Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle

James P. Morton,1 Louise Croft,1 Jonathan D. Bartlett,1 Don P. M. MacLaren,1 Thomas Reilly,1 Louise Evans,2 Anne McArdle,3 and Barry Drust1
1Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool; 2Stepping Hill Hospital, Stockport National Health Service Foundation Trust, Poplar Grove, Stockport; and 3School of Clinical Sciences, University of Liverpool, Liverpool, United Kingdom

Submitted 5 January 2009; accepted in final form 3 March 2009


HEAT SHOCK PROTEINS (HSPs) are a family of molecular chaperones that are upregulated in the skeletal muscle of both rodents and humans following acute and chronic exercise (24, 25, 30, 33, 36, 40, 42). HSPs can be classified according to their molecular weight, and the prominent HSPs expressed in muscle include the small HSPs (αB-crystallin and HSP27), mitochondrial HSP60, and the highly stress-inducible HSP70. HSP70 is the most conserved of the HSP family (49) and is the most widely studied to date. Given their chaperone properties, HSPs are involved in a number of remodeling processes associated with exercise training, such as facilitating mitochondrial biogenesis (21), regulating apoptotic pathways (46), and inducing improvements in insulin sensitivity (6). Furthermore, an increased muscle content of HSPs following non-damaging stresses (i.e., preconditioning) provides cytoprotection against subsequent periods of normally lethal or damaging stresses (16, 29, 34, 35). Transgenic approaches have also demonstrated that increased HSP content confers protection against contraction-induced damage in both adult and aged muscle (32). Collectively, such data suggest that increased muscle HSP content evident in trained muscle (42) may be a crucial component of the molecular mechanisms by which regular exercise provides improved cellular function and increased protection against stressful insults.

HSPs were first shown to be expressed following sublethal heat shock (44) although it is now known that stressors, including reduced pH, ischemia, oxidative stress, and depleted energy stores, also increase cellular HSP content (49). Given that these stressors are similar to the homeostatic perturbations occurring in contracting skeletal muscle, it is difficult to determine the precise stressor(s) that is responsible for mediating the exercise-induced production of HSPs. Nevertheless, several authors have proposed that reduced carbohydrate availability from both endogenous and exogenous sources (13) may be a contributing signal, although conclusive evidence is currently limited. Furthermore, no researchers have simultaneously investigated the putative roles of carbohydrate availability from both sources in modulating the exercise-induced stress response. Such research is warranted as it is possible that exercising in conditions of reduced carbohydrate availability may provide an enhanced stimulus for inducing the upregulation of the HSP family of cytoprotective proteins.

One experimental approach to chronically manipulate carbohydrate availability is to have subjects perform exercise training twice daily every second day vs. once daily (17, 54). In this way, all subjects can perform the same amount of work throughout a set training period, yet subjects who train twice every second day commence every second training session with reduced muscle glycogen stores. Interestingly, Hansen...
et al. (17) and Yeo et al. (54) have employed such a model (using one-legged knee extensor and cycling exercise, respectively) and have demonstrated that training in conditions of reduced carbohydrate availability induces significantly greater increases in citrate synthase activity. These data suggest that such training models may have implications for athletic preparation although the latter authors failed to observe increases in exercise performance in elite athletes. Furthermore, these authors have only investigated the effect of reduced muscle glycogen per se and have not simultaneously examined the role of exogenous glucose availability in influencing training adaptations.

The aims of the present study were twofold. Our primary aim was to test the hypothesis that training in conditions of reduced carbohydrate availability from both endogenous and exogenous sources augments the training-induced adaptation of the major HSP families in human skeletal muscle. Given recent studies examining the influence of carbohydrate availability on training-induced athletic performance (17, 54), our secondary aim was to investigate the role of reduced carbohydrate availability on markers of oxidative capacity and exercise performance. We chose a high-intensity intermittent running protocol as our intervention given the relevance of running to the general population (as a keep-fit activity) and also in an array of sporting activities. Furthermore, interval training is receiving increased recognition as an innovative training method compared with traditional methods of continuous running (19, 50).

MATERIALS AND METHODS

Subjects. Thirty recreationally active men volunteered to participate in the study. After giving written informed consent to participate, subjects were randomly assigned (according to Ref. 2) into one of three training groups to provide 10 subjects in each group. Seven subjects withdrew from the study during data collection, and their data were therefore discarded. The characteristics of the remaining subjects who completed the study are shown in Table 1. All subjects refrained from alcohol and caffeine intake for at least 24 h before any of the test sessions (excluding training sessions). None of the subjects had any history of neurological disease or musculoskeletal abnormality, and none was under any pharmacological treatment during the course of the study. The study was approved by the Ethics Committee of Liverpool John Moores University.

Due to subject drop out, there were therefore eight subjects who completed the study in groups 1 and 3, and seven subjects who completed the study in group 2. Using the NQuery statistical power software (Statistical Solutions, Cork, Ireland) and appropriate statistical guidelines (3), it was calculated that a sample size of 7 would still enable detection of a mean difference of 50% in basal protein levels assuming an SD of differences equal to 25% and statistical power of 80%. This effect size and SD is based on those values cited in previous studies that have examined training-induced increases in muscle HSP content in both longitudinal (30) or cross-sectional studies (42). Furthermore, with sample sizes of 7, these effect sizes have been abolished when the exercise protocol is performed following nutritional interventions such as reduced carbohydrate availability (14) or antioxidant supplementation (15, 22, 26).

Experimental design. After having initially been assessed for maximal oxygen uptake (V02 max) and running performance on Yo-Yo Intermittent Recovery Test 2 (YoYoIR2T) (see Assessment of physiological fitness), subjects completed 6 wk of high-intensity intermittent running occurring four times per week. Each group therefore performed a total of 24 training sessions throughout the 6-wk period. Groups 1 and 2 trained twice per day, 2 days/wk (once in the morning and once in the afternoon where training sessions were interspersed with a 3- to 4-h rest period), whereas group 3 trained once per day, 4 days/wk. In this way, each group performed the same amount of work throughout the training period, yet groups 1 and 2 performed every second training session with reduced preexercise muscle glycogen levels (pilot data demonstrate that subjects commence the afternoon training session with glycogen stores depleted by approximately 35 and 45% in the vastus lateralis and gastrocnemius muscle, respectively; see Table 2). Furthermore, to allow us to also examine the effects of exogenous glucose supplementation on influencing training adaptations, subjects in group 1 (Low + Glu) consumed a carbohydrate beverage (6.4%) immediately before and throughout every second (i.e., afternoon) training session, whereas subjects in group 2 (Low + Pla) consumed an identical amount of a taste-, consistency-, and odor-matched placebo solution at identical times. Subjects consumed 8 ml/kg of the relevant solution in a 10- to 15-min period immediately before exercise and a further 3 ml/kg in the active recovery periods following interval 1 and interval 3 (see High-intensity intermittent training protocol for a description of the training stimulus). In contrast, subjects in group 3 (Norm) commenced every training session with normal glycogen stores and consumed no beverages during any of their training sessions. Resting muscle biopsies were obtained from the vastus lateralis and gastrocnemius muscles immediately before the first training session (see Fig. 1, B1) and at 72 h after completion of the training program (see Fig. 1, B3). Both vastus lateralis and gastrocnemius muscle samples were analyzed for muscle content of HSP70, HSP60, HSP27, B-crystallin, Mn superoxide dismutase (MnSOD), cytochrome-c oxidase IV (COXIV), peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1-α), muscle glycogen concentration, and succinate dehydrogenase (SDH).

### Table 1. Subject characteristics of each training group

<table>
<thead>
<tr>
<th></th>
<th>Low + Glu (n = 8)</th>
<th>Low + Pla (n = 7)</th>
<th>Norm (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>20 ± 1</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.04</td>
<td>1.75 ± 0.06</td>
<td>1.79 ± 0.03</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>79 ± 7.5</td>
<td>74.5 ± 7.8</td>
<td>77 ± 10.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Low + Glu and Low + Pla groups trained twice per day, 2 days/wk, and consumed a 6.4% glucose or placebo solution, respectively, immediately before every second training session and at regular intervals throughout exercise and thus performed every second training session with reduced preexercise muscle glycogen levels. Norm group trained once per day, 4 days/wk, consumed no beverage throughout training, and commenced every training session with normal glycogen stores. See Experimental design for more details.

### Table 2. Muscle glycogen concentration of the gastrocnemius and vastus lateralis muscles immediately before and after the morning and afternoon training sessions

<table>
<thead>
<tr>
<th></th>
<th>Pre Morning Training</th>
<th>Post Morning Training</th>
<th>Pre Afternoon Training</th>
<th>Post Afternoon Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle glycogen, mmol/kg wet wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>96 ± 10</td>
<td>56 ± 8*</td>
<td>54 ± 5</td>
<td>25 ± 10*</td>
</tr>
<tr>
<td>Vastus lateralis</td>
<td>90 ± 18</td>
<td>61 ± 15*</td>
<td>59 ± 6</td>
<td>41 ± 13*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data were compiled from a pilot study (n = 4) and demonstrate that exercise induces significant glycogen depletion in both exercise bouts and that consumption of a low-carbohydrate lunch (consumed at the midpoint of the interval between morning and afternoon exercise) does not promote any glycogen resynthesis before the afternoon session. *Significant difference from respective preexercise values (P < 0.05), as assessed by a 1-way repeated measures general linear model.
activity. A schematic illustration of the experimental design is shown in Fig. 1.

Effects of training on the metabolic/physiological cost of a criterion exercise session. To evaluate the effects of training on the physiological and metabolic cost of a criterion exercise session, each subject performed an additional exercise session 3 days after completion of the training program (see Fig. 1, CE2). This session was performed at the same absolute intensities as the first training session (see Fig. 1, CE1). Heart rate was measured continuously during exercise (Polar S610i, Kempele, Finland). Muscle biopsies and venous blood samples were obtained immediately pre- and postexercise for both the first training session (see Fig. 1, B1 and B2) and this additional exercise session (see Fig. 1, B3 and B4). Biopsy samples were assessed for muscle glycogen concentration, and blood samples were analyzed for plasma glucose, lactate, nonesterified fatty acids (NEFA), glycerol, and serum insulin concentration.

Dietary interventions. On each day of testing or training, subjects arrived in the laboratory 2 h after having consumed a standardized breakfast (3.64 MJ, 863 kcal: 63% carbohydrate, 25% fat, 12% protein). For those groups who trained twice per day (i.e., Low + Glu and Low + Pla), the morning and afternoon training sessions were separated by a 3- to 4-h recovery period. At the midpoint of this interval (i.e., 1.5-2 h following completion of the morning exercise session), each subject consumed a standardized low-carbohydrate lunch containing <50 g of carbohydrate (1.29 MJ, 304 kcal: 52% carbohydrate, 39% fat, 9% protein). Pilot data demonstrated that this meal resulted in no significant muscle glycogen resynthesis immediately before the second exercise bout (see Table 2). This dietary intervention therefore allowed the afternoon session to be commenced under conditions of reduced muscle glycogen while simultaneously providing the subjects with some nutritional intake at “lunchtime.” Throughout the recovery period, subjects also consumed an adequate amount of water (150% of weight loss) to replace any fluid loss induced by the morning training session (47). To calculate weight loss, subjects were weighed while nude and fully dried immediately before and within 5–10 min after exercise (time scale allowing for biopsy/blood and “drying” procedures) using calibrated precision weighing scales (Seca). Weight loss was measured in kilograms and multiplied by 1.5 to give a value that was subsequently converted to liters (47). Following the completion of each daily training session (Norm subjects) or every second training session (Low + Glu and Low + Pla), subjects consumed 1.2 g/kg of carbohydrate per hour for 3 h to accelerate muscle glycogen resynthesis before the next exercise bout. These meals were given in the form of sports bars, gels, and drinks (GlaxoSmithKline Consumer Healthcare). After this 3-h period, subjects resumed their habitual diet.

On those days outside of “study” activities, subjects were instructed to consume their habitual dietary intake. To analyze the nutritional intake of such practices, each subject completed three 2-day food diaries at set periods throughout the study period. Subsequent dietary analysis was performed with the software program Microdiet (Downlee Systems). A breakdown of each group’s nutritional intake is shown in Table 3. These data demonstrated no significant difference between groups for total energy intake or macronutrient composition (as assessed by a 1-way between-groups general linear model). For 2 days before their pretraining biopsies, subjects consumed their habitual diet. This diet was photocopied, and subjects were instructed to consume an identical diet in the 2 days before their posttraining biopsies. Dietary analysis revealed no differences between groups (as assessed by a 1-way between-groups general linear model), and when taken together, the average composition per subject was ~6.5 ± 0.9 MJ (45 ± 4% carbohydrate, 35 ± 9% fat, 20 ± 7% protein).

To monitor physical activity levels outside of study activities, each subject completed a physical activity diary where they recorded the number of hours of physical activity performed throughout the 6-wk

Table 3. Nutritional analysis of each group throughout the study period

<table>
<thead>
<tr>
<th></th>
<th>Low + Glu</th>
<th>Low + Pla</th>
<th>Norm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate, %</td>
<td>50±5</td>
<td>53±4</td>
<td>51±3</td>
</tr>
<tr>
<td>Fat, %</td>
<td>30±6</td>
<td>27±5</td>
<td>31±5</td>
</tr>
<tr>
<td>Protein, %</td>
<td>20±3</td>
<td>20±4</td>
<td>18±4</td>
</tr>
<tr>
<td>Energy, MJ</td>
<td>7.5±1.2</td>
<td>8.3±3.2</td>
<td>8.2±2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Macronutrient composition is presented as % of total calorie intake. Data were compiled from three 2-day food diaries.
period. There was no significant difference between groups in terms of hours involved in this “additional” physical activity, which equated to approximately 0.5–1 h per subject per week (as assessed by a 1-way between-groups general linear model; data not shown). These activities consisted mainly of walking to and from their place of employment and, occasionally, recreational sporting activities at weekends. Indeed, the majority of subjects reported to the investigators that they did not perform any exercise outside of the study due to the need to recover from the intense nature of the training days.

Assessment of physiological fitness. All subjects were initially assessed for \( V\dot{O}_2\)max using an incremental exercise test performed on a motorized treadmill (HP Cosmos). Oxygen uptake (\( V\dot{O}_2\)) was measured continuously during exercise using an on-line gas analysis system (Metamax, Cortex). The test began with a 4-min stage at a treadmill speed of 10 km/h followed by 2-min stages at 12, 14, and 16 km/h. After completion of the 16 km/h stage, the treadmill inclined by 2% every 2 min thereafter until volitional exhaustion despite strong verbal encouragement. The \( V\dot{O}_2\)max was taken as the highest \( V\dot{O}_2\) value obtained in any 10-s period and was stated as being achieved by all of the following: 1) heart rate within 10 beats/min of age-predicted maximum, 2) respiratory exchange ratio > 1.1, and 3) plateau of oxygen consumption despite increasing workload. At least 24 h after completion of this test, subjects were also assessed for intermittent exercise performance using the YoYoIRT2 (27). This test consists of repeated two 20-m runs at a progressively increased running speed controlled by audio beeps from a tape recorder. Between each running bout, subjects have a 10-s rest period. When the subject twice failed to reach the finishing line in the required time, the distance covered was recorded and represented the test result. The test was performed on an indoor running surface on a 4-m-wide and 20-m-long running lane marked by cones. The coefficient of variation for test-retest reliability of this test in our laboratory is 8.8%. Each subject performed pre- and posttraining assessments of fitness at the same time of day so as to prevent any diurnal variations in performance (45).

High-intensity intermittent training protocol. The intermittent training protocol consisted of high-intensity running and was performed on a motorized treadmill (HP Cosmos). The protocol commenced with a 10-min warm-up at a running velocity corresponding to 70% of \( V\dot{O}_2\)max followed by five 3-min bouts at a running velocity corresponding to 90% of \( V\dot{O}_2\)max. The high-intensity efforts were separated by 3-min active recovery periods (1.5-min at a velocity corresponding to 25% \( V\dot{O}_2\)max followed by 1.5-min at velocity corresponding to 50% \( V\dot{O}_2\)max). Following the interval and recovery periods, subjects then performed a 10-min cool-down period at a running velocity corresponding to 70% of \( V\dot{O}_2\)max. The exercise protocol therefore gave a total of 15 min of interval exercise and 15 min of active recovery time, thus giving a total time for the intermittent training protocol of 30 min. When including the warm-up and cool-down times, the total duration of the exercise protocol was 50 min. Training intensities were increased by 5% of the original \( V\dot{O}_2\)max following 2 wk of training and by a further 5% after 4 wk of training. Heart rate was measured continuously during each training session (Polar S610i, Kempele, Finland).

Muscle biopsies and biochemical analysis. Considering that muscles of the lower limb are metabolically more active than those of the thigh during running (9), for comparative purposes we obtained biopsies from both the lateral portion of the gastrocnemius and the midportion of the vastus lateralis muscles. Samples from both muscles were obtained (\( \sim 30–50\) mg wet wt) under local anesthesia (0.5% maraine) using a Pro-Mag 2.2 biopsy gun (MD-TECH, Manan Medical Products, Northbrook, IL). Once the biopsy needle is inserted through the fascia, the “firing” of the biopsy gun operates with a feed-forward of up to 2.5–3.5 cm, depending on the angle of insertion of the needle. Previous data from our group have demonstrated that the process of serial muscle biopsies per se is insufficient to induce changes in HSP content (25). Moreover, there were \( \sim 45\) days between the pretraining and posttraining biopsy (both performed in the same limb and separated by approximately 2–3 cm), therefore further minimizing the impact of repeat biopsies on muscle protein content. Once obtained, samples were immediately frozen in liquid nitrogen and stored at \(-80^\circ\)C for later analysis.

For analysis of muscle protein content, samples from both the vastus lateralis and gastrocnemius muscles (\( \sim 10–15\) mg wet wt per muscle) were homogenized separately in a 1% solution of SDS in distilled H\(_2\)O containing protease inhibitors (33). Each sample was centrifuged at 4°C and the total protein content of the supernatant was measured using bicinchoninic acid (Sigma Chemical, Dorset, UK). This information was used to standardize the amount of sample loaded onto the gel. Fifty micromgrams of total protein was separated by SDS-PAGE using a 12% polyacrylamide gel and 4% stacking gel (National Diagnostics). Proteins were transferred onto a PVDF membrane as previously described (33). The muscle content of HSP70, HSP60, HSP27, \( \alpha\)B-crystallin, MnSOD, and PGC1-\( \alpha\) was analyzed using a panel of mouse monoclonal (HSP60, HSP70, and COXIV; Sigma, Stressgen and Abcam, respectively) or rabbit polyclonal antibodies (HSP27, \( \alpha\)B-crystallin, MnSOD, Stressgen; PGC1-\( \alpha\), Cell Signalling). Samples from each muscle group were run on separate gels and bands were visualized using an enhanced chemiluminescence detection system (Pierce, Rockford, IL, USA) and Chemi-doc image capture system with Quantity One software, where the densities of the entire band were analyzed (Bio-Rad, Hemel Hempsted, UK). Following training, the content of muscle proteins was expressed as a percentage of the pretraining content for each subject.

Muscle glycogen concentration in both the vastus lateralis and gastrocnemius muscles was determined in wet weight (from samples containing \( \sim 10–15\) mg wet wt per muscle) according to the hexokinase method (31). Muscle SDH activity was assessed in both the vastus lateralis and gastrocnemius muscles (from samples containing \( \sim 10–15\) mg wet wt per muscle) according to the method described by Kayani et al. (24) where data are presented as micromoles per minute per milligram of protein.

Venous blood samples and biochemical analysis. Blood samples were drawn from a superficial vein in the antecubital crease of the forearm using standard venipuncture techniques (Vacutainer Systems, Becton, Dickinson). Samples were collected into vacutainers containing EDTA, lithium heparin, or serum separation tubes and either stored on ice or at room temperature (serum samples, \( \sim 1\) h) until centrifugation at 2,000 rpm for 15 min at 4°C. Following centrifugation, aliquots of plasma/serum were stored at \(-80^\circ\)C for later analysis. Samples were analyzed for plasma glucose (Glucose oxidase, Instrumentation Laboratory, Monza, Italy), lactate and glycerol (Randox Laboratories, Antrim, UK), NEFA (NEFA-C, Wako Chemicals, Neuss, Germany), and serum insulin (Insulin ELISA, DRG Instruments) concentration. All postexercise blood samples were corrected for plasma volume changes according to Dill and Costill (11).

Statistical analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences software program (version 15). Changes in \( V\dot{O}_2\)max, YoYoIRT2, and resting muscle characteristics (i.e., stress/mitochondrial protein content, SDH activity, and muscle glycogen concentration) were assessed using a two-way mixed-design repeated-measures general linear model where the within factor was time (pretraining vs. posttraining) and the between factor was training condition (Low vs. Pla vs. Norm). Changes in the metabolic cost of the criterion exercise session were assessed using a three-way mixed-design repeated-measures general linear model where the within factors were acute exercise response (preexercise vs. postexercise) and training status (pretraining vs. posttraining) and the between factor was training condition (Low + Glu vs. Low + Pla vs. Norm). Where there was a significant interaction effect, Bonferroni post hoc tests were used to locate the differences. All data in text, figures, and tables are presented as means (SD) with \( P \) values \( \leq 0.05 \) indicating statistical significance.
When data from all subjects were pooled, there was a
index) with no difference between training conditions.

RESULTS

Changes in physiological fitness following training. A comparison of each group’s VO2 max, time to exhaustion on the VO2 max test, and distance covered on the YoYoIRT2 before and after training are displayed in Table 4. Training improved all performance parameters in all groups (P = 0.001 for all indexes) with no difference between training conditions. When data from all subjects were pooled, there was a significant correlation between VO2 max and distance covered on the YoYoIRT2 before and after training (P = 0.007 and 0.02, respectively).

Skeletal muscle adaptations following training. The posttraining protein content of HSP70, HSP60, HSP27, αB-crystallin, MnSOD, COXIV, and PGC1-α in the gastrocnemius muscle of each group. Data are expressed as a percentage of pretraining values. *Significant main effect of training, P < 0.05.

Table 4. VO2max, run time to exhaustion on VO2max test and YoYoIRT2 test performance of each group pre- and posttraining

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2max, ml·kg−1·min−1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low + Glu</td>
<td>51.4±4.7</td>
<td>56.5±5.7</td>
<td>10±6*</td>
</tr>
<tr>
<td>Low + Pla</td>
<td>56.6±6.7</td>
<td>61.8±4.1</td>
<td>10±8*</td>
</tr>
<tr>
<td>Norm</td>
<td>56.9±7.3</td>
<td>60.8±5</td>
<td>8±2*</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low + Glu</td>
<td>4.0±0.23</td>
<td>4.4±0.4</td>
<td>9±2*</td>
</tr>
<tr>
<td>Low + Pla</td>
<td>4.1±0.57</td>
<td>4.4±0.39</td>
<td>9±2*</td>
</tr>
<tr>
<td>Norm</td>
<td>4.2±0.5</td>
<td>4.4±0.52</td>
<td>7±2*</td>
</tr>
<tr>
<td>Time to exhaustion, min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low + Glu</td>
<td>8.3±1</td>
<td>10.2±0.9</td>
<td>24±11*</td>
</tr>
<tr>
<td>Low + Pla</td>
<td>8.7±1.8</td>
<td>10.5±1.4</td>
<td>22±13*</td>
</tr>
<tr>
<td>Norm</td>
<td>8.5±1.2</td>
<td>10.9±1.4</td>
<td>24±13*</td>
</tr>
<tr>
<td>YoYoIRT2, m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low + Glu</td>
<td>400±95</td>
<td>463±113</td>
<td>16±15*</td>
</tr>
<tr>
<td>Low + Pla</td>
<td>411±130</td>
<td>474±139</td>
<td>17±10*</td>
</tr>
<tr>
<td>Norm</td>
<td>393±132</td>
<td>480±199</td>
<td>20±26*</td>
</tr>
</tbody>
</table>

Values are means ± SD. VO2max, maximal oxygen uptake; YoYoIRT2, Yo-Yo Intermittent Recovery Test 2. *Significant main effect of training, P < 0.05.

Effects of training on the physiological and metabolic cost of a criterion exercise session. Muscle glycogen utilization of the vastus lateralis and gastrocnemius muscles during the criterion exercise session before and after training is shown in Fig. 5, A and B, respectively. Training reduced glycogen utilization in the gastrocnemius muscle (P = 0.04) in the vastus lateralis muscle (P = 0.01), MnSOD (P = 0.001), COXIV (P = 0.01), and PGC1-α content (P = 0.01) in the gastrocnemius muscle. Similarly, all of these proteins increased with training in the vastus lateralis muscle (HSP70, P = 0.01; HSP60, P = 0.02; αB-crystallin, P = 0.003; COXIV, P = 0.001; MnSOD, P = 0.001; PGC1-α, P = 0.01). There was, however, no difference between groups in the magnitude of the training-induced increase in the aforementioned proteins in either muscle. In contrast, HSP27 showed no change following training in either muscle.

Training increased SDH activity in both the gastrocnemius (P = 0.001) and vastus lateralis (P = 0.001) muscles in all groups (see Fig. 4, A and B, respectively). Specifically, SDH activity increased by 27, 76, and 53% in the gastrocnemius muscle for the Low and Pla, and Norm conditions, respectively. In the vastus lateralis muscle, SDH activity increased by 17, 70, and 19% in the gastrocnemius muscle for the Low and Pla, and Norm conditions, respectively. Furthermore, the training-induced increases in SDH activity of the gastrocnemius (P = 0.03) and vastus lateralis (P = 0.04) were significantly larger for subjects training in Low and Pla compared with the other conditions.

The responses of the blood metabolites to the criterion exercise session before and after training are shown in Table 5. Acute exercise increased plasma glucose (P = 0.03), lactate...
P/H11005 0.001), NEFA (P/H11005 0.001), and glycerol (P/H11005 0.001) and decreased serum insulin (0.002) in both the untrained and trained state. However, only the response of plasma lactate changed with training (P/H11005 0.001) where lactate values were lower following training. There was no difference between groups for any blood metabolite measured.

**DISCUSSION**

The primary aim of the present study was to test the hypothesis that training in conditions of reduced carbohydrate availability from both endogenous and exogenous sources augments the training-induced adaptation of the major HSP families in human skeletal muscle. Given recent studies examining the influence of carbohydrate availability on training-induced athletic performance (17, 54), our secondary aim was to investigate the role of reduced carbohydrate availability on markers of oxidative capacity and exercise performance. Using an ecologically valid exercise training protocol, our data provide novel findings as we have shown no effect of either endogenous or exogenous carbohydrate availability on modulating training-induced increases in HSP content of skeletal muscle. Furthermore, our data confirm and extend the recent findings of Yeo et al. (54) by utilizing a differing training intervention (i.e., high-intensity running vs. cycling) and also demonstrating that reduced muscle glycogen availability augments training-induced increase in oxidative enzyme activity in both vastus lateralis and gastrocnemius muscles. Finally, our data are also novel as we show that consuming exogenous glucose when glycogen stores are low can offset this enhanced training adaptation.

HSPs are a family of highly conserved chaperone-like proteins that protect against contraction-induced damage (32) and are also thought to be involved in facilitating the general remodeling processes inherent to exercise training (38). HSPs are upregulated in response to a multitude of cellular stressors, including hyperthermia, oxidative stress, acidosis, increased intracellular calcium, and energy depletion (49). Given that these stressors are similar to the homeostatic perturbations occurring in contracting skeletal muscle, it is difficult to isolate the precise stressor(s) that is responsible for initiating the exercise-induced induction of HSPs in humans. Using a two-legged knee extensor model in which one limb performed the exercise protocol under conditions of reduced muscle glyco-
In reviewing the present data in combination with existing literature, it is increasingly apparent that contraction-derived reactive oxygen species (ROS) may be the primary activator of the HSP response during endurance-type exercise protocols as opposed to energy depletion (14) or increases in contracting muscle temperature per se (41). Indeed, a series of studies in humans from our group (22, 25, 26) and others (15) have demonstrated that the exercise-induced upregulation of HSP70 and HSP60 is abolished or attenuated following antioxidant supplementation. In this regard, it is unlikely that carbohydrate availability would have modified the extent of redox signaling between the training conditions employed here. Evidence in support of this view is provided by the finding of a similar training-induced increase in MnSOD content of the gastrocnemius and vastus lateralis muscles between groups. It is suggested that ROS may mediate the exercise-induced induction of HSPs by an indirect effect of oxidation of muscle proteins (33) or a direct effect on heat shock transcription factor 1 (1).

In relation to our second aim, both Hansen et al. (17) and Yeo et al. (54) observed that training under conditions of reduced muscle glycogen availability induces significantly greater increases in citrate synthase activity and resting muscle glycogen concentration of the vastus lateralis muscle (of recreationally active and elite subjects, respectively). The latter authors, however, did not observe improved exercise performance (power output on a 60-min cycle test) as a result of these enhanced metabolic adaptations. The present data confirm and extend these findings as we show (using a high-intensity intermittent running training protocol) that performing 50% of training sessions with reduced muscle glycogen stores induces significantly greater increases in oxidative enzyme activity (as evidenced by SDH activity) of both the vastus lateralis and gastrocnemius muscles, which also does not translate to improvements in high-intensity exercise performance. The enhanced oxidative enzyme activity with no concomitant increase in performance is, however, not surprising given that high-intensity exercise performance is not likely to be limited by enzymes of the tricarboxylic acid cycle. The discrepancy between the previous two studies and no increased resting glycogen concentration in the Low + Pla subjects studied here may be related to subtle differences in methodological design between studies. Before their pretraining biopsy, subjects consumed their habitual diet for 2 days, and this was subsequently photocopied for the subjects to adhere in the 2 days before their posttraining biopsy. Dietary analysis on these diets indicated that the average carbohydrate content per subject was ~45% of 6.5 MJ. The finding of no increased resting glycogen content in Low + Pla subjects may therefore be related to this dietary protocol and the relatively low amount of carbohydrate consumed.

The increased SDH activity with no increase in COXIV protein is somewhat surprising and may be related to variations in mitochondrial location between samples used for each analysis procedure. Indeed, it is known that SDH activity is twofold greater than COX activity in subsarcolemmal (SS) compared with intermyofibrillar (IMF) mitochondria (8), thus potentially reflecting mitochondria at different stages of biogenesis. Also, SDH activity in the SS region of human vastus lateralis muscle is more responsive to endurance training than IMF region (5).
Our data also provide novel findings as we show that consuming exogenous carbohydrate in the specific low-glycogen training sessions can offset the apparent enhanced training response. This finding agrees with data from acute exercise demonstrating that carbohydrate ingestion during exercise blunts the expression of metabolic genes related to oxidative metabolism (7). Our data are also in agreement with a recent longitudinal training study demonstrating that increased glucose availability during training (achieved via consumption of a high-carbohydrate preexercise meal and consuming glucose during exercise) can attenuate oxidative adaptations of human skeletal muscle (10). Indeed, these authors observed that increased glucose availability before and during each training session completely inhibited the training-induced upregulation of FABPm content (10), a key protein involved in fatty acid metabolism.

To offer some mechanistic insight to the finding of enhanced SDH activity for subjects in Low + Pla, we also measured protein content of PGC1-α before and after training, given its proposed role as a “master” regulator of mitochondrial biogenesis (43). However, in agreement with Yeo et al. (54) our data do not provide definitive support for this pathway as we observed no effect of carbohydrate availability during training on muscle PGC1-α protein content. Furthermore, while Cluberton et al. (7) observed an attenuating effect of glucose ingestion during exercise on oxidative metabolic genes, they observed no effect of carbohydrate on muscle PGC1-α/H9251 expression. Berton et al. (7) observed an attenuating effect of glucose on muscle PGC1-α/H9251 expression. Our data provide novel findings as we show that carbohydrate ingestion during exercise on oxidative metabolic genes, they observed no effect of carbohydrate availability during training and consuming glucose during exercise can attenuate oxidative adaptations of human skeletal muscle (10). Indeed, these authors observed that increased glucose availability before and during each training session completely inhibited the training-induced upregulation of FABPm content (10), a key protein involved in fatty acid metabolism.

The discrepancy between muscles may be related to an enhanced sensitivity of the gastrocnemius muscle to detect local metabolic adaptations to this mode of exercise, likely due to the greater recruitment of the gastrocnemius muscle during running (9). Indeed, consistent with the only other invasive study to compare responses from both muscles (9), we also observed glycogen utilization to be significantly (P = 0.02) higher (−30%) in the gastrocnemius compared with the vastus lateralis muscle. Given that postraining SDH activity was significantly higher for subjects in the Low + Pla condition, one may also expect that the training-induced reduction in muscle glycogen utilization would be more pronounced in these subjects. However, despite the enhanced activity of oxidative enzymes, lipid oxidation cannot be concomitantly increased during high-intensity exercise (28).

In summary, the present data provide the first report that in whole body high-intensity exercise conditions, carbohydrate availability during training has no modulating effect on the training-induced upregulation of the heat shock protein family in human skeletal muscle. In contrast, training with reduced preexercise muscle glycogen levels and without the provision of exogenous carbohydrate provides an enhanced stimulus for inducing beneficial oxidative enzyme adaptations of skeletal muscle.

ACKNOWLEDGMENTS

We thank all of the subjects who took part in the study for their outstanding efforts throughout the intensive training period. We also extend our appreciation to Dave Stokes and Padraic Phibbs for technical assistance during data collection. Finally, we thank Professor Greg Atkinson for help with all statistical analysis.

GRANTS

This study was supported by a research grant from GlaxoSmithKline Nutritional Healthcare (UK).

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