This investigation studied the importance of muscle glycogen levels for body temperature regulation during cold stress. Physiological responses of eight euglycemic males were measured while they rested in cold (18°C, stirred) water on two separate occasions. The trials followed a three-day program of diet and exercise manipulation designed to produce either high (EMG) or low (LMG) pre-immersion glycogen levels in the muscles of the legs, arms and upper torso. Pre-immersion vastus lateralis muscle glycogen concentrations were lower during the LMG trial (144±14 m mol·kg dry weight) than the HMG trial (543±53 m mol·kg dry weight). There were no significant differences between the two trials in shivering as reflected by aerobic metabolic rate or in the amount of body cooling as reflected by changes in rectal temperature during the immersions. Post-immersion muscle glycogen levels remained unchanged from pre-immersion levels in both trials. Small but significant increases in plasma glucose and lactate concentration occurred during both immersions. Plasma glycerol increased during immersion in the LMG trial but not in the HMG trial. Plasma free fatty acid...
Concentration increased during both immersion trials, but the change was apparent sooner in the LMG immersion. It was concluded that human thermoregulatory response to cold stress is not impaired by a substantial reduction in the muscle glycogen levels of several major skeletal muscle groups. Furthermore, the data suggest that the availability of other metabolic substrates enables muscle glycogen levels to be spared during shivering in otherwise resting individuals.
THERMOREGULATION DURING COLD WATER IMMERSION IS UNIMPAIRED BY MUSCLE GLYCOGEN DEPLETION

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Running Head: Shivering Metabolism in Humans

Key Words: shivering, thermogenesis, body temperature regulation

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ABSTRACT
This investigation studied the importance of muscle glycogen levels for body temperature regulation during cold stress. Physiological responses of eight euglycemic males were measured while they rested in cold (18°C, stirred) water on two separate occasions. The trials followed a three day program of diet and exercise manipulation designed to produce either high (HMG) or low (LMG) pre-immersion glycogen levels in the muscles of the legs, arms and upper torso. Pre-immersion vastus lateralis muscle glycogen concentrations were lower during the LMG trial (144±14 m mol·kg dry weight) than the HMG trial (543±53 m mol·kg dry weight). There were no significant differences between the two trials in shivering as reflected by aerobic metabolic rate or in the amount of body cooling as reflected by changes in rectal temperature during the immersions. Post-immersion muscle glycogen levels remained unchanged from pre-immersion levels in both trials. Small but significant increases in plasma glucose and lactate concentration occurred during both immersions. Plasma glycerol increased during immersion in the LMG trial but not in the HMG trial. Plasma free fatty acid concentration increased during both immersion trials, but the change was apparent sooner in the LMG immersion. It was concluded that human thermoregulatory response to cold stress is not impaired by a substantial reduction in the muscle glycogen levels of several major skeletal muscle groups. Furthermore, the data suggest that the availability of other metabolic substrates enables muscle glycogen levels to be spared during shivering in otherwise resting individuals.
INTRODUCTION

Humans have two primary physiological mechanisms available to maintain normal body temperature during cold exposure. Cutaneous vasoconstriction increases tissue insulation and limits convective and conductive heat transfer from the body to the environment. Except for very mild cold-stress conditions, however, cutaneous vasoconstriction alone is insufficient to prevent a loss of body heat stores, and an increase in metabolic heat production is necessary to prevent body temperature from decreasing. In the absence of a voluntary increase in muscular activity, metabolic heat production will be increased during cold exposure by involuntary shivering. Like all types of muscular activity, shivering thermogenesis is dependent upon an adequate supply of metabolic energy substrates. Although the principal substrate has not been clearly identified, both blood glucose and muscle glycogen have been reported to be metabolized during shivering thermogenesis.

There is ample evidence that plasma glucose plays some role in shivering thermogenesis. Gale et al. (8) found that when insulin was infused into subjects exposed to cold air, shivering stopped as plasma glucose concentration fell below 2.5 mmol·l⁻¹; with the cessation of shivering, metabolic rate and core temperature decreased. Intravenous glucose administration rapidly restored shivering, but, even more interestingly, shivering was restored in both an arterially occluded leg as well as a nonoccluded leg, suggesting that the glucose effect on shivering was via the central nervous system as opposed to a peripheral mechanism (8). Haight and Keatinge (10) exposed subjects to cold air after a two-hour bout of heavy exercise followed by ethanol ingestion. This manipulation was employed to produce hypoglycemia, and resulted in less
visible shivering, lower metabolic rates and greater body temperature declines as compared to responses observed when glucose ingestion followed the ethanol and prevented hypoglycemia.

The importance of muscle glycogen as a substrate for shivering thermogenesis is not clear. Measurements of muscle glycogen utilization in shivering humans have not been reported. During the experiments discussed above by Haight and Keatinge (10), the exercise bouts undoubtedly produced, in addition to hypoglycemia, some degree of glycogen depletion in several large muscle groups. Thus, glycogen depletion may have, at least partially, accounted for the effects attributed to hypoglycemia. Animal experiments suggest that during shivering there is a decrease in plasma glucose oxidation, and a concomitant increase in muscle glycogenolysis as compared to rest (14). Other animal experiments suggest that the improvement in cold tolerance produced by cold adaptation may be related to a concomitant increase in liver and skeletal muscle glycogen levels (5). In addition, muscle glycogen utilization in humans performing low-intensity exercise was observed to be greater in a cold as compared to temperate environment, and the additional glycogen utilization was attributed to the energy cost of shivering (11).

Thus, the relative importance of these different metabolic substrates for satisfying the energy requirements of shivering thermogenesis remain unclear. Despite this lack of experimental evidence, one widely cited mathematical model of human thermoregulation bases its prediction of tolerance time in cold water on the rate of glycogen depletion in the skeletal muscle (18). The purpose of the present investigation was to study the importance of muscle glycogen for body temperature regulation during acute cold stress. Specifically,
the experimental aim was to measure thermoregulatory and metabolic responses during cold water immersion in resting euglycemic humans with high versus low muscle glycogen levels. It was hypothesized that the shivering and metabolic heat production would be impaired and body cooling would be greater when subjects were immersed in cold water in a glycogen depleted state. In addition, the experimental design offered an opportunity to determine whether or not a prolonged period of shivering during cold water immersion would result in a significant depletion of muscle glycogen stores in otherwise resting individuals.

METHODS

Subjects and experimental design. Eight male volunteers served as test subjects in this investigation after being completely informed as to the risks and requirements of participation. Prior to experimental testing, each subject's maximal oxygen uptake ($\dot{V}O_2$ max) was determined and anthropometric measures were assessed.

Each subject completed two experimental cold water immersion tests. The immersions were separated by at least fourteen days in order to control for possible effects of repeated cold water immersion (21). One cold water immersion was completed with the subjects having high muscle glycogen (HMG) levels and the other with low muscle glycogen levels (LMG). The subjects completed the trials in a randomized order, with three subjects undergoing the HMG trial prior to the LMG trial. The cold water immersions were performed on the same day of the week, and at the same (within one hour) time of day. A fasting period of at least ten hours
preceded the immersions, during which the subjects were permitted to ingest only water.

The cold water immersion tests were performed in a 36,000 liter pool. The water was continuously circulated and the temperature was maintained at 18 °C for all immersions. The subjects wore swimming trunks for the immersion tests. Approximately one hour before the scheduled immersion time, subjects were weighed, instrumented and had a catheter placed in the antecubital vein of one arm. A muscle sample was obtained from the vastus lateralis approximately 30-min before immersion, after which the subjects moved to a nylon-mesh lounge chair where they reclined in a semisupine position while wrapped in blankets. After 20-min of quiet rest in this position, a blood sample was obtained, and baseline measurements of rectal temperature ($T_{re}$) and respiratory exchange parameters were completed. The platform supporting the chair was then lowered into the pool, and the water level was adjusted to the base of the subjects' neck. The subjects rested quietly throughout the immersion, which was terminated after 180-min or when $T_{re}$ fell below 35.5 °C. During immersion, $T_{re}$ and skin heat flow were continuously measured, and respiratory exchange parameters were measured at five min intervals. The electrocardiogram was continuously monitored and heart rate was recorded at 10-min intervals. Blood samples were obtained at the tenth and sixtieth min of immersion. Immediately before removing the subjects from the water a final blood sample was obtained. Upon egressing from the water, the subjects were rapidly dried and wrapped in blankets. A final muscle sample was obtained from the vastus lateralis muscle within five min after completion of the immersion.
During the three days preceding the cold water immersion tests, the subjects resided in a dormitory where their diet and physical activity were strictly controlled, in order to achieve the desired muscle levels. Prior to the LMG trial, a low-carbohydrate, high-fat diet was consumed. In addition, the subjects completed an exercise regimen each day which consisted of three 60-min bouts of exercise at intensities sufficient to elicit 75% of their maximum heart rate. The three exercise bouts each employed a different mode of exercise (treadmill running, cycle ergometry, rowing ergometry), so as to deplete glycogen from several different major muscle groups of the arms, legs and upper torso. During the three days preceding the HMG trial, the subjects consumed a high-carbohydrate, low-fat diet, and they were not permitted to exercise or engage in any strenuous activity. The menus for the two diets were designed by a dietitian to provide approximately equivalent total caloric intake, and were adjusted according to individual preference. All meals were prepared and served in measured portions under the supervision of the dietician, and any food offered but not consumed was also measured, so that the individuals' actual intake could be determined. The subjects were instructed to consume only food prepared by the dietary staff during the three days preceding the cold water immersion tests. The diets and the procedures used to partition and quantify the subjects' food consumption have been described in detail elsewhere (3).

Experimental procedures. A continuous, progressive intensity treadmill test was used to determine $\dot{V}O_2\text{max}$ and the maximal heart rate (17). Measurements of $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}E$) were made at 15-sec intervals throughout this test using an automated
system (Sensormedics Horizon MMC). Heart rates were determined from the electrocardiogram, which was monitored from chest electrodes (CM-5 placement) connected to an oscilloscope-cardiotachometer (Hewlett-Packard). Body density, determined by hydrostatic weighing, was corrected for residual lung volume and used to calculate percent body fat (6). Measurements of skinfold thickness at 13 sites distributed on the arm, leg and torso were averaged to obtain mean skinfold thickness. Mean subcutaneous fat thickness was calculated by subtracting the thickness of two layers of skin (4 mm) from the mean skinfold thickness, and then dividing by two (1).

During cold water immersion, $\dot{V}_O_2$, $\dot{V}_CO_2$ and $\dot{V}_E$ were measured from the sixth through the tenth min of each consecutive ten min period using the same automated system used in the maximal aerobic power test. Heart rate was determined from electrocardiograms radiotelemetered to an oscilloscope-cardiotachometer. Skin heat flows ($h_c$), water temperature ($T_w$) and $T_{re}$ were continuously measured and recorded throughout the experiment using a computer-operated system. A thermister placed approximately 50 cm from the subject was used to monitor water temperature. A thermister inserted 10 cm beyond the anal sphincter was used to measure $T_{re}$. Measurements of $h_c$ were obtained at five sites (upper chest, upper arm, left thigh, calf and top of the foot) using heat-flow disks (RdF, Hudson, NH). The heat-flow disks were secured to the skin with one layer of double-backed adhesive tape. The disks were factory calibrated with a reported accuracy of 6-7% of actual heat flow. Mean weighted heat flow ($\bar{h}_c$) was calculated according to the formula $\bar{h}_c = 0.47 h_c(\text{chest}) + 0.14 h_c(\text{arm}) + 0.19 h_c(l \ \text{thigh}) + 0.13 h_c(\text{calf}) + 0.07 h_c(\text{foot})$. Tissue insulation was calculated as the difference between $T_{re}$ and skin temperature (assumed to equal to $T_w$) divided by $\bar{h}_c$. 
The pre- and post-immersion muscle samples were obtained by biopsy of the vastus lateralis using the method of Bergstrom et al. (2). Muscle samples were quickly dissected free of any connective tissue, divided into several pieces, and weighed, with correction made for evaporation of water (7). Muscle samples were stored in liquid N\textsubscript{2} for subsequent analysis of glycogen content. Glycogen concentration of the muscle pieces was determined after they were freeze dried for ~72 hours. The pieces were then weighed, acid hydrolysed (2 M HCl) and glucose concentration was determined by a standard enzymatic fluorometric assay (16). Blood samples were collected in chilled tubes containing either ethylenediaminetetraacetate (EDTA) or, in the case of samples to be analyzed for lactate, EDTA and sodium flouride. Blood samples were immediately analyzed in triplicate for hematocrit, hemoglobin concentration (Coulter Hemoglobinometer) and plasma protein concentration (American Optical refractometer). Aliquots were centrifuged at 4 °C, and the plasma separated. Plasma glucose and lactate concentrations were determined using automated analyzers (Yellow Springs Instruments), and the remaining plasma was frozen in liquid N\textsubscript{2} for subsequent analysis of free fatty acid (15) and glycerol (14) concentrations.

**Statistical procedures.** The data were analyzed using multifactor analysis of variance (ANOVA) for repeated measures. Significance of factor main effects and multifactor interactions was determined for the factors trial (i.e. HMG versus LMG) and immersion (i.e. pre-immersion and various times during the immersion). When factor main effects or multifactor interactions were found to be statistically significant (P <0.05), Tukeys critical difference was calculated and used to determine the location of significant differences between means. All data are reported as means±SE.
RESULTS

TABLE 1 ABOUT HERE

Descriptive characteristics of the eight subjects who completed the study are shown in Table 1. Table 1 shows that, although unintentional, the subjects could be separated into two discrete groups on the basis of their body composition. Four of the subjects were relatively lean (< 12% body fat) whereas the other subjects were fatter (> 17% body fat). Therefore, certain ANOVAs were repeated in order to determine whether or not responses differed between the two groups.

FIGURE 1 ABOUT HERE

The subjects' vastus lateralis glycogen concentrations before and after each of the two cold water immersions are shown in Figure 1. Pre-immersion glycogen concentrations (mmol glucose·kg dry tissue⁻¹) were lower (P<0.001) for the LMG (144±14) than the HMG (543±53) trial. Post-immersion glycogen concentrations were not significantly different from pre-immersion values for either the LMG (141±10) or the HMG (526±53) trials. There was no difference in glycogen concentrations between fat and lean subjects before or after either trial.

TABLE 2 ABOUT HERE

Several subjects did not complete the full 180 min of immersion during one or both of the trials because their T_re decreased below 35.5°C (the predetermined medical safety limit). However, there were no significant differences between trials in immersion durations which averaged 123±19 min during the LMG trial (range 49-180 min), and 137±49 min during the HMG trial (range 65-180 min). There were no significant differences between the two trials in
either pre- or post-immersion $T_{re}$ (Table 2). The individual changes in $T_{re}$ (Figure 2) were calculated and were not significantly different during the HMG trial from those during the LMG trial. When the data were evaluated in a single group, post-immersion $T_{re}$ were lower ($P<0.02$) than pre-immersion values during both trials. However, when the data were separated into two groups according to body composition, differences ($P<0.005$) were found between the $T_{re}$ responses of the fat as compared to lean subjects. There was no significant change in $T_{re}$ during either immersion for the fat subjects but the $T_{re}$ of the lean subjects decreased during both cold water immersions. Tissue insulation, whether expressed absolutely (Figure 3A) or normalized for differences in subcutaneous fat thickness (Figure 3B), was not significantly different between the two trials.

FIGURE 2 AND 3 ABOUT HERE

The aerobic metabolism and respiratory parameters measured during the two immersions are shown in Figure 4. There were no significant differences between the lean and fat subjects in these parameters. The $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$ all increased with time during cold water immersion with no differences between the HMG and LMG trials. Shivering was observed in all subjects during both cold water immersions, as indicated by the more than two-fold increase over resting values in $\dot{V}O_2$, which exceeded 1 l·min$^{-1}$ at the end of both trials. Respiratory exchange ratios were not altered by cold water immersion, but were lower ($P<0.001$) during the LMG than HMG trial.

FIGURES 4 AND 5 ABOUT HERE

The concentrations of selected plasma metabolites before and during the two immersions are shown in Figure 5. There were no significant differences
in the metabolite concentrations between the lean and fat subjects. Plasma glucose concentrations were higher (P<0.005) both pre-immersion and at all times during immersion in the HMG than LMG trial. During both HMG and LMG, there were small but statistically significant (P<0.05) increases in plasma glucose concentration from pre- to post-immersion. Lactate concentrations did not differ between HMG and LMG trials except at the tenth minute of immersion when lactates in the HMG trial were higher (P<0.005) than in the LMG trial. Plasma lactate concentration increased (P<0.001) by the 60th min of both cold water immersions, as compared to pre-immersion values. Plasma glycerol concentration increased (P<0.001) during immersion in the LMG trial, and glycerol levels were higher (P<0.005) than in the HMG trial when glycerol levels remained unchanged during immersion. Plasma free fatty acid concentrations were higher (P<0.01) before and during immersion in the LMG trial than in the HMG trial. In both trials, free fatty acids were significantly higher at the end of the immersion as compared to pre-immersion levels, but the increase was apparent earlier during the LMG than HMG immersion.

Changes in hematological parameters during the two cold water immersions are shown in Table 3. Concentrations of hemoglobin and plasma proteins as well as hematocrit increased (P<0.001) during both immersions. These changes correspond to approximately an 8% reduction in plasma volume, however, the validity of of estimating changes in plasma volume from changes in hematocrit and hemoglobin concentration during cold exposure is open to question (14). There were no differences between HMG and LMG for pre- or post-immersion hematocrits, plasma protein concentration, or hemoglobin concentration.
DISCUSSION

The diet and exercise manipulation employed in this investigation appears to have been successful in achieving the desired pre-immersion muscle glycogen states. Only the vastus lateralis glycogen content was assessed, and undoubtedly the absolute value for glycogen concentration in other muscles differed from that measured in the vastus lateralis. However, it seems reasonable to assume that the effects of the diet and activity regimen were, at least qualitatively, the same for the other muscle groups of the legs, arms, shoulders and back. Pre-immersion muscle glycogen levels were substantially different for the two trials. In addition, although somewhat lower in the low-than high-glycogen trial, plasma glucose levels were in the normal euglycemic range (9) during both trials.

It had been hypothesized that depletion of glycogen stores in several major skeletal muscle groups would impair thermoregulation during cold water immersion. Changes in body (rectal) temperature during cold water immersion were the same when immersion was preceded by a three day program of heavy exercise and low carbohydrate diet as when the immersion was preceded by a three day program of rest and high carbohydrate diet. One possible explanation for the failure to observe greater and more rapid body cooling during cold water immersion in the glycogen depleted state is that a reduction in shivering was compensated for by an improvement in the insulative value of the shell. Shivering decreases insulation derived from the non-fatty shell (i.e. the skin and subcutaneous muscle) of the body via two mechanisms (5). Muscle blood flow is increased, thus convective heat transfer between the body core and shell is increased; in addition, movement of the skin surface enhances
conductive heat transfer between the skin and surrounding medium. A decrease in shivering could, therefore, produce an improvement in shell insulation. As indicated in Figure 4, there was no evidence for such a compensatory improvement in insulation during the low glycogen trial. Furthermore, there were no differences between the two trials in the oxygen uptake during immersion strongly indicating that the magnitude of whole-body shivering during cold water immersion was unaffected by the pre-immersion muscle glycogen concentration.

The basis for the hypothesized impairment of thermoregulation was the assumption that muscle glycogen was the primary metabolic substrate for shivering thermogenesis. There have been studies reported which support this assumption. For example, LeBlanc and Labrie (13) observed an increase in cold tolerance of mice 24 hours following completion of an adaptation program consisting of repeated, short duration exposures to intense (-15 °C) cold air. The authors suggested that the improved cold tolerance was associated with the concomitant increase in liver and skeletal muscle glycogen concentration also observed. In contrast, the results of the present investigation indicate that human cold tolerance is not affected by major alterations in skeletal muscle glycogen levels. However, improved cold tolerance of cold-adapted rats and mice is primarily attributed to enhanced nonshivering thermogenesis and diminished shivering (13). Therefore, even if increased liver and muscle glycogen levels do account for improved cold tolerance in mice, shivering metabolism is not likely to be involved.

A greater depletion of vastus lateralis glycogen was observed by Jacobs et al. (11) when subjects exercised in cold air as compared to temperate
conditions, and it was concluded that the additional glycogen utilization was due to shivering. In contrast, no significant changes in vastus lateralis glycogen concentration were observed during either of the two cold water immersion trials in the present investigation. The cold water immersion elicited vigorous shivering, as indicated by the oxygen uptakes which increased to about the same level (1 l·min⁻¹) as that of the exercising subjects in the study of Jacobs et al. (11). However, the subjects studied during cold water immersion were, other than shivering, resting quietly. Thus, either the vastus lateralis does not participate in shivering, or shivering activity does not result in significant depletion of muscle glycogen stores. Whichever is the case, a mechanism other than shivering must explain the observation of greater glycogen utilization during exercise in the cold.

It has been documented by others (8) using electromyographic recordings that cold exposure does elicit shivering of the human quadriceps muscle, but the results of the present investigation cannot address this question. On the other hand, observations made in the present study do suggest that there are ample alternatives to muscle glycogen to serve as substrates for shivering thermogenesis. Plasma glucose concentrations were within normal levels and tended to increase slightly during both cold water immersions. Insulin-dependent uptake of glucose by skeletal muscle has been shown to be enhanced by cold exposure (19). Therefore plasma glucose may serve as a substrate during shivering either directly via glycolysis or indirectly by enabling glycogen resynthesis at a rate equal to glycogenolysis. Alternatively, the increased plasma glycerol during cold water immersion in the low-glycogen trial indicates that enhanced lipolysis is the source of the increased plasma free
free fatty acids observed in this trial. Assuming that metabolic control during shivering and exercise are analogous processes, then free fatty acid oxidation could easily support the relatively small (compared to moderate intensity exercise) increase in metabolic rate produced by shivering.

In summary, this investigation has shown that human thermoregulatory and metabolic responses to cold water immersion are not altered by a substantial reduction in the muscle glycogen levels of several major skeletal muscle groups of the body. Furthermore, vastus lateralis muscle glycogen depletion was not observed in vigorously shivering but otherwise resting individuals. The availability of other metabolic substrates for shivering thermogenesis appears to be sufficient to enable muscle glycogen levels to be maintained unchanged. The relative contributions of carbohydrate and fatty acid oxidation during shivering thermogenesis remain to be precisely quantified.
Acknowledgements

The authors express their appreciation to the volunteers whose participation made this study possible. The expert technical assistance of James Bogart, Leslie Levine, Robert Oster and Karen Speckman is gratefully acknowledged. Finally, this study would not have been completed without the very considerable efforts of members of the Military Nutrition Division, and in particular Joan Buchbinder, R.D.

The views, opinions and/or findings in this report are those of the authors and should not be construed as official Department of the Army position, policy or decision unless so designated by other official documentation. Human subjects participated in these studies after giving their free and informed consent. Investigators adhered to AR-70-25 and USMRDC Regulation 70-25 on Use of Volunteers in Research.
REFERENCES


Table 1. Test subject characteristics.

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<th>Subject #</th>
<th>Age, yrs</th>
<th>Height, cm</th>
<th>Mass, kg</th>
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<th>Mean Skin Fold, mm</th>
<th>$\dot{V}O_2$ max ml/kg*min</th>
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<td>12</td>
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TABLE 2. Effect of muscle glycogen levels on body cooling during cold water immersion.

<table>
<thead>
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<th>POST-IMMERSION</th>
<th>CHANGE</th>
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<td>37.20 ± 0.06</td>
<td>36.18 ± 0.26*</td>
<td>-1.03 ± 0.21</td>
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<td><strong>LMG TRIAL</strong></td>
<td>37.10 ± 0.05</td>
<td>36.04 ± 0.27*</td>
<td>-1.06 ± 0.25</td>
</tr>
</tbody>
</table>

(IMMERSION DURATION = 137 ± 17 MIN)

(IMMERSION DURATION = 123 ± 19 MIN)

Values are means ± SE of rectal temperatures (T<sub>rc</sub>) before (PRE-) and after (POST-) resting immersion in cold (18 °C) water. Cold water immersions were performed following dietary and activity manipulation designed to result in high (HMG) and low (LMG) muscle glycogen levels; * significantly different from pre-immersion.
Table 3. Changes in hematological parameters during cold water immersion.

<table>
<thead>
<tr>
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<td>HMG TRIAL</td>
<td>43.2 ± 0.8</td>
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<td>LMG TRIAL</td>
<td>43.4 ± 0.9</td>
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<tr>
<td>HMG TRIAL</td>
<td>15.0 ± 0.3</td>
<td>16.3 ± 0.3*</td>
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<td>LMG TRIAL</td>
<td>15.3 ± 0.2</td>
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<td>Plasma Protein, g * 100 ml⁻¹</td>
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<tr>
<td>HMG TRIAL</td>
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<tr>
<td>LMG TRIAL</td>
<td>7.5 ± 0.1</td>
<td>8.5 ± 0.1*</td>
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</table>

Values are means ± SE (N=8) of measurements made on venous blood samples obtained from subjects who had been resting in a semi-recumbent position for more than 30 min. There were no significant differences between the high (HMG) and low (LMG) muscle glycogen trials for any parameter; *indicates significant (P<0.001) difference between pre- and post-immersion.
FIGURE LEGENDS

Figure 1. Comparison of individual pre- and post-immersion vastus lateralis muscle glycogen concentrations during the low (LMG) and high (HMG) muscle glycogen trials. Dashes indicate the line of identity.

Figure 2. Comparison of individual changes in rectal temperature during cold water immersion in the low LMG) and high (HMG) muscle glycogen trials. Dashes indicate the line of identity.

Figure 3. Comparison of individual tissue insulation measurements made at the end of cold water immersion in the low (LMG) and high (HMG) muscle glycogen trials. Dashes indicate the line of identity. The data are shown uncorrected (A) and corrected (B) for differences between the individuals' subcutaneous fat thickness.

Figure 4. Mean±SE of oxygen uptake, carbon dioxide output, minute ventilation and respiratory exchange ratio before (time =0 min) and during cold water immersion in the low muscle glycogen (open symbol) and high muscle glycogen (closed symbol) trials. Asterisks indicate significant differences between the low and high muscle glycogen trials and daggers indicate significant differences from the pre-immersion value.

Figure 5. Mean±SE of plasma metabolite values before (time =0 min) and during cold water immersion in the low muscle glycogen (open symbol) and high muscle glycogen (closed symbol) trials. Asterisks indicate significant differences between the low and high muscle glycogen trials and daggers indicate significant differences from the pre-immersion value.
HIGH MUSCLE GLYCOGEN ○
LOW MUSCLE GLYCOGEN ○

O₂ UPTAKE

CO₂ OUTPUT

MINUTE VENTILATION

RESPIRATORY EXCHANGE RATIO

TIME (MIN)
HIEH MUSCLE GLYCOGEN  •
LOW MUSCLE GLYCOGEN  ○

**Plasma Glucose**

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**Plasma Lactate**

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**Plasma Glycerol**

<table>
<thead>
<tr>
<th>IMMERSION TIME (min)</th>
<th>FINAL</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

**Plasma Free Fatty Acids**

<table>
<thead>
<tr>
<th>IMMERSION TIME (min)</th>
<th>FINAL</th>
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</thead>
<tbody>
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</table>