	8.010	2.830	1.001
with, 35 min	8	7.551	2.309	.816
with, 45 min	8	8.819	2.028	.717
without, 5 min	8	4.957	4.006	1.416
without, 15 min	8	6.289	2.082	.736
without, 25 min	8	6.960	2.082	.736
without, 35 min	8	7.210	2.115	.748
without, 45 min	8	7.416	1.994	.705

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	17.999
Mean square	17.999
F-Value	37.796
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	6.956
Mean square	6.956
F-Value	14.608
P-Value	.0007
G-G	.0035
H-F	.0007

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	4.410
Mean square	4.410
F-Value	9.261
P-Value	.0050
G-G	.0137
H-F	.0050

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.466
Mean square	.466
F-Value	.978
P-Value	.3311
G-G	.2920
H-F	.3311

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	7.868
Mean square	7.868
F-Value	16.522
P-Value	.0004
G-G	.0023
H-F	.0004

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	3.469
Mean square	3.469
F-Value	7.284
P-Value	.0117
G-G	.0245
H-F	.0117

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	12.110
Mean square	12.110
F-Value	25.431
P-Value	.0001
G-G	.0004
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	16.040
Mean square	16.040
F-Value	33.683
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	24.182
Mean square	24.182
F-Value	50.780
P-Value	.0001
G-G	.0001
H-F	.0001

Group 2 CHO

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	80034.763	11433.538				
Trial	1	2405.392	2405.392	3.405	.1075	.1075	.1075
Trial * Subject	7	4944.986	706.427				
time	4	2291.994	572.999	1.016	.4159	.3698	.3796
time * Subject	28	15786.211	563.793				
Trial * time	4	1973.155	493.289	4.923	.0039	.0176	.0057
Trial * time * S...	28	2805.751	100.205				

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

Means Table

Effect: Trial

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with	40	175.558	35.049	5.542
without	40	186.524	39.200	6.198

Means Table

Effect: time

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
5 min	16	190.494	48.575	12.144
15 min	16	182.629	30.998	7.749
25 min	16	175.221	30.899	7.725
35 min	16	179.822	34.320	8.580
45 min	16	177.039	41.758	10.439

Means Table

Effect: Trial * time

Dependent: CHO oxidation ($\mu\text{mol/kg/min}$)

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	176.470	43.580	15.408
with, 15 min	8	177.061	28.832	10.194
with, 25 min	8	172.626	31.488	11.133
with, 35 min	8	180.784	34.889	12.335
with, 45 min	8	170.848	42.836	15.145
without, 5 min	8	204.518	52.032	18.396
without, 15 min	8	188.197	34.012	12.025
without, 25 min	8	177.816	32.233	11.396
without, 35 min	8	178.861	36.120	12.770
without, 45 min	8	183.230	42.592	15.058

Comparison 1

Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	3146.649
Mean square	3146.649
F-Value	31.402
P-Value	.0001
G-G	.0002
H-F	.0001

Comparison 2

Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	496.064
Mean square	496.064
F-Value	4.950
P-Value	.0343
G-G	.0552
H-F	.0389

Comparison 3

Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	107.744
Mean square	107.744
F-Value	1.075
P-Value	.3086
G-G	.2688
H-F	.3009

Comparison 4

Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	14.784
Mean square	14.784
F-Value	.148
P-Value	.7038
G-G	.5657
H-F	.6752

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	613.305
Mean square	613.305
F-Value	6.120
P-Value	.0197
G-G	.0381
H-F	.0234

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	59.098
Mean square	59.098
F-Value	.590
P-Value	.4489
G-G	.3677
H-F	.4317

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	126.450
Mean square	126.450
F-Value	1.262
P-Value	.2708
G-G	.2423
H-F	.2657

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	2851.827
Mean square	2851.827
F-Value	28.460
P-Value	.0001
G-G	.0004
H-F	.0001

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	1812.631
Mean square	1812.631
F-Value	18.089
P-Value	.0002
G-G	.0022
H-F	.0004

Group 2 RPE

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	108.122	15.446				
Trial	1	2.278	2.278	.617	.4579	.4579	.4579
Trial * Subject	7	25.847	3.692				
time	4	94.331	23.583	23.154	.0001	.0007	.0004
time * Subject	28	28.519	1.019				
Trial * time	4	1.269	.317	.539	.7084	.5502	.5811
Trial * time * S...	28	16.481	.589				

Dependent: RPE

Means Table

Effect: Trial

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
with	40	13.225	1.874	.296
without	40	13.562	1.878	.297

Means Table

Effect: time

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
5 min	16	11.781	1.303	.326
15 min	16	12.594	1.143	.286
25 min	16	13.562	1.424	.356
35 min	16	14.281	1.673	.418
45 min	16	14.750	2.082	.520

Means Table

Effect: Trial * time

Dependent: RPE

	Count	Mean	Std. Dev.	Std. Error
with, 5 min	8	11.688	1.335	.472
with, 15 min	8	12.312	.884	.312
with, 25 min	8	13.562	1.237	.438
with, 35 min	8	13.938	1.935	.684
with, 45 min	8	14.625	2.264	.800
without, 5 min	8	11.875	1.356	.479
without, 15 min	8	12.875	1.356	.479
without, 25 min	8	13.562	1.678	.593
without, 35 min	8	14.625	1.408	.498
without, 45 min	8	14.875	2.031	.718

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.141
Mean square	.141
F-Value	.239
P-Value	.6288
G-G	.4082
H-F	.4494

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	1.266
Mean square	1.266
F-Value	2.150
P-Value	.1537
G-G	.1508
H-F	.1548

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.0000
Mean square	.0000
F-Value	.0000
P-Value	1.000
G-G	1.000
H-F	1.000

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	1.891
Mean square	1.891
F-Value	3.212
P-Value	.0839
G-G	.1075
H-F	.1061

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	.250
Mean square	.250
F-Value	.425
P-Value	.5199
G-G	.3440
H-F	.3767

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	14.062
Mean square	14.062
F-Value	23.891
P-Value	.0001
G-G	.0033
H-F	.0019

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	34.516
Mean square	34.516
F-Value	58.639
P-Value	.0001
G-G	.0002
H-F	.0001

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	11.391
Mean square	11.391
F-Value	19.352
P-Value	.0001
G-G	.0058
H-F	.0036

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	36.000
Mean square	36.000
F-Value	31.160
P-Value	.0001
G-G	.0001
H-F	.0001

Group 2 Lactate

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	140.934	20.133				
Trial	1	.913	.913	.398	.5480	.5480	.5480
Trial * Subject	7	16.050	2.293				
time	4	36.539	9.135	7.256	.0004	.0047	.0009
time * Subject	28	35.252	1.259				
Trial * time	4	1.311	.328	.646	.6342	.5515	.6046
Trial * time * S...	28	14.206	.507				

Dependent: lactate (mM)

Means Table

Effect: Trial

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
with	40	3.442	1.626	.257
without	40	3.656	1.903	.301

Means Table

Effect: time

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
0 min	16	2.257	1.033	.258
15 min	16	3.510	1.870	.467
25 min	16	3.874	1.805	.451
35 min	16	4.070	1.688	.422
45 min	16	4.035	1.801	.450

Means Table

Effect: Trial * time

Dependent: lactate (mM)

	Count	Mean	Std. Dev.	Std. Error
with, 0 min	8	2.247	1.062	.375
with, 15 min	8	3.438	1.856	.656
with, 25 min	8	3.929	1.690	.598
with, 35 min	8	3.850	1.542	.545
with, 45 min	8	3.748	1.649	.583
without, 0 min	8	2.267	1.076	.381
without, 15 min	8	3.583	2.008	.710
without, 25 min	8	3.819	2.029	.717
without, 35 min	8	4.290	1.902	.672
without, 45 min	8	4.321	2.010	.711

Comparison 1
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	.002
Mean square	.002
F-Value	.003
P-Value	.9567
G-G	.8441
H-F	.9263

Comparison 2
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.085
Mean square	.085
F-Value	.167
P-Value	.6862
G-G	.5352
H-F	.6328

Comparison 3
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.048
Mean square	.048
F-Value	.095
P-Value	.7597
G-G	.5994
H-F	.7045

Comparison 4
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.774
Mean square	.774
F-Value	1.526
P-Value	.2269
G-G	.2087
H-F	.2222

Comparison 5

Effect: Trial * Time

With min 45 vs. Without min45	
df	1
Sum of squares	1.316
Mean square	1.316
F-Value	2.593
P-Value	.1185
G-G	.1310
H-F	.1250

Comparison 6

Effect: Trial * Time

With min 5 vs. With min 25	
df	1
Sum of squares	11.308
Mean square	11.308
F-Value	22.289
P-Value	.0001
G-G	.0013
H-F	.0002

Comparison 7

Effect: Trial * Time

With min 5 vs. With min 45	
df	1
Sum of squares	9.008
Mean square	9.008
F-Value	17.754
P-Value	.0002
G-G	.0028
H-F	.0007

Comparison 8

Effect: Trial * Time

Without min 5 vs. Without min 25	
df	1
Sum of squares	9.633
Mean square	9.633
F-Value	18.987
P-Value	.0002
G-G	.0022
H-F	.0005

Comparison 9

Effect: Trial * Time

Without min 5 vs. Without min 45	
df	1
Sum of squares	16.886
Mean square	16.886
F-Value	33.283
P-Value	.0001
G-G	.0002
H-F	.0001

Group 2 Temperature

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	G-G	H-F
Subject	7	18.281	2.612				
trial	1	.029	.029	.032	.8628	.8628	.8628
trial * Subject	7	6.405	.915				
time	6	47.198	7.866	168.904	.0001	.0001	.0001
time * Subject	42	1.956	.047				
trial * time	6	.100	.017	.397	.8766	.6714	.7312
trial * time * S...	42	1.763	.042				

Dependent: rectal temp

Means Table

Effect: trial

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
with	56	37.979	.844	.113
without	56	38.011	.814	.109

Means Table

Effect: time

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
0	16	37.038	.498	.124
5	16	37.170	.487	.122
15	16	37.711	.484	.121
25	16	38.162	.516	.129
35	16	38.496	.542	.135
45	16	38.781	.605	.151
10 min post	16	38.608	.507	.127

Means Table

Effect: trial * time

Dependent: rectal temp

	Count	Mean	Std. Dev.	Std. Error
with, 0	8	37.054	.490	.173
with, 5	8	37.172	.511	.181
with, 15	8	37.688	.511	.181
with, 25	8	38.130	.582	.206
with, 35	8	38.489	.645	.228
with, 45	8	38.789	.718	.254
with, 10 min post	8	38.529	.578	.204
without, 0	8	37.021	.539	.191
without, 5	8	37.168	.498	.176
without, 15	8	37.734	.489	.173
without, 25	8	38.194	.479	.170
without, 35	8	38.504	.462	.163
without, 45	8	38.773	.519	.184
without, 10 min post	8	38.686	.449	.159

Comparison 1
Effect: Trial * Time

With min 0 vs. Without min 0	
df	1
Sum of squares	.004
Mean square	.004
F-Value	.103
P-Value	.7495
G-G	.4488
H-F	.5285

Comparison 2
Effect: Trial * Time

With min 5 vs. Without min 5	
df	1
Sum of squares	8.100E-5
Mean square	8.100E-5
F-Value	.002
P-Value	.9652
G-G	.7075
H-F	.8018

Comparison 3
Effect: Trial * Time

With min 15 vs. Without min 15	
df	1
Sum of squares	.008
Mean square	.008
F-Value	.195
P-Value	.6609
G-G	.3910
H-F	.4601

Comparison 4
Effect: Trial * Time

With min 25 vs. Without min 25	
df	1
Sum of squares	.016
Mean square	.016
F-Value	.381
P-Value	.5403
G-G	.3250
H-F	.3795

Comparison 5
Effect: Trial * Time

With min 35 vs. Without min 35	
df	1
Sum of squares	.001
Mean square	.001
F-Value	.021
P-Value	.8862
G-G	.5727
H-F	.6670

Comparison 6
Effect: Trial * Time

With min 45 vs. Without min 45	
df	1
Sum of squares	.001
Mean square	.001
F-Value	.025
P-Value	.8747
G-G	.5593
H-F	.6527

Comparison 7
Effect: Trial * Time

With 10 post vs. Without 10 post	
df	1
Sum of squares	.099
Mean square	.099
F-Value	2.357
P-Value	.1322
G-G	.1325
H-F	.1382

Comparison 8
Effect: Trial * Time

With min 0 vs. With min 25	
df	1
Sum of squares	4.634
Mean square	4.634
F-Value	110.411
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 9
Effect: Trial * Time

With min 0 vs. With min 45	
df	1
Sum of squares	12.035
Mean square	12.035
F-Value	286.755
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 10
Effect: Trial * Time

With min 45 vs. With 10 post	
df	1
Sum of squares	.270
Mean square	.270
F-Value	6.430
P-Value	.0150
G-G	.0477
H-F	.0400

Comparison 11
Effect: Trial * Time

Without min 0 vs. Without min 25	
df	1
Sum of squares	5.499
Mean square	5.499
F-Value	131.023
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 12
Effect: Trial * Time

Without min 0 vs. Without min 45	
df	1
Sum of squares	12.268
Mean square	12.268
F-Value	292.292
P-Value	.0001
G-G	.0001
H-F	.0001

Comparison 13
Effect: Trial * Time

Without min 45 vs. Without 10 post	
df	1
Sum of squares	.030
Mean square	.0303
F-Value	.709
P-Value	.4045
G-G	.2599
H-F	.2981

Appendix III

Manuscript for submission to Research in Sports Medicine

Effects of Peripheral Cooling on Substrate Selection During Exercise

David E. Lankford, Brent C. Ruby, Steven E. Gaskill

The University of Montana

Department of Health and Human Performance

McGill Hall room 109

Missoula, MT 59812

Ph. (406) 243-4211

Fax (406) 243-2117

Email ruby@selway.umt.edu

Abstract

Purpose: To examine the effects of peripheral cooling via the rapid thermal exchange (RTX) cooling device (AVAcure Technologies Inc.) on whole body substrate oxidation during exercise at a moderate ambient temperature (28°C). **Methods:** Subjects (N=16) completed two 45-minute cycle ergometer trials at 90% of ventilatory threshold (VT) with RTX peripheral cooling and without (CON). Rectal temperature was continuously recorded throughout both trials. VO_2 and VCO_2 were monitored to compute whole body substrate oxidation. Significance was set at $p < 0.05$. **Results:** Despite no differences in rectal temperature between trials, mean CHO oxidation was lower during RTX (1453 ± 299 kJ) versus CON (1546 ± 291 kJ). Conversely, mean fat oxidation was higher during RTX (719 ± 248 kJ) versus CON (610 ± 197 kJ). HR and RPE were similar between trials. **Conclusion:** These data indicate the RTX device promotes a decrease in whole body carbohydrate (CHO) reliance despite no difference in rectal temperature. Supported by AVAcure Technologies, Inc.

Key words: Carbohydrate, fat, AVAcure.

Running Head: Effects of Peripheral Cooling on Substrate Selection.

Introduction

It has been recognized that a rise in body temperature during submaximal exercise will increase muscle glycogenolysis during exercise [1,2,3]. Previous research has shown that an attenuated rise in body temperature either by reducing ambient temperatures [2,4], or by cooling [5,6] will reduce temperature induced increases in glycogen flux and total carbohydrate oxidation.

Heat stress also influences lipid mobilization and oxidation, although the concentration of plasma free fatty acids are unaffected by heat stress [3]. Additionally, it has been shown that intramuscular triacylglycerol utilization [3] and free fatty acid uptake [7] are reduced under conditions of muscular thermal stress. These data may suggest a reduction in free fatty acid release by adipocytes during exercise in the heat [8]. It has also been suggested that elevated muscle temperature decreases phosphorylative efficiency in isolated skeletal muscle mitochondria, increasing the reliance on carbohydrate as the primary substrate [9]. Febbraio [1] suggests that elevated muscle temperature may impair mitochondrial function. Mills et al. [10] observed an increase of lipid peroxides, an indicator of oxidative stress, in the plasma of hyperthermic horses exercising to fatigue, possibly supporting the notion that heat stress may impair metabolic function.

To examine alterations in substrate oxidation in the heat, previous research has utilized the measurement techniques of indirect calorimetry [4,11], rates of glucose disposal (glucose Rd) [11], and muscle glycogen depletion [4,6]. It has been shown that heat stress stimulates an increase in hepatic glucose production (glucose Ra) without a concomitant rise in glucose Rd [11]. Febbraio [1] suggests that an increase in body

temperature and perceived effort promote a feed-forward effect resulting in increased blood catecholamines which in return increases muscle glycogenolysis.

Past research has consistently demonstrated increased whole body CHO oxidation from indirect calorimetry during heat stress [4,11,12]. Augmented glycogen utilization during heat stress was first reported by Fink et al. [3] while comparing exercise at ambient temperatures of 40°C and 9°C. Fink et al. also noted an increased respiratory exchange ratio and reduced intramuscular triglyceride use. Using water-perfused cuffs to cool one thigh and heat the other during submaximal exercise, Starkie et al. [6] found lower post exercise muscle glycogen levels in the heated leg compared with the cool leg.

Consistent with increased CHO oxidation, previous studies have consistently demonstrated an elevated blood lactate accumulation during exercise in the heat [3,4,11,12]. Hargreaves et al. [11] suggests that increased blood lactate is associated with increased rates of muscle glycogenolysis, since total carbohydrate oxidation is increased while glucose Rd is unaltered.

Past research on substrate use under conditions of heat stress can be categorized into two parts: 1) those comparing exercise in extremely different ambient temperatures or 2) those comparing exercise using cooling methods resulting in temperatures exceedingly lower than normal ambient working temperatures. Under normal circumstances exercise is most often performed within a relatively narrow temperature range of comfortable temperatures unlike the extreme temperature differences used for most prior research.

Slight to moderate core temperature cooling can be achieved through use of the AVAcore (AVAcore Technologies Inc.) rapid thermal exchange (called RTX) device.

The technology behind the RTX exploits the fact that under various surfaces of the body are arterio-venous anastomoses and venous plexuses involved in heat exchange from the body to the environment. These special exchange sites are found in high concentrations on the palms of the hands, soles of the feet and on the face. Blood flow is amplified to these areas with the AVAcore RTX device by using negative pressure to enhance heat exchange. The RTX's temperature is controlled via water flow at adjustable temperatures, which cools a metal surface on which the palm of the hand is placed.

The purpose of this study was therefore to evaluate the effect of exercise on substrate utilization at ambient temperatures commonly found in exercise facilities (28°C). We compared substrate utilization under conditions of slight cooling (22°C) of one hand using the RTX with negative pressure to a control trial (CON) without cooling. We hypothesized that the RTX cooling device would significantly reduce body temperature during exercise thereby reducing calculated rates of whole body CHO oxidation.

Methods

Sixteen recreationally active males participated in the study after completing a University IRB approved informed consent.

Exercise Testing

For the initial session subjects completed a graded exercise cycle test using a Monark 824E mechanically braked cycle ergometer (Varberg, Sweden) to determine ventilatory threshold (VT) and peak VO_2 work rates. The graded exercise consisted of 1-minute (min) stages starting at 70-watts and increased 15-watts for each subsequent stage

until volitional exhaustion. Cadence was maintained at 70 revolutions per minute. Expired gases were collected using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and analyzed at 15-second intervals. VT was determined using a combination of the V-slope method, the ventilatory equivalent method and the excess CO₂ method [13]. Subject's characteristics are outlined in Table 1.

Trials

Each subject was asked to refrain from strenuous exercise, caffeine, alcohol, and tobacco for 24-hours prior to each trial. All trials were performed in the morning following a 10-hour fast. Subjects cycled at an intensity equivalent to 90% of VT work rate for 45-min. Exercise chamber temperature was controlled at 28°C for both trials. Subjects received either 22°C cooling (RTX) from the AVAcore hand device (manifold T= 22°C, P=635mmHg) or no cooling (control=CON) using a random cross-over design. Core body temperature was continuously recorded on an Omega data logger using a Mon-a-therm (Mallinkrodt Medical, Inc.) rectal probe inserted to a depth of at least 10 cm. Heart rate was monitored with a telemetry system (Polar, Port Washington, NY) and recorded using a 2-min average every 10-min, starting at min 5. RPE was recorded every 10-min starting at min 5.

Whole blood samples (15µl) to measure blood lactate were collected from the fingertip at min 0, 15, 25, 35 and 45. Blood samples were mixed with 100 µl chilled perchloric acid and stored at -30°C until subsequent analysis. Blood lactate was analyzed using an enzymatic, spectrophotometric method. All samples were analyzed in duplicate.

Whole body carbohydrate and fat oxidation were calculated, using the Frayn equation [14], from average steady state VO_2 and VCO_2 measures collected for the 2-min prior to min 5, 15, 25, 35 and 45 of the trial. Following the completion of each trial, a passive recovery took place for 10-min in which time only rectal temperature was recorded.

Statistics

HR, RPE, fat oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and CHO oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) were compared across trials using apriori planned comparison for min 5, 15, 25, 35, and 45. Pre-planned comparisons were used to examine within trials differences in HR, RPE, fat oxidation and CHO oxidation at min 5 vs. 45. Temperature differences between trials were examined using apriori planned comparisons at min 0, 45, and 10-min post exercise. Apriori planned comparisons were also used to determine within trial changes in temperature (min 0 vs. 45 and min 45 vs. 10-min post). Differences in overall energy expenditure (total kJ), total fat kJ (fat kJ), and total CHO kJ (CHO kJ), were examined using 2-tailed paired t-tests. Blood lactate was examined using apriori planned comparisons between trials at min 15, 25, 35, and 45 and within trials across time (min 0 vs. 45). Statistical significance was set at $p < 0.05$. All data are shown as means $\pm$ standard deviation (SD).

Results

CHO Utilization

Within trials both RTX and CON demonstrated significant decreases in CHO oxidation with time from min 5 to 45 (RTX $\Delta\text{CHO} = -6.5 \pm 17.1 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p < 0.05$, CON $\Delta\text{CHO} = -22.5 \pm 26.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p < 0.001$). Between trials CHO oxidation was significantly decreased during RTX compared with CON at min 5, 15, 25, and 45 (see Figure 1).

Fat Utilization

Within trials both RTX and CON demonstrated significant increases in fat oxidation from min 5 to 45 (RTX $\Delta\text{FAT} = 1.5 \pm 2.4 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p < 0.001$, CON $\Delta\text{FAT} = 2.4 \pm 1.7 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p < 0.001$). Between trials RTX cooling resulted in a significant decrease in fat utilization at min 5, 15, 25 and 45 compared with CON (see Figure 2).

Heart Rate

Within trials heart rate increased significantly with time between min 0 and 45 for both trials (RTX $\Delta\text{HR} = 21.6 \pm 7.4$, $p < 0.001$, CON $\Delta\text{HR} = 18.3 \pm 8.3$, $p < 0.001$). Between trials, RTX resulted in a significantly lower heart rate only at min 5 compared to CON ($p < 0.01$, see Table 2).

RPE

Within trials there was a significant increased in RPE between min 0 and 45 for both trials (RTX $\Delta\text{RPE} = 2.3 \pm 2.1$, $p < 0.001$, CON $\Delta\text{RPE} = 3.2 \pm 2.4$, $p < 0.001$). RPE

during RTX cooling was significantly lower at min 15 ($p < 0.05$) and 35 ($p < 0.05$) compared with CON (see Table 2).

Rectal Temperature

There were no significant differences in rectal temperature between trials (see Figure 3). Both trials resulted in a significant increase in rectal temperature between min 0 and 45 (RTX $\Delta^{\circ}\text{C} = 1.3 \pm 0.6$, $p < 0.001$, CON $\Delta^{\circ}\text{C} = 1.4 \pm 0.5$, $p < 0.001$), and a significant decrease between min 45 and 10-min post (RTX $\Delta^{\circ}\text{C} = -0.2 \pm 0.5$, $p < 0.001$, CON $\Delta^{\circ}\text{C} = -0.2 \pm 0.6$, $p < 0.001$).

Blood Lactate

Both trials resulted in a significant increase in blood lactate (BLa^+) with time between min 5 and 45 (RTX $\Delta\text{BLa}^+ = 1.1 \pm 1.1$ mM, $p < 0.001$, CON $\Delta\text{BLa}^+ = 2.1 \pm 2.4$ mM, $p < 0.001$). During RTX, BLa^+ was significantly lower at min 35 ($p < 0.05$) and 45 ($p < 0.001$) compared with CON (see Table 3).

Energy Expenditure

Total energy expenditure was not significantly different between trials (RTX = 2172 ± 426 kJ, CON = 2156 ± 403 kJ, $p = 0.31$). RTX resulted in both greater total fat utilization than CON (719 ± 248 kJ vs. 610 ± 197 kJ respectively, $p < 0.01$) and lower total CHO utilization (1453 ± 299 kJ vs. 1546 ± 291 kJ respectively, $p < 0.05$) than CON (see Table 4).

Discussion

Previous studies have shown that cooling influences metabolic substrate selection during exercise [1,2,3,7]. Febbraio [8] suggests that changes in body temperature greater

than 0.4°C can cause decreased fat and increased carbohydrate metabolism. The present study differed from the results of Febbraio in that RTX hand cooling resulting in decreased CHO and increased fat utilization despite no significant differences in rectal temperature between trials. During the cooling trial with the AVAcore RTX device, the decreased reliance on CHO and increased reliance on fat occurred largely at the beginning of exercise (min 5 = 5.2 ± 3.6 vs. $3.5 \pm 3.2 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for RTX and CON respectively). We cannot explain the physiological mechanism responsible for this early decreased reliance in CHO. Some psychological or other feedback mechanism as a result of the hand on the cool surface of the RTX may have influenced catecholamine response early in the exercise, which was later modulated by the effects of the exercise. We have no evidence for these effects, but the pattern of reduced CHO dependence during early exercise in the RTX trial was seen across subjects.

Hargreaves and colleagues [11] suggest that during heat stress an increased blood lactate may be associated with an increased rate of muscle glycogenolysis, since blood glucose uptake is reduced with a concomitant rise in total carbohydrate oxidation. The present data demonstrates an increase in blood lactate during the last 10-min of exercise in the CON. Considering the difference in CHO oxidation across trials at the end of exercise, these results suggest a possible increase in muscle glycogenolysis during the later portion of CON. However, we are skeptical to conclude that glucose uptake was altered due to an increased heat load during the CON trial as there were no differences in rectal temperature. The subtle cooling provided by the RTX may have had other effects than cooling core temperature. An additional possibility is that the temperature in the working skeletal muscle was reduced due to increased perfusion of slightly cooled blood,

while the rectal temperature remained unaffected due to reduced blood flow to the gut during exercise.

At exercise intensities below VT it is common to note an increased reliance on fat oxidation over time. Given the mild temperature environment and the moderate exercise intensity of the present study, it is doubtful that muscle temperature increased to a level that would impair lipid metabolism through oxidative phosphorylation [9]. During the first 15 minutes of exercise the core temperature under both trials was less than 37.6°C and peak core temperature averaged near 38.4°C at the end of exercise. Why there would be a difference in substrate utilization at the lower temperatures early in exercise and no difference at the moderate core temperature after 45 minutes of exercise cannot be explained by previous research. Our data agree with others that there should be no difference during the later stages of exercise when core temperature values were not different, however the early differences do not appear to be a result of changes in core temperature. Although catecholamines have been shown to alter substrate selection and are increased during heat stress [2,11,15], it is unclear whether variations in epinephrine could explain the observed difference in substrate oxidation since we did not measure catecholamine levels in the present study. CON resulted in a significantly higher RPE at min 15 and 35, and approached significance at min 25 ($p = 0.09$) and 45 ($p = 0.07$). This may suggest an increase in sympathetic drive, which would stimulate both hepatic glucose production and carbohydrate oxidation [8]. However, increased catecholamines should also cause an increase in HR, and there were no differences in the HR response between trials with the exception of the initial 5 minutes (RTX 131 ± 17 , CON 134 ± 17). The decreased HR at min 5, combined with the lower CHO oxidation early in the RTX

trial possibly suggest that there was some reduction of catecholamines due to the cooling device though this possibility will require further study.

The similar total energy expenditure between trials verifies the similarity in exercise intensity across trials (see Table 4). That there were no differences in core temperature, no differences in energy expenditure yet early differences in HR and substrate utilization suggest some effect of the RTX device that is not linked to core temperature changes in this study. Use of the RTX may suppress muscle glycogenolysis during the early portion of an exercise session with continued small glycogen sparing over the course of the submaximal exercise bout. For long slow distance (low intensity) aerobic exercise sessions, or to enhance recovery between exercise sessions by reducing within session glycogen depletion, the RTX device may provide benefits to athletes if mobile units are developed.

Conclusion

In summary, the findings of our study suggest that the use of the AVAcore RTX to supply peripheral cooling influences substrate selection during exercise near VT, especially within the initial stages of an exercise session. However, the underlying physiological mechanism(s) for these results are unclear as there were no rectal temperature differences between trials.

Future research should incorporate measures of intramuscular temperature, patterns of muscle glycogen depletion, and substrate kinetics to further evaluate the physiological effects of the RTX device during exercise.

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Table 1. Descriptive characteristics of subjects (mean $\pm$ SD).

	Subject (N=16)
Age (yr)	25.5 $\pm$ 6.0
Height (in)	70.4 $\pm$ 2.2
Height (cm)	178.9 $\pm$ 5.7
Body Weight (kg)	85.7 $\pm$ 21.6
Fat Mass (kg)	14.6 $\pm$ 14.4
Fat Free Mass (kg)	71.1 $\pm$ 8.3
PeakVO ₂ (L $\cdot$ min ⁻¹)	3.9 $\pm$ 0.5
PeakVO ₂ (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	48.9 $\pm$ 14.5
VT (L $\cdot$ min ⁻¹)	2.5 $\pm$ 0.5
VT (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	31.6 $\pm$ 11.3
VT (%VO ₂ peak)	64.0 $\pm$ 5.4%

Peak VO₂ = peak oxygen uptake; VT = ventilatory threshold.

Table 2. Heart rate and rate of perceived exertion with RTX and CON.

Minute	5	15	25	35	45
HR					
RTX	131 ± 17*	141 ± 18	146 ± 17	151 ± 16	153 ± 16 [#]
CON	135 ± 17	142 ± 18	146 ± 17	150 ± 16	153 ± 16 [#]
RPE					
RTX	11.5 ± 1.3	12.4 ± 1.0*	13.3 ± 1.2	14.0 ± 1.8*	14.4 ± 2.0 [#]
CON	11.7 ± 1.2	12.9 ± 1.4	13.7 ± 1.7	14.5 ± 1.6	14.8 ± 2.1 [#]

Significant difference between RTX and CON *p < 0.05 .

Significant difference between minute 5 and 45 within trials #p < 0.05.

Table 3. Comparison of Blood Lactate (mM) between RTX and CON.

Minute	0	15	25	35	45
RTX	2.3 ± .9	3.5 ± 1.5	3.8 ± 1.4	3.9 ± 1.5*	3.5 ± 1.3* [#]
CON	2.4 ± 1.1	3.8 ± 1.8	3.8 ± 1.7	4.5 ± 2.0	4.5 ± 2.5 [#]

*p < 0.05 Significant difference between RTX and CON.

[#]p < 0.05 Significant difference between minute 0 and 45 within trials.

Table 4. Exercise trial data. The intensity of exercise is shown both as a percent of $\text{VO}_{2\text{max}}$ (% $\text{VO}_{2\text{max}}$) and as a percent of $\text{VO}_{2\text{vt}}$ (%VT). Total substrate oxidation in kilojoules for the 45 minutes of exercise is shown for carbohydrate (CHO kJ), fat (FAT kJ) and overall (Total kJ).

Trial	% VO_2 max	%VT	CHO kJ	Fat kJ	Total kJ
RTX	60.8 $\pm$.07	94.0 $\pm$.07	1453 $\pm$ 299*	719 $\pm$ 248*	2172 $\pm$ 426
CON	59.2 $\pm$.06	93.1 $\pm$.06	1546 $\pm$ 291	610 $\pm$ 197	2156 $\pm$ 403

* $p < 0.05$ Significant difference vs. CON.

Figures Legend

Figure 1. CHO oxidation of subjects during RTX and CON (Mean $\pm$ SE).

* $p < 0.05$ RTX vs. CON.

$p < 0.05$. vs. min 0 within trials.

Figure 2. Fat oxidation of subjects during RTX and CON (Mean $\pm$ SE).

* $p < 0.05$ RTX vs. CON.

$p < 0.05$. vs. min 0 within trials.

Figure 3. Rectal temperature of subjects during RTX and CON (Mean $\pm$ SE). There were no significant differences between trials.

$p < 0.05$ min 0 vs. 45 within trials.

† $p < 0.05$ min 45 vs. 10-post within trials.