TECHNICAL NOTE

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TIME COURSE OF BRAIN LACTATE AND GLUTAMINE CONCENTRATIONS FOLLOWING HIGH-INTENSITY PHYSICAL TRAINING: VALIDATING PROTOCOL OF A MAGNETIC RESONANCE SPECTROSCOPY STUDY

Stefan P. Gaździński[1], Anna Przewodzka[1], Michał Madeyski[2], Aleksandra Przybysz[4], Ewelina Maculewicz[3,4]

1 Creative Neuroscience Lab – CNS Lab, Military Institute of Aviation Medicine, Warsaw, Poland
2 Military Institute of Medicine – National Research Institute, Warsaw, Poland
3 Department of Molecular Biology, Military Institute of Aviation Medicine, Warsaw, Poland
4 Department of Biomedical Sciences, Józef Piłsudski University of Physical Education, Warsaw, Poland

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Author's address: S. Gaździński, Military Institute of Aviation Medicine, Krasińskiego 54/56 Street, 01-755 Warsaw, Poland, e-mail: sgazdzinski@wiml.waw.pl

Introduction: This technical note presents work performed to validate the magnetic resonance spectroscopy (MRS) protocol used in our previous study. Earlier studies using MRS have reported changes in the concentrations of glutamate (Glu) and lactate (Lac) following exercise of various intensities and performed by participants at different fitness levels. However, the time-course of these changes within a few hours after a single strenuous exercise bout is not known. This information was essential to decide if the procedures to measure Lac and Glu could be added before and after a standard radiological exam lasting about 90 minutes, which is performed on candidates to the Polish Air Force University in Dęblin, who undergo medical evaluation at Military Institute of Aviation Medicine and are at very high fitness level. This evaluation also includes a maximal exercise test. Here, we asked whether such exercise-induced changes of brain Lac and Glu return to resting values within 60 minutes post-exercise.

Method: Concentration changes of Glu, glutamate+glutamine (Glx), and Lac were measured in one person with outstanding performance. The MRS measurements were performed twice before the exercise and then within an hour, at 8-minute intervals, using MRS at 3T, within a large voxel encompassing bilateral occipito-parietal lobes.

Results: Glu, Glx, and Lac concentrations were elevated after the exercise, compared to pre--exercise concentrations. The Lac concentrations appeared to normalize (to pre-exercise values) within 60 minutes. However, Glu and Glx values seem to have larger concentrations after 60 minutes, compared to pre-exercise values. The general trend was the same for absolute concentrations and values scaled to total creatine signal (tCr), despite short-term deep in tCr concentration following the exercise.

Discussion: The results demonstrated the feasibility of using concentrations of Lac, Glu, and Glx obtained at about 60 minutes post-exercise to substitute for concentrations of these metabolites at rest.

Keywords: glutamate, lactate, magnetic resonance spectroscopy, exercise, brain, time-course

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INTRODUCTION

Physical exercise leads to a significant increase in neuronal activity across various brain regions, including the frontal cortex, parieto-occipital cortex, cerebellum, and subcortical nuclei [1,6], therefore, it affects the concentrations of neurotransmitters. Glutamate (Glu) serves as the primary excitatory neurotransmitter in the mammalian central nervous system, with approximately 60-80% of the brain's energy consumption in the non-stimulated cerebral cortex dedicated to supporting glutamatergic transmission [16]. The glutamate-glutamine cycle (Glu-Gln cycle) involves the uptake of glutamate from synapses by astrocytes, its conversion into glutamine (Gln) within the astrocytes, the transport of glutamine to neurons, and its reconversion into glutamate within the neurons [5,7]. In some circumstances there is a difficulty in numerical separation of Glu and Gln; therefore, the sum of concentrations of Glu and Gln: Glx is reported.

Despite the established role of glutamate in brain function, studies on the impact of acute physical exercise on brain concentrations of glutamate (or Glx) in humans are relatively few and have been performed among students, not athletes, and their findings have been inconsistent. For instance, in a 1H-MRS study using a 1.5T MRI scanner, Maddock et al. [11] reported an 18% increase in the Glx signal from the visual cortex following graded exercise to around 85% of the predicted maximal heart rate. A subsequent study from the same team, using a 3T MRI system, confirmed this glutamate increase in the visual cortex and also noted a similar rise in the anterior cingulate cortex. The researchers interpreted these results as evidence that exercise leads to an expansion of the cortical glutamate pool, a finding that aligns with the observed increase in whole-brain non-oxidative glucose consumption during exercise [15].

However, other researchers [4], using a 7T MRI system and analyzing 1H-MRS spectra from the occipital cortex, did not observe significant changes in glutamate or glutamine concentrations following vigorous exercise; these concentrations were normalized to the concentration of creatine. In contrast, for absolute concentrations of Glu and Gln, a decrease was noted. This result is unexpected, particularly since the parietal cortex, which includes the occipital-parietal region, is activated by a wide range of sensory and motor stimuli, including movement processing [3]. Interestingly, it has been shown that the occipito-parietal cortex is activated after repeated bouts of exercise [18].

Another metabolite whose concentration changes during/due to exercise is lactate (Lac). During acute aerobic exercise, glucose is metabolized through aerobic glycolysis, where it is broken down into pyruvate, which then either converts to lactate (or lactic acid) as exercise intensity increases. As exercise intensity transitions from aerobic to anaerobic, the "anaerobic threshold" is reached, and beyond this point, lactic acidosis can occur [1]. Lactate, a byproduct of muscle metabolism during exercise, can cross the blood-brain barrier [8], leading to an equilibrium between lactate concentrations in the brain and the bloodstream. Once lactate enters the brain, it can be utilized as an energy source [1,4]. Some of the lactate produced during exercise may be channeled into de novo synthesis of neurotransmitters via tricarboxylic cycle intermediates such as alpha-ketoglutarate [10]. Both neurons and astrocytes in the brain are capable of taking up and metabolizing lactate. In fact, lactate may serve as the primary energy substrate for neuronal activity, especially when it is supplied to neurons by glial cells during periods of intense activation [19,20].

Increase in lactate concentration in the brain following physical exercise has been consistently reported regardless of whether the glutamate (Glu) signal is found to be elevated [10,11] or unchanged [4,15]. There is also evidence suggesting a potential correlation between an increase in lactate concentration and changes in Glx, but the results are largely inconsistent due to differences in study methodology, such as the timing of measurements after exercise or the choice of brain regions [17]. Collectively, the studies that have investigated changes in Glu, Glx, Gln, and Lac during submaximal exercise typically included participants with varying fitness levels. As a result, their findings may not be directly applicable to individuals with very high fitness levels performing maximal aerobic exercise. Additionally, the time-course of changes in Lac and Glu concentrations after a single strenuous exercise bout is not known. As this information was essential to decide if the procedures to measure Lac and Glu could be added before and after a standard radiological exam lasting about 90 minutes, or they should be performed on other occasions.

MATERIALS AND METHODS

One male (40 years old) with high physical fitness participated in this study. The spectra were measured twice with long and short TE under rest-

ing conditions, before the exercise bout, and eight times after performing physical activity. The activity consisted of a 15-minute mixed effort (combining aerobic and anaerobic components), of high intensity (jumping rope). This exercise was meant only to change Lac, Glu, and Glx concentrations in the brain. The Military Institute of Aviation Medicine Ethics Committee approved the study. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Magnetic Resonance sessions

MRI studies were conducted using a 3T GE Discovery 750w scanner (General Electric, Fairfield, CT, USA) with a 70 cm wide bore, utilizing a body transmit coil for excitation and an eight-channel receiver coil for signal detection. The MR session involved both structural imaging for voxel placement and localized single-voxel proton spectroscopy.

The measurements were performed twice before the exercise and then within an hour, at 8-minute intervals (two measurements: 1) for Glu and Glx at TE=35ms, and for Lac at TE=288ms). The structural scan was performed using Steady State Fast Spin Echo with three slices in each orthogonal plane, with the following imaging parameters: TR/TE = 6.412/2.1 ms, and a duration of 30 seconds. For spectroscopy, the volume of interest (30×30×60 mm^3) was positioned in the occipitoparietal gray matter (Fig. 1), a region known to be activated during exercise (18) and offering adequate time resolution in high magnetic fields.

Spectroscopic data were collected using the PRESS PROBE-P single-voxel sequence, with two different TR/TE settings: TR/TE = 1500/35 ms for short echo time and TR/TE = 1500/288 ms for lactate (Lac) measurements, a bandwidth of 2367 Hz, 128 acquisitions, and a total duration of 3 minutes and 48 seconds. Chemical shift selective (CHESS) water suppression was applied during data acquisition. Concentrations of glutamate (Glu), glutamine (Gln), and glutamate+glutamine (Glx, Glu + Gln) metabolites, measured through in vivo 1H-MRS, are highly dependent on the echo time. The glutamate signal, which is tightly packed in synaptic vesicles, contributes to the signal at short echo times but becomes invisible at long echo times.

To measure Glu, Gln, and Glx, spectra were first acquired using a short echo time of 35 ms (Fig. 2), followed by acquisition with a long echo time of 288 ms for lactate (Fig. 3). A large volume of interest (VOI) was used to capture the smaller signals from Glu and Glx within a reasonable time frame. Unsuppressed water signals were acquired for each voxel to enable eddy current compensation and phase correction. Each spectroscopic measurement involved 4,096 complex data points. For each 1H-MRS session, two subsequent spectra were obtained—first with short TE (35 ms) for Glu and Glx (see Fig 3), as well as with long TE (288 ms) for Lac (see Fig 2). No spectra were collected at TE = 144 ms (9).

Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy (MRS) is a non-invasive in-vivo technique allowing measurement of concentrations of a small set of metabolites that are "visible" under the conditions used in human MR scanners. Due to their chemical surroundings, the metabolites resonate at magnetic field frequencies slightly different than water, therefore, they may be distinguished by the differences in local magnetic field, known as chemical shift. These differences are commonly measured in the local magnetic field differences (compared to a standard value) normalized to the magnet'sstatic field, i.e., in parts per million (ppm). The figure including all metabolites is called a spectrum; it includes all metabolites as a function of their chemical shifts. Fig. 2 and Fig. 3 show

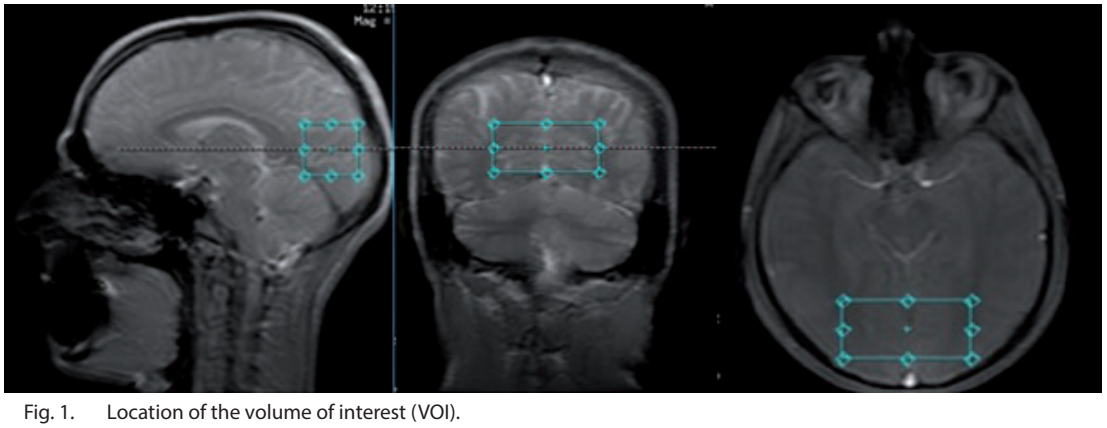

Fig. 1. Location of the volume of interest (VOI).

exemplary spectra. Please note that they differ according to some imaging parameters, here echo time TE, which reflects physical properties of the metabolites in tissue. The metabolite concentrations are calculated as the area under the curve (peak) corresponding to certain metabolites cut off from the bottom by so-called baseline (see captions of Fig. 2 and Fig. 3). The numerical calculations are performed by dedicated software. More basic information can be found at https://radiopaedia.org/articles/mr-spectroscopy-1.

Spectra quantification

Metabolite concentrations were estimated using the LCModel software [14], version 6.3. The default LCModel pre-processing pipeline was utilized, i.e., the sum of squares channel combined and phase cycle accumulation. The analysis window used with LCModel was 4.0 to 0.2 ppm. An example of the LCModel approximation of metabolite concentrations superimposed on the representative spectra obtained from the occipito-parietally localized VOI is shown in Fig. 2.

The 'standard' basis set for the 3T PRESS sequence with TE 35 ms or 288 ms was used, containing the following metabolites: alanine (Ala), aspartate, (Asp), creatine (Cr), phosphocreatine (PCr), glycerophosphocholine (GPC), phosphocholine (PCh), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), lactate (Lac), myo-Inositol (Ins), N-acetyl aspartate (NAA), N-acetyl aspartate glutamate (NAAG), scyllo-Inositol (Scyllo) and taurine (Tau). The simulated basis set was provided by S. Provencher on request. Specified timings and ideal pulses were used for gamma simulation. The signal-to-noise ratio (SNR) was derived from the LCModel. Relative standard deviation estimates of the resonance signals (Cramér-Rao lower bounds, CRLB) were used to establish the criterion for fur-

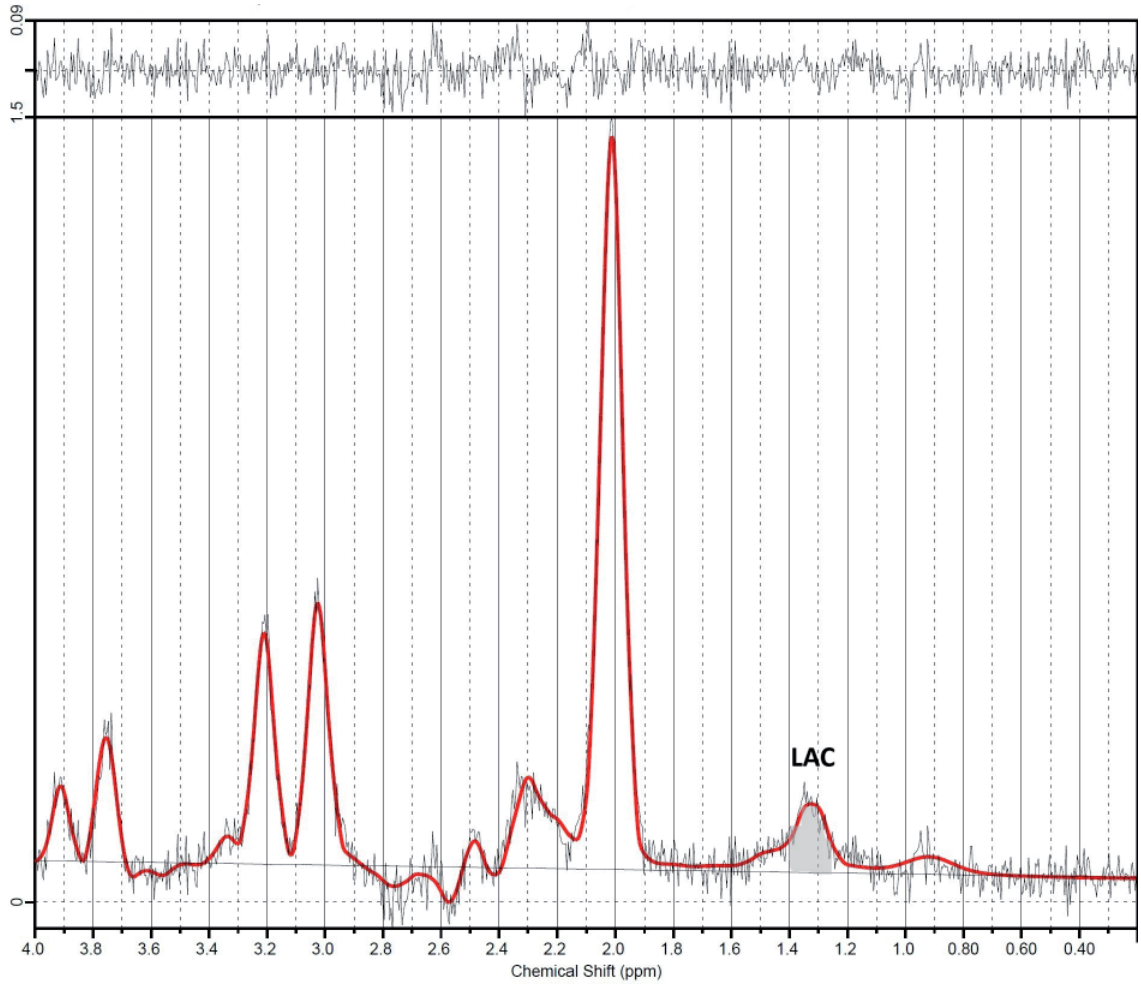

Fig. 2. Spectra acquired at TE=288ms that are used to calculate concentrations of Lac. The feature at 1.3 ppm originates from lactate (Lac). The red line depicts the calculated spectrum. The difference between acquired data and fitted spectrum, as a function of chemical shift, is presented in the top part of the figure. The concentration of Lac is proportional to the (shaded) area between the fitted spectrum (red line) and the baseline (black line).

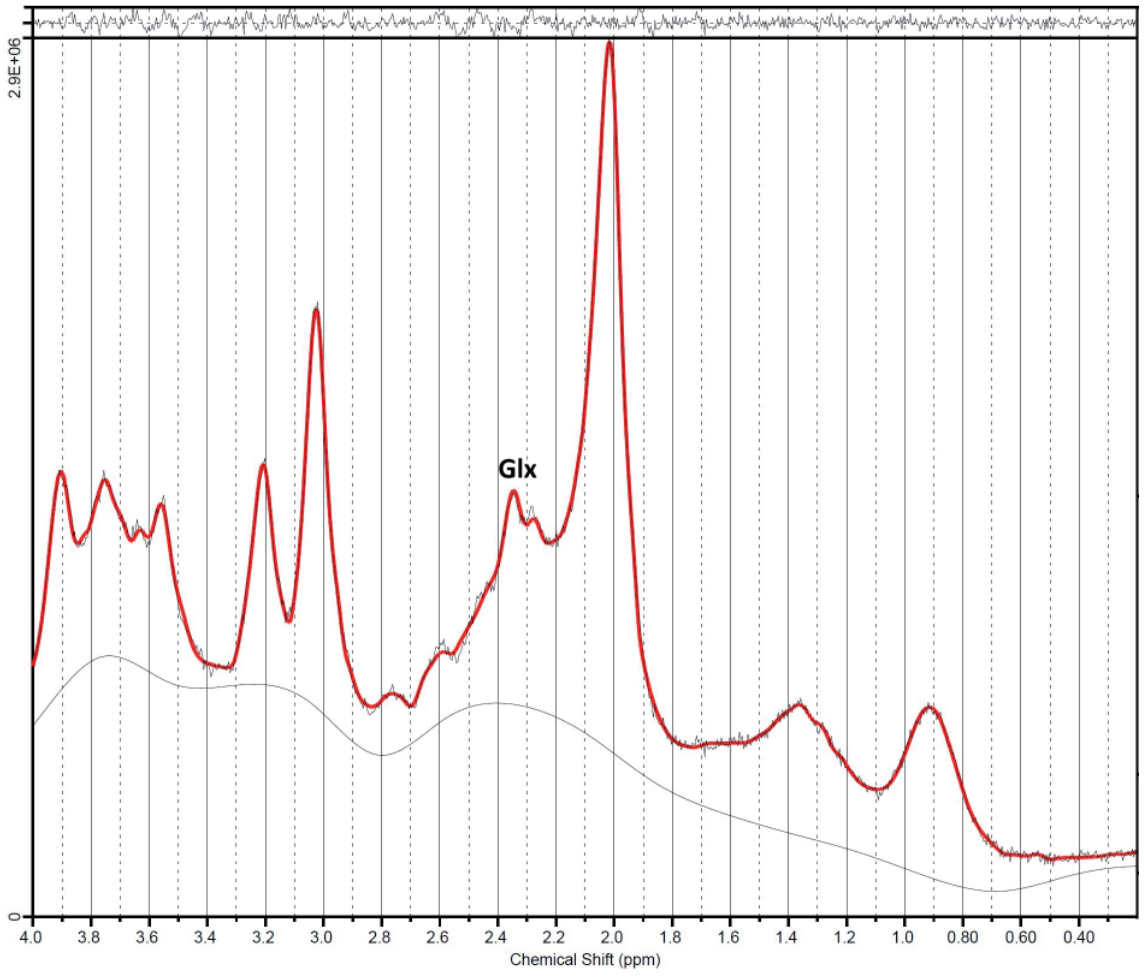

Fig. 3. Example spectrum for TE=35ms. Note that the signal at 1.3 ppm is contaminated by fat and macromolecules. Therefore, this spectrum is used only to calculate the concentration of Gln and Glu (Glx). The red line depicts the calculated spectrum. The difference between acquired data and fitted spectrum, as a function of chemical shift. is presented at the bottom part of the figure. The black line below the spectrum depicts so so-called baseline, which is non-zero at TE=35ms. The panel to the right depicts measured concentrations of metabolites.

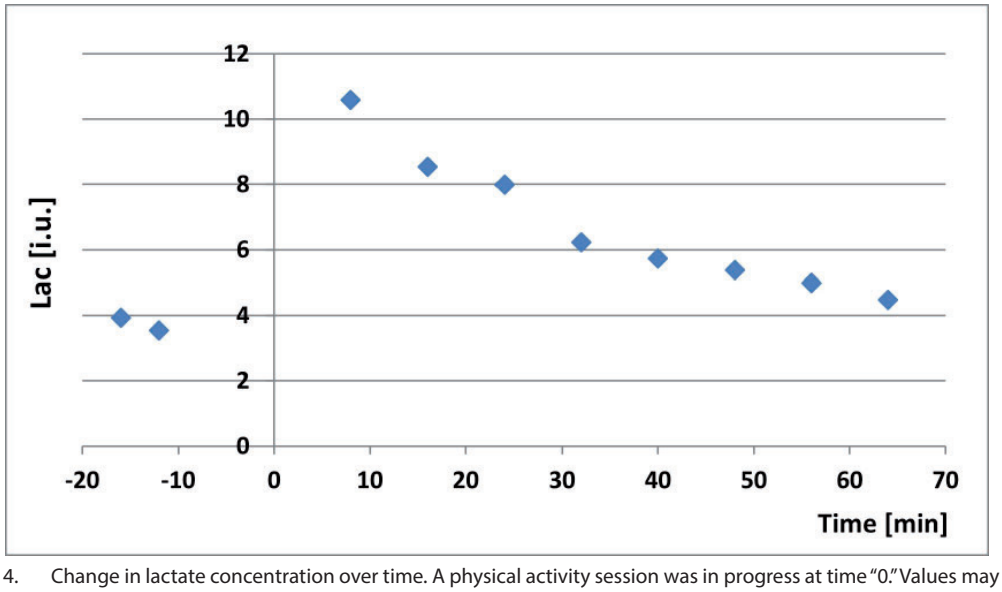

Fig. 4. Change in lactate concentration over time. A physical activity session was in progress at time "0." Values may be underestimated because the contribution of cerebrospinal fluid to VOI was not included in the calculations.

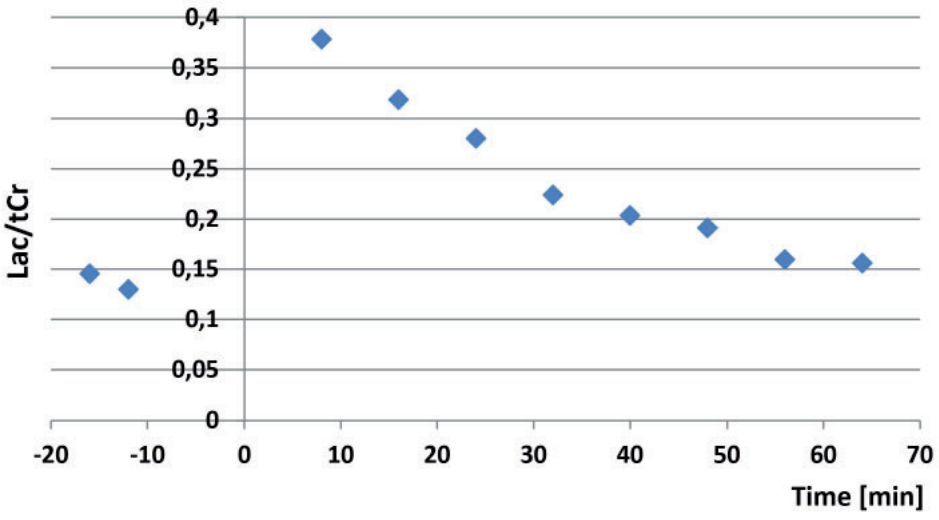

Fig. 5. As above, except that lactate concentrations were normalized to those of creatine-containing compounds.

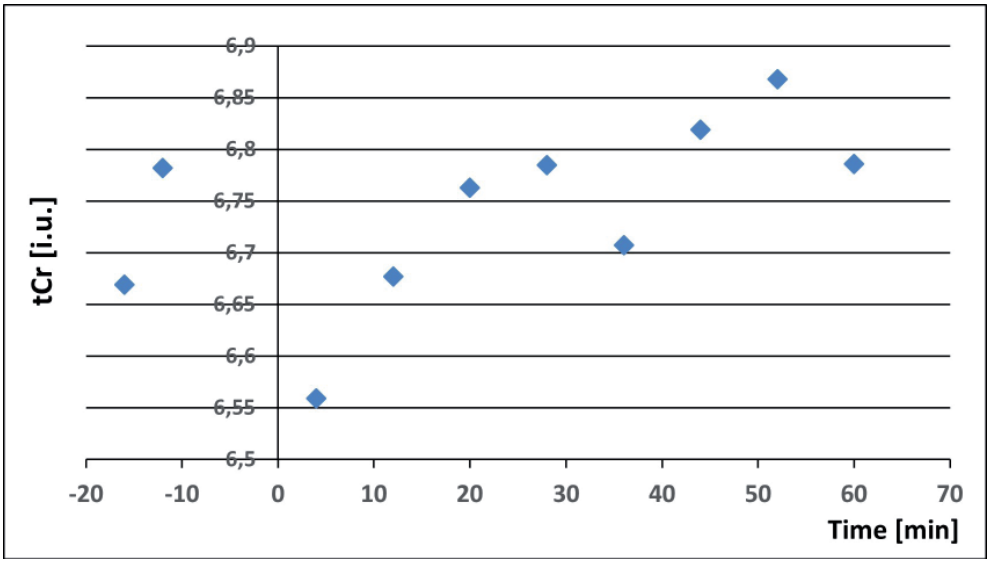

Fig. 6. Changes in concentration of creatine-containing compounds due to exercise and following recovery.

ther analysis. Resonance signals with mean CRLB ≤ 15% for metabolites and ≤ 30% for Lac (excluding macromolecules) were identified and used to obtain estimates of their respective concentrations. Metabolite signals in the acquired spectrum were additionally scaled to the signal of the sum of creatine and phosphocreatine (tCr); i.e., the concentrations were measured in institutional units, not in moles/grams of the neurotransmitter of interest per unit volume of the brain [13]. Metabolite concentrations were not corrected for brain tissue contributions to the volume of interest, by metabolite relaxation times, pulse profiles, or coil bias profiles.

RESULTS

Figures 4 and 5 show the changes in lactate concentration (Lac) and lactate concentration scaled to the concentration of creatine-containing compounds (Lac/tCr) following the exercise. As a result of physical activity, the lactate concentration reaches a value of 11mmol/L and then slowly decreases over time, reaching the pre-exercise value within an hour.

Figure 6 displays the changes in creatine-containing compounds (total creatine, tCr) due to exercise and their recovery with time. The changes are consistent with [4], but the concentration appears to normalize within 20 minutes. Therefore, the concentrations of Glu, Gln, and Glx scaled to tCr will be affected by these changes.

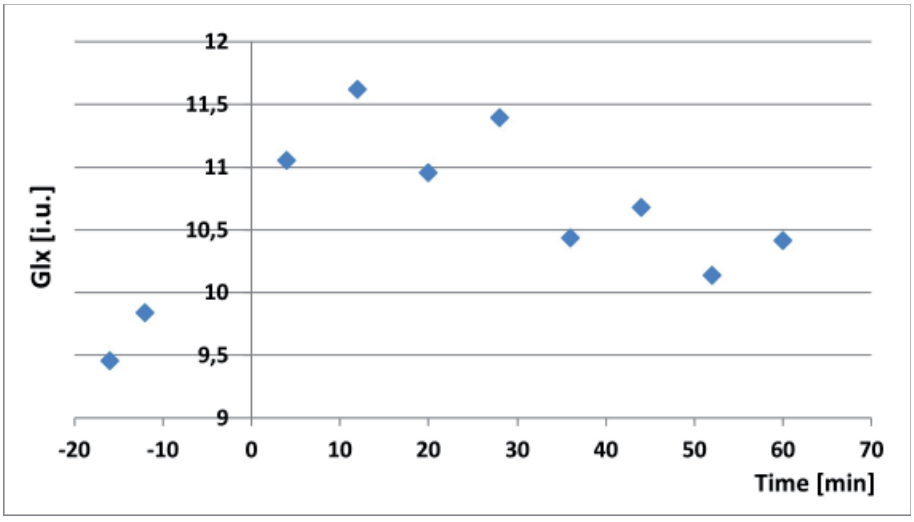

Fig. 7. Change in sum of glutamate (Glu) and glutamine (Gln) concentration over time. A physical activity session was in progress at time "0." Values may be underestimated because the contribution of cerebrospinal fluid to VOI was not included in the calculations.

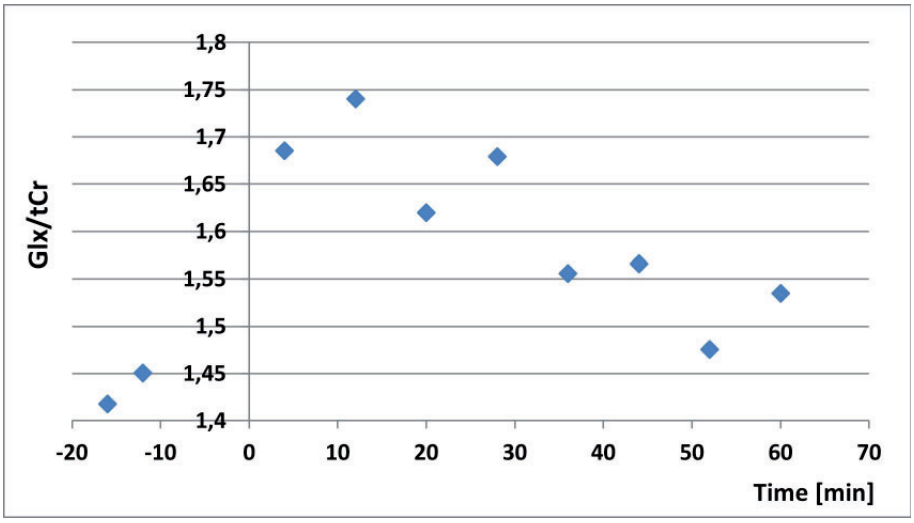

Fig. 8. As above, except that Glx concentrations were normalized to those of creatine-containing compounds.

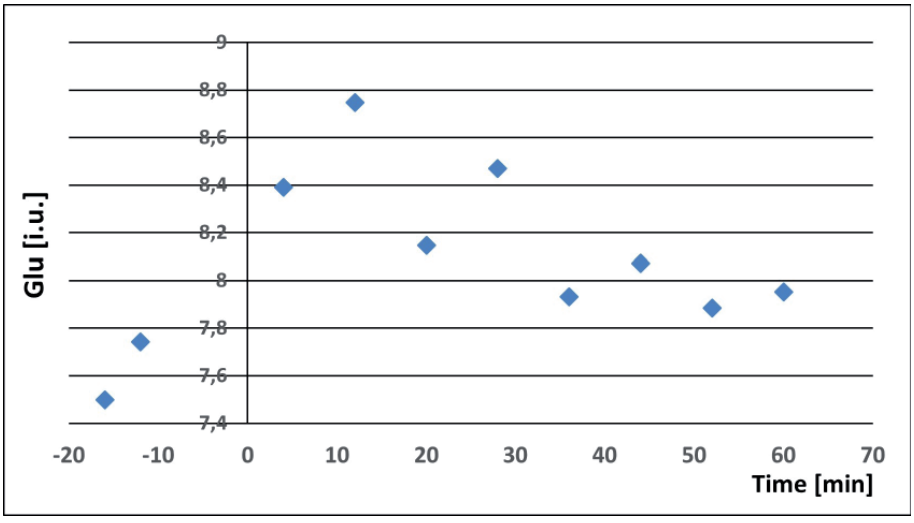

Fig. 9. Change in glutamate concentration over time. A physical activity session was in progress at time "0."

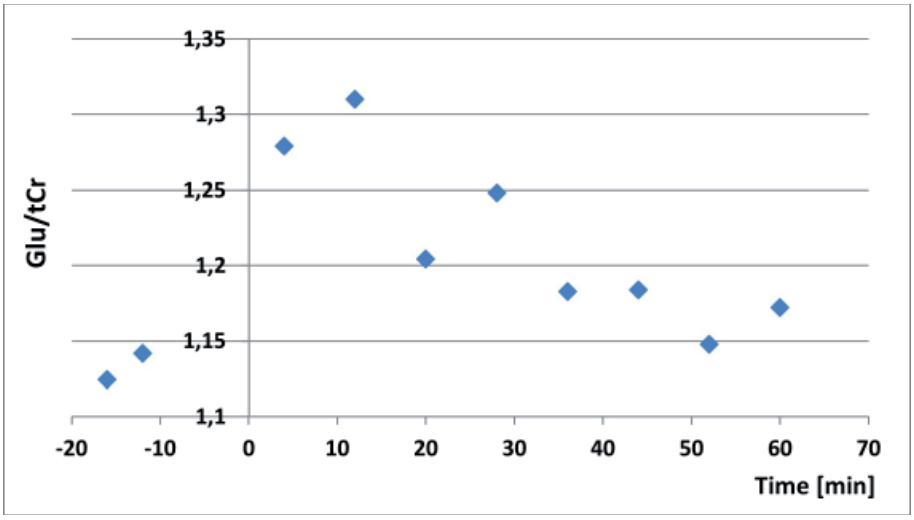

Fig. 10. As above, except that Glu concentrations were normalized to those of creatine-containing compounds.

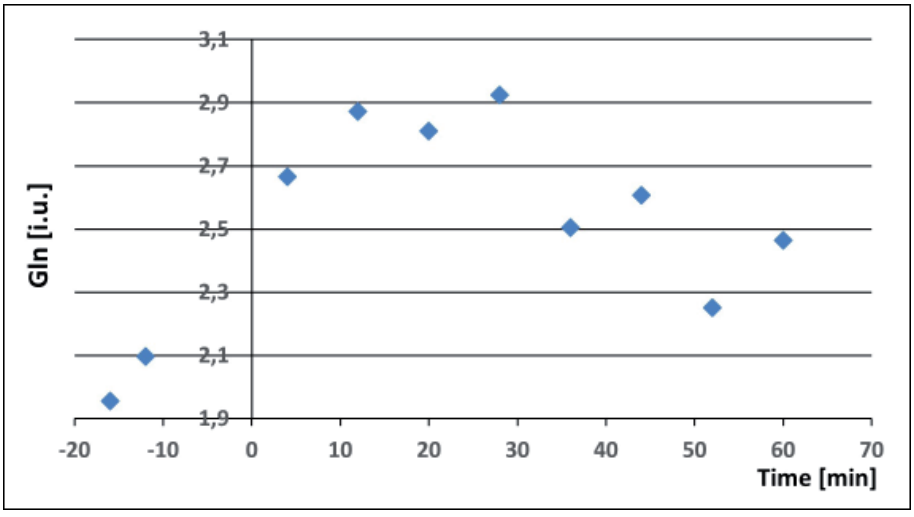

Fig. 11. Change in glutamine concentration over time. A physical activity session was in progress at time "0."

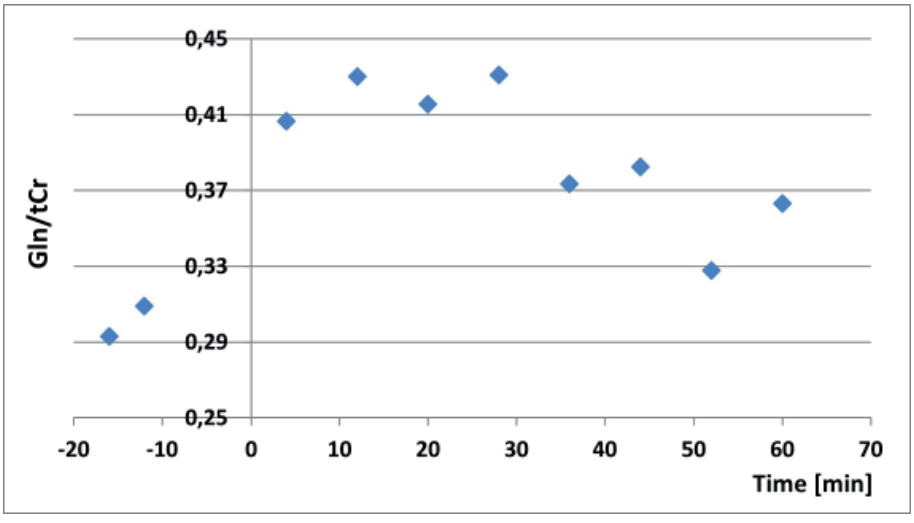

Fig. 12. As above, except that glutamine concentrations were normalized to those of creatine-containing compounds.

Figures 7-12 show changes of Glx, Glx/tCr, Glu, Glu/tCr, Gln, and Gln/tCt concentrations with time after the conclusion of the exercise. As a result of physical activity, their concentrations increase, then normalize over time.

The reader should note that Gln signal mirrors Glu, contrary to expectations. Based on the Glu-Gln cycle, we would expect decreases in Glu concentrations following Gln increases, and vice versa. Therefore, given the Glu and Gln spectral overlap, it appears that we observe a numerical artifact here. Taken together, Glx (Glu+Gln) should be reported (see [20]).

DISCUSSION

The study demonstrates the validity of performing spectroscopic measurements at about 60-minute interval post-exercise to serve as "at rest" reference values. These insights were used to refine the protocol for the study by Świątkiewicz and colleagues [20]. The use of the large volume-of-interest in the parieto-occipital lobe allows for increasing the time resolution of the measurement without introducing significant artifacts from magnetic field inhomogeneity. Therefore, the additional time needed to perform the spectroscopic measurements in the actual diagnostic setting is brought to a minimum.

Comparison of Glu, Gln, and Glx, to their respective values scaled to tCr demonstrated that the general trend is not changed by the effects of exercise on tCr concentrations.

Other studies reported that increasing brain Lac due to physical exercise affected the concentration of gamma-Aminobutyric acid (GABA) in sensorimotor cortex, but not in dorsolateral prefrontal cortex [2]. As GABA was not in focus of the study by Świątkiewicz et al [20], no attempt was made to establish how its concentration changes post-exercise with time.

Finally, post-exercise blood lactate concentration and its changes were demonstrated to be a performance marker in (elite and sub-elite) athletes [12]. However, the actual concentrations of lactate within the muscle are not known. Therefore, the protocol used in our study could be suitable to evaluate the concentrations of Lac in exercising muscles, both in members of the military and in athletes.

In conclusion, the results allow for the use of concentrations of Lac and Glx 60 minutes post-exercise as proxies for pre-exercise concentrations of these metabolites.

AUTHORS' DECLARATION:

Concept of the article: Stefan Gaździński and Ewelina Maculewicz. **Theoretical input:** Stefan Gaździński, Michał Madeyski, Aleksandra Przybysz, Ewelina Maculewicz. **Research methods:** Stefan Gaździński, Anna Przewodzka. **Execution of research:** Stefan Gaździński. **Data processing:** Stefan Gaździński, Anna Przewodzka. Analysis and interpretation of the results: Stefan Gaździński, Anna Przewodzka, Michał Madeyski, Aleksandra Przybysz, Ewelina Maculewicz. **Project administration:** Stefan Gaździński. The Authors declare that there is no conflict of interest.

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