QUANTIFICATION OF SUBSTRATE METABOLISM DURING 5 KM AND 20 KM RACES

Farahnaz Amirshaghaghi1, Fatemeh Shabkhiz2, Parisa Pournemati3
1Department of Exercise Physiology & Health Science, University of Tehran, Tehran, Iran.
E-mail: famirshaghaghi@ut.ac.ir
2Department of Exercise Physiology & Health Science, University of Tehran, Tehran, Iran.
3Department of Exercise Physiology & Health Science, University of Tehran, Tehran, Iran.

ABSTRACT

Background: Fat and carbohydrate are important fuels for aerobic exercise and there can be reciprocal shifts in the proportions of carbohydrate and fat that are oxidized. The exercise intensity and duration can affect the interaction between carbohydrate and fatty acid oxidation. Therefore, we investigated the quantification of substrate metabolism during 5 km and 20 km races.

Methods and Results: 6 male runners (age (years): 23.5 ± 2.9, weight (kg): 70.9 ± 4.5, and height (cm): 176 ± 5.6) volunteered to take part in the study. In a counter-balanced crossover design, each participant completed 5k and 20k trials separated by ≥1 wk. All participant completed each trials at maximum of their capacity. At the beginning and following the interventions blood samples were collected and analyzed. Total carbohydrate and fat oxidation rates (g/min) were calculated by using the stoichiometric equations of Jeukendrup and Wallis. The result of study indicated that 5 km running showed a higher absolute CHO than the first 5 km of longer (20 Km) running. Moreover, participants when performed 5 km running showed reduction of fat utilization compared to the first 5 km of 20 km. There was a significant difference between pre-race and post-race of glycerol, lactate, glucose and NEFA in both 5 km and 20 km (p≤0.05).

Conclusions: Our data highlight how the interplay between exercise intensity, and training duration can modulate the substrate utilization during exercise. In addition to informing future research design methodology, our data may be of practical significance in helping to formulate CHO and lipid requirements in relation to specific types of training sessions.

Keywords: Lipid, Carbohydrate, Aerobic Exercise, Metabolism.

I. INTRODUCTION

The primary aim of endurance training for the competitive athlete is to induce physiological and metabolic adaptations that increase the performance during training and competition (Lucia, 2000 #3970). Improving performance depend in part on the rate at which chemical energy [i.e., fat and carbohydrate (CHO)] can be converted into mechanical energy for skeletal muscle contraction (Hawley, 2002). Although in most endurance events, a mix of fat and carbohydrate are the principal substrates that contribute aerobic ATP synthesis in human skeletal muscle. The relative utilisation of fat and carbohydrate during exercise can vary enormously and depends strongly on exercise intensity and duration (van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001). In this regard, previous research suggested that plasma FFAs provide the majority of the substrate oxidised by skeletal muscle during low-and moderate-intensity (25 and 65 % of maximal oxygen uptake capacity, VO2,max) exercise. In addition, as exercise intensity increases from 65 to 85% of VO2,max FFA release into plasma and oxidation rates declined, as muscle glycogen became the main fuel source utilized (Romijn et al., 1993). Further, exercise a fixed intensity is prolonged (>60 min), an increasing energy contribution is derived from lipid oxidation. Consequently, the proportion of energy derived from muscle glycogen declines and is replaced by a progressive increase in plasma FFA oxidation (Romijn et al., 1993). Moreover, athletes pursue dietary strategies that increase the overall performance capacity that ensure optimal substrate availability to meet the energy cost of the event and training (Leckey, Burke, Morton, & Hawley, 2016). Hence, pre-exercise carbohydrate consumption decreases whole-body lipid oxidation in untrained and trained individuals, an effect mostly seen at moderate (40–60% VO2max) exercise intensities (Vieira, Costa, Macedo, Cocconcelli, & Kruehl, 2016). Additionally, Acute exercise in the fasted state, compared with the carbohydrate-fed state, for a given exercise intensity and duration, stimulates the oxidation of fatty acids from both intramyocellular and peripheral fat depots. Total carbohydrate oxidation rate is correspondingly decreased, but this is probably mainly, if not
entirely, due to reduced use of blood glucose because glycogen breakdown rate is well maintained or even increased (De Bock et al., 2008).

Although nutritional approaches for endurance training have typically promoted high carbohydrate availability before, during and after training sessions so as to fuel the energy requirements of high daily training intensities and volumes (Burke, Hawley, Wong, & Jeukendrup, 2011), there are a number of potential sites of control that can regulate the the CHO metabolism during endurance exercise. The breakdown of muscle glycogen to glucose-1-phosphate is under the control of glycogen phosphorylase (which is under the control of phosphorylation by phosphorylase kinase). It has been shown that, the percentage of phosphorylase does not appear to be increased with exercise intensity and, in actual fact, is decreased after only 10 min of high intensity exercise, which may be related to the reduced pH associated with intense exercise (Howlett et al., 1998). In addition to muscle glycogen, the contribution of plasma glucose to ATP production also increases with exercise intensity (Rose & Richter, 2005). Although glucose uptake is also regulated by GLUT4 content, GLUT4 is unlikely to play a role in this situation given that GLUT4 translocation to the plasma membrane is not increased with exercise intensity. Moreover, once glucose is transported into the cytosol, it is phosphorylated to glucose-6-phosphate under the control of hexokinase. During intense exercise at near maximal or supra-maximal intensity, glucose phosphorylation may be rate limiting to glucose utilization given that high rates of glucose-6-phosphate, secondary to muscle glycogen breakdown, can directly inhibit hexokinase activity. Finally, once glucose enters the glycolytic pathway, the rate limiting enzyme to glycolysis is considered to be phosphofructokinase (PFK) in the face of metabolic acidosis when PFK could be inhibited (Hearris, Hammond, Fell, & Morton, 2018). Moreover, the increase in fat availability resulted in key cellular changes including increased contents of muscle acetylcoenzyme A (CoA), citrate, and glucose-6-phosphate. These cellular changes downregulated carbohydrate metabolism at key regulatory sites (Spriet, 2014).

On the other hand, prolonged steady state exercise is characterized by a shift towards increased lipid oxidation and reduced CHO oxidation rates (Hearris et al., 2018). However, with regard to the rate of FFA utilization in skeletal muscle during exercise, there are several regulatory steps. Theoretically the main regulatory step may reside either in transport from the vascular to cytosolic compartment or in mitochondrial oxidation. Recently evidence has been presented that certain proteins, associated with the plasma membranes of both endothelial and muscle cells, contribute to the facilitated fatty acid uptake (Spriet, 2014). Thus, with the experimental evidence for a protein-mediated transport of fatty acids across the plasma membrane, the plasma membrane could be considered a subject of regulation for fatty acid utilization. Support for this notion is that fatty acid uptake and oxidation in human skeletal muscle during submaximal exercise did not increase linearly as a function of plasma fatty acid concentration (Jain et al., 2009) but rather displayed saturation kinetics. The regulation of IMTG hydrolysis involves the key enzymes ATGL and HSL, which each release a FFA from the IMTG complex (Watt & Spriet, 2010). Proteins known as perilipins also coat the lipid droplets and separate IMTG from ATGL and HSL, maintaining low rates of lipolysis. During moderate exercise, calcium- and epinephrine-related events phosphorylate HSL, and probably ATGL and AMP kinase phosphorylation of perilipin is involved in recruiting both HSL and ATGL to the lipid droplet, collectively enhancing rates of IMTG hydrolysis (van Loon et al., 2001). However, at higher power outputs it appears that AMP kinase phosphorylates additional sites on HSL that inhibit the phosphorylation by epinephrine and calcium, providing a potential mechanism for the lower rates of IMTG use (Watt & Spriet, 2004). It is not currently known if there are similar inhibitory events affecting ATGL and perilipin that may also decrease IMTG hydrolysis at higher exercise intensities and these remain to be investigated. Another possibility for the downregulation of fat transport at the mitochondrial membranes relates to the small reductions in muscle pH that are associated with exercise at intense aerobic power outputs. Studies in mitochondria isolated from resting human skeletal muscle showed that small decreases in pH from 7 to 6.8 caused large reductions in CPT I (the rate limiting enzyme that facilitates long chain fatty acid entry to the mitochondria) activity (Spriet, 2014).

In recent years, indirect/direct calorimetry in combination with isotopic tracer techniques have been used to evaluate the regulation of endogenous fat and CHO metabolism in relation to exercise of varying intensities and a number of nutritional interventions (Hawley & Leckey, 2015). With regard to the metabolic demands of endurance cycling training, Stepto et al. (Stepto et al., 2002) reported that during an interval session of cycling training (85 % of VO2peak), rates of CHO oxidation were 315 μmol/kg body mass/min while rates of fat oxidation were tenfold lower at 30 μmol/kg/min. Moreover, the results of O’Brien et al. (O’Brien, Viguie, Mazzeo, & Brooks, 1993) and others (Hawley & Leckey, 2015) clearly show CHO dependence during endurance running lasting up to 200 min.
While these studies provide valuable data, several caveats need to be considered within the framework of this paradigm. First, in the overwhelming majority of studies, subjects have only been tested at the same absolute (pretraining) work rate. Second, most studies employ previously untrained, predominantly male subjects to investigate the effects of short-term training interventions on patterns of substrate use. While there are major physiological and metabolic changes induced by the implementation of endurance training regimens in previously sedentary subjects, the results from these investigations bear little relevance to well-trained athletes with a history of many years of training. Finally, in the majority of studies, subjects are tested after a 10- to 12-h overnight fast. Notwithstanding the fact that competitive athletes are unlikely to commence the majority of training sessions and/or races with low CHO availability, such conditions would be expected to increase the contribution of fat-based fuels to total energy requirements, at least during exercise of low-to moderate intensity.

The aim of the present study was to investigate the quantitative effects of exercise intensity and duration on CHO and FFA mobilization and utilization. We hypothesized that lipolysis within adipocytes is stimulated maximally during 20km bout, since peripheral lipolysis is very sensitive to minimal sympathetic stimulation whereas lipolysis of triglycerides within muscle and + thus muscle triglyceride oxidation may be stimulated at higher exercise intensities. To put the data of FFA metabolism into the perspective of overall energy substrate metabolism, whole body carbohydrate metabolism was evaluated simultaneously. The information gained from this study is important to our understanding of the mechanisms responsible for the integration of carbohydrate and fat oxidation during exercise, and to our understanding of the metabolic defect(s) that cause(s) the impairment of fat oxidation in subjects.

II. PROCEDURES

1.1 Subjects
After providing informed written consent, 6 male runners (Age (years): 23.5 ± 2.9 , Weight (kg): 70.9 ± 4.5 , and Height (cm): 176 ± 5.6) volunteered to take part in the study. All procedures conformed to the standards set by the Declaration of Helsinki, and the study was approved by the University of Tehran.

1.2 Study Design
In a counter-balanced crossover design, each participant completed 5k and 20k trials separated by ≥1 wk. All participant completed each trials at maximum of their capacity. At the beginning and following the interventions blood samples were collected and analyzed. Each participant completed an incremental test to volitional fatigue on a motorized treadmill (Pulsar 3p; HP Cosmos, Nussdorf-Traunstein, Germany) to determine VO2max. Total carbohydrate and fat oxidation rates (g/min) were calculated by using the stoichiometric equations of Jeukendrup and Wallis (Jeukendrup & Wallis, 2005). Intake of daily nutrition a day before of trials and the morning of each trials was evaluated by a food frequency questionnaire and data were analyzed using the Nutritionist IV software.

1.3 Training Protocols
The subjects were studied on two days separated by ≥1 wk.

Day 1: Participants arrived at the laboratory on the evening (17.00) of Day 1 having avoided alcohol and vigorous physical activity for the previous 24 h. After a 10-min self-selected warm-up, participants then performed an 5 km running protocol on a motorised treadmill.

Fig. 1. Schematic figure of study design
**Day 2:** The same procedure like day 1 has been performed before the starting of running. After a 10-min self-selected warm-up, participants then performed an 20 km running protocol on a motorised treadmill.

On each occasion, stable isotopes were infused, and indirect calorimetry was used to determine oxygen consumption (\(\text{ire}_{\text{o}}\)) and carbon dioxide production (\(\text{irc}_{\text{co}}\)) during exercise. RPE using the scale proposed by Borg in 1973, heart rate (HR) (Polar Electro OY, Kempele, Finland), and expired gas were collected at km intervals. water was consumed ad libitum throughout exercise.

Participants were instructed to refrain from any vigorous physical activity in the 48 h before a performance trial and to abstain from exercise in the 24 h before a trial. Moreover, instructing them to abstain from caffeine (i.e., coffee, tea, energy drinks) and alcohol.

1.4 **Vo2max**

Oxygen uptake was measured continuously during exercise via breath-by-breath measurement using a CPX Ultima series online gas analysis system (Medgraphics, St. Paul, MN). The treadmill speed was increased by 1 km·h\(^{-1}\) every 3 min and during the final 30 s of each 3-min stage, blood lactate was assessed using capillary blood samples (Lactate Plus; Nova Biomedical, Waltham, MA). Part 1 of the test terminated once both lactate threshold and turnpoint had been visually identified (defined as \(\geq 0.4\) and \(\geq 1.0\) mmol·L\(^{-1}\) above resting values, respectively). After a 5-min resting period, part 2 of the test commenced at a velocity of 2 km·h\(^{-1}\) below lactate turnpoint, and the treadmill speed was increased by 1 km·h\(^{-1}\) every minute until volitional fatigue, after which point the treadmill inclined by 1% every minute until volitional fatigue. VO2 was taken as the highest VO2 value obtained in any 10-s period matching two of the following criteria: HR within 10 bpm of age-predicted maximum, RER > 1.1, and plateau of oxygen consumption despite increased workload (Impey et al., 2020).

1.5 **Carbohydrate and Fat Oxidation**

From the rate of carbon dioxide production and \(\dot{V'}\text{CO}_2\) (L/min), total carbohydrate and fat oxidation rates (g/min) were calculated by using the following stoichiometric equations of Jeukendrup and Wallis (Jeukendrup & Wallis, 2005), working under the assumption that protein oxidation during exercise is negligible and VO2 and VCO2 accurately reflect tissue O2 consumption and CO2 production:

- **Carbohydrate oxidation=4.210 V'CO2–2.962 V'O2**
- **Fat oxidation=1.695 V'O2–1.701 V'CO2**

1.6 **Anthropometric Data and Blood Sample Analyses**

Body weight was measured by a body composition analyzer (InBody 570, Korea) and height have been evaluated with a portable stadiometer (InBody, InLab S50, Korea).

Venous blood samples were collected in vacutainers containing K2 EDTA, lithium heparin, or serum separation tubes, and stored on ice until centrifugation at 1500g for 15 min at 4°C. Plasma samples were aliquoted and stored at −80°C until analysis. Plasma glucose, lactate, and glycerol were analyzed using the Randox Daytona spectrophotometer with commercially available kits (Randox, Crumlin, Ireland), as per the manufacturer’s instructions.

1.7 **Statistical Analysis**

Data analysis was performed by using SPSS for software (version 25, SPSS Inc, Chicago, IL). Data are expressed as means ± SEs unless otherwise stated. Variables over the course of the experimental trials were compared by using a 2-factor (time × trial) repeated-measures analysis of variance. Bonferroni post-hoc analysis was also used to identify significant group differences identified by two-way repeated ANOVA. Significance was set at \(P < 0.05\).

### III. RESULT

Mean energy and nutrients intakes of participants from the FFQ questionnaire are presented in table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CHO (g/kg)</th>
<th>FAT (g/kg)</th>
<th>PRO (g/kg)</th>
<th>Total EI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day before 5 k</td>
<td>5.1 ± 1.2</td>
<td>0.9 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>2565.3 ± 424</td>
</tr>
</tbody>
</table>
Physiological Responses to Exercise

The two-way repeated ANOVA exhibit substantial interaction effects (condition × time) for treadmill speed (F=5.724 P= 0.003). One-way repeated ANOVA analysis showed that there was no significant differences in treadmill speed between different stages of running, neither 20 km (p = .080) nor 5 km (p = .291).

The two-way repeated ANOVA exhibit substantial interaction effects (condition × time) for RPE (F= 227.047 P= 0.000). Simple main effects analysis showed that in RPE were significantly different (F=82 P= 0.000) in different stage of running in 5 km condition (p ≤ .05). Moreover, RPE were significantly different (F=10.739, P= 0.016) in between stage of running in 20 km condition. But, there were only significant difference between RPE in stage 2 by stage 3 (P= 0.041) and 5 (P= 0.029).

The results showed significant interaction effects (condition × time) for HR (F= 41.834 P= 0.001). One-way repeated ANOVA analysis showed that there was no significant differences in HR between different stages of running of 5 km. However, HR were significantly different (F=27.015, P= 0.001) in between stage of running in 20 km condition. In this regard, there were only significant difference between HR in stage 1 with all of remain stage (p ≤ .05).

The results showed significant interaction effects (condition × time) for VO2 (% VO2max) (F= 7.793 P= 0.022). One-way repeated ANOVA analysis showed that there was no significant differences in VO2 (% VO2max) between different stages of running of 5 km (F= 0.658, P= 0.501) and 20 km (F= 0.756 P= 0.506).

The results showed significant interaction effects (condition × time) for % Threshold (F= 5.558 P= 0.045). One-way repeated ANOVA analysis showed that there was no significant differences in % Threshold between different stages of running, 5 km (F= 2.367, P= 0.145) and 20 km (F= 3.303, P= 0.091).

The results showed significant interaction effects (condition × time) for % Turnpoint (ml/kg/min) (F= 8.388 P= 0.017). Further analysis showed that there was no significant differences in % Turnpoint (ml/kg/min) between different stages of running, 5 km (F= 0.503, P= 0.640) and 20 km (F= 0.745, P= 0.475).

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</tr>
</thead>
<tbody>
<tr>
<td>Day before 20 k</td>
<td>5.1 ± 1.2</td>
<td>0.9 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>2565.3 ± 424</td>
</tr>
<tr>
<td>In morning of 5 k</td>
<td>1.3 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>544 ± 221.1</td>
</tr>
<tr>
<td>In morning of 5 k</td>
<td>1.5 ± 0.4</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>626.2 ± 12.7</td>
</tr>
</tbody>
</table>
Figure 2. Physiological profile of participants during 5 km and 20 km races. Data are means ± SEM

**Plasma Metabolite Responses**

The two-way repeated ANOVA showed substantial interaction effects (condition × time) for Lactate ($F= 19.124\ P= 0.007$), paired samples T test analysis showed that there was significant differences in lactate between pre and post stages of 5 km ($P= 0.000$) and 20 km ($P= 0.001$) running.

The result showed significant interaction effects (condition × time) for Glucose ($F= 16.652\ P= 0.010$), paired samples T test analysis showed that there was significant differences in Glucose between pre and post stages in 5 km ($P= 0.007$) but not 20 km ($P= 0.241$) running.
The two-way repeated ANOVA showed significant interaction effects (condition × time) for Glycerol (F= 53.570 P= 0.001). Paired samples T test analysis showed that there was significant differences in Glycerol between pre and post stages of 5 km (P= 0.000) and 20 km (P= 0.001) running.

The result showed significant interaction effects (condition × time) for NEFA (F= 184.357 P= 0.000). Paired samples T test analysis showed that there was significant differences in NEFA between pre and post stages in 5 km (P= 0.049) but not 20 km (P= 0.000) running.

Substrate Oxidation Rates
The results of two-way repeated ANOVA showed no-significant main effect of time (F = 4.251, P= 0.060) and condition (F= 21.337, P = 0.060) on CHO Oxidation Rate.

The results of two-way repeated ANOVA showed no-significant main effect of time (F = 2.885, P= 0.114) and condition (F= 5.986, P = 0.058) on lipid Oxidation Rate.

The results of two-way repeated ANOVA showed no-significant main effect of time (F = 2.856, P= 0.117) and condition (F= 1.235, P = 0.317) on Energy Expenditure.
Figure 4. Quantification of substrate metabolism during 5 km and 20 km races. Effect of 5 km and 20 km exercise on the CHO and lipid oxidation rate. Each value is presented as the mean ± SE (n = 9)

Table 2 present the results of physiological responses at the two 20 km and 5 km running protocols at the end of protocols. Moreover, according to results, there was significant difference only in heart rate between among the 2 protocols.

<table>
<thead>
<tr>
<th>Variables</th>
<th>5 Km (Mean±SD)</th>
<th>20 Km (Mean±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treadmill Speed</td>
<td>13.7±1.3</td>
<td>11±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>RPE</td>
<td>19±0.9</td>
<td>17.3±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>189±10</td>
<td>178±15</td>
<td>S</td>
</tr>
<tr>
<td>VO2 (ml/kg/min)</td>
<td>40.5±4</td>
<td>36.5±7.2</td>
<td>NS</td>
</tr>
<tr>
<td>CHO Oxidation Rate (g/min)</td>
<td>4.19±0.89</td>
<td>3.07±1.57</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid Oxidation Rate (g/min)</td>
<td>-0.22±0.31</td>
<td>0.13±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Energy Expenditure (kcal/min)</td>
<td>14.7±1.7</td>
<td>13.3±2.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: No-significant, S: Significant

IV. DISCUSSION

Although numerous studies have addressed the mobilization and utilization of carbohydrate and lipiíd at different exercise intensities, little quantitative data are available regarding CHO and lipid kinetics. In this study, we quantify substrate metabolism during to different intensity and duration exercise. The result of study indicated that 1) 5 km running necessitates a higher absolute CHO requirement than the first 5 km of longer (20 Km) running, 2) participants when performed 5 km running showed reduction of fat utilization compared to the firs 5 km of 20 km. There was a significinficant difference between pre-race and post-race of glycerol, lactate, glucose and NEFA in both 5 km and 20 km. Moreover, according to the exercise intensity factors (VO2max, HR, RPE), subjects performed 5 km compared to the firs 5 km of 20 km with a higher intensity. Although, the pattern of substrate utilization observed here is, of course, specific to the characteristics of the chosen exercise protocols (duration and intensity), our data may help to inform practical guidelines in relation to fuelling strategies to promote both training intensity and metabolic adaptations.

It has become widely accepted that endurance exercise training reduce muscle glycogenolysis increase the fatty acid (FA) oxidation is compared with before training at a given exercise intensity (Hawley, Maughan, & Hargreaves, 2015). However, at a given exercise intensity and metabolic demand, there can be reciprocal shifts in the proportions of CHO and fat that are oxidized. The interaction between CHO and fatty acid oxidation is dependent on the intracellular and extracellular metabolic environments. The availability of substrate (both from inside and outside of the muscle), and the exercise intensity and duration can affect these environments. Recent data from Boorsma et al. (Boorsma, Whitfield, & Spriet, 2014) in elite runners clearly show CHO dependence when running at speeds typically undertaken by these athletes in training. Boorsma et al. determined rates of substrate oxidation from RER measures in eight male 1500-m runners during low- (50 % VO2peak), moderate- (65 % VO2peak) and highintensity (80 % VO2peak) treadmill running. For the entire group, RER values were 0.85, 0.89 and 0.92 when running at 50, 65 and 80 % of VO2peak. However, for the top three runners with the highest VO2peak values (83.4 mL/kg/min), RER was greater (0.94) when running at 80 % of VO2peak. At this intensity, CHO-based fuels contributed 81 % to the total energy cost of running. Another study reported that increasing the exercise intensity (from 40 to 80 % VO2max) reduced the uptake and oxidation of the transport-dependent LCFAs, but not the uptake and oxidation of membrane-independent medium chain LCFAs (Watt &
Spriet, 2010). This suggested an inhibitory effect of increased glycolytic flux on the transport of FFAs at the plasma membrane. However, there could also be inhibition of FFA transport into mitochondria at the higher exercise intensity.

Moreover, previous studies have investigated the different nutrient strategies on CHO and fat utilization pattern during endurance training. Bergman and Brooks (Brooks & Mercier, 1994) investigated the interaction of training status and pre-exercise nutritional state on rates of substrate oxidation during graded cycling exercise. RER values were significantly lower in well-trained compared with untrained individuals during low- (22 % of VO2max) and moderate-intensity (40 % of VO2max) cycling when fasted and also during moderate-intensity exercise when fed or fasted. However, there was no training effect (i.e., lower RER values), nor any training-nutrient interaction at higher exercise intensities (60 and 75 % of VO2max). These data demonstrate that because athletes train and compete at exercise intensities [40 % of VO2max, they will not oxidize a greater proportion of fat substrates compared with untrained subjects, regardless of nutritional state. Moreover, many studies have manipulated lipid availability before or during exercise and reported increased rates of fat oxidation. Stepto et al. (Stepto et al., 2002) reported that subjective RPE was significantly greater after just 4 days of a fat-rich diet compared with an isoenergetic high-CHO diet when well-trained cyclists/triathletes undertook a standardized laboratory-based bout of intense interval training.

Although we did not directly quantify muscle glycogen levels, our observation of capillary metabolite data (e.g. glucose, lactate, NEFA and glycerol) showed significant difference between pre and post values in both 20 km and 5 km running which are consistent with that previous research. The rate of whole body lipolysis can be assessed by measuring glycerol. Glycerol appears in the blood only as the product of lipolysis and can be reutilized only in tissues that contain glycerol kinase. The fat oxidation data in our study provide support for the contention that glycerol, is an accurate reflection of whole body lipolysis. During 20 km race, at which the highest fat oxidation rates were observed, the higher glycerol, reflecting the maximal availability of FFA derived from lipolysis, and fatty acid oxidation rates. In this regard Romijn et. al showed exercise at 65% of VO2max, the intensity at which they observed the highest fat oxidation rates, the rate of three times glycerol (Romijn et al., 1993). Moreover, in our study glucose concentration was higher after 5 km than 20 km running. It seems that, increasing the exercise power output above approximately 50 % VO2max also increases the use of muscle glycogen. Blood glucose levels and muscle glycogenolysis, glycolytic flux, PDH activation, and carbohydrate oxidation are all increased during exercise at higher, compared with moderate, exercise power outputs (Spriet, 2014).

Our data highlight how the interplay between exercise intensity, and training duration can modulate the substrate utilization during exercise. In addition to informing future research design methodology, our data may be of practical significance in helping to formulate CHO and lipid requirements in relation to specific types of training sessions.

Although the present study provided valuable information, it also had some limitations. Looking the inside of muscle through muscle biopsy can provide more specific data about the metabolism. In this regard, future research need to use muscle biopsy to study differences in glycogen and intramuscular triglyceride metabolism as well as the kinetics of lipid metabolism, as opposed to the limitations of whole muscle homogenate and static measures of postexercise NEFA and glycerol concentrations. In addition, a comparison of male and female participants at varying stages throughout the menstrual cycle (completing the types of exercise protocols studied here) is also a future research recommendation. Moreover, longitudinal data collected throughout an entire competitive season or during specific periodized training blocks are also needed to assess whether the energy (i.e., CHO) intakes of athletes fluctuate in accordance with alterations in training volume and load.

**V. CONCLUSION**

We presently lack detailed information on the metabolic demands of the endurance athletes. This applies to the fuel requirements of individual training sessions as well as the impact of undertaking several workouts a day and/or multiple sessions in different disciplines (i.e., triathletes). The result of study indicated that 5 km running necessitates a higher absolute CHO requirement than the first 5 km of longer (20 Km) running, participants when performed 5 km running showed reduction of fat utilization compared to the first 5 km of 20 km. There was a significant difference between pre-race and post-race of glycerol, lactate, glucose and NEFA in both 5 km and 20 km. Moreover, according to the exercise intensity factors (VO2max, HR, RPE), subjects performed 5 km compared to the first 5 km of 20 km with a higher intensity. Although such observations are specific to the training
status of the participants studied here, our data may provide a platform to help better inform CHO and lipid utilization for runners and will hopefully stimulate further research.

REFERENCES


