CONTRACT NUMBER DAMD17-96-C-6128

TITLE: Energy Metabolism in Cold-Stressed Females: Implications for Predictive Modeling

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REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012

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# 1. TABLE OF CONTENTS

1. TABLE OF CONTENTS	4
2. ABSTRACT	5
3. INTRODUCTION	6
3.1 Objectives	
3.2 Hypotheses	
4. METHODS	12
4.1 EXPERIMENTAL DESIGN	• •
4.2 STANDARDIZATION OF MENSTRUAL AND DIURNAL CYCLES	
4.3 EXERCISE AND EXPOSURE	
4.4 MUSCLE BIOPSIES	
4.5 BLOOD SAMPLING	
4.6 BIOCHEMISTRY	
4.7 IEMPERATURE MEASUREMENTS	
4.8 RESPIRATOR FORS EXCHANGE MEASOREMENTS	
4.10 STATISTICAL ANALYSES	
5. RESULTS 1	8
5.1 SUBJECT ATTRITION	
5.2 SUBJECT CHARACTERISTICS	
5.3 TEMPERATURE MEASUREMENTS AND HEAT FLOW	
5.4 METABOLIC HEAT PRODUCTION AND SUBSTRATE CONTRIBUTIONS	
5.5 MUSCLE GLYCOGEN CONCENTRATIONS	
5.6 BLOOD METABOLITES AND HORMONES	
6. DISCUSSION	22
6.1 Hypothesis A:	
6.2 Hypothesis B:	
7. SUMMARY AND CONCLUSIONS	24
8. REFERENCES	25
9. TABLES	31
10. FIGURES	45

# 2. ABSTRACT

This document is a progress report which describes the results from the second of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to compare substrate utilization during light (LI) and moderate intensity (MI) exercise performed in both cold and comfortable ambient temperatures. The objectives were to quantify metabolic rate (*M*), lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold versus comfortable ambient temperatures, and to compare these results with those previously collected in males who underwent the same protocol. Sixteen female subjects were divided into two groups matched for the submaximal exercise intensity corresponding to a blood lactate concentration of 4 mM (W4) during an incremental cycle exercise test. On two separate days subjects rested for 30 min at ambient temperatures of either 9 or 21°C, with the order of the trials balanced among subjects. Following rest a muscle biopsy was obtained from the m. vastus lateralis. Subjects in the MI group exercised for 30 min at 60%W4 while subjects in the LI group exercised for 30 min at 30%W4. Subjects exercised at the same power output for both trials. Another biopsy was taken immediately after exercise and both samples were assayed for glycogen concentration. M was significantly higher (13%) during exercise in the cold in LI but not MI. Muscle glycogen decreased significantly in MI (-28%) but not in LI; ambient temperature did not affect the magnitude of the change in muscle glycogen in either group. The relative contributions of carbohydrate, fat and protein oxidation to fueling Mwere similar in each exposure for both groups. These data provide further evidence of a more pronounced energy metabolism "predisposition" towards lipid metabolism in females than in males.

# 3. INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with lifethreatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of ST in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ( $\dot{M}$ ) by shivering in an attempt to balance heat loss. Existing models of ST in cold air or cold water are based on observations of factors which affect  $\dot{M}$  and the rate of heat loss from the body. In such models  $\dot{M}$  increases as a function of temperature signals from the core and skin. When cold exposure is too severe for  $\dot{M}$  to balance heat loss, ST is largely determined by the rate of heat loss from the body. Where there is a balance between  $\dot{M}$  and heat loss, ST is limited by the endurance time for shivering.

The physiological factors characterizing *M* are relatively complex. Until about a decade ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship

between *ST*, *M*, and heat loss is affected by the extent of the muscle mass involuntarily recruited during shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modeling of *ST* in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting subjects exposed to either cold air or cold water, carbohydrates and fat contribute

Page 4

approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6- or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal (Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected. Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the

total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975). It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985), and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989), cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such as those described above, i.e. quantification of the substrates used to fuel M during cold stress, nor in the development of ST predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990).

Page 7

There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984). In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995). Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of ST in the cold. However, even if the magnitude of the increase in M may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in *M* due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980). We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in M, are potentially of sufficient magnitude to warrant their consideration in a model of ST in cold stressed females.

#### 3.1 Objectives

This report is a progress report which describes the results from the second of a series of studies carried out to address the issues raised above. Specifically, this study was designed to compare substrate utilization during light and moderate exercise performed in both cold and comfortable ambient temperatures.

The objectives were: to quantify M, lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold; to compare the results to when the same exercise is performed a comfortable temperatures; to compare all results with those previously collected in males who underwent the same protocol.

## 3.2 Hypotheses

**a.** *M* will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature.

**b.** Contrasting with what has been reported for males, this increase will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature.

## 4. METHODS

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Jacobs et al. 1985). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Sixteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body

Page 9

density by hydrostatic weighing. Subjects performed an exercise test to near exhaustion on an electrically braked cycle ergometer to determine the power output at a lactate concentration of 4 mmol/l (W4). W4 is an index of aerobic fitness and exercise intensity (Jacobs et al. 1995, Jacobs, 1986) and was used in this study to determine the exercise intensity at which subjects in each group would perform. During this exercise test, subjects began cycling at 30 or 60 Watts and intensity was increased by 30 watts every 4 min. Blood was drawn from the ear lobe during the last 30 s of each 4 min interval. W4 was interpolated from a plot of lactate concentration against power output. Metabolic measurements were made throughout the test.

## 4.1 Experimental design

The subjects were divided into two groups, equally matched for the W4 scores. The low intensity (LI) group exercised at 30% W4 and the moderate intensity (MI) group exercised at 60% W4. On two subsequent visits the subjects exercised for 30 min following a 30 min supine rest, once at a room temperature of 9°C and once at 21°C, with identical procedures and measurements occurring for each exposure. The order of the exposures was counter-balanced among subjects with at least 7 days between trials.

## 4.2 Standardization of menstrual and diurnal cycles

There was no attempt to control for menstrual cycle phase. Mittelman et al. (personal communication, 1997) recently showed that there was no effect of menstrual cycle phase on body temperature regulation during cold stress. We therefore felt that risk of significant random experimental error introduced by a long interval between exposures was greater than the risk that the menstrual cycle phases would confound our data interpretation.

The subjects were tested at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each trial.

## 4.3 Exercise and Exposure

On the day of each exposure the subjects reported to the lab in a 12-h post absorptive state, clad in shorts, t-shirt socks and running shoes. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at either 9°C or 21°C. Their resting metabolic rate was recorded for 10 minutes beginning after 15 minutes of rest in a supine position using a semi-automated metabolic cart system. Rectal temperature, heat flow and skin temperatures were also measured during this time. The first biopsy was taken after these measurements were made, and after the muscle the subjects mounted the cycle ergometer and cycled for 30 min at an intensity of either 30% or 60% of W4 depending on the group to which they were assigned. Metabolic rate, heat flow and skin temperature were measured continuously during the 30 min of exercise. Heart rate and ratings of perceived exertion were recorded every 5 min.

#### 4.4 Muscle biopsies

Muscle samples were taken from the right *quadriceps femoris vastus lateralis* just before exposure (i.e. after the 30 minute rest period) and again from the same muscle within 45 seconds after completing exercise, employing the percutaneous needle biopsy technique (Bergström, 1962). Skin and the underlying fascia were anaesthetized with 3 mL of xylocaine (2% epinephrine) after cleansing with an antiseptic solution (Betadine surgical scrub, Purdue Frederick Inc.). Both pre- and post exposure samples were taken from the same incision. Incisions were closed using Steri-Strip® (3M, St. Paul, MN). An elasticized bandage was wrapped around the thigh in an attempt to exert some pressure on the biopsy site and hopefully reduce the soreness that is frequently experienced in the thigh for 2-3 days after the biopsy. This bandage was left on the leg during the exposure, removed for the post-exposure biopsy and then dry Steri-Strips® and a dry elasticized bandage were placed on the leg after the experiment. Subjects were instructed to leave the elasticized bandage on the leg for 3-4 hours; they were

instructed to leave the Steri-Strips<sup>®</sup> in place for 5 days. During the subsequent exposure, incisions were made on the same leg but at least 3 cm away from the previous incision.

No complications, such as subsequent infection, resulted from the biopsies. Subjects did, however, report varying intensities of muscle soreness in the thigh, sometimes lasting as long as 4-5 days after the biopsy. The intensities ranged from no soreness at all to some subjects who were in extreme discomfort for 24 hours after the experiment. No subject requested or required follow-up medical referral. One subject experienced some residual bleeding into her clothing because the Steri-Strips® did not adhere to the leg when she left the lab.

## 4.5 Blood sampling

The protocol called for venous blood samples to be obtained from an antecubital vein just before and after the 30 min rest and at 15 and 25 min during exercise. Difficulties in obtaining sufficient volume of blood were sometimes encountered during the cold exposure, probably due to the combination of vasoconstriction and decreased blood flow to the forearm. A heparin lock (10 U/mL) with the 20 gauge 1 inch catheter was used. A water-proof dressing (Tegaderm<sup>®</sup>) was placed over the site where the catheter pierced the skin to help stabilize the catheter. Ten mL blood samples were drawn and divided into 4 tubes which were kept on crushed ice: 5 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 5 mL were dispensed into a chilled, EDTA-treated tube (50  $\mu$ L were dispensed into tubes containing HCIO<sub>4</sub> for the subsequent determination of glucose and lactate; samples were taken to determine hematocrit and hemoglobin; the remainder was centrifuged and aliquots of the plasma was subsequently used for the determination of free fatty acids and glycerol). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

# 4.6 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge). Commercially available kits were used to measure concentrations of free fatty acids (WAKO<sup>™</sup> NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue<sup>™</sup>). After deproteinization samples were analyzed for glycerol (Boobis and Maughan, 1983) and lactate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

Muscle tissue samples were freeze dried for at least 8 hours. Glycogen was assayed as glucose units following hydrochloric acid hydrolysis using a fluorometric enzymatic method (Karlsson, 1971).

To facilitate calculations of protein oxidation during exposure, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the rest and exercise in the environmental chamber. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA).

#### 4.7 Temperature measurements

During 15 to 25 min of the rest period and continuously throughout the exercise, the following were measured with an automated data acquisition system and averaged each minute: rectal temperature (Pharmaseal® 400 Series, Baxter Healthcare Corporation, California), mean skin temperature and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For measurement of skin temperature and heat flow, the same twelve, calibrated heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F. Connecticut) were used throughout the entire experiment.

#### 4.8 **Respiratory gas exchange measurements**

Respiratory gases were monitored using a semi-automated metabolic cart system between 15 and 25 min of the 30 min rest period and continuously throughout the exercise. For this purpose the subject was connected to a mouthpiece, breathing valve, and hose, which directed the expired gases to a 5 litre mixing box, which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. Custom designed computer software (DCIEM/HPP Metabolic Measurement System V1.0, Keefe and Pope, 1997) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

#### **4.9** Calculation of metabolic heat production and substrate contributions

Metabolic heat production rates (M) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991).

The rates of carbohydrate and fat oxidation ( $CHO_{ox}$  and  $FAT_{ox}$ , respectively) were calculated using the non-protein oxygen consumption and the non-protein respiratory exchange ratio. Protein oxidation ( $PRO_{ox}$ ) was assessed using the urinary urea nitrogen excretion rates (Vallerand et al., 1993). Detailed descriptions of the calculations for substrate oxidation rates are available in Vallerand et al. (1995).

## 4.10 Statistical analyses

The reasons for the subject attrition are described in the Results. Comparisons between the LI and MI groups were made using a one-factor analysis of variance while intragroup comparisons were made using a repeated measures analysis of variance. Unless otherwise noted, data are presented as mean values  $\pm$  standard deviation. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 5. RESULTS

## 5.1 Subject attrition

Eighteen subjects signed consent forms and completed all familiarization and medical screening procedures. Of these, two subjects dropped out of the experiment due to scheduling conflicts.

## **5.2 Subject characteristics**

The physical characteristics of the subjects are presented in **Table 1**. Subjects had a mean age of 25 y and were of average height and weight. The mean relative body fat mass was normal (23%) and subjects were of average fitness (W4=121Watts). Subject characteristics were not significantly different between groups. Only three subjects took oral contraceptives, two in the LI group and one in the MI group. As stated earlier the phase of the menstrual cycle on the day of each immersion was not standardized, but it was recorded and this information is presented in **Table 2**.

## 5.3 Temperature measurements and heat flow

There was no significant difference in initial rectal temperature (Tre) taken upon arrival at the laboratory between groups for each exposure (**Table 2**), despite the fact that we did not control for menstrual cycle phase. **Table 3** shows the rectal temperature (Tre) responses for each subject during each exposure and **Figure 1** illustrates Tre during each exposure for each group for each minute of exercise. Ambient temperature during the rest period did not significantly affect Tre. Furthermore, during exercise, there was no effect of ambient temperature on Tre within each group at any time. The mean value for Tre in LI was higher at rest at 9°C than at 21°C but this was not statistically significant; one subject showed

an unusually high increase in Tre during the rest period at 9°C which was associated with an unusually high average metabolic rate.

To determine the effect of exercise intensity and ambient temperature on Tre, delta Tre was calculated as a change in mean Tre (averaged over each 10 min interval of exercise) from the Tre during the first minute of exercise. These values are illustrated in Figure 2. These data show that the increase in Tre during exercise was lowest in LI (0.123°C and 0.22°C during the 9°C and 21°C trials, respectively) and greatest in MI (0.31°C and 0.7°C during the 9°C and 21°C exposures, respectively. Delta Tre increased (p<0.03) between each time interval during each exposure except in LI at 9°C when there was no significant increase in Tre between 10 and 20 min. Delta Tre in LI was greater at 21°C(p<0.05) compared to 9°C at 20 and 30 min but not at 10 min. In MI, delta Tre was greater at 21°C than 9°C at all times. Table 4 shows mean heat storage for each group during each exposure. Heat storage was lower during rest at 9°C compared to 21°C (p<0.0001). There was a significant effect of both ambient temperature and exercise intensity on heat storage (p<0.02). Heat storage was lowest in LI at 9°C (28.9 $\pm$ 17.6 W·m<sup>-2</sup>) followed by LI at 21°C (58.1±15.6 W·m<sup>-2</sup>). The highest heat storage occurred in MI  $(78.9\pm28.4 \text{ and } 124.9\pm17.6 \text{ W}\cdot\text{m}^{-2} \text{ at } 9^{\circ}\text{C} \text{ and } 21^{\circ}\text{C}, \text{ respectively})$ . The mean heat flow for each group during each exposure is shown in Table 5. Heat flow was significantly lower during rest and exercise at 9°C compared to 21°C, however there was no effect of exercise intensity. Mean skin temperature for each group during each exposure is provided in **Table 6**. As expected, skin temperature was lower during rest at 9°C (28±.7 °C) than at 21°C (31±.7 °C). There was no effect of exercise intensity on skin temperature. When the groups were considered as one, skin temperature decreased with time at 9°C and increased with time at 21°C (p<0.0001).

## 5.4 Metabolic heat production and substrate contributions

The rate of metabolic heat production ( $\dot{M}$ ) for each subject during each exposure is provided in **Table 7**. Figure 3 illustrates the mean  $\dot{M}$  for each group

during each of the exposures. During the rest period,  $\dot{M}$  was significantly higher (p<0.005) at 9°C (94±25 J·s<sup>-1</sup> or 57±13 W·m<sup>-2</sup>) than at 21°C (77±15 J·s<sup>-1</sup> or 46±7 W·m<sup>-2</sup>). During exercise,  $\dot{M}$  was greater at 9°C compared to 21°C (p<0.02) in LI but not MI. LI elicited a mean  $\dot{M}$  of 308±55 J·s<sup>-1</sup> or 184±25 W·m<sup>-2</sup> at 21°C whereas at 9°C the same exercise intensity elicited a mean  $\dot{M}$  of 348±52 J·s<sup>-1</sup> or 209±30 W·m<sup>-2</sup>. There was no significant difference for the MI group between  $\dot{M}$  at 21°C (424±65 J·s<sup>-1</sup> or 261±37 W·m<sup>-2</sup>) and 9°C (432±54 J·s<sup>-1</sup> or 265±30 W·m<sup>-2</sup>).

Substrate oxidation for each subject during each exposure is provided in **Table 8**. Neither exercise intensity nor ambient temperature significantly affected the type of substrate used during each exposure. When substrates were grouped together, substrate oxidation rates were greater (p<0.005) at 9°C (31±25 J·s<sup>-1</sup>) than 21°C ( $26\pm16 \text{ J}\cdot\text{s}^{-1}$ ) during the rest period prior to exercise. During exercise, there was an effect of ambient temperature on substrate oxidation in LI but not MI (p<0.02). Substrate utilization was greater in LI at 9°C ( $116\pm92 \text{ J}\cdot\text{s}^{-1}$ ) than at 21°C ( $103\pm78 \text{ J}\cdot\text{s}^{-1}$ . **Figure 4** illustrates mean substrate oxidation as a percentage of  $\dot{M}$  in order to demonstrate the relative contributions of each of the substrates to fueling metabolism during each of the trials. There was no difference in the relative contributions of each of the substrates to fueling  $\dot{M}$  in either group during exercise at either 9°C or 21°C. Averaged over both trials, CHO<sub>0x</sub> contributed  $62\pm11\%$ , Fat<sub>ox</sub> contributed  $36\pm11\%$  and PRO<sub>ox</sub> contributed  $2.4\pm0.8\%$  to fueling  $\dot{M}$ .

## 5.5 Muscle glycogen concentrations

Muscle glycogen content before and after each of the trials is provided for each subject in **Table 9** and mean values are displayed in **Figure 5**. We were unable to obtain a post-exercise muscle sample in one subject in LI therefore her data were excluded from statistical analysis and data is presented as mean $\pm$ SE. There was no effect of ambient temperature on the magnitude of change in glycogen in either group. When the data from both the 9°C and the 21°C trials were pooled together, mean muscle glycogen content decreased by 26% in MI, from 390 $\pm$ 29 (mean $\pm$ SE) to 289 $\pm$ 21 mmol glucose kg dry muscle<sup>-1</sup> (p<0.03). The change in glycogen levels in LI

was not significant,  $434 \pm 18$  (mean $\pm$ SE) before exercise and  $405 \pm 19$  mmol glucose kg dry muscle<sup>-1</sup> after exercise.

# 5.6 Blood metabolites and hormones

Blood metabolite and hormone levels for each subject during each exposure are shown in **Tables 10 to 16**. As described previously, some difficulties were encountered in withdrawing blood samples in the cold air and therefore there are some missing data. Only those subjects for whom all samples were available were included in the statistical analysis. Hemoglobin (Hgb) increased significantly (p<0.01) during rest by 6% at 9°C and 3% at 21°C. During exercise, there was a significant effect of exercise intensity (p<0.01) but not ambient temperature. Hgb increased by 4% in LI and 7.7% in MI. Hematocrit (Hct) increased (p<0.01) during rest at 9°C (8.3%) but not 21°C. There was no effect of either ambient temperature or group on Hct during exercise however, when exposures and groups were averaged together, Hct increased (p<0.0001) during exercise by 10%. Lactate increased (p<0.05) during rest at 9°C (22%) but not 21°C. There was no effect of group on lactate concentrations. When data during exercise was pooled from the two groups, lactate increased (p<0.05) at both 21°C (104%) and 9°C (136%). At the end of exercise, lactate was greater (p<0.05) at 9°C compared to 21°C. There was no effect of either group or ambient temperature on NEFA or glycerol during rest or exercise. When groups and exposures were averaged together, NEFA increased (p<0.0001) by 25% during rest and by 48% during exercise. Glycerol increased (p<0.0001) by 38% during rest and 104% during exercise. There was no effect of ambient temperature on glucose, however, the MI group had pre-rest glucose levels that were significantly lower than the LI group and only the MI group showed a decrease during rest. When the data were pooled from both groups, there was a small, but significant decrease (p<0.02) in glucose.

These changes in blood metabolite and hormone concentrations should be considered in light of the hemoconcentration which is outlined for each subject during each exposure in **Table 10**. Changes in lactate, NEFA and glycerol were too large to be attributed only to hemoconcentration.

Catecholamine levels have not yet been analyzed but will be included in the Final Report.

## 6. DISCUSSION

This document is a progress report describing the results of the second in a series of projects designed to investigate whether gender differences in physiological responses to cold stress are of a sufficient magnitude to have implications for predictive models of human body temperature regulation. The objectives were: to quantify  $\dot{M}$ , lipid and carbohydrate oxidation rates, and muscle glycogen changes during light and moderate exercise in the cold; to compare the results to when the same exercise is performed at comfortable temperatures; to compare all results with those previously collected in males who underwent the same protocol.

Detailed analysis, interpretation of the results, and the implications for predictive modeling will be reserved for the Final Report. Some commentary follows below, however, regarding the testing of the specific experimental hypotheses for this particular project.

## 6.1 Hypothesis A:

"*M* will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature."

Shivering is the prime source of thermogenesis in humans during cold exposure. As such, it was expected that light exercise performed in the cold, at a metabolic rate which is typically lower than that observed during shivering, would not be sufficient to offset the requirement for additional shivering thermogenesis. As outlined in the results,  $\dot{M}$  was indeed greater during exercise at 9°C compared to 21°C in LI but not in MI. Compared to the 21°C exposure,  $\dot{M}$  was 13% higher at 9°C in LI and only 1.7% higher in MI. This would indicate that shivering was present when subjects performed only light intensity exercise at 9°C and that heat production during moderate intensity exercise was sufficient to preclude the need for shivering (Hong and Nadel, 1979). It is likely that shivering in LI was the result of significantly lower skin temperatures observed during exposures at 9°C rather than changes in core temperature.

The differences in the change in Tre during light intensity exercise between 9°C and 21°C can be explained by the differences in heat storage of about 30 W·m<sup>-2</sup>. These exposures resulted in differences in heat flow of about 50 W·m<sup>-2</sup> which were partially offset by the difference in  $\dot{M}$  of about 25 W·m<sup>-2</sup>. The difference in heat storage between the two exposures can be additionally attributed to respiratory heat loss. In MI the larger difference in the change in Tre between exposures can be attributed to a larger difference in heat storage of about 45 W·m<sup>-2</sup>. This was due to the inter-trial difference in the rate of heat loss that was not offset by a corresponding difference in  $\dot{M}$ . Thus, this hypothesis was accepted.

## 6.2 Hypothesis B:

"Contrasting with what has been reported for males, the increase in *M* seen during exercise in the cold will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature."

As discussed earlier, it has been demonstrated perviously that women have a greater tendency toward lipid metabolism than males during rest and exercise. In the current study, glycogen content decreased significantly in MI but not LI but there was no effect of temperature on the extent of the change in glycogen in either group. These results contrast with our earlier work with male subjects (Jacobs et al., 1985), where we observed a 23% decrease in muscle glycogen content in LI at 9°C but no change when the same exercise was performed at 21°C.

In the current study, during light intensity exercise, but not the higher intensity exercise, the higher  $\dot{M}$  indicates that more substrates were oxidized to fuel metabolism when the exercise was performed in the cold. Contrasting with

our earlier reports with male subjects, the current data do not suggest that there was a selective increase in carbohydrate oxidation to fuel  $\dot{M}$ . The results support acceptance of this hypothesis.

# 7. SUMMARY AND CONCLUSIONS

A. Data collection for the second phase of this project was completed in accordance with the experimental protocol. Vasoconstriction and lack of blood flow to the extremities resulted in some difficulty in obtaining venous blood samples during light exercise in the cold.

B. Shivering was induced during rest at 9°C and probably continued during light intensity exercise, which resulted in increases in metabolic heat production. The higher intensity exercise apparently resulted in sufficient heat production to eliminate shivering during exercise.

C. Females used muscle glycogen as one of the carbohydrate energy stores to fuel muscle contraction during exercise, as reported previously for male subjects. Contrasting with males, the increase in metabolic rate seen during low intensity exercise in the cold was not accompanied by an increase in muscle glycogen utilization. During light intensity exercise in the cold, both carbohydrate oxidation and fat oxidation increased to fuel  $\dot{M}$ . The relative contributions of carbohydrate, fat and protein oxidation to fueling  $\dot{M}$  were similar for both 21°C and 9°C trials, regardless of the intensity of exercise. These observations are consistent with a hypothesis that female skeletal muscle is less predisposed than males to selectively "prefer" carbohydrate oxidation to fuel increases in energy metabolic flux.

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Table 1. Physical characteristics of subjects.

Subject	Age	Height	Weight	Fat	BSA	Oral	W4	Exercise	Group
	( <u>v</u> )	(cm)	(kg)	(%)	( m²)	Contraceptives	(watts)	Intensity (W)	
				2					
BN	28	153.8	60.2	28.9	1.58	none	101	30	Ξ
ß	28	160.5	55.5	21.5	1.57	none	06	27	
EM	23	171.6	63.5	22.0	1.75	none	130	30	
کر ا	19	166.3	71.5	28.0	1.79	yes	120	36	
ML	20	157.2	50.5	23.1	1.48	yes	7 0	21	LI
ЪJ	35	168.0	62.4	31.1	1.70	none	94	28	-
с С	29	167.0	56.7	17.3	1.63	none	154	46	
8	22	170.0	69.7	23.6	1.80	none	159	48	LI
ß	34	170.0	73.1	30.1	1.84	none	112	67	īΨ
ð	19	175.6	63.8	14.3	1.77	none	177	106	μ
ც	23	169.0	62.5	20.7	1.71	none	159	91	MI
DL	34	163.4	60.5	24.9	1.65	none	95	57	Ϊ
Ш	23	159.0	54.0	15.0	1.54	none	88	53	M
Ŋ	20	138.0	55.6	34.3	1.41	none	144	86	MI
Z	20	156.9	52.7	15.0	1.51	none	129	77	Ξ
MM	20	157.0	62.0	25.3	1.62	yes	117	70	Ā
Mean LI	25.5	164.3	61.3	24.4	1.66		114.8	34.4	
±SD LI	5.4	6.4	7.1	4.6	0.12		31.6	9.5	-
mean MI	24.1	161.1	60.5	22.4	1.63		127.6	75.9	
±SD MI	6.3	11.5	9.9	7.5	0.14		30.9	17.9	
mean All	24.8	162.7	60.9	23.4	1.6		121.2	55.1	
±SD AII	5.7	9.2	6.6	6.1	0.1		30.9	25.5	

Page 28

Table 2. Day of menstrual cycle and intial rectal temperature before exposures

Subject	Group	Day of	f Cycle	Initial T	re (°C)
		21°C	9°C	21°C	0°C
BN	ΓI	15	9	37.05	37.10
EA	ΓI	19	22	37.30	37.06
BM	ΓI	4	18	36.90	36.80
٦V	ΓI	13	8	36.80	36.70
ML	ΓI	25	17	37.30	37.40
PJ	ΓI	9	13	37.20	missing
SP	ΓI	24	3	37.00	37.20
SS	ΓI	10	24	36.80	36.70
BS	IM	12	2	37.40	37.40
QM	IW	16	22	36.90	36.70
S	IW	24	12	37.50	37.10
DL	IW	14	28	37.07	37.30
Ш	IW	11	18	37.00	missing
IC	IW	13	19	37.00	36.90
Z	IW	15	22	36.60	36.70
MM	IW	28	4	36.80	37.10
			Mean, Ll	37.04	36.99
			± SD	0.21	0.27
			Mean, MI	37.03	37.03
			± SD	0.30	0.28

Page 29

Table 3. Mean core temperature before and during MI or LI exercise at ambient temperatures of 9°C or 21°C.

		0	30 min	37.01	37.27	36.79	37.68	37.14	37.27	37.83	36.85	38.04	36.80	36.93	37.11	37.02	37.27	36.83	37.38	37.23	0.37	37.17	0 40
	°C	Exercise	20 min	36.96	37.20	36.70	37.54	37.09	37.23	37.56	36.75	37.76	36.56	36.70	36.86	36.95	36.96	36.58	37.21	37.13	0.32	36.95	0.39
°C)	9 <sup>6</sup>		10 min	36.96	37.15	36.59	37.41	37.13	37.07	37.36	36.79	37.52	36.50	36.48	36.62	37.05	36.80	36.53	37.15	37.06	0.27	36.83	0.38
mperature (		Rest		37.21	37.13	37.06	37.19	37.41	36.92	37.24	37.09	37.45	36.64	36.79	36.89	37.12	36.71	36.85	37.10	37.16	0.14	36.94	0.26
an Core Te			30 min	37.33	36.72	36.14	37.52	37.41	37.31	37.58	36.92	37.76	37.30	37.60	37.23	37.22	37.97	37.28	37.40	37.12	0.49	37.47	0.28
Me	°C	Exercise	20 min	37.20	36.59	36.19	37.35	37.30		37.36	36.89	37.54	36.93	37.40	36.97	36.96	37.59	36.78	37.12	36.98	0.45	37.16	0.31
	21		10 min	37.12	36.35	36.40	37.12	37.25		37.15	36.87	37.15	36.70	37.28	36.64	36.80	37.05	36.40	36.72	36.89	0.37	36.84	0.29
		Rest	·	37.05	36.49	36.87	36.82	37.24	37.20	37.12	36.87	37.08	36.77	37.32	37.02	36.78	37.12	36.51	36.78	36.95	0.25	36.92	0.26
		Group										IM	M	M	M	M	IM	I	M				
		Subject		EA	Na	M	<u>ک</u> ر	ML	ЪJ	Ъ	SS	BS	S	8	Ч	Ξ	പ	Z	¥	Mean, LI	SD, LI	Mean, MI	

Table 4. Mean heat storage during moderate and low intensity exercise at 21 and  $9^{\circ}$ C (mean ± SD).

Page 31

		Mean heat s	storage (W·m <sup>-2</sup>	
	21	°C	6	)°C
Group	Rest	Exercise	Rest	Exercise
IM	-40.4 ± 6.7	124.4 ± 29.1	-87.6 ± 11.8	78.9 ± 28.5 *
	$-38.3 \pm 15.5$	$58.1 \pm 15.6$	$-82.6 \pm 13.3$	28.9 ± 17.6* +
Total	-39.3 ± 12	91.3 ± 41	-85.1 ± 12*	$53.9 \pm 35^{*}$

- Significantly different from 21°C + significantly different from MI

Table 5. Mean heat flow during moderate and low intensity exercise at 21 and  $9^{\circ}C$  (mean ± SD).

			~		.14	
		cise	± 8.8	± 8.	+ +	
		Exer	148.6	150.7	49.3	
	0°C		`	,		
<u>V·m<sup>-2</sup>)</u>		st	- 7.3	4.8	+6*	
√ ∕		Res	6.9	9.8 ∃	28.4	
t flo			12	12	12	
hea		se	9.5	10.7	- 10	
lean		xerci	2.0 ±	+ 9	0.3 ±	
2	ç	Ê	102	98.	10	
	21		9.3	3.8	6	
		Rest	5 <del>T</del> 0	1 ± 8	I.5 ±	
		-	71.	72.	7	
		dno	IV		tal	
		Grc	2		То	

Significantly different from 21°C

Table 6. Mean skin temperature during moderate and low intensity exercise at 21 and  $9^{\circ}C$  (mean  $\pm$  SD).

			Me	an skin ten	nperature ('	°C)		
		21	°C			<b>6</b>	с С	
Group	Rest		Exercise		Rest		Exercise	
		10 min	20 min	30 min		10 min	20 min	30 min
Z	31.0 ± .7	29.7 ± .5	29.9 ± .6	30.4 ± .8	27.5 ± .7	25.6 ± .9	24.9 ± .9	24.9 ± .9
	31.1 ± .7	29.9 ± .5	30.0 ± .6	30.3 ± .7	27.9 ± .7	25.3 ± .7	24.9 ± .8	<b>24.9 ± .8</b>
Total	31.1 ± .7	$29.8 \pm .5$	29.9 ± .6	$30.3 \pm .7$	27.7 ± .7 *	25.5 ± .8	<b>24.9 ± .8</b>	<b>24.9 ± .8</b>

\* different from  $21^{\circ}C$  (p< 0.001)

exercise in LI and MI at 9°C and 21°C. and Metabolic rate during rest Tahle 7

		200	M (W	·m <sup>-2</sup> )	
Subject	Group	Re	st	Exercise (	0-30 min)
	<b>.</b>	21°C	0°C	21°C	9°C
Na	L I	43.34	46.12	159.83	176.85
EA		48.33	47.23	171.38	211.28
EM		43.69	52.08	175.52	212.87
٦V		69.53	89.48	211.31	221.56
ML		46.03	48.03	160.67	201.28
ΡJ		41.03	74.42	170.49	189.24
SP		38.52	67.30	228.64	267.52
SS		50.12	64.54	197.83	194.00
BS	IW	43.14	54.15	212.78	234.30
Ø	Σ	40.92	48.12	310.63	307.57
g	Ξ	51.85	43.40	275.85	267.31
DL	Σ	42.96	53.70	213.66	225.98
Ш	Σ	49.92	56.30	235.42	244.90
ГC	Σ	43.43	73.05	309.73	298.49
Z	Z	47.01	47.12	286.63	287.34
M	M	43.49	43.95	245.38	257.03
	Mean, Ll	47.57	61.15	184.46	209.32*
	±SD, LI	9.63	15.59	25.27	27.54
	Mean, MI	45.34	52.47	261.26	265.36
	±SD, MI	3.84	9.60	39.96	30.17
	Total, Mean	46.46	93.87*	222.86	237.34
	± SD	7.18	13.29	51.15	40.20
* Significant	difference	from 21°C		·	

Muscle glycogen concentrations (mmol glucose•kg dry muscle<sup>-1</sup>) Table 9.

318.76 399.56 405.00 315.18 423.91 376.13 193.79 441.05 292.93 102.55 362.05 118.45 546.90 396.47 502.22 166.72 362.21 165.41 340.08 518.47 82.57 post 0°C 396.85 116.85 374.93 471.15 488.64 499.76 602.37 352.20 587.63 450.88 207.36 463.94 280.42 469.53 388.30 426.21 104.28 367.11 415.47 73.07 434.41 pre 279.80 316.16 372.56 398.76 398.00 227.38 416.94 319.88 368.70 324.04 383.88 363.70 312.00 253.43 258.64 284.97 66.10 211.71 29.52 347.81 65.21 post 21°C in MI and LI at 21°C and 9 °C. 335.98 511.29 399.02 391.39 123.30 394.95 445.45 389.14 313.66 396.00 390.64 448.54 409.74 380.85 636.42 328.94 329.82 249.91 357.91 45.07 92.13 pre Group MEAN, TOTAL Subject MEAN, MI\* SD, TOTAL ₹ 88  $\geq$ MEAN, LI SD, LI SD, MI

\* Significant pre-post difference in MI group only. No effect of temperature.

Page 34

Table 10. Changes in plasma volume during each exposure.

8.08 -23.24 N/A 3.10 2.77 -15.88 -17.33 -14.49 -19.02 -21.54 -17.68 -18.08 -20.45 -14.55-14.89-19.442.61 -19.27 -20.61 -18.41 -17.71 -14.71 exercise Шd γ----1 -14.95 -17.15 -22.72 -21.94 3.80 2.68 3.57 N/A -20.45 -14.89 -20.55 -16.44 -19.65 -11.70 -18.20 20.22 -22.32 -18.04-13.51 -19.27 -14.71 exercise 0°0 Mid Changes in blood plasma volume (%) -8.19 -2.36 -2.33 -6.66 -11.67 2.68 4.14 N/A N/A 5.17 -9.09 -11.70 -15.45-10.43 -11.63 -14.29 -10.28-10.29 -10.28 -12.94 -13.10 -14.14 exercise Pre -13.73 -9.85 4.86 4.86 5.76 -26.52 -17.68 0.69 -9.61 N/A -16.34-10.20 -11.36 -12.90 -11.87-19.89 -12.21 -13.89 -13.97 -13.51 -13.04-13.31exercise End -15.76 -12.70 -12.77 -25.54 -14.71 4.97 -10.13 -2.41 -11.59 -16.58-16.56 -15.08 -11.29 4.27 -16.524.37 -13.90-10.29 -10.43-12.52 -16.00 -19.37exercise 21°C Mid -4.52 3.85 -8.13 -0.75 -5.51 -7.43 -8.26 3.32 4.48 -4.57 -5.30 -5.77 -5.95 3.15 5.13 8.95 5.02 5.13 3.97 -4.01 3.87 6.11 exercise Рге ₹ M M Ξ SD, MI Mean, MI Mean, total ≣ Ξ ₹ Ξ SD, LI Mean, Ll \_\_\_\_ 1 SD, TOTAI Group ΜŊ 8 BS ≧ Ш Ц Subject

Page 35

Table 11. Changes in hemaglobin during each exposure.

					Hemaglob	in (g·dL <sup>-1</sup> )			
Subject	Group		21	°C			°6	C	
		Pre rest	Pre	Mid	End	Pre rest	Pre	Mid	End
			exercise	exercise	exercise		exercise	exercise	exercise
M	L1	12.6	13	14	13.6	13.1	•	14.2	14.6
<u>ک</u> ر		12.5	13	13.3	13.2	13.6	14.1	14.8	14.9
SS		13.6	14.2	14.4	14.6	13.1	13.6	14.2	14.4
EA		12.8	13.4	14.3	•	12.4	•	•	•
R		13.2	13.6	14	13.8	13.2	14.2	14	14.7
SP		13.5	14	14.1	14.5	12.4	13.6	14.4	14.4
ЪJ	_	12.4	12.6	13.5	13.4	12.6	13.2	13.6	13.8
ML	E	12.4	13	13.8	13.7	12.6	13.6	14.2	14.1
с ГС	M	13.4	13.9	15.3	15.4	12.6	14	14.4	14.6
ß	M	12.6	13.2	15.1	15.3	12.4	13.4	14.2	14.2
W	M	13.1	13.8	14.1	14.2	13.1	13.5	14.4	14.2
LZ	Z	13.2	13.3	14.4	14.6	13.4	14.2	•	14.8
Ξ	Z	11.7	12.2	13.2	13	11.4	12.7	13.4	13.5
<u>୪</u>	M	13.2	13.8	14.8	15	14.1	14.2	15.6	15.4
BS	M	13.1	12.6	14.4	14.2	12.8	12.9	14.1	14.1
Ы	M	12.5	13.2	13.8	13.7	12.6	14	14.6	14.4
	Mean, Ll	12.88	13.35	13.93	13.83	12.88	13.72	14.20	14.41
	SD, LI	0.49	0.55	0.38	0.53	0.44	0.37	0.37	0.37
	Mean, MI	12.85	13.25	14.39	14.43	12.80	13.61	14.39	14.40
	SD, MI	0.56	0.60	0.69	0.82	0.79	0.58	0.66	0.56
	Mean, Total	12.86	13.30	14.16	14.15	12.84	13.66	14.29	14.41
	SD, TOTAL	0.51	0.56	0.59	0.74	0.62	0.49	0.52	0.46

Table 12. Changes in hematocrit during each exposure.

					Hema	utocrit			
Subject	Group		21	°C			<sub>о</sub> б	°C	
		Pre rest	Pre	Mid	End	Pre rest	Pre	Mid	End
			exercise	exercise	exercise		exercise	exercise	exercise
Ma	ΓI	43	39	41	40	37	41	41	42
EA		35	36	39	•	34	•	•	•
M		35	36	37	37	36	•	40	40
<u>ک</u> ر		34	35	37	37	38 38	40	41	41
ML	_	36	37	40	6 8	36	3 G	41	42
ЪJ		37	38	40	40	37	40	42	41
SP		38	39	42	42	36	39	40	40
SS		38	39	41	41	36	39	41	40
BS	Þ	36	36	40	40	36	37	40	40
ð	Σ	35	38	42	42	34	37	41	40
8	Ā	38	40	42	42	40	41	45	44
Ы	Σ	36	38	40	49	37	40	42	42
Ξ	ž	32	33	36	3 5 3	31	34	37	35
C	Σ	37	38	42	42	34	38	40	40
Z	Z	37	37	40	40	37	41	•	43
M	Σ	38	40	41	41	35	40	43	42
	MEAN, LI	37.00	37.38	39.63	39.43	36.25	39.67	40.86	40.86
	SD, LI	2.83	1.60	1.85	1.90	1.16	0.82	0.69	0.90
	MEAN, MI	36.13	37.50	40.38	41.38	35.50	38.50	41.14	40.75
	SD, MI	1.96	2.27	2.00	3.85	2.67	2.45	2.54	2.76
	Mean, total	36.56	37.44	40.00	40.47	35.88	39.00	41.00	40.80
	SD, TOTAL	2.39	1.90	1.90	3.16	2.03	1.96	1.80	2.04

exposure.	
during each	
blood lactate	-
Changes in	
Table 13.	

					Lactate	(mmol·l <sup>-1</sup> )			
Subject	Group		21	°C			°6	ç	
		Pre rest	Pre	Mid	End	Pre rest	Pre	Mid	End
			exercise	exercise	exercise		exercise	exercise	exercise
R	-	0.76	0.69	1.29	0.99	0.75	1.34	1.63	1.66
EA		0.55	0.59	1.85	•	0.69	•	•	•
M	-	0.18	0.37	0.89	0.82	0.28	•	1.33	1.16
<u>ک</u> ر		1.31	1.12	1.17	0.96	0.71	0.64	1.26	0.92
ML	Ĺ	0.88	0.85	1.82	1.65	0.72	0.73	2.77	2.9
٢J		0.62	0.57	1.12	0.95	0.53	0.64	1.48	1.17
SP		0.25	0.45	1.32	1.11	0.42	0.38	2.06	1.48
SS	_	0.57	0.61	0.94	0.92	0.28	0.56	1.36	1.29
BS	M	0.49	0.55	1.88	1.49	0.54	0.54	1.84	1.71
ð	Ē	0.39	0.42	1.47	1.37	0.5	0.82	1.6	1.48
წ	ž	0.62	0.58	1.2	0.94	1.1	1.06	2.4	2.29
Ъ	Z	0.56	0.57	2.11	2.21	0.52	0.57	2.78	2.21
Ш	Ē	0.52	0.41	2.38	1.36	0.36	0.39	2.14	1.97
C	Z	1.02	0.93	1.88	1.73	0.72	1.06	1.39	1.42
Z	ΪΣ	0.43	0.36	1.97	1.62	0.35	0.47	•	1.88
W	IW	0.83	0.77	1.77	1.63	0.71	0.84	2.13	2.1
	MEAN, LI	0.64	0.66	1.30	1.06	0.55	0.72	1.70	1.51
	SD, LI	0.36	0.24	0.36	0.28	0.20	0.33	0.54	0.66
	MEAN, MI	0.61	0.57	1.83	1.54	0.60	0.72	2.04	1.88
	SD, MI	0.21	0.19	0.37	0.36	0.24	0.26	0.47	0.32
	Mean, total	0.62	0.62	1.57	1.32	0.57	0.72	1.87	1.71
	SD, TOTAL	0.29	0.21	0.45	0.40	0.22	0.28	0.52	0.52

Table 14. Changes in blood glucose levels during each exposure.

exercise 76.85 67.23 71.72 91.56 64.35 67.34 89.98 76.74 62.95 72.96 79.52 72.79 10.47 62.60 51.47 73.60 63.67 10.43 8.76 74.38 71.91 Шd exercise 71.35 91.56 68.93 72.79 72.55 75.96 65.06 74.09 73.25 78.59 77.49 86.32 84.92 76.49 67.87 75.17 65.76 75.21 8.40 7.54 4.22 Mid 0°0 exercise 91.56 51.12 70.64 53.28 61.58 68.72 72.84 62.20 67.17 72.09 63.12 67.17 75.17 72.20 68.55 62.77 63.41 66.11 9.15 6.90 9.48 Pre rest 70.46 71.86 71.86 59.26 68.26 68.38 91.56 64.35 71.50 72.09 70.34 70.29 77.00 66.64 62.97 74.47 (mg·dl<sup>-1</sup>) 61.01 72.31 9.19 5.18 7.49 71.91 Pre exercise Glucose 91.56 77.54 80.39 63.48 78.29 75.52 82.86 11.93 71.65 76.88 10.63 69.98 76.66 65.23 80.72 105.31 65.51 77.01 71.51 6.08 74.51 Б П exercise 72.09 67.33 79.75 74.08 76.73 91.56 72.15 77.95 91.08 77.72 77.16 77.72 79.00 67.34 67.34 83.01 73.77 75.71 7.99 7.52 6.11 Mid 21°C exercise 71.50 71.74 71.66 91.56 66.99 73.32 78.83 69.88 68.22 68.40 70.46 64.19 74.64 67.08 59.31 68.37 70.61 64.71 7.32 4.07 Pre 8.37 rest 77.08 76.03 91.56 64.88 65.76 68.43 71.74 66.32 75.52 69.22 72.84 77.19 80.57 72.03 67.17 68.02 70.81 76.45 72.27 7.72 3.28 6.84 Ъ Mean, total SD, TOTAL MEAN, MI MEAN, LI Group SD, MI SD, LI Ξ Ξ Ξ Ξ Ξ Ξ Ξ Ξ Ξ  $\Box$ Subject SS PJ K  $\mathbb{S} \mathbb{S} \mathbb{S} \mathbb{S} \mathbb{Z} \cong \mathbb{C} \mathbb{S} \mathbb{S} \mathbb{S}$ 2

Page 39

Table 15. Changes in non-esterified free fattty acid concentration during each

xnosure

exposur	e.								
					NEFA	(mmol·l <sup>-1</sup> )			
Subject	Group			1°C			°6	C	
		Pre	Pre	Mid	End	Pre rest	Pre	Mid	End
		rest	exercise	exercise	exercise		exercise	exercise	exercise
M	L	0.45	0.42	0.76	0.68	0.47	0.62	1.25	1.14
ß		0.34	0.43	0.67	•	0.53	•	•	•
Ð		0.16	0.17	0.27	0.35	0.36	•	0.30	0.40
<u>ک</u> ر		0.08	0.39	0.61	0.67	0.17	0.23	0.49	0.58
R		0.29	0.40	0.56	0.54	0.64	0.85	0.89	0.84
Ъ		0.58	0.65	1.20	1.15	0.74	0.95	1.06	1.22
ფ	-	0.38	0.37	0.39	0.41	0.30	0.52	0.57	0.45
SS		0.27	0.28	0.55	0.51	0.28	0.38	0.50	0.49
BS	MI	0.38	0.50	0.59	0.78	0.44	0.51	0.66	0.62
S	Ξ	0.45	0.63	0.72	0.69	0.24	0.39	0.40	0.43
8	E	0.53	0.75	1.24	1.09	0.33	0.41	0.55	0.63
Ц	Σ	0.49	0.54	0.71	0.78	0.49	0.52	0.52	0.63
Ш	I	0.33	0.52	0.59	0.57	0.57	0.67	1.16	1.54
Ŋ	Σ	0.14	0.27	0.50	0.57	0.16	0.41	0.98	0.94
Ы	Σ	0.37	0.71	1.02	1.00	0.56	0.76	•	1.55
M	MI	0.38	0.45	0.58	0.58	0.40	0.45	0.76	0.86
	MEAN, LI	0.32	0.39	0.63	0.62	0.44	0.59	0.72	0.73
	SD, LI	0.16	0.14	0.28	0.27	0.19	0.27	0.35	0.34
	MEAN, MI	0.38	0.55	0.74	0.76	0.40	0.52	0.72	06.0
	SD, MI	0.12	0.15	0.26	0.20	0.15	0.13	0.27	0.43
:	Mean, Total	0.35	0.47	0.68	0.69	0.42	0.55	0.72	0.82
	SD, TOTAL	0.14	0.16	0.27	0.24	0.17	0.20	0.30	0.38

Page 40

Changes in plasma glycerol during each exposure. Table 16.

exercise 0.16 0.12 0.27 0.19 0.21 0.08 0.22 0.07 0.24 0.20 0.28 0.15 0.23 0.22 0.33 0.30 0.35 0.12 0.15 0.24 0.07 End exercise 0.17 0.25 0.16 0.13 0.19 0.10 0.25 0.14 0.06 0.21 0.09 0.27 0.26 0.43 0.10 0.20 0.23 0.20 0.21 0.11 Mid • ဝ°ဝ exercise 0.16 0.10 0.06 0.12 0.12 0.12 0.03 0.10 0.09 0.12 0.03 0.03 0.15 0.10 0.14 0.17 0.11 0.07 0.11 0.11 Pre Plasma Glycerol (mmol·l<sup>-1</sup>) rest 0.06 0.10 0.06 0.05 0.06 0.09 0.06 0.10 0.09 0.08 0.02 0.02 0.08 0.02 0.06 0.10 0.10 0.07 0.09 0.07 0.11 Pre exercise 0.25 0.14 0.06 0.13 0.19 0.23 0.30 0.20 0.19 0.29 0.12 0.16 0.06 0.19 0.06 0.10 0.25 0.19 0.12 0.11 0.21 0.17 End exercise 0.16 0.15 0.06 0.16 0.25 0.13 0.12 0.14 0.16 0.32 0.19 0.25 0.12 0.16 0.06 0.19 0.18 0.09 0.23 0.13 0.07 0.20 Mid 21°C exercise 0.06 0.07 0.08 0.13 0.09 0.05 0.09 0.09 0.04 Pre 0.08 0.06 0.22 0.06 0.08 0.07 0.08 0.12 0.09 0.13 0.03 0.07 rest 0.05 0.06 0.08 0.07 0.07 0.07 0.05 0.06 0.12 0.02 0.06 0.05 0.12 0.05 0.08 0.03 0.02 0.07 0.06 0.07 0.07 Рге MEAN, TOTAL SD, TOTAL MEAN, MI MEAN, L Group SD, MI SD, LI ≣ Ξ ≣ Ξ Ξ Ξ Ξ Ī Ξ Subject  $\mathbb{R} = \mathbb{R} \xrightarrow{} \mathbb{R$ 















Figure 4. Relative contribution of substrate oxidation to M.

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Figure 5. Muscle glycogen content during moderate and low intensity exercise at 9°C and 21°C.

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