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Effects of raspberry ketone and green coffee bean extract on body mass and substrate utilisation

by

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Abstract

Dietary food supplements are becoming increasingly popular and while many claim to aid weight loss, there is limited evidence to support such claims. Raspberry ketone and green coffee bean extract have both been marketed for weight loss and are often sold in a combined form. The aim of the present study was to test whether raspberry ketone and green coffee bean extract, when consumed separately and in combination, affect substrate utilisation and body composition. In a single blind randomised control trial, 32 healthy recreationally active males and females (age: 30.4 ± 1.5 years; BMI: 26.7 ± 0.4 kg.m^{-2}; body mass: 79.0 ± 1.8 kg; $\dot{V}O_{2max}$: 37.7 ± 1.4 ml.kg^{-1}.min^{-1}) were randomly assigned to four different treatment groups for 8 weeks; raspberry ketone (RK) (200 mg/day), green coffee bean extract (CGE) (400 mg/day), raspberry ketone plus green coffee bean extract (RK+CGE) (200 mg/day plus 400 mg/day) or control (200 mg/day). Resting metabolic rate, anthropometry, body composition and metabolic responses during a 30-minute steady state exercise test (55% $\dot{V}O_{2max}$) were measured at baseline and repeated post intervention. There was no effect of either supplement alone, or in combination on body mass (Baseline vs post intervention - RK: 82.5 ± 3.8 vs 81.6 ± 3.9, CGE: 81.1 ± 3.0 vs 81.0 ± 3.2, RK+CGE: 75.9 ± 3.8 vs 74.5 ± 4.0, Control: 76.7 ± 4.1 vs 76.3 ± 4.2kg (p>0.05)) or body fat (Baseline vs post intervention - RK: 25.6 ± 3.3 vs 25.9 ± 2.7, CGE: 28.2 ± 4.3 vs 28.7 ± 4.2, RK+CGE: 33.0 ± 3.8 vs 32.4 ± 3.7, Control 25.8 ± 3.6 vs 25.8 ± 3.3% (p>0.05)). Similarly, no effect on substrate utilisation was observed either at rest (Baseline vs post intervention RER - RK: 0.83 ± 0.02 vs 0.84 ± 0.01, GCE: 0.84 ± 0.02 vs 0.83 ± 0.01, RK+GCE: 0.85 ± 0.03 vs 0.82 ± 0.03, Control: 0.82 ± 0.02 vs 0.84 ± 0.01 (p>0.05)) or during exercise (Baseline vs post intervention RER - RK: 0.89 ± 0.01 vs 0.87 ± 0.02, GCE: 0.90 ± 0.02 vs 0.90 ± 0.01, RK+GCE: 0.85 ± 0.01 vs 0.84 ± 0.01, Control: 0.86 ± 0.02 vs 0.87 ± 0.02 (p>0.05)). In conclusion, this study demonstrated that 8-weeks of supplementation with raspberry ketone, green coffee extract and a combination of these supplements had no effect on body mass, body composition or substrate utilisation.

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Completing my masters alongside working full time as a technician has been challenging at times, however I have found it to be a rewarding experience and something I am glad I finally got the chance to do.

Authors Declaration

I declare that, unless otherwise stated by acknowledgment or reference to published literature, the presented work in this thesis is the author's own and has not been submitted for any other degree at the University of Glasgow or another institution.

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Lynsey Johnston

Date:

Definitions/Abbreviations

AMPK	5' adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BMI	body mass index
CGA	cholorgenic acid
CHO	carbohydrate
EE	energy expenditure
EI	energy intake
FA	fatty acids
FFA	free fatty acids
FAD^+	flavin adenine dinucloetide
Fat_{max}	maximal fat oxidation
GCE	green coffee extract
HSL	hormone sensitive lipase
HHQ	hydroxyhydroquinone
IS	Insulin
LEP	Leptin
NAD^+	nicotinamide adenine dinucleotide
OGTT	oral glucose tolerance test

RK	raspberry ketone
RK + GCE	raspberry ketone + green coffee extract
RMR	resting metabolic rate
SEM	standard error of mean
TAG	triacylglycerol
TCA	tricarboxylic acid
T2D	type 2 diabetes
$\dot{V}O_2$	rate of oxygen consumption
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}O2_{max}$	maximal oxygen consumption
WHO	World Health Organisation
W_{max}	maximum power

1 Introduction

1.1 Obesity and Energy Balance

Overweight and obesity represent a rapidly growing threat to the health of populations in an increasing number of countries (World health organisation (WHO) 2000). Obesity is defined medically as a state of increased body weight, more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences. Worldwide obesity has almost tripled between 1975 and 2016 (WHO 2018). The epidemic of obesity took off from the 1980s and has been increasing inexorably ever since, however only in 1997 did the World Health Organisation (WHO) recognised the importance of obesity and it is now treated as a major public health problem around the world (James 2008). A commonly used scale to categorise the degree of excess weight is the body mass index (BMI) scale, this is calculated by dividing the weight in kilograms by the square of the height in meters (kg/m^2). The WHO defines overweight as a BMI equal to or more than 25 and obesity as a BMI equal to or more than 30 (World Health Organisation (WHO), 1995). According to the recent worldwide health survey in 2016, more than 1.9 billion adults, 18 years and over, were overweight, of these over 650 million were obese (WHO 2018). That equates to 39% of adults being overweight and 13% being obese. In Scotland alone, 65% of adults aged 16 and over are overweight (BMI > 25), including 29% who were obese (BMI > 30) (Scottish Health Survey. 2016). The prevalence is also seen in children, in 2016 it was reported 41 million children under the age of 5 were overweight or obese (WHO 2018). The UK's Foresight analysis has projected that 60% and 40% of men and women, respectively, will be clinically obese by year 2050 (James 2008). Obesity and physical inactivity are clearly associated with increased mortality and adverse health outcomes, particularly an increased risk of developing cardiovascular disease and type 2 diabetes (T2D) (Sullivan *et al*. 2005; Warburton *et al*. 2006).

Overweight and obesity are the result of an imbalance between energy intake and energy expenditure resulting in a positive energy balance. A negative energy balance is required for weight loss and this can be achieved by either decreasing energy intake or increasing energy expenditure, normally via lifestyle modifications such as diet and exercise. Energy intake consists of all food and

beverages with energy value and energy expenditure is represented by three major components; resting metabolic rate (RMR), physical activity and dietary induced thermogenesis (Spiegelman and Flier, 2001). Resting metabolic rate represents the largest portion of total energy expenditure, this is the energy associated with the maintenance of body functions and constitutes 60 to 75% of daily energy expenditure (Poehlman 1989). Thermogenesis is the energy expenditure associated with ingestion of food and constitutes around 10% of daily energy expenditure. Energy expenditure associated with physical activity is the most variable component and can constitute 15 to 30% of 24 hour energy expenditure (Poehlman 1989). When energy intake equals energy expenditure, energy balance is achieved and therefore body weight remains stable. Although, as simple as this concept may sound, it is often difficult for people to achieve this desired energy balance. However, it is not as simple as 'eat less and exercise more', other external factors such as stress and sleep quality/duration can also affect energy balance (Ogilvie *et al*. 2016).
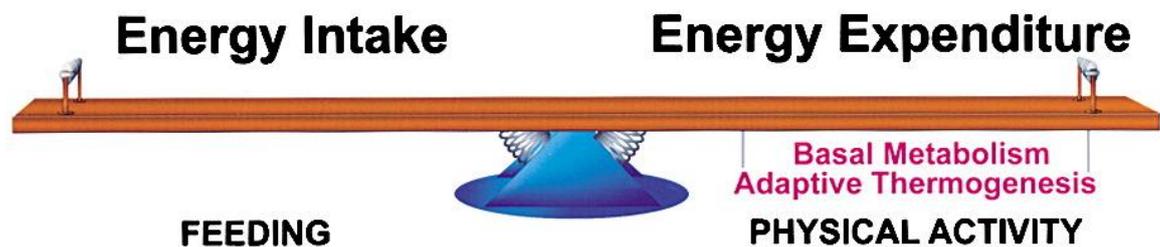
Figure 1. Key components of the energy balance system.
(Spiegelman and Flier, 2001)

1.1.1 Diet and Weight Loss

As previously mentioned, a reduction in energy intake is required for weight loss to occur and this can be achieved in a variety of ways. A systematic review and meta-analysis of weight loss clinical trials reported eight types of weight loss interventions; diet alone, diet and exercise, exercise alone, meal replacements, very-low energy diets, weight loss medication and advice alone. They reported diet alone (51 studies of the total 80 studies in the review), diet and exercise, meal replacements and weight loss medication with diet produced the most encouraging short-term results (Franz *et al*. 2007). They reported that during the

first 6 months, studies involving diet alone experienced a mean weight loss of 4.9kg, diet and exercise 7.9kg, meal replacements 8.6kg and weight loss medication 8.3kg, following this, weight loss begins to plateau across all interventions in the review. One of the 'diet alone' studies from the review investigated 78 obese females who followed either a 'diet' or 'non-diet' intervention for 6 months, with the 'diet' group reporting a reduction in body weight of 5.9 $\pm$ 6.3kg compared to -0.1 $\pm$ 4.8kg in the 'non diet' group (Bacon *et al*. 2002). A more recent systematic review and meta-analysis in 2017, investigating the effects of weight loss intervention in obese adults, also concluded weight reducing diets (usually low in fat), with or without exercise may reduce premature all-cause mortality in adults with obesity (Ma *et al*. 2017).

1.1.2 Physical Activity

At the other end of the scale, an increase in energy expenditure can result in a negative energy balance, this can be achieved by increasing physical activity/exercise. Exercise, either alone or in combination with diet, can be fundamental in tackling overweight and obesity and the prevention and management of cardiovascular and metabolic diseases. However for weight loss, exercise alone is not as effective when compared to diet alone, although it can enhance the effects of a weight loss programme or as a prevention mechanism. In the systematic review by Franz *et al*, 2007, it was concluded that clinical trials investigating exercise-alone and advice-alone interventions reported minimal weight loss at any time point, with exercise alone trials reporting a mean weight loss of only 2.4kg. Findings from epidemiological studies, however, have consistently shown that regular physical activity prevents unhealthy weight gain and obesity (Saris *et al*. 2010), although the amount of physical activity require to prevent long-term weight gain is unclear. In 2010, Lee *et al*, examined the association of different amounts of physical activity with long-term weight changes among women who consumed "usual" diet. From 1992-2007, a cohort of 34,079 healthy women took part in the trial. The women reported their physical activity and body weight at baseline and months 36, 72, 96, 120, 144 and 156. It was concluded that there was a significant interaction between BMI and physical activity levels, such that there was an inverse dose response relationship between activity levels and weight gain among women

with a BMI< 25, but no association in those with BMI>25. Over the 13 years follow-up, the women who maintained their normal body weight and gained fewer than 2.3kg were participating in, on average, 60 minutes of moderate intensity activity daily.

The WHO has set recommended levels of physical activity to promote 'healthy living' in adult's aged 18-64 years, their guidelines state that adults should do at least 150 minutes of moderate-intensity physical activity throughout the week or do at least 75 minutes of vigorous-intensity physical activity throughout the week or an equivalent combination of moderate and vigorous intensity activity and also do 2 sessions of muscle strengthening activities a week (WHO 2011). In 2016, a health survey in Scotland reported that only 64% of adults met these recommended health guidelines (Scottish Health Survey. 2016). For preventing weight gain or regain, these guidelines are likely to be insufficient for many individuals. It is thought that 60-90 minutes of moderate intensity exercise, or a lesser amount of vigorous exercise, per day is required for prevention of weight gain (Saris *et al*. 2003), although further work is needed to confirm this. The combination of aerobic and resistance training has greater health benefits than doing only one form of exercise, these include improving body composition, muscular strength, glycaemic control and cardiovascular fitness (Maiorana *et al*. 2002), and this would also be recommended for maintaining weight loss (Tsai and Bessesen, 2019). According to a report from the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society, the guidelines for management of overweight and obesity is a comprehensive lifestyle intervention including; prescription of a reduced-calorie diet, at least 150 minutes of moderate-intensity physical activity per week (with 200–300 minutes for maintenance of weight loss per week), and behavioural strategies to achieve diet and physical activity targets (Jensen *et al*. 2014).

1.1.3 Alternative Weight Loss Methods

As detailed above, commonly used methods for weight loss and control are dietary guidance, exercise and pharmaceutical approaches, which are often successful in the short term. However, long-term weight maintenance after weight loss is often unsuccessful due to lack of adherence to the lifestyle and

behavioural modifications. A recent study in 2016, investigated the long term effectiveness of a randomised, tailor-made lifestyle intervention consisting of diet (visits to the dietitian with tailor-made advice given, such as a low-fat or a low calorie diet, or both) and exercise (participation in physical activity classes such as dancing or low-intensity sports, such as Nordic walking or volleyball) (De Vos *et al*. 2016) in overweight females in primary care. The intervention lasted 2.5 years, after the first 6 month, weight loss was significantly higher in the intervention groups, however after 24 months the difference decreased and became non-significant (De Vos *et al*. 2016). Possible reasons for this is a lifestyle change involving a behavioural modification of diet and/or exercise often requires effort, is time consuming, compliance is often poor, and the effects are only visible in the longer term, thus people seek alternative methods. These methods include meal replacements, dietary and nutritional supplements such as vitamins, minerals and botanicals, with these becoming increasingly popular as a wide variety of these products are available over the counter.

In 2012, the dietary supplement industry in America reported sales greater than $32 billion (Garcia-Cazarin *et al*. 2014) and over $1.6 billion per year of this was due to weight-loss supplements alone (Spano and Antonio, 2008). Raspberry ketone and green coffee bean extract are reported as the most popular supplements for weight loss following heightened interest in the media (Bootsman *et al*, 2014). Some of the products available have been tested, individually, to assess their mechanisms of action and their ability to modulate fat metabolism. For example, green tea rich in phenolic compounds and caffeine have some evidence to support elevated fat oxidation at rest and during exercise (Hodgson *et al*. 2013, Hodgson *et al*. 2013). One of the often-cited mechanism regarding the effects of green tea on substrate utilisation is the metabolic pathway of green tea catechin degradation on O-methylation, which is catalysed by catechol-O-methyltransferase (COMT) (Hodgson *et al*. 2013). It is proposed that inhibition of COMT and the resulting increased and/or prolonged effects of norepinephrine, including increased energy expenditure and fat oxidation (Dostal *et al*. 2016). However, despite a number of studies observing positive effects in fat metabolism, overall the literature is inconclusive. A variety of nutritional supplements are often referred to as 'fat burners' and claim to

acutely increase fat metabolism or energy expenditure, increase weight loss, increase fat oxidation during exercise or somehow cause long-term adaptations that promote fat metabolism (Jeukendrup A.E and Randell R. 2011). However, the evidence supporting the effects of individual diet supplements on weight loss remains ambiguous and most marketing strategies for the supplements now focus on combined supplements to give 'enhanced' effects. Despite these claims there have been no studies to address the effects of combined supplement approaches to enhance rates of fat oxidation or weight loss. As many of these supplements purport to alter metabolism it is prudent, therefore, to briefly discuss the metabolic processes underlying the generation of energy.

1.2 Metabolism

Metabolism is typically subdivided into reactions in which chemical substances are broken down to generate energy, known as catabolism. Intracellular glucose and lipid metabolic homeostasis is vital for maintaining the basic life activities of a cell or an organism. Mitochondria are cellular organelles and are responsible for generating energy in the form of adenosine triphosphate (ATP). The generation of energy by the mitochondria involves several stages which are collectively known as cellular respiration, specifically aerobic respiration. In glucose metabolism, ATP is generated via glycolysis, the citric acid cycle, oxidative phosphorylation and the electron transport chain (Houston 1995). Lipid metabolism, involves lipid synthesis and oxidation through the processes known as lipogenesis and lipolysis respectively, and these will now be discussed in more detail. The interplay between glucose and lipid metabolism is summarised in figure 2.
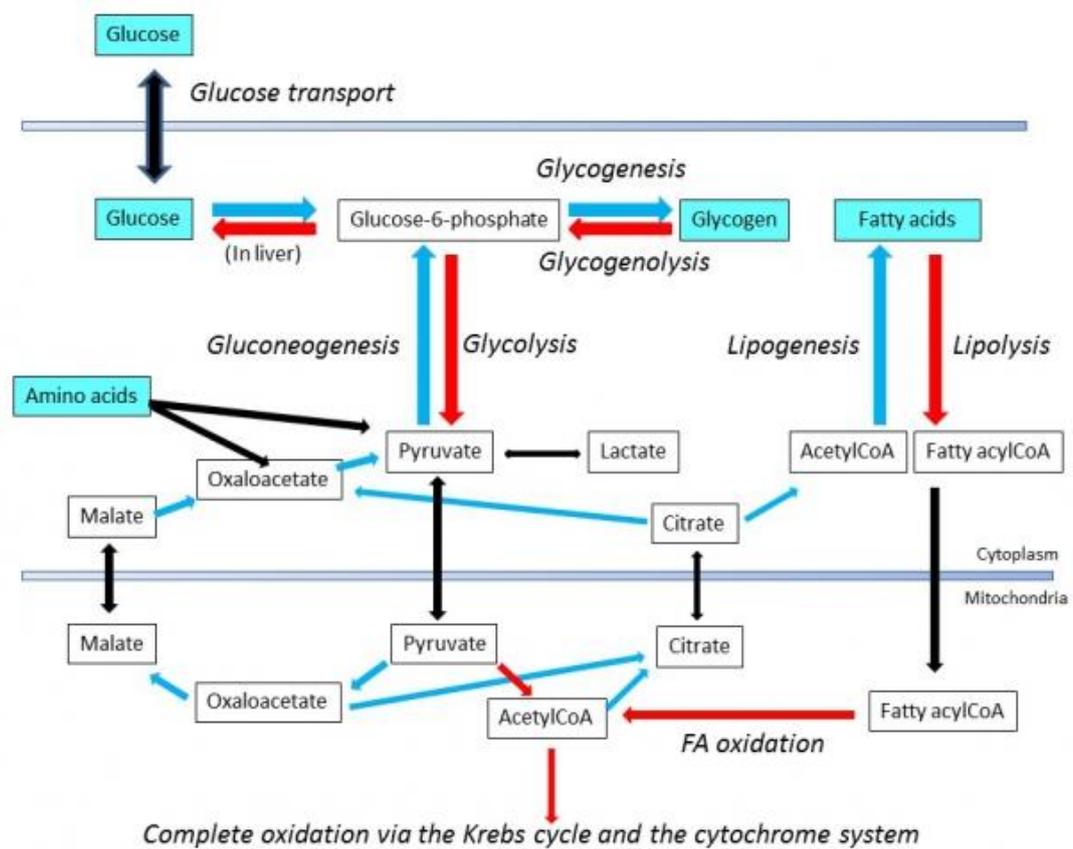
Figure 2. Interplay between glucose metabolism and lipid metabolism. (Ward, 2015).

1.2.1 Glycolysis

Carbohydrate, in the form of glucose, is broken down to produce pyruvate in the cell cytosol in a metabolic pathway consisting of a series of enzyme-catalysed reactions known as glycolysis. The glucose needed for glycolysis comes mainly from ingestion of carbohydrates or from stored glycogen. The body also has a limited ability to produce glucose from non-carbohydrate sources, such as amino acids, lactate and glycerol, when carbohydrate stores are low through a process known as gluconeogenesis.

Glycolysis is a pathway that can generate ATP, it generates a net production of 2 ATP, and it also plays a major role in providing the substrates for the citric acid cycle in the form of pyruvate. Glycolysis occurs in the cytoplasm of cells and is governed by the energy need of the cell tissues containing glycogen include liver, skeletal muscle and heart muscle. When either glucose or glycogen are initially broken down, glucose 6-phosphate is produced, this process is then

followed by a series of 10 reactions (see figure 3), finally resulting in production of pyruvate. Under anaerobic conditions, pyruvate is reduced to lactate, or in aerobic conditions enters the mitochondria membrane where it is used as a fuel for oxidative phosphorylation (Houston 1995).
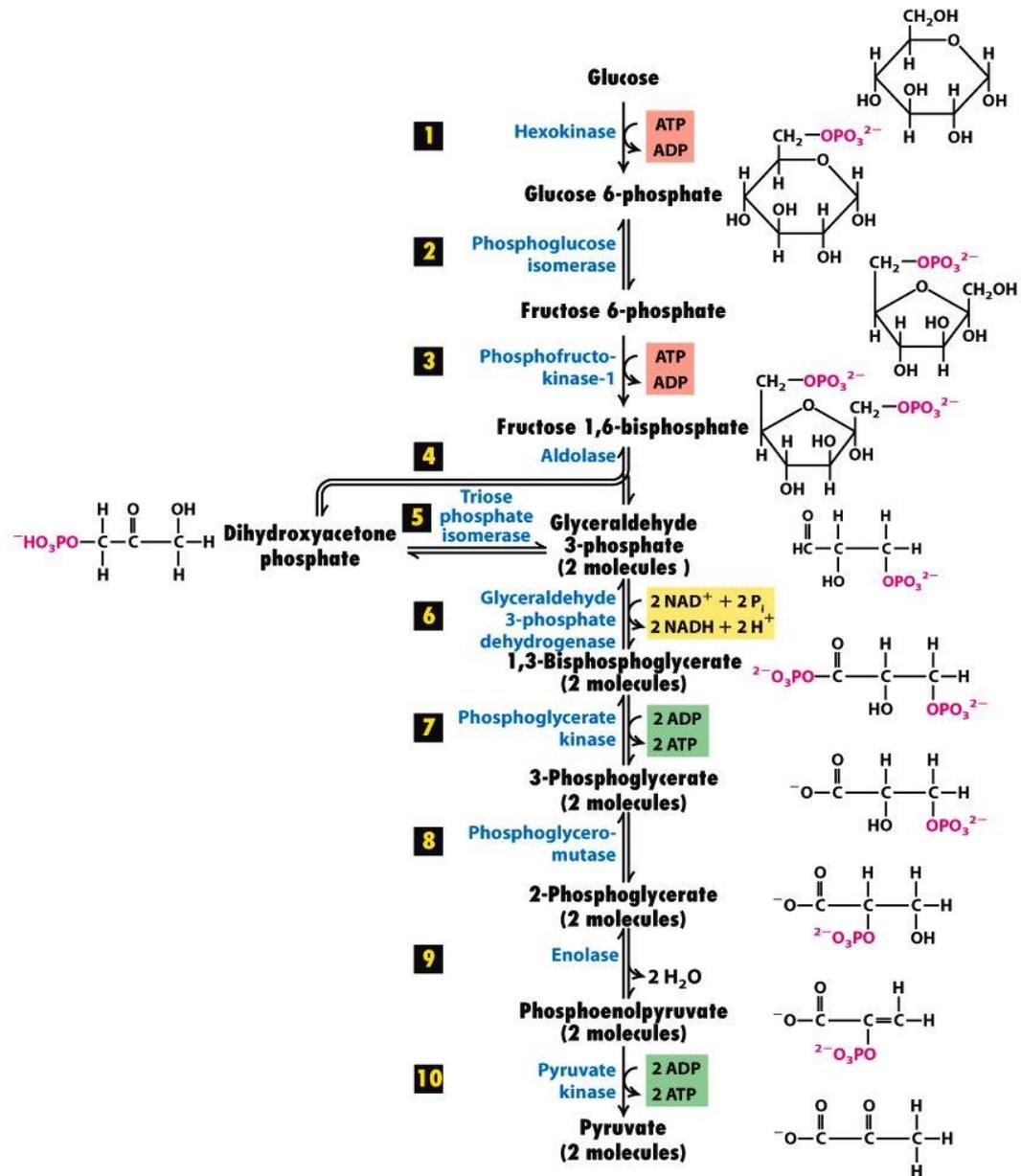
Figure 3. Stages of Glycolysis with the structures of all the intermediates. (Berk *et al.* 2008).

1.2.2 The Citric Acid Cycle

The citric acid cycle, also known as the tricarboxylic acid (TCA) or Krebs cycle, is a series of chemical reactions in which acetyl groups are oxidised to produce energy in the form of ATP. Under aerobic conditions, the pyruvate generated from glucose in glycolysis is converted to form acetyl CoA which then enters the citric acid cycle. The citric acid cycle is also tightly related to the electron transport chain as it removes electrons from the acetyl groups and attaches them to coenzymes NAD^+ and FAD^+, which are then fed into the electron transport chain, both these are linked to the rate of ATP utilisation (Houston 1995).

The citric acid cycle is a circular pathway catalysed by 8 enzymes (see figure 4), all of which are located in the mitochondrial matrix, except succinate dehydrogenase. It takes two turns of the cycle to process the equivalent of one glucose molecule. Acetyl groups containing two carbon atoms enter the pathway, one attaches to CoA and the other to oxaloacetate to form citrate. In the final reaction, malate results in the formation of oxaloacetate and NADH + H^+, due to the oxidation by enzyme malate dehydrogenase, this starting substrate forms a new round of the cycle. The cycle produces three NADH molecules, one flavin adenine dinucleotide ($FADH_2$) and ATP. The NADH and $FADH_2$ produced from the cycle can then enter the electron transport chain for oxidative phosphorylation (Houston 1995).
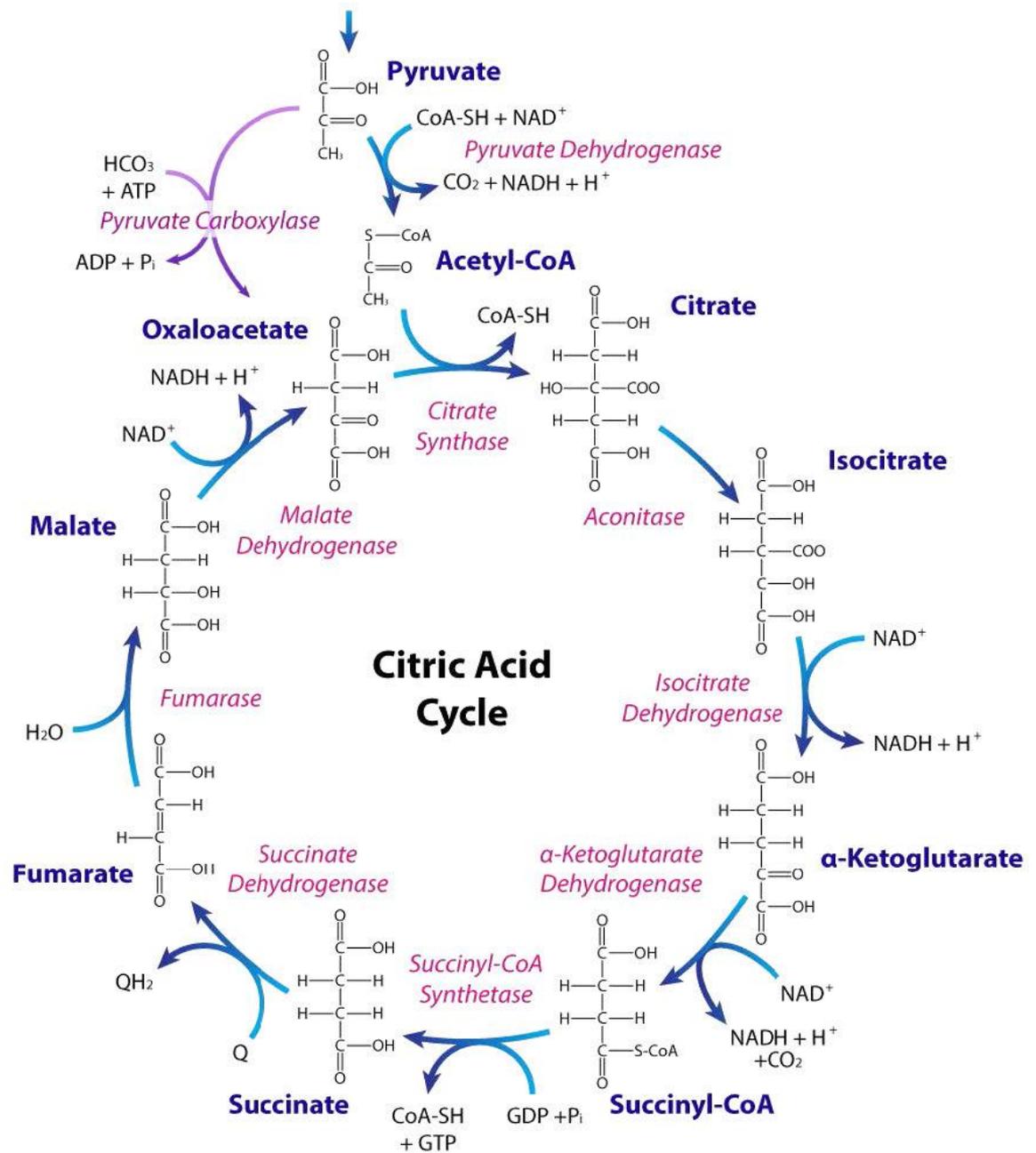
Figure 4. The citric acid cycle with the structures of all the intermediates. (Ahern *et al.* 2019).

1.2.3 Oxidative Phosphorylation

Oxidative phosphorylation occurs in the mitochondria and is the formation of ATP from ADP and inorganic phosphate (Pi) in association with the transfer of electrons from NADH or $FADH_2$ to oxygen by a series of electron carriers. It provides more that 85% of the ATP required, with the remaining arising from glycolysis. Oxygen is transported in the blood then delivered to the mitochondria following diffusion from the small capillaries into the cells. ATP synthesis occurs

in the inner mitochondrial membrane and is driven by the flow of protons back into the mitochondrial matrix in the electron transport chain (Houston 1995).

1.2.4 Electron Transport Chain

The electron transport chain is the last component of aerobic respiration and occurs on the inner membrane of the mitochondria. The chain comprises four complexes (I, II, III & IV) and a series of electron transporters, electrons are passed from donors to acceptors down the chain, releasing energy which is utilised to generate a proton gradient across the mitochondrial. Electrons from NADH and $FADH_2$ pass through the protein complexes in the electron transport chain, in the fourth complex where the electrons are accepted by oxygen which then undergoes a reduction reaction, producing water. If oxygen is not present, the reaction does not occur. Hydrogen ions diffuse through the inner membrane through an integral membrane protein known as ATP synthase. The force from the hydrogen ion diffusion through the membrane protein regenerates ATP from ADP, this flow of hydrogen ions across the membrane through ATP synthase is known as chemiosmosis (Houston 1995).

1.2.5 Glycogenolysis

Glycogenolysis is the breakdown of glycogen to glucose-1-phosphate and glycogen (n-1), this takes place in muscle and liver cells when energy demands need to increase due to low levels of ATP. Glycogen is broken down to glucose-1-phosphate, the enzyme phosphoglucomutase converts this to glucose 6-phosphate, which can then be used to produce ATP. In muscle cells glucose 6-phosphate is an immediate source for glycolysis and in the hepatocytes of the liver glucose is released into the bloodstream for uptake by other cells. Glycogenolysis is regulated by hormones in response to blood sugar levels, the hormones glucagon and insulin in the blood raise and lower glucose levels respectively. In addition, it is stimulated by epinephrine, a hormone produced during a time of stress or when the bodies 'fight-or-flight' response is activated (Houston 1995).

1.2.6 Lipolysis

Lipolysis is one of the most important metabolic pathways regulating adipose tissue mass and thus obesity. The stored form of fat (or lipids) found in adipocytes and striated muscle is known as triacylglycerol (TAG) and consists of a glycerol molecule that is bound to three long-chain fatty acids (FA). Fatty acid chains are carbon molecules with hydrogen atoms linking the chains together. Lipolysis is known as the breakdown process of these TAG to form FA and glycerol, this is regulated by hormone sensitive lipase (HSL), resulting in the release of FA, into the blood, which can then be transported to the working muscle for oxidation. When FA enter the blood stream they attach to a blood protein called albumin, these are known as free fatty acids (FFA). For this to occur the FA are converted to fatty acyl CoA then transported into the mitochondrial matrix by attaching to carnitine. Fatty acid molecules are broken down to generate acetyl-CoA in a process known as beta oxidation, this reaction releases acetyl CoA, $FADH_2$ and NADH, which can then feed in to the citric acid cycle. Lipolysis is promoted by epinephrine, norepinephrine, thyroid hormone and growth hormone, whereas it is inhibited by insulin. Lipogenesis is the process in which acetyl CoA is converted to form TAG. It encompasses the process of fatty acid synthesis and triglyceride synthesis, where FA are esterified to glycerol (Houston 1995).

1.2.7 Fat and Carbohydrate Oxidation

Fat and carbohydrate are the fuels most used as an energy source at rest and during exercise. Fat is stored in the form of TAG in subcutaneous adipose tissue and muscle and carbohydrate is stored as glycogen in muscle and liver. At rest, skeletal muscle is the main site of oxidation of fatty acids, especially in the fasted state, with carbohydrate oxidation making only a small contribution. Carbohydrate and fat are predominantly oxidised simultaneously however the relative contribution of these substrates depends on a number of factors, including exercise intensity, duration, diet and training status (Achten and Jeukendrup, 2004). Fat oxidation is relatively high during low-moderate intensity exercise, where metabolism is elevated. It has been reported that maximal fat oxidation (Fat_{max}) rates typically occurs at 45-65% $\dot{V}O_{2max}$ (Achten and Jeukendrup, 2004), after which a decline in the relative rate of fat oxidation is

observed at higher exercise intensities. This is in contrast to carbohydrate oxidation, which increases as a function of the aerobic work rate.

Over the past few decades research has focused on interventions and strategies to increase rates of fat oxidation both at rest and during exercise, these include exercise training interventions (Achten and Jeukendrup, 2004), high-fat diets and low carbohydrate diets (Kian Yeo *et al*. 2011), and more recently dietary food supplements (Jeukendrup and Randell, 2011). There is a plethora of dietary and food supplements that are available on the market that purport to enhance weight loss through suppression of appetite and enhance metabolism, although evidence in support of their use is often lacking. Many aspects of the aforementioned metabolic processes are hypothesised to be altered by commonly used weight loss supplements and some of the most common supplements will now be discussed.

1.3 Weight Loss Supplements

1.3.1 Raspberry Ketone

Raspberry Ketone [4-(4-hydroxyphenyl)-2-butanone] is one of the major aromatic compounds of red raspberry (*Rubus idaeus*) and has for a long time been used for nutritional and medicinal purposes. Raspberries contain an abundance of sugars vitamins, mineral, polyphenols and antioxidants, similar to that of the strawberry and blueberry. Raspberry ketone is widely used as a fragrance in perfumery and cosmetics and by the food industry as a flavouring substance in foodstuffs (Yurie 2003). The content of raspberry ketone in one kilo of raspberries can range from 0.009 to 4.3 mg/kg (Meister 2016), for an adult, the daily exposure of this from fruits and flavourings alone has been estimated to range between 1.8 and 3.8 mg/day (Crispim *et al.* 2010). With the concentration of naturally occurring raspberry ketone being limited and the extraction process being expensive, raspberry ketone can be synthesised chemically via the condensation of p-hydroxybenzaldehyde with acetone or biosynthesised in genetically modified micro-organisms such as bacteria or yeast (Beekwilder *et al.* 2007; Serra *et al.* 2005).

Raspberry ketone is marketed by food supplement suppliers with the daily dose ranging from 100 to 1400 mg/day, this is between 26 and 368 times higher than normally obtained from daily diet (Bredsdorff *et al.* 2015). The structure of raspberry ketone is similar to the structure of capsaicin and p-synephrine (see figure 5), compounds known to exert anti-obesity actions and alter lipid metabolism. Capsaicin (N-[(4-hydroxy-3-methoxyphenyl)-methyl]-8-methyl-6-nonamide), is a primary component of the red chilli pepper and has a thermogenic ingredient which stimulates energy expenditure. It was reported by Kawada *et al.* (1986), to decrease adipose tissue weight and serum TAG content by enhancing metabolism in rats through decreased glycogen in the liver, increased serum glucose concentrations and increased free fatty acids levels. In humans, several studies have shown that capsaicin stimulates thermogenesis by increasing energy expenditure (Lejeune *et al.* 2003; Yoshioka *et al.* 1998). In 1998, Yoshioka *et al*, investigated the effects of adding red pepper to a diet, under four diet conditions. Women were fed either a high fat diet, high fat diet plus red pepper, high carbohydrate diet or a high carbohydrate diet plus red

pepper. Following the allocated meal expired air samples were collected at every 10 min for the first hour after the experimental meals and then every 30 min from 90 min to 210 min after the experimental meals. Results showed the addition of the red pepper to the meal significantly increased diet-induced thermogenesis and fat oxidation, especially in the high fat diet group, resulting in a lower RQ compared with the control meals between 30 and 60 min postprandial. Furthermore, Lejeune *et al*, 2003 reported the effects of capsaicin on substrate oxidation and weight maintenance following a 4 week very-low-energy diet to induce weight loss (average of 7.8 $\pm$ 1.8 % weight loss). The randomised control trial had 91 overweight subjects split into two groups; capsaicin (135mg/day) or placebo group (225mg vegetable oil), subjects took the capsules for a 3-month weight maintenance period. At the end of the 3 month period there was no difference in mean percentage of weight regain, however there was a significant increase in fat oxidation in the capsaicin group. A more recent study by Janssens *et al.*, in 2013, investigated the effects of capsaicin on 24 hour energy expenditure, substrate oxidation and blood pressure, measured in a respiratory chamber. Subjects underwent four 36 hour sessions; eating 100% or 75% of their daily energy requirements plus the addition of either capsaicin (2.56mg) or control with every meal. They found that by reducing the negative energy balance by 25%, fat oxidation rates were higher in the 75% capsaicin group compared to the 100% control group.
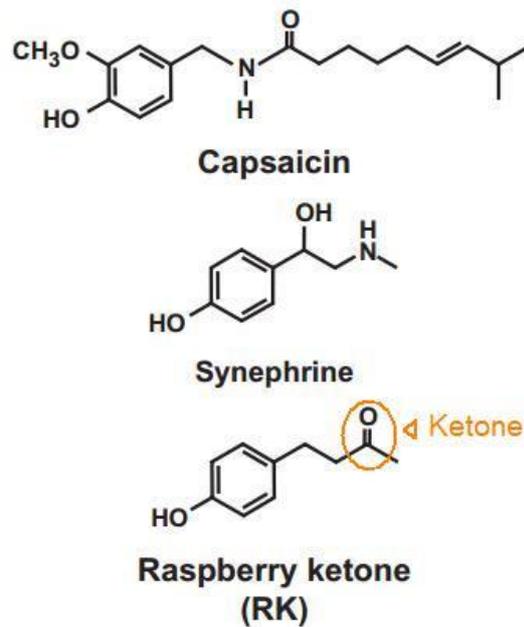
Figure 5. Structural compounds, comparing Capsaicine, P-Synephrine and Raspberry Ketone. (Morimoto et al. 2005)

Similarly, p-synephrine (I-(4-hydroxy-phenyl)-2-methylaminoethanol), a compound in citrus plants including bitter orange, exerts a lipolytic activity in fat cells (Carpéné *et al*. 1999). There have been a few studies investigating the effects of p-synephine when combined with exercise. A study in 2016, twelve healthy men performed a control resistance exercise protocol (6 × 10 repetitions of squats) and were randomly assigned (using a double-blind crossover design with random protocol sequencing) to a supplement sequence: p-synephrine (S; 100 mg), p-synephrine + caffeine (SCF; 100 mg of p-synephrine plus 100 mg of caffeine), or a placebo (P). The author reported that the number of repetitions during squat resistance exercise increased following the ingestion of 1.2 mg/kg of p-synephrine for 3 consecutive days. In addition, 30 minutes post-resistance exercise, energy expenditure increased throughout the 30 minutes (CT = 2.82 ± 0.3; P = 2.81 ± 0.5; S = 2.98 ± 0.3; SCF = 2.97 ± 0.3 kcal/min^{-1}) and fat oxidation rates increased between 25-30 minutes (S = 0.200 ± 0.04; SCF = 0.187 ± 0.06 compared to P = 0.166 ± 0.05 g/min^{-1}) (Ratamess *et al*. 2016). A recent study in 2018 investigated the dose-response effects of p-synephrine on maximal fat oxidation during exercise. The randomised control trial involved 17 subjects allocated to four experimental groups; 1, 2 or 3 mg/kg of p-synephrine or a placebo (cellulose). Subjects performed an incremental exercise test on a cycle ergometer until exhaustion with expired gas being continuously measured. All

the doses of p-synephrine were found to increase maximal fat oxidation during exercise compared with the placebo (0.47 ± 0.11 vs 0.35 ± 0.05 g/min), the highest effects were found with 2 and 3 mg/kg (0.55 ± 0.14 g/min), there was no change in the intensity at which $\dot{V}O_{2max}$ was obtained between the groups (Gutiérrez-Hellín and Del Coso, 2018). In addition, a previous study by Gutiérrez-Hellín and Del Coso, in 2016, reported that a similar dose of 3 mg/kg of p-synephrine did not result in any improvement in physical performance in elite sprinters during maximal running and jump tests. Therefore, with raspberry ketone being structurally similar to both capsaicin and p-synephrine, it could be hypothesised that raspberry ketone would also influence the lipid metabolism on adipocyte.

1.3.1.1 Current Literature

The current literature on raspberry ketones focuses on either *in vitro* or *in vivo* studies. An *in vitro* study using adipocytes (fat cells) investigated the effects of raspberry ketone on both lipolysis and fatty acid oxidation in 3T3-LI adipocytes, it was observed that treatment with 10 µM of raspberry ketone (99% purity), premixed with culture medium, increased lipolysis and fatty acid oxidation, suppressed lipid accumulation, and increased both the cellular and the secretion levels of adiponectin (Park 2010). The lipolytic effect of the cells treated with 10 µM of raspberry ketone induced a 3-fold greater release of glycerol into the culture medium compared to the control and the fatty acid oxidation levels of palmitic acid were assessed, reporting a significantly higher degree of oxidation in the cells pre-treated with raspberry ketone compared to the control (Park 2010). Similarly, a further study by Park, in 2015 investigated the effects of raspberry ketone on the adipogenic and liopgenic gene expression in adipocytes in mice and concluded raspberry ketone (10 µM) inhibits the process of adipocyte differentiation, as they reported a reduction in the expression of genes involved in adipogenesis and lipogenesis. In addition, it was reported that raspberry ketone enhanced the expression of genes involved in lipolysis, and fatty acid oxidation, including HSL and adipose triglyceride lipase (ATGL) (Park 2015), these finding are consistent with the previous finding from Park in 2010.

Meanwhile, *in vivo* studies in male mice, where mice were fed either a high-fat-diet including 0.5,1 or 2% of raspberry ketone (99% purity) for 10 weeks or a

high-fat-diet for 6 weeks and then fed the same high-fat-diet this time containing 1% raspberry ketone (HFD + (HFD + 1%) for the next 5 weeks (Morimoto *et al*. 2005). It was reported that raspberry ketone prevented high-fat diet-induced increases in body weight, visceral adipose tissue weight and the weight of the liver as shown in the HFD + (HFD + 1%) group. Raspberry ketone supplementation also decreased hepatic TAG content after they had been increased from the initial high-fat diet. They reported raspberry ketone supplementation significantly increased norepinephrine-induced lipolysis in white adipocytes and enhanced thermogenesis in brown adipose tissue, this was measured by the amount of glycerol released into the medium and could explain the findings of this anti-obesity effect (Morimoto *et al*. 2005). These results indicate that raspberry ketone prevents and improves obesity and fatty liver induced by feeding a high-fat-diet. Similarly, Meng *et al*. (2008) reported raspberry ketone could reduce the weight of obese rats fed high-fat-diets. They suggest that by mediating lipid disorders; like improving insulin resistance and reversing leptin resistance, this could exert an anti-obesity function. In 2011, Lili *et al* investigated the possible mechanism of raspberry ketone on obesity and the influence of resistance on obesity relevant insulin in rats. The study randomly divided 40 rats into 5 groups; normal fed with normal diet (NC), model control fed with high fat diet (MC), raspberry ketone low-does (RKL), raspberry ketone medium-does (RKM) and raspberry ketone high-does (RKH) groups fed high fat diet plus 0.5%, 1% and 2% of raspberry ketone for 4 weeks. After 8 weeks they reported the RKM and RKL observed significant decreases in FFA, LEP and tumor necrosis factor-α (TNT-α), and all RK groups showed significant reductions in blood glucose and decreased insulin levels compared to MC, therefore it was hypothesised the anti-obesity function was seen through combined effects of improving insulin resistance (Lili *et al*. 2011).

On the other hand, it has been reported that raspberry ketone improve leptin resistance and inhibit lipid peroxidation and modulating inflammatory responses when raspberry ketone is used as a treatment against non-alcoholic steatohepatitis ('fatty liver') (Wang *et al*. 2012). They investigated 5 groups of rats; normal control group (NC) (fed normal diet for 8 weeks), model control group (MC) (fed a high fat diet) and 3 groups with different doses of raspberry ketone (0.5% RKL, 1% RKM and 2% RKH + high fat diet for 4 weeks). The liver

tissues of rats in each group were analysed, comparing all 3 doses of raspberry ketone against the control group. All parameters measured reported significant improvements; these included, triglycerides, low-density lipoprotein cholesterol (RKL 18.6 ± 0.60; RKM 20.0 ± 1.00; RKH 18.7 ± 0.58 vs NC 21.2 ± 0.62 ng/L), , leptin RKL 2.83 ± 0.77; RKM 3.00 ± 0.83; RKH 4.77 ± 1.61 vs NC 2.41 ± 0.57 ng/mL), blood glucose (RKL 6.48 ± 1.10; RKM 6.31 ± 1.10; RKH 6.24 ± 1.36 vs NC 5.80 ± 1.44 mmol/L), insulin (RKL 3.49 ± 0.13; RKM 3.99 ± 0.12; RKH 3.96 ± 0.25 vs NC 3.49 ± 0.12 µIU/mL) and free fatty acids (RKL 87.35 ± 19.48; RKM 119.73 ± 24.92; RKH 132.00 ± 52.65 vs NC 83.63 ± 18.29 µg/L). They concluded raspberry ketone to be an effective intervention for fatty liver in rats who were fed a high-fat-diet.

These reports indicate raspberry ketone has a duel effect on fat reduction and liver protection. The mechanisms reported include decrease in adipose tissue degeneration in liver cells, reduction of liver inflammation, and correction of dyslipidaemia, reversal of leptin and insulin resistance and improved antioxidant capacity. A more recent study in 2017 investigated the effects of raspberry ketone on accumulation of adipose mass, hepatic lipid storage and levels of plasma adiponectin in mice fed a high fat diet. Firstly mice were fed a high fat diet for 2 weeks to induce weight gain, following this they were randomly assigned to four experimental groups; high-dose raspberry ketone (HRK, 1.74% RK wt/wt), low-dose raspberry ketone (LRK, 0.25% RK wt/wt), control and pair fed (PF) group, fed a similar food intake to the LRK mice. Mice were then fed for five weeks, results showed that mice fed LRK and HRK diets showed reduction in food intake and body weight compared to mice on the control diet, the HRK diet exhibited decreases in inguinal fat mass and increased liver mass compared to the control group. However, plasma adiponectin concentration levels were not affected by raspberry ketone, reporting no significant changes between the groups (Cotton *et al*. 2017), opposing the earlier findings by Park, 2010. Previous studies (Park 2010) have hypothesised the mechanism which results in raspberry ketone reducing body mass is from increasing adiopenctin levels, however Cotton *et al* (2017) reported reduction in body weight with no differences in plasma adiopnectin. While there have been some reported studies of the effects of raspberry ketone in rats and mice and their possible mechanisms, with the limited number of studies in humans the relevance could be debated.

1.3.1.2 Clinical Trials

In the last few years, raspberry ketone has been sold as an ingredient in food supplements where it is claimed to have a slimming effect and has been considered within alternative weight loss management; however, to date there has been no clinical evidence available on such claims. A randomised, placebo-controlled study by Lopez *et al* (2013) investigated a multi-ingredient weight loss supplement (METABO), with raspberry ketone being one of the ingredients, although the exact concentration is unclear, and other ingredients included caffeine, capsaicin, ginger and citrus. The trial investigated the effects of an 8-week diet and exercise program; a calorie restricted diet with a deficit of 500 kilocalories per day and performed three, 60-minute exercise sessions per week and included a daily supplementation (with either METABO or placebo). Significant differences in body composition were observed, compared to the placebo group, the participants in the METABO group reporting a -2% decrease in body weight with the placebo group reporting a -0.5% decease, fat mass (-7.8% versus -2.8% $P < 0.001$), waist girth (-2.0% versus -0.2% $P < 0.0007$) hip girth (-1.7% versus -0.4% $P < 0.003$) and an increase in lean mass (+3.4% versus +0/8% $P < 0.03$) (Lopez *et al*. 2013). Lopez *et al,* proposed a possible mechanism for the anti-obesity effects of raspberry ketone could be via an enhanced production of norepinephrine, resulting in an increase in the hormone-sensitive lipolytic action of lipolysis. Prior to the 2013 study by Lopez *et al,* the same research group carried out a preliminary trial and reported similar results. They carried out an 8 week intervention on 70 men and women who randomly ingested either METABO 4 x capsules/day or a placebo alongside a calorie restricted diet and supervised exercise training. The details of the pre and post testing was not described, however body composition, waist and hip girth and energy levels were measured. Significant difference were reported in the METABO group for body weight (-2.0 vs -0.5 %); fat mass (-7.8 vs -2.8 %); waist girth (-2.0 vs -0.2 %); hip girth (-1.7 vs -0/4 %) and energy levels (+29.3 vs +5.1 %) (Hofheins *et al*. 2012).

Furthermore, a more recent study in 2018 by Argent *et al,* investigated the same multi-ingredient weight loss supplement (METABO) in overweight men and women. The trial was a randomised, placebo controlled, double blind study where participants consumed 4 capsules/day of either METABO (n=18) or placebo (n=18). Following 8 weeks of supplementation alongside a calorie restricted diet

(500 Kcal < RMR) and supervised exercise training 3 times a week; body composition, girth and serum adipokines levels were assessed. Across both groups, significant decreases in weight (-2.6 ± 0.57 kg), fat mass (-1.8 ± 0.20 kg), percentage body fat (-3.7% ± 0.29%) and significant increase in lean body mass (1.5 ± 0.26 kg) were reported. They observed that only men in the METABO group reported increases in lean body mass (1.3 ± 0.38 kg) and only women in the METABO group reported reduced hip girth (-10.7 ± 2.15 cm). A significant increase in adiponectin in the METABO group was also reported. It was hypothesized that the raspberry ketone within the METABO supplement may have been responsible for the increase in adiponectin, following the report in 2010 by Park, in which raspberry ketone was reported to increase both the expression and the secretion of adiponectin in cell cultures. With most of the current research coming from either *in vitro* or *in vivo* studies, more evidence is needed to support these claims in humans and the mechanisms by which raspberry ketones can increases in fat oxidation and possible weight loss.

1.3.2 Green Coffee Bean Extract

Green coffee extract (GCE) is derived from green unroasted coffee beans, it is also found in both raw and roasted coffee, although much of the green coffee extract is destroyed in the roasting process of coffee. It is marketed in both caffeinated and decaffeinated forms. Polyphenols are well known antioxidants that protect body tissues against damage and prevent disease associated with oxidative stress and its related complications. The glycosylated derivate forms of polyphenols, chlorogenic acids (CGA) are the main polyphenols in green coffee bean extract, these can also be found in a variety of fruit and vegetables (Manach *et al*. 2004). Chlorogenic acids are a family of esters that are formed by quinic acid and several hydroxycinnamic acids, particularly caffeic, ferulic and p-coumaric acids. The most common chlorgenic acids are 5-O-caffeoylquinic acid, 2-O- caffeoylquinic acid and 4-O- caffeoylquinic acid. Chlorgenic acid is present, to some extent, in roasted coffee, although much of it is destroyed in the roasting process. The typical daily intake of chlorogenic acid from drinking coffee varies from 0.5 to 1g (Clifford 1999). Green coffee extract has been used as a weight-loss supplement and as an ingredient in other weight-loss products, it has been marketed under a variety of brand names such as "Coffee Slender", and "Svetol".
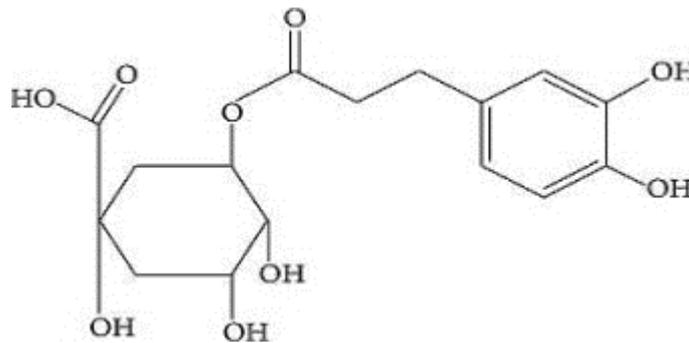
Figure 6. The chemical structure of chologenic acid (CGA) (Naveed *et al*. 2018)

1.3.2.1 Current Literature

There has been increasing evidence from animal studies regarding the use of green coffee extract as a weight loss supplement. Several studies reported green coffee extract (doses ranging from 50-400 mg/kg) to reduce visceral fat content and body weight after being administered to mice following both a standard fed diet and a high-fat diet. Shimoda *et al*. 2006 reported these results after 14 days of administration of green coffee bean extract (100, 200 and 400 mg/kg/day), Choi *et al*. 2016 similarly reported; after initially inducing obesity via four weeks of a high-fat diet, mice were then divided into five groups; fed only high fat diet, high fat diet with green coffee extract (50, 100 and 200 mg/kg) for six weeks alongside a high-fat diet, and a normal fat diet alone being the control group. In high fat induced obese mice it was reported that treatment with green coffee bean extract (100 and 200 mg/kg) significantly decreased weight gain, liver weight, FFA and white adipose tissue weights with regulation of adipose tissue hormones like leptin and adiponectin. Green coffee bean extract fed mice observed levels of adiponectin, HSL, ATGL and PPARα were significantly upregulated in a dose response manner. Compared to the mice fed only a high fat diet, the green coffee bean extract treated mice had a lower fat mass and relative body weight, it was thought that this lower body fat accumulation may be regulated by lipid metabolism related genes and adipogenesis

Another study comparing the efficacy of chlorogenic acid (0.02% wt/wt) in altering body fat in high-fat diet induced-obese mice compared to caffeic acid found comparable results to the previous studies, reporting both phenolic acids (caffeic acid and chlorogenic acid) improved body weight (body weight gain; high caffeic acid 15.45 ± 1.07; high chlorogenic acid 11.79 ± 0.97 vs high fat control 18.50 ± 1.32 g), , with chlorogenic acid seeming to be the more potent for body weight reduction and regulation of lipid metabolism. It was reported that both chlorogenic acid and caffeic acid significantly lowered plasma leptin (high caffeic acid 3.32 ± 0.34; high chlorogenic acid 2.52 ± 0.15 vs high fat control 5.58 ± 0.50 ng/mL), insulin levels (high caffeic acid 5.21 ± 0.33; high chlorogenic acid 4.86 ± 0.45 vs high fat control 6.93 ± 0.25 ng/mL), and fatty acid synthase when compared to the high fat control group, while they increased fatty acid ß-oxidation (high caffeic acid 5.43 ± 0.65; high chlorogenic acid 7.24 ± 0.47 vs high fat control 3.41 ± 0.54 nmol/min/mg/protein). Triglyceride content in the adipose tissue was lowered significantly, whereas the plasma concentration of adiponectin was increased in the chlorogenic acid group compared to the high fat control group. (Cho *et al*. 2010). Similar findings of Huang *et al*. (2015) reported that chlorogenic acid can supress high fat diet induced increases in body weight, visceral fat and serum lipid levels and serum and hepatic free fatty acids. Furthermore in 2014, Song *et al*. reported that decaffeinated green coffee extract significantly reduced visceral fat-pad accumulation in the mouse model of obesity, they concluded this may be due to a decrease in adipogenesis and inflammation in the white adipose tissues.

Caffeinated green coffee extract has also been reported to potentially reduce body weight, Tanaka *et al*. in 2009 investigated the effects of treating 4-week-old rats with green coffee extract (diet containing 1%) rich in caffeine (10%) and chlorogenic acid (27%) for 4 weeks. In the green coffee extract treated rats, body weight gain and white adipose tissue weight were significantly reduced compared to the control rats, although there was no difference in food intake between the diet of the control group without green coffee extract and the rats fed the green coffee extract. They reported serum and liver triglyceride concentration were significantly reduced in the green coffee extract fed rats compared to the control and further to this there was also an increase in fatty acid oxidative enzymes in the hepatic mitochondria, however these observations

could be due to the caffeine and not the green coffee extract. Moreover, a study investigated the effects of a decaffeinated green coffee extract 'Svetol' on diet-induced insulin resistance and brain energy metabolism dysfunction in a high-fat diet mouse model. In the study mice were assigned to one of four groups; high fat diet, high fat diet + Svetol, normal diet and normal diet + 'Svetol' for 5 months. Oral glucose tolerance tests determined changes in the circulating glucose levels and it was reported that supplementation with 'Svetol' significantly attenuated the development of high fat diet induced insulin resistance, reflected by improving glucose clearance. 'Svetol' treatment also improved brain energy metabolism as determined by oxygen consumption rate. However, unlike previous studies mentioned, it was observed that 'Svetol' had no effect on body weight under the context of both a normal diet and a high fat diet.

On the other hand, Li *et al*. in 2014 investigated the effect of green coffee extract on obesity, glucose tolerance, insulin resistance, systemic oxidative stress and endothelial dysfunction in a mouse model of the metabolic syndrome. Mice were randomly divided into 3 groups; normal diet (ND), high fat diet (HFD) or a high fat diet supplemented with green coffee extract rich in chlorogenic acid (0.5% w/w) (HFD + GCE) and fed this diet for 12 weeks. Both the HFD and HFD + GCE groups displayed symptoms of the metabolic syndrome compared to the ND groups, although no endothelial dysfunction was observed. GCE did not attenuate HFD induced obesity, glucose intolerance, insulin resistance or systemic oxidative stress. In 2008, a study by Bassoli et al, they investigated the effects chlorogenic acid on blood glucose levels and glucose tolerance in rats. It was found that chlorogenic acid promoted a significant decrease in the plasma glucose peak in the oral glucose tolerance test, most likely by attenuating intestinal glucose absorption, indicating a possible role for chlorogenic acid as a glycemic index lowering agent.

A study in 2014 investigated the lipolytic activity of varying concentrations of a decaffeinated green coffee extract known as 'Svetol' (0.01mg/mL, 0.1 mg/mL and 1.0mg/mL) on the liberation of free fatty acids from human adipocytes following short term (2 hours) or long term (192 hours) exposure (Flanagan *et al*. 2014). The results showed that long term exposure with 0.1 mg/mL & 1.0mg/mL

concentration of 'Sevtol' increased the release of free fatty acids from human adipocytes compared to the control.

1.3.2.2 Clinical Trials

Clinical trials investigating the effects of green coffee bean extract in humans are limited with mixed findings, however, a number of trials have demonstrated the effects of green coffee extract, containing chlorogenic acid, with positive reports with regards to weight loss and weight management as well as regulation of blood glucose levels (Thom 2007; Dellalibera *et al*. 2006). Thom, 2007 investigated the effects of chlorogenic acid enriched coffee on glucose absorption (study 1) in 12 healthy volunteers and the effects on body mass (study 2) on 30 overweight individuals (BMI 27.5 to 32 kg/m^2) over a 12-week period. The chlorogenic acid enriched coffee contained 200 mg per 2200 mg of the 'Coffee Slender', of this 200 mg, 90-100 mg contained chlorogenic acids (45-50%) compared to caffeinated and decaffeinated instant coffee containing 30-40 mg/g of chlorogenic acid (45-50%). Study 1 involved the intake of four different beverages for 12 weeks; glucose solution (control), 'Coffee Slender', instant coffee (caffeinated) and instant decaffeinated coffee. The result from the oral glucose tolerance test reported that the 'Coffee Slender' significantly reduced the absorption of glucose by 6.9% compared to the control, there was no significant difference reported between the two instant coffee groups compared to the control. Study 2 involved the intake of either 'Coffee Slender' of instant coffee (caffeinated) for 12 weeks, results reported average losses in body mass from the coffee slender group were 5.4kg compared to 1.7kg in the instant coffee (caffeinated) group with body fat reductions of 3.6% compared to 0.7%.

In addition, Dellalibera *et al*. 2006 investigated the effects of decaffeinated green coffee extract known as 'Svetol', a decaffeinated green coffee extract; that has high chlorogenic acid content, on weight loss and lean-to fat mass ratios. The intervention involved 50 overweight volunteers (BMI >25) consuming either 2 capsules per day of 'Svetol' (200 mg) or placebo for 60 days. Results showed a mean significant reduction in weight was observed in the 'Svetol' group (4.97 $\pm$ 0.32 kg, 5.7%) compared to the control group (2.45 $\pm$ 0.37 kg, 2.9%) ($P < 0.05$). The lean-to fat mass ratio was significantly increased in the 'Svetol' group (4.1 $\pm$ 0.7%) compared to the control group (1.6 $\pm$ 0.6%) ($P <$

0.05), consequently lowering the BMI. Moreover, the ratio of muscle mass to fat mass (MM/FM) was increased significantly in the 'Svetol' group compared to the control (4.1 ± 0.7 % vs 1.6 ± 0.6 %), which was determined by bioelectrical impedance. They stated that 'Svetol' "is able exacerbate the effects of a bland low calorie diet in volunteer who are overweight" and this could be due to the increasing consumption of fatty deposits, as shown by the changes in MM/FM ratio, and therefore preventing them from being accumulated.

Furthermore, a randomised pilot crossover trial of 18 healthy volunteers (BMI 24.23 ± 4.6 kg/m^2), investigated the effects of green coffee on body composition and blood pressure, where participants either consumed green coffee rich in chlorogenic acid (GC - 40g per cup per day, 4 times per day, % of chlorogenic acid unknown) or black coffee (BC -40g per cup per day, 4 times per day) for two weeks. Consumption of green coffee rich in chlorogenic acid resulted in significant effects on weight and BMI (P = 0.01 for both parameters), at baseline weight and BMI were 70.52 ± 15.89 kg and 24.23 ± 4.6 kg/m^2, this was significantly reduced to 69.13 ± 15.9 kg and 23.95 ± 4.78 kgm^2 in the green coffee group. GC group significantly reduced waist circumference (1.74 vs 0.89 cm P = 0.009), abdominal fat (1.75 vs 1.44 % P = 0.015), systolic blood pressure (2.65 ± 1.37 mmHg) and diastolic blood pressure (2.23 ± 1.44 mmHg) compared to the BC group (Revuelta-Iniesta and Al-Dujaili 2014).

A key observation with these studies is their investigation tends to involve overweight or obese individuals, there is less evidence to investigate the use of green coffee extract for weight loss on normal-weight individuals without a medical condition, for example hypertension (Kozuma *et al*. 2005, Watanabe *et al*. 2017). Although these studies have reported green coffee extract has promising for potentially preventing and treating overweight and obesity, two meta-analysis and systematic reviews on the use of green coffee extract as a weight loss supplement have identified considerable heterogeneity with some of the studies mentioned (Onakpoya *et al* 2011; Hausenblas and Huynh, 2014). Apart from the small sample size, there seems to be an association with a high risk of bias. Studies previously mentioned by Thom and Dellalibera were not clear on their blinding or how the randomisation was carried out and there was no information on blinding of outcome assessors, the internal validity of these

trials may prove doubtful. The 2006 study by Dellalibera *et al*, has two authors affiliated to the company which marketed 'Svetol', they did not declare any conflicts of interest.

On the other hand in a study by Kotyczka *et al*. (2011), no significant changes in body weight were reported following the consumption of chlorogenic acid-rich coffee. This was a randomized, longitudinally designed 12 week intervention where 30 volunteers firstly administered 500ml of a light roast coffee (with high concentrations of chologenic acid 4.5mmol/l) for 4 weeks, this was followed by a 2 week wash out period. Following the wash out period volunteers then administered the same volume of a dark roast coffee (chologenic acid concentration of 0.05mmol/l) for 4 weeks. Body mass was reported pre and post both 4 weeks interventions, they reported the chologenic acid rich coffee resulted in a significant increase in body mass (0.2 $\pm$ 0.5kg). The dark roast coffee which was rich in N-methylpyridinium ions, reported a significant decreass in body mass of 0.6 $\pm$ 0.4kg. Compared with previous studies this trial was relatively short with only 4-weeks of consumption been investigated.

The effects of green coffee bean/chlorogenic acid on energy metabolism in humans has not been sufficiently investigated. A study in 2013 by Soga *et al*, investigated the effects consuming chlorogenic acid on energy metabolism in 18 male subjects. The study was a double blind, placebo-controlled, crossover trial where subjects consumed two different beverages daily, one chlorogenic acid (329mg/185g) containing beverage (BMI 21.9 $\pm$ 0.6 kg/m^2) and one control beverage containing no chlorogenic acid (185g) (BMI 22.0 $\pm$ 0.5 kg/m^2) for 4 weeks. Energy metabolism was measured using indirect calorimetry pre and post intervention and up to 180 minutes postprandial (following a meal). Key findings observed following the 4 week intervention were that the beverage containing chlorogenic acid showed a significantly higher postprandial energy expenditure, especially for fat oxidation 120 minutes after the meal compared to the beverage containing no chlorogenic acid (0.958 $\pm$ 0.0064 vs 0.872 $\pm$ 0.046 mg/min/kg), there was no reduction in body weight (kg) or body fat (%). Resting fat oxidation rates were measured, however were not reported. Measures of blood glucose (mg/dL), were also recorded resulting in a significant reduction in fasting plasma glucose levels with the consumption of the chologenic acid

beverage versus the control following the intervention (99.0 $\pm$ 2.5 vs 97.8 $\pm$ 2.2 mg/dL).

Additionally, a more recent study by Katada *et al*, in 2018 investigated the effects of a chlorogenic acid enriched (CGA) and hydroxyhydroquinone (HHQ) reduced coffee on postprandial fat oxidation and anti-oxidative capacity in healthy men. HHQ is an antioxidant component produced during the coffee-bean roasting process. Similarly, the study was a randomised, double blind, placebo-controlled, crossover trial where 15 male volunteer (BMI 22.4 $\pm$ 1.5 kg/m^2) consumed either the CGA (382mg) with HHQ or CGA (428mg) without HHQ coffee for 4 weeks, it is unclear why the CGA concentration differed between the coffee beverages. Postprandial energy metabolism was evaluated 300 minutes following a test meal using indirect calorimetry and blood samples were collected. Results showed that after the 4 week intervention, postprandial fat oxidation (11 $\pm$ 17 vs 7 $\pm$ 16 mg/min) and (plasma biological antioxidant potential (BAP) and derivatives of reactive oxygen metabolites (d/ROM)) BAP/d-ROMs ratio (i.e., less oxidative stress) was significantly higher in the CGA without HHQ compared to the CGA with HHQ, there was no significant changes in body mass or plasma metabolites such as glucose, insulin and high-density lipoproteins cholesterol or low-density lipoprotein cholesterol. It seems that resting fat oxidation rates do not increase with cholorgenic enriched coffee however there is an increase in postprandial fat oxidation rates, while this was shown in the above studies there was no reduction in body fat, a possible reason for this discrepancy could be the short intervention period of only 4 weeks and the use of normal weight subjects, overweight or obese subjects may have accelerated the reduction.

Regarding the effects of consuming green coffee extract alongside performing exercise, the research is somewhat limited. Beam *et al*, in 2015, investigated the effects of post-exercise caffeine and green coffee extract consumption on blood glucose and insulin concentration on 10 male cyclists (BMI 24 $\pm$ 4.3 kg/m^2; $\dot{V}O_{2peak}$ 55.9 $\pm$ 8.4 ml/kg/min^{-1}). Following three bouts of 30-minute cycling at 60% of peak power, subjects consumed 75g of dextrose with either 5 mg/kg body weight of caffeine, 10mg/kg of green coffee extract or a placebo, immediately after exercise. An oral glucose tolerance test was performed immediately before

and after exercise, the results reported no significant difference in blood glucose and insulin between the two treatment groups.

Moreover, Ota *et al*, 2010 investigated the effect of coffee polyphenols on energy metabolism, this was a double blind, placebo-controlled, crossover trial involving 7 male subjects, BMI 21.8 $\pm$ 0.6 kg/m^2. Subjects either consumed a coffee polyphenol test beverage (chlorogenic acid concentration was 359mg/185g) or a control beverage containing no polyphenols for 1 week. Indirect calorimetry measurements were conducted pre and post intervention both after fasting and up to 3.5 hours postprandial, and during exercise. The exercise was performed on a cycle ergometer, 20 watt warm up for 3 minutes then the work rate was increased 15 watts per minute until 60% of maximal heart rate was achieved. They reported the coffee polyphenol beverage led to significantly higher oxygen consumption ($\dot{V}O_2$) pre and post meal ingestion and during the exercise period, compared to the control beverage. Postprandial $\dot{V}O_2$ was significantly higher at 30 and 60 minutes after the meal. Furthermore, the coffee polyphenol beverage reported a significantly higher fat oxidation rate 90 minutes after the meal compared to the control beverage. Following the 1 week intervention, during exercise, the $\dot{V}O_2$ in the coffee polyphenol beverage group was significantly higher in the warm up (20W for 3 minutes) and the first 2 minutes of the graded exercise session, similarly the anaerobic threshold was significantly higher, suggesting the potential to increase endurance capacity. There was a trend for fat oxidation during exercise to be higher in the coffee polyphenol beverage group, however there was no statistical difference.

The anti-obesity properties of green coffee extract and chlorogenic acid has been linked to the metabolism of glucose (Bassoli *et al*. 2008; Meng *et al*. 2013). A pilot prospective clinical study in 2007 investigated the effect of a green decaffeinated coffee extract (GDCE) on glycaemia, whereby 15 healthy men and women consumed 3 capsule/day of GDCE for 40 days with no diet and exercise changes. An oral glucose tolerance test was performed before and after the supplementation. The results reported significant decreases in post-load glycemia (147.8 ± 9.3 vs 133 ± 8.7 mg/dL) compared to pre-load. It was concluded that GDCE is able to modulate glucose metabolism (Blum *et al*. 2007). Moreover, a recent study investigated the effects of green coffee extract (GCE

400mg 2 x capsules/day) on anthropometric indices, glycaemic control, blood pressure and insulin resistance in patients with metabolic syndrome; the coexistence of risk factors predisposing to type 2 diabetes and cardiovascular disease. Following an 8 week placebo controlled intervention, GCE significantly reduced fasting blood glucose (-0.28 ± 3.34 vs 1.63 ± 2.22 mmol/l), waist circumference (-2.40 ± 2.54 vs -0.66 ± 1.17 cm) and appetite score (-1.44 ± 1.72 vs -0.2 ± 1.32). A reduction in body weight was reported significant (-2.08 ± 2.11 vs -0.95 ± 1.30 kg), however only marginally (p=0.05) (Roshan *et al*. 2018).

From the current research, suggestions of proposed mechanisms for these finding include that chlorogenic acid inhibits the release of glucose by preventing hepatic glucose-6-phosphatase activity and inhibition of glucose absorption in the small intestine by preventing glucose-6-phosphatase translocase. This limited release of glucose from glycogen through glycogenolysis therefore limits the release of glucose into the circulation and thus limits the release of insulin into the bloodstream. This, therefore forces lipids to be used as energy to compensate for the decrease in glucose and resulting in less fatty deposited in the adipose tissue owing to a reduction in insulin therefore inhibiting the fat accumulation and resulting in weight loss (as shown in Figure 7). An in vitro study in human liver cells investigated the effect of the natural decaffeinated green coffee extract 'Svetol' and chlorogenic acid on hepatic glucose-6-phosphatase. It was reported that 'Svetol' significantly inhibited glucose-6-phosphatase in the liver microsomes (Henry-Vitrac *et al*. 2010). Furthermore, it has been discerned that cholorgenic acid could increase adiponectin and adiponectin receptors and the phosphorylation of AMP-activated protein kinase (AMPK) and reduce the expression of hepatic glucose-6-phosphatase (Naveed *et al*. 2018). AMPK is an enzyme involved in cellular energy homeostasis, largely to activate glucose uptake and fatty acid oxidation when cellular energy is low. This upsurge in AMPK phosphorylation, adiponectin and adiponectin receptors and decrease in glucose-6-phosphatase has been associated with lowered levels of triglycerides, fasting glucose and cholesterol, inhibits fatty liver and an upsurge of glucose tolerance and insulin sensitivity is also observed (Naveed *et al*. 2018).
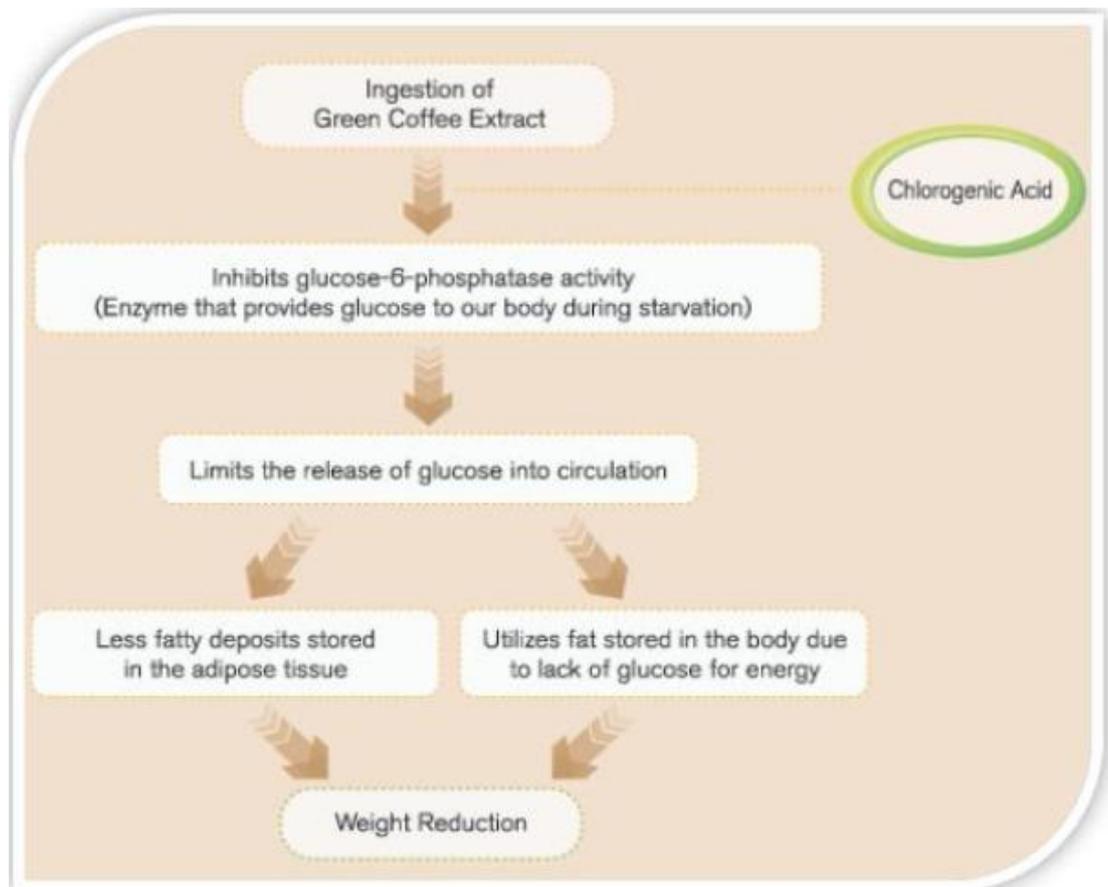
Figure 7. Chlorogenic acid; how does it work (Adapted from www.vitalhealth.com) (Naveed *et al*. 2018).

While the results from these trials are promising, the mechanisms of action on glucose and lipid metabolism have not yet been conclusively elucidate, therefore more research is needed to confirm these claims and potential mechanisms of action.

1.4 Study Aim

Raspberry ketone and green coffee bean extract are both marketed to have beneficial effects on weight loss and are often sold in a combined capsule form. Research on these supplements seems to be limited and their possible mechanisms of action remains unclear. The aim of the present study is to test whether these commonly used dietary food supplements have any effect on fat oxidation and body composition when consumed individually and in combination.

It was hypothesized that raspberry ketone and green coffee bean extract, either alone and/or in combination would reduce body mass and fat mass and increase the rate of fat oxidation at rest and during exercise.

2 Methods

2.1 Subject Recruitment and Screening

A total of 39 recreationally active ($\leq$ 3 exercise sessions per week) males and females between the age of 18 and 45 years were recruited to participate in the study. Subjects were required to have a body mass index (BMI) greater than or equal to 23 kg/m^2 and be non-smoking. Subjects were excluded if they had any previous history of a metabolic disorder or cardiovascular disease or were taking medications that may alter insulin sensitivity or antihypertensive drugs. Subjects were also excluded if they were currently following a diet plan or training for a sporting event, they were asked to complete an activity questionnaire (See appendix A). Subjects were recruited from the student and staff population of University of Glasgow via local advertising.

Subjects attended a screening visit at the university prior to participation, to ensure they met with the criteria for inclusion in the study. A health questionnaire detailing the subject's past, present health status and family history of disease were completed (see appendix A). Resting blood pressure was measured using an automated sphygmomanometer (Boso Medicus, Germany) and height and weight were measured to ensure BMI was in the appropriate range. They were provided with an information sheet describing the aim of the study, the experimental procedures involved and any potential risks or discomfort associated with these procedures which were also verbally explained to them. The study was approved by the MVLS ethics committee and written, informed consent was recorded for each subject.

2.2 Experimental Design

This study was a randomised, placebo-controlled, single blind design. The researcher was blinded to the randomisation of the study and the subjects were not told what treatment they were getting, however due to the distinct taste of some of the supplements they may have been able to distinguish which supplement they were allocated. The study intervention involved 8-weeks supplementation with a randomly assigned dietary food supplement. This was either a placebo, raspberry ketone, green coffee bean extract or a combination

of both raspberry ketone plus green coffee bean extract supplement (two capsules with a meal per day). Subjects were asked to attend the laboratory on three separate occasions; two baseline measurement visits and one post supplementation visit. For the duration of intervention subjects were asked to maintain their habitual dietary and physical activity regime
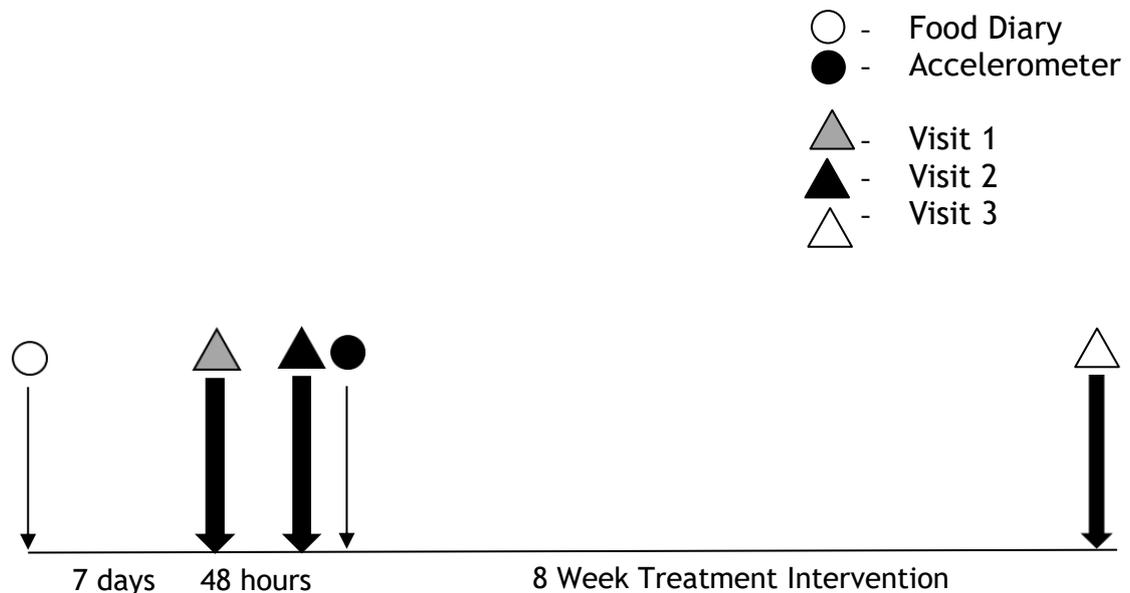
Figure 8. Experimental design – Visit 1 and 2 followed by the 8 week treatment intervention, then visit 3.

2.2.1 Food Diary

One week prior to starting the study, subjects completed a 7 day food diary. This was used to record habitual food intake, using a nutritional analysis program (WISP 4.0) and the last 3 days from the 7 day food dairy was repeated prior to future study visits.

2.2.2 Accelerometer

Subjects wore an activPAL accelerometer (activPAL 3c, PAL Technologies Ltd, Glasgow, UK) for a 7 day period during the first week of the intervention. This monitor was worn on the right thigh, 10cm above the knee during waking hours and removed for showering or bathing. This was used to quantify habitual physical activity and sedentary behaviour levels.

2.2.3 Baseline Measurements – Visit 1

Subjects arrived in the laboratory in the morning following a 12-hour overnight fast, they were asked to refrain from strenuous exercise 24 hours prior to this visit. Firstly a measure of resting metabolic rate (RMR) was performed followed by the anthropometric measures of height, body mass, circumference measurements and body composition. Finally a maximal incremental exercise test was performed on a cycle ergometer to measure maximum oxygen uptake ($\dot{V}O_{2max}$).

2.2.4 Baseline Measurements – Visit 2

On a separate visit to the laboratory, at least 48 hours after visit 1, subjects arrived in the laboratory in the morning following a 12-hour overnight fast, they were asked to refrain from strenuous exercise 24 hours prior to this visit. A 30 minute steady state exercise test was performed on a cycle ergometer at a work rate estimated to elicit 55% $\dot{V}O_{2max}$. Expired air and heart rate was measured and a blood sample was taken at 10 minute intervals throughout the test.

2.2.5 Intervention Treatment

Following the baseline measurements subjects were randomly assigned to one of the following supplements for an 8 week period: placebo (maltodextrose sugar – 400mg/day), green coffee bean extract (400mg/day) (standardised to contain 50% Chlorogenic Acids, 200mg), raspberry ketones (200mg/day) and combined green coffee bean extract (standardised to contain 50% Chlorogenic Acids, 200mg)/raspberry ketones (200mg/day and 400mg/day respectively) (see figure 9).

From doing some initial research into the recommended concentrations of the supplements it seemed that there was not much variation regarding green coffee bean extract and 400mg was a commonly used concentration. This is the concentration used in the market branded variety of green coffee bean extract, 'coffee slender' and 'svetol'. However, raspberry ketone concentrations in supplements seemed to vary from 100-500mg and with no other clinical trials to compare against a small pilot study (n=5) was carried out prior to the current study looking at the different concentration of raspberry ketone. Resting

metabolic rate was recorded at baseline following a 12 hour fast then repeated after 7 days on 3 different concentrations of raspberry ketone (100, 250 and 500mg – consuming 2 capsules per day or as instructed on the packaging), with a 7 day wash out period between supplements. It was concluded from this that there was no dose-response relationship, therefore 100mg (100mg x 2 per/day) was chosen as the investigative concentration for this study.

Two capsules of each supplement were taken daily alongside a meal. All supplements were purchased from well-known commercially available health food chains. The randomisation was performed by a colleague uninvolved in the research using a randomised computer program. During the intervention the researcher kept in regular contact (every 2 weeks throughout the 8 weeks) with the participant to check with their compliance to taking the supplement, maintaining their regular lifestyle and any illnesses (See Appendix B).
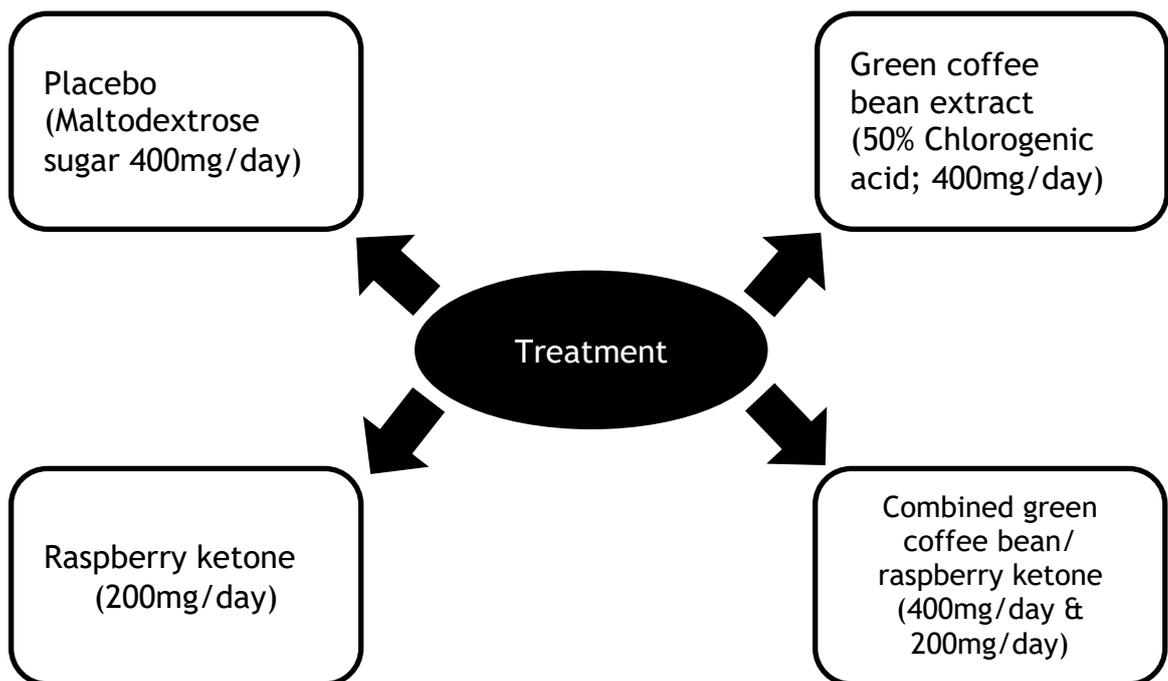
Figure 9. Intervention treatment. Subjects were assigned to either a placebo, raspberry ketone, green coffee bean extract or a combination of both raspberry ketone plus raspberry ketone supplement (two capsules with a meal per day) for 8 weeks.

2.2.6 Post Intervention – Visit 3

Following the 8-week intervention subjects arrived in the laboratory in the morning following a 12-hour overnight fast, having taken their last supplement with their last meal, they were also asked to refrain from strenuous exercise 24 hours prior to this visit. Prior to this visit subjects repeated the last 3 days from their 7 day food dairy. Post measurements of RMR, anthropometric measures and a steady state exercise test were repeated.

2.3 Experimental Measurements

2.3.1 Anthropometric Measurements

Anthropometric measurements were recorded pre and post intervention.

2.3.1.1 Height

Height was measured using the stretch stature method on a stadiometer (Seca, Hamburg, Germany). Measurement was recorded to the nearest 0.1 cm. Stature was recorded as the maximum distance from the floor to the highest point of the skull when the head is held in the Frankfort plane position following a full inspiration (Ross and Marfell-Jones 1991).

2.3.1.2 Body Mass

Body mass was measured in minimal clothing and without shoes to the nearest 0.01 kg. Body mass was measured using the same balanced scale for both pre and post measurements. BMI was calculated as weight in kilograms divided by the square of height in metres.

2.3.1.3 Circumference Measurements

Waist and hip circumference were measured in contact with the skin using a flexible, steel tape measure (Lufkin Executive thin line 2M, Mexico). Waist circumference was taken with subjects standing with feet shoulder-width apart and arms folded across the thorax and landmarked as the narrowest part of the torso, mid-way between the inferior margin of lowest rib and the iliac crest with the abdominal muscles relaxed. Hip circumference was taken with the subjects

standing with feet together and arms folded across the thorax and landmarked as the maximum circumference over the trochanters (buttocks) (Lean *et al.* 1995). With respect to both landmarks, the tape was placed horizontally directly on the skin. All measurements were taken at the end of a normal expiration, with repeated measurement. If the two measurements disagreed by more than 0.1 cm; a third measurement was made.

2.3.1.4 Body Composition

The BOD POD is an Air Displacement Plethysmograph (ADP) that uses whole body densitometry to determine body composition (fat vs. lean tissue). The BOD POD measures body mass (weight) using scales, and volume by sitting inside the BOD POD. Body density is then calculated; Density = Mass/Volume. Once the overall density of the body was determined, the relative proportions of body fat and lean body mass were calculated using the Siri equation (% Body Fat = (495/Body density) - 450). Prior to each measurement, the BOD POD was calibrated with a known volume cylinder. Participant were advised to wear minimal tight fitting clothing and remove any jewellery to minimise any errors.

2.3.2 Expired Air Measurements

2.3.2.1 Resting Metabolic Rate

Resting metabolic rate (RMR) was measured pre and post intervention using an open circuit indirect calorimetry system with a ventilated hood (Quark CPED, Cosmed, Italy). The apparatus included a high-speed differential paramagnetic O_2 sensor and a nondispersive infrared sensor (NDIR) for CO_2 analysis. Before each test, the gas analyser was calibrated using an automated calibration procedure, whereby a calibration gas mixture (16% O_2, 5% CO_2) was introduced to the system. A turbine flow-meter calibration was also performed using a calibrated 3-liter syringe where by a series of ten complete pumps of the syringe was repeated until the values were within the acceptability range. Weekly alcohol-burning tests were conducted on the system for validation purposes.

For RMR determination, subjects arrived in the laboratory in the morning following a 12-hour overnight fast. The last 3 days of their recorded 7-day food dairy was following prior to each measurement. Before the start of each

measurement, each subject was asked to lie quietly in a semi-recumbent position for 10 min in a temperature-controlled (21°C – 23 °C) environment. Next, a transparent ventilated plastic hood connected to the gas mixing chamber by corrugated flexible plastic tubing was placed over the subject's head. Rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were continuously measured then averaged for each 1 minute interval. The measurement was performed for 25 minutes and data from the last 15 minutes were averaged to determine RMR. Resting rates of fat and carbohydrate (g/min^{-1}) were also recorded.

Figure 10. Resting metabolic rate. Substrate utilisation measurement using an open-circuit indirect calorimetry system with a ventilated hood.

2.3.2.2 Measurements of Expired Air during Exercise

During exercise expired air was collected using the Douglas bag method, which involves the collection of exhaled air in large, impermeable 150 litre bags and measurements of gas fractions and expired volumes were obtained. The bags were completely emptied using an exhaust pump prior to usage. Participants were fitted with a rubber mouthpiece and breathed ambient air through a two-way non-rebreathing valve (Kansas City, MO) which was connected to a previously evacuated 150-liter Douglas bag (Hans Rudolph P/N 112377, Hans

Rudolph Inc., Kansas City, USA) on the expired side by a 1 m corrugated flexible plastic hose of a 3.2 cm diameter. A nose clip was worn to prevent nasal breathing. To determine fractions of O_2 and CO_2 in the bag, after gas collection, a small quantity of air was extracted from the used Douglas bag at a constant flow rate (500 ml/min^{-1}), measured by a flow meter and then passed into a gas analyser (Servomex, Sussex, UK). The analyser was calibrated prior to each test using certified reference gases (BOC Gases, Surrey, UK) of known concentration (e.g. 100% nitrogen, 18% O_2, 3% CO_2). The remaining volume of air in the used Douglas bag was vacuumed out using a dry gas meter (Harvard Apparatus, Kent, UK) and expired air volumes and temperature were recorded. To determine $\dot{V}O_2$ (STPD), $\dot{V}CO_2$ (STPD), and respiratory exchange ratio (RER), expired gas fractions and volumes were then corrected for standard temperature (ºC) and barometric pressure at sea level (760 mmHg). RER was calculated as $\dot{V}CO_2$ divided by $\dot{V}O_2$.

2.3.2.3 Expired Air Analysis

According to stoichiometric equations of Frayn, (1983), calculation of fat and carbohydrate oxidation rates, and energy expenditure were estimated from $\dot{V}O_2$ (STPD) and $\dot{V}CO_2$ (STPD). According to the formula, O_2 uptake and CO_2 production can be assumed as:

$$\dot{V}O_2 \text{ (l/min}^{-1}) = 0.746\ c + 2.03\ f + 6.04\ n$$

$$\dot{V}CO_2 \text{ (l/min}^{-1}) = 0.746\ c + 1.43\ f + 4.89\ n$$

Where; c = carbohydrate oxidation in grams per minute, f = fat oxidation in grams per minute, n = urinary nitrogen excreted in grams per minute.

For carbohydrate oxidation, a $\dot{V}O_2$ of 0.746 litres is associated with glucose oxidation but increases to 0.829 litres if glycogen is preferentially oxidised. Using equations based on glycogen as a fuel source in a situation where glucose oxidation predominates may lead to a substantial underestimation of carbohydrate oxidation (Jequier *et al*. 1987). Assuming that there is an absence of net lipogenesis, gluconeogenesis, or ketogenesis or any acid-base disturbances, these equations were derived. The equations were then solved for c grams of carbohydrate and f grams of fat oxidised per minute:

f (g/min^{-1}) = 1.67 $\dot{V}O_2$ – 1.67 $\dot{V}CO_2$ – 1.92 n

c (g/min^{-1}) = 4.55 $\dot{V}CO_2$ – 3.21 $\dot{V}O_2$ – 2.87 n

Based on data from similar studies in the literature, nitrogen excretion rate was assumed at 0.00011 g/kg^{-1}/min^{-1} throughout all tests (Melanson *et al*. 2005; Flatt *et al*. 1985).

Energy expenditure (EE), was calculated as the sum of each macronutrient oxidation rate (g/min^{-1}) multiplied by the appropriate conversion factor (glucose = 3.7 kcal/g^{-1}; fat = 9.3 kcal/g^{-1}) and expressed in kcal/min^{-1} then converted into kilojoules (Kj/min^{-1} and kj/day^{-1}), where 1 kcal is equal to 4.184 kj (Livesey and Elia, 1998).

EE (kcal/min^{-1}) = f (g/min^{-1}) x 9.3 + c (g/min^{-1}) x 3.7

2.4 Exericse Testing

2.4.1.1 Maximal Exercise Test

A maximal incremental exercise test was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) to calculate maximum oxygen consumption ($\dot{V}O_{2max}$) and maximum power (W_{max}), this was performed for each subject to calculate the work rate required for the steady state exercise test. 50% of W_{max} is equal to 55% $\dot{V}O_{2max}$ (Hodgson *et al*. 2013).

A 5 minute warm up at 20 watts was firstly performed on the cycle ergometer, following this the work rate was increased in steps of between 15-25 watts per minutes until volitional exhaustion (15 watts for females and 25 watts for males), the test was terminated when the subject was unable to maintain a revolution per minute (rpm) of greater than 50 rpm. Expired air was collected using the Douglas bag method during the last stages of the test in 1 minute intervals. Heart rate was continuously monitored throughout the test and the maximum heart rate at the end of the test was recorded.

Attainment of true $\dot{V}O_{2max}$ was achieved if 3 out of 4 of the essential criterial were met (Duncan *et al*. 1997): maximum heart rate within 10 bpm of the

predicted maximum heart rate (using the predicated maximum heart rate (HR$_{max}$) equation of 220-age), plateau in $\dot{V}O_2$ (change < 2.1 ml/kg^{-1}/min^{-1}), RER of 1.1 and blood lactate level of >8mmol^{-1}. Blood lactate was not measured post exercise, therefore the other 3 criteria were observed to confirm attainment of true $\dot{V}O_{2max}$.

2.4.1.2 Steady State Exercise Test

A 30 minute steady state exercise test was performed on an electronically braked cycle ergometer with the work rate set at approximately 55% $\dot{V}O_{2max}$ pre and post intervention. Before the start of the test a cannula was inserted into an antecubital or forearm vein and following a 10 minute rest period a 10ml blood sample was taken into an EDTA tube. Further blood samples were taken at 10 minute intervals throughout the 30 minute test to assess for metabolic markers. The cannula was flushed with saline solution after each sample and a total of 40mls of blood was taken during each test.

Expired air and heart rate were measured at 10 minute intervals throughout the test before the blood sample was taken. $\dot{V}O_2$ (l/min^{-1}), $\dot{V}CO_2$ (l/min^{-1}) and rates of fat and carbohydrate (g/min^{-1}) were calculated. An additional expired air sample was collected during minutes 3-4 of the test, $\dot{V}O_2$ (l/min^{-1}) was calculated to ensure the work rate was approximately set to 55% $\dot{V}O_{2max}$, the work rate was then adjusted accordingly if required.
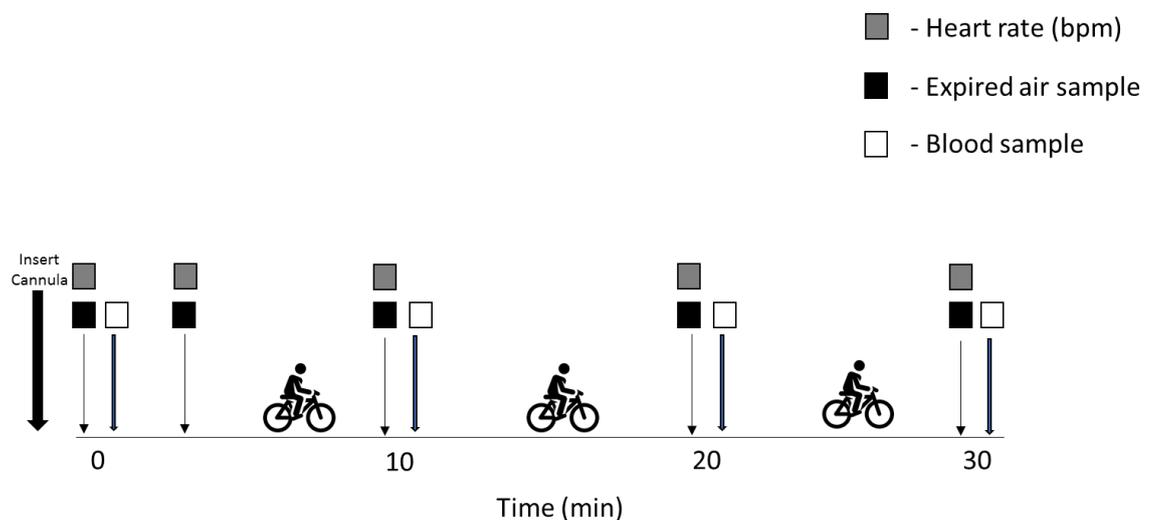
Figure 11. 30 minute steady state exercise test (55% $\dot{V}O_{2max}$).

2.4.1.3 Heart Rate Monitoring

Heart rate during exercise was monitored using a Polar® heart rate telemetry system which consisted of a heart rate transmitter and a wrist receiver (POLAR, Kempele, Finland) during the maximal exercise test and 30 minute steady state exercise test. Heart rate was continuously monitored during the maximal exercise test and obtained at 10 minutes intervals during the 30 minute steady state exercise test, averaged over the last minute of each interval.

2.4.1.4 Blood Analysis

Following collection, all blood samples were rested upon ice until the end of the experimental trial. Following this each tube was centrifuged at 4000 rpm for 15 minutes at 4 °C (Hettich Universal 320R Centrifuge, Germany). The plasma was aliquot into Eppendorf tubes then frozen at -80 °C for later analysis. Each blood sample taken throughout each experimental trial was analysed for plasma glucose and lactate.

Plasma glucose and lactate were analysed using a glucose & L-Lactate analyser (YSI 2300 STAT PLUS, USA). The analyser was calibrated using a glucose standard of 10.0 mmol/l and a lactate standard of 5.00 mmol/l. A self-calibration is performed every 5 samples or 15 minutes, if a difference of more than 2% between the present and previous net calibration occurred the calibration was repeated. In run mode the sipper pump piston retracts and draws in 25 microliters of sample, all samples were analysed in duplicate or until a difference of ≤ 0.02 mmol/l was determined.

2.4.2 Statistical Analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics 24, 2016). Where only baseline data was collected (e.g. physical activity and dietary data) differences between groups were compared via a one-way ANOVA. All other data were compared with a two-way (time and supplement) repeated measures ANOVA. Data are presented as mean SEM and significant differences were accepted at $p < 0.05$.

The current study was a pilot study and so the sample size had been calculated to detect changes in body mass (primary outcome) within each group but not to detect differences in changes between groups, (i.e. an interaction effect). Based on previous work with supplements containing raspberry ketones, which demonstrated a 2 ± 2 kg decrease in body mass (Lopez *et al*. 2013), we will require 10 participants per group to detect a similar change with 80% power at $P<0.05$. To allow for dropout we will recruit 12 participants to each group.

3 Results

3.1 Participants

All participants were recreationally active males and females between the age of 18 and 45 years, non-smoking and had a BMI $\geq$ 23 kg/m^2. Of the 39 participants initially recruited, 7 were lost due to poor compliance to the treatment [n=3], illness [n=1] and with no reason given [n=3]. Of the 32 subjects who completed the study there were 21 females and 11 males. Subject demographics were similar between treatment groups (all p>0.05); Raspberry Ketone (RK), Green Coffee Extract (GCE), Raspberry Ketone + Green Coffee Extract (RK+GCE) and Control Groups (Control). The ANOVA revealed no significant difference between the groups for Age (p=0.115), Height (p=0.531), Body mass (p=0.533), BMI (p=0.801) and $\dot{V}O_{2max}$ (p=0.811).

Table 1. Descriptive characteristics of the 32 recruited subjects and the 4 treatment groups.
Total of 11 males & 21 females (n=32). RK group: 5 males & 3 females (n=8); GCE group: 3 males & 5 females (n=8); RK+GCE group: 1 male & 7 females (n=8); Control group: 2 males & 6 females (n=8). Values are means ± S.E.M.

Variable	Total (n=32)	RK (n=8)	GCE (n=8)	RK+GCE (n=8)	Control (n=8)
Age (years)	30.4 ± 1.5	34.6 ± 2.9	24.8 ± 2.2	30.0 ± 3.2	32.2 ± 3.1
Height (cm)	171.8 ± 1.7	175.2 ± 3.4	172.6 ± 3.7	168.2 ± 3.0	171.1 ± 3.4
Body Mass (kg)	79.0 ± 1.8	82.5 ± 3.8	81.1 ± 3.0	75.9 ± 3.8	76.7 ± 4.1
BMI (kg/m^2)	26.7 ± 0.4	26.8 ± 0.8	27.2 ± 0.8	26.8 ± 1.0	26.2 ± 0.7
$\dot{V}O_{2max}$ (ml/kg/min)$^{-1}$	37.7 ± 1.4	39.2 ± 2.1	38.9 ± 2.9	35.8 ± 3.5	36.8 ± 2.9

3.2 Dietary Intake and Physical Activity

All participants wore an activPAL accelerometer for 7 days, and there was no difference in total physical activity between the groups (EE (MET/H/Week)p=0.762) (Table 2). One week prior to starting the study, subjects completed a 7 day food dairy.

For Energy Intake (EI) the ANOVA revealed no significant differences between the groups (p=0.825). Similarly there was no difference between all the dietary variables, Fat (p=0.866), CHO (p=0.580), Protein (p=0.596) and Alcohol (p=0.721).

Table 2. Total physical activity (MET/h/week) from 7 day accelerometer. Average EI (KJ), fat (g/day), CHO (g/day), protein (g/day) and alcohol (g/day) from 7 day food diaries.
Values are means ± S.E.M.

Variable	RK	GCE	RK+GCE	Control
EE (MET/H/Week)	238.8 ± 16.1	228.0 ± 5.2	226.1 ± 5.7	231.8 ± 5.7
EI (KJ)	8314.9 ± 914.2	9093.7 ± 784.4	7733.7 ± 656.0	8576.9 ± 1460.2
Fat (g/day)	73.5 ± 15.2	83.9 ± 5.6	76.4 ± 8.6	84.3 ± 12.8
CHO (g/day)	244.3 ± 26.5	261.8 ± 29.9	207.7 ± 15.0	221.5 ± 38.8
Protein (g/day)	73.8 ± 6.2	81.7 ± 8.1	75.2 ± 12.7	100.7 ± 25.4
Alcohol (g/day)	9.6 ± 3.3	4.8 ± 2.0	11.0 ± 5.2	9.3 ± 5.0

3.3 Anthropometric Measurements & Resting Metabolism

3.3.1 Anthropometric Measurements

The baseline and post-intervention anthropometric are presented in Table 3. For body weight the ANOVA revealed a significant effect of time (p=0.004), but no supplement (p=0.528) or interaction effects (p=0.548) were noted. For body fat no time (p=0.942), supplement (p=0.461) or interaction effects (p=0.933) were observed. Waist circumference revealed a significant effect of time (p=0.043), but no supplement (p=0.450) or interaction effects (p=0.503) were noted. For hip circumference no time (p=0.082), supplement (p=0.961) or interaction effects (p=0.912) were seen.

Table 3. Anthropometric variables of treatment groups. Values are means ± S.E.M.

Variable	RK		GCE		RK+GCE		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Body Mass (kg)	82.5 ± 3.8	81.6 ± 3.9	81.1 ± 3.0	81.0 ± 3.2	75.9 ± 3.8	74.5 ± 4.0	76.7 ± 4.1	76.3 ± 4.2
Body Fat (%)	25.6 ± 3.3	25.9 ± 2.7	28.2 ± 4.3	28.7 ± 4.2	33.0 ± 3.8	32.4 ± 3.7	25.8 ± 3.6	25.8 ± 3.3
Waist (cm)	87.3 ± 2.9	86.4 ± 2.7	83.8 ± 2.2	83.8 ± 2.1	83.6 ± 2.3	82.1 ± 2.1	81.0 ± 2.6	80.5 ± 2.7
Hip (cm)	105.4 ± 2.8	105.1 ± 2.7	106.9 ± 1.5	106.4 ± 1.7	106.2 ± 3.7	105.6 ± 3.9	104.9 ± 2.0	104.8 ± 2.0

3.3.2 Resting Metabolism

For resting fat oxidation rates the ANOVA revealed no significant effects of time ($p=0.992$) and no supplement ($p=0.809$) or interaction ($p=0.293$) effects were observed. Similarly, for resting carbohydrate (CHO) oxidation rates no time ($p=0.874$), supplement ($p=0.978$) or interaction ($p=0.348$) effects were seen.

For resting energy expenditure the ANOVA revealed no significant effects of time ($p=0.847$) and no supplement ($p=0.798$) or interaction ($p=0.579$) effects were observed. Similarly, energy expenditure during exercise no time ($p=0.703$), supplement ($p=0.342$) or interaction ($p=0.582$) effects were seen.

Table 4. Mean Fat & CHO oxidation rates (g/min) during rest and exercise and mean energy expenditure (EE) at rest (kj/min) and during exercise (kj/min).
Values are means ± S.E.M.

Variable	RK		GCE		RK+GCE		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Fat Oxidation (g/min) (Resting)	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.00	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00
CHO Oxidation (g/min) (Resting)	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.03	0.10 ± 0.02	0.10 ± 0.02	0.12 ± 0.01
Fat Oxidation (g/min) (Exercise)	0.35 ± 0.03	0.41 ± 0.04	0.33 ± 0.05	0.34 ± 0.04	0.40 ± 0.03	0.45 ± 0.04	0.41 ± 0.06	0.38 ± 0.05
CHO Oxidation (g/min) (Exercise)	1.53 ± 0.20	1.37 ± 0.22	1.61 ± 0.25	1.60 ± 0.21	0.97 ± 0.14	0.90 ± 0.14	1.17 ± 0.23	1.21 ± 0.22
Energy Expenditure (Kj/min) (Resting)	16.3 ± 1.0	15.8 ± 0.9	15.1 ± 1.0	15.7 ± 1.2	14.7 ± 0.8	14.9 ± 1.2	14.9 ± 1.0	14.9 ± 0.9
Energy Expenditure (Kj/min) (Exercise)	37.3 ± 3.3	37.3 ± 2.7	38.0 ± 3.5	38.2 ± 3.8	30.8 ± 3.1	31.8 ± 3.0	34.1 ± 3.8	33.8 ± 4.0

3.4 Exercise

3.4.1 Fat and Carbohydrate Oxidation

For fat oxidation rates during the steady state exercise test, the ANOVA revealed no significant effects of time ($p=0.368$) or supplement ($p=0.396$) and no interaction ($p=0.621$) effects were seen. Similarly, for carbohydrate (CHO) oxidation rates during exercise no time ($p=0.318$), supplement ($p=0.067$) or interaction ($p=0.712$) effects were observed (Table 4).
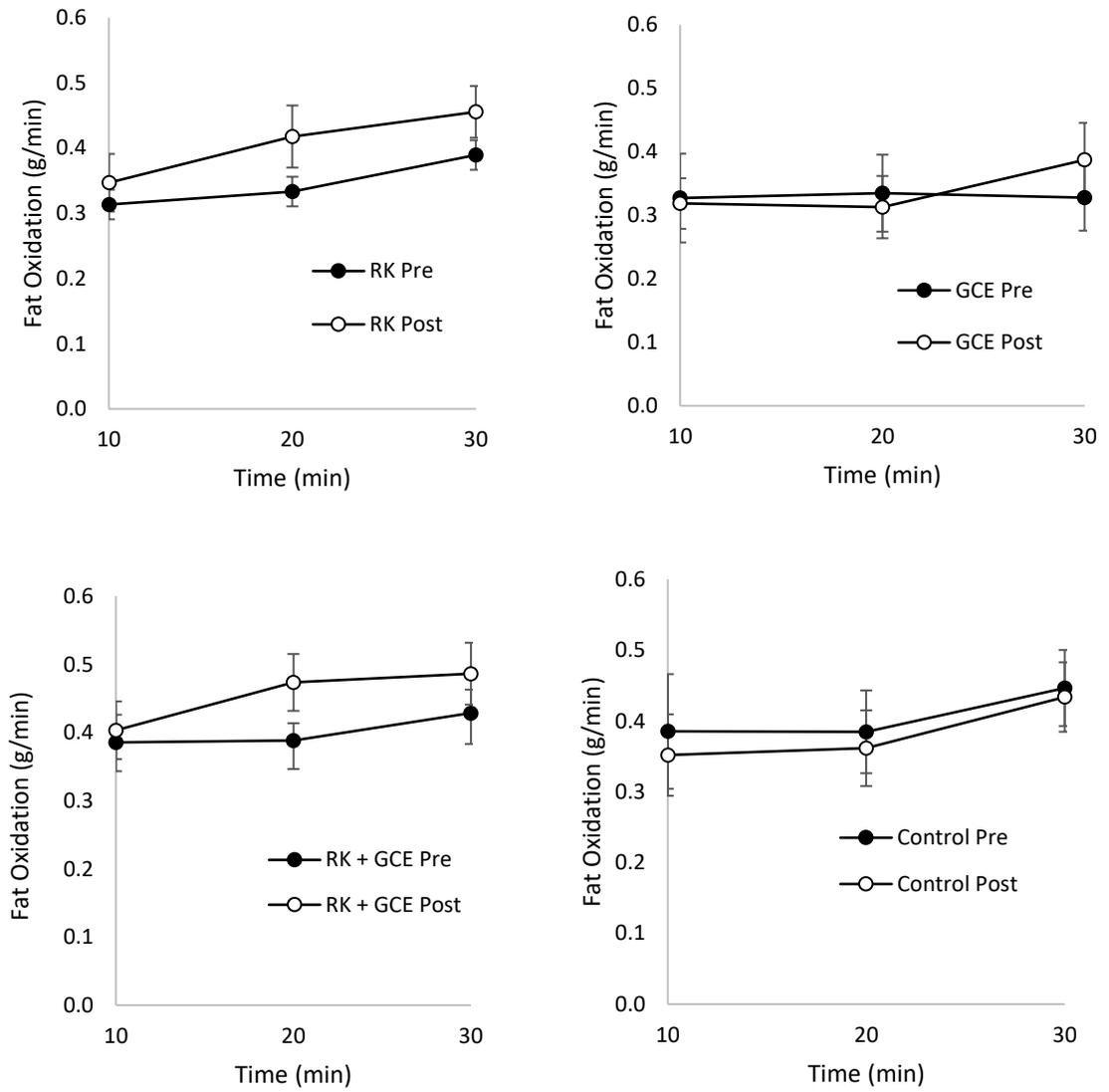
Figure 12. Fat oxidation rates (g/min) during 30 minute steady state exercise, pre (black circle) and post (white circle) intervention. Raspberry ketone (RK), Green coffee bean extract (GCE), Raspberry ketone + green coffee bean extract (RK + GCE) and Control. Values are means ± S.E.M.
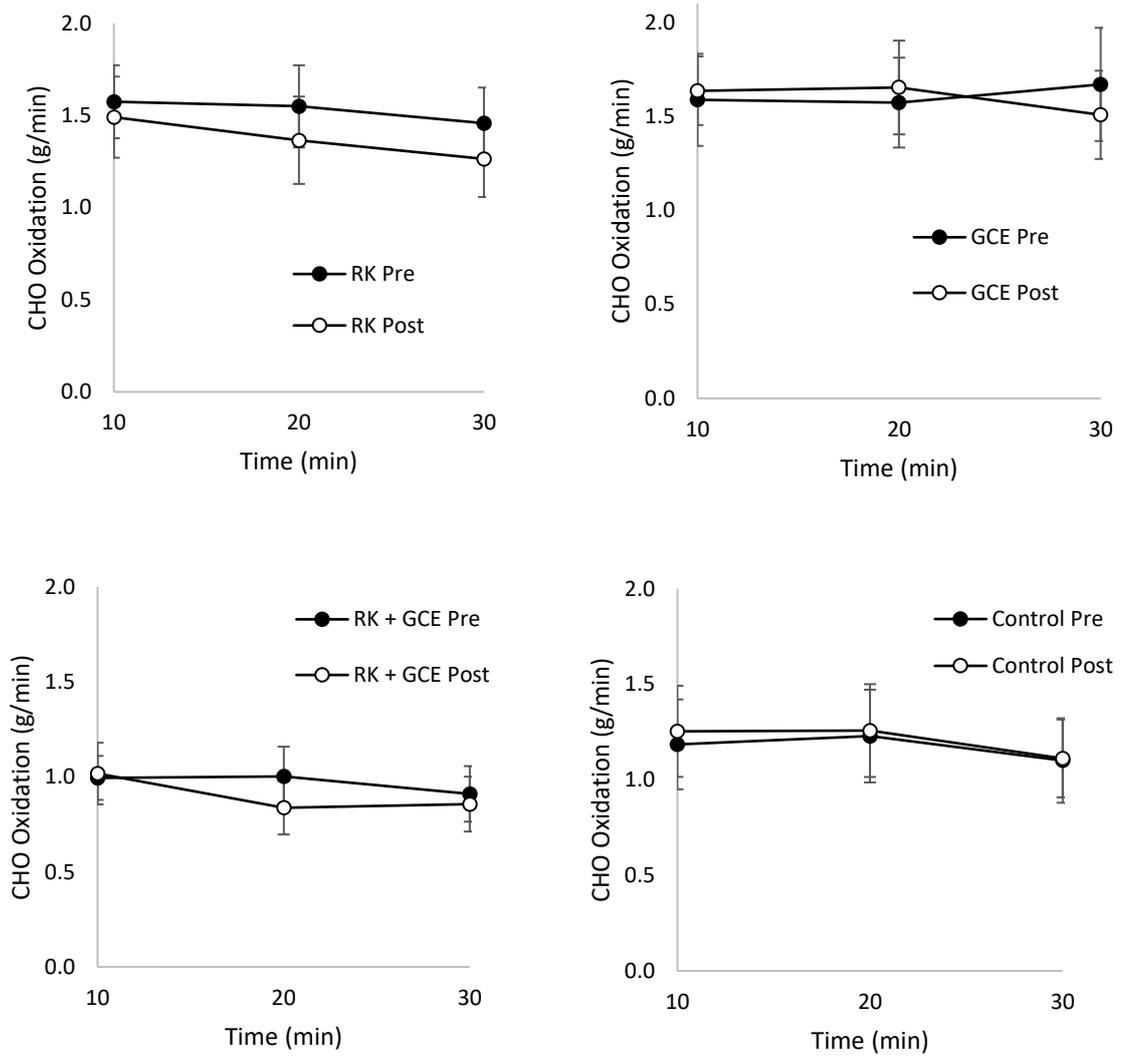
Figure 13. Carbohydrate (CHO) oxidation rates (g/min) during 30 minute steady state exercise, pre (black circle) and post (white circle) intervention. Raspberry ketone (RK), Green coffee bean extract (GCE), Raspberry ketone + green coffee bean extract (RK + GCE) and Control. Values are means ± S.E.M.

3.4.2 Steady State Exercise Test

Participants completed the steady state exercise test at a similar (p=0.431) workload, heard rate and % of $\dot{V}O_{2max}$ between the groups (Table 5).

Table 5. Treatment groups work rate (Watts), % $\dot{V}O_{2max}$ (ml/kg/min) & HR (bpm) during 30 minute steady state exercise test, pre & post intervention.
Values are means ± S.E.M

Variable	RK		GCE		RK+GCE		Control	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Work rate (Watts)	108 ± 10.6	108 ± 10.6	107 ± 12.1	107 ± 12.1	88 ± 11.5	88 ± 11.5	97 ± 13.4	97 ± 13.4
% $\dot{V}O_{2max}$ (ml/kg/min)	56 ± 1.9	57 ± 1.5	59 ± 0.9	59 ± 1.4	57 ± 0.8	59 ± 1.4	59 ± 1.2	59 ± 2.0
HR (bpm)	127 ± 4.0	128 ± 5.3	136 ± 4.9	145 ± 5.3	129 ± 5.2	136 ± 5.7	119 ± 4.2	124 ± 5.0

3.4.3 Plasma Metabolite Concentrations

Plasma metabolite responses at rest and exercise are displayed in figure 3 and 4. For plasma glucose concentrations (Figure 3) the ANOVA revealed no significant time (p=0.433), supplement (p=0.726) or interaction (p=0.905) effects. Similarly, for plasma lactate concentrations (Figure 4) during exercise reported, no time (p=0.259), supplement (p=0.943) or interaction (p=0.763) effects were observed.
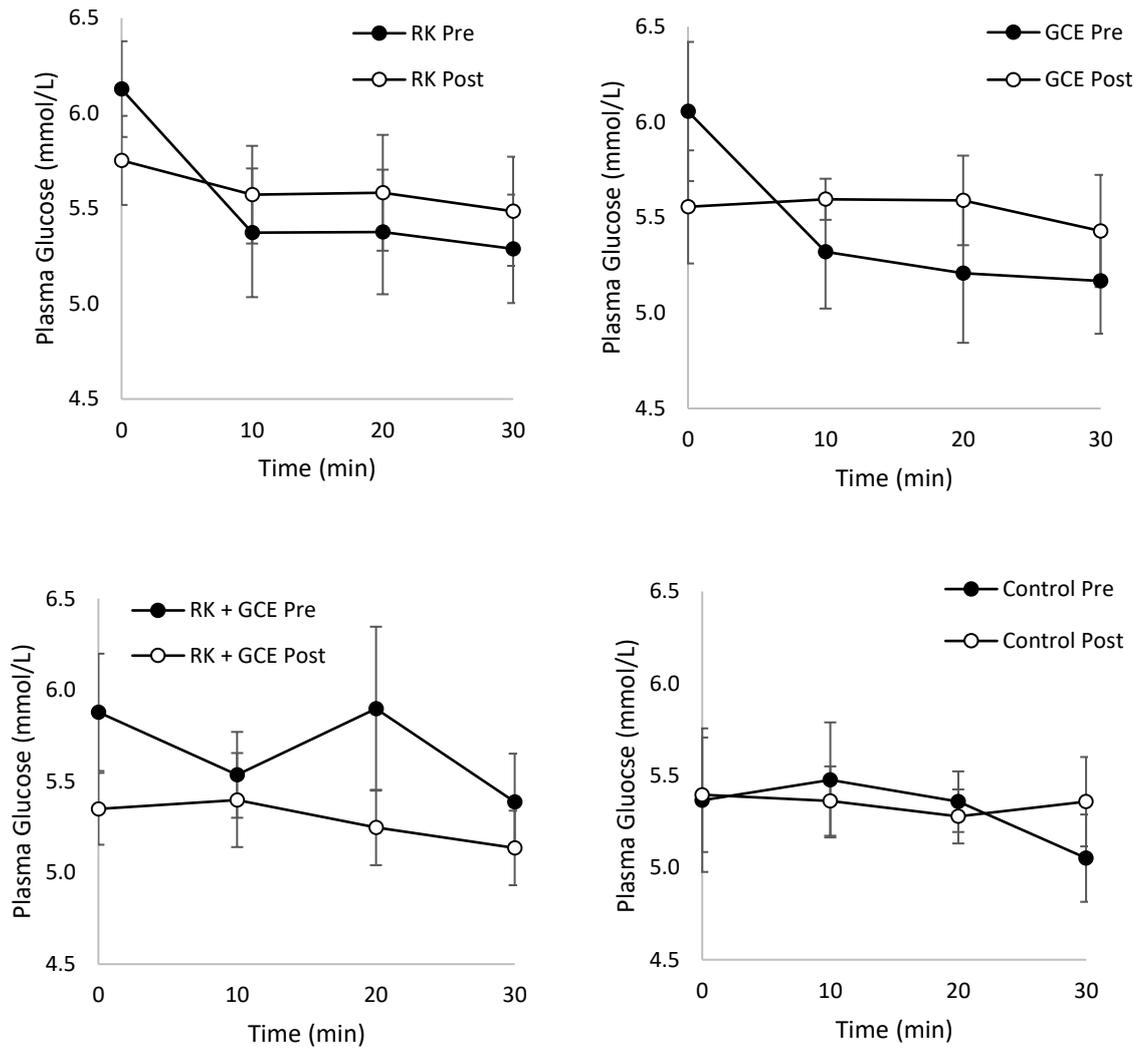
Figure 14. Plasma glucose concentrations (mmol/L) during 30 minute steady state exercise, pre (black circle) and post (white circle) intervention. Raspberry ketone (RK), Green coffee bean extract (GCE), Raspberry ketone + green coffee bean extract (RK + GCE) and Control. Values are means + S.E.M.
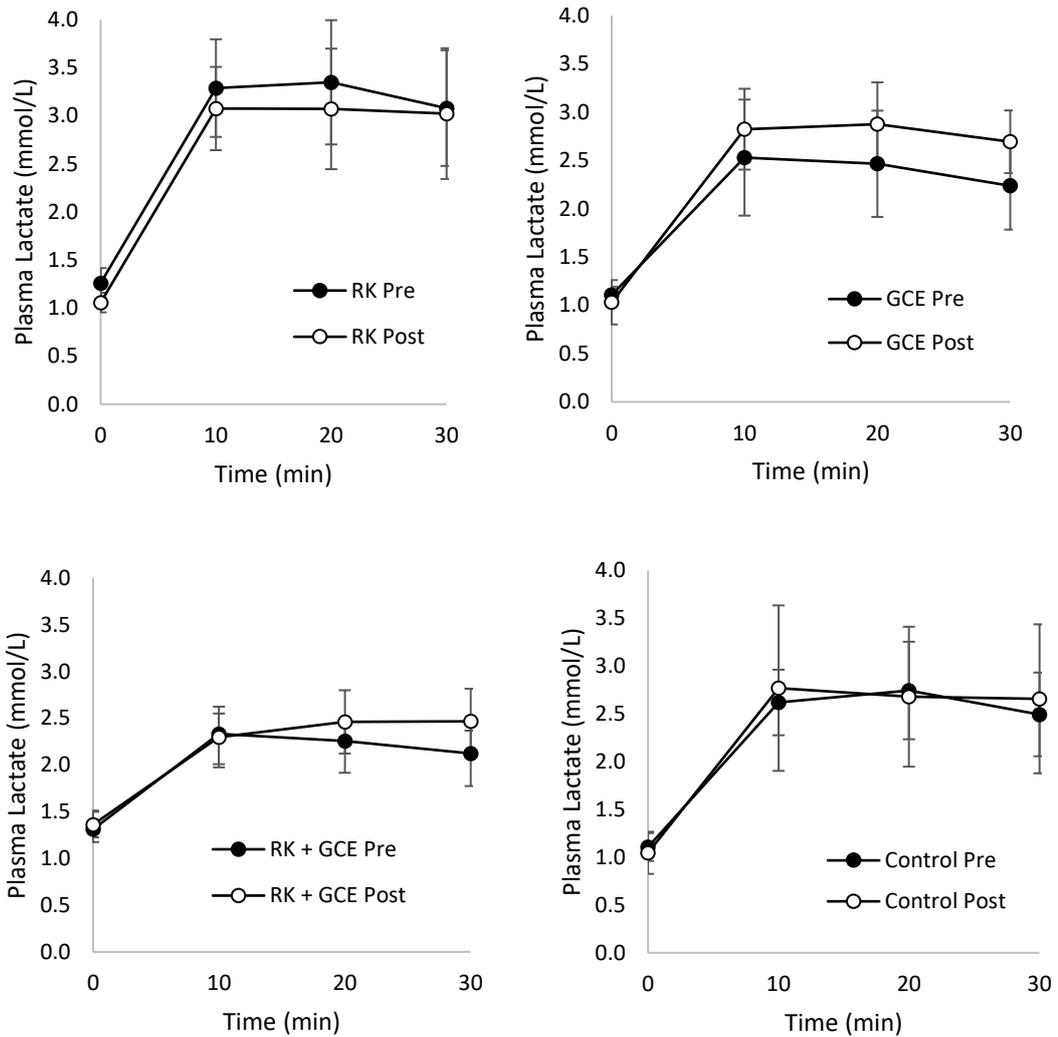
Figure 15. Plasma lactate concentrations (mmol/L) during 30 minute steady state exercise, pre (black circle) and post (white circle) intervention. Raspberry ketone (RK), Green coffee bean extract (GCE), Raspberry ketone + green coffee bean extract (RK + GCE) and Control. Values are means + S.E.M.

On completion of the 8 week intervention, participants were asked if they had experienced any adverse side effects, for example; reduced appetite, nausea, normal sleeping pattern, illness and general wellbeing. No adverse side effects were reported (See Appendix B).

4 Discussion

Despite the increasing popularity for the use of food supplements as an aid for weight loss, there is very limited clinical evidence to support such claims. The aim of the study was to determine whether commonly used dietary food supplements; Raspberry Ketone and Green Coffee bean extract, have any effects on substrate utilisation and body composition when consumed either individually or in combination following an 8 week treatment period. The current study found that following an 8 week treatment intervention, there was no significant effect of either supplement alone, or in combination on body mass (kg) or body fat (%). Similarly, there were no differences in substrate utilisation at rest or during exercise following the intervention. These results go against our hypothesis that these commonly used dietary food supplements would increase fat oxidation resulting in a possible weight loss.

4.1 Effects of Green Coffee Bean Extract

Very few studies have examined the effects of raspberry ketone on body weight, whilst there is some supporting evidence for green coffee bean extract to have a weight loss effect, studies do report conflicting observations. The present study involved 32 subjects and investigated 4 treatment groups; RK, GCE, RK+GCE and a control group, the participants were recreationally active and all had a BMI $\geq$23 kg/m^2, the findings reveal that for all treatment groups there was no effect of the supplements on body mass (kg). In comparing these finding to the literature, studies by Thom in 2007 and Dellalibera *et al*. 2006 both reported reductions in body mass following an intervention of consuming either 'Coffee Slender' or 'Svetol', both chlorogenic acid enriched coffee beverages, at similar concentration to the green coffee bean extract used in the current study. The main difference between these studies and the current work is that they studied overweight/obese individuals, BMI 25-32 kg/m^2, whilst the present study involved individuals with a BMI $\geq$23 kg/m^2 (mean BMI = 26.7 + 0.4 kg/m^2). Thom, 2007 investigated 30 overweight individual with BMI between 27.5-32 kg/m^2 with the average being 29 kg/m^2 following a 12 week intervention. In 2006, Dellalibera *et al*, studied 50 overweight individuals with BMI>25 kg/m^2, however the baseline average BMI was not reported. They reported a significant reduction in body weight in the 'Svetol' group compared to the control, 4.97 $\pm$ 0.32 kg

(5.7%) versus 2.45 $\pm$ 0.37 kg (2.9%) following an intervention of 60 days, moreover this study was not part of a weight loss programme, however the control group reported a 2.45kg reduction in body weight, and they did not give a possible explanation to account for this.

In a systematic review and meta-analysis of randomised control trials on the use of green coffee bean extract, it was reported that the studies above (Thom 2007 and Dellalibera *et al*. 2006) have been identified as having poor methodological quality with significant heterogeneity amongst the studies, for example small sample size and short-term supplement duration and limited descriptive information on the participants (Onakpoya *et al*. 2011; (Hausenblas and Huynh, 2014). The authors reported there was a high risk of bias, both papers were not clear on their blinding method or how the randomisation was carried out and there was no information on blinding of outcome assessors, the internal validity of these trials may prove doubtful. Dellalibera *et al*, also has two authors affiliated to the company which marketed 'Svetol', they did not declare any conflicts of interest and also did not state if the green coffee extract use in the 'Svetol' capsule was fortified with chlorogenic acid and if so what percentage was used. As well as that, the papers provided very little information regarding the baseline characteristic of their participants, such as weight or BMI. Despite stating that an objective of the study was to investigate if 'Svetol' could limit overweight, obesity development and secondary diseases associated, the author did not discuss any end point or results relating to these issues (Buchanan and Beckett, 2013). Thom et al, seemed to compare the resulting mean values with baseline data for each group individually, rather than directly comparing mean weight and BMI between the groups following the 12 weeks of treatment. Also, the title of the study suggests they are investigating the 'long term' effect of 'Coffee Slender' on body mass, however the intervention was only for 12 weeks, providing no information regarding the long-term efficacy or safety (Buchanan and Beckett, 2013).

On the other hand, as detailed above the present study investigated individuals with a BMI $\geq$23 kg/m^2, with the average BMI = 26.7 + 0.4 kg/m^2 (n=32), thus lower than the aforementioned studies, it could indicate that green coffee bean extract may be more effective in overweight or obese individuals with a higher

BMI in reducing body mass. But with this issue with the previous studies detailed above, this is far from certain. Studies carried out on normal weight individuals (BMI <25 kg/m2) have investigated the effects of green coffee extract on people with hypertension and reported no effects on BMI (Watanabe *et al*. 2006; (Kozuma *et al*. 2005). This was confirmed by the results of a recent systematic review and meta-analysis in 2014, who concluded green coffee bean extract supplementation resulted in significant weight loss for overweight/obese individuals but not for normal weight individuals with mild hypertension (Hausenblas and Huynh, 2014).

Similar findings to the current were reported by Soga *et al*, in 2013, who investigated the effects of consumption of chlorogenic acid on energy metabolism in normal weight male subjects; chlorogenic acid groups (BMI 21.9 $\pm$ 0.6 kg/m^2) and control beverage (BMI 22.0 $\pm$ 0.5 kg/m^2). They reported there was no reduction in body weight (kg) or body fat (%), also resting fat oxidation rates were measured, however data was not reported suggesting no changes were made. The findings from this study are in agreement with the current study. The study involved a 4 week intervention, this may have been too short to show any effects, although the current study finds the same when extending the duration to 8 weeks.

As previously mentioned, previous literature has shown that green coffee extract can potentially assist with the regulation of blood glucose levels. In a randomised crossover trial by Dijk *et al*, 2009, they investigated 4 supplements (12g decaffeinated coffee, 1g chlorogenic acid, 500mg trigonelline and placebo) in 15 overweight male subjects (BMI 25-35 kg/m^2) and reported chlorogenic acid significantly reduced glucose concentrations at 15 minutes of an OGTT, compared to placebo. Furthermore, several studies have reported chlorogenic acid enriched coffee to be beneficial for type-2 diabetes mellitus (T2D), this is a metabolic disorder that involves impaired metabolism of glucose and fat. A systematic review and meta-analysis on the effects of decaffeinated coffee in relation of incidents of T2D reported that those who drank 3-4 cups of decaffeinated coffee containing high levels of chlorogenic acid reduced the risk of T2D by 30% (Huxley *et al*. 2009). Such analysis is, however, at risk of reverse causality and residual confounding due to its epidemiological design.

In addition, a more recent study by Katada *et al*, in 2018 investigated the effects of two different coffees; a chlorogenic acid enriched (CGA) coffee and a hydroxyhydroquinone (HHQ) reduced coffee on postprandial fat oxidation in healthy men. HHQ is an antioxidant component produced during the coffee-bean roasting process. The study was a randomised, double blind, placebo-controlled, crossover trial where 15 male volunteer (BMI 22.4 $\pm$ 1.5 kg/m^2) consumed either CGA (428mg) coffee for 4 weeks or CGA (382mg) containing HHQ, it is unclear why the CGA concentration differed between the coffee beverages. Postprandial energy metabolism was evaluated 300 minutes following a test meal using indirect calorimetry and blood samples were collected. Results showed that after the 4 week intervention, postprandial fat oxidation rates (11 $\pm$ 17 vs 7 $\pm$ 16 mg/min) were higher in the CGA compared to the CGA containing HHQ, there was no changes in body mass or plasma metabolites such as glucose, insulin and high-density lipoproteins cholesterol or low-density lipoproteins cholesterol. According to the aforementioned studies above, resting fat oxidation rates do not seem to increase with chlorogenic enriched coffee however there is evidence for an increase in postprandial fat oxidation rates, although this does not result in a reduction in body fat. A possible reason for this discrepancy could be the short intervention period of only 4 weeks and the use of normal weight subjects, overweight or obese subjects may have accelerated the reduction.

There have also been *in vivo* studies in mice which have investigated the effects of green coffee bean extract on body weight. Choi *et al* in 2016 induced obesity in mice with a high fat diet for four weeks then five groups were investigated; three different concentrations of green coffee bean extract (50, 100 and 200 mg/kg) alongside a high fat diet, high fat diet alone and a control group which were fed a normal diet. The body weight gain in the high fat diet group alone were higher than in the control group, whereas, the mice fed a high fat diet alongside 100 and 200 mg/kg of green coffee extract reported reductions in body weight compared to the high fat diet alone. Similar findings were reported by Cho *et al*, 2010, they induced obesity in mice prior to their intervention where the mice were then randomly divided into four groups; normal fed diet, high fat diet, high fat diet plus 0.02% caffeic acid and a high fat diet plus 0.02% chlorogenic acid group and observed improvement in body weight. The caffeic acid and chlorogenic acid supplement groups reduced body weight by 8% and 16%

compared to the high fat diet alone group. Among the high fat diet fed groups, body weight gain was lower in the chlorogenic acid group compared to the high fat diet alone group or the caffeic acid supplement group. However, on the other hand, reports from Mubarak *et al*, 2013 and Li *et al*, 2014 observed that mice fed a high fat diet with green coffee extract failed to reduce body weight compared to a high fat diet alone. So as with the human data, the animal data is inconsistent in regards green coffee bean extract having any effect on body weight.

Fat oxidation rates during exercise are up to 10-fold higher than at rest, this is due to the increasing energy demand of the working muscle (Achten and Jeukendrup, 2004). Prior to this current investigation there was no studies in the literature examining the effects of raspberry ketone during exercise and with very limited evidence regarding the effects green coffee bean extract has during exercise, more research is therefore required.

Very few studies in the literature have examined the effects of green coffee extract with exercise. A study in 2015 by Beam *et al*, investigated the effects of post-exercise caffeine and green coffee bean extract consumption on blood glucose and insulin concentrations. Following the completion of a $\dot{V}O_2$ peak test, three bouts of 30-minute cycling were performed at 60% of peak power 1 week apart, subjects consumed 75g of dextrose with either 5 mg/kg body weight of caffeine, 10mg/kg of green coffee extract and 5 mg/kg body weight of dextrose as a placebo, immediately after exercise. A venous blood sample was taken pre exercise and an OGTT was performed post exercise. Indirect calorimetry was not measured during the OGTT, this could have been useful to see if the supplements had any changes in substrate utilisation. The results reported no differences in blood glucose and insulin between the two treatment groups. Although the current study did not perform an OGTT post exercise there was no difference in resting blood glucose or exercising blood glucose levels.

Furthermore, Ota *et al*, 2010 investigated the effect of coffee polyphenols on energy metabolism, this was a double blind, placebo-controlled, crossover trial involving 7 male subjects, BMI 21.8 $\pm$ 0.6 kg/m^2. Subjects either consumed a coffee polyphenol test beverage (chlorogenic acid concentration was 359mg/185g) or a control beverage containing no polyphenols for 1 week.

Indirect calorimetry was measured pre and post intervention both after fasting and up to 3.5 hours postprandial, and during exercise. The exercise was performed on a cycle ergometer, 20 watt warm up for 3 minutes then the work rate was increased 15 watts per minute until 60% of maximal heart rate was achieved. They found that the coffee polyphenol beverage led to significantly higher $\dot{V}O_2$ pre and post meal and during the exercise period, compared to the control beverage. Furthermore, the coffee polyphenol group had a higher fat oxidation rate 90 minutes after the meal compared to the control beverage. During exercise, the $\dot{V}O_2$ in the coffee polyphenol beverage group was higher in the warm up (20W for 3 minutes) and the first 2 minutes of the graded exercise session, compared to baseline. Fat oxidation during exercise tended to be higher in the coffee polyphenol beverage group, although there was no statistical difference, however the data was not reported, and this is in agreement with the present study. There was also no significant difference in fasting fat oxidation rates at rest between the treatment groups, this was similar to the current data.

From the literature there are several randomised control trial investigating green coffee bean extract as an individual supplement. To my knowledge there are no clinical trials investigating the combined effects of green coffee bean extract with raspberry ketone. Although, a study by Stohs *et al*, in 2016 investigated a novel weight loss supplement containing several ingredients including green coffee bean extract (300 mg & 45-50% chlorogenic acid), moringa oleifera leaf extract (200 mg), banana leaf extract (100 mg) and vitamin D3 (500 lU). The author claims the ingredients will suppress appetite, support weight management and weight loss and help maintain healthy blood sugar and lipid levels. This was a 60 day treatment intervention where 28 subjects consumed two capsules per day. The results demonstrated the product increased fat free mass and decreased body fat, but there were no changes in any of the blood measurements. A major limitation with this study was the failure to include a control group, if this was a randomised controlled trial the results would be more robust. Also subjects received a daily cash 'incentive fee' to ensure compliance.

Clinical trials investigating raspberry ketone are limited. Lopez *et al*, 2013 reported significant reduction in body mass and fat mass following 8 weeks of consuming METABO versus placebo. METABO was a multi-ingredient weight loss product with raspberry ketone being one of many ingredients, the exact concentration is not reported, and other ingredients included caffeine, capsaicin, ginger and citrus. This was a randomised, placebo controlled, double blind study in 45 overweight individuals over 8 weeks (METABO group BMI = 30.8 $\pm$ 2.5 kg/m^2 versus 31.5 $\pm$ 2.3 kg/m^2 in the placebo group), this was carried out alongside a diet and exercise program; a calorie restricted diet of 500 kilocalories less per day and performed three, 60-minute exercise sessions per week, and so the effect of the supplement alone is not clear. The participants in the METABO group reported a -2% decrease in body weight with the placebo group reporting a -0.5% decease, fat mass (-7.8% versus -2.8%), waist girth (-2.0% versus -0.2%) hip girth (-1.7% versus -0.4%) and an increase in lean mass (+3.4% versus +0/8%). No difference in glucose concentrations (mg/dL) between the METABO group and placebo were reported. Whether these effects were due to the raspberry ketones or one of the other supplement ingredients is not clear.

4.2 Effects of Raspberry Ketone

Most of the current literature investigating raspberry ketones has been carried out in animals, more evidence in humans is needed. A number of *in vivo* studies in the literature have shown similar observations to that of green coffee bean extract. Raspberry ketone has been shown to prevent and improve obesity in mice following a high fat diet. A study by Morimoto *et al*, 2005 reported that raspberry ketone prevented high fat diet induced increases in body weight versus a high fat diet alone. In the study mice were fed a high fat diet for 6 weeks then signed to five groups; normal diet fed group, high fat diet groups and high fat diet plus three difference concentrations of raspberry ketone (2%, 1% and 0.5%). They reported in the high fat diet plus 2% raspberry ketone group, the body weight increase that took place over the initial 6 weeks on the high fat diet was reduced. In addition, a more recent study in 2017 investigated the effects of raspberry ketone on accumulation of adipose tissue, hepatic lipid storage and levels of plasma adiponectin in mice fed a high fat diet. Firstly mice were fed a high fat diet for 2 weeks to induce weight gain, following this they were randomly assigned to four experimental groups; high-dose raspberry ketone

(HRK, 1.74% RK wt/wt), low-dose raspberry ketone (LRK, 0.25% RK wt/wt), control and pair fed (PF) group, fed a similar food intake to the LRK mice. Mice were then fed for five weeks, results showed that mice fed LRK and HRK diets showed reduction in food intake and body weight compared to the mice on the control diet, the HRK diet exhibited decreases in inguinal fat mass and increased liver mass compared to the control group. However, plasma adiponectin concentration levels were not affected by raspberry ketone, reporting no significant changes between the groups (Cotten *et al*. 2017). With these animal studies reporting the same outcome as the animal studies conducted on mice with regards to green coffee bean extract, such that the supplement is only effective following a high fat diet to induce obesity, it could be hypothesised that similar to clinical trials conducted on green coffee bean extract in humans, raspberry ketone may also be more effective in overweight/obese individuals with a higher BMI.

Regarding the effects raspberry ketone has on fat oxidation rates, there are no clinical trials in the current literature to my knowledge that have investigated this, either at rest or during exercise. There are a number of *in vitro* studies that report increases in fatty acid oxidation (Park 2010; Park 2015). Park in 2010 investigated the effects of raspberry ketone on both lipolysis and fatty acid oxidation in 3T3-LI adipocytes in mice, it was observed that treatment with 10 µM of raspberry ketone (99% purity), increased lipolysis and fatty acid oxidation, suppressed lipid accumulation, and increased secretion of adiponectin. These findings of increased secretion of adiponectin conflict with the study by Cotton *et al* in 2017. In 2015, Park investigated using 3T3-LI adipocytes, the effects of four different concentrations of raspberry ketone (1, 10, 20 and 50 µM) on adipogenic and liogenic gene expression. The results of the study showed that raspberry ketone inhibits the process of adipocyte differentiation as a results of a reduction in the expression of adipogenic and liopgenic genes. Raspberry ketone was also seen to enhance the expression of genes involved in lipolysis and the oxidative pathway, including adipose triglyceride lipase and hormone sensitive lipase. With some promising results from animal studies regarding the effects on fat oxidation, although the literature is still conflicting, you could proposed this effect may be seen in human populations, however more evidence is need in this area.

4.3 Study Limitations

As in a lot of randomised controlled trials there are number of limitations with the current study. During the trial period participants were asked to consume 2 capsules per day of the allocated supplement, maintain their regular lifestyle with regards to diet and exercise, and follow their food diary prior to the laboratory visits, the researcher kept in regular contact with the subjects however it is difficult to determine the level of compliance with these instruction. To ensure completion of the study in the allocated time, an intervention of 8 weeks was chosen, it may have been that a longer intervention is required before any effects are seen. In addition to ensure the target for the number subjects was achieved in the available time, a BMI $\geq$23 kg/m^2 was chosen, it may have been, as shown by previous studies (which were not without limitation) that in an overweight or obese population effects of the supplement may have been seen. Finally the dose chosen for both dietary supplements was in accordance with previous studies in the literature, however due to the limited research in some areas it may be that a higher or lower concentration of the supplements could elicit a different effect.

Due to time constraints, the current study, and other studies in the literature may be underpowered, increasing the subject numbers may prove successful in detecting significant differences in both substrate utilisation and body composition in normal weight individuals. The number of subjects for future studies should be based on an appropriately designed sample size calculation. A number of studies have investigated the effects of the supplements alongside a diet and exercise programme in overweight individuals, it is unlikely that supplementation alone will give the same reduction in body mass as seen in these previous studies. Therefore, it would be interesting to see if you get the same outcome with normal weight individuals by including a diet and exercise programme with the supplementation. It may be that an 8 week intervention is not long enough to see any positive results regarding weight loss, for example the study by Thom, in 2007 reported significant reduction in body mass following a 12 week intervention. Investigating different concentrations of the supplements may also be of interest to determine whether a dose-response relationship exist. The present study and other short term studies to date have reported no adverse events when consuming these food supplements,

intermediate and longer term effects are currently unknown, therefore a longer duration trial (exceeding 12 weeks) would be useful to determine how safe these supplements are. A better approach to the investigation of the impact on the regulation of glucose could be useful, an OGTT could be conducted pre (in the fasted state) and post exercise to give a greater understanding of this. In addition, the investigation of some metabolic health markers could be added to future studies, for example insulin, TAG, glycerol and non-esterified fatty acids. Further investigation into the effect these dietary supplements have during exercise, particularly raspberry ketone, is needed as there is limited research in this area. The steady state exercise test of 30 minutes may have been too short to identify any changes in energy substrate oxidation, therefore a longer exercise period could be considered. Also reporting the use of these supplements to improve performance could be of interest as research is limited.

4.4 Conclusion

In conclusion, this study demonstrated that following an 8 week treatment intervention of raspberry ketone, green coffee extract and a combination of these supplements together, alongside a control, there was no effect on body mass or body fat. Similarly, there was no difference in fat oxidation at rest or during exercise or plasma metabolites between the groups.

Appendices

Appendix A: Health Screen for study volunteers

UNIVERSITY OF GLASGOW
COLLEGE OF MEDICAL, VETERINARY & LIFE SCIENCE
SUBJECT QUESTIONNAIRE AND ASSENT FORM FOR EXERCISE TESTING

If you feel unwell on the day of a proposed test, or have been feeling poorly within the last two weeks, you are excluded from taking part in an exercise test. The considerations that follow apply to people who have been feeling well for the preceding two weeks.

NAME ……………………………………………..

SEX: M/F AGE: ……. (yr) HEIGHT: …..… (m) WEIGHT: …… (kg)

Details of last medical examination (where appropriate):

Date: ……………..….(*day/mo/yr*) GP Location…………………………….………

Exercise lifestyle:
What kind(s) of exercise do you regularly do (20 min or more per session), and how often?
(*Please circle the number of times per average week*):

Walking	1	2	3	4	5
Running	1	2	3	4	5
Cycling	1	2	3	4	5
Swimming	1	2	3	4	5
Skiing	1	2	3	4	5
Rowing	1	2	3	4	5
Gymnastics	1	2	3	4	5
Martial Arts	1	2	3	4	5
Zumba etc..	1	2	3	4	5
Aerobics	1	2	3	4	5
HIIT training	1	2	3	4	5
Weight Training	1	2	3	4	5
Field Athletics	1	2	3	4	5
Racket Sports	1	2	3	4	5
Rugby/soccer/hockey	1	2	3	4	5
Others*	1	2	3	4	5

*(*Please specify*)
.………………………………………………………..……………………

How long have you been exercising at least twice/week for at least 20 min/session?
...…………… years

Smoking: (*Please tick one*)   Never smoked ………
                              Not for > 6 months ………
                              Smoke < 10 per day ………
                              Smoke > 10 per day ………

Illnesses: Have you ever had any of the following? (*Please circle NO or YES*)

Anaemia	NO/YES	Asthma	NO/YES
Diabetes	NO/YES	Epilepsy	NO/YES
Heart Disease	NO/YES	High Blood Pressure	NO/YES
Other*	NO/YES		

*(*Please specify*)

……………………………………………………………..……………………….

Symptoms:

Have you ever had any of the following symptoms to a significant degree *at rest or during exercise*? That is, have you had to consult a physician relating to any of the following?

	Rest	*Exercise*
Breathlessness	NO/YES	NO/YES
Chest Pain	NO/YES	NO/YES
Dizzy Fits/Fainting	NO/YES	NO/YES
Heart Murmurs	NO/YES	NO/YES
Palpitations	NO/YES	NO/YES
Tightness in chest, jaw or arm	NO/YES	NO/YES
Other*	NO/YES	

*(*Please specify*)

……………………………………………………………..……………………….

Muscle or joint injury:
Do you have/or have had any muscle or joint injury which could affect your safety in performing exercise *(e.g. cycling or running),* strength testing or strength training?
        NO/YES*
*(*Please specify*)

……………………………………………………………..……………………….

Medication:
Are you currently taking any medication?   NO/YES*
*(*Please specify*)

……………………………………………………..………………………………….

Family History of Sudden Death:
Is there a history of sudden death in people under 40 years in your family?   NO/YES*
Can you think of any other reason why you should not take part in our tests?
(*Please specify*)

……………………………………………………………………...………………..

The following exclusion and inclusion criteria will apply to this study:
Exclusion Criteria

If you have any of the following, you will be excluded from the study:
(a) Asthma, diabetes, epilepsy, heart disease, a family history of sudden death at a young age, fainting bouts, high blood pressure, anaemia and muscle or joint injury.
(b) If you are taking any medication that may adversely affect your performance or health in this study, you will not be allowed to take part in the study.
(c) If you take recreational drugs, you will not be allowed to take part in the study.
(d) If you have ingested alcoholic drinks in the previous 48 hours, you will not be allowed to take part in the study.
(e) If you are pregnant.

Inclusion Criteria

(a) Male or female subject aged at least 18 years and ***normally no more than*** 45 years.
(b) In good health at the time of testing.

Signature …………………………………..…. Date ……………………

Body Weight and Blood Pressure:

Body Weight: ………………………………… Height: …………………
BMI.............................

BP (Resting) …………………………..……

Screened by: …………………….……………

Date: …………………………………..…...

Appendix B: Feedback form for study volunteers

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FEEDBACK FORM

Please circle

1. Have you been adhering to consuming 2 supplements per day with a meal?   *Y/N*
If no please note:

_____
_____
_____

2. Has your diet remained 'normal'?   *Y/N*
If no please note:

_____
_____
_____

3. Has your physical activity level remained 'normal'?   *Y/N*
If no please note:

_____
_____
_____

4. Have you been feeling generally well?   *Y/N*
If no please note:

_____
_____
_____

5. Have you had any problems with your given supplement?   *Y/N*
If yes please note:

_____
_____
_____

6. Have you had any adverse side effects with your given supplement?   *Y/N*
    (e.g. reduced appetite, nausea, abnormal sleeping pattern)

If yes please note:

_____
_____
_____

General comments

_____
_____
_____
_____
_____

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