EFFECTS OF CARBOHYDRATE QUANTITY AND GLYCAEMIC INDEX ON CENTRAL FATIGUE DURING ENDURANCE EXERCISE

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ABSTRACT

Central fatigue (CF) is the inability of the central nervous system to generate or maintain activation of muscle, measured using neurophysiological techniques such as twitch-interpolation techniques via nerve stimulation and neural responses recording of muscle activity. Reduction of muscle force alongside central activation has been associated with hypoglycaemia, indicating importance of carbohydrate (CHO) in preventing CF; one possible mechanism is through attenuation of serotonin, a neurotransmitter associated with sense of tiredness, produced during exercise when free-fatty acid (FFA) is high. In addition, the quality of CHO, categorised by glycaemic index (GI), influences fuel selection during exercise. Low GI (LGI) could improve endurance exercise by providing steady supply of CHO due to lower insulin response which promotes fat oxidation compared to high GI (HGI).

While peripheral benefits of CHO are well-documented, only few studies measured CF with neurophysiological techniques. The objective of this study was to investigate the effects of pre-exercise meals with different quantity and GI of CHO on CF. The neurophysiological measures employed were voluntary activation (VA), central-activation ratio (CAR) obtained during maximal voluntary contraction (MVC), alongside related variables, i.e. resting twitch and Mmax, blood serotonin, FFA, and insulin.

To elucidate the effects CHO and GI, three studies were designed; the first aimed at verifying the custom-made meals' post-prandial blood glucose (BG) response to conform with the established norm, the second investigated the effects of high and low CHO meals on CF following a 90-minute endurance exercise, while the third distinguished HGI and LGI on CF following similar exercise.

The first study, using a randomised, cross-over design with a 7-day washout-period, ten participants' post-prandial BG responses were analysed after consuming two sets of

meals: i) iso-caloric high CHO (HCHO, 1.5 g/kg body weight) and low CHO (LCHO, 0.8 g/kg body weight); and ii) iso-macronutrients; high CHO (1.5 g/kg body weight) with high (~75) and low (~40) GI. Consistent with the literature, HCHO and HGI meals induced a higher BG area-under-curve (AUC) compared to LCHO and LGI, respectively.

In study-2, fourteen participants, in a cross-over, randomised, double-blind design, consumed either HCHO or LCHO meals 1-hour prior to a 90-minute run at 65% VO_2max. HCHO preserved MVC post-running, mirrored by preservation of CAR and VA as well as an unchanged serotonin level. LCHO meanwhile showed lower insulin response (indicating lipolysis), and marked MVC loss with concurrent reduction in CAR and VA as well as increased serotonin.

Study-3 employed similar study design and found that with HGI meal, MVC was better maintained compared to LGI, but without concomitant changes in VA and serotonin, despite the differences in fuel selection. The results do suggest that HGI maintains the force production by larger magnitude compared to LGI due to preservation of CAR. The difference seen could be attributed to fuel availability as HGI provide higher CHO oxidation for MVC.

In conclusion, HCHO better attenuates CF compared to LCHO meal; and when amount of CHO is similar between meals, HGI better maintained CF following a 90-minute run compared to LGI meal.

ABSTRAK

Kepenatan sentral (*central fatigue*, CF) adalah ketidakupayaan sistem saraf pusat menjana atau mengekalkan pengaktifan otot. Ia diukur menggunakan teknik neurofisiologi seperti *twitch-interpolation* melalui rangsangan saraf dan rakaman aktiviti otot. Pengurangan daya otot bersama dengan pengaktifan sentral dikaitankan dengan hipoglisemia, dan ini menunjukkan kepentingan karbohidrat (CHO) dalam mengelakkan CF; satu mekanisme yang boleh dikaitkan dengan CHO adalah pengurangan serotonin, neurotransmiter berkaitkan dengan rasa penat, dan dihasilkan apabila tahap asid lemak bebas (FFA) tinggi semasa eksesais. Tambahan pula, kualiti CHO, dikategorikan mengikut indeks glisemik (GI), boleh mempengaruhi pemilihan sumber tenaga semasa eksesais. GI rendah (LGI) boleh meningkatkan prestasi eksesais daya tahan dengan membekalkan lebih banyak sumber CHO, memandangkan respon insulin yang lebih rendah meningkatkan oksidasi lemak berbanding dengan GI tinggi (HGI).

Walaupun kebaikan periferal CHO adalah diketahui, hanya beberapa kajian sahaja yang mengukur CF menggunakan teknik neurofisiologi. Objektif kajian ini adalah untuk menyiasat kesan makanan pra-eksesais terdiri daripada kuantiti dan GI CHO berbeza ke atas CF. Ukuran neurofisiologi yang digunakan adalah *voluntary activation* (VA), *central-activation ratio* (CAR) yang diperolehi sewaktu *maximal voluntary contraction* (MVC), serta unsur berkaitan seperti *resting twitch* and *Mmax*, serotonin, FFA, dan insulin.

Untuk menjelaskan kesan CHO dan GI ke atas CF, tiga kajian telah direka; kajian pertama bertujuan mengesahkan glukosa darah selepas pemakanan adalah mematuhi norma sedia ada; kajian kedua menyiasat kesan CHO dengan kuantiti banyak dan sedikit ke atas CF selepas eksesais daya tahan salama 90-minit; manakala kajian ketiga menyiasat kesan HGI dan LGI ke atas CF selepas eksesais yang sama.

Kajian pertama menggunakan kaedah secara rawak, *cross-over* dengan tempoh 7-hari antara sesi kajian, di mana 10 orang peserta diukur glukosa darah selepas pemakanan (*post-prandial*) dua set makanan iso-kalori tinggi CHO (HCHO, 1.5 g/kg berat badan) dan rendah CHO (LCHO 0.8 g/kg berat badan); dan ii) makanan iso-makronutrisi dengan GI tinggi (GI~75) dan rendah (GI ~40). Bersamaan dengan norma, makanan HCHO dan HGI menghasilkan kawasan-bawah-lekuk (AUC) yang lebih besar berbanding dengan LCHO dan LGI.

Dalam kajian kedua, empat belas peserta, melalui rekaan *cross-over,* secara rawak, *double-blind*, mengambil makanan HCHO dan LCHO 1-jam sebelum menjalani larian daya tahan 90-minit pada intensiti 65% VO_2max. HCHO mengekalkan MVC selepas larian, serentak dengan pengekalan CAR, VA, serta tahap serotonin. LCHO menunjukkan respon insulin yang lebih rendah (menunjukkan lipolisis), dan kehilangan MVC serentak dengan pengurangan CAR dan VA, serta peningkatan tahap serotonin.

Kajian ketiga menggunakan reka kajian yang serupa dan mendapati bahawa makanan HGI lebih mengekalkan MVC berbanding dengan LGI walaupun perubahan VA dan serotonin adalah serupa. Akan tetapi, CAR adalah lebih dikekalkan dengan HGI. Perbezaan dalam CF ini mungkin disebabkan kebolehan HGI menghasilkan pengoksidaan CHO yang lebih banyak untuk MVC.

Kesimpulannya, HCHO mengurangkan CF dengan lebih berkesan berbanding dengan LCHO. Apabila jumlah CHO dalam dua set makanan adalah sama, HGI mengekalkan CF secara lebih berkesan berbanding dengan LGI.

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List of abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AUC	Area under the curve
CAR	Central activation ratio
CF	Central fatigue
CHO	Carbohydrate
CMEP	Cervicomedullary motor evoked potential
CNS	Central nervous system
CV	Coefficient of Variation
FAT/CD36	Fatty acid translocase
FFA	Free fatty acid
GI	Glycaemic Index
GL	Glycaemic Load
GLUT-4	Glucose transporter type-4
IMTG	Intramuscular triglyceride
MEP	Motor evoked potential
Mmax	Maximal compound muscle action potential
MVC	Maximal voluntary contraction

RER	Respiratory exchange rate
SICI	Short-interval intracortical inhibition
SIT	Superimposed twitch
TG	Triglyceride
TMS	Transcranial magnetic stimulation
VA	Voluntary activation
VO_2	Volume of oxygen uptake
VO_2max	Maximal volume of oxygen uptake

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CHAPTER 1 INTRODUCTION

1.1 Central fatigue and endurance exercise

Fatigue is a physiological condition where the body's capability to do work is reduced. In sports and exercise, fatigue is induced by performing physical work, whether by training for a sport, for fitness, or by competing in a competition. Fatigue limits performance as it disables athletes from performing to their maximum capability, and athletes that can outlast their opponent before becoming fatigued has higher chances of winning a competition. This is especially true in endurance exercise, as this kind of work requires the body to continuously provide enough energy for a long period to enable the athlete to continue working at their desired work rate. If marathon runners can delay fatigue by just a minute compared to their opponents, they can take a big lead by being able to run at desired speed for a minute while others are slowing down. Is also beneficial for recreational athletes and exerciser to know how to prevent or delay fatigue to reduce their risk of getting injured due to fatigue.

It is also well known that fatigue may happen at different sites of the body, such as the working muscles and the central nervous system (CNS). While it is well established that fatigue at working muscles are due to factors such as depleted energy fuel sources, metabolic pathways failure, dehydration, among others; the mechanism and factors of fatigue at the CNS, known as central fatigue (CF), is less understood. Central fatigue (CF) is the inability of CNS to generate or maintain central activation to the muscle (Gandevia, 2001). It can induce fatigue due to factors within the CNS, such as reduced neural drive from the brain and reduced rate of neural firing, and/or either by depletion of fuel source in the brain, or rise in "fatigue hormone" such as serotonin concentration (Gandevia, 2001; Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006). While it has been observed that CF can occur during exercise, there has been not much consensus on

methods to prevent or delay it. Past studies have been limited by the fact that it was difficult to observe both peripheral fatigue and CF at the same time on exercising subjects, and this is especially true regarding CF studies as in the past, non-invasive methods are not common in measuring neural activities unlike present time.

1.2 Carbohydrate and central fatigue

An easily accessible method attenuate fatigue is through nutritional intervention before and during the exercise/sport events. Carbohydrate (CHO) consumption is a commonly applied method to prevent fatigue originating from the peripheral sites as it can replenish energy substrates for exercise and prevent depletion of muscle glycogen. The classical study by Romanowski & Grabiec (1974) suggested that increased free fatty acid (FFA) availability may delay CF by reducing brain serotonin concentration has also sparked the possibility of using CHO consumption to manipulate FFA metabolism to delay CF. However, most study had employed time to exhaustion as performance outcome, with only few measured neurophysiological activities with CHO feeding, and findings thus far are limited to only measurements of central activation ratio (CAR) during sustained maximum voluntary contraction (MVC) (Nybo, 2003) or voluntary activation (VA) during MVC (Del Coso, Estevez, & Mora-Rodriguez, 2008) in separate studies; and timing of feeding (i.e. during exercise). Neurophysiological measures typically involve force production and usage of superimposed twitches (electrical or magnetic) during the contraction to quantify the extra force one could produce involuntary following MVC. Using these neurophysiology methods to measure CF with pre-exercise feeding of different amount of CHO would strengthen the understanding on how CHO consumption can alter CF. There has been indication of the role of CHO feeding during exercise in CF, such as failure to generate desired muscle force due to hypoglycaemia (Nybo, 2003) with concurrent reduction in VA, a neurophysiological CF marker. A drop in MVC alongside VA has also been observed when CHO containing

drink were prescribed as opposed to plain water (Del Coso *et al.*, 2008). However, both studies prescribed CHO mid-exercise, and to date little is known on its effect on CF when provided as pre-exercise meal. These studies also did not measure serotonin, which could have given an additional information of possible effects of CHO on CF.

Furthermore, since relationships between CHO and CF has been established, and CHO feeding could alter insulin and possibly serotonin level, there will be a need to investigate whether the Glycaemic Index (GI) of CHO would influence CF. A same amount of CHO could cause vastly different rise in blood glucose and insulin level, and this difference is quantified by the GI of the CHO. Current literature clearly shows that GI affects the fuel selection during prolonged exercise, but its effects on performance outcome remains mixed (Wee, Williams, Gray, & Horabin, 1999; Wu & Williams, 2006). A high GI is expected to cause a higher rise in blood glucose and insulin level, which has been associated with promotion of CHO oxidation and inhibition of lipolysis; while low GI a smaller blood glucose and insulin spike with promotion of fat oxidation, which is speculated to improve endurance exercise (Wu *et al*., 2006). This promotion of fat oxidation could have a relation with the CF phenomenon, as increased FFA availability has been linked with increased serotonin, a possible inducer of CF (Meeusen *et al.*, 2006). This would be contradicting with the present knowledge that a higher fat oxidation should preserve muscle glycogen, allowing for longer exercise time prior to fatigue, further indicating a need to identify or distinguish effects of GI on CF. Hence this research aims to investigate the effects of CHO with different level of GI on CF during endurance exercise.

1.3 Problem statement

1. Some indication of CHO and CF has been shown in few studies that prescribed CHO during exercises, but none have employed neurophysiology techniques to measure central activity while designing a study that prescribe CHO as part of pre-exercise meal.

2. Pre-exercise intake of CHO has been shown to improve endurance performance by delaying fatigue, but the evidence to support its effect of CF is limited particularly due to inconsistent neurophysiological techniques employed.

3. Serotonin, a possible CF inducer which concentration is believed to be affected by FFA availability, has never been investigated in studies that employed CHO of different amount and GI, despite their known effects on insulin, and subsequently, FFA increment.

1.4 Objectives

1. To investigate how different amounts of CHO in iso-caloric pre-exercise meal would influence CF after endurance exercise among adult male runners.

2. To investigate whether the same amount of CHO with different GI would influence CF after endurance exercise among adult male runners.

3. To compare the changes in blood markers (insulin, glycerol, FFA) that could lead to changes in the blood CF marker, serotonin, during endurance exercise after consumption of iso-caloric meals with different amounts of CHO among adult male runners.

4. To distinguish effects of pre-exercise meals with same macronutrient but different GI on insulin, FFA, tryptophan and serotonin following an endurance exercise among adult male runners.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Role of substrates such as carbohydrate (CHO) in muscle metabolisms during exercise is well known, with depletion of the substrate causing muscular fatigue. For the muscles to work optimally during exercise, substrate availability must be maintained not only peripherally but also within the central nervous system (CNS), where adequate neural drive is required to produce the desired force. Central fatigue (CF) is the inability of CNS to generate or maintain central activation of muscle (Gandevia, 2001). There are evidences for the CF occurrence in many exercise modalities ranging from single joint exercise to whole body prolonged exercises which were well reviewed (Gruet *et al.*, 2013; Sidhu, Cresswell, & Carroll, 2013). The failure to generate desired muscular force due to hypoglycaemia has been shown, where the change in CAR is directly affected by CHO availability (Nybo, 2003). There are, however, only few studies to date that measure CF directly using neurophysiological measurements while employing CHO interventions (Del Coso *et al*., 2008; Nybo, 2003).

2.2 Central fatigue and role of carbohydrate

Few mechanisms may explain the development of CF as a result of CHO depletion. In working muscle, a reduction in CHO supplies leads to increased lipolysis to meet the energy demand by breaking down adipose tissue into free fatty acid (FFA) as readily-available fuel substrate (Klein, Coyle, & Wolfe, 1994). The increased plasma FFA exerts an influence on neurotransmitters availability which in turn affects CF. Specifically, as plasma FFA increases, tryptophan (a precursor to serotonin) bound to albumin is displaced by FFA, increasing the free-tryptophan concentration that is available to cross the blood-brain barrier, which in turn increases serotonin synthesis in the brain (Meeusen, *et al.*, 2006). Serotonin, a vasopressin group of stimulators, has been shown to cause a

sense of lethargy and fatigue (Romanowski & Grabiec, 1974). During prolonged exercise, the increase in serotonin seems to modulate fatigue (Romain Meeusen & De Meirleir, 1995).. Meanwhile study attempted to investigate the effects of ingesting different CHO concentrations (6 and 12% in 100 ml fluid) on cycling time to exhaustion at 68% VO_2max (maximal oxygen uptake) found that in the group not supplemented with CHO, a shorter time to exhaustion occurred alongside an increment in free-tryptophan, branched chain amino acid, and FFA concentrations; while these changes were attenuated in dose-related manner in the CHO group (Davis *et al*., 1992). It seems that CHO feeding suppresses free-tryptophan, and this has been speculated to have reduced serotonin levels and increase exercise performance (Meeusen *et al*., 2006). However, in human studies blood samples from the brain is difficult to obtain, and it is possible that serotonin outside the brain may influence analysis of blood samples obtained from other part of the body; although studies have shown blood serotonin is highly correlated with central activity (Mann *et al*., 1992).

Another mechanism is depletion of fuel source in the brain. A study using an animal model has shown that after 120-minute running, brain glycogen is reduced by ~37 - 60% (Matsui *et al*., 2011). In another study by the same authors, it is shown that similarly to the skeletal muscles, prolonged exercise causes super-compensation of brain glycogen, indicating the possibility of central adaptation to higher energy demand (Matsui & Soya, 2013). It is well known that insufficient CHO feeding during prolonged exercise leads to hypoglycaemia (Coyle *et al*., 1983; Coyle & Coggan, 1984; Nybo, 2003). Under such conditions, cerebral glucose is also likely depleted. This has been shown in parallel, a disrupted electroencephalogram activity, suggesting a possible cortical neuronal dysfunction (Jensen, Bøgh, & Lykkesfeldt, 2014). In addition, depletion of brain glycogen leads to concomitant increase in monoamines (e.g. tryptophan) (Matsui *et al*., 2011), supporting the notion that CHO supply must be kept at an optimal level to delay

CF. A decrease in oxidative CHO metabolism in the brain during exhaustive exercise is shown to occur alongside a reduction in cortical voluntary activation, indicating that the development of CF at supraspinal level is also associated with the brain's capability to metabolise CHO (Rasmussen *et al*., 2010). However, evidence to explain this phenomenon remains scarce because collecting blood samples from the jugular vein renders it difficult to conduct these experiments in humans. In any case, these samples might provide some insight into neurotransmitters interactions in the brain during development of CF. A study that employed CHO mouth rinse have also proposed that CHO receptors in the oral cavity may stimulate the central pathways associated with motivation and motor activity regulation (Carter, Jeukendrup, & Jones, 2004). Furthermore, it has been demonstrated that mouth rinse improved central motor drive as indicated by restored EMG amplitude when endogenous CHO availability is low (Ataide-Silva *et al*., 2016). In this case, it has been suggested that the taste buds send signals to the primary taste cortex which have projections to areas of the brain including dorsolateral prefrontal cortex, anterior cingulate cortex, and ventral striatum, which link the gustatory pathways to the improvement in high intensity exercise performance (> 75% VO_2max) (Jeukendrup & Chambers, 2010; Rolls, 2007). In brief, these studies highlight the importance of CHO on brain function during prolonged exercise, and its influence on CF.

2.2.1 Measurements in quantifying central fatigue

In order to measure CF, neurophysiological techniques have been used widely over the last 20 years. The reduction in central neural drive can be evaluated using the twitch interpolation technique via peripheral nerve stimulation which allows the measurement of conventional VA (Merton, 1954). Specifically, this is quantified as the ratio of superimposed interpolated twitch elicited during MVC to that elicited at rest, derived from the formula: 100 ($1-T_{interpolated}/T_{rest}$). A reduction in VA leads to a reduction in muscle force, indicating reduced neural drive to the motoneuron pool from at or above

the point of stimulation and could originate from spinal and/or supraspinal sites. The usage of VA can clearly dissociate between central and peripheral involvements in fatigue, however, it cannot distinct the precise location or sites and mechanism of the CNS involved (Gruet *et al.*, 2013). Analogous to this is the quantification of cortical VA, measured by eliciting superimposed twitch using transcranial magnetic stimulation (TMS) over the motor cortex. Neural drive measured from cortical VA is supraspinal in origin (Gandevia, 2001). An attenuation in drive measured from both variables reveals that the reduction in maximum muscle force following exercise is most likely due to inadequate motor unit recruitment and/or firing rates. Although the direct relationship between neural drive (i.e. measurements of VA and cortical VA) and responsiveness of the corticospinal pathway remains somewhat elusive (Hoffman & Logothetis, 2009; Smith, Martin, Gandevia, & Taylor, 2007; Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006), various neurophysiological techniques and methods have been used to probe influence of fatigue on input-output properties of central structures. Briefly, some of the responses that can be measured include muscle compound action potential (Mmax); Motor Evoked Potential (MEP); Cervicomedullary Motor Evoked Potential (CMEP); Maximal Compound Muscle Action Potential (Mmax); Silent Period and Short-Interval Intracortical Inhibition (SICI). Mmax is the excitability of the sarcolemma typically used as a peripheral index measured by electrical activity via electromyography (EMG) (Gruet *et al.*, 2013). It is vital that any stimulation intensity to elicit superimposed twitch to be stronger than that needed to reach Mmax, with supramaximal intensity (equal to or more than 120% of intensity to elicit Mmax) typically used (Lee, Gandevia, & Carroll, 2009). MEP and CMEP can be obtained by stimulating the brain and cervicomedullary junction using TMS/Transcranial electrical stimulation to reflect the excitability of the corticospinal and motoneuronal pathways respectively in a variety of tasks (Taylor, 2006). Meanwhile, Silent Period is the near silence period following a MEP elicited by

single pulse TMS, and SICI is induced via paired-pulse TMS reflects the magnitude of intracortical $GABA_A$-mediated inhibition is measured via MEP amplitude. The increased period of silent period and SICI could indicate fatigue as time to recover increases with increasing task duration (Gruet et al., 2013). While MEP reduction may indicate CF, significant MEP depression must be normalised to Mmax to provide a true reflection of the changes that occurred. Hence it is crucial to normalise MEP to concomitant Mmax to account for activity-dependent changes in peripheral neural activity (Gruet et al., 2013). Descriptions of the mentioned measurements are presented in Table 2.1.

Table 2.1: Methods used to measure central fatigue

Measurements	**Description**
Maximal Voluntary Contraction (MVC)	The largest muscle force that can be produced voluntarily.
Superimposed interpolated twitch	Muscular force increment when an external stimulation is imposed during MVC.
Voluntary activation (VA)	Ratio of superimposed interpolated twitch elicited from skeletal muscle during MVC to that elicited at rest.
Mmax/M-wave	Early direct electrical response of muscle to motor nerve stimulation; maximal response is termed the Mmax. As stimulation intensity increases, M-wave increases until it reaches Mmax.
Electromyography (EMG)	EMG is the measurement of skeletal muscle's neural activity, measured as– electrical sum of positive and negative action potentials recorded directly from muscle belly.

Motor Evoked Potential (MEP)	Elicited by single pulse TMS, is the measurement of entire corticospinal tract responsiveness, and can indicate corticospinal excitability.
Cervicomedullary Motor Evoked Potential (CMEP)	Elicited by cervicomedullary stimulation, is the measurement of spinal responsiveness, and can indicate motoneuronal excitability.
Short-interval Intracortical Inhibition (SICI)	Inhibition to MEP amplitude mediated by $GABA_A$ receptors.
Silent Period	Near-silence of the EMG signal following a MEP elicited by a single pulse TMS mediated by $GABA_B$ receptors.

Usage of these measurements together can give an insight to how the central activity reacts to different types of activity. For example, evidence from MEP (i.e. measurement of entire corticospinal tract responsiveness) and CMEP (measurement of spinal responsiveness) have revealed that whole body prolonged exercise disfacilitates motor cortical cell responsiveness (Sidhu, Cresswell, & Carroll, 2012), in contrast to that seen during single joint exercise (Gruet *et al.*, 2014). Conversely, using a subthreshold TMS techniques, an increase in intracortical inhibitory interneuron responsiveness has been indicated during cycling exercise (Sidhu, Lauber, Cresswell, & Carroll, 2013), similar to that seen during a sustained single joint exercise (Seifert & Petersen, 2010). This suggests that while responsiveness of some central structures (i.e. motor cortical cells) is task-dependent (i.e. single joint vs. whole body exercise), responsiveness of other neural structures within the human motor cortex, including the intracortical inhibitory interneurons, is likely due to the presence of exercise induced fatigue per se (independent

of type of exercise). These neurophysiological measures are useful to investigate contribution of central factors towards fatigue as they can mechanistically determine sites of fatigue and pattern of changes in neural activity; though these responses should be carefully interpreted. For example, changes in VA do not reflect changes in central neuronal responsiveness and hence should be interpreted independently.

2.2.2 Does carbohydrate attenuate central fatigue?

There have been few studies that attempted to measure the effect of CHO on CF using neurophysiological techniques, listed in Table 2.2. Few points can be synthesised from the present literature: first, prolonged endurance exercise performance is affected by nutritional intake, secondly; CHO alone or in combination with other ergogenic nutrients potentially affects force production and neurophysiological responses; and lastly, evidences from the neurophysiological techniques applied are insufficient and incomplete in addressing the role of CHO on CF.

The work by Gibson, Schabort, & Noakes (2001) was the earliest attempt to associate the amount of energy ingested and composition of CHO in the diet on neuromuscular outcome during prolonged exercise, and not just exercise performance alone. Interestingly, the integrated electromyography and exercise performance were not affected by the three days CHO intervention. It should however be acknowledged that the study lacked the application of neural stimulation methods/techniques such as Mmax and VA to determine the effects of CHO on CF.

Since then, five other studies have investigated the effects of CHO on CF via ingestion, while one has used mouth rinsing (Jeffers, Shave, Ross, Stevenson, & Goodall, 2015); all these studies measured force output through MVC before and after exercise. Out of the six studies, four studies (Del Coso *et al*., 2008; Jeffers *et al*., 2015; Nybo, 2003; Stewart *et al*., 2007) found that the reduction in MVC was attenuated with CHO intervention,

including rinsing. Two others (Cureton *et al*., 2007; Ganio *et al*., 2010) found no differences between the placebo control and CHO only intervention, but it is worth noting that in that two studies, MVC was only measured 20 minutes after exercise and may have missed larger drop in force immediately post exercise (Girard, Lattier, Micallef, & Millet, 2006). Although there is a consensus in four studies that MVC was reduced following prolonged exercise, there is a discrepancy in the site(s) that contribute to the attenuation. In other words, while most studies have shown that the reduction in MVC may be attributed to changes within the central nervous system (Nybo, 2003; Del Coso *et al*., 2008; Jeffers *et al*., 2015), another have attributed it mainly to peripheral mechanisms proximal to the neuromuscular junction (Stewart *et al*., 2007).

It is a well-known fact that prolonged exercise can lead to hypoglycaemia; however, it is unclear whether systemic hypoglycaemia reduces CNS activation. In Nybo's study, MVC remained close to the baseline following 3 hours of cycling at 60% VO_2max in the glucose fed group (2.67 g/kg body weight; blood glucose maintained), compared to the control group (blood glucose homeostasis was not maintained in this group), which saw reduction in MVC and subsequently CAR (Nybo, 2003). He concluded that since CAR was lower in the unfed group, fatigue was likely affected by CHO-induced changes at central level, and with CHO feeding, central activation was preserved. This study seems to suggest that hypoglycaemia directly influences CNS activity. The findings of Del Coso *et al.* (2008) is in agreement with Nybo's, whereby the MVC reduction in CHO group following exercise was less pronounced compared to control groups. In addition, the reduction of VA was also attenuated the supplemented group (Del Coso *et al*., 2008). However, it is worth noting that the CAR method doesn't take into account the potentiated twitch at rest, which when accounted for, would provide an absolute change in superimposed twitch (SIT). Nevertheless, in the context of a sustained contraction, identifying a resting twitch may not be feasible. Thus, a drop in CAR when comparing

between CHO and placebo as shown in Nybo's, can provide some indication on the involvement of central contribution.

Meanwhile, Stewart *et al.* (2007) measured MVC, SIT, and Mmax at pre-post 90-min cycling at 60% VO_2max, as well as after volitional fatigue. While MVC decreased across all groups, the reduction was least pronounced in the CHO fed group (1.23 g/kg body weight), and concurrently, a higher magnitude of Mmax was also observed. In contrast to the aforementioned studies by Nybo (2003) and Del Coso *et al.* (2008), this study did not find any changes in CF with glucose supplementation (Stewart *et al*., 2007). The authors only observed changes in M-wave and concluded that CHO affects fatigue peripherally but not centrally, whereby glucose possibly protects muscle membrane excitability, and hence increases time to fatigue (Stewart *et al*., 2007). Indeed, changes in M-wave alone is insufficient to infer fatigue arising exclusively at peripheral level (Gandevia, 2001). In addition, the authors depended on SIT alone when interpreting CF (Stewart *et al*., 2007). However, lack of changes in SIT despite reduction in MVC is no surprise since SIT alone does not account for changes in MVC, it should be considered together with resting twitches (Nybo, 2003; Del Coso *et al*., 2008). In other words, an unchanged SIT alongside a reduction in resting twitch would indicate a decrease in VA (increase in CF). It should also be noted that while both studies assessed the superimposed twitch during knee extension, Nybo (2003) stimulated the femoral nerve to measure conventional voluntary activation, while Stewart *et al*. (2007) stimulated the vastus lateralis muscle directly (motor point stimulation). Measuring M-wave from this muscle during transcutaneous stimulation could represent composition of action potentials from activation of fibres of different types and locations within the muscle of interest (Stewart *et al*., 2007). Finally, it is also worth noting that Nybo (2003) prescribed a higher amount (almost double) of CHO compared to Stewart *et al*. (2007) whereby serotonin secretion

could have been reduced as the need to break down adipose tissue into FFA would likely have decreased, potentially attenuating CF.

Table 2.2: Studies that employed neurophysiology measurements with CHO as nutritional intervention.

Reference	Participants' characteristics and exercise trial	Nutritional interventions	Main findings
Del Coso *et al.,* 2008	7 M, endurance-trained cyclists, VO_2max = 61±8 mL/kg/min 120 min cycling at 63% VO_2max	1) No fluid (NF) 2) Water (WAT) 3) 6% CHO-electrolyte solution (CES) 4) NF + 6 mg caffeine (CAF)/kg body weight 5) WAT + 6 mg of CAF/kg bw 6) CES + 6 mg of CAF/kg body weight In 3) and 6), an average of 146±21 g CHO was consumed.	• VA and MVC reduction were more pronounced in NF trials • VA and MVC reduction were more pronounced in WAT compared to CES • No differences in superimposed twitch (SIT) between interventions.
Cureton *et al.,* 2007	16 M, highly trained cyclists, VO_2max = 60.5 ± 7.2 mL/kg/min 135 min cycling, alternating between 60 and 75% VO_2max every 15 min for 120 min followed by 15 min performance ride	1) Placebo (PLA) 2) 6% CHO-electrolyte sports drink (CES) 3) 7% CHO-electrolytes sports drink + 6 mg of CAF/kg/bw (CES+CAF). CHO ingestion for 2) 127±12g, and 3) 149±15g.	• CES did not reported any beneficial effects compared to PLA
Stewart *et al.,* 2007	14 M, 1 F, Untrained, VO_2 peak = 45 ± 2 ml/kg/min Cycling to fatigue at ~60% VO_2 peak	1) No glucose (NG) 2) Oral glucose (G) at 30 min of exercise, administered every 15min. (total ingested was 1.23±0.11 g CHO/kg body weight)	• Time to fatigue increased in G • MVC reduced in G and NG at 90 min and fatigue, but the reduction was attenuated in G at fatigue • M-wave amplitude and area were higher in G at 90 min and fatigue.

Nybo, 2003	8 M, endurance-trained, VO_2max = 66 ± 2 ml/kg/min 180 min cycling at 60% VO_2max	1) PLA 2) CHO (6% glucose polymer) Ingestion every 15 min; 200±10g CHO and 3.3±0.2 L water was consumed	• MVC drop in PLA was higher than in CHO • CAR (VA) reduction were more pronounced in PLA
Gibson *et al.,* 2001	7 M, endurance trained cyclists, VO_2max = 63.9 ± 4.7 ml/kg/min Two 100-km TT	72 hours of 6 g/kg body weight CHO, followed by: 1) CHO (3 g/kg body weight CHO) 2) PLA (1200ml of low calorie drink)	• MVC and IEMG dropped in CHO and PLA • Performance was similar in CHO and PLA
Ganio *et al.,* 2010	14 M, trained cyclists, VO_2max = 60.4 ± 6.8 ml/kg/min 135 min cycling, alternating between 60 and 75% VO_2max every 15 min for 120 min followed by 15 min performance ride	3 conditions: 1) PLA 2) CHO (~120g)-electrolyte plus caffeine, carnitine, taurine, and B vitamins solution (CE+) 3) CHO (~120g)-electrolyte only solution (CE)	• CE did not reported any beneficial effects compared to PLA
Jeffers *et al.,* 2015	9 M, Cyclists, VO_2max = 61.5 ± 5 ml/kg/min 45 min cycling at 70% maximum power output (preload) followed by a 15-min TT.	2 Conditions: 1) 6.4% maltodextrin mouth-rinse (CHO) 2) Water (PLA) Mouth rinse before and every subsequent 7.5 min during 45 min preload; before and halfway through the 15 min TT.	• MVC reduction were more pronounced in PLA after TT • Mmax reduction was more pronounced in PLA compared to CHO • VA and TT performance similar in both conditions

2.3 Carbohydrate and fat oxidation during endurance exercise

As endogenous muscle glycogen is a primary fuel source during exercise, glycogen depletion is believed to induce fatigue during prolonged submaximal (< 85% VO_2max) endurance exercise (Bergström, Hermansen, Hultman, & Saltin, 1967; Coyle, Coggan, Hemmert, & Ivy, 1986; Ørtenblad, Westerblad, & Nielsen, 2013). In the classical study by Bergstrom *et al.* (1967), 9 subjects exhausted their glycogen store by hard exercise, and were then fed either a fat with protein diet (P), a CHO-rich diet (C), and a mixed diet (M) before they began the exercise trial on a cycle ergometer at a workload of 75% VO_2max until fatigue. The average time to fatigue was 59 minutes for diet P, 189 minutes for diet C, and 126 minutes for diet M, concluding that initial glycogen and its rate of utilisation content in working muscle is a determinant for long-term exercise capacity; hence maintaining sufficient muscle glycogen level during endurance exercise is needed to prevent fatigue. In response to these findings, nutritional strategy to boost endurance performance introduced by the International Olympic Committee had focused on increasing CHO availability by increasing CHO store in muscle and liver prior to exercise (CHO loading) and consuming CHO during exercise (Burke, 2003). Some findings, however, argued that consuming CHO immediately before and during exercise increased rate of whole body CHO oxidation leading to a faster glycogen depletion (Coyle *et al*., 1986).

Another way to conserve muscle glycogen is by increasing fat oxidation. A higher reliance on fat oxidation during endurance exercise has been observed to reduce glycogen oxidation (Burke *et al*., 2000; Lambert *et al*., 2001; Yeo *et al*., 2008). Yeo *et al*. (2008) had 8 endurance trained subjects undertook 2 separate 7-day trial where they were either fed a 6-day high-CHO (HCHO) diet, or 5-day high-fat diet followed by 1-day of HCHO restoration (fat-adapt). On day-7, subjects performed 1-hour cycling at an intensity of 70% VO_2max with muscle biopsies taken immediately before and after exercise. Results

showed that in the fat-adapt group, respiratory exchange rate (RER) and muscle glycogen utilisation were significantly lower during exercise, and exercised induced rise in AMP-activated protein kinase activities were attenuated compared to the HCHO group; these data concluded that increased rate of whole body fat oxidation spared muscle glycogen. Despite results proving that increased fat oxidation decreased muscle glycogen utilisation, the benefit of these adaptations on endurance performance were unclear, with some studies suggesting improved performance (Lambert *et al*., 2001) while some studies suggested no significant change (Burke *et al*., 2000; Phinney, Bistrian, Evans, Gervino, & Blackburn, 1983) with improved fat oxidation and reduced glycogen utilisation.

A more recent study (Ørtenblad *et al*., 2013) has further investigated the location in which glycogen depletion caused fatigue using electron microscopy to reveal that glycogen is localised in distinct pools instead of distributed evenly in muscle fibres, and the depletion of these localised glycogen within myofibrils that are in close contact with key proteins involved in calcium ions release and excitation-contraction coupling that affect muscle contractility and induced fatigue.

The notion of CHO being the dominant fuel and limiting factor during endurance exercise is consistent with the findings by O'Brien, Viguie, Mazzeo, & Brooks (1993) which stated that while all classes of energy substrate participated in marathon running, CHO, not lipid, is the primary fuel.

2.3.1 Metabolic pathways during endurance exercise

Endurance exercises are type of activity that typically involve large muscle mass (eg. running, cycling, swimming), of at least 50% VO_2max intensity and 20 minutes in duration (Pollock *et al*., 1998). The main metabolic pathway that is involved in producing ATP during an endurance event is the aerobic pathway, as anaerobic pathways cannot

support duration of muscle contraction for more than 2-3 minutes (Donhoffer & Chan, 2007). The aerobic pathway mainly uses fat and CHO as fuel source.

Energy substrate utilisation is mainly determined by the intensity and duration of exercise, with absolute rate of lipid oxidation increases from low to moderate intensities, and then decline as exercise becomes more intense, where rate of CHO oxidation increases (Achten, Gleeson, & Jeukendrup, 2002), particularly rate of glycogen and glucose oxidation (Vaughar & Kravitz, 2012), as indicated by increase in RER (Sahlin & Harris, 2008). Studies found that in endurance trained subjects, the maximal rate of fat oxidation is reached at exercise intensity of around 65% of VO_2max (Achten *et al*., 2002; Achten, Venables, & Jeukendrup, 2003; Jeukendrup, 2003). It was also found that among the exercise intensity of 25, 65 and 85% of VO_2max in 5 trained individuals, lipid oxidation increases from 25% exercise intensity towards 65%, and reduced as exercise intensity reached 85% (Romijn *et al*., 1993).

Glycogen has been identified as an important factor to maintain energy metabolism as glycogen depletion in the exercising muscle are associated with fatigue during very long duration (more than 2 hours) exercise (Bergström *et al*., 1967), and during work to exhaustion (Constantin-Teodosiu, Cederblad, & Hultman, 1992). In prolonged endurance exercise lasting three to four hours however, blood glucose plays a bigger role since glycogen storage in liver and muscle may be depleted by then, causing fatigue despite availability of lipid as fuel source; at this time blood glucose obtained from exogenous source could be the sole CHO source for energy metabolism (Coggan & Coyle, 1991).

The two modes commonly used as endurance exercise are running and cycling. A study found that in 12 trained male subjects, the intensity which elicits the highest lipid oxidation between treadmill test (59.2 ± 2.8% VO_2max) and cycle ergometer test (62.1 ± 3.1% VO_2max) is not significantly different, though rate of lipid oxidation were

significantly higher during treadmill test during the intensities from 55 to 80% of VO_2max (Achten *et al*., 2003). Another study, however, found that in 19 endurance trained male, there was a significantly higher normalised lipid oxidation in running compared to cycling (Knechtle *et al*., 2004).

The high rate of lipid oxidation during low to moderate intensities exercise decreases the CHO oxidation due to reduced glucose transporter, the GLUT-4, and changes in glycolytic enzymes including reduced hexokinase, reduced glycogen phosphorylase, reduced phosphofructokinase, and reduced pyruvate dehydrogenase (Spriet & Watt, 2003). The increase in rate of lipid oxidation as exercise intensity rises from low to moderate is mainly due to increased availability of FFA and increased rate of transport of FFA from adipose tissue to exercising muscle, which is a result of increased lipolysis and reduced rate of reesterification of FFA (Achten *et al*., 2002). Increased secretion of hormones such as catecholamine and adrenocorticotropic hormone (ACTH) during exercise also activate hormone sensitive lipase to promote of hydrolysation of TG into FFA in adipose tissue (Kraemer & Shen, 2002).

In short, lipid is always preferred at the onset of exercise, can be maintained as main fuel source at 25% VO_2max for 3 hours, while at 65% VO_2max, lipid oxidation starts to decline after 2 hours of exercise, and at 85% VO_2max, lipid oxidation is not only smaller, but also declines much earlier, from 30 minute onwards. When lipid oxidation declines, CHO oxidation increased and became more and more dominant.

2.3.2 Effects of pre-exercise meals on energy metabolism

As glycogen depletion is seen as factor leading to fatigue, nutritional techniques has been focusing on increasing pre-exercise glycogen concentration or increasing fat oxidation to spare muscle glycogen stores to improve endurance performance (Chryssanthopoulos, Williams, Nowitz, Kotsiopoulou, & Vleck, 2002; Murakami *et al*., 2012).

A study (Chryssanthopoulos *et al*., 2002) consisted of 10 male subjects who performed 3 treadmill runs at 70% VO_2max to exhaustion after they were fed, on different sessions, respectively: 1) CHO meal 3 hours prior to testing and given CHO solution during the test, 2) CHO meal 3 hours prior to testing and given water during the test, and 3) liquid placebo 3 hours prior to testing and given water during the test. Results showed that exercise time was longer in both sessions fed with CHO (125.1 ± 5.3 minutes in session 1; 111.9 ± 5.6 minutes in session 2) prior to exercise compared to the placebo group (102.9 ± 7.9 minutes). It should be noted, however, that feeding the control group with placebo liquid while the other 2 had meals might have affected the control group's motivation as a meal would be perceived as better energy provider. In fact, the placebo liquid contains no energy, which would have not provided any fuel for exercise. While this shows the importance of having CHO prior to an exercise. Furthermore, a study (Bussau, Fairchild, Rao, Steele, & Fournier, 2002) found that in 8 endurance trained athletes who were fed 10 g/day/kg body weight of high CHO over 3 days, their muscle glycogen content raised significantly after only 1 day in type I, IIa, and IIb muscle fibres and remained stable in the following 2 days, indicating that 1 day of high CHO feeding is enough to attain maximal muscle glycogen storage. In fact, even a meal intake prior to exercise is enough to improve exercise performance; 5 g/kg body weight of CHO intake 3-4 hours prior to exercise improved performance with a concurrent increase in CHO oxidation (Wright, Sherman, & Dernbach, 1991), while consumption of 1.0 - 2.2 g/kg body weight of CHO 1-hour or less prior to exercise has been observed to improve performance as well (DeMarco, Sucher, Cisar, & Butterfield, 1999; Sherman, Peden, & Wright, 1991; Thomas, Brotherhood, & Brand, 1991). It should be noted, however, that a review has shown that feeding of 1 g/kg body weight of CHO 1-hour or less prior to exercise produced a mixed results in exercise performance, while study that prescribed 1.5 g/kg body weight of CHO saw improvement (Donaldson, Perry, & Rose, 2010). This

could be attributed to how 1 g/kg body weight CHO intake is close to the daily recommended dietary allowance (RDA) of 0.8 g/kg body weight which was not designed as a pre-exercise prescription. Hence, when prescribing meals an hour before exercise, 1.5 g/kg body weight of CHO should be considered (DeMarco *et al*., 1999).

As CHO store in the body can reach a plateau and at this state the muscle cannot store more glycogen, any intake of extra CHO may lead to increased blood glucose level, stimulating an increase in insulin which will inhibit FFA mobilisation and oxidation, increased glycogenolysis, and increased muscle glucose oxidation (Murakami *et al*., 2012; Wee, Williams, Tsintzas, & Boobis, 2005). Some studies attempted to investigate the effects of high fat feeding to maximise benefits of exogenous nutrient intake.

Murakami *et al.* (2012) investigated the effects of pre-exercise high CHO and high fat meals on endurance performance. This study consisted of 8 male collegiate long-distance athletes who were fed high CHO (glycogen) meal for 3 days, before being randomly fed with a high CHO meal with placebo jelly, high fat meal with placebo jelly, or high fat meal with maltodextrin jelly 4 hours prior to the exercise test consisting of treadmill running exercise until exhaustion at lactate threshold (71.8 ± 1.7% VO_2max) speed. The high CHO meal group has an increased rate of glycolysis with very small effect on lactate production. The high fat meal groups showed that the meal induced a higher amount of lipid oxidation, an increased blood glucose, and an increased blood FFA level, compared to those who are fed a high CHO meal, and ultimately, a longer time to exhaustion in groups fed with high fat meal (100 ± 3.4 minutes in high fat meal + maltodextrin group; 92 ± 2.8 minutes in high fat meal + placebo group) compared to the group fed with high CHO meal (90 ± 1.7 minutes).

2.3.3 Glycaemic index of carbohydrate and its impact on fatigue

During the late 70s/early 80s, the tenet at the time was that simple CHO is more readily available for rapid absorption and induce a greater post-prandial plasma glucose and insulin response compared to complex CHO due to its structure that consists of only one or two sugar units (Crapo, Reaven, & Olefsky, 1977). However, there is also a wide range of plasma glucose and insulin response amongst simple and complex CHO food source. Cereals, which contains complex CHO, elicited similar glycaemic and insulin responses as glucose, a simple CHO, while fructose, another simple CHO, induced glycaemic and insulin raise that is only 20% of those induced by glucose, suggesting that categorising CHO into complex or simple CHOs are not enough to justify the post-prandial glycaemic and insulin effects of CHO (Crapo *et al*., 1977; Jenkins *et al*., 1981). Besides, various factors could cause dissimilarity in post-prandial effects of CHO, such as food form (eg. liquid, solid), dietary fibre in the food, and the influence of fat and protein in the food. The GI concept was first introduced by Jenkins *et al.* (1981) to quantify the degree of how quickly blood glucose rises after consumption of a food relative to a reference food (eg. white bread or glucose solution), by quantifying the amount (in gram) of available CHO (CHO minus fibre) in the food that raises the blood glucose level (Jenkins *et al*., 1981; Donaldson *et al*., 2010). Early GI researches mainly investigated its implication in health and medical science (especially in the nutritional management of type-2 diabetes), and as its effects towards glycaemic and insulin responses became clearer alongside a better understanding on how these responses affects exercise performance, the potential role of GI in optimising sports performance begun to be investigated during the 1990s (Thomas *et al*., 1991). Since then, various studies of CHO and GI has been conducted, where the effects of CHO and GI feeding prior to exercise (DeMarco *et al*., 1999; Febbraio & Stewart, 1996; Wee *et al*., 2005), during exercise (Earnest *et al*., 2004) on exercise performance was investigated with mixed results.

The GI value indicates how fast the food causes glycaemic reaction: the lower the GI value, the slower it will raise post-prandial blood glucose value, and vice versa (Donaldson *et al*., 2010). Low GI food produces a slower and more gradual glycaemic response by virtue of slower digestion and absorption of its CHO (Granfeldt, Wu, & Björck, 2005). High GI food on the other hand, produces a rapid spike in blood glucose after consumption (Burke, Collier, & Hargreaves, 1993).

The GI value of a single food is calculated by measuring the incremental area under the blood glucose response curve after ingesting the reference food (usually glucose solution or white bread) containing a certain amount (typically 50 g) of available CHO and a test food containing the same amount of CHO, the response to the test food is expressed in percentage of the mean response to the reference food (Wolever, Jenkins, Jenkins, & Josse, 1991). It is argued that food that are more palatable such as white bread is more suitable to be used as reference food as glucose solution maybe too concentrated and is not palatable too all (Sugiyama, Tang, Wakaki, & Koyama, 2003).

Meanwhile to calculate GI of a meal consisting of several foods, the following Equation 2.1 was derived (Wolever, Yang, Zeng, Atkinson, & Brand-Miller, 2006); where n was the number of CHO-containing foods in the meal, GIa = GI of the food a, gAvCHOa was grams of available carbohydrate in the food a, and GAvCHO was grams of available carbohydrate in the entire meal.

GI of meal = (Gia x gAvCHOa/GAvCHO) + (GIb x gAvCHOb/GAvCHO) +

(GIc x gAvCHOc/GAvCHO) + ……. *Equation 2.1*

Due to difference in glycaemic, lipolytic, and insulin reaction given by different type of CHO food, GI is used as guideline when prescribing CHO diet/supplementation during exercise (Chen *et al*., 2008; Wee *et al*., 2005). Studies had produced results that indicate low-GI CHO meal prior to exercise led to positive effects during subsequent endurance

exercise (DeMarco *et al*., 1999; Wee *et al*., 2005; Wu *et al.*, 2006) while high-GI meal prior to exercise led to negative or no beneficial effects during endurance exercise (DeMarco *et al*. 1999; Earnest *et al*., 2004; Febbraio *et al*., 2000). Wee *et al*. (2005) measured post-prandial plasma glucose response, plasma insulin response, and muscle glycogen concentration of 7 trained men on 2 separate occasions, once after they were fed low-GI CHO breakfast while the other time high-GI CHO breakfast 3-hour prior to exercise test (2.5 g/kg body weight, 30 minutes run at ~70% VO_2max). It was found that incremental areas under the 3-hour plasma glucose and serum insulin response curve were significantly greater in high-GI meal, with muscle glycogen concentration and utilisation greater in the high-GI as well; sparing of muscle glycogen observed in the low-GI is believed to be a result of better maintained fat oxidation (Wee *et al*., 2005). DeMarco *et al.* (1999) did a similar study measuring post-prandial glycaemic, insulin, and physiological response, and found that during 2 hours cycling (at 70% VO_2max), low-GI CHO (1.5 g/kg body weight) consumed 30 minutes prior to exercise led to significantly lower insulin rise and RER as well as higher plasma glucose concentration compared to consumption of high-GI CHO water or plain water. It is believed that these maintenances of plasma glucose coupled up with increased fat oxidation rate, which led to increased energy substrates availability and spared muscle glycogen, allowed an increased endurance exercise capacity (DeMarco *et al*., 1999; Wee *et al*., 2005; Wu & Williams, 2006), due to suppressed insulin response effect of low-GI CHO (Febbraio & Stewart, 1996; Wee *et al*., 1999).

Conversely, high-GI CHO led to increased insulin response, which promotes glucose and lipid absorption into cells away from working muscles, reducing energy substrate availability during endurance exercise (Febbraio & Stewart, 1996; Wee *et al.,* 1999). Febbraio *et al*. (2000) did a study on 8 trained man cycling for 120-minute on 70% VO_2max followed by 30-minute performance trial after ingesting high-GI, low-GI, (both

GI meals contain 1.5 g/kg body weight CHO) or placebo meal 30-minute before exercise, and found that the high-GI meal resulted in increased insulin response, increased muscle glycogen utilisation, reduced blood glucose, and reduced plasma FFA during exercise when compared to low-GI meal and placebo meal.

While metabolic effects of GI are well observed, its effects on endurance performance itself produced mixed results, as some studies reported improved time to exhaustion or time to completion when fed low-GI CHO (1-2 g/kg body weight CHO) (DeMarco *et al*., 1999; Thomas *et al*., 1991; Wu & Williams, 2006) while other studies reported no difference in time to exhaustion or time to completion when fed low or high GI CHO despite observing metabolic changes (Chen *et al*., 2008; Febbraio, Keenan, Angus, Campbell, & Garnham, 2000; Kirwan, Cyr-Campbell, Campbell, Scheiber, & Evans, 2001; Stannard, Thompson, & Miller, 2000; Wee *et al*., 1999). There are also studies that only measure metabolic changes induced by CHO with differing GI but did not directly observe endurance performance (Wee *et al*., 2005), making it difficult to make a consensus regarding effects of different GI on endurance performance. Chen *et al*. (2008) found that high-CHO, low-GI meal ingested 2-hour before exercise test (1-hour run at 70% VO_2max followed by 10-km performance run) induced higher post-prandial glycerol and FFA concentration during exercise compared to both high-CHO, high-GI meal and low-CHO, low-GI meal, though no difference in time to completion in the 10-km run was observed. Due to the relatively short distance (10-km vs. 42-km of a full marathon) of the test, however, a longer test distance may do more justice to the effects of GI (Chen *et al*., 2008). It is worth noting that most GI studies on exercise performances prescribed 70% VO_2max of intensity regardless of exercise mode. This could be due to the fact that as GI studies typically prescribe iso-CHO meals with amount of CHO larger than that of RDA (at least 1 g/kg body weight), an exercise intensity that is able to exert CHO as significant

fuel source while allowing lipid oxidation to be relevant, at least in the early part of exercise, is needed.

While the previously mentioned studies investigated effects of CHO with differing GI consumed before exercise, Earnest *et al*. (2004) investigated the effects during exercise. 9 subjects completed three 64-km time trial cycling tests, where riders ingested either low-GI CHO (honey; GI = 35), high-GI CHO (dextrose; GI = 100), or placebo drinks every 16-km. Both the CHO condition led to faster time to completion and higher wattage produced during the last 16-km though no significant difference in time to completion and higher wattage produced during the last 16-km was found between both CHO condition. This study concluded that CHO feeding during exercise regardless of GI improved time to completion and increased power output when compared to placebo condition. This study did not measure metabolic and physiological changes, which could provide more insight in changes induced by different GI.

The interest of how CHO with differing GI producing different metabolic adaptations are not only towards its implication on improving endurance performance, but also on how it may improve recovery from endurance exercise (Burke *et al*., 1993; Stevenson, Williams, McComb, & Oram, 2005). High-GI meal consumption after endurance exercise led to more rapid recovery in muscle glycogen compared to low-GI meal, owing to its capacity to induce high glycaemic and insulin responses (Burke *et al*., 1993), though this may not reflect the changes in endurance exercise performance capacity in subsequent exercise (Stevenson *et al*., 2005).

In the study by Burke *et al.* (1993), 5 trained cyclists performed glycogen depleting endurance exercise (~2-hour cycling at 75% VO_2max followed by four 30-second sprints) and were then fed either a high-GI meal or low GI-meal, on two separate occasions. Blood samples were collected before exercise, immediately after exercise, immediately before

meal, and 30, 60, 90 minutes after meal, while muscle biopsies were collected immediately after exercise and 24-hour after exercise. The high-GI meal produced a greater incremental of glucose and insulin area under the curve compared to low-GI meal after 24 hours, suggesting a more rapid increase in muscle glycogen.

Stevenson *et al.* (2005) questioned the relation between muscle glycogen content after a 24-hour recovery with subsequent endurance performance, and investigated the effects of CHO with differing GI on endurance capacity and metabolic responses during exercise on following day. In their study, 9 males depleted their muscle glycogen content on day 1 by running for 90-minute at 70% VO_2max, and were then fed either low-GI meal or high-GI meal. After an overnight fast, subjects ran to exhaustion at 70% VO_2max on the next day. The results showed that time to exhaustion was longer in the low-GI condition than the high-GI, with fat oxidation rate and plasma FFA concentration higher in low-GI condition than the high-GI, suggesting that low-GI recovery meal led to increased lipid oxidation and directly improved endurance exercise performance. This may also indicate that energy substrate availability (in this case, in the form of lipid) is a more important factor compared to higher muscle glycogen availability alone in improving endurance performance.

In conclusion, the effects of high and low GI meal on metabolic changes during and after endurance exercise are well documented, however, its effect on endurance performance is inconsistent. Moreover, there is no study that employed neurophysiology techniques to measure CF following consumption of CHO with high and low GI.

CHAPTER 3 BLOOD GLUCOSE RESPONSES FOLLOWING CONSUMPTION OF CUSTOM DESIGNED MEALS WITH DIFFERENT QUANTITY AND GLYCAEMIC INDEX OF CARBOHYDRATES

3.1 Introduction

The amount of carbohydrate (CHO) and glycaemic index (GI) within a meal has been shown to affect post-prandial blood glucose (Wolever & Bolognesi, 1996b). The insulin response is directly related to changes in blood glucose, with higher blood glucose triggering greater insulin release (Liu *et al.*, 2012; Wolever *et al.*, 1991). Typically, a higher CHO quantity in a meal lead to greater insulin response; and when CHO amount is the same, a higher GI produce higher response compared to a low GI CHO. In context of providing high CHO as pre-exercise meals, its objective is to maximise muscle glycogen storage, which in turn would provide sufficient fuel for endurance exercises, and maximising time to fatigue hence improving endurance performance. Both high CHO (DeMarco *et al*., 1999) and low CHO (Murakami *et al*., 2012) meals has been indicated to improve performances, hence to study effects of pre-exercise meals on exercise, both types of meals should be tested.

Besides differences with high and low quantity of CHO, the blood glucose reaction after consuming any custom designed iso-CHO test meals with different GI should also be tested prior to experiments. When designing meals, GI of food are usually based on published GI tables. However, plasma glucose and insulin responses to low and high GI meals prescribed using foods listed in the published GI tables may be different from expected. While some studies found that the GI of food can be used to predict the relative glycaemic effect of meals mixed with several types of food (Brand-Miller *et al*., 2003; Wolever *et al*., 2006); there are also findings that suggested no association between the

meal GI calculated solely based on the GI table (Alfenas & Mattes, 2005; Flint *et al*., 2004). In addition, GI of foods can also possibly be affected by protein and fat contents of meal (Wolever *et al*., 2006). Hence when designing a meal with low and high GI, protein and fat contents should be controlled, and GI of meals should be calculated and its post-prandial blood glucose reaction should be tested concurrently.

Objective

Present study aims to test the blood glucose responses following the test meals that would be used in the following studies in this thesis. These include ensuring significantly different responses in plasma glucose reactions produced with: 1) isocaloric meals with high and low amount of CHO, and 2) isocaloric meals with same amount of CHO but with high and low GI produce.

Hypothesis

1. Meal with higher amount of CHO will induce a higher blood glucose reaction compared to one with low amount of CHO.
2. Meal with high GI will induce a higher blood glucose reaction compared to low GI meal.

3.2 Literature review

When consumed, CHO is broken down into glucose through various process starting from the mouth up to the intestines, which is then absorbed into the blood stream. The increased blood glucose causes the pancreas to release insulin, which activates GLUT-4 receptors which transfer glucose into liver or muscle cells to be stored as glycogen (Thorell *et al*., 1999). In addition, insulin also functions as a lipolysis inhibitor, by stimulating uptake of fatty acids into adipose tissue to be stored as triglyceride (Chakrabarti, English, Shi, Smas, & Kandror, 2010).

Following meal consumption, blood glucose concentration is shown to peak 30-minute post-prandial, concurrent with the changes in insulin (Liu *et al*., 2012; Murakami *et al*., 2012; Shin, Park, & Choue, 2009). Furthermore, the rate of the rise in blood glucose differs with the amount of CHO ingested. High CHO meals caused a significantly higher blood glucose at 30 to 240 minutes compared to low CHO meal (Shin *et al*., 2009). Mean glucose area under the curve (AUC) was also larger with a high CHO meal compared to low CHO meal (Liu *et al*., 2012). In both situation, the insulin increased parallel with blood glucose (Liu *et al*., 2012, Shin *et al*., 2009). These results indicate that it is safe to assume that blood insulin rise occurs concurrently with blood glucose increase.

When prescribing meals with similar amount of CHO, blood glucose responses could be different between meals due to differences in the GI. GI is defined as the incremental area under the blood glucose response curve of a 50 g CHO portion of a test food expressed as percent of the response to the same amount of CHO from a standard food (usually glucose) taken by the same person (Jenkins *et al*., 1981). A high GI meal typically produce a higher glucose AUC compared to low GI meals (Liu *et al*., 2012; Wu *et al*., 2003). When taken as pre-exercise meals, a low GI meal has been shown to increase fat oxidation, while high GI increases CHO oxidation during the exercise (DeMarco *et al*.,

1999; Wee *et al*., 1999; Wee *et al*., 2005; Wu *et al*., 2003; Wu *et al*., 2006). The HGI meal also suppresses fat oxidation during exercise (Wu, Nicholas, Williams, Took, & Hardy, 2003), and this could be attributed to the possible lipolysis inhibition, as a higher glucose reaction would have led to a higher insulin release. The extent of post-prandial glucose response results mainly from the effect of GI and quantity of available CHO contained in the meals, which is why when calculating GI of a meal, the glycaemic load (GL, amount of CHO multiply by GI of a food) is also important determinant of glycaemic responses (Wolever & Bolognesi, 1996a). It was found that gradual increase in GL produced a predictable gradual increase in glycemic response, concluding that it would be more accurate to take the CHO amount into account alongside GI in estimation of glycemic response of the meal (Brand-Miller *et al*., 2003). Hence it cannot be advised to prescribe meals based on GI tables alone, as GL of meals has profound impact on postprandial metabolic responses (Brand-Miller *et al.*, 2003), and any custom designed meals should have its glucose response tested.

3.3 Methods

3.3.1 Participants

Ten physically active individuals (8 males, 2 females) aged between 20 to 25 years old were recruited, and all of them provided informed consent (Appendix D). This study was conducted with approval of University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC – 41) (Appendix C). Participants filled in the ACSM Risk Stratification Questionnaire, and only those who are free from metabolic disease are included in this study. Those with non-normal body mass index (BMI) (outside of 18.5 to 25.0) were excluded.

3.3.2 Study design

The present study employed a double blind, randomised crossover design with 7-day washout-period. The prescription meals were prepared by a lab technician independent of this study, with the order of meals prescribed being randomly arranged. Only at the end of the whole study that the order of meals given to each participant were informed to the researcher. Participants were prescribed one of either isocaloric: high CHO meal (HCHO, 1.5 g/kg body weight), low CHO meal (LCHO, 0.8 g/kg body weight); isocaloric, iso-CHO: high GI meal (HGI, GI ~75), or low GI meal (LGI, GI ~40). Participants were asked to refrain from consuming caffeine, alcohol, and participating in any vigorous physical activity 24 hr prior to the experimental days.

3.3.3 Experimental procedure

On experimental days, participants arrived at the lab after an overnight fast and their anthropometry information was collected. The fasting blood samples were obtained (details in Chapter 3.3.6), and the meal was served after that. Participants are required to finish consuming all the foods within 20 minutes. Post-prandial blood samples were

obtained at 15, 30, 45, and 60th minute after consumption of food. Participants were seated with minimal physical activity during the post-prandial period.

3.3.4 Development of experimental meals

Two sets of meals were developed, the first set is isocaloric meals with high amount of CHO (HCHO, 1.5 g CHO per kg body weight) or low amount of CHO (LCHO, 0.8 g CHO per kg body weight); while the second set is isocaloric, iso-macronutrient with 1.5 g CHO per kg body weight meals, distinguished by having high meal GI (HGI, GI ~80) or low meal GI (LGI, GI ~40).

All food was obtained from the same source, brand, and type of food in all experiment trials to assure that the foods' quality is controlled. All food was registered under the Food Act 1983 and Food Regulations 1985 of Malaysia. Nutrient information of all food items was retrieved from the nutrition label on the packaging, in and were keyed into and analysed by the NutriPro software (Axxya Systems). GI of meals were calculated using Equation 3.1 (Wolever *et al*., 2006); where n was the number of CHO-containing foods in the meal, GIa = GI of the food a, gAvCHOa was grams of available carbohydrate in the food a, and GAvCHO was grams of available carbohydrate in the entire meal.

$$GI = (GIa \times gAvCHOa/GAvCHO) + (GIb \times gAvCHOb/GAvCHO) \ldots \textit{Equation 3.1}$$

GI of each food was obtained from either nutrient label, or from the International Tables of Glycemic Index and Glycemic Load Values: 2008 (Atkinson, Foster-Powell, & Brand-Miller, 2008). The food items and nutrient information of HCHO and LCHO meals are presented in Table 3.1, while information for HGI and LGI meals are presented in Table 3.2. Calculations of HGI and LGI meals' GI are presented in Tables 3.3 and 3.4, respectively.

Table 3.1. Nutrient information of high carbohydrate (HCHO) and low carbohydrate (LCHO) test meals for a 70-kg participant. Values are mean ± SD (n = 10).

	HCHO	**LCHO**
Food	123 g bread, 30 g fruit jam, 138 g apple, 5 g butter, 70 g hardboiled egg	100 g bread, 25 g butter, 120 g hardboiled egg,
Energy (kcal)	619.6	624.8
% of calories from CHO	71.1	37.8*
% of calories from fat	23.2	56.7
% of calories from protein	5.6	5.6
Amount of CHO (g)	105	56.2*
Amount of fat (g)	15.5	38.1*
Amount of protein (g)	8.2	8.2

Note: *denotes significant difference ($p < 0.05$) compared to HCHO. HCHO = high carbohydrate, LCHO = low carbohydrate, CHO = carbohydrate.

Table 3.2. Nutrient information of high glycaemic index (HGI) and low glycaemic index (LGI) test meals for a 70-kg participant. Values are mean ± SD (n = 10).

	HGI Meal	**LGI Meal**
Food	20 g Jam, 250 ml skimmed milk, 40 g white bread, 50 g corn flakes,	160 g apple, 150 ml apple juice, 45 g bran flakes, 300 ml skimmed milk
GI	74.2	38.1
Energy (kcal)	619.26	622.89
% of calories from CHO	71.1	70.8
% of calories from fat	23.4	23.8
% of calories from protein	5.3	5.5
Amount of CHO (g)	106.5	105.4
Amount of fat (g)	15.6	15.9
Amount of protein (g)	7.9	8.1

Note: GI = glycaemic index, CHO = carbohydrate. HCHO = high carbohydrate, LCHO = low carbohydrate

Table 3.3. GI calculation of HGI meal for a 70-kg participant.

Food	**gAvCHO**	**GI**	**Glycemic Load**
Jam (20 g)	15.0	65	9.8
Skimmed Milk (250 ml)	16.1	32	5.2
White bread (40)	19.8	95	18.8
Corn Flakes (50 g)	42.3	84	35.5
Gatorade (200 ml)	13.3	75	10.0
		Total GL	79.0
GAvCHO	106.5	**GI of meal (total GL / Total CHO in meal)**	74.2

Note: GI = Glycaemic index of the food normalised to glucose, gAvCHO = grams of available carbohydrate in the food, GAvCHO = grams of available carbohydrate in the entire meal, GL = glycaemic load (AvCHO x GI), and GI of meal = Total GL / GAvCHO.

Table 3.4. GI calculation of LGI meal for a 70-kg participant.

Food	**gAvCHO**	**GI**	**Glycemic Load**
Apple Juice	28.5	45	12.8
Apple	21.0	28	5.9
Bran Flakes	35.6	42	15.0
Skimmed Milk	20.3	32	6.5
		Total GL	40.2
GAvCHO	105.4	**GI of meal (total GL / Total CHO in meal)**	38.1

Note: GI = GI of the food normalised to glucose, gAvCHO = grams of available carbohydrate in the food, GAvCHO = grams of available carbohydrate in the entire meal, GL = glycaemic load (AvCHO x GI), and GI of meal = Total GL / GAvCHO.

3.3.5 Blood glucose measurement

Blood glucose were obtained using the finger prick technique on the peripheral capillaries. Prick site were cleaned with alcohol swap and allowed to dry before being punctured using a lancet. Blood samples were collected using a capillary tube, before being transferred onto a blood glucose analysis strip (Reflotron, USA). The strip is analysed using the single test clinical chemistry system (Reflotron Plus, USA). It has a coefficient of variation (CV) of 0.25.

3.3.6 AUC calculation

The GI of the meals consumed over 60 minutes was calculated from the area under the plasma glucose response curve above the fasting plasma glucose concentration, ignoring any area beneath fasting values (Wolever *et al*., 1986; Wolever, 2004). The integrated area under the post-prandial glucose curve was calculated using the trapezoidal method (Wolever & Jenkins, 1986) and was used to assess the plasma glucose responses for the whole period of observation.

3.3.7 Statistical analysis

Statistical analysis was done using SPSS 20.0 (IBM Corp. Armonk, NYN). Normality was confirmed using the Shapiro-Wilks test. Paired t-test was used to determine the difference between means of two groups, between i) HCHO and LCHO, ii) HGI and LGI meals. Statistical significant were set and accepted at $p < 0.05$. Data are presented as mean values and standard deviation (mean ± SD).

3.4 Results

3.4.1 Participants' anthropometrics

The participants' anthropometrics data are presented in Table 3.5. The mean BMI of the participants were within the normal range (18.5 – 25.0 kg/m^2).

Table 3.5 Anthropometric measures of participants (n = 10).

Variables	Value
Age (year)	22.8 ± 1.3
Weight (kg)	61.3 ± 12.9
Height (cm)	167.30 ± 9.2
Lean Muscle Mass (kg)	40.7 ± 8.8
Body Fat Percentage (%)	14.4 ± 6.2
BMI (kg/m^2)	21.8 ± 3.2

Note: values are mean ± SD. BMI; body mass index.

3.4.2 Comparison between HCHO and LCHO meals

3.4.2.1 Nutrients consumed

The mean nutrient composition of the actual meals consumed by participants during the testing sessions are presented in Table 3.6. When comparing between HCHO and LCHO meals, amount of CHO consumed was significantly higher in HCHO, while amount of fat consumed was significantly higher in LCHO.

Table 3.6. Macronutrients, energy intakes, and percentage (%) of energy from macronutrients of HCHO and LCHO test meals consumed by participants. Values are mean ± SD (n = 10), and *denotes significant difference compared to HCHO.

	HCHO	**LCHO**
Energy (kcal)	584.5 ± 94.6	583.0 ± 94.4
Amount of CHO (g)	95.7 ± 15.5	51.9 ± 8.4*
Amount of fat (g)	15.9 ± 2.6	35.5 ± 5.8*
Amount of protein (g)	8.2 ± 1.3	8.2 ± 1.3

Note: CHO; carbohydrate, HCHO; high carbohydrate, and LCHO; low carbohydrate.

3.4.2.2 Post-prandial blood glucose

3.4.2.2.1 Post-prandial blood glucose kinetics for HCHO and LCHO meals

Fasting blood glucose were all in the reference range and similar throughout all sessions. Post-prandial blood glucose responses were higher in the HCHO compared to LCHO from 30-minute onward (Figure 3.1).

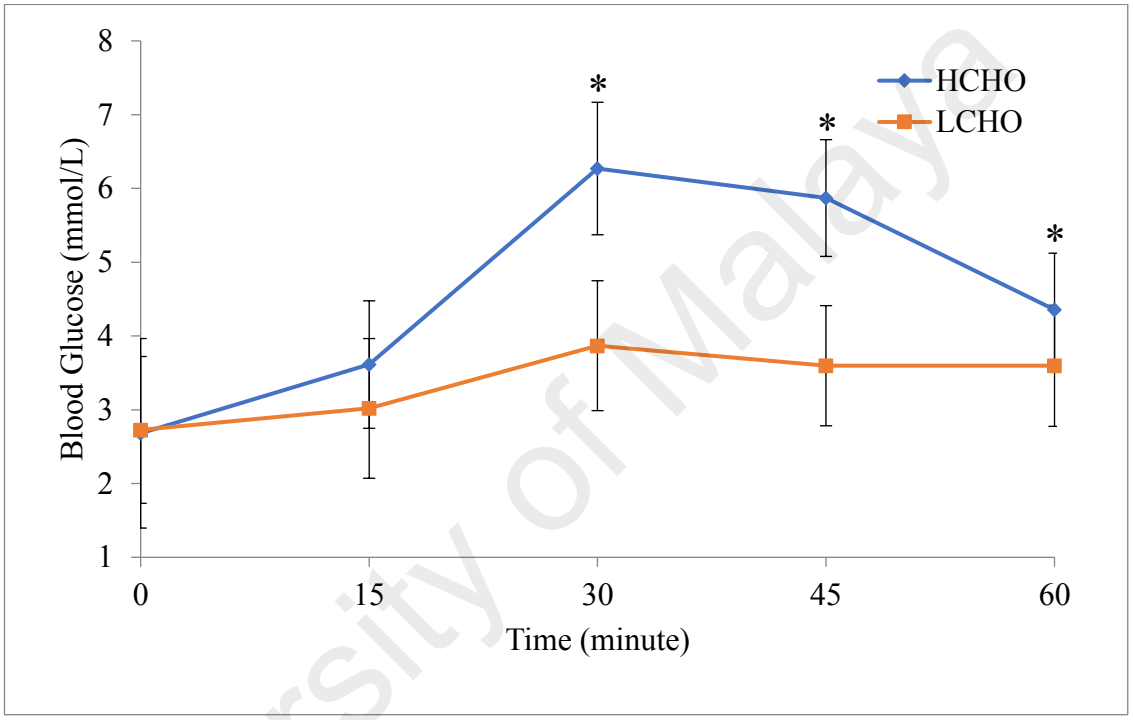

Figure 3.1 Post-prandial Blood glucose reaction for one hour following high carbohydrate (HCHO) and low carbohydrate (LCHO) meals consumption. Values are mean ± SD (n = 10). *denotes significant difference between groups.

3.4.4.2.2 Post-prandial blood glucose AUC for HCHO and LCHO meals

Post-prandial blood glucose AUC were higher in the HCHO (19.27 ± 3.28 mmol/L) compared to LCHO (13.65 ±3.43 mmol/L) (Figure 3.2).

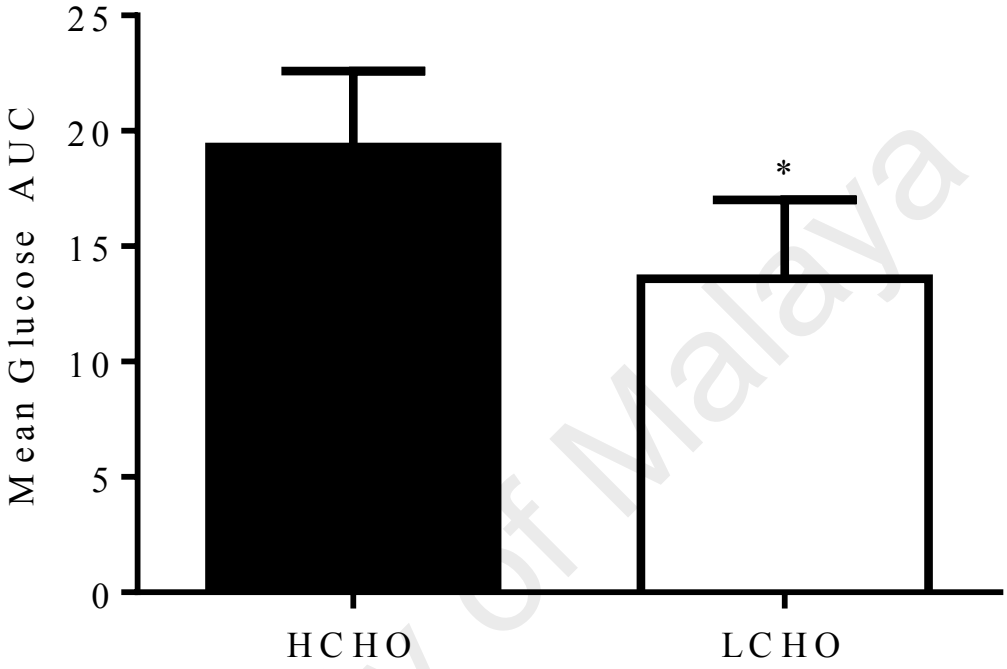

Figure 3.2 Post-prandial blood glucose area under curve (AUC) for one hour following high carbohydrate (HCHO) and low carbohydrate (LCHO) meals consumption. Values are mean ± SD (n = 10). *denotes significant difference compared to HCHO.

3.4.3 Comparison between HGI and LGI meals

3.4.3.1 Nutrients consumed

The mean nutrient composition of the actual meals consumed by participants during the testing sessions are presented in Table 3.7. There were no significant differences in CHO, fat, protein, and energy consumed between HGI and LGI meals. Only the GI values was different between meals.

Table 3.7 Macronutrients, energy intakes, and percentage (%) of energy from macronutrients of HGI and LGI test meals consumed by participants. Values are mean ± SD (n = 10).

	HGI Meal (GI ~ 75)	**LGI Meal (GI ~ 40)**
Energy (kcal)	547.8 ± 115.1	546.94± 115.0
Amount of CHO (g)	93.2 ± 19.6	92.3 ± 19.4
Amount of fat (g)	13.7 ± 2.9	13.9 ± 2.9
Amount of protein (g)	6.9 ± 1.5	7.1 ± 1.5

Note: CHO; carbohydrate, HGI; high glycaemic index, and LGI; low glycaemic index.

3.4.3.2 Post-prandial blood glucose for HGI and LGI meals

3.4.3.2.1 Post-prandial blood glucose kinetics for HGI and LGI meals

Fasting blood glucose were all in the reference range and similar throughout all sessions. Post-prandial blood glucose responses were higher in the HGI compared to LGI from 15-minute onward (Figure 3.3).

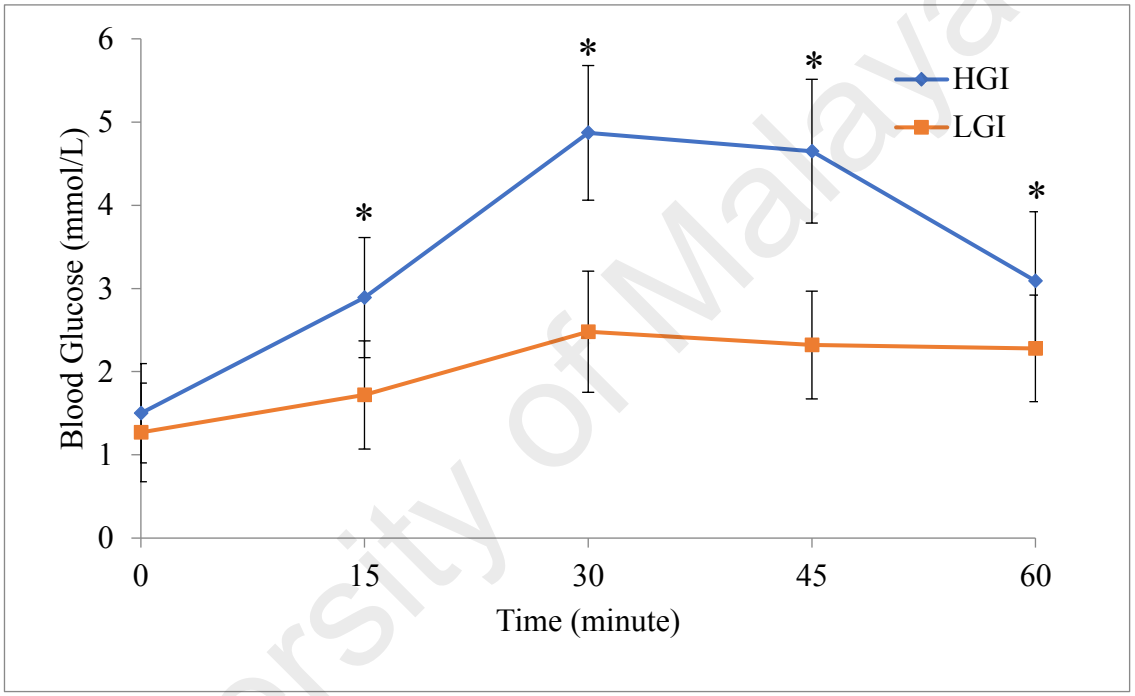

Figure 3.3 Post-prandial blood glucose reaction for one hour following high glycaemic index (HGI) and low glycaemic index (LGI) meals consumption.. Values are mean ± SD (n = 10). *denotes significant difference between groups.

3.4.3.2.2 Post-prandial blood glucose AUC for HGI and LGI meals

Post-prandial blood glucose AUC were higher in the HGI (21.25 ± 2.84 mmol/L) compared to LGI (17.90 ± 2.44 mmol/L) (Figure 3.4).

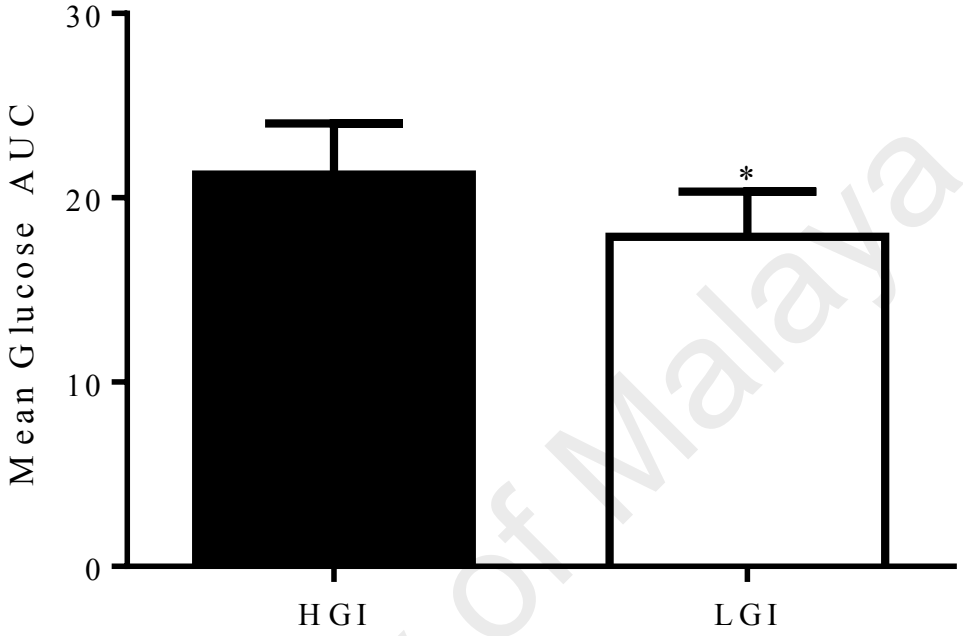

Figure 3.4. Post-prandial Blood glucose area under curve (AUC) one hour following high glycaemic index (HGI) and low glycaemic index (LGI) meals consumption. Values are mean ± SD (n = 10). *denotes significant difference compared to HGI.

3.5 Discussion

Present study compared the post-prandial blood glucose after consuming custom designed meals prepared using locally available foods. The first set of meals compared are composed of the same amount of protein but significantly different amount of CHO and fat content: HCHO meal has ~1.5 g/kg body weight of CHO, while LCHO meal has ~0.8 g/kg body weight CHO. Glucose reaction over time and AUC were greater in HCHO LCHO meal, which is in agreement with previous literatures (Shin *et al*., 2009). The higher CHO content in the HCHO meal would have led to a bigger amount of glucose broken down in the intestines, which is then absorbed into the blood stream, leading to a higher blood glucose response.

The second set of meals compared were the HGI (GI~74.2) and LGI (GI~38.1) meals, which composed of similar quantity of CHO, fat, and protein, and only differs in GI. The GI values of each meal were close to those prescribed in past studies that compare high (GI ~80) and low (~40) GI meals (Wee *et al*., 1999; Wee *et al*., 2005; Wu *et al*., 2003; Wu *et al*., 2006). Glucose reaction over time and AUC was greater in the HGI compared to LGI meal, conforming to past studies (Liu *et al*., 2012; Wee *et al*., 1999; Wee *et al*., 2005; Wu *et al*., 2003; Wu *et al*., 2006). The higher glucose reaction with HGI meal despite similar macronutrients content can be attributed to the meal's composition of high GI food such as white bread and corn flakes, which when compared to the bran flakes in LGI meal, are composed of mostly simple CHO that is fast to digest and absorbed into the blood stream. Bran flakes, on the other hand composed of mostly complex CHO such as starch which is slow and difficult to digest, producing less glucose to be absorbed.

HCHO meal was compared with LCHO so that these meals can be used in later study that investigate effects of meals with high and low amount of CHO on CF following exercise, while HGI and LGI meals was compared with each other so that it can be used to

investigate effects of iso-macronutrient meals with low and high GI on CF. While present study did not measure blood insulin level, studies have shown that insulin is consistently increased concurrently with blood glucose (Liu *et al*., 2012, Murakami *et al*., 2012, Shin *et al*., 2009), so it can be safely assumed that post-prandial concentration.

3.6 Conclusion

Present study showed that the custom designed HCHO and LCHO meals as well as HGI and LGI meals were able to induce the post-prandial blood glucose reaction that were similar to established findings, and the differences between meals in each set were significant.

CHAPTER 4 CENTRAL FATIGUE FOLLOWING ENDURANCE EXERCISE AND INFLUENCE OF CARBOHYDRATES

4.1 Introduction

Fatigue during exercise is a phenomenon where the body physiological system can no longer produce the desired effort. Fatigue is caused by several factors, with those due to processes within the motoneurons and central nervous system termed as central fatigue (CF) (Carroll, Taylor, & Gandevia, 2017). While Depletion of carbohydrates (CHO) storage has long been cited as the main factor causing fatigue during endurance exercises (Coyle *et al*., 1986; Ørtenblad *et al*., 2013), only few studies have investigated the effects of CHO on the central processes of fatigue using neurophysiological measures. Exercise-induced hypoglycaemia attenuates central nervous system (CNS) activation, while CHO supplementation during exercise seems to preserve it as reflected through changes in central activation ratio (CAR) (Nybo, 2003). Del Coso *et al*., (2008) later found similar results, where a 6% CHO-electrolyte solution attenuated the loss of maximal voluntary contraction (MVC) and voluntary activation (VA) compared to when the participants were fed only water. However, both studies prescribed CHO during mid-exercise, and to date little is known on its effect when provided as pre-exercise meal despite it being a strategy in improving endurance performance (Chryssanthopoulos *et al*., 2002, Sherman *et al*., 1991; Wright *et al*., 1991). CHO could suppress CF by providing metabolic fuel to the brain, or through other indirect way such as suppressing fatigue inducing neurotransmitter, serotonin. During endurance exercise, lipolysis increases free-fatty acid (FFA) which is hypothesised to displace tryptophan and increases its chances in crossing the blood-brain barrier and being converted to serotonin (Meeusen *et al.,* 2006).

Objective/Justification

Since benefits of CHO prescription before exercise on endurance performance and attenuation of CF with CHO supplementation during exercise have been reported, there is a need to investigate if the pre-exercise feeding affects CF as well. It is worth noting that CHO feeding generally affects insulin reaction which in turn alters FFA availability, influencing serotonin synthesis; therefore, the interactions between these biomarkers, the quantity of CHO fed, and CF warrant to be determined. Hence, the present study aims to investigate the effects of a pre-exercise meals with different CHO quantity on CF. Two meals: high CHO and low CHO were prescribed and CF was measured by quantifying the changes in muscle force generation and VA after a 90-minute endurance running at 65% VO_2max. This study's primary objective is to investigate the effects of high CHO and low CHO pre-exercise meals on CF using neurophysiological measurements, with investigation of changes in blood markers including serotonin as secondary objective.

Hypothesis

1. High CHO pre-exercise meal will preserve MVC better than LCHO pre-exercise meal.
2. The preservation of MVC will be due to preservation of CF markers i.e. CAR and VA.
3. The preservation of MVC and CF markers will be accompanied by a less pronounced rise in serotonin.

4.2 Literature review

4.2.1 CHO and CF

Nybo (2003) was the earliest to measure CF with superimposed twitches during MVC, and found that CHO supplement during an endurance exercise preserved the post-exercise MVC and VA compared to placebo. Del Coso *et al*., (2008) later found similar results, where a 6% CHO-electrolyte solution attenuated the loss of MVC and VA compared to when the participants were fed only water. However, both these studies prescribed CHO as mid-exercise supplementation, instead of as pre-exercise meal, and compared against placebo/water; and in Del Coso's studies, against other ergogenic aids i.e. caffeine. Hence the results gave a picture of how small quantity of CHO affects CF when prescribed during exercise; little is known about effects of a pre-exercise meal with typically recommended amount of CHO on MVC and CF indicator like VA.

The importance of pre-exercise meals has been recognised and its benefits were widely reported (Ormsbee, Bach, & Baur, 2014). Nutritional prescription before exercise focuses on providing enough energy for the upcoming activity. CHO rich meal prior to exercise typically increase blood glucose, and increased insulin level to promote uptake of CHO from food source into muscle to be stored as glycogen. A study found that CHO meal prior to exercise improves performance with or without CHO supplementation during exercise compared to when one is not given any pre-exercise meal, signifying importance of starting an exercise with sufficient CHO availability (Chryssanthopoulos *et al*., 2002). While this justifies the need to maximise glycogen storage, a contrasting finding shown that high level of insulin inhibits FFA oxidation, increased glycogenolysis, and increased glucose oxidation which subsequently led to shorter time to exhaustion (Murakami *et al*., 2012). This has been echoed as being counter-productive for endurance exercises (>1-hour duration), which are typically low to medium intensity hence metabolised high

amount of fat (fat oxidation reached its peak at 65% of VO_2max (Romijn *et al*., 1993), which in turn would spare muscle glycogen usage (Burke *et al*., 2000; Lambert *et al*., 2001). However, performance tests had returned mixed results. Feeding of CHO at 1 to 4 hours before exercise had been unanimously reported to increase insulin level (Chryssanthopoulos *et al*., 2002; Febbraio & Stewart, 1996; Hargreaves, Costill, Fink, King, & Fielding, 1987; Sherman *et al*., 1991; Whitley *et al*., 1998; Wright *et al*., 1991), but its effect on performance and substrate oxidation was mixed. Hargreaves *et al*. (1987) reported that CHO (1 g/kg body weight) feeding 45-minute prior to exercise did not led to performance change. When CHO was prescribed 1-hour before exercise, 1 g/kg body weight CHO led to an increment of insulin accompanied by reduction in blood FFA but not glycogen oxidation and performance (Febbraio *et al*., 1996), while 1.1 to 2.2 g/kg body weight CHO improved performance with increased CHO oxidation (Sherman *et al*., 1991). When CHO was prescribed 3-4 hours before exercise, a 0.7 to 3 g/kg body weight CHO caused hypoglycemia and lowered blood FFA as a result of insulin spike, but substrate oxidation and performance was not affected (Whitley *et al*., 1998); while 5 g/kg body weight CHO improved performance as a result of larger CHO oxidation (Wright *et al*., 1991). Both performance increment was accompanied by increased CHO oxidation, and a relatively larger amount of CHO compared to other studies with similar feeding time (Sherman *et al*. 1991, Wright *et al*., 1991). Possible role of CHO in influencing CF may be explained by the serotonin hypothesis (Meeusen *et al*., 2006). Serotonin is a vasopressin group of neurotransmitter, and causes the sense of fatigue and lethargy in the brain (Romanowski & Grabiec, 1974). During endurance exercise, raise in FFA could cause an increase of serotonin synthesis in the brain (Meeusen *et al*., 2006). Davis *et al*. (1992) demonstrated that time to exhaustion was inversely proportional with increased free-tryptophan and FFA levels. The time to exhaustion was increased with CHO feeding in a dose-related manner, suggesting that CHO feeding suppresses free-tryptophan level

and subsequently serotonin level in the brain. Since CHO feeding during exercise was found to reduce FFA and tryptophan increase (Davis, 1995) and pre-exercise feeding have been shown to significantly influence insulin and FFA availability, it is of interest to investigate if pre-exercise CHO in an amount large enough to affect substrate oxidation would affect serotonin level, and/or CF, which could have contributed to the performance improvement.

4.2.2 Measurement of CF

While time to exhaustion could indicate fatigue during endurance exercises, neuromuscular fatigue is typically observed through the drop in force generating capacity. Specifically, CF studies have been using neurophysiological techniques and measurements such as superimposed twitch (SIT), VA, CAR and input-output properties of CNS (i.e MEP, CMEP, SICI – refer Chapter 2.2.1) assessed together with force capacity (Nybo, 2003). These measurements can provide information on the contribution of the nervous system (including the sites and likely mechanisms involved) during neuromuscular fatigue. A reduction in VA indicates lowered neural drive that could originate from spinal and/or supraspinal sites. CAR is the MVC produced divided by MVC with extra force stimulated; when there is no extra force, CAR equals to 1 and any value less than 1 indicates a failure in the central activation (Kent-Braun *et al*., 1996). CAR and VA can be obtained during MVC, whether brief or prolonged. While VA can be optimum during brief MVC, its progressive decline alongside corresponding increment in force produced via stimulation has been observed during sustained contraction (Gandevia, Allen, Butler, & Taylor, 1996). This suggests that measuring VA during a brief instead of a sustained MVC might lead to observers missing the occurrence of CF. A study which prescribed high CHO supplementation during exercise has found that sustained MVC loss was attenuated in conjunction with preserved CAR during a sustained contraction (Nybo, 2003), but no information regarding changes VA was

reported. Furthermore, the current literature does not provide information regarding changes in VA and CAR following consumption of a full meal with high amount of CHO, despite its reported ergogenic benefits on prolonged performance (Sherman *et al*., 1991; Wright *et al*., 1991). Changes in VA during sustained contraction with CHO supplementation was also never reported, despite its application in observing CF using similar method (stimulation during a 90-second MVC) among hypoxic subjects (Szubski, Burtscher, & Loscher, 2007).

4.3 Participants and methods

4.3.1 Participants recruitment and criteria

The studies were conducted with the approval of University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC – 41) (Appendix C). Male participants were recruited via advertisement in posters and social media. Volunteers were given the study information sheet (Appendix E) and were informed of the study procedures, possible risks and discomforts. Those who agreed to participate signed a consent form (Appendix D) and completed an ACSM risk stratification screening questionnaire (Appendix F), to ensure that the participants are safe to participate in exercise studies, as well as are physically active.

The sample size was determined using the PS Power and Sample Size Calculation software (Version 3.0, Tennessee, United States) (Dupont & Plummer Jr, 1998) matched to Nybo's (2003) study, where: (a) alpha value was 0.05, (b) power was 0.80, (c) difference in population mean was 25, (d) standard deviation was 20.5, and the (e) ratio is 1. Overall sample size calculated in this study was 12 participants.

Fourteen participants (n = 14) with maximal oxygen consumption (VO_2max) of at least 50 ml/kg/min were finally included. All participants were not: smokers for at least the last six months, on weight reducing diet, diagnosed with cardiovascular or metabolic disease and consuming medication or drugs known to influence lipid or carbohydrate metabolisms. They underwent a VO_2max test and familiarisation sessions.

4.3.2 Study design

In a double-blind, randomised cross-over design with 7-day washout period, participants underwent two experimental trials where they consumed HCHO or LCHO. The prescription meals were prepared by a lab technician independent of this study, where the order of meals prescribed to each participant were randomly arranged. The researcher was only informed of the order at the end of the study. Participants also filled in the 24-hour diet recall questionnaire, and were instructed to consume the same menu prior to each experimental trial. They were restrained from intense exercise, caffeine, and alcohol 24-hour prior to the trials. All running trials were performed during similar time of the day in a controlled laboratory environment (0800). Prior to the experimental trials, participants underwent a VO_2max test was conducted to determine their maximum aerobic capacity and running speed for the trials, followed by a familiarisation session which simulated the actual trial.

4.3.3 Preliminary measurements (Anthropometry and VO_2max test)

Preliminary measurements included measurement of height (SECA, USA); measurement of participants' body composition and BMI using the InBody 370 Body Composition Analyzer (USA); and measurement of VO_2max.

Participants underwent VO_2max test using a submaximal protocol of continuous, incremental running test to determine their VO_2max on a treadmill (H/P Cosmos Quasar). Participants ran 4 stages, beginning at 8 km/h, with an increment of 2 km/h every 3-minute. Gaseous exchange data was collected using a cardiopulmonary exercise testing device (COSMED Quark CPET, Rome, Italy). Heart rate was collected using heart rate monitor (Polar, Finland) strapped at the chest. VO_2max was extrapolated based on VO_2 and heart rate plot. Running speed for their respective trials was then determined from this VO_2max value.

4.3.4 Development of experimental diets

The prescriptions for the CHO study were isocaloric meals with identical amount of protein (~5% of total calories). The high CHO meal (HCHO) consisted of 1.5 g/kg body weight of CHO; low CHO meal (LCHO) which consisted of ~0.8 g/kg body weight CHO. The post-prandial blood glucose response of the meals were tested in Chapter 3. The food items are listed in Table 4.1 below.

Table 4.1 Sample meals for a 70-kg participant:

	HCHO	**LCHO**
Food	123 g bread 30 g fruit jam 138 g apple 5 g butter 70 g hardboiled egg	77 g bread 25 g butter 120 g hardboiled egg

Note: HCHO; high carbohydrate, and LCHO; low carbohydrate

4.3.5 Experimental design

Upon arrival at the lab, participants had their fasting blood collected. After that, neurophysiological measurements (see subchapter 4.3.6 below for detail) were collected. They were then provided with the designated meals which were consumed within 20-minute, and additional one hour to digest the food. Participants were seated during this one hour with minimal physical activity. Then, another blood sample was obtained right before the exercise trials. Participants were also required to consume 500 ml of mineral water 15-minute prior to exercise testing to prevent dehydration. A 1-minute gaseous exchange was collected every 15-minute during the run. Upon completion, blood sample and neurophysiological measurements were collected within 5 minutes of running cessation. The flow of this experiment is presented in Figure 4.1.

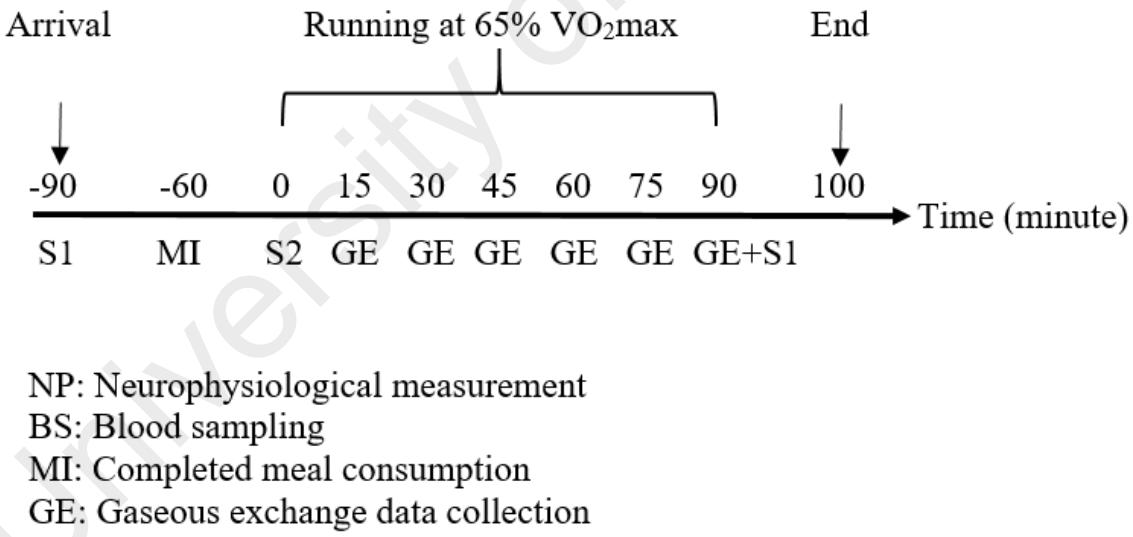

Figure 4.1. Experimental flow.

4.3.6 Neurophysiological data collection procedures

EMG. After preparation of the skin surface via shaving, abrasion, and cleaning with alcohol, Delsys Trigno Wireless self-adhesive electrodes were positioned on the lateral gastrocnemius muscles at 1/3 of the line between the head of the fibula and the heel (SENIAM guidelines) (Stegeman & Hermens, 2007) of the dominant leg. **M-wave recording**. The optimum stimulation site for tibial nerve was initially located using a hand-held stimulation probe, before placing a stimulation electrode on this site. One-ms pulse was delivered by a constant-current stimulator beginning at 30 mV, followed by 5-mV increments until there was no further increase in EMG response from the efferent fibre in 3 consecutive stimuli, and the average value of those 3 stimuli was established as Mmax. **MVC.** The participants were lied down on a prone position on the dynamometer (HUMAC NORM device, Massachusetts, USA) with their ankles at an anatomically zero position and secured with straps to prevent inversion and/or eversion movements. Participants performed one set of maximal isometric ankle plantarflexion contraction with the dominant leg, followed by a minute of rest. A sustained maximal isometric ankle plantarflexion was then performed for 90 seconds, during which the participants were verbally encouraged to maintain their maximal effort. **VA, CAR, and resting twitch.** Electrical stimulation was superimposed at 5, 30, 60, and 90th seconds of the sustained MVC, and right after contraction ceases in sustained MVC to obtain the superimposed resting twitch. The electrical stimulus used was 20% higher than that used to stimulate Mmax, with the same intensity used throughout the trials. CAR was measured by dividing highest force achieved voluntary over sum of highest force achieved voluntary + superimposed twitch at each time point. VA was measured by 1-(superimposed twitch at 90th second/resting twitch).

4.3.7 Serum preparation and analysis

Blood was withdrawn from participants' superficial vein on the dorsal side of hand using a butterfly catheter (BD, USA), and transferred into plain serum vacutainer (BD, USA). Blood sample was centrifuged at 3000 rpm for 15 minutes at 4 °C (Heraeus™ Multifuge™ X1R Centrifuge, USA). Samples were aliquoted into 1500 ml Eppendorf tubes and stored at -80 °C for later analysis of FFA, glycerol, insulin, and serotonin.

4.3.8 ELISA analysis for FFA, glycerol, insulin, and serotonin

The concentration of various blood markers was assayed and determined using commercially available enzyme-linked immunosorbent assays (ELISA) kits for FFA (EnzyChrom, BioAssays, USA), glycerol (EnzyChrom, BioAssays, USA), insulin (Insulin ELISA Kit, LDN, Germany), and serotonin (Serotonin ELISA Kit, LDN, Germany), based on manufacturers' guidelines. The assays were analysed using a microplate spectrophotometer (EPOCH, BioTek), and results were plotted against a standard concentration which forms a linear AUC to obtain the marker's concentration value. The CV of those kits are 0.79, 0.31, 0.29, and 0.61 respectively.

4.3.9 Statistical analysis

All statistical analyses were performed using SPSS 20.0 (IBM Corp. Armonk, NYN). Data normality was examined and confirmed with $p > 0.05$ using the Shapiro-Wilks test. A two-way analysis of variance (ANOVA) and post-hoc analysis (Bonferoni test) was used to determine the effect of measured parameters and time and the changes of parameters over the time. Paired t-test was used when comparing only means of two groups. Statistical significant were set and accepted at $p < 0.05$. Data are presented as mean values and standard deviation (mean ± SD).

4.4 Results

4.4.1 Participants' anthropometrics

The participants' anthropometrics data are presented in Table 4.2. The mean body mass index (BMI) of the participants were within the normal range (18.5 – 25.0 kg/m^2).

Table 4.2 Anthropometric measures of participants (n = 14).

Variables	Value
Age (year)	24.9 ± 3.7
Weight (kg)	63.8 ± 10.3
Height (cm)	171.70 ± 6.8
Lean Muscle Mass (%)	47.4 ± 6.2
Body Fat Percentage (%)	12.4 ± 4.0
BMI (kg/m^2)	21.6 ± 2.6
VO_2max (ml/kg/min)	58.6 ± 5.6

Note: values are mean ± SD. BMI; body mass index.

4.4.2 Nutrient consumed

The mean nutrient composition of the actual meals consumed by participants during the testing sessions are presented in Table 4.3. Amount of CHO was significantly higher in the HCHO, while amount of fat was significantly higher in the LCHO.

Table 4.3 Macronutrients, energy intakes, and percentage (%) of energy from macronutrients of HCHO and LCHO meals consumed by participants 1-hour prior to the 90-minute run at 65% VO_2max. Values are mean ± SD, and * denotes significant difference between groups.

	HCHO	**LCHO**
Energy (kcal)	584.5 ± 94.6	583.0 ± 94.4
% of calories from CHO	65.5	35.6
% of calories from fat	25.5	56.7
% of calories from protein	5.9	5.9
Amount of CHO (g)	95.7 ± 15.5*	51.9 ± 8.4
Amount of Fat (g)	15.9 ± 2.6*	35.5 ± 5.8
Amount of Protein (g)	8.2 ± 1.3	8.2 ± 1.3

Note: HCHO; high carbohydrate, LCHO; low carbohydrate, CHO; carbohydrate.

4.4.3 VO_2 during 90-minute Run

The participants' VO_2 at every 15-minute interval during the 90-minute run are presented in Table 4.4, and illustrated in Figure 4.2. There was no significant difference between LCHO and HCHO at all time points; and there was also no significant difference within each group at all time point. VO_2 was maintained at a similar level (~64 to 67% of VO_2max) throughout the 90-minute run at both sessions for all participants.

Table 4.4.1 VO_2 of the LCHO and HCHO group at every 15-minute interval during 90-minute run. Values are mean ± SD (n = 14).

Time point (minute)	**VO_2 (ml/kg/min)**	
	LCHO	**HCHO**
15	37.62 ± 6.06	38.17 ± 5.58
30	39.80 ± 5.05	39.03 ± 5.90
45	38.33 ± 5.55	38.73 ± 5.80
60	39.47 ± 5.76	38.60 ± 5.93
75	37.99 ± 5.88	38.99 ± 5.14
90	38.76 ± 5.92	39.77 ± 4.89

Note: VO_2, volume of oxygen uptake, HCHO; high carbohydrate, and LCHO; low carbohydrate.

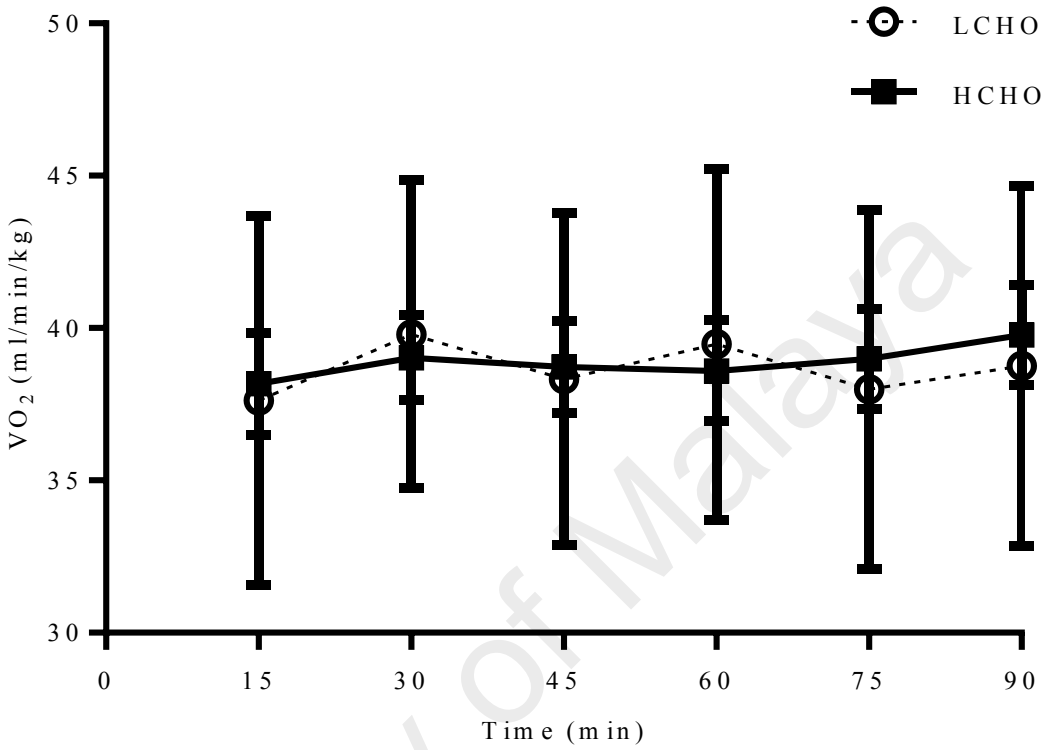

Figure 4.2 Oxygen consumption (VO_2; ml/kg/min) at 15-minute interval throughout the 90-minute run at 65% VO_2max in the LCHO (•) and HCHO (■). Values are mean ± SD (n = 14). Note: HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.4 CHO and fat oxidation during 90-minute run

4.4.4.1 Rate of CHO and fat oxidation

Rate of CHO and fat oxidation at every 15-minute interval during the 90-minute run are presented in Table 4.5. Changes of CHO and fat over time are illustrated respectively in Figures 4.3 and 4.4. The participants had similar CHO and fat oxidation rate during the first 60 minutes. At minutes 75 and 90, the CHO oxidation rate was significantly higher in the HCHO, while fat oxidation rate was higher in LCHO group. The total fat oxidised in HCHO was significantly lower than LCHO (Figure 4.5)

Table 4.5 Rate of CHO and fat oxidation at 15-minute interval throughout the 90-minute run at 65% VO_2max in the LCHO and HCHO.

Time point (minute)	**CHO oxidised (g/min)**		**Fat oxidised (g/min)**	
	LCHO	**HCHO**	**LCHO**	**HCHO**
15	1.12 ± 0.41	1.12 ± 0.37	0.74 ± 0.27	0.73 ± 0.21
30	1.05 ± 0.86	1.10 ± 0.37	0.86 ± 0.37	0.73 ± 0.29
45	0.87 ± 0.35	1.01 ± 0.34	0.88 ± 0.30	0.79 ± 0.33
60	0.84 ± 0.34	1.04 ± 0.44	1.00 ± 0.37	0.68 ± 0.32
75	0.81 ± 0.32	1.07 ± 0.31*	0.89 ± 0.29	0.75 ± 0.28*
90	0.77 ± 0.34	1.15 ± 0.48*	1.08 ± 0.49	0.80 ± 0.35*

Note: values are mean ± SD (n = 14), and *denotes significant difference ($p < 0.05$) between group. CHO; carbohydrate, HCHO; high carbohydrate, LCHO; low carbohydrate.

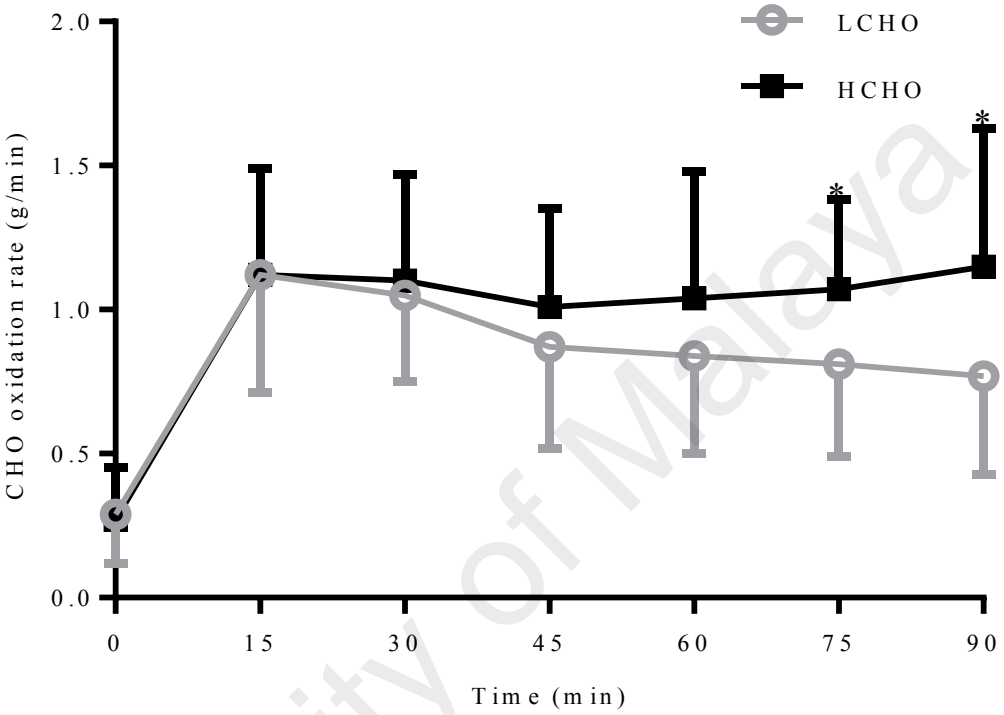

Figure 4.3 Rate of CHO oxidation (g/min) at 15-minute interval throughout the 90-minute run at 65% VO_2max in the LCHO (-⊖-) and HCHO (-■-). * significant difference ($p < 0.05$) from LCHO. Values and mean ± SD (n = 14). HCHO; high carbohydrate, LCHO; low carbohydrate.

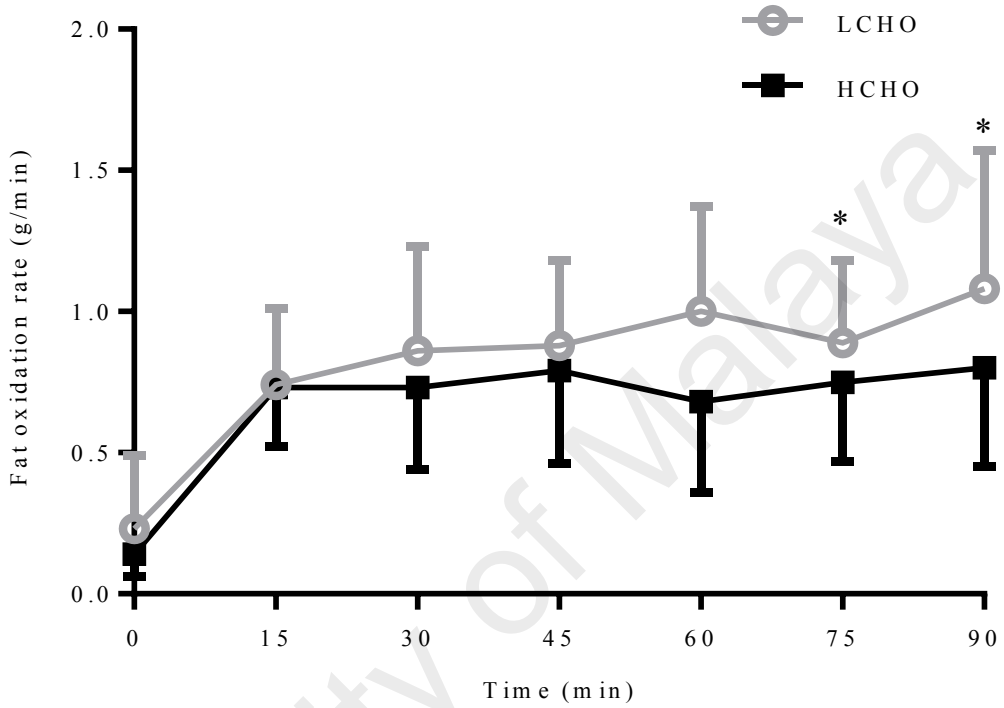

Figure 4.4 Rate of fat oxidation (g/min) at 15-minute interval throughout the 90-minute run at 65% VO_2max in the LCHO (-⊖-) and HCHO (-■-). * significant difference ($p < 0.05$) from HCHO. Values are mean ± SD (n = 14). HCHO; high carbohydrate, LCHO; low carbohydrate.

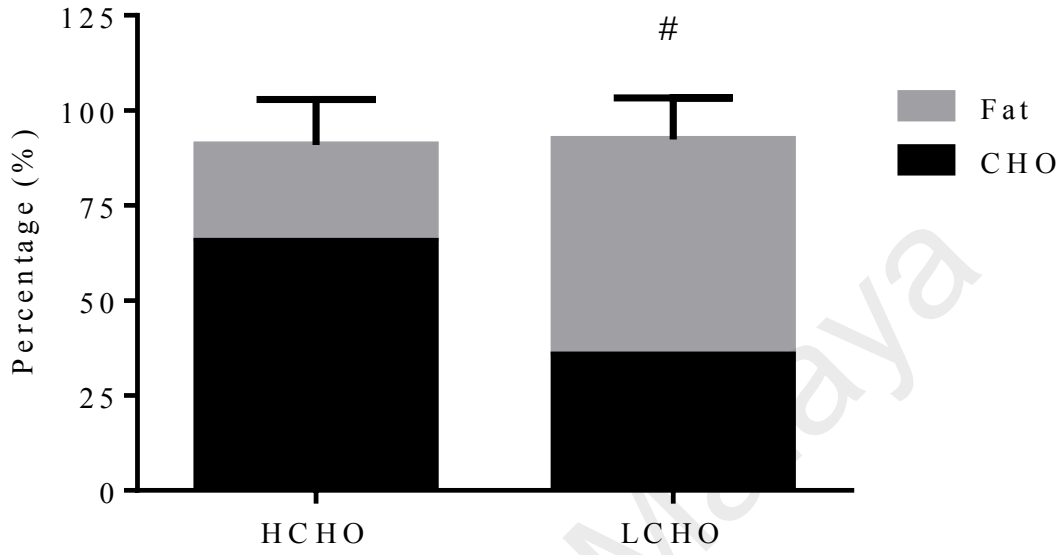

Figure 4.5 Percentage (%) of calories derived from fat and CHO during the 90-minute run at 65% VO_2max in the LCHO and HCHO. #fat significantly higher than HCHO. Values are mean (n = 14). HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.4.2 Respiratory exchange ratio (RER)

RER at every 15-minute interval during the 90-minute run are presented in Table 4.6. RER was significantly higher in the HCHO than LCHO group at 60th, 75th, and 90th minute of the run.

Table 4.6 Respiratory exchange ratio (RER) at 15-minute interval throughout the 90-minute run at 65% VO_2max in LCHO and HCHO.

Time point (minute)	**RER**	
	LCHO	**HCHO**
Baseline	0.81 ± 0.03	0.80 ± 0.06
15	0.83 ± 0.03	0.84 ± 0.05
30	0.81 ± 0.07	0.84 ± 0.06
45	0.81 ± 0.05	0.82 ± 0.05
60	0.80 ± 0.05*	0.87 ± 0.04
75	0.80 ± 0.04*	0.87 ± 0.04
90	0.79 ± 0.04*	0.85 ± 0.05

Note: values are mean ± SD (n = 14), and *denotes significant difference ($p < 0.05$) between group. HCHO; high carbohydrate, LCHO; low carbohydrate; RER, respiratory exchange ratio.

4.4.5 MVC

The participants' force at the 5th and every 30-second interval during the 90-second sustained MVC are presented in Table 4.7, and illustrated in Figure 4.6. In both HCHO and LCHO groups, MVC at the 5th second was significantly lower post 90-minute running. However only in LCHO group that the MVC at 30, 60, and 90th seconds were significantly lower post 90-minute running. MVC at 30, 60, and 90th seconds were relatively maintained post 90-minute running in the HCHO compared to LCHO group.

Table 4.7 MVC force generated during the 90-second sustained MVC for HCHO and LCHO measured at fasted state (Pre) and immediately after 90-minute run at 65% VO_2max (Post).

Time point (second)	**MVC (N/m)**			
	HCHO		**LCHO**	
	Pre	**Post**	**Pre**	**Post**
5	80.57 ± 16.76	68.02 ± 17.88*	81.14 ± 20.11	66.49 ± 20.97*
30	56.73 ± 21.93	49.72 ± 18.44	57.39 ± 19.12	43.93 ± 16.44*
60	48.80 ± 18.81	42.56 ± 16.51	49.38 ± 16.92	36.94 ± 11.66*
90	44.50 ± 17.28	37.96 ± 15.62	44.01 ± 15.32	32.85 ± 11.17*

Note: Values are mean ± SD (n = 14). *denotes significant difference ($p < 0.05$) between Pre and Post. MVC; maximal voluntary contraction, HCHO; high carbohydrate, LCHO; low carbohydrate.

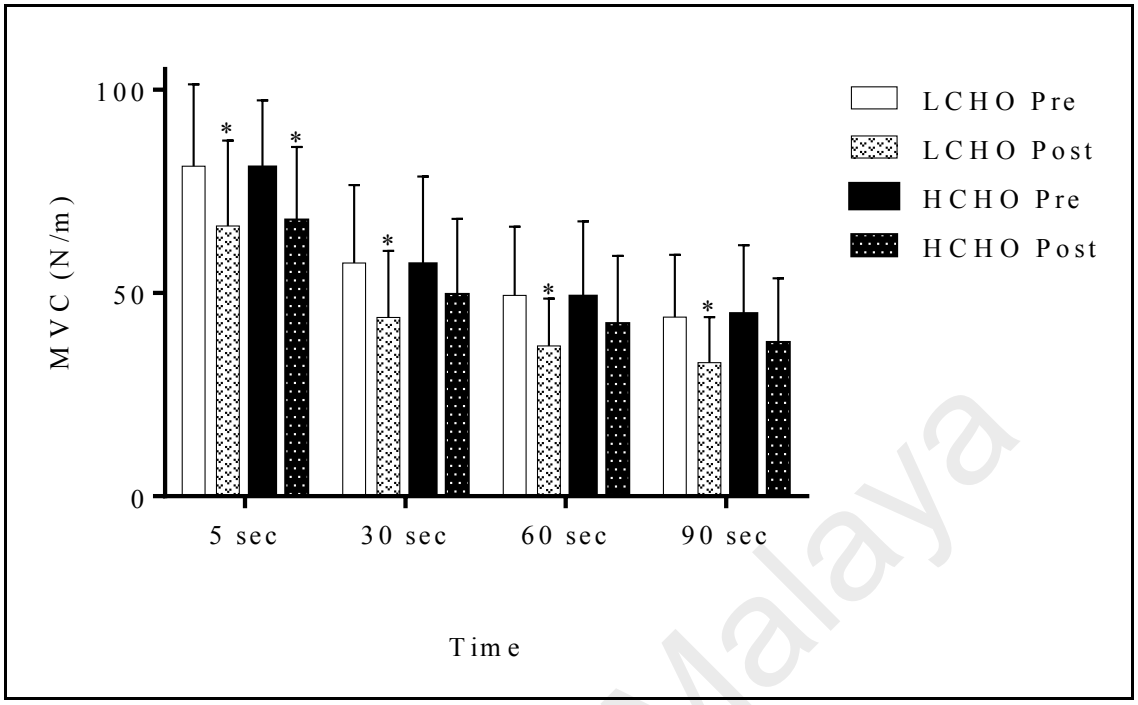

Figure 4.6 MVC force generated during the 90-second sustained MVC for HCHO and LCHO measured at fasted state (Pre) and immediately after 90-minute run at 65% VO_2max (Post). Values are mean ± SD (n = 14). *significant difference ($p < 0.05$) compared to the corresponding Pre value. MVC; maximal voluntary contraction, HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.6 CAR, VA, resting twitch, and Mmax

The participants' CAR and VA during the 90-second sustained MVC; as well as Mmax and resting twitch before and immediately after the sustained MVC are presented in Table 4.8. There was a main effect between various time points in both groups ($F_{3,64} = 3.68$, $p = 0.03$). In the HCHO, there was no changes in the CAR after the 90-minute running, while in the LCHO CAR was significantly lower at the 30, 60, and 90th seconds of sustained MVC post 90-minute running (Figure 4.7) with a main effect across time ($F_{3,78} = 2.23$, $p = 0.01$). While VA measured at 90th second shows no difference between trials at both fasting and post-running; VA was significantly reduced at Post-running compared to fasting only in the LCHO (main effect across time, $F_{3,78} = 3.22$, $p = 0.03$). Resting twitch was reduced while Mmax did not change in both groups.

Table 4.8 CAR and VA during the 90-second sustained MVC; VA and CAR for HCHO and LCHO measured at fasted state (pre) and immediately after 90-minute run at 65% VO_2max (post).

Time point (second)	HCHO		LCHO	
	Fasting	Post-running	Fasting	Post-running
	CAR			
5	0.96 ± 0.03	0.95 ± 0.03	0.95 ± 0.03	0.94 ± 0.05
30	0.95 ± 0.03	0.94 ± 0.03	0.95 ± 0.04	0.92 ± 0.06*
60	0.94 ± 0.03	0.93 ± 0.04	0.94 ± 0.04	0.92 ± 0.05*
90	0.94 ± 0.03	0.93 ± 0.04	0.94 ± 0.05	0.92 ± 0.05*
	VA			
90	0.63 ± 0.14	0.56 ± 0.22	0.70 ± 0.17	0.53 ± 0.24*
	Resting twitch			
	10.64 ± 2.56	8.72 ± 3.45*	11.74 ± 3.92	9.08 ± 4.32*
	Mmax			
	6.92 ± 1.88	6.66 ± 1.81	6.82 ± 1.48	6.12 ± 1.85

Note: Values are mean ± SD (n=14). *significant difference ($p < 0.05$) between Post value against its corresponding Pre value. HCHO; high carbohydrate, LCHO; low carbohydrate, CAR; central activation ratio, VA; voluntary activation, Mmax Maximal compound muscle action potential.

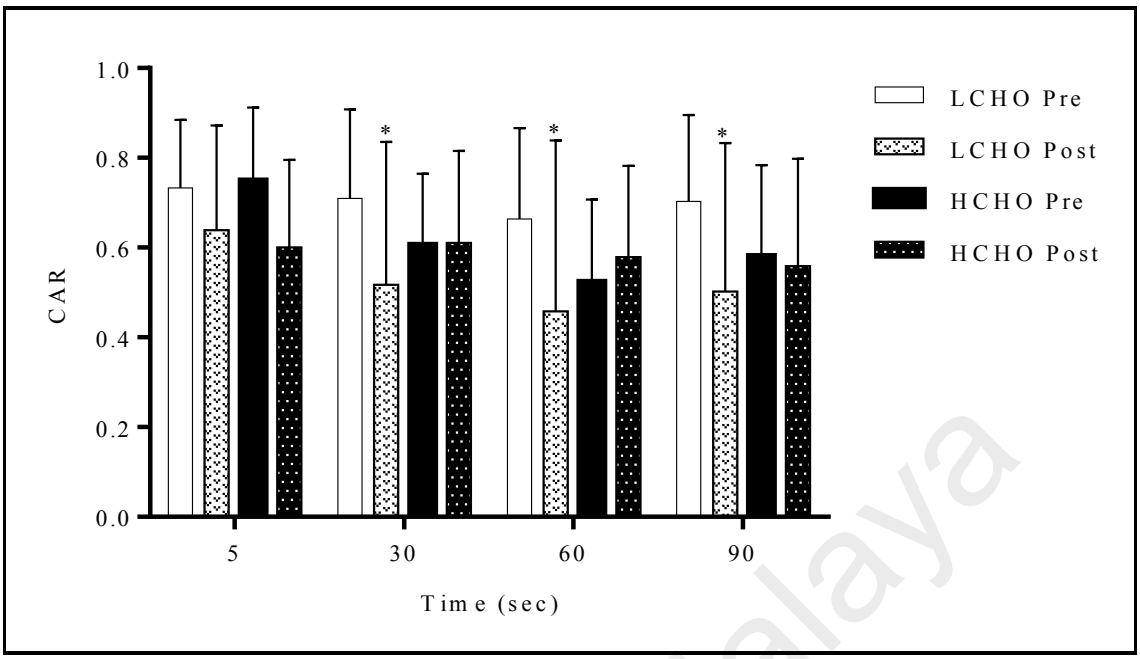

Figure 4.7 CAR during 90-second sustained MVC sustained MVC for HCHO and LCHO measured at fasted state (pre) and immediately after 90-minute run at 65% VO_2max (post). Values are mean ± SD (n=14). *significant difference ($p < 0.05$) compared to the corresponding pre value. VA; voluntary activation, HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.7 Serum FFA, glycerol, insulin, serotonin

The concentration of blood markers at all time points analysed are presented in Table 4.9.

Table 4.9 Serum FFA, glycerol, insulin, and serotonin concentration at fasting state (T0), 1 hour post-prandial and immediately prior to 90-minute run (T1), and at the end of the run (T2).

Marker	HCHO			LCHO		
	T0	T1	T2	T0	T1	T2
FFA (μM)	246.36 ± 121.79	407.28 ± 249.82	345.69 ± 188.71	209.87 ± 86.51	521.40 ± 412.28*	381.10 ± 262.53
Glycerol (mg/dl)	0.94 ± 0.29	1.14 ± 0.46	1.56 ± 0.66*	1.03 ± 0.25	1.36 ± 0.51	20.9 ± 0.71**
Insulin (IU/ml)	7.24 ± 1.47	16.21 ±4.63*	8.17 ± 1.57***	7.60 ± 1.61	9.71 ± 2.33*#	8.88 ± 2.26***
Serotonin (nmol)	304.45 ± 210.17	509.87 ± 301.88	593.46 ± 242.76	237.76 ± 187.96	555.58 ± 343.59	1236.43 ± 749.16**

Note: Values are mean ± SD (n=10). *significant difference ($p < 0.05$) than T0, and **significant difference ($p < 0.05$) than T0 and T1, and ***significant difference ($p < 0.05$) than T1, and #significant difference than corresponding values in the HCHO group. FFA; free fatty acid, HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.7.1 FFA

FFA concentration was significantly increased at 1 hour post-prandial and immediately prior to 90-minute run (T1) compared to fasting value (T0) in the LCHO group, while in the HCHO group FFA concentration did not differ significantly across all time points (Figure 4.8). Main effect between time points were found ($F_{2,41} = 3.65$, $p = 0.01$).

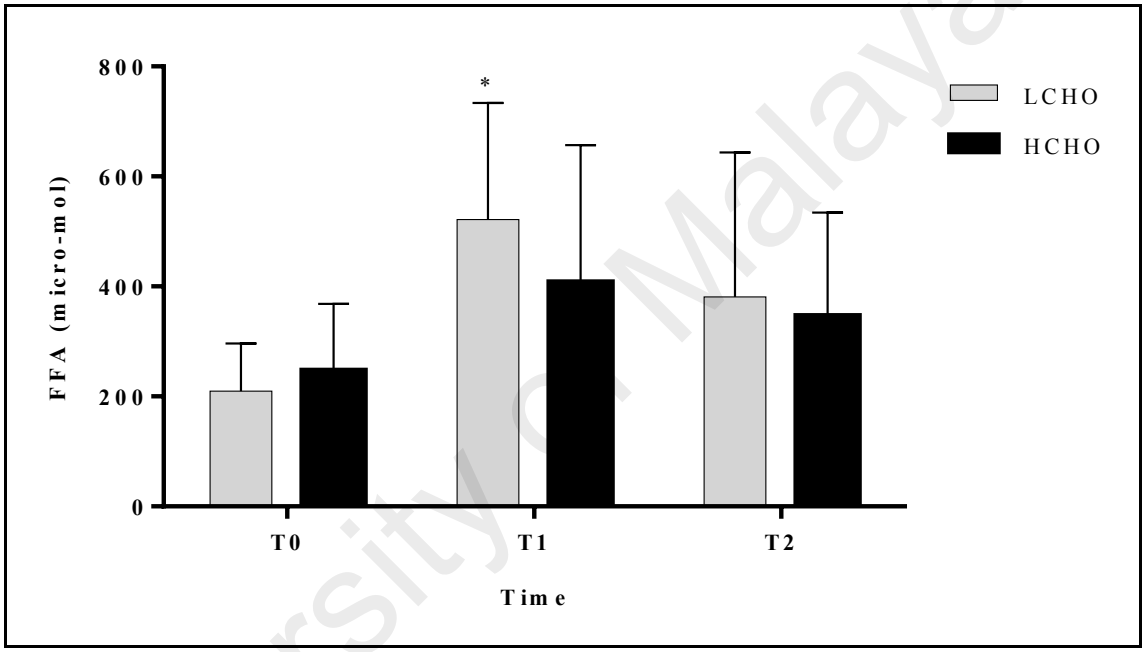

Figure 4.8 FFA concentration (µmol) at T0, T1, and T2 for HCHO and LCHO. Values are mean ± SD (n = 10). *significant difference ($p < 0.05$) compared to T0. FFA; free fatty acid, HCHO; high carbohydrate, LCHO; low carbohydrate

4.4.7.2 Glycerol

Serum glycerol concentration was significantly increased at the end of 90-minute run at 65% VO_2max (T2) from fasting value (T0) in both LCHO and HCHO; the glycerol concentration of LCHO group at T2 is also significantly increased from 1-hour post-prandial values (T1) (Figure 4.9). Main effect between time points were found ($F_{1,38}$ = 2.92, p = 0.00).

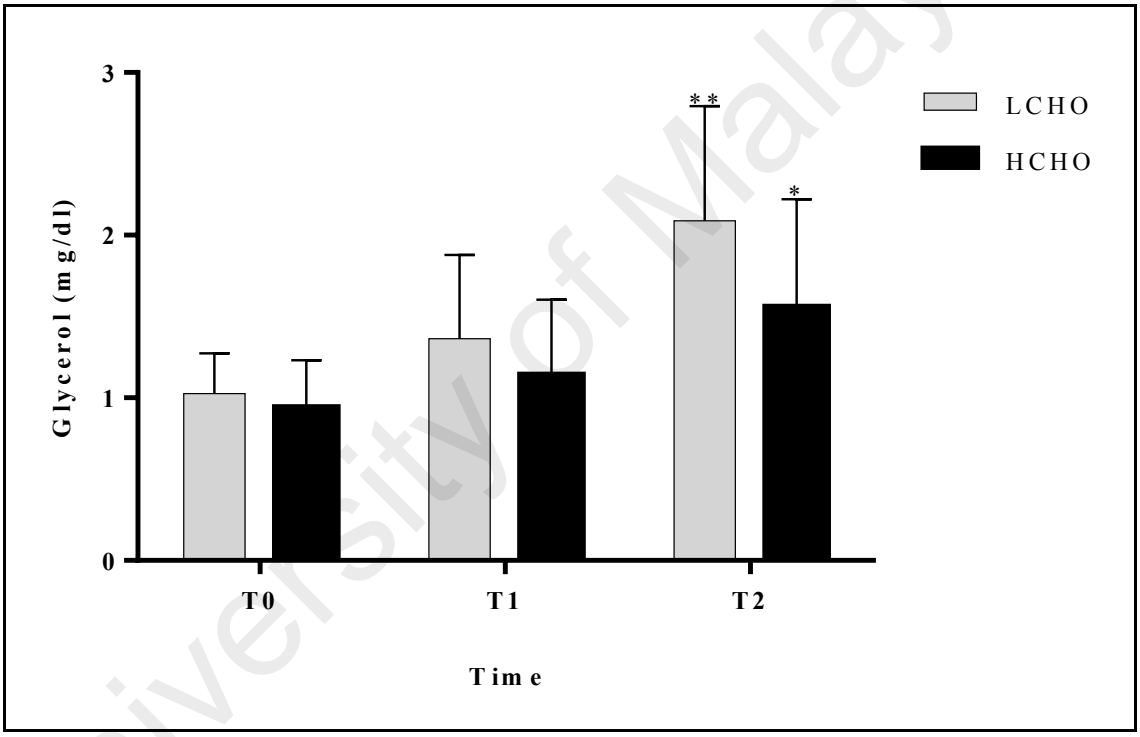

Figure 4.9 Glycerol concentration (mg/dl) at T0, T1, and T2 for HCHO and LCHO. Values are mean ± SD (n = 10). *significant difference ($p < 0.05$) compared to T0; **significant difference ($p < 0.05$) compared to T0 and T1. HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.7.3 Insulin

Both LCHO and HCHO groups had a similar change in insulin concentration where there was a significant increase at 1 hour post-prandial (T1) compared to fasting value (T0), and thereafter significantly reduced at post 90-minute running at 65% VO_2max (T2) compared to T1. However, the magnitude of increase from Fasting value (T0) to T1 was significantly higher in the HCHO group. (Figure 4.10). Main effect between time points were found ($F_{3,78} = 3.22$, $p = 0.03$).

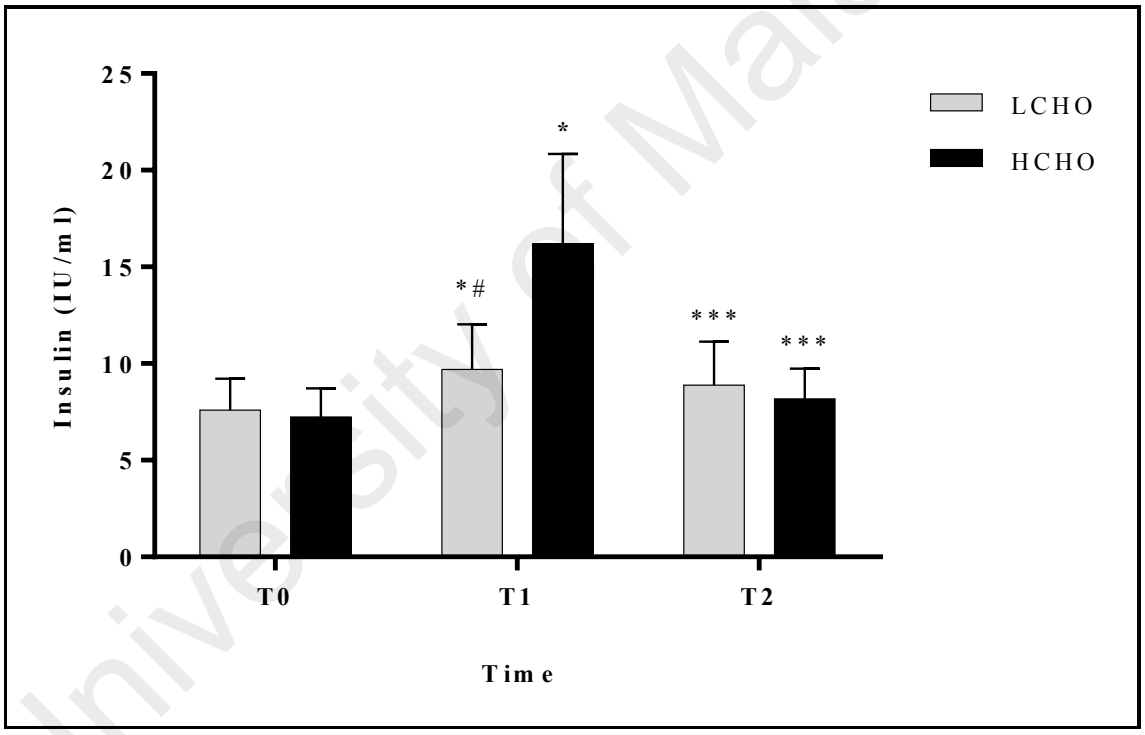

Figure 4.10 Insulin concentration (IU/ml) at T0, T1, and T2 for LCHO and HCHO. Values are mean ± SD (n = 10). *significant difference ($p < 0.05$) compared to T0; ***significant difference ($p < 0.05$) compared to T1; #significant difference ($p < 0.05$) compared to HCHO. HCHO; high carbohydrate, LCHO; low carbohydrate.

4.4.7.4 Serotonin

Serotonin concentration remained at a similar level from fasting values (T0) to 1 hour post-prandial (T1) in both trials, but while in the HCHO the level remained similar again at post 90-minute run at 65% VO_2max (T2), the concentration increased significantly in the LCHO at T2 compared to both T0 and T1 (Figure 4.11). Main effect between time points were found ($F_{4,82} = 4.52$, $p = 0.04$).

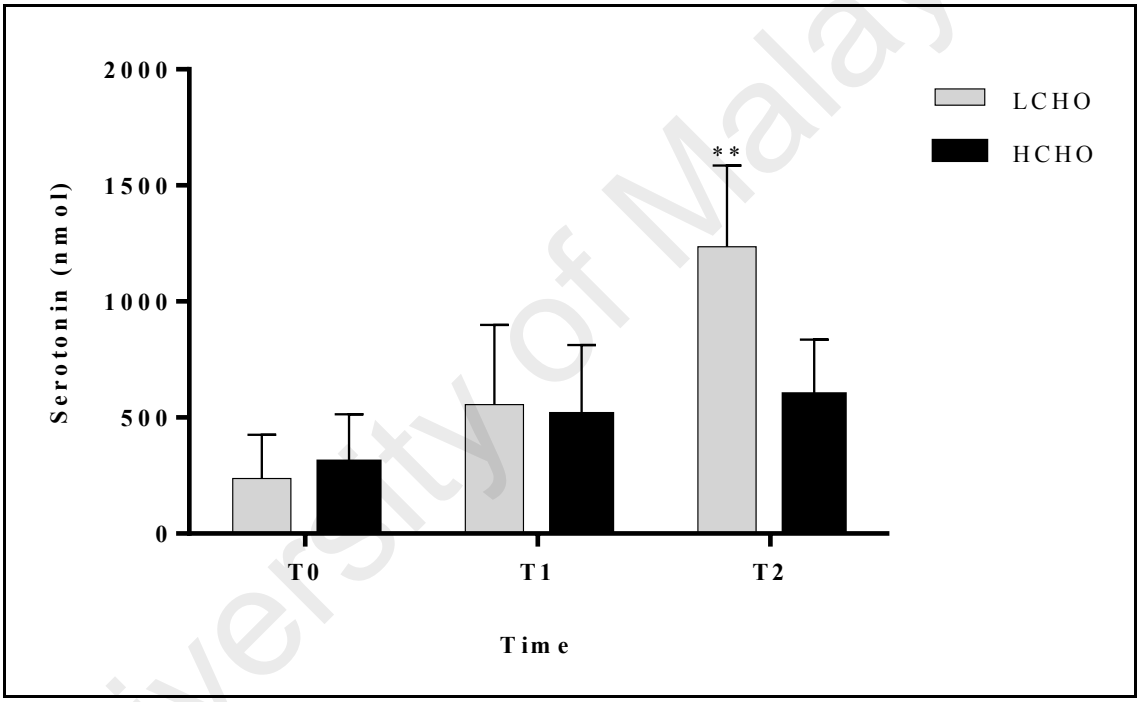

Figure 4.11 Serotonin concentration (nmol) at T0, T1, and T2 for LCHO and HCHO. Values are mean ± SD (n = 10). **significant difference ($p < 0.05$) compared to T0 and T1. HCHO; high carbohydrate, LCHO; low carbohydrate.

4.5 Discussion

Present study aimed to determine the effects of isocaloric pre-exercise meals with high and low CHO content on MVC and VA following an endurance exercise. The findings demonstrated that HCHO pre-exercise meal relatively maintained the muscle's force generating capability compared to LCHO meal, confirming the first hypothesis. Furthermore, it was indicated that these effects could have central contributions as shown by the differences in CAR, VA and indirectly by serotonin responses, confirming the second and third hypotheses, respectively.

4.5.1 Changes in MVC, CAR, VA, resting twitch and Mmax after the 90-min run with different CHO quantity

Reduction in MVC post dynamic exercise has been used as an indicator of muscle fatigue, with VA being used in conjunction to determine if the muscular fatigue is of central origins (Del Coso *et al*., 2008; Jeffers *et al*., 2015; Nybo, 2003). Present study found that while MVC in both trials were lower post 90-minute running compared to pre, differences were observed at different time points during the sustained MVC in both trials. Both trials' MVC at the 5th second were significantly lower, but HCHO meal was found to have attenuated the loss of MVC thereafter compared to LCHO meal, which saw a significant progressive drop at subsequent time points compared to the pre-running values (Table 4.7). The drop in force is predominantly contributed by CF since CAR showed concomitant reductions at 30, 60, and 90th seconds alongside the significant drop in sustained MVC. Since there was no difference in CAR from 30th second onwards in HCHO, this supports the hypothesis that high CHO supplementation preserves CNS activation, thus preventing CF (Nybo 2003). It was also noted that while there was no change in Mmax, a reduction in resting twitch was observed in both groups indicating possible involvement at peripheral level which may have contributed to the reduction of

MVC seen at the 5th second. Since, the MVC reductions were not observed at subsequent time points in HCHO and only LCHO, the reduction could not have been contributed solely by peripheral factor. These further strengthen the involvement of CF particularly in LCHO based on concomitant reductions of CAR and VA alongside MVC. In addition, as FFA and insulin levels were both significantly increased after a LCHO meal in the present study, this supports the notion of the serotonin hypothesis (Davis *et al*., 1992; Meeusen *et al*., 2006), where serotonin synthesis is promoted. This is most evidence at post-exercise, which is thought to exert effects on increased perception of tiredness and lethargy in the brain, thus reducing CNS activation level.

Studies in favour of CHO improving endurance performance have generally been emphasising on its effect in increasing glycogen and glucose availability. For CHO storage to deplete, a very long duration exercise (> 3 hours) on at least moderate intensity (> 60% VO_2max) was required (Bergstrom *et al*., 1967). The present study showed that HCHO meal could benefit endurance athletes by preserving the muscle force production capability during 90-minute running at moderately high intensity. This could be of importance especially in sports where sustained force production is required.

4.5.2 CHO and fat oxidation during the 90-minute run at 65% VO_2max

Present study showed that CHO oxidation was favoured in the HCHO, while fat oxidation was favoured in LCHO. This is in line with the understanding that high CHO meals promote CHO oxidation, while low CHO (high fat) meals promote lipid oxidation (Murakami *et al*., 2012). It has been shown that when CHO was consumed within 1 hour prior to exercise, the insulin surge (20-40 minutes after consumption) would activate GLUT-4 transporters to facilitate glucose uptake into muscle, reducing blood glucose concentration (Jeukendrup & Killer, 2011). This would probably reduce the amount of CHO available for oxidation, but present study did not observe this phenomenon in both

HCHO and LCHO despite increased insulin level at 1-hour post-prandial especially in the HCHO. In fact, CHO oxidation was higher in the HCHO particularly towards the end of the 90-minute run (Table 4.5), indicating that the glucose level could have returned to normal values by 1-hour post-prandial, and HCHO pre-exercise meal successfully provided more CHO as fuel source compared to LCHO meal. This is consistent with previous finding where CHO had been prescribed 1-hour before exercise increased total CHO oxidation and insulin concentration at the start and during exercise, which led to improved performance trial following a 90-minute cycling at 70% VO_2max (Sherman *et al*, 1991). As insulin increase was significantly greater in the HCHO, fat oxidation was lower compared in this trial compared to LCHO (by ~16 to 26%), especially towards the end of the 90-minute run. The difference in insulin in HCHO (~9 IU/ml) compared to LCHO (~2 IU/ml), metabolically tilt the balance as insulin is believed to reduce fat oxidation. It was suggested that even a small (10 - 30 μU/ml) elevation of plasma insulin concentration suppresses lipolysis, thus reducing fat oxidation (Horowitz *et al*., 1997). Since the insulin level in LCHO was much lower in response to lower CHO content, lipolysis of triacylglycerol was evident as the blood FFA and glycerol availability was larger and hence promoted fat metabolism, which could spare CHO as fuel source during the exercise. This is in line with multiple studies that found higher blood FFA availability, either by fat feeding (Costill *et al*., 1977; Helge, Watt, Richter, Rennie, & Kiens, 2001) or infusion (Dyck *et al*., 1996), accounted for an increased fat oxidation during exercise when CHO oxidation was suppressed. However, despite the difference in fuel selection, there was no difference in total energy expenditure between both trials. As VO_2 was relatively maintained at similar level between both trials, the pre-exercise meals' composition seems to be the only factor affecting fuel selection in the present study. The similar energy expenditure is achieved by a compensatory reaction where the body up-regulates fat oxidation when there is a decrease in CHO oxidation, and *vice-versa*.

While it has been shown that administration of a serotonin stimulant before endurance exercise caused shorter time to exhaustion (Davis, Bailey, Jackson, Strasner, & Morehouse, 1993; Wilson & Maughan, 1992), those studies did not establish that the reductions in performance were due to central factor. Despite lack of evidence they still attributed to central contribution since they did not observe changes in typical peripheral markers involving cardiovascular, thermoregulatory, and metabolic functions (Davis, Alderson, & Welsh, 2000). To our knowledge, present study is the first to correlate serotonin with CF measures in humans following pre-exercise meals of different quantity of CHO. In accord with the serotonin hypothesis (Meeusen *et al*., 2006), present study found that its concentration was higher in LCHO alongside an increased FFA concentration. It is shown that a correlation exists between serotonin concentration and CF: the CAR in LCHO was decreased at the end of exercise when serotonin concentration at its highest, while in the HCHO CAR was relatively maintained when serotonin concentration remained constant throughout the whole experimental trial. It should be noted that present study measured serotonin in blood samples collected from the hand instead of jugular vein due to ethical and methodological issues, but still do not best represent the changes in the brain.

4.6 Conclusion

To our knowledge, present study is the first to examine the effects of iso-caloric pre-exercise meals with different quantity of CHO on CF, as previous studies had only measured either performance, or CF with CHO prescribed during the endurance exercise. While peripheral factors were involved in the reductions of MVC in both groups as shown by changes in resting twitch, CF is more pronounced in LCHO indicated by CAR and VA. Besides that, present study is also the first to attempt to measure VA during a sustained MVC (90-second). While Nybo (2003) demonstrated that CAR was maintained post-exercise with glucose supplementation, present study measured the resting twitch immediately at the cessation of 90-second MVC, which allowed the calculation of VA. The sustained MVC assess the degree of fatigue after an endurance run when the muscle is tasked to perform work continuously, much like how runners must keep running continuously. Of interest to note in the present study, the CAR and MVC dropped in both trials at the 5th second time point of the sustained MVC, which could have typically been concluded that there are no differences between ergogenic effects of HCHO or LCHO pre-exercise meal. However, by extending the MVC to 90 seconds, it was found that the CAR and MVC was maintained close to pre-running level with HCHO, while they continue to drop progressively in LCHO. This is further reinforced when VA was calculated using the MVC at 90th second which shows a reduction post 90-minute running only in the LCHO. The effects of CHO took place only after some time (at least 30 seconds) into a sustained effort, where continuous firing of neural drive was needed from the brain. This is coupled with the increased serotonin in LCHO indicates that the perceived increased sense of tiredness and lethargy might have reduced the capability of the brain to maintain said drive.

CHAPTER 5 EFFECTS OF GLYCAEMIC INDEX ON CENTRAL FATIGUE FOLLOWING PROLONGED EXERCISE

5.1 Introduction

In the past, simple carbohydrate (CHO) was believed to be more readily absorbed by the body, and produced higher post-prandial plasma glucose and insulin compared to complex CHO due to its lesser number of basic sugar units (Crapo *et al*., 1977). However, it was later found that even simple CHO such as fructose elicited lesser glucose and insulin response compared to complex CHO such as cereals, indicating a need for better way to categorise CHOs based on their post-prandial effects on glucose and insulin (Crapo *et al*., 1977; Jenkins *et al*., 1981). Consequently, glycaemic index (GI) was introduced to quantify the degree of how quickly blood glucose rises relative to a reference food such as white bread or glucose (Jenkins *et al*., 1981). While GI was introduced as a tool to advise the clinical population especially diabetics on how to control their blood glucose level, it has since been applied in exercise and sports researches as a strategy to optimise performance. Low GI CHO especially has been proposed to provide better ergogenic aid compared to high GI CHO (DeMarco *et al*., 1999; Wu *et al*., 2006).

As high amount (1.5 g/kg body weight) of CHO consumed one hour before exercise has been proven to attenuate the central fatigue (CF) in Chapter 4, a follow up study to investigate whether GI plays a role in influencing CF is warranted. A higher rise in blood glucose level accompanied by a higher insulin surge is expected with high GI which also promotes CHO oxidation. In contrast, low GI promotes fat oxidation which would allow longer period of exercise as it could spare muscle glycogen, whereby depletion has been associated with fatigue (Bergstrom *et al*., 1967). Ironically, higher fat oxidation lead to higher FFA availability, which in turn increase tryptophan (precursor of serotonin) and

subsequently serotonin, hence LGI may induce CF greater than HGI. It is worth noting that muscle glycogen is typically depleted following an exhaustive exercise (3 hours or more), however when glycogen storage is not depleted the role of LGI may not be as beneficial to maintain central drive compared to HGI. While current literature shows that GI affects the fuel selection during prolonged exercise, its effect on performance outcome remains mixed, and to date, no study has employed neurophysiological measures to explore the effects of GI on CF. The main possible mechanisms of how CHO meals with different GI could affect CF stems from two factors: fuel supplies and possible neurotransmitter's (serotonin) influence.

Objective

Since benefits of GI on endurance performance has been reported earlier with mixed results and high CHO pre-exercise meal has been shown to improve CF in previous Chapter 4, there was a need to investigate whether this high-CHO pre-exercise meal with different GI would produce similar effects. Secondarily this chapter aimed to distinguish changes in MVC, 90-second MVC, CAR, and VA after a 90-minute endurance running at 70% VO_2max between high and low GI pre-exercise meals.

Hypothesis

1. High GI pre-exercise meal preserves MVC better than LGI pre-exercise meal.
2. The preservation of MVC is accompanied by preservation of CF markers i.e. CAR and VA.
3. The preservation of MVC and CF markers are accompanied by an increased FFA and reduced tryptophan.
4. The preservation of MVC and CF markers are accompanied by a less pronounced rise in serotonin.

5.2 Literature review

Since maintaining muscle CHO (glycogen) level, as well as maximising lipid as main energy source have been recognised as fatigue delaying strategies, meal intake prior to endurance exercise is a proven strategy in achieving those means. While CHO-rich meal is commonly consumed as pre-exercise meal, GI has been used as guideline to select the best CHO to induce the desired glycaemic, lipolytic, and insulin responses. Proponents of low GI as ergogenic aid have put-forth that low GI meal produced a smaller insulin and glucose reaction curve compared to high GI meal, which is believed to delay fatigue by promoting fat oxidation, thus sparing muscle glycogen, resulting in longer time to exhaustion (DeMarco *et al*., 1999; Wee *et al*., 2005). A study has showed that after consuming a low GI meal, serum insulin was lower while plasma FFA higher post-prandial and during exercise, indicating less inhibition of lipolysis as well as higher availability of lipid for oxidation (Wee *et al*., 2005). However, there are also studies which demonstrated that while low GI meals are consistent in inducing this change in fuel selection, it did not translate to improved prolonged exercise performance (Chen *et al*., 2008; Febbraio *et al*., 2000; Wee *et al*., 1999). One study demonstrated that higher blood glycerol and FFA during exercise did not lead to a longer time to exhaustion during a 70% VO_{2max} run (Wee *et al*., 1999); while another showed that while a high GI meal did manage to induce a higher blood glycerol and FFA concentration, there was no difference in time to complete a 10-km run compared to a low GI meal (Chen *et al*., 2008). The discrepancies of the exercise performance could be attributed to the lack of a standardised protocol to measure fatigue; the performance tests used were not sufficient to induce fatigue, or that the measurements used could not objectively quantify fatigue when subjects were not exercised till volitional fatigue. Changes in fatigue using neurophysiological measures such as VA, CAR, and even MVC following GI interventions would better distinct the contribution of CHO towards CF (Khong,

Selvanayagam, Sidhu, & Yusof, 2016). Studies that did these measures typically employed high GI CHO such as glucose, dextrose, and sucrose (Del Coso *et al*., 2008; Nybo, 2003; Stewart *et al*., 2007). Those studies found a less pronounced loss of muscle force production capability with indication of CF involvement after CHO supplementation during exercise against placebo and water. Sustained MVC reduction post-exercise was attenuated with CHO supplementation, with improvement to CF indicated with attenuation of CAR (Nybo, 2003); while MVC reduction post-exercise was attenuated with a corresponding attenuation of VA reduction (Del Coso *et al*., 2008).

To the best of our knowledge, there is no study that has compared high and low GI meals, or prescribed low GI meal alone while employing these measurements. This makes it difficult to hypothesise whether/how GI plays a role in the development of CF. Possible way that GI could affect CF is via providing more energy source to the brain through different fuel selection by increasing blood glucose (as seen in higher glucose AUC following HGI meal in Chapter 3) to be transported to the brain; and subsequently through the suppression of fatigue inducing neurotransmitter, serotonin. Results from Chapter 4 have shown that serotonin increases during exercise, but with a high amount of CHO consumed prior to the exercise, the rise was blunted. Since there were no difference in energy expenditure despite significant differences in fat oxidation between HCHO and LCHO, it doesn't seem that the fuel supply was affected by those meals. However, when insulin was increased significantly in HCHO compared to LCHO, there was also an increase in serotonin concurrent with reduction of VA and CAR only in the HCHO. While this data is too little to conclude role and relationship of CHO and serotonin on CF, it is interesting to investigate if similar amount of CHO with different GI would illicit similar responses. Since high GI meal promotes insulin surge (DeMarco *et al*., 1999; Wee *et al*., 2005), lipolysis and serotonin could be suppressed, possibly affecting serotonin in the same manner that HCHO meal does (see Chapter 4).

5.3 Methodology

5.3.1 Participants' recruitment

The University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC – 41) (Appendix C) approved this study. Male participants were recruited via advertisement in posters and social media. Volunteers were informed of the study procedures, possible risks, discomforts, and provided written information sheet (Appendix E). Those who agreed to participate signed a consent form (Appendix D) and completed an ACSM risk stratification screening questionnaire (Appendix F), to ensure that the participants are not in risk to conduct exercise testing.

The sample size was determined using the PS Power and Sample Size Calculation software (Version 3.0, Tennessee, United States) (Dupont & Plummer Jr, 1998) matched to Nybo's (2003) study, where: (a) alpha value is 0.05, (b) power was 0.80, (c) difference in population mean was 25, (d) standard deviation was 20.5, and the (e) ratio is 1. Overall sample size calculated in this study was 12 participants.

Sixteen participants (n = 16) whose maximal oxygen consumption (VO_2max) was at least 50 ml/kg/min were recruited for this study. All participants were not smokers for at least the last six months, on weight reducing diet, diagnosed with cardiovascular or metabolic disease and consuming medication or drugs known to influence lipid or carbohydrate metabolisms. They underwent both VO_2max test followed by a familiarisation session after being included in the study. During the familiarisation, each participant went through the same procedure of the experimental trials, including 90-minute running at 70% VO_2max, MVC, electrical stimulation, and body weight measurements. Two participants withdrew from the study midway, leaving fourteen participants (n = 14) who completed the experiments.

5.3.2 Study design

In a double-blind, randomised, cross-over design separated by 7-day washout-period, participants underwent two experimental trials. Participants were given LGI and HGI meals in random order prepared by a lab technician independent of this study. The researcher were only given the order for each participants at the end of this study. Participants also fill in the 24 hours' diet recall questionnaire and replicate the same meal intake, and restrained from intense exercise, caffeine, and alcohol 24 hours prior to the test. All running trials were performed during similar time of the day in a controlled laboratory environment.

5.3.3 Preliminary measurements (anthropometry and VO_2max test)

Preliminary measurements included measurement of height (SECA measurement scale, USA); measurement of participants' body composition and BMI (InBody 370 Body Composition Analyzer, USA); and measurement of VO_2max.

Participants underwent VO_2max test using a submaximal protocol of continuous, incremental running test to determine their VO_2max on a treadmill (H/P Cosmos Quasar). Participants ran 4 stages, beginning at 8 km/h, with an increment of 2 km/h every 3-minute. Gaseous exchange data were collected using a cardiopulmonary exercise testing device (COSMED Quark CPET, Rome, Italy). Heart rate was collected using a heart rate monitor (Polar, Finland) strapped at the chest. VO2max was extrapolated based on VO_2 and heart rate plot. Running speed for their respective trials was then determined from this VO_2max value.

5.3.4 Pre-exercise meals

The meals prescribed in this study are high and low GI isocaloric meals with identical amount of CHO (1.5 g/kg body weight), fat, and protein: LGI (GI: ~40) meal consisting of slow, steadily absorbed CHO; and HGI (GI: ~75) meal consisting of fast reacting and absorbed CHO. Meal compositions were calculated using the NutriPro software (Axxya Systems), GI of the food were obtained from the International Table of Glycemic Index and Glycemic Load Values (Foster-Powell, Holt, & Brand-Miller, 2002) and manufacturer; while GI of meals were calculated using Equation 5.1 (Wolever *et al*., 2006); where n was the number of CHO-containing foods in the meal, GIa = GI of the food a, gAvCHOa was grams of available carbohydrate in the food a, and GAvCHO was grams of available carbohydrate in the entire meal.

GI of meal = (GIa x gAvCHOa/GAvCHO) + (GIb x gAvCHOb/GAvCHO) +

(GIb x gAvCHOb/GAvCHO) …*Equation 5.1*

The meals were selected randomly and prepared by a nutritionist, with the researcher not knowing the order of meals taken during the experiment. All participants finished the meals.

Table 5.1. Sample Meals for a 70-kg participant:

	HGI Meal	**LGI Meal**
Food	20 g jam, 250 ml skimmed milk, 40 g white bread, 50 g corn flakes, 200 ml Gatorade©	160 g apple, 150 ml apple juice, 65 g bran flakes, 300 ml skimmed milk

Note: HGI; high glycaemic index, and LGI; low glycaemic index.

5.3.5 Experimental trials

Upon arrival at the lab, participants had their fasting neurophysiological measurements (see subchapter 5.3.6 for details) and fasting blood (see subchapter 5.3.7) collected. They were then provided with the designated meals (consumed within 20-minute) and after that rested an hour. Participants were seated with minimal physical activity during this one-hour post-prandial period. After the rest, a blood sample was obtained right before the commencement of the 90-minute run at 70% VO_2max. The intensity chosen was higher than that used in Chapter 4 after it was found during pilot tests that with 65% VO_2max, there were no differences in post exercise MVC between groups; 70% VO_2max intensity induced the differences. Participants were also required to consume 500-ml of mineral water 15-minute prior to exercise testing to prevent dehydration. A 1-minute gaseous exchange was collected every 15-minute during the run. Upon completion of the run, blood sample and neurophysiological measurements were again collected immediately. The flow of this experimental trial is presented in Figure 5.1.

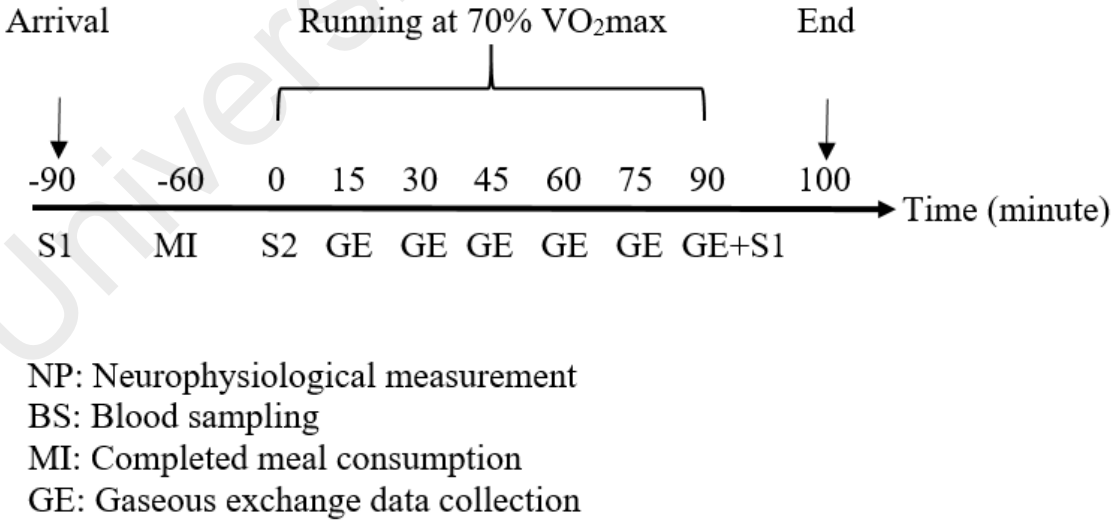

Figure 5.1. Experimental flow.

5.3.6 Neurophysiological data collection procedures

EMG. Skin surface was prepared by shaving, abrasion, and cleaning with alcohol. After that, Delsys Trigno Wireless self-adhesive electrodes were positioned on the lateral gastrocnemius muscles at 1/3 of the line between the head of the fibula and the heel of the dominant leg as recommended by surface EMG for non-invasive assessment of muscles (SENIAM) guidelines (Stegeman & Hermens, 2007). **M-wave recording**. The stimulation site for tibial nerve was located using a hand-held stimulation probe, where the location with the highest EMG reading being selected to place the stimulation electrode. One-ms pulse was delivered by a constant-current stimulator beginning at 30 mV, followed by 5-mV increments until there was no further increase in EMG response from the efferent fibre in 3 consecutive stimuli, and the average value of these 3 stimulations was established as Mmax. **MVC.** The participants were placed in the prone position on a dynamometer (HUMAC NORM device, Massachusetts, USA) with their ankles at an anatomically zero position, and secured with straps to avoid inversion and/or eversion movements. Participants performed one set of maximal isometric ankle plantarflexion contraction, followed by a minute of rest. The highest torque produced from 3 sets was established as MVC. A sustained maximal isometric ankle plantarflexion was then performed for 90 seconds, during which the participants were verbally encouraged to maintain their maximal effort. **VA, CAR, & resting twitch.** Electrical stimulation was superimposed at the peak force of MVC, and at the 5, 30, 60, and 90th seconds of the sustained MVC, and right after contraction ceases in both MVC and sustained MVC to obtain the superimposed resting twitch. The electrical stimulus used was 20% higher than that used to stimulate Mmax, with the same intensity used throughout the trials. CAR was measured by dividing highest force achieved voluntary over sum of highest force achieved voluntary + superimposed twitch at each time point.

VA was measured in MVC by 1-(superimposed twitch at MVC/resting twitch); and in the sustained MVC by 1-(superimposed twitch at 90th second/resting twitch).

5.3.7 Serum preparation and analysis

Blood samples were drawn from participants' superficial vein on the dorsal side of hand using a butterfly catheter, and transferred into plain serum vacutainer (BD, USA). Blood samples was centrifuged at 3000 rpm for 15 minutes at 4 °C (Heraeus™ Multifuge™ X1R Centrifug, USA) and subsequently aliquoted into 1500 ml Eppendorf tubes.

5.3.8 ELISA analysis for FFA, insulin, serotonin, and tryptophan

The concentrations of markers were assayed and determined using commercially available enzyme-linked immunosorbent assays (ELISA) kits for FFA (EnzyChrom, BioAssays, USA), insulin (Insulin ELISA Kit, LDN, Germany), serotonin (Serotonin ELISA Kit, LDN, Germany), and tryptophan (Tryptophan ELISA Kit, LDN, Germany) based on the manufacturers' instructions. The assays were analysed using a microplate spectrophotometer (EPOCH, BioTek), and results were plotted against standard concentration which forms a linear AUC to obtain the marker's concentration value. The CV of those kits are 0.79, 0.29, 0.61, and 0.53 respectively.

5.3.9 Statistical analysis

All statistical analyses were performed using SPSS 20.0 (IBM Corp. Armonk, NYN). Data normality was examined and confirmed with $p > 0.05$ using the Shapiro-Wilks test. A two-way analysis of variance (ANOVA) and post-hoc analysis (Bonferoni test) was used to determine the effect of measured parameters and time and the changes of parameters over the time. Paired t-test was used when comparing only means of two groups. Statistical significance was set and accepted at $p < 0.05$. Data are presented as mean values and standard deviation (mean ± SD).

5.4 Results

5.4.1 Participants' anthropometrics

The anthropometrics data of participants who completed the study (n = 14) are presented in Table 5.2. The mean body mass index (BMI) of the participants (21.6 ± 2.6 kg/m^2) were within the normal range (18.5 – 25.0 kg/m^2).

Table 5.2 Anthropometric measures of participants. Values are mean ± SD (n = 14).

Variables	Value
Age (year)	27.1 ± 2.9
Weight (kg)	65.7 ± 9.0
Height (cm)	171.5 ± 6.6
Lean Muscle Mass (%)	45.8 ± 4.6
Body Fat Percentage (%)	11.3 ± 7.3
BMI (kg/m^2)	22.3 ± 1.8
VO_2max (ml/kg/min)	54.6 ± 5.2

Note: values are mean ± SD, and BMI; body mass index.

5.4.2 Nutrients consumed

The mean nutrient composition of the meals consumed by participants during the testing sessions are presented in Table 5.3.

Table 5.3 Macronutrients, energy intakes, and percentage (%) of energy from macronutrients of HGI and LGI meals consumed by participants 1-hour prior to the 90-minute run at 70 % VO_2max.

	HGI Meal	**LGI Meal**
GI	40	75
Energy (kcal)	528.9 ± 72.1	525.3 ± 71.6
% of calories from CHO	78.4	77.9
% of calories from fat	4.6	4.9
% of calories from protein	17.0	17.1
Amount of CHO (g)	97.5 ± 13.3	97.5 ± 13.3
Amount of fat (g)	21.2 ± 2.9	21.4 ± 2.9
Amount of protein (g)	2.6 ± 0.4	2.8 ± 0.4

Note: values are mean ± SD (n = 14), and GI; glycaemic index, and CHO; carbohydrate.

5.4.3 VO_2 during 90-minute run

The participants' VO_2 at every 15-minute interval during the 90-minute run are presented in Table 5.4. There was no significant difference between and within group at all time points; VO_2 was maintained at a similar level (~70 ± 3% of VO_2max) throughout the 90-minute run at both trials for all participants (Figure 5.2).

Table 5.4 VO_2 of the LGI and HGI group at every 15-minute interval during 90-minute run. Values are mean ± SD (n = 14)

Time point (minute)	**VO_2 (ml/kg/min)**	
	LGI	**HGI**
15	39.9 ± 5.1	37.1 ± 4.7
30	39.8 ± 3.3	37.6 ± 3.7
45	39.1 ± 3.7	39.0 ± 3.3
60	39.7 ± 4.1	38.3 ± 3.7
75	37.5 ± 4.6	37.5 ± 3.8
90	38.5 ± 4.2	38.5 ± 3.8

Note: LGI; low glycaemic index, and HGI; high glycaemic index.

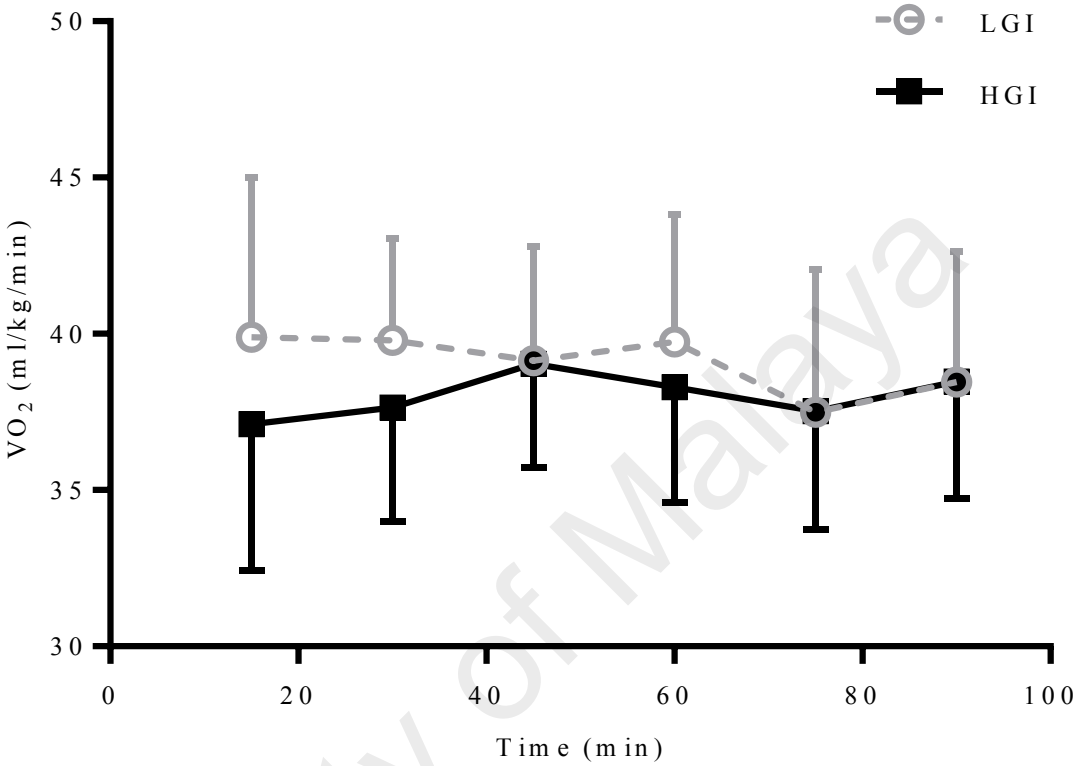

Figure 5.2 Oxygen consumption (VO_2; ml/kg/min) at 15-minute interval throughout the 90-minute run at 70% VO_2max in the LGI; low glycaemic index (-⊖-) and HGI; high glycaemic index (—■—). Values are mean with SD (n = 14). VO_2; Volume of oxygen uptake.

5.4.4 CHO and fat oxidation during 90-minute run

5.4.4.1 Rate of CHO and fat oxidation

Rate of CHO (Figure 5.3) and fat oxidation (Figure 5.4) at every 15-minute interval during the 90-minute run are presented in Table 5.5. Total energy expenditure was significantly higher in HGI (1144.11 ± 187.40 kcal) compared to LGI (1066.19 ± 151.14 kcal). The fat oxidation was significantly higher in LGI group, while CHO oxidation was significantly higher in the HGI group (Figure 5.5).

Table 5.5 Rate of CHO and fat oxidation at 15-minute interval throughout the 90-minute run at 70% VO_2max in the LGI and HGI.

Time point (minute)	**CHO oxidised (g/min)**		**Fat oxidised (g/min)**	
	LGI	**HGI**	**LGI**	**HGI**
Baseline	0.43 ± 0.47	0.39 ± 0.28	0.27 ± 0.28	0.24 ± 0.11
15	1.54 ± 0.62	2.01 ± 0.65*	0.62 ± 0.28	0.42 ± 0.17*
30	1.32 ± 0.60	1.92 ± 0.42*	0.73 ± 0.26	0.48 ± 0.19*
45	1.23 ± 0.44	1.81 ± 0.36*	0.77 ± 0.24	0.55 ± 0.18*
60	1.28 ± 0.51	1.75 ± 0.54*	0.75 ± 0.31	0.53 ± 0.19*
75	1.23 ± 0.48	1.56 ± 0.55*	0.73 ± 0.30	0.55 ± 0.19*
90	1.21 ± 0.31	1.55 ± 0.41*	0.74 ± 0.18	0.59 ± 0.16*

Values are mean ± SD (n = 14). *denotes significant difference ($p < 0.05$) compared to LGI. CHO; carbohydrate, LGI; low glycaemic index, and HGI; high glycaemic index.

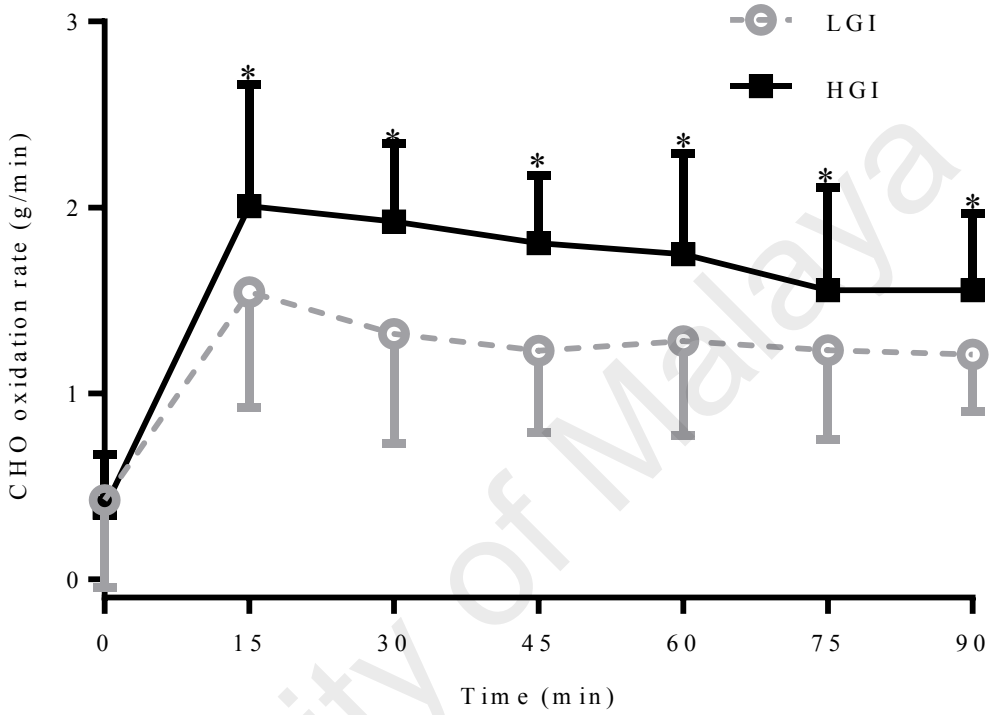

Figure 5.3 Rate of CHO oxidation (g/min) at 15-minute interval throughout the 90-minute run at 70% VO_2max in the LGI; low glycaemic index (-⊖-) and HGI; high glycaemic index (—■—). Values and mean (n = 14), *denotes significant difference ($p < 0.05$) compared to LGI.

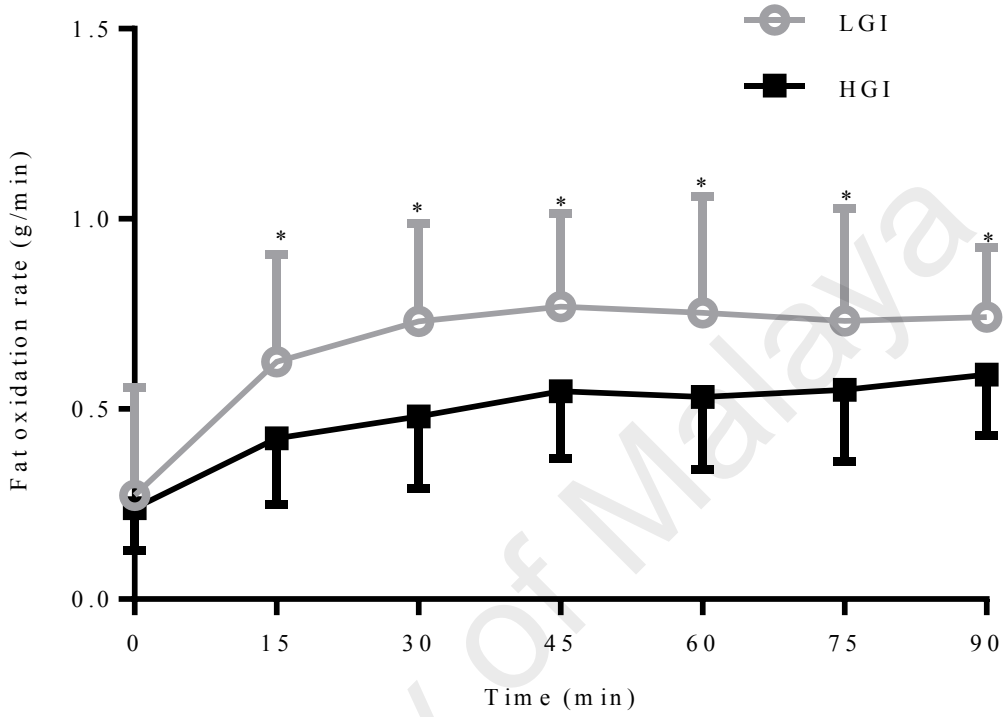

Figure 5.4 Rate of fat oxidation (g/min) at 15-minute interval throughout the 90-minute run at 70% VO_2max in the LGI; low glycaemic index (-⊖-) and HGI; high glycaemic index (—■—). #denotes significant difference ($p < 0.05$) compared to HGI.

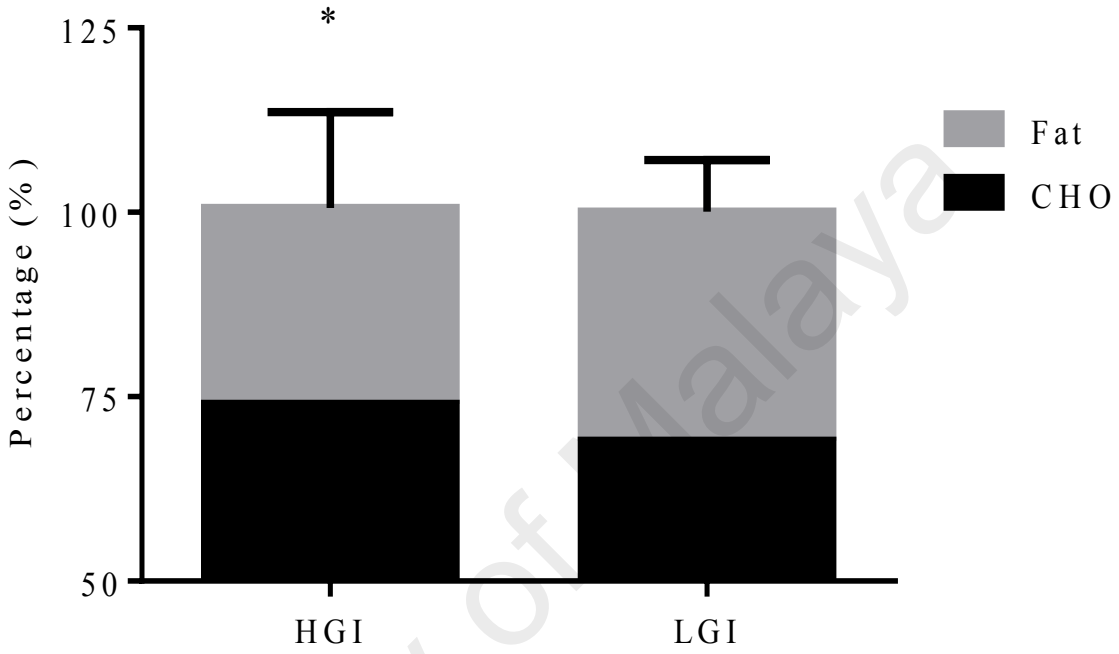

Figure 5.5 Percentage (%) of calories derived from fat and carbohydrate (CHO) during the 90-minute run at 70% VO_2max in the high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean (n = 14). *denotes significant difference ($p < 0.05$) compared to LGI.

5.4.4.2 Respiratory exchange ratio (RER)

RER at every 15-minute interval during the 90-minute run are presented in Table 5.6. RER was significantly higher in the HGI than LGI group at 75th and 90th minute of the run.

Table 5.6 Respiratory exchange ratio (RER) at 15-minute interval throughout the 90-minute run at 65% VO_2max in LCHO and HCHO.

Time point (minute)	**RER**	
	LGI	**HGI**
Baseline	0.80 ± 0.05	0.81 ± 0.05
15	0.87 ± 0.04	0.87 ± 0.06
30	0.87 ± 0.05	0.86 ± 0.06
45	0.87 ± 0.04	0.85 ± 0.03
60	0.86 ± 0.05	0.86 ± 0.06
75	0.86 ± 0.05*	0.90 ± 0.03
90	0.85 ± 0.05*	0.92 ± 0.04

5.4.5 MVC

The participants' force at MVC, and at the 5th and every 30-second interval during the 90-second sustained MVC, are presented in Table 5.7 and illustrated in Figure 5.6. In both HGI and LGI groups, MVC and MVC at the 5th second was significantly lower post 90-minute running. However only in LGI group that the MVC at 30th second was significantly lower post 90-minute running. Main effect across time was found ($F_{3,64}$ = 2.87, p = 0.02).

Table 5.7 MVC force generated during the 90-second sustained MVC for HGI and LGI measured at fasted state (Pre) and immediately after 90-minute run at 70% VO_2max (Post).

Time point (second)	**MVC (N/m)**			
	HGI		**LGI**	
	Pre	**Post**	**Pre**	**Post**
MVC				
	90.08 ± 31.07	77.77 ± 32.86*	91.46 ± 31.00	74.85 ± 28.50*
90-second sustained MVC				
5	69.45 ± 18.56	56.50 ± 12.95*	66.63 ± 15.24	54.64 ± 15.91*
30	48.43 ± 14.66	43.27 ± 12.33	51.21 ± 15.03	42.70 ± 16.28*
60	42.97 ± 12.31	40.52 ± 11.66	45.60 ± 15.09	39.69 ± 15.62
90	40.34 ± 13.20	36.25 ± 11.01	41.85 ± 13.86	35.46 ± 14.62

Note: values are mean ± SD, *denotes significant difference (p < 0.05) from Pre. MVC; maximum voluntary contraction, HGI; high glycaemic index, and LGI; low glycaemic index.

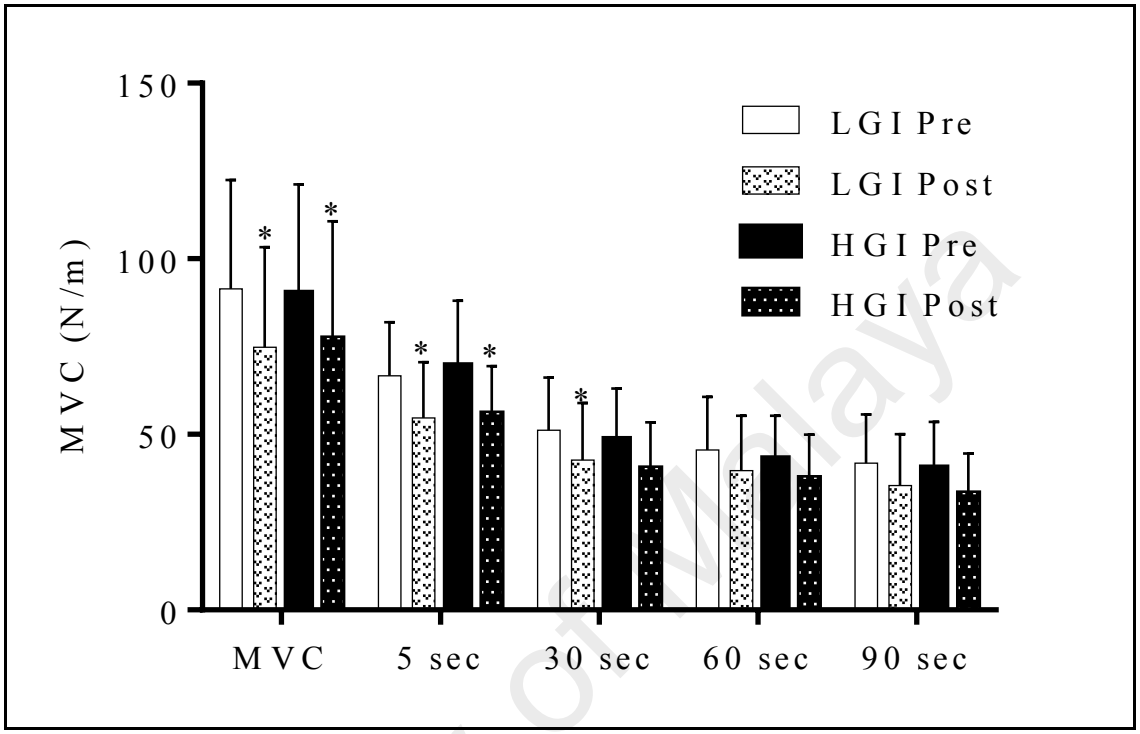

Figure 5.6 Maximum voluntary contraction (MVC) force generated during the 90-second sustained MVC for high glycaemic index (HGI) and low glycaemic index (LGI) measured at fasted state (pre) and immediately after 90-minute run at 70% VO_2max (post). *Denotes significant difference ($p < 0.05$) compared to Pre.

5.4.6 CAR, VA, resting twitch, and Mmax

The participants' CAR at the 5th and every 30-second interval during the 90-second sustained MVC; VA of MVC and at 90th second of sustained MVC; VA at MVC; resting twitches at right after sustained MVC; and Mmax are presented in Table 5.8. There was no significance difference in VA between both groups, though both groups' VA at Post was lower compared to Pre when measured at MVC (Figure 5.7). VA when measured at 90th second of sustained MVC did not produce between group differences. In LGI group, the CAR was reduced at 5 and 30th seconds (Figure 5.8). Resting twitch was also reduced in both groups.

Table 5.8 CAR and VA during the 90-second sustained MVC; VA, resting twitch, and Mmax for HGI and LGI trials measured at fasted state (pre) and immediately after 90-minute run at 70% VO_2max (post).

Time point (second)	HGI		LGI	
	Pre	Post	Pre	Post
	CAR			
5	0.97 ± 0.03	0.96 ± 0.03	0.97 ± 0.02	0.94 ± 0.03*
30	0.93 ± 0.04	0.94 ± 0.04	0.93 ± 0.03	0.91 ± 0.03*
60	0.93 ± 0.03	0.93 ± 0.04	0.93± 0.03	0.91 ± 0.04
90	0.94 ± 0.04	0.93 ± 0.05	0.93 ± 0.03	0.91 ± 0.04
	VA			
90	0.55 ± 0.23	0.50 ± 0.25	0.42 ± 0.29	0.39 ± 0.30
At MVC	87.2 ± 7.75	71.50 ± 18.91*	91.28 ± 4.30	79.58 ± 15.25*
	Resting twitch			
90	13.26 ± 5.22	10.21 ± 4.13*	13.26 ± 3.33	11.61 ± 2.68*
At MVC	13.51 ± 5.48	10.27 ± 4.46*	13.51 ± 3.57	11.94 ± 3.57*
	Mmax			
	7.12 ± 1.81	6.98 ± 1.72	7.28 ± 1.89	6.79 ± 1.67

Note: values are values are mean ± SD (n = 14). *denotes significant difference ($p < 0.05$) compared to Pre. HLGI; high glycaemic index, LGI; low glycaemic index, CAR; central activation ratio, VA; voluntary activation, and Mmax; maximal compound muscle action potential.

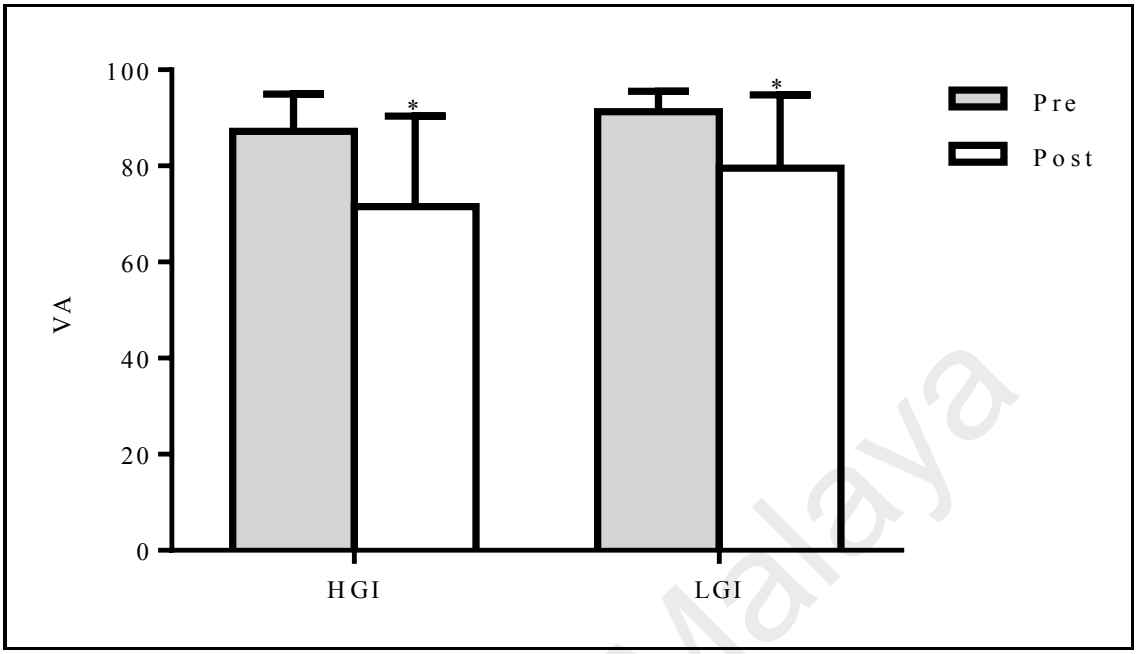

Figure 5.7 Voluntary activation (VA) obtained during maximum voluntary contraction (MVC) at pre and post for high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean ± SD (n = 14). *denotes significant difference ($p < 0.05$) compared to pre.

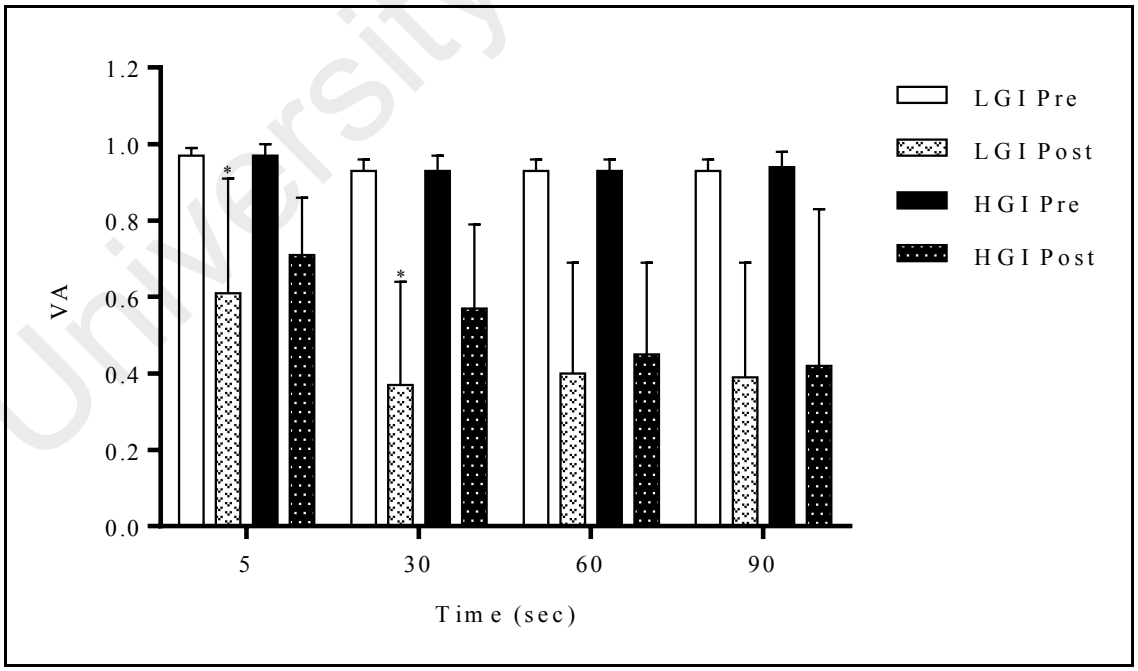

Figure 5.8 Central activation ratio (CAR) during 90-second sustained maximum voluntary contraction (MVC) sustained MVC for high glycaemic index (HGI) and low glycaemic index (LGI) measured at fasted state (pre) and immediately after 90-minute run at 70% VO_2max (post). Values are values are mean ± SD (n = 14). *denotes significant difference ($p < 0.05$) compared to pre.

5.4.7 Serum FFA, insulin, serotonin, and tryptophan

The concentration of blood markers at all time points analysed are presented in Table 5.9. Four participants had difficulty drawing blood during at least on one of the experimental trials, and their samples were excluded from the blood analysis. There were ten (n = 10) participants' blood samples included in the final analysis.

Table 5.9 Serum FFA, insulin, serotonin, and tryptophan concentration at fasting state (T0), 1-hour post-prandial and immediately prior to 90-minute run (T1), and post-running (T2).

Marker	HGI			LGI		
	T0	T1	T2	T0	T1	T2
FFA (μM)	264.75 ± 38.82	355.5 ± 91.03	314.17 ± 67.34	279.25 ± 40.62	665.50 ± 324.44*#	579.75 ± 382.36*#
Insulin (IU/ml)	6.83 ± 1.00	33.07 ± 13.12*^	10.95 ± 8.34	8.52 ± 5.10	22.07 ± 7.18*^#	9.98 ± 3.90
Serotonin (nmol)	271.97 ± 63.73	424.25 ± 68.65	440.72 ± 68.42	251.60 ± 58.24	404.43 ± 94.52	427.81 ± 72.08
Tryptophan (μmol/L)	53.48 ± 11.31	53.33 ± 11.59	42.64 ± 14.89**	50.02 ± 12.46	45.39 ± 17.69	34.99 ± 18.56**

Note: values are mean ± SD. *denotes significant difference ($p < 0.05$) compared to T0, and **significant difference ($p < 0.05$) compared to T0 and T1, and ^significant difference ($p < 0.05$) compared to T2, and #significant difference ($p < 0.05$) compared to HGI. FFA; free fatty acids, HGI = high glycaemic index, and LGI; low glycaemic index.

5.4.7.1 FFA

FFA concentration was significantly increased at 1 hour post-prandial and immediately prior to 90-minute run (T1) compared to fasting value (T0) in the LGI group, while in the HGI group FFA concentration did not differ significantly across all time points (Figure 5.9). Main effect across time was found ($F_{3,78} = 3.17$, $p = 0.04$).

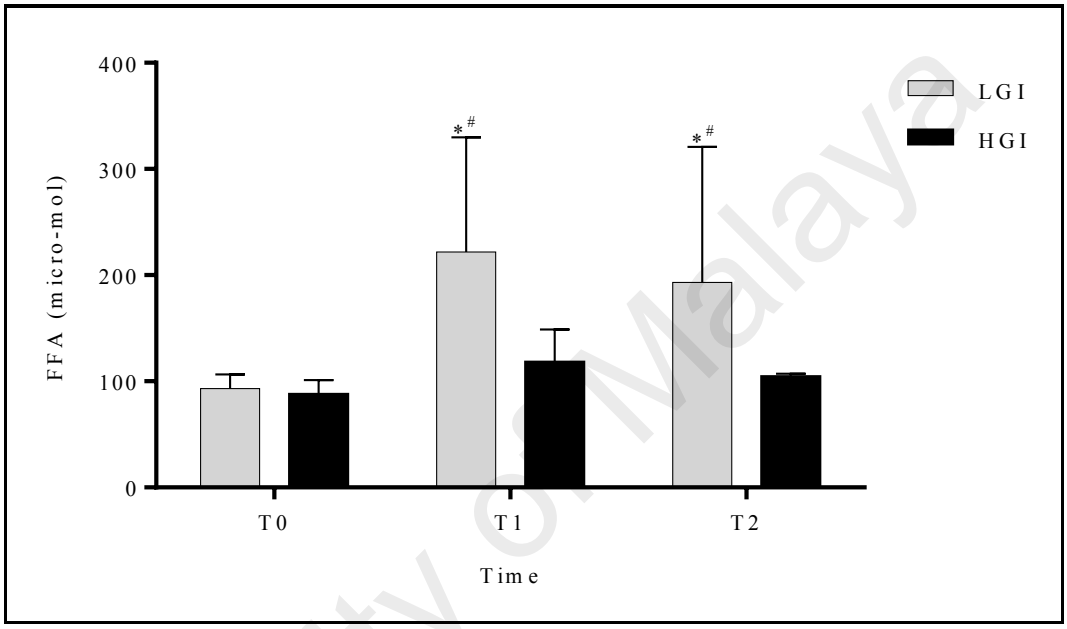

Figure 5.9 Free fatty acids (FFA) concentration (µmol) at T0 (fasting state), T1 (1-hour post-prandial and immediately prior to 90-minute run), and T2 (post-running) for high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean ± SD (n =10), and *denotes significant difference ($p < 0.05$) compared to T0, and #significant difference compared to HGI.

5.4.7.2 Insulin

Both LGI and HGI groups had a similar change in insulin concentration where there was a significant increase at 1-hour post-prandial (T1) compared to fasting value (T0), and thereafter significantly reduced at post 90-minute running at 70% VO_2max (T2) compared to T1. However, the magnitude of increase from Fasting value (T0) to T1 was significantly higher in the HGI group (Figure 5.10). Main effect across time was found ($F_{2,58} = 2.46$, $p = 0.02$).

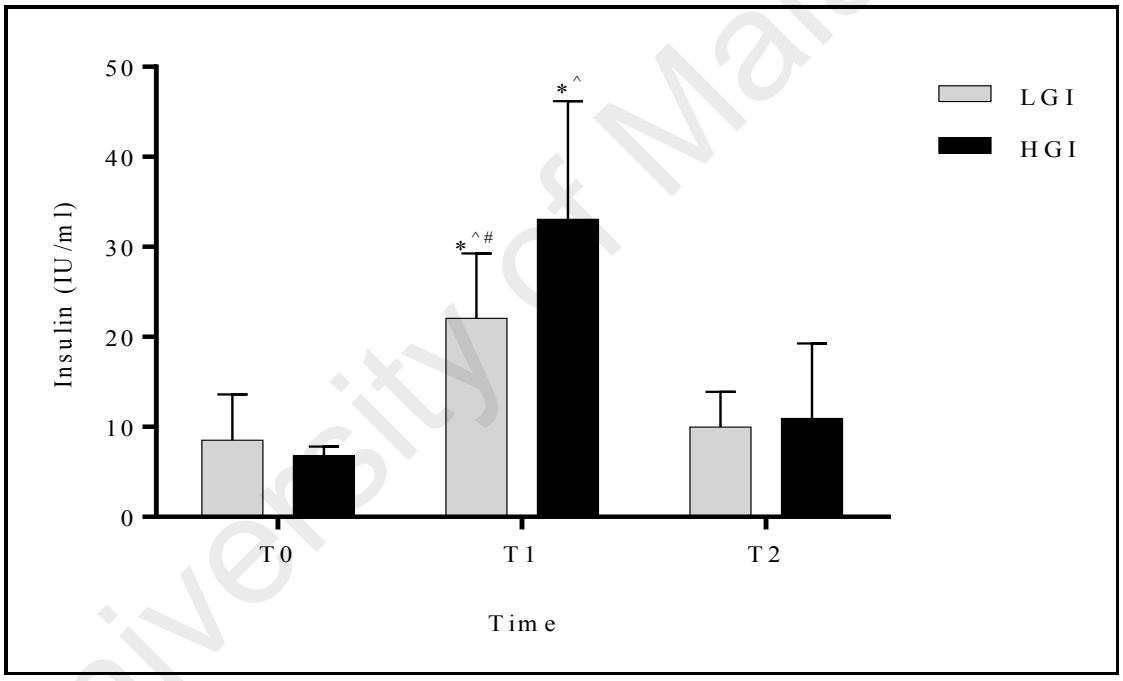

Figure 5.10 Insulin concentration (IU/ml) at T0 (fasting state), T1 (1-hour post-prandial and immediately prior to 90-minute run), and T2 (post-running) for high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean ± SD (n = 10), *denotes significant difference ($p < 0.05$) compared to T0; ^denotes significant difference ($p < 0.05$) compared to T2; while # denotes significant difference compared to HGI.

5.4.7.3 Serotonin

Serotonin concentration did not change significantly across fasting value (T0), to post 90-minute running at 70% VO_2max (T2), although the changes were bigger from T0 to 1-hour post-prandial (T1) (increment of 35.90% for HGI and 37.80% for LGI) compared to from T1 to T2 (increment of 3.74% for HGI and 5.46% for LGI) (Figure 5.11).

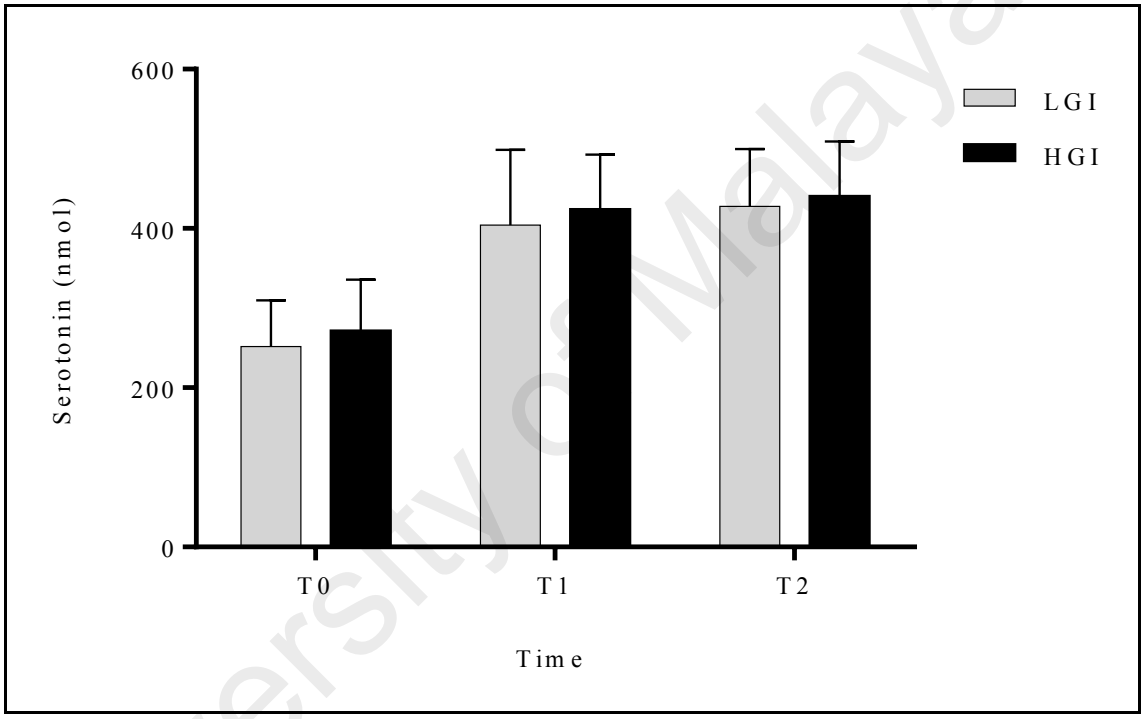

Figure 5.11 Serotonin concentration (nmol) at T0 (fasting state), T1 (1-hour post-prandial and immediately prior to 90-minute run), and T2 (post-running) for high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean ± SD (n = 10).

5.4.7.4 Tryptophan

Tryptophan concentration in both LGI and HGI changed in the same manner, where it remained the same from fasting value (T0) to 1-hour post-prandial (T1), but dropped significantly at post 90-minute running at 70% VO_2max (T2) (Figure 5.12).

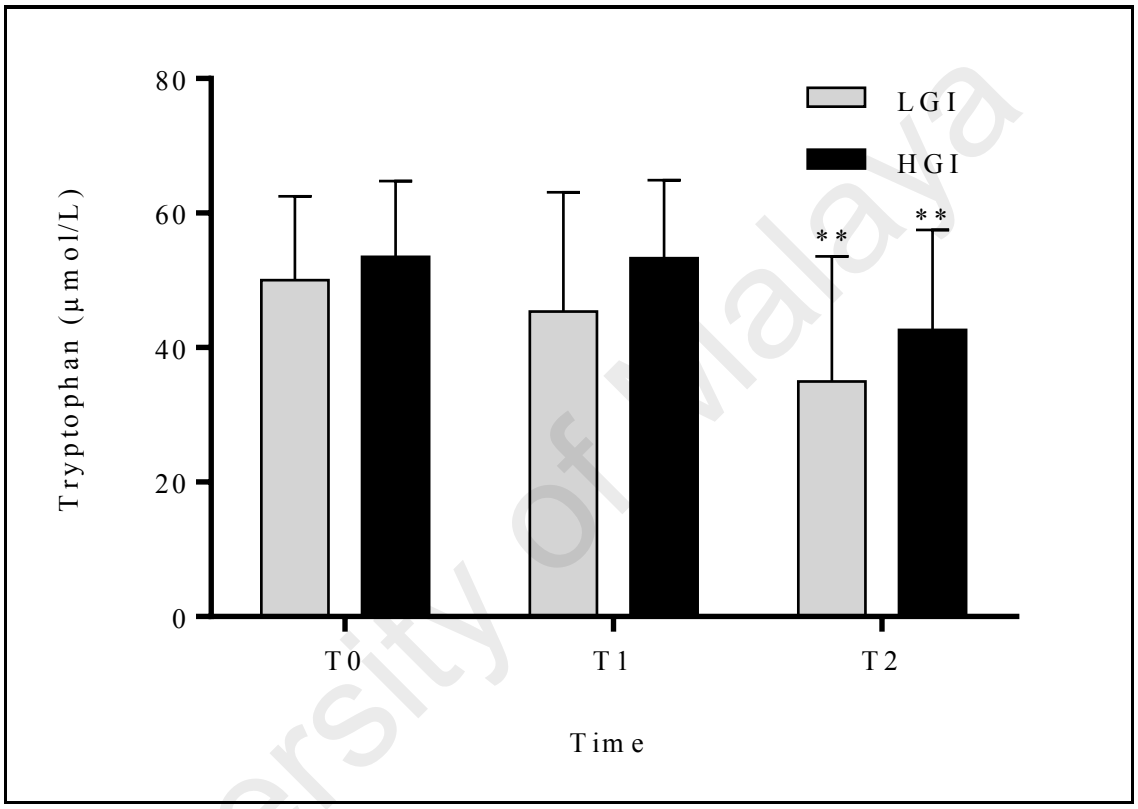

Figure 5.12 Tryptophan (nmol) at T0 (fasting state), T1 (1-hour post-prandial and immediately prior to 90-minute run), and T2 (post-running) for high glycaemic index (HGI) and low glycaemic index (LGI). Values are mean ± SD (n = 10). **denotes significant difference ($p < 0.05$) compared to T0 and T1.

5.5 Discussion

The objective of this study was to determine the effects of pre-exercise meal with similar quantity of macronutrients with high amount of CHO but differing GI on CF following an endurance exercise. The results demonstrated that after 90-minutes running at 70% VO_2max, MVC was better maintained in HGI, proving the first hypothesis; while VA at MVC and sustained MVC were both similar between groups, CAR was better maintained with HGI, proving some part of the second hypothesis. There was a markedly increased rise in FFA alongside the drop in tryptophan, proving the third hypothesis; however, serotonin did not change across the whole experiment in both groups, nullifying the fourth hypothesis.

5.5.1 Changes in MVC, CAR, VA, resting twitch and Mmax after the 90-min run with different GI

Loss in maximal force generation capacity during MVC is an indication of fatigue (Bigland-Ritchie *et al*., 1981). Present study found that at 70% VO_2max, reduction of MVC occurred regardless of GI. However, during the sustained MVC, maximal torque was reduced in LGI at the 5 and 30th seconds, while HGI preserved MVC during the contraction at 30, 60 and 90th seconds which is similar to Chapter 4 despite the higher intensity (70 compared to 65%) (Figure 5.6). This is interesting considering the similar nutrient content of both meals which consisted of mainly HGI foods. The reduction of MVC in both groups can be attributed to a mixture of peripheral and central factors; even though Mmax of the gastrocnemius muscle remain the same, resting twitch was reduced indicating a loss in the cumulative muscles activation capability, while VA measured at MVC was reduced, signifying reduced neural drive to the motoneuron pool which could originate from spinal and/or supraspinal sites. However, CF seems to be more pronounced in the LGI group: when tested on 90-second sustained MVC, force was reduced at 5 and

30th second in concomitant with CAR, while in HGI group MVC was only reduced at the 5th second without any CAR reduction. Serotonin, another possible CF indicator, did not change throughout the experimental period, despite the decrease in tryptophan for both trials which could have led to synthesis of more serotonin. It seems that serotonin level increased following rise in FFA via a bigger exogenous fat consumption (Chapter 4), but not through promotion of fat oxidation via a LGI meal with high amount of CHO. The tryptophan could have been used instead for other purposes such as formation of nicotinamide-adenine-dinucleotides (NAD, NADH) in the kynurenine pathway (Badawy, 2017). In addition, the better MVC preservation in HGI could also not be attributed to the conventional believe where LGI improves endurance exercise performance. While LGI has been proposed to improve endurance performance via promotion of fat oxidation and thus sparing glycogen, the benefits can only be observed if the test involved is endurance in nature such as time to exhaustion of time trial. Hence changes in MVC that seems to benefit from HGI pre-exercise meal could be due to the fact that HGI promotes CHO oxidation, which is the major energy source during maximal muscular contraction. Since present study is unlikely to exhaust the muscle glycogen content as the running session was less than 3 hours (Bergstrom *et al*., 1967), it can be speculated that when muscle glycogen is still available, the HGI better preserves force production capability compared to LGI, which could translate to better speed production during running (Silvers, Bressel, Dickin, Killgore, & Dolny, 2014). It seems that at 70% VO_2max, CF was preserved with a high CHO, high GI meal similar to Chapter 4, but when the type of CHO was changed to LGI, the CF was more pronounced. The fact that pre-exercise HGI consumption improved force production capability post 90-minute running could indicate a need for more meticulous GI selection for an event based on time and intensity. If an exercise is not lengthy or intense enough to exhaust glycogen, HGI could be more beneficial as it allows for higher muscle drive after 90-minutes of exercise.

5.5.2 CHO and fat oxidation during the 90-minute run at 70% VO_2max

Present study showed that CHO oxidation was favoured in the HGI, while fat oxidation was favoured in LGI. The HGI also produced a higher insulin response post-prandial compared to LGI. These results are consistent with the literature which showed that LGI meal promotes fat oxidation (Chen *et al*., 2008; Febbraio *et al*., 2000; Wee *et al*., 1999) with smaller insulin reaction curve (DeMarco *et al*., 1999; Wee *et al*., 2005). As both HGI and LGI provided similar amount of CHO, changes in the fuel selection could only be attributed to the difference in insulin response. The increased fat usage in LGI was deduced from higher fat oxidation observed in the gaseous exchange data, accompanied by increased blood FFA with a lesser insulin surge compared to HGI. As CHO-induced insulin rise has been shown to reduce the rate at which long-chain fatty acid entrance into mitochondria for oxidation (Coyle, Jeukendrup, Wagenmakers, & Saris, 1997), the lower insulin rise with LGI better promotes fat oxidation compared to HGI. This also shows that GI causes the change in fuel selection by strictly affecting the insulin rise, as there were no differences in amount of macronutrients provided between HGI and LGI pre-exercise meals.

In present study, the energy expenditure was significantly higher in the HGI, with concurrently higher CHO oxidation compared to LGI. While this is different from a past study that found no difference in energy expenditure during a 70% VO_2max run after consuming low or high GI pre-exercise meals (Wee *et al*., 2005), said study only had its participants ran for 30-minute, while present study had a 90-minute running. The longer duration might have caused the body to oxidise more fuel to keep up with the muscle's energy demand, and if the run was prolonged, the higher energy expenditure in HGI might cause exhaustion of glycogen supplies earlier than LGI, as reported by several studies (Demarco *et al*., 1999; Wu *et al*., 2006; Thomas *et al*., 1991). Should that happened, a bigger difference would be detected in MVC, neurophysiological measures, and blood

markers, hence a longer exercise duration, or an exercise to exhaustion protocol could be employed in future study to further examine whether GI plays a role in CF during prolonged exercise. It is also worth noting that while the post-prandial insulin was lower and post-prandial FFA and serotonin was higher after LCHO in Chapter 4, the LGI did not led to higher serotonin despite observing similar FFA and insulin responses. This is despite an increase in serotonin's precursor, tryptophan too. This tryptophan could have been used for other purpose, such as formation of nicotinamide-adenine-dinucleotides (NAD, NADH) in the kynurenine pathway (Badawy, 2017). This in some way indicates that there might be other mechanisms that caused the changes in serotonin, which could have led to the more pronounced CF seen in Chapter 4. At current stage it seems that amount, CHO influences serotonin and CF much more than GI.

5.6 Conclusion

Present study is the first to investigate changes in MVC, CAR, VA, resting twitch and Mmax with low and high GI pre-exercise meals. While past studies on GI and prolonged exercise had focused on measuring time to exhaustion as performance outcome (DeMarco *et al*., 1999; Wu *et al*., 2006); no data is available for any CF markers. There were some hints about effects of high GI CHO on CF using CAR and VA as measured by Nybo (2003) and Del Coso *et al*. (2008), but those studies employed single type of CHO food (CHO drink and sports drink respectively), and did not have a low GI CHO food to contrast the findings.

In the present study, VA during a normal MVC was also collected alongside VA at 90th second of the sustained contraction. The results indicated that a sustained contraction is needed to access the loss of muscle force production capability, as no between group differences was detected with typical MVC, and during the first 5 seconds of sustained MVC. This is a valuable information for studies going forward in employing MVC alongside superimposed twitches, as the presence of CF could be missed if only a short, quick MVC is employed as measurement. This is further reinforced with the results from Chapter 4, where between group differences in MVC and CAR was only detected after 5-second. A sustained MVC also allows the measurement of not only VA but CAR, and the nature of the sustained effort also better represent a prolonged exercise's nature where one should be producing muscular force continuously. Also, serotonin seems to be affected only by amount of CHO but not GI, and it seems that the loss of MVC with LGI compared to HGI was due to central factor, as other peripheral factors were similar between those groups.

CHAPTER 6 GENERAL CONCLUSION

This PhD investigated the effects of pre-exercise meals with different quantity and glycaemic index (GI) of carbohydrate (CHO) on central fatigue (CF) following an endurance exercise. The meals were first designed and verified to induce the desired post-prandial blood glucose responses that were significantly different from each other (LCHO with HCHO, and LGI with HGI), and in accordance to the established norms in (Chapter 3). Chapter 4 investigated the effects of consuming iso-caloric LCHO and HCHO pre-exercise meals towards CF after an endurance run, and found that when high amount (1.5 g/kg body weight) of CHO was consumed, muscular force production was better preserved post-running, by preserving the central contribution as indicated by attenuated loss of central activation ratio (CAR) and voluntary activation during a 90-second sustained maximal voluntary contraction (MVC). There was also a significantly higher amount of serotonin with higher fat oxidation when the low CHO meal (0.8 g/kg body weight) was consumed, suggesting a more pronounced CF. Finally, Chapter 5 investigated effects of iso-caloric, iso-macronutrient high CHO pre-exercise meals (1.5 g/kg body weight) with high (~75) and low (~40) GI towards CF following endurance exercise. It was found that the high GI meal preserved CF similar to HCHO meal in previous chapter: sustained MVC was better preserved concurrently with CAR during sustained MVC compared to the low GI meal. However, there was no increment in serotonin despite a higher tryptophan concentration with the low GI meal, indicating that serotonin regulation is not the only way CHO could affect CF.

To synthesise the findings, it was shown that a meal with high amount of CHO consumed 1-hour pre-exercise better preserve CF compared to meal with low amount of CHO; while a high CHO, high GI also preserve CF even after a higher (70% VO_2max) intensity, when compared to meal with similar amount of CHO but composed of low GI food. In conclusion, regardless of GI, a high CHO meal better preserve CF compared to low CHO

meal, and when the CHO amount is similar, high GI meal better preserve CF compared to low GI meal.

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Appendix A – Papers published

1. Khong, T. K., Selvanayagam, V. S., Sidhu, S. K., & Yusof, A. (2016). Role of carbohydrate in central fatigue: a systematic review. *Scandinavian Journal of Medicine & Science in Sports*. Published ahead of print.

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Review

Role of carbohydrate in central fatigue: a systematic review

T. K. Khong, V. S. Selvanayagam, S. K. Sidhu, A. Yusof

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Abstract

Carbohydrate (CHO) depletion is linked to neuromuscular fatigue during exercise. While its role at peripheral level is relatively well understood, less is known about its impact centrally. The aim of this systematic review was to critically analyze the effects of CHO on central fatigue (CF) assessed by various neurophysiological techniques. Four databases were searched using PRISMA guidelines through February 2016. The inclusion criteria were: CHO as intervention against a placebo control, fatigue induced by prolonged exercise and assessed using neurophysiological measures [voluntary activation (VA), superimposed twitch (SIT), M-wave, electromyography], alongside maximal voluntary contraction (MVC). Seven papers were reviewed, where exercise duration lasted between 115 and 180 min. CHO improved exercise performance in three studies, whereby two of them attributed it to CF via attenuation of VA and SIT reductions, while the other indicated peripheral involvement via attenuation of M-wave reduction. Although a few studies suggest that CHO attenuates CF, data on its direct effects on neurophysiological outcome measures are limited and mixed. Generally, measures employed in these studies were inadequate to conclude central contribution to fatigue. Factors including the techniques used and the lack of controls render additional confounding factors to make definitive deductions. Future studies should employ consistent techniques and appropriate neurophysiological controls to distinguish CHO effect at central level. The use of pharmacological intervention should be incorporated to elucidate involvement of central mechanisms.

2. Khong, T. K., Selvanayagam, V. S., Hamzah, S. H., Lim, P. J., & Yusof, A. (2016, September). Effect of High and Low Carbohydrate Meals on Sustained Maximum Voluntary Contraction (MVC) after Prolonged Exercise. In *International Conference on Movement, Health and Exercise* (pp. 133-135). Springer, Singapore.

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Effect of High and Low Carbohydrate Meals on Sustained Maximum Voluntary Contraction (MVC) after Prolonged Exercise

T. K. Khong ✉ , V. S. Selvanayagam, S. H. Hamzah, P. J. Lim, A. Yusof

Abstract

Fatigue is a natural physiological phenomenon where the body's capability to do work is reduced, and in general carbohydrate (CHO) depletion has been attributed to the aetiology of this condition. However, its composition in iso-caloric meals has never been investigated on capacity of sustained force production following a prolonged exercise. Aim: To investigate the effects of high and low CHO meals on sustained maximal voluntary contraction (MVC) capability after a 90-minute run. Methods: Ten (n=10) moderately trained runners (age: 25±3.7 years, and VO2max: 51.42±4.78 ml/min/kg) were prescribed, in a cross-over, randomised, and double blind design, either one of these iso-caloric meals: high CHO meal (1.5 g/kg body weight), or a low CHO meal (0.8 g/kg body weight) prior to the 90-minute run at 65% of VO2max. A 90-second sustained MVC was measured before and after the run (represented at 30, 60, and 90 seconds respectively). Results: MVC dropped significantly (p<0.01) after the prolonged running exercise in both groups. The difference in the sustained MVC was significant between the two groups at all time points (p<0.01). Conclusion: While both groups completed the 90-minute running task successfully, the high CHO meal allowed a higher sustained force production (MVC) post-exercise, suggesting physiological changes that allows better neuromuscular functions. Theoretically, several factors could be attributed to this phenomenon, such as preservation of fuel source, and/or alteration of brain neurotransmitter concentrations that affect neural drive.

Appendix B – Conference presentation

Effect of High and Low Carbohydrate Meals on Sustained Maximum Voluntary Contraction (MVC) after Prolonged Exercise. Presented in: 3rd International Conference on Movement, Health, & Exercise.

ID#257

Effect of High and Low Carbohydrate Meals on Sustained Maximum Voluntary Contraction (MVC) after Prolonged Exercise

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Fatigue is a natural physiological phenomenon where the body's capability to do work is reduced, and in general carbohydrate (CHO) depletion has been attributed to the aetiology of this condition. However, its composition in iso-caloric meals has never been investigated on capacity of sustained force production following a prolonged exercise. The aim of this study was to investigate the effects of high and low CHO meals on sustained maximal voluntary contraction (MVC) capability after a 90-minute run. Ten (n=10) moderately trained runners (age: 25±3.7 years, and VO2max: 51.42±4.78 ml/min/kg) were prescribed, in a cross-over, randomised, and double blind design, either one of these iso-caloric meals: high CHO meal (1.5 g/kg body weight), or a low CHO meal (0.8 g/kg body weight) prior to the 90-minute run at 65% of VO2max. A 90-second sustained MVC was measured before and after the run (represented at 30, 60, and 90 seconds respectively). MVC dropped significantly (p<0.01) after the prolonged running exercise in both groups. The difference in the sustained MVC was significant between the two groups at all time points (p<0.01). While both groups completed the 90-minute running task successfully, the high CHO meal allowed a higher sustained force production (MVC) post-exercise, suggesting physiological changes that allows better neuromuscular functions. Theoretically, several factors could be attributed to this phenomenon, such as preservation of fuel source, and/or alteration of brain neurotransmitter concentrations that affect neural drive.

Keywords— muscular force, carbohydrate, sports nutrition