Comparison of metabolic adaptations between endurance- and sprint-trained athletes in two different calf muscles using a multi-slice $^{31}$P spectroscopic sequence

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Abstract: The effects of different training strategies were investigated by simultaneous monitoring of exercise-induced changes of high-energy phosphates and pH values in two muscles revealing heterogeneous metabolic adaptations. In addition, the results were linked to global parameters of the physiological regulation, which are commonly used to monitor the training status of athletes. This enables comprehensive evaluation of training interventions by supporting the gold standard methods of sport science with knowledge of local metabolic changes in single muscles.


Motivation
Different orientations in sports lead to muscle fiber adaptations to meet the performance requirements of the specific discipline, and thus the ability to either produce extremely high peak forces within a short time or to sustain submaximal intensities for long time periods up to several hours (1). Measurements of exercise-induced metabolic adaptations, like oxygen consumption ($V_{O2}$), carbon dioxide exhalation ($V_{CO2}$), or lactate concentration, are important indicators for assessing the current performance level of athletes in training science. Apart from these global physiological parameters, it is well known that differently trained athletes, e.g., sprinters and endurance athletes, also show distinct variations of local muscular quantities related to constitutional, metabolic and biomechanical aspects as well as different perfusion patterns and muscle fiber composition (2,3). Several authors suggested an association between changes in global, whole-body parameters, like ventilation, and local, high-energy metabolic adaptations (4), but also between exercise termination and accumulation of fatigue-related intramuscular metabolites (5). Therefore, a more comprehensive characterization of metabolite kinetics during muscular performance with non-invasive histochimical interventions has always been an important aim of sport science studies (1,6).

Against this background, the aim of this study was to evaluate both global (whole-body) as well as muscle-specific metabolic local adaptations in two groups of endurance-trained and sprint-trained athletes. Both athlete groups performed an exhausting all-out calf exercise, during which localized $^{31}$P-MRS data of two calf muscles (m. soleus - SOL and m. gastrocnemius medialis - GM) were continuously acquired together with spirometry data and blood lactate concentrations. Due to the heterogeneous muscle fiber compositions not only between the muscles, but also between the athlete groups, we hypothesized different metabolic adaptations for the SOL and GM muscles as well as training-specific differences of the metabolic outcome between sprinters and endurance athletes.

Experimental
In total, 21 male subjects practicing either sprint sports (100 m, long- and high jump, hurdles, n = 11) or endurance sports (triathlon and long distance runners, n = 10) participated in this study. Prior to the main MR examination, all subjects performed initial exercises with the in-house developed, MR-compatible pedal ergometer (7) to familiarize with the setup and to determine the individual maximum voluntary contraction force (MVC) of the right calf muscle.

The exercises in the MR scanner consisted of a series of unilateral plantar flexions performed in supine position with 20% of the subject’s in-
individual MVC and with a frequency of 0.8 Hz. During load, subjects were encouraged to sustain the exercise as long as possible and verbal instructions were given to ensure constant load conditions until total fatigue of the calf muscle was reached. Fig. 1a illustrates the protocol of the multi-modal data acquisition (total duration: 30 min), which consisted of a rest phase (2 min), a load phase (3 - 15 min), and a recovery phase after the exercise (up to 25 min).

Figure 1 (a) Scheme of the exercise time regime of $^3$P-MRS, spirometry and blood lactate samplings during rest, load and recovery phase. (b) Transverse image of the right calf with the yellow marked slices covering the SOL and GM muscles. Right: Representative ‘stack plots’ of dynamic $^3$P-MR spectra series showing a reduced metabolic activation in the SOL compared to the GM.

Dynamic series of $^3$P-MR spectra were acquired continuously on a clinical 3 T whole-body MR scanner (Magnetom PRISMA fit; bore size, 60 cm; VE11B, Siemens Healthcare, Erlangen, Germany) by using a flexible double-tuned $^1$H/$^3$P MR coil wrapped around the subject’s calf (Rapid BioMedical; Rimpar, Germany). An in-house developed $^3$P-MRS pulse sequence, dubbed MUSCLE (MUlti SiCe Localized Excitation (8)), was used to acquire $^3$P spectra in two parallel, 16 mm thick slices covering the GM and SOL muscle, respectively (Fig. 1b) The sequence combines slice selective excitation with fast spectroscopic FID data sampling and enables interleaved measurements in multiple, spatially shifted slices (temporal resolution: TR = 5 s). For each spectroscopic slice, 24 spectra were measured during rest, followed by the acquisition of 36 to 180 spectra during load, and up to 300 spectra during recovery.

Oxygen uptake (VO$_2$ in l/min) and carbon dioxide production (VCO$_2$ in l/min) were monitored continuously by using the spirometry system PowerCube (LF8, Ganshorn, Medizin Electronic GmbH, Niederlauen, Germany), which had been adapted to enable measurements in the MR scanner (9). Small blood samples were collected at specific time points during rest, exercise and the recovery phase by stitching the earlobe with safety lancets (Extra 18G/ Penetration Depth 1.8 mm, Sarstedt AG&Co, Nürmbrecht, Germany). Blood lactate concentrations were immediately quantified with a portable lactate SCOUT unit (EKF Diagnostics GmbH; Cardiff, UK).

All MR spectra were quantified with an AMARES routine, included in the jMRUI 5.2 software (www.jmrui.eu). Intra-muscular pH values were calculated by inserting the corresponding measured chemical shifts between the PCR and Pi peaks into the Henderson-Hasselbalch equation (10). The PCR time courses, PCR(t), were fitted in four sections (see Fig. 2) including a constant term for the pre-load level (resting state PCR concentration), an initially decaying mono-exponential function (amplitude PCR$_{dec}$ and time constant of PCR decay $\tau$), a mono-exponential increasing function for the partial recovery during the subsequent load phase (amplitude A$_{init}$) and, finally, a bi-exponential function described by a weighted sum of fast and slow PCR recovery during the post-load phase (time constant of post load PCR recovery $\tau$). Similar to the analysis of the PCR time courses, the kinetics of VO$_2$ and VCO$_2$ were fitted in sections by three functions representing the constant resting phase, the mono-exponential increase during load and a bi-exponential decay after the exercise (see Fig. 3). Besides determining the amplitudes and time constants of the respiratory changes, the resulting fits were also used to assess the exercise-induced overshoot of CO$_2$ over the O$_2$ volume (excess CO$_2$ in l), which was determined from the difference between the areas under the superimposed fitted VO$_2$ and VCO$_2$ curves during load and recovery (9).

Finally, the blood lactate evolutions (see Fig. 3) were fitted using a simple kinetic model based on pharmacokinetic theory, which describes the lactate on release from the muscle into the blood compartment as well as the lactate elimination due to dilution and oxidative depletion (11).

For comparison between the athlete groups and between the calf muscles, the two-sided Wilcoxon rank sum was used (p < 0.05). The
relationships between muscle-related and global metabolic changes were analyzed by using Pearson’s correlation coefficient.

Results

Fig. 1b shows representative spectra series in GM and SOL muscles, and Table 1 summarizes the mean metabolic parameters derived from the 31P-MR spectra series in both muscles in sprinters and in endurance-trained athletes. During the exercise, distinctly weaker exercise-induced PCr depletions and smaller Pi frequency shifts were seen in SOL compared to GM, which, in turn, showed a faster and stronger PCr decrease ($t_{PCr_d}$ and $PCr_{dec}$) as well as a strongly pronounced acidification at the end of the load phase ($pH_{end}$). Endurance-trained athletes revealed distinctly higher PCr depletion in the SOL compared to sprinters. In the GM, almost full PCr depletion was reached in all subjects. Inspection of the whole PCr time courses resulted in interesting findings that depended on the overall load duration: predominantly endurance athletes ($n = 8$, both muscles) but only few sprinters ($n = 2$, both muscles) showed a biphasic PCr profile with a partial PCr increase during the load phase. In the GM, this was usually accompanied by an increase of pH (Fig. 2).

This pH increase was less pronounced in the SOL. Significantly lower $pH_{end}$ values were detected in sprinters compared to endurance athletes in the GM. Finally, PCr recovery was significantly faster in endurance athletes compared to sprinters (see Table 1).

The attained O2 consumption, CO2 exhalation and accumulated lactate indicate high pulmonary adaptation, when considered against the small loaded muscle group.

The sprinters yielded apparently faster VO2 increases during load ($340 \pm 103$ s vs. $405 \pm 155$ s; $p = 0.11$). After the exercise, VO2 decreased instantly for both groups, with a faster time constant in endurance athletes ($60 \pm 30$ s vs. $51 \pm 13$ s; $p = 0.16$). The difference between the areas under the VCO2 and VO2 curves during the load and post-load phases was positive for all subjects, indicating high accumulation of non-metabolic CO2 ($3.3 \pm 1.8$ s vs. $1.9 \pm 0.8$ s; $p = 0.03$). These anaerobic adaptations were mirrored in considerable individual blood lactate increases, which were measured within the first minutes of the post-load phase (Fig. 3). Sprinters had a significantly higher mean lactate peak value ($3.1 \pm 0.8$ mmol/l vs. $2.5 \pm 0.5$ mmol/l; $p = 0.047$). During the post load phase, clearance of lactate dominated over the release, with concentration depletion in sprinters being significantly slower compared to endurance athletes ($t_{Lac}$ $606 \pm 162$ s vs. $360 \pm 186$ s; $p < 0.01$).
Global anaerobic adaptations were mirrored in considerable lactate increases and CO₂ overshoot, which both were significantly correlated with the acidification of GM muscles (R = 0.59 and R = -0.61, respectively and both p ≤ 0.05).

**Discussion**

In this work, a comprehensive analysis of metabolic adaptations in all-out exercised calf muscles of two differently trained athlete groups was performed by using localized dynamic 31P-MR spectroscopy. Respiratory parameters VO₂ and VCO₂ as well as blood lactate concentrations were monitored simultaneously with the spectroscopic measurements in order to assess the effects of local metabolic adjustments in the loaded muscles on global physiologic parameters. By recruiting two opposing athlete groups we intended to differentiate the impact of specific metabolic energy supply pathways, whose kinetics are assumed to be affected by specific training orientations. Applying an advanced 31P-MRS sequence with time-resolved spectra acquisition in multiple slices enabled interleaved tracking of the metabolic time courses in two different calf muscle parts. A reduced energy demand of the SOL confirms the different working domains of both muscles, which is also evident from the higher load-induced acidification in the GM. Thus, the mono-articular SOL acts as a supporter in the applied exercise of this study, whereas the poly-articular main contributor GM is able to generate higher forces. Based on these findings we also assumed heterogeneous fiber compositions with higher amount of type I fibers in the SOL and higher amount of type II fibers in the GM (12). This, in turn, could affect the preference of either aerobic or anaerobic energy supply in the SOL and GM, respectively (13). Furthermore, activation of the supportive SOL was more heterogeneous, especially among the endurance athletes, who tend to use it to a greater extent. This, in turn, may represent an adaptive strategy to cope with this unusually stressful exercise. An interesting, even unexpected finding of our study was the apparent different PCR kinetics, which was observed in both muscles during the load phase in athletes exceeding the load duration of 350 s. Sprinters showed fully (GM) or partly (SOL) drained PCR stores, whereas most of the endurance athletes revealed a partial PCR recovery during the load phase. This indicates that the GM of sprinters utilized the anaerobic pathway to a greater extent, whereas the endurance athletes were obviously able to switch to the aerobic pathway and thus oxygenate lactate, which led to diminished PCR turnover and partial elevation of the PCR concentration (14). Such metabolic recovery behavior reflects the effectiveness of the metabolism in highly-trained athletes. Since long-term aerobic training is known to increase fatigue resistance a selective recruitment of muscles and motor units in endurance athletes might be a way of dealing with the particular exercise type of our study (15).

It is well known that sport orientation-driven adaptations in the local muscle metabolism, such as changes in mitochondrial density, oxygen turnover or muscle fiber fractions, are accompanied by global adjustments, including changes in perfusion, efficiency of capillary gas exchange or intermediate turnover rates (16). Consequently, it was not surprising to find distinctly different respiration and blood lactate characteristics between our investigated athlete groups as indicated, e.g., by the almost twice as large excess CO₂ levels in sprinters, which are related to the elevated anaerobic energy supply and are also mirrored in distinctly higher amounts of accumulated lactate in the blood. This association was confirmed by significant correlations between excess CO₂ and blood lactate peak values in both groups but also in muscular acidification. As an intermediate, lactate, which is directly related to the ac-

**Table 1** Muscle-specific metabolic parameters extracted from the 31P-MR spectra series for the two athlete groups. **PCR** represents the maximum percentage drop in PCR concentration during the load phase normalized to rest. Mean values are listed together with corresponding SD and p-values. Significant group differences with p<0.05 are marked in bold.

<table>
<thead>
<tr>
<th></th>
<th>sprint-trained athletes</th>
<th>endurance-trained athletes</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SOL</td>
<td>GM</td>
<td>SOL</td>
</tr>
<tr>
<td><strong>pH-rest</strong></td>
<td>7.05 ± 0.03</td>
<td>7.04 ± 0.03</td>
<td>7.03 ± 0.03</td>
</tr>
<tr>
<td><strong>PCRdec [%]</strong></td>
<td>47 ± 17</td>
<td>&gt;90</td>
<td>33 ± 20</td>
</tr>
<tr>
<td><strong>τPCR_d [s]</strong></td>
<td>46 ± 18</td>
<td>31 ± 14</td>
<td>60 ± 33</td>
</tr>
<tr>
<td><strong>pH-end</strong></td>
<td>6.90 ± 0.16</td>
<td>6.29 ± 0.20</td>
<td>6.86 ± 0.12</td>
</tr>
<tr>
<td><strong>τPCR_f [s]</strong></td>
<td>57 ± 25</td>
<td>125 ± 38</td>
<td>52 ± 30</td>
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cumulation of an equimolar amount of $\text{H}^+$ limits muscle performance and is buffered by bicarbonate, causing the production of non-metabolic $\text{CO}_2$ (17). This implies that pH might be a good indicator for anaerobic metabolism.

**Conclusion**

In summary, our study demonstrates the capability of *in vivo* $^{31}\text{P}$-MRS to resolve effects of different training strategies by monitoring exercise-induced changes of pH values and high energy phosphates in two muscle groups, which contribute to the mechanical output to different extents. The observed heterogeneous adaptations might be assigned to appropriate muscle fiber distributions as they make it possible to deal with different load domains during exhaustive exercises. In addition, by combining local MRS measurements with acquisition of respiratory and blood lactate data we were able to link metabolic processes in the loaded muscles with global physiological adaptations, which are commonly used to monitor the training status of athletes. Therefore, the results of this work might provide useful input for designing training interventions by supporting the sport science golden standard methods with knowledge of local adjustments in the trained muscles.

**References**


