**Post-lunch resting and exercise metabolism following high-fat and high-carbohydrate breakfast**

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**Declaration**

I declare that the research and subsequent write up in this MPhil Thesis is solely the work of myself and is within the guidelines and requirements of the University of Stirling Faculty of Health Sciences and Sport.

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**Introduction**. The aim of this research was to identify the effect of breakfast macronutrient composition on second meal and evening exercise substrate utilisation. The hypothesis of this study was that a high fat breakfast would lead to increased fat utilisation throughout the rest of the day in both resting and exercise, and a high carbohydrate breakfast would lead to increased carbohydrate utilisation throughout the rest of the day in both resting and exercise. **Methods.** 17 subjects completed a baseline trial and two feeding trials; high carbohydrate (porridge) and high fat (avocado based smoothie). The baseline trial consisted of resting energy expenditure using indirect calorimetry and VO2 max test. The feeding trials consisted of the trial breakfast (carbohydrate or fat), a standardised lunch, followed by resting indirect calorimetry and blood glucose measurements, and then finished with an early evening graded exercise test. Substrate utilisation was estimated during rest and during exercise tests with indirect calorimetry. **Results.** Following carbohydrate breakfast RER was significantly higher during rest after lunch (carbohydrate:0.89±0.07 vs fat:0.85±0.07; p=0.026). Blood glucose was significantly higher up to 2 hours after lunch following the high fat breakfast (carbohydrate:6.29±0.79mmol/L vs fat:6.94±1.15 mmol/L; p=0.018). There were no significant differences between breakfast compositions in RER during evening exercise. **Conclusion.** An isocaloric high fat breakfast alters substrate utilisation at rest following a second meal but does not influence fuel selection during early evening exercise. A high fat breakfast impairs glucose tolerance up to 2 hours after the second meal.

**Key Words**

Carbohydrate Oxidation. Fat Oxidation. Blood Glucose. Indirect Calorimetry. Fatmax Exercise Test.

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# Introduction

Breakfast has long been considered the most important meal of the day as its consumption significantly contributes to whole diet nutrient adequacy (Rampersaud et al, 2005). The body requires adequate and appropriate fuel to work effectively and what we eat can influence the way in which our body performs. The benefits of consuming breakfast have been well documented.  Skewing caloric intake towards breakfast improved overall glucose control for the day (Jakubowicz et al 2015). Data suggests that skipping breakfast may impair glucose tolerance later in the day and there is evidence that daily consumption of breakfast can help maintain insulin sensitivity and glycemic control in lean individuals (Betts et al, 2014) and healthy adults (Pereira et al, 2011). Clayton et al (2015) suggested that skipping breakfast may negatively impact exercise performance later in the day due to the reduced calorie intake. Subjects who skipped breakfast did not fully compensate for lost calorie intake at lunch, resulting in a lower daily calorie intake and thus potentially reduced fuel supply for exercise later in the day. It was noted that total work completed during the performance test was greater on post lunch exercise when subjects had eaten breakfast (Clayton et al, 2015). Energy expenditure has been shown to be greater during steady-state exercise in the breakfast omission condition (Clayton et al, 2015), suggesting that breakfast reduces energy expenditure in steady-state exercise. It has been identified that eating breakfast can have a positive effect on metabolism during exercise and throughout the day (Edinburgh et al, 2018). In order to identify the most suitable type of breakfast to maximise training effect and improve performance levels in competition it is necessary to consider macronutrient composition and its impact on metabolism.

Metabolic flexibility is the body’s ability to switch between the two major fuel sources (lipid and carbohydrate) based on availability and needs (Stephenson et al, 2014; Battaglia et al, 2012). Metabolic flexibility is vital as the availability of a fuel source and the body’s requirement can change dramatically depending on the situation. Metabolic flexibility is the capacity to switch from predominantly lipid oxidation and high rates of Fatty Acid (FA) uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation and storage under insulin stimulated conditions (Kelley & Mandarino, 2000). To quantify metabolic flexibility the switch from carbohydrate to lipid oxidation (drop in RER) during an overnight fast or in response to a high fat feeding should be assessed (Galgani et al, 2008). In terms of physical performance, improved metabolic flexibility will result in the body being able to switch quickly between fuel sources and will allow fats to be used at an increased intensity of exercise (Battaglia et al, 2012). Metabolic inflexibilty is the consequence of impaired cellular glucose uptake, which in the face of high lipid supply may lead to increased muscle fat accumulation and insulin resistance (Galgani et al, 2008). A number of exercise nutrition strategies have been extensively researched in this area as a tool to improve metabolic flexibility and glycemic control. One common strategy is to modify breakfast composition (Proeyen et al, 2011).

A mechanism that can be used to explain metabolic flexibility is Randle’s Cycle. Randle’s Cycle is explained by the significant reduction in the uptake and utilisation of glucose that occurs in the muscle when fat oxidation is increased (Randle et al, 1963) (Figure 1). The main features of the model were that increased fat oxidation in muscle would inhibit both pyruvate dehydrogenase and phosphofructokinase by accumulation of acetyl CoA and citrate, respectively. These blocks in the glycolytic pathway would lead to increased glucose-6-phosphate, inhibiting hexokinase and resulting in reduced glucose uptake and oxidation. Randle’s Cycle is also observed in adipose tissue (Blanco & Blanco, 2017). When blood glucose is high and the pancreas secretes insulin, lipolysis is depressed and lipogenesis is stimulated, which reduces the concentration of free FAs in plasma. After a meal, when glucose and insulin levels are elevated, the muscle tends to predominantly use glucose instead of fatty acids. In contrast, during periods of fasting, blood glucose is close to baseline levels, insulin secretion decreases and the concentration of free FAs increases (Blanco & Blanco, 2017).

*Figure 1: Metabolism Summary*

Whether carbohydrate or fat is the dominant fuel during exercise is dependent on a variety of factors, such as background diet, intensity of exercise, aerobic fitness level and duration of exercise (Jeukendrup, 2002). FAs are usually the dominant fuel in low to moderate intensity exercise, whilst during high intensity exercise carbohydrates become the main fuel source (Jeukendrup, 2003) (Figure 2). Fat oxidation increases as exercise intensity increases from low to moderate. In the transition from low to moderate exercise intensity, the increased fat oxidation is a direct result of increased energy expenditure. As the intensity of exercise increases to a higher level (>75% VO2 max) fat oxidation is inhibited and both the relative and the absolute rates of fat oxidation are decreased to very low values (Jeukendrup, 2003). Previous studies observed this relationship in a group of trained subjects over a wide range of exercise intensities, finding that, on average, maximal rates of fat oxidation were observed at 62-63% of VO2 max (Achten & Jeukendrup, 2003; Achten et al, 2003). Substrate utilisation at rest and during exercise can be influenced by the ingestion of a meal. Previous studies have identified that meals containing different proportions of carbohydrate and fat can affect the contributions of carbohydrate and fat to substrate utilisation at rest (Whitley et al, 1998; Murakami et al, 2012) and during exercise (Costil et al 1977; Lambert et al, 1994; Whitley et al, 1998; Pitsiladis & Maughan, 1999; Burke et al, 2000; Rowlands & Hopkins, 2002a; Murakami et al, 2012).

*Figure 2: Fuel source in relation to exercise intensity*

## Pre-exercise carbohydrate ingestion

A number of studies have addressed the impact of pre-exercise carbohydrate feeding on substrate metabolism and performance (Table 1). In terms of performance most studies found either no effect or improved exercise performance following pre-exercise consumption of carbohydrates (100g – 215g) as identified by the systematic review by Colombani et al (2013).  The ACSM Position Stand identifies that endurance athletes require 6-12g/kg BM per day of carbohydrates (ASCM, 2016).

In terms of fuel selection, pre-exercise (45min-4hours) carbohydrate consumption causes an increase in plasma glucose during exercise which causes the pancreas to release insulin (Coyle et al, 1985; Ahlborg & Felig, 1976) reducing hepatic glucose output (Marmy-Conus & Fabris, 1996). The release of insulin increases muscle glucose uptake when at rest (DeFronzo et al, 1983). Increased glucose uptake results in increased glucose availability in the muscle, which stimulates glucose oxidation and glycolysis (Coyle et al, 1997; Febbraio & Stewart, 1996). At the same time, insulin release suppresses fat oxidation via insulin-mediated suppression of lipolysis (Coyle et al, 1985). The result is a reduction of FA availability (Horowitz et al, 1997). These previous studies identify the metabolic process following the ingestion of carbohydrate.

The metabolic effects of carbohydrate ingestion on exercise can be influenced by the timing of ingestion in relation to exercise. It has been shown that blood glucose and insulin elevations are greater when carbohydrate ingestion occurs in close proximity to the onset of exercise (Moseley et al, 2003). Previous studies identified that when carbohydrate is ingested 1-4 hours prior to exercise, insulin and glucose levels decline to near base-levels prior to the exercise (Coyle et al, 1985; Chryssanthopoulos et al, 2002; Chen et al, 2009). However, after carbohydrate consumption ≤60 minutes prior to exercise, insulin and glucose levels are elevated prior to exercise (Marmy-Conus & Fabris, 1996; Chryssanthopoulos et al, 1994; Febbraio et al, 2000; Koivisto et al, 1981). Regardless of the time of ingestion, and increased levels of insulin and glucose, metabolic perturbations associated with carbohydrate ingestion cause an initial reduction in blood glucose at the start of exercise (Coyle et al, 1985; Marmy-Conus & Fabris, 1996). This reduction is temporary and blood glucose levels return to base levels within approximately 20 minutes (Marmy-Conus & Fabris, 1996; Sparks et al, 1998; Moseley et al, 2003; Kuipers et al, 1999). The level at which blood glucose is reduced as exercise begins is associated with the proximity of the meal ingestion, with meals ≤60 minutes prior to exercise causing a greater reduction (Moseley et al, 2003). Moseley et al (2003) identified that regardless of the changes in blood glucose prior to and during the onset of exercise, the effect of timing had no effect on physical performance. This suggests that the timing of carbohydrate ingestion, in relation to exercise, can influence blood glucose prior to exercise and at the onset of exercise.

In addition to the effect on blood glucose levels, the proximity to exercise in which carbohydrate is ingested can also have a marked effect on substrate utilisation during exercise. It has been identified that carbohydrate ingested 1 hour before exercise reduced both lipolysis and fat oxidation, while plasma FA concentrations decreased to very low levels (0.16±0.01mM) during exercise (Horowitz et al, 1997). These findings indicate that a reduced availability of FAs is indeed a factor that limits fat oxidation. When a large amount of glucose was ingested 1 hour prior to exercise, plasma insulin levels were very high (42µU/ml) at the start of exercise, whereas plasma FA concentrations were very low (317±19µM), which resulted in a 30% reduction in fat oxidation compared with no carbohydrate intake (Coyle et al, 1997). This finding suggests that a high carbohydrate meal, taken in as little as 1 hour prior to exercise, could result in a reduction of fat oxidation.

*Table 1: The effect of pre-exercise carbohydrate ingestion on human metabolism and exercise performance*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Year** | ***n*** | **Treatment** | **Time Prior** | **Protocol** | **Results** |
| **Coyle et al** | 1985 | 7 | HCM (2g/kg BM CHO)Control (fasted )(C) | 4 hrs | 105 min at 70% VO2 max (Bike) | RER (12min) HCM > C (0.95 vs 0.87)RER (after 12 min) = No differenceCHO Oxi = CHO > C (291±20 vs 221±21; g) (32% difference) |
| **Cryssanthopoulos et al** | 1994 | 9 | 75g Glucose (G)Placebo (P) | 30 min | TTE at 70% VO2 max (Treadmill) | RER = No differenceTTE Performance = No difference |
| **Sparks et al**  | 1998 | 8 | HGI (1g/kg BM)LGI (1g/kg BM)Placebo (P) | 45 min45 min | 50 min at 70% VO2 max15 min TT(Bike) | RER = HGI > LGI & P(HGI=0.9±0.01; LGI=0.86±0.01; P=0.85±0.01)CHO Oxi = HGI > LGI & P(HGI=2.80±0.018; LGI=2.35±0.15; P=2.16±0.12; g)TT Performance = No difference |
| **Cryssanthopoulos et al**  | 2002 | 10 | HCM+G (2.5b/kg BM + 6.9% CHO drink)HCM+P (2.5b/kg BM + Placebo)Placebo + Placebo (P+P) | 3hrs | TTE at 70% VO2 max(Treadmill) | RER & CHO Oxi = HCM+G & HCM+P > P+PTTE Performance = HCM+G > HCM+P P+P(HCM+G=125.1±5.3; HCM+P=111.9±5.6; P+P=102.9±7.9; min) |
| **Chen et al**  | 2009 | 8 | 100g HGI CHO (HGI)100g LGI CHO (LGI)Placebo (P)  | 2hrs | 5km at 70% VO2 max16km TT(Treadmill) | RER, CHO Oxi, Fat Oxi = No differenceTT Performance = HGI < P(HGI=91.5±2.2 vs P=93.6±2.1; ,min) |
| **Coyle et al** | 1997 | 6 | 1.4g/kg BM Glucose (G)Control (fasted) (C) | 60 min & 10 min | 40 min at 50% VO2 max (Bike) | RER (25-30min) = G > C(G=0.91±0.01; C=0.86±0.01)RER (35-40min) = G > C(G=0.90±0.01; C=0.86±0.01)CHO Oxi = G (24-28%) > CFat Oxi = G (29-34%) < C |
| **Schabort et al**  | 1999 | 7 | 100g CHO (CHO) Control (fasted) (C) | 3hrs | TTE at 70% VO2 max (Bike) | RER, CHO Oxi, Fat Oxi = No differenceTTE Performance = CHO > C(CHO=135.7±13.9; C=109±12.5; min) |
| **Moseley et al**  | 2003 | 8 | 75g Glucose | 15 min (15G)45 min (45G)75 min (75G) | 20 min at 65% VO2 max40 min TT(Bike) | RER = No differenceTT Performance = No difference |
| **Neufer et al**  | 1987 | 10 | 45g Liquid CHO (LCHO)45g Solid CHO (SCHO)Placebo (P)200g CHO meal + SCHO (M+SCHO)  | 5 mins4hrs + 5 mins | 45 min at 77% VO2 max15min TT(Bike) | RER (15 & 30 min) = M+SCHO > PTT RER = M+SCHO > LCHO, SCHO, P |
| **Rauch et al** | 1995 | 8 | Control Diet (C) + standardised breakfastHCD (78% CHO) + standardised breakfast | 3 days + 3hr | 120min at 65% VO2 max (60sec sprint at 20, 40, 60,80, 100 min)60min TT | Power Output = HCD > C(HCD=233±15; C=219±17; W)Speed = HCD > C(HCD=38.02±1.1; C=36.74±1.29; km/hr) |

*Notes: CHO, Carbohydrate; HGI, High Glycaemic Index; LGI, Low Glycaemic Index; HCM, High Carbohydrate Meal; HFM High Fat Meal; HCD, High Carbohydrate Diet; BM, Body Mass; Fat Oxi; Fat Oxidation; CHO Oxi, Carbohydrate Oxidation; TT, Time Trial; TTE, Time To Exhaustion*

As identified above, carbohydrate ingestion can affect metabolism in as little as 1 hour. Previous studies have also identified the effects of carbohydrate ingestion 3-5 hours prior to exercise. Ingestion of a carbohydrate-rich meal 3-5 hours prior to exercise increases muscle glycogen content by 42% (114.6±13.2 to 163.3±19.5mmol glucosyl units ·kg­­-1) (Coyle et al, 1985) and improves exercise time to exhaustion performance (Chryssanthopoulos et al, 2002) compared to ingestion of a pre-exercise placebo. The enhanced performance observed by Chryssanthopoulos et al (2002) is likely related to increased pre-exercise muscle glycogen (Hargreaves et al, 2004). As muscle glycogen and liver glycogen are combined during exercise, replenishing the liver glycogen level may be as important as this is reduced by approximately 80% after an overnight fast (Jeukendrup, 2004). Ingestion of carbohydrate increases liver reserves and contributes to the maintenance of blood glucose concentration during the subsequent exercise bout (Jeukendrup, 2004). Any performance improvement following pre-exercise carbohydrate consumption is likely due to an increase in glycogen stores (Coyle et al, 1985). It was reported that following a mixed meal, approximately 20% of consumed carbohydrate is stored directly as liver glycogen (Taylor et al, 1996). It has also been found that following pre-exercise ingestion of carbohydrates, there was a 42% increase in muscle glycogen storage (Coyle et al, 1985). Following carbohydrate ingestion, carbohydrate fuel stores are maximised, increasing carbohydrate availability for subsequent exercise tasks. This suggests that exercise performance could potentially improve due to increased carbohydrate availability.

Narrowing down the 3-5 hour time window, Coyle et al (1985) looked at the effect of a high carbohydrate meal 4 hours prior to an endurance exercise test. The findings indicated that consuming a high carbohydrate meal 4 hours prior to exercise increased initial muscle glycogen concentrations which caused an increased reliance on carbohydrates and a decreased reliance on lipids. The pre-exercise meal limited fat oxidation and increased muscle glycogen utilisation (Coyle et al, 1985). In another study, the effect of a pre-exercise carbohydrate meal on time-to-fatigue was assessed using a time to exhaustion cycle test (Schabort et al, 1999). The most significant finding regarding the ingestion of a pre-exercise carbohydrate meal was the significant increase in time to fatigue. It was concluded that the important finding was the increased time to fatigue following the carbohydrate meal without the negative effect of increased insulin concentrations and decreased FA concentrations at the start of exercise. However, the metabolic mechanisms for this could not be identified (Schabort et al, 1999).

This section has identified the metabolic changes, and their effects, that occur following the ingestion of a high carbohydrate diet or meal prior to exercise. It has been identified that the timing of ingestion in relation to the onset of exercise is an important factor as it does have an effect on exercise metabolism (Moseley et al, 2003; Coyle et al, 1985; Neufer et al, 1987; Schabort et al, 1999). There is evidence to suggest that pre-exercise ingestion of carbohydrate could improve exercise performance (Chryssanthopoulos et al, 2002; Chen et al, 2009). However, carbohydrate is not the only macronutrient to have been researched in relation to exercise metabolism and exercise performance.

## Pre-exercise fat ingestion

As noted earlier carbohydrate and fat are oxidised as a combination, although in varying proportions. Fat is a valuable energy source for the body and the role it plays in improving performance is an ongoing consideration for sports coaches, nutritionists, athletes and sport scientists (Lowery, 2004). A number of studies have investigated the effect of pre-exercise high fat meals (Table 2) and compared high fat ingestion with high carbohydrate ingestion (Table 3). Previous studies have shown significantly increased FA availability as a result of a single high fat meal, although this was in humans with heparin administration (which causes the release of lipolytic enzymes into blood circulation) (Costil et al, 1977; Dyck et al, 1993; Dyck et al, 1996; Vukovich et al, 1993).

A high fat meal consumed pre-exercise alters the substrate supply prior to exercise which leads to an increase in FA availability in the blood (Whitley et al, 1998). The increased FA levels cause an increase in fat oxidation during exercise, which either preserves carbohydrate stores or reduces the normal rates of carbohydrate depletion (Costil et al, 1977; Vukovich et al, 1993). These findings suggest that a high fat meal could lead to improved exercise performance due to increased fat oxidation in the early stages of exercise and sparing carbohydrates for use in the later stages of exercise. Murakami et al (2012) examined the exercise performance effect of consuming either a high fat meal 4 hours pre-exercise + maltodextrin jelly 3 minutes pre-exercise, high fat meal 4 hours pre-exercise + placebo jelly 3 minutes pre-exercise, and a high carbohydrate meal 4 hours pre-exercise + placebo jelly 3 minutes pre-exercise, following 3 days of a high carbohydrate diet. It was identified that time to exhaustion was significantly longer following the high fat meal + maltodextrin jelly (100±3.4min vs 92±2.8min and 90±1.7min). In the initial 60 minutes of a time to exhaustion cycle, fat oxidation increased and carbohydrate oxidation decreased, which lead to sparing of the glycogen stores in the early stages of exercise and increased availability at the end of exercise, leading to improved time to exhaustion (Murakami et al, 2012). The findings suggest that prior carbohydrate intake is an important factor in improved performance following a high fat meal prior to endurance exercise. However, only 8 long distance trained athletes participated, which suggests that such a small number of subjects could lead to greater variability in the findings. It would be expected that carbohydrate loading over the 3 days prior to trial day should impact performance with or without a high fat meal, although this is not identified in the research by Murakami et al (2012).

One strategy to increase fat availability is to use a chronic (7-28 days) high fat diet. Previous studies identified metabolic adaptations resulting from chronic high fat diets (Hollowszy & Coyle, 1984; Goedecke et al, 1999; Lambert et al, 1994; Rowlands and Hopkins, 2002a). These metabolic adaptations included increased fat oxidation and the sparing of carbohydrate stores following a high fat diet when compared with high carbohydrate diets (Goedecke et al, 1999; Lambert et al, 1994). The findings suggest that sparing of carbohydrate stores could improve exercise performance. However, another study identified that while fat-adaptation strategies did improve glycogen sparing, there was no benefit on subsequent performance tests (Burke et al, 2000). A possible reason for these differences is the intervention period. Goedecke et al (1999) used a 15 day intervention and Lambert et al (1994) used a 14 day intervention, whilst Burke et al (2000) used a 5 day intervention.

*Table 2: The effect of pre-exercise fat ingestion on human metabolism and exercise performance*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Year** | ***n*** | **Treatment** | **Time Prior** | **Protocol** | **Results** |
| **Dyck et al**  | 1996 | 11 | CHO + Control (saline) (C)CHO + Intralipid Infusion (20%) (IL) | 3hrs + 0 min | 15 min at 85% VO­2 max (Bike) | Plasma FFA = IL > C(IL=0.21±0.04; C=0.94±0.09; mM) |
| **Vukovich et al** | 1993 | 5 | HFM (90g fat) + HeparinIntralipid TG emulsion (IL) + HeparinControl (meal) (C) | 3hrs + 15 min1hr + 15 min3hrs | 60 min at 70% VO2 max (Bike) | RER = IL < HFMPlasma FFA = HFM & IL > C(HFM=1.97±0.37; IL=1.34±0.25; C=0.33±0.04; mmol/l) |
| **Dyck et al**  | 1993 | 6 | Intralipid (IL) + Heparin x3Control (Saline (C) + Heparin x 3 | 30 min + 30, 15, 0 min | 15 min at 85% VO2 max | Plasma FFA (30min rest) = IL > CPlasma FFA (exercise) = IL > C |
| **Goedecke et al**  | 1999 | 16 | HFD (20% CHO, 70% Fat)Control (55% CHO, 30% Fat) (C) | 15 day diet | 150 min at 63%Wpeak40km TT(Bike) | Fat Oxi (Day 5) = HFD Increased(D0=0.67±0.16; D5=0.91±0.20; g/min)Fat Oxi (Day 5) = HFD > CCHO Oxi (Day 5) = HFD < C |

Notes: CHO, Carbohydrate; HGI, High Glycaemic Index; LGI, Low Glycaemic Index; HCM, High Carbohydrate Meal; HFM High Fat Meal; HCD, High Carbohydrate Diet; HFD, High Fat Diet; BM, Body Mass; Fat Oxi; Fat Oxidation; CHO Oxi, Carbohydrate Oxidation;TT, Time Trial; TTE, Time To Exhaustion; FFA, Free Fatty Acids

*Table 3: The effect of pre-exercise fat vs carbohydrate ingestion on human metabolism and exercise performance*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Year** | ***n*** | **Treatment** | **Time Prior** | **Protocol** | **Results** |
| **Costil et al** | 1977 | 7 | 75g Glucose (G)Fatty meal (F)Control (fasted) (C)  | 45 min5 hrs | 30 min at 70% VO2 max (Treadmill) | CHO Oxi (g)= G > F & C(G=76.3±3.7; F=56.1±4.2; C=67.7±5.0; g)Fat Oxi (g)= F > G & C(G=12.9±1.6; F=21.7±1.4; C=16.5±1.7; g) |
| **Okano et al**  | 1996 | 10 | HCM (79% CHO, 10% Fat)HFM (30% CHO, 61% Fat) | 4hrs | 120 min at 65% VO2maxTTE at 80% VO2 max(Bike) | RER (initial 40mins) = HCM > HFMRER (after 40mins) = No differenceTTE Performance = No difference |
| **Lambert et al** | 1994 | 5 | HFD (70% Fat)HCD (70% CHO) | 2 weeks | TTE at 90% VO2 maxTTE at 60% VO2 max(Bike) | RER (high intensity) = No differenceRER (mod intensity) = HFD < HCD(HFD=0.87±0.03; HCD=0.92±0.02)CHO Oxi (mod intensity) = HFD < HCD(HFD=1.41±0.7; HCD=2.23±0.4; g/min)Fat Oxi (mod intensity) = HFD > HCD(HFD=0.6±0.12; HCD=0.32±0.07; g/min) |
| **Pitsiladis & Maughan** | 1999 | 6 | HFD (65% Fat)HCD (80% CHO) | 4 days | TTE at 60% VO2 max (10°C and 30°C)(Bike) | RER (10°C) = HFD < HCDFat Oxi (10°C) = HFD > HCD(HFD=87±49; HCD=78±17; g)CHO Oxi (10°C) = HFD < HCD(HFD=201±36; HCD=434±77; g)TTE Performance = HFD < HCDRER (30°C) = HFD < HCDFat Oxi (30°C) = HFD > HCD(HFD=34±9; HCD=20±11; g)CHO Oxi (30°C) = HFD < HCD(HFD=96±19; HCD=187±42; g)TTE Performance (30°C) = HFD < HCD |
| **Burke et al**  | 2000 | 8 | HFD (>65% Fat) + HCD (10g/kgBM CHO)HCD (70% CHO) + HCD (10g/kgBM CHO) | 5 day + 1 day | Day 6 = 20min at 70% VO2 maxDay 7 = 120 min at 70% VO2 max; 7kj/kg TT(Bike) | RER (Day 6) = HFD < HCD(HFD=0.82±0.01; HCD=0.88±0.01)Fat Oxi (Day 6) = HFD > HCD(HFD=1.04±0.07; HCD=0.63±0.06; g/min)CHO Oxi (Day 6) = HFD < HCD(HFD=1.73±0.18; HCD=2.59±0.19; g/min)Fat Oxi (Day 7) = HFD > HCDCHO Oxi (Day 7) = HFD < HCD |
| **Rowland & Hopkins** | 2002a | 7 | HFD (70% Fat) + HFMHCD (70% CHO) + HCM | 2 week + 90 min | 15 min TT45 min at 50% VO2 maxIncremental 6x 10min stage100km TT(Bike)  | Fat Oxi (Incremental) = HFD > HCDFat Oxi (100km TT) = HFD > HCD |
| **Rowlands & Hopkins** | 2002b | 12 | HFM (85% Fat, 5% CHO, 10% Protein)HCM (85% CHO, 5% Fat, 10% Protein) | 90 mins | 60 min at 55% peak powerIncremental 5x10 min stage50km TT (1 & 4km sprints)(Bike) | Fat Oxi = HFM > HCMCHO Oxi = HFM < HCMTT Performance = No difference |
| **Starling et al**  | 1997 | 7 | HFD (68% Fat)HCD (83% CHO) | 12hrs + 12hr fast | 1600kJ TT(Bike) | TT Performance = HFD > HCD(HFD=139.3±7.1; HCD=117.1±3.2; mins) |
| **Murakami et al** | 2012 | 8 | HFM (55% Fat, 30% CHO) + MaltodextrinHFM (55% Fat, 30% CHO) + PlaceboHCM (70% CHO, 21% Fat)+ Placebo | 4hrs + 3 mins | 80min at 72% VO2 maxTTE at 72-80% VO2 max(Bike) | RER (Rest) = HFM+P & HFM+M < HCM+PFat Oxi (Rest) = HFM+P & HFM+M > HCM+PCHO Oxi (Rest) = HCM+P > HFM+P & HFM+MRER (Exercise) = HFM+P < HCM+PFat Oxi (Exercise) = HFM+P > HCM+PCHO Oxi (Exercise) = HFM+P < HCM+PTTE Performance = HFM+M > HFM+P & HCM+P(HFM+M=100±3.4; HFM+P=92±2.8; HCM+P=90±1.7; mins) |
| **Paul et al** | 2003 | 8 | HCM (3g/kg BM)HFM (1.3g/kg BM)Placebo (P) | 3.5hrs | 30 min at LT+25W20km TT | CHO Oxi = No differenceFat Oxi (TT) = HFM & P > HCM(HFM; 16.5±2.7; HCM; 4.8±2.1; P=17±3.2; µmol.kg.min) |
| **Whitley et al** | 1998 | 8 | HCM (215g CHO, 3g fat)HFM (50g CHO, 80g fat)Control (fasted )(C) | 4hrs | 90 min at 70% VO2 max10km TT (Bike) | Start exercise RER = HCM > HFM & CStart exercise CHO Oxi = HCM > HFM & C(HCM=27.8±1.7; HFM=22.3±1.35; C=22.2±2.2; g )Start exercise Fat Oxi = HCM < HFM & C(HCM=3.09±0.49; HFM=4.83±0.65; C=5.55±0.8; g)TT Performance = No difference |

Notes: CHO, Carbohydrate; HGI, High Glycaemic Index; LGI, Low Glycaemic Index; HCM, High Carbohydrate Meal; HFM High Fat Meal; HCD, High Carbohydrate Diet; HFD, High Fat Diet; BM, Body Mass; Fat Oxi; Fat Oxidation; CHO Oxi, Carbohydrate Oxidation;TT, Time Trial; TTE, Time To Exhaustion; FFA, Free Fatty Acids; LT, Lactate Threshold

Burke et al (2000) compared 5 days high fat diet followed by one day high carbohydrate diet with a 5 day high carbohydrate diet followed by one day high carbohydrate diet. It was identified that a 5 day high fat, low carbohydrate diet followed by a one day high carbohydrate diet resulted in increased fat oxidation. The results indicate that some chronic effects of diet cannot be directly explained by substrate availability. Muscle glycogen levels significantly decreased following the 5 day high fat diet (451±32 to 255±24 mmol/kgBM), whilst there was no difference in the 5 day high carbohydrate trial (470±24 to 464±42 mmol/kgBM). The one-day high carbohydrate intake increased glycogen stores compared to day 1 to similar levels in the high fat diet (554±45mmol/kgBM) and high carbohydrate diet (608±51mmol/kgBM). Although the liver stores glycogen, no measurements were taken. If this research had investigated liver glycogen levels in addition to muscle glycogen levels it may have provided a more detailed analysis of changes to whole body glycogen levels and the way in which they are affected by diet.

Large differences existed in substrate utilisation between the two diets. Following 5 days, the decrease in RER with the high fat diet was greater than with the high carbohydrate diet (0.07±0.01 vs 0.03±0.01). After consuming a high carbohydrate diet for one day, RER was still significantly lower compared with baseline values in the high fat trial (0.87±0.01 vs 0.90±0.01). Carbohydrate oxidation significantly decreased following the 5 day high fat diet and was significantly lower compared to the 5 day high carbohydrate diet (1.73±0.18 vs 2.59±0.19 g/min). Following one day high carbohydrate diet, carbohydrate oxidation increased in both trials, but the high fat trial remained lower than the high carbohydrate trial (2.48±0.14 vs 3.21±0.14 g/min). The increase of 0.62g/min in carbohydrate oxidation from day 5 to day 6 of high carbohydrate feeding may be due to the higher carbohydrate content of the meals on the 6th day compared to the initial 5 days. Fat oxidation significantly increased following the 5 day high fat diet and was significantly higher compared to the 5 day high carbohydrate diet (1.04±0.07 vs 0.63±0.06 g/min). Following one day high carbohydrate diet, fat oxidation decreased in both trials, but remained significantly higher in the high fat trial (0.70±0.05 vs 0.37±0.04 g/min). It was unclear whether the metabolic changes identified following the high fat diet involved the upregulation of fat oxidation, downregulation of carbohydrate oxidation or a combination of both (Burke et al, 2000). Other studies have identified that in trained subjects, 1-28 days of a high fat diet increases muscle triglyceride stores, which could provide additional substrate to account for the additional fat utilised (Janssen & Kaijser, 1982; Starling et al, 1997). However, the precise mechanism to explain the use of fat substrates, even when there is plentiful glycogen stored, is still unknown (Burke et al, 2000).

Compared to a long-term fat adaptation approach, a different method for performance improvement could be to increase fat availability by consuming a high fat meal ≤4 hours prior to exercise (Whitley et al, 1998; Rowlands and Hopkins, 2002b). Studies that observed the effect of long term (≥1 week) consumption of high fat diets identified either no effect on performance (Stellingwerff et al, 2006) or impaired endurance performance due to reduced endogenous carbohydrate stores (Pitsiladis & Maughan, 1999; Starling et al, 1997). However, the consumption of a single high fat meal prior to exercise may allow immediate fatty acid availability as well as maximum endogenous carbohydrate stores (Whitley et al, 1998; Rowlands & Hopkins, 2002b; Murakami et al, 2012; Rauch et al, 1995). Despite the potential metabolic benefits, previous studies reported no performance improvements from the consumption of a high fat meal prior to exercise when compared to a high carbohydrate meal (Whitley et al, 1998; Okano et al, 1996; Rowlands & Hopkins, 2002b; Paul et al, 2003).

Many of the papers mentioned in both pre-exercise carbohydrate and fat ingestion use small subject groups, with the largest group used being n=12. The sample size should reflect the target population and include a suitable number of subjects to ensure validity in the results. The small sample size seen in many papers could account for varied results. Another possible reason for variable results could be the equations used for calculating oxidation rates. A number of papers have used a variety of different equations which could cause varying results from these papers (Costill et al, 1977; Coyle et al, 1997; Schabort et al, 1999; Burke et al, 2000; Hawley et al, 2000; Betts et al, 2007; Murakami et al, 2012).

Whilst many studies identify the effect of breakfast versus no breakfast and pre-exercise high fat or high carbohydrate feeding, there are very few studies that identify the effect of different breakfast compositions on substrate utilisation throughout the day. One study observed the time of day effect of high fat feeding over a continuous 9 day period in mice (Joo et al, 2016). Researchers investigated the effects of time of day dependent high fat feeding on substrate metabolism and metabolic plasticity in mice (Joo et al, 2016) using a human meal pattern, although the degree to which a human meal pattern with mice can provide valid results reflecting human metabolism must be considered. Results indicated that mice fed a high fat (45% of calories from fat) or very high fat (60% of calories from fat) diet early in their waking cycle (EHF and EVHF respectively), which is equivalent to human breakfast, demonstrated a significant reduction in average daily RER, suggesting increased FA oxidation when compared to those fed a high fat diet or a very high fat diet late in the waking cycle (LHF and LVHF respectively). Although mean RER values for the EHF mice were lower than the LHF mice they were not statistically significant (0.887±0.003 vs 0.900±0.003). A significant reduction in mean RER was identified in EVHF mice compared to LVHF mice (0.848±0.002 vs 0.876±0.002), suggesting an extended period of increased FA oxidation as a result of a very high fat diet taken early in the waking cycle. Results provide evidence to suggest that reduced RER and thus increased fatty acid oxidation are dose dependent in terms of amount of fat ingested.

Joo et al, (2016) identified a second meal effect in mice. The research included a low fat meal (10% of energy from fat) given either early or late in the 12 hour waking phase. EVHF and EHF mice received their low fat meal in the third 4 hour period of the waking phase (second meal of the day following an early very high fat or high fat meal). LVHF and LHF mice received their low fat meal in the first 4 hour period of the waking phase (as a first meal of the day). The low fat first meal for the LVHF and LHF mice resulted in increased RER values after the meal. However, following this group’s LHF and LVHF meal, RER values reduced significantly, and remained lower than the EVHF and EHF mice throughout the 12 hour resting phase. It is suggested that the lower RER in this phase was possibly due to the high fat or very high fat meal at the end of the waking phase accelerating the shift into FA oxidation associated with an extended fasting period (the resting period).  This suggests that the last meal before sleeping has the greatest influence on energy metabolism in the resting/fasting phase. RER values for the EVHF and EHF mice increased markedly as a result of the low fat meal in the third 4 hour period of the waking phase, suggesting that a second low fat meal in the waking phase affects substrate metabolism as it results in decreased FA oxidation and increased oxidation of carbohydrates.

An unexpected finding in the research was that mice fed a very high fat diet at the start of their waking cycle demonstrated lower energy expenditure for a similar level of physical activity compared to mice fed a late very high fat diet (9.97±0.12 vs 10.57±0.18 kcal/day), establishing a link between energy expenditure, timing of food intake and circadian cycles. The key findings of the Joo et al (2016) study were that the high fat first meal increased fat utilisation through the rest of the waking phase and that the total amount of fat and timing of intake does indeed impact on substrate metabolism.

High fat feeding affects not only substrate utilisation as mentioned above, but can also impact glucose tolerance (Roden et al, 1996). One of the metabolic changes that occurs following a high fat meal is a change in blood glucose levels. A study by Beaudoin et al (2011) identified that after the ingestion of a high fat meal blood glucose levels are increased. The study used a specifically prepared drink containing a blend of palm stearine and soybean oil and was chemically interesterified to achieve a random distribution of FAs, which enabled researchers to observe changes in glucose tolerance when fat is ingested. Healthy males ingested 1g/kg body mass of lipids followed 6 hours later by a glucose drink. Usually when glucose is ingested the body produces insulin, which removes glucose from the blood and distributes it to the muscles and the liver. However, this study found that fat affected the body’s ability to remove glucose from the blood. Blood glucose levels were 32% higher following a high fat meal compared to the control group (Beaudoin et al, 2011). Roden et al (1996) identified that following a high fat meal, the level of FAs in the bloodstream rises which causes the liver to become more resistant to insulin. Roden et al (1996) identified that the elevation in plasma FA concentration causes insulin resistance by the inhibition of glucose transport and/or phosphorylation with a subsequent reduction in rates of glucose oxidation and muscle glycogen synthesis. When the liver is resistant to insulin it produces and secretes more glucose than usual, increasing the blood glucose level steadily as liver glucose output increases. While these studies identified the effect of a single dose of fat, the effect of fat ingestion on meals consumed later in the day was not observed.

As noted above, a high fat meal can increase blood glucose following carbohydrate ingestion. Small increases (48% of energy from fat) in fat intake at breakfast can influence adversely glucose tolerance for up to 6 hours later in the day and following a second meal (Frape et al, 1998). Research by Frape et al (1998) identified that increases in the proportion of fat at breakfast resulted in reduced insulin sensitivity at lunch, a second meal effect on glucose metabolism. The reduced insulin sensitivity resulted in an elevated insulin response, more insulin being required to keep blood glucose levels in the normal range.

Whilst fat ingestion can affect glucose tolerance for up to 6 hours (Frape et al, 1998), carbohydrate ingestion only affects glucose tolerance up to 2-3 hours (Horowitz et al, 1993). Gentilcore et al (2006) identified that the lengthened effect could be due to slower gastric emptying following fat ingestion. Gastric emptying is a significant determinant of postprandial glycemia, as shown by the link between increases in blood glucose following oral carbohydrate intake and gastric emptying. The rate at which nutrients are delivered to the small intestine from the stomach is approximately 2-4kcal/min. As fat is has higher energy density (9kcal/g) compared to carbohydrate (3.75kcal/g), gastric emptying is slowed and therefore the blood glucose response is slowed (Gentilcore et al, 2006). As well as slowing the blood glucose response, it was also identified that the effect of fat ingestion on the glycemic response to oral carbohydrate was greater when the fat was consumed by itself before a meal containing carbohydrate (Gentilcore et al, 2006).

## Aim and Hypothesis

As previous research has identified, both high fat and high carbohydrate meal ingestion does affect metabolism during rest and during exercise. Previous research has observed changes directly after ingestion and up to 5 hours post ingestion. There is limited research identifying the effect of a high fat or high carbohydrate breakfast on metabolism following lunch and during early evening exercise. The aim of this research is to identify the influence of breakfast macronutrient composition on second meal metabolism and early evening exercise metabolism. The hypothesis of this study is that a high carbohydrate breakfast will lead to increased carbohydrate utilisation throughout the day and during evening exercise while a high fat breakfast will lead to increased fat utilisation throughout the day and during evening exercise. In addition to metabolism, high fat and high carbohydrate can affect blood glucose. A previous study identified that a moderately high fat (48%) meal can affect blood glucose up to 6 hours after ingestion and following a second meal. Another study identified a large increase in blood glucose immediately after high fat ingestion. There is limited research on the effect of high fat ingestion on blood glucose after a second meal. Therefore, a second hypothesis is that the fat content of the breakfast will increase blood glucose levels after the second meal.

# Methodology

## Subject Characteristics

After completing power calculations, based on achieving significance between breakfast compositions in RER and blood glucose it was identified that 12 was the minimum subject number required. A total of 24 subjects were recruited, seven subjects did not complete the study for various reasons. Seventeen subjects (twelve males and five females) completed the study (Table 4). Due to the maximal exercise included in the trials participants in the study were required to be healthy individuals whose weekly exercise exceeded the standard governmental guidelines of 150 minutes moderate activity per week (Department of Health, 2011). Subjects completed a Pre-Participation Health Screen Questionnaire to identify medical risk factors (Appendix A). Subjects had no prior history of cardiovascular or pulmonary disease, diabetes or recent musculoskeletal injury (prior six months). The study protocol was approved by the University of Stirling Ethics Committee and once fully informed of the nature of the study, all participants provided written consent in keeping with university requirements.

*Table 4: Subject characteristics. All values are mean ± SD.*

|  |  |
| --- | --- |
| **Age (yrs)** | 24.9 ± 5.2 |
| **Weight (kg)** | 74.5 ± 9.8 |
| **Height (cm)** | 177.6 ± 10.6 |
| **BMI (kg/m­­­­2)** | 23.6 ± 2.4 |
| **VO2 Max (ml/min/kg)** | 49.5 ± 6.8 |

## Experimental Design

The study comprised of three trials with 7 days between each trial. Prior to the first trial, subjects were given a 3-day food diary and a 3-day training diary to complete and return on the first trial. Subjects were required to replicate their normal diet and exercise regimes on the day before each trial. On the day of each trial subjects were required to arrive fasted for 12 hours, have no alcohol for 24 hours and to be in a rested state. During the first trial subjects completed a 15 minute indirect calorimetry using an OxyCon Pro to calculate Resting Metabolic Rate (RMR), followed by a VO2 max test (Figure 3). The second and third trial had a similar protocol design, differing only in the breakfast composition (Figure 3). Upon arrival at the second and third trial, subjects completed a 15 minute resting indirect calorimetry, followed by the trial breakfast (carbohydrate or fat). Subjects rested for a 3 hour period, with appetite scales completed every 60 minutes. A standardised lunch was then provided. Subjects rested for 3 hours following lunch with a 15 minute indirect calorimetry carried out at 60 minute intervals following lunch, blood glucose tests completed at 15 minute intervals for the first hour and 30 minute intervals for the second hour, and appetite scales completed at 60 minute intervals. Following a rest of 3 hours after lunch, subjects completed a graded exercise test. Subjects remained seated at all times during the rest periods unless they required the bathroom. All subjects were allocated 30 minutes to complete their meals. Water was given *ad libitum* throughout the trials.

**Trial 1:**

^ = Appetite scales

**RER**

° = Blood Glucose

RER—15 Minutes resting

Meal—30 minutes for meal

Exercise Test

**8**

**9**

 **RER**

**Trial 2 and 3:**

**8 9 10 11 12 1 2 3 4 5**

**Rest**

**RER**

**RER**

**RER**

**RER**

**Rest**

° ° ° ° ° ° °

˄ ˄ ˄ ˄ ˄ ˄ ˄ ˄

*Figure 3: Trial Protocols*

***Meal*** *–* The total calorie content of each meal was calculated using one-third of the subjects’ RMR as suggested by Verboeket-van de Venne and Westerterp (1991). RMR was used as participants remained seated throughout the trial until the exercise trial. The energy content for each meal was split down depending on the composition of the meal (Table 5) (Appendix B & Appendix C).

*Table 5:* *The energy content and weight of the total and each macronutrient of each meal. All values are mean ± SD.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Total** | **Carbohydrate** | **Fat** | **Protein** |
| Kcal | g | Kcal | g | Kcal | g | Kcal | g |
| **Carbohydrate breakfast** | 569.7 ± 98.7 | 133.2 ± 23.1  | 398.8 ± 69.1 | 106.3 ± 18.4 | 113.9 ± 19.7 | 12.6 ± 2.2 | 57.0 ± 9.9 | 14.2 ± 2.5 |
| **Fat** **breakfast** | 569.7 ± 98.7 | 88.8 ± 15.4 | 113.9 ± 19.7 | 30.37 ± 5.3 | 398.8 ± 69.1 | 44.2 ± 7.7 | 57.0 ± 9.9 | 14.2 ± 2.5 |
| **Lunch** | 569.7 ± 98.7 | 128.8 ± 22.3 | 379.3 ± 64.2 | 98.7 ± 17.1 | 142.4 ±24.7 | 15.8 ± 2.7 | 57.0 ± 9.9 | 14.2 ± 2.5 |

***Breakfast*** – The second and third trial had different breakfast compositions. In a randomised order, subjects consumed either a high fat or high carbohydrate breakfast. The high fat breakfast consisted of 70% of total calories from fat, 20% from carbohydrate and 10% from protein in the form of a high fat smoothie (Table 6). The high carbohydrate breakfast consisted of 70% of total calories from carbohydrate, 20% from fat and 10% from protein in the form of porridge (Table 6).

***Lunch*** – The second and third trial both had a standardised lunch meal consisting of 65% of total calories from carbohydrate, 25% from fat and 10% from protein in the form of pasta and sauce (Table 6).

*Table 6: Content of each meal based on a 2000kcal/day RMR*

|  |  |
| --- | --- |
| **Carbohydrate Breakfast** | **Fat Breakfast** |
| Tesco easy oats (Golden syrup) | 78g | Tesco Frozen berry smoothie mix | 120g |
| Lyle’s Golden syrup | 25g | Avocado | 130g |
| Whole Milk | 280ml | Onken Natural Yoghurt | 120g |
| Banana | 90g | Whole Milk | 230ml |
| **Standardised Lunch** |
| Fusilli Pasta | 100g |
| Tesco creamy tomato and herb sauce | 100g |
| Tesco Olive Oil | 5g |
| Grated Parmesan | 5g |
| Garlic bread | 31g |

***Appetite Scale*** *–* Visual analogue scales were used for ratings of hunger, fullness and nausea, which were assessed by marking line analogues on a 100mm scale (Sepple & Read, 1989) (Appendix D). Appetite Scale measurements were taken after the breakfast meal at time points 0, 60, 120, and 180 minutes, and after the lunch meal at time points 0, 60, 120, and 180 minutes.

***Indirect calorimetry*** – The OxyCon Pro, a gas analyser linked to a facemask identified breath-by-breath changes in the composition of the expired air. Indirect calorimetry was used to determine the subject's oxygen consumption and carbon dioxide production which the Oxycon Pro used to estimate substrate utilisation (Simonson and DeFronzo, 1990). Indirect calorimetry was tested once the subjects had arrived at each trial to measure RMR, during the VO2 max test, the FatMax test and once every hour during the rest break between lunch and the FatMax test. During the VO2 max test and the FatMax test, the mouthpiece was used. During resting indirect calorimetry, the hood was used.

***Blood Glucose*** – Blood glucose was tested using a finger prick test with a hand-held blood glucose monitor (One-Touch Ultra mini). Although not as valid as using a cannula, finger prick monitors have been widely used as an alternative (Olansky & Kennedy, 2010). Blood glucose was tested post lunch intake at time points 0, 15, 30, 45, 60, 90, and 120 minutes after the 30 minute time slot for lunch.

***Rate of Perceived Exertion (RPE) Scale*** *–* A numbered scale was used to measure how hard the subject worked during exercise (Borg, 1970) (Appendix E). Rate of Perceived Exertion was measured at the end of each stage during the VO2 max test and the FatMax exercise test.

***FatMax Test*** – The FatMax test, a graded exercise, followed the protocol set by Achten et al (2002). The test ran for 10 to 30 minutes, the time dependent on the individual subject’s fitness level. The test started with a setting of 90W and the work rate increased 35W every 3 minutes until volitional fatigue. Heart rate was recorded throughout the test using a Polar heart rate monitor and Rate of Perceived Exertion was recorded at the end of each stage using the RPE Scale. Breath-by-breath measurements were recorded throughout the test using an OxyCon Pro gas analysis system to measure substrate utilisation.

***VO2 max* *Test*** – The VO2 max test, a graded exercise, ran for 6 to 12 minutes using a Watt cycle ergometer, the time dependent on the individual subject’s fitness level. The test started at moderate intensity (80W) and every minute the workload increased (30W) until exhaustion, following the test 1 protocol by Carta et al (1991). During the test, expired gases and substrate utilisation were measured using indirect calorimetry, heart rate was recorded throughout the test and RPE was recorded at the end of each stage.

## Statistical Analysis

All data were expressed as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to evaluate the normality of the data. For non-normal data, the data was transformed using a Box-Cox transformation. All time-based data were analysed for time (post breakfast or post lunch) and breakfast effects by repeated-measure 2-way ANOVA using the Jamovi software. In exercise trials, time-based date were analysed for breakfast and intensity. Tukey Honestly Significant Difference Post-Hoc tests were carried out on significant results identified in the ANOVA. Area under the curve (AUC) was calculated using the trapezoid method for resting indirect calorimetry and blood glucose. Differences between breakfasts for AUC were analysed by Paired T-tests. Effect sizes Cohen’s d were calculated for t-test results and Eta squared (ƞ2) for ANOVA results. Effect sizes were interpreted using values from Cohen, 1988. Differences between means with a p-value of ≤0.05 were considered statistically significant.

# Results

## Appetite Scales

During the post breakfast period, hunger significantly increased from 10.09±11.36mm to 66.62±15.28mm (p<0.001) with a Cohen’s effect size (ƞ2=0.816) which showed a large practical significance (Figure 4A). Post breakfast fullness significantly decreased from 76.95±12.68mm to 16.59±10.90mm (p<0.001) with a Cohen’s effect size (ƞ2=0.805) which showed a large practical significance (Figure 4B). However, there were no significant differences between the two breakfast compositions in hunger or fullness, with Cohen’s effect sizes (ƞ2=0.007 and 0.001 respectively) which showed a very small practical significance.

During the post lunch period, hunger significantly increased from 23.00±20.46mm to 58.00±20.86mm (p<0.001) with a Cohen’s effect size (ƞ2=0.596) which showed a large practical significance (Figure 5A). Post lunch fullness significantly decreased from 64.68±14.68mm to 25.64±18.56mm (p<0.001) with a Cohen’s effect size (ƞ2=0.691) which showed a large practical significance (Figure 5B). There were no significant differences between the two breakfast compositions in hunger or fullness, with Cohen’s effect sizes (ƞ2=0.004 and 0.022 respectively) which showed a very small practical significance.

*◊*

*◊*

*abc*

*ab*

*a*

*abc*

*ab*

*a*

A.

B.

*Figure 4: Post Breakfast visual analogue scales of Hunger and Fullness. A. Appetite scales measuring hunger at time points 0, 60, 120, and 180 minutes. B. Appetite scale measuring fullness at time points 0, 60, 120, and 180 minutes. Data are means ± SD. ◊ (p<0.05) across time (a significantly different from 0; b significantly different from 60; c significantly different from 120); \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

A.

B.

*◊*

*◊*

*abc*

*ab*

*a*

*ab*

*ab*

*a*

*Figure 5: Post Lunch visual analogue scales of Hunger and Fullness. A. Appetite scales measuring hunger at time points 0, 60, 120, and 180 minutes. B. Appetite scale measuring fullness at time points 0, 60, 120, and 180 minutes. Data are means ± SD. ◊ (p<0.05) across time (a significantly different from 0; b significantly different from 60; c significantly different from 120); \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

## Resting Indirect Calorimetry

Carbohydrate oxidation significantly increased from 0.124±0.01 to 0.163±0.04 (p<0.001) with a Cohen’s effect size (ƞ2=0.114) which showed a small practical significance (Figure 6A). No significant differences were identified in carbohydrate oxidation between breakfast compositions or between AUC of the two breakfast compositions. No interaction effect was identified.

Fat oxidation significantly decreased across time from 0.058±0.005 to 0.037±0.005 (P<0.001) with a Cohen’s effect size (ƞ2=0.309) which showed a moderate to large practical significance (Figure 6B). No significant differences were identified in fat oxidation between breakfast compositions or between AUC of the two breakfast compositions. No interaction effect was identified.

RER identified a significant increase across time from 0.836±0.01 to 0.885±0.01 (p<0.001) with a Cohen’s effect size (ƞ2=0.144) which showed a small to moderate practical significance (Figure 6C). RER was significantly higher following the carbohydrate breakfast in RER (0.886±0.02 vs 0.858±0.02; p=0.026) as well as a significantly greater in RER AUC (107.02±6.32 vs 103.55±6.77; p=0.044). No interaction effect was identified.

*◊\**

A.

B.

*◊*

*◊*

*a*

*a*

*a*

*a*

*a*

C.

*Figure 6: Resting Indirect Calorimetry.* *A. Carbohydrate Oxidation during the post lunch resting indirect calorimetry at time points 0, 60 and 120 minutes. B. Fat Oxidation during the post lunch resting indirect calorimetry at time points 0, 60 and 120 minutes. C. RER during the post lunch resting indirect calorimetry at time points 0, 60 and 120 minutes. Data are means ± SD. ◊ (p<0.05) across time (a significantly different from 0; b significantly different from 60); \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

## Blood Glucose

A significant difference between the two breakfast compositions was identified (p=0.018) with blood glucose following the high fat breakfast being significantly higher than the high carbohydrate breakfast (Figure 7). A Cohen’s effect size (ƞ2=0.172) was identified which showed a small to moderate practical significance. A significant difference between AUC was identified with AUC following the high fat breakfast being greater (833.52**±**106.23 vs 754.25**±**66.22mmol/l; p=0.002). There was no significant time effect or interaction identified in blood glucose with Cohen’s effect size (ƞ2=0.063 and 0.023 respectively) which showed a very small practical significance.

*\**

*Figure 7: Blood glucose during the post lunch rest at time points 0, 15, 30, 45, 60, 90 and 120 minutes. Data are means ± SD. ◊ (p<0.05) across time; \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

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## Exercise Indirect Calorimetry ­

A significant increase was identified in carbohydrate oxidation as intensity increased during the Fatmax exercise test from 1.04**±**0.33g/min to 3.08±0.36g/min (p<0.001) with a Cohen’s effect size (ƞ2=0.863) which showed a large practical significance (Figure 8A). There was no significant difference between the breakfast compositions in carbohydrate oxidation.

Fat oxidation significantly decreased as intensity increased during the Fatmax exercise test from 0.32**±**0.11g/min to 0.02±0.01g/min (p<0.001) with a Cohen’s effect size (ƞ2=0.698) which showed a large practical significance (Figure 8B). A trend was identified between the two breakfast compositions, for fat oxidation to be higher following the high fat breakfast (0.17±0.07 vs 0.18±0.09g/min; p=0.094) with a Cohen’s effect size (ƞ2=0.821) which showed a large practical significance. A significant interaction between breakfast and fat oxidation at different intensities was identified (p=0.002) at 50-60% (p=0.011) and at 60-70% (p=0.025) with a Cohen’s effect size (ƞ2=0.241) which showed a moderate practical significance.

RER significantly increased as intensity increased during the Fatmax exercise test from 0.89**±**0.06g/min to 1.10±0.04 (p<0.001) with a Cohen’s effect size (ƞ2=0.644) which showed a large practical significance (Figure 8C). A trend was identified between the two breakfast compositions was identified with RER following the high carbohydrate breakfast being higher than following the high fat breakfast (1.03±0.06 vs 1.02±0.05;p=0.05). A Cohen’s effect size (ƞ2=0.421) was identified between the breakfast compositions which showed a large practical significance.

## Exercise

Percentage of heart rate maximum significantly increased as the intensity increased from 58.31±3.97%hrm at 30-40%VO2 to 93.38±1.51%hrm at 90-100%VO2 (p<0.001) with a Cohen’s effect size (ƞ2=0.896) which showed a large practical significance (Figure 9A). No differences were identified between the breakfast compositions and there was no interaction effect.

RPE significantly increased as intensity increased from 8.30±1.44 at 30-40%VO2 to 19.33±0.44 at 90-100%VO2 (p<0.001) with a Cohen’s effect size (ƞ2=0.848) which showed a large practical significance (Figure 9B). No significant differences were identified between the two breakfast compositions and there was no interaction effect.

There was no significant difference in time to exhaustion during the Fatmax test between the high carbohydrate trial and the high fat trial (16.89±5.05 vs 15.85±4.09; p=0.201).

There was no significant difference in the time to reach RER of 1 between the high carbohydrate trial and the high fat trial (60.85±12.54 vs 61.09±12.57; p=0.955).

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C.

A.

B.

*Figure 8: Indirect Calorimetry during graded exercise A. Carbohydrate Oxidation during the Fatmax trials against the percentage of VO2max. B. Fat Oxidation during the Fatmax trials against the percentage of VO2max. C. RER during the Fatmax trials against the percentage of VO2max. Data are means ± SD. ◊ (p<0.05) across time (a significantly different from 30-40; b significantly different from 40-50; c significantly different from 50-60; d significantly different from 60-70); \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

*◊*

*◊*

A.

B.

*abc*

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*Figure 9: A. Percentage of Heart Max during Fatmax trials against percentage of VO2max. B. RPE during the Fatmax trials compared to the percentage of VO2max. Data are means ± SD. ◊ (p<0.05) across time (a significantly different from 30-40; b significantly different from 40-50; c significantly different from 50-60; d significantly different from 60-70; e significantly different from 70-80); \* (P<0.05) between the two breakfast compositions; Ω (P<0.05) time\*breakfast interaction.*

# Discussion

The main findings of this study were that ingestion of a high fat breakfast followed by a standardised lunch resulted in a significant reduction in the blood glucose up to two hours post lunch ingestion. It was identified that there was a significantly lower area under the curve for RER during the post lunch resting period after the high fat breakfast.

The main aim of this study was to identify how the macronutrient composition of breakfast influences metabolism following a second meal and during early evening exercise. The hypothesis of this study was that a high fat breakfast would increase fat oxidation throughout the day and during early evening exercise. As the results from RER area under the curve identified, substrate utilisation at rest following a second meal can be affected by the breakfast composition as RER was significantly lower following the high fat breakfast. RER during evening exercise indicated that breakfast composition does not affect substrate utilisation. However, at rest and during exercise breakfast composition had no effect on carbohydrate and fat oxidation.

## Resting indirect calorimetry

Following a standardised lunch, in both RER and AUC RER, a significant difference was observed which provides evidence to indicate that breakfast composition does have an effect on substrate metabolism up to 2 hours following the ingestion of a second meal. However, no significant differences were identified in carbohydrate oxidation or fat oxidation. RER is known as a surrogate of substrate utilisation and can be used to give an indication of relative oxidation of both carbohydrates and lipids although these are not absolute (Farinatti et al, 2016).

RER gives an estimation of fuel selection. A RER of 0.70 indicates predominantly fats being oxidised and a RER of 1.0 indicates predominantly carbohydrates being oxidised (Burke et al 2000). At rest there is an expectation that the body will oxidise less carbohydrates, giving a lower RER (Simonson and DeFronzo 1990). Results in this study identified a significantly lower RER during the post lunch rest period following the high fat breakfast when compared to a high carbohydrate breakfast. RER did increase at time points 60 minutes and 120 minutes from the 0 minute RER. These results indicate that despite a rest period following the breakfast meal and a subsequent standard lunch, RER was lower in subjects after the high fat breakfast than after the high carbohydrate breakfast throughout the post lunch resting period. As RER is an indicator of substrate metabolism, the result suggests that a single high fat breakfast can affect subsequent metabolism, which could suggest increased fat oxidation following such a meal over an extended period of time on the same day. However, the fat oxidation and carbohydrate oxidation data did not conform with the RER data.

An unexpected finding in this current study was that whilst a significant effect was identified in RER, there was no effect identified in fat oxidation or carbohydrate oxidation. A significant difference was identified in both the RER and AUC RER following lunch between the high carbohydrate and high fat trials. As fat oxidation and carbohydrate oxidation, as well as RER, were calculated using the VO2 and VCO2 data, it was expected that fat oxidation and carbohydrate oxidation would also be significantly different between the breakfast trials. However, no significant difference was identified in fat oxidation and carbohydrate oxidation. Using the equations identified in previous papers for RER, carbohydrate, and fat oxidation, results still identified significance in RER but not in carbohydrate or fat oxidation (Lusk, 1924; Frayn, 1983; Livesey & Elia, 1988; Peronnet & Massicotte, 1991). When using the equations identified with the date from the current study, the results were quite varied. It is unknown what equations the Oxycon Pro uses. However, although neither carbohydrate oxidation nor fat oxidation identified significance, RER alone is commonly used to identify substrate utilisation in a number of papers (Cryssanthopoulos et al, 1994; Farinatti et al, 2016; Neufer et al, 1987; Okano et al, 1996).

There are few studies that observed fat and carbohydrate oxidation in addition to RER at rest. One study by Murakami et al (2012) identified that, following a high fat meal, RER and carbohydrate oxidation were significantly lower while fat oxidation was significantly higher. Following a high carbohydrate meal, RER and carbohydrate oxidation were significantly higher and fat oxidation was significantly lower during rest (Murakami et al, 2012).  In another study by Whitley et al (1998) both RER and carbohydrate oxidation were significantly higher and fat oxidation was significantly lower following the high carbohydrate meal before the onset of exercise. Following a high fat meal, fat oxidation was greater and RER and carbohydrate oxidation were lower (Whitley et al, 1998). These results provide evidence to indicate that even after one high fat meal or high carbohydrate meal, RER can be affected. However, these studies observed RER and substrate utilisation after the meal ingestion without a second meal. It has been identified that ingestion of a standardised second meal can have an effect on RER. Although it was a multi-day study in mice, Joo et al (2016) identified that RER is reduced to a greater degree after the ingestion of a high fat meal, and increases slightly after the ingestion of a standardised meal. This might suggest that RER and carbohydrate oxidation in this current study’s fat trial increased after the standardised lunch, which could explain why significance was identified in RER, yet the fat and carbohydrate oxidation did not.

All meals were based on subject’s RMR, without considering diet-induced thermogenesis (DIT). DIT is an increase in metabolic activity after a meal, as a result of the energy cost of digestion and absorption, and the energy cost of synthesising reserves of glycogen, triacylglycerol and protein. This may be as much as 10-15% of the energy yield of the meal (Weststrate, 1993). Weststrate (1993) identified that DIT can be assessed with good accuracy within 3 hours of ingestion, but has considerably larger intraindividual variation which is not reduced when the diet was controlled. As measures of indirect calorimetry were not taken until after lunch which was 4 hours after the ingestion of the breakfast, DIT was not measured.

Whilst the difference in RER following lunch is statistically significantly different between trials, Cohen’s effect size identified the difference as being a small change. Therefore, in general population, this difference may not be physiologically relevant. However, in elite athletes, where marginal gains are crucial, a small difference in RER could potentially be vital in training and competition (Slater, 2012).

## Blood Glucose

Higher blood glucose levels and a later peak were identified following the high fat breakfast. The significant impact on blood glucose levels following the high fat breakfast is in keeping with previous studies (Frape et al 1998, Robertson et al 2002, Beaudoin et al, 2011). This can be accounted for due to slower gastric emptying and absorption of high fat meals and the increased FA and triglycerides in the bloodstream. A high fat meal requires more time than a carbohydrate meal to be digested and absorbed (Robertson et al, 2002), it can take between 4 and 6 hours to be fully metabolised and will result in the blood glucose level taking up to 6 hours to peak (Frape et al, 1998). Fat takes longer to digest due to FAs taking longer to break down compared to sugars (Gibney et al, 2009). The sugars in carbohydrates start to be broken down in the mouth by the enzyme amylase, found in the saliva and stomach, and converted into maltose, which are then broken down into separate glucose molecules. Other similar enzymes repeat these interactions for lactose, sucrose and complex sugars in carbohydrate. The glucose molecules pass through the intestine lining into the bloodstream. The breakdown of fats is started with bile acids secreted by the liver, which bond with water at one end and lipids at the opposing end, and are then slowly broken down into smaller droplets. These small droplets are fragmented in the small intestine by the enzyme lipase into their individual chains of FAs and diffuse through the lining of the intestines into the bloodstream. Although the breakdown of fats is not a complex process, the slow emulsification of fats by the bile acids takes longer than the quicker enzymatic action that breaks down the sugars in carbohydrates (Gibney et al, 2009). This may explain why the blood glucose was at higher levels for longer following the high fat breakfast compared to the high carbohydrate breakfast.

As identified by Robertson et al (2002), it takes 4-6 hours for ingested fat to be fully metabolised, which Gentilcore et al (2006) found to be caused by slowed gastric emptying. Gentilcore et al (2006) identified that the extent of slower gastric emptying was likely to be greater when high fat olive oil was given 30 minutes before a meal containing carbohydrate. The effects of fat on the glycemic response to oral carbohydrates were likely to be greater if the fat was consumed on its own and before a carbohydrate-based meal, as replicated in the current study protocol. The delayed glycemic response can be identified in the blood glucose after lunch as it was still increasing following the high fat breakfast, whilst blood glucose was decreasing following the high carbohydrate breakfast. Horowitz et al (1993) identified that blood glucose will have increased to peak within 30 minutes of ingestion. During this study, blood glucose measures started 30 minutes after the subjects were given food. As subjects finished the meal within the first 10 minutes of receiving their meal, it is possible that blood glucose had increased before measures were taken. This can be seen in the results as the blood glucose was already decreasing at the start of the measurements on the high carbohydrate trial. However, in the high fat trial, blood glucose was still increasing at the end of the 30 minutes allocated for lunch, which could be due to the slowed gastric emptying and glucose response after the high fat breakfast followed by the carbohydrate content of the lunch.

As mentioned above, a high fat meal can affect blood glucose and insulin resistance for up to 6 hours after ingestion (Robertson et al, 2002). As lunch was ingested 3 hours after breakfast, blood glucose and insulin resistance could still have been affected by the fat content in the high fat breakfast, as it has been identified in previous research that glucose and insulin responses are affected to a greater degree by fat ingestion followed by carbohydrate ingestion (Gentilcore et al, 2006). As the lunch was carbohydrate based, it was expected that blood glucose would increase rapidly following consumption. Although this rapid increase was not seen in the current study, blood glucose measures did not occur until 30 minutes after subjects were given food. Since subjects finished lunch within the first 10 minutes, blood glucose could have already increased before blood glucose measurements were taken. This seems to be supported by Horowitz et al (1993) who identified that blood glucose can peak within the first 15-30 minutes following ingestion. However, as previous research indicates, the liver could still be insulin resistant following the high fat breakfast (Robertson et al, 2002), therefore it would be feasible for blood glucose levels to increase further.

The response of the body to fat is also dose-dependent, suggesting that when more fat is ingested, the liver becomes more insulin resistant and more glucose is produced which causes the blood glucose level to be higher. The fat content of the breakfast in the current study was 70% of caloric meal composition, which was higher than previous studies (Wuesten et al, 2005). Since the percentage of fat used in the current study was higher, it would suggest that the response should have been similar or greater than Wuesten et al (2005). During the high carbohydrate trial it is evident that blood glucose following lunch declined across the 2 hour period. However, during the high fat trial, blood glucose levels increased in the first 30 minutes then plateaued across the 90 minute period following lunch. This provides evidence that blood glucose levels are increased for up to 5-6 hours following the ingestion of fat and that the ingestion of carbohydrates can delay the glucose increase from the fat ingestion in the blood.

The type of fat in the diet also appears to have an effect, with saturated fats causing more insulin resistance than mono-saturated or polyunsaturated (Rivellese et al, 2002). The majority (~60%) of the fat content in the high fat breakfast used in the current study was mono-unsaturated from the avocado (Appendix B), with added saturated fat from the yoghurt and milk. This could explain why this current study did not identify as great an increase in blood glucose as the study by Beaudoin et al (2011) following a high fat meal. Beaudoin et al (2011) used a blend of palm stearine and soybean oil to achieve a ratio of polyunsaturated FAs : saturated FAs of 0.2, which is a higher content of saturated FAs than the current study.

The RER result identifying the increase in fat oxidation following a high fat breakfast correlates with the result identifying increased blood glucose following the high fat breakfast. During fat oxidation, FAs in the blood are increased which have been shown to cause insulin resistance in the liver (Shulman, 2000; Robertson et al, 2003; Kraegen & Cooney, 2008). This insulin resistance results in the liver continuing to produce and secrete glucose into the bloodstream. As a result of increased blood glucose due to fat utilisation and as a result of glucose being secreted from the liver, blood glucose levels rise. The results from the current study suggest this as a possible mechanism as the RER is decreased in the high fat trial in addition to increased blood glucose levels.

## Appetite Scales

The current study did not identify any significant differences between the high fat breakfast and the high carbohydrate breakfast in terms of appetite up to two hours post meal. Holt et al (1999) identified that a high fat breakfast has a weaker satiating power when compared to a high carbohydrate breakfast. Each meal used by Holt (1999) was energy matched but not weight matched, which could account for the increased hunger experienced following a high fat breakfast. Whilst the current study also used meals that were energy matched and based on individual energy requirements, Holt et al (1999) used a standardised energy intake for all subjects. A standardised energy intake for all subjects could cause an intake to be too little or too much for specific individuals as it is not matched to individual requirements. A different study used individual daily energy requirements and based their meals on those requirements (Brennen et al, 2012). Brennen et al (2012) results suggested that there was no difference between high fat and high carbohydrate breakfast in terms of regulating appetite. The results of the current study are similar to the study by Brennen et al (2012), perhaps due to the fact that each meal was based on the subject’s energy requirements rather than a standardised meal, as each subject received the correct amount of energy required. In addition to the energy content of the meal, it has been found that different macronutrients have different satiating powers for different lengths of time (Poppitt and Prentice, 1996).It has been observed that while carbohydrates have a greater satiating power over shorter periods of time, such as 2-4 hours, fats can have a greater satiating power over longer periods of time, such as 4-6 hours. These time frames could indicate that if appetite was measured for a longer period of time, a difference could have been noted. However, in situations where fat has a greater satiating power compared to carbohydrate, the amount of fat ingested needs to be higher in terms of weight and therefore energy content (Holt et al, 1999).

## Exercise

The current study did not identified a significant difference during exercise in RER, which does not correlate with findings in previous studies (Murakami et al, 2012; Okano et al, 1996). Okano et al (1996) identified that a pre-exercise high fat meal caused decreased RER, but only in the initial stages of exercise (first 40 minutes), whilst Murakami et al (2012) identified that a pre-exercise high fat meal decreased RER throughout the time to exhaustion trial (90-92 minutes).However, it was also identified that there was an interaction effect in fat oxidation between the breakfast and the increasing intensity, which shows that the composition of breakfast influences fat oxidation depending on the intensity at which the subjects are working. It was expected that the exercise fat oxidation results would look similar to the fat oxidation graph shown by Achten et al (2002), with an increase at the onset of exercise followed by a decrease. However, the current studies fat oxidation curve decreases from the onset of exercise.

Some previous studies identified that pre-exercise ingestion of a high carbohydrate meal, such as the standardised lunch, can increase muscle glycogen levels before the onset of exercise (Costill, 1988; Coyle et al, 1985). It has been reported that ingestion of a high carbohydrate meal increases carbohydrate oxidation, especially muscle glycogen breakdown during exercise, due to enhanced insulin action or increased glycogen levels (Costill et al, 1977). Hawley et al (2000) identified that the ingestion of high fat meals in combination with heparin administration (which causes the release of lipolytic enzymes into the blood circulation) is effective in raising plasma FA concentrations and has been associated with reduced muscle glycogen utilisation and carbohydrate oxidation. Hawley et al (2000) identified that following a high fat meal, the level of FAs in plasma increase above the level normally observed during moderate intensity exercise which could have a positive effect on exercise capacity due to the slowed depletion of the remaining muscle glycogen. It was identified that the slowing of glycogen depletion prolongs the period during which moderate intensity exercise can be performed (Hawley et al, 2000).

One of the hypotheses of this study was that following a high fat breakfast, subjects would utilise fat for a longer period during the exercise trial. However, there was no significant difference in total exercise time or in time before RER reached 1. Gollnick (1985) identified that during prolonged exercise hepatic and skeletal muscle glycogen depletion occurs. Regardless of training state, a reduction in glycogen reserves, as a result of prolonged exercise and inadequate pre-exercise nutrition, may limit glycolytic flux (the rate at which molecules go through the glycolytic pathway) during subsequent exercise. As the intensity of exercise increases, there is a reduction in fat oxidation and an increase in carbohydrate oxidation, which is known as the crossover concept (figure 10). According to the crossover concept, the effect of glycogen depletion on the balance of substrate utilisation during exercise would be identified by a leftward shift in the carbohydrate oxidation curve and a corresponding rightward shift in the fat oxidation curve. Glycogen stores can be depleted by ~80% following an overnight fast, with muscle glycogen levels increasing by 42% following ingestion of food (Coyle et al, 1985). Glycogen depletion, for both trained and untrained individuals, is accompanied by a decrease in power output that can be sustained over time (Brooks & Mercier, 1994). In addition to prolonged exercise leading to glycogen depletion and a consequent shift toward fat metabolism, it is also known that a high fat diet containing adequate calories can result in physiological adaptations to increase lipid oxidation during rest and exercise (Phinney et al, 1983). Instead of using power output like previous studies, the current study used percentage of VO2 max. Although, the current study did not identified that RER was lower following the high fat breakfast, it was identified that fat oxidation was affected by intensity. This suggests that lower fat oxidation identified at different intensities in the high fat trial could be a result of subjects having lower levels of glycogen. However, without measuring glycogen, this is uncertain.

*Figure 10: Crossover concept (Brooks and Mercier, 1994)*

Unlike previous studies, the current study based the meal plans on the subjects’ RMR which likely caused subjects to be in an energy deficit. RMR was used since subjects would be remaining seated throughout the trial until the exercise, therefore not requiring additional food energy to compensate for energy used. A study by McMurray et al (1991) observed the effect of exercise performance and substrate utilisation following an energy deficit. It was identified that while there was no effect on the ability to complete an 8 minute run at 85% of maximal capacity, there was an increase in fat utilisation during the run. This suggests that the energy deficit experienced by subjects would not affect the performance of the trial, although there could be an effect on substrate utilisation. If this were true then it would be expected that fat oxidation in both carbohydrate and fat trials would be greater than carbohydrate oxidation. However, in the trials for this study, RER was not affected by the high fat breakfast which would indicate that fuel utilisation during early evening exercise is not affected by the change in breakfast composition.

The current study identified that a high fat meal had no significant effect on exercise performance which is in keeping with previous research by Whitley et al (1998), Okano et al (1996), Rowlands & Hopkins (2002b), and Paul et al (2003). Although there are no studies that identify improved performance due to a single high fat meal, there are many more studies that identify improved performance due to a high carbohydrate meal (Sherman et al, 1991; Chryssanthopoulos et al, 2002; Chen et al, 2009). It has been suggested that this improvement in performance is due to a higher level of muscle glycogen at the onset of exercise and/or the sparing of muscle glycogen during exercise contributing to the maintenance of higher carbohydrate availability later in the exercise which can delay the onset of fatigue (Hickson et al, 1977).

Studies observing the effect of a high carbohydrate meal identified either no effect or improved performance. The current study identified no significant effect on performance (exercise time), which is similar to previous studies researching the effect of a high carbohydrate meal (Cryssanthopoulos et al, 1994; Sparks et al, 1998; Whitley et al, 1998). Studies that identified an improvement in performance used well-trained subjects (Chryssanthopoulos et al, 2002; Chen et al, 2009), whereas the current study used active general population. Perhaps if the current study had used trained athletes there may have been a significant effect on the exercise results, although this is uncertain.

Previous studies that observed the effect of diet composition on performance used either time trial or time to exhaustion exercise tests (Sparks et al, 1998; Whitley et al, 1998; Chryssanthopoulos et al, 2002; Chen et al, 2009; Okano et al, 1996; Burke et al, 2000). Pitsiladis and Maughan (1999) used a time to exhaustion exercise test at 70% of VO2max to observe substrate utilisation and performance during exercise. This current study used a Fatmax graded exercise test, which was designed to identify fat oxidation at a wide range of intensities instead of at a single intensity (Achten et al, 2002). It was not designed to measure performance, which could explain why no significant effect was identified in exercise time to exhaustion.

The high fat feeding strategy may show benefits for moderate-intensity, ultra-distance endurance performance, which favours fat as the main fuel source (Muoio et al, 1994; Robins et al, 2005; Murakami et al, 2012). These previous studies identified that high fat diets improve the performance of endurance athletes, which suggests that ingesting high fat diets to improve performance is more suited to endurance athletes. However, the current study used a graded exercise test, where the intensity increases past the intensity where fat is the main fuel source and is not best suited for measuring performance. The graded exercise test was necessary to measure the FatMax of subjects, with performance subsequently measured in the same test using the time from start to exhaustion. If this research was to be repeated, a time trial or time to exhaustion exercise test would be best for measuring performance. This would better suit an investigation using trained endurance athletes as subjects rather than active general population.

## Limitations and Future research

A limitation of this current study is that resting indirect calorimetry was not measured between breakfast and lunch. As mentioned, the second meal influences RER. Measuring substrate utilisation in the period after breakfast and before lunch, would perhaps have provided a more detailed and accurate view of ongoing changes, which may have identified greater significance. Fat and carbohydrate are not the only macronutrients that are metabolised. Whilst it is not a major contributor to fuel selection, protein can be metabolised to give energy when required. It was assumed that protein metabolism was at a minimum, although nitrogen levels in urea were not measured, therefore protein metabolism was an unknown factor.

One limitation in relation to blood glucose is that measures were only taken following lunch. No measures were taken before breakfast or between breakfast and lunch to obtain a baseline or to identify changes in blood glucose following the breakfast. Blood glucose measures did not start until 30 minutes after subjects being given lunch. Measurements should be taken as soon as subjects have consumed their meal as blood glucose starts to change as soon as food is ingested. In future research, blood glucose should be measured throughout the day to help track glucose tolerance following each meal and help to identify the mechanisms that cause these changes in relation to the macronutrient consumed. Measuring blood glucose throughout the full day, using Continuous glucose monitoring (CGM) instead of finger prick, may provide a more accurate and frequent measure of blood glucose and also allow testing of insulin levels to measure insulin resistance.

Following the findings of this current study, there is clearly a place for further research into the second meal effect following high carbohydrate and high fat breakfasts. Substrate utilisation and blood glucose need to be measured throughout the day, from before breakfast, in order to establish a baseline, until the end of the exercise test. Further research could incorporate both a single high carbohydrate and high fat breakfast and multiple days of high carbohydrate and high fat breakfasts. This would help determine whether one or more breakfasts are required to bring about a significant effect on substrate utilisation following a second meal and during evening exercise. Once changes to substrate metabolism have been identified there is a need to identify the period of time in which these changes are maintained.

Different techniques such as tracer technology with labels (13C-Glucose) and spectroscopy could be used to identify the metabolic mechanisms that cause the impairment to utilise carbohydrates and also identify the metabolic changes that occur in the body following fat ingestion in relation to both substrate utilisation and blood glucose. A theoretical method by Coggen (1999) suggests the use of stable isotope tracers in conjunction with indirect calorimetry to estimate utilisation of muscle glycogen and FA stores which could circumvent the need for muscle biopsies. By using tracers, there is the possibility of determining if the affected glucose tolerance is caused by skeletal muscle glucose uptake or hepatic glucose output.

In addition to gaining a better understanding of the mechanisms at work, there is a need to identify ways in which these dietary manipulations could be applied as part of training preparation. Whilst breakfast alone would not influence training, nutrition as a whole is vital. Such research could identify feeding strategies to be used in training programmes for endurance athletes where the increased utilisation of fats could help glycogen sparing and thus improve performance.

## Conclusion

In summary, consuming a high fat breakfast influenced substrate utilisation in terms of RER following a second meal, but no effect was identified on fat oxidation or carbohydrate oxidation. However, whilst significant, the change in RER may not be physiologically relevant for general population. A high fat breakfast increased the blood glucose levels up to 2 hours after the second meal. There was no significant difference in RER or fat and carbohydrate oxidation during evening exercise. However, fat oxidation was affected by breakfast composition in relation to the percentage of VO2 max at which the subjects were cycling.References

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# Appendix A: Pre-Participation Health Screen Questionnaire

**Pre-Participation Health Screen Questionnaire (PPHS-Q)**

|  |
| --- |
| PPHS-Q is an exercise – specific checklist for classification of training categories.**Accuracy in completion of the PPHS-Q is of the utmost importance**The purpose of the pre-participation health screen is:* To optimise safety during exercise testing and programme description.
* To identify medical risk factors which may contra-indicate exercise.
* To identify those with special exercise needs.
 |

Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Age:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Gender:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Address\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Tel:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_(H) \_\_\_\_\_\_\_\_\_\_\_\_\_\_(W)

Doctor’s name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Tel:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_(H)\_\_\_\_\_\_\_\_\_\_\_\_\_\_(W)

|  |
| --- |
| **Section A: Medical History Summary and Recommendations** |
| Date: |  |
| Date: |  |
| Date: |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  **Section B: Coronary Heart Disease Risk Index** | **Date** | **Date** | **Date** |
| Group |  |  |  |  |
| 1. 1-10 | No supervision required – exercise at will |  |  |  |
| 2. 10-17 | No supervision required – use general exercise guidelines |  |  |  |
| 3. 18-27 | No supervision required – use prescribed programme only |  |  |  |
| 4. 28-40 | Use prescribed programme – Personal Training recommended |  |  |  |
| 5. 41+  | Use prescribed programme – Personal Training and re-test within 8 weeks recommended |  |  |  |

|  |
| --- |
|  **Section C: Physical Activity Index** |
|  | **Date:** |  |  |
| **Activity Level** | **Times per week** | **Risk level** |  |  |
| Inactive | 0 occasional | Very High |  |  |
| Semi-active | 1 | High |  |  |
| Active | 2-3 | Moderate |  |  |
| Very active | 4 or more | Low |  |  |

**SECTION A MEDICAL HISTORY**

Have you ever been told that you have had or have any of the following conditions? If yes, please mark with an X in the appropriate box:

|  |  |  |
| --- | --- | --- |
| **CARDIAC (Heart Related Diseases)** |  |  |
|  | Heart Attack |  | High blood pressure |
|  | Coronary thrombosis (blood clot) |  | Rheumatic fever |
|  | Narrowing of arteries |  | Angina / Chest Pain |
|  | High cholesterol |  | Congenital Heart Disease  |
|  |  |  |  |
|  | Further / comments |  |  |

|  |  |  |
| --- | --- | --- |
| **PULMONARY (Lung Diseases)** |  |  |
|  | Asthma |  | Exercise-induced asthma |
|  | Chronic Bronchitis |  | Emphysema |
|  | T.B. |  |  |
|  |  |  |  |
|  | Other / comments |  |  |

|  |  |  |
| --- | --- | --- |
| **OTHER** |  |  |
|  | Type I Diabetes (insulin dependent) |  | Type II Diabetes (non-insulin dependent) |
|  | Anaemia (ion deficiency) |  | Rheumatic fever |
|  | Kidney disease |  | Angina / Chest Pain |
|  | Rheumatoid Arthritis |  | Congenital Heart Disease  |
|  |  |  | Pregnant  |
|  | Other / comments |  |  |

|  |  |  |
| --- | --- | --- |
| **ORTHOPAEDIC SURGERY (Musculo Skeletal)** |  |  |
|  | Neck |  | Hip |
|  | Back |  | Knee |
|  | Shoulder |  | Ankle |
|  | Arm |  | Foot  |
|  | Other / comments |  |  |

|  |
| --- |
| **INJURY**Have you suffered any of the following injuries? If so, how long ago? |
|  | Neck vertebrae |  | Back vertebrae |
|  | Rotator cuff |  | Impingement Syndrome (shoulder) |
|  | Tennis elbow |  | Runner’s knee |
|  | ITB |  | Lower leg  |
|  | Achillies Tendonitis |  | Plantar Fascitis  |
|  | Other / comments |  |  |

|  |
| --- |
| **MEDICATION**Do you use medication at present for any of the following? (If yes, please state the drug) |
|  | Heart rhythm |  | Blood pressure |
|  | Blood clotting. |  | Blood circulation |
|  | Asthma |  | Bronchitis |
|  | Emphysema |  | Flu |
|  | Diabetes |  | Thyoid dysfunction |
|  | Cholesterol |  | Anaemia |
|  | Kidney |  | Liver |
|  | Arthritis |  | Muscle injury |
|  | Other / comments |

**SECTION B CARDIOVASCULAR DISEASE RISK INDEX**

Please read the following questions carefully and answer each accurately. Mark your choice with an X.

|  |
| --- |
| **History of heart attack or bypass surgery / angioplasty** |
| 0 |  | None | 5 |  | 1 – 2 years ago |
| 2 |  | Over 5 years ago | 8 |  | < 1 year ago |
| 4 |  | 3 – 5 years ago |  |  |  |

|  |
| --- |
| **Family history of heart disease** |
| 1 |  | No known history |
| 2 |  | 1 relative with cardiovascular disease over the age of 60 |
| 3 |  | 2 relatives with cardiovascular disease over the age of 60  |
| 4 |  | 1 relative with cardiovascular disease under the age of 60  |
| 6 |  | 2 relatives with cardiovascular under the age of 60 |
| 8 |  | Heart – related sudden death: |
|  |  | Male, first degree relative before the age of 55 |
|  |  | Female, first degree relative before the age of 65 |

|  |  |
| --- | --- |
| **Age / Gender Index** | **Smoking status** |
| 0 |  | Male / female under 30 years of age | 0 |  | None |
| 1 |  | 30 – 40 years of age | 1 |  | Pipe |
| 2 |  | Female 40 - 50 years of age | 2 |  | 1 – 10 cigarettes daily |
| 3 |  | Male 40 – 50 years of age | 3 |  | 11 – 20 cigarettes daily  |
| 3 |  | Female 50 – 60 years of age | 4 |  | 21 – 30 cigarettes daily |
| 4 |  | Male 50 – 60 years of age | 5 |  | 31 – 40 cigarettes daily |
| 4 |  | Male / female 60+ years of age | 6 |  | 41 – 60 cigarettes daily |
|  |  |  | 8 |  | + 60 cigarettes daily |
|  |  |  | State how long you have smoked for:Years\_\_\_\_\_\_\_\_\_\_\_\_\_\_months\_\_\_\_\_\_\_\_\_\_\_\_ |

|  |  |
| --- | --- |
| **How would you describe your bodyweight?** | **Total Cholesterol**  |
| 0 |  | Ideal weight | 0 |  | < 5 mmol / L |
| 2 |  | 0 – 5kg overweight  | 1 |  | 5.0 – 5.2 mmol / L |
| 4 |  | 6 – 10kg overweight | 3 |  | 5.3 – 5.9 mmol / L |
| 6 |  | 11 – 15kg overweight | 5 |  | 6.0 – 6.2 mmol / L |
| 8 |  | + 15kg overweight | 6 |  | 6.3 – 6.9 mmol / L |
| 10 |  | Underweight | 7 |  | 7.0 – 7.5 mmol / L |
|  |  |  | 8 |  | > 7.5 mmol / L |
|  |  |  |  |  | Not sure |

|  |  |
| --- | --- |
| **Systolic Blood Pressure** | **Diastolic Blood Pressure** |
| 0 |  | < 130 mmHg | 0 |  | < 80 mmHg |
| 1 |  | 130 – 140 mmHg | 1 |  | 81-90 mmHg |
| 2 |  | 141 – 150 mmHg | 2 |  | 91 – 100 mmHg |
| 3 |  | 151- 160 mmHg | 3 |  | 101 – 110 mmHg |
| 4 |  | > 160 mmHg | 4 |  | > 110 mmHg |
|  |  | Not sure |  |  | Not sure |

|  |  |
| --- | --- |
| **Diabetes** | **Occupational activity level** |
| 0 |  | None | 1 |  | Intense physical labour |
| 1 |  | Type 11 (non-insulin dependent) | 2 |  | Moderate (walk often etc.) |
| 2 |  | Type 1 (insulin dependent) | 3 |  | Sedentary |

|  |
| --- |
| **Work Stress Tension** |
| 0 |  | No stress, very relaxed |
| 1 |  | Moderate work stress and relaxed personality |
| 2 |  | High work stress but cope well |
| 3 |  | Very high work stress and tense personality |
| 4 |  | Very high work stress, highly strung personality |

|  |
| --- |
| **Physical Activity Status** (for a minimum of 30 minutes a session) |
| 1 |  | Exercise 4 or more times per week |
| 2 |  | Exercise 2 – 3 times per week |
| 3 |  | Recreational sport once a week |
| 4 |  | Recreational sport occasionally or complete lack of exercise |

**SECTION C: EXERCISE PARTICIPATION**

|  |
| --- |
| Do you participate in any of the activities more than twice weekly?(Please tick all relevant activities) |
|  | Jogging more than 5 km |  | Aerobic classes 45 min |
|  | Cycling more than 45 min. |  | Tennis 90 min |
|  | Swimming more than 600 m |  | Squash 45 min. |
|  | Gym (Combined strength / aerobic) |  | Team sport (outdoor) – rugby hockey, soccer |
|  | Gym (weights only) |  | Team sport (indoor) – basketball, netball, etc |
|  | Gym (aerobic only) |  | Canoeing / Rowing 45 min |

**SECTION D**

I have read, understood and completed this questionnaire to the best of my knowledge.

I am aware of the risk involved in fitness testing and understand the test procedures that I will perform. I give consent to participate in this assessment.

**Participant’s Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_­\_\_\_\_\_\_\_**

**Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

# Appendix B: Macronutrient content of meal ingredients

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Carbohydrate (g)** | **Fat (g)** | **Protein (g)** |
| Total | Sugars | Total | Saturated | Poly-unsaturated | Mono-unsaturated | Trans-Fat |  |
| **Carbohydrate Breakfast** |  |  |  |  |  |  |  |  |
| Tesco easy oats (Golden syrup) (100g) | 70.7 | 18.6 | 5.5 | 1.0 |  |  |  | 7.0 |
| Lyle’s Golden syrup (100g) | 80.5 | 80.5 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| Whole Milk (100ml) | 4.5 | 4.5 | 3.9 | 2.51 | 0.14 | 1.03 | 0.14 | 3.3 |
| Banana (100g) | 23.2 | 20.9 | 0.3 | 0.1 | 0.1 | Tr | 0 | 1.2 |
| **Fat Breakfast** |  |  |  |  |  |  |  |  |
| Tesco Frozen berry smoothie mix (100g) | 7.9 | 7.2 | 0.2 | 0 |  |  |  | 0.9 |
| Avocado (100g) | 1.9 | 0.5 | 19.5 | 4.1 | 2.2 | 12.1 | 0 | 1.9 |
| Onken Natural Yoghurt (100g) | 4.8 | 4.5 | 10.2 | 6.75 | 0.28 | 2.53 | 0.21 | 5.7 |
| Whole Milk (100ml) | 4.5 | 4.5 | 3.9 | 2.51 | 0.14 | 1.03 | 0.14 | 3.3 |
| **Standardised Lunch** |  |  |  |  |  |  |  |  |
| Fusilli Pasta (100g) | 55.5 | 1.5 | 2.4 | 0 | 0 | 0 | 0 | 11.5 |
| Tesco creamy tomato and herb sauce (100g) | 7.6 | 5.5 | 7.5 | 0.8 |  |  |  | 1.7 |
| Tesco Olive Oil (100ml) | 0 | 0 | 99.9 | 14.3 | 8.2 | 73 | Tr | Tr |
| Grated Parmesan (100g) | 0.9 | 0.9 | 29.7 | 19.25 | 1.12 | 7.67 | 1.14 | 36.2 |
| Garlic bread (100g) | 45 | 2.7 | 18.3 | 9.69 | 1.46 | 5.53 | 0.85 | 7.8 |

# Appendix C: Macronutrient content of meal based on 2000kcal/day RMR

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Carbohydrate (g)** | **Fat (g)** | **Protein (g)** |
| Total | Sugars | Total | Saturated | Poly-unsaturated | Mono-unsaturated | Trans-Fat |  |
| **Carbohydrate Breakfast** |  |  |  |  |  |  |  |  |
| Tesco easy oats (Golden syrup) (78g) | 55.15 | 14.51 | 4.29 | 0.78 |  |  |  | 5.46 |
| Lyle’s Golden syrup (25g) | 20.12 | 20.12 | 0 | 0 | 0 | 0 | 0 | 0.12 |
| Whole Milk (280ml) | 12.6 | 12.6 | 10.92 | 7.03 | 0.39 | 2.88 | 0.39 | 9.24 |
| Banana (90g) | 20.28 | 18.81 | 0.27 | 0.09 | 0.09 | Tr | 0 | 1.08 |
| **Fat Breakfast** |  |  |  |  |  |  |  |  |
| Tesco Frozen berry smoothie mix (60g) | 9.48 | 8.64 | 0.24 | 0 |  |  |  | 1.08 |
| Avocado (130g) | 2.47 | 0.65 | 25.35 | 5.33 | 2.86 | 15.73 | 0 | 2.47 |
| Onken Natural Yoghurt (120g) | 5.76 | 5.4 | 12.24 | 8.1 | 0.34 | 3.04 | 0.25 | 6.84 |
| Whole Milk (230ml) | 10.35 | 10.35 | 8.97 | 5.77 | 0.32 | 2.37 | 0.32 | 7.59 |
| **Standardised Lunch** |  |  |  |  |  |  |  |  |
| Fusilli Pasta (100g) | 55.5 | 1.5 | 2.4 | 0 | 0 | 0 | 0 | 11.5 |
| Tesco creamy tomato and herb sauce (100g) | 7.6 | 5.5 | 7.5 | 0.8 |  |  |  | 1.7 |
| Tesco Olive Oil (5ml) | 0 | 0 | 4.99 | 0.71 | 0.41 | 3.65 | Tr | Tr |
| Grated Parmesan (10g) | 0.05 | 0.05 | 1.48 | 0.96 | 0.06 | 0.38 | 0.06 | 1.81 |
| Garlic bread (31g) | 13.95 | 0.84 | 5.67 | 3.0 | 0.45 | 1.71 | 0.26 | 2.42 |

# Appendix D: Visual Analogue Scale

Volunteer code: \_\_\_\_\_\_\_ Date:DD/MM/YYYY \_\_\_/\_\_\_\_/­­\_\_\_\_\_

Please place a vertical mark on the horizontal line depending on how you feel now. It is important that you rate how you actually feel now, and not how you might think you should feel, or how someone else might expect you to feel. Please consider the extreme labels – for example, ‘not at all hungry’ and ‘as hungry as I have ever felt’ – as the least hungry and the most hungry you have ever felt, respectively.

1. How hungry do you feel?

**Not at all hungry** **Extremely hungry**

|  |
| --- |
|  |
|  |

2. How full do you feel?

**Not at all full** **Extremely full**

|  |
| --- |
|  |
|  |

3. How nauseated do you feel?

**Not at all nauseated** **Extremely nauseated**

|  |
| --- |
|  |
|  |

# Appendix E: Rate of Perceived Exertion Scale

