FACTORS AFFECTING FAT OXIDATION IN EXERCISE

By

Rebecca Randell

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ABSTRACT

Increasing fat oxidation rates during exercise may be beneficial for the athletic population. At rest, ingestion of Green Tea Extract (GTE) has been found to augment fat oxidation, but there are limited data on the effects during exercise. This thesis systematically investigated the effects of GTE ingestion on exercise metabolism in physically active males. We set out to determine if longer-term feeding of GTE could increase fat oxidation rates during a steady state exercise bout to a greater degree than an acute dose. However, irrespective of the length of ingestion no measureable change in substrate metabolism was found.

Due to the large individual differences in fat oxidation at a given absolute and relative exercise intensity, we investigated the effects of acute GTE ingestion during a graded exercise test. Again, no change in fat metabolism was found over a wide range of exercise intensities. Finally, we collected fat oxidation data from a large cohort of athletes. From these data we set new criteria to define individuals as either a fat or carbohydrate metabolic type. Although it is still not known fully what determines metabolic type, the use of a nutritional intervention may be more effective in one type over the other.

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PUBLICATIONS

Published

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- **Randell RK**, Hodgson AB, Lotito SB, Jacobs DM, Boon N, Mela DJ, et al. No effect of 1 or 7 days Green Tea Extract ingestion on fat oxidation during exercise. *Med Sci Sports Exerc*. 2013;45(5):883-91.
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- Hodgson AB, **Randell RK**, Jeukendrup AE. The Effect of Green Tea Extract on Fat Oxidation at Rest and during Exercise: Evidence of Efficacy and Proposed Mechanisms. *Adv Nutr*. 2013;4(2) 129-140.

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Oral Presentations

- No effect of 1 or 7 days Green Tea Extract ingestion on fat oxidation during exercise at European College of Sports Science (ECSS), Bruges, Belgium, 2012.

Poster Presentation

- Nutritional intake and gastrointestinal problems during competitive endurance event at American College of Sports Medicine (ACSM), Denver, USA, 2011.
- Effects of variable duration green tea extract ingestion on fat metabolism at ECSS, Barcelona, Spain, 2013.

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ABBREVIATIONS

Adipocyte Fatty Acid-Binding Protein **(aP2)**; Adipose Triglyceride Lipase **(ATGL)**; Analysis of covariance **(ANCOVA);** Analysis of variance **(ANOVA);** Beats per minute **(bpm);** β-hydroxyacyl-coenzyme A dehydrogenase **(βHAD)**; Body fat **(BF);** Body mass index **(BMI);** Body weight **(BW);** Carbohydrate **(CHO);** Carbohydrate Metabolic Type **(CMET)**; Carbon Dioxide **(CO_2);** Carnitine Palmitoyltransferase 1 **(CPT1)**; Catechol-*O*- Methyl- Transferase **(COMT)**; CCAAT Enhancer-Binding Protein-α **(C/EBP-α)**; Citrate Synthase **(CS);** Coefficient of variation **(CV);** Coenzyme A **(CoA)**; Degrees Celsius **(°C);** Decaffeinated Green Tea Extract **(dGTE);** Dual-energy X-ray absorptiometry **(DXA)**; Epigallocatechin-3-Gallate **(EGCG)**; Energy Expenditure **(EE);** Fat free mass **(FFM);** Fatty Acid Binding Protein **(FABPpm)**; Fatty Acids **(FAs)**; Fatty Acid Synthase **(FAS)**; Fatty Acid Translocase CD36 **(FAT/CD36)**; Fatty Acid Transport Protein **(FATP)**; Fat mass **(FM)**; Fat metabolic type **(FMET)**; Intensity at which maximal rates of fat oxidation occur **(FATMAX);** Gatorade Sports Science Institute **(GSSI);** Green Tea Extract **(GTE)**; Gram **(g);** Heart rate **(HR);** Heart rate at FATMAX **(HR_{fatmax})**; Heart rate max **(HRmax);** Hormone Sensitive Lipase **(HSL)**; Intramuscular Triglycerides **(IMTGs)**; Kilocalorie **(Kcal);** Kilogram **(kg);** Kilojoules **(kJ);** Lipoprotein Lipase **(LPL)**; Litre **(L);** Maximal Fat Oxidation **(MFO)**; Medium-chain acyl-CoA dehydrogenase **(MCAD);** Messenger RNA **(mRNA)**; Metre **(m);** Microlitre **(µL);** Micromol **(µmol);** Millilitre **(ml);** Millimol **(mmol);** Minute **(min);** Noradrenaline **(NA);** Number of participants **(N);** Oxygen **(O_2);** Percent body fat **(%BF);** Peroxisome Proliferator-Activated Receptor- γ **(PPARγ)**; Rating of perceived exertion **(RPE);** Regulatory Element-

Binding Protein-1c **(SREBP-1c)**; Respiratory Exchange Ratio **(RER)**; Respiratory Quotient **(RQ)**; Revolutions per minute **(rpm);** Standard Deviation **(SD);** Standard error of means **(SEM);** Time **(t);** Volume of carbon dioxide **($\dot{V}CO_2$);** Volume of Oxygen **($\dot{V}O_2$);** Maximal oxygen uptake **($\dot{V}O_2max$);** Watt **(*W*);** Maximal work load **(*W*max);** Maximal work output **(*W*out);** Year **(*y*).**

Chapter 1: INTRODUCTION

1.1 Overview

During exercise skeletal muscle is the main site for fat oxidation and an increased capacity to utilise fats is often associated with health and exercise performance benefits. This introduction firstly describes the regulation of fat metabolism in skeletal muscle under exercise conditions. Then the concept of metabolic flexibility and the importance of fat oxidation for the prevention of disease are briefly explained. The focus of this introduction then shifts to the benefits of increasing fat metabolism during exercise in healthy athletic populations, as well as discussing the exercise protocol used to measure fat oxidation and the potential determinants.

Manufacturers often claim that their food/ beverage/ supplement can increase fat oxidation rates. Thus, this introduction reviews the evidence for some of these nutritional interventions. However a more detailed discussion is presented on the potential role of green tea and green tea extract (GTE) ingestion on increasing fat oxidation rates and, in light of the current literature, highlights what is still left to be determined in this area of research.

1.2 Regulation of Fat Metabolism in Skeletal Muscle

The heart and skeletal muscle are proficient in the handling of fatty acids (FAs) for oxidation (energy) (25). Skeletal muscle also has the ability to store small amounts of FAs however adipose tissue is the main site for FA storage (25). At rest, and during periods of fasting, energy is mainly derived from the hydrolysis (lipolysis) of adipose tissue triglyceride (ATGL) stores, subsequently increasing plasma FA concentration for uptake into other tissues for oxidation (Please refer to Frayn et al (26) for a detailed review on the regulation of fat metabolism in adipose tissue). Thus under resting conditions, especially when in a fasting

state, FAs are the predominant fuel used by the skeletal muscle (46). At the onset of exercise there is an increased utilization of FAs in skeletal muscle, as a result of increased blood flow and energy demand of the contracting muscles. The available FAs oxidised by the skeletal muscle are derived from lipolysis of ATGLs, lipolysis of the skeletal muscles own triglyceride stores (intramuscular triglycerides; IMTGs) and plasma FAs.

Using blood sampling and isotope tracers Romijn et al (78) quantified fat and carbohydrate (CHO) kinetics and oxidation during exercise performed at different intensities. On three consecutive days five endurance trained cyclists performed three exercise bouts at 25%, 65% and 85% maximal oxygen uptake ($\dot{V}O_2max$). The authors observed that during the exercise bout performed at low intensity (25% $\dot{V}O_2max$) plasma FAs were the predominant energy source and on average fat oxidation rates were ~27 μmol·kg·min^{-1}. During the moderate intensity exercise bout (65% $\dot{V}O_2max$) fat oxidation rates increased to ~43 μmol·kg·min^{-1}. Rates of plasma FA oxidation were still relatively high during this moderate exercise bout however, there was an increase in IMTG derived FA oxidation (~500% increase). With further increases in exercise intensity (85% $\dot{V}O_2max$) fat oxidation rates decreased (~30 μmol·kg·min^{-1}) and substrate use shifted towards predominately CHO sources (muscle glycogen and plasma glucose), despite maintaining high levels of whole body lipolysis. This study by Romijn et al (78) is well cited as it clearly shows the change in substrate utilisation with increases in exercise intensity however the low sample size (N=5) is a major limitation. In 2001 Van Loon et al (96) confirmed these findings when taking muscle biopsies, in addition to isotopic tracers, during three separate exercise bouts performed at 40%, 55% and 75% maximal work load (Wmax). Thus, it appears that when exercise intensity increases from low to moderate there is a shift in substrate from predominately plasma FAs to a greater utilisation of FAs derived from IMTG lipolysis. However, with further increases in exercise

intensity fat oxidation decreases, despite high rates of whole body lipolysis, and CHO sources become the predominant energy substrate.

There are several sites which may regulate the utilisation of FAs in the skeletal muscle during exercise these include: 1) the transport of FAs into the myocyte (skeletal muscle cell); 2) the uptake of FAs across the mitochondrial membrane and 3) the release of FAs from IMTG. Each of these potential rate limiting steps will be discussed.

It was once believed that uptake of FAs into the skeletal muscle was by simple diffusion (31). However, with advances in scientific technology it has been established that several transport mechanisms are responsible for FA uptake into the myocyte (12, 41, 56). Thus, the trafficking of FAs into the muscle may be a potential rate limiting step in FA oxidation (42, 47). The proteins involved in the shuttling of FAs into the skeletal muscle are: FA binding protein (FABPpm) (41); FA translocase CD36 (FAT/CD36) (12) and FA transport protein (FATP) (56).

Unlike FABPpm, which is membrane bound protein, FATCD/36 is primarily located in the sarcoplasm. In 2000, Bonen et al (14) extracted rat skeletal muscle and found that with acute muscle contraction FATCD/36 translocated from the intracellular pool to the plasma membrane. In the same study, contraction mediated translocation of FATCD/36 was accompanied with increased uptake of palmitate (14). Furthermore, overexpression of FATCD/36 in mice only increased FA oxidation during acute muscle contraction and not a rest (38). These studies highlight the role of FATCD/36 for fat oxidation during acute muscle contraction. Multiple exercise bouts have also been found to increase plasma FA uptake and oxidation (94). In 1997 Kiens et al (49) found increases in FABPpm with endurance training. In addition, chronic electrical stimulation of rat skeletal muscle (indicative of endurance

training in humans) found increased fat oxidation which was associated with increases in the FA transporter FAT/CD36 protein (13). Taken together the literature suggests that FA uptake into the myocyte is regulated by FA transporter proteins but, due to the increases in translocation (resulting in increases in fat oxidation rates) with acute exercise bouts and increases in FA transporter protein expression with exercise training, FA uptake may not be a rate limiting step.

IMTG stores are an important fuel source during exercise (97). As already mentioned rates of FA oxidation derived from IMTGs are increased during exercise performed at moderate intensity (78, 96). The breakdown of IMTGs is regulated by the neutral enzyme hormone sensitive lipase (HSL). One known activator of HSL is noradrenaline, via increases in β-adrenergic activation (54). However, when a β-adrenergic blockade was used in rat muscle no impairment in HSL activation was found during electrical stimulation (53). Thus other HSL activators have been reported including calcium (Ca^{2+}) (52), translocation of HSL to the lipid droplet (19) and the energy status of the cell (free AMP and ADP) (27). Watt et al (107) measured HSL activity during three 10 min exercise bouts differing in intensity (30%, 60% and 90% $\dot{V}O_2$peak). After one min of exercise HSL activation rapidly increased at all exercise intensities but was maintained in only the low (30%) and moderate (60%) exercise bout. Given that HSL activation was highest in the first min of exercise, a time at which fat oxidation is negligible, other factors such as FA uptake into the mitochondria must play a crucial role in the regulation of fat metabolism.

Once in the sarcoplasm FAs are activated by the enzyme acyl-CoA synthase to form acyl-CoA. Carnitine is involved in transporting this activated FA (acyl-CoA) across the otherwise impermeable inner mitochondrial membrane for oxidation to take place. Carnitine

palmitoyltransferase I (CPTI) is an important oxidative enzyme that catalyses the esterifaction of carnitine with the acyl moiety forming acyl-carnitine to allow transportation into the mitochondrial matrix. The acyl-carnitine complex is then shuttled through the inner mitochondrial membrane into the matrix where it is converted into acyl-CoA and free carnitine (catalysed by Carnitine palmitoyltransferase II; CPTII). The activated FA is cleaved by the β-oxidation pathway and oxidised and the free carnitine is released back into the sarcoplasm and becomes available to transport more FAs across the mitochondrial membrane.

Another function of carnitine is the buffering of acetyl-CoA, forming acetlycarnitine. With increases in exercise intensity glycolytic flux is heightened subsequently increasing the formation of acetyl-CoA and reducing the availability of free carnitine. Van Loon et al (96) found that exercise performed at 75% *W*max increased the acetylation of carnitine reducing the free carnitine pool compared to exercise performed at 55% *W*max. Therefore it has been suggested that the reduction in free carnitine may reduce the formation of fatty acyl-CoA and decrease the uptake of FAs into the mitochondria (96, 105).

Malonyl coenzyme A (CoA), an enzyme involved in FA synthesis, may also play a role in limiting fat utilisation during exercise. Malonyl CoA allosterically binds to CPT1 inhibiting the enzyme (59) and subsequently preventing the uptake of FAs into the mitochondria. At rest Rasmussen et al (75) found, as expected, suppressed fat oxidation and increased CHO oxidation during a 5 h period of hyperglycemia and hyperinsulemia. Interestingly the authors found skeletal muscle concentrations of malonyl-CoA to be increased with no change in FA uptake, suggesting that the FAs were being shunted towards storage than oxidation. During exercise malonyl-CoA kinetics are less clear. Odland et al (69) measured skeletal muscle malonyl-CoA concentrations in eight volunteers at rest and during three 10 min exercise bouts performed at 35, 65 and 90% $\dot{V}O_2$max. Respiratory data reflected previous literature with fat

utilisation the predominant fuel during the low and moderate exercise bouts (RER 0.84 and 0.92 respectively) and the high intensity exercise bout indicating a large reliance on CHO (RER > 1.0). However the authors found no difference in skeletal muscle malonyl-CoA concentrations at rest and during all three of the exercise bouts. An additional study from Copenhagen also found no difference in skeletal muscle malonyl-CoA concentrations during a one legged exercise protocol performed at 60%, 85% and 100% $\dot{V}O_2$max (20). Thus the role of malonyl-CoA on inhibiting fat oxidation still remains unclear.

In summary, despite an abundance of studies investigating the mechanisms on fuel selection it is not entirely known what the main regulator(s) of fat oxidation during exercise are. In a recent study fat oxidation rates during steady state exercise were increased when skeletal muscle carnitine concentrations were augmented over a 24 week period (104). However more intervention studies are needed to fully elucidate the role of carnitine on regulating fat oxidation.

1.3 The Role of Fat Oxidative Capacity for Health

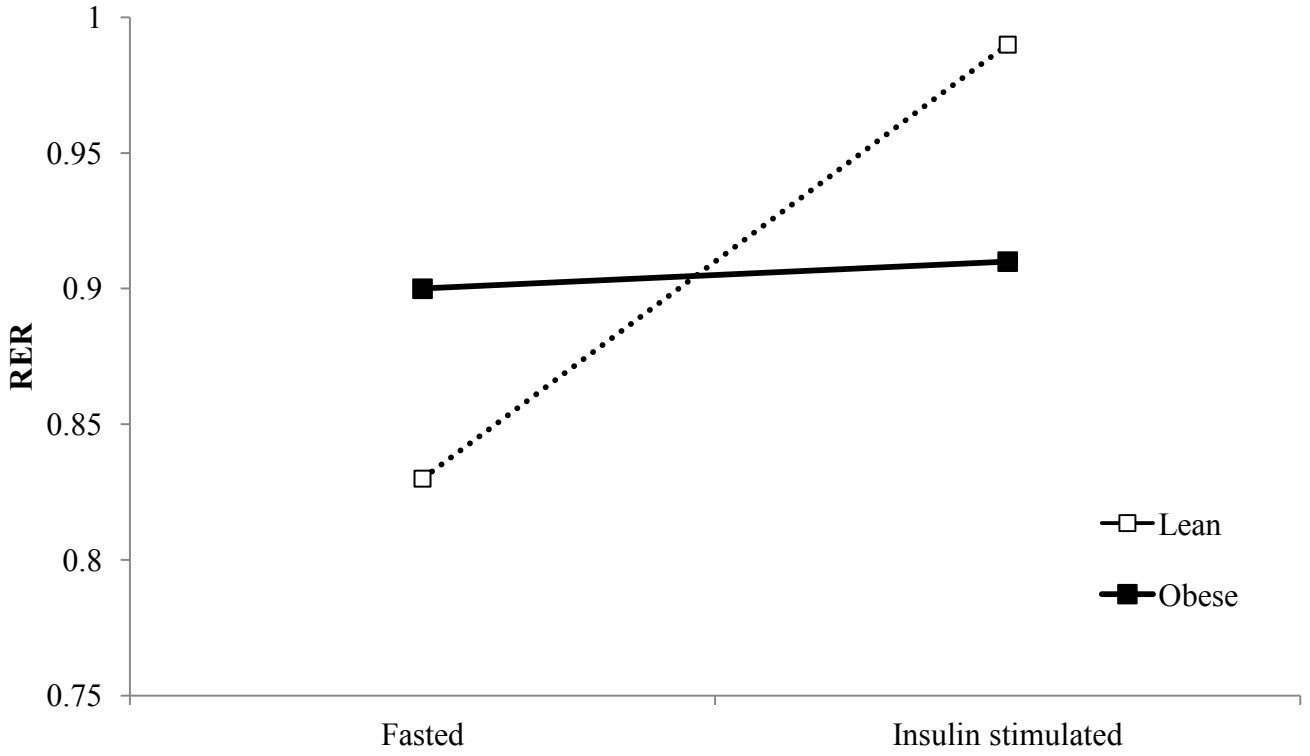
Skeletal muscle of healthy individuals has the capacity to respond to metabolic and environmental stimuli resulting in changes in substrate metabolism. For example, during periods of fasting, FAs are the predominant fuel for skeletal muscle (6). In the postprandial state (a situation where plasma insulin is elevated) fuel selection shifts from fat oxidation to predominately CHO (in the form of glucose) (45). The ability to alter substrate use is often referred to as skeletal muscle metabolic flexibility (45, 46). Obese individuals are less able to response to environmental and metabolic changes and therefore metabolic flexibility is impaired (46). For instance, under fasting conditions CHO oxidation is higher in obese skeletal muscle compared to lean, despite comparable rates of skeletal muscle FA uptake (46).

Thus, fat oxidation rates are lower leading to accretion of IMTGs (46). Furthermore, during periods of elevated plasma insulin (e.g in a postprandial state), CHO uptake, oxidation and storage is blunted (46) (Figure 1.1). Thus, the dynamic metabolic flexibility of skeletal muscle is diminished which may have a negative impact on health.

Increased delivery of FAs to the skeletal muscle (a tissue not suited for fat storage) leads to the synthesis of long-chain acyl-CoAs (LCACoAs) and other FA metabolites such as diacylglycerols (DAGs) and ceramides (82). In brief, these named FA metabolites are known to activate protein kinase C (PKC) and ceramide- activated protein kinase (CAPK), which phosphorylate and inactivate the insulin signalling pathway (51). This in turn reduces glucose uptake resulting in skeletal muscle insulin resistance and if untreated may lead to Type II diabetes. For a detailed review see (82).

Goodpaster et al (28) compared skeletal muscle insulin sensitivity and IMTG content in lean, obese (with and without type 2 diabetes) and endurance trained individuals. As expected, histochemical analysis of muscle samples showed the highest IMTG content in the obese subjects with Type II diabetes (28). These individuals also displayed the lowest insulin sensitivity. Therefore interventions to increase fat oxidation, in order to reduce plasma FAs and IMTGs, in obese populations have been the topic of investigation in recent years (29, 84). Low and moderate intensity exercise training has been found to elicit higher fat oxidation rates in obese individuals compared to high intensity training (95, 100). This increase in fat oxidation, may aid body fat loss in the long term, if coupled with a negative energy balance, and result in improved health (76).

Figure 1.1.Metabolic flexibility



Graph adapted from Kelley et al (46) comparing the metabolic differences in lean and obese individuals in a fasted and insulin stimulated state.

1.4 The Importance of Fat Oxidation for Athletes and Exercise Performance

Interestingly, endurance trained individuals are also characterised by having high IMTG content (28). This apparent "paradox" provides the trained individual with IMTGs which are an important substrate during endurance type exercise (97). Using magnetic resonance spectrometry (MRS) Van Loon et al (97) collected data on mixed muscle IMTG content before, immediately after and 48 h after a 3 h cycle exercise bout at moderate intensity (~55% *W*max). The authors observed a 21% reduction in IMTG content immediately following the exercise bout, which had fully recovered to pre-exercise levels in the 48 h recovery period. In an additional study IMTG content was reduced by 49% and 67% (depending on analytical technique) following a 3 h cycle (85). Taken together these findings emphasise the importance of IMTGs as a substrate and an insight into the time course of IMTG repletion. This cycle of IMTG depletion and repletion, as a result of repeated exercise bouts, is thought to reduce the accumulation of FA metabolites interfering with the insulin signalling cascade; thus in this athletic population insulin sensitivity is not compromised (28).

Endurance exercise training is also associated with a change in IMTG density and intracellular location. Tarnopolsky et al (91) investigated the effects of a seven week endurance exercise regimen (60 min cycle exercise 5 days/week) in 12 untrained male and females. Muscle biopsies were obtained before and after training to quantify changes in IMTG content, density, location as well as changes in mitochondria and oxidative enzymes. Endurance exercise training was found to significantly increase skeletal muscle mitochondria content as well as mitochondrial size, which was accompanied with increases in measured oxidative enzymes (citrate synthase (CS) and β-hydroxyacyl-coenzyme A dehydrogenase

(βHAD) (91). Furthermore, a strong trend for an increase in IMTG density was observed following training, attributed to changes in IMTG number not size (91). Interestingly the authors also found a higher proportion of IMTGs in close proximity of mitochondria which was associated with higher fat oxidation during exercise (91). This structural/functional relationship may reflect the high exercise capacity (74) and efficiency to oxidise fats often seen in endurance athletes.

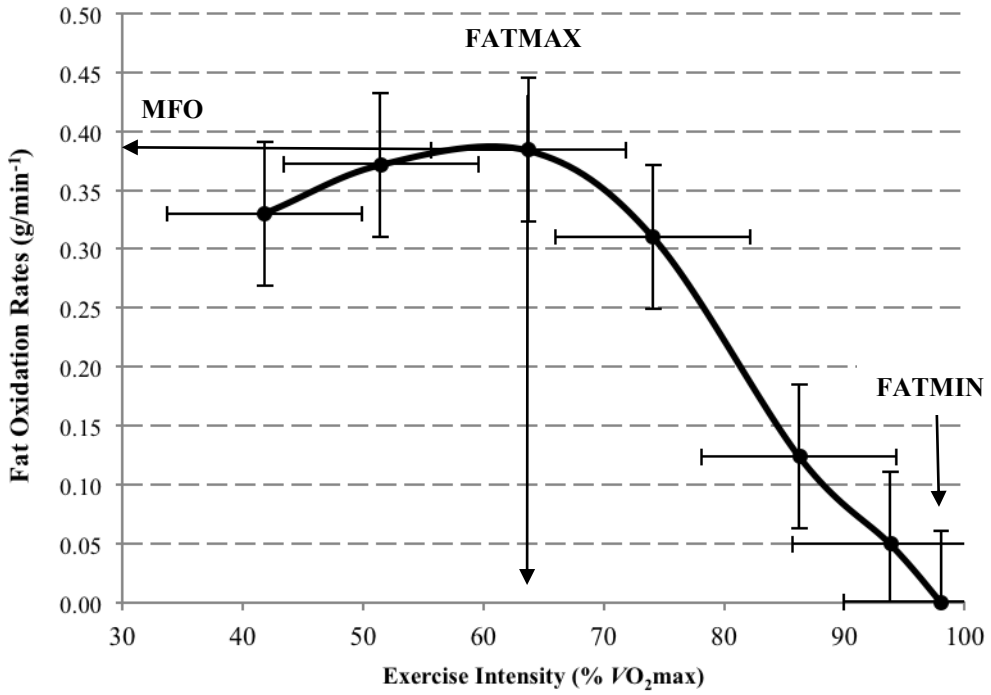
Studies in the 1960s demonstrated the crucial role of glycogen for endurance capacity (9, 10, 32). Unlike endogenous fat stores, endogenous glycogen stores are limited and can be depleted in the first 30-90 min of intense exercise (32). Therefore research suggested that any intervention to reduce glycogen breakdown (sparing glycogen) may potentially increase endurance capacity (34). More specifically this led to the theory that any method to increase fat oxidation could spare glycogen use and in turn enhance endurance performance. Odland et al (68) infused intralipid and heparin during exercise to increase plasma FA concentration and found an increase in fat oxidation and a concurrent decrease in CHO oxidation. However these increases in fat oxidation were relatively small (68). Several nutritional manipulations including high fat feeding, starvation and the use of various dietary constituents are alternative ways to alter fat oxidation. However, in all these studies comparisons were made during exercise pre- and post intervention at one exercise intensity. Thus day to day variation in substrate metabolism, which has been found to vary from 15 – 25% (109), cannot be accounted for. It is possible that changes may only occur at lower or higher intensities and therefore it was essential to develop methods that measured fat oxidation over a wide range of intensities.

1.5 Validation of the FATMAX test

In 2002 Achten et al (2) developed the FATMAX test to accurately establish the relationship between exercise intensity and fuel selection on an individual level. This FATMAX test, performed either on a cycle ergometer or treadmill, increases the work rate in relatively small incremental steps. During each stage of the test breath by breath samples are collected and using indirect calorimetry (44) whole body rates of fat and CHO oxidation rates are estimated. The estimated rates of fat oxidation are then used to construct a fat oxidation curve (See Figure 1.2 for an example of a typical fat oxidation curve). To describe fat oxidation over a wide range of intensities several parameters relating to fat metabolism can be determined including maximal fat oxidation (MFO) rates, the exercise intensity at which MFO occurred (FATMAX; expressed as a percentage of $\dot{V}O_2$max) and the exercise intensity where fat oxidation rates are negligible/ can no longer be estimated (FATMIN; RER > 1.0) (2) (Figure 1.2). The determination of fat oxidation rates at high exercise intensities cannot be estimated due to increases in bircarbonate production, from an increase in glycolytic flux, resulting in excess non-oxidative CO_2. As CO_2 production is used to calculate fat oxidation rates (using indirect calorimetery), the determination of fat oxidation rates at high exercise intensities is flawed (44).

Achten et al (2) first developed the FATMAX test in 18 healthy males. Using a cycle ergometer the test started at 95 *W,* every 5 min the work rate increased by 35 *W* until an RER of 1 was reached (indicating negligible fat oxidation rates), hereafter the workload was increased every 2 min until exhaustion. In these 18 subjects the average MFO rate was 0.60 ± 0.07 $g \cdot min^{-1}$ occurring at 64 ± 4% $\dot{V}O_2$max (2). Although maximal rates of fat oxidation were determined, the authors also acknowledged that MFO and FATMAX may be influenced by

Figure 1.2. Typical fat oxidation curve



A typical fat oxidation curve illustrating the change in fat oxidation rates (g/min^{-1}) with increases in exercise intensity (expressed as % $_2$max). MFO; maximal fat oxidation rates. FATMAX; exercise intensity at which maximal fat oxidation rates occur. FATMIN; the exercise intensity at which fat oxidation rates are zero.

the exercise performed in the previous stages of the test. To validate the test, the same subjects performed steady state exercise trials (lasting between 80 to 35 min = 2.8 MJ) at exercise intensities corresponding to the stages of FATMAX test where RER was < 1 (between 95 to 270 *W*). During these exercise trials substrate metabolism was measured every 5 min. On average, maximal fat oxidation rates during the steady state exercise bouts were not statistically different from the stages of graded exercise tests (2). Furthermore, the authors found no statistical difference in FATMAX between the graded and steady state exercise trials (2). Pérez-Martin et al (72) conducted a similar study in which 10 healthy males performed an initial incremental exercise test consisting of four workloads (30, 40, 50, 60% *W*max), calculated from predicted *W*max. The same subjects then completed four, six min, constant workload exercise trials corresponding to the exercise intensities of each stage of the incremental test. In accordance with Achten et al (2), Perez-Martin found no differences in ventilatory response or fat oxidation in each stage of the incremental test when compared to the steady state exercise trials (72). These findings suggest that substrate metabolism, measured in the latter stages of the FATMAX test, is not influenced by the early intensities. Therefore the FATMAX test is an accurate tool to measure MFO rates and FATMAX.

Achten et al (2) wanted to develop the test protocol so that it was as practical as possible, without reducing validity. Therefore seven subjects, on separate occasions, completed three variations of the FATMAX tests differing in duration of stage and increment of work load. The protocols were as follows 1) 35 *W* increments every 5 min until the RER reached 1.0, after which the work rate was increased by 35 *W* every 2 min until exhaustion 2) 35 *W* increments every 3 min until exhaustion and 3) 20 *W* increments every 3 min until exhaustion. All tests started at 95 *W*. All three test protocols elicited maximal oxygen uptake ($\dot{V}O_2$max) and maximal heart rate (HRmax). Stage duration and workload increment did not affect

FATMAX (61, 59 and 65% $\dot{V}O_2$max for the three test protocol described above). Therefore the authors concluded that 35 *W* increments every 3 min until exhaustion was the most practical test protocol, as it reduces the total test time, and allows for valid assessment of FATMAX (2).

The reliability of the FATMAX test is also of importance if this test is to be used to track changes in MFO and FATMAX over time. In a follow up study by Achten et al (4) 10 trained males ($\dot{V}O_2$max 60.1 ± 0.3 ml·kg·min^{-1}) completed a FATMAX test on three separate occasions. All tests were completed following a standardized diet and at the same time of day to avoid circadian variance. The FATMAX test protocol was performed on a cycle ergometer and was identical to that described above (35 *W* increments every 3 min). The average coefficient of variation (CV) was 3.7%, 4.5%, 3.2%, 9.6% and 9.4% for $\dot{V}O_2$, $\dot{V}CO_2$, RER, FATMAX and FATMIN respectively. However, the 9.6% variation in FATMAX equated to a difference of ~10 beats per min (BPM), when expressed as percentage of maximal heart rate. Similarly, Pérez-Martin et al (72) found a CV of ~11.4% in FATMAX when an incremental exercise test (using specific exercise intensities) was repeated twice. Therefore, detecting small changes in FATMAX (if used as an outcome variable in an intervention study) may be difficult due to the variation in the measurement.

Meyer et al (61) in 2009 recognised the need for more reliability studies on the FATMAX test protocol. In this study subjects performed a baseline FATMAX test during which the protocol was modified depending on body weight, gender and training history (initial stage 50 or 100 *W* with either 25 or 50 *W* increments). Subjects then completed two further individualised graded exercise tests, based on the results from initial FATMAX, consisting of five incremental stages lasting six min. On average the authors found no difference in the stage at which maximal rates of fat oxidation occurred between the two exercise trials (60). However,

large intraindiviual variation in FATMAX was found which may be partly explained by the lack of control on standardising physical activity and diet prior to the exercise trials (60). Both of these factors can have a significant impact on substrate metabolism (3)

There are other factors to take into consideration when using the FATMAX test protocol to establish variables of fat oxidation. The test protocol was originally developed in relatively well-trained individuals (2). It could be argued that the 35 *W* increments may be too large resulting in missed MFO rates and FATMAX when testing other populations (i.e sedentary or children). Thus, it is vital that the FATMAX test, when performed on the cycle ergometer, is modified and validated before use in other populations (110). Furthermore, performing a FATMAX test on a cycle ergometer may not be the suitable for all individuals. Cycling economy is a measure of oxygen consumed per unit of work (63). In some individuals the amount of oxygen consumed is higher than what is needed to perform the work. This is common in individuals who are unaccustomed to cycling and as a result cycling economy is low. Since fat oxidation rates are calculated using oxygen consumption this can result in an overestimation of fat oxidation rates. In these circumstances it may be beneficial to perform the FATMAX test on a treadmill.

A treadmill FATMAX protocol was also developed by Achten et al (5). On two separate occasions 12 healthy males completed the incremental FATMAX exercise test on a cycle ergometer and a treadmill (5). Using the same principle, of incremental increases in work rate, the treadmill protocol started with subjects walking at 7.5 $km \cdot h^{-1}$ at a 1% gradient. The gradient of the treadmill was increased with 2% every 3 min until an RER of 1.0 was reached. The subjects then started running at 10 $km \cdot h^{-1}$ at 10% and speed was further increased by 2 $km \cdot h^{-1}$ every 3 min until exhaustion. FATMAX occurred at ~62% $\dot{V}O_2$max when the test was performed on the cycle ergometer and ~59% $\dot{V}O_2$max on the treadmill; there were no

significant differences between the two protocols. However, MFO were significantly higher in treadmill test compared to the cycle test (0.65 ± 0.05 and 0.47 ± 0.05 g·min^{-1} respectively), this difference was explained by more muscle fibre recruitment during running compared to cycling (5).

In conclusion it appears that the FATMAX test is an accurate and valid means to measure FATMAX and MFO. The variation in FATMAX is ~10% (when the test is repeated) however there is a lack of reliability studies available. Finally, when measuring FATMAX and MFO the test protocol should be specific and valid to the population being tested.

1.6 Determinates of Fat Oxidation rates

Since the development of the FATMAX test it is has been adopted in many research studies in order to establish what may predict MFO and FATMAX (3-5, 67, 90, 98). Achten et al (4) recruited 55 endurance trained individuals (average $\dot{V}O_2$max ~65 ml·kg^{-1}·min^{-1}) who underwent a FATMAX test after an overnight fast. The average MFO in this cohort of individuals was 0.52 g·min^{-1} reached at an average of ~70% $\dot{V}O_2$max (4). Despite the subjects being classed as endurance trained the authors observed a wide spread of $\dot{V}O_2$max values. Therefore further analyses of these results were completed to establish if fitness level (according to $\dot{V}O_2$max) altered MFO or FATMAX. Subjects were separated into two groups; those who had a $\dot{V}O_2$max above and below the mean. The authors found no difference in the exercise intensity at which maximal rates of fat oxidation occurred (FATMAX equalled ~ 63% $\dot{V}O_2$max in both groups) (4). However, the MFO rates were significantly higher in the high $\dot{V}O_2$max group when compared to the low $\dot{V}O_2$max group (0.56 and 0.48 g·min^{-1}

respectively) (4). Additionally the authors found a positive correlation (r=0.64) between MFO rates and $\dot{V}O_2$max (4). However only a small subgroup of subjects (moderately to highly trained athletes) were used in this study meaning that the results could not be extrapolated to other groups of individuals. Using a similar study design Nordby et al (67) took a group of 16 individuals who all completed a FATMAX test. However in this study subjects were either classified as untrained or trained (average $\dot{V}O_2$max ~47 and 57 ml·kg^{-1}·min^{-1} in the untrained and trained group respectively). Once more it was found that on average the trained individuals had higher rates of MFO compared to their untrained counterparts. In addition, the exercise intensity at which peak rates occurred was also significantly higher in the trained group compared to the untrained (67). Although this is in contrast to the findings by Achten (4) it could be explained by the larger difference in $\dot{V}O_2$max between the two groups. However, another study found no difference in MFO rates and FATMAX in eight endurance trained and nine untrained females (90). The low sample size in this study may explain these findings.

In order to elucidate what predicts fat oxidation rates a large scale study was completed in 2005 (98). In this cross-sectional study 300 volunteers, differing in training status, age, weight, body mass index (BMI) and gender underwent a FATMAX test (at least 4 h fasted). The authors found that on average the maximal rate of fat oxidation was 0.46 ± 0.01 g·min^{-1} occurring at 48 ± 1% $\dot{V}O_2$max (98). Interestingly, what the authors also observed was the large inter individual variation in both of these variables. Peak oxidation rates in these 300 volunteers ranged from 0.18-1.01 g·min^{-1} occurring at exercise intensities as low 22% $\dot{V}O_2$max up to 77% $\dot{V}O_2$max (98). Correlation analysis of these results showed that fat free mass, self-reported physical activity, $\dot{V}O_2$max, gender and fat mass were all significant predictors of peak fat oxidation rates accounting for 34% of the variance (98). However the

authors could not elucidate what accounted for the remaining 66% variance in maximal fat oxidation rates.

Wade et al (103) found lower RER (indicative of higher fat oxidation rates), during steady state exercise, in individuals who had a higher proportion of oxidative slow twitch muscle fibres (slow twitch are characterised as having high mitochondria content and capillary network). Additionally habitual diet may play a role in determining fat oxidation rates. A low CHO diet, consumed for five days, has been found to significantly increase rates of fat oxidation compared to a one day high fat diet (89). Thus, individuals who habitually consume a low CHO and/or high fat diet may display higher rates of fat oxidation. However to date it is still unknown what the main predictors of maximal fat oxidation rates are.

1.7 Fat Oxidation in Young Children and Adolescence

An adaptation of the FATMAX test protocol has also been used to measure FATMAX and MFO in young adolescent boys and girls. The FATMAX test protocol described above (see section 1.4 Exercise and Fat Oxidation) was developed in adults therefore Zakrzewski et al (110) set out to validate an exercise test protocol suitable for children. In this study 26 children aged 8 -10 *y* completed an incremental exercise test on a cycle ergometer starting at 0 *W* and increasing by 6-8 *W* every 3 min until a respiratory exchange ratio (RER) of 0.95 was reached. Furthermore, on two separate occasions the same children performed six 10 min exercise bouts at intensities corresponding to the stages in the incremental test. On average MFO rates were similar in the incremental and constant exercise bouts (110). Additionally the type of exercise test (incremental vs. constant) did not influence the exercise intensity at

which MFO occurred (~55% $\dot{V}O_2$peak for both test protocols). Therefore the authors concluded that an incremental exercise test is valid for measuring fat oxidation variables in children.

Using this incremental test protocol Tolfrey et al (93) performed FATMAX tests on a group of 19 children (8 boys and 11 girls) with an average age of 14. On average peak fat oxidation rates were 0.50 $g \cdot LBM^{-1} \cdot min^{-1}$ which occurred at 35% $\dot{V}O_2$peak (93). The authors found that the boys displayed higher MFO rates than girls (93). However, the boys had higher $\dot{V}O_2$peak than the girls and as mentioned above this may predict fat oxidation rates to some extent. The results from this study and others also show that there is inter individual differences in MFO and FATMAX in young children similar to what is observed in adults (93, 111).

The studies described in this section have used healthy, but not exercise trained, children. In adults, exercise training is an effective intervention in increasing absolute and relative fat oxidation rates (73). As previously mentioned highly trained endurance athletes have higher MFO than their less trained counterparts (4). Therefore there is a gap in the literature comparing fat oxidation rates in exercise trained children and adolescents.

A few factors have been suggested which may predict fat oxidation rates in young children. A cross sectional study found FATMAX and MFO lower in obese than in non-obese pubertal boys (112). Pubertal status may also influence rates of fat oxidation in young and adolescent children. A cross sectional study investigated the influence of puberty on fat oxidation (86). Stephens et al (86) found FATMAX occurred at a higher exercise intensity (40% $\dot{V}O_2$peak) in early and mid-pubertal boys compared to late pubertal boys (30% $\dot{V}O_2$peak). Furthermore, fat oxidation rates at the same relative exercise intensity, were found to be higher in pre-pubertal

boys than pubertal boys (86). Although these findings provide an insight into pubertal status on substrate utilisation it is limited in the fact that it is a cross-sectional study.

In 2008 Riddell et al (77) completed a longitudinal study which measured fat oxidation using a graded exercise test in a cohort of pre-pubertal healthy boys (aged 11 – 12 *y*). These young boys were tested annually, as they developed through puberty. The results obtained during each annual test were compared in addition to a group of healthy male adult subjects (aged 22 – 26 *y*). Riddell et al observed peak oxidation rates in pre-pubertal boys to be 2-fold greater when compared to the male adults (77). Furthermore, as the boys progressed through puberty there was a significant decrease in peak fat oxidation rates, despite no change in aerobic capacity (77). It may be that changes in gender hormones during puberty could explain the change in substrate metabolism. Thus, similar to the adult population, it appears that the main determinants of MFO and FATMAX are still yet to be determined in adolescent individuals.

1.8 Exercise training and Fat Oxidation

In the long term endurance training can promote skeletal muscle adaptations favourable for fat metabolism. In 1967 it was first observed that endurance exercise training promoted skeletal muscle adaptations (34). Rats that underwent treadmill running 5 day/ week increased skeletal muscle mitochondrial content which subsequently increased exercise capacity (34). A few years later it was discovered that the enzymatic activity of mitochondrial enzymes, involved in fat metabolism (CPT1, palmityl CoA dehydrogenase, and palmityl CoA synthetase), were upregulated following the same exercise protocol in rats (62).

Endurance training has the same effect on human skeletal muscle (48). Following thirty-one days of endurance exercise training (2 h cycle 5-6 times/ week) muscle oxidative capacity,

estimated from succinate dehydrogenase activity, was found to increase by 41% (73). In fact as little as five endurance training sessions has been found to alter skeletal muscle metabolism, evidenced by reductions in lactate production and glycogen depletion (73). Endurance training is also associated with an upregulation of fatty acid transporter proteins (35, 49). Thus, endurance exercise training *per se* is an effective means of increasing fat oxidation. However, individuals are always striving to achieve additional gains in metabolism above that of exercise training alone.

1.9 Increasing Fat Oxidation: Nutritional interventions

There is an abundance of the nutritional foods, beverages and supplements (often known as 'fat burners') that claim to improve health, increase fat metabolism at rest and during exercise and promote weight loss (43). The reasons for their popularity is that they can easily be incorporated into habitual diet, little effort is required and they are often marketed using too good to be true before and after photographs. Some examples of 'fat burners' include L-carnitine, fucoxanthin and green tea and are often sold on their own or as a combination in the hope to produce additive effects (43). Although these supplements often have a proposed mechanism on how they may upregulate fat metabolism, the scientific evidence for the efficacy and practicality is often lacking.

L-carnitine (carnitine) has received a lot of interest over the recent years. Carnitine is involved in the shuttling of FAs across the mitochondrial membrane (see section 1.1 Regulation of Fat Metabolism for more detail). Therefore it was assumed that carnitine supplementation may increase muscle carnitine stores, increasing the uptake of FAs into the mitochondria for oxidation. This theory was originally flawed when many studies found that carnitine supplementation alone did not increase muscle carnitine content (8, 101, 102). However in

2006 Stephens et al (87, 88) were able to increase muscle carnitine content by simultaneously increasing plasma insulin levels. Since this discovery, 24 weeks of carnitine supplementation, alongside consuming 80 g of CHO (to increase plasma insulin), was found to reduce muscle glycogen breakdown by 50% during exercise (indicating higher rates of fat utilisation) (104). Therefore, it appears possible that carnitine supplementation (in addition to consuming large quantities of CHO) may be effective in increasing fat metabolism, however this is a relatively new area and research and there are currently limited studies. Furthermore the practicality of supplementing with carnitine could be questioned as it requires several months of ingestion for muscle carnitine concentrations to increase and consuming a relatively large dose of CHO may not be practical for all athletes, especially those who need to make weight.

Fucoxanthin has also been associated with having fat burning properties. A carotenoid found in brown seed, fucoxanthin has been found to significantly reduce adipose tissue of mice fed 0.4% body mass after a 4 week period (57). The authors of this study proposed that the fat loss was due to increases in metabolism. However, when applying this to humans this would result in an impractical amount of fucoxanthin consumed daily (for an 80 kg man this would equate to 320 g/ day). A recent study, conducted in overweight females, found that just 2.4 mg of fucoxanthine consumed daily for 16 weeks resulted in significant weight loss (1). The weight loss observed in the fucoxanthine supplemented women was accompanied with increases in resting energy expenditure (EE). In this study fat metabolism was not measured therefore it cannot be established if the change in EE was due to increases in fat oxidation. To date there is limited data on fucoxanthine and fat oxidation and no study has investigated the effects under exercise conditions. Therefore if it unknown if fucoxanthine ingestion would be of benefit to an athletic population.

Green tea and/ or green tea extract (GTE) is one nutritional intervention with promising metabolism enhancing effects at rest and during exercise. The potential role of green tea/GTE ingestion on metabolism will be discussed in the more details in the following sections.

1.10 Green Tea

Tea originates from the leaves of *Camellia Sinensis L* a species of the Theaceae family. The leaves are processed as green, oolong and black tea, differing in composition due to differences in the fermentation process. Green tea is processed from non-oxidised/ non-fermented leaves, therefore it contains high quantities of catechin polyphenols which are absent in black tea. The most abundant of the catechin polyphenols are epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate (EGCG), the latter being the most abundant and pharmacologically active. Caffeine is present in all teas regardless of the fermentation process.

1.11 Green Tea: Fat Oxidation at rest (Acute)

In recent years tea has received a growing interest in the literature partly because of its potential ability to stimulate fat oxidation. In a short term human study, encapsulated green tea extract (GTE) plus caffeine (120 mg GTE/50 mg caffeine), caffeine (50 mg) or placebo were consumed three times a day on three separate occasions (23). Relative to placebo, consumption of GTE/caffeine significantly increased 24 h fat oxidation (RER 0.88 and 0.85 for placebo and GTE respectively). Interestingly the increase in 24 h fat oxidation, seen following ingestion of GTE/caffeine, exceeded that which was observed when subjects received caffeine alone (20% higher) (23). Similar observation have been found when oolong tea (EGCG + caffeine, 244 and 270 mg/d respectively) was consumed three days prior to a 24

h calorimetry measurement (12% increase in fat oxidation) (80). However in this study the authors found no difference when a lower dose (~122 mg/d EGCG, 135 mg/d caffeine) was consumed (80). Similarly, Gregersen et al (30) administered capsules containing either EGCG or a mixture of all catechins(enriched with caffeine (25 mg/ capsule)) in addition to a caffeine and placebo control. In this study the authors measured fat oxidation rates during a 13 h period in a respiratory chamber. Irrelevant of the nutritional intervention no change in fat metabolism was found compared to placebo (30). However, this finding might be explained by the feeding protocol employed. Subjects received low doses of catechins (40-101 mg/capsule EGCG) intermittently throughout the day. Suggesting that ingestion of ≤ 100 mg EGCG (in one serving) may be beneath the threshold to elicit the substrate enhancing effect seen in other studies. In addition, no dose-dependent relationship exists between the amount of catechins consumed and the degree of change in fat metabolism. Berube-Parent et al (11) found no change in 24 h fat oxidation when subjects ingested GTE differing in EGCG content (270-1200 mg plus 600 mg of caffeine) However, the authors speculated that the relatively high caffeine content (600 mg) may have masked the effects of GTE on enhancing fat oxidation. However, these studies seem to suggest that a moderate amount of catechins (EGCG = 244-270 mg/d) is required in order to augment fat oxidation rates at rest.

The aforementioned studies investigate the effects of GTE ingestion on resting metabolism in healthy normal weight adults. A recent study by Thielecke et al (92) investigated the potential substrate enhancing effects of GTE in overweight/ obese volunteers. In a cross over, placebo controlled study ten overweight/ obese adults consumed a moderate dose of EGCG (300 mg/ d), a high dose of EGCG (600 mg/ d), caffeine (moderate dose of 200 mg/ d) and a EGCG/caffeine mixture (300 and 200 mg/ d respectively) for three days. On the third day following an overnight fast substrate metabolism was measured over a 4 h period. Under

fasting conditions the moderate and high dose of EGCG did not alter fat metabolism (92). However, ingestion of EGCG/caffeine increased fat metabolism by 35% compared to placebo (92). Furthermore, fat oxidation was significantly increased in all GTE trials in a postprandial state when compared to placebo. These findings suggest that GTE may have different effects in different populations.

Many studies have investigated the effects of acute GTE/caffeine feeding on resting fat oxidation rates (Table 1.1). Therefore, Hursel et al (36) recently conducted a meta-analysis to determine the size of the effect. Of the six studies included in this meta-analysis the authors found that consumption of GTE/caffeine to increase fat oxidation rates by 16%. Although it appears that GTE consumption does have the potential to increase fat oxidation at rest. This meta-analysis was conducted on a small and select number of studies therefore the results should be interpreted with caution.

1.12 Green Tea: Fat Oxidation during exercise (Acute)

Venables et al (99) is the only study which has investigated the effects of acute GTE ingestion (24 h before plus an additional dose one hour before exercise ~366 mg/d EGCG) on substrate metabolism during moderate intensity exercise in humans. The authors found fat oxidation, during a 30 min cycling at 60% $\dot{V}O_2$max, were significantly higher (17%) following GTE ingestion compared to placebo (99). Although this provides promising data on the effects of acute GTE ingestion on increasing fat oxidation rates during exercise the literature is limited to this one study.

1.13 Green Tea: Mechanisms (Acute)

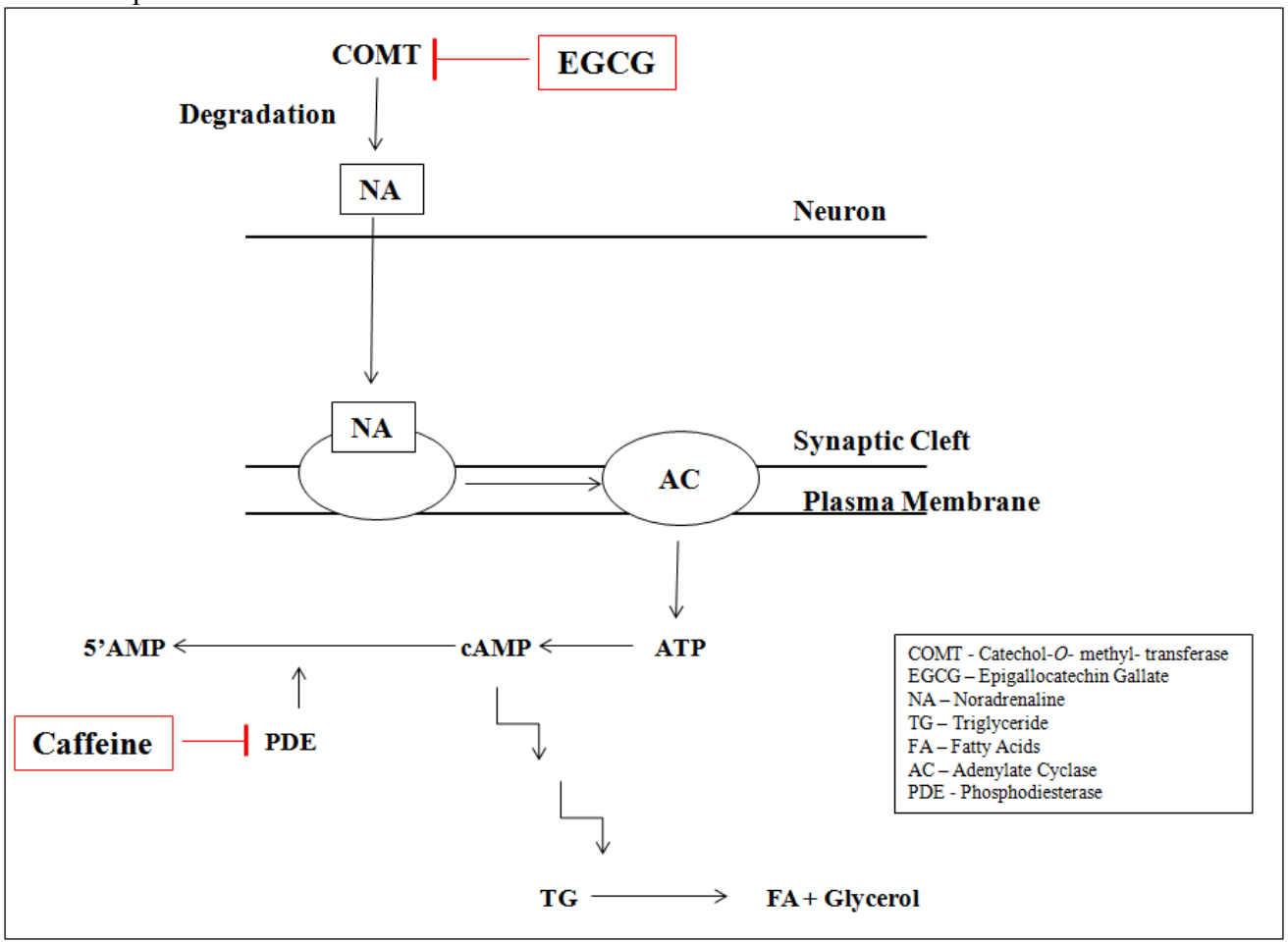
Catechol-*O*- methyl- transferase (COMT) is a membrane bound and soluble enzyme found ubiquitously in all cells of the human body. COMT is involved in the *O*-methylation of free noradrenaline (NA) causing degradation to NA metabolites (58, 70). Catechins, more specifically EGCG, are thought to directly inhibit COMT (15). It is suggested that this acute increase in sympathetic nervous system stimulation results in elevated activation and concentration of NA. As NA is indirectly involved in lipolysis, it is believed that FA mobilization may be upregulated increasing the availability for oxidation. This is the most referenced mechanism explaining GTE potential effect on upregulating fat oxidation albeit no convincing evidence. An *in vitro* study by Borchardt et al is the most often cited study to support the theory (15). However this study did not find a direct inhibitory effect of EGCG on COMT.

Plasma concentration of NE is a good indicator of sympathetic nervous activity. However, the majority (16, 22, 39, 92, 99) of the studies investigating the role of GTE on fat oxidation have not measure plasma NE. Dulloo et al found 24 h urinary NA to be significantly higher during an acute GTE trial (24 h ingestion) compared to placebo and caffeine (23). However, others have found no difference in urinary NA concentration following consumption of GTE or EGCG (79, 80).

Caffeine ingestion is also associated with the upregulation of fat metabolism. Caffeine is known to reduce degradation of cyclic AMP (cAMP), by inhibition of phophodiesterase, and increase NA release through the antagonism of adenosine receptors. It is believed that the increase in cAMP, which is an important intracellular mediator for lipolysis, and NA will result in higher rates of IMTG breakdown and in turn increase FA availability for oxidation.

Taken together, these two theories provide a rationale for the synergist effect of GTE and caffeine on increasing fat oxidation (Figure 1.3). However there is no human study to support this theory.

Figure 1.3 Schematic of the potential acute GTE mechanism



Schematic of the potential synergistic effect of acute GTE and caffeine ingestion on upregulating fat metabolism.

Table 1.1 Human studies investigating the effects of green tea ingestion on resting fat oxidation

Authors	Total Catechins/ day (mg)	EGCG/ day (mg)	Caffeine/ day (mg)	Ingestion period	Fat oxidation rates
Rumpler et al. (80)	662	244	270	3 days	↑ 12% *
Rumpler et al. (80)	331	122	135	3 days	↔
Dulloo et al. (23)	375	270	150	24 h	↑ 35% *
Rudelle et al. (79)	540	282	300	3 days	↔
Berube-Parent et al. (11)	EGCG only	270	600	24 h	↔
Berube-Parent et al. (11)	EGCG only	600	600	24 h	↔
Berube-Parent et al. (11)	EGCG only	900	600	24 h	↔
Berube-Parent et al. (11)	EGCG only	1200	600	24 h	↔
Boschmann et al. (16)	EGCG only	300	~0.3	2 days	↓ in postprandial RQ only *
Gregersen et al. (30)	EGCG only	645	150	13 h	↔
Gregersen et al. (30)	684	EGC only	150	13 h	↔
Gregersen et al. (30)	493.8	242.4	150	13 h	↔
Diepvens et al. (22)	1125	264	225	87 days	↔
Auvichayapat et al. (7)	750	100.7	86.6	12 weeks	↓ in RQ at 8 weeks only †
Westerterp-Plantenga et al. (108)		270	150	3 months	↓ in RQ in low caffeine consumers †

* statistically different from placebo, † statistically different from baseline

1.14 Green tea: Fat Oxidation at rest (Chronic)

The above studies all investigated the acute/short term effects of GTE feeding (24 h – 3 days). However, it is possible that effects may be greater if GTE is consumed over an extended period of time.

Long term GTE ingestion is reported to have positive effects on reducing and maintaining body weight (17, 33, 65, 66, 106). Westerterp-Plantenga et al (108) administered a GTE to subjects during a weight maintenance period of 3 months (270 mg/ d EGCG). Compared to placebo, body weight regain was significantly smaller in the group that received the GTE but only in those who were low habitual caffeine consumers. In contrast, Diepvens et al (22) found that a high dose of GTE (1125 mg/ d catechins) ingested alongside a low energy diet had no effect on any body composition parameters. However when a low dose of 300 mg/ d catechins was consumed alongside a hypocaloric diet substantial weight loss of 14 kg was observed, compared to 5 kg lost in the diet only group (21). In this study (21) catechin intake was low and the authors did not report what proportion of the supplement was EGCG. Therefore, the results of this study should be interpreted with caution.

However, a recent meta-analysis found a favourable effect of catechin ingestion on weight loss and weight maintenance. It was estimated from the results of 11 studies, that subjects in a green tea intervention group lost on average 1.31 kg more weight, over a 12-13 week supplementation period, than a control group. Furthermore, the effect size was larger in populations with a low regular caffeine intake compared to moderate-to-high (mean weight loss -1.63 and -0.27 kg respectively). Interestingly this meta-analysis also highlighted the interaction of ethnicity as a moderator. Studies that used Asian subjects had a larger effect

size than Caucasian (37). Factors such as ethnicity and caffeine consumption should be taken into account when conducting future studies.

Although it appears that GTE ingestion may have favourable effects on maintaining and reducing body mass these findings cannot be entirely attributed to increases in fat metabolism. In the aforementioned study by Westerterp-Plantenga (108) RQ was significantly lowered (indicative of increase fat oxidation) in those subjects who consumed GTE during the weight maintenance period. Furthermore, Auvichayapat et al (7) found the RQ of subjects ingesting a GTE (~130 mg/d) was significantly lower after 8 weeks compared to placebo (0.81 and 0.83 respectively). However, when a second measurement was taken at 12 weeks this effect had diminished by week 12 (7). Diepvens et al (22) also found no change in postprandial RQ when GTE was ingested for 32 days compared to baseline measures. Thus more studies are needed to establish if long term/ chronic GTE ingestion can increase fat metabolism at rest (Table 1.1).

1.15 Green Tea: Fat Oxidation during exercise (Chronic)

Few studies have investigated the effects of chronic green tea ingestion in combination with an exercise intervention. Murase et al (64) subjected mice to a 10 week intervention of dietary GTE ingestion (0.2% and 0.5% GTE) in combination with exercise training. Following the intervention, β-oxidation activity significantly increased in the GTE+exercise group above that of exercise alone (74% and 36% respectively). Furthermore Shimotoyodome et al (83) found that high fat fed mice supplemented with GTE and undergoing exercise training for four weeks showed higher fat utilisation at rest and during exercise.

In humans when a low dose of catechins (70 mg/d EGCG) was ingested over 3 weeks no change in fat oxidation rates were observed compared to a baseline trial (24). However, Ota et al (71) supplemented 14 healthy male subjects with a placebo or GTE beverage (570 mg/ d catechins of which 218 mg was EGCG) three times a week for 2 months. In addition to the prescribed GTE feeding volunteers underwent regular low intensity treadmill exercise (5 km/ h for 30 min 3 times a week). The authors found that following the 2 month period the subjects who had consumed the GTE test beverage had 24% higher fat oxidation rates during exercise than the placebo group (71). A more recent study investigated the effects of 10 weeks GTE consumption in combination with a training regimen in humans (60 min cycle at 60% $\dot{V}O_2$peak 3 days/ week) (39). The GTE group in this study showed lowered respiratory exchange ratio during a 90 min cycle exercise bout from 0.84 to 0.82 pre to post GTE feeding/training (see Table 1.2 for all human studies investigating the effects of GTE ingestion on fat oxidation rates during exercise). Thus, longer term supplementation may be more effective in increasing fat metabolism during exercise. However, both of the studies which found an effect of GTE were performed in healthy untrained individuals. Therefore it is unknown if the same effects would be seen in a physically active population.

Table 1.2 Human studies investigating the effects of green tea on fat oxidation during exercise

Authors	Total Catechins/ day (mg)	EGCG/ day (mg)	Caffeine/ day (mg)	Ingestion period	Fat oxidation rates during exercise
Venables et al. (99)	890	366	-	24 h	↑ 17% *
Eichenberger et al. (24)	169	68	28	3 weeks	↔
Ota et al. (71)	570	175	-	10 weeks (plus exercise training)	↑ 24% *
Ichinose et al. (40)	572	125	77	10 weeks (plus exercise training)	↓ in RQ †

* statistically different from placebo, † statistically different from pre-training

1.16 Green Tea: Mechanism (Chronic)

Regular consumption of GTE may increase fat metabolism as a result of the mechanism described above (See section 1.13). However skeletal muscle and adipose tissue adaptations may also occur if GTE is ingested over an extended period. Lee et al (55) demonstrated that in high-fat diet-induced obese mice, diets supplemented with EGCG resulted in reductions of body weight and mass of adipose tissues at various sites in a dose-dependent manner. These findings were accompanied with changes in adipose proteins involved in fat metabolism. In the epididymal white adipose tissue of EGCG diet-fed mice, the mRNA levels of adipogenic genes such as proliferator-Activated Receptor- γ (PPAR-γ), CCAAT enhancer-binding protein-α (C/EBP-α), regulatory element-binding protein-1c (SREBP-1c), adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL) and fatty acid synthase (FAS) were significantly decreased (55). In addition, the mRNA levels of CPT-1 and uncoupling protein 2 (UCP2), as well as lipolytic genes such as hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), were significantly increased (55). These findings suggest a shift from FA storage to oxidation. This theory was confirmed by Kim et al who found suppression of lipogenic enzymes in hepatic and adipose tissue (50).

Additionally, Chen et al (18) found increased expression of UCP-2 and PPAR- γ in perirenal fat tissue in rats fed EGCG for six months (18). Interestingly there were no changes in enzyme expression of the quadriceps muscle in any of the treatment groups when compared to a control group. A recent study investigated the effects of 16 weeks EGCG ingestion on skeletal muscle adaptation in high fat-fed mice (81). The authors observed increased mRNA levels of mitochondrial enzymes (MCAD, NRF1, UPC3 and PPARα) involved in fat metabolism in the EGCG mice (81). Furthermore body weight gain was lower in the EGCG

mice compared to the high fat fed mice. This suggests that long term ingestion of GTE may have favourable effects on different tissues throughout the body at rest. However no study has investigated this in humans.

Similar results are observed when GTE catechins are ingested alongside exercise training. Murase et al (64) performed a study in which over a 10 week period mice underwent an exercise training program in addition to consuming a diet containing 0, 0.1, 0.2 or 0.5% EGCG. Firstly the authors found that the swimming time to exhaustion was increased in the mice fed 0.5% EGCG compared to an exercise only group. This increase in endurance capacity was accompanied with higher β-oxidation activity in the muscle as well as increased expression of the FA transporter enzyme FAT CD/36. In addition mRNA expression of MCAD, an enzyme involved in mitochondrial β-oxidation, was also increased in the EGCG fed mice. Again this has not been studied in humans however the two available studies which have combined exercise training with GTE ingestion (in humans) have found favourable effects on fat oxidation (40, 71). Although highly speculative, these results suggest that the augmentation of fat metabolism found with chronic GTE ingestion in combination with exercise may due to increased expression of certain fat metabolism enzymes.

1.17 Aims

It appears that GTE ingestion may be effective in increasing fat oxidation under resting conditions. However there is a lack of research investigating the effects of GTE ingestion on fat oxidation during exercise. Only one acute study has been conducted and although long term ingestion appears to increase fat oxidation rates during exercise it is unknown if the same effects would be observed in physically active individuals.

Furthermore the precise mechanism into how GTE may exert its fat metabolism enhancing effects on the human body is highly speculative. Therefore the main aim of my thesis is to extend and contribute to the already existing scientific literature on the effects of GTE on fat oxidation during exercise conditions. Firstly I set out to determine if longer term (7 days) ingestion of a caffeinated GTE beverage could further increase fat metabolism in physically active males during a moderate intensity exercise bout compared to an acute (24 h) dose **(Chapter 3).** Here I hypothesized that 7 days of ingestion would increase fat oxidation rates more than an acute 24 hour supplementation period. In addition, to gain a deeper insight into the possible acute and chronic mechanisms I designed a study which investigated the effects of a single bolus, 7 days and 28 day decaffeinated GTE (dGTE) ingestion on substrate metabolism **(Chapter 4)**. It was hypothesized that ingestion of dGTE, at all time points, will alter fat oxidation during a 30 min steady state exercise bout compared to placebo. Furthermore, I hypothesized that 28 days dGTE ingestion will result in greater alterations of fat oxidation compared to a single bolus and 7 days. In **Chapter 5** I employed the FATMAX test protocol (described above) to study the effects of GTE on substrate metabolism over a wide range of intensities. I hypothesized that ingestion of a dGTE would elicit changes in fat oxidation rates and the exercise intensity at which maximal rates occur, compared to placebo acute. A second aim of this study was to develop and use a new mathematical model to analyse individual fat oxidation curves. Finally, in **Chapter 6,** FATMAX data were collected from athletes differing in age, gender, body composition and sport. The main purpose of **Chapter 6** was to establish MFO and FATMAX from a large heterogeneous sample of athletes, with a focus on team sports. A second aim of **Chapter 6** was to use the new and validated mathematical model (from **Chapter 5**) to determine the fat oxidation profile of each athlete and to use these profiles to classify athletes by metabolic type.

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Chapter 2: GENERAL METHODS

2.1 Respiratory gas analysis

2.1.1 Oxycon Pro

Breath by breath data was collected in **Chapter 3-5** using an Online Gas Analyser (Oxycon Pro, Jaeger, Wuerzburg, Germany). The Oxycon system can be used to measure a variety of cardiopulmonary variables during exercise and at rest. The Oxycon Pro was primarily used in this thesis to measure ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) production during exercise at a range of exercise intensities. Participants breathed through a mouth piece and a nose clip was placed to eliminate any air leaking from the nasal passage. The inspired air is passed through a sensitive volume transducer (Triple V) to determine $\dot{V}E$. Additionally the expired air is passed through a sample tube (twintube) and analysed by paramagmatic and infrared absorption principles to determine concentrations of $\dot{V}O_2$ and $\dot{V}CO_2$.

2.1.1.1 Oxycon Pro Calibration

Prior to using the Oxycon Pro, a three step calibration process was always completed. Flow volume was calibrated using a calibrated 3-L syringe. This calibration process ensured that the mouth piece was set up correctly and that the Triple V was working. The calibration required the mouth piece to be placed onto the end of the syringe. Six smooth, slow pumps of the syringe were then performed, each time the system recorded the volume of each pump. This process was completed twice and the system measured the percent difference between the two. If the percent difference was more than 2% the calibration was repeated.

An automated volume calibration was also completed, this time the mouth piece was inserted onto the system. The system automatically passed a flow through the mouth piece at a low and high volume. This process was completed twice and the percent difference was

calculated. As before, if the percent difference was more than 2% the automatic volume calibration was repeated.

Finally a calibration of the gas analyser was performed. This was an automated calibration procedure where a cylinder containing a mix of known gases (~21.00% Oxygen (O_2) and 0.03% Carbon Dioxide (CO_2)) was passed through the Oxycon and the analyser adjusted the concentration accordingly. This procedure was repeated until there was < 2 % difference in the current and previous data.

2.1.2 Moxus Modular

In **Chapter 6** breath by breath data was collected using the Moxus Modular VO_2 system (AEI technologies, Pittsburgh, USA). The Moxus system can be used to measure a variety of cardiopulmonary variables during exercise and at rest. The Moxus was used in **Chapter 6** to measure ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) production during an incremental exercise test.

In more detail, participants breathed through either a face mask or a mouth piece, if the latter a nose clip was placed to eliminate any air leaking from the nasal passage. The data was acquired on a breath-by-breath basis. The mouth piece/ mask were fitted to a two-way breathing valve. During the exercise tests the inhaled and exhaled air was passed through two separate tubing connectors. Breath-by-breath exhaled gas concentrations were measured (by a mixing chamber) and saved after incorporating an appropriate phasing delay. This delay is necessary because of two factors: 1) the time necessary for the expired air to transverse the volume of the non-rebreathing valve, tubing and mixing chamber and 2) the gas analyser tubing and response delays (O_2 Analyzer Delay and CO_2 Analyzer Delay). Following the end

of each breath the system waits until the participant has exhaled. It then waits a specified number of seconds for each of the analyzer time delays. It then phases the Mixed O_2 and Mixed CO_2 data with the breath that produced that data.

2.1.2.1 Moxus Modular Calibration

Prior to using the system a stringent calibration process was completed. Firstly a gas calibration was performed. Two calibration gas cylinders were used for the gas analyser calibration: typically 21.00% O_2 and 0.03% CO_2 for one cylinder; and typically 16.00% O_2 and 4.00% CO_2 for the other. The system performed the calibration automatically the values displayed for O_2 and CO_2 on the calibration screen should track the values displayed on the individual analyser panel meters. The acceptable tolerance for this correlation is +/- 0.02% for the individual channels. If this is not the case for either channel, the calibration sequence was run again.

Secondly a gas verification calibration was completed. This gas calibration is performed to verify against the calibration gas cylinders. On the calibration screen 'Calibrate Air' was selected. Approximately one min was waited to allow for the analysers to stabilise. On the calibration screen the Mix O_2 Average Data should display the value (± 0.01%) for the high calibration value (~21.00%) and the Mix CO_2 Average Data should display the low calibration value (0.03%). The 'Calibrate Exp' option was then selected. This time, following a one min period, the Mix O_2 Average Data should display the low calibration value (~16.00%) and the Mix CO_2 Average Data should display the high calibration value (~4.00%). Again this value should be within ± 0.01%. The dials on the front of the analyser

panels were adjusted if the values were ±0.01%. If the dials were adjusted the gas cylinder calibration (see above) was repeated.

Finally flow calibration was performed. The two way breathing valve was attached to a 3- L syringe. Seven smooth and consistent pumps of the syringe were performed, after the first two pumps the system recorded the volume. This process was completed twice; an initial calibration followed by verification. The desired average error was defined as ±2%. If the error was greater than ±2% the volume calibration was repeated.

2.2 Calculations

2.2.1 Indirect Calorimetry

Research undertaken in as early as 1920 measured pulmonary gas exchange to calculate energy expenditure at rest and during exercise (7). Since this early work it was established that breath- by-breath measurements could be used to determine the contribution of fat and carbohydrate oxidation to total energy expenditure. Indirect calorimetry is a method to determine substrate oxidation at a whole body level using measurements of O_2 consumption and CO_2 excretion. Carbohydrate (CHO), fat and protein, all differ in chemical structure thus the ratio of O_2 needed relative to CO_2 production is different in order for oxidation to take place. In all chapters of this thesis it was assumed that during exercise the contribution of protein to overall energy expenditure was negligible.

In **Chapter 3-5** fat and CHO oxidation was calculated using indirect calorimetry with the following equations proposed by Jeukendrup and Wallis (6):

$$\textbf{Fat oxidation (g·min}^{-1}\textbf{)} = 1.65 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2$$

$$\textbf{CHO oxidation (g·min}^{-1}\textbf{)} = 4.21 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2$$

In **Chapter 6** fat and CHO was calculated using the following equations (5):

$$\textbf{Fat oxidation (g·min}^{-1}\textbf{)} = 1.718 \cdot \dot{V}O_2 - 1.718 \cdot \dot{V}CO_2$$

$$\textbf{CHO oxidation (g·min}^{-1}\textbf{)} = 4.170 \cdot \dot{V}CO_2 - 2.965 \cdot \dot{V}O_2$$

The variation in fat oxidation rates from using different equations is as small as ~3% (6).

2.2.2 Respiratory Exchange Ratio (RER):

The ratio of CO_2 produced relative to the O_2 consumed at tissue level.

$$\textbf{RER} = \dot{V}CO_2 / \dot{V}O_2$$

RER is an indirect measure of substrate utilisation. To oxidise one mol of glucose 134 L of O_2 are needed and 134 L of CO_2 are produced therefore an RER of 1.0 (134 L/134 L = 1.0) is indicative of predominately CHO oxidation. In order to oxidise one mol of palmitate 515 L of O_2 are needed, producing 358 L of CO_2. Thus an RER of 0.7 (358 L/515 L = 0.7) indicates that fat is the predominant fuel (6).

2.2.3 Maximal power output

*W*max was calculated in **Chapter 3 and 4** in order to set the exercise intensity for subsequent trials (~50% *W*max). The following equation was used to calculate *W*max (8):

$$\boldsymbol{W}\textbf{max} = \boldsymbol{W}\textbf{out} + [(\boldsymbol{t}/180)\cdot 35]$$

Where *W*out is the power output of the last stage completed during the test, and *t* is the time spent, in seconds, in the final stage.

2.2.4 Cycling Economy

In **Chapter 5** cycling economy was calculated for each work rate (*W*atts (*W*)) performed by the all participants. Economy is defined as a measure of oxygen consumption per unit of power output (9):

$$\textbf{Cycling economy} = \dot{V}O_2 / W$$

If economy at a certain workload was lower than the average minus two times the standard deviation then the whole trial was excluded from the final data set.

2.3 FATMAX test protocol

2.3.1 Test protocol: Cycle Ergometer

In **Chapters 3, 4 and 5** all participants completed a FATMAX test (1) during the preliminary trial to establish maximal oxygen uptake ($\dot{V}O_2$max). In more detail, the test protocol involved a 5 min warm up at 75 *W* on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The test started at 95 *W*, every 3 min the effort was increased in incremental steps of 35 *W*, until voluntary exhaustion was reached. During each stage of the test respiratory gas measurements ($\dot{V}O_2$ and $\dot{V}CO_2$) were collected using an Online Gas Analyser (Oxycon Pro, Jaeger, Wuerzburg, Germany). $\dot{V}O_2$ was considered maximal ($\dot{V}O_2$max) and the test was stopped if 2 out of the 4 following criteria were met. 1) if $\dot{V}O_2$ did not increase even when workload increased (< 2 mL· kg^{-1}·min^{-1} increase from the previous stage) 2) a respiratory exchange ratio (RER) of >1.05 3) a heart rate within 10 beats per min of age predicted maximal heart rate 4) a cadence of 50 rpm could not be maintained. Heart rate (HR) was recorded during each stage of the test using a HR monitor (Polar RS800CX, Polar Electro (UK) Ltd, Warwick, United Kingdom). In **Chapters 5** participants did not complete a warm up however the first test stage was set at 60 *W*.

In **Chapter 4** after 15 min of rest participants completed a steady state cycle. This involved participants cycling for 20-min at a pre-determined exercise intensity of 50% *W*max (55% $\dot{V}O_2$max; calculated from the $\dot{V}O_2$max test). To ensure the correct intensity was set (*W*) a 4-min measurement of $\dot{V}O_2$ was obtained, using an Online Gas Analyzer (Oxycon Pro, Jaeger, Wuerzburg, Germany), every 5 min. If the recorded $\dot{V}O_2$ values did not equate to 55% $\dot{V}O_2$max (± >5%) the resistance on the cycle ergometer was adjusted accordingly.

2.3.2 Test protocol: Treadmill

In **Chapter 6** FATMAX tests were completed on a treadmill. A standardised protocol was used for all treadmill FATMAX tests. In more detail, the test started at 5.0 $km \cdot h^{-1}$ and at a gradient of 1% for three min. The speed then increased to 7.5 $km \cdot h^{-1}$. Speed was increased by 1 $km \cdot h^{-1}$ every 3 min until an RER of 1 was reached thereafter the speed remained constant and the gradient was increased by 1% every 1 min until voluntary exhaustion. Respiratory gas measurements ($\dot{V}O_2$ and $\dot{V}CO_2$) were collected continuously using the Moxus Modular $\dot{V}O_2$ system (AEI technologies, Pittsburgh, USA). Furthermore, HR was measured throughout the whole test and rating of perceived exertion (RPE) was recorded during each stage.

In **Chapter 6** $\dot{V}O_2$ was considered maximal and the test was stopped if 2 out of the 4 following criteria were met. 1) if $\dot{V}O_2$ did not increase even when workload (gradient) increased 2) a respiratory exchange ratio (RER) of >1.01 3) a heart rate within 10 beats per min of age predicted maximal heart rate. If 2 out for the 4 criteria were not met (i.e $\dot{V}O_2$max was not reached) the data was not included in the average FATMAX curve.

2.4 Construction of average fat oxidation curve

2.4.1 Manual Analysis

Previous studies have used the FATMAX test protocol, under different conditions, to produce an average fat oxidation curve (1-4, 10). In these studies, in order to construct an average fat oxidation curve, each individual fat oxidation curve was analyzed manually. In more detail, the maximal rate of fat oxidation (MFO) and the intensity at which it occurred was determined for each participant. The authors then calculated fat oxidation rates which were 5,

10 and 20% below the maximal rate. Each individual graph was then used to determine the work rate ($\dot{V}O_2$) at each of these fat oxidation rates. However, this is a time consuming process and as a result the authors only calculated fat oxidation rates 5, 10 and 20% below the maximal rate. Furthermore this method of analysis was subjective and therefore there is a greater chance of human error.

In this thesis **(Chapter 5-6)**, a computer programming software (Matlab) was used for the first time to analyze each participant's fat oxidation curve to created an average curve.

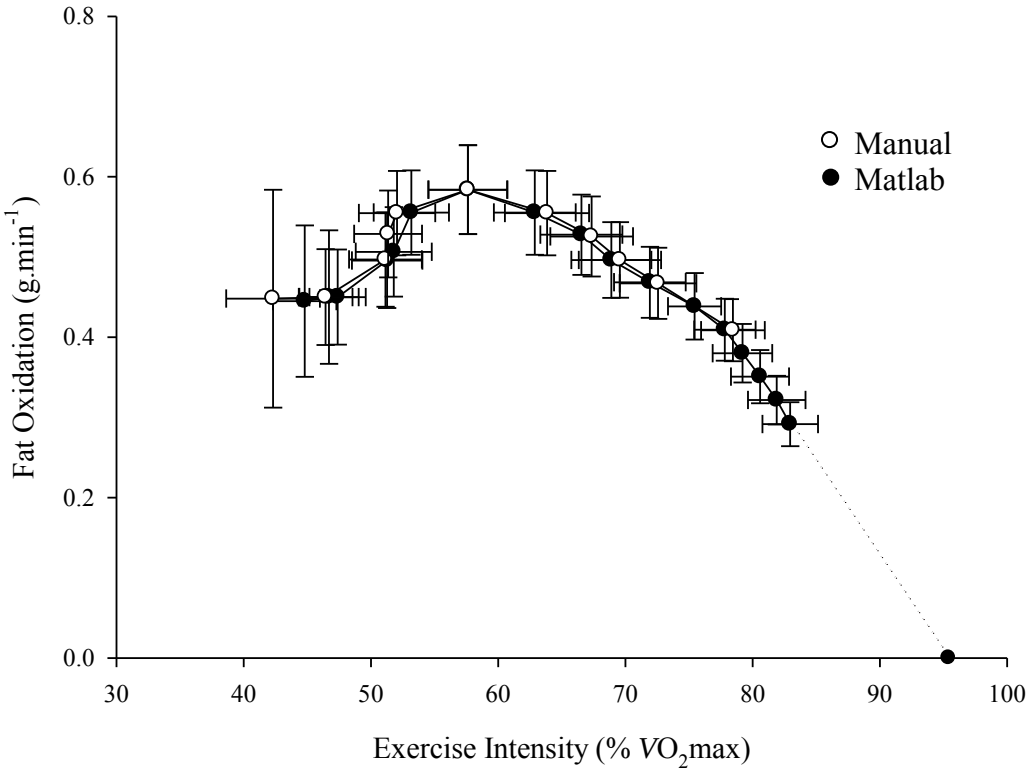
2.4.2 Matlab analysis

MFO for each individual was determined as the highest rate ($g \cdot min^{-1}$) estimated using the stoichiometric equation described above. Using Matlab (MathWorks Matlab 2011a, Natick, Massachusetts, U.S.A) 95% to 50% of MFO was calculated in intervals of 5% (i.e. 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60% 55%, and 50%). To calculate the absolute $\dot{V}O_2$ (mL) associated with these 11 fat oxidation rates, linear interpolation was applied between subsequent intercepts of the measured fat oxidation and $\dot{V}O_2$ (mL) values. Each interpolated link consisted of 1000 hypothetical intercepts of $\dot{V}O_2$ (mL) and fat oxidation. Depending on the $\dot{V}O_2$ at MFO a hypothetical $\dot{V}O_2$ value, for each of the 11 fat oxidation rates intervals, could be determined. These values of absolute $\dot{V}O_2$ were converted to a percentage of each individual's $\dot{V}O_2$max. This interpolation process was then repeated for heart rate and carbohydrate oxidation at the calculated fat oxidation rates.

In order to compare the agreement between the manual analysis and Matlab analysis 13 FATMAX tests were analysed using both techniques. For the manual analysis the maximal rate of fat oxidation and the intensity at which it occurred was determined for each

participant. Fat oxidation rates which were 5, 10, 15, 20 and 30% of the maximal rate were then calculated. Each curve was printed and by hand the $\dot{V}O_2$ (mL) corresponding to the each of the fat oxidation rates was recorded. The Matlab analysis was run automatically using the method described above. There was no difference in fat oxidation rates, at any exercise intensity, when the manual analysis was compared to Matlab (Figure 2.1).

Figure 2.1Fat Oxidation curve: Matlab verses Manual



Average fat oxidation curve constructed using manual and Matlab analysis.

2.5 Biochemical analyses

2.5.1 Plasma metabolites

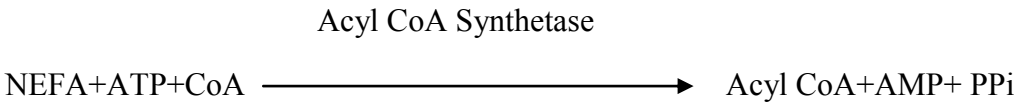
Plasma metabolites were measured using the ILAB 650 (Instrumentation laboratory, UK), unless otherwise stated. The ILAB 650 is an automated blood analyser used commonly in clinical chemistry laboratories to quantify plasma metabolites by spectrophotometry or turbidity. Enzyme catalysed assays are commonly used, and typically absorbance values correlate with the concentration of the blood metabolite in question.

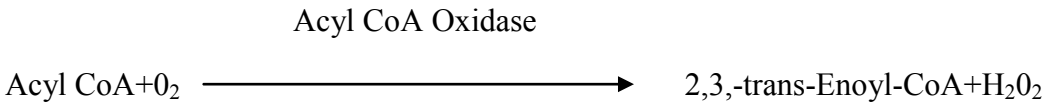
2.5.2 Plasma FA

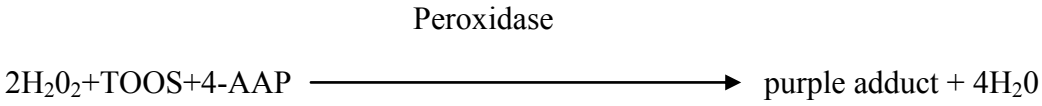
Plasma FA concentrations were analysed using an ILab 650. In **Chapter 3** the human plasma samples obtained were analysed using the NEFA-HR 1and 2 reagent (Wako Diagnostics, Richmond, USA)

The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide, in the presence of peroxidase (POD) permits the oxidative condensation of 3-methy-N-ethyl-N(β-hydroxyethyl)-aniline (MEFA) with 4-aminoantipyrine to form a purple coloured adduct which can be measured colorimetrically at 550 nm.

In **Chapter 4 and 5** the NEFA reagent was supplied by RANDOX. The enzymatic method is described:

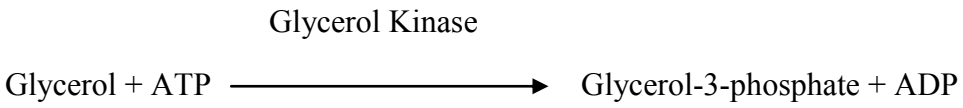


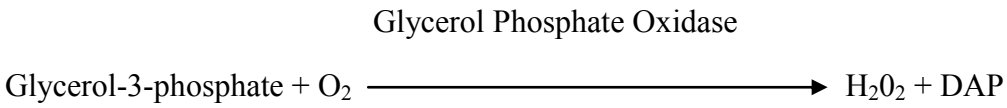


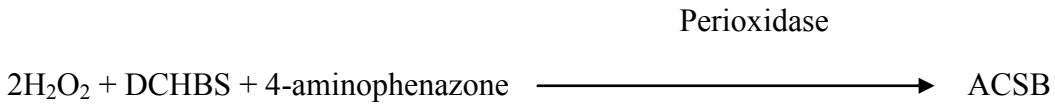


2.5.3 Plasma glycerol

Plasma glycerol was quantified using a colorimetric method on the ILAB 650 using a glycerol reagent (Randox, County Antrium, UK). Three enzymatic reactions took place involving glycerol kinase, glycerol phosphate oxidase and perioxidase. The resultant intensity of n-(4-antipyryl)-3-chloro-5-sulphonate-pbenzoquinoneimine (ACSB: red dye) was measured colorimetrically.







2.5.4 Plasma EGCG

Plasma EGCG was measured on a mass-spectrometer, this analysis was undertaken off site at Unilever, Vlaardingen, The Netherlands.

To measure the concentrations of deconjugated EGCG, EDTA plasma (200 µL), stabilizer solution (20 µL, 10 % ascorbic acid containing 0.1 % EDTA), sodium acetate (20 µL of 1.5 $mol \cdot L^{-1}$ NaOAc, pH 4.8), and β-glucuronidase (10 µL, 50k $U \cdot L^{-1}$ in acetate buffer) were mixed and incubated at 37 °C for 45 min. From the supernatant, 5 µL was injected into the high-performance liquid chromatography multiple-reaction monitoring mass spectrometer (HPLC-MRM-MS) system (Agilent 6410 mass spectrometer equipped with an Agilent 1200SL HPLC (Agilent Technologies, Amstelveen, The Netherlands) and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). Samples were analysed batch-wise and controlled by two quality control samples (QCs) per sample batch. EGCG was quantified in plasma by means of 10-point calibration curves. The peak areas of the internal standards as well as the target compounds were determined using Agilent's MassHunter Quantitative Analysis software (version B.03.02, Agilent Technologies, Santa Clara, CA).

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Chapter 3: NO EFFECT OF 1 OR 7 DAYS GREEN TEA EXTRACT INGESTION ON FAT OXIDATION DURING EXERCISE

Rebecca K. Randell, Adrian B. Hodgson, Silvina B. Lotito, Doris M. Jacobs, Niels Boon, David J. Mela and Asker E. Jeukendrup. No effect of 1 or 7 days Green Tea Extract ingestion on fat oxidation during exercise. *Med Sci Sports Exerc*: 2013;45(5):883-91.

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Chapter 4: EFFECTS OF VARIABLE DURATION GREEN TEA EXTRACT INGESTION ON FAT METABOLISM

Rebecca K. Randell, Adrian B. Hodgson, Silvina B. Lotito, Doris M. Jacobs, Matthew Rowson, David J. Mela, and Asker E. Jeukendrup Med Sci Sports Exerc (in submission).

The work presented in this chapter was funded by Unilever R&D.

Chapter 5: ACUTE DECAFFEINATED GREEN TEA EXTRACT (dGTE) INGESTION ON FAT OXIDATION RATES DURING A GRADED EXERCISE TEST

Rebecca K. Randell, Carlijn Vernooij and Asker E. Jeukendrup

This study did not receive any external funding

5.1 Abstract

Purpose: The aim of this study was to investigate the effects of acute decaffeinated green tea extract (dGTE) ingestion on fat oxidation rates over a range of exercise intensities during a graded exercise test (FATMAX test).

Method: Twelve moderately trained male participants completed four FATMAX tests on a cycle ergometer. In a counterbalanced, cross over, design two trials were performed following acute (24 hour prior to, plus 2 hour before the exercise bout) ingestion of dGTE (1141 ± 21 mg total catechins/day), the other two trials were completed following placebo ingestion. Breath-by-breath samples were collected during each stage of the FATMAX test to calculate rates of whole body fat oxidation. Blood samples were collected at rest and following the exercise bout to determine plasma fatty acids (FAs) and glycerol concentrations.

Results: On average acute ingestion of dGTE did not alter fat oxidation rates at any exercise intensity compared to placebo. In addition, average maximal rates of fat oxidation (MFO) were not statistically different following acute dGTE ingestion, compared to placebo (0.49 ± 0.03 and 0.50 ± 0.03 $g \cdot min^{-1}$, respectively). Plasma concentrations of FAs and glycerol were unchanged in the dGTE trial at rest and at cessation of the exercise bout compared to placebo.

Conclusion: Acute ingestion of dGTE did not result in measureable changes in fat oxidation, over a range of exercise intensities, during a graded exercise type test in physically active males.

5.2 Introduction

Most studies that investigate the effects of a nutritional intervention on exercise metabolism make comparisons at one single exercise intensity (15, 17, 19, 20). It is known, however, that fat oxidation during exercise displays large intra-individual variation with some showing maximal fat oxidation rates at 22% $\dot{V}O_2$max and others at 77% $\dot{V}O_2$max (18). There are also large individual differences in absolute rates of maximal fat oxidation (3, 18) . Therefore it is likely that responses to an intervention are also variable at different exercise intensities.

For these reasons it may be preferred to make comparisons, not at a single intensity, but over a wide range of intensities. This was recognised by Achten (1) and colleagues who developed the FATMAX test. This test, under taken on a cycle ergometer or treadmill, increases the work load (watts or speed) every 3 min. In order to estimate rates of whole body fat and carbohydrate (CHO) oxidation (using indirect calorimetry (10)) during each stage of the test breath-by-breath samples are collected. As a result, individual maximal fat oxidation rates (MFO), the exercise intensity at which MFO occurred (FATMAX: expressed as percentage of $\dot{V}O_2$max) as well as fat oxidation rates above and below FATMAX can be established (1).

The ability to utilise fats and subsequently spare muscle glycogen stores is one determinant for exercise performance (9); therefore, measuring fat oxidation is of interest to athletes. In the present study we wanted to investigate the effects of a green tea extract (GTE) on fat metabolism during exercise. Although in an early study Venables et al (19) observed an increase in fat oxidation with acute GTE ingestion, in **Chapter 3** and **Chapter 4** we were unable to replicate these findings. In these studies, fat oxidation was compared at the same relative submaximal intensity (~55% $\dot{V}O_2$max) with no information about lower or higher

exercise intensities. Therefore in this study we investigated the effects of acute decaffeineated GTE ingestion on fat oxidation during the FATMAX test.

Thus, the primary aim of this study was to determine if acute ingestion (24 hour) of a dGTE can alter fat oxidation rates at any given exercise intensity during a FATMAX test in physically active males. We hypothesized that compared to placebo acute ingestion of a dGTE would elicit changes in fat oxidation rates and the exercise intensity at which this occurs will be determined. A second aim of this study was to develop and use a new mathematical model to analyse individual fat oxidation curves.

5.3 Participants and Methods

Participants

Participants were all physically active males recruited from the student population at the University of Birmingham. All volunteers gave written informed consent to participate in this study and were healthy according to the results of a general health questionnaire. Inclusion criteria included habitual caffeine intake of ≤ 400 mg/day (approximately ≤ 4 cups coffee/ day) and participation in exercise 3-5 times/ week for 30-90 min. All procedures and protocols were approved by the Life and Sciences Ethical Review Committee at the University of Birmingham, UK.

Preliminary Testing

At least 1 week prior to the first experimental trial, all participants reported to the Human Performance Laboratory, at the University of Birmingham, where they were familiarised with the equipment and testing procedures. During this visit all volunteers performed a FATMAX test (adapted from Achten et al (1)) on a cycle ergometer. This test was identical to the test performed during the experimental trials and will be explained in more detail below and in **Chapter 2**.

Following the preliminary trial participants were asked to fill out a weighed three day diet diary, including two week days and one weekend day. Verbal and written instructions were given to the participants and they were also provided with a set of food weighing scales (SALTAR, ARC Electronic Kitchen Scale, Kent, UK) to weigh all foods where appropriate.

General Design

This double blind, cross over, counterbalanced study involved 4 exercise trials (1 trial per week) which were completed over a 4 week period. The exercise trials were all identical and consisted of a 2 hour rest period followed by a FATMAX test. In the 24 hours prior to each exercise trial participants ingested dGTE or placebo. Participants completed two dGTE trials and two placebo trials. The order of the trials assigned to each participants was randomised. Each exercise trial was separated by a 5 day wash out period.

Exercise Trial

Participants arrived at the Human Performance Laboratory at a prearranged time following a 10-12 overnight fast. The exercise trials were always completed on the same day and time to avoid any circadian variation.

On arrival body weight was recorded (Seca Alpha, Hamburg, Germany) and a flexible 20-gauge Teflon catheter (Venflon; Becton Dickinson, Plymouth, United Kingdom) was inserted into an antecubital vein. A 3-way stopcock (Connecta; Becton Dickinson, Plymouth, United Kingdom) was attached to the catheter to allow for repeated blood sampling during the whole trial. An initial 15 mL (5 mL collected in Sodium Fluoride-containing tubes and 10 mL collected into EDTA-containing tubes) blood sample was collected (t=0). Participants then consumed two capsules (of either dGTE or PLA) with at least 200 mL of water and rested for 2 hours in a seated position. Participants were allowed to consume water *ad libitum* throughout the whole trial. During the 2 hour rest period blood samples (15 mL) were obtained every 30 min. The catheter was kept patent by flushing with 4-5 mL isotonic saline (0.9% w·v; B Braun, Sheffield, United Kingdom) after every blood sample. A final resting blood sample was taken at 120 min (t=120). Participants then mounted the cycle erogmeter to undergo a FATMAX test.

The test protocol was adapted from Achten et al (1). In more detail, the test started at 60 *W,* this stage also acted as a warm up. Every 3 min the workload was increased in incremental steps of 35 *W* until voluntary exhaustion was reached. During the last two min of each stage respiratory gas measurements ($\dot{V}O_2$ and $\dot{V}CO_2$) were collected using an Online Gas Analyser (Oxycon Pro, Jaeger, Wuerzburg, Germany). Once RER had reached 1.0 the mouth piece remained in and breath-by-breath measurements were collected continuously until participants reached exhaustion. Heart rate (HR) was recorded during each stage of the test using a HR

monitor (Polar RS800CX, Polar Electro (UK) Ltd, Warwick, United Kingdom) as well as cadence and Rating of Perceived Exertion (RPE).

$\dot{V}O_2$ was considered maximal ($\dot{V}O_2$max) and the test was stopped if 2 out of the 4 following criteria were met. 1) if $\dot{V}O_2$ did not increase even when workload increased 2) a respiratory exchange ratio (RER) of >1.05 3) a heart rate within 10 beats per min of age predicted maximal heart rate 4) a cadence of 50 rpm could not be maintained. *W*max was calculated using the following equation (11):

$$W\text{max} = W\text{out} + [(t/180) \text{ X } 35]$$

Where *W*out is the power output of the last stage completed during the test, and *t* is the time spent, in seconds, in the final stage.

Immediately following cessation of exercise a final 15 mL blood sample was obtained. Participants were then free to leave the laboratory and the exercise trial was repeated the following week.

Diet

Food diaries were analysed using an online food analysis website (12). Average total energy intake and the percent contribution of carbohydrate (CHO), protein and fat over the three day period were calculated. On average participants consumed a total of 2898 ± 569 kilocalories (kcal)/ day consisting of 48% CHO, 18% protein and 34% fat. Short term (5 days) ingestion

of a low carbohydrate diet has been found to increase fat oxidation rates during exercise (6). Therefore we used the food diary data to plan individual 24 hour diets which were similar in composition and total energy intake to each participant's habitual diet.

The diet included three main meals plus snacks. All food was weighed and prepared at the testing facility and was given to the participant to consume in the 24 hours before each exercise trial. Participants were given strict instructions to only eat what they had been provided, they were allowed to self select the quantity of liquid they consumed throughout the day but were asked to refrain from drinking any caffeinated or alcoholic beverages. Exercise was also prohibited in the 24 hours prior to each exercise test.

Nutritional Intervention

In addition to the control diet in the 24 hours before each exercise trial participants consumed four capsules. Participants received the capsules in white (opaque) pots. The pots were labelled with a number (corresponding to the trial which was unknown to the experimenters and participants) and instructions on when to consume the capsules.

The dGTE capsules contained 285 ± 5 mg total catechins/ capsule, of which 157 ± 3 mg was EGCG (1141 ± 21 mg total catechins/day, 627 ± 12 mg EGCG/day). A negligible amount of caffeine was present in the dGTE (~ 3mg/ capsule). The placebo capsules contained cellulose (~310 mg/capsule). All capsules were identical in colour (blue and white) and size (Size 0). Two capsules were consumed an hour before lunch and the additional two capsules were consumed an hour before dinner. Participants were instructed to consume the capsules with at least 200 mL of water. On the morning of each exercise trial, following an initial blood sample, participants ingested two more capsules (~570 mg total catechins) in a fasted state.

Indirect calorimetry and calculations

Breath-by-breath data were collected for two min during each 3 min stage of the FATMAX test. Of the data collected, values for $\dot{V}O_2$ and $\dot{V}CO_2$ in the last 30 sec of each stage were discarded. Thus, the remanding $\dot{V}O_2$ and $\dot{V}CO_2$ values were averaged and used to calculate fat and CHO oxidation using the following stoichiometric equations (10).

$$\text{Fat Oxidation} = 1.65 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2$$

$$\text{CHO Oxidation} = 4.210 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2$$

For each trial the results from the exercise test were used to construct individual fat oxidation curves. Fat oxidation rates, during each stage of the test, were plotted against exercise intensity (expressed as percent of $\dot{V}O_2$max). This graph allowed us to determine a number of variables such as MFO, FATMAX and FATMIN.

MFO = maximal fat oxidation rate ($g \cdot min^{-1}$)

FATMAX = the exercise intensity (expressed as % $\dot{V}O_2$max) at which the highest rate of fat oxidation was observed.

FATMIN = the exercise intensity (expressed as % $\dot{V}O_2$max) where fat oxidation becomes negligible and carbohydrate becomes the predominant fuel source (i.e. RER $\geq$ 1.0).

These data were then used to create an average fat oxidation curve for the dGTE and placebo trials. If participants did not complete 4 stages of the test where RER was < 1 data was excluded from the average curve analysis. In total 2 trials were excluded from the final data set **(Chapter 2)**.

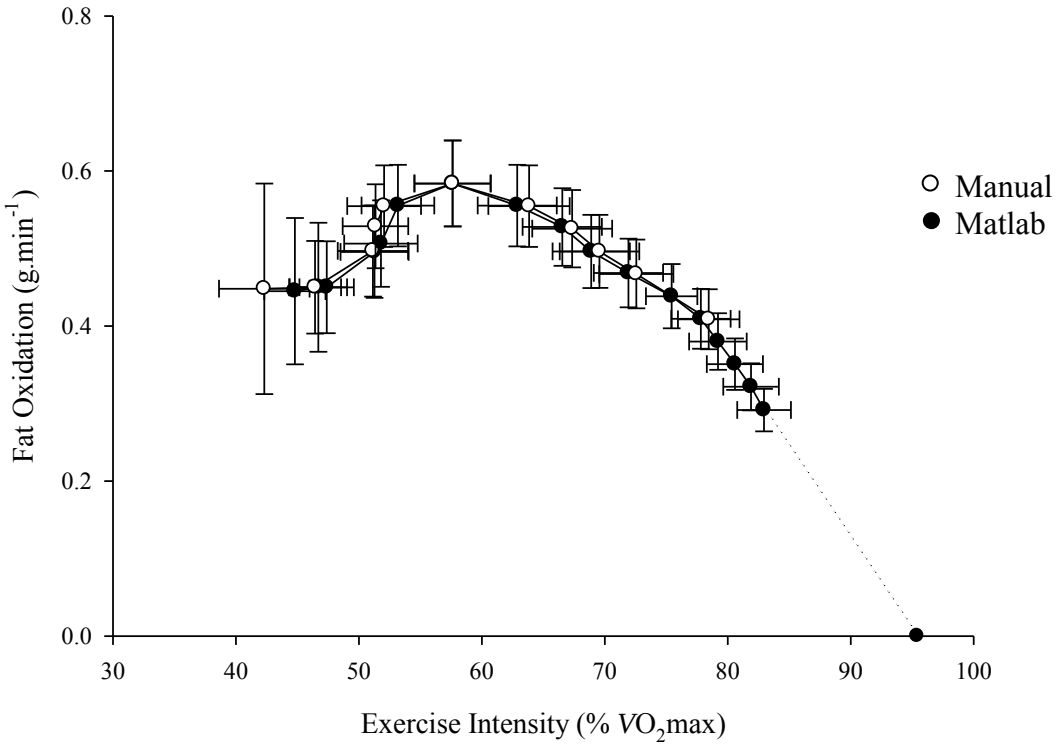
To produce the average fat oxidation curve for the dGTE and placebo trial the data was analysed using Matlab (MathWorks Matlab 2011a, Natick, Massachusetts, U.S.A.). MFO for each individual was determined as the highest rate ($g \cdot min^{-1}$) calculated using the stoichiometric equation described above. For each trial 95% to 50% of the MFO rate was calculated in intervals of 5% (i.e. 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60% 55%, and 50%). To calculate the absolute $\dot{V}O_2$ ($mL \cdot min^{-1}$) associated with these 11 fat oxidation rates, linear interpolation was applied between subsequent intercepts of the measured fat oxidation and $\dot{V}O_2$ ($mL \cdot min^{-1}$) values. Each interpolated link consisted of 1000 hypothetical intercepts of $\dot{V}O_2$ ($mL \cdot min^{-1}$) and fat oxidation. Depending on the $\dot{V}O_2$ at MFO a hypothetical $\dot{V}O_2$ values, for each of the 11 fat oxidation rates intervals, could be determined. These values of absolute $\dot{V}O_2$ were converted to a percentage of each individuals $\dot{V}O_2$max

Previous studies have used the FATMAX test to produce an average fat oxidation curve (1-4, 18). In these studies average fat oxidation curves were constructed manually. In more detail, the maximal rate of fat oxidation and the intensity at which it occurred was determined for each participant. The authors then calculated fat oxidation rates which were 5, 10 and 20% below the maximal rate. Each individual graph was then used to determine the work rate ($\dot{V}O_2$) at each of these fat oxidation rates. However, this is a time consuming process and as a result the authors only calculated fat oxidation rates 5, 10 and 20% below the maximal rate.

Furthermore this method of analysis was subjective and there was a greater chance of human error.

In order to compare the agreement between the manual analysis and Matlab analysis 13 FATMAX tests were analysed using both techniques. For the manual analysis the maximal rate of fat oxidation and the intensity at which it occurred was determined for each participant. Fat oxidation rates which were 5, 10, 15, 20 and 30% of the maximal rate were then calculated. Each curve was printed and by hand the $\dot{V}O_2$ corresponding to the each of the fat oxidation rates was recorded. The Matlab analysis was run automatically using the method described above. We found no difference in fat oxidation rates, at any exercise intensity, when the manual analysis was compared to Matlab (Figure 5.1). Thus the use of this mathematical model is a valid technique for the analysis of fat oxidation curves **(Chapter 2)**.

Figure 5.1 Fat Oxidation curve: Matlab verses Manual



Mean (±SD) absolute fat oxidation rates (g·min^{-1}) plotted against exercise intensity (expressed relation to $\dot{V}O_2$max

Economy is defined as the amount of energy (i.e oxygen) required to perform work at a given workload (13). For all trials cycling economy, at each stage of the exercise test, was calculated (13).

$$\text{Cycling economy } (W/\text{L}) = \text{Work rate } (W) \,/\, \dot{V}O_2 \text{ (mL·min}^{-1}\text{)} \cdot 1000$$

The average economy for each given workload was calculated. If economy at a certain workload was lower than the average minus two times the standard deviation then the whole trial was excluded from the final data. In total four trials were excluded from the final data set.

Blood Variables

All tubes were centrifuged at 3500 rpm for 15 min at 4 °C. Aliquots of plasma were stored at -80 °C for later analysis. Plasma FAs (NEFA-C; Wako Chemicals, Neuss, Germany), and glycerol (Glycerol; Randox, England) were analysed on an ILAB 650 (Instrumentation Laboratory, Cheshire, United Kingdom) **(Chapter 2)**.

Statistical Analysis

For each individual the median was calculated for fat oxidation rates in the two placebo and dGTE trials. This data was used in the statistical analysis. Data analysis was performed by using SPSS for WINDOWS software (version 19; SPSS Inc, Chicago, IL). Data are expressed as means ± SEMs unless otherwise stated. Statistical differences between FATMAX, FATMIN, $\dot{V}O_2$max, *W*max, RERmax and HRmax in the dGTE and placebo trials were analysed using a Student's paired samples *t*-test. Differences in the fat oxidation rates at

different exercise intensities between trials were identified using a general linear model for repeated measures. For all statistical analyses significance was set at $p < 0.05$. Bivariate correlations were carried out between maximal fat oxidation ($g \cdot min^{-1}$) and FATMAX (% $\dot{V}O_2max$) with habitual CHO intake expressed in absolute (g) and relative terms ($g \cdot kg \cdot bw^{-1}$) as well as a percentage of total energy intake.

5.4 Results

Twelve healthy, physically active males participated in the study. Participant anthropometric information can be found in Table 5.1.

Habitual CHO Intake and Fat Oxidation

No significant correlation was found between maximal fat oxidation rates, measured in the placebo trials, and habitual CHO intake. There was however a significant negative correlation between FATMAX and habitual CHO intake when expressed and as a percent of total energy intake ($r = -0.32$, $p < 0.001$), accounting for 10.2% of the variance.

Table 5.1. Participant Characteristics

	Mean (± SD)
Age (y)	19 ± 1
Height (m)	1.79 ± 0.07
Weight (kg)	74.4 ± 8.7
BMI ($m \cdot kg^{-2}$)	23.2 ± 2.2
$\dot{V}O_2$peak ($mL \cdot min^{-1} \cdot kg^{-1}$)	52.6 ± 7.6
*W*max (*W*)	281 ± 37

Mean (± SD) of participant anthropometric and physical characteristics

dGTE ingestion and Fat Oxidation

In some individuals a decrease in fat oxidation rates was observed after the first and lowest exercise intensity. As a consequence, the exercise intensities below FATMAX could not be determined for all participants. Therefore, the graph in Figure 5.2 contains data from all participants but does not display any data points at exercise intensities below FATMAX.

On average acute dGTE ingestion did not alter substrate metabolism at any exercise intensity during the graded exercise test. Thus, the average FATMAX curve was similar in the dGTE and placebo trials. On average, there was no statistical difference in FATMAX in the dGTE trials compared to placebo (Table 5.2). In addition acute ingestion of dGTE did not alter maximal rates of fat oxidation compared to placebo (0.49 ± 0.03 $g \cdot min^{-1}$and 0.50 ± 0.03 $g \cdot min^{-1}$ for the dGTE and placebo trial respectively).

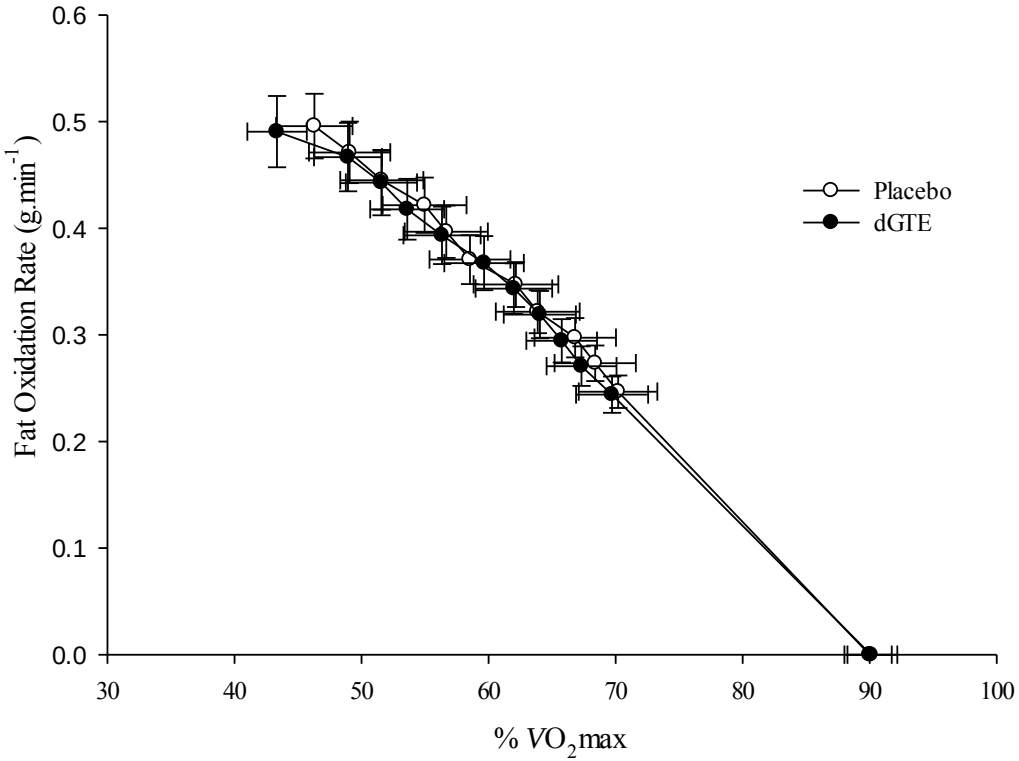
Plasma FA and Glycerol

Under resting conditions on the morning of the exercise trials, at t=120, plasma FAs and glycerol were unchanged compared to the plasma trial (Figure 5.3 A and B). In addition, there were no differences in circulating concentrations of plasma FAs (p= <0.05) and glycerol (p= <0.05) immediately post exercise between the two conditions.

Maximal Oxygen Uptake ($\dot{V}O_2max$)

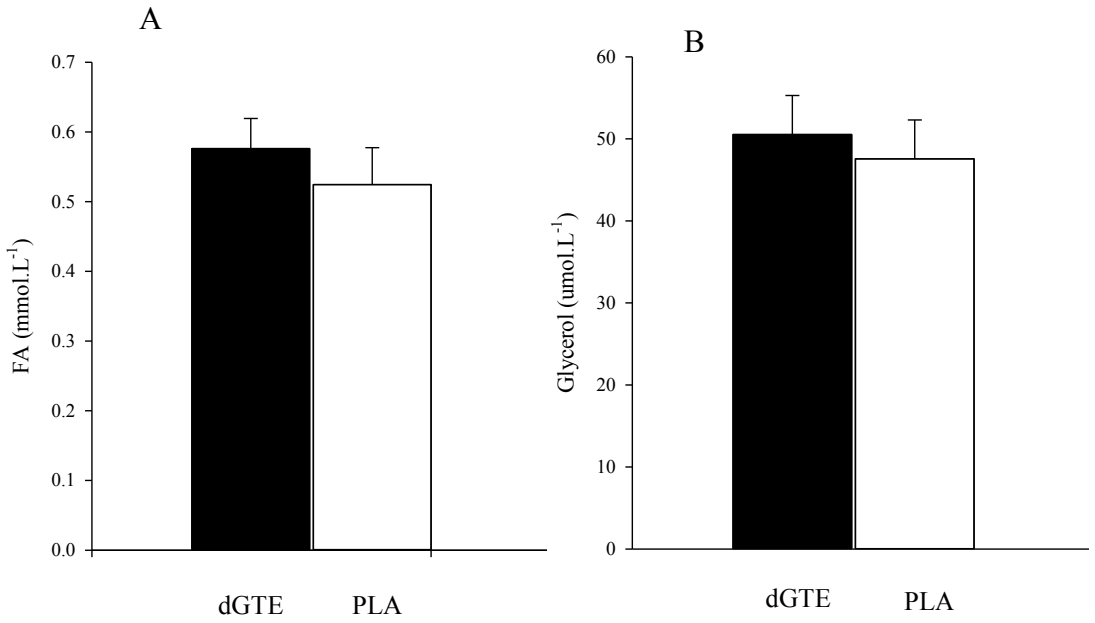
Each FATMAX test was completed until voluntary exhaustion. Therefore, measurements of maximal oxygen uptake ($\dot{V}O_2$max), RER and HR were recorded following both dGTE and placebo ingestion. These results can be found in Table 5.2. Ingestion of dGTE did not alter maximal work output (*W*max) during the graded exercise test compared to placebo (P = <0.05).

Figure 5.2 dGTE and placebo fat oxidation curve



Mean (±SEM) Fat oxidation rates (g·min^{-1}) versus exercise intensity (%$\dot{V}O_2$max) in the placebo and dGTE trial. N=10

Figure 5.3 Effects of dGTE on plasma fatty acids and glycerol



Mean (±SEM) plasma FA (A) and glycerol (B) measured at rest (t=120) in the dGTE (black bar) and placebo (white bar) trials.

Table 5.2 Exercise metabolism in dGTE and Placebo trials

	Placebo	dGTE
$\dot{V}O_2$max (L·min^{-1})	3.98 ± 0.09	3.99 ± 0.10
RERmax	1.09 ± 0.01	1.09 ± 0.01
HRmax (beats·min^{-1})	187 ± 2	184 ± 2
*W*max (*W*)	279 ± 13	281 ± 14
FATMAX (% $\dot{V}O_2$max)	46 ± 3	43 ± 2
FATMIN (% $\dot{V}O_2$max)	91 ± 2	89 ± 2

Mean (±SEM) $\dot{V}O_2$max (L·min^{-1}), RERmax, HRmax (beats·min^{-1}), *W*max, FATMAX (% $\dot{V}O_2$max) and FATMIN (% $\dot{V}O_2$max) in the placebo and dGTE trials.

MFO and FATMAX variation

All participants completed each condition twice as a result the variation in MFO and FATMAX could be calculated. Data was analysed if complete data sets of all four trials were available (N= 9). The average variation in MFO for placebo and dGTE was 19% and 6% respectively. Furthermore the variation in FATMAX was similar in the two interventions (10% and 9% for the placebo and dGTE trials respectively).

5.5 Discussion

All previous studies (including **Chapter 3** and **Chapter 4**) investigating the effects of GTE ingestion on substrate metabolism have used a steady state exercise protocol at a fixed intensity (8, 14,19). In the present study we investigated the effects of acute decaffeinated GTE ingestion on fat oxidation during a graded exercise test in physically active males. We found that dGTE did not alter whole body fat oxidation rates at any given exercise intensity compared to placebo.

Our findings are in line with **Chapter 3** and **Chapter 4** which found no effect of acute (24 hour or a single bolus) caffeinated or decaffeinated GTE ingestion on substrate utilisation during exercise in physically active males. Furthermore, we observed no change in plasma FA and glycerol concentrations during rest and following the exercise bout. It is often proposed that GTE exerts an effect on fat metabolism by indirectly increasing FA availability through

augmentations in sympathetic nervous activity (5). However, in Chapter 4 I also found no effect of acute caffeine free GTE ingestion on plasma FA and glycerol. Taken together these studies question the potency of acute (or chronic) GTE ingestion on augmenting fat oxidation rates in this population of individuals.

GTE and maximal oxygen consumption

In humans, a three day period of EGCG ingestion was found to significantly increase absolute maximal oxygen uptake ($\dot{V}O_2$max) compared to placebo (16). The authors speculated that EGCG was effective in increasing $\dot{V}O_2$max by attenuating the degradation of noradrenaline and as a result increasing heart rate and stroke volume. In the present study, the exercise test protocol allowed us to determine $\dot{V}O_2$max. However, we found no difference in $\dot{V}O_2$max following acute dGTE ingestion compared to placebo. Furthermore performance (defined as change in *W*max) was also unaffected by acute dGTE which is in line with previous work by Richards et al (16). Although research is limited these data suggest that GTE ingestion may not be effective in improving certain aspects of performance.

Habitual CHO Intake and Fat Oxidation

Fat free mass, fat mass, gender, $\dot{V}O_2$max and self reported physical activity levels have been found to account for 34% of the intra-individual variance in MFO (18). However, habitual diet also plays a role in substrate utilisation during exercise (6, 7). Many studies have found that manipulating daily carbohydrate intake can influence fat utilisation (6, 7). In the present study habitual macronutrient intake was predicted using information obtained from 3-day diet

diaries. From this we could establish if diets that are low/high in CHO (resulting in high/low fat diets, respectively) can predict MFO rates and FATMAX. Analysis of the results obtained in the placebo trials, show a significant negative correlation between CHO intake and FATMAX (r= -0.32). Although our sample size was relatively small (N=12) these data suggest that habitual diet may explain ~10% of the variance of the exercise intensity at which fat oxidation rates are maximal. Therefore future studies investigating the effects of a nutrition intervention on exercise metabolism should record habitual dietary intake and be aware of the implications that habitual diet may have on exercise metabolism when undertaking exercise trials.

Conclusion

In conclusion, acute ingestion of a decaffeinated GTE did not alter fat oxidation rates at any given exercise intensity in physically active males. Furthermore $\dot{V}O_2$max and performance (indicated by *W*max) was unchanged by decaffeinated GTE consumption. However, our mathematical model is a valid and comprehensive tool to use for the analysis of fat oxidation curve.

5.6 References

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Chapter 6: MAXIMAL FAT OXIDATION RATES IN AN ATHLETIC POPULATION

Rebecca K. Randell, James Carter, Ian Rollo, JohnEric Smith, Timothy Roberts, Kortney Dalrymple, Justin Dobson, John Stofan, Carlijn Vernooij and Asker. E. Jeukendrup

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6.1 Abstract

Purpose: The aim of this study was to establish maximal fat oxidation rates (MFO) and the exercise intensity at which it occurred (FATMAX) in a large athletic population. In addition, on the basis of each athlete's fat oxidation-exercise intensity relationship, athletes were classified as a fat or carbohydrate metabolic type (FMET and CMET).

Method: For the purpose of this study 281 athletes, from a variety of sports and competitive level, undertook a graded exercise test to exhaustion on a treadmill in a fasted state (≥ 5 h fasted). Rates of fat and carbohydrate (CHO) oxidation during each stage of the test were determined using indirect calorimetry. Fat oxidation curves were constructed for each individual using mathematical modelling.

Results: On average the MFO of all 281 athletes was 0.59 ± 0.17 g·min^{-1} occurring at an average exercise intensity of 53 ± 15% $\dot{V}O_2$max. Using set criteria 187 athletes were classified as FMET and the remaining 94 as CMET. MFO were significantly greater (0.63 ± 0.17 versus 0.51 ± 0.13 g·min^{-1}) and occurred at a higher exercise intensity (61 ± 10% versus 38 ± 8% $\dot{V}O_2$max) in the FMET group compared to CMET. sex, age, FFM, $\dot{V}O_2$max, body mass, and percent body fat may account for 33% of the variation.

Conclusion: Here we propose new criteria to group individuals as either FMET or CMET. CMET elicit MFO at the first exercise intensity, whereas individuals who showed an increase in fat oxidation followed by a decreased were classed as FMET. FMET appear to have higher MFO rates compared to CMET. Sex, training and body composition may explain some of the differences between the two groups (~33%).

6.2 Introduction

Carbohydrate (CHO) and fat are the predominant energy sources during exercise (10). However, the absolute and relative contributions of CHO and fat are, amongst other factors, dependent on exercise intensity. In fact exercise intensity may be the single most important factor influencing substrate utilisation. In 2002, Achten et al (1) developed a graded exercise test protocol that allowed the determination of substrate metabolism over a wide range of intensities. This test provides a measure of maximal fat oxidation (MFO) as well as the exercise intensity at which fat oxidation is maximal (FATMAX). This test is unique in that an individual has to perform only one bout of exercise to determine differences in metabolism at various intensities. Other studies have investigated substrate metabolism during prolonged exercise at different intensities where each exercise bout was performed on a separate occasion (8, 18, 21). However, day-to-day variation in metabolism (as a result of diet and other factors) could increase the variability (24) and make interpretation of results more difficult.

Since the development of the FATMAX test, it has been used in numerous studies to determine fat oxidation profiles in trained (2, 13, 19), untrained (13, 19), obese (14, 22) and sedentary (22) individuals, as well as in children (25, 26) and adults (2, 3, 14, 19, 22). A common finding from all these studies is that large inter-individual differences exist in MFO and FATMAX, yet reproducibility seems to be good (intra- individual variation is small) (1, 2, 14). In 2005 Venables et al (22) performed a cross sectional study of 300 individuals, ranging in body composition and aerobic capacity, and described MFO and FATMAX as well as the factors that influenced these parameters. The authors observed that on average MFO was 0.46 ± 0.01 $g \cdot min^{-1}$ with a wide range of 0.18 – 1.01 $g \cdot min^{-1}$ (22). MFO was reached at an

exercise intensity of 48 ± 1 % $\dot{V}O_2$max again with a wide range (25 – 77% $\dot{V}O_2$max) (22). Fat free mass (FFM), self reported physical activity, $\dot{V}O_2$max, sex and fat mass (FM) accounted for 34% of the variance however the remaining 66% was unaccounted for. Some have suggested that young age may also play a role in the body's ability to oxidise fats. Riddell et al (16) found higher (2-fold) peak fat oxidation rates in young boys (aged 11 – 12 *y*) compared to male adults (aged 22 – 26 *y*). Furthermore, as the boys progressed through puberty the authors reported a decrease in peak oxidation rates, despite no change in aerobic capacity (reported as $\dot{V}O_2$max expressed relative to body mass) (16).

Athletes typically have higher rates of fat oxidation compared to untrained individuals at a given relative and absolute exercise intensity (13, 19). This is because exercise training promotes skeletal muscle adaptations as well as whole body changes that favour fat oxidation (11). For example, trained individuals have high intramuscular triglyceride (IMTG) content located close to the mitochondria suggesting an increased efficiency in oxidation (15). Exercise training also promotes increases in mitochondrial mass which will allow fat oxidation and reduce the need for energy production through glycolysis (20). Irrespective of sporting activity, competitive athletes may benefit from increasing fat oxidation rates. The ability to utilise fats and spare muscle glycogen is often associated with delays in fatigue and potentially improving endurance performance (9) and because higher rates of fat oxidation reflect an enhanced oxidative capacity it may also impact on recovery in team sports athletes in between high intensity exercise bouts.

Previously we observed that fat oxidation in some individuals start high but decline as soon as the exercise intensity increases (**Chapter 5**). In other individuals, fat oxidation increases with exercise intensity until a certain point where it then declines. Reliability of this data is good (2), and results within an individual seem reproducible. However, it appears that inter-

individual differences in fat oxidation are large (2, 8). Generally there may be different metabolic types i.e those that have a higher capacity to oxidise fat during exercise and those who have a reduced ability to increase fat oxidation, despite similarities in some physical and physiological attributes. It may be possible to develop criteria to divide individuals into different categories based on metabolic type. This may help to elucidate why such differences exist and what health (and performance) implications this may have.

Currently there are no normative data on MFO rates and FATMAX from an athletic population. Furthermore, no study to date has compared MFO and FATMAX of competitive athletes ranging in age, body mass, $\dot{V}O_2$max and sporting activity. Therefore the purpose of the present study was to establish MFO and FATMAX from a large heterogeneous sample of athletes, with a focus on team sports. A second aim of this study was to use a new and validated mathematical model to determine the fat oxidation profile of each athlete and to use these profiles to classify athletes by metabolic type.

6.3 Participants and Methods

General Design

Data were collected from two separate exercise physiology laboratories; 1) The Gatorade Sports Science Institute (GSSI), IMG Academy, Bradenton, Florida, US (GSSI US) and 2) GSSI, Loughborough University, Loughborough, UK (GSSI UK). The two separate laboratories tested 281 male and female athletes in total, 216 athletes were tested at GSSI US and the remaining 74 were tested at GSSI UK. All athletes performed a FATMAX test during a single visit to either testing location. The exercise protocol and equipment used were identical between the two sites. The graded exercise test was performed on a treadmill

(h/p/cosmos sports & medical, Germany). Whole body rates of fat and carbohydrate were calculated during each stage of the exercise test, using indirect calorimetry, to establish MFO and FATMAX. Environmental conditions varied slightly across the research sites but remained constant for all trials (20 – 23 °C and 41 – 37% relative humidity for GSSI UK and GSSI US respectively).

Participants

All volunteers were recruited via e-mail, personal visits/ meetings, telephone calls or the athlete personally contacting the testing facility. The majority of the athletes were recruited from the student pool at the IMG academy, the student pool at Loughborough University and athletes local to the GSSI UK and GSSI US area.

The 281 athletes recruited for the purpose of this study ranged in competitive level however the inclusion criteria were the same for all athletes with the exception of age which was 16 – 60 *y* in GSSI UK and 13 – 60 *y* in GSSI US. Additional inclusion criteria included regular training or participation in sporting activity (≥ 1 session per week), healthy (assessed by completion of a general health questionnaire) and no known cardiovascular or metabolic disorders. Local ethical approval was obtained for each of the study sites. For GSSI UK the study was approved by the South Birmingham NHS National Research Ethics Committee (West Midlands, UK). For GSSI US the study was approved by The Sterling Institutional Review Board, Atlanta, Georgia.

On initial contact the purpose and nature of the study was explained to all athletes. Informed consent was signed on-site, prior to the exercise test and following a more in-depth explanation of the testing protocol. Parental consent was obtained from volunteers who were

under the age of 18 (N=144). All volunteers were healthy as assessed by a general health questionnaire. Prior to testing medical clearance was obtained for all participants who completed the testing at GSSI US.

Experimental Design

Each athlete reported to the laboratory in a fasted state (≥ 5 h) having consumed their normal habitual diet and abstained from strenuous physical activity, alcohol and caffeine consumption in the preceding 24 h. Before the initiation of the FATMAX test body composition was measured and height and nude body weight were recorded. Different techniques were used to measure body composition at the two different testing locations. Athletes underwent air displacement plethysmography (BODPOD, COSMED, Chicago, IL, US) at GSSI UK. Whereas Dual-energy X-ray absorptiometry (DXA) (Lunar iDXA, GE Healthcare, Buckinghamshire, UK) was used to measure body composition at GSSI US.

The exercise test protocol was adapted from a previously described and validated protocol (1, 14, 22). These studies demonstrated that 3 min stages can result in accurate and valid measurements of MFO and FATMAX. In the present study the exercise test was performed on a treadmill (h/p/cosmos sports & medical, Germany). The test started at 5.0 km/h^{-1} and at a gradient of 1% for three min. The speed then increased to 7.5 km/h^{-1}. From this point, speed was increased by 1 km/h^{-1} every 3 min until an RER of 1 was reached. The speed then remained constant and the gradient was increased by 1% every 1 min. The test ended when athletes reached voluntary exhaustion or the test was stopped if two out of the three following criteria were reached: 1) levelling off in $\dot{V}O_2$ with further increases in workloads (< 2 $mL \cdot kg^{-1} \cdot min^{-1}$); 2) Heart rate within 10 beats/min (bpm) of age predicted max or 3) Respiratory

exchange ratio (RER) of >1.05. Respiratory gas measurements ($\dot{V}O_2$ and $\dot{V}CO_2$) were collected continuously using a Moxus Modular VO_2 system (AEI technologies, Pittsburgh, USA). Furthermore, HR (Polar RS800CX, Polar Electro Ltd, Kempele, Finland) was measured throughout the whole test and Rating of Perceived Exertion (RPE) was recorded during each stage **(Chapter 2)**.

Indirect calorimetry and Calculations

To calculate substrate metabolism the breath-by-breath data was averaged in 10 s increments, this was calculated automatically by the Moxus Modular $\dot{V}O_2$ system (AEI technologies, Pittsburgh, USA) system. These raw data were then analysed manually for each athlete. In more detail, the first min and last 30 s of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) recorded during each stage of the test were excluded from analysis. The remaining 90 s of data was averaged for each stage. Using this averaged data whole body fat and carbohydrate oxidation rates were calculated using Stoichiometric equations (7) assuming that protein oxidation was negligible throughout the test.

$$\text{Fat oxidation } (g \cdot min^{-1}) = 1.718 \cdot \dot{V}O_2 - 1.718 \cdot \dot{V}CO_2$$

$$\text{CHO oxidation } (g \cdot min^{-1}) = 4.170 \cdot \dot{V}CO_2 - 2.965 \cdot \dot{V}O_2$$

For each athlete MFO, FATMAX and FATMIN (FATMIN; exercise intensity (expressed as % $\dot{V}O_2$max) where fat oxidation becomes negligible and carbohydrate becomes the

predominant fuel source (i.e. RER ≥ 1.0)) were established. Furthermore, a metabolic profile was constructed where substrate oxidation rates were plotted against exercise intensity (expressed as a percentage of $\dot{V}O_2$max) **(Chapter 2)**.

Determining Metabolic Type

Each individual metabolic profile was used to determine the metabolic type of the athletes. From visual analysis of the metabolic profiles it became apparent that individuals could be split into two groups depending on the exercise intensity at which rates of fat oxidation were maximal. Firstly, we found a high proportion of athletes who had the highest fat oxidation rate (MFO) at the lowest exercise intensity of the test (e.g walking at 5.0 km/h^{-1}). In these individuals any increase in exercise intensity resulted in a decrease in absolute fat oxidation. Therefore we have classified these individuals as having a CHO metabolic type (CMET). The metabolic profiles of the remaining athletes displayed an increase in fat oxidation with increases in exercise intensity until a certain threshold. Thus we classified these individuals as having a fat metabolic type (FMET). This classification was independent of sex, age and sport. These differences in the fat metabolism-exercise intensity relationship has been found by others (8, 22) however this is the first study to group individuals based on their metabolic profile.

Construction of Average Fat Oxidation Curves

Further analysis of the data was performed, using Matlab (MathWorks Matlab 2011a, Natick, Massachusetts, U.S.A.), to construct average fat oxidation curves for both FMET and CMET.

In more detail, MFO for each individual was determined as the highest rate ($g \cdot min^{-1}$) calculated using the stoichiometric equation described above. Then, 95% to 50% of MFO was calculated in intervals of 5% (i.e. 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60% 55%, and 50%). To calculate the absolute $\dot{V}O_2$ (mL) associated with these 11 fat oxidation rates, linear interpolation was applied between subsequent intercepts of the measured fat oxidation and $\dot{V}O_2$ (mL) values. Each interpolated link consisted of 1000 hypothetical intercepts of $\dot{V}O_2$ (mL) and fat oxidation. Depending on the $\dot{V}O_2$ at MFO a hypothetical $\dot{V}O_2$ values, for each of the 11 fat oxidation rates intervals, could be determined. These values of absolute $\dot{V}O_2$ were converted to a percentage of each individual's $\dot{V}O_2$max. This interpolation process was then repeated for heart rate and carbohydrate oxidation at the calculated fat oxidation rates. This mathematical model for calculating rates of fat oxidation has been validated in **Chapter 5**.

Statistical Analysis

Data analysis was performed using SPSS for WINDOWS software (version 19; SPSS Inc, Chicago, IL). Data are expressed as means ± standard deviations (SDs) unless otherwise stated. Sex, age and metabolic type differences in any anthropometric characteristics, MFO, MFO/FFM and FATMAX were identified using an independent t-test. Differences in anthropometric characteristics, MFO, MFO/FFM and FATMAX between sports were identified using a one-way ANOVA. Differences in substrate utilization across different exercise intensities between the FMET and CMET were identified by using a repeated-measured ANOVA.

Bivariate correlations were undertaken between MFO with the following as independent variables; age, sex, body mass, percent body fat (%BF), FFM, FM and $\dot{V}O_2$max. Hierarchical linear regression analysis was then used to predict MFO with all the significant independent variables found in the bivariate analysis. Bivariate correlations, between MFO and the dependant variables mentioned above, were also performed on the FMET and CMET groups separately.

It should be noted that a different technique (air displacement plethysmography and DXA) was used to assess body composition (FFM, FM and %BF) between the two testing locations. Lockner et al (12) found air displacement plethysmography (using a BODPOD) to under-report %BF by 2.9% when compared to DXA in children aged between 10-18 *y*. Furthermore, a significant difference has been found between reported %BF obtained from DXA and BODPOD measurements in collegiate females (5). Therefore, to investigate whether the body composition technique used would impact the outcome of this study statistical analysis was performed once on the whole data set and also excluding the data where body composition was measured using air displacement plethysmography. No differences were found in any of the outcome measures, expressed relative to FFM, when air displacement plethysmography measurements had been removed. Therefore the statistical analysis was performed on the whole data set.

6.4 Results

Athlete Characteristics

The data presented in this study are from a diverse cohort of athletes including those who participate in team sports and individual sports/events. The competitive level of the athletes ranged from recreational all the way though to elite/professional. The physical characteristics of all athletes can be found in Table 6.1.

Substrate Metabolism

The average relative (MFO/FFM) and absolute MFO of the combined 281 athletes was 10.0 ± 2.6 mg·kg·FFM^{-1}·min^{-1} and 0.59 ± 0.17 g·min^{-1} respectively, occurring at a FATMAX of 53 ± 15% $\dot{V}O_2$max. Using our set criteria, 94 athletes were deemed CMET and 187 FMET. Anthropometric characteristics of the two groups can be found in Table 6.1. The two groups did not differ in body mass or HR_{max}. However the FMET group had significantly lower body fat percentage and fat mass and significantly greater FFM and $\dot{V}O_2$max compared to CMET ($P < 0.01$). On average, MFO (0.63 ± 0.17 versus 0.52 ± 0.13 g·min^{-1}) and MFO/FFM (10.4 ± 2.6 versus 9.2 ± 2.3 mg·kg·FFM^{-1}·min^{-1}) were significantly greater and occurred at a higher exercise intensity (61 ± 10 versus 38 ± 8% $\dot{V}O_{2max}$) in the FMET compared to CMET.

The average fat and CHO oxidation curves for the two groups can be found in Figure 6.1 A and B. The "cross-over" point of substrate metabolism (the point at which CHO becomes the predominant fuel over fat) was found at approximately 65% $\dot{V}O_2$max in the FMET and 50% $\dot{V}O_2$max in the CMET.

Sex Differences

Of the 281 athletes tested 47 were females and 234 were males. Age was similar between the two groups (Table 6.3). The males were significantly heavier, had higher FFM and lower body fat percentage. In addition, $\dot{V}O_2$max expressed per kg body mass and per FFM, was significantly higher in the males compared to females ($P < 0.01$) (Table 6.3). During the graded exercise test average absolute MFO rates ($g \cdot min^{-1}$) were significantly greater in the males. However when MFO was expressed relative to FFM no sex difference was found. FATMAX did not differ between the males and females (Table 6.3).

Table 6.1 Participant characteristics in combined group, CMET and FMET

Variable	Combined Group (N=281)	CMET (N=94) Males (N=70) Females (N=24)	FMET (N=187) Males (N=164) Females (N=23)
Age (y)	20 ± 7 (13 – 52)	18 ± 6 (13 – 45)	20 ± 7 (13 – 52)*
%BF	18 ± 7 (4 – 37)	20 ± 7 (4 – 37)	16 ± 6 (4 – 36)*
Body Mass (kg)	72 ± 13 (38 – 116)	71 ± 13 (39 – 116)	73 ± 13 (38 – 104)
FFM (kg)	59 ± 12 (27 – 93)	56 ± 11 (27 – 83)	61 ± 12 (32 – 93)*
FM (kg)	13 ± 5 (3 – 36)	15 ± 6 (3 – 36)	12 ± 5 (3 – 29)*
$\dot{V}O_2$max (ml·kg^{-1}·min-$^{-1}$)	51 ± 6 (34 – 71)	48 ± 6 (34 – 60)	53 ± 6 (34 – 71)*
$\dot{V}O_2$max (ml·kg FFM^{-1}·min-$^{-1}$)	62 ± 6 (42 – 78)	60 ± 5 (47 – 71)	63 ± 6 (42 – 78)*
HR_{max} (bpm)	191 ± 9 (164 – 216)	192 ± 10 (167 – 210)	191 ± 9 (164 – 216)
MFO (g·min^{-1})	0.59 ± 0.17 (0.11 – 1.09)	0.51 ± 0.13 (0.11 – 0.87)	0.63 ± 0.17 (0.25 – 1.09)*
MFO/FFM (mg·kg FFM^{-1}·min^{-1})	10.0 ± 2.6 (1.9 – 17.6)	9.3 ± 2.3 (1.9 – 14.8)	10.4 ± 2.6 (4.3 – 17.6)*
FATMAX (% $\dot{V}O_2$max)	53 ± 15 (25 – 91)	38 ± 8 (25 – 73)	61 ± 10 (35 – 91)*
HR_{fatmax} (bpm)	130 ± 26 (77 – 180)	105 ± 16 (77 – 171)	142 ± 20 (99 – 180)*

Values are mean (±SD), ranges are in parentheses, of body mass Fat Free Mass; FFM, Fat Mass; FM, Maximal oxygen uptake; $\dot{V}O_2$max (relative to body mass and FFM), Maximal heart rate; HR_{max}, Absolute (g·min^{-1}) and relative (mg·kg FFM^{-1}·min^{-1}) maximal fat oxidation (MFO), FATMAX and heart rate at FATMAX (HRfatmax) in the total athletes (N=281) and when grouped depending on metabolic type (CMET and FMET). * significantly different (p = <0.05) to CMET

Age Differences

Athletes were grouped into two age categories; over 18 *y* (using the assumption that these individuals had reached Tanner stage 5) and under 18 *y*. Of the 281 athletes tested, 144 were under 18 *y* and 137 were over 18 *y*. The average age in these two groups was 15 ± 1 and 24 ± 7 *y* respectively. The over 18 *y* group was significantly heavier, had a higher FFM and %BF compared to the under 18s. Furthermore, absolute $\dot{V}O_2$max was significantly higher in the over 18 *y* group when compared to the under 18 *y* (3991 ± 754 and 3381 ± 622 mL ($P < 0.01$). However, when $\dot{V}O_2$max was expressed relative to body mass and FFM there was no difference between the two age groups (Table 6.2). MFO rates were significantly greater in the over 18 *y* however, when expressed relative to FFM no differences between the two groups were found (Table 6.2). Furthermore, there was no significant difference in FATMAX or HR_{FATMAX} (Table 6.2).

Table 6.2 Participant characteristics of males, females, under 18s and over 18s

Variable	Males (N=234)	Females (N=47)	Under 18s (N=144) Males N=123 Females N=21	Over 18s (N=137) Males N=111 Females N=26
Age (y)	19 ± 6 (13 – 52)	21 ± 8 (14 – 45)	15 ± 1 (13 – 17)	24 ± 7 (18 – 52)†
%BF	16 ± 6 (4 – 37)	25 ± 7 (9 – 37)*	19 ± 6 (6 – 37)	16 ± 7 (4 – 37)†
Body Mass (kg)	74 ± 13 (38 – 116)	64 ± 9 (39 -82)*	67 ± 12 (38 – 102)	77 ± 12 (39 – 116)†
FFM (kg)	62 ± 11 (27 – 93)	48 ± 6 (33 – 55)*	55 ± 10 (27 – 81)	65 ± 12 (33 – 93)†
FM (kg)	12 ± 5 (3 – 36)	16 ± 6 (5 – 29)*	13 ± 5 (3 – 36)	12 ± 5 (3 – 33)
$\dot{V}O_2$max (ml·kg^{-1}·min-$^{-1}$)	53 ± 5 (34 – 71)	44 ± 6 (34 – 63)*	50 ± 6 (34 – 64)	52 ± 7 (34 – 71)†
$\dot{V}O_2$max (ml·kg FFM^{-1}·min-$^{-1}$)	63 ± 6 (42 – 78)	59 ± 5 (47 – 75)*	62 ± 6 (42 – 78)	62 ± 6 (47 – 78)
HR_{max} (bpm)	192 ± 9 (164 – 216)	191 ±10 (167 – 205)	194 ± 8 (174 – 216)	189 ± 9 (164 – 209) †
MFO (g·min^{-1})	0.61 ± 0.16	0.50 ± 0.17*	0.54 ± 0.14	0.64 ± 0.18†
MFO/FFM (mg·kg FFM^{-1}·min^{-1})	9.9 ± 2.4	10.4 ± 3.2	10.0 ± 2.4	10.0 ± 2.7
FATMAX (% $\dot{V}O_2$max)	54 ± 15	52 ± 13	53 ± 17	54 ± 12
HR_{FATMAX} (bpm)	130 ± 20	128 ± 24	131 ± 28	129 ± 23

Values are mean (±SD), ranges are in parentheses of body mass, Fat Free Mass; FFM, Fat Mass; FM, Maximal oxygen uptake; Percent body fat; %BF, $\dot{V}O_2$max (relative to body mass and FFM), Maximal heart rate; $HR_{max.,}$ maximal fat oxidation; MFO, FATMAX and heart rate at FATMAX; HRfatmax. * $P < 0.05$ compared to males. † $P < 0.05$ compared to under 18s.

Sporting Activity

Athletes were divided into different subgroups depending on the energy demand of their sporting activity. Using the metabolic equivalent (MET) intensity levels, as proposed by Ainsworth et al (4), sports were grouped as 1) Low (LOW; 3 – 6 METS) 2) Moderate (MOD; 8 – 10 METS) and 3) High (HIGH; > 10 METS). The sporting activities associated with the three groups can be found in Table 6.3.

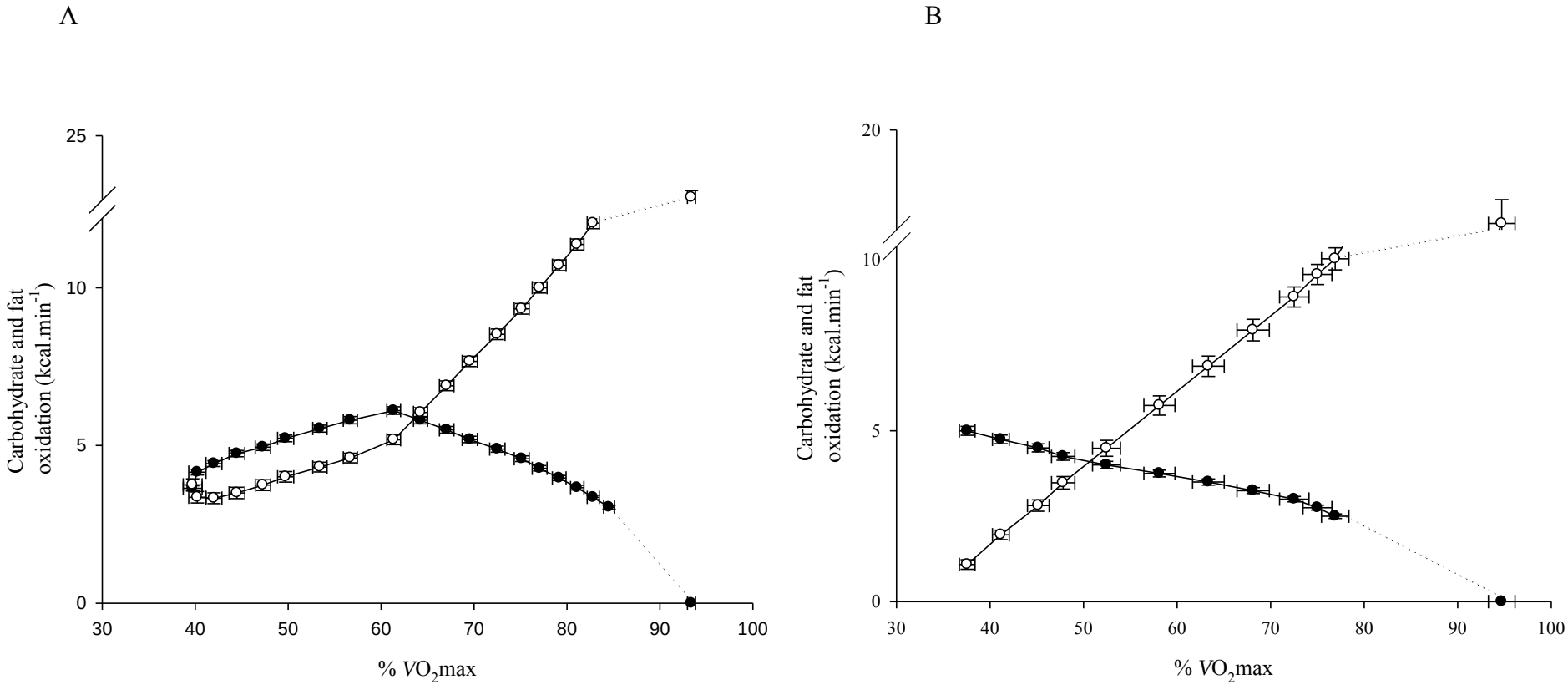
Anthropometric data of the three groups (LOW, MOD and HIGH) can be found in Table 6.4. On average absolute MFO rates were significantly greater in the MOD and HIGH group compared to LOW (Table 6.4). However when MFO was expressed relative to FFM there were no differences between any of the groups. FATMAX was significantly higher in the HIGH group compared to LOW. However there was no difference in FATMAX in the MOD group when compared to LOW and HIGH (Table 6.4).

Table 6.3 Sport/Activity classification

LOW	MOD	HIGH
Baseball (N=27)	Basketball (N=32)	Decathlon (N=1)
Golf (N=31)	Beach Volleyball (N=2)	Rowing (N=1)
Polevault (N=1)	Boxing (N=2)	Running (N=5)
Weight Lifting (N=1)	Jump Rope (N=1)	Track (N=8)
	Lacrosse (N=10)	Triathlon (N=7)
	Rugby League (N=11)	Ultra Endurance (N=9)
	Rugby Union (N=16)	
	Soccer (N=70)	
	Taekwondo (N=1)	
	Tennis (N=35)	

Sport/ Activity classification based on the energy demand of the sport (Low, Moderate and High) (4). Number (N) of athletes who participate in each sport are shown in parenthesis.

Figure 6.1 Fat and carbohydrate profile for FMET and CMET



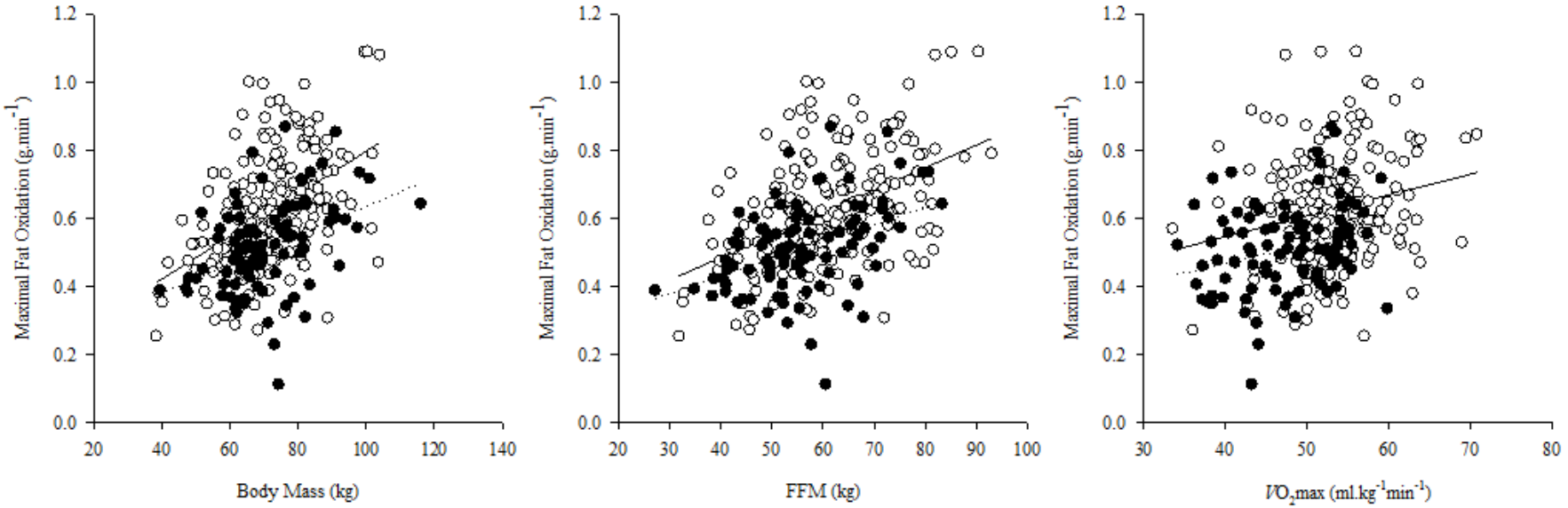
Mean absolute (kcal·min^{-1}) substrate oxidation over a range of exercise intensities in the FMET (A) and CMET (B) athletes. Values are means ± SE; fat and carbohydrate oxidation represented by filled and open circles respectively.

Table 6.4 Participant characteristics of athletes grouped by sporting activities

Variable	LOW (N=60)	MOD (N=181)	HIGH (N=32)
Age (*y*)	17 ± 2 (13-27)[a]	18 ± 4 (13–36)[a]	29 ± 8 (17–52)[b]
%BF	20 ± 6 (9–37)[a]	17 ± 7 (4–37)[b]	16 ± 6 (6–29)[b]
Body Mass (kg)	71 ± 11 (50–102)	72 ± 13 (38–116)	70 ± 13 (39–97)
FFM (kg)	57 ± 10 (40–81)	60 ± 12 (27–93)	60 ± 12 (33–78)
FM (kg)	14 ± 5 (5–27)[a]	12 ± 5 (3–36)[a,b]	11 ± 4 (5–22)[b]
$\dot{V}O_2$max (ml·kg^{-1}·min-$^{-1}$)	48 ± 6 (34–60)[a]	52 ± 6 (34–64)[b]	54 ± 8 (38–71)[b]
$\dot{V}O_2$max (ml·kg FFM^{-1}·min-$^{-1}$)	60 ± 5 (52–74)[a]	63 ± 6 (50–78)[b]	64 ± 7 (47–78)[b]
HR_{max} (bpm)	193 ± 9 (170–212)[a]	192 ± 9 (167–216)[a]	187 ± 9 (164–202)[b]
MFO (g·min^{-1})	0.53 ± 0.12[a]	0.60 ± 0.17[b]	0.62 ± 0.20[b]
MFO/FFM (mg·kg FFM^{-1}·min^{-1})	9.4 ± 2.0	10.2 ± 2.6	10.5 ± 3.3
FATMAX (% $\dot{V}O_2$max)	49 ± 16[a]	54 ± 14[b]	57 ± 12[b]
HR_{Fatmax} (bpm)	125 ± 30	132 ± 24	127 ± 22

Values are mean (±SD), ranges are in parentheses, for body mass, percent body fat; %BF, Fat Free Mass; FFM, Fat Mass; FM, Maximal oxygen uptake; $\dot{V}O_2$max (expressed relative to body mass and FFM), Maximal heart rate; HR_{max} maximal fat oxidation; MFO, FATMAX and heart rate at FATMAX; $HR_{fatmax.}$ Means with different superscript letters are significantly different from each other, $P < 0.05$.

.Figure 6.2 Correlation analysis



Correlations between absolute maximal fat oxidation rates (g·min^{-1}) and body mass (kg), FFM (kg) and $\dot{V}O_2$max (ml·kg·min^{-1}). Open and filled circles represent the FMET and CMET respectively. Linear regression lines are shown as a solid and dashed line for the FMET and CMET respectively.

Table 6.5 Predictors of maximal fat oxidation

					Coefficients		Correlations		
Dependant Variable	Independent Variable	R	R^2	Adjusted R^2	β	Sig.	Zero order (r value)	Partial	Part
MFO	FFM	0.58	0.34	0.33	0.62	0.16	0.50	0.09	0.07
	Sex				-0.10	0.13	0.25	-0.09	-0.08
	$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)				0.43	0.00	0.32	0.35	0.30
	%BF				0.28	0.13	-0.25	0.09	0.07
	Body Mass				-0.02	0.95	0.46	-0.04	-0.03
	Age				-0.00	0.99	0.15	0.00	0.00

Multiple regression analyses of the whole data set (N=281) with MFO ($g \cdot min^{-1}$) as the dependant variable.

Determinants of MFO in an athletic population

Bivariate correlation analysis was performed on the whole data set with MFO as the dependant variable. FFM, body mass, age, sex, $\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$) and %BF were significantly correlated (Table 6.5). No correlation was found between absolute FM and MFO. In addition, multiple regression analysis was performed with MFO as the dependant variable. The predicator variables included in the analysis were those variables that were significantly correlated with MFO in the bivariate correlations (Table 6.5). The results show that FFM, body mass, $\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$), sex, age and percent body fat account for 33% of the variance in MFO.

Furthermore, correlation analyses were performed on the CMET and FMET data sets with MFO as the dependant variable (Figure 6.2). MFO rates in the FMET group were significantly correlated with age (r=0.17, < 0.05), body mass (r = 0.46, $P < 0.01$), %BF (r = -0.21, $P < 0.01$), $\dot{V}O_2$max (r = 0.21, < 0.01), and FFM (r = 0.47, $P < 0.01$). MFO rates in CMET individuals were correlated to body mass (r = 0.42, $P < 0.01$), FFM (r = 0.42, $P < 0.01$) and FM (r = 0.26, $P < 0.05$).

6. 5 Discussion

These data confirmed previous data (8, 22) that individuals have different metabolic profiles. Here we defined criteria to divide participants into two groups based on the shape of their fat oxidation curve. This is also the first time that fat oxidation rates have been described in a diverse and large athletic population (N=281). On average we found absolute and relative MFO rates of 0.59 ± 0.17 $g \cdot min^{-1}$ and 10.0 ± 2.6 $mg \cdot kg \cdot FFM^{-1} \cdot min^{-1}$ respectively, occurring at

an exercise intensity of 53 ± 15% $\dot{V}O_2$max. However as reported previously we also observed large inter-individual variation in MFO and FATMAX (Table 6.1).

Using a similar graded exercise test, only one other large scale (N=300) study has investigated fat oxidation rates in healthy adults. (22). This study (22) described fat metabolism in a population including the opposite ends of the spectrum in terms of body composition and aerobic capacity. On average, MFO was 0.46 ± 0.01 g·min^{-1} reached at a FATMAX of 48 ± 1% $\dot{V}O_2$max (22). Averages however only tell part of the story, as a large inter-individual variation in both MFO (0.18 – 1.01 g·min^{-1}) and FATMAX (25 – 77% $\dot{V}O_2$max) was found. Only a fraction of this variation was explained by body composition and aerobic capacity (34%). In other words, a highly trained professional cyclist in that study did not necessarily have higher fat oxidation rates than an untrained and overweight individual.

In the present study we investigated fat metabolism in an athlete population. This population included athletes that ranged in body mass (range 38 – 116 kg) and aerobic capacity ($\dot{V}O_2$max ranged from 34 – 71 ml·kg^{-1}·min^{-1}). In the previous study by Venables et al (22) body mass ranged from 46 – 132 kg and the $\dot{V}O_2$max of the sample population ranged from 21 – 82 ml·kg^{-1}·min^{-1}. However on average we observed greater MFO rates, occurring at a higher FATMAX. One explanation for this could be that the majority of athletes in our sample population were classed as having a fat metabolic type.

This is the first study to group individuals depending on the exercise intensity at which MFO occurred introducing two new terms; 1) fat metabolic type (FMET) and 2) carbohydrate metabolic type (CMET). We defined CMET as individuals who displayed highest rates of fat oxidation at the first stage of the exercise test. In the context of the present study this related to a slow walk (5.0 km/h). In these individuals any increase in exercise intensity resulted in a

decrease in absolute rates of fat oxidation. On the other hand, FMET were defined as individuals who displayed an increase in fat oxidation with increases in exercise intensity until FATMAX occurred. Hereafter, absolute rates of fat oxidation decreased and CHO became the predominant fuel. We found that absolute and relative rates of MFO were significantly higher in the FMET when compared to CMET. In addition the "cross-over" point (a concept introduced by Brooks and Mercier (6)), where the relative contribution of lipids and CHO equally contribute to energy expenditure occurred at a higher exercise intensity in the FMET athletes compared to the CMET (~65 vs. 50% $\dot{V}O_2$max respectively). In the present study we observed a higher proportion of individuals as FMET (N=187) than CMET (N=94). Thus accounting for the differences in average MFO between the current study and the previous study by Venables et al (22).

It is obvious that there are different metabolic types however it is still unclear what exact variables predict fat metabolism during exercise. In the present study we have shown that MFO is positively correlated with $\dot{V}O_2$max (r = 0.32). Investigating the role of $\dot{V}O_2$max on fat oxidation Achten et al (2) divided 55 trained cyclists into two groups; 1) $\dot{V}O_2$max higher and 2) $\dot{V}O_2$max lower than the group average (~80 vs. ~59 ml·kg^{-1}·min^{1} for the high and low group respectively). Interestingly Achten et al (2) found that MFO rates in the high $\dot{V}O_2$max group were significantly greater (0.56 ± 0.14 g·min^{-1}) compared to the low $\dot{V}O_2$max group (0.48 ± 0.15 g·min^{-1}). When looking at our two metabolic type groups, the average $\dot{V}O_2$max in the FMET individuals was significantly higher than CMET.

In addition we found FFM to be positively correlated with MFO (r = 0.50). Again when comparing our two metabolic groups, FFM was significantly higher in the FMET group compared to CMET. It was previously found that individuals with a higher proportion of type

I (oxidative) muscle fibre had a great capacity to oxidise fats (23). However several years later, Goedecke et al (8) found no link between skeletal muscle composition and substrate metabolism. These authors found that it was the activity of oxidative enzymes and the ratio of oxidative to glycolytic enzymes which determined exercise metabolism at different workloads (25, 50, and 70% of peak power output). Increases in oxidative enzyme activity may be a result of current training volume (which has been found to negatively correlate with exercise RER (8) and subsequent skeletal muscle adaptations). Training volume and skeletal muscle enzyme activity were not measured in the present study but it could be suggested that both of these predictor variables may have been higher in the FMET group.

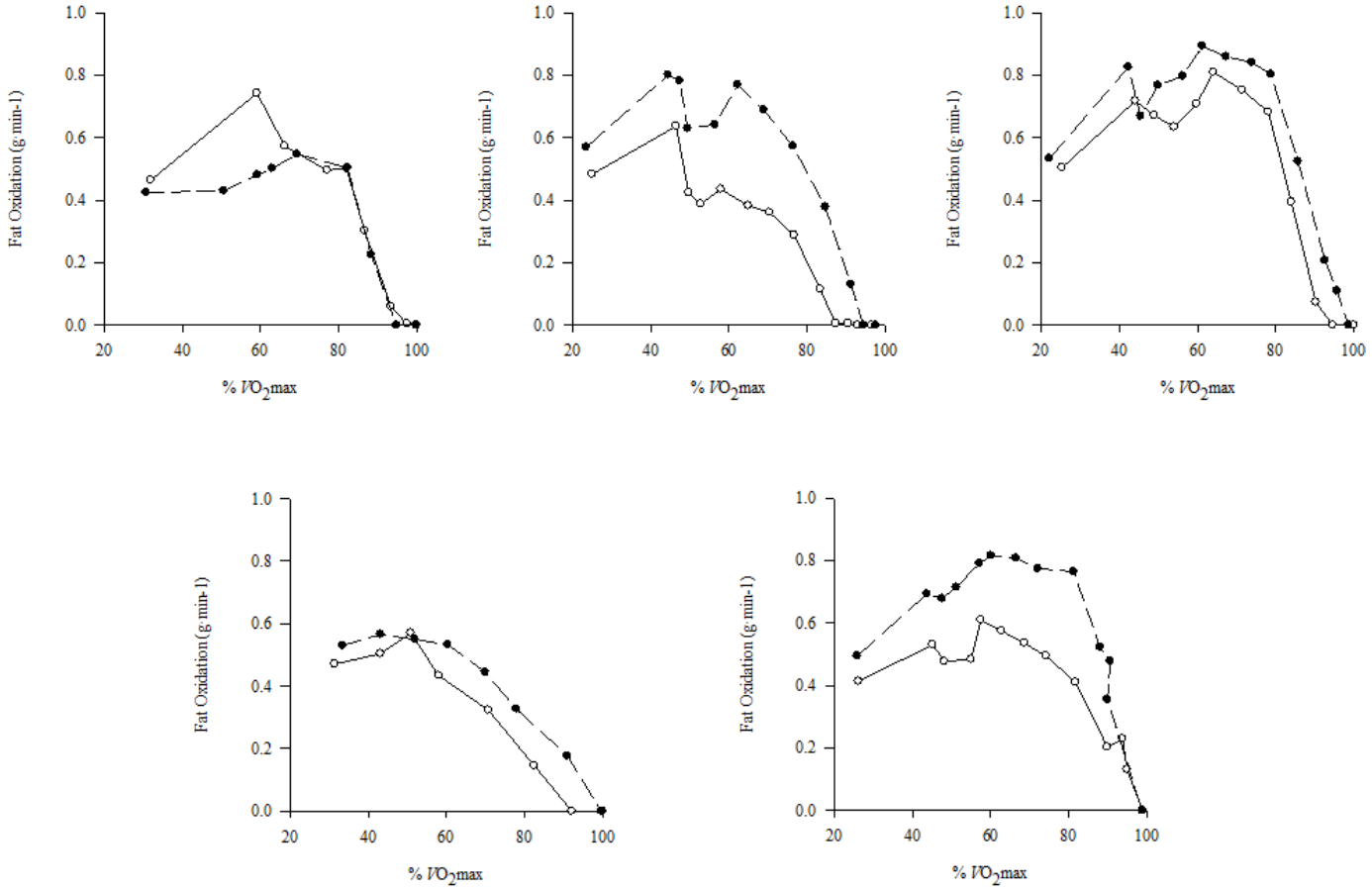
We also observed a small but significant positive correlation between MFO rates with age (r = 0.15) and sex (r = 0.25). It has been reported that peak fat oxidation rates decrease with age (17). However in the present study the FMET individuals, who displayed higher MFO, were significantly older than the CMET. In regards to sex differences in substrate metabolism, previous research has found higher MFO in females compared to males (22). However we found no sex differences in fat oxidation (when expressed relative to FFM). Furthermore there were equal numbers of females in the two metabolic type groups. Therefore using the current data, taking into account the relatively low female sample size, we cannot fully determine what impact sex has on fat metabolism in this athletic population.

However, we can be confident that the differences we observe in metabolic type/metabolic profiles are not due to day-to-day variation. Figure 6.3 shows fat oxidation curves of 5 athletes who undertook a FATMAX test on two occasions, under identical testing conditions. From these graphs it is apparent that our metabolic type and metabolic profile is individual and does not vary from day-to-day or from test-to-test. Therefore taken together, we have found body composition (FFM, body mass, %BF) $\dot{V}O_2$max, age and sex to account for 33% of

the variance in MFO. These observations are in agreement to Venables et al (22) who found similar predictor variables to account for 34% of the variance in MFO, in a group of healthy adults. Thus, factors which may explain the remaining ~60% of variance are still left to be determined.

One of these factors is likely to be the habitual diet. In 2001, Helge et al (9) manipulated the diet and training regime of 13 healthy males. During a 7 week period participants consumed a low-CHO diet (21% CHO and 62% fat) or a moderate-CHO diet (65% CHO and 20% fat), whilst following the same training protocol. After the 7 week period participants completed a steady state exercise bout during which substrate metabolism was measured. Helge et al (9) found that the respiratory exchange ratio (RER) was significantly lower (indicative of higher fat utilisation) in the participants who had consumed the low-CHO diet. Furthermore, Coyle et al found a 27% decrease in fat oxidation rates during exercise when participants consumed a high CHO diet (of which 88% of the total energy intake was CHO and <2% fat) compared to a moderate CHO diet (68% CHO and 22% fat). Also, when a very large amount of glucose was ingested pre-exercise, fat oxidation was suppressed by 34% compared with no carbohydrate ingestion .This figure would represent the largest expected reduction in fat oxidation. What can be taken from this finding is that habitual diet may be responsible for an additional ~30% of the variance observed in fat oxidation.

Figure 6.3 Fat oxidation curves: multiple tests



Metabolic profiles (absolute fat oxidation on the y axis and exercise intensity on the x axis) of five different athletes who completed a FATMAX test on two separate occasions Open and filled circles represent test 1 and test 2 respectively. .

Goedecke et al (8) found, in 61 endurance trained cyclists, that resting muscle glycogen content was positively correlated to RER during exercise at 25% of peak workload (W_{peak}). At the same exercise intensity and when the intensity was increased to 50% W_{peak} a negative correlation was found between plasma FA concentrations and RER (8). Furthermore, a positive association was found between plasma lactate levels and RER at 50 and 70% W_{peak} (8). This highlights the influence of endogenous substrate availability on substrate oxidation.

In conclusion, this is the first study to present MFO from an athletic population. In addition, we have established that individuals can be grouped as having a fat metabolic type (FMET) or CHO metabolic type (CMET). FFM, percent body fat, age, $\dot{V}O_2$max may account for some but not all of the difference between these two groups. Future research should investigate the role of habitual diet, current training program and endogenous substrate availability on fat oxidation rates in athletic and healthy populations.

6.6 References

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Chapter 7: GENERAL DISCUSSION

7.1 General Discussion

Green tea extract ingestion has been found in some (8, 24) but not all studies (5, 23) to increase fat oxidation rates under resting conditions. On balance, it is reported that resting 24 h fat oxidation may be increased by up to 16% through ingestion of caffeinated GTE (14). Under exercise conditions the effects of GTE ingestion on upregulating fat metabolism is equivocal at best and there are a limited number of studies. Therefore, the main aim of the series of studies in this thesis (**Chapters 3-5**) was to systematically investigate if GTE ingestion increases fat oxidation during exercise. In addition, these studies were designed to elucidate if the duration of ingestion and the composition of GTE could alter metabolism to a greater degree.

Venables et al (27) were the first to investigate acute (24 h) GTE (~890 mg total catechins) ingestion on fat oxidation during steady state exercise (30 min cycle at ~60% $\dot{V}O_2$max). In this study the authors found GTE to increase whole body rates of fat oxidation by 17% compared to placebo. The first study presented in this thesis (**Chapter 3**) was designed to expand on this previous work and investigated differences between short term (24 h) and longer term (7 days) ingestion on substrate metabolism during exercise. In **Chapter 3**, one group of physically active males received a placebo for 6 days followed by GTE on the final day (~1200 mg total catechins). A second group of participants ingested the same GTE beverage for a total of 7 days. Both groups undertook a steady state exercise trial (60 min cycle at ~55% $\dot{V}O_2$max) before and after the ingestion period.

In contrast to Venables et al (27) we found no difference in fat oxidation in the one day GTE group compared to the baseline trial. This was unexpected as the study design and sample population was similar between the two studies (27). Following 7 days of GTE ingestion, plasma concentrations of fatty acids (FAs) and glycerol were elevated at rest and during

exercise compared to baseline. However despite this increase in fuel availability, fat oxidation rates during the exercise bout were unchanged.

Hodgson et al (13) performed metabolomics analysis on the plasma samples collected in **Chapter 3** (from the placebo and the 7 day GTE group only). It was found that ingestion of GTE did not enhance adrenergic stimulation (indicated by no change in plasma adrenaline or noradrenaline) during exercise. Thus, not providing support for the often proposed acute GTE ingestion mechanism (For more information refer to Introduction 1.13). We also found in the GTE group an increase of glycolytic metabolites during exercise compared to placebo. Therefore, taking together the metabolomics data with the substrate metabolism data obtained in **Chapter 3**, it appears that acute/ longer term ingestion of GTE did not result in the expected metabolic changes.

One of the differences between the study described in **Chapter 3** and the previous work by Venables et al (27) is that in **Chapter 3** the GTE contained a moderate dose of caffeine whereas Venables et al (27) used a decaffeinated extract. In fact in **Chapter 3**, when the participants were consuming GTE, daily caffeine intake by the participants was moderate (240 mg/ day, equivalent to ~3-4 cups of coffee) and on the morning of the exercise trial an additional 120 mg of caffeine was consumed. Interestingly, Hodgson et al (13) also found increases in plasma lactate during exercise in the GTE group, which was not observed in the placebo group. Caffeine has been found to increase glycolysis and has been associated with subsequent inhibition of fat metabolism (12). It is likely that the effect of caffeine is dose dependent, with lower doses stimulating predominantly fat metabolism and higher doses stimulating carbohydrate metabolism more. If this is the case, the moderate caffeine intake in **Chapter 3**, may have counteracted any effect of GTE on fat oxidation and could explain why fat oxidation rates were unchanged.

Therefore, in the next study (**Chapter 4)** we investigated the effects of decaffeinated GTE (dGTE: containing ~1140 mg total catechins/ day) on exercise metabolism (during a 30 min steady state cycle at ~55% $\dot{V}O_2$max) following a single bolus and 7 days of ingestion. We also changed the design of the study from a parallel design with 2 groups to a cross over study as was the case in study by Venables et al (27). One final addition in this study was that participants continued to consume the extract for a total of 28 days, following which a further exercise test was performed. This was designed to gain insight in the mechanisms that would be responsible for potential effects of long term GTE ingestion (reader is referred to section 1.16 of the introduction). In this cross-over placebo controlled study we again found no effect of GTE ingestion on exercise metabolism at any of the measured time points (single bolus, 7 days and 28 days). Furthermore, no change was found in plasma metabolites (FA and glycerol) at rest or during exercise when compared to the placebo trials.

These findings are in agreement with a recent study by Eichenberger et al (9) in which endurance trained males ingested GTE (160 mg total catechins and 30 mg caffeine/ day) and placebo for three weeks, in a cross-over study design. Following both intervention periods participants completed a 2 hour steady state cycle ride. Similar to our findings (**Chapter 4**), Eichenberger et al (9) found no change in fat related plasma metabolites or substrate metabolism compared to placebo. Thus, our data (**Chapter 3 and 4**) and the data reported by Eichenberger et al (9) suggests that irrespective of feeding duration, GTE ingestion does not change substrate metabolism during steady state exercise in physically active males.

It is well known that large inter-individual variation exists in absolute fat oxidation during exercise performed at the same absolute and relative intensity (1, 10, 25, 26). In addition, the exercise intensity at which fat oxidation is maximal (FATMAX) can also vary significantly between individuals (10, 26). Therefore it may be difficult to determine the true effect of a nutritional intervention on fat metabolism during steady state exercise (set at a standardised

intensity), as it may result in some people exercising above or below FATMAX. It is also possible that effects of an intervention are dependent on the exercise intensity and whether fat oxidation is close to maximal fat oxidation in a control condition. In **Chapter 5** we employed a graded exercise test (FATMAX test) to measure substrate metabolism over a wide range of exercise intensities following acute (24 h) dGTE and placebo ingestion. In this study physically active males consumed a standardised diet, in the 24 h prior to the exercise trial, which was similar in composition to their habitual diet. This was implemented in order to eliminate any effects that change in habitual diet may have on substrate metabolism (in the previous chapters all participants consumed the same standardised diet) (6, 7). Participants also consumed dGTE or placebo in this 24 h period and an additional dose two hours before the exercise trial.

We constructed fat oxidation curves (absolute fat oxidation rates plotted against exercise intensity) for both of these intervention groups and observed no difference in the shape of the fat oxidation curves between the dGTE and placebo trials. Furthermore there were no statistical differences in absolute fat oxidation rates at any given exercise intensity between the two interventions. These findings in addition to our previous findings suggest that GTE has no effect on fat oxidation during exercise.

In **Chapter 5** I used, for the first time, a mathematical model in order to construct an average metabolic profile (absolute fat oxidation rates plotted against exercise intensity) for both interventions. I validated this mathematical model against manual analysis (the analytical technique used in previous studies (1, 3, 26)) and found that methods produced similar fat oxidation curves (**Chapter 2** and **Chapter 5**). Therefore I used this model in **Chapter 6** to describe the fat oxidation-exercise intensity relationship of a large athletic population.

Using this mathematical model I was able to construct a fat oxidation curve for each individual. As I had observed in **Chapter 5**, some individuals have fat oxidation curves that are distinctly different from others. Based on these observations, we distinguished two categories, and using well defined (but arbitrary) criteria we introduced two new terms (metabolic types): fat metabolic type (FMET) and carbohydrate metabolic type (CMET). FMET individuals displayed an increase in fat oxidation with increases in exercise intensity until FATMAX occurred. On the other hand, METC individuals displayed the highest rates of fat oxidation at the lowest exercise intensity (walking a 5 km/h^{-1}) and any increase in exercise intensity resulted in a decrease in absolute rates of fat oxidation. To our knowledge this is the first study to categorise individuals based on metabolic type according to set criteria. Although we found body composition, age, gender and aerobic capacity to explain some of variance, and diet may explain another part, a relatively large component of the variation is still unexplained. The next step for this area of research would be to run metabolomic analysis to establish if CMET and FMET individuals differ in their blood metabolic response during a FATMAX test. This may provide more systematic criteria to define the two metabolic types.

7.2 Limitations and Future Directions

Collectively the three studies presented in **Chapter 3, 4 and 5** question the efficacy of GTE ingestion on increasing fat oxidation rates. It is possible that different effects may be found in other populations as these studies were all performed in physically active males. It cannot be excluded that a population with a lower aerobic capacity would display different results. In this thesis we recruited healthy, physically active males who were exercising at least 3 days a week for 30 – 90 minutes. The average $\dot{V}O_2$max of these participants was ~50 $ml \cdot kg^{-1} \cdot min^{-1}$, indicative of a moderately trained population. Exercise training results in skeletal muscle

adaptations in favour of fat metabolism. Therefore, it could be assumed that the individuals in our studies already had a greater capacity to oxidise fats and any increase in fat oxidation from a nutritional intervention may go undetected. To support this proposed theory, in **Chapter 4** we performed additional analysis and separated participants into two groups; 1) individuals who had a $\dot{V}O_2$max higher and 2) individuals who had a $\dot{V}O_2$max lower than the mean average. Although no statistical difference was found between the two groups the graph in Figure 7.1 illustrates that the percent change in fat oxidation following dGTE ingestion was greater in the low $\dot{V}O_2$max group compared to the high group. Furthermore, studies which have found chronic (10 weeks) GTE ingestion to increase fat oxidation rates during exercise have been undertaken in healthy but sedentary individuals (16, 21). However, in these studies they combined 10 weeks of GTE ingestion with an exercise training program. Future studies should investigate the effects of chronic GTE ingestion on sedentary individuals (not undertaking an exercise program) to establish if GTE ingestion has an effect in this population.

Individuals can now be classed into one of two metabolic types: FMET or CMET (using the metabolic type criteria described in **Chapter 6)**. Using this classification system future research may want to explore the effects of a nutritional intervention on certain metabolic types. From revisiting the data in **Chapter 5** it is apparent that the majority of the participants were CMET (illustrated in Figure 5.2). In these individuals acute dGTE feeding did not alter fat oxidation rates. There are many nutritional beverages, foods, supplements which claim to increase 'fat burning' however there is often a lack of conclusive literature to confirm these effects (17). It may be possible that the metabolic type of an individual may impact the effectiveness of some of these 'fat burners'. Therefore, future research may wish to screen and group individuals based on metabolic profile to establish if a nutritional intervention has more or less of an effect depending on metabolic type.

Future research may also wish to explore the possible fat metabolism enhancing effects of GTE in aging and overweight populations, and the implications this may have on disease prevention and health. Overweight/ obese individuals have high levels of plasma FAs and IMTGs. However, they have a reduced capacity to oxidise these available fat stores (18), a situation which can lead to the development of Type II diabetes (18). Therefore, endurance exercise is often prescribed to obese individuals to promote weight loss and also increase fat oxidation rates (11). One study has found 12 weeks ingestion of GTE catechins, in conjunction with exercise training, to promote weight loss in overweight individuals (19). However, these findings cannot be attributed to increases in fat metabolism as substrate metabolism was not measured. Therefore, it would be of great interest to investigate if GTE ingestion could further augment fat oxidation rates in obese/ overweight individuals undergoing an exercise training program. In 2008, Murase et al (20) observed, in accelerated aging mice, that the age related decline in endurance capacity was prevented when fed a 0.35% GTE diet (combined with exercise training). This finding was paralleled with greater skeletal muscle β oxidation rates (20). It is unknown if the same effects would be found in aging humans and the implications this may have on healthy aging.

Ethnicity may also determine the potency of GTE ingestion. The enzyme COMT is thought to play a crucial role in augementating fat oxidation following GTE ingestion (see introduction for more detail). However, COMT activity levels have been found to vary between individuals, with heredity and ethnicity playing a crucial role in determining the enzymatic activity (22, 28). The frequency of low activity COMT allele in Caucasian individuals is as high as 50% compared to 20-30% in Asian individuals and only 6% observed in Ghanaians (4, 22). A recent meta-analysis reported that GTE ingestion had a greater effect on weight loss in Asian populations (1.51 kg) than Caucasian individuals (0.82 kg) (15). In addition, studies that have found chronic ingestion of a GTE to increase fat

oxidation rates during exercise were conducted in Asian countries (16, 21). In this thesis all participants were Caucasian; therefore, it could be suggested that GTE was less potent in this sample population. Therefore, GTE may have a more potent effect in Asian populations or populations with a high COMT activity level.

Finally, future research should investigate the optimal time point at which to measure fat oxidation. At the onset of exercise, substrate metabolism is upregulated several fold compared to resting conditions (please refer to the introduction for a more detailed explanation). Day-to-day variation in fat oxidation rates during exercise have been found to be around 10% (2) (Randell et al, **Chapter 5**). Therefore, the fat metabolism enhancing effects of GTE may be small and go undetected, especially when fat oxidation rates are elevated as a result of exercise. Despite consistently finding no effect of GTE on exercise metabolism, we did find 7 days of GTE to increase plasma FA metabolites under pre-exercise resting conditions and during the exercise bout (**Chapter 3**). Unfortunately, more sophisticated measures (isotopic tracers or muscle biopsies) were not used to determine the fate of these FAs.

In summary, to elucidate if GTE ingestion does have any substrate enhancing effects future studies may wish to investigate:

1) GTE ingestion on sedentary, overweight/obese and aging individuals

2) The impact of metabolic type on the effects of GTE ingestion

3) The interaction between ethnicity and GTE

4) Use blood metabolomic analysis to systematically define metabolic type

7.3 References

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