



Levels of intra and extra cellular triglycerides in human muscles by means of ¹H-ERM – A case study

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ABSTRACT

The objective of this study was to analyze the intra (IT) and extra cellular (ET) triglycerides consumption in the soleus, tibialis anticus, vastus internus muscles, after 4 hours training ride on the road. This study used a top-level cyclist as a case study. Magnetic resonance studies used the following spectroscopy parameters imposed to adjust the time, such as the distance of the frequency between the signs IT and ET of the similar chemical nature. We were able to conclude that the vastus internus was the muscle which demonstrated the largest triglyceride consumption as the energetic substrate after a 4 hour bike ride on the road. Thus, we can infer that a workout at 80% of the $\dot{V}O_{2max}$ enabled the consumption of intra muscle triglyceride during exercise.

INTRODUCTION

The constant advance of results of the high-level sportive practice is grounded on the improvement and optimization of the athlete's motricity based on a series of environmental, biomechanical, psychological and physiological factors. The interest of the study on physiological factors includes the direct or indirect stimulation of the energy stores as well as their availability, transportation and utilization during exercise.

We know that the hydrolysis of the adenosine-triphosphate (ATP) is required for the muscle contraction to occur and that the aerobic and anaerobic processes will deplete the substrates that will allow maintaining the ATP level required to perform repeated contractions. During many years, the only way to valorize the biochemical and exercise-induced physiological modifications consisted of analyzing the cardiovascular and metabolic adaptation kinetic (cardiac frequency, oxygen intake, lactatemia, etc.). However, a new methodology called as nuclear magnetic resonance (NMR) has recently emerged, what allows the direct study of the biochemical modifications produced in the muscle during contraction.

The NMR functions as a real local chamber that enables visualizing in a non-invasive way the variation of the elements that influence the ATP resynthesis. This non-invasive featured NMR allows performing an unlimited number of measurements, what favors the attainment of data with physiological interest including situations close to important competitions. As result, the use of this new methodology to valorize the physiological intramuscular modifications produced during exercise and during the recovery phase opens new horizons for the functioning comprehension of the most intimate mechanisms of the muscular function.

The magnetic resonance (MR) methods are more and more used to investigate the physiology of the human muscle. Although the

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MR image (MRI) reveals the muscle volume and the orientation of its fibers, the MR spectroscopy (MRS) provides information with regard to the tissue chemical composition. Depending on the nucleus observed, the MRS allows the observation of phosphorylated metabolites involved in the muscular bioenergetics (³¹P-MRS), glycogen (¹³C-MRS) or intramyocellular lipids (¹H-MRS).

The triglycerides metabolism in the fat tissue and its regulation has been studied in details for many years. However, the knowledge of the triglycerides (TG) metabolism regulation in muscle is limited.

However, it was estimated that the contribution of the muscular TG for the total production of energy during exercise (65% of the $\dot{V}O_{2max}$) was 15-35%⁽¹⁻⁴⁾. The energetic contribution of distinct substrates during exercise at three distinct intensities was also investigated⁽⁵⁾. The intramuscular TG contribution was of 7%, 26% and 8% during exercise performed at 25%, 65% and 85% of the $\dot{V}O_{2max}$, respectively. This suggests that there is an optimal point in the utilization of intramuscular TG between 25% and 85% of the $\dot{V}O_{2max}$. Highly trained athletes seem to use more their intracellular triglycerides (IT) stores. In this context, other study demonstrated that the training increases the contribution of the IT consumption to the total energetic expenditure⁽⁶⁾.

Generally, one may conclude that the muscular TG are consumed during the submaximal exercise as an important substrate for the contracting muscle⁽⁷⁾. It was also recently described that the intra (IT) and extra (ET) muscular triglycerides may be quantified in a non-invasive and non-destructive way by proton magnetic resonance spectroscopy (¹H-MRS)^(4,8-13). One may observe some advantages in the utilization of the magnetic resonance non-destructive method in the biopsy, once it presents higher volume of accessible sample, uses the same investigated zone before and after exercise, allows the distinguishing between IT and ET and, finally, enables higher access to elite athletes, who do not easily agree with the performance of tests by means of biopsies.

Therefore, this study had as objective analyzing the intra and extra muscular triglycerides levels in the soleus, tibialis anticus, vastus internus muscles through proton magnetic resonance spectroscopy before and after four hours of training ride on the road.

METHODOLOGY

Case study

This research is characterized as case study with an international level cyclist who had been previously informed about the type of experiment he would participate in and signed the consent form. The protocols and the consent form were previously approved by the Ethics Commission of the Federal University of Paraná.

Magnetic resonance (MR) procedures for ¹H-MRS

The athlete arrived in the magnetic resonance unit of the Diagnostic and Image Center (DAPI) two hours after his last meal. The magnetic resonance studies were performed in a superconductor magnet that generates a magnetic field of 1.5 Tesla by means of the utilization of a knee coil (figure 1).

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Fig. 1 – Knee coil for proton spectroscopy

During the experimental protocol, the athlete remained lying at supine position with his right leg immobilized and aligned with the magnet longitudinal axis. The left leg was supported with cushions out of the coil. A mark on the skin was performed and the distance from the mark up to the patella was measured with a tape measure to know the correct distance in the medium portion of the following muscles: soleus (S), tibialis anticus (TA), vastus internus (VI) in order to have the same region as reference for the performance of the experimental protocol before and after four hours training ride on the road and this mark was positioned at the magnet isocenter.

After positioning, the athlete was reminded not to move while the protocol of ¹H-MRS was performed (figure 2).

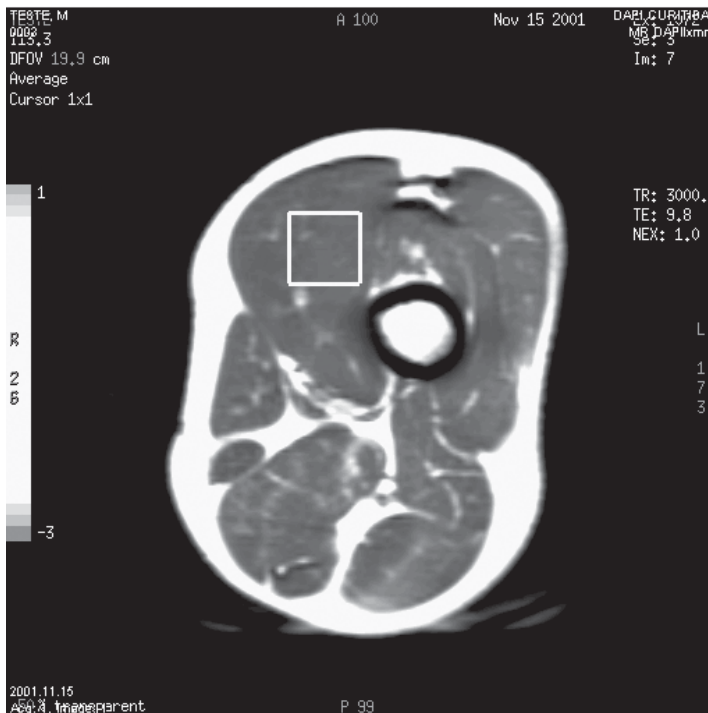


Fig. 2 – Voxel localization images of the vastus internus muscle to obtain the spectra

The spectra were processed using the *Magnetic Resonance User Interface* (MRUI) software, which is a mathematic program that enables the application of algorithms of signals in time domain for the extraction of the spectra parameters that may express biochemical information.

The spectral parameters imposed for the adjustment in time domain were: the frequency distance between IT and ET signals from protons ($-\text{CH}_2$)_n or $-\text{CH}_3$, which was assumed as cause of the magnetic susceptibility effects⁽⁸⁻¹¹⁾, being considered as equivalent for both resonances ($\delta_{\text{ET,CH}_2} - \delta_{\text{IT,CH}_2} = \delta_{\text{ET,CH}_3} - \delta_{\text{IT,CH}_3}$). However, the chemical dislocations interval for groups $-\text{CH}_3$, $(-\text{CH}_2)$ _n of extra cellular triglycerides (ET), $=\text{C}-\text{CH}_2$, and intra cellular triglycerides (IT) $\text{HOOC}-\text{CH}_2$, were restricted to intervals between 1.0-1.2, 1.4-1.7, 1.7-1.9 and 2.3-2.5 ppm, respectively. The smoothing factors (R) and the amplitudes (a) were fixed as $R_{\text{ET,CH}_3} = 0.842 R_{\text{ET,CH}_2}$ and $R_{\text{IT,CH}_3} = 0.842 R_{\text{IT,CH}_2}$, and $a_{\text{ET,CH}_3} = 0.130 a_{\text{ET,CH}_2}$ and $a_{\text{IT,CH}_3} = 0.124 a_{\text{IT,CH}_2}$, respectively.

The phase correction of order zero and one was estimated by AMARES in the MRUI program. However, the relative phase of the resonances between each other remained zero. The water resonance was quantified by AMARES and the water FID without being suppressed was obtained at the same voxel using the sine curve exponentially smoothed (corresponding to a Lorentzian signal in the frequency domain). In this last case, all adjustment parameters were left with no restriction. The areas calculated were corrected by the differential saturation effects using the longitudinal (T_1) and transversal (T_2) relaxation times of the resonances of interest in lipids and water (figure 3).

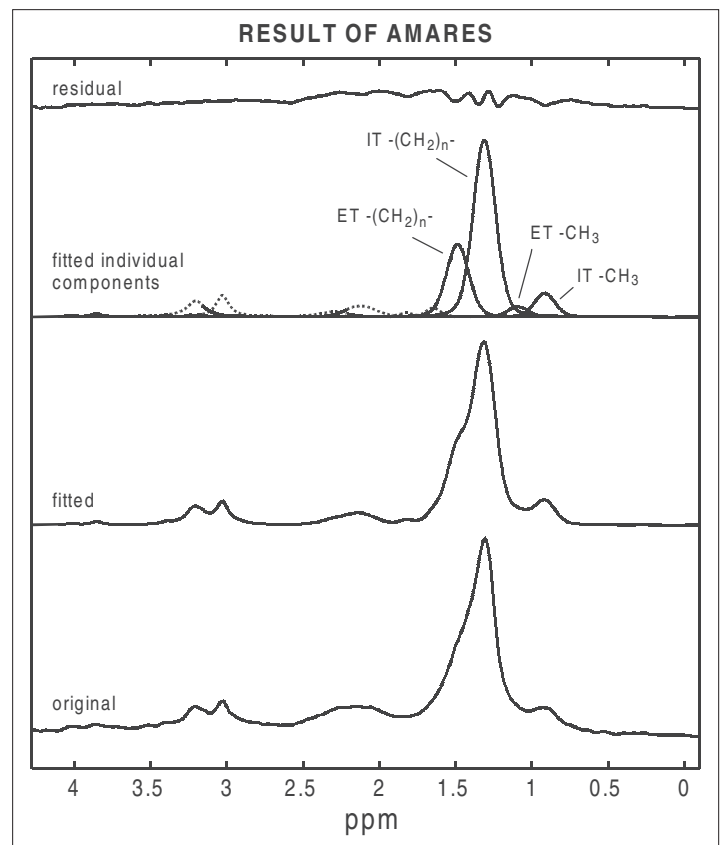


Fig. 3 – Typical ¹H-MRS spectrum of the cyclist's vastus internus muscle by means of the MRUI program

RESULTS

Table 1 presents the results of the intra and extra cellular triglycerides of soleus, tibialis anticus, vastus internus muscles. A higher consumption of intracellular triglycerides was observed after training ride on the road.

DISCUSSION

The possible alterations of the intra and extracellular triglyceride concentration in the soleus, tibialis anticus, vastus internus mus-

TABLE 1
Intra and extracellular triglyceride values

	CH2 extra/water	CH2 intra/water	CH2 extra/intra/water
Tibialis anticus			
before	0.0879	0.0199	–
after	0.0843	0.0200	–
Vastus internus			
before	0.1574	0.1325	–
after	0.1611	0.0837	–
Soleus			
before	–	–	0.8266
after	–	–	0.8594

cles after four hours of training ride on the road in a highly trained athlete were investigated through non-invasive measurements in this part of the study. The results demonstrated IT consumption of 63% in the vastus internus muscle after four hours of training ride.

The IT metabolism was studied during continuous exercises of several intensities and durations by means of biopsies. The different results showed disagreements with regard to the use of IT during exercise. Thus, the IT contribution in the energetic metabolism of the vastus internus muscle in humans during intermittent exercises was evaluated^(14,15). Researchers reported a decrease on IT of approximately 25% of the rest value after 60 minutes of intermittent bike exercise, alternating 15 s at 100% of the $\dot{V}O_2$ max with 15 s of recovery⁽¹⁴⁾. After 15 minutes of exercise, these authors observed IT decreasing tendency. In this context, a decrease of 29% in IT after 30 s of exercises performed until fatigue alternated with 5 minutes of recovery was also observed⁽¹⁵⁾.

The utilization of IT and ET in soleum, gastrocnemius and tibialis anticus muscles in humans was studied by means of ¹H-MRS in two high-intensity continuous exercise protocols alternated with periods of lower intensity⁽¹⁰⁾.

A consumption of IT and ET during exercise protocols performed was not found in this study. However, some researchers described IT depletion ranging from 20 to 50% of the rest value in exercises performed from 55 to 65% of the $\dot{V}O_2$ max^(1,2,6,16,17). In this regard, a decrease of 40% in IT was found through ¹H-MRS in a subject after 3 hours pedaling mountain bike^(4,9). However, our study is in agreement with studies previously mentioned because it presented consumption of 63% of IT in the vastus internus muscle after four hours of training ride on the road at intensity of 80% of the maximal cardiac frequency.

Contrarily to these results, other researchers reported no alterations in IT concentrations during bike exercise of 25 to 120 minutes at intensity of 50 to 65% of the $\dot{V}O_2$ max or leg extension dynamic exercises at intensity of 68% of the $\dot{V}O_2$ ⁽¹⁸⁻²²⁾. However, it has been demonstrated that the highest utilization of IT occurs at intensity of 65% of the $\dot{V}O_2$ max⁽¹⁷⁾.

An additional reason for the disagreements existing in literature with regard to the utilization of the IT during exercises is the existence of possible methodological problems. A variation coefficient of 35% was observed in IT values in prepared sample biopsies combining with the same type of fibers (fibers I or II)⁽²²⁾, however, other study observed variation coefficient of 24% in skeletal muscle biopsies⁽²¹⁾.

The main problem of the utilization of biopsy analysis methodologies seems to be related to the difficulty in separating the ET situated next to the muscular fibers through dissection methods. On the other hand, using ¹H-MRS, the variation coefficient described for the IT determination in the different studies was of 6%⁽⁹⁾, 14%⁽¹⁰⁾ and 12%⁽¹¹⁾.

Several studies proposed that an important factor to detect IT muscular alterations is the exercise protocol duration^(14,19,20,24,25). In this regard, it was observed by means of biopsies that during ex-

tended exercises of up to seven to eight hours, a decrease of 53% to 63% on IT^(26,27) occurs. This IT depletion occurs when the muscular glycogen or the surrounding glucose diminishes with the progressive exercise protocol duration^(28,29).

Another important cause of the IT decrease is the intensity of the exercise performed^(5,29,30). Studies in animal model verified reduction of IT during intermittent contractions of 30 s at 5 Hz followed 60 s of rest in female rat muscle^(29,30). However, results in humans verified that the peripheral lipolysis was stimulated when subjects worked at 25% of the $\dot{V}O_2$ max and the IT consumption was stimulated at intensities between 65% and 85% of the $\dot{V}O_2$ max⁽⁵⁾.

During short and intense exercises, it seems clear that IT consumption is not detected and, therefore, the energetic supply is mainly provided from the PCr and glycogen hydrolysis in both types of muscular fibres^(31,32).

However, we cannot disregard a possible contribution of the surrounding fatty acids and those released by the lipoprotein lipase from endothelial capillaries to the muscular energetic metabolism during exercise protocol^(25,33). Thus, the results obtained by means of the palmitate marking with ¹⁴C demonstrate an active turnover of the IT pool when exercises at 45% of the $\dot{V}O_2$ max are performed with depletion and resynthesis quantitatively comparable resulting in IT pool apparently constant⁽³⁴⁾.

CONCLUSION

The intra (IT) and extracellular (ET) triglycerides in the soleus, tibialis anticus, vastus internus muscles of a cyclist were studied by means of ¹H-MRS in a non-invasive way.

We concluded that the vastus internus muscle of the cyclist presented higher triglyceride consumption after four hours of training ride on the road; these results are in agreement with a biomechanical analysis that reports that the vastus internus muscle is one of the main muscles responsible for the movement performed by the cyclist. Therefore, one concludes that a work with intensity of 80% of the maximal cardiac frequency allowed triglyceride consumption during exercise.

All the authors declared there is not any potential conflict of interests regarding this article.

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