Fuelling the fire: assessment of muscle metabolism during submaximal endurance exercise

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Athletes from around the world congregated this summer for the Tokyo 2020 Summer Olympic Games, eager to participate following months of uncertainty following the COVID-19 pandemic. The peak athletic performance showcased at this event is a culmination of decades of training, physiologically priming the cardiovascular, respiratory and musculoskeletal systems, while psychologically preparing to face this daunting sporting event. An ATP supply for cellular processes surrounding motor unit contraction in skeletal muscle is the basis of any physical activity, from high-powered sprints lasting seconds to marathons lasting hours. Although ATP stores are relatively limited, intramuscular metabolic pathways, such as substrate-level and oxidative phosphorylation, generate energy to sustain contractility for extended periods (Hargreaves & Spriet, 2020). Together, carbohydrates and fats fuel the ATP-dependent enzymatic processes of contraction, including membrane depolarization, sarcoplasmic reticulum calcium handling and myofilament cross-bridge formation. The degree of utilization of each substrate varies with the type of activity, type of fibre recruited, athlete training status, diet and sex (Hargreaves & Spriet, 2020). Type I muscle fibres are selectively engaged in long duration exercise and proportionally higher in athletes training for stamina (De Bock et al. 2005). Conversely, type II fibres are involved in shorter contractile, anaerobic events, and are more abundant in power-trainers (De Bock et al. 2005). Depletion of muscle glycogen correlates with muscle fatigue, and therefore athletes often increase dietary carbohydrate intake prior to exercise to sustain excitation-contraction coupling (Johnson et al. 2004).

In addition to commencing exercise with high muscle glycogen, athletes are also often advised to consume carbohydrate (CHO) during exercise as so to maintain CHO availability and improve exercise capacity and performance. The ergogenic effects of CHO feeding during exercise are likely not underpinned by muscle glycogen sparing but rather maintenance of plasma glucose concentration and high rates of CHO oxidation (Hargreaves & Spriet, 2020). Whilst the absence of a glycogen sparing effect is well documented, the effect of CHO feeding on intramuscular triglyceride (IMTG) utilization during exercise is less well documented. During exercise, this fuel source is dynamic, with lipolysis occurring alongside esterification, and in the past, quantification of subcellular MTG changes via biopsy have proved challenging and inconsistent (Johnson et al. 2004). Although CHO feeding during exercise is ergogenic to performance, there is some literature suggesting that CHO feeding may attenuate cell signalling pathways with regulatory roles in training adaptation. Accordingly, there is an emerging practice of withholding CHO intake during exercise in an attempt to promote cell signalling and potentiate components of training adaptation.

An article recently published in The Journal of Physiology by Fell et al. (2021) examined such interactions in an ecologically valid model of carbohydrate feeding and exercise on muscle metabolism and endurance capacity. Specifically, the authors aimed to determine whether consuming carbohydrates during submaximal exercise could alter IMTG/lipid droplet morphology, glycogen stores or molecular regulation of mitochondrial biogenesis. Fell and colleagues enlisted eight male amateur cyclists to complete 180 min of cycling at submaximal effort following carbohydrate feeding. Experimental trials were preceded by a 36-h CHO-loading regiment (12 g kg⁻¹) and a 3-h CHO pre-exercise meal (2 g kg⁻¹). Study participants then completed four cycling endurance protocols in which CHO was ingested at rates of 0, 45 and 90 g h⁻¹. Initially, a familiarization trial (FAM) mimicking the 0 g h⁻¹ condition was implemented to allow participants to become familiar with the study design while also validating that participants have the required capabilities to complete the trials. The particular carbohydrate feeding intervals used in this study mimic commonly used practices by high performance athletes. Vastus lateralis muscle biopsies, blood and exhaled gas samples were taken for analysis of muscle biology, serum metabolites and energy expenditure, respectively.

**Results**

During the training regimen, CHO feeding elicited effects on circulating metabolites and whole-body substrate handling. Heart rate, oxygen uptake and rate of perceived exertion increased during exercise, irrespective of the trials. Plasma lactate, non-esterified fatty acids (NEFA), glycerol and β-hydroxybutyrate (β-OHB) exhibited significant changes during exercise. Furthermore, increasing CHO dose elicited larger differences in plasma glucose, NEFA, glycerol and β-OHB. Next, exercise decreased IMTG content in type I and IIA fibres; however, CHO had no observed impact on this reduction, and no differences were noted in net IMTG breakdown. Additionally, exercise reduced lipid droplet count in type I and IIA fibres, with negligible impact from CHO feeding. Exercise did induce significant decreases in muscle glycogen content, with a greater decline in type I fibres, irrespective of CHO feeding. In terms of cell signalling, exercise-induced phosphorylation of AMP-activated protein kinase (AMPK)Thr172, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII)Thr286 and p53Ser15 was noted, regardless of CHO availability. Finally, CHO feeding increased exercise capacity in a dose-dependent manner. Of the four trials (FAM, 0, 45, 90 g h⁻¹), the 90 g h⁻¹ experimental group exhibited greatest exercise capacity, and the 45 g h⁻¹...
cohort had a greater exercise capacity compared to the FAM and 0 g h⁻¹ groups. A schematic representation of the results is shown in Fig. 1.

Discussion

The study by Fell et al. aimed to determine the effects of CHO feeding on IMTG and glycogen metabolism, skeletal muscle cellular signalling and athletic performance during submaximal exercise. More specifically, it was the first to assess the effects of CHO feeding on IMTG content in both central and peripheral regions of type I and IIa fibres. Ultimately, the study provided novel evidence that exercise, rather than CHO feeding during endurance activity, was responsible for changes in IMTG content in both these fibres. This is in contrast to previous studies which report IMTG alterations in only central regions of type I fibres. Fell et al. attributes this phenomenon to the combination of an increased oxidative role of muscle fibres in endurance trained athletes and raised energy requirements associated with intense exercise. This may propose a positive correlation between prior levels of endurance training achieved and the increased recruitment of muscle fibres for IMTG oxidation during exercise. An absence of an IMTG sparing effect after CHO feeding was also observed. It was proposed that this may have been due to the pre-exercise meal causing an interruption of CHO-mediated regulatory effects in the first hour of exercise, which is when IMTG degradation is found to be at its highest. Similarly, glycogen sparing upon CHO feeding was not observed in either muscle fibre type. This is in contrast to a study by De Bock et al. (2007) which observed glycogen sparing in type IIa fibres 2 h post-exercise. Fell et al. suggest that various factors including the longer 3-h duration of exercise in their study may have conferred these changes and warrant further investigation. From a cell signalling perspective, it was observed that AMPK- and p53-related signalling were not influenced by CHO feeding, but rather exercise was the driving force behind the elicited changes in this signalling cascade. Most importantly, it was observed that trials with higher CHO doses increased the duration of CHO oxidation before occurrence of the crossover point at which lipid oxidation became predominant. This demonstrates that CHO feeding improves exercise capacity in a dose-dependent manner. In addition, prior to this study it was believed that only through CHO restriction can endurance athletes improve skeletal muscle adaptation during training. To the contrary, the conclusion made by Fell et al. suggests that CHO intake before and during activity avoids disturbing cellular signalling pathways involved in training adaptations like mitochondrial biogenesis. This may exempt CHO consumption from being exclusive to only intensity-based training diets as it may begin to be incorporated into diets promoting training adaptation as well.

It is important to highlight the utilization of BODIPY 493/503 dye in the present study to assess IMTG content. Previously, studies used Oil Red O (ORO)dye to estimate IMTG content (Strauss et al. 2020). ORO dye does not exclusively stain IMTG, as neutral lipids, such as phospholipids within membranes, are also stained. This means that the IMTG content observed in the present study is in fact more accurate than previously determined values.

As the current study focused on IMTG metabolism, future studies could investigate the effects of CHO feeding on intracellular glycogen utilization and its role in improving exercise capacity. Next, further exploration of the effects of CHO feeding may be warranted in a female cohort. As females have a greater proportion of type I fibres and a heavier reliance on CHO rather than solid. This may avoid the possibility of participants altering their exercise performance based on knowledge of their intake.

Through investigating evidence-based nutritional regimens on skeletal muscle biology during endurance exercise, the study by Fell et al. provides valuable insight into maximizing performance for high-level athletes. The effect of nutrition on athletic performance is a growing field within sports medicine. This paper addresses key points surrounding CHO feeding on muscle metabolism, and calls for further research to be conducted to optimize feeding regimens for professional athletes allowing them to continue to break records, whether it be in amateur sporting events or the 2020 Tokyo Olympics.

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**Additional information**

**Competing interests**

None declared.

**Author contributions**

All contributors to the conceptualization, drafting and editing of the paper. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**Supporting information**

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