Intramuscular triacylglycerol: an important energetic substrate for endurance exercise

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ABSTRACT

Free fatty acids represent an important source of energy during endurance exercise. Plasmatic fatty acids are delivered to skeletal muscle as free fatty acids bounded to albumin or associated to triacylglycerol (TAG) found in lipoproteins. However, besides these plasmatic sources, hydrolysis of intramuscular TAG also contributes to increase fatty acids availability during endurance exercise. The objective of the present study was to access the role of intramuscular TAG as an energetic substrate to endurance exercise. The present data suggests that the contribution of endogenous intramuscular TAG supplies is quite relevant. Furthermore, it is possible to conclude that endurance training induces an increase of these intramuscular supplies. After endurance training, it is also observed an increase in the utilization of intramuscular TAG. Although the intramuscular TAG seems to be an important energetic substrate, there is a controversy on the actual relative participation of this fuel during endurance exercise. The apparent discrepancy in literature is associated to methodological limitations that have been associated with the strategies used to estimate its oxidation during exercise. In order to deplete this issue, more research using new methods (e.g., utilization of stable isotope methodology, magnetic resonance spectroscopy, and electron microscopy) should be conducted.

Key words: Triacylglycerol. Muscle. Endurance exercise. Lipid metabolism.

INTRODUCTION

Although knowledge on the lipid metabolism in the skeletal muscle has spread considerably only in the last decades[1], the contribution of lipids as substrate for musculature under effort has been discussed since the beginning of the XVIII century[2].

The relation between lipid metabolism and physical activity has always been object of controversy. In the middle of 1800, Chaveau suggested that fat should be converted into sugar before being extracted by the muscle under effort[3]. In 1911, Zuntz demonstrated that fat was directly oxidized as energetic substrate[3]. The use of fat as fuel for musculature during exercise in humans was firstly demonstrated in 1939[4]. However, only in the middle of decades of '50 and '60, studies determined that fat was transported into the muscular fiber under the form of free fatty acids (FFA) in experiments using fatty acid marked with radioisotopes[5,6].

Nowadays, one knows that fat is an important substrate for muscle during exercise[1,7]. However, the proportion of energy derived from the oxidation of fatty acids during exercise is highly variable and influenced by many factors, including the nutritional state, the hormonal profile, the type, intensity and duration of exercise as well as the training level[8].

The triacylglycerol molecule (TAG – three molecules of fatty acids associated to one molecule of glycerol) represents the form how fat is stored. In human beings, most part of TAG is stored in the fat tissue (~9 to 15 kg in an adult man weighting ~70 kg), but the triacylglycerol is also present in small amounts in the plasma and in the skeletal muscle[9,10]. These large fat stocks in human beings and in other mammals are developed in order to assure survival in long fasting periods or in periods of rare supplies.

At first, the body energy storage capacity is limited to carbohydrates and unlimited to fat. The total amount of energy stored as TAG (80,000-140,000 kcal) is sometimes 60 times higher than that stored under the form of glycogen (1,700-2,000 kcal)[9,10]. Therefore, it became evident that during the performance of physical exercise, the amount of available fat is not a limiting factor to its oxidation. The use of this energy stock allows physical activity to be maintained for long periods and glycogen depletion and hypoglycemia to be delayed[9,11]. Other limiting steps such as the mobilization processes and the peripheral adaptations related to the lipid oxidation are vital for the attainment of energy during physical activity.

Undoubtedly, fatty acids are important energetic substrate for the performance of low intensity and long duration physical exercise (endurance exercise). However, the contribution of the intramuscular TAG supply has been neglected. The objective of the present work was to perform an extended literature review with the objective of determining the relevance of the energetic contribution of the intramuscular TAG supply during endurance exercises.

SUPPLY AND EXTRACTION OF LIPIDS BY THE SKELETAL MUSCLE

Once the capacity of muscular fibers to synthesize fatty acids is limited, this substrate has to be supplied by extracellular sources. In the organism, the fat present in the blood stream is found available for the muscular fibers under the form of fatty acids associated to albumin (also known as free fatty acids – FFA) or under the form of TAG found in lipoproteins (chylomicron, VLDL, LDL, IDL and HDL)[10].

In resting conditions, the blood flows into the muscle in a ratio of approximately 0.05 ml of blood per gram of muscle per minute. Considering that the FFA arterial plasma concentration is of 0.4 μmol per ml of blood in the hematocrit of 40%, the arterial fatty acids supply will be of approximately 12 μmol per gram per minute[12].

Based on those values, Owen and Reichard[12] estimated that the muscle in the resting situation would use approximately 5 ηmol of fatty acids per gram per minute, indicating that, in this condition, less than half of the fatty acids available in plasma will be extracted during the passage through the muscular capillaries. During exercise, the FFA availability in the muscle increases up to 600-900 ηmol of fatty acids per gram of muscle per minute. De-

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spite the sudden increase on the FFA availability, only a small parcel of this substrate is extracted by the muscular fiber during exercise[13]. Hagenfeldt and Wahren[13] demonstrated that the FFA extraction by the muscle during exercise is approximately 10-20% of the amount in circulation.

The arterial fatty acids supply associated to TAG from lipoproteins is far higher than the FFA. Calculations indicate that 90 and 1,800-2,700 ηmol of fatty acids associated to TAG per gram of muscle per minute are supplied to musculature in rest and during exercise, respectively. However, just as the FFA, only a small parcel of fatty acids present in the circulating TAG will be extracted during the blood passage in the capillary[14]. Therefore, the availability of fatty acids far exceeds the muscular fiber extraction and oxidation capacity.

The FFA associated to plasma albumin is originated from fat stored in the peripheral fat tissue as ester, that, once hydrolyzed by the hormone sensitive lipase (HSL), releases two moles of fatty acid and one mol of monoacylglycerol per TAG molecule[15]. Besides the fatty acids stored in the fat tissue, those originated from diet compose other source of substrate for the muscle during exercise. In order for the energy found in these fatty acids to be available, they must undergo many stages, including digestion, degradation into two fatty acids and monoacylglycerol due to the action of pancreatic and enteric lipases, emulsification due to the action of biliary salts, and lecitin and adsorption by the enterocyte, where they are converted into TAG through re-esterification for subsequent formation of chylomicrons. These lipoproteins reach the lymphatic circulation through the thoracic duct and then the venous system[16]. The TAG component of these particles may follow many paths, such as the storage in the fat tissue, the guiding to the energy attainment routes or even as substrate for the synthesis of other lipoproteins (such as VLDV) in the liver.

Considering that the vascular endothelium is impermeable to the circulating lipoproteins, the TAG present in these particles has to be hydrolyzed into glycerol and fatty acids in order for the transendothelial transport to occur. The hydrolysis of the TAG contained in lipoproteins is intermediated by the catalytic action of the lipoprotein lipase enzyme (LPL)[17]. This enzyme is found in the endothelium, more specifically in the luminal surface of the endothelium cell. The fraction of LPL enzyme that attaches to the proteoglycan composes its catalytic site[18].

Some reports in literature indicate a small contribution of TAG associated to lipoproteins in exercise or after training[19]. These studies indicate that no more than 10% of the total lipid oxidation is a result of the hydrolysis of TAG originated from lipoproteins in these conditions[15], although researchers have found difficulties in evaluating whether the measured plasma fatty acids concentration is a result of the lipoproteins hydrolysis or the release of fatty acids from adipocytes.

It is known that the lipoproteins profile is strongly influenced by exercise, which is recommended in the prevention of vascular diseases[20]. Trained individuals present low TAG plasma concentration, both in fasting state and in the postprandial period[21]. This is a result of the higher TAG extraction rate by the skeletal musculature caused by the increase on the muscular LPL activity[22].

Therefore, the differential regulation of the LPL activity in the fat tissue and muscle presents important implications in the distribution of the circulating TAG. The activity of this enzyme in the fat tissue is related to the storage of the circulating TAG. In the muscle, the LPL favors the utilization of TAG as source of energy during exercise. While the LPL activity and its RNAm concentration in the fat tissue decrease, the opposite behavior is observed in the cardiac and skeletal muscle during physical activity and after endurance training[23].

Besides the hydrolysis capacity of bridges between fatty acid residues and the glycerol in the TAG molecule, the LPL yet presents A2 phospholipase activity. The hydrolysis of phospholipids that compose the lipid layer that encapsulates the TGA from lipoproteins allows the interaction of LPL with its substrate, in this case the TAG. From the hydrolysis of this TAG found in lipoproteins, one parcel of the released fatty acids is immediately extracted by the muscular tissue cells. The rest attaches to the plasma albumin and reaches the blood stream[24].

Regardless the origin, either from the FFA derived from the fat tissue or from the TAG found in the lipoproteins, once extracted by the muscle, the fatty acid is transported through the plasmatic membrane by specific transporters[2]. After its extraction, the fatty acid reaches the cytoplasm through fatty acid-binding proteins (FABP)[25,26]. Following, the fatty acid may, then, be re-esterified or suffer oxidation in the mitochondria. In this last case, the fatty acid is activated through the acyl-CoA synthetase in order to form acyl-CoA. Once activated, the acyl group is now transported through the mitochondria membrane due to a carnitine-dependent enzymatic system[27]. Due to the catalytic action of the enzyme found in the outer mitochondria membrane, the carnitine palmitoil transferase (CPT I), the acyl combines with carnitine and the coenzyme A is released. The acyl-carnitine complex crosses the membrane due to the action of the translocase (carnitine acylcarnitine translocase) and the carnitine palmitoil transferase II (CPT II), found at the inner mitochondria membrane, is responsible for the dissociation of the acyl-carnitine complex with consequent acyl-CoA and carnitine regeneration[28,29].

After the catalytic action of the CPT II, the acyl-CoA is available for the β-oxidation system that will originate acetyl-CoA. The acetyl-CoA will then be able to be oxidized through the Krebs cycle in the intramitochondrial compartment. The activity of the CPT complex composes the main fatty acids oxidation regulation site[30].

Therefore, the lipids extracellular sources, regardless the origin, have to overcome barriers such as the mobilization (either through HSL in the fat tissue or through the LPL in the endothelium), extraction, cytoplasmatic and intramitochondrial transportation up to its final destination: the β-oxidation and the Krebs cycle.

THE INTRAMUSCULAR NEUTRAL LIPIDS SUPPLIES

Besides the plasmatic lipids sources, the muscle may also count on an additional lipid supply found in the own tissue. The intramuscular TAG is mostly found in the cytoplasm of low-contraction oxidative fibers[8,10,31,32], under the form of lipid droplets in the mitochondria neighborhood. Theoretically, this disposition would increase the capacity of the intramuscular TAG to supply fatty acids to be oxidized by mitochondrias.

Once some physical barriers such as the endothelium and the sarcolemma become irrelevant, the use of the intramuscular TAG would be an alternative to attend the energetic demand imposed by exercise[33,34]. Some works relate the increase on the mitochondria density in function of the endurance training to the increase on the use of these TAG intracellular supplies[35]. Other important adaptation to the endurance training is the increase on the storage capacity of these TAG intramuscular supplies[8,10] (figure 1 – A and B – micrographs given by Aoki[31]).

Historically, one believes that the transported FFA associated to the albumin, derived from stocks of TAG from the peripheral fat tissue, would supply most additional fat oxidized in trained individuals[3]. However, this concept is in disagreement with the idea of attenuating the neuroendocrine mechanisms induced by the endurance training. This mechanism regulates the lipolysis and hence the FFA availability during exercise. More recent proposals support the alternative hypothesis, that the endurance training increases the metabolism of these intramuscular TAG supplies and reduces the role of FFA as source of energy during exercise[58].

Investigations conducted for over than 40 years demonstrated that during moderate-intensity extended exercise, the intramuscular TAG is the preferential substrate of oxidative muscular fibers of

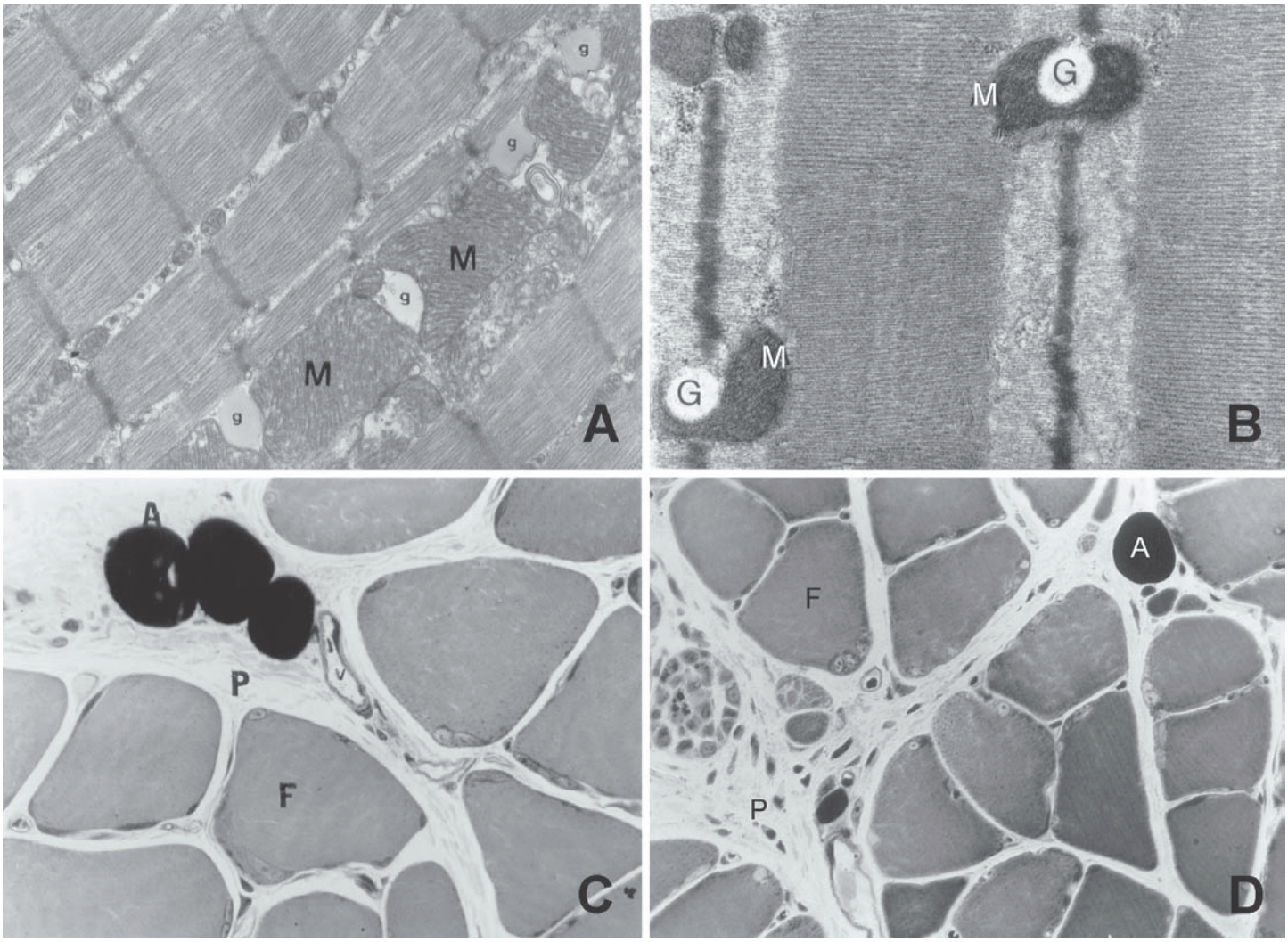

***Fig. 1** – Micrographs of the soleus muscle (**A** and **B**) – Observe several lipid droplets surrounded by mitochondrias (**M**) – given by Aoki (2000)[31]. Transversal section of the soleus muscle (**C** and **D**). Observe adipocytes (**A**) in the perimysium (**P**) between muscular fibers (**F**) – given by Belmonte et al. (2004)[54].*

guinea-pigs[37]. This seems to be the same for several species of birds and fishes, suggesting that the intramuscular TAG utilization and storage processes developed during the evolution of the more able species to use the body fat as main substrate. These migratory animals accumulate large amounts of TAG within locomotive muscular fibers. This makes them prepared to their journeys and these supplies are found depleted after they arrive to their destinations[38,39]. If migratory birds had to store the same amount of energy as carbohydrates, they probably could not fly due to the weight excess[38,39].

Consistent evidences of the physiological importance of the intramuscular TAG as energy source in non-migratory species became available about 20 years ago through studies in rats. A single exercise session until exhaustion resulted in depletion of 30-70% of the muscular TAG supplies of rats when compared to the pre-exercise value[40].

Yet in that study, Reitman *et al.*[40] used rats led to exhaustion after a swimming session and demonstrated that the magnitude of the intramuscular TAG depletion was dependent on the type of fiber, being about 70% in the fast-contraction oxidative fibers (type IIa) of the quadriceps and about 25% in the slow-contraction oxidative fibers (type I) of the soleus[40]. Back then, it was observed that the TAG concentration in the *vastus lateralis* muscle homogenate of human beings had decreased 25% after 90 minutes of cycling and 50% after some hours of a cross-country skiing event[41].

Recently, we have demonstrated that the high and chronic ingestion of lipids associated to the endurance training increased significantly the supplies of intramuscular TAG under the form of scattered droplets in the sarcoplasma (figure 1 – A and B – micrographs given by Aoki[31]). Despite the depletion on the glycogen supply (~50%) induced by the high lipid ingestion, animals supplemented with lipids presented the same muscular glycogen content as the control animals after 60 minutes of treadmill exercise, indicating the occurrence of the glycogen-saver effect[42]. A possible explanation for the induction of the glycogen-saver effect was the increase on the use of the intramuscular TAG in animals supplemented with lipids[42].

Turcotte *et al.*[43] corroborate our findings, reporting that the use of intramuscular TAG increases when the carbohydrates availability is dramatically reduced. In our study, the glycogen availability was smaller (~50%) in groups supplemented with lipids. The increase on the intramuscular TAG content through the endurance training would be opportune, once it would allow to save glycogen and, thus, would extend the time for the peripheral fatigue installation.

However, the contribution of this source of fatty acids derived from intramuscular TAG is not yet quite explained and seems to range from 5 to 70% of the pool of fatty acids oxidized during submaximal effort[8,10,44]. This discrepancy in the contribution values of this substrate may be explained when one considers: the intra-

muscular TAG supply before exercise, the heterogeneous distribution of these supplies in the different types of fibers, and the differences in the use of TAG between muscular groups. All these factors influence the use of fat during moderate-intensity extended exercises[45].

Romijn *et al.*[46] observed that in low intensity (25% of the $\dot{V}O_{2max}$), the intramuscular TAG contributes with less than 10% of the total oxidized fat. On the other hand, in intensity equivalent to 65% of the $\dot{V}O_2$max, the intramuscular TAG supplies 50% of the total fat metabolized during the first 60 minutes of activity in cycle ergometer. With the increase on the exercise duration at 65% of the $\dot{V}O_{2max}$, the intramuscular TAG represents about 30% of the total fat metabolized after 120 minutes. In higher intensities (85% of the $\dot{V}O_{max}$), the intramuscular TAG is responsible for about 40-50% of all oxidized fatty acid, but for only 10-15% of the total metabolized substrates[46]. Based on these results, one concludes that the contribution of these intramuscular TAG supplies is highly dependent on the exercise intensity and duration. Other factor that also determines its contribution is the training level[8-10].

Despite being limited, this TAG supply found in the cytoplasm of muscular fibers (2 to 10 μmol/g.wet weigh of tissue^{-1}) is equivalent to approximately 2,000 kcal[10,47]. Considering that its content is reduced in function of its higher oxidation[3,10,35,48-51], the importance of this substrate in the energy supply during endurance exercise is emphasized[10,52].

Unlike the glycogen, the intramuscular TAG is not homogeneously stored in the muscle[53]. The precise localization of this lipid source in the muscle is not yet quite conclusive. The intramuscular denomination implicates the fact of being TAG supplies stored as droplets within the muscular fiber[53]. However, it is not clear whether these supplies are only found in fibers or else in adypocites in the perimysium between the muscular fibers, and which would be the contribution of each compartment during extended exercise[3].

In another recent study, we verified through histological techniques the localization and participation of the intramuscular TAG during acute session of exercise and after endurance training[54]. In that study, it was observed that the lipid droplets found in the cytoplasm of the muscular fibers both in gastrocnemius and soleus presented decreased area in sedentary and also in trained animals after the performance of an exercise session at 65% of the $\dot{V}O_2$max.

Still in that study, the participation of adypocites found in the conjunctive tissue that covers the muscle was evaluated. With regard to the area occupied by adypocites (situated at the perimysium – figure 1 – C and D – transversal section of the soleus muscle – material given by Belmonte *et al.*[54]), a reduction only in the gastrocnemius muscle of trained rats was observed after an exercise session at 65% of the $\dot{V}O_{2max}$ with duration of 60 minutes[54].

Based on these results[54], we could demonstrate that the lipid droplets found in the fiber's cytoplasm served as energetic substrate both in trained and untrained animals. However, according to our histological observations, the mobilization capacity of the perimysium adypocites was muscle-specific (only in the gastrocnemius) and only observed after the endurance training. It is worth emphasizing that the extraction and oxidation of fatty acids released in the interstitial space of the adypocites found in the perimysium, without attaching to the plasma albumin, had never been demonstrated[15]. Therefore, in that study, it was demonstrated for the first time that the adypocites found in the perimysium are mobilized to supply energy during the endurance exercise[54].

INTRAMUSCULAR TAG METABOLISM CONTROL

The intramuscular TAG metabolism control (synthesis rate vs. degradation rate) is still unclear. This pool of muscular fatty acids is depleted during exercise and its content depends on the balance between the influx of the circulating FFA and the efflux caused by the muscle energetic demand[48].

However, it is important mentioning that the circulating fatty acids extracted by the muscle may come to be re-esterified. During exercise, this process is not very active and probably most hydrolyzed fatty acids are used by the muscle[48]. Other studies, however, indicate that 70% of fatty acids released from the fat tissue in rest are re-esterified in the muscle, and this value decreased down to 25% in the beginning of the submaximal exercise at 40% of the $\dot{V}O_{2max}$. Therefore, the increase on the oxidative rate could be a result not only of the higher intramuscular TAG mobilization, but also of the reduction on the re-esterification rate[55].

Recently, Sacchetti *et al.*[56] demonstrated that the incorporation capacity of fatty acids was reduced four times during exercise. Anyhow, the final result of the TAG intracellular supply turnover is a result of the sum of the hydrolysis and synthesis processes continuously and simultaneously underway[57].

There are several mechanisms that participate in the TAG regulation and utilization that seem to be highly dependent on the type of exercise, duration, intensity and training level[15]. The activity of the muscular HSL enzyme is an important stage for the intramuscular TAG utilization[36,58]. The lipolytic process that takes place in the skeletal muscle is regulated by means of neuroendocrine action, just as in the fat tissue; however, only type β2 adrenergic receptors are involved[57,59].

It is surprising that the intramuscular TAG metabolism is increased in trained individuals in which the exercise-induced sympathetic-adrenergic response is lower when compared with untrained individuals. In the study of Buckenmeyer *et al.*[60], the density of the β2 adrenergic receptors is increased in the oxidative fibers of slow (type I) and fast (type IIa) contraction in rats after a 12-week endurance training program. Therefore, the increase on the amount of β-adrenergic receptors could be mediating the increase on the hydrolysis of the intramuscular TAG, despite the decrease on the sympathetic-adrenergic stimulation observed during exercises in trained individuals[61].

Recent evidences indicate that, besides the activation of the muscular HSL via adrenaline, the muscle contractile activity itself is important for the activation of this enzyme[62]. These factors would be responsible for unchaining the phosphorylation of the muscular HSL in different sites, thus explaining the partly additive effect that they perform[62].

In a recent literature review[36], it was verified that during the initial minute of low-intensity aerobic exercise in the absence of adrenaline elevation, the HSL is activated through the muscular contraction. On the other hand, during high-intensity exercise, the initial HSL activation depends on the adrenaline[36]. With a few minutes of aerobic exercise performance at low intensity, the adrenaline starts playing an important role in the HSL activation[36]. It has also been demonstrated that after one-two hours of moderate-high intensity exercise, despite the adrenaline increase, the HSL activity is attenuated possibly due to the AMP accumulation[36].

The HSL regulation in the muscle is not yet quite clear; however, this enzyme plays an important role on the control of the intramuscular TAG utilization[62-64].

METHODOLOGICAL DIFFICULTIES AND FUTURE RESEARCHES

Some works that used the ^{14}C incorporation method in the CO_2 collected (calorimetric method) have demonstrated that the amount of oxidized FFA from the peripheral fat tissue is overestimated and, hence, data underestimate the contribution of the TAG oxidation from other sources[35]. This occurs because it is always necessary to take into account that part of the fatty acids extracted by the muscle may come to be re-esterified[65].

Many are the difficulties to estimate the oxidation rate of FAA from peripheral supplies and intramuscular TAG, as well as the amount of FAA from the fat tissue that may come to be re-esteri-

fied in the contracting muscle. The study becomes even more complicated when it is necessary to quantify the hydrolysis and oxidation of the intramuscular TAG during physical activity. Some studies with muscle of humans estimate the intramuscular TAG oxidation through the difference between the total fat oxidation (calorimetric method) and the exogenous FAA oxidation (marked FAA disappearing method). However, as already mentioned, this indirect method to evaluate the intramuscular TAG contribution in the oxidation of this substrate in the muscle during physical activity takes into account that all FAA extracted by the musculature is being oxidized and, therefore, is not a good indicative of reliable data.

Although the decrease on the intramuscular TAG content is frequently observed based on direct measurements, the large variability (~23%) between biopsies exceeds the reduction observed in untrained individuals, initially leading to inconclusive results[66].

Currently, most studies performed with different techniques (biopsies and histological analysis, marked isotopes and magnetic resonance) indicate a significant, but variable energetic contribution for the endurance exercise performance. Future and more refined studies shall use muscles isolated in incubation and magnetic resonance in order to obtain intramuscular TAG contribution values closer to reality.

FINAL CONSIDERATIONS

The literature review indicates great participation of the TAG endogenous supplies in the performance of endurance exercises. Furthermore, one may conclude that an important adaptation generated by the endurance training is the increase on the intramuscular TAG supplies and the higher capacity of utilization of these supplies. Although the recognition of the contribution of these intramuscular supplies as energetic substrate is clear, the methodological limitations make difficult the precise calculation of the intramuscular TAG contribution during exercise. Further studies using new technologies shall quantify more precisely the participation of this substrate and this will be vital for the understanding of the lipid metabolism in exercise.

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