



## Further glycogen decrease during early recovery after eccentric exercise despite a high carbohydrate intake

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■ **Summary** *Background* Delayed onset muscle soreness (DOMS) is a well-known phenomenon of athletes. It has been reported from muscle biopsies that the rate of muscle glycogen resynthesis is reduced after eccentric compared to concentric exercise. *Aim of the study* Try to compensate by a carbohydrate (CHO)-rich diet the decelerated glycogen resynthesis after eccentric exercise, measured by magnetic resonance spectroscopy. *Methods* Glycogen, phosphocreatine, ATP, and Pi were measured in the human calf muscle. Twenty athletes divided into two groups (DOMS and CONTROL), reduced glycogen in M. gastrocnemius during two different running protocols. Additionally, 12 DOMS subjects performed an eccentric exercise while the CONTROL group rested. Subsequently, subjects consumed a CHO-rich diet (> 10 g/kg body mass/24 h). *Results* In both groups, glycogen has been reduced by about 50%. The first 2 h

after exercise, glycogen dropped further ( $-15.6 \pm 15.7$  mmol/kg ww) in the DOMS but rose by  $+18.4 \pm 20.8$  mmol/kg ww in the CONTROL group ( $P < 0.001$ ). CONTROL subjects reached resting glycogen within 24 h ( $137 \pm 47$  mmol/kg ww), while DOMS subjects needed more than one day ( $91 \pm 23$  mmol/kg ww;  $P < 0.001$ ). Pi and Pi/PCr, indicators of muscle injury, rose significantly in the DOMS but not in the CONTROL group. *Conclusion* The diet rich in CHO's was not able to refill glycogen stores after eccentric exercise. Glycogen decreased even further during the beginning of recovery. This loss, which to our knowledge has not been measured before is probably the consequence of muscle cell damage and their reparation.

■ **Key words** magnetic resonance spectroscopy – muscle injury – inorganic phosphate – delayed onset of muscle soreness

### Introduction

Delayed onset muscle soreness (DOMS) is caused by exercise of unaccustomed vigor or intensity, especially by exercise involving eccentric contractions [1]. Manifestations of DOMS are muscle damage including inflammation, loss in muscle strength and range of motion, swelling release of muscle proteins in the blood, and

decrement in motor control [2]. In addition, it has been reported from muscle biopsies that the rate of muscle glycogen resynthesis is reduced after eccentric compared to concentric exercise [3].

In our study, we were interested in determining if a carbohydrate (CHO)-rich diet is able to compensate for the delayed glycogen resynthesis after eccentric exercise. So far, subjects were only investigated through muscle biopsies and received less than 8.5g CHO/

kg BM/24 h during recovery in studies showing a delayed glycogen resynthesis after eccentric muscle contractions [4–9]. This lack of sufficient CHO in the diet could explain a part of the delayed glycogen resynthesis after eccentric exercise because it was shown that 10 g CHO/kg BM/24 h is necessary for a complete replenishment of glycogen stores within 24 h after glycogen-depleting concentric exercise [10–14].

As mentioned above, glycogen resynthesis studies have mainly been conducted by the method of muscle biopsies, which have a damaging effect on muscles. Therefore, the number of measurements is limited and it is not possible to closely follow the time course of the glycogen resynthesis with this method. Furthermore, it is impossible to investigate the same muscle specimen several times because muscle biopsy sampling induces focal damage (trauma) which is not completely repaired after two weeks, and also retards the rate of glycogen resynthesis [15–17], i. e., it would interfere with the aim of our study. Finally, the major muscle damage from eccentric exercise occurs in the deepest extensor muscles and may only affect a small percentage of the fibers. It would be relatively easy to miss these sites of injury in biopsy samples from human muscles [18]. The noninvasive method of  $^{13}\text{C}$ -magnetic resonance spectroscopy (MRS), which we have applied in our study, can use an adequate diameter of the surface coil for complete measurements of fibers, which are located at some distance from the body surface and are part of the analysis. This method also allows as many measurements from the same muscle specimen as it is necessary to follow the time course of glycogen resynthesis after glycogen-reducing exercise.

Eccentric exercise followed by DOMS does not only delay glycogen resynthesis but it also affects phosphate variables. Phosphocreatine (PCr), ATP, and inorganic phosphate (Pi) are destabilized after muscle fiber injuries which occur after eccentric exercise [19]. The increase in the resting Pi/PCr ratio in muscles, indicating a structural and cellular damage, reaches its peak 1 to 2 days after an exercise-induced injury as analyses with  $^{31}\text{P}$ -MRS have shown [20–23]. The Pi/PCr ratio inversely reflects the oxidative capacity of the mitochondria, which is decreased after muscle damage. In addition, a decreased metabolic efficiency of ATP may occur after eccentric contractions in human muscle [19].

The aim of our study was 1) to induce muscle soreness by eccentric exercise, 2) to investigate with  $^{13}\text{C}$ -MRS whether a CHO-rich diet can reduce a delayed glycogen resynthesis during DOMS, and 3) to measure with  $^{31}\text{P}$ -MRS phosphate variables, which possibly emerge as disturbing factors for glycogen resynthesis.

## Methods

### ■ Subjects

Twenty well-trained, non-smoking men volunteered to participate in the study. The risks and benefits were explained, and written informed consent was obtained from each subject. All subjects were instructed to refrain from exercise and to follow the nutritional guidelines during the course of the study starting 48 h before the glycogen-reducing protocol (see below) and lasting until the last MRS measurement 48 h after exercise. The University of Zurich Ethic's Committee of Physiology and Pharmacology approved the experimental protocol. The subjects were randomly assigned to one of the two groups (DOMS 12 subjects, CONTROL 8 subjects). The average ( $\pm$ SD) age, height, and weight in the DOMS group were 34 ( $\pm$ 9) years, 179.4 ( $\pm$ 5.4) cm, and 71.0 ( $\pm$ 6.2) kg and in the CONTROL group 37 ( $\pm$ 11) years, 178.9 ( $\pm$ 5.0) cm, and 73.4 ( $\pm$ 3.8) kg, respectively. None of the parameters showed any difference between the two groups.

### ■ Experimental design

At least one week before the experiment, each subject performed a Conconi test [24] on a treadmill (H-P-Cosmos, Nussdorf-Traustein, Germany). Depending on the training status, the subject started running with a velocity of 9 or 10 km/h. Every 200 m, the velocity increased by 0.5 km/h until the subject was exhausted. To supervise the cardiac frequency, each subject was equipped with a heart rate monitor (Polar Electro, Kempele, Finland). From the individual maximal velocity, we calculated the 65% velocity.

On the test day, the subjects ingested a CHO-rich breakfast and consumed their lunch (7 g CHO/kg BM), including 0.5 L of an isotonic drink, at least 1.5 h before their arrival in the laboratory at 1.30 p. m. To reduce glycogen stores in the fast twitch fibers, each subject performed several bouts of sprints. Each bout corresponded to 6 sprints of 15 m length. As soon as a subject was more than 10% below his maximal sprint velocity, the sprints were stopped. The 65% velocity of the Conconi test was then used for a 1 h tread-mill run, in order to reduce the glycogen stores in the slow twitch fibers of the calf muscles. After these glycogen-reducing protocols, 12 subjects (DOMS group) performed a single-leg toe-raise exercise to induce muscle soreness. Standing in an erect position with fully extended knees and with the forefoot on a board 7 cm thick, the subject lifted and lowered his body by plantar and dorsi flexion of the ankle joint. Each subject completed 10 bouts of 20 s toe-raise exercise (1/s) followed by 40 s of rest. To increase the intensity of this exercise, each subject had to

lift + 25% of his body mass in form of dumb-bells around the hips. Instead of the eccentric exercise, the CONTROL group rested for the same amount of time.

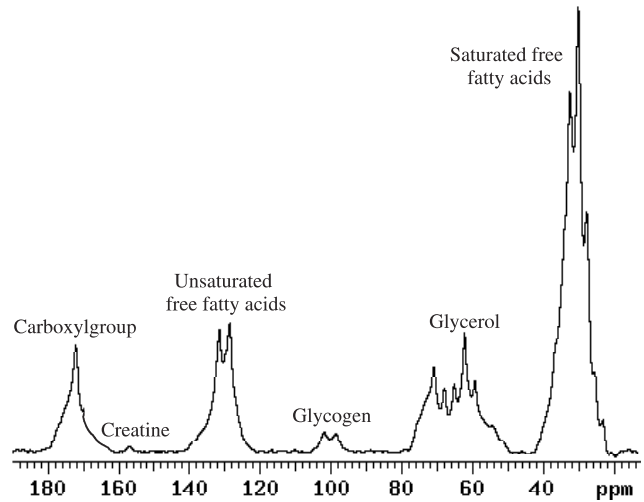
### ■ Magnetic resonance spectroscopy

A 4.7 Tesla 30 cm-bore spectrometer (Varian, Palo Alto, CA, USA) and a standard Varian spectral processing software were used for the *in vivo* MRS measurements. Two concentric, radiofrequency (rf) surface coils in 6 cm of diameter for  $^{31}\text{P}$  and of 10 cm in diameter for  $^{13}\text{C}$  were used to transmit and receive the signals. Both coils were placed under the center of the right calf muscle (*M. gastrocnemius* and *M. soleus*) of each subject and were frequency-tuned to 80.98 MHz for the  $^{31}\text{P}$ - and to 50.31 MHz for the  $^{13}\text{C}$ -MRS measurements. To assure the same coil position in the subjects for each measurement, a fit for the right calf and heel was constructed. Additionally, waterproof marks on the skin provided an exact repositioning until the end of the experiment. A sticky tape tagged each subject's leg to the fit to inhibit any movement.

Small external reference samples of phenylphosphoric acid for  $^{31}\text{P}$  and of formic acid for  $^{13}\text{C}$  were placed at the center of the two concentric coils. First, they had to control the stability of the RF signal and of its amplification during the whole experiment. Second, they optimized the RF pulse power in order to achieve a precise  $180^\circ$  flip angle in the center of the RF coil. This  $180^\circ$  pulse guaranteed that the main signal was collected from the deeper part of *M. gastrocnemius* and not the one adjacent to the coil. Before each measurement, the homogeneity of the static magnetic field was adjusted with an automatic shimming procedure ( $x$ ,  $y$ ,  $z$ ,  $x^2$ ,  $y^2$ ,  $z^2$ , and  $x^2-y^2$ ) using proton signals from water.

### ■ $^{13}\text{C}$ -spectra: acquisition and quantification

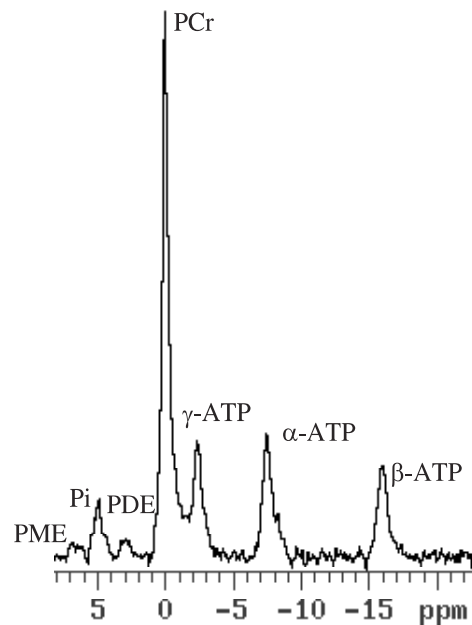
Glycogen concentration was measured in the calf muscle before (Pre exercise) and 0 (Post exercise for the DOMS, Post resting for the CONTROL group), 2, 15, 24, and 48 h after exercise by  $^{13}\text{C}$ -MRS (Fig. 1). The inter-pulse delay (repetition time =  $T_r$ ) was 0.15 s and the partial saturation of the glycogen signal was corrected by determining its relaxation time ( $T_1$ ) in the calf muscle at 4.7 Tesla. For a better signal-to-noise ratio, 6000 free induction decays were sampled before Fourier transformation. The spectra were collected using a simple pulse-acquire sequence.



**Fig. 1** Natural abundance, undecoupled  $^{13}\text{C}$ -MR spectrum of the *M. gastrocnemius* and *M. soleus* of a subject before exercise. Resonances 104 to 98 ppm are from C1 of muscle glycogen

### ■ $^{31}\text{P}$ -spectra: acquisition and quantification

PCr, ATP, and Pi concentrations were determined after the glycogen measurements by  $^{31}\text{P}$ -MRS (Fig. 2). The inter-pulse delay  $T_r$  was 4 s. The  $T_1$  values of the muscle metabolites were obtained in four subjects to correct the partial saturation effect. In order to increase the signal-



**Fig. 2** Typical resting  $^{31}\text{P}$ -MR spectrum of the *M. gastrocnemius* and *M. soleus* of a subject. Resonances are from phosphomonoesters (PME, 6.5 ppm), inorganic phosphate (Pi, 5 ppm), phosphodiester (PDE, 3 ppm), phosphocreatine (PCr, 0 ppm) and the three different adenosine triphosphates ( $\gamma$ -,  $\alpha$ -,  $\beta$ -ATP, -2.5 to -16 ppm)

to-noise ratio, 100 free induction decays were sampled, phased, and baseline corrected.

### ■ Processing of the $^{31}\text{P}$ - and $^{13}\text{C}$ -spectra and fitting of the peaks

At the end of each measurement, the spectra were phased, baseline corrected and finally fitted in order to determine the peak areas of the resonance lines of the investigated metabolites. In order to avoid severe systematic errors due to baseline distortion and peak overlapping, integration is not valid. Therefore we fitted our data in the time as well as in the frequency domain. The time domain fitting was performed with an iterative least-squares method. Exponentially decreasing model signals were fitted to the experimental signals. These model signals were converted into Lorentzian lines by Fourier transformation. Iteration was started with a list of model signals, one signal for each *in vivo* resonance. The first two milliseconds of the experimental signal were omitted for fitting in order to avoid the strong but fast dephasing signal of the almost immobile membrane molecules.

### ■ Quantification of the glycogen and phosphate peaks

$^{13}\text{C}$ -quantification was achieved with a calibration phantom used as a concentration standard. A cylindrical 2 L bottle was filled with a solution of 100 mmol/L of oyster glycogen buffered to a pH of 7.2 and supplemented with sodium acid to impair bacterial influence. The natural abundance of  $^{13}\text{C}$  was identical in the oyster glycogen and the *in vivo* muscle glycogen. As the signal intensity of partially relaxed glycogen depends on the temperature of the solution, the glycogen phantom was warmed up to 37 °C. The *in vivo* (muscle glycogen) and *in vitro* (phantom oyster glycogen) measurements were identical with respect to all measurement parameters, to the area investigated in the tissue respectively phantom, and to the load of the RF-coil. The coil load for the calibration measurement was adjusted to match the one of the muscle examination in the following way: after the *in vivo* measurement, the matching capacitance of the RF-coil was left unchanged. In contrast, the coil load of the calibration measurement was successively increased with a small saline bottle, which was gradually brought closer to the phantom-loaded RF-coil until identical matching was achieved. The electrical conductivity of the phantom solution was improved by adding NaCl ( $\sigma = 0.65 \text{ S/m}$ ) and was slightly kept below the one of human tissue ( $\sigma = 0.69 \text{ S/m}$ ) in order to allow the described fine adjustment of the coil load with the small saline bottle.

In a pilot study, the accuracy and reproducibility of the glycogen quantification with and without proton de-

coupling was examined. The calibration strategy using an external phantom was significantly more accurate if proton decoupling was not applied. The reason therefore is probably the heat deposition pattern, which develops during decoupling and differs greatly between a phantom and human tissue. As a high field 4.7 Tesla spectrometer has been used, an excellent S/N ratio ( $> 10$ ) was obtained for glycogen and a decrease in peak height by a factor of two could be afforded. Thus, peak fitting was not a problem. It was rather more accurate as a lifelike  $^{13}\text{C}$ -spectrum shows two peaks of glycogen instead of one after decoupling.

The quantification of the  $^{31}\text{P}$ -spectra, using a replacement phantom, was done by the same procedure as described above. Immediately after the *in vivo* session, an identical *in vitro* measurement was performed with a 2 L calibration phantom containing a solution of 50 mmol/L of  $\text{KH}_2\text{PO}_4$ .

In order to compare quantified glycogen, PCr, ATP, and Pi with results from other human studies, muscle metabolite concentrations were converted from mmol/L to mmol/kg wet weight (ww), using the constant value of 1.04 kg/L for the specific gravity of muscle tissue [25].

### ■ Blood sampling and analyses

Blood samples for the examination of glucose, insulin, free fatty acid (FFA),  $\beta$ -hydroxybutyrate ( $\beta$ -Hb), creatine kinase (CK), myoglobin (Mb), and lactate were drawn from an antecubital vein of the left arm. The first blood sample was drawn before exercise (after resting 30 min in a supine position), the following samples 0, 2, 15, 24, and 48 h after exercise. The blood for the analyses of insulin, FFA, CK, and Mb was filled in a 7.5 ml EDTA tube containing  $\text{NH}_4$ -heparin (Sarstedt, Nümbrecht, Germany). The blood for the analyses of glucose,  $\beta$ -Hb, and lactate was collected in a 2.7 ml NaF tube. Following centrifugation (4800 revolutions/min at 4 °C for 15 min) the plasma was separated from the erythrocytes and stored until analysis at -80 °C. Lactate and glucose were analyzed with an enzymatic calorimetric method,  $\beta$ -Hb, CK, and FFA by a photometric-enzymatic method. Lactate was measured with a test kit of Bergmeyer (New York, USA), glucose,  $\beta$ -Hb, and CK with the Cobas Mira (Roche, Basel, Switzerland), and FFA by a kit of WAKO (Neuss, Germany). Insulin and Mb were determined by radioimmunoassay. Insulin with a test kit of INSI-PR Cis bio international (Sur-Yvette Cedex, France) and Mb by a test kit of immuno diagnostic center (Dallas, USA).

### ■ Diet analyses

Starting two days before the experiment, subjects were told to eat foods selected from a list of CHO-rich meals

and to additionally ingest 1 L of an isotonic drink every evening, made of maltodextrine powder, to ensure full muscle glycogen stores. If the subjects were not able to eat all of the suggested food, they had to record it. The subjects were not allowed to ingest any drink containing caffeine from 24 h before the start until the end of the experiment. At least 1.5 h before their arrival in the laboratory on the experimental day, the subjects consumed a CHO-rich lunch, including again 0.5 L of the isotonic drink. From the moment the subjects were in the laboratory and started the experiment, they were allowed to eat and drink only the food prepared by us to ensure that every subject ingested at least 10 g CHO/kg BM/24 h during recovery. To optimize glycogen resynthesis after the reducing exercise, the meals were composed of dried fruits, gingerbread, rice pudding, pasta, rice, etc., and an isotonic drink. The snack given immediately after exercise (Table 1) and the dinner on the testing day were consumed in the laboratory. On the next day, the subjects consumed the delivered breakfast at home, 1.5 h before the 15 h post exercise MRS measurements. From 15 until 24 h after exercise, the subjects stayed in the laboratory again and consumed the morning snack, lunch, and afternoon snack under our supervision (Table 1). The subjects took the dinner, which they consumed after the 24 h MRS measurements, and all the other prepared foods for the second day with them. If anything was left over, they brought it back to the laboratory before the last MRS measurements (48 h after exercise). The food records were analyzed using a standard nutrition analysis software package (EBIS 2.0; E + D Partner, Stuttgart, Germany).

### ■ Perceived muscle soreness

Muscle soreness in the calf muscle was rated according to a modified Borg scale ranging from 0 (nothing at all sore) to 10 (maximally sore) and was determined by ask-

ing the subject while walking slowly. Always the same investigator performed the soreness recording before each withdrawal of blood.

### ■ Statistical analyses

The results are presented as mean value  $\pm$  standard deviation (SD). Differences over time between the two groups (DOMS and CONTROL, respectively) were analyzed by multiple analysis of variance (MANOVA) and, if significant, indicated with an asterisk (\*). Differences within each group were tested by one-way analysis of variance (ANOVA-factorial) followed by Fisher's testing for significance. Within a group, a plus (+) shows significant differences from the base value before exercise. Linear regression analysis was performed with standard procedures.

## Results

### ■ Glycogen-reducing exercises

The DOMS group sprinted for  $6.1 \pm 3.3$  min with an average heart rate of  $155.4 \pm 11.0$  beats/min and the CONTROL group for  $7.9 \pm 5.2$  min with a heart rate of  $158.5 \pm 11.4$  beats/min. Afterwards, all subjects finished the 1 h running protocol at 65 % maximal performance (DOMS:  $12.0 \pm 1.1$  km/h; CONTROL:  $11.1 \pm 1.6$  km/h), determined by the Conconi test previously. The average heart rate amounted to  $151.7 \pm 14.0$  beats/min in the DOMS and  $154.4 \pm 11.5$  beats/min in the CONTROL group. None of the comparisons between the two groups showed a significant difference.

**Table 1** Food ingested by the subjects after the exercise until the end of the experiment 48 h later

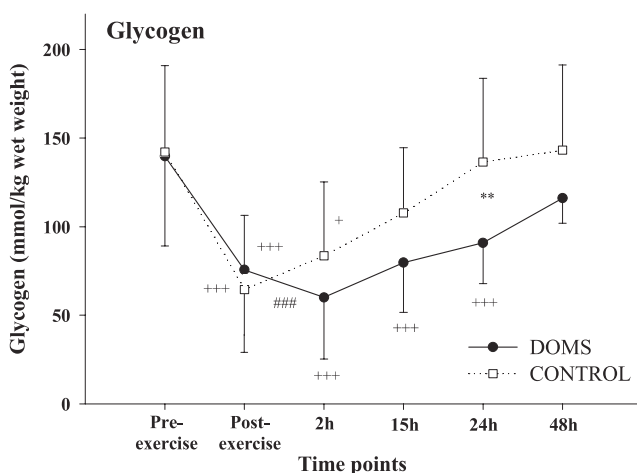
When	Food	g CHO/Portion
Snack after exercise	0.5 L isotonic drink, 150 g apple puree	112
Dinner after exercise	75 g apricot pie, 280 g rice, 160 g mixed vegetables, 2 dl mushroom sauce, water ad libitum	161
Day 1 and 2 after exercise Breakfast	132 g toast (6 slices), 40 g honey, 150 g banana chips yogurt, 3 dl orange juice	171
Morning snack	100 g dried fruit, 75 g gingerbread stuffed with almond paste, 3 dl apple juice	135
Lunch	5 dl herbal ice tea, 132 g toast (6 slices), 75 g pear bread, 200 g rice pudding	199
Afternoon snack	100 g dried fruit, 42 g crunchy chocolate bar, 3 dl orange juice	110
Dinner	180 g pasta, 75 g pear bread, 0.5 L isotonic drink	246

## Nutrition

Table 2 presents the average results of the daily-ingested macronutrients and energy in both groups, which were similar. The intake of more than 10 g CHO/kg BM/24 h was achieved during recovery as intended.

## Glycogen metabolism

Fig. 3 shows the different <sup>13</sup>C-MRS measurements of muscle glycogen. Sprinting and running in both groups, and the additional eccentric exercise performed by the DOMS group, significantly reduced the glycogen concentrations identically by about 50% ( $P < 0.001$ ). The concentration dropped further by  $7.8 \pm 7.9$  mmol/kg ww/h in the first 2 h of recovery in the DOMS group and increased by  $9.2 \pm 10.4$  mmol/kg ww/h during the same time period in the CONTROL group ( $P < 0.001$ ). In the following hours, there was a trend for lower glycogen



**Fig. 3** Muscle glycogen concentrations of the DOMS (N = 12) and CONTROL (N = 8) group. Significant differences between the two groups are indicated with an asterisk (\*). Within a group, a plus (+) shows significant differences between Pre and any Post exercise concentration. In addition, a pound sign (#) shows significant differences between the glycogen resynthesis rates of the two groups. +:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; +++, ###:  $P < 0.001$ . Values are means  $\pm$  SD

**Table 2** Average 24 h-intake of macronutrients and energy during 48 h before and after glycogen-reducing exercise with additional eccentric contractions (DOMS) or without eccentric exercise (CONTROL)

Nutrients/Energy	Group	-48 h	-24 h	+24 h	+48 h
Carbohydrates (g/kg BM/24 h)	DOMS	9.1 $\pm$ 2.4	10.0 $\pm$ 2.7	12.4 $\pm$ 0.8	11.8 $\pm$ 1
	CONTROL	7.4 $\pm$ 3.3	8.2 $\pm$ 2.2	11.9 $\pm$ 0.7	11.0 $\pm$ 1
Fat (g/kg BM/24 h)	DOMS	1.1 $\pm$ 0.5	0.9 $\pm$ 0.3	1.0 $\pm$ 0.1	0.9 $\pm$ 0.2
	CONTROL	0.9 $\pm$ 0.5	0.8 $\pm$ 0.2	1.1 $\pm$ 0.2	0.8 $\pm$ 0.1
Protein (g/kg BM/24 h)	DOMS	1.4 $\pm$ 0.6	1.5 $\pm$ 0.5	1.2 $\pm$ 0.2	1.1 $\pm$ 0.2
	CONTROL	1.1 $\pm$ 0.6	1.2 $\pm$ 0.2	1.2 $\pm$ 0.2	1.1 $\pm$ 0.2
Energy (MJ/24 h)	DOMS	15.9 $\pm$ 4.3	16.7 $\pm$ 3.8	19.4 $\pm$ 1.43	18.5 $\pm$ 1.4
	CONTROL	13.9 $\pm$ 6.1	14.4 $\pm$ 2.8	20.1 $\pm$ 1.2	17.7 $\pm$ 1.3

The results are presented as mean value  $\pm$  standard deviation. N = 12 (DOMS) and 8 (CONTROL), respectively

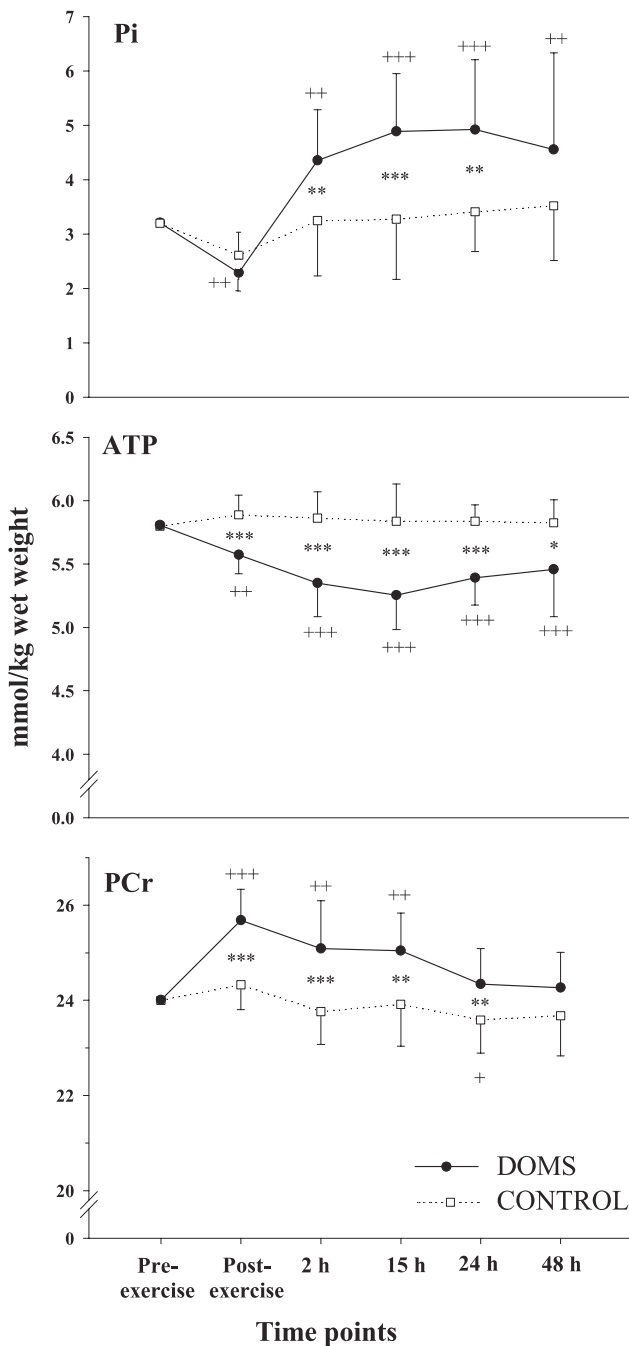
concentrations in the DOMS compared to the CONTROL group (15 h:  $P = 0.07$ ; 24 h:  $P < 0.01$ ; 48 h:  $P = 0.08$ ). The glycogen concentration of the DOMS group was still significantly ( $P < 0.001$ ) below the resting concentration 24 h after exercise. Only after 48 h was the glycogen concentration no different from the resting level, whereas the CONTROL group achieved this already after 15 h.

## Phosphate metabolism

Whereas the PCr, ATP, and Pi concentrations (Fig. 4) of the DOMS group differed significantly from the base value, this was not the case in the CONTROL group (one exception: PCr at 24 h;  $P < 0.05$ ). In the DOMS group, the PCr concentration was significantly higher ( $P < 0.01$ ) compared to the resting level up to 15 h after exercise, which resulted in a significant difference between the two groups ( $P < 0.01$ ). The ATP concentration declined immediately after exercise and remained significantly below the resting concentration until the end of the study ( $P < 0.001$ ). Therefore, the DOMS group significantly differed from the CONTROL group during most of the recovery period ( $P < 0.001$ ). The Pi drop during exercise in the DOMS group and the following rise over 48 h were significant ( $P < 0.01$ ) and showed a significant difference to the CONTROL group during the first 24 h ( $P < 0.01$ ).

## Blood parameters

None of the blood concentrations of CK, Mb, lactate, glucose, insulin, FFA, and  $\beta$ -Hb showed a difference between the two groups over time (Fig. 5). Subsequently, only CK and Mb, which are consistently mentioned in connection with DOMS, will be described in more detail. CK rose until it was significantly above the base value after 15 h ( $P < 0.01$ ) in both groups. It remained elevated ( $P < 0.01$ ) until the end of the study in the DOMS group and up to 24 h in the CONTROL group ( $P < 0.01$ ). Contrary to CK, Mb was already significantly increased after exercise in both groups, remained increased up to



**Fig. 4** Pi, ATP, and PCr concentrations of the DOMS (N = 12) and CONTROL (N = 8) group. Significant differences between the two groups are indicated with an asterisk (\*). Within a group, a plus (+) shows significant differences between Pre and any Post exercise concentration. \*, +:  $P < 0.05$ ; \*\*, ++:  $P < 0.01$ ; \*\*\*, +++:  $P < 0.001$ . Values are means  $\pm$  SD

2 h ( $P < 0.001$ ), and fell back to normal values thereafter. Mb started to increase again after 24 h in the DOMS group. However, this slight increase did not reach a significant level. Additionally, a few correlations were observed between Mb and perceived soreness (after 24 h:

$r^2 = 0.27$ ;  $P < 0.08$ ) as well as Mb/CK and Pi/PCr ratio (after 15 h:  $r^2 = 0.4$ ;  $P < 0.05$ / $r^2 = 0.5$ ;  $P < 0.01$ ) in the DOMS but not in the CONTROL group.

### ■ Muscle soreness

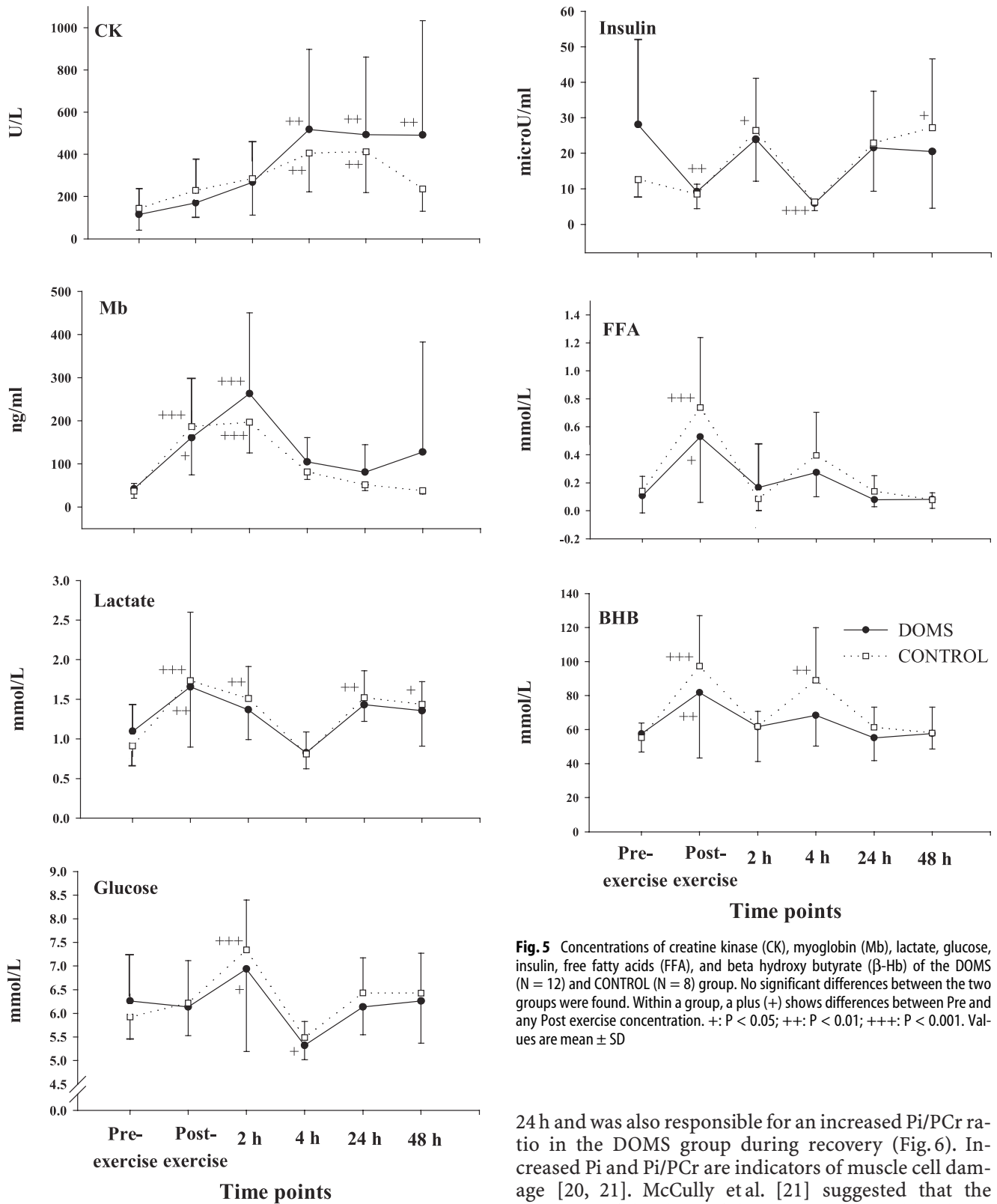
Both groups showed a significant increase of the perceived soreness immediately after exercises ( $P < 0.001$ ; Fig. 6). Testing of the differences between the two groups revealed a significantly higher perceived soreness rating in the DOMS group after 48 h ( $P < 0.05$ ), which started with a trend after 24 h ( $P = 0.07$ ). The toe-raise exercise additionally significantly increased the Pi/PCr ratio after 15 h ( $P < 0.001$ ; Fig. 6) in the DOMS group. This ratio remained higher than the resting ratio until the end of the study, whereas in the CONTROL group it always showed resting values. Comparing the two groups, the DOMS group had a significantly higher Pi/PCr ratio after 15 and 24 h ( $P < 0.01$ ). After 2 and 48 h, but not after 15 and 24 h, there was a significant correlation between perceived soreness and Pi/PCr ratio in the DOMS (Fig. 7) but not in the CONTROL ( $r^2 = 0.03$ ;  $P = 0.67$  and  $r^2 = 0.03$ ;  $P = 0.68$ , respectively) group.

### Discussion

Muscle glycogen concentration was equally lowered after running and sprinting irrespective of whether subjects rested or performed an additional eccentric exercise. Eccentric exercise of the DOMS group successfully induced muscle soreness. The CHO-rich diet immediately consumed after exercise not only failed to prevent delayed glycogen resynthesis. Glycogen even further decreased during the first 2 h of recovery in the DOMS subjects ( $-15.6 \pm 15.7$  mmol/kg ww) in contrast to the CONTROL group, where glycogen started to refill ( $+18.4 \pm 20.8$  mmol/kg ww). This decrease in glycogen concentration after eccentric exercise is reported here for the first time and has not been seen in previous studies, which measured glycogen after 4 to 6 h of exercise [e. g. 6, 9, 12]. The further glycogen loss during early recovery is especially disadvantageous because the first 2 h of exercise recovery are normally characterized by a rapid, insulin independent, glycogen resynthesis phase [26]. The averaged glycogen resynthesis rate of  $9.2 \pm 10.4$  mmol/kg ww/h found in the CONTROL group corresponded to what is described in other studies [8, 26].

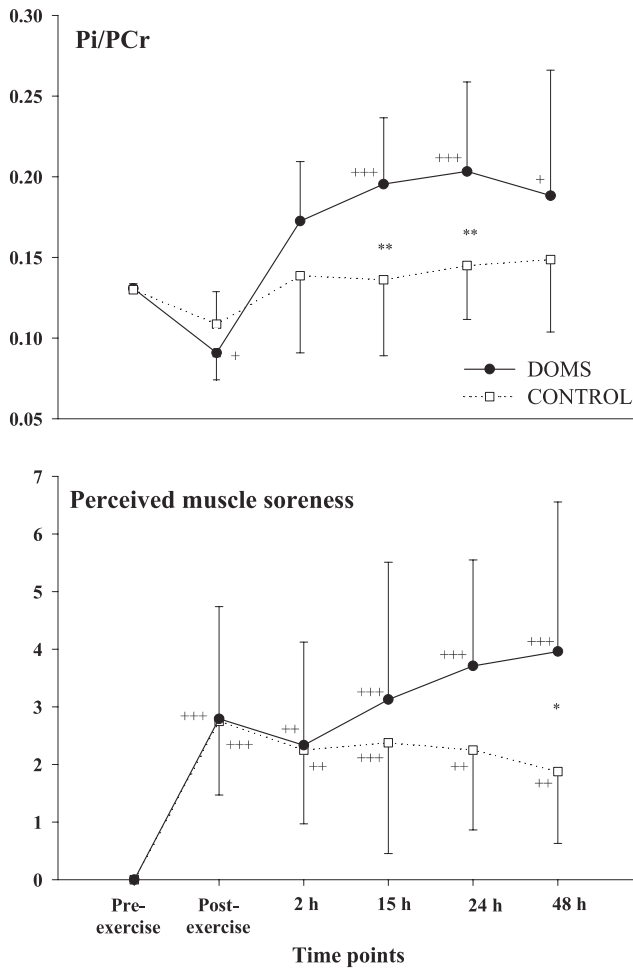
Several factors may account alone or in combination for the further decrease in glycogen concentration found during the first 2 h after vigorous eccentric exercise followed by muscle soreness. A significant rise of Pi ( $P < 0.001$ ) was measured during the first 2 h (Fig. 4). This elevated Pi concentration persisted during at least





**Fig. 5** Concentrations of creatine kinase (CK), myoglobin (Mb), lactate, glucose, insulin, free fatty acids (FFA), and beta hydroxy butyrate ( $\beta$ -Hb) of the DOMS (N = 12) and CONTROL (N = 8) group. No significant differences between the two groups were found. Within a group, a plus (+) shows differences between Pre and any Post exercise concentration. +: P < 0.05; ++: P < 0.01; +++: P < 0.001. Values are mean  $\pm$  SD

24 h and was also responsible for an increased Pi/PCr ratio in the DOMS group during recovery (Fig. 6). Increased Pi and Pi/PCr are indicators of muscle cell damage [20, 21]. McCully et al. [21] suggested that the steepness of the slope of the Pi/PCr ratio could indicate the severity of the exercise-induced muscle injury. In addition, a shallower Pi/PCr ratio curve points to a preser-



**Fig. 6** Pi/PCr ratio and perceived muscle soreness of the DOMS (N = 12) and CONTROL (N = 8) group. Significant differences between the two groups are indicated with an asterisk (\*). Within a group, a plus (+) shows significant differences between Pre and any Post exercise value. \*, +:  $P < 0.05$ ; \*\*, ++:  $P < 0.01$ ; +++, +++:  $P < 0.001$ . Values are means  $\pm$  SD

vation of PCr as it also happened in our CONTROL group. In the present study, the slope of the Pi/PCr ratio was highest during the first 2 h after eccentric exercise (DOMS group:  $+0.09 \pm 0.05$ ; CONTROL group:  $+0.03 \pm 0.06$ ;  $P < 0.05$ ; Fig. 6). This result of the DOMS group is in close relation with the result of McCully et al. [20], where 24 h after repeated lengthening contraction exercise resulting in muscle injury, the subjects showed a maximum Pi/PCr ratio increase (+0.1). Also, Lund et al. [27] described the elevated Pi/PCr as a reliable indicator for muscle injury after eccentric exercise. Therefore, we assume that in the present study the eccentric exercise, followed by DOMS, caused muscle fiber injury observable already early during recovery. This assumption is realistic because O'Reilly and colleagues [28] showed numerous ultra-structural abnormalities in muscle cells immediately after eccentric exercise as, e. g.,

focal myofibrillar lysis or damage to the sarcoplasmic reticulum.

During eccentric toe-raise exercise with fully extended knees, M. gastrocnemius is predominately strained. M. gastrocnemius contains mainly fast-twitch fibers. These fibers are characterized by an increased intramuscular Pi concentration after muscle injury [29]. Pi is one of the substrates for phosphorylation and activated by glycogenolysis, especially in fast-twitch muscles [29]. Moreover, phosphorylase a is also activated by elevations in intracellular  $Ca^{2+}$  levels, which occur after fiber injury and result in glycogenolysis [30].

Furthermore, Hultman and Greenhaff [31] described that eccentric exercise causes metabolic perturbations in muscle fibers. Type II fibers were unable to maintain a high rate of ATP resynthesis for energy provision, as it is necessary, e. g., for glycogen resynthesis. This happened most likely also in the present study because the DOMS group had a significantly lower ATP concentration ( $P < 0.001$ ) than the CONTROL group after exercise (Fig. 4). The diminished ATP concentration and its negative consequences for glycogen resynthesis in the DOMS group may be also explained by the evoked microruptures. The result is an uptake of  $Ca^{2+}$  into the sarcoplasm, followed by a disturbance of the oxidative phosphorylation, which reduces ATP resynthesis [32].

Ryschon et al. [19] estimated the efficiency of ATP utilization for concentric, eccentric, and isometric muscle action. A higher metabolic efficiency during an eccentric compared to a concentric action followed a lower ATP resynthesis rate after eccentric action. An explanation for this is the recruitment of more efficient fibers during eccentric compared to concentric exercise [19]. Therefore, we could assume that the ingested CHO in the DOMS group was rather used to provide energy for, e. g., the regeneration of the hydrolyzed ATP than being used to restore muscle glycogen during the initial 2 h of recovery.

The influence of eccentric exercise on phosphate metabolism and glycogen resynthesis was not only seen after 2 h. The trend for different glycogen concentrations between the two groups after 15 h ( $P < 0.07$ ) and the significant difference after 24 h ( $P < 0.01$ , Fig. 3) was paralleled by a significant difference in the Pi/PCr ratio ( $P < 0.01$ , Fig. 6). The higher Pi/PCr ratios in the DOMS group can still reflect the disrupted muscle fibers. Damaged muscle fibers lead in turn to a defect in oxidative metabolism, i. e., in our study to a perturbation of the phosphate equilibrium, which enables an influx of plasma Pi [33]. Additionally, it can also be a sign of an increased oxidative capacity of mitochondria [21], which evokes increased energy demand. This increased energy demand might be a result of the healing process of the injured fibers. Therefore, also after 2 h glucose could provide energy for the ATP resynthesis used for

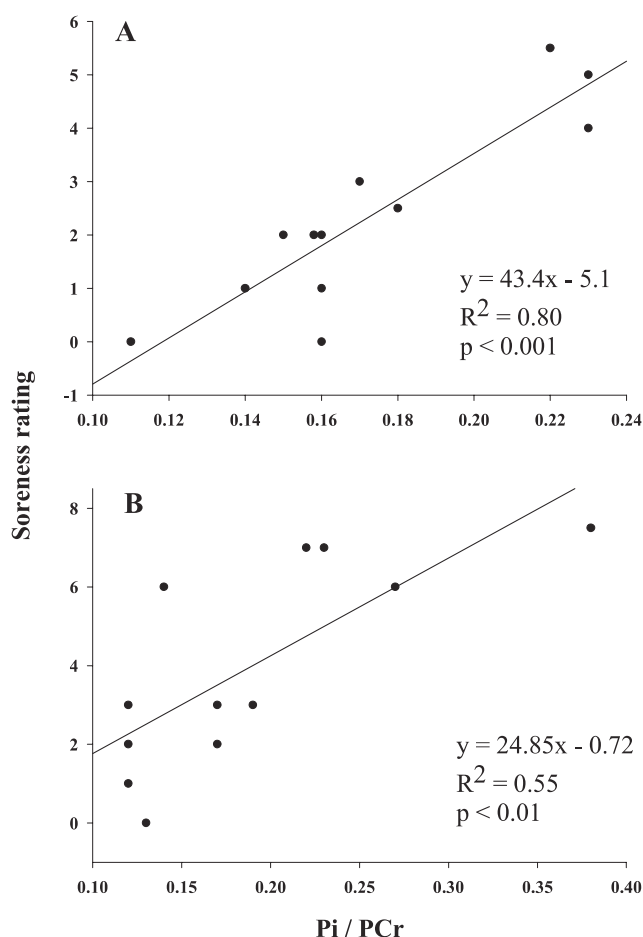
the healing process rather than for the glycogen resynthesis. This is in accordance with a reduced glycogen resynthesis between post exercise and 15 h of only  $4 \pm 28$  mmol/kg ww in the DOMS compared to  $43 \pm 26$  mmol/kg ww in the CONTROL group (Fig. 3).

Additionally, different studies [4, 34–36] investigated the influence of glucose transport proteins (GLUT 4) in connection with eccentric exercise and impaired glycogen resynthesis. They found, on the one hand, a reduced amount of GLUT 4 in damaged muscle cells [4], on the other hand, a diminished function, caused by a relatively short-term insulin resistance after eccentric exercise [35, 36]. In a newer study, Asp et al. [34] measured a delay of muscle glycogen resynthesis after a muscle damage inducing marathon run although GLUT 4 concentration was unaltered. Therefore, they concluded that factors other than GLUT 4 concentration must be involved in the slow glycogen resynthesis. Compared to our study, the subjects consumed less CHO (only 7 g CHO/kg BM/24 h) during the recovery from the marathon and it is not clear whether the subjects received CHO immediately after exercise. Therefore, one could argue if “immediately administered” or more CHO would have reduced or prevented the delayed glycogen resynthesis. However, from the previous insights of our study this option has to be abandoned.

From Kapur et al. [37] and Schmidt and Walter [38] a further potential explanation arises for the diminished glycogen resynthesis of the DOMS compared to the CONTROL group. It concerns the cytokine-inducible enzyme, one of the three different nitric oxide synthase isozymes, which is most evident in fast-type extensor muscles [39]. On the one hand, the enzyme is enhanced during inflammation and, on the other hand, its activity causes impaired insulin-stimulated glucose uptake. The impaired insulin activity would reduce muscle glucose uptake and therefore glycogen resynthesis.

Besides Pi, Pi/PCr ratio, and probably the cytokine-inducible enzyme, muscle soreness is another reliable indicator for muscle damage. The Pi/PCr ratio of the DOMS group correlated significantly with the perceived muscle soreness 2 h post exercise ( $P < 0.001$ ; Fig. 7A). Soreness follows muscle injury, which is mostly caused by eccentric exercise [e.g., 4]. McCully et al. [20] reported a ratio of Pi/PCr peaking one or two days after exercise, which is similar to the results found in our study (Fig. 6). Additionally, the Pi/PCr ratio correlated significantly with the perception of muscle soreness ( $P < 0.01$ , Fig. 7B). After 48 h, the perception of muscle soreness achieved its maximum value [as reported by others, e.g., 40] and differed significantly from the CONTROL group ( $P < 0.05$ , Fig. 6). This correlation makes perceived soreness rating an additional possible candidate for the judgement of the severity of the muscle fibers injury after eccentric exercise.

Often, CK [e.g., 41, 42] and Mb [e.g., 42] are used to



**Fig. 7** Correlation between perceived muscle soreness and Pi/PCr ratio in the DOMS group 2 h (A) and 48 h (B) after eccentric exercise

detect injured muscle fibers after eccentric exercise and to judge the severity of the damage. In our study, CK and Mb were both elevated in the course of recovery, but not significantly different in the two groups (Fig. 5). From the shown results and other studies [3, 8, 43, 44] one can question the suitability of CK and Mb as indicators or predictors of muscle injury. Additionally, there is also a large inter-subject variability in the rise of CK [e.g., 45, 46]. However, 48 h after exercise, CK concentration was still elevated in the DOMS but not in the CONTROL group (Fig. 5). At the same time, the amount of glycogen in the DOMS group reached approximately resting concentration, which is a sign that CK does not negatively influence glycogen resynthesis (Fig. 3).

As a critique of the MRS method, one might argue that after eccentric exercise, swelling and storage of water in the contracted muscle can occur and could lead to a diminished glycogen signal in the  $^{13}\text{C}$ -spectra. However, substantial swelling and edema occur only 24 h after eccentric exercise [23, 30, 47, 48]. The extent of swelling and edema after eccentric exercise was about 3

to 9% [49]. This low fluid expansion is within the accuracy of MRS measurements [50] and would therefore hardly disturb such measurements.

## Conclusion

Despite a high carbohydrate supply during recovery (> 10 g CHO/kg BM/24 h), the delay of muscle glycogen resynthesis after eccentric exercise could not be prevented. An important new finding was a further reduction of the glycogen concentration during the initial 2 h of recovery after eccentric exercise instead of a high resynthesis rate. Even 24 h after eccentric exercise, muscle glycogen concentration in the DOMS group was still

35% below the resting concentration. The initial, further glycogen reduction and the delayed glycogen resynthesis were most likely an effect of muscle cell damage as indicated by increased Pi concentration and Pi/PCr ratio. In consideration of this fact, it is probably impossible to eliminate the negative effect of muscle injury on the glycogen resynthesis after eccentric exercise even with a large supply of CHO during recovery.

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