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Alsabih, Ahmed O.N. (2010) *Effects of prior resistance or endurance exercise on metabolism during and after moderate intensity exercise.* MSc(R) thesis.

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EFFECTS OF PRIOR RESISTANCE OR ENDURANCE EXERCISE ON METABOLISM DURING AND AFTER MODERATE INTENSITY EXERCISE

By

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A thesis submitted in fulfilment of the requirements for the degree of

Master of Science by Research

February 2010

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Author's Declaration

Unless otherwise indicated by acknowledgment or reference to published literature, the presented work in this thesis is the author's own and has not been submitted for a degree at another institution.

Ahmed O. N. Alsabih …….. **Date** ……

Some of the findings from the thesis have been presented at the 2009 British Association of Sport and Exercise Sciences (Bases) annual student conference, University of Hull, 31 March and 1 April:

A.O.N Alsabih., J.M.R Gill., N.G. MacFarlane (2009). Effects of prior resistance or moderate intensity on metabolic responses and fat oxidation during and after subsequent moderate intensity exercise.

Acknowledgement

First and foremost, I must thank God for all the guidance and success in all things in my life. I am eternally thankful to Almighty “Allah”, who rewarded my efforts in the successful accomplishment of this work.

This thesis would not have been possible without considerable help, support, and endless efforts of a number of individuals and groups.

I am indebted to my government (Kingdom of Saudi Arabia) for funding the research described in this thesis.

I owe my deepest gratitude to my supervisors Drs’ Jason Gill and Niall MacFarlane, for their supervision, guidance, great efforts and encouragement throughout the duration of this work.

I would also like to extend my sincere thanks to the following honours students: Steven Charter, Kathryn Cooke, Ryan Morrison and Gemma Strange for their assistance with the experiment.

I would also like to extend my gratitude to the lab technicians; Ian Watt, Heather Collin, John Wilson and Paul Paterson; and my friends and colleagues, especially Dr. Carrie Ferguson, Yahya, Abdulrahman and Anas, for their help and assistance.

I would like to say a special thank-you to all my subjects, for their time and their participation in this work.

Finally, I would like to deeply thank my family, especially my wife and my parents for their constant encouragement, continued prayers and moral support throughout my life.

Abstract

Loss of body fat requires a negative energy balance, thus, interventions that increase fat oxidation are likely have beneficial effects in the prevention and management of obesity. Recent studies have indicated that prior resistance exercise increases fat oxidation during subsequent moderate intensity exercise. However, it is not clear from the data presented in these studies whether *any* prior exercise would increase subsequent fat oxidation, or whether this enhanced fat oxidation continues in the post-exercise period. Therefore, the purpose of this study was to compare the effects of prior resistance and prior endurance exercise (moderate- and high-intensity exercise) on metabolic responses and fat oxidation both during and after a subsequent bout of moderate intensity exercise.

With institutional ethics approval, nine healthy men (mean ± SD: Age 26.3 ± 5.7 years; body mass index (BMI) 26.0 ± 3.0 $kg.m^{-2}$, body mass 76.9 ± 7.7 kg, fat free mass (FFM) 62.0 ± 6.1 kg, maximal oxygen uptake $\dot{V}O_2$ max 47 ± 5.6 ml kg^{-1} min^{-1}) participated. Following preliminary tests, volunteers undertook three main experimental trials in a random order, separated by a week. For each trial volunteers attended in the fasted state; consumed a standard 600 kcal breakfast; then undertook either 30 min moderate exercise at 40% $\dot{V}O_2$ max reserve, prior moderate trial (M), 15 min high intensity exercise at 80% $\dot{V}O_2$ max reserve prior high trial (H), or 30 min resistance exercise comprising 3 x 10 reps of six exercises working the major muscle groups, prior resistance trial (R), [Ex.1]; following 30 min of rest [post-Ex.1] and a further 60 minutes of moderate-intensity exercise [Ex.2] was completed. Volunteers were then provided with a standard test lunch (800 kcal) and observations were continued during a further 4.5 hours of rest [post-Ex.2]. Mixed expired air and blood samples were taken at regular intervals throughout the trial. Data were compared using two-way repeated-measures ANOVA, with *post-hoc* Tukey test and are presented as mean ± SEM unless otherwise stated. Significance was accepted at $p < 0.05$.

Area under the curve (AUC) over the total study duration 7.5 hours (0-450 min), for oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and respiratory exchange ratio (RER) did not differ between the different interventions. However, a summary measurements of the AUC during different phases of the study showed that during Ex.1, $\dot{V}O_2$ was lower in the (R) = 42.0 ± 2.5 l/min compared with the $\dot{V}O_2$ in the (H) = 49.2 ± 2.5 l/min, ($P < 0.05$). Post Ex.1 $\dot{V}O_2$ was significantly higher in (H) = 21.4 ± 1.0 l/min, compared with the (M) = 15.0 ± 0.8 l/min, ($P < 0.05$). During the first exercise session Ex.1, RER was significantly different between trials (M vs. H vs. R: 0.88 ± 0.01 vs. 0.95 ± 0.01 vs. 1.01 ± 0.02, $P < 0.01$). During the second exercise session Ex2, RER was significantly lower in (R) = 0.84 ± 0.01 than (M) = 0.88 ± 0.01, ($P < 0.05$). Area under the curve over the total study duration 7.5 hours (0-450 min), for plasma glucose, insulin, non-esterified fatty acid (NEFA) and triglyceride (TG), were not different between the different trials. However, at the end of Ex.1 at the 60 min time point, glucose and insulin concentrations were significantly lower in (M) compared to (H) ,$P < 0.05$. Non-esterified fatty acid concentration was significantly lower in (R) compared to (M) at the 180 time point ($P < 0.01$). Triglyceride concentrations were significantly lower in (M) compared to (R) at the 75 min time point ($P < 0.01$), at the 180 min time point ($P < 0.05$), and at the end of the trial at the 390 min and 450 min time points ($P < 0.01$). Furthermore, TG concentrations were significantly higher in (H) than in (M) at the end of the trial at time points 390 min ($P < 0.05$), 450 min ($P < 0.01$)

These data demonstrate that the respiratory exchange ratio (RER) is lower during moderate intensity exercise when this is preceded by resistance compared to moderate and high intensity exercise. This is consistent with the previous observations. However, RER during Ex.1 was significantly higher (exceeding 1.0, which suggests a non metabolic source of CO_2) during resistance exercise compared to both moderate and high intensity exercise, and RER was not different between the trials over the total study period. This indicates that, compared to prior moderate and high intensity exercise, prior resistance exercise offers no clear additional benefits for increased fat oxidation during and after subsequent moderate intensity exercise. With

regards to triglyceride, the data from this thesis suggests that prior moderate intensity exercise may have a slight advantage, with lower triglyceride plasma concentration during and after subsequent moderate intensity exercise and 3-4 hours postprandial, compared to prior high and resistance exercise. In addition, this work has shown that there is no difference in fat oxidation over the course of the day, between the different interventions.

Table of contents

List of Tables

List of Figures

Abbreviation

AE	Aerobic exercise
BMI	Body mass index
CHO	Carbohydrate
DNL	*de novo* lipogenesis
EE	Energy expenditure
EPOC	Excess post-exercise oxygen consumption
CO_2	Carbon dioxide
FFA	Free fatty acid
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
H_2CO_3	Carbonic acid
HR	Heart rate
HSL	Hormone sensitive lipase
LDL	Low density-lipoprotein
LPL	Lipoprotein lipase
NEFA	Non-esterified fatty acid
O_2	Oxygen
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
RPE	Rate perceived exertion
TG	Triglyceride
$\dot{V}CO_2$	Rate of pulmonary carbon dioxide output
$\dot{V}O_2$	Rate of pulmonary oxygen output
$\dot{V}O_2$ **max**	Maximum rate of pulmonary oxygen uptake

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Obesity

Obesity is defined as an abnormal accumulation of body fat that frequently leads to negative effects on health and results from a long-term positive fat balance because the fat intake from nutrition exceeds fat oxidation. Obesity is generally defined according to body mass index (BMI), which is based on body mass (kg) and height in square$(m)^2$ as follows: BMI = body mass (kg) / stature $(m)^2$ (WHO, 2000). It is generally accepted that if the BMI is between 25-29.9 kg/m^2, an individual is considered to be overweight, whereas if the BMI is 30 kg/m^2 or more, an individual is considered to be obese (WHO, 2000).

In recent decades, obesity has become an international public health epidemic in wealthy societies (Silventoinen *et al.*, 2004) as well as in low-income countries (Popkin, 1994). The prevalence of obesity has increased rapidly in developed countries (Schrauwen & Westerterp, 2000;Gellner & Domschke, 2008); for instance, from 1960-1991, adults in the U.S. aged 20 to 74 have become increasingly overweight (Kuczmarski *et al.*, 1994). Flegal *et al.* (2002) reported that about 30% of residents in the U.S. are obese (Flegal *et al.*, 2002). Gellner and Domschke (2008) stated that 15.7% of adults and 4% of children and adolescents in Europe are obese, and around 23% of adults and 6.3% of children and adolescents are obese in Germany. Other studies have also shown an increase in the incidence of obesity in Brazil, Canada, and Australia (Monteiro *et al.*, 1995;Millar & Stephens, 1993;Boyle *et al.*, 1994).

Likewise, the incidence of obesity has reportedly increased in developing countries; almost one-third of the populations in the Middle East, North Africa, Latin America, and the Caribbean are considered to be overweight (Delpeuch & Maire, 1997). Previous figures and statistics have indicated the prevalence of obesity and its emergence as a serious global issue; obesity is known to lead to reduced general health and quality of life and increased mortality because it precipitates various chronic diseases, such as Type II diabetes (Colditz *et al.*,

1995;Carey *et al.*, 1997), cardiovascular disease (Dhaliwal & Welborn, 2009;Jousilahti *et al.*, 1996), and muscle skeletal pain (Han *et al.*, 1997). Therefore, control and prevention of obesity has become a cardinal health priority. In order to achieve this purpose, it is very important to understand the energy and macronutrient balance of the metabolism; by doing so, researchers and scientists will understand more about the aetiology of obesity and the factors affecting it, consequently enabling them to prevent and treat this global problem.

1.2 Energy Balance

Energy balance is defined as the difference between the energy input (number of calories taken in by nutrition) and the energy output (number of calories expended). Therefore, the energy balance equation is:

Energy Balance = Total energy intake – Total energy expenditure (Equation 1) (Swinburn & Ravussin, 1994).

Thus, to maintain a healthy and stable body weight, the energy balance must remain neutral. This means that the amount of energy intake must be equal to the amount of energy expenditure, as in the following equation:

Stable Energy Balance : energy intake = energy expenditure (Equation 2) (Swinburn & Ravussin, 1994).

Hence, according to Equation 2, the positive energy balance is due to energy intake being greater than the energy expenditure. This result in weight gain, largely in the form of increased fat storage, and essentially because body carbohydrate stores are limited and quickly saturated. An extra 7700 kcal from food that is greater than the expended energy will lead to a gain of 1 kg of fat (McArdle *et al.*, 2006). On the contrary, a negative energy balance results from

energy expenditure exceeding energy intake, leading to weight loss, and mobilization of the adipose tissue to compensate for the deficiency in calories. Astrup *et al* (2000) and Sumiyoshi *et al* (2006) studied the role of dietary fat and suggested that another definition for obesity would be a situation of change in one's fat balance (fat stored = dietary fat intake – fat oxidation) (Sumiyoshi *et al.*, 2006;Astrup *et al.*, 2000). Consequently, the loss of body weight requires a negative energy balance. This mechanism occurs by increasing energy expenditure, decreasing energy intake, or both.

Energy expenditure during the day consists of three components: basal energy expenditure or resting metabolic rate (RMR), physical activity (PA), and the thermic effect of food (TEF) (McArdle *et al.*, 2006;Schutz, 1995). RMR is defined as the amount of energy expended by a person at rest in the morning after fasting 10–12 hours; this amount of energy is sufficient for the physiological functions of the body organs, such as the brain, heart, lungs, kidneys, liver, and muscles. RMR comprises approximately 60-70% of a person's daily energy expenditure (Ravussin *et al.*, 1986). Increased lean body mass leads to increased RMR, and vice versa; a loss of lean body mass will decrease a person's RMR.

The thermic effect of food accounts for approximately 10% of a person's daily energy expenditure (Schutz *et al.*, 1984), and it is affected by the composition and the size of the meal ingested. Physical activity is the most inconsistent component of energy expenditure, and it makes up approximately 20-30% of the total energy expenditure in sedentary adults. Consequently, obesity could build up slowly from a small, continued positive energy balance produced by some combination of increased energy intake and decreased physical activity (Bouchard, 1991). However, regulating body weight is complex and requires more than merely regulating energy balance; it also requires regulation of the macronutrient balance.

1.3 Substrate Balance

To maintain a steady body weight and body composition, energy and macronutrient balance must be achieved. Maintaining energy and macronutrient balance in the body relies on a complex regulating system. The three macronutrient energy sources are carbohydrate, protein, and fat. To balance the macronutrients, each macronutrient intake should equal its oxidation (Swinburn & Ravussin, 1994). Therefore, any alterations in the macronutrient balance, whether in intake or oxidation, may lead to a change in the body tissue stores of that macronutrient (Hill *et al.*, 1991). Hence, any disruption that occurs in energy and macronutrient balance, such as over-consuming food, over-fasting, and a chronic change in one's level of physical activity, incites the body to restore a situation of stability. The concept of macronutrient balance is valid when every macronutrient does not convert into another macronutrient for storage and has independent oxidation and storage in the body (Swinburn & Ravussin, 1994).

1.3.1 De Novo Lipogenesis (DNL)

De novo lipogenesis is the metabolic process wherein a macronutrient is converted to another macronutrient, particularly in the enzymatic pathway for synthesizing fat from dietary carbohydrates (by acetyle-coenzyme A, glucose converted to fatty acid and triglyceride) (Hellerstein, 1999). It is widely believed that de novo lipogenesis is one of the mechanisms that results in the accumulation of fat in the body. Its high thermogenic cost means that this process requires more energy than the direct storage of exogenous lipids (dietary fat intake) and consumes about 28% of the CHO energy content (Hellerstein *et al.*, 1996).

The DNL enzymatic pathway occurs in all organisms (Hellerstein, 1999). However, lipogenic enzyme activities are much higher in the livers of birds or rats than in the livers of humans (Zelewski & Swierczynski, 1990). Additionally, the conversion of carbohydrates into fat in human adipose tissue is slight

(Bjorntorp & Sjostrom, 1978). To display the whole body net DNL in humans (as indicated by a respiratory quotient greater than 1.0) and to produce small amounts of net triglycerides, a massive amount of CHO must be ingested, for example during peritoneal dialysis (Manji *et al.*, 1990) or intravenous feeding (Just *et al.*, 1990).

Acheson *et al.* (1987) demonstrated that only a very small amount of fat is produced after acute ingestion of a large amount (> 8MJ) of simple sugar (500g of dextrin maltose), (Acheson *et al.*, 1987). Acheson *et al.* (1988) investigated glycogen storage capacity and de novo lipogenesis during large amounts of carbohydrate overfeeding. They found that only a net production of about 9g/day of lipids is produced; after several days of overfeeding of a huge amount of carbohydrates, about 20MJ of post-saturation of glycogen stores occurs (Acheson *et al.*, 1988). Hellerstein (Hellerstein *et al.*, 1991) measured DNL in humans by using a non-invasive stable isotopic method. They reported that the fraction of very low-density lipoprotein-palmitates derived from DNL was only 0.9% in the fasted status and 1.6–2.0% in the fed status after a mixed breakfast containing a high level of carbohydrates. Therefore, de novo hepatic lipogenesis is a quantitatively unimportant pathway in humans. The major pathways to dispose of ingested carbohydrates are either direct oxidation or storage as glycogen in liver and muscles.

1.3.2 Protein Balance

Protein intake generally comprises about 15% of a person's total daily calories, and approximately 10-20% of a body's total stored calories are made of protein stores (Bray & Gray, 1988). Normal daily protein intake represents about 1% of the total protein stores in the body (Galgani & Ravussin, 2008). To achieve protein balance, protein intake must be equal to protein expenditure. The protein balance equation is:

Rate of change of protein stores = Rate of protein intake – Rate of protein oxidation (Equation 3)

Protein stores in the body do not increase directly from protein intake; however, they increase in mass in response to exercise training, growth stimulation from growth hormones, and weight gain (Swinburn & Ravussin, 1994). Therefore, protein stores are closely controlled, and protein balance is achieved during a short-term period (Abbott *et al.*, 1988).

Ingested protein has several functions, including restoring and maintaining structural proteins, hormones, and enzymes. A continuous energy imbalance resulting from a non-equilibrium state of protein balance occurs in response to growth stimulation and does not initiate an increase in the lipid mass; therefore, it cannot be directly implicated as a factor in the improvement of obesity (Jequier, 1993). Any excess protein intake leads to a stimulation of protein oxidation and only causes a temporary positive nitrogen balance (CALLOWAY & SPECTOR, 1954). However, protein intake may influence fat balance (Abbott *et al.*, 1988).

1.3.3 Carbohydrate Balance

The carbohydrate balance equation is:

Rate of change of carbohydrate stores = Rate of carbohydrate intake – Rate of carbohydrate oxidation (Equation 4).

Carbohydrates are usually the major source of dietary calories. A normal daily intake of carbohydrates amounts to about 50 - 100% of the carbohydrate stores in the body (Galgani & Ravussin, 2008). After eating a meal, insulin and glucose plasma levels will increase; this leads to a stimulation of glucose uptake and oxidation in muscles and other tissues, as well as an increase in glycogen storage in liver and muscles. On the other hand, insulin inhibits lipolysis,

plasma free fatty-acid levels reduce, and fat oxidation decreases (Frayn, 2003). Carbohydrate oxidation is balanced by carbohydrate intake after hours, since carbohydrate stores in the body are small in contrast to fat stores and normally do not change from day to day (Jequier, 1993). Moreover, short-term variations in glycogen stores lead to suitable responses in food intake, which helps to maintain a steady carbohydrate balance in the body (Tremblay *et al.*, 1991).

It has been demonstrated that carbohydrate intake results in suppressed lipid oxidation and enhanced glycogen synthesis and glucose oxidation (Felber *et al.*, 1987;Acheson *et al.*, 1982). However, glycogen stores in the body are very limited, with as little as 50 - 500 mmol glycosyl residues/kg in liver and 60 - 120 mmol glycosyl residues/kg in the muscles, depending on the previous food intake and the muscle investigated (Acheson *et al.*, 1988), and are very closely controlled (Thomas *et al.*, 1992). Carbohydrate oxidation responds rapidly to carbohydrate overfeeding (Aarsland *et al.*, 1997). Therefore, carbohydrates and proteins are regulated according to three common principles. Firstly, they have restricted and controlled storage capacities (Acheson *et al.*, 1988;Abbott *et al.*, 1988). Secondly, the net of de novo lipogenesis DNL in humans happens only during extreme situations (Just *et al.*, 1990;Manji *et al.*, 1990). Thirdly, the oxidation process increases and decreases in accordance with intake. Thus, a chronic incompatibility between intake and the oxidation of carbohydrates and proteins does not take place in humans and therefore cannot be implicated as the reason for the chronic positive energy imbalance that leads to obesity.

1.3.4 Fat Balance

In significant contrast to the other macronutrients, fat stores in the body are very large, and fat intake has little effect on fat oxidation; thus, eating a meal that contains a mixture of food results in an increase in carbohydrate oxidation and a decrease in fat oxidation (Flatt *et al.*, 1985;Schutz *et al.*, 1989). As with other macronutrient balance equations, the fat balance equation is:

Rate of change in fat stores = Rate of fat intake – Rate of fat oxidation (Equation 5)

Normally, daily fat intake represents a smaller amount of total intake, approximately 0.8% of the total fat stores in the body. This percentage is close to the percent of daily protein intake to total protein stores, which is about 1% (Bray, 1991). But the amount of energy in the protein stores is six times smaller than the amount of energy in the fat stores (Bray, 1991). Abbott *et al.*(1988) demonstrated that oxidation adjusts to the intake of protein and carbohydrate stores, which are usually regulated; nevertheless, fat, in contrast to carbohydrates and proteins, is virtually used or stored according to the energy balance vacillation during daily activity (Abbott *et al.*, 1988).

To achieve fat balance, fat oxidation should equal fat intake, so any increase of fat intake greater than fat oxidation leads to positive fat balance and then to weight gain. When fat oxidation exceeds fat intake, a negative fat balance appears and reduces body weight. Therefore, any over-consumption of food or restricted physical activity or both leads to a positive fat balance. Again, the reverse is true; when physical activity is increased or food consumption is decreased or both, this will result in a negative fat balance. Several studies have investigated the role of dietary fat in the bodies of overweight humans and animals (Stefanick *et al.*, 1998;Siggaard *et al.*, 1996;Pritchard *et al.*, 1996;Weststrate *et al.*, 1998;Sumiyoshi *et al.*, 2006). They suggested a better definition for obesity as a state of change in fat balance. They reported that a limitation of dietary fat could reduce the epidemic of obesity and its related health consequences.

Thomas *et al.* (1992) investigated the effects of diet composition on nutrient balance and energy expenditure in 21 weight-stable subjects (10 obese and 11 lean). They fed subjects two experimental diets for one week each in random

order, separated by one month for washing out. One of the experimental diets contained a high-fat composition, and the other was of a high-carbohydrate composition. They found that the correlation between oxidation and intake of carbohydrates and proteins was accurate, whereas the fat oxidation was not correlated to intake. These findings confirm that obesity increases with a high-fat diet more than with a high-carbohydrate diet.

In 1992, Rising *et al*. (Rising *et al.*, 1992) studied the effect of food intake on the energy balance using a special automated food selection system. They reported that the ad libitum food intake resulted in seven days of spontaneous overfeeding, leading to a gain of about 2.3 kg. Nearly all of this excess weight came from the excess fat intake, which was stored as body fat.

Flatt *et al*. (Flatt *et al.*, 1985) investigated the effects of dietary fat on postprandial metabolic responses and nutrient balance in lean subjects. Three standard breakfasts were given to the subjects. One breakfast as (control) had low fat content (482 kcal: 11% fat, 62% CHO and 27% protein) and two breakfasts, containing the same amounts of carbohydrates, proteins and high amount of fat, in two different forms (858 kcal: 50% fat, 35% CHO and 15% protein). The authors reported that during a period of nine hours post-breakfast, the addition of fat to the meal did not stimulate fat oxidation and did not influence the oxidation rate of protein, carbohydrate, or fat. In addition, a fat supplement of about 106 ± 6g fat was added to the diet, and this excess amount of fat did not promote fat oxidation over a 24-hour period (Schutz *et al.*, 1989).

The major factor influencing fat balance is energy balance (Abbott *et al.*, 1988;Zurlo *et al.*, 1990). In other words, fat balance is affected by the amount and composition of the diet eaten and the total amount of daily physical activity. From the previous observations and findings, it can be concluded that fat balance is almost equivalent to energy balance, and that there is clear

evidence for a chronic mismatch between fat intake and fat oxidation in states of positive energy balance.

1.4 Substrate Metabolism in Response to Feeding

After the consumption of a normal meal containing carbohydrate, protein and fat, the body begins to store the substrate. During this postprandial state a number of changes in hormonal balance and metabolic activities take place (Frayn, 2003).

Plasma glucose concentrations increase above the normal stable value of 5 $mmol.l^{-1}$, gaining peak concentrations at around 1 hour after a meal (Frayn *et al.*, 1993). Insulin is released by the pancreas in response to the rise of glucose. The levels of insulin release are dependent on the quantity and type of carbohydrate (Lineback, 2005). This change in hormonal balance has direct consequences on metabolic activities in different tissues.

The major organ involved in dealing with elevated glucose concentrations is the liver. During the postprandial state the liver increases uptake of glucose into hepatocytes through GLUT-2 transporters and hence begins glycogen synthesis (Klover & Mooney, 2004).

Glycogen synthesis in the liver is promoted by increased insulin and glucose concentrations which in turn activates glycogen synthase and inhibits glycogen phosphorylase (Felber & Golay, 1995). Other tissues influenced by the change in hormonal balance are the adipose tissues and skeletal muscles. In contrast to the liver, the rise in glucose and insulin concentrations cause glucose uptake by GLUT – 4 transporters (Klover & Mooney, 2004). The fate of glucose once it has entered skeletal muscle cells is that it may either be oxidised to provide energy or stored as glycogen (Frayn, 1998). On the other hand, in adipose tissue, glucose is anaerobically metabolised and this causes the adipose tissue to release lactate into the blood which is sent back to the liver and used as one of the substrates involved in gluconeogenesis (Felber & Golay, 1995).

Lipid metabolism is also affected by the rising insulin and glucose concentrations during the postprandial state. In particular, hormone sensitive lipase (HSL) activity is inhibited, although not completely, by elevated circulating insulin. The inhibition of this enzyme results in a fall in non esterified fatty acids (NEFA) concentration in the blood (Frayn, 2003). The fall in NEFA concentrations explains why, during the postprandial state, skeletal muscle is more inclined to utilise glucose to provide energy rather than lipids. In hepatocytes the oxidation of fatty acids and the formation ketone bodies fall after consumption of a meal (Frayn, 2003).

The postprandial metabolism of triglyceride is greatly increased and is also of great importance during this state. The metabolism and circulation of triglyceride (TG) is much slower than glucose and peak concentrations in the blood occur around 4 hours after a meal (Frayn, 2003). Exogenous TG, along with apolipoprotein A1, apolipoprotein B48 and cholesterol esters are secreted into circulation as a chylomicron particle by the intestine through the thoracic duct (Cohn, 1998). Once in the blood stream, chylomicrons are still not able to be lypolised by the enzyme lipoprotein lipase (LPL) until the addition of other structures such as apolipoprotein C II. This is then available for hydrolysis by LPL located in the capillaries of adipose tissue (Cohn, 1998). Lipoprotein lipase is also expressed by skeletal muscle and the heart, although to a lesser extent compared to adipose tissue (Frayn, 2003). However in adipose tissue rising insulin concentrations stimulate LPL activity, whereas in skeletal muscle the enzyme is adversely affected. Therefore in the postprandial state, the uptake of fatty acids in skeletal muscle is reduced compared to adipose tissue (Frayn, 2003).

Triglyceride also circulates in the blood in the form of very low-density lipoprotein VLDL secreted by the hepatic duct of the liver. However, during the postprandial state VLDL concentrations increase in the blood (Gill *et al.*, 2001a) due to LPL favouring chylomicron particles for lipolysis (Brunzell *et al.*, 1973). Thus, mechanisms are in place to deal with the shift in equilibrium.

Hepatic secretion of VLDL is reduced which is affected by rising plasma insulin (Karpe, 1999). Also NEFA uptake by hepatocytes is constrained due to its low plasma concentration. Therefore there is reduced secretion of VLDL (Frayn, 2003).

Actually, the metabolic pathways described previously are normal metabolic responses after a single meal. Normally, within three to four hours following a meal, plasma insulin and glucose concentrations will return to baseline values (Frayn *et al.*, 1993), and it has been reported that within eight hours after the meal, TG concentration return to normal base line values (Gill *et al.*, 2004). In fact, during everyday life, there are other meals or snacks normally consumed and those following meals will affect the metabolic pathways. Therefore, plasma insulin and glucose will be elevated again, and if this happens before a return to baseline values, an additional stimulation of glycogenesis in the liver and skeletal muscles may occur. Plasma lipid will accumulate by adding of new dietary fat, then TG concentrations will continue to increase in response to the following meals (Silva *et al.*, 2005).

1.5 Substrates Oxidation Measurements and the Principles of Indirect Calorimetry

Indirect calorimetry is a method to non-invasively measure metabolic rate and allows calculation of substrate utilisation under different conditions (Frayn, 1983). The basic principles of this technique are based on the measurement of the oxygen consumption and carbon dioxide production at the level of the lungs, nitrogen excretion and the knowledge of the stoichiometry of carbohydrate, protein and fat oxidation.

Respiratory exchange ratio (RER) is the ratio of the volume of carbon dioxide released to the volume of oxygen taken up into the lungs at the same time. Respiratory exchange ratio normal ranges are between 0.7 and 1.0 and this factor is used as an indicator of substrate oxidation, where 1.0 indicates total carbohydrate oxidation, while 0.7 refers to total fat oxidation (Ferrannini,

1988). Frayn (1983) chose glucose and palmitoyl-stearoyl-oleoyle-glycerol (PSOG) to represent carbohydrate and fat oxidation, respectively. The stoichiometry of glucose and PSOG is as follows:

Glucose ($C_6H_{12}O_6$) + 6 O_2 → 6 H_2O + 6 CO_2

PSOG ($C_{55}H_{104}O_6$) + 78 O_2 → 52 H_2O + 55 CO_2

From the above equations, oxidation of 1 mol of glucose, requires an uptake of 6 mol of oxygen and a release of 6 mol of carbon dioxide, thus, RER will be 6/6 =1.0. Also, the oxidation of 1 mol of PSOG requires an uptake of 78 mol of oxygen and a release of 55 mol of carbon dioxide, thus, RER will be 0.70.

There are different techniques used in indirect calorimetry to measure fuel oxidation, and the most common one is the Douglas bag method which has been called the gold standard method (Jeukendrup & Wallis, 2005). In this method the volunteer is required to wear a nose clip and breathe through a mouth piece into a large, air-tight bag. Expired air can be analysed to calculate the fraction of oxygen and carbon dioxide by passing aliquots of expired air into a gas analyser. The indirect calorimetry methods are considered as an attractive way to conduct research due to the non-invasive nature and the quick response time (Jequier *et al.*, 1987). Nevertheless, indirect calorimetry depends on some assumptions, which are very important to understand, together with the limitations of this technique, so that appropriate interpretations of data can be made.

The amount of oxygen used and carbon dioxide produced during substrate oxidation are different for different carbohydrates, proteins and fats because of their different chemical composition. Metabolic rate and energy nutrient oxidations are determined by equations which are normally extracted from single values of oxygen uptake and carbon dioxide produce related with the complete oxidation of one gram of carbohydrate, protein and fat (Jequier *et al.*, 1987). However, these values are altered depending on the type of fuel source being oxidised. Therefore, the amount of oxygen consumed for carbohydrate oxidation will differ according to the type of sugar, whether the

oxidised sugar is a mono-, di-, or poly saccharide (Jequier *et al.*, 1987). Thus, to oxidise one gram of glucose and lactose requires the consumption of 746 and 786 ml of oxygen, respectively. But, for starch (Glycogen) oxidation, the volume of oxygen used will be raised to 829 ml (Jequier *et al.*, 1987). Consequently, when using equations derived from glucose as a source of energy in a situation where glycogen oxidation is dominant, an overestimation of carbohydrate oxidation may result. In contrast, using equations based on glycogen oxidatio in a state where glucose oxidation is dominant, may cause an underestimation of carbohydrate oxidation (Jequier *et al.*, 1987).

Frayn (1983) used a triglyceride called palmytoyl-stearoyl-oleoyl glycerol (PSOG) as this gives a representation of the composition found within normal human adipose tissue. This value is used if the exogenous lipid oxidised is of a comparable composition or if fat oxidation is expected to be mostly within the tissue. Therefore, the determination and the assumption of the appropriate type of fuel source should rely on the physiological situation under study (Ferrannini, 1988).

Under normal physiological conditions the metabolic rate for protein oxidation is relatively low. Protein oxidation is calculated by measuring the total urinary nitrogen (TUN). An alternative method is by measuring urinary urea nitrogen (UUN), however the former is generally recommended as UNN may underestimate TUN (Skogerboe *et al.*, 1990).

Other researchers assume protein oxidation is constant or zero (Tsetsonis *et al.*, 1997;Gill *et al.*, 2004;Wallis *et al.*, 2007). This is due to the fact that, under normal physiological conditions, even if protein oxidation was miscalculated by 50%, this would only produce an error of 13 - 15% in fat and carbohydrate oxidation. During exercise such as aerobic and resistance exercise, the protein oxidation rate may vary slightly. However, during periods such as energy restriction, protein oxidation appears to be fairly stable (Melanson *et al.*, 2002a;Schneiter *et al.*, 1995;Votruba *et al.*, 2002). Thus, for most situations, most researchers do not undertake the measurement of

urinary nitrogen in the experiment, and assume that protein is oxidized at a constant rate, or assume that protein oxidation does not lead to big errors in substrate calculations.

The use of indirect calorimetry is based on the assumption that intermediate metabolic processes have no consequence on the final outcome. Metabolic intermediates will not have an effect on the final outcome if their end products are H_2O or CO_2 (Frayn, 1983); however, if this is not the case then this assumption may not be correct. Intermediate processes such as gluconeogenesis, lipolysis, and ketone body formation or utilisation may affect $\dot{V}O_2$ and $\dot{V}CO_2$, and therefore the obtained values of RER may not represent valid substrate oxidation rates. An increased RER value above 1.0 may be due to the process of lipogenesis and ketone body utilisation. On the other hand, gluconeogenesis and ketone body formation may decrease the RER value below 0.7 (Frayn, 1983). However, the effect of such intermediate metabolic processes is insignificant except in extreme cases. Therefore, generally and in most cases, indirect calorimetry provides an accurate representation of substrate oxidation (Frayn, 1983).

The final assumption is that the equilibration of the body oxygen and carbon dioxide pools during the measurement of substrate oxidation are essential to make a value data estimation from indirect calorimetry (Ferrannini, 1988). For oxygen, this assumption may be less important and unlikely to be problem because there is no oxygen reserve inside the body. Therefore, oxygen uptake will reliably reflect whole body oxygen consumption (Ferrannini, 1988).

In order for RER to be used to estimate substrate oxidation during exercise, the subject must have reached the steady state, which means that physiological function remains relatively stable (Jequier *et al.*, 1987). This is important because it is only during steady state exercise that the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$ reflects the metabolic gas exchange in the body tissues (Jeukendrup & Wallis, 2005).

Basically, during low to moderate exercise intensities this does not present a problem, because the lactate concentration in the circulation during low to moderate intensity exercise may be matched or slightly higher than the baseline value, but it is still in steady rate (Jeukendrup & Wallis, 2005). Therefore, lactate production in the muscles is mostly in equilibrium with lactate elimination, due to oxidation and conversion to glucose in the liver (Jeukendrup & Wallis, 2005).

However, during high intensity exercise, where lactate acid production can be greater than oxidation, lactate will accumulate in the muscle (Jeukendrup & Wallis, 2005), resulting in hydrogen ions release and increased in plasma. These ions are may buffered by bicarbonate [HCO_3^-], and finally extra non-metabolic CO_2 will be excreted on the breath (Frayn, 1983). Thus, during heavy exercise when lactate excretion is high, estimation of substrate utilisation by indirect calorimetry technique may be flawed (Frayn, 1983).

Hyperventilation occurs during high intensity exercise and leads to CO_2 being eliminated at rate greater than O_2 is consumed, and this may result in an increase in RER values above 1.0 (Jequier *et al.*, 1987), and this can substantially affect the estimated values of carbohydrate and fat oxidation. Nevertheless, bicarbonate reserve in the body are replenished during a compensatory period called hypoventilation after hyperventilation, and to ensure the calculations of substrate utilisation are correct, the measurement period must be continued and include both phases (hypoventilation and hyperventilation) (Jequier *et al.*, 1987).

In summary, if these assumptions and limitations are considered properly by the researcher, the measurements of substrate oxidation using gas exchange and indirect calorimetry will be valid under different experimental situations.

1.6 Carbohydrate and Fat Oxidation

Fat is the primary source of energy in the fasted state at rest and during low to moderate physical activity (Frayn *et al.*, 2006). The sources of lipid substrates, such as fatty acids, or triglycerides contained by lipoproteins (primarily very low-density lipoproteins (VLDL), and chylomicrons), are present in the bloodstream and come directly from dietary foods which are absorbed in the small intestine, or derived from the lipolysis of adipose tissues as fatty acids (Hansen *et al.*, 2005). In addition, there are other sources for triglyceride called intramuscular triglycerides located in the muscles and can be metabolized as a substrate when required, and can contribute considerably to total energy metabolism (van Loon *et al.*, 2003).

Stored fat or triglyceride in adipose tissues is broken down into fatty acids and glycerol by a hormone called hormone sensitive lipase (HSL) as follows:

$$\text{Triglyceride} + 3\ H_2O \xrightarrow{\text{HSL}} \text{Glycerol} + 3\ \text{Fatty acids}$$

Hormone sensitive lipase is activated by growth hormone and catecholamines and is inhibited by insulin. Almost all of the fatty acids in the blood circulation bind to plasma albumin for transport around the body's active tissues as free fatty acids (FFA). Through beta (ß)-oxidation, FFA then transforms to acetyl-CoA for direct entry into the citric acid cycle metabolism in the mitochondria to be used as energy. The glycerol either transforms as a 3-phosphoglyceraldehyde, breaks down to a pyruvate, and it is metabolized through glycolysis, or it can be used to make glucose in the tissues (McArdle *et al.*, 2006).

Oxidation mostly depends on energy requirements because fat intake does not stimulate fat oxidation (Schutz *et al.*, 1989). Therefore, an excess amount of dietary fat is almost completely stored in adipose tissue, whilst extra amounts of protein and carbohydrate are quickly utilized, so the oxidation adjusts to

the intake (Abbott *et al.*, 1988; Acheson *et al.*, 1988). Swinburn & Ravussin (1994) stated that a person who has a high rate of fat oxidation can succeed in losing weight and resisting weight gain (Swinburn & Ravussin, 1994). Further, lower rates of fat oxidation and high respiratory exchange ratios (RER) may provide a positive fat balance, which can result in body weight gain (Zurlo *et al.*, 1990;Marra *et al.*, 1998;Seidell *et al.*, 1992). Therefore, people should oxidise more fat to prevent weight gain and maintain a healthy body weight.

Regular exercise, which increases fat oxidation and total daily energy expenditure, has been suggested as one of the most critical body weight management factors. Much debate in the literature has centred around identifying an optimal level of exercise intensity to elicit a maximal fat oxidation rate (Meyer *et al.*, 2007). Exercise type, intensity, and duration have been identified as the main factors of fat oxidation (Achten & Jeukendrup, 2004). Understanding the influencing factors that inhibit or promote fat oxidation is essential to controlling excess weight. Consequently, using previous studies, researchers are working to find the best exercise strategies leading to maximal fat oxidation, whether low-intensity (< 40% $\dot{V}O_2$ max), moderate-intensity (40-70% $\dot{V}O_2$ max), high-intensity (> 70% $\dot{V}O_2$ max), resistance exercise, or any combination thereof, and with different durations.

1.7 Effects of Exercise on Fat Oxidation and Metabolic Responses

Currently, it is commonly accepted that one of the most effective strategies for managing body weight and improving general and metabolic health is exercise training, which increases energy expenditure (Pate *et al.*, 1995). Fat and carbohydrate are the main sources of energy used by the muscles during aerobic exercise in well-fed humans (Spriet, 2002). There are certain factors that affect the contribution of these sources of energy, including an alteration in dietary intake and composition, a person's training status (Coyle *et al.*, 2001;Horowitz *et al.*, 1997;Ainslie *et al.*, 2002;Bergman & Brooks, 1999),

muscle glycogen content (Weltan *et al.*, 1998), and exercise intensity and duration (Romijn *et al.*, 1993;Friedlander *et al.*, 1998a;Friedlander *et al.*, 1998b;Achten *et al.*, 2002). In 2001, van Loon *et al*. reported that exercise intensity is one of the most important factors affecting the consumption of fat as a major fuel in producing energy adenosine tri-phosphate (ATP) in human skeletal muscles during aerobic exercise.

As previously mentioned, the source of energy selection during exercise is reliant on a number of factors, including duration and intensity of the exercise. Post-exercise substrate oxidation and metabolism are altered by exercise. Most research has focussed on the effects of exercise on metabolic responses during the exercise period. However, it is essential to also consider the recovery and post-exercise stages because they represent a much longer period of time during which metabolic responses and fat oxidation can be influenced (Saris & Schrauwen, 2004).

Several studies have demonstrated that after endurance exercise, in the post-exercise phase, fat oxidation rates have been increased (Gill *et al.*, 2001b;Horton *et al.*, 1998;Gill & Hardman, 2000;Malkova *et al.*, 2000).

1.7.1 Effect of Exercise Intensity and Duration on Fat Oxidation and Metabolism

According to two widely-cited studies by (Romijn *et al.*, 1993;Romijn *et al.*, 2000) fat oxidation increases with increasing exercise duration. Romijn *et al* (1993) studied exercise intensity and substrate utilisation during prolonged exercise using indirect calorimetry and isotopes (tracer techniques). They examined trained subjects, five males in the first study and eight females in the second. Subjects exercised at 25%, 65%, and 85% of maximum volume oxygen consumption ($\dot{V}O_2$max). They found that the rate of fat oxidation increased from low-intensity exercise (25% of $\dot{V}O_2$max) to moderate-intensity (65% of $\dot{V}O_2$max), and then reduced at high-intensity (85% of $\dot{V}O_2$max). Fat

oxidation rates increased from rest to low- to moderate-intensity exercise and plasma fatty acid was the main substrate utilised for energy. However, When the exercise intensity exceeded 75-85% of $\dot{V}O_2$ max, carbohydrate oxidation became the major source of energy provision and fat oxidation was suppressed; this suppression is probably the result of the stimulation of glycolysis and glycogenolysis during high-intensity exercise (Jeukendrup, 2003).

Some studies have reported that endurance exercise stimulates fat oxidation at rest in young, trained individuals (Romijn *et al.*, 1993) and young, normal, healthy subjects (Calles-Escandon *et al.*, 1996). During exercise, low- to moderate-intensity exercise leads to maximum fat oxidation rate (Achten *et al.*, 2002). In this study, Achten and colleagues investigated different exercise intensities and their effects on fat oxidation using indirect calorimetry. They concluded that the maximum rate of fat oxidation occurred at 64% of $\dot{V}O_2$ max, corresponding to a 74% heart rate.

Moreover, in a study investigated the substrate utilisation during different exercise intensities in 26 normal-weight and 32 overweight subjects, subjects completed a submaximal exercise test consisting of four six-minute gradual phases at 30%, 40%, 50%, and 60% of maximal power (Wmax). The researchers reported that there were no significant differences were found between the two groups in plasma glucose concentrations at rest and during exercise. However, significantly lower maximal fat oxidation rates happened at significantly lower exercise intensities (30.5 ± 2.3 versus 44.6 ± 3.3 Wmax) (Perez-Martin *et al.*, 2001).

By contrast, some researchers have found that neither exercise nor intensity affects the resting fat metabolism in obese women and men (Nicklas *et al.*, 1997;Aggel-Leijssen *et al.*, 2001;van Loon *et al.*, 2001). In 2001, van Aggel-Leijssen *et al.* studied the effects of the addition of low-intensity exercise sessions to a diet restriction programme in obese males. They found no

significant difference in resting metabolism rate (RMR) between the diet restriction only and the diet restriction plus exercise session groups. However, the addition of exercise in the second group counteracted the decrease in fat oxidation which was produced in the period following the diet due to the loss of body weight. Another study found almost a similar result among older obese women; the baseline RMR of the subjects was similar in the hypocaloric diet group compared to the other group, which added endurance exercise to the same hypocaloric diet programme, but the declines in fat oxidation and adipocyte lipolysis caused by weight loss, were counteracted by the additional endurance exercise (Nicklas *et al.*, 1997).

High-intensity exercise has a beneficial impact on cardiorespiratory fitness (Laursen & Jenkins, 2002), and may lead to a chronic increase of RMR and thus total energy expenditure (Poehlman *et al.*, 1986;Phelain *et al.*, 1997). By contrast, there is evidence from several studies to suggest that low- and moderate-intensity exercise may lead to greater health benefits than high-intensity exercise of the same work load because the incidence rates of musculoskeletal injuries, cardiovascular episodes, and muscle damage in high-intensity exercise is greater compared with low- and moderate-intensity, especially in obese individuals (Siscovick *et al.*, 1984;Hootman *et al.*, 2001). Additionally, fat oxidation rat during exercise increases with low and moderate intensity more than with high intensity (Romijn *et al.*, 2000;Aggel-Leijssen *et al.*, 2002). But, it must be taken into account the changes in fat oxidation and metabolism in the period after exercise (Saris & Schrauwen, 2004;Phelain *et al.*, 1997;Gill *et al.*, 2006;Horton *et al.*, 1998).

Furthermore, Melanson *et al.* (2001) investigated the effects of low- and high-intensity exercise on 24-hour energy expenditure (EE) and nutrient oxidation. Sixteen lean, healthy subjects (8 women and 8 men) participated in the study. They were studied in three trials: a control day with no exercise, a low-intensity exercise trial expending 400 kcal at 40% of $\dot{V}O_2$ max, and a high-intensity exercise trial expending 400 kcal at 80% of $\dot{V}O_2$ max, using indirect

calorimetry. The researchers found that the 24-h EE and the CHO oxidation significantly increased in the exercise trials compared to the control, whereas 24-hour fat oxidation was not significantly different between trials (Melanson *et al.*, 2002b).

Moreover, in a similar study, Saris and Schrauen (2004) examined the effects of different intensities of exercise in 24-hour substrate oxidation. They studied 8 obese males who followed two different exercise protocols (High and Low intensity) in random order, separated by two weeks and using respiratory chambers. In the low intensity protocol, subjects cycled three times for 60 minutes at 38% of Wmax, and in the high intensity protocol, they cycled three times for 30 minutes at alternative bouts of 2.5 minutes at 80% and 50% of Wmax. The energy expenditure of the high and low intensity protocols was matched. Researchers reported that the 24-h EE and RER were not significantly different between the protocols. During exercise, the RER was lower in the high intensity protocol compared to low intensity protocol, whereas in the recovery phase of exercise, the RER tended to be higher in the low intensity protocol compared to the high intensity protocol. However, the differences during the exercise trials were compensated through the post-exercise period, which resulted in a comparable substrate oxidation pattern over 24 hours (Saris & Schrauwen, 2004).

Furthermore, fat oxidation rate and $\dot{V}O_2$ uptake were similar during six hours in the recovery phase following 90 minutes at 33% of $\dot{V}O_2$ max or 45 minutes at 66% of $\dot{V}O_2$ max of isocaloric exercise (Thompson *et al.*, 1998). It has been reported that in healthy obese males, fat oxidation during exercise increases after an intervention of 12 weeks of moderate-intensity exercise at 40% of $\dot{V}O_2$ max, while high-intensity exercise at 70% of $\dot{V}O_2$ max for 12 weeks with a similar caloric expenditure does not significantly affect fat oxidation during exercise after this intervention (Aggel-Leijssen *et al.*, 2002).

1.7.2 Effect of Training Status and Feeding on Fat Oxidation and Metabolism

In addition to the previous factors affecting fat oxidation and metabolism, numerous studies have reported that training status and food intake may also play important roles in altering fat oxidation and metabolism. Bergman and Brooks (1999) investigated RER during different intensities of exercise in trained and untrained males in a fasted state and after feeding. Seven trained and seven untrained healthy male subjects were involved in this study. After preliminary tests and a determination of $\dot{V}O_2$ max, volunteers were instructed to exercise at a target intensity of 22% and 40% of $\dot{V}O_2$ max for two hours, 59% $\dot{V}O_2$ max for 90 minutes, and 80% $\dot{V}O_2$ max for 30 minutes for untrained subjects and 45 minutes for trained subjects after either a 12-hour overnight fast or three hours after a standard breakfast containing 550 kcal (2% fat, 87% CHO, and 11% protein). The interesting findings were in the fasted state; trained subjects had the highest fat oxidation rates at 40% of $\dot{V}O_2$ max, compared to 59% $\dot{V}O_2$ max for the untrained subjects. However, in the fed state, in both trained and untrained participants, the maximal fat oxidation was noted at 40% of $\dot{V}O_2$ max (Bergman & Brooks, 1999).

Bassami and colleagues (2007) investigated substrate metabolism during different intensities of exercise in older men. Seven trained and six untrained, healthy, older males performed three exercise trials, including 30 minutes of ergometer cycling at 50%, 60%, and 70% of $\dot{V}O_2$ max, and two other trials at 60% and 70% of $\dot{V}O_2$ max, in which the total energy expenditure was calculated to be equal to 30 minutes at 50% of $\dot{V}O_2$ max. They demonstrated that exercise intensity significantly affects the CHO and fat oxidation. The percentages of fat oxidation during constant durations at 50% and 60% $\dot{V}O_2$ max were significantly higher than trial at 70%. However, the CHO oxidation rates at 50% and 60% $\dot{V}O_2$ max were significantly lower compared to exercising at 70% $\dot{V}O_2$ max. Plasma insulin and glucose concentrations did not change

significantly at the different intensities of exercise either with similar durations or with the trials with equal energy expenditure. Plasma NEFA and Glycerol concentrations were increased significantly immediately after the three exercise intensities when the durations were constant (Bassami *et al.*, 2007).

Ainslie et al. (2002) studied the metabolic responses to high fat or CHO meals and snacks during 7.5 hours of prolonged, intermittent walking at different intensities between 25-30% and 50-55% of $\dot{V}O_2$ max in eight males using indirect calorimetry. The investigators reported that fat oxidation, NEFA, and glycerol were increased by a high-fat diet more than by a high-CHO diet or a mixed diet (Ainslie *et al.*, 2002).

Furthermore, in a study investigating the effects of high-CHO and low-fat diets on metabolism during exercise, researchers demonstrated that fat oxidation lowered by 27% with the high-CHO, low-fat diet (88% CHO, 2% fat, and 10% protein) versus the other diet containing high CHO and high fat (68% CHO, 22% fat, and 10% protein) in seven well-trained cyclists who trained for 2 hours per day at 70% $\dot{V}O_2$ max for 1 week during each diet programme (Coyle *et al.*, 2001).

The ingestion of CHO before exercise has a strong inhibitory influence on fat utilisation. It has been suggested that the ingestion of 50-100g of CHO before exercise will reduce lipolysis and inhibit fat oxidation by about 30 - 40% (Coyle *et al.*, 1997;Achten & Jeukendrup, 2003). In 2000, Hawley *et al.* examined seven well-trained male cyclists who consumed a high-fat meal 90 minutes before intense cycling. The researchers found that the fat oxidation and plasma fatty acid concentrations were elevated during the 20 minutes of cycling (Hawley *et al.*, 2000). Additionally, in a similar pre-exercise feeding study, Whitley *et al.*, (1998) investigated the effects of prior high-fat and high-CHO meals on metabolic responses during endurance exercise. Eight trained

cyclists were given an isoenergtic high-CHO or high-fat diet four hours before 90 minutes of cycling at 70% $\dot{V}O_2$ max. The researchers noted that higher fat oxidation rates occurred after high-fat meals, which were most significant during the first 15 minutes of the 90-minute cycling sessions (Whitley *et al.*, 1998).

If CHO loads were ingested several hours before exercise, the rate of fat oxidation decreased and the NEFA concentrations were suppressed. In fact, CHO ingestion several hours prior to exercise also led to an increase of CHO oxidation during the exercise and an elevation of plasma insulin concentration before exercise (Coyle *et al.*, 1985;Montain *et al.*, 1991).

Votruba *et al.*, (2002) studied the effects of prior intensities of exercise on subsequent oxidation of dietary fat using whole-room indirect calorimetry. Seven female subjects were given a typical western diet containing 55% CHO, 30% fat, and 15% protein after performing cycle ergometery during two different exercise treatments. The exercise was performed at low intensity (25% of $\dot{V}O_2$ max) and high intensity (85% of $\dot{V}O_2$ max), which was calculated to expend ~300 kcal above RMR. In both treatments, exercise was then followed by multiple meals containing 30% fat. The investigators reported that both intensity exercises (low and high) had a similar increase in fat oxidation rates above the resting rates for 11.5 hours after exercise (Votruba *et al.*, 2002).

In a similar study looking at fat oxidation, Gill *et al.*, (2001) investigated the effects of prior moderate exercise on dietary fat metabolism. Eleven subjects participated in two trials including two oral fat tolerance tests and tracer-containing emulsion (1,1,1- 13C), preceded by two different situations the day before: a control with no exercise, and exercise with 90 minutes of walking at 60% of $\dot{V}O_2$ max. Data and samples were collected over 8 hours after the mixed high-fat meal and intermittently thereafter at 15 hours and 24 hours after ingestion of the test meal. Gill and colleagues reported that exogenous fat

oxidation rates significantly increased at about 20 hours post-exercise (Gill *et al.*, 2001a).

1.7.3 Effect of Resistance Exercise on Fat Oxidation and Metabolism

Many health organisations and associations, such as the American College of Sports Medicine, the American Heart Association, and the American Association of Cardiovascular and Pulmonary Rehabilitation, recommend resistance exercise (R), categorised by high-intensity and low-velocity with short durations, to improve health and prevent and treat chronic conditions like cardiovascular disease, obesity, and diabetes (Tresierras & Balady, 2009), and to maintain a successful fitness and exercise programme (Haskell *et al.*, 2007;Donnelly *et al.*, 2009;Pollock *et al.*, 2000). It has been reported that resistance exercise can improve body composition by increasing total and specific-region body lean mass and decreasing total and regional fat mass in middle-aged and older men (Treuth *et al.*, 1994;Ormsbee *et al.*, 2009).

1.7.4 Effect of Chronic and Acute Resistance Exercise on Fat Oxidation and Metabolism

Many studies suggest that resistance exercise may have a positive impact on metabolic responses; increasing post-exercise oxygen consumption (EPOC) (Thornton & Potteiger, 2002;Schuenke *et al.*, 2002;Melby *et al.*, 1993;Binzen *et al.*, 2001) and the rate of fat oxidation (Goto *et al.*, 2007; Melby *et al.*, 1993; Binzen *et al.*, 2001), suggesting that (R) may offer more health benefits with regard to body-weight balance.

Moreover, (R) exercise has been shown to reduce resting RER immediately after exercise (Melby *et al.*, 1993) and at 15 and 16 hours following an (R) session compared to a control day with no exercise, which demonstrates an increase in the fat oxidation rate after exercise in males and females (Gillette *et al.*, 1994;Melby *et al.*, 1993;Osterberg & Melby, 2000).

It has been reported that intramuscular triglycerides stores and glycogen contents were reduced after 45 minutes of (R) in untrained healthy men (Koopman *et al.*, 2006) and after 30 minutes of heavy (R) in nine body builders (Essen-Gustavsson & Tesch, 1990). Additionally, it has been demonstrated that the basal, VLDL-TG production rate in healthy, untrained, non-obese young men was decreased after a single bout of (R) more than after a single bout of endurance exercise with matched total energy expenditure (Magkos *et al.*, 2008).

Furthermore, some studies have investigated the effects of chronic and acute resistance exercise on substrate oxidation and EE using indirect calorimetry. Kirk *et al*. (2009) recently examined the effects of chronic slight resistance training (using one set of 11 minutes, 3 days/week for 6 months) on daily EE and fat oxidation. Thirty-nine overweight young adults participated randomly in two groups: a resistance training group (R, N = 22), and a control group (CO, N = 17). They completed all assessment tests at baseline and after 6 months. The training programme consisted of one session of three to six repetition maximum RM (nine exercises total) 3 days/week for 6 months. Then, 3 days after the last (R) session, subjects were asked to enter the whole-room indirect calorimetry to measure the 24 EE and substrate oxidation. The investigators found that 24-hour fat and CHO oxidation were not changed after (R) compared to the CO, nor were there any significant changes within groups at baseline in comparison to after 6 months. However, the increase in the 24-hour EE in the (R) group was about double that of the increase in the 24-hour EE in the CO group (Kirk *et al.*, 2009).

Likewise, another study examined the effects of acute resistance (R) and aerobic exercise (AE) on 24-hour EE and macronutrient oxidation in 10 non-obese, healthy male subjects by using whole-room indirect calorimetry. The subjects participated on three occasions: (AE) at 70% of $\dot{V}O_2$ max (cycle ergometry), (R) at 70% of exercise-specific 1 RM, and a non-exercise control

day (CO). Researchers observed that the 24-hour RER, fat, and protein oxidation rates were almost the same in all three treatments. However, the CHO oxidation was significantly increased in the R and the (AE) compared to the CO. In the post-exercise period, EE and nutrient oxidation rates did not differ in all conditions. Therefore, the investigators concluded that (R) and (AE) have mostly the same effects on 24-hour EE and substrate oxidation (Melanson *et al.*, 2002a).

Moreover, acute and chronic combination exercise programmes which include high-power and low-velocity exercises (such as resistance exercise) combined with low-power and high-velocity exercises are strongly recommended to obtain good health and a suitable body weight (Izquierdo *et al.*, 2004;Haskell *et al.*, 2007;Donnelly *et al.*, 2009;Pollock *et al.*, 2000). Marzolin *et al.* 2008 (Marzolini *et al.*, 2008) recently demonstrated that combining resistance training sessions and aerobic training sessions induced greater improvements in cardiovascular fitness compared to a single aerobic training session. Metabolic responses during submaximal exercise might be influenced by elevated lipolysis proceeding the same session of (AE) (Stich *et al.*, 2000).

Drummond *et al.* (Drummond *et al.*, 2005) studied the effects of aerobic and resistance exercise sequences on EPOC. They reported that the treadmill $\dot{V}O_2$ was significantly higher for trials involving resistance exercise followed by running at 70% of $\dot{V}O_2$ max compared to reverse sequence trial (running at 70% of $\dot{V}O_2$ max followed by resistance exercise). They demonstrated that EPOC was greatest after running followed by resistance exercise. They recommended performing resistance exercise after aerobic exercise, for a combined training programme including aerobic and resistance exercises.

Recent studies indicate that a prior session of resistance exercise can significantly increase fat oxidation during subsequent moderate intensity exercise (Kang *et al.*, 2009;Goto *et al.*, 2007b). In the Goto *et al.* study,

subjects participated in three trials, separated by approximately seven days in random order: a trial with only submaximal continuous exercise using a cycle ergometer at around 50% $\dot{V}O_2$ max for 60 minutes (control) and two trials with the same submaximal exercise preceded by resistance exercise, 20 minutes of rest or resistance exercise, and 120 minutes of rest. Kang et al. (2009) executed approximately the same 3 trials and order of exercises, but with different intensities of resistance exercise at 60% and 90% of 8-RM and only 20 minutes of aerobic exercise with 5 minutes of rest in between.

However, there are a number of important limitations in the Goto et al. (2007) and Kang et al. (2009) studies. They examined only the effect of prior resistance exercise on lipolysis during subsequent submaximal exercise and did not consider metabolism during the resistance exercise session itself. To fully understand the effects of resistance exercise on metabolism, it is necessary to evaluate metabolism during the resistance exercise session itself. A further limitation was that in the control trial, subjects did no exercise prior to the moderate exercise bout. It is possible that other types or intensities of prior exercise could conceivably also influence subsequent metabolism; for example, prior moderate intensity exercise at 50% of $\dot{V}O_2$ max for 1 hour leads to enhanced lipid mobilization and decreased plasma insulin concentrations during a second session of moderate exercise at the same intensity and duration performed 1 hour later (Stich et al., 2000). Subjects in the prior resistance exercise trial would have done a longer exercise session and will show increased fat oxidation, a process which increases with exercise duration (Romijn *et al.*, 1993). Therefore, it might be expected that fat oxidation would be higher, irrespective of the exercise mode. Thus, it is not clear if any prior exercise, rather than resistance exercise *per se* would lead to an increase in fat oxidation during subsequent exercise. This requires investigation.

A third limitation was they did not follow the metabolic responses and fat oxidation throughout the recovery stage. This is crucial since the post-exercise period may also play an important role, because exercise alters post-exercise

substrate oxidation and metabolism. Thus, including the post-exercise phase is necessary, as understanding the total effect of exercise on metabolism, requires evaluation of changes made following, as well as during exercise (Gill *et al.*, 2001b;Horton *et al.*, 1998;Saris & Schrauwen, 2004;Phelain *et al.*, 1997;Gill & Hardman, 2000;Malkova *et al.*, 2000). This is illustrated by the study of Saris and Schrauwen, (2004) which investigated the differences over 24 hour in nutrient oxidation between moderate and high intensity exercise which were matched for energy expenditure. They found that the 24-h EE and RER were not significantly different between the two trials. However, during exercise, the RER was lower in the high intensity trial compared to moderate intensity trial, whereas in the recovery phase of exercise, the RER tended to be higher in the moderate intensity protocol compared to the high intensity protocol.

1.7.5 Summary and Objectives

This chapter reviewed current knowledge and provides the reader with the information required to understand energy and macronutrient balance, substrate oxidation and metabolism and the effects of different types of exercise on metabolism and fat oxidation.

Obesity and its associated diseases are major public health concerns, and there is an essential need to understand how to prevent and treat such diseases. Exercise is offered as an important method for protecting against and treating obesity through its effects on fat oxidation. Many investigators have suggested that maximal fat oxidation occurs during moderate-intensity exercise. However, in the post exercise period, fat oxidation tends to be higher following high-intensity exercise than exercise which is of a moderate-intensity. Furthermore, several studies have reported that different modalities of prior exercise may affect metabolism and fat oxidation during subsequent moderate-intensity exercise. However, it remains unclear which exercise strategy is best for maximising fat oxidation.

During moderate- to high-intensity exercise, the source of energy is a mixture of both carbohydrate and fat. During exercise at 80% $\dot{V}O_2$max, the rate of carbohydrate oxidation is relatively high compared to fat oxidation, and it is well known that prolonged endurance exercise can be limited by glycogen stores. Therefore, an increase in the rate of fat oxidation, increasing the proportional contribution of fat metabolism to the total energy requirement, has the potential to preserve intramuscular glycogen stores and thus improve performance. This suggests subjects can exercise for a greater duration at the same work rate, or can exercise for the same duration but at a higher work rate.

The present study addresses some limitations that have been discussed above in Section 1.7.4., with the aim of this study being to further investigate the effects of prior resistance, moderate-, and high-intensity exercise on metabolism and fat oxidation during and after subsequent moderate-intensity exercise.

2 METHODOLOGY

2.1 Overview

The present study was reviewed and approved by the Faculty of Biomedical and Life Sciences Ethics Committee at the University of Glasgow (Appendix A). Subjects undertook three preliminary visits. These included screening visits involving information about the study and consent forms (Appendix B), inquiries, health history questionnaire (Appendix C), physical activity questionnaire (Appendix D), $\dot{V}O_2$ max test performed on a Woodway motorised treadmill and a familiarisation session for resistance exercise.

Each subject participated in three main trials: a prior moderate-intensity exercise (M), a prior high-intensity exercise (H), and a prior resistance exercise (R), with an interval of approximately one week in random order. In (M) trial, subjects performed 30 minutes of treadmill walking M (Ex.1), at a speed corresponding to 40% $\dot{V}O_2$ max reserve ($\dot{V}O_2$ max reserve = $\dot{V}O_2$ max - $\dot{V}O_2$ at rest), followed by rest for 30 minutes and then a treadmill walking at the same speed, which was 40% $\dot{V}O_2$ max reserve for 60 minutes M (Ex.2). For (H) trial, subjects performed 15 minutes of treadmill running at a speed corresponding to 80% $\dot{V}O_2$ max reserve H (Ex.1) (to expend the same amount of energy as in the (M)), followed by rest for 30 minutes before performing treadmill walk at speed of 40% $\dot{V}O_2$ max reserve for 60 minutes H (Ex.2). For the (R) trial, subjects performed 30 minutes of different resistance exercises of large muscle groups, such as squats, lunges, lat pull-downs and shoulder presses R (Ex.1), followed by rest for 30 minutes and then a treadmill walk at a speed of 40% $\dot{V}O_2$ max reserve for 60 minutes R (Ex.2). A standard breakfast and lunch were given to the subjects at the start, and after the second exercise session Ex.2, respectively. In all trials, blood and expired air samples were taken throughout the tests and four hours after the lunch. Heart rate and rate of perceived exertion (RPE) were taken during Ex.1 and Ex.2, in the three different interventions.

Subjects abstained from physical activity and alcohol consumption for the two days prior to the start of each trial. In addition, subjects weighed and recorded their food and drink intake for the two days before each trial. This diet was replicated as closely as possible for all the trials. An overview of the experimental designed is depicted in Figure (1).

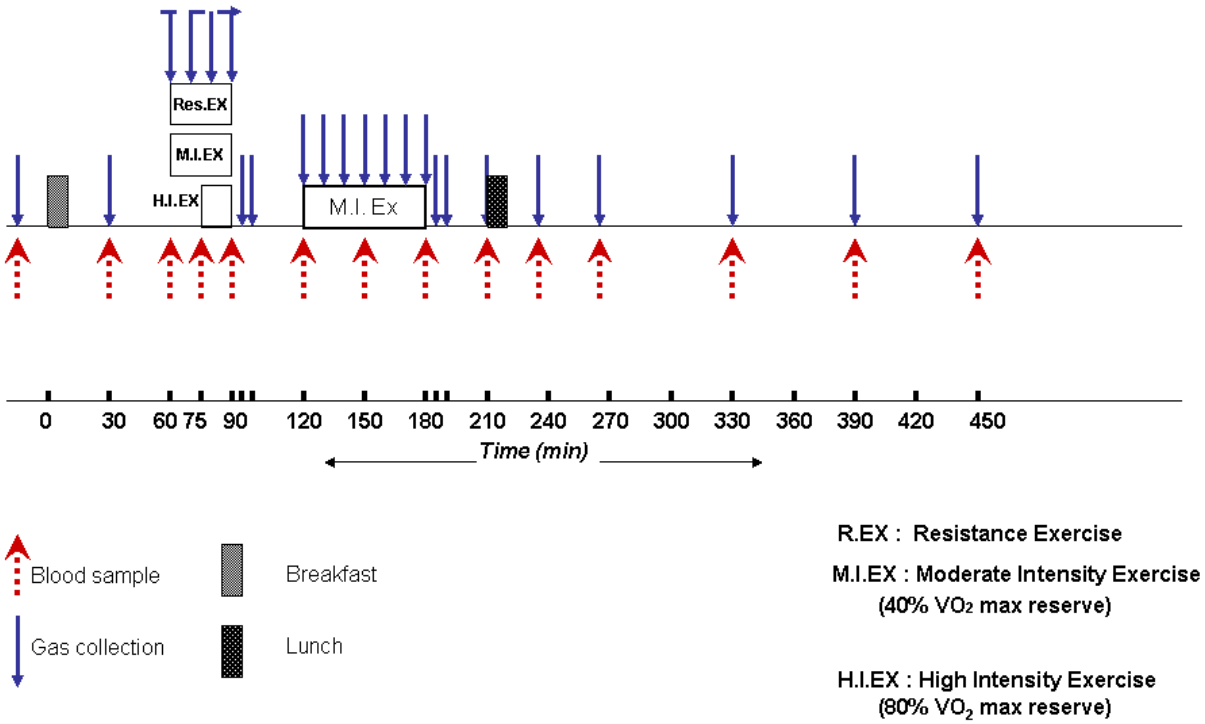

Figure 1: Schematic representation of the study protocol.

2.2 Subjects

Nine healthy males volunteered to participate in this study. Their physical characteristics : age, 26.3 ± 5.7 years; body mass, 76.9 ± 7.7 kg; height, 1.73 ± 0.10 m; body mass index (BMI), 26 ± 2.8 $kg.m^{-2}$; free fat mass, 62 ± 6.1kg; and maximal oxygen uptake ($\dot{V}O_2$max), 47 ± 5.6 ml kg^{-1} min^{-1} (Mean ± S.D). Exclusion criteria included current smoking (within the past 6 months), uncontrolled hypertension (>160/90 mm Hg on anti-hypertensive medication), a previous history of established chronic heart disease (e.g. myocardial infarction, stroke), acute illness or active, chronic systemic disease, family history of early cardiac death (less than 40 years), and a body mass index greater than 35 $kg.m^{-2}$ Each subject gave written, informed consent and completed a healthy history questionnaire before participation.

2.3 Equipment and Procedures

2.3.1 Motorised Treadmill

A motorised treadmill (Woodway Gmbh, Weil am Rhein, Germany) was used in the second preliminary test and for all the treadmill walking and running in the main trials to determine the $\dot{V}O_2$max. Speed and gradient were monitored via the treadmill's digital display (Figure.2).

Figure 2: Motorised treadmill (Woodway Gmbh, Weil am Rhein, Germany)

2.3.2 Multi-Use Gym Machine

In the prior resistance trial, subjects were instructed to use a multi-use gym machine (Body Craft Jones Maxrack 3D Machine) for all the resistance exercises (Figure.3).

Figure 3: Multigym machine (Body Craft Jones Maxrack 3D Machine).

2.3.3 Heart Rate

The subjects' heart rates were monitored continuously during exercise and in the recovery stages by short-range telemetry (Polar S610i, Polar Electro, Finland).

2.3.4 Rate of Perceived Exertion (RPE)

Rate of perceived of exertion values were obtained from the subjects using the Borg scale (1973) during the $\dot{V}O_2$ max test, prior to moderate-intensity exercise,

prior to high-intensity exercise, and prior to resistance exercise. The Borg scale ranged from 6 as ‘minimum,’ and 7 as ‘very, very light’ to 19 as ‘very, very hard’ and 20 as ’maximum’ (Borg, 1973)

2.3.5 Expired Air Collection and Analysis

For the $\dot{V}O_2$ max test, expired air collections were taken at rest, before, and during the test, using Douglas bags. Also, in all of the main trials, oxygen intake and carbon dioxide production were measured throughout the trial, before, during, and after exercise. Samples of expired air were collected in 100 or 150 L air-tight Douglas bags. Subjects wore a nose clip to make sure all expired air was collected in the bag while breathing through a mouthpiece fitted to a lightweight large 2-way respiratory valve (2700 series, Hans Rudolph Inc., USA), which was connected to a lightweight, wide-bore tube (35mm diameter). The tubing ends at a two-way valve, which opens and closes the Douglas bag.

Expired air was analysed to measure the fraction of oxygen and carbon dioxide by passing aliquots of expired air through a gas analyser (Servomex 1400, Servomex Group Ltd., East Sussex, England) for 1 minute.

The measurement of the volume of expired air in the Douglas bags was determined by emptying the contents through a dry gas meter (Harvard apparatus, supplied by Cranlea and Co., Birmingham, England). The temperature of expired air was determined during evacuation using a thermistor (Kane-May KM 330). Barometric pressure was obtained from a barometer, and the measured expired gas volume was corrected to standard temperature and pressure (STPD) for a dry gas.

2.3.6 Gas Analyser Calibration

The calibration of machines is very important in obtaining accurate and consistent results; therefore, the gas analyser used and described above was calibrated before every use with reference gases from BOC Ltd, Surrey, UK. A rubber tube connected the reference gas cylinder and the gas analyser, and a 'zero gas' containing 100% nitrogen was passed through the analyser, followed by a calibration gas containing 16% oxygen, 6% carbon dioxide, and atmospheric air. The adjustments of the screw to zero and the span of the meters were made after passing the gases. This calibration was repeated every few hours to minimise the errors.

2.4 Preliminary Testing

2.4.1 Questionnaires

Subjects were asked to fill out two questionnaires, one for health history (Appendix C) and the other for a physical activity (Appendix D) and also completed a consent form (Appendix B) on their first preliminary visit. The health history questionnaire was designed to determine whether the subjects were taking any medicine or had any previous sickness or injuries that might affect their exercise performance, their blood lipid metabolism and their breathing. Moreover, the health questionnaire was used to exclude subjects who suffered during exercise testing or cannulation. Also, each subject completed a questionnaire concerning his physical activity level in order to identify the actual level of his activity.

2.4.2 Body Composition and Anthropometric Measurements

Subjects' body mass was measured in (kg) to the nearest 0.10 kg using a digital weighing machine (SECA) while subjects were wearing light clothing. Subjects, without wearing shoes, stood on a wall-mounted stadiometer to measure height to the nearest 0.1 cm. Body fat percentage was assessed by the total body density equation using the skin fold method from four sites in the body: triceps, biceps, iliac crest, and subscapular (Durnin & Womersley, 1974).

2.4.3 $\dot{V}O_2$ Max Test

Maximal oxygen uptake ($\dot{V}O_2$ max) test was assessed using a motorised treadmill (Woodway Gmbh, Weil am Rhein, Germany) and the protocol for the Vo_2 max test was a modified version of the Taylor protocol (Taylor *et al.*, 1955); while the subjects walked on the treadmill, the speed was increased until it elicited a heart rate of 140b.min^{-1}. Then the incline increased 2% every 2 minutes until the subjects were exhausted and could no longer continue. The speed and gradient required to elicit 40% and 80% $\dot{V}O_2$ max reserve were calculated from the ACSM equations (section 2.4.5), and after 30 minutes of recovery subjects performed 10 minutes of exercise at each of these intensities to verify work rates.

2.4.4 Familiarisation of Resistance Exercise

In this visit, each subject was asked to do one set of 10 repetitions for each resistance exercise to ensure that the subject become familiar with resistance exercise. The machine and the weights were optimised for each subject according to his percentage of fat free mass FFM (squat at 90% FFM, bench, lunge, high pull at 70% FFM, lat pull-down at 60% FFM, and bicep curl at 35% FFM).

2.4.5 Equations

American College of Sports Medicine (ACSM) equations:

Running (speeds of 5mph or if person is truly jogging/running):

$$\dot{V}O_2 = (0.2 * S) + (0.9 * S * G) + 3.5$$

Walking (speeds of 1.9-3.7mph):

$$\dot{V}O_2 = (0.1*S) + (1.8*S*G) + 3.5$$

S = speed in m/min

G = Gradient as a decimal

(ACSM, 1998)

2.5 Main Trials

2.5.1 Prior Moderate-Intensity Exercise Trial

After an overnight fast, subjects arrived at the exercise laboratory, rested for 10 minutes, then had two resting gas samples taken. A cannula was inserted into the upper forearm (antecubital vein) to make blood collection easy throughout the trial. After a 10-minute interval, a fasting blood sample was taken (0 time point). Immediately, breakfast was served; it included a bagel (75g) with margarine (17g) and Complan (57g) with whole milk (191ml), which equated to 600 kcal see Table 1. Thirty minutes after breakfast, a second series of gas and blood samples were collected. Subjects again rested for 30 minutes, then gas and blood samples were taken before the first exercise (moderate intensity) at 40% of $\dot{V}O_2$ max reserve begun at the 60 min time point for 30 minutes (M) Ex.1. During this session of exercise, gas samples were taken for two minutes at 68-70 mins, 78-80 mins, and 88-90 mins time points, in addition to a blood sample being collected at the 75 min time point. Immediately after finishing exercise at the 90 min time point, another blood sample was collected, and two five-minute post exercise gas collections were taken (Figure.1).

The subjects were asked to relax until the time point of 113 min, when another five-minute gas sample was taken (113-118 mins). Then two minutes (118-120 mins) were allowed for taking a blood sample before the second exercise session started. Subject started the second session (subsequent moderate intensity) (M) Ex.2 at the 120-minute time point for 60 minutes at 40 % of $\dot{V}O_2$ max reserve, with the last two minutes including gas samples collected every 10 minutes at 128-130 mins, 138-140 mins, 148-150 mins, 158-160 mins, 168-170 mins, and 178-180 mins time points. Additionally, a blood sample was collected at the 150-minute time point.

Immediately after the second session of exercise, at the 180-minute time point, a blood sample was obtained, and two five-minute post-exercise gas

samples were collected (180-185 mins and 185-190 mins). At the 205-minute time point, another five minute gas sample was taken, along with a blood sample. Then lunch was served to the subjects at the 210-minute time point. The lunch meal contained the same foods as breakfast, but the meal was equal to 800 kcal see Table 2. Thirty minutes after lunch, gas and blood samples were then taken. The subjects were asked to relax in a comfortable room. Thirty minutes after lunch, gas and blood samples were collected at the 240-minute time point, and repeat samples were taken at the 270-minute time point and for every hour until the conclusion of the trial. Subjects' heart rates were monitored continuously during exercise and during the recovery stage by short-range telemetry (polar S610i, Polar Electro, Finland). Rate of perceived exertion values were obtained from the subjects using the Borg scale (1973) during the $\dot{V}O_2$ max test and all of the exercise sessions in the main trials.

Breakfast Meal				*FAT (g)*			*CHO (g)*	
	Portion Size	**Energy (kcal)**	**Protein (g)**	**SFA (g)**	**MFA (g)**	**PFA (g)**	**Starch (g)**	**Sugar (g)**
Bagel Toasted (plain)	75	194.3	7.4	0.3	0.4	0.9	34.8	2.9
Margarine	17	90.3	0.0	2.2	3.0	4.8	0.0	0.0
Complan (powder)	43	189.6	6.6	2.8	1.8	1.8	6.4	19.9
Whole Milk (ml)	191	126.1	6.1	4.6	2.3	0.6	0.0	9.2
Total		**600.21**	20.084	9.932	7.448	7.996	41.207	31.884

Table 1. Breakfast meal ingredients and its nutritional components.

Lunch Meal					*FAT (g)*		*CHO (g)*	
	Portion Size	**Energy (kcal)**	**Protein (g)**	**SFA (g)**	**MFA(G)**	**PFA (g)**	**Starch (g)**	**Sugar (g)**
Bagel Toasted (plain)	100	259	9.8	0.4	0.5	1.2	46.4	3.8
Margarine	23	122.1	0.0	3.0	4.0	6.4	0.0	0.0
Complan (powder)	57	251.4	8.8	3.8	2.4	2.3	8.5	26.3
Whole Milk (ml)	254	167.6	8.1	6.1	3.0	0.8	0.0	12.2
Total		**800.14**	26.7	13.3	9.9	10.7	54.9	42.3

Table 2. Lunch meal ingredients and its nutritional components

2.5.2 Prior High-Intensity Exercise Trial

The high-intensity exercise protocol followed the same methodology as the moderate-intensity protocol, except the high-intensity exercise began 15 minutes later at the 75-minute time point. At the 70-minute time point, the subjects began a warm-up on the treadmill for five minutes at 40% $\dot{V}O_2$ max reserve. Gas and blood samples were taken during the last two minutes of exercise, and heart rate and RPE were also taken at each further point when a gas collection was made. At the 75-minute time point, the treadmill was then put to 80% $\dot{V}O_2$ max reserve for 15 minutes (H) Ex.1. Gas samples were taken for two minutes at 78-80 mins, 83-85 mins, and 88-89 mins time points. After 30 minutes, subject did exactly the same session of 60 minutes of subsequent moderate intensity at 40% of $\dot{V}O_2$ max reserve (H) Ex.2, and they completed the remainder of the experiment under identical conditions as the prior moderate-intensity trial.

2.5.3 Prior Resistance Exercise Trial

The prior resistance exercise trial protocol was exactly the same as the prior moderate- and high-intensity exercise trials, except the first session included six types of resistance exercises (R) Ex.1. In this trial, subjects warmed up on the treadmill for 5 minutes at 40% $\dot{V}O_2$ max reserve, before start the resistance exercise at the 60-minute time point, and for 30 mins on the multi-gym machine. The resistance exercises included six different exercises: lat pull-downs, squats, bench presses, lunges, bicep curls, and high pulls. Subjects performed three sets of ten repetitions of each type of exercise with over 30 seconds of exercise followed by one minute of rest before the start of the next exercise. The weight for each exercise was determined by a percentage of fat free mass (squat at 90% FFM, bench, lunge, high pull at 70% FFM, lat pull-down at 60% FFM, and bicep curl at 35% FFM). After 30 minute, subjects did exactly the same second session of 60 minutes of subsequent moderate-intensity exercise at 40% of $\dot{V}O_2$ max reserve (R) Ex.2, and they completed the finished

the experiment in a manner identical to the prior moderate and high-intensity trials.

2.5.4 Blood Separation and Analysis

Blood samples were collected in tubes containing EDTA as an anticoagulant (BD, Plymouth, UK), and were immediately placed in ice. Plasma was separated within 10 minutes by centrifugation for 15 minutes at 3000 rpm at 4°C, in a refrigerated centrifuge (Universal 320 R, Hettich, Germany). Plasma samples were dispensed into Eppendorf tubes and were immediately stored at -80°C, for further analysis. Plasma samples were analysed for triglyceride, Glucose, and non-esterified fatty acid (NEFA) by the spectrophotometeric method, using a clinical chemistry analyser (Cobas Mira Plus, ABX Horiba, France). Insulin was determined using a commercially-available enzyme–linked immunoassay (ELISA) with <0.01 cross reactivity with pro-insulin (Mercodia AB, Uppsala, Sweden).

2.6 Statistical Analysis

Data analysis was performed with Statistica (version 6.0, StatSoft Inc., Tulsa, OK, USA) and Minitab (version 14.0, Minitab Inc., Stat College, PA, USA). Prior to the analysis, all data were tested for normality using the Anderson-Darling normality test and, if required, logarithmically transformed. The total area under 7.5 hours variable versus time curves (AUC) was calculated using the trapezium rule and the incremental area under curve (IAUC) was calculated as the increment in AUC above the baseline fasting concentrations. Throughout the trials, differences in blood parameters concentrations, RER, $\dot{V}O_2$, and $\dot{V}CO_2$, were compared using a two-way (trial vs. time) analysis of variance (ANOVA) with repeated measures, followed by Tukey's post hoc test; for comparison of the mean of total time (0 - 450) and values of different parameters, a paired t-test was used. Data values are reported as mean ± SEM unless otherwise stated. The level of statistical significance was set at $P < 0.05$.

3 RESULTS

3.1 Responses of Oxygen Uptake, Carbon Dioxide Output And Respiratory Exchange Ratio (RER) To Exercise

3.1.1 Oxygen Uptake across Whole Trials

The O_2 uptake over the 7.5 hours (0 - 450 min) of each of the three trials, moderate (M), high (H) and resistance (R) is shown in Figure 4. Statistical analysis was performed on the summary measurement of the area under the curve (AUC), providing a measure of total volume as oxygen utilised during the different phases of the study: pre exercise session.1 (Pre Ex.1 0-60 min), during the first exercise session (Ex.1 60-90 min), following the first exercise session (Post Ex.1 90-120 min), during the second exercise session (Ex.2 120-180 min), following the second exercise session (Post Ex.2 180-450 min) and the total volume for the study as a whole (total 0-450 min) is shown in Figure 5. As expected, the baseline $\dot{V}O_2$ values were similar for all the three treatments. During the first exercise session Ex.1, $\dot{V}O_2$ was significantly lower in (R) = 42.0 ± 2.5 l/min than (H) = 49.2 ± 2.5 l/min, ($P < 0.05$). Post Ex.1 $\dot{V}O_2$ was significantly higher in (H) = 21.4 ± 1.0 l/min, than (M) = 15.0 ± 0.8 l/min, ($P < 0.05$). However, there were no significant differences between all trials in the remaining phases of the study. In addition, as can be seen from Table.3, $\dot{V}O_2$ was a not significant difference between trials over the total study period (0–450 min): (M) = 268 ± 15.0 l, (H) = 275 ± 14.0 l, (R) = 274 ± 12.0 l; (M) vs. (R) P = 0.95, (M) vs. (H) P = 0.93, (H) vs. (R) P = 1.0.

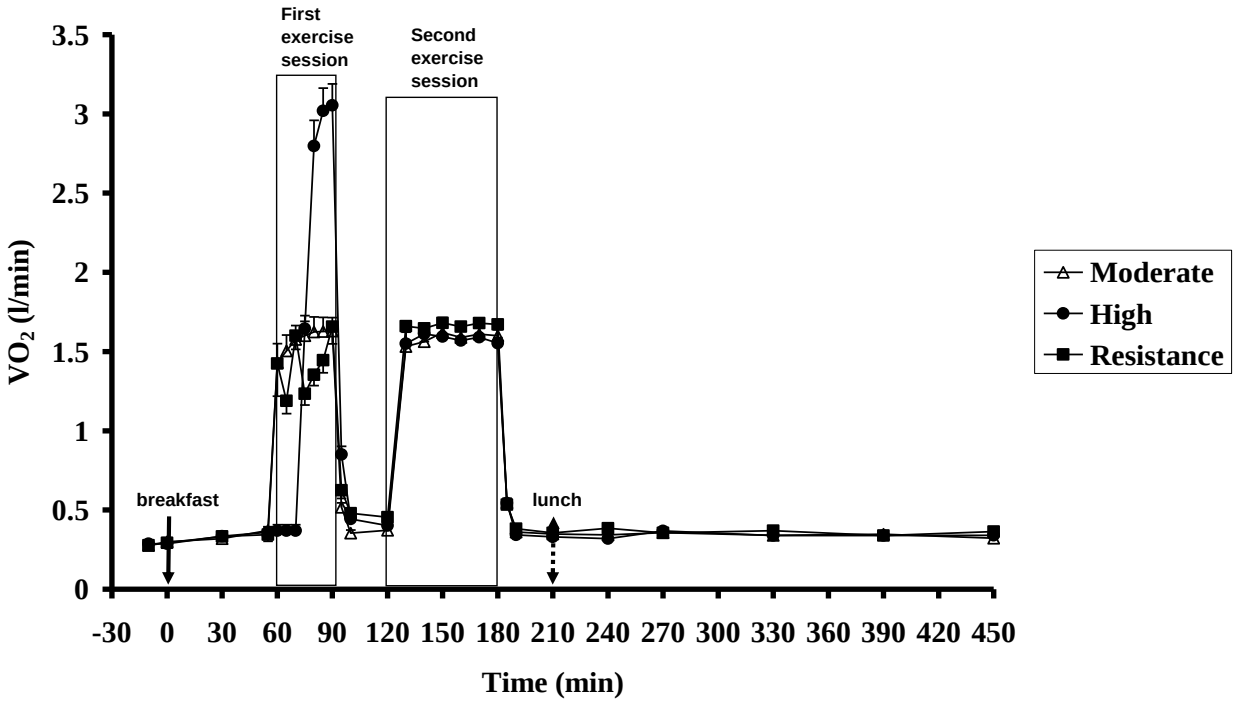

Figure 4: Oxygen uptake ($\dot{V}O_2$) measurements taken over 7.5 hours period (0-450 min) for, moderate (M), high (H) and resistance (R) trial.

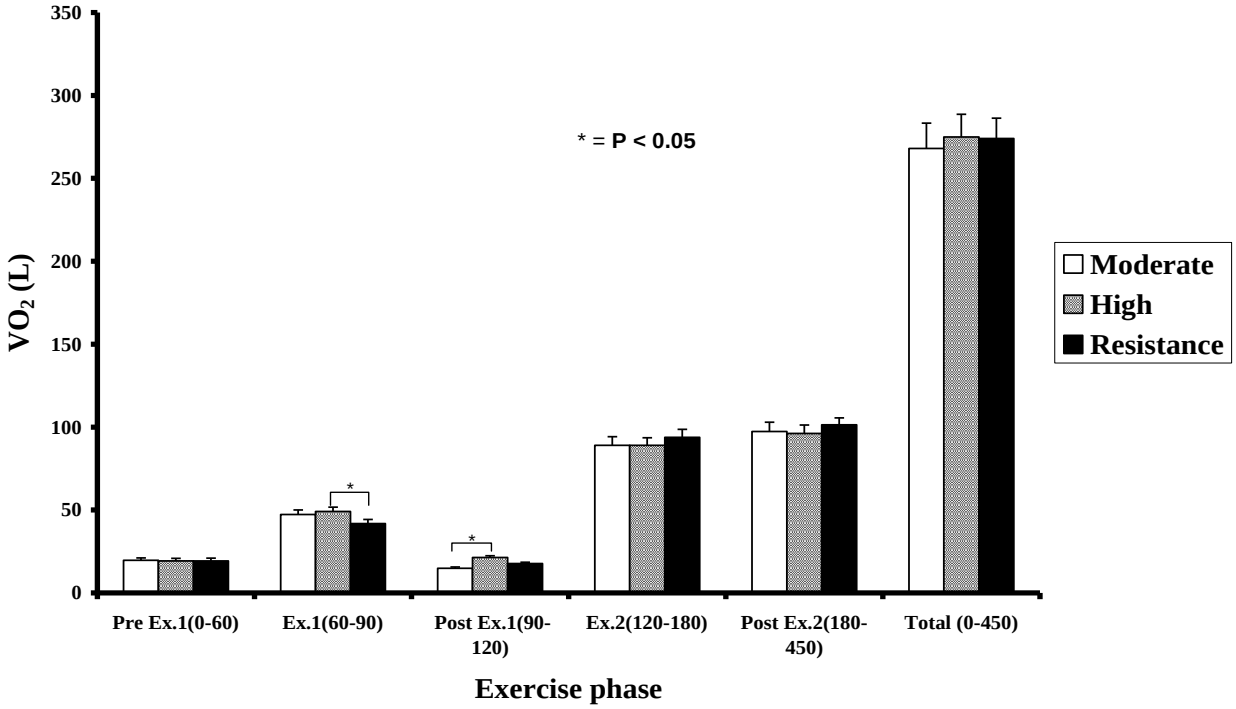

Figure 5: Total area under curve (AUC) for oxygen uptake ($\dot{V}O_2$) during the different phases of the study and over the total time period for the moderate (M), high (H) and resistance (R) trial.

3.1.2 Carbon Dioxide Output across Whole Trials

Figure 6 shows carbon dioxide output over the trial duration 7.5 hours (0-450 min), of each of the three trials, moderate (M), high (H) and resistance (R). A summary response of the AUC for carbon dioxide output during different phases of the trials is shown in Figure 7. As expected, the baseline $\dot{V}CO_2$ values were similar for all the three trials. There were no significant changes in AUC $\dot{V}CO_2$ volumes in (M), (H) and (R) trials, during all different phases and over the total study period (0-450 min) : (M) = 213 ± 13.0 l,(H) = 237 ± 12.7 l, (R) = 234 ± 9.6 l ; (M) vs. (R) P = 0.98, (M) vs. (H) P = 0.95, (H) vs. (R) P = 1.0. Table.3.

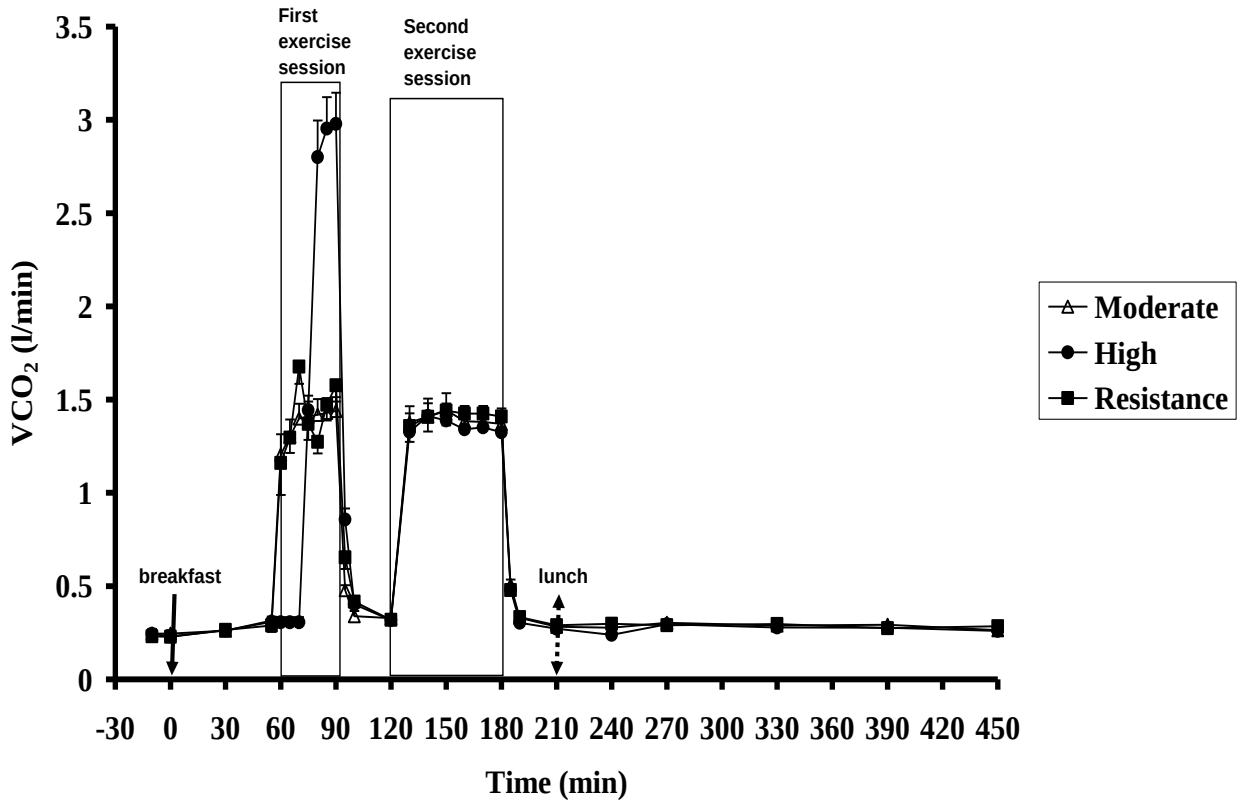

Figure 6: Carbon dioxide ($\dot{V}CO_2$) production measurements taken over 7.5 hours period (0–450 min) for, moderate (M), high (H) and resistance (R) trial.

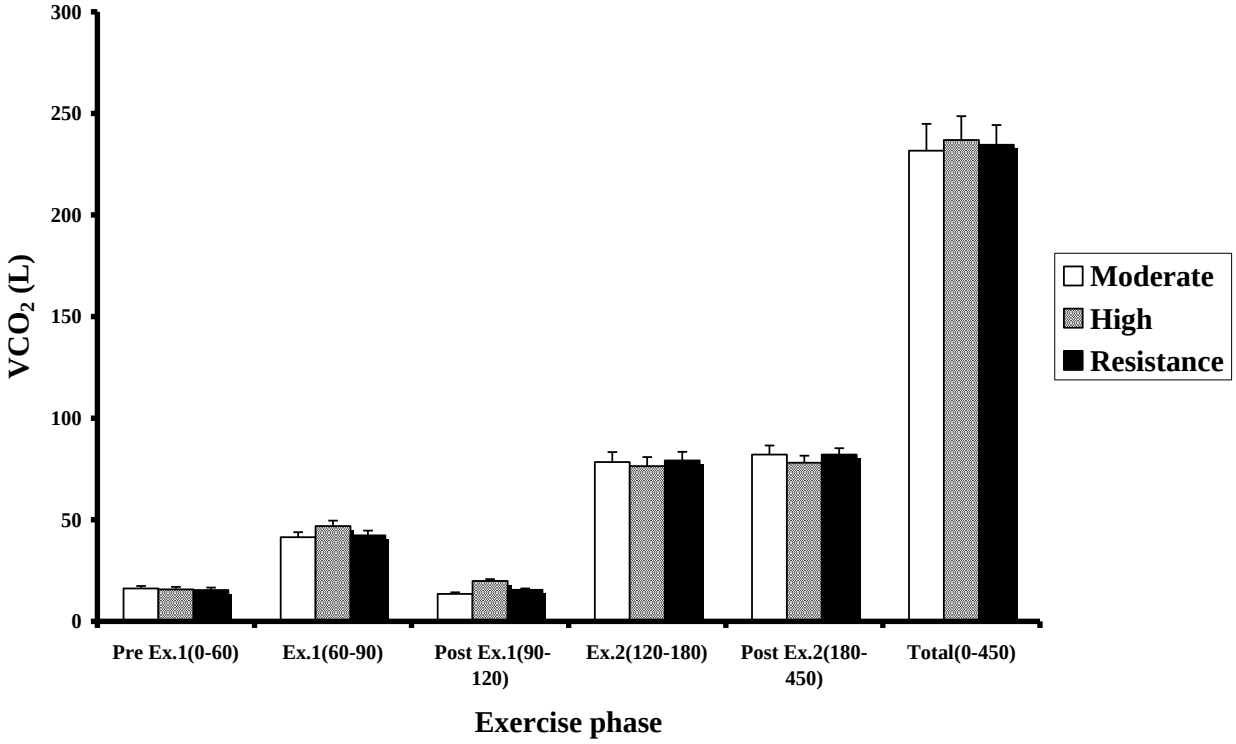

Figure 7: Total area under curve (AUC) for carbon dioxide output ($\dot{V}CO_2$) during the different phases of the study and over the total time period for moderate (M), high (H) and resistance (R) trial.

Table.3 Summary of different variables responses for the total time period (0-450) for moderate (M), high (H) and resistance (R) trial.

Variables	Moderate (M)	High (H)	Resistance (R)
Total VO_2 (l)	268 ± 15	275 ± 14	274 ± 12
Total VCO_2 (l)	213 ± 13	237 ± 12.7	234 ± 9.6
RER	0.866 ± 0.015	0.863 ± 0.011	0.858 ± 0.015
Plasma Triglyceride AUC ($mmol.l^{-1}.min$)	651 ± 80	741 ± 104	793 ± 128
Plasma Triglyceride IAUC ($mmol.l^{-1}.min$)	149 ± 25	237 ± 51	212 ± 28
NEFA AUC ($mmol.l^{-1}.min$)	176 ± 10.4	175 ± 21	170 ± 11
NEFA IAUC ($mmol.l^{-1}.min$)	-92 ± 21	-78 ± 32	-100 ± 29
Glucose AUC ($mmol.l^{-1}.min$)	2645 ± 65	2652 ± 82	2629 ± 99
Glucose IAUC ($mmol.l^{-1}.min$)	112 ± 60	189 ± 113	149 ± 88
Insulin AUC ($mU.l^{-1}.min$)	11689±1643	14001 ± 2487	12056±2056
Insulin IAUC ($mU.l^{-1}.min$)	7487 ± 1199	10243 ± 2143	8288 ± 1545

Table 3. Abbreviations: AUC, area under the (0-450 min) concentration vs. time curve, IAUC, incremental AUC; RER, respiratory exchange ratio; NEFA, non-esterified fatty acid. N = 9, values are mean ± s.e.m. No significant differences were found between the different trials for all the above variables.

3.1.3 RER Response across Whole Trials

Figure 8 shows RER measurements over the 7.5 hours (0 - 450 min) of each of the three trials, moderate (M), high (H) and resistance (R). A summary response of the AUC for the respiratory exchange ratio RER during different phases of the study is shown in Figure 9. These summary statistics were calculated as total $\dot{V}CO_2 / \dot{V}O_2$ for each of study phases. Total Baseline RERs were approximately equal in all the different trials. There were significant differences in RER during the first exercise session between the different trials (Figure.9). During Ex.1 RER was significantly different between trials (M vs. H vs. R: 0.88 ± 0.01 vs. 0.95 ± 0.01 vs. 1.01 ± 0.02 , $P < 0.01$). Interestingly, during (R) Ex.1, RER was greater than 1.0, which suggests a non metabolic source of CO_2. Throughout post Ex.1, RER was significantly higher for (H) compared to (R), ($P < 0.01$). Contrary to Ex.1, throughout the second exercise session Ex2, RER was significantly lower in (R) = 0.84 ± 0.01 than (M) = 0.88 ± 0.01, ($P < 0.05$). However, RER was not different during post Ex.2 periods and over the total trial time (0-450 min); (M) = 0.866 ± 0.015, (H) = 0.863 ± and (R) 0.858 ± 0.015; (M) vs. (R) P = 0.93, (M) vs. (H) P = 0.95, (H) vs. (R) P = 1.0. Table.3

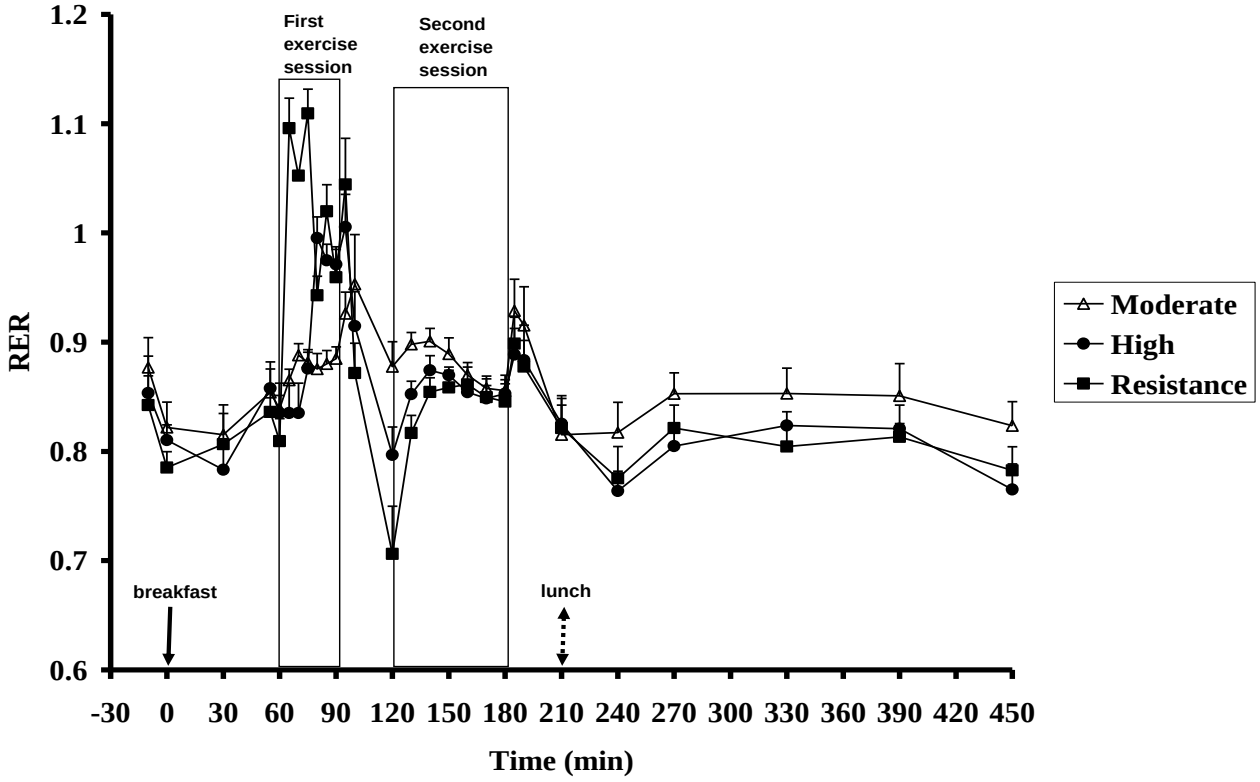

Figure 8: RER measurements taken over 7.5 hours period (0–450 min) for, moderate (M), high (H) and resistance (R) trial.

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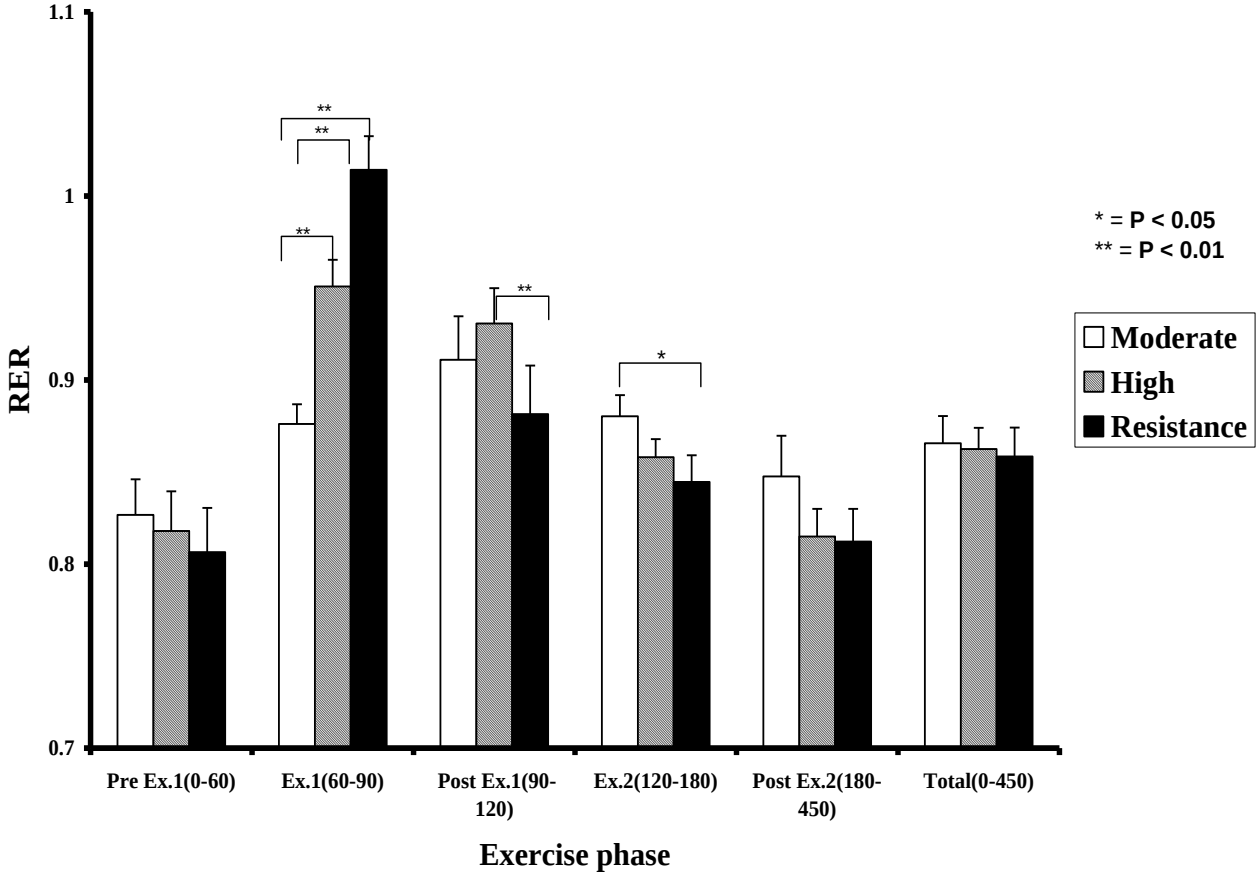

Figure 9: Total area under curve (AUC) for respiratory exchange ratio (RER) during different phases of the study and the total time period for moderate (M), high (H) and resistance (R) trial.

3.2 Metabolic Responses to Exercise

3.2.1 General Observations

Mean values of heart rate (HR) during Ex.1 were as follows: (M) = 122 ± 4 $b.m^{-1}$, (H) = 175 ± 5 $b.m^{-1}$ and (R) = 152 ± 5 $b.m^{-1}$. HR was significantly different between all the different trials; (M) vs. (H) vs. (R), ($P < 0.01$). However, during Ex.2, the mean values of HR were as follows: (M) = 121 ± 3.5 $b.m^{-1}$, (H) = 129 ± 4 $b.m^{-1}$, and (R) = 128 ± 4 $b.m^{-1}$, with no significant differences between the different trials; (M) vs. (H), ($P = 0.34$), (M) vs. (R), ($P = 0.44$), and (H) vs. (R), ($P = 1.0$). RPE during Ex.1 as follows: (M) = 8.0 ± 0.7, (H) = 12.0 ± 0.8 and (R) = 14.2 ± 0.6. RPE was significantly higher during (R) and (H) compared to (M); (M)

vs. (H), ($P < 0.01$), (M) vs. (R), ($P < 0.01$). However, during Ex.2, RPE was as follows: (M) = 10 ± 1.6, (H) = 9.2 ± 1.1, (R) = 9.0 ± 1.0, and there were no significant differences in RPE between the different trials; (M) vs. (H), ($P = 0.19$), (M) vs. (R), ($P = 0.20$), (R) vs. (H), ($P = 0.30$).

3.2.2 Mean Plasma Triglyceride Responses

Figure 10 shows the Plasma triglyceride concentrations (TG) at the different time points throughout the trials: moderate (M), high (H), and resistance (R). TG concentrations were significantly lower in (M) compared to (R) after Ex.1 at the 75 min time point ($P < 0.01$), at the end of Ex.2 at the 180 min time point ($P < 0.05$), and at the end of the trial at the 390 min and 450 min time points ($P < 0.01$). Furthermore, TG concentrations were significantly higher in (H) than in (M) at the end of the trial at time points 390 min ($P < 0.05$), 450 min ($P < 0.01$). No significant differences were observed between the trials in the area under the curve, and the incremental area under the curve for the total time (0 – 450 min) in triglyceride responses Table 3. P values for TG (AUC) are as follows: (M) vs. (H), ($P = 0.82$), (M) vs. (R), ($P = 0.62$), (H) vs. (R), ($P = 0.94$), respectively. P values for TG (IAUC) are as follows: (M) vs. (H), ($P = 0.23$), (M) vs. (R), ($P = 0.45$), (H) vs. (R), ($P = 0.88$), respectively.

3.2.3 Mean Plasma Non-Esterified Fatty Acid (NEFA) Responses

Figure 11 compares the plasma NEFA concentration among all the three trials, (M), (H) and (R) for every time points during the experiment. From Figure 11 we can see that the only significant difference observed was at the end of Ex.2 at the 180 min time point in the (M) which was significantly lower than NEFA in the (R), ($P < 0.01$). No significant differences were observed between the trials in AUC, and IAUC for the total time (0–450 min) in NEFA responses (Table 3). P values for NEFA (AUC) are as follows: (M) vs. (H), ($P = 1.0$), (M) vs. (R), ($P = 0.94$), (H) vs. (R), ($P = 1.0$) respectively. P values for NEFA (IAUC) were as follows: (M) vs. (H), ($P = 0.94$), (M) vs. (R), ($P = 1.0$) (H) vs. (R), ($P = 1.0$), respectively.

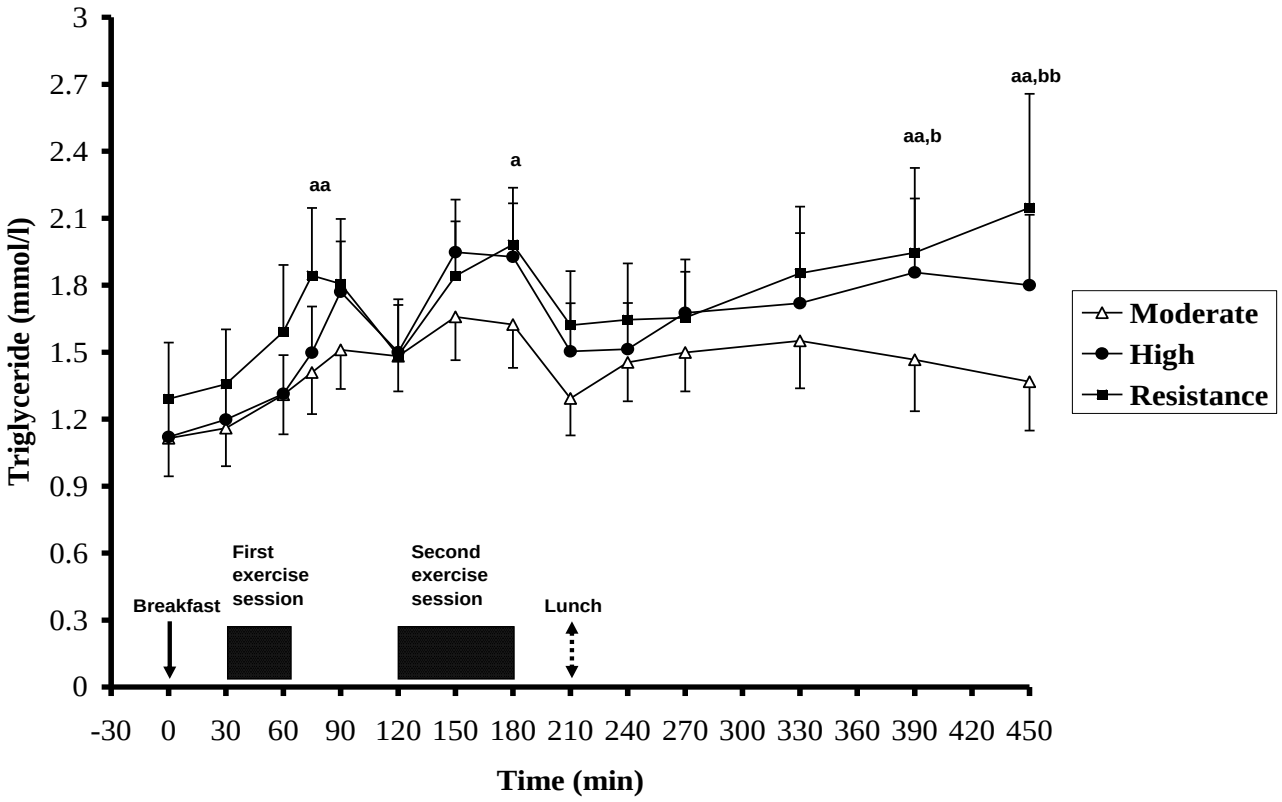

Figure 10, Plasma triglyceride concentrations (TG) over all the study period in different trials: moderate (M), high (H), and resistance (R). a = significantly different between M & R, p < 0.05, aa = significantly different between M & R, p < 0.01, b = significantly different between M & H, p < 0.05, bb = significantly different between M & H, p < 0.01.

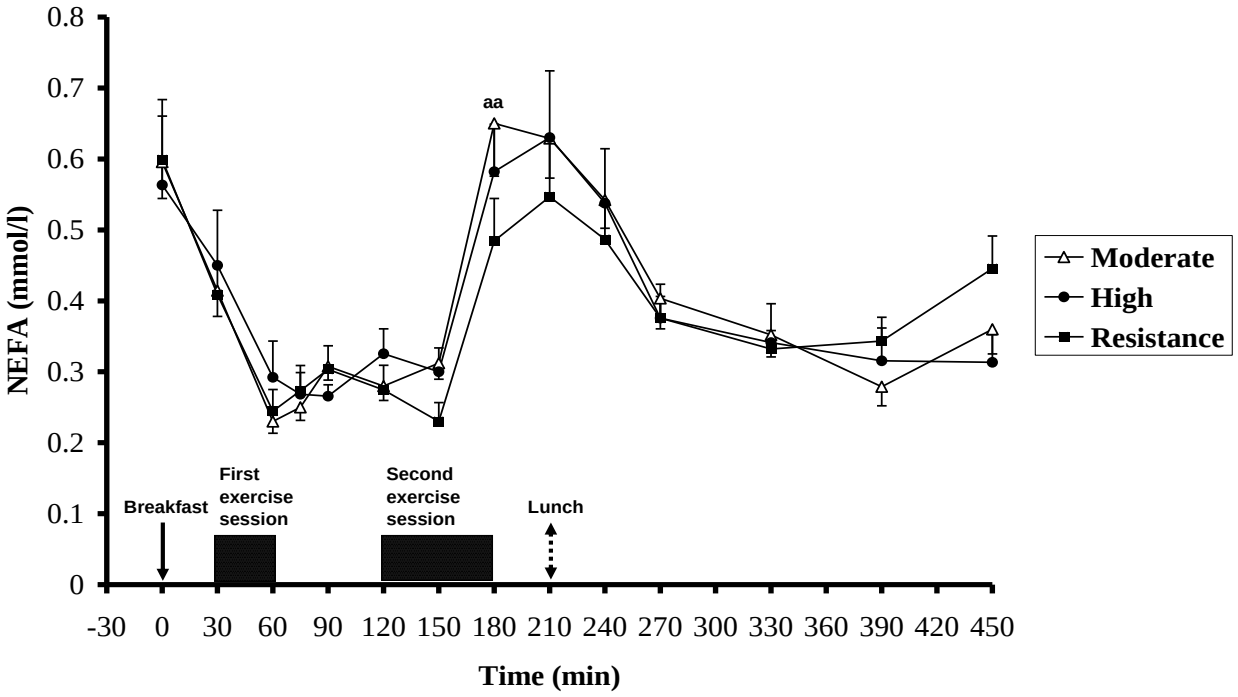

Figure 11, Plasma Non-Estrefied Fatty Acid concentrations (NEFA) over all the study period in different trials: moderate (M), high (H), and resistance (R). aa = significantly different between M & R, p < 0.01.

3.2.4 Mean Plasma Glucose Responses

Figure 12 presents the plasma glucose concentrations at all the different time points throughout all the trials: moderate (M), high (H), and resistance (R). As can be seen from Figure 12, there were no significant changes in glucose concentration between the different trials, except at the end of Ex.1 at the 60 min time point, where the glucose concentration was significantly lower during the (M) compared to (H), ($P < 0.05$). Moreover, no significant differences were observed between the trials in the area under curve AUC, and incremental area under curve IAUC for the total time (0–450 min) in GLU responses (Table 3). P values for glucose (AUC) as follows: (M) vs. (H), ($P = 1.0$), (M) vs. (R), ($P = 1.0$), (H) vs. (R), ($P = 1.0$) respectively. P values for glucose (IAUC) were as follows: (M) vs. (H), ($P = 0.82$), (M) vs. (R), ($P = 1.0$) (H) vs. (R), ($P = 0.95$), respectively.

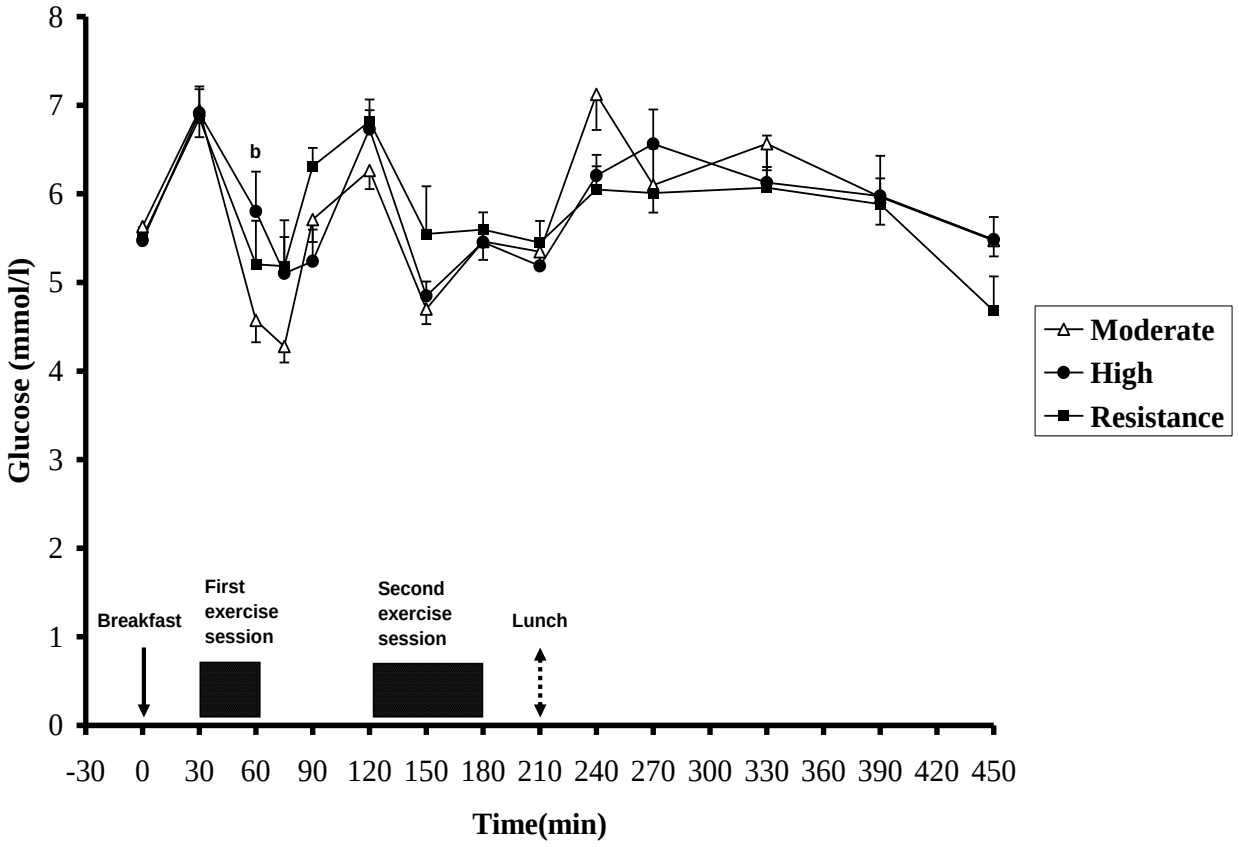

Figure 12, Plasma glucose concentrations over all the study period in different trials: moderate (M), high (H), and resistance (R). b = significantly different between M & H, p < 0.05.

3.2.5 Mean Plasma Insulin Responses

Figure 13 provides the plasma insulin concentrations throughout all the trials: (M), (H), and (R). As can be seen from Figure 13, there were only two time points at which insulin concentrations were different throughout the different trials, at the end of Ex.1 at the 60 min time point insulin level was significantly lower during the (M) compared to the (H), ($P < 0.05$). Moreover, there were no significant differences between the trials in AUC, and IAUC for the total time (0–450 min) in insulin responses (Table.3). P values for insulin (AUC) are as follows: (M) vs. (H), ($P = 0.72$), (M) vs. (R), ($P = 1.0$), (H) vs. (R), ($P = 1.0$) respectively. P values for insulin (IAUC) were as follows: (M) vs. (H), ($P = 0.50$), (M) vs. (R), ($P = 0.94$) (H) vs. (R), ($P = 0.70$), respectively.

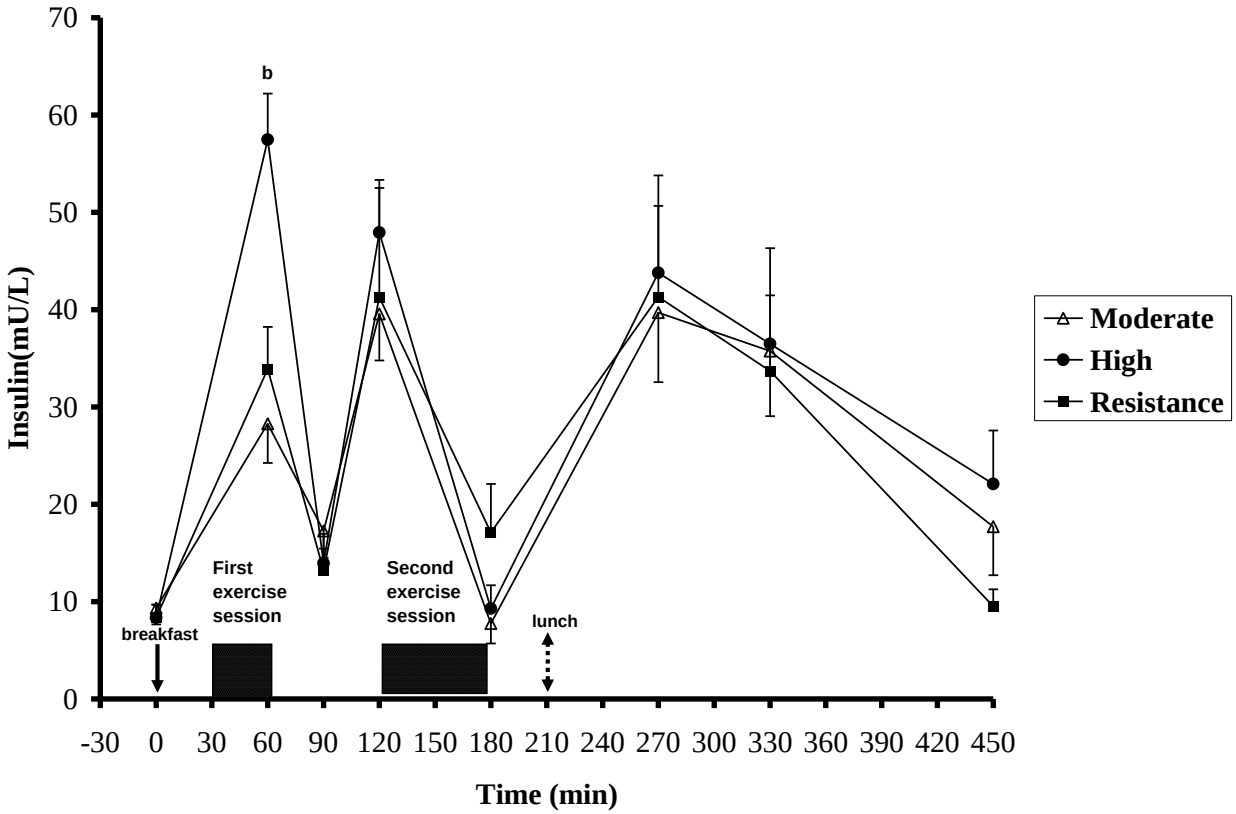

Figure 13, Plasma Insulin concentrations over all the study period in different trials: moderate (M), high (H), and resistance (R). b = significantly different between M & H, $p < 0.05$.

4 DISCUSSION

The main findings from the present study indicate that the respiratory exchange ratio (RER) during (Ex.1) was significantly higher (exceeding 1.0) during (R) when compared with both (M) and (H) intensity exercise at 40% and 80% of $\dot{V}O_2$max reserve, respectively. Furthermore, RER was significantly lower during the subsequent moderate intensity exercise (Ex.2) when it was preceded by resistance exercise compared to moderate intensity exercise. This suggests that when moderate intensity exercise is preceded by resistance exercise, RER is lower during the subsequent moderate bout, which could be interpreted as indicating an increase in fat oxidation during subsequent moderate intensity exercise.

Results from the present study are in partial agreement with previous research, which indicated that prior resistance exercise resulted in significant elevations in the rate of fat oxidation during subsequent moderate intensity exercise (Goto *et al.*, 2007b;Kang *et al.*, 2009). However, as mentioned previously (Refer to introduction 1.7.4), Goto et al. (2007b) completed three trials including a trial with subsequent submaximal exercise as control and two trials with the same subsequent submaximal exercise preceded by resistance exercise with 20 min and 120 min of rest in between. Kang *et al* (2009) conducted approximately the same trials, but with a different intensity of resistance exercise and only 5 min of rest in between. However, they examined only prior resistance exercise, not taking into account what happened before and after the subsequent submaximal exercise (Ex.2) in the recovery phase. Accounting for these phases is critical since the post-exercise period following the initial exercise and the subsequent bout may also play an important role in determining substrate utilisation, as exercise alters post-exercise substrate oxidation and metabolism. Therefore, to understand the total impact of exercise on metabolism, researchers need to assess the changes occurring not only during, but also following exercise (Saris & Schrauwen, 2004). Goto *et al*. (2007b) and Kang *et al*. (2009) reported that prior resistance exercise led to an increase in the rate of fat oxidation during subsequent aerobic exercise,

interpreting the low RER during the subsequent moderate intensity exercise (Ex.2) as an increase in fat oxidation. However, the present findings suggest that their interpretation of data is incorrect.

Interestingly, as shown in Figure 9, RER during Ex.1 (which was not assessed in the previous studies) was significantly higher in (R) compared with (M), and slightly higher than (H) and was greater than 1.0. This has significant implications for interpreting the results during the subsequent moderate intensity exercise (Ex.2) as an RER above 1.0 suggests there is an additional source of CO_2, in addition to that produced by metabolism (Roecker *et al.*, 2000;Patessio *et al.*, 1992;Zhang *et al.*, 1994). This high RER suggests that during resistance exercise the intensity was high and there was not enough energy produced by oxidative pathways to support the required work rate, this therefore, resulted in the release of lactate + H^+ in to the blood (i.e. the shortfall in the aerobic energy supply was compensated for by anaerobic pathways with the consequent production of lactate + H^+). Consequently, bicarbonate buffering of H^+ ions occurs to constrain the fall in pH, a by-product of which is CO_2:

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

This excess CO_2 is called "nonmetabolic" CO_2 (Roecker *et al.*, 2000;Patessio *et al.*, 1992) or "buffer" CO_2 (Zhang *et al.*, 1994). Thus, the volume of CO_2 at the level of the lung becomes greater than the volume of metabolically produced CO_2 leading to a high RER.

The consequence of this is the bicarbonate reserve is depleted. Thus, after resistance or high intensity exercise during the recovery and the second exercise Ex.2, as the bicarbonate reserve are depleted, some of the CO_2 produced metabolically at this lower level of exercise is stored, replenishing the bicarbonate stores and is therefore not excreted in the breath. Therefore, the volume of CO_2 at the level of the lung becomes less than the volume of that metabolically produced, and RER is lower than the ratio of CO_2 production

to O_2 utilisation in the muscle itself, potentially resulting in the overestimation of the rate of fat oxidation. In other words, gas exchange at the mouth is not equilibrated with that in the muscle. Thus, RER at the mouth is lower than muscle respiratory quotient, and if used, the indirect calorimetry method would lead to an overestimation of the rate of fat oxidation. By making measurements during the initial resistance exercise which was not done in the previous published studies (Goto *et al.*, 2007b;Kang *et al.*, 2009), the present findings suggest that the interpretation of the lower RER during subsequent moderate intensity exercise following resistance exercise, of an increase in the rate of fat oxidation, is an artefact of blood buffering. This is further supported by the current study findings, that the total RER over the course of each trial including both exercise and recovery phases, was not different between the exercise interventions.

Our results show, as can be seen from Figure 5 and 7, that baseline and Pre Ex.1 volumes for $\dot{V}O_2$ and $\dot{V}CO_2$ were identical in the three treatments and the area under curve (AUC) of $\dot{V}CO_2$ were not significantly different between the trials during the different phases of the study and for the total time of the study as well. However, we found that during Ex.1, $\dot{V}O_2$ during (H) was significantly higher than (R), and (M), with $\dot{V}O_2$ 13% higher than (R) . These results during Ex.1 may be explained by a number of different factors; the mode and intensity of exercise, duration and the source of energy that has been consumed during the exercise. Moderate and high intensity exercise can be considered endurance, steady-state constant-load exercise, while the resistance is strength exercise. In the current study, resistance exercises were non–steady state intermittent exercise.

As mentioned in the literature review, during moderate to high intensity exercise the source of energy is a mixture of both carbohydrate and fat, and because the work done during the moderate and high intensity exercise bouts were constant (resulting in different durations), it would expected that $\dot{V}O_2$ during the exercise would be similar.

During the prior resistance trial, subject did no more than 9 min of resistance exercise with the rest of the 21 minutes taken up by recovery between different resistance exercises; thus, the source of energy and the O_2 requirements would be expected to be different. Intermittent resistance exercise is fuelled mainly by creatine phosphate (CP), adenosine triphosphate ATP and glycogen, due to a reliance on anaerobic glycolysis. Therefore, under these conditions in the present study, it would be expected the $\dot{V}O_2$ during (R) Ex.1 would be lower than (H) Ex.1. In contrast, excess post-exercise oxygen consumption EPOC after (H) Ex.1 was significantly higher than (M) Ex.1, and EPOC after (R) Ex.1 was 18% greater than (M) Ex.1. This result may explained by the fact that during anaerobic exercise which includes high exercise intensities, the body requires more energy in response to this high intensities, and perhaps a longer duration for recovery and this may take much longer than moderate intensity to reach a steady state.

In addition, these results from the present study support many researchers who have investigated the relationship between EPOC, exercise intensity and duration, and they have demonstrated that the volume of EPOC increases with exercise intensity (Gore & Withers, 1990;Knuttgen, 1970;Bahr & Sejersted, 1991). Some of these findings in the Post Ex.1 are consistent with previous investigations. Burleson *et al*. (1998) reported no difference in $\dot{V}O_2$ during a bout of 27 min weight training and a bout of 27 min treadmill walking at matched rates of $\dot{V}O_2$. However, the total $\dot{V}O_2$ during the first 30 min into recovery was higher in the weight training session compared with treadmill walking. Schuenke *et al*, (2002) reported that EPOC can remain elevated for up to 48 hours post resistance exercise (Schuenke *et al.*, 2002), and for at least 24 hours after both resistance and aerobic exercise characterised by the same relative intensity (Jamurtas *et al.*, 2004).

Another study has reported that treadmill $\dot{V}O_2$ was significantly higher for a combination trial that involved resistance exercise followed by running at 70%

of $\dot{V}O_2$ max compared to another trial with the reverse sequence (running at 70% of $\dot{V}O_2$ max followed by resistance exercise), and they found that EPOC was significantly higher after the Run-Resistance trial than the Resistance-Run trial. Therefore, they suggested that EPOC was greater after running followed by resistance exercise (Drummond *et al.*, 2005).

Almuzaini *et al.* (1998) investigated the effects of a split exercise session on EPOC and resting metabolic rate RMR, and they found that $\dot{V}O_2$ was not significantly different between a continuous trial (30 min at 70% of $\dot{V}O_2$ max followed by 40 min EPOC) and a split trial (two × 15 min trial at 70% of $\dot{V}O_2$ max followed by 20 min of EPOC). On the other hand, they reported that the split trial significantly increased the overall magnitude of EPOCs compared to the continuous trial, but did not change RMR (Almuzaini *et al.*, 1998). It is interesting to note in the current study that even though there are some significant differences in $\dot{V}O_2$ during the different types of prior exercises Ex.1; (M), (H) and (R), this did not influence the $\dot{V}O_2$ during the subsequent moderate intensity exercise Ex.2 and in the recovery Post Ex.2.

The present study observed that the heart rate (HR) during Ex.1 was significantly higher during (H) and (R) than (M) and during (H) than (R). However, during the subsequent moderate intensity exercise Ex.2, although HR was slightly higher following both (H) and (R) compared with (M), this was not significant. This slight elevation in HR during the (H) Ex.2 and (R) Ex.2 might be due to the enhancement of nervous sympathetic activity in response to prior resistance and high intensity exercise.

Our HR results are in a partial agreement with (Goto *et al.*, 2007b), who found that the mean value of HR during submaximal endurance exercise was significantly higher when it was preceded by resistance exercise with 20 min or 120 min of rest compared to trial with only submaximal exercise. In another study by (Burleson *et al.*, 1998), they reported that mean values of HR and

RER were significantly greater during weight training than during treadmill exercise.

Insulin influences the breakdown of fat in adipose tissue and during exercise lipolytic responses are elevated by lower insulin levels in the circulation (Hirsch *et al.*, 1991;Wasserman *et al.*, 1989). An increase in the insulin and glucose concentrations after breakfast in all the trials was expected in response to the metabolism of nutrients provided in the meal after the fasted state. However, after approximately three hours of consuming the meal, glucose and insulin concentrations return to the normal baseline volumes (Frayn *et al.*, 1993).

During Ex.1, glucose concentrations were decreased in all trials, and at the end of Ex.1 glucose and insulin concentrations were significantly lower in (M) than (H). This might be the result of the high intensity exercise, which is likely to stimulate glycogenolysis in the muscles (Romijn *et al.*, 1993). Post Ex.1 insulin and glucose levels were increased until the start of Ex.2, and then they were decreased throughout Ex.2 in all the three trials (M), (H) and (R) in response to the enhanced glucose uptake as source of energy, until the end of the exercise session.

As expected after lunch, insulin began to rise in response to rising blood glucose after the meal, then four hours postprandial, insulin concentrations returned to near baseline levels. This suggests that the three different prior exercises did not have any influence, having almost the same effects on the glucose and insulin concentrations during and after the subsequent moderate intensity exercise. Insulin and glucose results in (R) in the present study, are consistent with the results of (Goto *et al.*, 2007b), they found approximately the same insulin and glucose patterns following resistance exercise with 20 min of rest before 60 min of submaximal subsequent exercise. With regards to prior moderate trial (M), a previous study by (Stich *et al.*, 2000), was in agreement with our results demonstrating that plasma insulin concentrations during moderate exercise were decreased significantly by prior exercise of the same

period and intensity, separated by 60-min of rest. During Ex.2 in all the trials of our investigation, there were apparent decrease in insulin concentrations and this might be due to the enhancement of lipolysis.

During low and moderate intensity exercise energy is derived mainly from adipose tissue triglyceride stores (Klein *et al.*, 1994). As demonstrated in Figure 10, in the Post Ex.1 at the 75 min time point triglyceride was significantly higher in (R) compared to (M). In the recovery period following Ex.1, triglyceride decreased in both trials, returning to the same the level by the 120 min time point. At the onset of Ex.2, triglyceride rose until the end of the exercise at the 180min time point, (M) was significantly lower than (R), then triglyceride decreased during recovery until lunch. After the lunch, triglyceride concentrations were elevated and three and four hours later at the 390 and 450 time point, respectively, triglyceride concentrations were significantly lower in (M) compared to (H) and (R) trials. These findings might be a consequence of the lower insulin concentration at the corresponding time points and due to LPL activity in the muscles and adipose tissue. Lipoprotein lipase activity is three fold higher in adipose tissue in the postprandial state, peaking approximately 5 hours after the consumption of a meal (Frayn *et al.*, 1995), whereas in the skeletal muscle, LPL activity falls after a meal (Lithell *et al.*, 1978). There is a general belief that aerobic exercise is more effective than resistance exercise in reducing triglyceride plasma concentration (Hurley, 1989). The depletion of intramuscular TG stores during high intensity strenuous exercise may lead to increases in LPL activity in the skeletal muscle after exercise (Kiens & Richter, 1998). In several studies it has been reported that the postprandial triglyceride concentrations were decreased by moderate intensity exercise (Gill *et al.*, 2001a;Gill *et al.*, 2002;Aldred *et al.*, 1994). The current study is in agreement with previous studies, and suggests that prior moderate intensity exercise is slightly beneficial compared with prior high and resistance exercise in reducing fat. This is demonestrated by lower triglyceride plasma concentration during and after subsequent moderate intensity exercise and 3 or 4 hours postprandially. Present finding shows that RPE was significantly higher during the (R) Ex.1 and high (H) Ex.1, compared to (M)

Ex.1, and during Ex.2, RPE was not different between the different trials. This indicates that the prior resistance and high intensity exercise were more difficult and the effort required was higher, while the moderate intensity exercise was easier and required less effort, and this is further support to our suggestion that the prior moderate intensity exercise may have more benefits with less effort compared to high and resistance exercise, in terms of fat oxidation and reduced postprandial triglyceride.

As expected, Plasma NEFA concentrations were reduced after breakfast and lunch in all trials. The rapid decline in NEFA concentrations after the meal, may be a consequence of the elevated postprandial glucose and insulin concentrations which has been implicated in suppressing HSL (Frayn, 2003), and the activity of LPL in the skeletal muscle (Seip & Semenkovich, 1998) and impairing the mobilisation of NEFA from adipose tissue (Ferrannini *et al.*, 1983). Non-estrefied fatty acid concentrations increased during (Ex.2) in all three trials and at the end of (Ex.2) at the 180 min time point. Non-estrefied fatty acid concentrations were significantly lower as result of (R) Ex.2 compared with that during (M) Ex.2. These results are in partial agreement with previous investigations. Goto *et al.*(2007b) have reported that FFA concentrations were elevated during subsequent submaximal exercise and was significantly higher at the end of this session when it is preceded by resistance exercise and 20 min of rest compared with control trial (Goto *et al.*, 2007b).

Moreover, some studies which used repeated exercise bouts have shown an increase in lipolysis (Stich *et al.*, 2000;Goto *et al.*, 2007a). Plasma NEFA concentrations were increased significantly during moderate intensity exercise by prior moderate exercise of equal duration and intensity, with 60 min of rest between the two bouts (Stich *et al.*, 2000). Goto *et al.* (2007a) compared a single bout of 60 min prolonged moderate endurance exercise at 60% of $\dot{V}O_2$ max with two repeated 30 min exercise bout at the same intensity separated by 20 min of rest, and they reported that increases in plasma fatty acids concentrations were higher during the second 30 min exercise bout compared to the single trial. The increase of plasma NEFA concentration during exercise

is the result of lipid mobilization which is facilitated by decreases in the concentration of insulin during exercise (Hirsch *et al.*, 1991;Wasserman *et al.*, 1989). After exercise, there is a sudden release of fatty acids from adipose tissue, which increases the level of fatty acids in the circulation. This might happen because there is an imbalance between the supply and demand of fatty acids after the termination of the exercise (Bahr *et al.*, 1991).

5 CONCLUSION

In conclusion, the current study suggests that different modes and intensities of prior exercise; moderate, high intensity were equal in oxygen utilisation (therefore, energy expenditure) and resistance exercise with less oxygen utilisation, have almost the same effect on metabolism. There was no difference in fat oxidation and metabolism during and after subsequent moderate intensity exercise, and over the day between the different interventions. In contrast, previously published researches (Goto *et al.*, 2007b;Kang *et al.*, 2009), resistance exercise prior to moderate intensity exercise, offered no clear additional benefits in term of increased fat oxidation during subsequent moderate intensity exercise across these conditions in men (compared to prior moderate and high intensity exercise). With regards to triglyceride, the data from this thesis suggests that prior moderate intensity exercise may have a slight positive benefit, with lower triglyceride plasma concentration during and after subsequent moderate intensity exercise and 3 - 4 hours postprandial, compared to prior high and resistance exercise. However, more research on this topic needs to be undertaken because the association between exercise and metabolism (including its relationship to fat oxidation) is not fully understood. Our conclusion cannot be extended to women, because in women, the total energy expenditure elicited from fat oxidation is greater (Horton *et al.*, 1998). In addition, post-exercise, men maintained high rates of fat oxidation, whereas women do not. This gender variation seems to be greater with higher intensity exercise. Therefore, investigating the effects of prior resistance and endurance exercise on fat oxidation and metabolic responses during and after moderate intensity exercise in women might be a further study.

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7 APPENDICES

Appendix A: Application Form for Ethical Approval

UNIVERSITY OF GLASGOW
FACULTY OF BIOMEDICAL AND LIFE SCIENCES

ETHICS COMMITTEE FOR NON CLINICAL RESEARCH INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

A submission to this Committee does not automatically result in approval. Investigators must wait for written approval before commencing data collection. Disciplinary measures will be taken if work commences without ethical approval being in place. The matter will be referred to the Dean for appropriate action.

THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.

ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.

Project Title_

Effects of prior resistance or endurance exercise on metabolism during and after moderate-intensity exercise

Is this project from a commercial source? No

If yes, give details and ensure that this is stated on the Informed Consent form.

Date of submission __24 August 2007_____
_____

Name of all person(s) submitting research proposal
Dr Jason Gill
Dr Niall Macfarlane
Mr Ahmed Alsabih
_____
_____

Position(s) held
Senior Lecturer
Senior Lecturer
MSc student_

_____

Division_____

_____

Address for correspondence relating to this submission
Dr. Jason Gill
Integrative and Systems Biology
West Medical Building
FBLS
University of Glasgow
G12 8QQ

Telephone: 0141 3302916
Email: j.gill@bio.gla.ac.uk

_____

_____

Name of Principal Researcher (if different from above e.g., Student's Supervisor)

_____

_____

Position held

_____

_____

1. Describe the purposes of the research proposed.

Loss of body fat requires the imposition of a negative fat balance and there is evidence that individuals who are successful in weight loss and/or are resistant to weight gain have high rates of fat oxidation (Swinburn & Ravussin, 1994). Exercise increases the rate of fat oxidation, the extent of this depends on a number of factors including exercise intensity (greater proportions of fat are oxidized during lower intensities of exercise), feeding status (pre-ingestion of carbohydrate increases carbohydrate oxidation and reduces fat oxidation) and the training status of the exerciser (well-trained individuals oxidize greater amounts of fat during exercise than their untrained peers). Recent evidence also indicates that a prior session of resistance (i.e. weight lifting type) exercise can increase fat oxidation during subsequent moderate intensity exercise (Goto *et al.*, 2007), suggesting that this could potentially be a strategy for maximizing fat loss during exercise. However, there are a number of factors relating to this which are currently unresolved. Firstly, since the control condition in Goto and co-workers' study involved no exercise prior to the moderate intensity session, it is possible that any prior exercise, rather than specifically resistance exercise, would have caused the observed subsequent elevation in fat oxidation, as it is well-established that fat oxidation increases progressively with time during prolonged exercise (Romijn *et al.*, 1993). If it is the case that any prior exercise would increase fat oxidation during subsequent exercise, it would be helpful to understand whether this effect is a function of the intensity of the prior exercise or just the energy expended. Furthermore, it is unclear whether the increased fat oxidation observed during the moderate intensity exercise after prior resistance exercise would have persisted into recovery. It has been shown previously that although low intensity exercise elicits greater fat oxidation than high intensity exercise (for a given total energy expenditure), this pattern is reversed post-exercise, so that total fat oxidation for low and high intensity exercise is similar when both the exercise and post-exercise periods are taken into account (Saris & Schrauwen., 2004). Thus, it is conceivable that prior resistance exercise could increase fat oxidation *during* subsequent moderate intensity exercise, but reduce fat oxidation during the hours following the exercise, leading to no net increase. The purpose of the present study is therefore to determine the effects of prior resistance exercise, moderate intensity exercise and high intensity exercise on metabolism during and following a subsequent moderate intensity exercise session.

2. Please give a summary of the design and methodology of the project. Please also include in this section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made. (If in doubt, please obtain statistical advice).

12 healthy men (see power calculation below), aged 18-35 years will be recruited. Exclusion criteria will include; current smoking, physician diagnosed diabetes, uncontrolled hypertension (> 160/90 mm Hg on anti-hypertensive medication), previous history of established CHD (e.g. MI, CABG, PTCA), body mass index > 35 $kg.m^{-2}$, family history of early cardiac death (< 40 years). They will be recruited through posters, internet and local advertising.

Experimental design:
Following preliminary testing (described in section 3), each subject will undergo three main experimental trials, with an interval of approximately 1 week, in random order as described below.

a) **Prior moderate exercise** - Subjects will arrive at the Exercise Laboratory after an overnight fast and an expired air sample will be taken to assess metabolic rate and rate of fat and carbohydrate oxidation. A cannula will be inserted into an antecubital or forearm vein (by Dr Gill, Dr MacFarlane (trained phlebotomists) or another suitably qualified person (i.e. a medical practitioner or other trained phlebotomist) and a blood sample taken. Subjects will then be given a standard breakfast. One hour after breakfast, subjects will walk or run on a treadmill for 30 minutes at 40% of their maximal oxygen uptake (VO_2max) reserve (i.e. 40% of the difference between resting metabolic rate and VO_2max, plus resting metabolic rate. This is typically ~50% of VO_2max). On completion of the walk or run, subjects will rest for 30 minutes before undertaking a second treadmill walk or run for 60 minutes at 45-50% of VO_2max reserve. On completion of the second exercise session, subjects will be given a standardized test lunch. Blood and expired air samples will be taken and heart rate recorded at intervals throughout this time and for a further four hours following completion of the test lunch. Throughout this observation period will also be collected for the determination of urinary nitrogen excretion, which enables calculation of protein oxidation (Frayn, 1983). No more than 200 ml of blood will be taken over the course of this trial (less than half the amount given during a blood donation).

b) **Prior high intensity exercise** – This trial will be identical to the prior moderate exercise trial described above, except the first treadmill exercise session will involve walking or running on a treadmill for 15 minutes at 80% of their maximal oxygen uptake (VO_2max) reserve. This session has been designed to elicit the same net energy expenditure as the first exercise session in the prior moderate exercise trial.

c) **Prior resistance exercise** - This trial will be identical the two trials described above except that the first exercise session will involve 30 minutes of resistance exercise of large muscle groups (e.g. squats, lunges, shoulder press, lat pull downs, etc). To ensure that this can be accomplished safely in subjects who may be unfamiliar with resistance exercise, a 'Jones Lateral Smith machine' will be used. This is a guide-rail system in which the weights are supported, which automatically catches the weight if subject becomes too tired to lift further. Loads will be individually prescribed (based on their body % of fat free mass FFM). This session will be designed so that subjects lift the largest total load that they can manage over the 30-minute period. The energy expenditure of this session will be lower than the first exercise session in the prior moderate and prior high intensity exercise trials.

Blood will be analysed for substrates related to metabolism (e.g. lipids, glucose, insulin) to help understanding of the physiological mechanisms underpinning any differences in substrate utilisation between trials.

Based on published data that the within-subject reproducibility of 24-hour substrate utilization which preceding diet has been controlled is <7% for fat oxidation and <6% for carbohydrate oxidation (Toubro et al., 1995), 12 subjects would provide us with sufficient power to detect differences between trials in fat and carbohydrate rates of <10% with 90% power at α = 0.05.

3. Describe the research procedures as they affect the research subject and any other parties involved.

Volunteers will be asked to attend the laboratory on six occasions:

1) A screening visit during which subjects will be provided with written and oral information about the study and given the opportunity to ask questions; will complete a health history questionnaire; have their blood pressure measured; and provide written informed consent.
2) An incremental treadmill VO_2max test. Basic anthropometry (height, body mass, waist and circumferences and skinfold measurements) will also be performed at this visit.
3) A familiarization session for resistance exercise, during which subjects will be instructed how to perform the resistance exercises and their % of FFM for each exercise will be determined

4-6) The three main trials described above.

Tests will be conducted according to the 'Code of Practice for Conducting Experiments on Non-patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum)', re-approved by the University Ethics Committee on October 26, 2001. Subjects will be supervised at all times to ensure well-being, and emergency equipment and CPR-trained staff will be present.

The risks associated with performing maximal and near-maximal exercise in healthy young adults are minimal when subjects are appropriately instructed and familiarized with the equipment and protocols prior to participation, and are appropriately supervised during the experiment. All maximal and near-maximal exercise sessions will be preceded by an adequate "warm-up" and finish with a "cool-down" period. The latter is of particular importance during high-intensity exercise, when the local accumulation of exercise metabolites can cause an "expansion" (or vasodilatation) of the blood vessels in the lower limbs, which can impair the adequate return of blood to the heart – predisposing to fainting on ceasing exercise. This risk is minimised by having the subject exercise at a mild level during recovery to "wash away" these metabolites and therefore to restore the capacity of the involved blood vessels to their resting levels.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Blood sampling
Blood will be taken by venous cannulation. These incur a minor risk of bruising. There is also a risk of thrombophlebitis (inflammation of the vein) but this risk is small in non-smokers with no history of coagulation disorders. Plastic or air embolism can occur if incorrect cannulation technique is employed but good practice minimizes this risk. Some individuals may feel faint when giving blood.

Exercise testing
Some exercise testing will at maximal and near-maximal levels. This is of negligible risk in healthy young adults, although maximal exercise has a small risk of inducing myocardial ischaemia. Preliminary screening will exclude any subjects with a history of cardiovascular problems and those known to exhibit major risk factors for CHD. Heart rate will be continuously monitored during the tests, at least one person trained in CPR will be present during testing and a telephone to contact emergency services is available in the exercise laboratory, in the unlikely event of a problem arising. CPR-trained individuals likely to be present include Mr John Wilson, Mr Ian Watt, Mr Paul Patterson, Mrs Heather Collin, Dr Jason Gill, Dr Niall Macfarlane or Dr Lesley Hall (medical doctor). Mr Ahmed Alsabih will undergo training in CPR shortly.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

The risks associated with participating in this study are very small. Subjects will receive feedback on their fitness level and metabolic health profile (e.g. insulin, glucose, blood pressure), so will benefit from the study personally. The results will help our understanding of how different exercise protocols influence substrate metabolism so may be beneficial in the design of exercise strategies to facilitate weight loss, which is important in the current context of increasing obesity in society.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Drs Jason Gill and Niall MacFarlane have extensive experience in carrying out human studies including organization and planning of studies, blood sampling, biochemical analysis of samples and dietary assessment. Dr Gill has ~12 years of experience in conducting human metabolic and exercise studies, without incident and is trained and has ~7 years experience in venepuncture and cannulation. Dr Macfarlane has over 20 years experience of exercise testing, venepuncture and cannulation. Dr Gill, Dr MacFarlane or another appropriately trained person (i.e. a medical practitioner or trained phlebotomist) will perform all blood sampling or cannulations for this study. Ahmed Alsabih is a MSc student, working under the supervision of Drs Gill and MacFarlane. He will be trained in all of the procedures prior to commencement of the study. A number of undergraduate Honours project students (Ms Kathryn Cooke, Ms Gemma Strang, Mr Ryan Morrison, Mr Steven Charters) (supervised by Drs Gill and MacFarlane) will assist with aspects of testing and will be fully trained in any procedures that they undertake.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

The risks associated with the procedures are extremely small. Tests will be conducted according to the 'Code of Practice for Conducting Experiments in Non-Patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum)', re-approved by the University Ethics Committee on October 26, 2001. The exercise laboratories all have telephones for contacting emergency services.

All exercise tests will be supervised by at least two personnel experienced in the procedures involved, at least one of whom will be trained in CPR. In the event of an emergency, the previously approved emergency protocols will be followed.

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which

Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

9. Specify whether subjects will include students or others in a dependent relationship.

It is likely that some subjects will be students. However, they will not be directly recruited by Dr Gill or Dr Macfarlane (Recruitment will be performed principally by Mr Alsabih and by Honours Project students working on the project) and will be placed under no pressure to participate.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.

No

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.

No

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Subjects will be provided with a 'Volunteer Information Sheet' to read and will be given a verbal explanation of the study and the opportunity to ask questions before providing written consent to participate in the study.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

Subjects will be men. This is because in physiological studies of this nature, men and women often respond differently and therefore need to be considered separately for analysis. Thus, studying both sexes would double the size of the study. Men were chosen for this initial study, as studying women is complicated by the need to control for menstrual cycle. This would result in the three main trials needing to be conducted at intervals of ~4 weeks (rather than 1 week) which would prolong the length of the study. Depending on the findings, we may choose to repeat this study in women at a later time.

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised?

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

Not for the duration of the study.

16. Date on which the project will begin 1 October 2007 and end 1 December 2008

17. Please state location(s) where the project will be carried out.

IDEAL Laboratories, West Medical Building.

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All samples will be handled according to the 'Code of Practice for Conducting Experiments on Non-patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum)'.

Signed _____ Date _____

(Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

Signed _____ Date _____

Supervisor of student)

Email the completed form to: S.Morrison@bio.gla.ac.uk

And send the signed hard copy to:

Stuart Morrison
Faculty Research Office
Faculty of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Gilmorehill
Glasgow
G12 8QQ

Appendix B: Subject Information Sheet and consent Form

UNIVERSITY
of
GLASGOW

Version 2, 07th December 2007

VOLUNTEER INFORMATION SHEET

Effects of prior resistance or endurance exercise on metabolism during and after moderate-intensity exercise

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?
In order to lose body fat, your body must burn off more fat than you eat. The main way to increase the amount of fat your body burns is to exercise. However, different types of exercise have different effects on the amount of fat that you burn. In addition, the amount of fat that you burn during exercise may be influenced by whether you have done another exercise session earlier in the day. This study will determine the effects of a prior session of moderate intensity exercise, high intensity exercise or resistance (weight lifting) exercise on fat burning and metabolism during and following a second moderate intensity exercise session. This will help us to understand more about how exercise can help people to maintain a healthy body weight.

Why have I been chosen?
You have been chosen because you are a healthy man aged between 18-35 years.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any

time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Preliminary procedures

Before enrolling in the study you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history and physical activity level
- measure your blood pressure
- measure your height and weight
- provide an opportunity for you to ask questions

These preliminary procedures will enable us to determine whether you fall into the group of people we wish to study and will also ensure that it is perfectly safe for you to participate in this study.

Experimental procedures

A. Preliminary Treadmill Exercise Test

At the beginning of the study, a treadmill exercise test will be undertaken. This will involve running on a motorised treadmill. If you are not used to running on a treadmill, we will familiarise you with this before any 'real' sessions are performed. The test is designed to determine your body's ability to use oxygen (VO_2max) and enables us to find the correct speed and gradient for the exercise sessions later in the study. During the test the speed and/or gradient of the treadmill will increase progressively until you decide that you have reached your maximum capacity, when the test will stop. Heart rate will be monitored and recorded throughout using a heart rate monitor and expired air will be collected at intervals using a mouthpiece and respiratory valve.

B. Preliminary Resistance Exercise Session

This session will familiarise you with the weight lifting equipment we will be using in the study and teach you how to lift the weights safely. This session will also be used to determine how much weight you should lift in the in the resistance exercise session later in the study.

C. Body Composition

The amount and distribution of your body fat will be determined using callipers to measure skin fold thickness at four different sites (a sophisticated version of "pinch an inch"). Your height, weight and waist and hip circumferences will also be recorded. In order for these measurements to be as accurate as possible we will ask you to wear minimal clothing. All of the above measurements are taken

in private for your comfort. These measurements only take a few minutes and can be made on the same day as other tests.

D. Main experimental trials

We will ask you to undertake 3 main experimental trials, with about 1 week between each trial, in random order.

a) **Prior moderate exercise** - We will ask you to come to the University after an overnight fast (i.e. having eaten nothing for 12 hours) and spend the day with us (~8 hours). On arrival we will collect an expired air sample to measure how many calories and how much fat you are burning. We then collect a small blood sample from a tiny plastic tube called a 'cannula' which will be placed in a vein in your forearm. This is no more painful than a simple blood test. You will then be given a test breakfast and then, one hour later, we will ask you to walk or run (depending on your fitness level) on a treadmill for 30 minutes at a moderate intensity. We will then ask you to rest for 30 minutes, before asking you to walk or run on a treadmill for a further 60 minutes at a moderate intensity. After this you will be given a test lunch. We will take blood samples and expired air samples throughout this time and continue for four hours after your test lunch. We will also collect the urine that you produce over the course of the day (this will help us to calculate how much protein you are burning). A total of 200 ml (a small cupful, or less than half the amount taken when you donate a "pint" of blood) will be taken over the course of the day. During the times during the day when you are not exercising or eating, you will be free to relax, and can read, watch TV or DVDs, listen to music or use a computer.

b) **Prior high intensity exercise** – This trial will be exactly the same as the prior moderate exercise trial except that the first treadmill exercise session will involve walking uphill or running on a treadmill for 15 minutes at a high intensity (~80% of your maximum capacity).

c) **Prior resistance exercise** – This trial will be identical the two trials described above except that the first exercise session will involve 30 minutes of resistance exercise using large muscle groups. This session will be designed so that you lift the largest load that you can manage over 30-minute period.

What do I have to do?

Other than the specific tasks described above, we ask you to maintain your usual lifestyle (i.e. don't change your diet or exercise habits) for the duration of this study. We also ask you to weigh and record everything that you eat and drink for the two days before your first main experimental trial (we will provide you with scales and record sheets to do this) and not to undertake any planned exercise or drink alcohol on these days. We will ask you to repeat this

diet and activity pattern before your second and third main experimental trials.

What are the possible disadvantages and risks of taking part?

- Some of the exercise testing will be at maximal and near-maximal intensities. This poses a negligible risk in healthy young adults with not history of cardiac problems but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.
- Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high blood pressure. If such a problem is revealed, we will seek your permission to inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?

There may be no benefits to you but as a result of being involved in this study you will receive health and fitness information about yourself including fitness tests, a dietary assessment and body fat measurement. The findings of this study will be published in scientific journals so that understanding about how exercise can help people to improve their cardiovascular health and control their weight can be increased. This information may contribute towards improved exercise guidelines.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain our findings and discuss their implications with you.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the University, will have your name and address removed so that you cannot be recognised from it.

What will happen to my samples after the study has finished?
The blood samples that you provide for this study may be useful for future research into the prevention and treatment of obesity and related conditions such as diabetes and heart disease; this may involve analysis of certain genes associated with these diseases. Any use of your samples for future research will require further approval from a Research Ethics Committee and samples will be analysed in such a way that the results will not be directly traceable to you. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has reviewed the study?
This study has been reviewed and approved by the Institute of Biomedical and Life Sciences Ethics committee at the University of Glasgow.

Contact for Further Information
Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting either Ahmed Al-Sabih on 01413302915, email: a.alsubeih.1@research.gla.ac.uk, Dr Jason Gill on 0141 3302916 or j.gill@bio.gla.ac.uk or Dr Niall MacFarlane on 0141 3305965 or n.macfarlane@bio.gla.ac.uk.

You will be given a copy of this information sheet and a signed consent form to keep for your records.

Volunteer Identification Number for this trial:

CONSENT FORM

Title of Project: **Effects of prior resistance or endurance exercise on metabolism during and after moderate-intensity exercise**

Name of Researcher:_____

Please initial box

1. I confirm that I have read and understand the information sheet dated 7th December 2007 (version 2) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

4. I agree for my samples to be used for future research into the prevention and treatment of obesity and related conditions such as diabetes and heart disease. This may involve analysis of genes associated with these diseases.
   Yes ☐
   No ☐

_____	_____	_____
Name of Volunteer	Date	Signature
_____	_____	_____
Name of Person taking consent **(if different from researcher)**	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1for volunteer; 1 for researcher

Appendix C: Health History Questionnaire

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name: …….

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present**, do you have any health problem for which you are:
   - (a) On medication, prescribed or otherwise — yes [ ] no [ ]
   - (b) Attending your general practitioner — yes [ ] no [ ]
   - (c) On a hospital waiting list — yes [ ] no [ ]
2. **In the past two years,** have you had any illness which required you to:
   - (a) Consult your GP — yes [ ] no [ ]
   - (b) Attend a hospital outpatient department — yes [ ] no [ ]
   - (c) Be admitted to hospital — yes [ ] no [ ]
3. **Have you ever** had any of the following:
   - (a) Convulsions/epilepsy — yes [ ] no [ ]
   - (b) Asthma — yes [ ] no [ ]
   - (c) Eczema — yes [ ] no [ ]
   - (d) Diabetes — yes [ ] no [ ]
   - (e) A blood disorder — yes [ ] no [ ]
   - (f) Head injury — yes [ ] no [ ]
   - (g) Digestive problems — yes [ ] no [ ]
   - (h) Hearing problems — yes [ ] no [ ]
   - (i) Problems with bones or joints — yes [ ] no [ ]
   - (j) Disturbance of balance/co-ordination — yes [ ] no [ ]
   - (k) Numbness in hands or feet — yes [ ] no [ ]
   - (l) Disturbance of vision — yes [ ] no [ ]
   - (m) Thyroid problems — yes [ ] no [ ]

(n) Kidney or liver problems	yes [ ]	no [ ]
(o) Chest pain or heart problems	yes [ ]	no [ ]
(p) Any other health problems	yes [ ]	no [ ]

4. **Have any of your immediate family** ever had any of the following: (if yes please give details including age of first diagnosis)

(a) Any heart problems	yes [ ]	no [ ]
(b) Diabetes	yes [ ]	no [ ]
(c) Stroke	yes [ ]	no [ ]
(d) Any other family illnesses	yes [ ]	no [ ]

5. Do you currently smoke	yes [ ]	no [ ]
Have you ever smoked	yes [ ]	no [ ]

If so, for how long did you smoke and when did you stop? ……

6. How many units of alcohol do you typically drink in a week? …….

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……

……

……

Name and address of GP ……

……

Blood pressure measured at screening……..mm Hg

Appendix D: Physical Activity questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ **No** → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.