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**Abstract**

**Purpose:** The effect of hyperglycaemia on exercise with low and elevated muscle glycogen on glucose utilization (GUR), carbohydrate and fat oxidation, hormonal and metabolite responses as well as rating of perceived exertion (RPE) were explored. **Methods:** Five healthy trained males were exercised for 90 minutes at 70% V̇O2max in two trials while glucose was infused intravenously at rates to “clamp” blood glucose at 12 mM. On one occasion participants were ‘loaded’ with carbohydrate (CHO-L) whilst on a separate occasion participants were glycogen depleted (CHO-D). Prior exercise and dietary manipulations produced the ‘loaded’ and ‘depleted’ states. **Results:** The CHO-L and CHO-D conditions resulted in muscle glycogen concentrations of 377 and 159 mmol/g dw, respectively. Hyperglycaemia elevated plasma insulin concentrations with higher levels for CHO-L than for CHO-D (*P<*0.01). Conversely, CHO-D elevated plasma adrenaline and noradrenaline higher than CHO-L (*P<*0.05). Plasma fat metabolites (NEFA, β-hydroxybutyrate, and glycerol) were higher under CHO-D than CHO-L (*P<*0.01). The resultant was that the rates of total carbohydrate and fat oxidation were elevated and depressed for loaded CHO-L vs CHO-D respectively (*P<*0.01), although no difference was found for GUR (*P>*0.05). The RPE over the exercise period was higher for CHO-D than CHO-L (*P<*0.05). **Conclusion:** Hyperglycaemia during exercise, when muscle glycogen is reduced, attenuates insulin but promotes catecholamines and fat metabolites. The effect is a subsequent elevation of fat oxidation, a reduction in CHO oxidation without a concomitant increase in GUR, and an increase in RPE.

**Keywords:** *hyperglycaemia; hormones; glucose utilisation; carbohydrate oxidation; muscle glycogen; RPE*

**Abbreviations**

ANOVA; analysis of variance

CHO-D; carbohydrate depleted

CHO-L; carbohydrate loaded

CHO; carbohydrate

GUR; glucose utilization rate

NEFA; non-esterified fatty acids

RIA; radioimmunoassay

RPE; rating of perceived exertion

V̇O2max; maximal oxygen update

β-HB; β-hydroxybutyrate

**Introduction**

The carbohydrate (CHO) loading diet was popularised in the late 1960’s after it had been found that muscle glycogen content could be increased by a high-CHO diet (~70% CHO) which led to an improvement in sporting performance (Ahlborg et al. 1967; Karlsson and Saltin 1971). This ergogenic effect may be due to the elevated muscle glycogen stores resulting in a delay to the depletion of muscle glycogen (Coyle et al. 1986). Indeed, CHO availability has been shown to limit the performance of prolonged sub-maximal and intermittent high intensity exercise capacity (Hargreaves 1999). The restoration of muscle and liver glycogen through CHO ingestion is also of importance when athletes are recovering in between periods of intensified workload (Burke et al., 2018). CHO loading elevates plasma insulin concentrations during exercise, whereas CHO depletion results in a diminished response (Galbo 1983). The resultant is a higher rate of CHO oxidation, reduced fat oxidation, attenuated responses of catecholamines, non-esterified fatty acids (NEFA), β-hydroxybutyrate (β-HB), and glycerol when carbohydrate loaded (Galbo 1983).

Over the last number of years, investigations have been reported on the effects of ketogenic diets and the effect on performance and metabolism (Chang et al., 2017). Invariably, the findings from these investigations report on lowered muscle glycogen content and changes in circulating hormones and metabolites leading to enhanced fat oxidation and diminished carbohydrate oxidation. The majority of these studies have usually been conducted when participants had undergone the dietary manipulation for some weeks rather than a few days.

Prolonged, intense exercise results in an increase in plasma concentrations of catecholamines and a concomitant reduction in insulin (Viru 1992). These changes favour the subsequent release of fatty acids from triglyceride stores and their subsequent use as an energy source (Jeukendrup et al. 1998). CHO ingestion before or during exercise, on the other hand, attenuates this response (Holloszy and Kohrt 1996). Infusion of glucose during prolonged exercise elevates plasma insulin and attenuates the catecholamine response, which leads to a high rate of CHO oxidation and a decrease in plasma NEFA and fat oxidation (MacLaren et al. 1999). Previous work investigating differing muscle glycogen status has found lower glycogen content does not cause increased glucose oxidation compared to normal glycogen status (Weltan et al. 1998). However, the investigation did not evaluate the hormonal and metabolite response to maintained hyperglycaemia during exercise when in a CHO loaded state, particularly when hyperglycaemia is apparent before the onset of exercise.

Another effect of reducing CHO availability prior to and during exercise is that the rating of perceived exertion (RPE) is elevated when compared to a CHO loaded state (i.e. increased perception of effort for the same work rate) (Kang et al., 1996). This is likely to be due to the reduced circulating glucose being detected by the brain and thereby resulting in modifying the perception of effort (Burgess et al. 1991). The concept of the ‘Central Governor’ has been proposed as a brain-mediated fatigue detection mechanism (Noakes et al. 2001). The model suggests that it is the process from the brain which hinders exercise tolerance as opposed to the peripheral fatigue model typically associated with low muscle glycogen content during exercise (Arkinstall et al. 2004). Therefore, it would be of interest whether starting exercise with a low or high muscle glycogen content but maintaining hyperglycaemia throughout exercise has an impact on the subsequent RPE scores. If so, then hyperglycaemia *per* *se* is not a factor in influencing central perception of effort during exercise.

The present investigation examined the hormonal and metabolic response to maintained hyperglycaemia during exercise when CHO loaded and depleted. The investigation also aimed to determine the rates of glucose infusion which were oxidised and the influence of pre-exercise muscle glycogen status. Since hyperglycaemia would be prevalent throughout our trials, would the effect of the exercise/diet manipulation lead to variations in muscle glycogen level prior to exercise and thereby have an impact on subsequent metabolic measures? We hypothesized that there would be no significant differences between CHO-L and CHO-D with regard to hormonal, metabolite, and metabolic measures, nor for RPE due to maintained hyperglycaemia during exercise.

**Methods**

**Participants**

Five healthy, well-trained, male participants gave informed written consent in accordance with the procedures approved by the Ethics Committee of the Royal Liverpool University Hospital and of Liverpool John Moores University. Mean age, body mass and max oxygen update (V̇O2max) were 26.8 ± 9.6 years, 69.6 ± 7.7 kg and 4.4 ± 0.3 l.min-1, respectively. None of the participants had a history of neurological disease or skeletal muscle abnormality and none were under pharmacological intervention during the study.

**Preliminary testing**

V̇O2max was determined using a progressive incremental exercise test on an electrically braked cycle ergometer (Bosch ERG 551, Robert Bosch GMBH, Berlin, Germany). After a 5-minute warm-up at a work load of 90W, the test commenced at 180W and the power output increased by 30W increments every 2 minutes until volitional exhaustion. Expired air was analysed using an automated gas analysis system (P.K. Morgan, Chatham UK). The results were used to calculate the power outputs corresponding to 70% of V̇O2max. After a suitable rest period, the participants underwent a familiarization exercise where power outputs were adjusted to produce V̇O2, as measured, corresponding to 70% of V̇O2max. This power output was subsequently used during the experimental procedures.

**Experimental design**

A randomised counterbalanced, cross-over design was implemented in which participants exercised at 70% V̇O2max for 90 minutes in a state of maintained hyperglycaemia. In one condition, the participants had undergone a CHO loading treatment (CHO-L) over a 3 day period prior to testing. Conversely, participants undertook a second condition in which CHO levels were depleted prior to exercise (CHO-D). The variation in muscle glycogen was achieved by the participants exercising to fatigue at 70% V̇O2max three days prior to the test and then resting over the subsequent two days whilst consuming either a high (~500 g day-1; CHO-L) or low CHO diet (~200 g day-1; CHO-D). All food was weighed for analysis of the mass of CHO consumed. A computer program using the McCance and Widdowson Food Composition Tables was employed (Microdiet, Salford University). In order to check whether appropriate levels of muscle glycogen had been achieved, three of the participants, on occasions after the experimental trials, repeated the exercise and dietary interventions and then had a muscle biopsy taken from the quadriceps using a conchotome after administration of a local anaesthetic and after an incision of the skin and muscle fascia (MacLaren et al. 1999). The muscle sample was placed in a sterile eppendorf tube before being plunged into liquid nitrogen. The sample was then stored at −70°C until analysed for glycogen content.

**Experimental protocol**

Participants arrived at the laboratory in the morning at 08.00 hours after an overnight fastand without having engaged in physical activity during the previous 48 h. After each participant voided urine, a 16-gauge IV cannula was inserted into a forearm vein of the left hand, while the right hand was placed in a box heated to 65°C to “arterialize” the blood. After 20 min, another cannula was inserted retrogradely into a dorsal vein of the right hand to take blood samples. Slow infusion of 0.9% saline was used to maintain patency. An IMED 928 volumetric infusion pump (IMED, Abingdon, UK) was used to infuse the glucose. After insertion of the lines, the participants rested for 20 min before a resting blood sample was drawn. A priming infusion of 20% dextrose was then started, according to DeFronzo et al. (1979) to raise the plasma glucose concentration to 12mM. The prime infusion period of 30 min was followed by the withdrawal of a further blood sample before a 90-min bout of exercise began at an intensity corresponding to 70% V̇O2max. During the prime infusion and throughout the exercise bout, plasma glucose was maintained at close to 12mM by variation of the rate of infusion every 5 min, according to the arterialized plasma glucose concentrationusing an Analox GM7 analyzer (Analox Instruments, London, UK). Changes in infusion rate were calculated by using a Sharp MZ-80B computer. Fig. 1 provides an overview of the experimental protocol.

Two 10-ml blood samples were taken in lithium-heparin syringes (Monovette, Sarstedt, Leicester, UK) at rest (−30 min), after the prime infusion (0 min), and at 15, 30, 60, and 90-min during the exercise. The blood was centrifuged at 3,000 rpm and divided into aliquots. Plasma was stored at −20°C before analysis for lactate, NEFA, glycerol, β-HB and insulin. Plasma samples for the determination of adrenaline and noradrenaline were stored at −70°C. An on-line gas-analysis system (P.K. Morgan) was used for the determination of V̇O2 and respiratory exchange ratio over a 5-min period before the prime infusion, in the last 5 min of the prime infusion, and at 15, 30, 60, and 90-min of exercise. These values were used for the calculation of carbohydrate and fat oxidation rates. RPE was determined at 15, 30, 60, and 90 mins during exercise. Immediately after the exercise bout, participants were requested to lie supine while the infusion rate was slowly decreased, so as to prevent rebound hypoglycaemia. participants repeated the opposite CHO condition 3 weeks after the first trial.

**\*\*\*Insert Figure 1 Around Here\*\*\***

**Blood and muscle analyses**

Plasma NEFA values were determined by an enzymatic spectrophotometric method, while a portion of the plasma was deproteinised with perchloric acid (7% wt/vol) before assay for lactate, glycerol, and β-HB by using enzymatic methods. Analyses were performed on a Cobas-Bio centrifugal analyzer (Roche Products, Welwyn Garden City, Herts, UK). Plasma insulin was determined using radioimmunoassay (RIA) (IM.78, Amersham International, Amersham, UK). Plasma adrenaline and noradrenaline concentrations were determined using high-performance liquid chromatography with electrochemical detection via an in-house method (Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, UK). Freeze-dried muscle biopsy samples were prepared and analysed for glycogen according to the method of Edwards et al. (1980).

**Calculations**

Exogenous glucose utilization rate (GUR) was calculated as the glucose infused, corrected for glucose lost in the urine (DeFronzo et al. 1979), and averaged over 20 minute epochs. Carbohydrate and fat oxidation rates were calculated from the gas exchange data using stoichiometric equations (Frayn 1983).

**Statistics**

A two-way repeated measures analysis of variance (ANOVA) was employed to determine differences between the trials and the time points. ﻿Where the ANOVA revealed a significant effect, post hoc tests were completed using the Bonferroni correction method. Alpha significance level was set at 0.05 for all analyses. All data were analysed using Statistical Package for Social Sciences (version 24.0, SPSS Inc., Chicago, IL, USA). Assumptions for all statistical analyses were explored according to the methods of Field (2009). Values are reported as mean and SD.

**Results**

**Glucose utilisation and hyperglycaemia**

Hyperglycaemia was maintained in both trials (Figure 2a), with no significant difference in plasma glucose values (*P >* 0.05). The rate of glucose utilisation was similar between the CHO-L and CHO-D conditions (*P >* 0.05) (Figure 2b).

**\*\*\*Insert Figure 2 Around Here\*\*\***

**Oxidation rates**

The rate of total CHO oxidation was higher across all time points under the CHO-L compared to CHO-D condition (*P <* 0.05) (Figure 3a). Conversely, fat oxidation rates were higher in the CHO-D condition compared to CHO-L (*P <* 0.05) (Figure 3b).

**\*\*\*Insert Figure 3 around Here\*\*\***

**Hormonal response**

Plasma insulin concentrations became elevated as a result of the prime infusion and exercise under both conditions (*P <* 0.01) (Figure 4a). Mean values at rest were 7.2 ± 2.5 µU mlˉ¹ for CHO-L and 4.1 ± 2.0 µU.ml-1 for CHO-D. However, CHO-L produced significantly higher insulin than for CHO-D across all time points during exercise (*P <* 0.05). Plasma adrenaline were significantly higher at 30, 60 and 90 mins during exercise in the CHO-D compared to CHO-L (*P <* 0.05) (Figure 4b). Plasma noradrenaline was also significantly elevated at baseline and up to 60 mins during exercise in CHO-D (*P <* 0.05) (Figure 4c).

**\*\*\*Insert Figure 4 around Here\*\*\***

**Metabolite response**

Plasma NEFA (Figure 5a) and β-HB (Figure 5b) concentrations were elevated under CHO-D compared with CHO-L across all time points (*P <* 0.05). Plasma glycerol was elevated during exercise forCHO-D compared to CHO-L (*P <* 0.05) (Figure 5c). Plasma lactate values were elevated during CHO-L at baseline and up to 30 minutes during exercise compared to CHO-D (*P <* 0.05) (Figure 5d), with values not significantly different at 60 and 90 mins (*P >* 0.05).

**\*\*\*Insert Figure 5 around Here\*\*\***

**Energy oxidation contribution**

When the GUR is examined in relation to the rate of total carbohydrate oxidised (Figure 6), it is observed that as the rate of utilisation is increased over the 90 min, the rate of total carbohydrates oxidised is reduced, resulting in an increased reliance on exogenous carbohydrate. The contributions from the glucose infused during CHO-L was 47%, 58%, 66%, and 80% at 20-40, 40-60, 60-80, and 80-90 min respectively. The corresponding values during CHO-D were 58%, 77%, 88% and 100%.

**\*\*\*Insert Figure 6 around Here\*\*\***

**Rating of perceived exertion**

The RPE were elevated at 30, 60 and 90 mins during exercise under CHO-D compared to CHO-L (*P <* 0.05) (Figure 7).

**\*\*\*Insert Figure 7 around Here\*\*\***

**Dietary analysis**

Examination of the weighed food intakes of the participants showed that CHO-L resulted in 561 ± 48 g.day-1 of CHO being consumed whereas CHO-D resulted in 177 ± 56 g.day-1 being consumed. These diets led to muscle glycogen concentrations of 377 mmol/g dw for CHO-L and 159 mmol/g dw for CHO-D, thereby reflecting the exercise and dietary strategy for modifying muscle glycogen status prior to testing.

**Discussion**

The present investigation identified differences in CHO and fat oxidation, plasma metabolites, hormones and ratings of perceived exertion during exercise between glycogen loaded and depleted states under conditions of maintained hyperglycemia. CHO oxidation was elevated throughout the loaded state, whereas fat oxidation was elevated during the depleted state. The increased catecholamine response and reduction in insulin during CHO-D probably accounted for the increased lipolysis and in the elevation of circulating NEFA, glycerol and β-HB. On the other hand, the reduction in catecholamines and increase in insulin during CHO-L attenuated levels of NEFA, glycerol and β-HB and thereby favoured CHO oxidation. However, the enhanced CHO oxidation occurred without a concomitant increase in GUR. The overall effect of these metabolic changes may have resulted in the higher RPE observed during CHO-D irrespective of the fact that hyperglycemia was maintained. Furthermore, significant changes in metabolites (with the exception of glycerol) and hormones were evident at baseline, reflecting the effects of the dietary manipulation.

The significantly reduced resting levels of NEFA and β-HB, have been previously observed in studies examining the effect of low carbohydrate/high fat manipulation. The invariable consequence is elevated fat oxidation and reduced carbohydrate oxidation. How is this possible? Several studies have demonstrated that following a 5 to 7-day adaptation to a high-fat low-carbohydrate diet there is an increased reliance on skeletal muscle fat oxidation and decreased carbohydrate oxidation both at rest and during exercise (Bigrigg et al., 2009). The decrease in skeletal muscle carbohydrate utilization is accompanied by decreased transformation of pyruvate dehydrogenase (PDH) to the active form (PDHa). PDH is a mitochondrial enzyme that regulates skeletal muscle carbohydrate oxidation and catalyzes the irreversible decarboxylation of pyruvate to acetyl-CoA. As such, it is crucial in regulating oxidative carbohydrate disposal in skeletal muscle. PDH activity is decreased through increased phosphorylation by PDH kinases (PDKs) and increased by PDH phosphatases (Bigrigg et al., 2009). Following adaptation to a 3-day high-fat diet total PDK activity have been shown to increase in human skeletal muscle and PDH activity to decrease (Peters et al 2001).

However, PDH activity is not the only consideration, a 5-day high-fat/low-carbohydrate dietary was shown to upregulate the fatty acid transport protein, CD36, as well as a key enzyme involved in fatty acid oxidation, β-hydroxyacyl CoA dehydrogenase (Cameron-Smith et al 2003). So, enhanced fatty acid uptake and oxidation by skeletal muscle is up-regulated by short periods of dietary manipulation. But is this upregulation maintained when hyperglycaemia is evident? Our findings would suggest that this is the case.

The maximum rates of glucose utilisation of 1.82 and 1.99 g minˉ¹ for the loaded and depleted conditions respectively were similar to MacLaren et al. (1999). Muscle cells are incapable of taking up exogenous glucose at a rate greater than these levels, in spite of significantly elevated concentration of insulin. It is possible that this rate represents maximum transport kinetics for glucose across the plasma membrane of skeletal muscle.

Comparisons of the GUR with the rate of total carbohydrate oxidation reveals that as exercise progresses there is an increase in the contribution from the infused glucose, 47%, 58%, 66%, and 80% under CHO-L compared with 58%, 77%, 88%, and 100% under CHO-D at 20-40, 40-60, 60-80, and 80-90 minutes respectively. In addition, these findings illustrate that during CHO-D the reliance on exogenous glucose is more pronounced with a 20% increased rate observed during the final time point due to the lack of endogenous glycogen stored with the muscle. In agreement with Weltan and colleagues (1998), glucose oxidation during both trials does not increase. Conversely, an increased rate of fat oxidation is observed for both conditions regardless of hyperglycaemic, and that CHO-D significantly elevates fat oxidation above that seen during CHO-L. Although not expected, but in line with the increased fat oxidation observed, were the significant increases in plasma NEFA, β-HB, and glycerol measured throughout CHO-D. These findings occurred in spite of maintained hyperglycaemia. In contrast, there was a marked reduction in NEFA, β-HB, and glycerol when participants were glycogen loaded. The resting concentration of β-HB for participants under glycogen depletion reflects a ketotic state, and this is mirrored on examination of pre-exercise levels of NEFA but not for glycerol. The concentrations of these indicators of lipid metabolism during exercise were equivalent to those found under saline infusion (MacLaren et al. 1999). Moreover, these increased levels of lipid metabolism were found with participants exercising at similar intensities with low or normal glycogen availability prior to exercise (Weltan et al. 1998).

An elevation in the lipid metabolites and fat oxidation may also be linked to the attenuation of insulin observed under CHO-D. Weltan et al. (1998) suggested that muscle glycogen content influences plasma insulin concentrations, even during hyperglycaemia, andthat this was possibly achieved via muscle afferent pathways to the hypothalamic region of the brain. An inhibition of plasma insulin could be a result of the increased levels of adrenaline and noradrenaline (Christensen and Galbo 1983). Catecholamines are known to stimulate hormone sensitive lipase and augment NEFA release and lipid oxidation, conversely lowering the rate of glucose oxidation. In contrast, during CHO-L plasma insulin was significantly increased during exercise.

The elevation in plasma lactate concentrations both before and during exercise under CHO-L is an established phenomenon (Jacobs 1986), whereby an increase in muscle glycogen favours glycolysis with a concomitant enhanced production of pyruvate, and thereby lactate. Since the exercise is relatively strenuous, an increase in plasma concentration of lactate is inevitable, at least for 30-60 min. The observation that lactate levels failed to decrease to pre-exercise levels can be attributed to the availability of significant amounts of glucose, even in the glycogen depleted trial.

During CHO-D, blood lactate levels were significantly reduced up to 30 mins during exercise compared to CHO-L. This also coincided with a significant increase in RPE scores at 30 mins for CHO-D. The importance of peripherally produced blood lactate as fuel for the brain after traumatic injury has been reported (Glenn et al., 2015), and this is in line with the original concept of the 'lactate shuttle'. In fact prior to this finding, blood lactate as a fuel for the brain during high intensity exercise was observed (Van Hall et al., 2009), and is particularly noteworthy when it is appreciated that cerebral use of glucose diminishes during high intensity exercise whereas the use of lactate increases. Indeed, the brain becomes dependent on lactate delivery during high intensity exercise (Quistorff et al., 2008), especially for cognitive function. Recently, Hashimoto et al. (2018) highlighted the importance of lactate as a cerebral energy supply following exercise in order to maintain brain executive function (i.e. cognitive function). It is feasible to suggest that the increase in RPE during CHO-D could be due to a reduced systemic availability of lactate to regulate brain function during the exercise bout, even though the level of exercise intensity in our study was not as high as those reported above. However, the fact that blood lactate was similar between conditions at 60 and 90 minutes whilst RPE was different does not support that contention.

Whether the rate of glucose infusion would have been capable of sustaining this intensity of exercise for a longer period under CHO-D cannot be answered, although the RPE at the end of the depleted CHO-D was 18 compared with a value of 12 at the end of the CHO-L. As the current investigation required participants to complete an exercise bout at a set intensity of 70% V̇O2max, no pacing strategy was employed. Rauch et al. (2005) suggested that muscle glycogen has a metabolic signalling role and supports a pacing strategy to ensure that an exercise end point is safely achieved. Following a fixed work rate of cycling, which included intermittent sprints, participants were required to complete a 1-hour time trial. Irrespective of starting muscle glycogen, the concentration left in the muscles at the end of the exercise was near identical. This suggests a critical glycogen level exists and participants self-paced themselves in accordance to an internal physiological feedback mechanism that supports their chosen power output to ensure safe completion of the exercise. This mechanism has been referred to as the central governing model/theory (Noakes et al. 2001) and suggests that the brain regulates performance to balance and therefore slowing down or pacing is the result of this regulation. As the participants were not able to self-pace with a lack of anticipation, it may be that the metabolic signalling during the CHO-D trial increased RPE regardless of hyperglycaemic conditions due to the lower glycogen content within the muscles.

Increased availability of glucose in the ventromedial and ventrolateral cells of the hypothalamus reduces sympathetic activity and hence reduces adrenaline release. However, what is of interest in this context is that hyperglycaemia was maintained throughout exercise in both trials yet there were differences observed for carbohydrate and fat oxidation as well as the hormonal and metabolite responses in relation to pre-exercise levels of muscle glycogen. If the brain detects changes in the circulation of glucose and so brings about changes to circulating hormones such as the catecholamines, why is there a difference between these trials when the blood glucose concentration is consistent (i.e.12mM)? Could it be that, in some way, signals are reaching the CNS from the muscle in response to muscle glycogen content and so bringing about the changes we observed with the hormonal and consequent metabolite responses? Indeed, if blood glucose levels are an important factor in modifying RPE scores during exercise, why is there a variation in the RPE scores between the trials in spite of similar blood glucose levels? These questions are difficult to answer at present, although they clearly demonstrate a link between muscle and brain. It is possible that the link is via afferent muscle neurones as suggested by Weltan et al (1998), although other factors such as muscle-derived cytokines and myokines, which play a crucial role in the skeletal muscle crosstalk with other tissues, or brain-derived neurotrophic factors may have a role (Delezie & Handschin,2018). It is beyond the scope of this article to speculate as to which of these (if any) are a likely influence. Indeed, further exploration is invited.

There is another possible consideration with regard to the findings of variations in hormonal and metabolite concentrations, as well as in RPE despite similar blood glucose levels and that may be in relation to brain glycogen. Recently, Matsui et al (2019) proposed that glycogen stores in the astrocytes (akin to that in muscle) plays an energetic role in the brain during exercise to maintain endurance capacity through lactate transport. The astrocytes are recognised as having monocarboxylate transporters to shuttle lactate within the brain, and that lactate thereby provides an energy source for brain function. Albeit in rats, they provide direct evidence that astrocytic glycogen-derived lactate fuels the brain to maintain endurance capacity during exhaustive exercise and suggested that brain ATP levels maintained by glycogen might serve as a possible defence mechanism for neurons in the exhausted state.

**Conclusion**

The essential findings from our study clearly demonstrate that an exercise bout to fatigue followed by a 3-day has a significant impact on hormonal, metabolite, and metabolic measures during a subsequent bout of exercise during maintained hyperglycaemia. The rate of total carbohydrate oxidation and the contribution of infused glucose to total carbohydrate oxidation was greater for the depleted state than the loaded state, even though the rates of glucose utilisation were similar. This would imply that glucose uptake by muscle may have reached saturation at 1.8-1.9 g minˉ¹. Furthermore, irrespective of the robust hyperglycaemic conditions, the reduction in carbohydrate intake over 3 days and the concomitant reduced pre-exercise glycogen within the skeletal muscle promotes fat oxidation and attenuates CHO oxidation, elevates fat metabolites, and leads to reduced plasma insulin and an elevation in catecholamines. The precise mechanism as to how this is achieved was not undertaken in this investigation but points to the possibility of some link between muscle and brain as well as the likelihood of downregulation of PDH and upregulation of fatty acid transporters and oxidative enzymes. A final result of note is the augmented RPE observed during CHO-D in spite of similar blood glucose levels, which could support the central governor theory of fatigue and that muscle glycogen has a signalling role within the body.

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**Figure Captions**

**Figure 1.** Overview of the experimental protocol.

**Figure 2.** Plasma glucose (A) and glucose utilisation rate (GUR) (B) during exercise under carbohydrate loaded (CHO-L) and depleted (CHO-D) conditions.

**Figure 3.** Total carbohydrate (A) and fat (B) oxidation rates during exercise under carbohydrate loaded (CHO-L) and depleted (CHO-D) conditions. \* denotes between conditions (*P <* 0.05).

**Figure 4.** Plasma insulin (A), adrenaline (B) and noradrenaline (C) response during exercise under carbohydrate loaded (CHO-L) and depleted (CHO-D) conditions. \* denotes between conditions (*P <* 0.05).

**Figure 5.** Plasma non-esterified fatty acids (NEFA) (A), β-Hydroxybutyric acid (β-OH) (B), glycerol (C) and lactate (D) response during exercise under carbohydrate loaded (CHO-L) and depleted (CHO-D) conditions. \* denotes between conditions (*P <* 0.05).

**Figure 6.** The relative contribution of fuel substrates to total energy during 90 min cycling at 70% V̇O2max using the hyperglycaemic clamp when carbohydrate loaded (A) and depleted (B).

**Figure 7.** Rating of perceived exertion (RPE) response during exercise under carbohydrate loaded (CHO-L) and depleted (CHO-D) conditions. \* denotes between conditions (*P <* 0.05).