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David Bishop, Johann Edge, Claire Thomas, Jacques Mercier. High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. *Journal of Applied Physiology*, American Physiological Society, 2007, 102 (2), pp.616 - 621. <10.1152/jappphysiol.00590.2006>. <hal-01585662>

HAL Id: hal-01585662

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Submitted on 11 Sep 2017

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High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle

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Bishop D, Edge J, Thomas C, Mercier J. High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. *J Appl Physiol* 102: 616–621, 2007. First published November 2, 2006; doi:10.1152/jappphysiol.00590.2006.—The regulation of intracellular pH during intense muscle contractions occurs via a number of different transport systems [e.g., monocarboxylate transporters (MCTs)] and via intracellular buffering ($13m_{in\ vitro}$). The aim of this study was to investigate the effects of an acute bout of high-intensity exercise on both MCT relative abundance and $13m_{in\ vitro}$ in humans. Six active women volunteered for this study. Biopsies of the vastus lateralis were obtained at rest and immediately after 45 s of exercise at 200% of maximum O_2 uptake. $13m_{in\ vitro}$ was determined by titration, and MCT relative abundance was determined in membrane preparations by Western blots. High-intensity exercise was associated with a significant decrease in both MCT1 (-24%) and MCT4 (-26%) and a decrease in $13m_{in\ vitro}$ (-11%; 135 ± 3 to 120 ± 2 $\mu\text{mol H}^+\cdot\text{g dry muscle}^{-1}\cdot\text{pH}^{-1}$; $P < 0.05$). These changes were consistently observed in all subjects, and there was a significant correlation between changes in MCT1 and MCT4 relative abundance ($R^2 = 0.92$; $P < 0.05$). In conclusion, a single bout of high-intensity exercise decreased both MCT relative abundance in membrane preparations and $13m_{in\ vitro}$. Until the time course of these changes has been established, researchers should consider the possibility that observed training-induced changes in MCT and $13m_{in\ vitro}$ may be influenced by the acute effects of the last exercise bout, if the biopsy is taken soon after the completion of the training program. The implications that these findings have for lactate (and H^+) transport following acute, exhaustive exercise warrant further investigation.

buffer capacity; lactate transport; hydrogen ions

THE REMOVAL OF H^+ DURING INTENSE skeletal muscle contractions occurs via a number of different transport systems and via intracellular buffering (23, 24). Intracellular buffer capacity is typically estimated by titrating a muscle homogenate with a fixed acid ($13m_{in\ vitro}$) and measures the contribution of physicochemical buffering by proteins, dipeptides, and phosphates within the muscle (i.e., nonbicarbonate buffering). Lactate uptake and release is largely mediated by monocarboxylate transporters (MCTs) that are stereo-selective for L-(+)-lactate and pH dependent (25, 35). Sarcolemmal lactate transporters have also been suggested to have an important role in the regulation of intracellular pH (23).

Although 14 MCTs are known to exist (21), lactate/ H^+ transport in skeletal muscle is currently attributed to two

isoforms (MCT1 and MCT4; Refs. 25, 33, 45). Chronic skeletal muscle activity exerts a strong influence on the expression of MCTs. Inactivity (denervation and hindlimb suspension) reduces MCT1 and MCT4 expression (45), while training increases MCT1 and MCT4 expression (4, 33). This altered density of membrane transporter proteins has been reported to be of functional importance for the translocation of H^+ and lactate from the muscle to the blood during high-intensity exercise (22). Training has also been reported to increase $13m_{in\ vitro}$ (2, 14–16, 39).

In contrast to the many training studies, very few studies have investigated the effects of an acute bout of muscle activity on MCT abundance. However, increases in MCT have been observed 2–6 days after a 5- to 6-h training session in humans (19) and immediately following a 2-h treadmill session in rats (8). Changes in MCT following an acute bout of high-intensity muscle activity are more controversial. In the only study to date, it was reported that, in rats, immediately following 10 min of high-intensity electrical stimulation, there was a decrease in the plasma membrane MCT1 (-10%) and MCT4 (-25%) (44). However, in an additional experiment (conducted as part of the same study), the 10-min contraction period reduced MCT4 (-20%), but not MCT1. To date, the results of this study have not been replicated in humans or in response to high-intensity exercise.

The aim of this study was to investigate the effects of an acute bout of high-intensity exercise on MCT relative abundance in humans. We hypothesized that a high-intensity exercise stimulus would decrease the relative abundance of both MCT1 and MCT4 in human skeletal muscle.

METHODS

Subjects. Six active women [age: 19 ± 3 yr, mass: 63.1 ± 3.2 kg, peak O_2 uptake ($\dot{V}O_{2\ peak}$): 46.7 ± 3.2 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$] volunteered to participate in the study. Each subject was involved in an intermittent sport at club level (2–3 times/wk of hockey, netball, tennis, basketball, or football). All of the subjects completed a familiarization trial of a graded exercise test (GXT) and a 45-s constant-intensity exercise test (200% $\dot{V}O_{2\ peak}$; CIT₄₅) before the main trial (where muscle biopsies were taken pre- and immediately postexercise). Subjects were informed of the study requirements, benefits, and risks before giving written, informed consent. Approval for the study's procedures was granted by the University of Western Australia Research Ethics Committee. Subjects were required to consume no food or beverages (other than

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water) 2 h before testing and were asked not to consume alcohol or caffeine, or to perform vigorous exercise, in the 24 h before testing.

Graded exercise test. The graded exercise test (GXT) was performed on an air-braked track-cycle ergometer (Evolution Pty., Adelaide, Australia) and consisted of graded exercise steps (4-min stages), using an intermittent protocol (1-min break between stages). The test commenced at 50 W, and, thereafter, intensity was increased by 30 W every 4 min until volitional exhaustion.

Gas analysis during the GXT. During the GXT, expired air was continuously analyzed for O₂ and CO₂ concentrations using Ametek gas analyzers (SOV S-3A11 and COV CD-3A, Applied Electrochemistry, Pittsburgh, PA). Ventilation was recorded every 15 s using a turbine ventilometer (225A, Morgan, Kent, UK). The gas analyzers were calibrated immediately before and verified after each test using three certified gravimetric gas mixtures (BOC Gases, Chatswood, NSW, Australia); the ventilometer was calibrated preexercise and verified postexercise using a 1-liter syringe, in accordance with the manufacturer's instructions. The ventilometer and gas analyzers were connected to an IBM PC, which measured and displayed variables every 15 s. The sum of the two highest consecutive 15-s values was recorded as the subject's $\dot{V}O_{2\text{ peak}}$.

CIT₄₅. The high-intensity cycle test consisted of 45 s of continuous cycling at a set power output (408 ± 34 W) on an air-braked, front-access cycle ergometer (model Ex-10, Repco). Toe clips and heel straps were used to secure the feet to the pedals, and the test was performed in the seated position. Strong verbal encouragement was provided to each subject during the test. The power output for the CIT₄₅ was set as 200% of the mean power output at $\dot{V}O_{2\text{ peak}}$ intensity.

Muscle sampling and analysis. On the day of the CIT₄₅ cycle test, incisions were made under local anesthesia (5 ml, 1% xylocaine) into the vastus lateralis of each subject (midway between the anterior, superior iliac spine, and the patella). The first muscle sample was taken (before warm up) during supine rest. The second muscle sample was taken immediately (within 10 s) following the cessation of the CIT₄₅, while the subject remained on the cycle ergometer. The samples were then removed from the biopsy needle and immediately stored at -80°C until subsequent analysis.

ATP, PCr, and La⁻ determination. Freeze-dried rest and postexercise muscle samples (2–3 mg) were dissected free from visible blood, fat, and connective tissue and then enzymatically assayed for ATP, phosphocreatine (PCr), and lactate (La⁻) content. ATP, PCr, and La⁻ were extracted from muscle samples by the addition of 6% perchloric acid, before being centrifuged (10,000 g × 10 min). The supernatant was removed and neutralized by the addition of 2.4 mol/l KOH and 3 mol/l KCl. Samples were centrifuged again, and the supernatant was stored at -80°C. ATP, PCr, and La⁻ were measured as previously described (1).

Nonbicarbonate muscle buffering capacity; titration method (13m_{in vitro}) and H⁺. Muscle samples were weighed pre- and post-freeze drying to determine water content. Freeze-dried muscle samples (1.8–2.5 mg) were then homogenized on ice for 2 min in a solution containing sodium fluoride (10 mM) at a dilution of 30 mg dry muscle (dm)/ml of homogenizing solution (29). The pH measurements were made at 37°C with a microelectrode (MI-415, Microelectrodes, Bedford, NH) connected to a pH meter (SA 520, Orion Research, Cambridge, MA). After initial pH measurement, muscle homogenates for both pre- and postexercise samples were adjusted to a pH of 7.2 with a sodium hydroxide (0.02 M) solution and then titrated to a pH of 6.2 by the serial addition of 2 µl of hydrochloric acid (10 mM). From the fitted titration trend line, the number of moles of H⁺ (per gram dm) required to change the pH from 7.1 to 6.5 was interpolated. This value was then normalized to the whole pH unit for final display as micromoles H⁺ per gram dm per unit pH (µmol H⁺·g muscle dm⁻¹·pH⁻¹) and determined as the subject's 13m_{in vitro}.

Sample preparation for Western blotting. Proteins were isolated from muscles for Western blotting by a standard method previously described by McCullagh et al. (30) with slight modifications that our

laboratory has previously described (10, 43). Briefly, total muscle membranes were obtained from 40 mg of frozen skeletal muscle and gently homogenized, using a glass mortar and pestle, in 1 ml of ice-cold buffer (in mM: 30 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4, 210 sucrose, 2 ethylene glycol-bis(13-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 40 NaCl, and 5 EDTA). The homogenate was then centrifuged at 800 g for 10 min at 4°C to remove red blood cells. The supernatant was subsequently mixed with 750 µl of 1.167 M KCl plus 58.3 mM pyrophosphate and centrifuged at 40,000 g for 105 min at 5°C to remove myosin and contractile proteins. The pellet was solubilized in 16.5 µl of 16% SDS and 50 µl of 10 mM Tris and 1 mM EDTA, pH 7.4. Total membranes were recovered by centrifugation at 20°C for 20 min at 1,100 g. The supernatant was stored in aliquots at -70°C for subsequent determinations of protein and Western blotting. Muscle protein concentrations were determined in duplicate by bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with the use of BSA as a standard. There was no significant difference between pre- and postexercise muscle samples for the amount of muscle homogenized (33.7 ± 7.8 vs. 39.6 ± 10.7 mg; *P* > 0.05) or the normalized protein yield (6.7 ± 1.4 vs. 6.5 ± 1.9 mg/g tissue; *P* > 0.05).

Western blotting of the MCT1 and MCT4. MCT abundance was determined using previously described methods (43). Polyclonal antibodies yielded a single band on a Western blot that corresponded to 43 kDa, consistent with the molecular mass reported earlier (30). Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4. Membranes were washed as previously described, and MCT1 or MCT4 expression was detected by enhanced chemiluminescence (Biomax MR films, Kodak, Reuil-Malmaison, France). Films were developed and processed using a Hyperprocessor, RNP 1700 (Amersham, Les Ulis, France). MCT1 and MCT4 protein band densities were determined by scanning the blots on a scanner (AGFA Duo Scan T1200, New York, NY) and Scion Image software (Scion, Frederick, MD). Results were expressed in arbitrary optical density units, as used by others (43).

Statistical analysis. All values are reported as means ± SD. One-way ANOVA with repeated measures for time were used to test for main effects for measurements. Least squares linear regression analysis was used to calculate correlation coefficients between changes in MCT1, MCT4, and 13m_{in vitro}, using Pearson's product moment (*r*). Significance was accepted at *P* < 0.05.

RESULTS

Muscle metabolites and pH. Muscle metabolite and pH data measured before and after the 45-s cycle test are summarized in Table 1. Postexercise, there was a significant decrease in ATP, PCr, and pH and a significant increase in La⁻. There were no significant changes in muscle water content from pre- to postexercise (77.0 ± 0.4 to 76.9 ± 0.3%; *P* > 0.05).

MCT1/MCT4 and 13m_{in vitro}. High-intensity exercise was associated with a significant decrease in both MCT1 (24%, Fig. 1) and MCT4 relative abundance (26%, Fig. 2). Individual

Table 1. Skeletal muscle metabolites and pH at rest and immediately after the 45-s cycle test

Variable	Preexercise	Postexercise
ATP, mmol/kg dm	20.3±2.0	14.7±2.2*
PCr, mmol/kg dm	82.6±12.3	49.0±14.2*
La ⁻ , mmol/kg dm	6.4±2.4	70.0±21.2*
pH	7.11±0.07	6.84±0.14*

Values are means ± SD. PCr, phosphocreatine; La⁻, lactate; dm, dry muscle. *Significantly different from preexercise (*P* < 0.05).

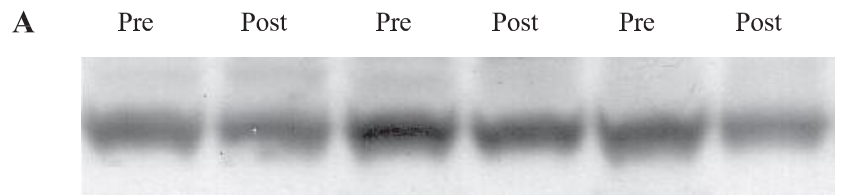
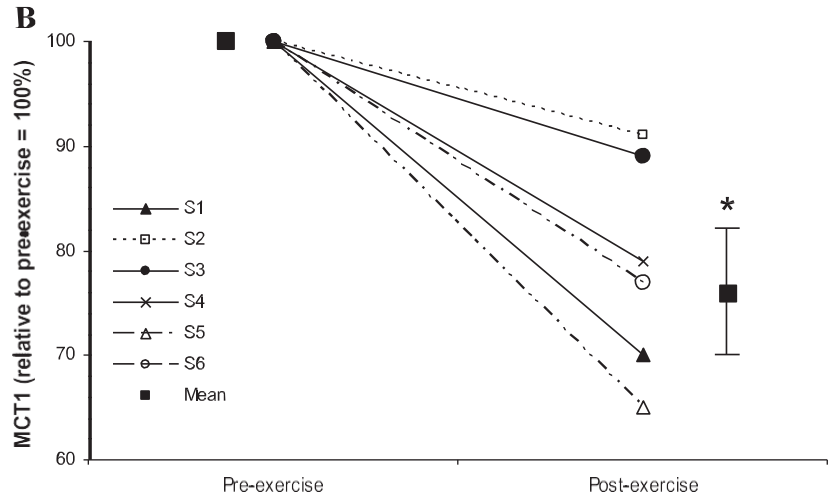


Fig. 1. A: representative monocarboxylate transporter (MCT) 1 blots in skeletal muscle biopsies taken from women before (Pre) and immediately after (Post) 45 s of high-intensity exercise. B: means \pm SD and individual changes in MCT1 relative abundance pre- and postexercise. S1–S6, subjects 1–6. *Significant difference between pre- and post-exercise ($P < 0.05$).



changes in both MCT1 and MCT4 are also plotted in Figs. 1 and 2, respectively. There was a significant correlation between changes in MCT1 and MCT4 relative abundance ($R^2 = 0.92$; $P < 0.05$).

Individual changes in $13m_{in\ vitro}$ (along with mean values) are shown in Fig. 3. High-intensity exercise was associated with a significant decrease in $13m_{in\ vitro}$ (135.4 ± 10.8 to $119.2 \pm 5.1 \mu\text{mol H}^+ \cdot \text{g dm}^{-1} \cdot \text{pH}^{-1}$; $P < 0.05$). There were no significant correlations between changes in MCT1 and MCT4 relative abundance and changes in $13m_{in\ vitro}$ ($P > 0.05$).

DISCUSSION

This is the first study to report changes in MCTs and the nonbicarbonate, physicochemical buffer capacity following an acute bout of high-intensity exercise (45 s at $200\% \dot{V}O_{2\ peak}$) in humans. The observed large changes in muscle metabolites support the intense nature of the chosen protocol. The main results are that high-intensity exercise was associated with a significant decrease in both MCT1 (-24%) and MCT4 (-26%) and a decrease in $13m_{in\ vitro}$ (-11%).

Despite limited research, it appears that prolonged exercise (>2 h) can acutely increase MCT relative abundance in rats (8) and in humans (19). In contrast, in the only published study to date, 10 min of high-intensity electrical stimulation in rats was reported to decrease sarcolemmal MCT1 (-10%, $P < 0.05$) in one experiment but not another (percentage not reported, $P > 0.05$) (44). There was also a consistent reduction in MCT4 (20–25%, $P < 0.05$) in both experiments, which was of a similar magnitude to that observed in the present study. Thus our findings are consistent with the only previous rat study and support the hypothesis that brief, high-intensity exercise can acutely decrease relative MCT abundance in humans.

While 45 s represents a very brief stimulus, a recent study, using quantitative proteomics, has reported an acute decrease in the level of another protein (creatine kinase) in response to only 3 min of intense swimming exercise in rats (20). Furthermore, increases in enzyme activities of 200–2,500% have been reported in humans after 10 min of moderate-intensity exercise (27). In addition, the exercise-induced activation of enzymes reported by Krook et al. (27) was limited to working muscle, indicating that local rather than systemic factors activate these signaling cascades. Therefore, the combined results of these studies, along with the previous findings in rats (44), suggest that muscle proteins are able to respond to local changes in the muscle, during high-intensity exercise, within the time frame observed in our study.

Dismissing the 10% decrease in MCT1 in one part of their experiment, Tonouchi et al. (44) proposed that no change in MCT1 and an acute decrease in MCT4 could be attributed to their earlier observation that MCT4, but not MCT1, exists in an intracellular pool (5). Thus they hypothesized that MCT4, but not MCT1, may translocate to an intracellular pool. To date, no other research group has verified the existence of intracellular pools of MCT4 (or MCT1). However, other similar sized proteins (e.g., GLUT-4) do exist in intracellular pools and have been reported to reversibly translocate following exercise (26). While the existence of intracellular pools could not be verified from our protein preparation, it is possible that an intracellular pool of MCT1 is yet to be discovered or that changes in MCT can occur without a preexisting intracellular pool. A decrease in plasma membrane MCT1 has previously been reported to occur without the detection of a preexisting intracellular pool (44).

Loss of lactate transporters could also result from free-radical-induced lipid peroxidation (11). Exhaustive exercise

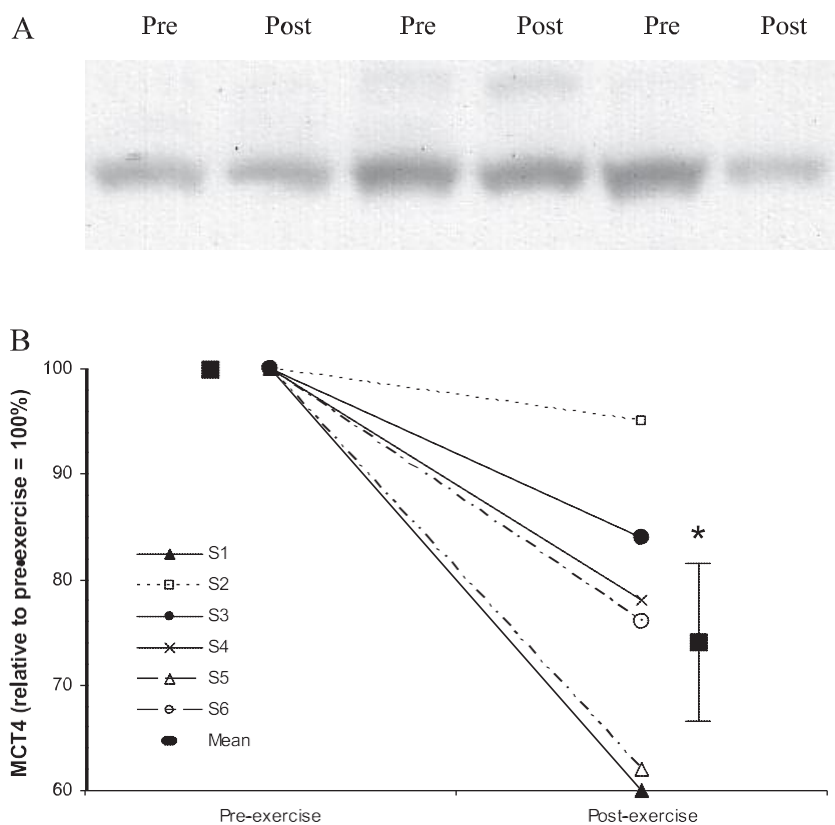


Fig. 2. A: representative MCT4 blots in skeletal muscle biopsies taken from women before and immediately after 45 s of high-intensity exercise. B: means \pm SD and individual changes in MCT4 relative abundance pre- and post-exercise. *Significant difference between pre- and postexercise ($P < 0.05$).

increases free-radical concentration (9), and it has been suggested that peroxidation of polyunsaturated fatty acids may lead to fluidity and permeability alterations in the membrane (9, 38). The decrease in the relative abundance of MCT could, therefore, result from its release out of muscle due to exercise-induced muscle damage.

The short time frame in which these changes occurred raises the possibility that our results represent an artifactual effect due to a contraction-induced vascular fluid shift into muscle (36, 40) or an alteration in the degree of protein extraction (34).

However, an acute change in muscle water content appears unlikely to explain our results, as the same amount of protein was loaded on all gels and there was no significant change in muscle water content. While previous human studies have reported significant exercise-induced increases in muscle water content (77–79%), these studies have involved exhaustive exercise of much longer duration than the present study (6–15 min; Refs. 28, 36, 40).

Alternatively, changed levels of a given protein could result from an alteration in the degree of protein extraction, since the

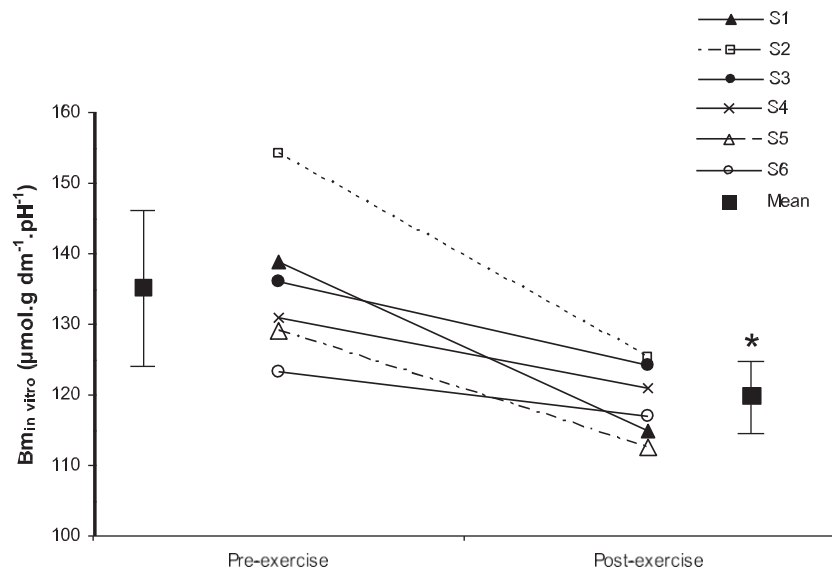


Fig. 3. Individual changes in titrated muscle buffer capacity [$B_{m_{in\ vitro}}$; $\mu\text{mol} \text{H}^+ \cdot \text{g} \text{muscle dry muscle (dm)}^{-1} \cdot \text{pH}^{-1}$] in skeletal muscle biopsies taken from women before and immediately after 45 s of high-intensity exercise. Also plotted are the mean values. *Postexercise mean significantly different from preexercise mean ($P < 0.05$).

degree of extraction of some muscle proteins is sensitive to muscle physiological state and level of activity (34). While the protein isolation technique that we used (30) is a well-established technique, which has been used in many published studies, it does involve fractionation steps with several steps of centrifugation and isolation of supernatant and unquestionably results in the incomplete recovery of protein. More importantly, it is not known if prior high-intensity exercise affects the recovery of MCT proteins using this technique. As the only other study to report an acute decrease in MCT also used this technique (44), we cannot exclude the possibility that these results reflect a reduced recovery of MCT protein postexercise. However, the absence of a significant difference in the concentration of protein isolated from pre- and postexercise muscle samples strongly argues against this possibility. Furthermore, if there were a decrease in MCT protein extraction postexercise, it would have to have been matched by a similar increase in the extraction of other unknown proteins, an unlikely scenario in our view.

The physiological significance of these findings is unclear, as the relationship between MCT abundance and lactate transport is complex. It has previously been reported that the rate of lactate flux into and out of the muscle is correlated with the content of MCT1 and MCT4 in the muscle (5, 6, 30, 31). Thus the decrease in MCT1 and MCT4 in the present study may be expected to decrease rates of lactate (and H⁺) removal from the cell. Furthermore, a decrease in lactate transport capacity has been reported following exhaustive exercise (11, 18). However, concomitant changes in lactate transport cannot be assumed in the present study, as changes in MCT1 and MCT4 content have not always been strongly associated with changes in muscle lactate concentration (19) or lactate transport (17, 44).

While previous research has investigated changes in 13m_{in vitro} in response to training (2, 15, 32), this is the first study to report a decrease in 13m_{in vitro} (-11%) following an acute bout of exercise in humans. The mechanisms underlying the acute decrease in 13m_{in vitro} cannot be determined from the results of this study, but are likely to be due to changes in the intracellular buffers that are measured using the *in vitro* titration technique (i.e., phosphates, proteins, and dipeptides). It has previously been reported that acute, intense exercise results in a small (◆10%), but significant, loss of carnosine from the muscle (13). Furthermore, a significant increase (+20%) in plasma carnosine was observed following severe exercise in horses (12), indicating a loss of carnosine from the muscle. Due to its capacity to buffer H⁺ within the typical physiological range experienced during high-intensity exercise (pK_a = 6.83), carnosine is an important intracellular buffer, and any loss from the muscle should cause a decrease in 13m_{in vitro}. Another potential mechanism underlying the decrease in 13m_{in vitro} observed in the present study is a decrease in protein buffering, as metabolically induced acidosis has been reported to increase protein breakdown (7). It appears unlikely that the decrease in 13m_{in vitro} can be attributed to an acute change in muscle phosphate concentration following high-intensity exercise. It has previously been reported that there is no significant decrease in the total adenine nucleotide pool (ATP + ADP + AMP) plus IMP following a 30-s maximal sprint (42) and no significant change in total muscle phosphate following exercise to exhaustion at 75% of maximum O₂ uptake (44–108 min) (37).

In conclusion, a single bout of high-intensity exercise decreased the membrane content of both MCT1 and MCT4 and also 13m_{in vitro}. Future studies should verify our findings and also examine the time course of the acute changes that we have reported. Until the time course of these changes has been established, researchers should consider the possibility that observed training-induced changes in MCT and 13m_{in vitro} may be influenced by the acute effects of the last exercise bout, if the biopsy is taken soon after the completion of the training program. Further research is also required to investigate the implications that these findings have for lactate (and H⁺) transport following acute, exhaustive exercise.

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