INFLUENCE OF SKELETAL MUSCLE GLYCOGEN ON PASSIVE REWARMING AFTER HYPOTHERMIA(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA P D NEUFER ET AL
Influence of Skeletal Muscle Glycogen on Passive Rewarming After Hypothermia

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Running Head: Muscle Glycogen and Rewarming

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LMG and NMG in the duration of afterdrop (14.5±5.3 vs 9.3±7.4 min, respectively) or time to maximum afterdrop (10.8±3.8 vs 5.5±2.8 min, respectively). Independent of treatment, afterdrop responses were evident only in those individuals (body fat <15%) whose body core cooled during immersion (T = 35.50–35.81°C) supporting the contention that afterdrop is a function of the kinetics of heat flow through a mass of tissue. Furthermore, these data indicate that low muscle glycogen levels do not impair rewarming time nor alter afterdrop responses during passive rewarming following mild to moderate hypothermia.
Influence of skeletal muscle glycogen on passive rewarming after hypothermia

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(U) Individuals performing work and athletes competing in cold conditions may experience, in addition to muscle glycogen depletion, mild to moderate hypothermia. It has been hypothesized that muscle glycogen may provide the substrate for shivering thermogenesis. To examine the influence of muscle glycogen on the thermal responses to passive rewarming subsequent to mild hypothermia, eight subjects completed two cold (18°C) water immersions followed by 75 min of passive rewarming (24°C air, resting in blanket). The experiments followed several days of different exercise/diet regimens eliciting either low (LMG; 141.0±8.8 mmol·kg⁻¹ dry wt.) or normal (NMG; 526.2±44.2 mmol·kg⁻¹ dry wt.) prewarming muscle glycogen levels. Cold water immersion was performed for 180 min or to a rectal temperature (T<sub>re</sub>) of less than 35.5°C. Rewarming increased T<sub>re</sub> similarly during both LMG (0.50±0.13°C) and NMG (0.45±0.15°C). No differences were found for the magnitude of afterdrop (continued decline in T<sub>re</sub> during the initial period of rewarming) during LMG (0.08±0.04°C) or NMG (0.10±0.08°C). Furthermore, differences were not observed between...
Abstract

Individuals performing work and athletes competing in cold conditions may experience, in addition to muscle glycogen depletion, mild to moderate hypothermia. It has been hypothesised that muscle glycogen may provide the substrate for shivering thermogenesis. To examine the influence of muscle glycogen on the thermal responses to passive rewarming subsequent to mild hypothermia, eight subjects completed two cold (18°C) water immersions followed by 75 min of passive rewarming (24°C air, resting in blanket). The experiments followed several days of different exercise/diet regimens eliciting either low (LMG; 141.0 ± 8.8 mmol·kg⁻¹ dry wt.) or normal (NMG; 528.2 ± 44.2 mmol·kg⁻¹ dry wt.) prewarming muscle glycogen levels. Cold water immersion was performed for 180 min or to a rectal temperature (T<sub>re</sub>) of less than 35.5°C. Rewarming increased T<sub>re</sub> similarly during both LMG (0.50 ± 0.13°C) and NMG (0.45 ± 0.15°C). No differences were found for the magnitude of afterdrop (continued decline in T<sub>re</sub> during the initial period of rewarming) during LMG (0.08 ± 0.04°C) or NMG (0.10 ± 0.08°C). Furthermore, differences were not observed between LMG and NMG in the duration of afterdrop (14.5 ± 5.3 vs 9.3 ± 7.4 min, respectively), or time to maximum afterdrop (10.8 ± 3.8 vs 5.5 ± 2.8 min, respectively). Independent of treatment, afterdrop responses were evident only in those individuals (body fat < 15%) whose body core cooled during immersion (T<sub>re</sub> 35.50-35.81°C) supporting the contention that afterdrop is a function of the kinetics of heat flow through a mass of tissue. Furthermore, these data indicate that low muscle glycogen levels do not impair rewarming time nor alter afterdrop responses during passive rewarming following mild to moderate hypothermia.

Index terms. cold water immersion; afterdrop; endurance athletes; glycogen depletion; shivering thermogenesis
Introduction

Spontaneous rewarming following mild to moderate hypothermia is accomplished primarily through shivering activity of the skeletal musculature (10). The involuntary increase in contractile activity and corresponding metabolic heat production serves to warm the perfusing blood which, in turn, circulates back to the core of the body. Although increased oxygen uptake ($VO_2$) during exercise in a cold as compared to warm environment has been attributed to the additional metabolic demand of shivering (19,22), the relative contribution and importance of the various metabolic substrates to shivering activity is unknown.

Recently, Jacobs et al. (11) demonstrated that low intensity (30% of maximal oxygen uptake, $VO_2$ max) cycling exercise performed in a cold environment (air, $0^\circ$C) resulted in a greater glycogen utilization as compared to exercise in a neutral environment ($21^\circ$C). The authors (11) attributed the greater muscle glycogen use in the cold to the increased metabolic demand of shivering. Moreover, LeBlanc and Labrie (12) have reported reductions in the cold resistance of rats with low glycogen concentrations, suggesting that glycogen may serve as a rate-limiting substrate for shivering thermogenesis.

Endurance athletes competing in cold environmental conditions may experience, in addition to muscle glycogen depletion, mild to moderate hypothermia (4,14,16,20,22). Likewise, military and occupational demands may occasionally require sustained physical work in cold environments. Numerous investigators (3,8,9,13,15,17,18) have examined the efficacy of various rewarming techniques with regards to rewarming rate and avoidance of afterdrop (defined as a continued decline in core temperature during the initial period of rewarming). However, little information is available on the effects of
metabolic substrate levels on the ability to rewarm following cold exposure. The purpose of the present study was to examine the influence of depleted muscle glycogen levels on the passive rewarming responses subsequent to mild hypothermia. Our hypothesis was that glycogen depleted subjects would require a greater rewarming time.

Methods

Subjects. Ten males volunteered to participate in this study after being informed of the requirements and possible risks associated with this research. The characteristics (mean ± SE) of the subjects were age 20 ± 1 yr, height 179 ± 3 cm, weight 78 ± 5 kg, body fat 16 ± 2% (determined by hydrostatic weighing), and maximal aerobic power (ref. 23, \( V_{\text{O2 max}} \), treadmill) 51 ± 1 ml·min\(^{-1}\)·kg\(^{-1}\). Total skinfold thickness of the arm, leg, and torso regions was determined from the appropriate measurement sites.

Experimental design. Mild hypothermia was induced on two separate occasions by whole-body immersion in cold water (18°C, stirred). The subjects wore only swim shorts for all testing sessions. A thermistor placed ~10 cm beyond the anal sphincter was used for the measurement of rectal temperature (\( T_{\text{re}} \)). The participants remained immersed in the water for 180 min or until their \( T_{\text{re}} \) fell below 35.5°C. After exiting the water, the subjects quickly moved (~2 m) to a second reclining chair and were wrapped in several blankets with the exception of the thigh region of one leg. A muscle biopsy was then obtained from the vastus lateralis as described by Bergstrom et al. (1) after which the exposed leg was covered. Rewarming was commenced within 2 min of the end of immersion and was carried out passively at room temperature (27°C) for a period of 120 min or until \( T_{\text{re}} \) had returned to pre-immersion values. Because of variations in the total rewarming time among subjects, only data obtained during the
initial 75 min of rewarming is reported.

To vary the skeletal muscle glycogen levels prior to rewarming, each of the two cold water immersions was preceded by several days of different diet/exercise regimens. Three days before one of the cold water immersions (trial LMG), the subjects completed three 60 min work bouts at ~50% VO2 max, each employing a different mode of exercise (running, rowing, or cycling). Each 60 min bout consisted of three cycles of 20 min exercise/10 min rest periods with all work bouts being separated by 3-5 h. In addition to the exercise protocol, the carbohydrate (CHO) intake of the subjects was limited to ~120 g per day in a total caloric intake of ~2900 kcal (17% CHO, 65% fat, & 18% protein). This diet/exercise regimen was implemented on 3 successive days and designed to deplete glycogen levels within the leg, arm, and torso muscle groups.

Three days before the other cold water immersion (trial NMG), the subjects diets were manipulated to deliver 600 g CHO per day in a total caloric intake of 2500 kcal (64% CHO, 24% fat, 12% protein). The subjects consumed the high CHO diet and were instructed to refrain from any strenuous physical activity during each of the 3 days preceeding trial NMG. This procedure was designed to insure muscle glycogen content was at or above normally reported resting levels. Trial LMG and NMG were completed in a counterbalanced fashion with at least two weeks separating each cold water immersion. The subjects were restricted from any physical activity or food during the 16 h prior to each trial.

Biochemical analysis. A venous blood sample (~4 ml) was collected in tubes containing ethylenediaminetetraacetate (EDTA) and 10 mg of sodium floride from the arm within 1 min prior to the end of the cold water immersions. Samples were then centrifuged at 4°C, and the plasma separated for the subsequent measurement of blood glucose using an automatic analyser (Yellow Springs
Instruments). Muscle biopsy samples obtained within 5 min after cold water immersion were quickly dissected free of connective tissue, divided into 10-20 mg pieces, and frozen in liquid nitrogen. All samples were freeze dried for 3 days, weighed, and hydrolyzed in 2 M HCl. Glycogen concentrations were determined in triplicate for all muscle samples using a standard enzymatic technique (21).

Statistics. Paired t-tests were used to locate differences in means between trials. A significance level of P<0.05 was set for all tests.

Results

Although all 10 subjects completed both rewarming trials, 2 of the 10 subjects failed to adhere to the exercise/dietary regimen during trial LMG. As such, data from these two subjects was excluded from the statistical analysis unless otherwise noted.

There was no difference in the initial $T_{re}$ at the beginning of the rewarming period between trials NMG (36.19°C) and LMG (36.04°C). Despite the subjects being towel dried, wrapped in blankets and seated at room temperature (24°C) within 2 min of the end of immersion, $T_{re}$ continued to fall during the initial rewarming period. Such a response is characteristic of passive rewarming after cold water immersion and has been collectively referred to as afterdrop (10).

Fig. 1 illustrates the various parameters examined associated with the afterdrop response including magnitude of afterdrop, duration of afterdrop (total time $T_{re}$ remained below the final immersion $T_{re}$ during rewarming), and the time to attain maximum afterdrop.

Insert figure 1 about here

The exercise/dietary regimens elicited significant (p<0.05) differences in the pre-immersion glycogen contents within the vastus lateralis muscle (Table
1). Although glycogen content was not determined in any other muscles, it would be expected that the effects of the diet/exercise regimen on the glycogen content of the shoulder, upper arm, chest, and calf muscle regions would be similar to the effects on the vastus lateralis. Also presented in Table 1 are the plasma glucose values obtained immediately prior to the end of the cold water immersions. Glucose concentrations in trial LMG, although significantly lower (p<0.05) compared to trial NMG, remained well above the hypoglycemic level (<3.5 mM) in all subjects.

Insert table 1 about here

The passive rewarming responses were not significantly different between trials NMG and LMG (Fig. 2). Following the initial afterdrop phase, the rate of increase in $T_{re}$ was similar for both trials, averaging $\sim 0.46^\circ C/h$. As a result, the change in $T_{re}$ following 75 min of rewarming was not significantly different between trials NMG and LMG (Fig. 3).

Insert figure 2 and 3 about here

No significant difference in the magnitude of afterdrops were noted between trials (Fig. 4). Furthermore, the different exercise/dietary regimens did not significantly influence the duration of afterdrop nor the rewarming time at which the maximum afterdrop occurred (Fig. 4).

Insert figure 4 about here

Because no overall differences were observed in the rewarming responses between trials NMG and LMG, data from both trials of all 10 subjects was pooled to examine any possible relationship between body composition measurements and the afterdrop responses. Somewhat surprisingly, 4 of the 10 subjects exhibited no afterdrop response. These individuals completed the 180 min cold water immersions with a mean final immersion $T_{re}$ of $36.72 \pm 0.15 ^\circ C$. Rewarming for 75 min resulted in an increase in $T_{re}$ of only $0.12 \pm 0.06^\circ C$. Body fat for these 4 subjects ranged from 17 to 22% with a mean of $20 \pm 1%$. In contrast,
final immersion $T_{re}$ (mean ± SE) for the 6 subjects who displayed afterdrop responses averaged $35.51 ± 0.04^\circ C$ and increased $0.72 ± 0.14^\circ C$ after 75 min of rewarming. Body fat for this group ranged from 9 to 14% with a mean of 12 ± 1%. However, a regression analysis of the percent body fat vs the change in $T_{re}$ after 75 min of rewarming for all ten subjects revealed a nonsignificant correlation of $-0.61$ (p=0.06). In addition, within the 6 leaner subjects, no significant correlations were found between % body fat, the sum of arm, leg, or torso skinfold measurements and any of the afterdrop parameters.

Discussion

The development of mild to moderate hypothermia during certain occupational and military tasks as well as athletic events performed in cold environmental conditions represents a potential medical concern for both the civilian and military communities. Recent reports have described incidents of hypothermia in competitive triathletes (14), marathon runners (16,20), wheelchair athletes (4), mountain climbers (22) and divers (10). Reductions of 1-2°C in core temperature result in sensations of extreme discomfort, light-headedness and, with further declines, the possible impairment of circulatory and cardiovascular functions (5,9,22). Accordingly, considerable attention has been given to the efficacy of various rewarming techniques. However, from a practical standpoint, passive rewarming techniques will be the first and/or only treatment available to persons experiencing mild to moderate hypothermia. It is well recognized that shivering represents the primary metabolic means for heat production during passive rewarming (10). In addition, it has been suggested that muscle glycogen represents an important substrate for shivering thermogenesis (11,12). However, no information is available on the passive rewarming characteristics of individuals in which skeletal muscle glycogen
stores may have been compromised by their previous activities. The results of the present study suggest that low skeletal muscle glycogen levels do not significantly impair passive rewarming following mild to moderate hypothermia.

The importance of muscle glycogen as a rate-limiting substrate in exercising muscle is well known (1). However, shivering is a relatively low intensity form of physical activity. The role muscle glycogen plays in the contribution to the energy demand, therefore, remains unclear. In the present study, the participants completed an intensive 3 day exercise/diet regimen designed to severely compromise the muscle glycogen stores in the major muscle groups of the legs, arms and upper torso. As evident from Table 1, muscle glycogen stores were nearly depleted in the vastus lateralis. Comparable reductions in the glycogen stores of other muscle groups would be expected. Blood glucose levels were well above hypoglycemic levels prior to rewarming in trial LMG, although significantly lower than in trial NMG (Table 1). In view of the nearly identical rewarming responses observed between trials LMG and NMG (Fig 2), it appears that, if passive rewarming does depend on shivering, then muscle glycogen is not a major substrate for shivering thermogenesis. Considering the relatively low form of activity (in terms of maximal aerobic power) shivering represents, it seems likely that other metabolites (free fatty acids and/or glucose) serve as the primary substrates for muscle during passive rewarming.

The degree of afterdrop occurring during the initial period of rewarming from hypothermia has been a major concern in both the laboratory and clinical settings. This continued decline in core temperature has predominately been attributed to a return of cold blood from the limbs as a result of a rewarming-induced peripheral vasodilation. The influx of cold blood presumably further cools the myocardium, thereby potentiating the risk of ventricular fibrillation (2,4). Accordingly, several rewarming techniques have been evaluated based on
their afterdrop responses \((3, 8, 9, 13, 15, 17, 18)\). Because many athletic and military first aid facilities are limited in the availability of immediate treatment, it was a secondary intent of the present study to examine the influence of varied muscle glycogen levels on the afterdrop responses to passive rewarming. As evident from Fig. 4, the magnitude of afterdrop, duration of afterdrop and the rewarming time at which the maximum afterdrop occurred were not affected by the different exercise/diet regimens. In view of these findings, we subsequently attempted to examine the possible mechanisms responsible for the observed afterdrop responses.

Although much of the previous research has focused on the avoidance of afterdrop in the treatment of hypothermia \((3, 9, 13, 15, 17, 18)\), several more recent investigators have questioned the validity of the circulatory explanation of afterdrop \((7, 23, 25)\). Golden and Hervey \((7)\), using pigs noted that afterdrop did not appear to correspond to an influx of cold blood from the periphery, but could be explained simply by a thermal conduction mechanism. To examine this hypothesis, Savard et al. \((23)\) measured the forearm, calf, and foot blood flows of humans during hot \((40^\circ C)\) water bath rewarming. These authors \((23)\) found no differences in forearm and calf blood flows, and only a minimal increase in foot blood flow during the afterdrop phase, suggesting that afterdrop did not result from a large vasodilation of the periphery during rewarming. Most recently, Webb \((25)\) observed the typical afterdrop responses only when subjects were rapidly cooled \((1-2 \text{ h})\) and immediately rewarmed. Afterdrops in core temperature were not found when cooling was slow \((6-8 \text{ h})\), or when, after cooling \((1-2 \text{ h})\), rewarming was delayed \((\sim 2 \text{ h})\). Moreover, Webb \((25)\) demonstrated in two cooled physical models (a bag of gelatin and a leg of beef) placed in a rewarming bath that temperatures measured at the center of the mass continued to decline while surface temperatures equilibrated immediately with the water temperature. This afterdrop of the central compartment continued as
long as the layer surrounding it remained cooler, i.e. as long as rewarming did not progress to the center of the mass. Taken together, these findings provide little support for the circulatory explanation of the afterdrop phenomenon. Alternatively, the afterdrop occurring in humans during rapid rewarming appears to be simply a function of the kinetics of heat flow through a mass of tissue (25).

If the initiation of central rewarming is dependent upon the rate of heat flow from the periphery to the body core, then it would be expected that individuals with a greater degree of subcutaneous fat would display a more pronounced afterdrop. In the present study, however, afterdrop was observed only in those 6 subjects whose body fats were below 14%. Moreover, the 4 subjects whose body fats were 17-22% showed no afterdrop in $T_{re}$ during rewarming. Inasmuch as these data appear to contradict the thermal conduction explanation of afterdrop (7, 23, 25), it is important to note that the 4 subjects with the greater percentage of body fat began rewarming with a mean ($\pm$ SE) $T_{re}$ only slightly below normal body temperature (36.72 ± 0.15 vs ~37.00°C, respectively). Thus, cooling did not progress to the core region of the body during cold water immersion in these 4 subjects. However, cold water immersion in the 6 leaner subjects elicited marked reductions in $T_{re}$ with further declines evident during the initial phase of rewarming. These findings, therefore, appear to support the notion that $T_{re}$ afterdrop responses are evident during rewarming only when prior external cooling has reached the body core (7, 23, 25). Further research should be directed toward examining the rewarming responses of individuals with varying body compositions when cooled to identical core temperatures.

The most obvious and relevant question of both physicians and first-aid personnel remains; what is the most effective means of rewarming mild to moderately hypothermic individuals? A number of investigators (3, 8, 15) have
compared several rewarming techniques on subjects passively cooled by cold water immersion and concluded that immersion in warm water offers the most effective method. However, the limitations of this technique become evident when multiple hypothermic victims require assistance or when rewarming must be initiated under field conditions. In such situations, heating pads, inhalation of warm air, and spontaneous rewarming in blankets represent more practical treatments with little or no difference in the relative effectiveness between techniques (17,18). In contrast to passively cooled subjects, relatively little information is available concerning the impact of hypothermia in combination with exercise fatigue. In addition to hypothermia, marathon runners, for example, may also be somewhat hypoglycemic and hypovolemic at the completion of a race. Moreover, it is important to note that the perception of cold discomfort is greatly reduced as blood glucose approaches hypoglycemic levels, possibly due to the reduced shivering activity (6). In view of these possible mitigating factors, there remains considerable question as to the most effective and practical treatment for rewarming mild to moderately hypothermic individuals.

In summary, this study has demonstrated that passive rewarming subsequent to mild to moderate hypothermia is not impaired by low skeletal muscle glycogen levels. Furthermore, afterdrop responses occurring during the initial period of rewarming are not altered by differing muscle glycogen content. Afterdrop responses appear to occur during rewarming only when prior external cooling has progressed to the body core, supporting the contention that afterdrop is a function of thermal conduction through a mass of tissue (24). The implications of these findings suggest that individuals suffering from mild hypothermia and fatigue following prolonged activities will spontaneously rewarm despite having low muscle glycogen levels.
Acknowledgements.

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 voluntary consent. Investigators adhered to AR 70-25 and USMRDC Regulation 70-25 on Use of Volunteers in Research.
References


Table 1. Mean (±SE) values for plasma glucose (determined 1 min before the end of cold water immersion) and muscle glycogen concentration prior to rewarming. *

* Significantly (p<0.05) different from corresponding trial.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Plasma Glucose (mmol/l)</th>
<th>Muscle Glycogen (mmol·kg⁻¹ dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Muscle Glycogen</td>
<td>5.48 ±0.30</td>
<td>526.2 ±44.2</td>
</tr>
<tr>
<td>Low Muscle Glycogen</td>
<td>4.58* ±0.17</td>
<td>141.0* ±8.8</td>
</tr>
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Figure legends.

FIG. 1. Characteristics of the core temperature responses to cold water immersion and passive rewarming. Delta (Δ) TRE corresponds to the change in rectal temperature occurring during a given amount of time.

FIG. 2. Rectal temperature (°C) values (mean ± SE) during 75 min of passive rewarming after cold water immersion for trial NMG (normal muscle glycogen) and trial LMG (low muscle glycogen).

FIG. 3. Values (mean ± SE) for the change in rectal temperature (°C) following 75 min of passive rewarming for trial NMG (normal muscle glycogen) and trial LMG (low muscle glycogen).

FIG. 4. Values (mean ± SE) for the magnitude of afterdrop (°C), duration of afterdrop (°C) and the time to maximum afterdrop (min) during passive rewarming for trials NMG (normal muscle glycogen) and trial LMG (low muscle glycogen).
FIG 4

Tre AFTERDROP

Tre AFTERDROP DURATION

TIME TO MAXIMUM Tre AFTERDROP

NORMAL MUSCLE GLYCOGEN LOW MUSCLE GLYCOGEN
END
DATE
FILMED
JAN
1988