INTERACTION OF CARBOHYDRATE INTAKE AND EXERCISE INTENSITY ON CARBOHYDRATE SPARING AFTER EXERCISE

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``You have powers you never dreamed of. You can do things you never thought you could do. There are no limitations in what you can do except the limitations in your own mind as to what you cannot do.

Don’t think you cannot. Think you can”.

– Darwin P. Kingsley.
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**EXECUTIVE SUMMARY**

It is well established that an increase in exercise intensity is generally associated with a decrease in the proportion of CHO oxidised during recovery, thus promoting the sparing of CHO for subsequent exercise bouts. In contrast, the proportion of CHO oxidised in response to a meal ingested after exercise has been reported to be unaffected by exercise intensity. This latter finding might be explained on the basis that large amounts of CHO were fed after exercise in some studies, thus raising the possibility that this may have overridden any CHO sparing during the post-exercise period. Moreover, since the temporal pattern of change in CHO and fat oxidation after meal ingestion was not examined in previous studies, it is possible that CHO sparing at particular points in time might have gone undetected. For these reasons, this study re-examines the extent to which the amount of CHO fed post-exercise affect over time the impact of exercise intensity on the proportion of CHO and fat oxidised during recovery.

Twelve physically active males completed four experimental treatments following a counterbalanced randomised design: 1) High intensity exercise (cycling at 70% $\dot{V}O_{2\text{peak}}$ for 60 min) followed by a low-CHO meal (LCHE), 2) low intensity exercise (cycling at 35% $\dot{V}O_{2\text{peak}}$ for 120 min) followed by a low-CHO meal (LCLE), 3) high intensity exercise (cycling at 70% $\dot{V}O_{2\text{peak}}$ for 60 min) followed by a high-CHO meal (HCHE) and 4) low intensity exercise (cycling at 35% $\dot{V}O_{2\text{peak}}$ for 120 min) followed by a high-CHO meal (HCLE). Before, during and after exercise, respiratory gases and blood were sampled at selected time intervals. During the first hour of recovery without food, the rates of CHO oxidation were significantly lower following high compared to low intensity exercise ($P<0.05$). During the 2-h period after meal ingestion, there were no differences between all trials for relative and absolute rates of fat and CHO oxidation.
However, later into recovery, rates of CHO oxidation were greater in HCLE, HCHE and LCLE than LCHE ($P<0.05$), with higher fat oxidation rates in LCHE compared to the other trials ($P < 0.05$). Moreover, exercise intensity had little effect on the rates of CHO and fat oxidation after a high-CHO meal was ingested post-exercise. The rise in blood glucose and insulin concentrations was higher after high-CHO compared to low-CHO meal trials ($P < 0.05$), with the least response following LCHE. FFA levels decreased across trials after meal ingestion then rose rapidly in LCHE compared to LCLE, HCHE and HCLE ($P < 0.05$).

In conclusion, this study does not support earlier findings that exercise intensity has no effect on the proportion of carbohydrate oxidised in response to a CHO-rich meal ingested during recovery. It shows that an increase in CHO sparing occurs at the expense of an increase in the oxidation of the body’s endogenous fat stores especially when a low-CHO meal is ingested following high intensity exercise. Given the limited size of the body’s CHO stores and the importance of CHO in supporting muscle energy demands, such a sparing of CHO is clearly advantageous for maintaining our capacity to engage in ‘fight or flight’ behaviours.
Candidate Declaration

I am the author of the thesis entitled:

*Interaction of Carbohydrate Intake and Exercise Intensity on Carbohydrate Sparing during Recovery.*

Submitted for the degree of:

*Master of Science of The University of Western Australia*

I certify that the thesis is the result of my own research, except where otherwise acknowledged, and that this thesis in whole or in part has not been submitted for an award, including a higher degree, to any other university or institution.

In addition, this thesis does not contain work that I have published, nor work under review for publication.

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Full Name: Mohamad Haiyum Jaafar

Signed: [Signature]

Date: 8th October 2009
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ABBREVIATIONS

ATP  Adenosine triphosphate
BMI  Body mass index (weight/height$^2$)
$^{13}$C  Carbon 13
CHO  Carbohydrate
CPT  Carnitine palmitoyl transferase
DAG  Diacylglycerol
EE  Energy expenditure
EI  Energy intake
EPOC  Excess post-exercise oxygen consumption
FA-CoA  Fatty acyl-coenzyme A
FATP-1  Fatty acid transporter protein-1
FFA  Free fatty acid
FFM  Fat free mass
GLU  Glucose
GLUT-4  Glucose transporter 4
GLY  Glycogen
G6P  Glucose-6-phosphate
g  Gram
h  Hour
HC  High carbohydrate
HR$_{\text{max}}$  Maximal heart rate.
HGI  High-glycaemic index
HIE  High intensity exercise
HK  Hexokinase
HSL  Hormone-sensitive lipase
IMTG  Intramyocellular triacylglycerol
IRS  Insulin receptor substrate
kJ  KiloJoule
LC  Low carbohydrate
LCFA  Long chain fatty acid
LGI  Low-glycaemic index
LIE  Low intensity exercise
LPL  Lipo-protein lipase
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<tr>
<td>mmol.L$^{-1}$</td>
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<tr>
<td>Na$^+/K^+$</td>
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</tr>
<tr>
<td>NAD</td>
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</tr>
<tr>
<td>NFK-B</td>
<td>Nuclear factor kB</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyruvate dehydrogenase complex</td>
</tr>
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CHAPTER I

INTRODUCTION

&

LITERATURE REVIEW
1.1 Introduction

1.1.1 Rationale

It is well documented that during high intensity exercise, CHO is the major oxidative substrate for contracting muscle, whereas fat is predominantly oxidised during low to moderate intensity exercise. Exercise intensity also affects the proportion of fat and CHO oxidised during recovery without food, with CHO being oxidised to a lesser extent following high intensity exercise. However, whether exercise intensity affects the pattern of fuel oxidation after a meal ingested during recovery remains to be determined. In particular, there is a need to further investigate the acute effect of exercise intensity on the partitioning of fat and CHO oxidation at time intervals following a meal ingested during recovery, and to determine if low CHO intake and high intensity exercise elicits a higher fat oxidation rate during recovery, thus promoting the sparing of the body CHO stores. For this reasons, the purpose of this thesis is to investigate the acute effect of low and high CHO intake during recovery following low or high intensity exercise on the rate of fat and CHO oxidation, and to determine if CHO sparing increases with a fall in CHO intake.

1.1.2 Research hypotheses

It was hypothesised that:

1. When CHO intake post-exercise is lower than that oxidised during exercise, CHO sparing increases with a rise in exercise intensity.

2. When CHO intake post-exercise is greater than that expended during exercise, CHO sparing is not affected by a rise in exercise intensity.
1.1.3 Significance

Previous research on the effect of exercise intensity on the partitioning of carbohydrate and fat oxidation after a CHO-rich meal ingested during recovery suggests that the extent to which CHO are spared under these condition is not affected by exercise intensity. It is expected that this study will show that there are conditions where exercise intensity has a marked effect on the extent to which CHO are spared in response to a meal ingested post-exercise.

1.1.4 Delimitations and limitations

Delimitations

1. Only active, healthy male participants between the ages of 18-35 years will be recruited for this study to control for any possible gender-base physiological differences and because of the difficulty in standardising the phases of the menstrual cycle for female participants.

2. Participants will be healthy and free of any underlying diseases or medical conditions that would prevent them from performing exercise testing.

3. All participants will be fasted before and during exercise, and for this reason the results thus obtained are unlikely to be representative of fed individuals.

Limitations

1. The volunteers from this study might not be representative of the 18-35 yrs old male population.

2. Lack of compliance with pre-testing instructions (e.g. diet, physical activity level, changes in life style habits) may affect our findings although every effort will be made to minimise lack of compliance.
Fat and carbohydrate oxidation during exercise

Fat and carbohydrate (CHO) are the major fuels recruited for the aerobic production of adenosine triphosphate (ATP) in human skeletal muscle. The oxidation of fat and CHO during exercise can vary considerably depending on both the intensity and the duration of exercise (Romijn et al., 1993; Brooks & Mercier, 1994; Coyle, 1995; van Loon et al., 2001). For instance, during high-intensity exercise, CHO is the predominant energy source, with a smaller contribution from fat since humans cannot oxidise fat at high enough rates. Unfortunately, the body’s CHO stores are limited and can be exhausted within a few hours of intense aerobic exercise (Coyle, 1995; Coyle et al., 1997; van Loon et al., 2001; Ivy, 2004). This in turn can adversely affect exercise performance of both moderate and high intensity, and may impair the ability to engage in ‘fight-or-flight’ responses (Bangsbo et al., 1997; Fairchild et al., 2003; Raja et al., 2003), emphasising the importance of CHO for energy production (Ivy, 1991a; Romijn, et al., 1993; Coyle, 1995; Hargreaves et al., 1995; Balsom et al., 1999; Coyle, 2000). For these reasons, the sparing of CHO during and after exercise is expected to be highly advantageous for subsequent bouts of activity (Bangsbo et al., 1997; Balsom et al., 1999; Fournier et al., 2004; Hargreaves, 2006). As discussed in the following review of the literature, a number of factors influence the proportion of fat and CHO oxidised during both exercise and recovery, thus affecting the extent to which CHO stores are spared.

Exercise is a potent stimulator of muscle metabolism with muscle glycogen, blood glucose, free fatty acid (FFA), and intra-muscular triglycerides contributing to meet the energy needs of the working muscle (Romijn et al., 1993; Coyle, 1995; van Loon et al., 2001; Jeukendrup, 2003). The extent to which CHO and fat are oxidised during exercise depends on a number of factors such as exercise intensity and duration (Romijn et al., 1993; Brooks & Mercier, 1994; Coyle, 1995; van Loon et al., 2001), as well as the
training state (Friedlander et al., 1998; Bergman & Brooks, 1999; Coyle, 2000; Romijn et al., 2000), and dietary status of the individual (Turcotte et al., 1992; Coyle, 1995; Coyle et al., 1997, Bergman & Brooks, 1999). With respect to exercise intensity, it is well established that during low intensity exercise (25% \(\dot{V}O_{2\text{max}}\)), the oxidation of FFA derived from triglycerides stored in adipocytes supports most of the energy needs of contracting muscles, with muscle glycogen and blood glucose contributing little under these conditions (Figure 1.1; Wolfe et al., 1990; Romijn et al., 1993; Klein et al., 1994; Coyle, 1995; Jeukendrup et al., 1997; van Loon et al., 2001). In addition, there is growing evidence that intra-muscular triglycerides (IMTG) also contribute to energy provision during low intensity exercise (Romijn et al., 1993; Guo et al., 2000; van Loon et al., 2001; Watt et al., 2002a; Watt et al., 2002b).

As exercise intensity increases, there is a rise in the absolute rate of fat oxidation together with a concomitant increase in the absolute and relative rate of CHO oxidation (Figure 1.1; Romijn et al., 1993; Brooks & Mercier, 1994; Brooks, 1997; Jeukendrup et al., 1998; Romijn et al., 2000; van Loon et al., 2001; Greenhaff et al., 2002). As exercise continues and blood flow to muscle increases, blood borne substrates become increasingly important sources of energy. The total body turnover of glucose is increased two, three and five times during mild, moderate and severe exercise respectively. Depending on the exercise intensity and duration, muscle glucose uptake increases substantially (Wahren et al., 1971; Coyle, 2000) and accounts for 30 to 40 % of the total oxygen consumed by muscle. The exercise intensity at which the predominant fuel being oxidised shifts from fat to CHO has been described as the "crossover point" by Brooks (1994). This crossover point may occur at \(~50\%\ \dot{V}O_{2\text{peak}}\) in untrained or mildly trained individuals and between 60-70\% \(\dot{V}O_{2\text{peak}}\) in trained athletes (Brooks & Mercier, 1994; Brooks, 1997; Bergman & Brooks, 1999).
FIGURE 1.1. Contribution of the four major substrates to energy expenditure at rest and after 30 min of exercise at 25%, 65% and 85% of maximal oxygen consumption (\(\text{VO}_2\text{max}\)) in well-trained fasted men. Adapted from Romijn et al., 1993 and van Loon et al., 2001.

One limitation with the ‘crossover point’ concept, however, is that during prolonged exercise of moderate intensity, the proportion of energy derived from muscle glycogen and blood glucose decreases progressively with time, while that of fat rises (Romijn et al., 1993; Brooks & Mercier, 1994; Romijn et al., 1995; Coyle et al., 1997; van Loon et al., 2001). This progressive increase in fat oxidation has been explained on the grounds that the lowering of CHO stores as exercise progresses results in a decreased CHO oxidation (Jansson et al., 1982; Romijin et al., 1995; Hargreaves et al., 1995; Coyle et al., 1997; Coyle, 2000). One factor likely to contribute to this rise in fat oxidation is the increase in the rate of FFA uptake into the mitochondria and/or increased β-oxidation in response to low muscle glycogen levels (Jansson et al., 1982; Turcotte et al., 1992; Sidossis & Wolfe, 1996, Odland et al., 1998; Turcotte, 2006). Other factors that may contribute to the time-dependent rise in FFA oxidation during exercise include the progressive increase in
circulating catecholamines and the associated increases in plasma FFA levels which promote the increased FFA uptake by skeletal muscle. It is also likely that reduced insulin levels as the exercise duration increases may increase FA oxidation (Hargreaves et al., 1988; Sidossis et al., 1996; Coyle et al., 1997; Horowitz et al., 1997; Jeukendrup, 2002; Horowitz, 2006). Finally, IMTG hydrolysis may increase in response to low muscle glycogen given that fat oxidation rates and plasma glycerol levels during exercise are higher when muscle glycogen levels are low despite the absence of changing plasma FFA concentrations (Starling et al., 1997; Coyle et al., 1997; Blomstrand & Saltin, 1999, van Loon et al., 2001). Consistent with this observation, intramuscular lipolysis is regulated by hormone-sensitive lipase (HSL), which in turn is activated by muscular contraction and epinephrine (van Loon et al., 2001; Watt et al., 2003; van Loon et al., 2003; Prats et al., 2006). As a result, IMTG may account for as much as 20-50% of energy expenditure during prolonged submaximal exercise (Romijn et al., 1993; Romijn et al., 1995; Friedlander et al., 1998; Romijn et al., 2000; Guo et al., 2000; van Loon et al., 2001; Watt et al. 2002a; van Loon., et al, 2003).

As exercise intensity increases to ~85% of $\dot{V}O_{2max}$, there is a decrease in both the proportion of total energy derived from fat oxidation and the absolute rate of fat oxidation (Figure 1.1; Romijn et al., 1993; Coyle, 1995; Brooks, 1997; Bergman & Brooks, 1999; Romijn et al., 2000; van Loon et al., 2001; Jeukendrup, 2002). Under these conditions, the rate of oxidation of both blood glucose and muscle glycogen increases, with blood glucose providing ~10 to 15% of total energy, while muscle glycogen provides the majority (60% or more) of the energy required for strenuous exercise (Romijn et al., 1993; Coyle, 1995; Jeukendrup et al., 1997; Romijn et al., 2000; van Loon et al., 2001). The remaining contribution is accounted for by FFA and IMTG (Romijn et al., 1993; Coyle, 1995; van Loon et al., 2001).
A number of mechanisms have been proposed to explain the lower rate of fat oxidation during high intensity exercise. Of interest, this decline in fat oxidation occurs despite high rates of adipose tissue lipolysis (Hodgetts et al., 1991; Romijn et al., 1995). This is explained by the fact that during high intensity exercise, part of the FFA originating from triglyceride breakdown becomes trapped within adipose tissue in response to a decline in adipose tissue blood flow resulting from sympathetic-related vasoconstriction (Hodgetts et al., 1991; Romijn et al., 1993, 1995; Bülow et al., 2006; Turcotte, 2006). As a result of this decreased release of FFA from adipose tissue, plasma FFA concentrations may not change (van Loon et al., 2001) or even decrease (Romijn et al., 1993), thus reducing both FFA availability during high intensity exercise and the rate of fat oxidation. This is likely
to be worsened under conditions where lactate levels are elevated since a rise in lactate levels may increase the rate of FFA re-esterification (Wolfe et al., 1990; Romijn et al., 1993; Brooks & Mercier, 1994; Romijn et al., 2000; van Loon et al., 2001, Achten & Jeukendrup, 2004). However, this decreased availability of FFA only partially explains the fall in fat oxidation rate associated with high intensity exercise.

There is compelling evidence that the lower rate of fat oxidation during high intensity exercise is also related to a decline in the transport of FFA into the mitochondria (Figure 2.2; Coyle et al., 1997; Sidossis et al., 1997; Winder 1998). This is mediated, in part, by an increase in malonyl-CoA, an allosteric inhibitor of carnitine palmityl transferase I (CPT-1), the rate-limiting enzyme that plays an important role in the transport of long-chain fatty acids inside the mitochondrial matrix (Odland et al., 1998; Winder, 1998; Achten & Jeukendrup, 2004; Roepstorff et al., 2005, Turcotte, 2006). In this regard, it has been suggested that the increased glycolytic flux during high intensity exercise elevates the concentration of acetyl-CoA, thus enhancing the production of malonyl-CoA and its inhibition of FFA transport into the mitochondria (Rasmussen & Winder, 1997; Odland et al., 1998; Winder, 1998, Bergman & Brooks, 1999; Roepstoff et al., 2005). In addition, the rise in H⁺ ions during intense exercise may also inhibit the activity of CPT-1 (Mills et al., 1984; Starritt et al., 2000; van Loon et al., 2001; Achten & Jeukendrup, 2004; Turcotte, 2006), since this enzyme binds to malonyl CoA more efficiently at a lower pH than at a more neutral pH (Mills et al., 1984, Starritt, et al., 2000).

Finally, there is evidence that the decreased reliance on fat oxidation during intense exercise is related to the decreased availability of free carnitine, a substrate of CPT-1, thereby limiting the activity of CPT-1 (Costantin-Teodosiu et al., 1991; Odland et al., 1998; van Loon et al., 2001; Achten & Jeukendrup, 2004; Roepstoff et al., 2005). Consequently, fat oxidation and muscle carnitine concentration in humans are tightly
coupled during incremental exercise; with both decreasing with intense exercise while carbohydrate oxidation rates increases (Odland et al., 1998; van Loon et al., 2001; Achten & Jeukendrup, 2004; Roepstorff et al., 2005).

1.3 Effect of exercise intensity on post-exercise oxygen consumption and energy expenditure

During the recovery period after exercise, there is an increase in oxygen uptake above that required to support resting metabolic processes (Bielinski et al., 1985; Maelum et al., 1986; Bahr et al., 1987; Gore & Withers, 1990; Bahr & Sejersted, 1991a; Dawson et al., 1996; Phelain et al.; 1997; Speakman & Selman, 2003; LaForgia et al., 2006; Warren et al., 2009), a phenomenon referred to as excess post-exercise oxygen consumption (EPOC). Immediately after intense aerobic exercise, oxygen consumption is elevated, but decreases rapidly within one hour to levels slightly above pre-exercise levels, where it remains elevated for up to several hours before returning to baseline. The transient high EPOC early during recovery is referred to as the rapid component of EPOC, whereas the subsequent long lasting EPOC is referred to as the slow component (Bahr, 1992; Borsheim & Bahr, 2003). Overall, EPOC has the capacity to affect the total energy cost of an exercise bout. For instance, after one hour of prolonged strenuous exercise at 70% $\dot{V}O_{2\text{peak}}$, EPOC can remain elevated for 12-24 hours and may represent an additional 10–15% of the exercise-related energy expenditure (Maelum et al., 1986; Withers et al., 1991; Bahr & Sejersted, 1991a; Bahr, 1992; LaForgia et al., 1997; LaForgia et al, 2006).

A number of reasons have been proposed to explain the fast and slow components of EPOC. In response to a short bout of high intensity exercise, the level of anaerobic metabolism is a key predictor of the magnitude of the rapid component of EPOC. This is
because the rapid component of EPOC is related to the replenishment of ATP, phosphocreatine and muscle glycogen stores post-exercise (Gasser & Brooks, 1984; Bielinski et al., 1985; Bangsbo et al., 1990; Bahr & Sejersted, 1991a; Bahr, 1992; Borsheim & Bahr, 2003). The proportion of EPOC accounted for by rephosphorylation of creatine and ADP during recovery is approximately 10% after maximal exercise (Bangsbo et al., 1990). Furthermore, the rate of muscle glycogen synthesis is directly linked to the intensity of the exercise, with the rate of muscle glycogen synthesis following short duration high intensity exercise (100% of $\dot{V}O_{2\text{max}}$) being greater than that after prolonged exercise of moderate intensity exercise (15.1-33.6 mmol/kg/h for high intensity exercise vs. 1.5-2 mmol/kg/h for moderate intensity exercise; Pascoe & Gladden, 1996). Since the energy supporting prolonged aerobic exercise originates from oxidative metabolism resulting in little or no ATP/phosphocreatine depletion or lactate accumulation, it is not surprising that the magnitude of the early component of EPOC is low under these conditions (Bangsbo et al., 1990; Bahr & Sejersted, 1991a).

The slow component differs from the rapid component of EPOC in that it can last for up to 24 hours and the mechanisms involved are less well understood. Increased triglycerides/fatty acid (TG/FA) cycling rates and a shift from carbohydrate to fat as fuel source may explain part of the slow component of EPOC after exhaustive submaximal exercise (Bahr et al., 1990; Bahr & Sejersted, 1991a; Trost et al., 1997, Borsheim et al., 1998a; Borsheim & Bahr, 2003). Since the energy equivalent of oxygen is lower for fat than for carbohydrate, an increase in lipid oxidation (and hence oxygen requirements) after exercise is probably one of the major contributors to EPOC (Trost et al., 1997, Borsheim et al., 1998a). In support of this, Trost et al. (1997) studied the effect of nicotinic acid, a potent inhibitor of FFA mobilisation on lipid oxidation and energy expenditure during recovery from exercise. In this study FFA levels were significantly lower during both the
exercise and recovery following nicotinic acid administration and this resulted in a significant reduction in the magnitude of EPOC compared to a control trial.

To a lesser extent, the magnitude of the slow component of EPOC and associated rates of substrate oxidation are partly related to the sustained elevation in plasma catecholamine concentrations, ventilation and body temperature post-exercise (Frey et al., 1993; Borsheim & Bahr, 2003; Laforgia et al., 2006). In particular, it has been proposed that catecholamines may have an indirect effect on the slow component of EPOC in several ways, namely via increasing heart rate and rates of respiration, blood flow, glycogenolysis, gluconeogenesis and lipolysis in adipose tissue (Bahr et al., 1990; Bahr & Sejersted, 1991b; Frey et al., 1993; Borsheim et al., 1998a, Borsheim & Bahr, 2003). Moreover, norepinephrine facilitates the Na+/K+ pump, which in turn requires ATP and thus increased oxygen consumption for active transport. Finally, the plasma concentration of catecholamines increases linearly with exercise duration and exponentially with exercise intensity (Bahr, et al., 1990; Leuenberger et al., 1993). These relationships are similar to the relationship between EPOC and exercise duration and intensity (Maehlum et al., 1986; Bahr et al., 1990; Borsheim & Bahr, 2003; Laforgia et al., 2006). Another additional effect of catecholamines is their stimulatory effect on FA oxidation via an acetyl-CoA mediated increased in FA mobilisation (Wolfe et al., 1990; Bahr et al., 1991a; Bahr, 1992; Coyle, 2000). Interestingly, despite this supportive evidence, the potential role of the sympathoadrenal system in prolonged EPOC is still a controversial issue as some researchers have reported no significant role for catecholamine and adrenergic control in the prolonged component of EPOC (Borsheim et al., 1998a, 1998b; Pritzaff et al., 2000).

The magnitude and duration of EPOC are affected by the intensity of exercise (Table 1.1, Maehlum et al., 1986; Bahr et al., 1987; Gore & Withers, 1990; Bahr & Sejersted, 1991a;
Laforgia et al., 1991; Frey et al., 1993; Dawson et al., 1996; Phelain et al., 1997; Dooly et al., 1997; Speakman & Selman, 2003; Borsheim & Bahr, 2003; Laforgia et al., 2006, Warren et al., 2009). More specifically, there is a positive curvilinear relationship between the magnitude of EPOC and the intensity of exercise (Bahr et al., 1991b; Borsheim et al., 1998a). It is important to note, however, that although high-intensity exercise has been shown by many to have a more pronounced effect on EPOC than low-intensity exercise (Gore & Withers, 1990; Smith & McNaughton, 1993; Trueth et al., 1996; Phelain et al., 1997; Warren et al., 2009), others have not found the same trend between exercise intensity and EPOC duration (Sedlock et al., 1989; Sedlock, 1991, Dawson et al., 1996). These discrepancies may be attributed to the narrower spectrum of exercise intensities investigated in some of these studies (e.g. Sedlock, 1991) and their lack of statistical power to detect significance difference (Dawson et al., 1996). In this respect, it has been proposed that significant EPOC differences occur when moderate- to high intensity exercise treatments (> 70% of \( \dot{V}O_{2\text{max}} \)) are compared with iso-energetic low intensity exercise treatments (35-50% of \( \dot{V}O_{2\text{max}} \)), and as the differences in exercise intensities between iso-energetic work bouts diminishes, so does the resultant EPOC magnitude (Borsheim & Bahr, 2003; Laforgia et al., 2006).
Table 1.1. Studies investigating the effect of exercise intensity and duration on excess post-exercise oxygen consumption (EPOC).

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Exercisea</th>
<th>Post-exercise monitoring</th>
<th>EPOC duration</th>
<th>EPOC magnitude</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hagberg et al. (1980)</td>
<td>18 M</td>
<td>cycle, 10 min at 50, 65 and 80%</td>
<td>15 min</td>
<td>100 min</td>
<td>50%: 0.7 litre 80%: 1.4 litre</td>
<td>No EPOC was found beyond 35 mins of recovery.</td>
</tr>
<tr>
<td>Sedlock et al. (1989)</td>
<td>10 M</td>
<td>cycle, 30 and 60 min at 50%, 20 min at 75%</td>
<td>35 min</td>
<td>50%: 20,</td>
<td>50%: 60, 51 kJ 75%: 123 kJ</td>
<td>Energy expenditure during the 30- and 20-min treatments was both 300 kcal, whereas that during the 60-min treatment was 600 kcal.</td>
</tr>
<tr>
<td>Bielinski et al. (1986)</td>
<td>10 M(T)</td>
<td>Treadmill 3h at 50%</td>
<td>10 h</td>
<td>75 min</td>
<td>RMR ↑ 9% at 4.5 RMR ↑ 4.7% at 18h</td>
<td>Food given 30 min after exercise.</td>
</tr>
<tr>
<td>Gore and Withers (1990)</td>
<td>9 M</td>
<td>treadmill, 20, 50 and 80 min at 30, 50 and 70%</td>
<td>24 h</td>
<td>30%: &lt;1 h</td>
<td>30%: 1.0, 1.4 and 1.0 litres</td>
<td>EPOC increased with duration for intensities &gt;30%. Exercise intensity accounted for 45.5% of the EPOC variance, while duration and the interaction of intensity and duration only accounted for 9 and 8% respectively.</td>
</tr>
<tr>
<td>Bahr and Sejersted (1991)</td>
<td>6 M</td>
<td>cycle, 80 min at 29, 50 and 75%</td>
<td>14 h</td>
<td>18 min, 1.3 h 10.5 h</td>
<td>1.3, 5.7 and 30.1 litres</td>
<td>EPOC increased exponentially with exercise intensity.</td>
</tr>
<tr>
<td>Sedlock (1991)</td>
<td>7 F</td>
<td>cycle, 41 min at 40%, 27 min at 60%</td>
<td>to baseline</td>
<td>28 and 18 min</td>
<td>30 and 36 kJ</td>
<td>Total work was equated. No significant EPOC difference.</td>
</tr>
<tr>
<td>Dawson et al. (1996)</td>
<td>8 F</td>
<td>cycle, 34 min at 65%, 41 min at 55%, 49 min at 45%</td>
<td>to baseline</td>
<td>13 - 14 min</td>
<td>45%: 50 kJ 55%: 53 kJ 65%: 74 kJ</td>
<td>The 65% exercise produced a significantly greater EPOC even though the work was equated (1290 kJ) with the other exercise bouts.</td>
</tr>
<tr>
<td>Phelain et al. (1997)</td>
<td>8 F</td>
<td>cycle, 78 min at 50%, 51 min at 75%</td>
<td>3 h</td>
<td>50%: 1.5 h    75%: &gt;3 h</td>
<td>50%: 4.8 litres 75%: 9.0 litres</td>
<td>Exercising energy expenditure was 2026 kJ for both treatments. Higher intensity exercise produced significantly higher EPOC.</td>
</tr>
<tr>
<td>LaForgia et al. (1997)</td>
<td>8 M</td>
<td>treadmill, 30 min at 70%, 20 min at 105%</td>
<td>9 h</td>
<td>70%: 1 h 105%: 8 h</td>
<td>70%: 6.9 litres 105%: 15 litres</td>
<td>Higher intensity work produced double the EPOC of equated lower intensity work.</td>
</tr>
<tr>
<td>Warren et al. (2009)</td>
<td>14 M</td>
<td>Intensity: Cycle, isoenergetic at 50% and 85%</td>
<td>90 min</td>
<td>N.A</td>
<td>Intensity: 50% 24 ml/kg 85%: 79 ml/kg</td>
<td>Exercise duration did not affect EPOC. High intensity exercise significantly increases EPOC and EE.</td>
</tr>
</tbody>
</table>

a. Exercise duration and relative intensity (percentage of maximal aerobic power) were reported unless otherwise specified.

Abbreviations: RMR = resting metabolic rate, EE = Energy expenditure
There is also compelling evidence that the magnitude of EPOC is related to exercise duration (Table 1.1). However, there is a threshold of exercise intensity that has to be attained before exercise duration has an effect on EPOC (Bahr et al., 1987; Chad et al., 1988; Sedlock et al., 1989; Gore & Withers, 1990; Quinn et al., 1994; Borsheim et al., 1998a; Laforgia et al., 2006). For instance, Gore and Withers (1990) compared EPOC in nine men after 20, 50 and 80 minutes of exercise each at 30%, 50% and 80% of $\dot{V}O_{2max}$ and found no difference in EPOC among the 20-, 50- and 80-minute walks at 30% of $\dot{V}O_{2max}$, whereas EPOC increased significantly with exercise duration when running at 80% of $\dot{V}O_{2max}$. In agreement with these findings, Bahr and colleagues (1987) compared EPOC in six healthy male participants after they had exercised at 70% of $\dot{V}O_{2max}$ for 20, 40 and 80 minutes and showed that the magnitude of the 12-h EPOC had a positive linear relationship with exercise duration. Based on these findings, it has been suggested that exercise intensity must be above 70% of $\dot{V}O_{2max}$ for exercise duration to affect EPOC (Bahr, et al., 1987; Gore & Withers, 1990; Borsheim et al., 1998a).

1.4 Effect of exercise on fat and CHO oxidation during recovery.

The increase in EPOC following exercise implies that fat and CHO oxidation rates are also affected during recovery. In support of this view, many studies have shown a fall in post-exercise respiratory exchange ratio (RER) compared to pre-exercise or control levels, suggesting a greater reliance on fat as a fuel source during recovery (Bielinski et al., 1985; Maehlum et al., 1986; Gore & Withers, 1990; Bahr & Sejersted, 1991a; Bahr & Sejersted, 1991b; Broeder et al., 1991; Quinn et al., 1994; Phelain et al., 1997; Kiens & Richter, 1998; Yoshioka et al., 2001, Gill et al., 2001; Kimber et al., 2002; Henderson et al., 2007; Malatesta et al., 2009; Warren et al., 2009). More specifically, others have shown an increase in post-exercise lipid oxidation and lower CHO oxidation rate after
endurance (Horton et al., 1998; Pritzaff et al., 2000; Folch et al., 2001; Kuo et al., 2005; Tarnopolsky et al., 2006; Warren et al., 2009), resistance (Osterberg & Melby, 2000; Binzen et al., 2001; Petitt et al., 2003; Ormsbee et al., 2007) and interval type exercises (Laforgia et al., 1997; Christmass et al., 1999; McGarvey et al., 2005; Malatesta et al., 2009) compared with either pre-exercise or time-matched no-exercise control period values.

The preferential oxidation of fat after exercise is believed to prevent further depletion of glycogen, thus sparing the body’s limited CHO stores and facilitating subsequent muscle glycogen repletion, which represents a priority during recovery (Maelum et al., 1986; Wolfe et al., 1990; Bahr & Sejersted, 1991a, Bahr & Sejersted, 1991b; Kiens & Richter, 1998; Mulla et al., 2000; van Loon et al., 2001; Kimber et al., 2002; Jamurtas et al., 2004; Kuo et al., 2005; Malatesta et al., 2009). In support of this view, the fall in CHO oxidation rate after exercise is closely associated with the magnitude of the decline in muscle glycogen stores (Romijn et al., 1993; Blomstrand & Saltin, 1999; Mulla et al., 2000; Kimber et al., 2002; Watt et al., 2004; Kuo et al., 2005; Warren et al., 2009). Some of the factors that are likely to contribute to the increased fat utilisation rate post-exercise include the decreased insulin and glucose levels after exercise observed in some studies (Bielinski et al., 1986; Dionne et al., 1999; Kimber et al., 2002; Henderson et al., 2007; Holtz et al., 2008; Long et al., 2008), both of which are being anti-lipolytic and inhibiting fat oxidation. In addition, the rapid increase in plasma FFA released from the adipose tissue after intense exercise provides a large source of potential energy (Hodgetts et al., 1991; Romijn et al., 1993; Mulla et al., 2000) that may increase fat oxidation rate and in turn restrict the rate of glycolysis via operation of the glucose-fatty acid cycle until the depleted CHO stores are replenished (Figure 1.3; Brooks & Gasser, 1980; Bielinski et al., 1985; Wolfe et al., 1990; Bahr & Sejersted, 1991c; Brooks, 1997; Pritzaff et al., 2000;
Hargreaves, 2004; Henderson et al., 2007). Recently, Henderson and colleagues (2007) have shown that lipolysis and FFA mobilisation are elevated during recovery and plasma FFA oxidation are also increased, leading to an elevation of total lipid oxidation after exercise. It has been suggested that noradrenaline and growth hormone are involved in the elevated postexercise lipolysis (Bahr et al., 1991a; Holloszy & Kohrt, 1996). Finally, even when no CHO or food is ingested after exercise, a proportion of muscle glycogen stores are replenished, with lactate being the most likely carbon source after high intensity exercise (Bangsbo et al., 1990; Bangsbo et al., 1997; Fournier et al., 2002; Fairchild et al., 2003; Raja et al., 2003; Fournier et al., 2004) and amino acids derived from protein breakdown or lactate during recovery from moderate intensity exercise (Brooks & Gasser, 1980). Under these conditions, the body relies predominantly on fat oxidation to provide the energy demands for glycogen repletion from these endogenous carbon sources (Maehlum et al., 1978; Brooks & Gasser, 1980; Bangsbo et al., 1997; Raja et al., 2003; Fournier et al., 2004).
**FIGURE 1.3.** Interaction of free fatty acids (FFA) with glucose (GLU) oxidation in skeletal muscle and the glucose acid cycle. FFA are taken up by fatty acid transporter protein-1 (FATP-1) and activated to fatty acyl-coenzyme A (FA-CoA), whereas GLU is taken up by glucose transporter-4 (GLUT-4) and phosphorylated by hexokinase-II (HK). FA-CoA oxidation increases the ratios of acetyl-CoA/CoA and of NADH/NAD+, which inhibit the pyruvate dehydrogenase (PDH) complex. Increased citrate further inhibits phosphofructokinase (PFK). These changes would slow down oxidation of GLU and pyruvate (PYR) and increase glucose-6-phosphate (G6P), which in turn stimulates glycogen (GLY) storage, inhibits hexokinase (HK), and decreases glucose transport. Adapted from Roden, 2004.

### 1.5 Effect of exercise intensity on fat and CHO oxidation during recovery in the absence of food intake

The effect of exercise intensity on the proportion of CHO and fat oxidised during recovery without food intake has been examined in a number of studies. It has been reported by most (Bielinski et al., 1986; Bahr & Sejersted, 1991c; Broeder et al., 1991; Phelain et al., 1997, Yoshioka et al., 2001; Warren et al., 2009) but not all studies (Mulla et al., 2000; Kuo et al., 2005; Henderson et al., 2007; Malatesta et al., 2009), that high-intensity exercise is associated with a more pronounced rise in post-exercise fat oxidation than moderate or low intensity exercise. The reason for this lack of agreement is likely due to
the intensity of exercise being compared. For instance, Mulla et al. (2000) reported no effect of exercise intensity on fat oxidation rate during recovery when two levels of isoenergetic exercise (40% versus 60% \(\dot{\text{VO}}_{2\text{peak}}\)) were compared in fasted volunteers, although fat oxidation rates increased during recovery relative to pre-exercise. Similarly, Kuo and colleagues (2005), who matched the energy expenditure of exercise performed at 45% and 65% \(\dot{\text{VO}}_{2\text{peak}}\) in healthy young men and women, reported that there was a substantial post-exercise increase in fat oxidation rate compared to pre-exercise levels, but no differences between exercise intensities. Recently, Malatesta and colleagues (2009) reported that there was no significant differences between two isoenergetic exercise bouts of different forms and intensities (INT, 1-min intervals at 80% \(W_{\text{max}}\) with 1 min of active recovery at 40% \(W_{\text{max}}\) and C45%, 60-min moderate-intensity continuous exercise at 45% \(\dot{\text{VO}}_{2\text{peak}}\)) in substrate turnover and oxidation. These authors suggested that isoenergetic exercises result in similar elevations in postexercise fat oxidation with no significant effect of exercise intensity on substrate oxidation in recovery. Furthermore, it could be due to the narrow spectrum of exercise intensities being compared (e.g. Kuo et al., 2005). In support of this view, the rates of fat oxidation and oxygen consumption have been reported to be greater after high-intensity exercise of 75% \(\dot{\text{VO}}_{2\text{max}}\) compared to exercise performed at 50% \(\dot{\text{VO}}_{2\text{max}}\) (Phelain et al., 1997). Furthermore, Warren and colleagues (2009) have recently showed that higher rates of fat oxidation following high- versus low-intensity exercise (85% and 50% \(\dot{\text{VO}}_{2\text{max}}\), respectively) was a reflection of both augmented EPOC and a lower post-exercise RER suggestive of a higher relative fat oxidation. Moreover, many others have observed a more pronounced impact of high intensity exercise on the rate of fat oxidation after exercise (Gore & Withers, 1990; Broeder et al., 1991; Bahr & Sejersted, 1991a; Frey et al., 1993; Tremblay et al., 1994, Yoshioka et al., 2001). This has been explained on the basis that the elevated plasma FFA levels during early recovery
from high intensity exercise result in increased uptake and oxidation of FFA within glycogen depleted muscle, sparing CHO to facilitate glycogen repletion (Sidossis & Wolfe, 1996; Phelain et al., 1997; Kuo et al., 2005; Henderson et al., 2007).

1.6 Fat and CHO oxidation during recovery with food intake

Exercise alters not only the proportion of fat and CHO oxidised during recovery without food, but also after a subsequent meal, and in a manner that is affected by the fat and CHO content of the meal itself (Bielinski et al., 1986; Ivy et al., 1988; Tremblay et al., 1994; Coyle et al., 1997; Schrauwen et al., 2000, Folch et al., 2001; Votruba et al., 2002; Horowitz, et al., 2004; Stevenson et al., 2005; Holtz et al., 2008).

1.6.1 Effect of the amount of CHO intake.

The ingestion of excess CHO during recovery not only increase CHO storage capacity but may lead to an increase in CHO oxidation and elevated energy expenditure (Bielinski et al 1986; Ivy et al., 1988; Folch et al., 2001). In support of this view, Ivy and colleagues (1988) found that increasing CHO consumption increased the rate of CHO oxidation during recovery. Likewise, Folch and colleagues (2001) found that after the ingestion of either a large (400g CHO) or small (150 g CHO) pasta meal following exercise, the amount of CHO oxidised over the 8 h recovery period was greater after the large meal. In contrast, the amount of fat oxidised was significantly lower following the larger meal.

The effect of the CHO content of the food ingested post-exercise on the pattern of fuel oxidation during recovery is also illustrated by the decrease in post-exercise fat oxidation rate and increase in CHO oxidation normally observed when a CHO-rich mixed meal is ingested during recovery from exercise (Ivy et al., 1988; Folch et al., 2001, 2003; Coyle et al., 2001; Dionne et al., 2001; Long et al., 2008; Holtz et al., 2008). However, the
ingestion of large amounts of CHO in a mixed diet during recovery from prolonged or exhaustive exercise only decreases, without suppressing completely fat oxidation despite elevated plasma insulin levels (Kzentowski et al., 1982; Bielinski et al., 1986; Kiens & Richer, 1998; Folch et al., 2001, 2003; Kimber et al., 2002). The mechanism for this increased fat oxidation in the present of elevated insulin and glucose is not readily apparent (Kiens & Richter, 1998; Kimber et al., 2002).

The inhibitory effect of a high-CHO meal on fat oxidation post-exercise may be explained on the basis of the insulin response to CHO intake. Insulin plays an important role in activating glucose uptake into insulin-sensitive tissues such as skeletal muscle, liver and adipose tissue (Young et al., 1983; Campbell et al., 1992; Derave et al., 2000; Rose et al., 2001; Jenjens & Juekendrup, 2003; Holtz et al., 2008). In addition, insulin decreases the release of FA from adipose tissue by inhibiting hormone-sensitive lipase (HSL) and by stimulating triacylglycerol uptake in adipose tissue via activation of lipoprotein lipase (LPL), thus resulting in a fall in plasma FFA levels (Lithell et al., 1981; Kiens, et al., 1987; Kiens & Richter, 1998). For these reasons, the rise in glycaemia and insulinaemia in combination with reduced plasma FFA concentrations following a CHO-rich meal results in an increase in the proportion of energy derived from CHO oxidation together with a decrease in fat oxidation from the body (Kiens & Richter, 1998; Long et al., 2008).

1.6.2 Type of CHO intake

The glycaemic index (GI) of the CHO ingested after exercise is another factor likely to affect fuel partitioning. This has been explained on the grounds that a high-glycaemic index (HGI) meal increases serum insulin and glucose concentrations to a greater extent than a low-glycaemic index (LGI), thereby promoting increased carbohydrate oxidation and reduced fat oxidation (Ludwig, 2002, Diaz et al., 2005) together with an enhanced
restoration of muscle glycogen (Burke et al., 1993; Jozsi et al., 1996; Burke, 2004). However, recent studies have shown that the ingestion of a LGI or HGI meal after exercise does not affect fat and CHO oxidation during recovery as predicted. For instance, Tittelbach and colleagues (2000) reported that the quantity of CHO oxidised after a high fructose diet (LGI) was significantly higher than a high glucose diet (HGI) in obese females. Furthermore, Stevenson and colleagues (2005) reported that prior exercise did not elicit any significant differences in fat or CHO oxidation during the recovery period after HGI or LGI CHO ingestion. Similarly, Bernard and Doucet (2006) showed that following an overnight fast, the total amount of fat and CHO oxidised following exercise and post-exercise carbohydrate breakfast is not affected by the breakfast GI (LGI or HGI).

1.6.3 Type of food intake

There is evidence that the ingestion of a high fat/low CHO meal during recovery from exercise increases the rate of post-exercise fat oxidation (Tremblay et al., 1994; Schrauwen et al., 1997; Schrauwen et al. 1998; Gill et al., 2001; Peters et al., 2001; Horowitz et al., 2004; Hansen et al., 2007) due to both increased oxidation of meal-derived fat and also endogenous fat stores (Schrauwen et al., 1997; Gill et al., 2001, Votruba et al., 2002; Horowitz et al., 2004; Hansen et al., 2005). One explanation for the above finding is that the low glycogen stores after exercise together with the elevated plasma FFA levels in response to a high fat meal contribute to the increased in fat oxidation (Schrauwen & Westrap, 2000; Hansen et al., 2005; Hansen et al., 2007). Indeed, there is evidence that the post-exercise elevation of plasma FFA concentrations and subsequent increases in FFA uptake are responsible, in part, for the increase in fat oxidation rate after a high-fat/low-CHO meal ingested after exercise (Schrauwen et al., 1997; 1998; Gill et al., 2001; Horowitz et al., 2004). It has been shown that the increased fatty acid uptake either via infusion of FFA or the ingestion of a high fat meal interferes
with the insulin signalling pathway responsible for glucose transporter (GLUT) 4 translocation, resulting in the reduction of insulin-stimulated glucose disposal and decreases the oxidation of CHO (Figure 1.4; Yu et al., 2000; Horowitz et al., 2004; Roden, 2004). This is mediated in part, through the increase of FA-CoA resulting in the diacylglyceride mediated activation of protein kinase C (PKC). This in turn contributes to insulin resistance by increasing phosphorylation-mediated inhibition of the insulin receptor substrate (IRS) proteins (Yu et al., 2000; Itani et al., 2002; Roden, 2004, Howlett et al., 2006; Frøsig et al., 2009). In addition, the accumulation of ceramides within the cytosol of the muscle cell can disrupt the insulin signal within the cell and ultimately inhibits glucose transport (Figure 1.4; Ramussen et al., 1998; Yu et al., 2000; Itani et al., 2002; Roden, 2004; Frøsig et al., 2009).

**Figure 1.4:** FFA interaction with glucose uptake. FA-CoA primarily activates isoforms of protein kinase C (PKC) and NFκ-B, which inhibits insulin signal transduction, resulting in decreased glucose transport/phosphorylation and glycogen synthesis. IRS, insulin receptor substrate; DAG, diacylglycerol; PI3K, phosphoinositide 3-kinase. Adapted from Roden, 2004.
It is interesting to note that since the amount of fat stored in the adipose tissue is relatively large compared to CHO stores, even in the leanest athlete, the replacement of the fat oxidised during an exercise session is often not perceived as important. However, there is emerging evidence that the consumption of a diet low in fat during recovery from prolonged exercise may fail to provide sufficient recovery of IMTG (Decombaz et al., 2000, Decombaz et al., 2001; van Loon et al., 2003). Decombaz and colleagues (2000) reported that the replenishment of IMTG after prolonged exercise increased significantly after a high fat/low CHO meal compared to a high CHO/low fat meal, with IMTG repletion being prevented when fat intake was small. Other investigators have demonstrated that in response to a high-fat meal ingested after exercise, IMTG concentration were 30-45% higher than pre-exercise levels, whereas they remained 5-17% lower following a low-fat meal after prolonged exercise (Starling et al., 1997; Johnson et al., 2003). On the other hand, meals with a combination of high fat and low CHO are associated with slower recovery of muscle glycogen levels (Starling et al., 1997; Decombaz et al., 2001; Johnson et al., 2003). Taken together, these findings show that the type and amount of macronutrients in the recovery diet appears to be critical to extent to which CHO and fat are oxidised and the replenishment of both glycogen and IMTG stores.

1.7 The effect of exercise intensity on fat and CHO oxidation after a meal during post-exercise recovery

The observation that exercise affects the pattern of fuel oxidation during a subsequent meal raises the issues of whether the intensity of exercise also influences the pattern of substrate oxidation under these conditions. To date, there is a general agreement that exercise intensity does not affect the pattern of CHO and fat oxidation in response to a meal ingested after exercise (Table 1.2, Trueth et al., 1996; Thompson et al., 1998; Folch
et al., 2001; Melanson et al., 2002; Votruba et al., 2002). Treuth and collaborators (1996) did not find any significant differences in post-exercise 24-hour CHO or fat oxidation between high intensity interval (100% $\dot{V}O_{2\text{max}}$) and low intensity exercise (50% $\dot{V}O_{2\text{max}}$) individuals fed after exercise. Likewise, Thompson and colleagues (1998) reported no significant differences in post-exercise CHO oxidation between low (33% $\dot{V}O_{2\text{max}}$) and moderate (66% $\dot{V}O_{2\text{max}}$) intensity exercise over a 6-hour recovery period during which a mixed meal was ingested (4400 kJ; 65% CHO, 20% fat, and 15% protein). In another study, Folch et al. (2000) showed that exercise intensity did not affect the post-exercise rates of CHO and fat oxidation, irrespective of the meal size ingested during recovery. More recently, Votruba and colleagues (2002) examined the proportion of fat and CHO oxidised post-exercise in female participants that were fed an average western diet (55% CHO, 30% fat, and 15% protein) during recovery from two different exercise intensities (25% versus 85% $\dot{V}O_{2\text{peak}}$) matched for total energy expenditure (1250 kJ). Under these conditions, low and high intensity exercise affected fat and CHO oxidation to a similar extent over 11.5 hours of recovery. Likewise, Melanson and collaborators (2002) reported no difference in 24 hour substrate oxidation following a bout of exercise either at 40% or 70% $\dot{V}O_{2\text{peak}}$.

A potential confounding factor shared by most of these aforementioned studies is that the temporal pattern of change in fat and CHO oxidation immediately after meal ingestion was not reported (Trueh et al., 1996; Thompson et al., 1998; Folch et al., 2001; Melanson et al., 2002; Votruba et al., 2002), thus overlooking the possibility that the early responses of fat and CHO oxidation to food ingestion might have been affected by exercise intensity. In support of this, Yoshioka and colleagues (2001) showed an increase in fat oxidation rate after a mixed meal diet over a 3-h recovery period following high compared to low
intensity exercise. However, in their study, the rate of CHO oxidation after exercise was not examined.

Another potential limitation shared by many of the aforementioned studies is the amount of CHO ingested during recovery in these studies being well in excess of the total amount of CHO oxidised during exercise (Table 1.2, Thompson et al., 1998; Folch et al., 2001; Votruba et al., 2002; Melanson et al., 2002). To date, only one study has investigated the combined effects of exercise of various intensities and durations, and CHO intake on the metabolic fate of dietary carbohydrates, and energy and substrate balance. Folch and colleagues (2001) studied the metabolic response to a 150 or 400 g $^{13}$C-labelled pasta meal for 8 h following rest (no exercise) or exercise at low (37% $\dot{V}O_{2max}$) or moderate (57% $\dot{V}O_{2max}$) iso-energetic workloads. The total amount of fat oxidised during recovery was higher after moderate exercise following both the small and large meal compared to low intensity exercise. However, there were no difference between low- and moderate intensity exercise following each meal for total amount of CHO oxidised, although glucose oxidation was higher after the large meal compared to the small meal. The authors proposed that the compensatory increase in fat oxidation following prolonged exercise is not blunted by the ingestion of CHO, even in large amounts, when the exercise intensity is comparatively high due to the preferential conversion of glucose into glycogen for the increase in glycogen stores following exercise. This raises the possibility that CHO intake might have been high enough to override the body’s CHO sparing state. In this regard, it remains to be determined whether exercise intensity affects CHO sparing when CHO intake during recovery is lower than that oxidised during exercise.
Table 1.2. Studies designed to investigate the effect of exercise intensity and diet on substrate metabolism during post-exercise period.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Dietary intake</th>
<th>Performance protocol*</th>
<th>Effect on substrate metabolism</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broeder et al.</td>
<td>5 lean, 5 obese males</td>
<td>3000 kJ liquid meal (F)</td>
<td>30% &amp; 60% EE equated to 3000kJ</td>
<td>↓ RER in HI (NF) &gt; HIE (F) &gt; LIE (NF) &gt; LI (F). No diff. btw group.</td>
<td>Exercise intensity may play a role independent of total EE in increasing EPOC and post-exercise substrate utilisation up to 180 min.</td>
</tr>
<tr>
<td>(1991)</td>
<td></td>
<td>No-kcal liquid (NF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trueht et al.</td>
<td>8 females</td>
<td>EI: 1.3 x RMR/day</td>
<td>50% : 60 min 100% : 60 min (2min/2 rest)</td>
<td>CHO oxidation : 204 vs 207 g/day Fat Oxidation : 79 vs 79 g/day</td>
<td>24 hr EE was higher after HIE. No differences in substrate oxidation over 24 hr period.</td>
</tr>
<tr>
<td>(1996)</td>
<td></td>
<td>CHO: 65%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson et al.</td>
<td>10 males</td>
<td>EI: 4020 kJ</td>
<td>LIE: 33% - 90 min HIE: 66% - 45 min EE: 4470 kJ</td>
<td>Recovery: CHO use Low-142.5g &lt; Mod – 188.8g Fat use Low – 42g &gt; Mod – 24g</td>
<td>Substrate use during post-exercise recovery not significantly different due to differences during exercise</td>
</tr>
<tr>
<td>(1998)</td>
<td>(active)</td>
<td>CHO: 66%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folch et al.</td>
<td>18 males</td>
<td>Small: 150 g (2400 kJ)</td>
<td>37%: 180 min 57%: 90 min</td>
<td>CHO oxidation: Small: Rest &gt; LIE &gt; HIE Large: Rest &gt; LIE = HIE Fat oxidation: Small: Rest &gt; LIE &gt; HIE Large: Rest &gt; LI = HIE</td>
<td>Rise in fat oxidation following prolonged exercise is not blunted by ingestion of CHO, even in large amounts, when the exercise intensity is comparatively high</td>
</tr>
<tr>
<td>(2000)</td>
<td>(3 groups)</td>
<td>Large: 400 g (6400 kJ)</td>
<td>13C labelled pasta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoshioka et al.</td>
<td>8 males</td>
<td>EI: 3757 kJ</td>
<td>HIE: 77% - 33 min LIE: 38% - 65 min</td>
<td>Fat oxidation rate: HIE=LIE &gt; Rest (0 -120 min post meal) HIE&gt;LIE&gt;Rest (120 - 240min post meal) CHO oxidation rate : NA</td>
<td>High intensity exercise favours increased in postexercise energy metabolism mediated by β-adrenergic stimulation.</td>
</tr>
<tr>
<td>(2001)</td>
<td>CHO: 45%</td>
<td>33min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Votruba et al.</td>
<td>7 females</td>
<td>EI: 9490 kJ/day</td>
<td>CON: no Ex – 33min EE:2088kJ</td>
<td>CHO use 17 % for heavy than light exercise. Fat use for light exercise &gt; heavy.</td>
<td>Dietary fat oxidation and whole body fat utilization during recovery was similar between both exercise session.</td>
</tr>
<tr>
<td>(2002)</td>
<td>(healthy)</td>
<td>CHO: 55%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanson et al.</td>
<td>8 males</td>
<td>EI: 1.5-1.8 x RMR/day + 2000 kJ</td>
<td>LIE : 40% HIE: 70% EE: 1600 kJ</td>
<td>Both 24-h EE and CHO oxidation elevated during ex. day (CON &lt; LI = HI).24-h fat oxidation no different across trials(CON = LIE = HIE)</td>
<td>Exercise intensity has no effect on 24-h EE and substrate oxidation. Possible hormonal response attenuated fat oxidation.</td>
</tr>
<tr>
<td>(2002)</td>
<td>8 females</td>
<td>CHO: 55%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exercise duration and relative intensity (percentage of maximal aerobic power) were reported unless otherwise specified.

*Abbreviations: RMR = resting metabolic rate, EE = energy expenditure, EI = energy intake, LIE = low intensity, HIE = high intensity
1.8 Conclusion

It is well documented that during high intensity exercise, CHO is the major oxidative substrate for contracting muscle, whereas fat is predominantly oxidised during low to moderate intensity exercise. Exercise intensity also affects the proportion of fat and CHO oxidised during recovery without food, with CHO being oxidised to a lesser extent following high intensity exercise. However, whether exercise intensity affects the pattern of fuel oxidation after a meal ingested during recovery remains to be determined. In particular, there is a need to further investigate the acute effect of exercise intensity on the partitioning of fat and CHO oxidation at time intervals following a meal ingested during recovery, and to determine if low CHO intake and high intensity exercise elicits a higher fat oxidation rate during recovery, thus promoting the sparing of the body CHO stores. For this reasons, the purpose of this thesis is to investigate the acute effect of low and high CHO intake during recovery following low or high intensity exercise on the rate of fat and CHO oxidation, and to determine if CHO sparing increases with a fall in CHO intake.
REFERENCES


CHAPTER II

INTERACTION OF CARBOHYDRATE INTAKE AND EXERCISE INTENSITY ON CARBOHYDRATE SPARING AFTER EXERCISE

(formatted for publication in Journal of Applied Physiology)
ABSTRACT

AIM: To re-examine the extent to which the amount of CHO fed post-exercise affects over time the impact of exercise intensity on the proportion of CHO and fat oxidised during recovery. METHODS: Twelve active males completed four experimental treatments following a counterbalanced randomised design: High-intensity exercise (70% \( \dot{\text{VO}}_{2\text{peak}} \) for 60 min) with a low-CHO meal (LCHE), low-intensity exercise (35% \( \dot{\text{VO}}_{2\text{peak}} \) for 120 min) with a low-CHO meal (LCLE), high-intensity exercise with a high-CHO meal (HCHE) and low-intensity exercise with high-CHO meal (HCLE). Before, during and after exercise, respiratory gases and blood were sampled at selected time intervals. RESULTS: Early in recovery, CHO oxidation rates were significantly lower following high compared to low intensity exercise \((P < 0.05)\). For 2-h after meal intake, there were no differences between trials for relative and absolute rates of fat and CHO oxidation. Later into recovery, CHO oxidation rates were lower and fat oxidation rates higher in LCHE compared to other trials \((P < 0.05)\). Exercise intensity had little effect on rates of CHO and fat oxidation after high-CHO meal ingested post-exercise. Rise in blood glucose and insulin concentrations was higher after high-CHO than low-CHO trials \((P < 0.05)\) but lowest in LCHE. Free fatty acids (FFA) concentrations were higher after LCHE than other trials \((P < 0.05)\). CONCLUSION: Exercise intensity affects the proportion of carbohydrate oxidised in response to a CHO-rich meal ingested during recovery and this CHO sparing is more pronounced and occurs at the expense of an increase in the oxidation of the body’s endogenous fat stores if a Low-CHO meal is ingested after intense exercise.

Keywords: substrate utilisation, metabolism, post-exercise CHO sparing.
2.1 INTRODUCTION

Carbohydrate and fat provide important sources of energy during exercise, with their relative contribution depending on exercise intensity and duration (Romijn et al., 1993; Brooks & Mercier, 1994, van Loon et al., 2001), training status and dietary state (Brooks & Mercier, 1994, Coyle et al., 1997; Achten & Jeukendrup 2004). At higher exercise intensities, carbohydrate (CHO) supports most of the energy needs of skeletal muscle, but since the body only stores a limited amount of CHO, the depletion of these stores can adversely affect exercise performance of both moderate and high intensity exercise (Ivy, 1991a, Bangsbo et al., 1997; Balsom et al., 1999). In turn, this may impair an individual’s ability to respond optimally to situations eliciting ‘fight-or-flight' responses (Balsom et al; 1999; Fournier et al., 2002; Fairchild et al., 2003; Raja et al., 2003). For these reasons, the ‘sparing’ of CHO during and after exercise is expected to be highly advantageous for prolonged exercise or subsequent bouts of physical activity.

During recovery from exercise, CHO sparing is favoured by an increase in fat oxidation (Bielinski et al., 1986; Phelain et al., 1997; Horton et al., 1998; Kuo et al., 2005; Henderson et al., 2007; Malatesta et al., 2009). This post-exercise increase in fat oxidation and CHO oxidation are acutely enhanced by increasing exercise intensity (Phelain et al., 1997; Coyle, et al., 1997; Kuo et al., 2005; Warren et al., 2009). There is a general agreement that post-exercise nutrient oxidation in response to a meal ingested during recovery is not affected differently by the intensity of exercise (Trueth et al., 1996; Thompson et al., 1998; Folch et al., 2001; Votruba et al., 2002; Melanson et al., 2002). One potential confounding factor with some of these studies is that the temporal pattern of change in fat and CHO oxidation immediately after meal ingestion was not reported (Thompson et al., 1998; Folch et al., 2000; Votruba et al., 2002, Melanson et al., 2002). In addition, the amount of CHO ingested during recovery in many of these studies was in
excess of the total amount of CHO oxidised during exercise and consequently might have been high enough to override the body’s CHO sparing state (Thompson et al., 1998; Folch et al., 2001; Melanson et al., 2002). This is because the consumption of a meal high in CHO and the concomitant increase in blood insulin concentrations would be expected to stimulate CHO disposal and increased contribution of CHO oxidation to energy expenditure while suppressing lipolysis during the post-exercise recovery period (Trueth et al., 1996; Thompson et al., 1998; Folch et al., 2000; Dionne et al., 2001; Long et al., 2008).

For these reasons, the purpose of this study is to investigate the acute effect of low- and high-CHO intake during recovery from low or high intensity exercise on the partitioning of fat and CHO oxidation over time, and to determine if CHO sparing increases if CHO intake is less than that oxidised during exercise. We hypothesise that if CHO intake post-exercise is lower than that oxidised during exercise, CHO oxidation during recovery will be lower following high intensity exercise, but not if CHO intake post-exercise is far greater than that oxidised during exercise.
2.2 METHODS

2.2.1 Participants

Twelve healthy, physically active male volunteers between the ages of 19 and 35 years were recruited for the study (see Table 2.1 for descriptive characteristics). The criterions for a participant to be considered active are (1) aerobic capacity (\( \dot{V}O_{2\text{peak}} \)) must be above 42 ml.kg\(^{-1}\).min\(^{-1}\) (fair to excellent) and (2) participant must be involved in moderate to vigorous activity level on most days of the week (ACSM, 2007). All participants were non-smokers, with no reported use of medications or history of metabolic or cardiovascular disorders. The purpose, nature and potential risks of the study were explained to each participant and written consent was obtained prior to participation. The study protocol was approved by the Human Research Ethics Committee of The University of Western Australia.

2.2.2 Power analysis

For the proposed study, sample size estimation was determined based on an effect size of 0.9 and an expected average standard deviation similar to that of Thompson et al. (1998). With a statistical power of 0.8 and \( p < 0.05 \), we calculated that a minimum of 9 participants would be required, with this number raised to 12 subjects to increase further the statistical power of this study.

2.2.3 Experimental design

Each participant visited the laboratory on six separate occasions. First, they attended a familiarization session during which anthropometric measurements, determination of \( VO_{2\text{peak}} \), and familiarization with the low intensity and high intensity exercise protocols were performed. This visit was followed by a second session where the amounts of fat
and CHO oxidised during exercise at 35% and 70% VO$_{2\text{peak}}$ were determined to calculate the amount of CHO to be given for the subsequent testing trials.

Upon completing these preliminary sessions, participants were subjected to four experimental trials administered in a randomised counterbalanced order: 1) High intensity exercise (cycling at 70% VO$_{2\text{peak}}$ for 60 min) followed by a low-CHO meal (LCHE), 2) low intensity exercise (cycling at 35% VO$_{2\text{peak}}$ for 120 min) followed by a low-CHO meal (LCLE), 3) high intensity exercise followed by a high-CHO meal (HCHE) and 4) low intensity exercise followed by a high-CHO meal (HCLE). A minimum of one-week separated each experimental condition. A schematic diagram of the study design is shown in Figure 2.1.

**Screening test and familiarisation**

On their first visit, participants completed a health history questionnaire, and their body mass and height were measured. Body composition (percent body fat and lean body mass) was determined by dual energy X-ray absorptiometry (DEXA; Lunar Prodigy, GE 2004, Madison, Wisconsin). Next, aerobic capacity (VO$_{2\text{peak}}$) was determined using a graded exercise test (GXT) on the same cycle ergometer (Evolution Pty. Ltd., Adelaide, Australia) as that used for the subsequent experimental trials. The initial workload was set at 70 watts and subsequently increased by 30 watts every 3 minutes until volitional exhaustion. During the test, arterialised capillary blood samples were collected every 3 min for the assessment of lactate levels and lactate threshold determination (Fairchild et al., 2003), and heart rate was also continuously throughout the test (Polar advantage, Kempele, Finland). Respiratory gases were collected throughout the test and recorded as described below. This required participants to breathe through a mouthpiece attached to a two-way Hans-Rudolph valve which was connected to a computerised gas analysis
system. This consisted of a turbine ventilometer (Morgan, 225A, Kent, England) to assess ventilation and Ametek gas analysers (Applied Electrochemistry, SOV S-3A11 and COV CD-3A, Pittsburgh, PA, USA) to measure oxygen (O₂) and carbon dioxide (CO₂) concentration in expired air. The volume was calibrated by using a 3L calibration syringe. The gas analysis system was calibrated immediately before and verified after each test using a certified beta-grade gravimetric gas mixture of a known concentration (O₂:16.02% and CO₂:4.52%; BOC Gases, Chatswood, Australia). \( \dot{V}O_{2\text{peak}} \) was considered to be reached when at least two of the three following criteria were met: (1) a levelling off with increasing workload (increase of no more than 2 ml kg\(^{-1}\) min\(^{-1}\)), (2) a heart rate within 10 beats min\(^{-1}\) of predicted maximum (heart rate 220-age), and (3) a respiratory exchange ratio (RER) > 1.1. \( \dot{V}O_{2\text{peak}} \) was calculated as the average VO₂ over the last 60 s of the test.

After \( \dot{V}O_{2\text{peak}} \) determination and a 15-min recovery period, participants were familiarised with the exercise protocol to be used in the subsequent experimental trials. This involved cycling for 15 min at 70% \( \dot{V}O_{2\text{peak}} \) while breathing through a mouth piece for respiratory gas collection. Before leaving the laboratory, each participant was provided with a physical activity and dietary diary for recording activity level and food intake over 24 h before each subsequent trial in order to standardise these variables across testing sessions. Furthermore, all participants were instructed not to change their normal diet and physical activity habits for the whole duration of the study, and not to engage in any strenuous activity or consume caffeine/alcohol 24 h prior to each testing session.

### 2.2.4 Experimental protocol

Each participant was tested on five separate occasions. On the first visit, participants were required to visit the laboratory at 0800 h after a 10-12 h overnight fast to determine the
approximate amount of fat and CHO oxidised during exercise at 35\% and 70\% \( \dot{V}O_{2\text{peak}} \).

This visit commenced with a 30-min rest period in a darkened room kept at 22 ± 1\^\circ C, after which a sample of expired air was collected for determination of baseline resting metabolic rate (RMR), \( \dot{V}O_2 \) and RER (Kuo et al., 2005), followed by 15 min of cycling at 35\% \( \dot{V}O_{2\text{peak}} \) then 45 min at 70\% \( \dot{V}O_{2\text{peak}} \). The results obtained were used to calculate the absolute amount of CHO oxidised in response to the two workloads in order to determine the amount of CHO to be given to each participant during the subsequent experimental trials to replace either 50\% or 110\% of the CHO oxidised during exercise at 70\% \( \dot{V}O_{2\text{peak}} \).

Participants then completed four experimental trials in a randomised counterbalanced design, with testing of any given participant performed on the same day of the week with a week between trials. For these sessions, participants arrived at the laboratory at 0800 h in a fasted state before resting quietly in a supine position while expired air was collected for 30 min, with the final 5 min used to calculate baseline RMR, \( \dot{V}O_2 \) and RER. Then, participants cycled either for 60 min at 70\% of \( \dot{V}O_{2\text{peak}} \) or 120 min at 35\% of \( \dot{V}O_{2\text{peak}} \). The duration of the exercise trials was intended to achieve similar energy expenditure (isoenergetic) between exercise protocols. Following exercise, participants were weighed to determine the amount of fluid required to replace that lost during exercise, and a 6-h recovery period was initiated in a seated position. During this period, participants were allowed to read or watch television. At selected time intervals, respiratory gases and blood were sampled as described below.

### 2.2.5 Post-exercise meal

Sixty minutes following the completion of the exercise, participants were given a meal to consume within 15 minutes. The meal was either high or low in CHO and consisted of
100% glucose polymer mix (Polycose; Ross Laboratories, Columbus, OH; GI of 94).

Solution ratio: 15g CHO/100 mL H2O). The High-CHO meal was designed to replace
110% of carbohydrate expended during high intensity exercise (2.2 ± 0.14 g/kg) and the
Low-CHO meal provided 50% of the expended carbohydrate (0.98 ± 0.21 g/kg).

**Figure 2.1.** Experimental protocol. After an overnight fast, participants exercised either
at 35% \( \dot{V}O_{2\text{peak}} \) (LIE) or 70% \( \dot{V}O_{2\text{peak}} \) (HIE), followed after one hour of recovery by
ingestion of either 50% CHO (LC) or 110% CHO (HC) liquid meal based on CHO
expended during exercise.

### 2.2.6 Determination of substrate oxidation

Substrate oxidation rate (relative and absolute) was determined from the expired air
collected during the last 5 min of the 30-min resting period as described by Peronnet and
Massicotte (1991). Likewise, expired air was collected intermittently throughout exercise
to ensure that participants were exercising at the appropriate intensity and to calculate
energy expenditure and fuel utilisation. During the first hour following exercise, expired
air was collected every 15 min for 5 min until the ingestion of the CHO meal. During the
post-meal period, 5-min expired air samples were collected every 15 min for the first two
and half hours and then every 30 min till the end of the recovery period. All participants
breathed through a mouthpiece that was connected to a chain-suspended 120 litre Tissot gasometer tank (Collins Inc, Braintree, Massachusetts) for 10 min prior to the collection of the expired air. At each relevant time-point, the expired air was analysed for oxygen and carbon dioxide concentrations and substrate oxidation was calculated accordingly. Energy derived from CHO and fat oxidation was calculated as described by Kuo et al. (2005). Total carbohydrate and fat oxidation were calculated from the $\dot{V}O_2$ and $\dot{V}CO_2$ (L/min) as described in Péronnet and Massicotte (1991).

### 2.2.7 Blood sampling and analytical procedures

Capillary blood samples (300 µl) were obtained from the fingertips of a preheated hand immersed for five minutes in warm water (42 ± 2°C) immediately before and at various time intervals during (30 and 60 min at 70% $\dot{V}O_2$peak and 60 and 120 min at 35% $\dot{V}O_2$peak) and after exercise (30, 60, 90, 120, 150, 180, 270, and 330 min). A portion of blood was analysed with a blood gas analyser (125µl; EML105, Radiometer, Denmark) to determine blood glucose, lactate, pH and pCO$_2$ levels. The remaining blood was collected into tubes containing 10 µl of ethylenediaminetetraacetic anhydride (EDTA) solution (25 mg/mL), with the plasma removed after centrifugation (7000 rpm for 7 min) and frozen at -80°C until analysis. From these samples, plasma insulin was analysed by radioimmunoassay using a Human Insulin ELISA kit (LINCO Research, Missouri, USA). Plasma free fatty acids (FFA) were determined spectrophotometrically using an assay kit from Wako Bioproducts (Wako Chemicals Inc, Japan) with CV of 4.3%.

### 2.2.8 Statistical analyses

Comparison between exercise intensities and diet trials across time were made for $\dot{V}O_2$, energy expenditure, RER, substrate oxidation and blood-related parameters using a two-
way within subject repeated-measures analysis of variance (ANOVA) followed by a Tukey post-hoc test. This also allowed the testing of exercise intensity x diet interaction. When significant main effects were found or when significant interaction between diet and exercise intensity was found, one way ANOVA with a Tukey post-hoc test was conducted in the event of significant $F$ ratio to locate differences at specific time points. The data were analysed using the Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL). All data are presented as means ± SEM and statistical significance set at $P < 0.05$. 
2.3 RESULTS

2.3.1 Participant characteristics

The physical characteristics of the participants are shown in Table 2.1. Habitual dietary energy intake and macronutrient composition, as determined by multiple 3-day diet records, did not vary significantly between the four experimental trials and therefore average values are reported. There were no significant changes in body mass, BMI or time spent in habitual exercise (approximately 5-h of moderate exercise) between trials.

2.3.2 Rate of oxygen consumption, RER and energy expenditure

Oxygen consumption (\(\dot{V}O_2\)) increased significantly in response to exercise compared to baseline. However, the increase in \(\dot{V}O_2\) was higher during the high intensity exercise trials (LCHE and HCHE) compared to low intensity exercise trials (LCLE and HCLE; \(P < 0.01\); Figure 2.2A). Following exercise, \(\dot{V}O_2\) remained above pre-exercise levels for the whole 6-hour recovery period for all trials \((P < 0.05)\), with \(\dot{V}O_2\) being significantly higher during the first hour following high intensity exercise (HCHE and LCHE) compared to low intensity exercise trials (HCLE and LCLE; \(P < 0.01\)). Meal ingestion did not result in a transient change in \(\dot{V}O_2\) across conditions, although \(\dot{V}O_2\) remained elevated above resting levels.

During exercise, the rate of energy expenditure was similar for both high intensity exercise trials and significantly higher than the low intensity trials \((P < 0.05; \) Figure 2.2B), whereas total energy expenditure during low intensity exercise trials (3421 ± 125 kJ) was similar to high intensity exercise trials (3299 ± 177 kJ). During the first hour of recovery, energy expenditure was higher following the high intensity compared to the low
intensity exercise trials ($P < 0.05$). After meal ingestion, energy expenditure was significantly higher than baseline but remained similar across all trials.

Baseline RER prior to exercise was similar between trials. However, during exercise, average RER was higher in the high compared to low intensity trials ($P < 0.05$; Figure 2.2C). During early recovery, RER were significantly lower following high intensity exercise compared to both baseline and the corresponding low intensity trials. There were no significant differences between trials for the first two hours after meal ingestion. However, over the remaining recovery period, the mean RER was significantly higher in both high-CHO meal conditions compared to the other trials ($P < 0.05$). Furthermore, RER was significantly lower in LCHE than the other trials throughout this period ($P < 0.05$), and at some time points RER was lower in HCHE than HCLE ($P < 0.05$).
Table 2.1

Descriptive characteristics of the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (n = 12)</th>
<th>± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>70.74</td>
<td>10.1</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>22.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>13.38</td>
<td>4.9</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>62.03</td>
<td>8.4</td>
</tr>
<tr>
<td>(\dot{V}O_{2\text{peak}}) (ml.kg⁻¹.min⁻¹)</td>
<td>58.4</td>
<td>4.3</td>
</tr>
<tr>
<td>(l.min⁻¹)</td>
<td>3.9</td>
<td>1.8</td>
</tr>
<tr>
<td>HRmax</td>
<td>184</td>
<td>7</td>
</tr>
<tr>
<td>Daily food intake (kJ/day)</td>
<td>12,250</td>
<td>1,060</td>
</tr>
<tr>
<td>RMR (kJ.day⁻¹)</td>
<td>6,802</td>
<td>668</td>
</tr>
<tr>
<td>Exercise (hr/week)</td>
<td>5.1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

All values are expressed as means ± standard deviation (SD), n =12. BMI: Body mass index (weight/height²); FFM: Fat free mass; \(\dot{V}O_{2\text{peak}}\): Peak oxygen consumption; RMR: Resting metabolic rate; HRmax: Maximal heart rate.
FIGURE 2.2. Effect of exercise intensity on (A) oxygen consumption, (B) energy expenditure and (C) respiratory exchange ratio (RER). \( ^{a} \) significant difference between LCHE and HCHE, \( ^{b} \) significant difference between LCLE and HCLE, \( ^{c} \) significant difference between HCHE and HCLE, \( ^{d} \) significant difference between LCHE and LCLE, and \( ^{e} \) significant difference from pre-exercise \((P < 0.05)\). All results are expressed as means ± SEM \((n = 12)\).
2.3.3 *Relative and absolute rates of substrate oxidation*

The average relative contribution of CHO oxidation during exercise was greater during the high intensity trials compared to the low intensity trials ($P < 0.05$; Figure 2.3A). Accordingly, the relative contribution of fat oxidation during exercise was reduced during high intensity compared to low intensity exercise trials ($P < 0.05$; Figure 2.3B). In absolute terms, average CHO oxidation rate was threefold higher during high compared to low intensity exercise ($P < 0.05$; Figure 2.4A), and although the absolute rate of CHO oxidation fell progressively during both high intensity exercise trials, the absolute rate of CHO oxidised remained significantly greater compared to the low intensity exercise ($P < 0.05$). The total amount of fat oxidised (Figure 2.5B) during exercise was significantly higher during the low intensity exercise compared to high intensity exercise trials ($P < 0.05$), but the rate of fat oxidation did not differ over the last 20 min of exercise across all trials.

During the first hour of recovery, the average relative and absolute CHO oxidation rates were significantly lower after the high compared to low intensity exercise trials ($P < 0.05$; Figure 2.3A and 2.4A). In contrast, the relative contribution of fat to energy expenditure during the first hour of recovery from high intensity exercise was higher than low intensity exercise ($P < 0.05$; Figure 2.3B). Consequently, the total amount of CHO (gram) oxidised during the first hour of recovery was significantly lower for the high intensity exercise compared to low intensity exercise ($P < 0.05$; Figure 2.5A), whereas the absolute amount of fat oxidised over this period was higher following high compared to low intensity exercise trials ($P < 0.05$; Figure 2.5B).

Over the first two hours following meal ingestion (180 - 300 min), the average relative and absolute contribution of CHO and fat oxidation rates were similar between all trials.
(Figure 2.3 and Figure 2.4). However, over the next 3-h period (300 - 480 min), the patterns of change in relative CHO and fat oxidation rates were markedly different ($P < 0.05$). Correspondingly, relative CHO oxidation was significantly lower during the low CHO trials (LCLE and LCHE) compared to the high CHO trials (HCLE and HCHE). In addition, relative CHO oxidation was lower in LCHE compared to LCLE ($P < 0.05$). At some time points, the relative and absolute CHO oxidations rates were lower in HCHE than HCLE ($P < 0.05$).

During the last 3-h of recovery, relative and absolute fat oxidation was higher in LCHE compared to the other trials ($P < 0.05$). At most time points during this period, there were no difference between HCHE and HCLE, whereas the relative fat oxidation rates were higher in LCHE than LCLE ($P < 0.05$). Overall, the total amount of CHO oxidised after meal ingestion (post-absorptive 1- 6 h) was significantly lower in LCHE than the other trials ($P < 0.05$; Figure 2.5A), whereas the total amount of fat oxidised during the same period was higher in LCHE compared to the other trials ($P < 0.05$; Figure 3.4B). The amount of CHO and fat oxidised in HCHE was lower and higher, respectively, than HCLE ($P < 0.05$) during this period.
FIGURE 2.3. Effect of exercise intensity on the relative contributions of (A) CHO and (B) FAT oxidation during exercise and recovery. \textsuperscript{a} significant difference between LCHE and HCHE, \textsuperscript{b} significant difference between LCLE and HCLE, \textsuperscript{c} significant difference between HCHE and HCLE, \textsuperscript{d} significant difference between LCHE and LCLE ($P < 0.05$). All results are expressed as means ± SEM ($n = 12$).
FIGURE 2.4 Effect of exercise intensity on the absolute rate (kJ.min\(^{-1}\)) of (A) CHO and (B) Fat oxidation. \(^a\) significant difference between LCHE and HCHE, \(^b\) significant difference between LCLE and HCLE, \(^c\) significant difference between HCHE and HCLE, \(^d\) significant difference between LCHE and LCLE \((P < 0.05)\). The figure insert highlights the 180 min to 480 min recovery period.
FIGURE 2.5. Effect of exercise intensity and recovery on total fuel utilisation for (A) CHO and (B) Fat oxidation. \(^{a}\) significant difference between LCHE and HCHE, \(^{b}\) significant difference between LCLE and HCLE, \(^{c}\) significant difference between HCHE and HCLE, \(^{d}\) significant difference between LCHE and LCLE \((P < 0.05)\). All results are expressed as means ± SEM \((n = 12)\).
2.3.4 Metabolite and hormonal responses

In response to high intensity exercise, blood lactate levels were several-fold higher compared to low intensity exercise trials (Figure 2.6A; \( P < 0.05 \)). Post-exercise lactate levels returned to near pre-exercise levels within 30 min of recovery. However, 3 h after meal intake, there was a slight increase in lactate above baseline which remained stable till the end of the recovery period. During exercise, blood pH (Figure 2.6B) fell to levels that were significantly lower in the high intensity exercise compared to low intensity exercise trials \( (P < 0.05) \). During the recovery period, blood pH returned to baseline levels within 30 min of recovery and remained stable across all trials.

Despite the large differences in exercise intensity, blood glucose concentrations during exercise were similar across all trials (Figure 2.7A; \( P > 0.05 \)). However, during first hour of recovery, blood glucose concentrations were lower in high intensity exercise compared to low intensity exercise \( (P < 0.05) \). In response to the CHO meal, the blood glucose profile showed a similar pattern across trials, with an increase blood glucose above pre-meal and baseline values before returning to near baseline values approximately 3 h later (Figure 2.7A). Peak plasma glucose concentrations were significantly higher after the High-CHO diet compared to the Low-CHO diet, with lowest levels attained in the LCHE trials \( (P < 0.05) \). During the remainder of the recovery period, blood glucose concentrations decreased progressively to pre-meal levels within 3 h.

Insulin concentrations (Figure 2.7B) significantly increased above pre-exercise and pre-meal values following meal ingestion \( (P < 0.05) \), peaking at 210 min and decreasing progressively before returning to pre-meal values by the end of the recovery period. The response of plasma insulin concentration to LCHE was significantly lower than the other
trials \((P < 0.05)\). During the last hour of the recovery period, insulin concentrations returned to near pre-meal values in all four trials with no differences between conditions.

After exercise, free fatty acid (FFA) concentrations (Figure 2.7C) increased to a greater extent in response to high intensity exercise compared to the low intensity exercise trials \((P < 0.05)\). Within the first hour after meal ingestion, FFA declined rapidly across all conditions and remained below baseline levels over the following two hours. However, FFA concentrations were higher for LCHE compared to the other three conditions \((P < 0.05)\). After 3-h of recovery (2-h after meal ingestion), FFA concentration increased across all trials, with FFA levels in LCHE being significantly higher compared to LCLE, HCHE and HCLE, with no differences between the two High-CHO trials \((P > 0.05)\).
FIGURE 2.6 Effect of exercise intensity and recovery on (A) lactate and (B) pH.

\(^{c}\) significant difference between HCHE and HCLE, \(^{d}\) significant difference between LCHE and LCLE, and \(^{e}\) significant difference from pre-exercise \((P < 0.05)\). All results are expressed as means ± SEM \((n = 12)\).
FIGURE 2.7. Effect of exercise intensity and recovery on (A) glucose, (B) insulin and (C) free fatty acid (FFA). a significant difference between LCHE and HCHE, b significant difference between LCLE and HCLE, c significant difference between HCHE and HCLE, d significant difference between LCHE and LCLE, and e significant difference from pre-exercise (P < 0.05). All results are expressed as means ± SEM (n = 6).
2.4 DISCUSSION

It is well established that an increase in exercise intensity is generally associated with a decrease in the proportion of CHO oxidised during recovery, thus promoting the sparing of CHO for subsequent exercise bouts. In contrast, the proportion of CHO oxidised in response to a meal ingested after exercise has been reported to be unaffected by exercise intensity (Trueth et al., 1996; Thompson et al., 1998; Folch et al., 2001; Votruba et al., 2002; Melanson et al., 2002). This latter finding might be explained on the basis that large amounts of CHO were fed after exercise in some studies (Thompson et al., 1998; Folch et al., 2001; Melanson et al., 2002), thus raising the possibility that this may have overridden any CHO sparing during the post-exercise period. Moreover, since the temporal pattern of change in CHO and fat oxidation after meal ingestion was not examined in any of these studies (Trueth et al., 1996; Thompson et al., 1998; Folch et al., 2001; Melanson et al., 2002; Votruba et al., 2002), it is possible that CHO sparing at particular points in time might have gone undetected. For these reasons, this study re-examined the extent to which the amount of CHO fed post-exercise affects over time the impact of exercise intensity on the proportion of CHO and fat oxidised during recovery. Our results indicate that irrespective of the amount of CHO ingested after exercise, exercise intensity has no effect on the rates of CHO and fat oxidation within 2-h after meal ingestion. Later, however, less CHO and more fat are oxidised in response to high compared to low intensity exercise, particularly when less CHO is ingested. These findings are consistent with previous studies that reported following a post-exercise meal, the body shifts towards fat oxidation and spare CHO, especially after intense exercise when CHO preservation or storage becomes a priority (Bielinski et al., 1986, Kiens & Richter, 1998; Yoshioka et al., 2001; Kimber et al., 2002). In reality, after a mixed meal or glucose meal, fat oxidation is suppressed due to the effect of insulin on lipolysis.
The delayed inhibitory effect of intense exercise on CHO oxidation after the post-exercise ingestion of a meal does not support earlier findings that CHO and fat oxidation rates under these conditions were unaffected by exercise intensity. Indeed, Votruba and colleagues (2002) reported that meal ingestion following low and high intensity exercises has a similar effect on the average rate of CHO oxidation over an 11.5-h recovery period. Likewise, Melanson et al. (2002) did not observe any effect of exercise intensity on the total amount of fat or CHO oxidised over 24 h in individuals fed post-exercise. Thompson and colleagues (1996) also reported no significant differences in post-exercise CHO oxidation between low and moderate intensity exercise over a 6-h recovery period during which a meal was ingested. Moreover, Treuth and collaborators (1996) did not find any significant differences in post-exercise 24-h CHO or fat oxidation between high intensity and low intensity exercise. Although, Yoshioka and colleagues (2001) reported that fat oxidation rate during the 3-h recovery period was higher after the high compared to low intensity exercise condition following the ingestion of a mixed meal, they did not examine the response of CHO oxidation rate post-exercise. Finally, Folch and colleagues (2001) showed that exercise intensity has no effect on the post-exercise rates of CHO oxidation, irrespective of the meal size ingested during recovery.

The earlier reports of exercise intensity having no effect on the amount of CHO oxidised in response to a meal ingested during recovery might be due to the excessive amounts of CHO ingested in some studies (Thompson et al., 1998; Folch et al., 2001; Melanson et al., 2002), with CHO intake exceeding the amount of CHO oxidised during exercise. In support of this view, our results shows that when post-exercise CHO intake is large and exceeds that oxidised during exercise, exercise intensity has little effect on the extent to which CHO is oxidised after a meal, with only an extra $2.13 \pm 1.65$ g of CHO spared when CHO is ingested after high compared to low intensity exercise. In contrast, when
the amount of ingested CHO is below that oxidised during exercise, high intensity exercise inhibits CHO oxidation and increases fat oxidation, thus causing the extra sparing of CHO. The hyperinsulinaemia together with the marked hyperglycaemia and decreased levels of FFA associated with the high CHO intake might be sufficient to counter any increase in CHO sparing that would normally occur after high intensity exercise, thus resulting in diminished fat oxidation (Trueth et al., 1996; Thompson et al., 1998; Melanson et al., 2002). In support of this view, a number of studies have reported that the consumption of a high CHO meal increases CHO oxidation and decreases fat oxidation due to a rise in glucose delivery and an increase in insulin-mediated inhibition of lipolysis and associated reduction in plasma FFA level (Bielinski et al., 1986; Dionne et al., 2001; Rose et al., 2001; Long et al., 2008).

Another factor that might explain why most of the aforementioned studies did not report significant effects of exercise intensity on CHO and fat oxidation rates is that they did not examine the temporal pattern of change in fat and CHO oxidation rates (Trueth et al., 1996; Thompson et al., 1998; Folch et al., 2001; Votruba et al., 2002; Melanson et al., 2002). As a result, it is possible that those studies missed the time periods during which fat and CHO oxidation rates might have been affected by exercise intensity. In support of this view, this study shows that later during recovery, the rates of carbohydrate and fat oxidation after meal ingestion were affected by exercise intensity but not during the initial 2-h following meal ingestion, irrespective of the amount of CHO ingested. Also, our current findings showed that had only the five-hour post-meal period been examined here as a period of time, we would have reported minimal effect of exercise intensity on total CHO oxidation in individuals fed a high CHO meal, which is in agreement with previous findings of Thompson et al. (1998), Folch et al. (2000) and Votruba et al.,
(2002), thus highlighting the importance of examining fuel oxidation across time rather than as an end point measure.

The patterns of change in plasma insulin, glucose and FFA levels in response to exercise and meal intake might explain, at least in part, the time-dependent effect of exercise intensity on CHO and fat oxidation described above. During the first 2 h after meal ingestion, the absence of any effect of both exercise intensity and CHO meal content on CHO and fat oxidation rates could be explained on the grounds that the elevated plasma glucose and insulin concentrations during that time were probably high enough across all conditions to favour optimal rates of transport, storage, and oxidation of glucose in skeletal muscle (Ivy et al., 1988; Ivy, 1991a, 1991b; Rose et al., 2001, Long et al., 2008). Moreover, the absence of difference in CHO sparing during that time might be related to a marked insulin-mediated inhibition of lipolysis and increased fatty acid reesterification and storage as triglycerides (Horowitz, et al., 1997; Dionne et al., 2000; Long et al., 2008) as suggested by the fall in plasma FFA levels across all conditions. However, late during recovery, the lower rate of CHO oxidation during that time when CHO is ingested after high compared to low intensity exercise might be explained by the rapid decrease in plasma insulin and glucose levels together with the progressive and higher rise in plasma FFA concentrations after intense exercise (Yoshioka et al., 2001). In addition, the lower muscle glycogen levels after high intensity exercise is likely to play some role, since there is evidence that low glycogen levels inhibit muscle CHO metabolism and stimulate fat oxidation after exercise (Kiens & Richter, 1998; Kimber et al., 2002). This could involve the activation of carnitine palmitoyl transferase 1 (Sidossis & Wolfe, 1996; Sidossis et al., 1997; Ramussen et al., 1998; Henderson et al., 2007), a process likely to be favoured by the higher levels of circulating FFAs after exercise in agreement with previous studies (Bielinski et al, 1986; Wolfe et al., 1990; Bahr et al., 1991a; Yoshioka et al., 2001;
Henderson et al., 2008; Long et al., 2008). The progressive rise in muscle glycogen concentrations during recovery would eventually be expected to favour the return of CHO oxidation to basal rates, whereas CHO restriction that maintains glycogen depletion should slow this process (Ivy et al., 1988; Wolfe et al., 1990; Kimber et al., 2002). Since CHO oxidation and pyruvate dehydrogenase (PDH) activation have been shown to decrease despite the marked elevation in plasma glucose and insulin levels following the ingestion of high-CHO meals ingested after intense exercise (Kimber et al., 2002), a different pattern of PDH response to exercise intensity might also explain some of our present findings. Finally, high FFA levels have been shown to compromise glucose transport, as indicated by infusion of lipids and heparin during hyperinsulinemic, euglycemic clamp studies (Itani et al., 2002; Ramussen et al., 2002). The increased FFA levels late after meal intake in individuals recovering from intense exercise might have resulted in less CHO available for oxidation (Bielinski et al, 1986, Bahr et al., 1991; Yoshioka et al., 2001; Kimber et al., 2002; Long et al., 2008).

In agreement with other studies, our study shows that high intensity exercise may have an effect on CHO sparing during early recovery without food despite the absence of marked differences in plasma insulin, FFA and glucose levels (Bielinski et al, 1986; Broeder et al., 1991; Phelain et al., 1997; Kuo et al., 2005). Indeed, during the first hour of recovery without food, we showed that the higher rates of fat oxidation and lower rates of CHO oxidation following high compared to low intensity exercise are consistent with the body sparing its limited stores of CHO by increasing the proportion of fat oxidised during recovery. It is important to note that the magnitude of the increase and fall in the rates of fat and CHO oxidation, respectively, during early recovery from intense aerobic exercise is unlikely to have been overestimated by the replenishment of the body’s bicarbonate pool during recovery because plasma pH and CO₂ levels remained relatively stable during
that time. Therefore, our results corroborate those of others that CHO oxidation rates fall during recovery without food compared to pre-exercise or control conditions (Bielinski et al., 1986; Horton et al., 1998; Kiens & Richter, 1998; Kimber et al., 2002; Kuo et al., 2005; Henderson et al., 2007; Malatesta et al., 2009), with higher fat oxidation rates during recovery from high compared to low intensity exercise (Bahr & Sejerstead, 1991b; Bahr et al., 1991; Broeder et al., 1991; Phelain, et al., 1997; Yoshika et al., 2001; Kuo et al., 2005; Warren et al., 2009). This increase in fat oxidation rate together with the sparing of the body’s CHO stores from further depletion is favourable to the repletion of muscle glycogen stores from endogenous carbon sources. Indeed, although humans depend to a large extent on CHO intake to replenish their stores of muscle glycogen after exercise, they also have the capacity to replenish at least part of their muscle glycogen without food intake, using amino acids derived from muscle protein breakdown or lactate as a carbon source for glycogen repletion (Maehlum & Hermansen, 1978; Bangsbo et al., 1999; Fournier et al., 2002; Fairchild et al., 2003; Raja et al., 2003).

The importance of sparing CHO with or without a meal after intense exercise is clearly advantageous, particularly considering that the relative and absolute rates of CHO oxidation during exercise increase with exercise intensity as shown here and by others (Romijn et al., 1993, 2000; Brooks & Mercier, 1994; Horowitz et al., 1997; Coyle et al., 1997; van Loon et al., 2001; Kuo et al., 2005; Malatesta et al., 2009). Despite such increased reliance on CHO oxidation with exercise intensity, it is noteworthy that, as shown in earlier studies, the rates of CHO oxidation during exercise fall progressively with time, whereas those of fat oxidation rate increase, thus promoting some sparing of CHO (Wolfe et al., 1990; Horowitz et al., 1997; Coyle et al., 1997; Sidossis et al., 1997; van Loon et al., 2001).
2.5 CONCLUSION

In conclusion, this study does not support earlier findings that exercise intensity has no effect on the proportion of carbohydrate oxidised in response to a meal ingested during recovery. Although the results indicate that irrespective of the amount of CHO ingested after exercise, exercise intensity has no effect on the rate of CHO and fat oxidation within 2 hours of meal intake, later during recovery, less CHO and more fat were oxidised in response to high compared to low intensity exercise, particularly when less CHO was ingested. This increased CHO sparing occurs at the expense of an increase in the oxidation of the body’s endogenous fat stores. However, when CHO intake during recovery is large enough to match or exceed that oxidised during exercise, as has been the case in many previous studies, exercise intensity has little effect on the rates of CHO and fat oxidation after a meal. Overall, given the limited size of the body’s CHO stores and the importance of CHO in supporting muscle energy demands, the sparing of CHO across a range of physiological conditions is clearly advantageous for maintaining our capacity to engage in fight or flight responses.

This study raises a number of unanswered questions since it was limited to physically active males, and it remains to be seen whether highly trained athletes, females or obese individuals would respond in a similar way. The findings also suggest a potential dose-response relationship between exercise intensity and post-exercise fuel metabolism response to meal intake. In addition, it is still unclear to what extend the ingestion of a mixed meal rather than the 100% CHO meal adopted here would affect our findings. Furthermore, given that the total amount of fat oxidised during exercise and recovery is at its lowest when high intensity exercise is followed by high carbohydrate intake, this might be a factor to take into consideration in the prevention of weight gain. Finally, the molecular mechanisms underlying the pattern of fat oxidation by the skeletal muscle in
response to a meal ingested after exercise of varying intensity remains to be elucidated as well as those determining the partitioning of CHO oxidation between endogenous CHO stores and ingested CHO.
2.6 REFERENCES


Appendix A

Human Ethics Approval
Our Ref: RA/4/3/1508
2009

Dr P Fournier
Sport Science, Exercise & Health - M408
UWA

Project: Effect of carbohydrate intake after low or high intensity exercise on carbohydrate sparing during recovery
Student: Mohamad Jaafar - Masters - 10450025

Please be advised that ethical approval of the above project has been granted in accordance with the procedures of the Human Research Ethics Committee at the University of Western Australia.

It is the responsibility of the researcher to advise the Committee of any departure from the original protocol. The Committee requires that all Chief Investigators report immediately any adverse or unexpected events that might affect ethical approval of the project.

Approval should be sought in writing in advance from the Human Research Ethics Committee if any change to the procedures or the number of participants in the original application is envisaged. Should this change require amendments to an Information Sheet or Consent Form related to the project; the amended version of the forms should be submitted for review. The application for the amendment should give the rationale behind and justification for the amendment. You are also required to inform the Committee, giving reasons, if the research project is discontinued before the expected date of completion. Correspondence should be submitted to the Secretary, Human Research Ethics Committee, Research Services.

The Committee is bound by NHMRC Guidelines to monitor the progress of all approved projects until completion to ensure that they continue to conform to approved ethical standards. An Annual Report form will be sent to you twelve months after the initial approval date.

Please note that approval has been granted for a period of four years. Initial approval is for a period of one year, and, thereafter for future periods of one year at a time subject to the receipt of satisfactory annual reports. At the end of the four-year period you will be required to complete a new "Application to Undertake Research Involving Human Subjects" should you wish to continue with your research. However, in special circumstances, the Chair has the authority to extend the approval period in order to complete a project. Failure to submit a final report may result in delays for future applications.

Please quote Project No RA/4/3/1508 on all correspondence associated with this study.

Yours sincerely

KATE KIRK
Executive Officer
(Human Research Ethics Committee)
Appendix B

Participant information sheet

Participants consent form
EFFECT OF CARBOHYDRATE INTAKE AFTER LOW OR HIGH INTENSITY EXERCISE ON CARBOHYDRATE SPARING DURING RECOVERY

- Participant Information Sheet -

Dear Participant,

As part of my Master’s thesis, I would like to invite you to participate in a research study performed in the School of Sport Science, Exercise & Health at the University of Western Australia. To be eligible for this study, healthy physically active male volunteers between the ages of 18 – 35 years old will be considered. All participants will be required to have maintained a stable weight over past 3 months (+/- 2 kg) and will be screened for any sickness including evidence of metabolic or cardiovascular disorders.

Purpose:

The purpose of this study is to investigate the effect of carbohydrate (CHO) intake on the rates of fat and carbohydrate oxidation during recovery from low and high intensity exercise, and to determine if CHO sparing increases with a rise in exercise intensity. CHO is an important fuel source for contracting muscle; therefore the ‘sparing’ of CHO during and after exercise is expected to be highly advantageous in subsequent exercise performance.

Procedures:

You will be required to attend our Exercise Physiology laboratory on six separate occasions. The first visit is a familiarisation session. During this session, your height, weight and skinfolds at eight sites will be measured. In addition, your peak aerobic capacity ($\dot{V}O_{2peak}$) will be determined using a graded exercise test on a cycle ergometer. This will require you to cycle at a constant number of revolutions per minute (70–90), while the resistance is gradually increased until volitional exhaustion. The workload will commence at 70 W and increased by 30 W after every 4 minutes (3 minute work period followed by 1 minute rest period). During this test you will breathe through a mouth piece to allow for the collection of your expired air. Blood samples will be taken from the ear lobe with a sterilised lancet at rest and during the 1 minute rest period of each stage for the measurement of blood lactate concentration. After a short rest, you will then be required to perform a familiarisation ride for 15 minutes before the end of this session. Then you will be briefed on the subsequent testing trials and be given a 3-day physical activity and food diary to be completed before the next testing session. It is important to replicate the physical activity and food intake as closely as possible to maintain consistency throughout the rest of the study period.

For the second session, you will be required to visit the lab at 8.00am after an overnight fast. After a 30-min rest period, you will cycle at low intensity (35% $\dot{V}O_{2peak}$) for 15 min, followed by 45 min at moderate intensity (70% $\dot{V}O_{2peak}$). Your respiratory gases will be collected at time intervals
for measurement of fat and carbohydrate oxidation rates by breathing through a mouthpiece during exercise

The subsequent 4 sessions will occur approximately 1-2 weeks later and will be randomly assigned. For each testing session, you will be asked to perform one of the following cycling tasks on four separate occasions:

- Low intensity exercise at 35% $\dot{V}O_{2peak}$ for approximately 120 minutes followed by a low CHO meal
- High intensity exercise at 70% $\dot{V}O_{2peak}$ for 60 minutes followed by a low CHO meal
- Low intensity exercise at 35% $\dot{V}O_{2peak}$ for approximately 120 minutes followed by a high CHO meal
- High intensity exercise at 70% $\dot{V}O_{2peak}$ for 60 minutes followed by a high CHO meal

After exercise, you will be required to stay at the laboratory for six hours. An experimental CHO meal will be provided one hour after exercise. Your respiratory gases and blood samples will be taken during rest, exercise and recovery period every 15-30 minutes for measurements of energy expenditures. During this time, you will breathe through a mouthpiece connected to Tissot gasometer tank or a metabolic cart for the collection of expired air (volume & concentration of $O_2$ and $CO_2$). Blood samples (200µl per sampling) will be taken from your fingertips at time intervals before, during and after exercise. To facilitate blood sampling, your hand will be placed in a plastic/rubber glove and submerged in water maintained at 40°C. Then the finger is alcohol swabbed and finger prick with a sterilised disposable lancet before drawing the blood into the capillary tubes. These samples will be used for the measurement of insulin, lactate, glucose, free fatty acids, pCO$_2$, bicarbonate and pH levels.

Benefits:

You will receive a free physiological assessment of your body composition (Body fat % and fat free mass) and aerobic capacity ($\dot{V}O_{2peak}$).

Possible Risks:

The study examines how fast your body burns fat and carbohydrate during and after exercise. Some discomfort may arise from the physical exercise sessions. Nevertheless, you will be under full supervision for the duration of the testing period. You may also experience some delayed-onset-muscle-soreness and fatigue 24-72 hours following testing sessions. For this reason, you will be asked to stretch and massage your muscles after each of your visits to the laboratory. Blood withdrawal may cause minimal discomfort, pain and temporary bruising at the site sampling.

Confidentiality of Data:

Personal details and test results will be treated confidentially at all times. Record keeping of your data will be kept and viewed only by authorised personnel at the School of Human Movement and Exercise Science. Individual data will not be identifiable, but collective results may be published.
Subject Rights:

Participation in this research is voluntary and you are free to withdraw from the study at any time without prejudice. You can withdraw for any reason and you do not need to justify your decision.

*If you withdraw from the study and you are an employee or student at the University of Western Australia (UWA) this will not prejudice your status and rights as employee or student of UWA. If you withdraw from the study and are a patient recruited from one of the affiliated clinics your treatment will not be prejudiced or affected in any way.*

If you do withdraw we may wish to retain the data that we have recorded from you but only if you agree, otherwise your records will be destroyed. Your participation in this study does not prejudice any right to compensation that you may have under statute of common law.

Your participation in this study is greatly appreciated. If you have any questions, I can be contacted directly on 0421 775 990 or via e-mail at jaafam02@student.uwa.edu.au. Queries can also be directed to my supervisors, Dr Paul Fournier on 6488 1356 or Dr Kym Guelfi on 6488 2606 in the School of Sport Science, Exercise & Health.
Effect of exercise intensity on fat and carbohydrate oxidation after a carbohydrate-rich meal ingested early during recovery

Consent Form

I ___________________________ have read the information provided and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time without reason and without prejudice.

1. I understand that my participation is entirely voluntary.
2. I understand that, at my request, I can receive additional explanation of the study at any time.
3. I understand that blood sampling is essential to this study and the risks associated with this study are minimal, but may include minimal discomfort and pain.
4. I have been fully briefed on the experimental protocols, benefits and possible risks associated with this study.

I understand that all information provided will be treated as strictly confidential and will not be released by the investigator unless required to by law. I have been advised as to what data is being collected, what the purpose is, and what will be done with the data upon completion of the research.

I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

______________________________  _______________________
Participant Name (please print)  Date

______________________________  _______________________
Participant Signature  Participant Contact No.

The Human Research Ethics Committee at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee, Registrar’s Office, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (telephone number 6488-3703). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records.
Appendix C

Medical screening form
Medical Screening Sheet

All information given is personal and confidential. The information will give us to better understanding of your health and fitness level.

Name ___________________________________________ Age ______________________________

Current address ________________________________________________________________________

Phone (home, business) ________________________________________________________________

E-mail address _______________________________ ____________________________________________

Emergency contact information (name, address, phone)
____________________________________________________________________________________

1. Do you have chest pain or discomfort in the chest, neck, jaw and arms during exercise? YES NO

2. Do you have shortness of breath at rest or with mild exertion? YES NO

3. Have you ever fainted or experienced dizziness? YES NO

4. Do you have trouble breathing when you lie down or sleep? YES NO

5. Do you have swollen ankles? YES NO

6. Do you have a fast heart beat? YES NO

7. Do you experience intermittent cramps in your legs while walking? YES NO

8. Do you have a known heart murmur? YES NO

9. Do you feel unusual fatigue or shortness of breath with usual activities? YES NO

Family History
Has your father had myocardial infarction, coronary revascularization or sudden death before age 55 or your mother before age 65? YES NO

Cigarette smoking
Do you smoke cigarettes or did you quit in the previous 6 months? YES NO

Hypertension
Has your doctor ever told you that you have high blood pressure? YES NO

Hypercholesterolemia
Has your doctor ever told you that you have abnormal cholesterol levels? YES NO

Impaired fasting glucose
Has your doctor ever told that you have high blood glucose (sugar)? YES NO

Obesity
Is your BMI more than 30 or your waist circumference more than 100cm? YES NO
**Sedentary lifestyle**

Do you participate in an exercise program or recreational activity regularly? **YES** **NO**

Do you have any other reasons that limit your exercise program? (Orthopedic problems, metabolic problems)? If so, please list them below:

________________________________________________________________________

________________________________________________________________________

Do you currently exercise? **Yes** **NO**

If yes, how often do you exercise and how long have you been exercising?

________________________________________________________________________

________________________________________________________________________

What type of exercise do you do? (e.g. running, bike, rowing, free weights, machine etc...)

________________________________________________________________________

Do you take any medication or supplements? **Yes** **NO**

If yes, please list the names of drugs, purpose of drugs, and prescribed or OTC?

________________________________________________________________________

________________________________________________________________________

I have answered the above questions honestly, and to the best of my knowledge.

Name __________________

Signature________________

Date___________
Appendix D

Physical activity and food intake diary
EFFECT OF EXERCISE INTENSITY ON FAT AND CARBOHYDRATE OXIDATION AFTER A CHO-RICH MEAL INGESTED EARLY DURING RECOVERY

Participant Handbook
A Note to Participants:
Thank you for your participation in this research project. Your willingness to commit your time as a volunteer is very much appreciated. Through this project, we hope to enhance our understanding of the way the body system works during and after exercise. This handbook contains some information on the project along with a physical activity and food intake diary that you are required to fill up daily before each test session. Your diligence in adhering to the instructions given below will help towards the success of this project.

Reminders:

- Please do not alter your lifestyle throughout your participation in this study.
  The physical activity and food diary is provided to closely match your food intake and energy expenditures over the study period.

- Do not consume alcohol or food 12-hours prior to the exercise session.

- Avoid any type of physical activity 24-hours before any testing session.

- Avoid cycling, walking or running to the lab on the morning of every testing session. Pick-up arrangements can be made days in advance with the researcher.

- If you have any difficulties or further enquiries, please feel free to contact me at 0421 775 990 (hp) or email: jaafam02@student.uwa.edu.au.

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Mohamad Jaafar
Researcher
**Physical Activity Diary Directions**

This diary is for you to keep a record of all your physical activity 3 days before testing. This includes activities that feel easy (or light), hard (or vigorous) and activities that feel neither easy or hard (moderate):

- **Easy (light)** activity involves little effort (e.g. walking, bowling or snooker)
- **Neither easy or hard (moderate)** activity makes you warm and slightly out of breath but not exhausted (e.g. brisk walking, steady swimming, cycling, dancing)
- **Hard (vigorous)** activity involves lots of effort and makes your heart beat fast (e.g. basketball, football, jogging/running, energetic dancing, aerobics or circuit training).

Activity you do in school and activity you might do in the playground, street or park (such as playing chase, roller skating, or skate boarding) also counts.

**Please:**

1. Complete all sections of the diary for each day and weekend day.
2. Record how long you spent doing the activity (in minutes).
3. Record how hard the activity was.
4. Try to fill your diary in regularly and at least once a day. Otherwise, you might forget things you have done.
5. Only perform the activities that you would normally do during a normal week.

At the end of each day or at the end of the week, add up the number of minutes you have spent being active each day

**Food Diary Directions**

1. Three days before your testing session, you need to keep a food diary to help you replicate your food intake before subsequent testing sessions.
2. Do not change your eating habits during these three days.
3. Complete the food diary. Be as specific as possible (i.e. brand or type of food, how it was prepared, and the amount you ate/drank). You need to include everything (i.e. mayonaisse, butter, jelly, etc.), including your drinks (water, beer, wine, diet coke, pepsi, etc.).

**PORTION SIZE: VISUAL GUIDES** Based on the size of an average adult

- 1/2 teaspoon = Fingertip
  - Butter, oil, margarine

- 1 teaspoon = Tip of thumb
  - Peanut butter, butter

- 1 tablespoon = 3 thumbtips
  - Peanut butter

- 1 ounce = Tip of thumb
  - Cubed meat, cheese

- 3 ounces = Palm of hand
  - (Meat, fish)

- 1/4 cup = 1 layer on palm of hand
  - (Mixed nuts)

- 1/2 cup = Rounded handful
  - (Cooked pasta, rice, ice cream)

- 1 cup = 2 cupped hands, two handfuls
  - (Cereal, popcorn, fruit)

- 1 cup = Tight fist
  - (Vegetables, berries)

Sources: "The Portion Teller" by Lisa R. Young, Ph.D., R.D.; USDA dietary guidelines
Food Diary Directions

1. Three days before your testing session, you need to keep a food diary to help you replicate your food intake before subsequent testing sessions.
2. Do not change your eating habits during these three days.
3. Complete the food diary. Be as specific as possible (i.e. brand or type of food, how it was prepared, and the amount you ate/drank). You need to include everything (i.e. mayonnaise, butter, jelly, etc.), including your drinks (water, beer, wine, diet coke, pepsi, etc.).

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<th>Date:</th>
<th>Day 1</th>
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<td><strong>Meal</strong></td>
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# PHYSICAL ACTIVITY DIARY

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<th>Activity</th>
<th>Time Spent</th>
<th>Remarks</th>
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