DOCTOR OF PHILOSOPHY

Metabolic adaptation on muscular and whole body levels in response to altered nutrient availability and energy demand

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Award date:
2014

Awarding institution:
Bangor University

METABOLIC ADAPTATION ON MUSCULAR AND WHOLE BODY LEVELS IN RESPONSE TO ALTERED NUTRIENT AVAILABILITY AND ENERGY DEMAND

By

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A Thesis submitted to the school of Sport, Health & Exercise Sciences, Bangor University in fulfillment of the requirements of the degree of Doctor of Philosophy

March, 2014

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SUMMARY

In the modern day lifestyle, two factors have been primarily associated with the rising prevalence of obesity, the increasing consumption of energy dense food and the decline in physical activity (Varo et al. 2003). With an estimated 500 million adults thought to be obese worldwide (WHO 2013), it is clear we need to stem this epidemic. Much emphasis on research has already been placed on developing strategies to manipulate these two factors and reduce the incidence of obesity but at present an effective and sustainable solution seems elusive.

One thing that is clear, with the increase in the cases of obesity, there has also been a concomitant rise in our intake of high refined carbohydrates and sugars. Moreover, the consumption of sugar-sweetened beverages (SSB) has increased over the past four decades (ERS 2004) and consequently, their association with obesity has been well documented (Nissinen et al. 2009, Olsen, Heitmann 2009, Hu, Malik 2010). To undo the damage of diets high in refined carbohydrates and sugars, exercise has been thought to be the answer by many but as yet this has not yielded convincing success in promoting weight loss and repairing the alterations to metabolism associated with chronic hyperglycaemia and obesity.

In the first experimental chapter, an in vivo and in vitro approach was used to investigate the effects of high glucose availability on skeletal muscle metabolism. In our in vivo study the effects of 4 weeks sugar-sweetened beverage (SSB) supplementation was investigated on lean, healthy, lightly active individuals with very little or no previous consumption of SSB. Muscle biopsies were taken from each participant pre and post 4 week intervention and through western blotting and real time reverse-transcriptase polymerase chain reaction (RT-PCR), protein and gene expression of several metabolic markers and glucose regulating factors was measured. In the in vitro study, primary human muscle cell cultures were exposed to chronic hyperglycaemia and compared to cultures with normal glucose concentrations for 7 days. Analyses revealed both the in vivo and in vitro studies demonstrated a shift towards increased glycolytic activity and reduced oxidative activity, similar to that found in type 2 diabetes mellitus patients. Furthermore, in both studies an increase in MondoA expression was observed and in the in vitro cell cultures TXNIP expression

was also increased. The fact that the findings from in vivo study are comparable to those in the in vitro study, demonstrates the potency of high glucose availability on skeletal muscle. The results are even more alarming as the participants were young healthy individuals, not overweight or obese and with very little previous history of SSB consumption, highlighting just how damaging the effects of SSBs can be and the significant role they play in the development of obesity.

In chapter 3 and 4, long term exercise interventions were used to investigate the effects of chronic exercise on metabolism, body composition and energy balance of lean and overweight or obese (Ov/Ob) sedentary women. The aim of these studies was to investigate the compensatory mechanisms in Ov/Ob individuals preventing adaptations to exercise. A novel approach to long term exercise with *ad libitum* energy intake was used in both of these studies, where participants were not recruited with a desire to lose weight and they were also naïve to the true aims of the study. In chapter 3 a 4 week exercise training intervention was used and in chapter 4 an 8 week training intervention was used. Both studies utilized a group based circuit training format 3 days per week, the 4 week intervention was at a moderate intensity and the 8 week intervention had both a high and low intensity exercise group. Dual-energy x-ray absorptiometry (DXA) and indirect calorimetry, at rest and during exercise, were used to give and overview of participants' anthropometric and metabolic profiles, before and after inventions. Blood samples were collected for analysis of several key hormones regulating metabolic adaptations and energy homeostasis in response to exercise. Diet records were also collected from participants to measure any alterations in energy and macronutrient intake.

Neither the 4 week nor the 8 week exercise intervention demonstrated significant weight loss of either the lean or Ov/Ob individuals. However, in both the interventions lean participants displayed losses in percentage body fat, even though no weight loss was seen overall. These findings reaffirm those reported in previous literature that during an increase in exercise induced energy expenditure, Ov/Ob individuals with respond with a concomitant increase in energy intake, negating any possible weight or fat loss and possibly even promote weight gain. On the contrary lean individuals do not display the same over-compensation to the increased energy expenditure and there energy intake is unchanged. It is hypothesized that the lean

participants were able to display a reduction in body fat through the ability to exercise at a higher absolute intensity. Based on analysis of blood samples, it was possible to further investigate possible regulators that may control a homeostatic mechanism, preventing weight/fat loss in obese individuals. In the 8 week study a significant drop in amylin concentrations was observed in the Ov/Ob participants, compared to no change in the lean subjects. It was proposed that Ov/Ob individuals, who also displayed heightened insulin resistance and hyperleptinemia, have developed an overreliance on amylin for satiety signalling. Moreover, in conjunction with the inability to achieve an absolute intensity high enough to induce adaptive responses to exercise, Ov/Ob individuals with reduced amylin secretion, increase energy intake after exercise and prevent any possible weight or fat loss.

Additionally, adaptive responses to exercise were seen in both the 4 week and 8 week exercise studies, Ov/Ob participants displayed a positive movement away from a preference to rely on anaerobic metabolism at a low exercise intensity pre-intervention, towards more anaerobic metabolism post-intervention. In addition, following the 8 week exercise intervention, the inter-individual variability in response to exercise was investigated. Participants were separated based on their heart rate response, into responders and non-responders and it was revealed that those who clearly demonstrated adaptations, also tended to lose more weight, maintain a negative energy balance, and avoid the maladaptive decrease in amylin concentrations. Suggesting these individuals do not develop the hypothesized leptin resistance, and overreliance on amylin signal, meaning they do not overcompensate for increased exercise induced energy expenditure and can lose weight.

In conclusion, it is clear that an exercise only or a one size fits all approach is not the answer in the treatment of obesity. A more multi-dimensional approach is necessary and in order to reduce many of the associated lifestyle disease markers, both diet and levels of physical activity must be addressed in the obese. Recommendations for future intervention studies must address this and combine a diet low in refined carbohydrates and sugars, with high intensity interval training and resistance exercise.

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ACKNOWLEDGEMENTS

Firstly and most notably, I would like to thank Dr. Hans-Peter Kubis for his supervision and guidance throughout my PhD and the years prior, which has been invaluable. I would also like to thank my colleagues at the School of Sport, Health & Exercise Sciences; in particular I would like to acknowledge Dr. Francesco Sartor and Fardin Fatahi for their assistance and friendship during my PhD. I also wish to express a special thanks to Kevin Williams and Jason Edwards, for their technical expertise during the data collection of the studies presented. Additionally, I would like to thank all the masters and undergraduate students who assisted with the implementation of interventions and data collection.

Finally, I wish to express my gratitude to my parents for their continued support and encouragement, without whom none of this would be possible, and my friends Ryan Iddon, Tom Lancaster and Helen Graham for their moral support.

Contributions

Chapter 2: The studies presented in chapter 2 were carried out by myself and another PhD student for the publication listed below. My contribution to this publication and presented in this thesis, was conduction of the study, data collection, all lab experiments, tissue processing and RT-PCR analysis. Other data included in the publication are presented here for a more complete understanding and explanation of the findings.

Chapter 3 & 4: All data was collected by myself or in collaboration with a further PhD student (Chapter 4) and MSc students. In both chapters because of the size of the interventions, a team of MSc and BSc students assisted with the implementation of interventions and data collection, under supervision.

Publications

Chapter 2: Francesco Sartor, Matthew J Jackson, Cesare Squillace, Anthony I Shepherd, Donald E Ayer, and Hans-Peter Kubis. Adaptive metabolic response to 4

weeks sugar-sweetened beverage consumption in healthy, lightly active individuals and chronic high glucose availability in primary human myotubes. *European Journal of Nutrition*, 52(3), 937-948.

Published abstracts

Chapter 3: Matthew Jackson, Kholoud Alabduljader, Kirstie Tew and Hans-Peter Kubis (2012). Effects of a 4 week exercise intervention on body composition, energy balance and metabolism in lean and overweight/obese women. *Proceedings of the Physiological Society* 27, PC113.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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STATEMENT 1

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CHAPTER I

GENERAL INTRODUCTION

Introduction to obesity

The rise in the prevalence of obesity is a worldwide problem, no longer just for high income countries but obesity is now rising in low and middle income countries also, with an estimated 1.4 billion adults thought to be overweight and of those 200 million men and 300 million women were obese (WHO 2013). Furthermore, in England alone over 60 % of people are overweight or obese and 28% of children aged between 2 and 15, with the associated health problems costing the National Health Service (NHS) more than £5 billion every year (Department of Health 2013). In addition, obesity has been identified as a risk factor for a wide range of co-morbidities including, type 2 diabetes mellitus, hypertension, coronary heart disease, osteoarthritis, respiratory problems and some forms of cancer (Neary, Goldstone & Bloom 2004). Two key factors in our modern day lifestyle have been associated with this epidemic, the first is an increase in the consumption of energy dense food and the second is a decline in physical activity (Varo et al. 2003). Naturally, these two variables have been the focus of research attempting to outline the causes, treatment and prevention of obesity and a greater understanding of how these contributing factors influence metabolism is required, to ensure progress in the fight against the ever increasing prevalence of obesity. Moreover, with a more complete understanding it will be more possible to develop effective strategies for weight loss, significantly reducing the morbidity and mortality associated with obesity (Murphy, Bloom 2004).

To date intervention studies have investigated how either restricting energy intake, manipulating energy expenditure or both simultaneously affect weight loss and so far have produced no clear intervention that has been able to achieve long-term sustainable weight loss or curb the increasing incidence of obesity, more research needs to focus on the dysregulation of homeostatic mechanisms regulating energy balance (Hafekost et al. 2013). In Hafekost *et al*'s review (2013) all intervention studies investigating weight loss through energy manipulation with follow up tests post-intervention, demonstrated significant weight regain and in Jakicic *et al*'s (Jakicic et al. 2008) study after 6 months of lifestyle alteration and weight loss, an 18 month follow up was conducted, 72% had regained weight and a further 31% regained weight over and above their previous baseline. The issue with these previous intervention studies is that by way of approach they do not take into account

compensatory mechanisms that may exist and assume that energy intake and expenditure can be altered independently from one another (Stubbs et al. 2002). The mechanisms causing these compensatory changes and the coinciding alterations in metabolic factors need further investigation, in order to develop more successful and effective strategies to promote and maintain weight loss in the growing overweight and obese population.

The role of high refined carbohydrates and sugars, and the incidence of obesity

In recent years, one thing is clear that with the increase in the cases of obesity worldwide, there has been a concomitant rise in our intake of high refined carbohydrates and sugars. Since industrialisation, the availability of sugar and refined carbohydrates has increased significantly and in modern times, corn syrup has become the leading source of added sugar in our diets (Bray 2008). Corn syrups can be fructose, combination of fructose and glucose or glucose (100% glucose) based but whatever their make-up they are the main sweetener used in sugar sweetened beverages (SSB) today and it is the consumption of these which this thesis is specifically concerned with. Furthermore, the consumption of SSBs has increased over the past four decades, with the percentage of adults drinking SSBs in the US rising from 58% between 1988 and 1994 to 63% between 1999 and 2004 (ERS 2004). Subsequently, the association between obesity and refined carbohydrate and SSB consumption has been reported in many investigations recently (Nissinen et al. 2009, Olsen, Heitmann 2009, Hu, Malik 2010, Ludwig, Peterson & Gortmaker 2001, Ebbeling, Pawlak & Ludwig 2002, Schulz et al. 2002, St-Onge, Keller & Heymsfield 2003, Berkey et al. 2004, Bray, Nielsen & Popkin 2004, Gross et al. 2004, James, Kerr 2005, Bes-Rastrollo et al. 2006, Malik, Schulze & Hu 2006, Dhingra et al. 2007, Vartanian, Schwartz & Brownell 2007, Palmer et al. 2008, Bleich et al. 2009, Fung et al. 2009). Not all of the evidence surrounding SSB consumption and obesity is damning however, there are studies with conflicting evidence (Drewnowski, Bellisle 2007, Gaesser 2007, Forshee, Anderson & Storey 2008, Gibson 2008, Gomez-Martinez et al. 2009, van Baak, Astrup 2009). Although as recognized by a recent review (Vartanian, Schwartz & Brownell 2007) these articles which try to discredit the association between obesity and SSBs are food industry funded and adjust the energy intake when supplementing SSBs to reduce the relationship with weight gain.

Nonetheless, more evidence is needed to prove that increasing SSB consumption is directly associated with obesity (Mattes et al. 2011). Moreover, obesity is greatly associated with type 2 diabetes mellitus and insulin resistance (Wannamethee, Shaper 1999) and consequently a diet high in refined carbohydrates and sugars has been seen shown to be related to the development of this condition (Hu, Malik 2010, Gross et al. 2004, Vartanian, Schwartz & Brownell 2007, Palmer et al. 2008, Bleich et al. 2009, Liu 2002, Yoshida et al. 2007, Mohan et al. 2009, Stanhope et al. 2009).

Additionally, offering further support for the negative effects of diets high in refined carbohydrates, low carbohydrate diets have been proven to promote weight loss in overweight and obese (Ov/Ob) individuals (Brehm et al. 2003, Foster et al. 2003, Boden et al. 2005, Dansinger et al. 2005, Westman et al. 2006, Ebbeling et al. 2007). They have also been demonstrated to lessen the symptoms of insulin resistance in Ov/Ob people, reducing both plasma glucose and insulin concentrations (Foster et al. 2003, Dansinger et al. 2005). The same has also been reported in patients with type 2 diabetes mellitus (Boden et al. 2005, Samaha et al. 2003, Yancy et al. 2005).

Regulation of blood glucose

In healthy individuals, blood glucose levels are tightly regulated, primarily by insulin and glucagon, as part of a hormonal regulatory system. During postprandial hyperglycaemia after carbohydrate ingestion, glucose is absorbed in the gut promoting high intracellular glucose availability in the pancreatic β-cells; as a result insulin secretion is stimulated to decrease circulating blood glucose levels. Under normal conditions, insulin then stimulates glucose uptake of the peripheral tissues and blood glucose levels are reduced. In contrast, under hypoglycaemic conditions, glucagon is secreted by the α-cells of the Langerhans; in the liver it stimulates the release of glucose through glycogenolysis and gluconeogenesis.

In skeletal muscle, during hyperglycaemia glucose uptake is facilitated by insulin-dependent active transport (GLUT4) (Klip, Paquet 1990). In order for this to happen, first insulin must bind to the insulin receptors at specific sites of the muscle cells (Burant et al. 1986). This binding of insulin induces the phosphorylation of tyrosine (Hubbard et al. 1994) and in turn the phosphorylation of insulin-receptor substrate

(IRS-1) (Gual, Le Marchand-Brustel & Tanti 2005). This activates phosphatidylinositol- 3-kinase (PI3-K) (Gual, Le Marchand-Brustel & Tanti 2005) which interacts with the IRS-1 docking site, phosphorylating phosphatidylinositol-4,5-biphosphate (PIP2), subsequently generating phosphatidylinositol-3,4,5-triphosphate (PIP3) (Saltiel, Pessin 2002). PIP3 binds to Akt (also known as protein kinase B; PKB) and after phosphorylation by 3-phosphoinositide-dependent protein kinase (PDK-1) (Corvera, Czech 1998), Akt activates its substrate AS160 which induces the translocation of GLUT4 to the plasma membrane (Sano et al. 2003), increasing glucose uptake. Simultaneously, Akt while promoting the translocation of GLUT4 to the membrane for glucose uptake, also inhibits glycogen synthase kinase-3 (GSK-3) (Cross et al. 1995) which allows glycogen synthase to direct much of the glucose entering the cell towards storage through glycogen synthesis.

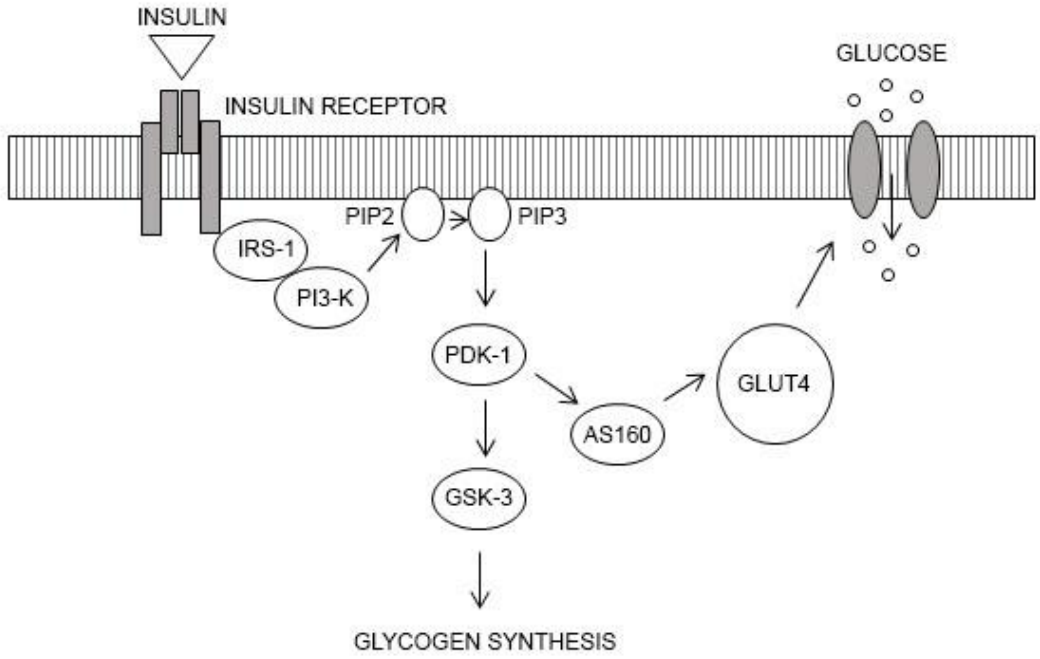

Fig. 1.1 Regulation of insulin stimulated glucose transport and glycogen synthesis in skeletal muscle.

After ingestion, most glucose is disposed of in skeletal muscle tissue through insulin dependent glucose uptake. It has previously been demonstrated that 67% enters glycolysis and 33% is stored as glycogen, of the 67% glycolysis, 65% goes through oxidative glycolysis and the rest non-oxidative glycolysis (Woerle et al. 2003).

Additionally, the existing levels of glycogen stores, depending on diet and physical activity, can also influence the amount of glycogen synthesis (Fery, Plat & Balasse 2003). Furthermore, glycogen has been demonstrated to influence more than just glucose storage it has also been shown to regulate glucose flux also (Jensen et al. 2006). Subsequently, high glycogen levels have been shown to reduce glycogen synthase activity (Danforth 1965) and glucose uptake into the muscle (Derave et al. 2000, Aslesen et al. 2001). It is possible that under conditions of high glucose availability, the function of glycogen synthase may be reduced, leading to increased glucose metabolites, lipogenesis and finally insulin resistance.

Glucose sensing

After refined carbohydrate and sugar ingestion, glucose is absorbed by the gut and there is an increase in blood glucose levels, through insulin action this glucose is taken up by skeletal muscle and used in any one of numerous cellular processes. Recently, new evidence has demonstrated that glucose and its metabolites are sensed in the muscle cell by specific molecules stimulating regulatory gene expression (Desvergne, Michalik & Wahli 2006). Inside the muscle, hexokinase (HK) converts glucose into glucose-6-phosphate (G6P). G6P is important in the regulation of L-pyruvate kinase (L-PK) in the hepatic and adipose tissues (Desvergne, Michalik & Wahli 2006). A region on the L-PK gene has been shown to interact with glucose (Diaz Guerra et al. 1993), as in other genes and these have been termed carbohydrate response elements. ChREBP or MondoB as it's also known, has since been identified as a protein which binds to the ChoRE of the L-PK gene (Yamashita et al. 2001) but only in the liver. Under hyperglycaemic conditions MondoB translocates to the nucleus of hepatic cells and activates acetyl-CoA carboxylase (ACC) and L-PK, promoting lipogenesis (Ma, Tsatsos & Towle 2005). The paralog of MondoB, MondoA is expressed in skeletal muscle (Billin et al. 2000), it has been found to translocate from the mitochondrial membrane to the nucleus (Sans et al. 2006) and here it accumulates, like MondoB, through G6P activity (Peterson et al. 2010). In the muscle cell, MondoA and its heterodimerisation partner max-like protein x (Mlx) form a complex (Billin et al. 2000) which activates the transcription of lactate dehydrogenase-A (LDH-A), phosphofructosekinase 2-fructose biphosphatase 2 (PFK2-FBPase2) and HK genes, highlighting its importance in the regulation of

glucose in and out of the muscle cell (Sans et al. 2006). Moreover, MondoA has been shown to activate thioredoxin-interacting protein (TXNIP) (Stoltzman et al. 2008). This is significant because TXNIP inhibits thioredoxin-NADPH-dependent reduction of protein disulfides (Nishiyama et al. 1999), making cells more susceptible to oxidative stress by reactive oxygen species (Nordberg, Arner 2001). TXNIP has also previously been shown to reduce glucose uptake (Parikh et al. 2007). Other glucose sensors present in skeletal muscle include, sterol regulatory element binding-protein 1c (SREBP-1c) and liver X receptor (LXR). During periods high glucose availability SREBP-1c up regulates lipogenesis by activating HK, FAS, ATP-citrate lysase and ACC (Guillet-Deniau et al. 2004). LXR is also an important regulator of lipid and cholesterol metabolism in skeletal muscle (Muscat et al. 2002).

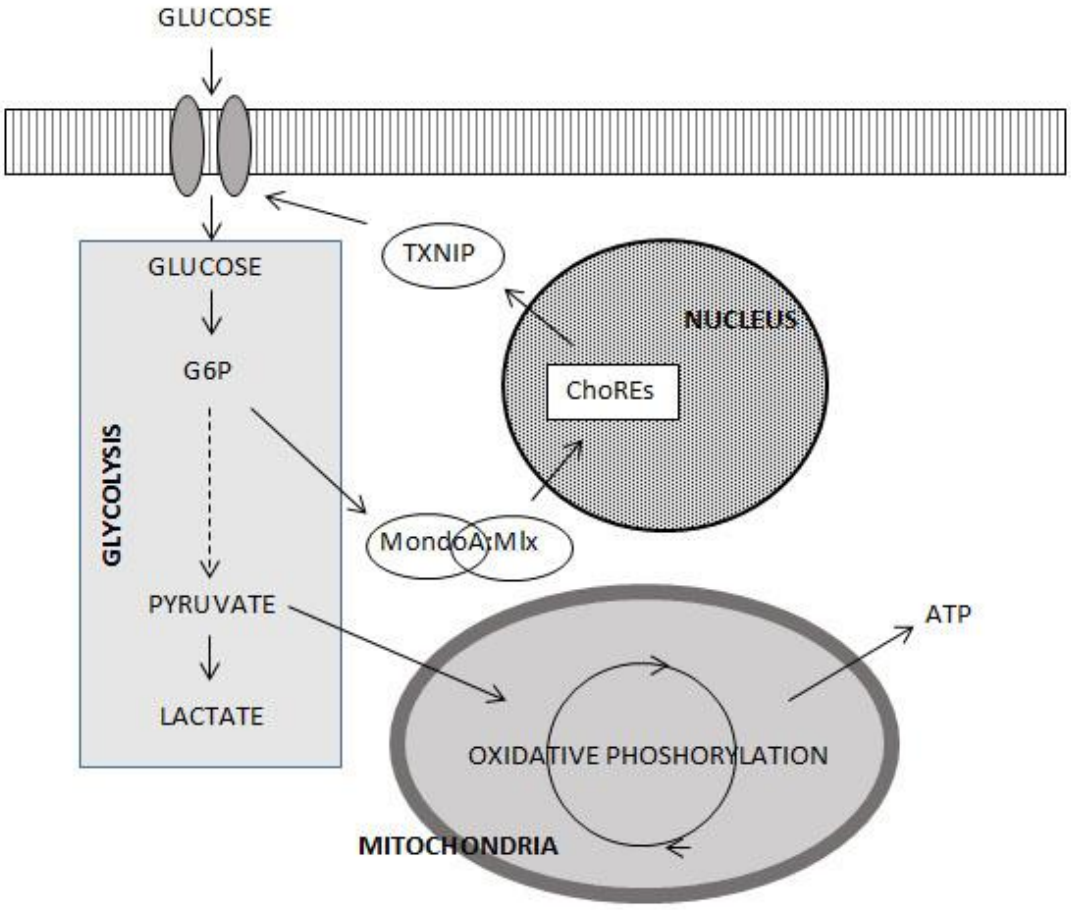

Fig. 1.2 Glucose sensing in skeletal muscle, highlighting the influence of MondoA and TXNIP in controlling glucose uptake.

High glucose availability and skeletal muscle

The increase in dietary refined carbohydrates and sugars produces a large rise in glucose availability (Foster-Powell, Miller 1995) and continuous high energy food intake and a lack of energy expenditure, challenge the normal regulation of blood glucose levels (Manson et al. 1992), eventually weakening the system so that periods of hyperglycaemia following carbohydrate ingestion are extended. In turn this can influence several signalling pathways, causing adaptations to skeletal muscle which contribute to reduced oxidative capacity and insulin sensitivity. Reduced glucose uptake (DeFronzo, Simonson & Ferrannini 1982) and high intracellular glucose levels have been observed in diabetic patients during hyperglycaemia and hyperinsulinaemia (Bonadonna et al. 1996). This leads to the assumption that high carbohydrate diets combined with low physical activity can cause prolonged periods of hyperglycaemia even in individuals with normal glucose tolerance and if continued they can lead to development of a low oxidative capacity and impaired insulin signalling pathways, usually described as a diabetic phenotype. Moreover, high glucose availability has been widely recognized as a cause of insulin resistance in skeletal muscle (Fell et al. 1982, Richter, Hansen & Hansen 1988a, Richter, Hansen & Hansen 1988b, Hansen et al. 1992, Davidson et al. 1994, Gulve et al. 1994, Kawanaka et al. 2001, Kawanaka et al. 1999, Oku et al. 2001, Han, Chen & Holloszy 2003). Some of the mechanisms, through which high glucose availability leads to insulin resistance include, UDP-N-acetylhexosamine accumulation (Marshall, Bacote & Traxinger 1991b, Marshall, Bacote & Traxinger 1991a, Robinson, Sens & Buse 1993, Baron et al. 1995, Virkamaki et al. 1997), protein kinase C activation (Pillay, Xiao & Olefsky 1996, Filippis, Clark & Proietto 1997, Laybutt et al. 1999) and increased glycogen content (Fell et al. 1982, Kawanaka et al. 1999, Jensen et al. 1997).

High glucose availability and gene expression

Based on findings of previous investigations, there is sufficient evidence to suggest that high glucose availability, in skeletal muscle, can alter numerous transcription factors, including, MondoA, SREBP-1c, LXR, and proliferator-activated receptor coactivator 1 alpha (PGC1α), which have all been implicated in the metabolic alteration of skeletal muscle towards a diabetic phenotype, through decreasing

oxidative capacity and increasing glycolytic capacity (Sans et al. 2006, Guillet-Deniau et al. 2004, Mitro et al. 2007, Hanke et al. 2008). Changes in metabolic gene expression are also likely to occur very early in the development of this phenotype, as previously seen in human muscle cell cultures, changes in glucose uptake and glycogen synthesis were seen after just two days of exposure to high glucose availability (Aas et al. 2004). Of all of these MondoA has been found to be transcribe many of the genes regulating of glucose, one of the most dependent genes is TXNIP (Stoltzman et al. 2008). As previously discussed, TXNIP is known to be involved the formation of ROS and is important in the regulation of glucose uptake by negative feedback (Parikh et al. 2007). For these reasons, it is apparent that MondoA is crucial to the regulation of glucose homeostasis and high glucose availability is likely to destabilise this relationship. Furthermore, as a result of the inhibition of MondoA during chronic high glucose availability, downstream complications include the inhibition of glyceradehyde-3-phoshate dehydrogenase (GAPDH), through the ROS stimulated mitochondrial dysfunction, having profound effects on glycolytic capacity (Bouche et al. 2004). Moreover, it is highly likely that the high glucose availability will reduce oxidative capacity of skeletal muscle, as seen in obese and diabetic or insulin resistant individuals (Kelley, Mandarino 1990, Kelley et al. 1999). Other impairments of skeletal muscle in this population include, decreased mitochondrial ATP synthesis, less oxidative fibres compared to glycolytic fibres, decreased glycogen synthesis and significantly lower expression of PGC1α (Mootha et al. 2003, Patti et al. 2003, Lowell, Shulman 2005). Finally, it should be possible to see these alterations in sedentary individuals' with diets high in refined carbohydrates and sugars, typically seen in SSBs, as they will not be able to combat the damage caused by periodic high glucose availability with increased glucose clearance through of physical activity.

Benefits of physical activity

In order to undo the damage to the regulation of glucose and hence, the metabolic profile of the obese population, typically with diets high in refined carbohydrate, many investigations have been conducted to find the best cure. As previously mentioned low carbohydrate diets have been employed by many and have been demonstrated to promote weight loss and lessen the symptoms of insulin resistance

associated with obesity (Foster et al. 2003, Dansinger et al. 2005) but to date they have been unsuccessful in sustaining long term weight loss and returning glycaemic control back to normal function (Boden et al. 2005, Dansinger et al. 2005, Samaha et al. 2003, Yancy et al. 2005).

Exercise has been thought to be the answer by many but as yet this has not yielded convincing success in promoting weight loss and repairing the alterations to metabolism associated with chronic hyperglycaemia and obesity. In many exercise studies even with reported weight loss, this is usually lower than expected (King et al. 2007). In addition, where follow up tests have been conducted after the exercise intervention and weight loss was achieved, it is not uncommon to see individuals regain some of the weight and regain over and above original pre-intervention weight has also been well documented (Hafekost et al. 2013, Jakicic et al. 2008, Wadden et al. 1998, Doucet et al. 2011). Moreover, it has previously been demonstrated that interventions using exercise alone only show minimal weight loss (2.4 kg), compared to diet interventions (4.9 kg) and a combined approach (7.9 kg) (Franz et al. 2007), suggesting that exercise may cause a compensatory increase in energy intake in response to the added energy expenditure. In addition, previous literature demonstrates a large variability in the individual responses to exercise interventions, particularly regarding bodyweight and fat mass. Ranges in fat mass change of Ov/Ob individuals post intervention, was reported between -9.5 and +2.6 kg in one study, after a 12 week programme (King et al. 2007) and weight change varied between -5.3 and +2.1 kg in another study after 7 weeks training (Barwell et al. 2009). These studies clearly demonstrate that responses to long term exercise interventions vary greatly between individuals but what is not clear are the underlying mechanisms which control this response, more research is needed here.

The question of exercise intensity is also an issue when prescribing exercise, previous research demonstrates to produce greater weight loss, increased fat oxidation, as opposed to carbohydrate oxidation, is more favourable and this is dependent on exercise intensity (Achten, Jeukendrup 2004). Exercise at moderate intensities (55% $\dot{V}O_{2MAX}$) has been shown to use equal amounts of energy from fat and carbohydrate sources, and with increasing intensity (75% $\dot{V}O_{2MAX}$) more energy is derived from

carbohydrate oxidation than from fats, with low intensity (40% $\dot{V}O_{2MAX}$) this relationship is reversed (van Loon et al. 2001). Furthermore, even though a greater percentage of energy is derived from fat oxidation at low intensity (25% $\dot{V}O_{2MAX}$), absolute amounts of fat oxidation at the end of exercise is significantly higher using a moderate intensity (65% $\dot{V}O_{2MAX}$) during exercise (Romijn et al. 1993), suggesting that a moderate intensity exercise intervention may be the most suitable for weight and fat loss. However, to increase glycogen depletion through exercise and reduce the effects of chronic hyperglycaemia on skeletal muscle, higher intensity exercise may be more advantageous. Muscle glycogen depletion has been seen to be greater in high intensity (90% $\dot{V}O_{2MAX}$) exercise than low intensity (60% $\dot{V}O_{2MAX}$) exercise (Gollnick, Piehl & Saltin 1974, Vollestad, Blom 1985). Finally, high intensity exercise has been seen to increase glucose clearance and improve insulin sensitivity of both healthy and insulin resistant individuals (Kjaer et al. 1990, Mourier et al. 1997, Loimaala et al. 2003, Dela et al. 2004, DiPietro et al. 2006).

Compensatory mechanisms influencing energy balance

The basic principle of energy balance is that, energy storage will only increase if energy intake exceeds energy expenditure, where energy expenditure includes physical activity, basal metabolism and thermogenesis (Spiegelman, Flier 2001). The central nervous system (CNS) controls energy balance and body weight via three mechanisms, which are; effects on behaviour, including intake and physical activity; effects on autonomic nervous system, which regulates expenditure and metabolism; and effects on the neuroendocrine system, including secretion of hormones (Spiegelman, Flier 2001). All three of these mechanisms interact and form a complex network of pathways regulating energy balance.

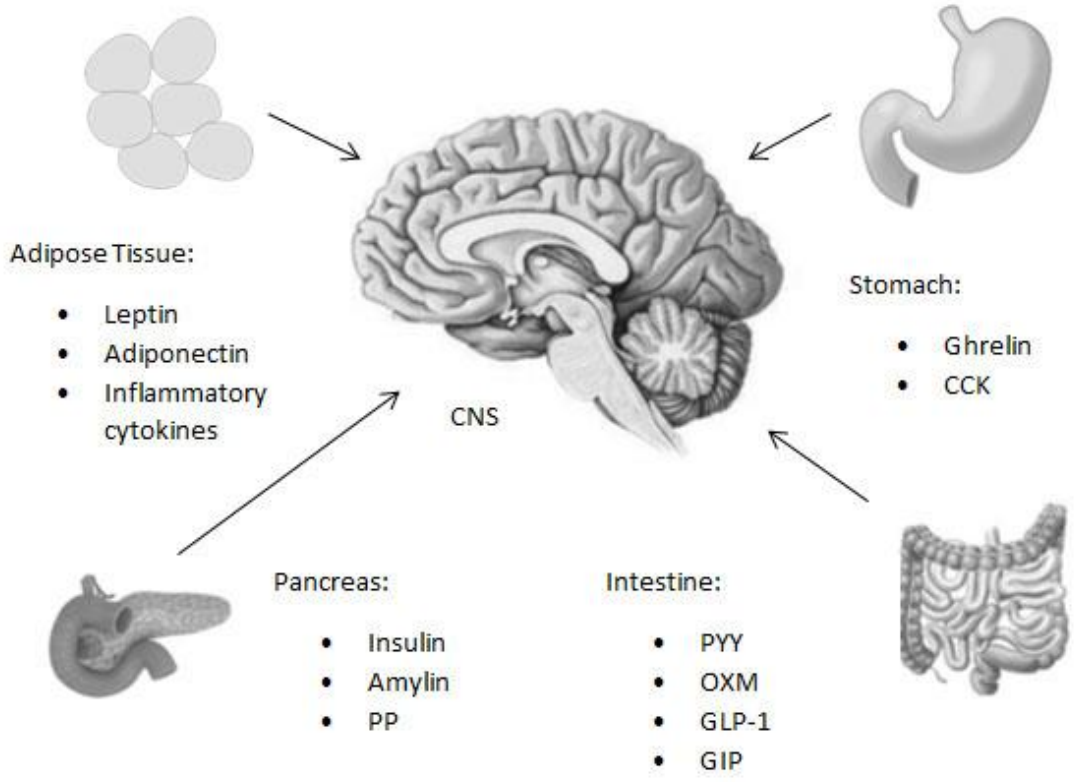

Fig. 1.3 Peripheral factors contributing to energy balance regulation via the central nervous system (CNS). PP – pancreatic polypeptide, PYY – Peptide YY, OXM – oxyntomodulin, GLP-1 – glucagon like peptide 1, GIP – gastric inhibitory polypeptide and CCK – cholecystokinin.

As previously discussed, recent research using long term exercise interventions to promote weight loss in the Ov/Ob, seem to suggest a compensatory mechanism exists within this population, causing an increase in energy intake after an initial exercise induced energy deficit (Stubbs et al. 2002). Consequently, this implies energy balance may be homeostaticly regulated to maintain a set bodyweight, understanding the regulation of this mechanism may be the key to enabling Ov/Ob individuals to lose weight. Previous research documenting this compensatory response to increased energy expenditure, shows it takes a number of weeks for exercise to stabilise energy balance (Doucet et al. 2011, King et al. 2008), explaining why larger discrepancies in energy balance may be seen in the earlier stages of exercise interventions. Moreover, it is also possible that the type of exercise may influence the compensatory response to exercise. As mentioned earlier, obesity is associated with impaired fat oxidation and a greater emphasis on carbohydrate oxidation, influenced by the higher blood

glucose and muscle glycogen levels. In addition, individuals with high levels of carbohydrate oxidation have been shown to have significantly higher energy intake post exercise than high fat oxidation levels (Almeras et al. 1995). As high intensity exercise also promotes carbohydrate oxidation, it is reasonable to assume high intensity exercise will result in a larger compensatory intake following exercise.

Furthermore, even though there is strong evidence to suggest that a compensatory mechanism exists in obese individuals preventing response to exercise and weight loss; research also shows that not all individuals display this phenomenon. Both compensators and non-compensators were identified in a recent study, where energy intake was compared after exercise against intake without exercise and compensators tended to eat more after exercise and non-compensators consumed the same amount of food or less (Finlayson et al. 2009). In this study, the authors attributed this variation in compensation of energy expenditure to individual's hedonic response to exercise, indicating this may be an important mediating factor influencing response to exercise.

Leptin

It is well documented that leptin plays a key role mediating; fat mass, bodyweight and energy balance (Adami et al. 2002). It is also known that leptin is produced and stored in adipose tissue and high circulating levels are known to correlate with elevated adiposity in obese individuals (Considine et al. 1996). When administered to obese leptin deficient mice and humans, it has been shown to induce significant fat loss by decreasing appetite and increasing energy expenditure (Zhang et al. 1994, Farooqi et al. 2002). Although, in humans the leptin administration only produced modest weight loss, it is evident that obese individuals experience a leptin resistance (Munzberg et al. 2005). It has been suggested that this leptin resistance is caused by impairment in the transport of leptin across the blood brain barrier; demonstrating a reduced efficiency in brain leptin delivery and CNS leptin uptake (Nam et al. 2001).

To date there is little evidence to suggest that exercise alters leptin concentrations independent of changes in body composition; in many studies which have displayed a reduction, the findings can be explained by other extraneous variables. Acute leptin

concentrations have been reported to decrease after a graded exercise test to exhaustion (Elias et al. 2000) but this reduction was attributed to an increase in non-esterfied fatty acids (NEFA) during exercise which has been negatively associated with leptin concentrations previously (Duclos et al. 1999). In a further study, a significant decrease in leptin levels was reported after a 30 minute high intensity exercise bout however, with the presence of a control group where a significant decrease was also observed, meant the reduction was explained by changes brought on by circadian rhythms (Kraemer et al. 1999). Moreover, a further explanation of decreased leptin concentrations following exercise may be an alteration in hemoconcentration which may cause leptin levels to appear greater due to changes in plasma volume (Kraemer, Chu & Castracane 2002). After a 9 week exercise programme no alterations in leptin concentrations were found, indicating the training had no impact on leptin levels although this was unsurprising as no significant changes in fat mass were reported (Kraemer et al. 1999). In contrast, a 12 week exercise intervention did reveal a significant reduction in leptin but this was accompanied by a significant drop in fat mass also (Ozcelik et al. 2004). Both of these studies support the notion that changes in leptin levels with exercise are reliant on changes with fat mass. Having said that it may be possible to alter leptin concentrations with exercise irrespective of fat mass if insulin sensitivity is improved (Martins, Robertson & Morgan 2008)

Insulin and leptin

As previously described, insulin is secreted by the pancreatic β-cells in response to food intake and in an effort to regulate plasma glucose concentrations. Like leptin, increased insulin concentrations are known to decrease appetite and increase energy expenditure (Porte, Baskin & Schwartz 2005). Insulin concentrations are also known to be higher in obese individuals (Moore, Cooper 1991), often leading to insulin resistance. Moreover, suppression of insulin has been shown to reduce leptin resistance (Blaak, Saris & van Baak 1993), combined with the associated hyperglycaemia and hyperlipidaemia, this suggests that hyperinsulinaemia may be one of the key causes of leptin resistance (Lustig 2006). Both, insulin and leptin are controlled by the CNS in similar ways, sharing signalling pathways that regulate adiposity and energy homeostasis (Niswender, Schwartz 2003) but it is not yet known

whether a chronic increase in insulin levels, seen in the obese, can cause a resistance here in the same way as leptin.

Amylin

Amylin is co-secreted with insulin by the pancreatic β-cells following food intake, to regulate glucose levels (Qi et al. 2010). However, even though amylin and insulin are co-secreted together, they have different effects on energy homeostasis. Amylin concentrations peak one hour after food ingestion and remain high for up to 4 hours (Roth et al. 2009), they are also typically elevated in obese individuals (Moore, Cooper 1991). Moreover, amylin receptors are found within the area postrema of the brain (Grill, Kaplan 2002) and amylin receptor agonism has been found to suppress appetite (Pullman, Darsow & Frias 2006), slow gastric emptying (Smith et al. 2008) and reduce postprandial glucagon release (Roth et al. 2008), together these processes slow the release of glucose into the blood to improve the effectiveness of insulin. Furthermore, when amylin antagonist AC187 was centrally administered to rats it increased food intake, glucagon concentrations, gastric emptying, and glycaemia after glucose ingestion (Riediger et al. 2004, Gedulin et al. 2006).

At present, few studies have investigated the effect of exercise on amylin response. In a study with progressive intermittent exercise from moderate to maximal intensities (60%, 75%, 90% and 100% $\dot{V}O_{2MAX}$), well trained men demonstrated no change at the moderate intensities but at 90 and 100% amylin was elevated with the peak in amylin at 100% (Kraemer, Chu & Castracane 2002). This increase was maintained till one hour after exercise and the same changes were observed in glucose and insulin. Interestingly, in a further study prescribing resistance exercise no significant increase was seen in amylin concentrations but insulin was significantly elevated (Kraemer et al. 2004). Furthermore, in a final study by the same authors, a reduction in amylin of healthy young men was seen during prolonged moderate intensity exercise for 90 minutes (Kraemer et al. 2011). To date, no long term exercise interventions have investigated the effects on amylin concentrations.

Amylin/leptin mechanism

As discussed earlier there is strong evidence to suggest that obese individuals are not only insulin resistant but they are also likely to be leptin resistance (Munzberg et al. 2005). Moreover, it has been suggested that insulin resistance is one of the major factors contributing to the development of leptin resistance (Lustig 2006). Therefore, it seems logical that amylin is important in this mechanism, as it is co-secreted with insulin from the pancreas and both play a pivotal role in energy homeostasis. In a study by Roth *et al.* (Roth et al. 2006), obese leptin resistant rats administered amylin, reduced food intake, bodyweight and body fat. The authors postulate this may be through improved leptin sensitivity via altered hypothalamic activity and metabolic alterations. Complimentary to these findings, in another study by the same authors (Roth et al. 2008), obese humans were administered amylin and leptin together for a 20 week period, these were compared to amylin alone and leptin alone treatment groups. The combined amylin and leptin treatment induced a 12.7% loss in bodyweight, compared to the amylin and leptin groups where an 8% loss was observed. Although the mechanisms for this change are not fully understood it is clear amylin is very important in mediating leptin sensitivity and therefore any potential weight loss in obese individuals. Possible metabolic mechanisms which may explain the amylin/leptin mediated weight loss include; reduced energy intake without the concomitant reduction in energy expenditure, improved substrate utilization towards preferred fat oxidation and reduced lipid biosynthesis (Trevaskis et al. 2008).

Adiponectin and pro-inflammatory cytokines

Adiponectin is another chemical which has strong links to obesity and may contribute to the response to exercise in these individuals. It is produced solely by adipocytes (Scherer et al. 1995) and a strong negative correlation has been found between adiponectin and BMI, with obese individuals demonstrating significantly lower concentrations than their lean counterparts (Arita et al. 1999, Cnop et al. 2003, Weyer et al. 2001). Additionally, adiponectin has been repeatedly shown to be negatively correlated with insulin resistance (Cnop et al. 2003, Matsubara, Katayose & Maruoka 2003, Yamamoto et al. 2002) and in support of this increased insulin sensitivity has been proven to be associated with high adiponectin levels, independent of adiposity

(Baratta et al. 2004, Tschritter et al. 2003).Furthermore, after a 4 week exercise intervention sufficient enough to induce weight loss, adiponectin concentrations and insulin sensitivity were significantly elevated, this increase in adiponectin was also associated with increased glucose uptake and decreased free fatty acid levels (Bluher et al. 2006). In further support of this, in exercise studies without weight loss no significant alterations in adiponectin concentrations were observed (Hulver et al. 2002, Boudou et al. 2003). It is suggested that the primary mechanism by which adiponectin enhances insulin sensitivity is likely through increased fatty acid oxidation and inhibition of hepatic glucose production (Lihn, Pedersen & Richelsen 2005).

Pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) have also been shown to be elevated in obese individuals and to influence insulin sensitivity (Hotamisligil 1999, Senn et al. 2002, Kern et al. 2001); they have also been implicated in the regulation of adiponectin. Under in vitro conditions TNF-α has been shown to down regulate and inhibit adiponectin gene expression (Fasshauer et al. 2002, Maeda et al. 2001). Additionally, these findings have been replicated in vivo with humans (Lihn et al. 2003, Kern et al. 2003) where negative associations were observed between adiponectin and TNF-α in plasma concentrations and mRNA in adipose tissue. Consequently, this suggests TNF-α and adiponectin act against each other in an antagonistic manner, influencing insulin action accordingly and in the case of obesity both TNF-α and IL-6 are elevated causing adiponectin to be down regulated, reducing insulin sensitivity (Lihn, Pedersen & Richelsen 2005). (Kern et al. 2003)(Kern et al. 2003)(Kern et al. 2003)

Other mechanisms regulating energy intake

Energy intake is regulated at two levels, both centrally and peripherally, to maintain energy homeostasis. The CNS and more specifically, the arcuate nucleus in the hypothalamus is responsible for the central control of food intake and the gastrointestinal tract, adrenal glands, pancreas and adipose tissue, all combine to regulate food intake in the peripheral organs (Neary, Goldstone & Bloom 2004). Ghrelin is secreted by the gut and is the only known hormone to stimulate food intake (Cummings, Foster 2003). When administered to both lean and obese individuals

before an *ad libitum* buffet test meal, participants ate significantly more and reported higher subjective ratings of hunger than controls (Wren et al. 2001, Druce et al. 2005, Druce et al. 2006). Furthermore, ghrelin concentrations are thought to be suppressed by insulin (Flanagan et al. 2003, Shiiya et al. 2002), suggesting that the associated hyperinsulinaemia seen in obesity is responsible for decreased ghrelin in this populations and a possible mechanism for bodyweight regulation also (McLaughlin et al. 2004). There are two types of ghrelin, acylated and desacylated or unacylated, acylated ghrelin is considered to be the more active form and has the greatest influence on intake (Asakawa et al. 2005). Acylated ghrelin is thought to control short term energy balance as it is reportedly greater in a fasting state and suppressed postprandially, in proportion to the size of meal ingested (Callahan et al. 2004).

Moreover, differing effects of exercise have been observed on both acylated and total ghrelin. Acute exercise has been seen to lower acylated ghrelin up to one hour post exercise (Broom et al. 2007, Broom et al. 2009) but not alter concentrations of total ghrelin (Dall et al. 2002, Burns et al. 2007). However, chronic exercise has reported no effects on acylated ghrelin (Kim et al. 2008) but increased total ghrelin concentrations have been seen after a one year exercise intervention, alongside significant weight loss (Foster-Schubert et al. 2005). This suggests that the ghrelin concentrations were increased in response to weight loss as part of feedback mechanism regulating body weight and without weight loss exercise would have no effect on total ghrelin concentrations (Borer 2008).

Peptide YY (PYY) is another hormone that has a significant effect on energy intake and homeostasis. It is secreted in the small and large intestine by the L cells of the mucosa after food ingestion. Once again there are two forms of PYY, PYY_{1-36} and $PYY_{3-36,}$ PYY_{3-36} is the most common form found in the gut and circulation (Neary, Goldstone & Bloom 2004). PYY_{3-36} is secreted after food intake and concentrations begin to rise 15 minutes after ingestion, with peak concentrations reached up to two afters eating (Guo et al. 2006). Increased levels of PYY_{3-36} have been shown to suppress food intake through receptor action in the hypothalamus (Neary, Goldstone & Bloom 2004), demonstrated by exogenous infusion (Batterham et al. 2002) where food intake was reduced by 33% when compared to a saline control. Batterham *et al*. (2002) also observed lower fasting total PYY concentrations in obese individuals but

no differences have been seen in PYY_{3-36} concentrations between lean and obese participants (Pfluger et al. 2007), suggesting obesity is not characterised by impaired PYY action.

Previous investigations have shown that PYY concentrations are significantly increased during exercise but this increase is short lived as no differences were seen one hour after exercise; both lean (Martins et al. 2007) and obese subjects (Ueda et al. 2009) displayed this same effect. Additionally, long term exercise interventions have revealed significant decreases in PYY concentrations but these studies also showed significant weight loss (Roth et al. 2005, Jones et al. 2009). Research investigating the effects of exercise on PYY_{3-36} is limited and at present the findings have been contradictory. Previously, 60 minutes of moderate intensity cycling (60% $\dot{V}O_{2MAX}$) did not reveal any significant changes in PYY_{3-36} concentrations (Cheng et al. 2009). However, in a another study 90 minutes of running at 70% $\dot{V}O_{2MAX}$ increased PYY_{3-36} concentrations by as much as 27% (King et al. 2011). The conflicting results here could be explained by the differences in either intensity or duration in exercise but the findings suggest higher intensity or longer duration exercise is needed to increase PYY_{3-36} levels. To date, the long term effects of exercise on PYY_{3-36} have not been investigated.

Chronic exercise and energy homeostasis

It has previously been stated that energy storage will only increase if energy intake exceeds total energy expenditure (Spiegelman, Flier 2001). This assumes that if we increase energy expenditure through increasing physical activity we will create an energy deficit and reap the much reported benefits of exercise. However, as we have discussed in this chapter there are many different factors which can influence both sides of the equation, altering the responses to exercise. Research investigating the long term effects of exercise on the regulation of energy balance has been limited, with many studies employing dietary restriction to achieve weight loss. Based on previous findings it is evident that in the case of long term exercise with ad libitum energy intake, participants will subsequently increase energy intake to compensate the increased expenditure. However, this does not take into account the influence of

potential training adaptations which may alter one of the many mechanisms discussed and thus their response to exercise.

To date, it seems that chronic exercise has very little effect on leptin concentrations, where training effects have been seen, these have been dependent on reduced fat mass (Kraemer et al. 1999, Ozcelik et al. 2004). Moreover, training load seems to have no effect either, as varying training levels showed no differences in reported leptin concentrations (Ishigaki et al. 2005, Desgorces et al. 2004). However, in a yearlong training intervention study with overweight men and a combination of resistance and aerobic exercise, a significant reduction in leptin was observed (Miyatake et al. 2004). This coincided with significant gains in strength and decreases in weight, fasting insulin and fat mass, indicating significant metabolic and endocrine responses to training may be dependent on training modality.

Furthermore, from the long term exercise interventions with significant metabolic and endocrine alterations post training, it seems evident that resistant training has been key to achieving these outcomes (Miyatake et al. 2004, Brooks et al. 2006). Studies utilising aerobic exercise interventions have been shown to improve insulin sensitivity but these have implemented some kind of dietary restriction (van der Heijden et al. 2012) making it difficult to attribute these findings to exercise and not diet. In a further study, investigating exercise modality and insulin resistance (Davidson et al. 2009)(Davidson et al. 1994)(Davidson et al. 1994)(Davidson et al. 1994), to isolate the effect of exercise, sedentary obese men and women were prescribed a minimum energy diet to maintain baseline bodyweight and participants were encouraged to eat healthily with 4 weeks familiarisation. Participants were split into aerobic training, resistance training and combined training groups and after 6 months the combined training group saw the greatest improvements in insulin resistance, the aerobic group also improved but the resistance group did not. It has been suggested that the contractile activity during exercise is the significant driver behind the improved insulin sensitivity and not just the increase in lean mass (Holloszy 2005).

The effect of chronic exercise on amylin concentrations is yet to be examined but 2 weeks of exercise and diet restriction did bring about a 36% reduction in amylin post intervention (Izadpanah et al. 2012). It seems logical that the effects of a long term

exercise intervention would be similar to those observed in insulin. In support of this, the findings after acute exercise in amylin concentrations follow those seen in insulin; moderate intensity (<75% $\dot{V}O_{2MAX}$) exercise had no effect on either amylin (Kraemer et al. 2004) or insulin (Kreisman et al. 2000) but high intensity (>85% $\dot{V}O_{2MAX}$) exercise increased both amylin (Kraemer et al. 2004) and insulin levels (Kraemer, Chu & Castracane 2002). Evidently, more research is needed to understand the role of amylin in the regulation of energy balance during chronic exercise.

Aim of thesis

Worldwide we our suffering from an obesity epidemic and this can be primarily attributed to an increased consumption of refined carbohydrates and sugars, as well as an ever increasing sedentary population. Now more than ever, we need to understand how and why this is happening in order to reduce the considerable negative effects on our society.

The general aim of this thesis is to improve the current knowledge of the mechanistic pathways involved in the development of obesity. More specifically, to investigate the damaging effects of high glucose availability on skeletal muscle metabolism, typically seen in sedentary individuals with a diet high in refined carbohydrates and sugars. In particular, this thesis will focus on the contribution of high sugar-sweetened beverage consumption and their role in the growing prevalence of obesity. Gene expression analysis has been used to identify alterations in metabolic enzyme expression and transcription factors important in the regulation of glucose. In vivo and in vitro studies have been used to identify both the periodic and chronic effects of high glucose availability on metabolism.

Furthermore, it is also the aim of this thesis to investigate the influence of chronic exercise on metabolism of lean and obese sedentary populations. Experiments have been designed to improve the understanding of how lean and obese individuals respond to exercise and how they differ in key regulatory processes of whole body and cell metabolism. A novel approach to long-term exercise interventions has been utilised, where participants are naïve to nature of the experiments and its end goals, this has been combined with *ad libitum* energy intake to gain a more accurate view of the mechanisms controlling energy homeostasis in both populations.

Research questions

Chapter 2: How does 4 weeks periodic high glucose availability (sugar-sweetened beverage consumption) alter metabolism of young, healthy, lightly active individuals? How does chronic hyperglycaemia affect the cell metabolism of human primary skeletal muscle cells?

Chapter 3: How does 4 weeks moderate intensity exercise training, with ad libitum energy intake, affect the body composition, metabolism and energy balance, of lean and obese sedentary women?

Chapter 4: How does 8 weeks high and low intensity exercise training, with ad libitum energy intake, affect the body composition, metabolism and energy balance, of lean and obese sedentary women?

CHAPTER II

METABOLIC ALTERATIONS IN RESPONSE TO 4 WEEKS SUGAR-SWEETENED BEVERAGE CONSUMPTION IN HEALTHY, LIGHTLY ACTIVE INDIVIDUALS AND CHRONIC HIGH GLUCOSE AVAILABILITY IN PRIMARY HUMAN MUSCLE CELLS

Introduction

The consumption of SSBs has increased over the past four decades, with the percentage of adults drinking SSBs in the US rising from 58% between 1988 and 1994 to 63% between 1999 and 2004 (ERS 2004) and the association between obesity and SSB consumption has been well documented (Nissinen et al. 2009, Olsen, Heitmann 2009, Hu, Malik 2010, Ludwig, Peterson & Gortmaker 2001, Ebbeling, Pawlak & Ludwig 2002, Schulz et al. 2002, St-Onge, Keller & Heymsfield 2003, Berkey et al. 2004, Bray, Nielsen & Popkin 2004, Gross et al. 2004, James, Kerr 2005, Bes-Rastrollo et al. 2006, Malik, Schulze & Hu 2006, Dhingra et al. 2007, Vartanian, Schwartz & Brownell 2007, Palmer et al. 2008, Bleich et al. 2009, Fung et al. 2009). Moreover, obesity is greatly associated with type 2 diabetes mellitus and insulin resistance (Wannamethee, Shaper 1999) and consequently a diet high in refined carbohydrates and sugars has been seen shown to be related to the development of this condition (Hu, Malik 2010, Gross et al. 2004, Vartanian, Schwartz & Brownell 2007, Palmer et al. 2008, Bleich et al. 2009, Liu 2002, Yoshida et al. 2007, Mohan et al. 2009, Stanhope et al. 2009).

The increase in dietary refined carbohydrates and sugars such as those in SSBs, produces a large rise in glucose availability (Foster-Powell, Miller 1995) and continuous high energy food intake and a lack of energy expenditure, challenge the normal regulation of blood glucose levels (Manson et al. 1992), eventually weakening the system so that periods of hyperglycaemia following carbohydrate ingestion are extended. In turn this can influence several signalling pathways, causing adaptations to skeletal muscle which contribute to reduced oxidative capacity and insulin sensitivity. This leads to the assumption that high carbohydrate diets combined with low physical activity can cause prolonged periods of hyperglycaemia even in individuals with normal glucose tolerance and if continued they can lead to development of a low oxidative capacity and impaired insulin signalling pathways, usually described as a diabetic phenotype. Moreover, high glucose availability has been widely recognized as a cause of insulin resistance in skeletal muscle (Fell et al. 1982, Richter, Hansen & Hansen 1988a, Richter, Hansen & Hansen 1988b, Hansen et al. 1992, Davidson et al. 1994, Gulve et al. 1994, Kawanaka et al. 2001, Kawanaka et al. 1999, Oku et al. 2001, Han, Chen & Holloszy 2003).

After refined carbohydrate and sugar ingestion, glucose is absorbed by the gut and there is an increase in blood glucose levels, through insulin action this glucose is taken up by skeletal muscle and used in anyone of numerous cellular processes. Recently, new evidence has demonstrated that glucose and its metabolites are sensed in the muscle cell by specific molecules stimulating regulatory gene expression (Desvergne, Michalik & Wahli 2006). One of these molecules, the transcription factor MondoA, has been demonstrated to be a master regulator of glycolytic genes, activating the transcription of many genes encoding metabolic enzymes (Sans et al. 2006). In the muscle cell, MondoA and its heterodimerisation partner max-like protein x (Mlx) form a complex under high glucose conditions (Billin et al. 2000), which up regulates glycolytic gene expression. Moreover, the MondoA:Mlx complex has been shown to activate thioredoxin-interacting protein (TXNIP) (Stoltzman et al. 2008), which has previously been shown to reduce glucose uptake (Parikh et al. 2007). TXNIP mRNA has also been shown to be expressed more highly in pre-diabetic and diabetic patients (Parikh et al. 2007). This suggests, chronic high glucose availability leads to metabolic alterations in skeletal muscle through the promotion of MondoA activity. These alterations may include the inhibition of glyceradehyde-3-phoshate dehydrogenase (GAPDH), causing profound effects on glycolytic capacity (Bouche et al. 2004). Moreover, it is highly likely that the high glucose availability will reduce oxidative capacity of skeletal muscle, as seen in obese and diabetic or insulin resistant individuals (Kelley, Mandarino 1990, Kelley et al. 1999). Other impairments of skeletal muscle in this population include, decreased mitochondrial ATP synthesis, less oxidative fibres compared to glycolytic fibres, decreased glycogen synthesis and significantly lower expression of PGC1α (Mootha et al. 2003, Patti et al. 2003, Lowell, Shulman 2005). Finally, it should be possible to see these alterations in sedentary individuals' with diets high in refined carbohydrates and sugars, typically seen in SSBs, as they will not be able to combat the damage caused by periodic high glucose availability with increased glucose clearance through of physical activity. In this chapter, both in vitro and in vivo studies have been conducted to analyses the impact of chronic and periodic high glucose availability on skeletal muscle metabolism.

In vitro study

The effects of chronic hyperglycaemia were examined on primary muscle cell cultures for 7 days and compared to control conditions. The in vitro effects of the high glucose conditions on metabolic gene expression were measured using real-time RT-PCR. Additionally, western blot analyses were used to scrutinize the impact of the chronic hyperglycaemia on protein expression of the same markers. It was assumed that the in vitro chronic hyperglycaemia would display similar metabolic alterations seen in type 2 diabetes patients; elevated glycolytic and lipogenic activity and reduced oxidative activity. Finally, it was also hypothesised that the chronic hyperglycaemic conditions would increase MondoA and TXNIP expression.

Methods

Muscle biopsies

Muscle biopsies were taken with a 14-gauge needle (14ga x 10cm, Tru·Core® II Biopsy instrument, Angiotech, Gainesville, FL, USA). Following local anaesthesia (1% lignocaine), 2 needle muscle biopsies were collected from the vastus lateralis of the left leg, at a mid-distance between the greater trochanter and the femorotibial joint. Upon collection biopsies were snap-frozen and stored in liquid nitrogen until later use.

Primary cell cultures

Satellite cells were isolated from four muscle biopsies of healthy male subjects (age 38 ± 13 yrs, height 1.79 ± 0.06 m, weight 72 ± 6 kg). After collection, biopsies were immediately transferred to RT skeletal muscle growth medium (SMGM) with 5% foetal calf serum (FCS), 1% L-glutamine with pencillin/streptomycin plus supplements (PromoCell, Heidelberg, Germany), and washed twice in medium. Disintegration was then performed with surgical blades and 0.05% trypsin (PAA, Pasching, Austria) in PBS for 30 minutes, trypsination was stopped by trypsin inhibitor (Sigma-Aldrich, St. Louis, MO, USA) to give a final concentration of 0.14 mg/ml. Cells were centrifuged at 800 x g for 5 minutes at 10^{o}C, pellets were then re-

suspended in accutase (PAA, Pasching, Austria) and incubated for 20 minutes at 37ºC. Following sedimentation, the cell suspension was centrifuged once more at 800 x g for a further 5 minutes at 10ºC, cell pellets were then re-suspended in SMGM with 5% FCS, and 1% L-glutamine with pencillin/streptomycin plus supplements (PromoCell, Heidelberg, Germany). This cell suspension was then transferred into 75 cm^2 tissue culture flasks (Greiner, Frickenhausen, Germany) and incubated at 37ºC, with 5% CO_2 and 95% humidity until passage. Half-medium change was performed every 2nd day and 3 passages were completed before the initiation of differentiation. After initiation of differentiation in Dulbecco's modified eagle medium (DMEM; PAA, Pasching, Austria) with 2% FCS for 4 days, the primary myotubes were tested for creatine kinase activity. For the cultivation of differentiated myocytes, grown on microcarriers, 5 x 10^6 cells per 10 ml medium were seeded on microcarriers in suspension (0.015g microcarriers, CultiSpher- GL; Percell Biolytica, Astorp, Sweden) using 25 cm^2 flasks. To guarantee sufficient O_2 supply to the cells and to prevent the cells and microcarriers from settling down, flasks were kept on a circular shaker at 53rpm. After 12 days of differentiation myotubes were exposed to high glucose, DMEM with 2% FCS , 15 mM D-glucose (Sigma-Aldrich, St. Louis, MO, USA) and 10 µg/ml insulin (Actrapid, Novo Nordisk A/S, Bagsvaerd, Denmark). Control myotubes were cultivated in DMEM with 2% FCS, 5 mM D-glucose and 10 µg/ml insulin. Exposure time for both conditions was 7 days.

Cell harvest and western blotting

Myotubes on microcarriers were washed with PBS, lysed and denatured using SDS-PAGE sample buffer at 95ºC for 3 minutes and then cooled on ice. Samples were cleared by centrifugation at 16000 x g in QIAshredder columns (Qiagen, Hilden, Germany) and the eluates were frozen in liquid nitrogen and stored at -80ºC until later use. Nuclear and cytoplasmic fractions of myotubes, from both high glucose and control conditions, were prepared using a nuclear extraction kit, according to the manufacturer's protocol (Nuclear extraction kit (No400010/No40410), Active-Motif, Rixensart, Belgium). Protein content of samples was assessed by Lowry protein assay (Sigma-Aldrich, St. Louis, MO, USA). 5 or 10% SDS-PAGE was used to separate equal amounts of protein per lane; these were then transferred to nitrocellulose membrane (Hybond ECL 6 x 8cm, GE Healthcare, Amersham, Slough, UK). India

ink staining of membranes was used for loading control. Once membranes were blocked in PBS (containing, 0.2% tween, and 5% low-fat dry milk), blots were probed with primary antibodies for 4 hours, for ACC (H-76; sc-30212) with a 1:250 dilution, FUM (J-13; sc-100743) at 1:2000, and GAPDH (0411; sc-47724) at 1:10000 (Antibodies provided by Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). MondoA (Sans *et al*., 2006) at at1:500 and TXNIP (MBL, Nakaku Nagoya, Japan) at 1:1000 were incubated overnight. Goat anti-rabbit, IgG-HRP, 1:15000 (Sigma-Aldrich, St. Louis, MO, USA) and goat anti mouse, IgG-HRP, 1:5000 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) were used. ECL (Amersham Hyperfilm ECL, 18 x 24cm, GE Healthcare Life Sciences, Little Chalfront, UK) detection was performed using SuperSignal West Pico and Femto ECL kits (Pierce, Rockford, IL, USA). For re-probing membranes were stripped for 45 minutes at 50^{o}C in striping buffer (10% SDS, 0.5M Tris, pH 6.8, and 0.8% β-mercaptoethanol), and washed for 1 hour. Protein bands were quantified using densitometry (Gel Doc 2000 and software Quantity One 4.6.3, Bio-Rad, Hercules, CA, USA).

Real-time RT-PCR

Total RNA was isolated from frozen muscle biopsies using RNeasy kit (Qiagen Ltd, Crawley, West Sussex, UK). RNA concentration was measured at 260nm and 280nm using spectrophotometry (Double-Beam Spectrophotometer (U-2800A), Hitachi High-Technologies Pte Ltd, Singapore) and purity of RNA was assessed using a ratio of 260/280nm. cDNA was synthesised from 50ng RNA using sensiscript reverse transcription kit (Qiagen) and thermal cycler (Auto Q Server Gradient Thermal Cycler, Quanta Biotech, Byfleet, Surrey, UK) at 37^{o}C for 60 minutes, after heating for 5 minutes at 65^{o}C. cDNA was stored at -80^{o}C until use. Real-time RT-PCR was performed using Applied Biosystems 7900HT fast real-time PCR system (Applied Biosystems, Carlsbad, California, USA) and 2x Quantitect SYBR green PCR master mix (Qiagen). Primers for each gene were selected from published sources or designed using reference mRNA sequences (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, USA) and Primer3 software (http://frodo.wi.mit.edu/primer3/). All primers were confirmed for homologous binding to desired mRNA targets by conducting a BLAST search (http://blast.ncbi.nlm.nih.gov/), once chosen primers were synthesised (Eurofins

MWG Operon, Ebersberg, Germany). Table 2.2, shows details of each primer set. Each gene was measured in all samples simultaneously, and all samples were measured in duplicate. 18s rRNA was used as an endogenous control to normalise gene expression. Each reaction contained PCR master mix, forward and reverse primers (0.3 µM), RNase free water and 2.5 µl cDNA in 25 µl final volume. Thermal cycling conditions were as follows: PCR initial activation step 15 min at 95^{o}C, then 3 step cycling of 15 s denaturation at 94^{o}C, 30 s annealing 50-60^{o}C (varies with primer) and 30 s extension at 72^{o}C for 45 cycles. Melting curves performed after all runs showed single products in each case. Critical threshold (C_T) values were recorded for target gene and endogenous control and used to quantify mRNA expression. Standard curve method was used to quantify relative amounts of mRNA for each gene, this was done by preparing a 10x dilution series of template cDNA for both target gene and endogenous control and run simultaneously with all samples.

Gene	Accession No.	Primer Sequence	Product Size (bp)
MondoA	NM_014938	F: TGACTTTGGCCTACAGTGGG R: TTGCGCTTCTCCAGATACTGC	139
TXNIP	NM_006472	F: CTGGCGTAAGCTTTTCAAGG R: AGTGCACAAAGGGGAAACAC	185
PGC1α	NM_013261	F: CCTGCATGAGTGTGTGCTCT R: GCAAAGAGGCTGGTCTTCAC	164
ACC α	NM_198834	F: AGTGAGGATGGCAGCTCTGGA R: TGAGATGTGGGCAGCATGAAC	132
GAPDH	NM_002046	F: GAAGGTGAAGGTCGGAGT R: GAAGATGGTGATGGGATTTC	226
Citrate Synthase	NM_004077	F: CCATCCACAGTGACCATGAG R: CTTTGCCAACTTCCTTCTGC	186
18s	NR_003286	F: GTAACCCGTTGAACCCCATT R: CCATCCAATCGGTAGTAGCG	151

Table 2.2. Primer pairs for each gene analysed in the present study including, forward and reverse primer sequences, accession numbers and product sizes for each gene.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 20. Differences between conditions were analysed by non-parametric Mann-Whitney *U* tests. Pearson's correlations were used to analyse relationships between variables. All data are reported as means ± SD unless otherwise stated. Statistical significance was set at $P<0.05$.

Results

Primary human myotubes were isolated from biopsies of healthy male subjects and grown on microcarriers. After 12 days of differentiation, cells were exposed to either high glucose (15mM glucose, 10µg/ml insulin) or control conditions (5mM Glucose, 10µg/ml insulin) for 7 days. Cell harvest was performed and western blotting and real time RT-PCR was used to assess any potential metabolic alterations at protein and gene level.

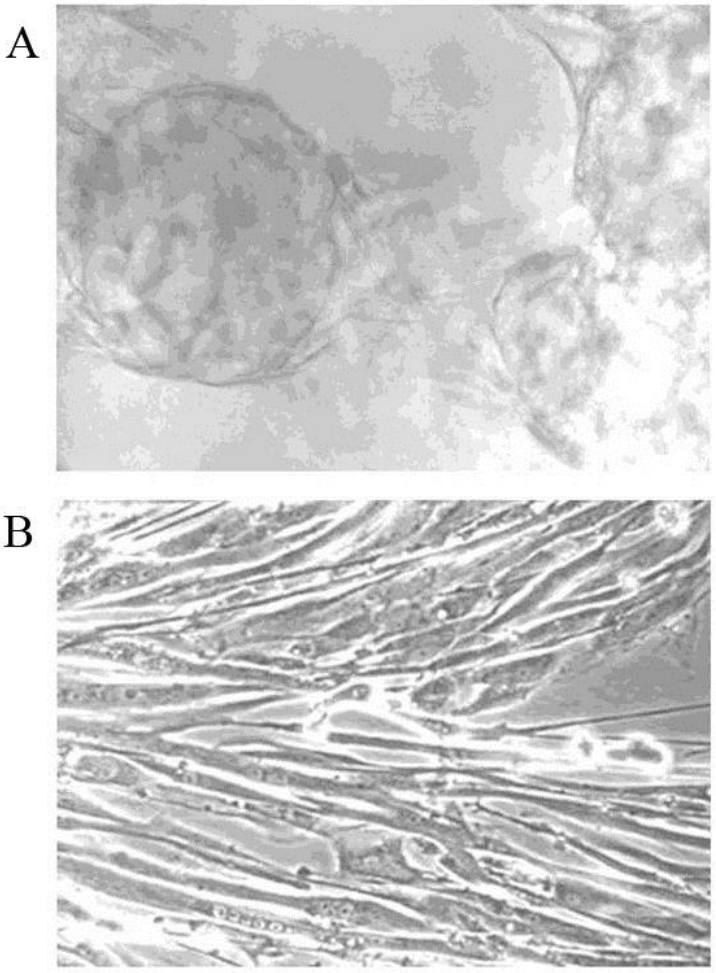

Fig. 2.5. Primary human myotubes grown on microcarriers in culture (A) and seeded in culture flasks (B). Image captured with light inverted microscope (Eclipse TS100, Nikon, Kawasaki, Japan) 20x magnification.

Protein expression in primary human myotubes

Western blot analysis of cell homogenates showed that seven days of high glucose availability significantly increased protein levels of glycolytic marker GAPDH and lipogenic marker ACC when compared to control conditions (173 ± 32 % and 177 ± 18 % of control, *P* = <0.05, respectively). Oxidative marker FUM levels were unaffected by the 7 days hyperglycaemia (91 ± 30 % of controls, *P* = 0.44). Both MondoA and TXNIP expression levels were also elevated in the cells under high glucose conditions (190 ± 30 % and 352 ± 47 % of control, P = < 0.05, respectively). A significant positive correlation between protein levels of MondoA and TXNIP was also observed (r = 0.87, *P* = 0.011).

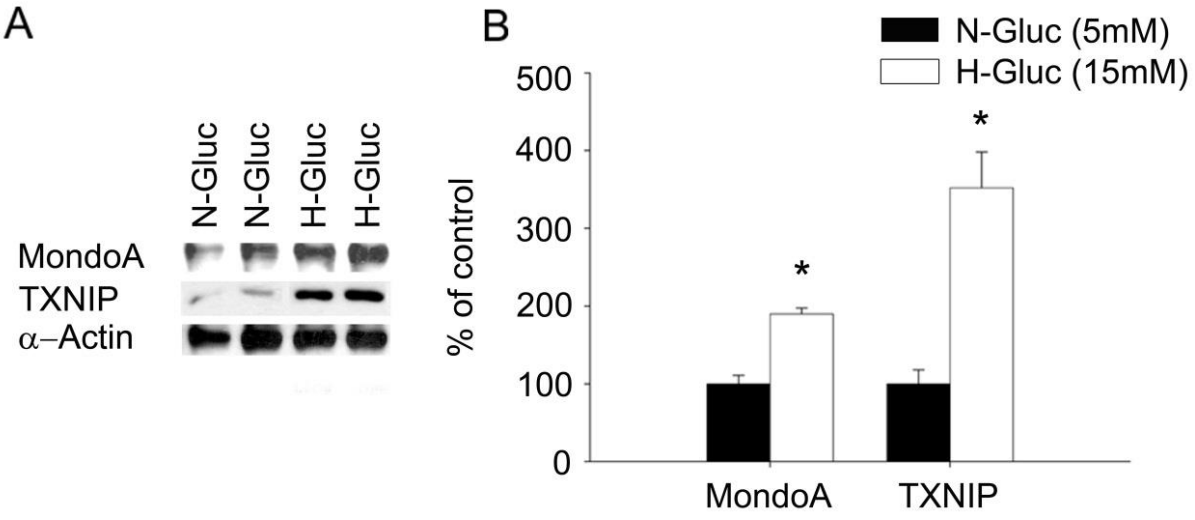

Fig. 2.6 A. Western blot analysis of MondoA in primary muscle cell cultures, under conditions of normal glucose (N-Gluc (5mM)) and high glucose (H-Gluc (15mM)) with loading control alpha-actin. B. Quantification of MondoA and TXNIP western blots under normal and high glucose conditions, expressed as percentage of control (Sartor et al. 2013). Data are presented as means ± SEMs. * *P* = < 0.05 for differences between conditions.

Gene expression in primary human myotubes

Real time RT-PCR analysis of cell homogenates revealed no significant difference in either MondoA or TXNIP gene expression between high and normal glucose conditions (83 ± 8 % and 111± 16 % of controls, respectively). Further analysis of metabolic enzyme gene expression under differing glucose concentrations also displayed no significant differences between measurements of GAPDH and ACC (129 ± 27 % and 128 ± 41 % of controls, respectively), although a decrease in the marker of mitochondrial gene expression PGC1α was observed under high glucose availability (70 ± 14 % of control, $P = 0.05$).

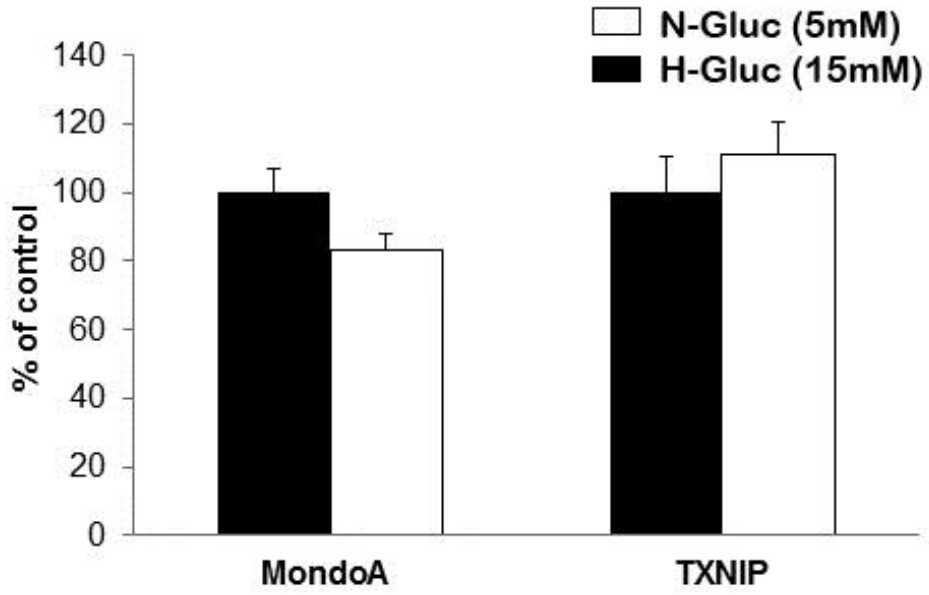

Fig. 2.7 MondoA and TXNIP gene expression under conditions of normal glucose (N-Gluc (5mM)) and high glucose (H-Gluc (15mM)). Data are presented as means ± SEMs.

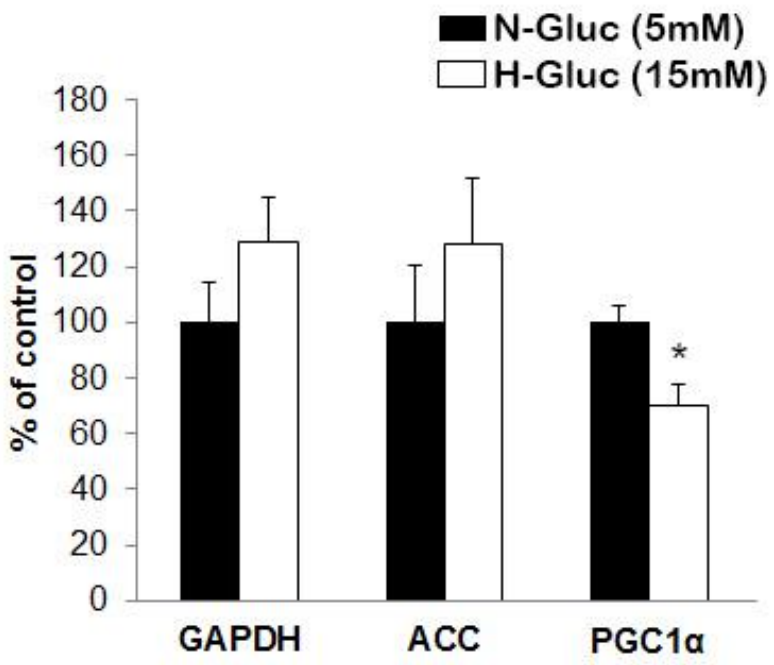

Fig. 2.8 Metabolic gene expression under conditions of normal glucose (N-Gluc (5mM)) and high glucose (H-Gluc (15mM)). GAPDH glyceraldehyde-3-phosphate dehydrogenase, ACC acetyl-CoA carboxylase α, PGC1α peroxisome proliferator-activated receptor-gamma coactivator 1 α,. Data are presented as means ± SEMs. * *P* = < 0.05 for differences pre and post between conditions.

In vivo

In this chapter, the effects of periodic high glucose availability in the form of a 4 weeks sugar-sweetened beverage (SSB) consumption was investigated on the metabolism of healthy, lightly active, normal weight individuals, with very little or no history of previous soft drink consumption. To analyse the effects of the SSB intervention in vivo, muscle biopsies were taken from vastus lateralis of participants and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted to measure metabolic gene expression. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and acetyl-CoA carboxylase (ACC) were used as markers of glycolytic and lipogenic markers respectively, citrate synthase (CS) and peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC1α) were used to reflect mitochondrial activity. Additionally, glucose sensing transcription factors MondoA and thioredoxin-interacting protein (TXNIP) gene expression was also analysed to measure the impact of SSB consumption on glucose metabolism and regulation. It was expected that the periodic high glucose availability would shift the participants' metabolism towards more carbohydrate use, promoting an elevation in glycolytic and lipogenic gene expression, GAPDH and ACC. Subsequently, it was hypothesised that mitochondrial gene expression, CS and PGC1α, would reduce during the SSB consumption and it was also expected that MondoA and TXNIP expression would be elevated following the intervention. Finally, the effects of the SSB consumption on whole body metabolism and protein expression in skeletal muscle were also investigated.

Methods

Participants and study design

Eleven (5 male and 6 female) healthy subjects with low physical activity were recruited to take part in the study after an initial screening for lifestyle and (sugar-sweetened beverage) SSB consumption via qualitative questionnaire. Only people consuming less than 500ml of SSB per week were considered eligible to take part in the study (32 out of 213 people who completed the questionnaire). Baseline anthropometric characteristics for participants are presented in table 2.1. Participants

were informed they would receive £100 for compensation of their time upon completion of the study. All subjects in the study underwent a 4-week SSB supplementation period and various pre and post anthropometric, metabolic, blood and muscle biopsy measures were carried out. Tests were conducted across two visits to the laboratory and before each test participants were asked to refrain from any exercise for a 24 hour period. All post-tests were completed at least 36 hours after the last SSB supplementation.

Parameters (units)	Baseline	Post-intervention
Age (yr)	26 ± 7	
Weight (kg)	65.9 ±10	66.8 ± 11
BMI (kg/m^2)	21.6 ± 1.5	22.0 ± 1.8$^{\#}$
DXA		
Fat mass (kg)	15.2 ± 5.1	16.2 ± 4.7*
Lean mass (kg)	48.1 ± 12.6	48.0 ± 13.0
Blood Lipids		
Total Cholesterol (mmol/L)	4.27 ± 0.89	4.42 ± 0.73
Triglycerides (mmol/L)	1.01 ± 0.36	1.02 ± 0.33
HDL (mmol/L)	1.30 ± 0.21	1.29 ± 0.22
LDL (mmol/L)	2.52 ± 0.75	2.67 ± 0.64

Table 2.1. Anthropometric data of all participants before and after the 4 weeks SSB supplementation. Data are presented as means ± standard deviation. * $P < 0.05$, $^{\#}$ $P < 0.10$.

Study intervention

Lucozade Energy (Lucozade energy, GlaxoSmithKline plc, Harlow, UK; Ingredients: carbonated water, glucose syrup, orange juice from concentrate, citric acid, preservatives (sodium benzoate, sodium bisulphate, flavourings (including caffeine), stabiliser (acacia gum), antioxidant (ascorbic acid) and colour (beta carotene)) was the chosen SSB for the 4 weeks SSB supplementation, this was provided to participants by the experimenters on top of their habitual diet. Supplementation was calculated on

the basis of 2 grams per kilogram bodyweight carbohydrate intake per day. On average this equated to 760 ml SSB per subject per day.

Anthropometry

Body mass was measured to using a beam balance scale (Seca, Hamberg, Germany), participants were instructed to wear minimal clothing, remove footwear and empty their pockets whilst being weighed. Height was measured using a wall mounted stadiometer (Bodycare products, Southam, United Kingdom). Body composition (lean mass, fat mass and percentage body fat) bone mineral density were measured using dual-energy x-ray absorptiometry (DEXA; QDR 4500, Hologic, Bedford, MA, USA).

Indirect calorimetry

Participants were instructed to arrive at the laboratory after a 12 hour overnight fast and well rested, having refrained from any exercise for 48 hours prior to measurement. The meal prior to indirect calorimetry and blood and muscle sampling was controlled according the World Health Organisation (WHO 2006) recommendations for oral glucose tolerance tests. Respiratory exchange ratio (RER; $\dot{V}CO_2/\dot{V}O_2$) was measured by indirect calorimetry (Metalyzer 3B, Cortex Biophysik, GMBH, Leipzig, Germany) following published guidelines (da Rocha, Alves & da Fonseca 2006) as participants lay awake in a supine position for 30 minutes. Substrate oxidation rates were also calculated based on indirect calorimetry (Frayn 1983). Ambient temperature (20^{o}C) and humidity (40%) were kept constant during measurement through use of a climate chamber.

Energy and macronutrient intake

Participants were asked to maintain their normal diet and complete a 7 day diet diary before and after the 4 weeks SSB supplementation, using food record sheets provided by the researchers. Additionally, a 14 day diet diary was completed during the 4 weeks supplementation. Participants were instructed to complete the diet diaries using standardised instructions (Gibson 1993) and the importance of accuracy and precision

when reporting was emphasised. The United States Department of Agriculture, National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) and the Tesco Supermarket online database (http://www.tesco.com/groceries/) were used to assess energy intake and macronutrient composition.

Blood sampling and analysis

Overnight fasting venous blood (4ml) was collected from the antecubital vein of each participant in heparinised vacutainers, a 75g oral glucose load was then administered and precisely 1 hour after consumption a second venous blood sample was collected. Following collection whole blood was centrifuged at 3000rpm for 10 minutes at 4^{o}C and plasma was separated. Plasma glucose (YSI 2300 STAT, Incorporated Life Sciences, Yellow Springs, OH, USA) and lipid profile (Reflotron®, Roche Diagnostics, Mannheim, Germany) were measured in fasting plasma. Plasma samples were then stored at -40^{o}C for later insulin analysis by ELISA (Ultrasensitive human insulin ELISA, Mercodia, Uppsala, Sweden). The Homeostasis Model Assessment (HOMA) was used to calculate beta cell function (%B), insulin resistance (IR) and insulin sensitivity (%S) from fasting plasma insulin and glucose (Wallace, Levy & Matthews 2004).

Muscle biopsies

Muscle biopsies were taken one hour after glucose load with a 14-gauge needle (14ga x 10cm, Tru·Core® II Biopsy instrument, Angiotech, Gainesville, FL, USA). Following local anaesthesia (1% lignocaine), 2 needle muscle biopsies were collected from the vastus lateralis of the left leg, at a mid-distance between the greater trochanter and the femorotibial joint. Post-test biopsies were taken 0.5-1cm from the pre-test biopsies. Upon collection biopsies were snap-frozen and stored in liquid nitrogen until later use.

Real-time RT-PCR

Frozen muscle biopsies (15.6 ± 0.3 mg) were pulverized (1900 rpm for 15 seconds) at liquid nitrogen temperature using a micro-dismembrator (Sartorius-Stedim Biothec,

Goettingen, Germany). Total RNA was isolated from frozen muscle biopsies using RNeasy fibrous tissue kit (Qiagen Ltd, Crawley, West Sussex, UK). RNA concentration was measured at 260nm and 280nm using spectrophotometry (Double-Beam Spectrophotometer (U-2800A), Hitachi High-Technologies Pte Ltd, Singapore) and purity of RNA was assessed using a ratio of 260/280nm, total RNA concentrations averaged 108 ± 37 µg/ml. Following RNA extraction the same protocol for real-time RT-PCR from the cell culture experiments was replicated as stated earlier in the chapter

Western blotting and myosin heavy chain (MHC) analysis

Frozen muscle biopsies and 150µl frozen buffer containing, 10% PBS, 5% protein phosphatase inhibitors, 0.1% 1M DTT, 0.05% protease inhibitor and 0.1% detergent (Nuclear Extraction Kit, Acrivemotif, Rixensart, Belgium) were pulverized (1900 rpm for 15 seconds) using a micro-dismembrator (Mikro-Dismembrator S, Sartorius-Stedin Biotech, Goettingen, Germany), all equipment was cooled to liquid nitrogen temperature. Pulverized muscle and buffer was thawed on ice and centrifuged at 20000 x g at 4^{o}C for 5 minutes and the resulting supernatant was used to measure ACC, FUM, GAPDH and TXNIP expression levels. Pellets were extracted using an ultrasonic processor (VCX 130, Sonics & Materials INC, Newton, CT, USA) with myosin extraction buffer (0.6M KCL, 1mM EGTA, 10mM sodium phosphatedibasic, 1mM PMSF, pH 6.8) on ice at 0^{o}C. Following this extracts were centrifuged at 20000 x g for 20 minutes at 4^{o}C, crude nuclear membrane fractions were then re-suspended in SDS sample buffer, these were used to measure MondoA expression levels. As per real-time RT-PCR, western blotting procedure was replicated using the same procedures from the in vitro study. Furthermore, MHC extraction and electrophoresis was carried out as previously described in (Sartor et al. 2010).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 20. Differences pre and post intervention of the in vivo study were analysed by paired samples *t*-tests. Differences between male and female participants were analysed at baseline by independent samples *t*-tests before pooling genders. Pearson's correlations were used

to analyse relationships between variables. All data are reported as means ± SD. Statistical significance was set at $P<0.05$. Due to a viral infection one participant in the in vivo study was unable to attend the post-test oral glucose tolerance test and muscle biopsy, because of this only body composition, energy and macronutrient intake data were used for this participant in the statistical analysis.

Results

11 healthy, lightly active, normal weight individuals, with very little or no previous soft drink use, consumed SSBs for 4 weeks (2 grams of carbohydrate per kilogram bodyweight), on top of their habitual diet. DXA scans, indirect calorimetry, venous blood and muscle biopsies were collected to assess any potential metabolic alterations.

Body composition, diet diary analysis and indirect calorimetry

The analysis of the body composition by DXA revealed a significant increase in fat mass post SSB supplementation but lean mass and weight remained unchanged (Table 2.1). Diet diary analysis showed no alteration in energy intake during the SSB supplementation however, a significant increase in carbohydrate (pre, 264 ± 72 g/d, post, 347 ± 65 g/d, $P = 0.005$) and sugar intake (pre, 98.6 ± 40.2 g/d, post, 183.9 ± 32.1 g/d, $P = <0.001$) were reported, this coincided with a significant decrease in protein (pre, 83.4 ± 21.1 g/d, post, 71.0 ± 23.2 g/d, $P = 0.008$), total fat (pre, 93.1 ± 33.5 g/d, post, 72.5 ± 34.8 g/d, $P = 0.05$) and PUFA (pre, 10.7 ± 5.1 g/d, post, 7.4 ± 4.6 g/d, $P = 0.04$). SFA showed a trend towards a decrease (pre, 41.8 ± 19.0 g/d, post, 31.7 ± 16.9, $P = 0.08$) and MUFA and dietary fibre remained unchanged (pre, 40.6 ± 15.7 g/d, post, 33.3 ± 17.6 g/d, $P = 0.12$; pre, 19.6 ± 5.9 g/d, post, 18.5 ± 5.8 g/d, $P = 0.29$, respectively). Analysis of resting substrate metabolism by indirect calorimetry showed a significant increase in RER (pre, 0.75 ± 0.09 AU, post, 0.87 ± 0.08 AU, $P = 0.039$) (Fig. 2.1 B) and further analysis of substrate oxidation rates revealed a shift in metabolism of preferred substrate, from a high level of fat oxidation at baseline to an altered high level of carbohydrate oxidation after the SSB supplementation period (Fat: pre, 67.5 ± 48.9 %, post, 22.7 ± 23.8 %; Carbohydrate: pre, 32.5 ± 48.9 %, post, 77.3 ± 23.8 %) (Fig. 2.1 A).

Glucose levels and insulin sensitivity

The 4 weeks SSB supplementation significantly increased fasting plasma glucose levels and a trend towards increased plasma insulin levels was also observed (pre, 4.83 ± 0.43 mmol/L, post, 5.13 ± 0.38 mmol/L, $P < 0.05$; pre, 4.95 ± 1,90 mU/L, post, 6.40 ± 1.62 mU/L, $P = 0.09$, respectively) (Fig. 2.2). In addition, HOMA 2 calculations demonstrated a trend towards a decrease in %S and an increase in IR (pre, 158 ± 47, post, 126 ± 32, P = 0.06; pre, 0.68 ±0.19, post, 0.84 ± 0.20, P = 0.08, respectively), %B remained unchanged (pre, 76.6 ± 28.4, post, 81.3 ± 21.8, $P = 0.57$). Blood lipids were unaffected by the SSB intervention (Table 2.1).

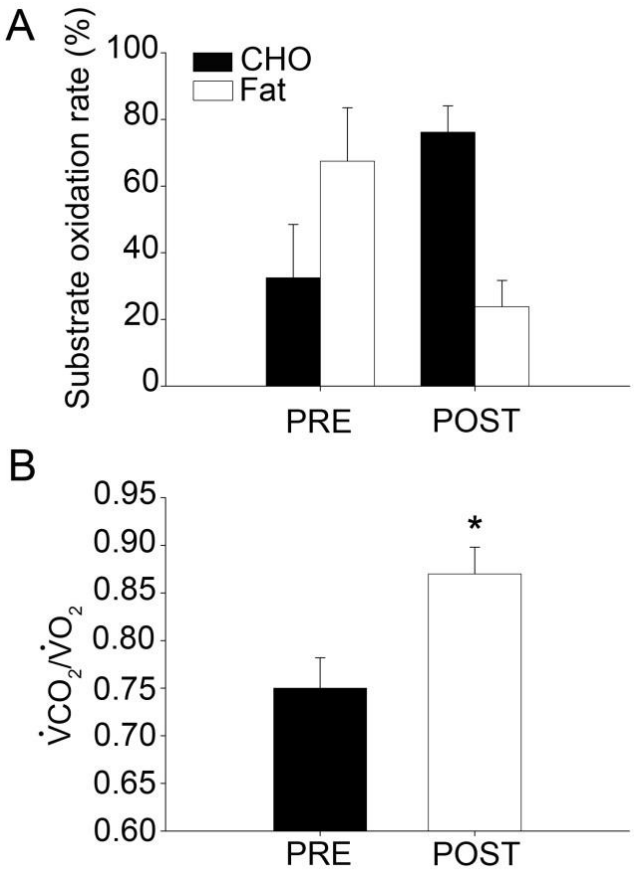

Fig. 2.1 A. Substrate oxidation rate derived from RER during indirect calorimetry after an overnight fast, pre and post 4 week SSB intervention. B. RER (VCO_2/VO_2) pre and post 4 week SSB intervention measured by indirect calorimetry after an overnight fast (Sartor et al. 2013). Data are presented as means ± SEMs. * $P = < 0.05$ for differences pre and post intervention.

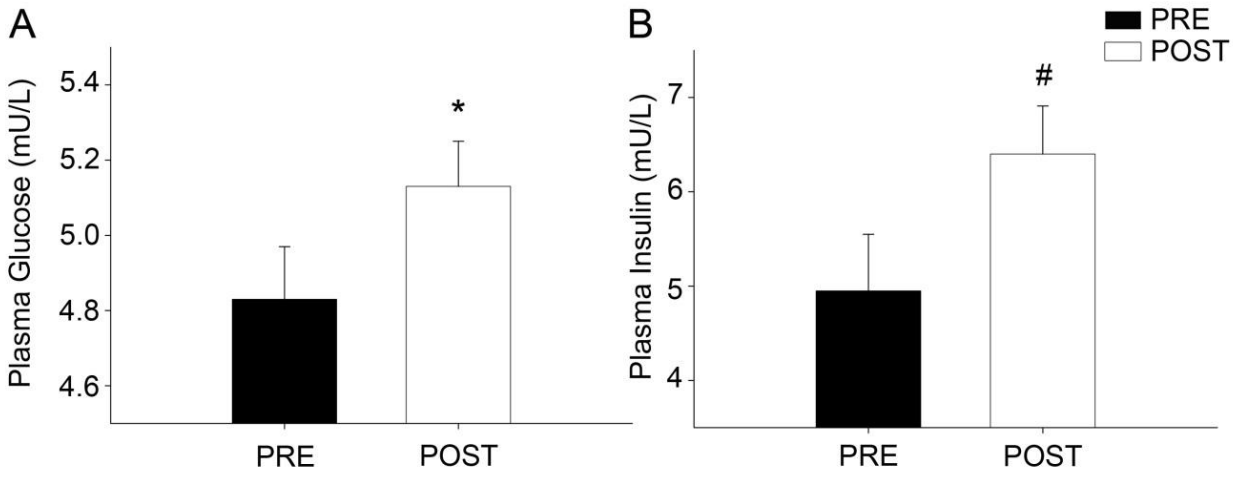

Fig. 2.2 A. Plasma glucose levels after an overnight fast pre and post 4 week SSB intervention. B. Plasma insulin levels after an overnight fast pre and post 4 week SSB intervention (Sartor et al. 2013). Data are presented as means ± SEMs. * P = < 0.05 and # P = < 0.10 for differences pre and post intervention.

Myosin heavy chains and protein expression in skeletal muscle biopsies

Western blot analysis of metabolic markers, GAPDH, ACC and FUM showed no significant changes post supplementation (pre, 1.00 ± 0.24 AU, post, 0.93 ± 0.37 AU, P = 0.393; pre, 1.00 ± 0.55 AU, post, 0.84 ± 0.47 AU, P = 0.328; pre, 1.00 ± 0.35 AU, post, 0.79 ± 0.41 AU, P = 0.179, respectively). The transcription factor MondoA, in the crude nuclei membrane fraction of the muscle, demonstrated a clear trend towards an increase in protein content (pre, 1.00 ± 1.08 AU, post, 2.58 ± 2.46 AU, P = 0.08) (Fig. 2.3 A, B). Conversely, no significant alteration in TXNIP protein expression was observed (pre, 1.00 ± 0.38 AU, post, 1.24 ± 0.49 AU, P = 0.21) (Fig. 2.3 C, D). However, there was a positive correlation between changes of TXNIP and MondoA at the protein level (r = 0.88, P < 0.01). MHC analysis by electrophoresis did not show any difference in fibre type composition pre to post SSB supplementation (MHC I: pre, 52.2 ±17.0 %, post, 50.4 ± 15.5 %; MHC II: pre, 45.4 ± 19.3 %, post, 46.8 ± 17.6 %; MHC IIx: pre, 2.4 ± 7.6 %, post, 2.8 ± 8.9 %), it can therefore be assumed the origin of muscle biopsies did not affect the outcomes of the study.

Gene expression in skeletal muscle biopsies

Real time RT-PCR analysis pre and post SSB intervention revealed a significant alteration in metabolic gene expression of the muscle. GAPDH gene expression, which was used as a glycolytic marker, increased significantly (pre, 1.00 ± 0.74 AU, post, 1.94 ± 1.52 AU, $P = 0.03$) and a trend towards an increase in the lipogenic marker ACC was also observed (pre, 0.99 ± 0.52 AU, post, 1.21 ± 0.78 AU, $P = 0.09$). The co-transcription factor and marker of mitochondrial gene expression PGC1α, decreased significantly (pre, 0.96 ± 0.44 AU, post, 0.79 ± 0.53 AU, $P = 0.04$) and no change in CS expression was seen (pre, 1.00 ± 0.38 AU, post, 0.98 ± 0.66 AU, $P = 0.94$). A strong trend towards increased MondoA mRNA transcription was also observed after the 4 week SSB intervention (pre, 0.36 ± 0.25 AU, post, 0.44 ± 0.34 AU, $P = 0.06$) but no alteration in TXNIP mRNA was seen (pre, 0.51 ± 0.55 AU, post, 0.31 ± 0.41 AU, $P = 0.37$).

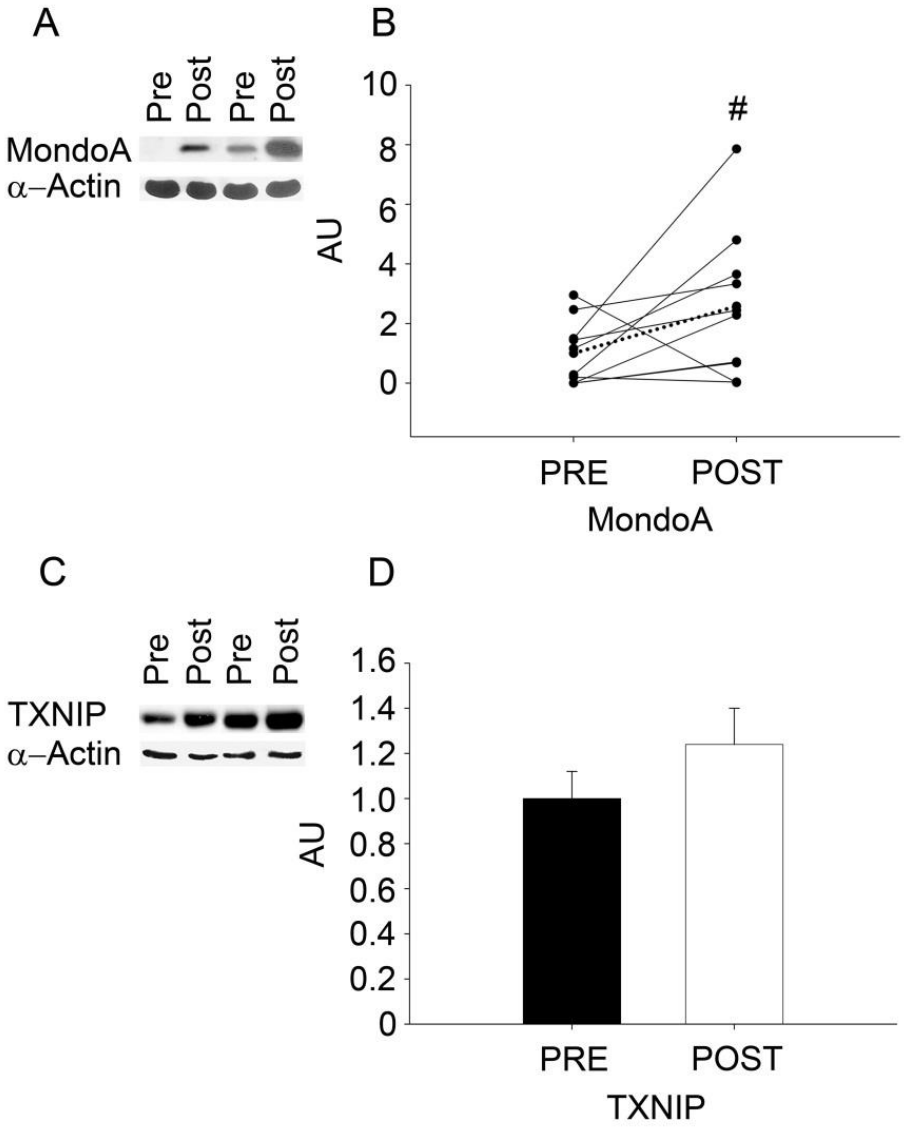

Fig. 2.3 A. Western blot analysis of MondoA in muscle biopsies pre and post 4 week SSB intervention with loading control alpha-actin. B. Quantification of MondoA western blots pre and post intervention for all participants. C. Western blot analysis of TXNIP in muscle biopsies pre and post 4 week SSB intervention with loading control alpha-actin. D. Quantification of TXNIP western blots pre and post intervention (Sartor et al. 2013). Data are presented as means ± SEMs. # $P = < 0.10$ for differences pre and post intervention.

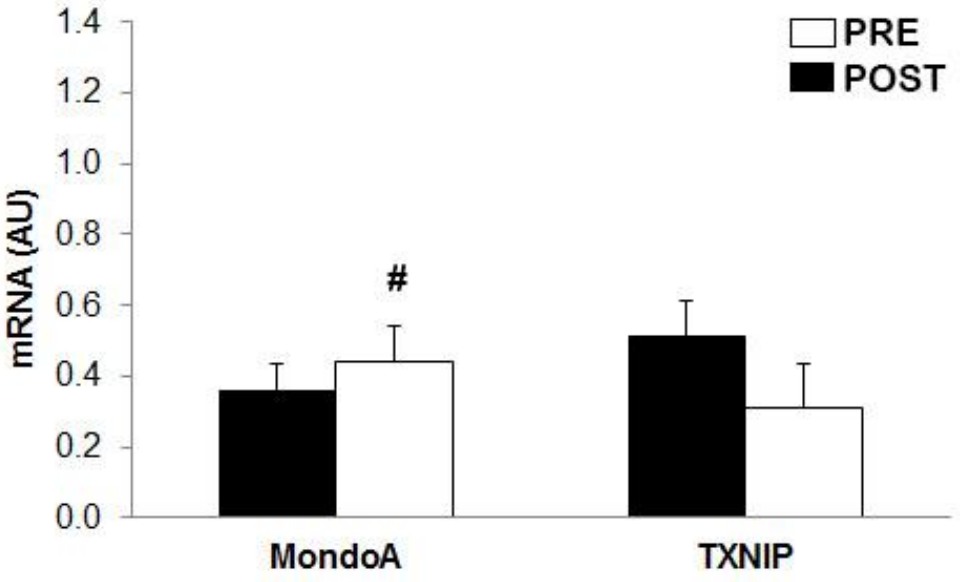

Fig. 2.4 A. MondoA and TXNIP gene expression pre and post 4 week SSB intervention. Data are presented as means ± SEMs. # *P* = < 0.10 for differences pre and post intervention.

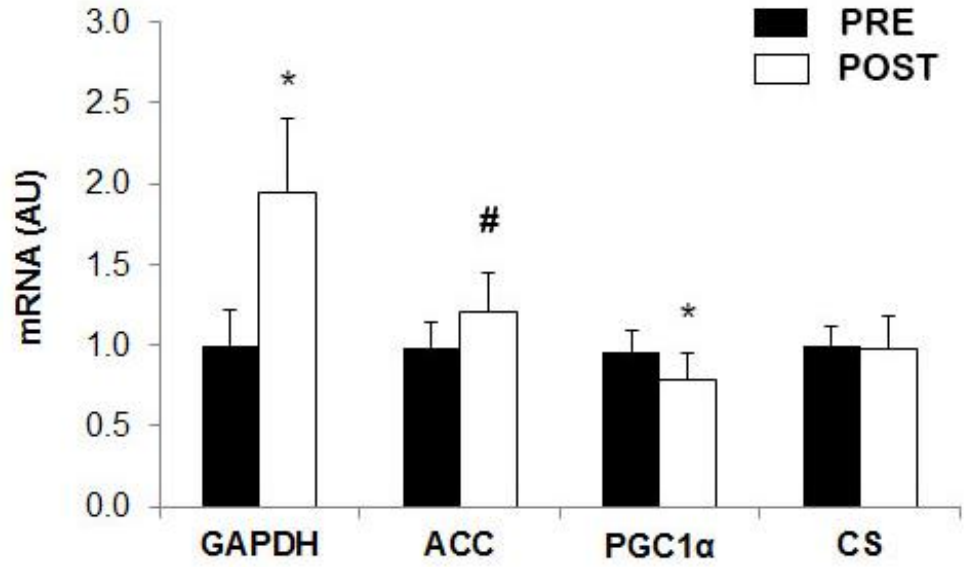

Fig. 2.4 Metabolic gene expression pre and post 4 week SSB intervention. GAPDH glyceraldehyde-3-phosphate dehydrogenase, ACC acetyl-CoA carboxylase α, PGC1α peroxisome proliferator-activated receptor-gamma coactivator 1 α, CS citrate synthase. Data are presented as means ± SEMs. * *P* = < 0.05 and # *P* = < 0.10 for differences pre and post intervention.

Discussion

From the findings of these studies, it is evident that 4 weeks of high glucose availability through regular SSB consumption is sufficient to bring about metabolic alterations similar to those, in muscle cells under chronic hyperglycaemic conditions. These findings were seen in vivo mainly at gene expression level but under in vitro conditions alterations at the protein level were also visible.

After the 4 weeks SSB intervention and in line with previous research (Reiser et al. 1981, Reiser et al. 1979), a significant increase in plasma glucose levels and a trend towards increase plasma insulin levels was observed. This was also accompanied by a trend towards decreased insulin sensitivity and increased insulin resistance of participants, which were lean and healthy with very little previous exposure to SSB (less than 500 ml per week). A further alteration caused by the SSB intervention was a significant increase in fat mass; this is most likely attributed to the changes in the insulin sensitivity, although alterations in insulin sensitivity have been reported without the concomitant changes in fat mass (Black et al. 2005). Furthermore, previous investigations have shown that high glucose intake or consumption of high glycaemic index foods at night does not increase overnight fasting levels of blood glucose or insulin (Nilsson et al. 2006, Stevenson, Williams & Nute 2005, Wolever et al. 1988). Consequently, this demonstrates that the alterations we have observed can be attributed to consistently altered glucose homeostasis and metabolism caused by the 4 week SSB intervention. Additionally, changes to glycogen storage during the periods of high glucose availability may be responsible for these alterations, as glycogen storage reached capacity, to compensate the high intracellular glucose, glycolysis, glucose oxidation and lipogenesis would have been enhanced (Acheson et al. 1988). The increase in resting RER after an overnight fast also supports this explanation, after the 4 week intervention lipid oxidation reduced and conversely carbohydrate oxidation increased by 40%. This increase in RER following high carbohydrate intake has been shown in previous research (Aitken, Thompson 1989). Furthermore, it has also previously been proven that carbohydrate ingestion during exercise inhibits lipid oxidation (Horowitz et al. 1997, Trenell et al. 2008). These findings have also been replicated in animal studies using rodents, after only 3 weeks

of a high glycaemic index diet, later preceding to obesity, fat oxidation was lowered significantly.

One of the major outcomes of this study was influence of the SSB consumption on skeletal muscle metabolic gene expression. Increases in the glycolytic marker glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and a trend towards increased lipogenic enzyme acetyl Co-A carboxylase (ACC) gene expression were both reported, in line with previous findings seen in individuals with type 2 diabetes mellitus (He, Kelley 2004). Reduced expression of peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC1α), the oxidative marker and metabolic regulator was also observed as expected (Puigserver, Spiegelman 2003). This ratio of increased glycolytic to oxidative enzyme activity has also been reported in insulin-resistant and type 2 diabetes mellitus skeletal muscle (Simoneau, Kelley 1997). Although, no change in citrate synthase (CS) gene expression was seen, this may be caused by the low level of physical activity in our participants, in turn resulting in calcium transients and calcium-dependent signal transduction (Kubis et al. 2003) which has previously been shown to limit the impact of glucose signalling on CS activity (Hanke et al. 2011). Our in vitro study also showed similar results with a shift towards increased GAPDH and ACC levels at a protein level, although no differences were observed in gene expression of these, a significant reduction in PGC1α was seen under hyperglycaemic conditions, in line with findings from results seen in rabbit primary muscle cell cultures (Hanke et al. 2011).

This shift in the metabolic gene expression of skeletal muscle towards that found in type 2 diabetes mellitus may be helped by alterations in glucose dependent signalling, such as the transcription factor MondoA. MondoA has recently been recognized to be important in regulating glycolytic gene expression and further metabolic responses to high glucose availability (Sans et al. 2006, Stoltzman et al. 2008). In our vivo study a trend towards increased MondoA protein expression was observed in skeletal muscle after the 4 weeks SSB consumption. Furthermore, this trend was amplified under hyperglycaemic conditions in the primary muscle cell cultures of the in vitro study, providing convincing evidence that MondoA plays an important role in the metabolic adaptations of hyperglycaemia. Further support for this explanation is seen in the expression of thioredoxin interacting protein (TXNIP) which has been shown to be

upregulated by MondoA (Stoltzman et al. 2008).TXNIP is in involved in the inhibition of peripheral glucose uptake of skeletal muscle (Parikh et al. 2007) and has been found to be important in the regulation of glucose production in the liver also (Chutkow et al. 2008). Furthermore, TXNIP transcription has been demonstrated to be heightened in pre-diabetic and type 2 diabetes mellitus patients, suggesting it may play a role in the development of this condition (Parikh et al. 2007, Muoio 2007). In the in vivo study, the change in MondoA protein expression was significantly correlated with the changes in TXNIP although no significant change in expression was reported, a significant increase in TXNIP expression was however seen under hyperglycaemic conditions in the in vitro study. A significant increase in TXNIP expression in the in vivo study may have been prevented by the nature of SSB intervention which only exposed participants to short periods of high glucose availability. In addition, the increase in insulin levels during the intervention may have led to increased glutamine in the muscle (Rennie et al. 1996) which in turn has been shown to influence the activity of MondoA and consequently inhibit TXNIP (Kaadige et al. 2009). Moreover, the amplified alterations present in the in vitro study compared to the in vivo study are expected as the exposure of the high glucose conditions to cell cultures was continuous and at a greater concentration than the in vivo study which was lower and periodic in contrast. Glucose in the in vitro study was comparable to those reported in post-prandial type 2 diabetes mellitus patients (Matsuda, DeFronzo 1999, Monnier et al. 2006) and in the vivo study; SSB supplementation was chosen to be similar to daily amounts consumed by the average individual in the UK, in 2010 (BSDA 2011). Therefore, we can assume that the results of the in vitro study are more comparable to what may result from long term exposure to SSB. However, it is important to note that after only 4 weeks of SSB consumption in otherwise healthy individuals, with a history of very minimal soft drink consumption, significant changes can begin to be seen in metabolic alterations experienced in the development of type 2 diabetes mellitus.

In summary, the findings from the present study provide further evidence for the important role SSBs play in the development of obesity and type 2 diabetes mellitus (Hu, Malik 2010, Malik et al. 2010a, Malik et al. 2010b). The chronic and periodic exposure of hyperglycaemia was enough to influence metabolic phenotype and alter substrate preference which could lead to alterations in metabolism of skeletal muscle

similar to those in type 2 diabetes mellitus. Finally, it is suggested that MondoA and TXNIP are important mediators in these adaptations of skeletal muscle to high glucose availability but more research is needed to fully understand their role.

CHAPTER III

EFFECTS OF 4 WEEKS MODERATE INTENSITY EXERCISE ON BODY COMPOSITION, METABOLISM AND ENERGY BALANCE OF LEAN AND OVERWEIGHT/OBESE SEDENTARY WOMEN

Introduction

To overcome the prevalence of obesity and undo much of the damage to the regulation of glucose and the metabolic profile of the obese population, many investigations have suggested exercise as the answer but as yet this has not yielded convincing success in promoting weight loss and repairing the alterations to metabolism associated with obesity. In many exercise studies even with reported weight loss, this is usually lower than expected (King et al. 2007). Moreover, it has previously been demonstrated that interventions using exercise alone only show minimal weight loss (2.4 kg), compared to diet interventions (4.9 kg) and a combined approach (7.9 kg) (Franz et al. 2007), suggesting that exercise may cause a compensatory increase in energy intake in response to the exercise induced energy expenditure but what is not clear are the underlying mechanisms which control this response.

The suggestion of a compensatory mechanism implies energy balance may be homeostaticly regulated to maintain a set bodyweight and understanding the regulation of this mechanism may be the key to enabling Ov/Ob individuals to lose weight. Previous research and opinion documenting this compensatory response to increased energy expenditure, shows it takes a number of weeks for exercise to stabilise energy balance (Doucet et al. 2011, King et al. 2008), explaining why larger discrepancies in energy balance may be seen in the earlier stages of exercise interventions. Moreover, it is also possible that the type of exercise may influence the compensatory response to exercise. One of the many characteristics of obesity is an impaired fat oxidation and a greater emphasis on carbohydrate oxidation, influenced by the higher blood glucose and muscle glycogen levels. In addition, individuals with high levels of carbohydrate oxidation have been shown to have significantly higher energy intake post exercise than high fat oxidation levels (Almeras et al. 1995). As high intensity exercise also promotes carbohydrate oxidation, it is reasonable to assume high intensity exercise will result in a larger compensatory intake following exercise, suggesting that a high intensity intervention may not be the best approach to exercise with this population.

Leptin is likely to play a key role in mediating a compensatory mechanism as it is well known to regulate fat mass, bodyweight and energy balance (Adami et al. 2002). It is also known that leptin is produced and stored in adipose tissue and high circulating levels are known to correlate with elevated adiposity in obese individuals (Considine et al. 1996). When administered to obese individuals, leptin administration only produced modest weight loss, suggesting its effects have been dampened in these individuals causing a leptin resistance (Munzberg et al. 2005). This leptin resistance is likely to be central to obese individuals' inability to lose weight through exercise with *ad libitum* energy intake.

In further support of this, there is little evidence to suggest that exercise alters leptin concentrations independent of changes in body composition. After a 9 week exercise programme no alterations in leptin concentrations were found, indicating the training had no impact on leptin levels, although this was unsurprising as no significant changes in fat mass were reported (Kraemer et al. 1999). In contrast, a 12 week exercise intervention did reveal a significant reduction in leptin but this was accompanied by a significant drop in fat mass also (Ozcelik et al. 2004). Both of these studies support the notion that changes in leptin levels with exercise are reliant on changes with fat mass. However, there is evidence to suggest that it may be possible to alter leptin concentrations with exercise irrespective of fat mass if insulin sensitivity is improved (Martins, Robertson & Morgan 2008).

Adiponectin may also contribute to the lack of response to exercise in obese individuals. Obese individuals have been shown to have significantly lower concentrations than their lean counterparts (Arita et al. 1999, Cnop et al. 2003, Weyer et al. 2001). Additionally, adiponectin has been repeatedly shown to be negatively correlated with insulin resistance (Cnop et al. 2003, Matsubara, Katayose & Maruoka 2003, Yamamoto et al. 2002). Previously, a 4 week exercise intervention demonstrating significant weight loss, also showed adiponectin concentrations and insulin sensitivity were significantly elevated (Bluher et al. 2006). Moreover, exercise studies without weight loss have shown no significant alterations in adiponectin concentrations (Hulver et al. 2002, Boudou et al. 2003).

In this chapter, a 4 weeks moderate intensity (70-80% heart rate max) exercise training intervention (60 min/day, 3 days/week), in the form of group based circuit training was used to investigate the effects of chronic exercise with *ad libitum* energy intake on body composition, metabolism and energy balance of previously sedentary lean and overweight or obese (Ov/Ob) women. A female only sample was chosen as hormonal responses have been shown to vary with gender (Hickey et al. 1997). Participants were recruited after expressing no desire to lose weight and during the study participants were naïve to the true purpose of the study and informed only that the study aimed to investigate the effect of exercise on metabolism and reaction time. Lean and Ov/Ob individuals were then assigned to either exercise or control groups. Dual-energy X-ray absorptiometry (DXA) was used to assess body composition at baseline and post intervention, and incremental exercise testing with indirect calorimetry was used to assess cardiovascular fitness, metabolism and exercise tolerance. Moreover, diet records were collected across the intervention to monitor energy intake and capillary blood samples were collected to analyse plasma leptin and adiponectin concentration, before and after the exercise intervention.

It was anticipated that Ov/Ob participants would respond to the increase in energy expenditure from exercise by adapting energy intake and therefore compensating for any possible weight loss from exercise induced energy expenditure. This compensatory mechanism would also be supported by no changes in the concentrations of leptin and adiponectin, responsible for regulating this mechanism and maintenance of bodyweight. Furthermore, it was expected the lean exercise group would not display this mechanism and this would be reflected by positive alterations in body composition following the 4 week program. Significant differences in the regulatory hormones and metabolic data of lean and Ov/Ob participants would also offer further support for this argument. Finally, although significant alterations in bodyweight or fat mass were not expected from exercise in the Ov/Ob subjects, it was hoped that analysis of metabolic data would shed more light on the regulation of compensatory mechanisms, explaining how and why these populations differ in their response to exercise.

Methods

Ethical approval

The study design was approved by the School of Sport, Health and Exercise Sciences, Bangor University ethics committee. Before taking part in the study all participants were given an information sheet describing all procedures and any potential risks involved in experiments. As well as this all procedures were explained verbally and once satisfied all participants provided written informed consent.

Participants and study design

Twenty-nine healthy and sedentary women were recruited for this study through an advertising campaign which included emails and posters placed around Bangor University and the surrounding area. During the recruitment process and throughout testing participants were informed that the aim of the study was to investigate the effects of exercise on metabolism and cognitive performance (reaction time), they were not aware that weight loss was being investigated. Participants were also asked during the screening process if they wished to lose weight and those which did were excluded. This was necessary to prevent participants from consciously altering their diet in any way. Upon completion of the study participants were debriefed and informed the true purpose of the study.

Following initial interest potential participants were selected to take part in the study based on their responses to a pre-screening questionnaire. All subjects were; aged between 18 and 35 years; BMI between 18 and 24.9 kg/m^2 (lean) or 27 and 39.9 kg/m^2 (overweight/obese); in good health; sedentary; not pregnant or breast feeding; and not consuming any type of specialised diet. In good health was defined as having no chronic health complaints, currently not receiving any medication other than the oral contraceptive pill, and non-smoking (Martins et al. 2010). Sedentary lifestyle was defined as not engaged in regular strenuous physical activity more than once a week or light exercise more than 20 minutes a day on 3 days per week (Martins et al. 2010).

In this study there were three experimental conditions; lean exercise (LEx), overweight/obese exercise (OEx) and lean control (LC). An overweight/obese (Ov/Ob) control group was intended but due to a smaller number of willing Ov/Ob recruits, it was not possible. Participants in both exercise groups completed a supervised 4 week exercise training intervention and participants in the control group remained sedentary, only maintaining their current level of physical activity. Twenty lean women were randomly assigned to either LEx (n=10) or LC (n=10) and 7 overweight/obese women made up the OEx (n=7) group. Baseline anthropometric characteristics for each group are presented in table 3.1. All participants went through a 2 week pre-testing period prior to the training intervention during which several measurements were taken including, anthropometrical measures, maximal oxygen consumption and blood sampling. All of these measures were repeated after the intervention during a 2 week post-testing period.

Parameter (units)	Lean Control (n=10)	Lean Exercise (n=10)	Ov/Ob Exercise (n=7)
Age (yr)	22.5 ± 2.2	22.4 ± 4.6	26.9 ± 3.9
Weight (kg)	57.3 ± 9.8	58.1 ± 6.3	77.0 ± 9.9
BMI (kg/m^2)	21.2 ± 2.5	22.7 ± 2.1	31.1 ± 5.6
Fat mass (kg)	17.5 ± 4.5	19.3 ± 4.8	34.5 ± 8.1
Lean mass (kg)	38.6 ± 6.1	37.5 ± 3.7	42.3 ± 4.4
Fat Percentage (%)	29.9 ± 4.1	32.6 ± 5.9	42.6 ± 6.9
VO_{2PEAK} (ml/kg/min)	30.5 ± 5.6	32.5 ± 8.3	26.6 ± 7.2
Leptin (ng/ml)	29.7 ± 25.1	57.6 ± 44.1	85.0 ± 31.5
Adiponectin (µg/ml)	11.3 ± 3.9	15.5 ± 7.2	7.6 ± 3.7

Table 3.1. Anthropometric, oxygen uptake and hormonal data of participants in all conditions pre training intervention. Data are presented as means ± standard deviation.

Study intervention

Those participants assigned to LEx and OEx groups underwent a 4 week supervised exercise training intervention (60 min/day, 3 days/week). Exercise training was

performed in groups and a circuit based exercise intervention was used. Training intensity was set at 70-80% maximal heart rate, obtained during $\dot{V}O_{2MAX}$ assessment pre intervention. Heart rate was kept within range using heart rate monitors (Polar F1, Polar Electro Oy, Kempele, Finland) fitted to all participants during each exercise session; monitors were assigned and programmed to individual participants and alarmed to notified researchers if heart rate was outside of set range.

Anthropometry

Body mass was measured to using a beam balance scale (Seca, Hamberg, Germany), participants were instructed to wear minimal clothing and remove footwear. Height was measured using a wall mounted stadiometer (Bodycare products, Southam, United Kingdom). Body composition (lean mass, fat mass and percentage body fat) bone mineral density were measured using dual-energy x-ray absorptiometry (DXA; QDR 4500, Hologic, Bedford, MA, USA).

Peak oxygen consumption

To determine peak oxygen uptake ($\dot{V}O_{2PEAK}$; $ml.kg^{-1}.min^{-1}$) a progressive exercise test was performed on an electronically braked cycle ergometer (Corival 400, Lode, Groningen, Netherlands). After a 3 minute seated measurement, participants began a graded exercise test with 1 minute stages, starting at 25 watts (w), then increasing to 50w and plus 20w every minute following, until the point of volitional exhaustion. Expired gas fractions of oxygen and carbon dioxide were measured breath by breath via online spirometry analysis (Metalyzer 3B, Cortex Biophysik, GMBH, Leipzig, Germany; Zan 600 CPET, Meβeräte, Germany). Respiratory exchange ratio (RER) and energy expenditure ($Kcal.min^{-1}$) were also obtained via metabolic cart. Energy expenditure was calculated using the Weir equation. Resting energy expenditure was also calculated using the Mifflin equation for women (Frankenfield et al. 2003). These were used in conjunction with energy intake data to produce two ratios for energy balance across the intervention, energy intake and measured total energy expenditure (EIEE) and secondly, energy intake and theoretical total energy expenditure (EITEE). Heart rate (Polar F1, Polar Electro Oy, Kempele, Finland) and ratings of perceived

exertion (RPE; Borg, 1973) were collected at the end of every stage and at the point of exhaustion. $\dot{V}O_{2PEAK}$ was achieved when one of three criteria was met: RER greater than 1.1, RPE of 20 or cadence less than 60rpm.

Energy and macronutrient intake

During the 8 week duration of the study (2 weeks pre-testing period, 4 weeks training intervention and 2 weeks post-testing period) all participants were asked to maintain their normal diet and report 3 days (2 week days and 1 weekend day, chosen at random) diet each week using food record sheets. Participants were instructed to complete the diet diaries using standardised instructions (Gibson 1993) and the importance of accuracy and precision when reporting was emphasised. Each participant was provided with a set of cup measures to improve the estimation of portion sizes. The United States Department of Agriculture, National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) and the Tesco Supermarket online database (http://www.tesco.com/groceries/) were used to assess energy intake and macronutrient composition.

Blood sampling and analysis

Capillary blood was collected from the earlobe of participants following a 4 hour fast and mixed with 3.4% sodium citrate solution (using a 1:4 dilution factor). Samples were centrifuged at 3400rpm for 10 minutes at 4^{o}C; plasma was separated and stored at -80^{o}C for hormone measurement at a later date. Plasma leptin and adiponectin were assessed by enzyme-linked immunosorbent assay (ELISA; BioVendor Research and Diagnostic Products, BioVendor – Laboratorni medicina a.s., Czech Republic). All samples were batch analysed and assayed in duplicate using the same microplate reader (Opsys MR, Dynex Technologies, Chantilly (VA), USA). Hormone concentrations are reported for 17 participants (3 LEx, 5 LC, and 7 OEx) as blood samples were not obtained from 12 participants (7 LEx and 5 LC).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 20. Differences pre and post intervention were analysed by two-factor mixed model ANOVA with repeated measures, after all relevant assumptions had been met and outliers removed. Due to the difficulty obtaining blood samples, means and standard deviations for concentrations are presented in the results section below but due to the low sample size, these results should not be considered representative of the population. Bonferroni *Post hoc* tests were conducted where appropriate. Pearson's correlations were used to analyse relationships between variables. All data are reported as means $\pm$ SD. Statistical significance was set at $P<0.05$.

Results

To recap, lean and overweight or obese individuals were selected to take part in the present study, after having already expressed no desire to lose weight. Participants were also not informed about the aims of the study, to prevent them from consciously altering energy intake. Furthermore, lean participants were randomly assigned to either an exercise or control group, those in the exercising group completed 4 weeks of circuit training based exercise, at a moderate intensity and controls were informed to keep to their normal habitual levels of physical activity. The effects of the exercise intervention with ad libitum energy intake on body composition were measured by DXA and $\dot{V}O_{2PEAK,}$ leptin and adiponectin plasma concentrations were also obtained pre and post intervention. Finally, diet records were collected before during and after the intervention to analyse participants' energy and macronutrient intake.

Anthropometry and plasma concentrations of leptin and adiponectin

Comparison of anthropometric measurements at baseline showed significant differences in weight, BMI, fat mass and fat percentage between lean participants in both groups and obese participants as expected (See table 3.2, $P < 0.05$). When comparing differences pre and post training intervention by two factor mixed model ANOVA no significant differences in bodyweight, BMI or fat mass of either lean exercise (LEx) or overweight/obese exercise (OEx) groups were reported. However, although no change in body weight was seen, a significant reduction of body fat percentage and increase in lean mass of LEx was observed post intervention (Table 3.2, $P < 0.05$); this was not repeated in OEx. Both plasma leptin and adiponectin concentrations were found to be significantly different between lean and obese groups as expected, overweight/obese participants were found to have significantly higher plasma leptin concentrations and significantly lower plasma adiponectin levels than their lean counterparts (Table 3.2, $P < 0.05$). However, no significant alterations were observed after the 4 week training intervention in either of the exercise groups plasma leptin or adiponectin levels. Total energy expenditure during the 4 week training intervention was not significantly different between both exercise groups (LEx, 1769.89 ± 616.6 kcal, OEx, 1431.34 ± 620.5 kcal, $P = 0.764$).

	Lean Exercise (n = 10)		Obese Exercise (n = 7)		Lean Control (n = 10)	
Parameter (units)	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	58.13±6.27	58.66±5.73	77.00±9.85†	77.2±10.95	57.34±9.78	58.22±10.10
BMI (kg/m^2)	22.70±2.14	22.92±2.09	31.12±5.60†	31.21±5.99	21.22±2.46	21.55±2.70
Fat mass (kg)	19.30±4.83	18.71±4.56	35.22±8.61†	33.74±9.83	17.52±4.51	17.75±4.95
Fat Percentage (%)	32.60±5.91	31.39±5.84*	43.93±6.43†	42.54±7.05	29.90±4.09	29.80±4.54
Lean mass (kg)	37.49±3.67	38.56±3.85*	42.30±4.81	42.51±4.67	38.59±6.08	39.19±6.26
VO_{2PEAK} (L/min)	1.87±0.47	1.87±0.36	2.07±0.65	1.99±0.51	1.77±0.49	1.69±0.57
VO_{2PEAK} (ml/kg/min)	32.47±8.34	31.85±6.33	26.63±7.15	25.31±6.26	30.49±5.64	28.77±7.21
Leptin (ng/ml)	57.55±44.08	58.77±44.18	84.98±31.52†	64.30±39.06	29.71±25.07	51.52±48.86
Adiponectin (µg/ml)	15.53±7.18	11.49±3.42	7.58±3.68†	7.06±3.10	11.34±3.87	13.39±3.43

Table 3.2. Anthropometric, oxygen uptake and hormonal data of participants in all conditions pre and post 4 week training intervention. Data are presented as means ± standard deviation. * P = < 0.05 for differences pre and post intervention. † P = < 0.05 for differences between lean and obese subjects.

$\dot{V}O_{2PEAK,}$ and individual stage analysis of $\dot{V}O_{2PEAK}$ assessment

The 4 week training intervention had no significant effect on absolute or relative $\dot{V}O_{2PEAK}$ (Table 3.2) of participants in either of the exercise groups, nor were any differences seen between groups. Nevertheless, significant alterations were observed in certain parameters at the individual stages of $\dot{V}O_{2PEAK}$ assessment after the exercise intervention. Heart rate was significantly reduced post intervention in exercise groups during loaded stages of 25, 50, 70, 90 and 110 watts (Table 3.3, all P's < 0.05) but again no differences were reported between groups. RER during the exercise test was unaffected by the training intervention but overweight/obese subjects were found to display significantly higher values during 50, 70 and 110 watt stages (Table 3.3, all P's < 0.05), this trend was also seen at 90 watts and during rest but these were not significantly different. Both groups displayed improved cycling efficiency post training, evident through a decline in energy expenditure during the $\dot{V}O_{2PEAK}$ assessment but this was only found to be significant during the 70 and 130 watt stages (Table 3.3, all P's < 0.05). Perhaps more interestingly, OEx and LEx groups were seen to respond differently during the early stages of the exercise test, the LEx group had significantly higher energy expenditure than OEx pre intervention at the 25 watt

stage but after the intervention no between group differences were seen. This shows a normalisation and improvement of the OEx energy expenditure towards the LEx group post intervention. RPE was also measured during each stage of the $\dot{V}O_{2PEAK}$ measurement, this was seen to be significantly reduced post training in the later stages of the exercise test, the 110 watt stage, of the LEx group (pre, 15.89 ± 1.62, post, 14.00 ± 1.41, $P = 0.004$). Additionally, participants RPE in the LEx group was significantly greater than those in the OEx and LC groups across the 25, 50, and 70 stages (all P's < 0.05).

	Lean Exercise		Obese Exercise		Lean Control	
Parameter (units)	Pre	Post	Pre	Post	Pre	Post
HR (bpm)						
Rest	89.20±12.30	85.20±12.76	86.83±8.18	91.86±13.01	86.00±10.90	86.60±12.22
25 w	112.00±13.44	106.80±12.50*	119.17±17.39	111.86±11.44*	105.80±10.70	105.30±12.26
50 w	122.50±13.70	115.11±15.72*	129.67±19.37	121.57±14.06*	116.20±10.57	114.80±14.47
70 w	135.90±12.62	129.33±15.26*	139.67±20.91	134.14±13.66*	132.20±15.07	130.50±16.17
90 w	151.22±14.73	142.00±17.05*	152.67±19.89	146.57±14.33*	144.80±17.97	145.20±17.43
110 w	162.67±15.58	153.75±17.30*	160.83±17.90	157.29±15.96*	158.50±17.66	156.50±17.78
130 w	171.89±14.30	163.38±16.86	163.25±2.63	170.29±13.40	169.22±18.16	167.33±17.43
RER (VCO_2/VO_2)						
Rest	0.84±0.05	0.84±0.05	0.85±0.04‡	0.88±0.04	0.81±0.05	0.82±0.06
25 w	0.79±0.03	0.77±0.04	0.82±0.05	0.81±0.04	0.79±0.04	0.80±0.07
50 w	0.77±0.07	0.77±0.06	0.85±0.08†	0.88±0.05	0.76±0.11	0.79±0.08
70 w	0.82±0.11	0.83±0.08	0.97±0.08†	0.94±0.06	0.80±0.13	0.82±0.09
90 w	0.91±0.10	0.91±0.07	1.03±0.09‡	1.00±0.07	0.87±0.14	0.91±0.10
110 w	0.95±0.10	0.97±0.07	1.12±0.10†	1.07±0.08	0.95±0.10	0.98±0.09
130 w	1.03±0.11	1.01±0.09	1.11±0.10	1.12±0.11	1.04±0.10	1.01±0.10
EE ($kcal.min^{-1}$)						
Rest	1.56±0.30	1.45±0.30	1.14±0.15	1.18±0.20	1.55±0.25	1.53±0.32
25 w	2.80±0.90	2.78±1.07	2.04±0.67†	2.36±0.84	2.92±0.43	2.38±0.99
50 w	3.07±0.98	3.09±1.01	2.85±1.31	2.88±0.75	3.24±0.81	2.60±1.22
70 w	4.20±0.86	4.03±0.64*	4.02±1.22	3.51±0.72*	4.08±0.88	3.50±1.28
90 w	4.77±0.86	5.07±0.84	4.97±1.16	4.62±1.22	5.09±0.87	4.61±1.51
110 w	6.14±0.93	6.27±0.97	5.76±2.30	5.47±1.33	6.04±0.99	5.50±1.53
130 w	7.26±0.73	7.11±1.10*	6.37±2.77	6.02±1.59*	7.04±1.28	6.58±2.00

Table 3.3. Heart rate, RER and energy expenditure data during $\dot{V}O_{2PEAK}$ assessment pre and post 4 week training intervention. Data are presented as means ± standard deviation. * $P = < 0.05$ for differences pre and post intervention. † $P = < 0.05$ and ‡ $P = < 0.10$ for differences between lean and obese subjects.

Energy and macronutrient intake

After removal of participants with implausible dietary intake (less than 600 or greater than 4000 kcal per day; (Willet 1998)) analysis was conducted on the remaining nineteen participants (8 LEx, 7 OEx and 4 LC). Total energy intake was not significantly altered across the duration of the study, including when expressed relative to body surface area no differences were reported (all *P*'s > 0.05). When macronutrient intake was also compared across the duration of the study as percentage intake per body surface area, carbohydrate, sugar, total fat, and saturated fat were not significantly altered (all *P*'s > 0.05). However, a significant alteration in protein intake was observed in both exercise groups during the intervention, percentage protein per body surface was significantly lower in week 3, the first week of the exercise training, compared to week 6, the last week of the training (Figure 3.1, $P = 0.013$). Furthermore, percentage protein per body surface area was significantly different between OEx and LEx groups, with obese participants consuming less protein than their lean counterparts (Figure 3.1, $P = 0.027$). When diet records were grouped into 2 week blocks; pre-test (weeks 1 and 2), early exercise (weeks 3 and 4), late exercise (weeks 5and 6) and post-test (weeks 7 and 8), again no differences in sugar, total fat or saturated fat were observed (Table 3.4, all *P*'s > 0.05). Total energy intake and energy intake per body surface area however, were significantly reduced from pre-test to post-test in the OEx group (pre, 1717.63 ± 237 kcal, post, 1361.65 ± 283 kcal, $P = 0.014$; pre, 971.51 ± 184 kcal.m^{-2}, post, 771.39 ± 198 kcal.m^{-2}, $P = 0.016$), as well as a significant reduction in reported amounts of carbohydrate pre to post intervention were also seen in this group only (Table 3.4, P = 0.007). Protein percentage intake per body surface area in all groups tended to be higher during late exercise when compared to early exercise but this difference was not significant (Table 3.4). Protein percentage intake per body surface area showed further grouped differences, even though both exercise groups tended to increase protein intake further into the training, LEx consumed significantly more than OEx throughout the study (Table 3.4). Pearson's correlations were used to investigate any potential relationships

between changes (post-test minus pre-test) in energy intake and physiological characteristics. Significant trends were noticed between changes in energy intake and changes in both leptin and body weight (r = -0.524, *P* = 0.081; r =-0.410, *P* =0.081, respectively). Furthermore, a significant positive correlation was observed between changes in energy intake and percentage body fat (r = 0.578, *P* = 0.010) and finally a significant negative correlation was also reported for lean mass change (r = -0.577, *P* = 0.010).

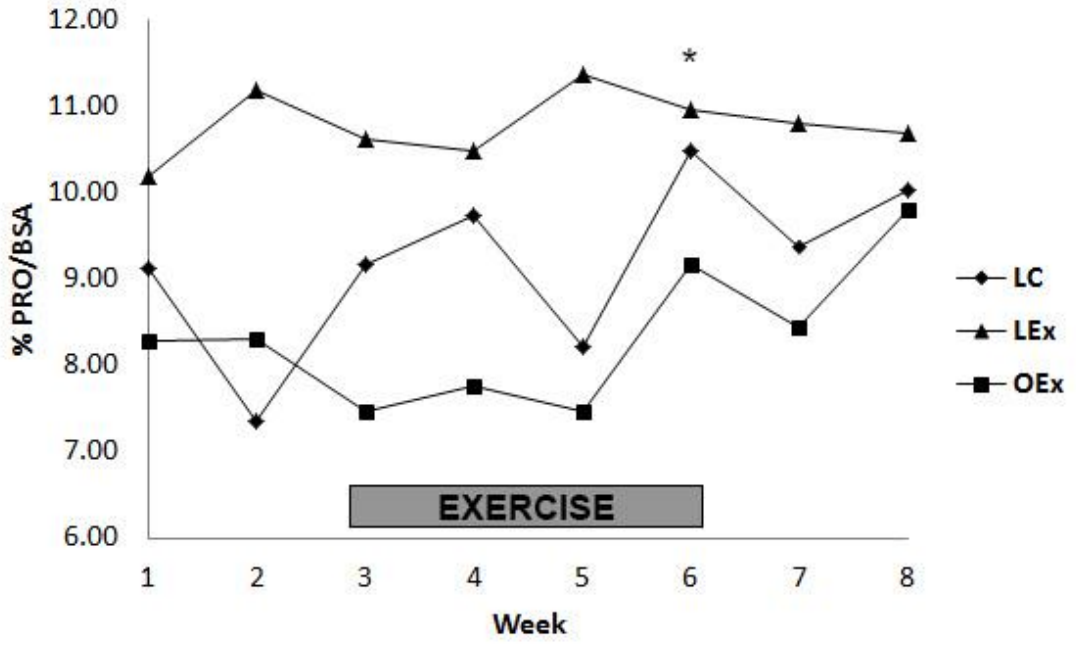

Fig. 3.1. Percentage protein intake relative to body surface area each week of the 8 week study. Data are presented as mean values. * P < 0.05 when compared to week 3, beginning of training intervention.

	Pre-test			Early Exercise			Late Exercise			Post-test		
	LEx	OEx	LC	LEx	OEx	LC	LEx	OEx	LC	LEx	OEx	LC
Energy Intake ($kcal.day^{-1}.m^{-2}$)	1108.4±259	971.5±184	827.2±148	1111.6±231	887.2±259	888.3±139	1074.4±278	850.5±186	980.2±52	1068.0±300	771.4±198*	838.4±74
Protein ($\%.day^{-1}.m^{-2}$)	10.1±1.9	8.0±1.2	7.9±1.3	9.3±2.3	7.5±1.1	8.6±1.4	10.7±1.6	8.1±1.0†	9.3±1.4	10.1±1.3	9.1±2.6	9.58±2.1
Carbohydrate ($\%.day^{-1}.m^{-2}$)	33.1±5.3	29.8±3.6	37.8±6.9	32.1±5.0	31.3±3.2	37.2±3.3	31.4±4.1	30.2±1.8	37.1±7.1	31.5±4.1	32.4±3.4*	37.0±5.3
Sugar ($\%.day^{-1}.m^{-2}$)	11.31±3.8	10.2±2.8	15.4±4.9	11.6±2.6	11.3±2.3	14.1±3.7	10.4±3.1	9.7±2.7	13.8±3.1	11.1±2.9	13.1±3.4	13.3±4.7
Fat ($\%.day^{-1}.m^{-2}$)	17.8±4.8	16.7±3.1	19.0±5.2	19.5±3.2	17.0±3.5	19.9±3.5	18.6±2.0	16.7±3.1	18.3±1.5	18.4±3.2	14.9±3.7	17.8±2.1
Saturated fat ($\%.day^{-1}.m^{-2}$)	6.7±1.5	5.3±0.7	6.3±2.8	7.2±1.5	6.3±1.5	5.8±2.0	6.5±1.1	5.4±1.2	5.2±1.8	6.5±1.1	4.8±1.02	5.8±1.9

Table 3.4. Total energy intake (kcal) and macronutrient intake (%) expressed using body surface area, in two weeks blocks. Data are presented as means ± standard deviation. * $P < 0.05$ when compared to pre-test. † $P < 0.1$ when compared to early exercise.

Energy balance

Energy balance was expressed as the ratio between calculated energy intake and energy expenditure (EIEE). EIEE was calculated on a week by week basis and compared throughout the study using a two factor mixed model ANOVA. EIEE did significantly alter over time, as a significant main effect for time was observed (P = 0.005) and on closer inspection using Bonferroni *post hoc* test, EIEE was found to be significantly lower in week 6 than week 1 (Figure 3.2, P = 0.006), trends were also seen towards lower EIEE in weeks 3 and 4 (Figure 3.2, P = 0.08 and 0.074, respectively), all during the exercise intervention. As energy expenditure was not measured in a true resting state theoretical energy expenditure was also calculated as outlined earlier in the methods section. This was also used to give a new energy balance ratio, energy intake and theoretical energy expenditure (EITEE). When EITEE was compared a significant interaction between group and time was observed (Figure 3.3, P = 0.02), and using a Bonferroni *post hoc* test once again, trends were observed in both OEx and LEx. When compared to week 1 lower EITEE was seen in weeks 2, 3, 5, and 6 of LEx, and lower in weeks 2, 4, 5, 6, 7, and 8 of OEx. In spite of this, only weeks 1 and 6 in OEx displayed a significant difference after a multiple comparisons correction (Figure 3.3, P = 0.004). Moreover, as with energy intake, weeks were separated into 2 week blocks and energy balance was analysed once more by ANOVA. A significant difference in EIEE over time was observed and after further analysis a significant difference between EIEE pre-test and post-test was revealed in OEx (pre, 1.01 ± 0.09, post, 0.82 ± 0.13, P = 0.027). EITEE also tended to be lower post-test but this difference was not significant (pre, 1.19 ± 0.23, post, 0.94 ± 0.25, P = 0.062). Finally, changes in EITEE from pre-test to post-test were correlated with changes in percentage body fat (r = 0.619, P = 0.005), and lean mass (r = -0.598, P = 0.007).

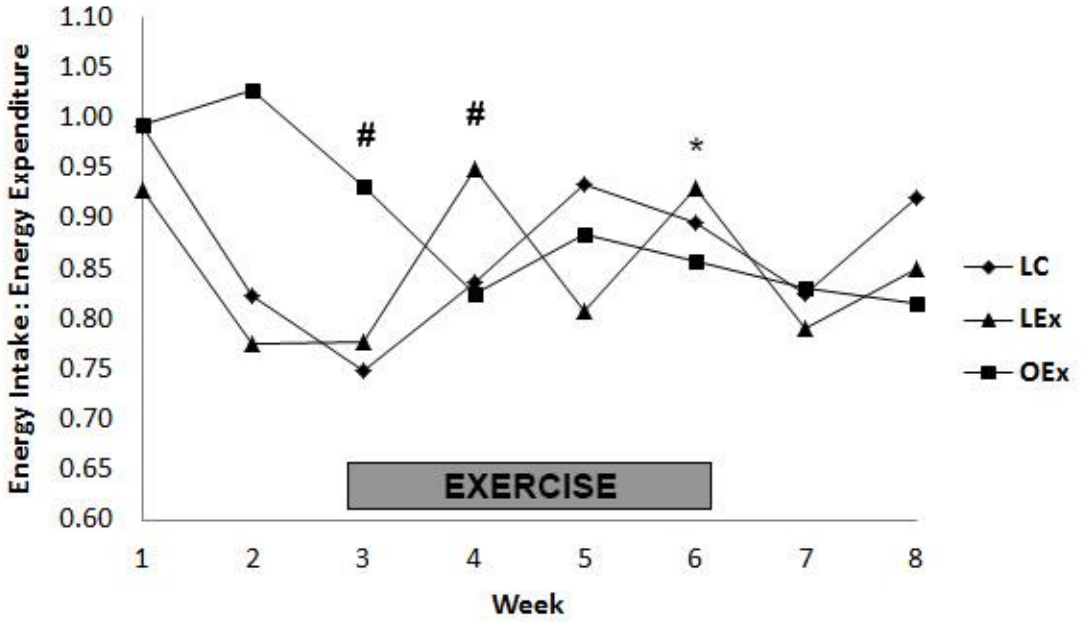

Fig. 3.2. The ratio between energy intake and energy expenditure (EIEE) during each week of the study. Data are presented as mean values. * $P < 0.05$ and # $P < 0.1$ when compared to week 1 of the study.

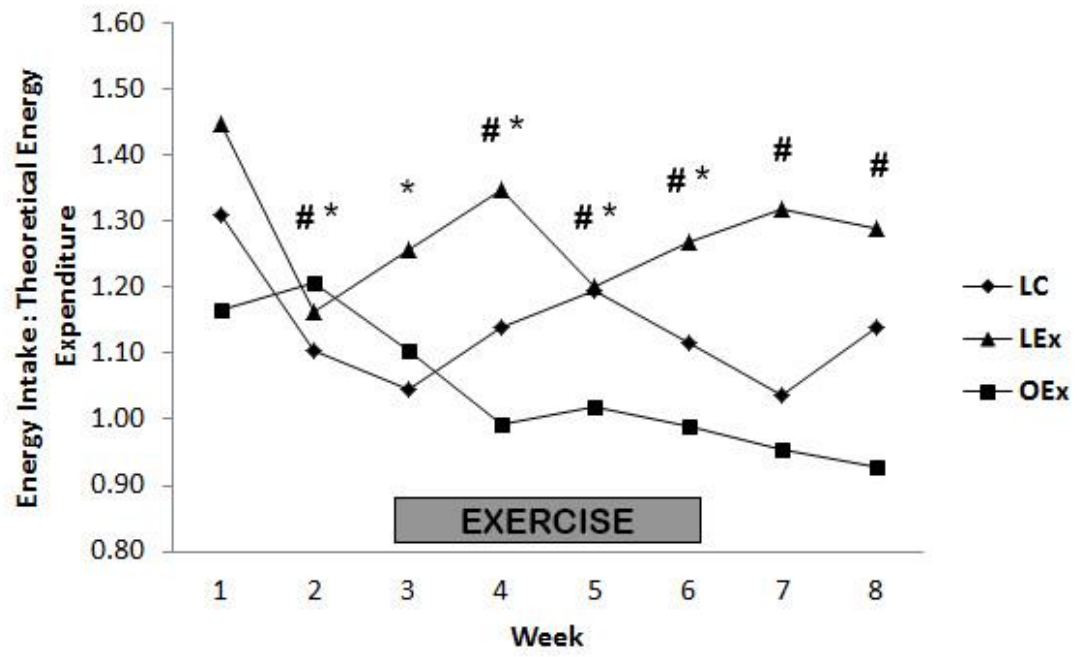

Fig. 3.3. The ratio between energy intake and theoretical energy expenditure (EITEE) during each week of the study. Data are presented as mean values. * Trend towards lower EITEE than week 1 ($P < 0.1$) and # trend towards lower EITEE than week 1 ($P < 0.1$).

Discussion

The main outcomes of the present study are that a 4 week exercise training intervention (3d/wk, 60min/d, 70-80% HR_{peak}) with ad libitum energy intake, where participants were naïve to the purpose of the study, did not results in weight or fat loss of Ov/Ob but did lead to a reduction in percentage fat and increase in lean mass of lean participants. Although, no improvements were seen in $\dot{V}O_{2PEAK}$ after the 4 week intervention, significant adaptations to exercise were seen in energy expenditure of Ov/Ob at lower intensities, towards those measured in their lean counterparts. Furthermore, the 4 week training intervention did seem to improve the coupling of energy intake and expenditure in both sets of exercise groups and even though no changes were observed in macronutrient composition of participants, protein intake was significantly different between lean and Ov/Ob participants, which may support the lack of changes in lean mass with exercise in the Ov/Ob participants.

The lack of weight loss and the maintenance of bodyweight in both Ov/Ob and lean as a result of completing the 4 week training intervention suggest that both groups compensated the increase in energy expenditure from exercise by adjusting their energy intake. The significant changes in percentage fat and lean mass of the lean group indicates that even though they were able to respond to the exercise with improved body composition, their overall body weight remained unaffected in line with Ov/Ob subjects. However, the analysis of energy balance across the whole study does show a trend towards a reduction in energy balance suggesting that over time, exercise did improve the previously sedentary participants' ability to balance their energy intake and expenditure. At the beginning of exercise the Ov/Ob group tended to over-compensate their intake and by the end of the exercise there ability to more accurately compensate their expenditure was improved. In support of these findings regular exercisers have been shown to more accurately compensate a high carbohydrate preload when compared to non-exercisers (Long, Hart & Morgan 2002). Previous investigations have also shown that increased physical activity in sedentary individuals can improve sensitivity to eating behaviour and therefore reduce their capacity to overcompensate increased energy expenditure with higher energy intake (Martins, Robertson & Morgan 2008, Martins, Truby & Morgan 2007). It has been

put forth that this improvement in sensitivity of eating behaviour through exercise is caused by an improved satiation in response to food and control of appetite (Martins et al. 2010, King et al. 2009).

Although trends were seen towards adjusted energy balance at the end of the exercise intervention, little differences were detected in macronutrient intake across the intervention. These findings reflect those from further investigations where prolonged exercise training of previously sedentary Ov/Ob participants did not affect macronutrient composition (Martins, Robertson & Morgan 2008, Donnelly et al. 2003, Tremblay, Drapeau 1999). However, even though few changes were observed over time, significant differences were reported between lean and Ov/Ob participants. Both sets of exercise groups tended to consume more protein later on in the intervention when compared to the onset of exercise and lean participants consumed significantly more protein across the intervention than their Ov/Ob counterparts. Ov/Ob participants did consume less carbohydrate post intervention than pre intervention; this is likely due to the increase in protein replacing excess carbohydrate. Past research has shown that obese individuals consuming a diet high in protein resulted in greater weight than those who consumed a high carbohydrate diet (Skov et al. 1999). This can be explained primarily by increased satiety and diet-induced thermogenesis (Jequier 2002). Energy expenditure has also been demonstrated to be significantly greater when a diet high in carbohydrate was exchanged for a high protein diet (Mikkelsen, Toubro & Astrup 2000). The increase in diet-induced thermogenesis may explain why the lean participants managed to increase lean mass and lose percentage body fat by consuming higher amounts of protein than their Ov/Ob counterparts, whose body composition remained unaltered. In addition, it may also explain the trend towards a lower energy balance during the later stages of the exercise intervention through an increase in energy expenditure by diet-induced thermogenesis.

The 4 week circuit training intervention had no effect on leptin concentrations in either of the exercise groups; however this is not surprising as fat mass remained unaltered by the intervention also. It is well established that leptin concentrations are linked to adiposity (Yannakoulia et al. 2003) and higher leptin levels are known to be associated with the prevalence of obesity (Hellstrom et al. 2004, Hagobian et al.

2009). Additionally, it has previously been reported that leptin concentrations remain unchanged without weight loss (Martins, Robertson & Morgan 2008, Christiansen et al. 2010, Perusse et al. 1997). Moreover, small decreases in leptin as a result of changes to energy balance have been seen to increase fat mass in obese subjects, in an attempt to restore levels to some 'set point' (Farooqi et al. 2001). The above not only explain why leptin levels were higher in Ov/Ob individuals but it may also offer an explanation why participants did not lose weight during the exercise intervention, even under changes in energy balance through increased energy expenditure, leptin forms part of a homeostatic mechanism that tightly regulates body weight and adiposity, maintaining an inherent set point.

Following the same trend as leptin, adiponectin levels were not significantly altered by the exercise intervention, however levels were significantly different between lean and Ov/Ob participants. In line with previous investigations plasma adiponectin was lower in Ov/Ob subjects compared their lean counterparts (Arita et al. 1999). Adiponectin has also been shown to be positively associated with weight loss; individuals with the greatest weight loss have been identified with greater concentrations of circulating adiponectin (Christiansen et al. 2010). Furthermore, it has been previously been demonstrated that after exercise training where weight loss did not occur, adiponectin levels remained unchanged (Hulver et al. 2002). This association with weight loss is most likely due to the fact that adiponectin reportedly stimulates mitochondrial biogenesis and henceforth if individuals are low in adiponectin their ability to make more mitochondria, reduce fatty acid oxidation and increase carbohydrate metabolism is impaired (Borer 2008, Civitarese et al. 2006).

A positive outcome of the study in terms of adaptation to exercise was the significant shift in energy expenditure of the Ov/Ob group at the early onset of exercise, Ov/Ob subjects had significantly lower energy expenditure pre intervention but after the intervention no significant difference was seen. Energy expenditure has previously been reported to be higher in obese individuals because of their increased mass (Westerterp 1998) however, as this was not the case in our energy expenditure data based on oxygen consumption; it would seem that these participants obtain energy through other mechanisms/pathways. It has previously been found that due to their reduced mitochondrial capacity and physical inactivity, obese individuals tend to

depend more on anaerobic glycolysis during exercise and a glycolytic pathway that may not function optimally, this is likely caused by an increase in phosphomonesters (PME) that impair normal glycolytic function (Hunter et al. 2006). This suggests that the participants in the Ov/Ob group of the present study were dependent on anaerobic metabolism during the early stages of exercise pre intervention but through an exercise induced improvement in oxidative capacity this was no longer the case post intervention.

Additionally, because of the potential increase in anaerobic glycolysis of the Ov/Ob women in this study it is likely that blood lactate levels were also increased (Gladden 2004), this has previously been seen in overweight diabetic individuals (Metz et al. 2005). Furthermore, when lactate levels are increased, some of the lactate may be buffered by bicarbonate which can release more CO_2 into the blood and raise RER (Steffan et al. 1999). This was likely the case in our Ov/Ob group as RER did tend to be higher in these individuals. Moreover, although only seen in animal studies, due to the increased levels of lactate in the blood, some of the lactate may be converted to fatty acid stores in the liver (Shreeve et al. 1967). This suggests the increased lactate metabolism in Ov/Ob individuals may contribute to the higher rates of carbohydrate oxidation seen in this population and increased fatty acid storage.

In the present study, the initial difference in energy expenditure, during exercise at low intensity, between lean and Ov/Ob subjects was no longer apparent after the exercise intervention, suggesting that the increase in physical activity reduced the Ov/Ob participants dependence on anaerobic metabolism. Previously, a reduction in monocarboxylate transporter 1 (MCT1) expression has been associated with a decreased capacity to oxidise exogenous lactate in oxidative muscle fibres (Crawford et al. 2010) and alterations in MCT1 expression have been linked with obesity (Py et al. 2001). In support of this explanation, endurance training has previously been demonstrated to decrease lactate levels by an increase in lactate clearance, through increased MCT1 and MCT4 expression of sarcolemmal and mitochondrial membranes, in turn promoting the action of the oxidative enzyme citrate synthase (Dubouchaud et al. 2000). In the present study, it is possible the exercise training increased oxidative capacity of the Ov/Ob individuals, by an exercise induced increase in lactate clearance.

To conclude, whilst participants were naive to the true purpose of the study, the 4 weeks exercise training intervention did not lead to weight/fat loss in Ov/Ob subjects but was enough to reduce percentage body fat and increase lean mass in lean subjects. This suggests that a body weight set point may exist in both groups but on different weight levels. It is suggested that leptin may form part of a homeostatic mechanism that tightly regulates body weight and adiposity, maintaining an inherent set point. Protein intake may also play an important role in the response to exercise and control of energy balance, regulating body weight. Finally, the results of the present study do indicate that 4 weeks circuit training at a moderate intensity can improve aerobic metabolism, of Ov/Ob individuals during exercise by enhancing the oxidative capacity of skeletal muscle.

CHAPTER IV

EFFECTS OF 8 WEEKS HIGH AND LOW INTENSITY EXERCISE ON BODY COMPOSITION, METABOLISM AND ENERGY BALANCE OF LEAN AND OVERWEIGHT/OBESE SEDENTARY WOMEN

Introduction

At present, intervention studies have investigated how either restricting energy intake, manipulating energy expenditure or both simultaneously affect weight loss and so far have produced no clear intervention that has been able to achieve long-term sustainable weight loss, more research needs to focus on the dysregulation of homeostatic mechanisms regulating energy balance (Hafekost et al. 2013). In Jakicic *et al.* (2008) study after 6 months of lifestyle alteration and weight loss, an 18 month follow up was conducted, 72% had regained weight and a further 31% regained weight over and above their previous baseline. Stubbs *et al.* (2002) described the issue with intervention studies so far, that they do not take into account compensatory mechanisms that may exist and assume that energy intake and expenditure can be altered independently from one another (Stubbs et al. 2002).

Both the regulatory processes of leptin and insulin in the regulation of energy balance have been well documented. They are both known to decrease appetite and increase energy expenditure (Porte, Baskin & Schwartz 2005). Insulin concentrations are also known to be higher in obese individuals (Moore, Cooper 1991), often leading to insulin resistance. Moreover, suppression of insulin has been shown to reduce leptin resistance (Blaak, Saris & van Baak 1993), combined with the associated hyperglycaemia and hyperlipidaemia, this suggests that hyperinsulinaemia may be one of the key causes of leptin resistance (Lustig 2006). Both, insulin and leptin are controlled by the CNS in similar ways and they share signalling pathways that regulate adiposity and energy homeostasis (Niswender, Schwartz 2003) but more research is needed to further understand how they interact to regulate a compensatory mechanism in obese individuals.

Amylin which is co-secreted with insulin could form part of a compensatory mechanism is obese individuals. Amylin concentrations are also typically elevated in obese individuals (Moore, Cooper 1991). Moreover, amylin receptor agonism has been found to suppress appetite (Pullman, Darsow & Frias 2006), slow gastric emptying (Smith et al. 2008) and reduce postprandial glucagon release (Roth et al. 2008), together slowing the release of glucose into the blood to improve the effectiveness of insulin. Furthermore, when amylin antagonist AC187 was centrally

administered to rats it increased food intake, glucagon concentrations, gastric emptying, and glycaemia after glucose ingestion (Riediger et al. 2004, Gedulin et al. 2006).

There is strong evidence to suggest that insulin resistance is one of the major factors contributing to the development of leptin resistance (Lustig 2006). Therefore, it seems logical that amylin may be important in this mechanism, as it is co-secreted with insulin and they both are known to regulate energy homeostasis. Furthermore, obese leptin resistant rats administered amylin, reduced food intake, bodyweight and body fat (Roth et al. 2006) and obese humans that were administered amylin and leptin together for a 20 week period, were compared to amylin alone and leptin alone treatment groups (Roth et al. 2008). The combined amylin and leptin treatment induced a 12.7% loss in bodyweight, compared to the amylin and leptin groups where an 8% loss was observed. Although the mechanisms for this change are not fully understood it is clear amylin is very important in mediating leptin sensitivity and therefore any potential weight loss in obese individuals.

At present, there are few studies which have investigated the effect of exercise on amylin response. In a study with progressive intermittent exercise from moderate to maximal intensities (60%, 75%, 90% and 100% $\dot{V}O_{2MAX}$), no change at the moderate intensities was seen but at 90 and 100% amylin was elevated with the highest at 100% (Kraemer, Chu & Castracane 2002). Additionally, in a further study with resistance exercise no significant increase was seen in amylin concentrations but insulin was significantly elevated (Kraemer et al. 2004) and in a final study, a reduction in amylin was seen during prolonged moderate intensity exercise for 90 minutes (Kraemer et al. 2011). What is more, the effects of chronic exercise on amylin are so far unknown as no studies have investigated this but 2 weeks of exercise and diet restriction have been seen to reduce amylin concentrations by 36% (Izadpanah et al. 2012). Although, the effects of a chronic exercise are likely to be similar to those observed in insulin and in support of this, the findings after acute exercise in amylin concentrations follow those seen in insulin (Kraemer, Chu & Castracane 2002, Kraemer et al. 2004, Kreisman et al. 2000). However, it is evident more research is needed to understand the role of amylin in the regulation of energy balance during chronic exercise.

Taking into consideration chapter 3 and expanding on its findings, in this chapter an 8 week high (80-90% $\dot{V}O_{2MAX}$) and low (50-60% $\dot{V}O_{2MAX}$) intensity exercise intervention (60-90 min/day, 3 days/week) was prescribed to lean and Ov/Ob sedentary individuals, to analyse the effects of chronic exercise with *ad libitum* energy intake on body composition, metabolism and energy balance. The aim of this study was to improve the understanding of regulatory mechanisms controlling energy homeostasis and response to exercise of both lean and Ov/Ob populations. As with the 4 week study, participants were recruited with no intention to lose weight and kept unaware of the objectives of the study throughout the intervention. Participants were notified that the aim of the study was to investigate the effects of exercise on cardiovascular health and cognitive function. Once agreeing to take part, subjects were randomly assigned to either a high or low intensity exercise group, both groups exercised together in a circuit training fashion and a telemetric heart monitoring system was used to set intensity and match energy expenditure between groups. To analyse the effects of exercise, pre and post intervention body composition was assessed by dual-energy x-ray absorptiometry (DXA). Metabolism, cardiovascular function and response to exercise were assessed between and within groups by indirect calorimetry during incremental exercise to exhaustion and at rest in a fasted state. Furthermore, venous blood was collected before and after a test meal also and lipid profile, glucose, insulin, leptin and amylin plasma concentrations were measured to gain a comprehensive view of participants' metabolic profile. Finally, diet records were collected from all participants to measure energy and macronutrient intake before, during and after the 8 week exercise intervention.

It was hypothesised that during the exercise intervention Ov/Ob participants would display compensatory tendencies in response to the exercise induced energy expenditure, adapting energy and macronutrient intake, thus preventing any weight or fat loss. This was expected to be facilitated by a reduction in amylin concentrations, explained by reduced insulin and leptin sensitivity in this population. However, this was not anticipated in the lean participants with normal metabolic function, because of this it was expected lean participants would demonstrate a stronger response to exercise, primarily seen in significant changes to body composition.

Methods

Ethical approval

The study design was approved by the School of Sport, Health and Exercise Sciences, Bangor University ethics committee and the North Wales Research Ethics Committee – West (Ysbyty Gwynedd Hospital, Betsi Cadwaladr University Health Board). Before taking part in the study all participants were given an information sheet describing all procedures and any potential risks involved in experiments. As well as this all procedures were explained verbally and once satisfied all participants provided written informed consent.

Participants and study design

Thirty-five healthy and sedentary women were recruited for this study through an advertising campaign which included emails and posters placed around Bangor University and the surrounding area. During the recruitment process and throughout testing participants were informed that the aim of the study was to investigate the effects of exercise on cardiovascular health and cognitive function, they were not aware that weight loss was being investigated. Additionally, only participants who had expressed no desire to lose weight in the screening process were recruited to take part in the study. This was necessary to prevent participants from consciously altering their diet in any way. Upon completion of the study participants were debriefed and informed the true purpose of the study. As an extra incentive participants were offered a pair of trainers up to the value of £100 at the end of the study.

Following initial interest potential participants were selected to take part in the study based on their responses to a pre-screening questionnaire. All subjects were; aged between 18 and 40; BMI between 18 and 24.9 kg/m^2 (lean) or 25 and 39.9 kg/m^2 (overweight/obese); in good health; sedentary; not pregnant or breast feeding; and not consuming any type of specialised diet. In good health was defined as having no chronic health complaints, currently not receiving any medication other than the oral contraceptive pill, and non-smoking (Martins et al. 2010). Sedentary lifestyle was

defined as not engaged in regular strenuous physical activity more than once a week or light exercise more than 20 minutes a day on 3 days per week (Martins et al. 2010).

All participants completed an 8 week supervised exercise intervention and both lean and overweight/obese participants were randomly allocated to either a high intensity exercise or a low intensity exercise programme, giving rise to four experimental conditions; lean high intensity exercise (LH; n=6), overweight/obese high intensity exercise (OH; n=11), lean low intensity exercise (LL; n=6) and overweight/obese low intensity exercise (OL; n=12). Baseline anthropometric characteristics for each group are presented in table 4.1. During the week before the training intervention and the week following participants came into the laboratory on three separate occasions for pre and post testing. Participants completed anthropometric measures on day 1, metabolic measures and blood sampling on day 2, and maximal oxygen uptake assessment on day 3.

Parameter (units)	Lean high intensity (n=6)	Lean low intensity (n=6)	Overweight high intensity (n=11)	Overweight low intensity (n=12)
Age (yr)	25.0 ± 7.54	23.5 ± 6.35	24.0 ± 6.62	22.8 ± 4.95
Weight (kg)	65.39 ± 6.91	62.99 ± 4.20	86.16 ± 12.99	79.70 ± 10.37
BMI (kg/m^2)	23.34 ± 1.24	21.67 ± 2.50	30.72 ± 4.56	29.87 ± 2.76
Fat mass (kg)	19.28 ± 3.35	18.62 ± 3.75	34.13 ± 8.73	30.85 ± 6.86
Lean mass (kg)	43.84 ± 6.17	42.44 ± 3.09	50.88 ± 5.40	47.54 ± 5.20
Fat Percentage (%)	29.77 ± 5.52	29.40 ± 4.84	39.15 ± 5.06	38.38 ± 4.91
VO_{2PEAK} (ml/kg/min)	44.80 ± 7.84	46.43 ± 5.71	31.17 ± 6.5	33.87 ± 6.06
RMR (kcal/d)	1386.42 ± 247.23	1404.31 ± 182.21	1761.92 ± 344.23	1488.28 ± 237.80
RER	0.79 ± 0.06	0.79 ± 0.08	0.77 ± 0.07	0.78 ± 0.05

Table 4.1. Anthropometric, oxygen uptake, and metabolic data of participants in all conditions pre intervention. Data are presented as means ± standard deviation.

Study intervention

Participants were randomly assigned to either a high intensity (80-90% $\dot{V}O_{2MAX}$) or a low intensity (50-60% $\dot{V}O_{2MAX}$) 8 week exercise training intervention. Exercise

sessions were circuit based and completed 3 times a week, length of sessions was dependent on the intensity of exercise. Energy expenditure during exercise sessions was matched between groups. All sessions were completed in groups and supervised by at least 3 members of the research team. Training intensity was controlled by a telemetric monitoring system (Activio, Activio Sport System, Sweden) and projector, used to display the live heart rates of each participant. Participants were asked to increase or decrease their effort where necessary to remain in the correct zones, corresponding to $\%\dot{V}O_{2MAX.}$

Anthropometry

Body mass was measured to using a beam balance scale (Seca, Hamberg, Germany), participants were instructed to wear minimal clothing. Height was measured using a wall mounted stadiometer (Bodycare products, Southam, United Kingdom). Body composition (lean mass, fat mass and percentage body fat) bone mineral density were measured using dual-energy x-ray absorptiometry (DEXA; QDR 4500, Hologic, Bedford, MA, USA).

Resting metabolic measurements

Participants were instructed to arrive at the laboratory after a 12 hour overnight fast and well rested, having refrained from any exercise for 48 hours prior to measurement. Resting metabolic rate ($kcal.min^{-1}$) and respiratory exchange ratio (RER; $\dot{V}CO_2/\dot{V}O_2$) were measured by indirect calorimetry (Oxycon Pro, Erich Jaegar, Germany) as participants lay awake in a supine position for 30 minutes, temperature of the room was kept constant (19^{o}C) and to drown out ambient noise subjects listened to classical music through headphones. Heart rate (Polar RS800CX, Polar Electro Oy, Kempele, Finland) was also recorded during resting metabolic rate measurement and electro-cardiography (ECG) was monitored for pre-screening purposes.

Test meal

Following an overnight fast participants were given a liquid test meal (Resource® Energy Vanilla 200ml, Nestle, Switzerland), providing 300kcal of which 55% was carbohydrate, 30% fat and 15% protein. Blood samples were taken prior to the test meal and precisely 1 hour after consumption. Fasting and postprandial blood samples were then analysed to measure the response of various hormones to the test meal.

Maximal oxygen consumption

To determine maximal oxygen uptake ($\dot{V}O_{2MAX}$; $ml.kg^{-1}.min^{-1}$) a progressive exercise test was performed on an electronically braked cycle ergometer (Corival 400, Lode, Groningen, Netherlands). After a 3 minute seated measurement, participants began a graded exercise test with 1 minute stages, starting at 25 watts (w), then increasing to 50w and plus 20w every minute following, until the point of volitional exhaustion. Expired gas fractions of oxygen and carbon dioxide were measured breath by breath with a metabolic cart (Oxycon Pro, Erich Jaegar, Germany). RER and energy expenditure ($kcal.min^{-1}$) were also obtained by metabolic cart. Energy expenditure was calculated using the Weir equation. Heart rate (Polar RS800CX, Polar Electro Oy, Kempele, Finland) and ratings of perceived exertion (RPE; Borg, 1973) were collected at the end of every stage and at the point of exhaustion. As a plateau in $\dot{V}O_2$ was not reached for many of the participants $\dot{V}O_{2PEAK}$ will be used henceforth. $\dot{V}O_{2PEAK}$ was achieved when one of three criteria was met: RER greater than 1.1, RPE of 20 or cadence less than 60rpm.

Energy and macronutrient intake

During the 10 week duration of the study (1 week pre-testing period, 8 weeks training intervention and 1 week post-testing period) all participants were asked to maintain their normal diet and report 3 days (2 week days and 1 weekend day, chosen at random) diet each week using food record sheets. Participants were instructed to complete the diet diaries using standardised instructions (Gibson 1993) and the importance of accuracy and precision when reporting was emphasised. Each participant was provided with a set of cup measures to improve the estimation of portion sizes. The United States Department of Agriculture, National Nutrient

Database for Standard Reference (http://ndb.nal.usda.gov/) and the Tesco Supermarket online database (http://www.tesco.com/groceries/) were used to assess macronutrient composition. Energy balance (EB), energy expenditure (EE) and energy intake (EI) were calculated based on body composition data obtained by DEXA scan according to methods previously outlined (Thomas et al. 2010).

Blood sampling and analysis

Under both fasting and postprandial conditions 12ml (2 x 6ml vacutainers™ containing ethylenediaminetetraacetic acid) of venous blood was collected from the forearm of participants. Glucose was measured by the Accu-Chek Aviva glucose meter (Accu-Chek® Aviva, Mannheim, Germany), and total cholesterol, triglycerides, and high-density lipoproteins were measured using the Reflotron Plus system (Reflotron® Plus, Roche, Germany). Low-density lipoproteins were calculated using the formula LDL = TC – HDL - (TG/5) (Friedewald, Levy & Fredrickson 1972). For further hormone measurements, whole blood was centrifuged at 3000rpm for 10 minutes at 4^{o}C, plasma was then separated, aliquoted and stored at -80^{o}C for later analysis. Hormone measurement was carried out by enzyme-linked immunosorbent assay (ELISA) and plate reader (Fluostar Omega, BMG Labtech, Germany). ELISA's were carried out to measure amylin (Millipore, St. Charles, MO, USA), insulin (Mercodia, Uppsala, Sweden), and leptin (BioVendor Research and Diagnostic Products, BioVendor – Laboratorni medicina a.s., Czech Republic). The Homeostasis Model Assessment version 2 (HOMA2) (www.dtu.ox.ac.uk/homacalculator/) was used to calculate beta cell function (%B), insulin resistance (IR) and insulin sensitivity (%S) from fasting plasma insulin and glucose (Wallace, Levy & Matthews 2004). All samples were batch analysed and assayed in duplicate.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 20. All anthropometry, metabolic, exercise and blood data were analysed by three-factor mixed model ANOVA, with one within factor (pre versus post intervention) and two between factors (high intensity versus low intensity and lean versus obese), after all

relevant assumptions had been met and outliers removed. Pearson's correlations were used to analyse relationships between variables. All data are reported as means $\pm$ SD. Statistical significance was set at $P<0.05$.

Results

In the present study, an 8 week high and low intensity exercise intervention was prescribed to lean and overweight/obese (Ov/Ob) sedentary individuals, to analyse the effects of chronic exercise with *ad libitum* energy intake on body composition, metabolism and energy balance. The aim of this study was to improve the understanding of regulatory mechanisms controlling energy homeostasis and response to exercise of both lean and Ov/Ob populations. Participants were recruited and completed the intervention in 3 phases, the 8 week exercise and recruitment process was conducted twice with Ov/Ob participants and once again with lean participants. It was not intended to run the intervention twice with Ov/Ob participants but because of a high attrition rate it was necessary to do so. All participants expressed no desire to lose weight via qualitative questionnaire and during the course of the study, participants were not made aware we were investigating weight loss, to prevent them from knowingly altering energy intake. Pre and post intervention, body composition was assessed by dual-energy x-ray absorptiometry (DXA), along with peak oxygen uptake ($\dot{V}O_{2PEAK}$), resting metabolic rate (RMR) and fasting and postprandial blood sampling to gain a comprehensive view of participants' metabolic profile before and after the 8 week intervention. Diet diaries were also collected throughout the study to monitor participants' energy and macronutrient intake.

Anthropometry

Three factor mixed model ANOVA with two between group factors (Intensity: high versus low and body type: lean versus Ov/Ob) and one repeated measures factor (pre versus post 8 week training intervention), revealed no significant differences in weight, BMI, lean mass and fat mass pre to post 8 week training intervention (Table 4.2, all *P*'s > 0.05). However a significant effect for time ($P = 0.011$) was seen in percentage body fat, along with significant interactions between time and intensity ($P = 0.041$) and time and body type ($P = 0.03$). Both lean groups decreased in percentage body fat with LH decreasing considerably more than LL (Table 4.2), compared to OH and OL which were unaltered. Body fat was also separated into two different categories, trunk fat and limb fat and although there was no significant change in

either of these after the training, a trend towards an interaction between time and body type was observed. Both trunk fat and limb fat decreased slightly in lean participants and this was not seen in the Ov/Ob subjects (Table 4.2, all P's < 0.1). Significant differences were also reported between lean and Ov/Ob participants for weight, BMI, lean mass, fat mass, percentage body fat, trunk fat and limb fat (All P's < 0.05).

Metabolic, aerobic and exercise tolerance measures

Absolute $\dot{V}O_{2PEAK}$ was unaffected by the training intervention and no group differences were observed between groups (Table 4.2, all *P's* > 0.05). Relative $\dot{V}O_{2PEAK}$ was also unaffected by the intervention but a significant difference was observed between lean and Ov/Ob participants, with lean participants reporting significantly greater levels (Table 4.2, *P* = 0.000). RMR, RER and resting HR were also unchanged by the 8 weeks exercise training but a significant difference was seen between body types once again with Ov/Ob participants showing a significantly higher RMR.

Heart rate (HR), ratings of perceived exertion (RPE), respiratory exchange ratio (RER) and energy expenditure (EE) were all monitored and recorded during every watt stage of $\dot{V}O_{2PEAK}$ assessment. After conducting three factor mixed model ANOVA's of HR at each stage no main effects were seen for any of the three factors, although significant interactions for intensity and body type were observed at every stage from rest to 130 watts, it seems that the LH group had the greatest response to exercise, lowering HR post intervention at every stage, LL had a reduced response but HR was still lower at all stages, OH had little to no response during stages and OL actually increased HR in most stages of assessment (Table 4.3, all *P*'s < 0.05 where significant).

RPE data did not display normal distribution between groups; therefore a log transformation was used to achieve this. A significant effect of time was observed in RPE at 25 watts, with all groups rating the first stage lower. Significant interactions were also illustrated for time and intensity at 50, 70 and 110 and also time, intensity and body type, a trend was also seen in these at 90 watts, both high intensity groups

displayed greater reductions in RPE post intervention than low intensity groups, with lean participants showing the biggest reductions (Table 4.3, all *P*'s < 0.05 except trend where *P* < 0.1).

RER showed no alterations post intervention and no group effects or interactions were seen either (Table 4.4, all *P*'s > 0.05). Also, EE did not display any significant main effects but a significant interaction was reported between time and body type at the onset of exercise (25 watts). Ov/Ob participants displayed significantly lower EE than lean participants at 25 watts pre intervention but following exercise this difference was no longer visible, meaning Ov/Ob participants energy expenditure normalised during exercise towards that seen in lean participants. A significant interaction was also observed between time and intensity for EE at 90 and 110 watts, including a trend at 70 watts, where high intensity groups increased EE and low intensity was unaffected (Table 4.4, all *P*'s < 0.05 except trend where *P* < 0.1).

Blood parameters

Analysis of lipid profile showed no change in total cholesterol after the training intervention and no effect of intensity or body type was reported (Table 4, all *P*'s > 0.05). HDL showed no significant differences but LDL did show a significant effect of time and body type, with a significant interaction between intensity and body type also. LDL increased over the intervention in all groups apart from in the LH group and Ov/Ob levels were significantly greater than their lean counterparts (Table 4.2, all *P*'s < 0.05).

	Lean High		Lean Low		Ov/Ob High		Ov/Ob Low	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	65.39±6.91*	64.42±6.75	62.99±4.20*	63.36±4.36	86.16±12.99	86.72±12.99	79.70±10.37	80.01±10.68
BMI (kg/m^2)	23.34±1.24*	23.00±1.44	21.67±2.50*	21.78±2.46	30.72±4.56	30.92±4.52	29.87±2.76	29.99±2.93
Lean mass (kg)	43.84±6.17*	46.20±5.24	42.44±3.09*	42.79±3.13	50.88±5.40	51.38±5.78	47.54±5.20	47.36±5.21
Fat mass (kg)	19.28±3.35*	17.23±4.33	18.62±3.75*	18.27±3.90	34.13±8.73	34.18±8.53	30.85±6.86	31.33±7.14
Fat percentage (%)	29.77±5.52*	26.12±5.96†	29.40±4.84*	28.70±5.17†	39.15±5.06	39.01±5.02	38.38±4.91	38.80±5.16
Trunk fat (kg)	8.24±1.99*	7.15±2.54#	8.16±2.40*	8.02±2.73#	17.26±5.07	17.45±5.02	14.73±3.95	14.94±4.22
Limb fat (kg)	10.17±1.62*	9.21±1.84#	9.57±1.50*	9.37±1.34#	15.93±4.29	15.79±3.79	15.19±3.36	15.45±3.27
VO_{2PEAK} (L/min)	2.91±0.60	2.80±0.62	2.94±0.52	2.65±0.58	2.61±0.46	2.70±0.43	2.66±0.53	2.68±0.51
VO_{2PEAK} (ml/kg/min)	44.80±7.84*	43.08±9.34	46.43±5.71*	42.32±7.38	31.17±6.50	32.15±4.53	33.87±6.04	34.15±5.48
RMR (kcal/d)	1386±247*	1441±144	1404±182*	1542±267	1762±344	1763±305	1488±238	1574±285
RER (VCO_2/VO_2)	0.79±0.08	0.83±0.06	0.79±0.08	0.80±0.13	0.77±0.07	0.81±0.05	0.78±0.05	0.80±0.07
Tchol (mmol/L)	3.38±0.60	3.36±0.53	3.80±0.40	4.13±0.82	3.90±0.79	4.13±0.70	3.81±0.90	4.12±0.69
HDL (mmol/L)	1.70±0.47	1.80±0.55	1.67±0.34	1.64±0.38	1.24±0.26	1.23±0.23	1.75±0.50	1.56±0.34
LDL (mmol/L)	1.52±0.58*	1.40±0.37	1.97±0.58*	2.31±0.69†	2.42±0.74	2.64±0.63†	2.05±0.65	2.34±0.63†

Table 4.2. Physiological characteristics pre and post intervention of all groups, including anthropometric, metabolic and lipid data. Data are presented as means ± SD. * $P < 0.05$ for differences between body type. † $P < 0.05$ for differences pre and post intervention. # $P < 0.1$ for interaction between time and body type.

	Lean High		Lean Low		Ov/Ob High		Ov/Ob Low	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HR (bpm)								
25 w	126.00±17.23	121.67±9.67	103.83±10.17	103.33±12.66	112.27±13.39	112.00±11.39	116.58±14.96	121.17±9.12
50 w	133.33±17.42	128.33±10.75	113.17±10.78	109.17±12.29	119.00±12.97	119.82±12.32	125.42±14.64	127.25±12.02
70 w	144.83±16.55	141.33±8.41	127.33±14.09	123.33±14.31	127.45±13.36	128.45±15.12	135.42±13.24	136.50±11.07
90 w	155.67±13.71	151.67±10.88	139.17±13.96	136.17±16.61	137.82±14.79	139.00±15.09	146.42±13.03	146.75±11.99
110 w	165.00±13.15	161.33±12.29	149.00±13.99	148.83±16.08	147.27±13.61	149.00±16.22	156.00±14.02	155.67±11.71
130 w	174.50±11.04	169.50±12.80	159.83±12.35	159.67±14.49	155.70±12.01	158.18±16.02	165.42±12.68	164.42±11.78
RPE								
25 w	7.67±1.21	6.33±0.52†	7.17±1.17	7.00±0.63†	7.09±1.64	6.82±1.25†	7.25±1.08	6.92±0.67†
50 w	9.83±2.23	8.33±2.25*	7.50±1.38	9.00±1.41	8.36±1.80	7.64±1.29*	8.75±2.01	8.58±1.68
70 w	11.75±2.52	10.17±3.06*	9.17±2.32	11.33±1.97	9.82±2.09	9.36±1.80*	10.38±2.48	10.00±1.91
90 w	13.17±2.56	12.83±2.86	10.67±1.37	13.00±2.19	11.18±1.78	11.27±1.62	11.54±2.19	11.58±2.11
110 w	15.33±2.25	14.17±2.93*	13.00±0.89	14.83±2.23	12.64±1.12	12.82±1.60	13.42±2.11	13.50±2.11
130 w	17.17±2.14	16.67±3.20	14.83±1.33	16.50±2.59	14.10±1.52	14.59±1.69	14.92±2.15	15.17±2.21

Table 4.3. Heart rate (HR) and ratings of perceived exertion (RPE) during each watt (w) stage of $\dot{V}O_{2PEAK}$ assessment. Data are presented as means ± SD. * $P < 0.05$ for differences between time and intensity. † $P < 0.05$ for differences pre and post intervention.

	Lean High		Lean Low		Ov/Ob High		Ov/Ob Low	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
RER (VCO_2/VO_2)								
25 w	0.84±0.09	0.81±0.08	0.80±0.05	0.82±0.11	0.82±0.08	0.83±0.09	0.84±0.13	0.83±0.10
50 w	0.77±0.11	0.77±0.10	0.76±0.03	0.76±0.09	0.75±0.09	0.77±0.08	0.76±0.11	0.78±0.09
70 w	0.79±0.14	0.81±0.13	0.78±0.03	0.80±0.10	0.77±0.12	0.76±0.08	0.76±0.11	0.79±0.10
90 w	0.86±0.15	0.85±0.13	0.87±0.06	0.88±0.12	0.83±0.13	0.81±0.09	0.82±0.11	0.86±0.11
110 w	0.92±0.17	0.91±0.15	0.94±0.08	0.97±0.13	0.90±0.15	0.87±0.10	0.90±0.13	0.93±0.12
130 w	1.00±0.18	0.97±0.18	1.01±0.11	1.04±0.16	0.96±0.18	0.93±0.10	0.96±0.15	0.99±0.14
EE ($kcal.min^{-1}$)								
25 w	2.06±0.81	2.07±0.67	2.79±0.89	1.93±1.08	1.97±0.69	2.31±0.76*	1.81±0.60	2.12±0.66*
50 w	2.70±1.04	3.46±0.73	3.62±1.39	3.31±0.73	3.41±0.66	3.61±0.81	3.26±0.55	3.43±0.91
70 w	4.11±0.94	4.48±0.73	4.89±1.45	4.37±0.69	4.53±0.52	4.78±0.66	4.47±0.72	4.52±0.78
90 w	5.14±0.66	5.38±0.78†	5.92±1.08	5.37±0.89	5.43±0.55	5.83±0.58†	5.32±0.64	5.39±0.77
110 w	5.95±0.67	6.57±0.66†	6.83±1.03	6.42±0.74	6.35±0.53	6.67±0.44†	6.21±0.62	6.33±0.54
130 w	7.02±0.72	7.44±0.81	7.96±1.14	7.71±1.17	7.58±0.46	7.89±0.63	7.23±0.57	7.36±0.50

Table 4.4. Respiratory exchange ratio (RER) and energy expenditure (EE) during each watt (w) stage of $\dot{V}O_{2PEAK}$ assessment. Data are presented as means ± SD. * $P < 0.05$ for differences between time and body type. † $P < 0.05$ for differences between time and intensity.

	Lean High		Lean Low		Ov/Ob High		Ov/Ob Low	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Fasting Glucose (mmol/l)	4.68±0.42	4.83±0.27	4.37±0.41	4.73±0.19*	4.78±0.34	4.66±0.26	4.37±0.41	4.63±0.31*
Postprandial Glucose (mmol/l)	4.03±0.55	3.93±1.09	5.58±0.57	5.22±0.96	4.78±0.82	4.78±0.85	4.33±0.90	4.20±0.67
Fasting Insulin (mU/L)	4.36±1.67†	4.55±2.15	3.33±2.52†	4.45±3.03	8.02±3.53	8.43±3.85	7.07±2.92	7.48±3.66
Postprandial Insulin (mU/L)	22.25±6.84	26.68±10.98#	38.61±20.12	47.60±27.35#	41.01±22.48	52.01±17.30#	40.97±17.20	43.32±21.10#
Beta Cell Function (%B)	73.52±19.26†	69.95±17.06	73.48±31.69†	71.28±32.82	105.46±29.54	114.10±33.14	116.73±32.86	106.07±28.51
Insulin Sensitivity (%S)	201.82±74.05†	191.62±58.02	284.88±163.03†	236.88±117.68	114.70±47.48	112.47±53.21	134.80±62.32	123.43±46.23
Insulin Resistance (IR)	0.56±0.22†	0.59±0.28	0.48±0.32†	0.57±0.38	1.03±0.46	1.07±0.50	0.89±0.37	0.97±0.48

Table 4.5. Glucose and insulin levels both in fasting and postprandial conditions, with HOMA 2 calculations for beta cell function, insulin sensitivity and insulin resistance. Data are presented as means ± SD. * $P < 0.05$ for differences between time and intensity. † $P < 0.05$ for differences between body types. # $P < 0.1$ for differences pre to post intervention.

Fasting glucose also proved to be significantly affected by the intervention, a significant effect of intensity was also reported, along with a significant interaction between time and intensity, fasting glucose increased in all groups apart from OH (Table 4.4, all *P*'s < 0.05). Postprandial glucose revealed no effects of time on circulating levels, one hour after a test meal but a significant interaction was observed between intensity and body type ($P = 0.000$), as levels seemed to decrease in all groups except from OH once again.

Fasting insulin demonstrated no significant effects of time, intensity or interactions although a significant difference was measured between body types with Ov/Ob participants displaying greater concentrations than lean participants (Table 4.4, $P = 0.002$). Postprandial insulin showed no significant differences but a trend towards increased levels post intervention did occur and Ov/Ob tended to display significantly higher levels than lean participants (Table 4.4, all *P*'s < 0.1). When fasting values were subtracted from postprandial values to reflect the response to the test meal, Ov/Ob participants showed significantly greater differences than lean participants but no training effects were observed.

HOMA 2 calculations, combining fasting measurements of glucose and insulin, were used estimate insulin sensitivity (%S), resistance (IR) and beta cell function (%B). %S data was not normally distributed and to resolve this data was transformed using a logarithm. Post intervention no differences were observed in %S, %B, or IR, no effect of intensity or any interactions were seen either but differences between body types were present, Ov/Ob participants displayed significantly greater %B and IR, while lean participants showed significantly greater %S (Table 4.4, all *P*'s < 0.05).

Leptin and amylin concentrations were also measured in plasma samples before and after the 8 week exercise programme. Leptin was only measured in fasting samples as this was not expected to alter with a food challenge but amylin was measured both in a fasting and postprandial state, as with glucose and insulin. Leptin showed no significant differences when pre and post intervention levels were compared or between intensities (Fig. 4.1, all P's > 0.05). However, a significant effect of body type as expected was observed, with Ov/Ob showing significantly greater amounts than lean subjects (Fig. 4.1, $P = 0.000$).

When fasting amylin values were compared significant main effects for body type and intensity were both seen, a trend was also observed for time as well as a significant interaction between time and body type (Fig. 4.2, all *P*'s < 0.05, trend *P* < 0.1). Figure 4.2 illustrates a significant decrease in both Ov/Ob groups post intervention, with a greater reduction in the high intensity group, compared to no reduction in the lean participants. Postprandial amylin also showed a significant effect of intensity as well as, significant interactions between time and body type, intensity and body type (Fig. 4.2, all P's < 0.05), no main effects of time or body type were observed though. As with fasting amylin, postprandial amylin is reduced post intervention in Ov/Ob compared to lean which increased over time. Amylin response to test meal, where fasting levels were subtracted from postprandial concentrations, revealed no significant training effects or group differences (All P's > 0.05).

Moreover, pre intervention fasting amylin levels were significantly correlated with pre leptin levels (r = 0.435, *P* = 0.013), showing individuals with higher leptin concentrations also displayed higher amylin concentrations. When pre intervention concentrations were subtracted from post intervention concentrations (Δ) of fasting amylin, this was negatively correlated with pre leptin also (r = -0.567, *P* = .001), demonstrating participants with higher leptin concentrations also showed the greatest reductions in amylin. Furthermore, leptin concentrations were significantly correlated with Δ bodyweight (r = 0.4, 0.017), showing individuals with the greatest weight gain displayed higher leptin levels and those with the greatest weight loss tended to have lower leptin levels.

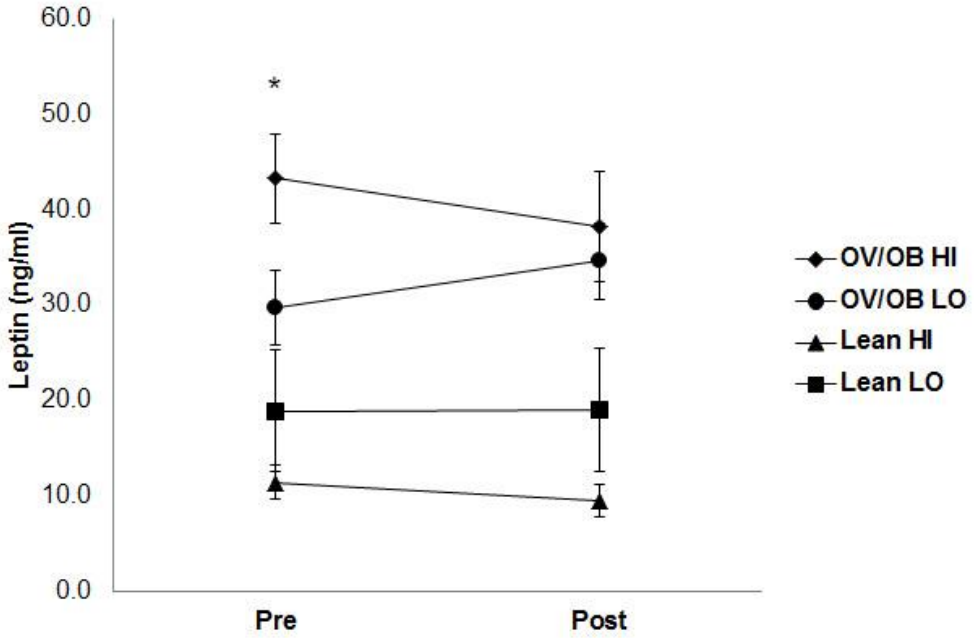

Fig. 4.1. Plasma leptin concentrations after an overnight fast, pre and post 8 week training intervention. Data are presented as means ± SEM. * $P < 0.05$ for differences between body types.

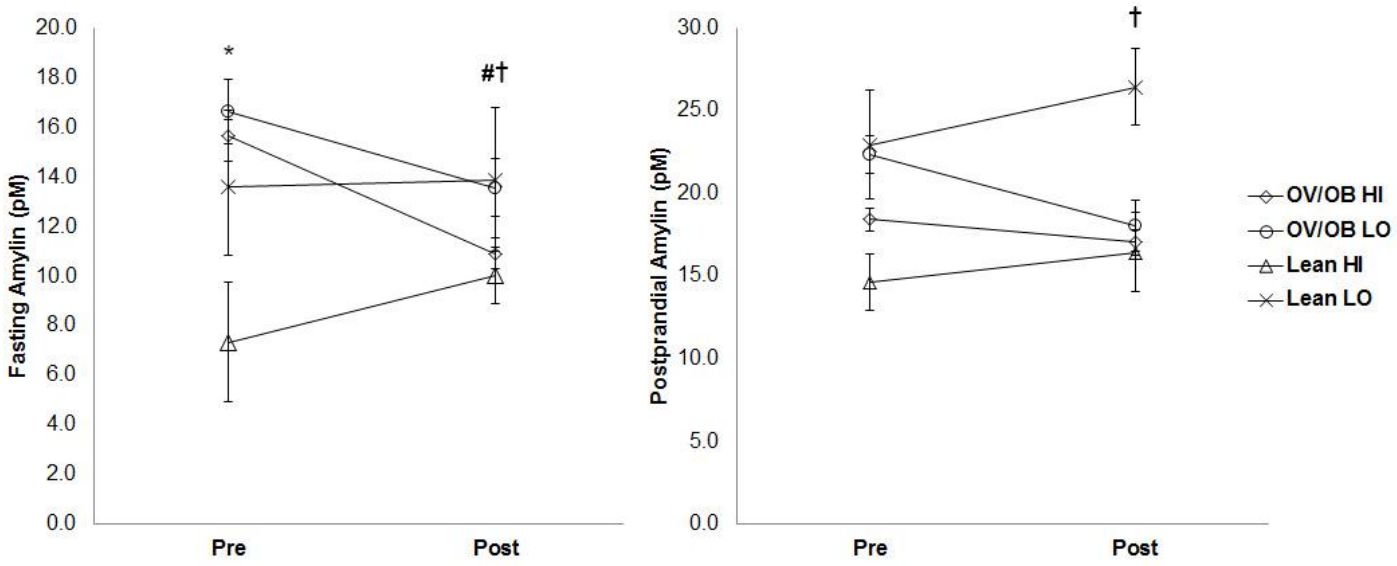

Fig. 4.2. Plasma amylin concentrations after an overnight fast and one hour following a test meal. Data are presented as means ± SEM. * $P < 0.05$ for differences between body types. # $P < 0.1$ for differences pre to post intervention. † $P < 0.05$ between intensities.

Energy intake, expenditure and balance

Total energy expenditure for the exercise training intervention was calculated using a telemetric heart rate monitoring system, as described in the methods section, to keep energy expenditure equal between groups. To test this assumption a one way ANOVA was used and no significant difference was found between groups (OH, 3317.09 ± 1217.83 kcal, OL, 3331.60 ± 948.90 kcal, LH, 2747.42 ± 554.68 kcal, LL, 3453.90 ± 1799.12 kcal, $P = 0.712$). Average training intensity in wattage was also calculated in the same way, from the telemetric system during training (OH, 121.56 ± 22.77 w, OL, 60.96 ± 14.96 w, LH, 86.82 ± 15.75 w, LL, 68.48 ± 19.81 w). Both total energy expenditure from exercise and average training intensity were shown to be positively correlated with changes in lean mass ($r = 0.344$, $P = 0.046$; $r = 0.370$, $P = 0.031$, respectively), meaning participants with the highest expenditure and training load showed the greatest gains in lean mass.

Total energy expenditure (kcal/d), total energy intake (kcal/d) and energy balance (kcal/d) were calculated for the duration of the 8 week training intervention based on DEXA data, as described in the methods section, and groups were compared with a one way ANOVA. Total energy expenditure showed a significant difference between groups ($P = 0.044$) and Tukey's *post hoc* test only revealed a significant trend between OH and LH groups (OH, 2173.92 ± 345.80 kcal/d, OL, 1896.67 ± 293.52 kcal/d, LH, 1745.60 ± 210.96 kcal/d, LL, 1829.65 ± 249.95 kcal/d, $P = 0.098$). Total energy intake also proved to be significantly different between groups ($P = 0.022$) and *post hoc* analysis showed a significant difference between OH and LH groups only (OH, 2192.98 ± 363.25 kcal/d, OL, 1973.55 ± 501.97 kcal/d, LH, 1508.35 ± 201.00 kcal/d, LL, 1778.29 ± 340.60 kcal/d, $P = 0.018$). Finally, energy balance was also significantly different between groups (Fig. 4.3, $P = 0.045$) but only between OL and LH ($P = 0.031$), OH and LH showed a trend towards being significantly different ($P = 0.092$).

Percentage macronutrient intake of protein, carbohydrate, sugar and fat were also calculated from the diet diaries of participants across the 8 week training intervention and this was not shown to be significantly different between groups (all P's > 0.05). However, even though no significant differences were reported in macronutrient

intake, percentage carbohydrate, sugar and fat intake were all significantly correlated with changes in lean mass (r = -0.541, *P* = 0.002; r = -0.451, *P* 0.014; r = 0.499, *P* = 0.006, respectively), demonstrating high carbohydrate and sugar intake was associated with low lean mass and conversely, higher fat intake was associated with higher lean mass.

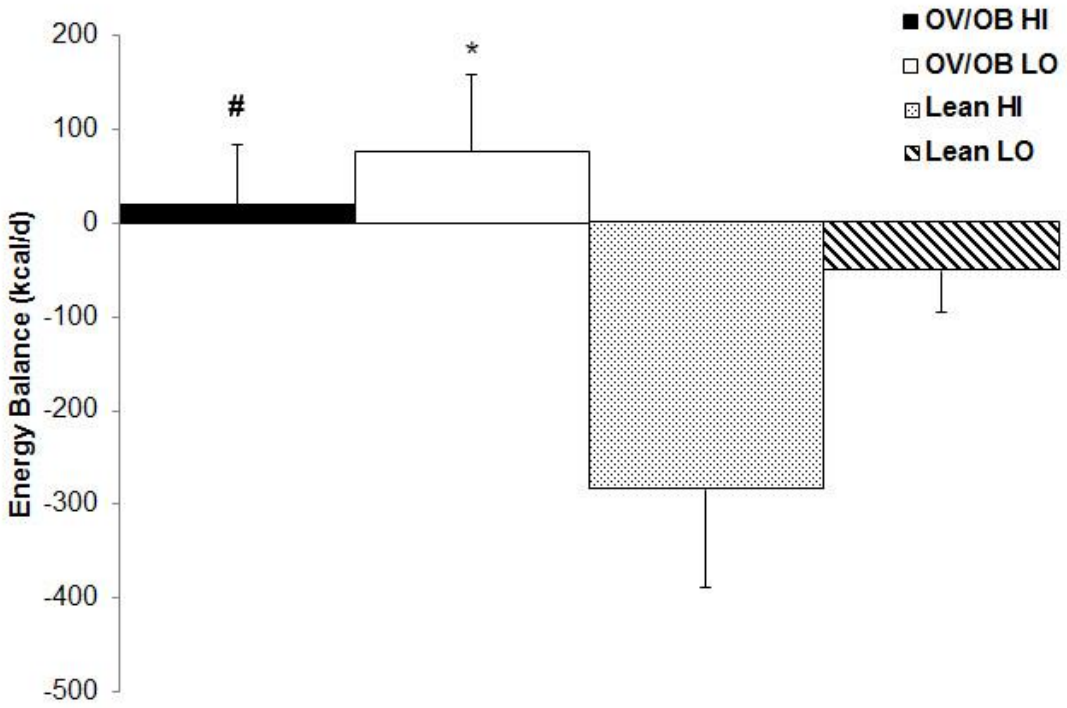

Fig. 4.3. Energy Balance (kcal/d) during the 8 week training intervention, calculated from DEXA scans. Data are presented as means ± SEM. * *P* < 0.05 and # *P* < 0.1 for differences between LH.

Responders versus Non-Responders

Although no alterations were observed in $\dot{V}O_{2PEAK}$ after the 8 week intervention, it was noted that some individuals did show shifts in some exercise related outcomes during the $\dot{V}O_{2PEAK}$ assessment. Most notably, a reduction of HR in the concomitant stages of the test post training. These individuals who reduced heart rate during each stage of $\dot{V}O_{2PEAK}$ assessment from 25 to 130 watts were separated from those who did not and henceforth are referred to as responders; those who did not show this response are referred to as non-responders. Participants were split into four groups; Ov/Ob responders (n = 8), lean responders (n = 5), Ov/Ob non-responders (n = 14) and lean non-responders (n = 7). For statistical analysis a three factor mixed model ANOVA with two between group factors (Response: responder versus non-responder and body type: lean versus Ov/Ob) and one repeated measures factor (pre versus post 8 week training intervention) was used to compare groups.

Anthropometry

When comparing the results of the DEXA scans between responders and non-responders to exercise, no significant differences were seen between groups, for weight, BMI, lean mass, fat mass, body fat percentage or distribution of fat (Table 4.5, all *P*'s > 0.05).. However, trends were observed for differences in body weight and BMI when looking at the interaction between responders and non-responders over time, responders had a trend towards decreased body weight and BMI post intervention, whereas non-responders show no alterations post intervention (Table 4.5, all *P*'s < 0.1).

	Ov/Ob responders		Lean responders		Ov/Ob non-responders		Lean non-responders	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	88.07±13.59	87.69±14.43*	63.99±6.34	63.17±6.32*	80.56±10.39	81.35±10.53	64.34±5.52	64.39±5.20
BMI (kg/m^2)	31.57±3.87	31.42±4.13*	22.96±1.42	22.68±1.62*	29.89±3.42	30.18±3.47	22.18±2.49	22.19±2.38
Lean mass (kg)	51.39±5.76	51.25±6.40	42.22±6.28	44.30±6.01	47.95±5.25	48.20±5.48	43.80±3.63	44.36±3.71
Fat mass (kg)	34.97±9.42	34.72±9.53	19.56±2.24	17.98±3.48	31.56±6.72	32.11±6.79	18.52±4.17	17.7±4.42
Fat percentage (%)	39.19±5.27	39.05±5.20	30.96±4.73	27.93±5.35	38.88±4.80	39.19±5.02	28.60±5.23	27.30±5.88
Trunk fat (kg)	16.83±5.40	16.87±5.75	8.55±1.47	7.57±2.53	15.72±4.28	16.04±4.20	7.95±2.55	7.66±2.77
Limb fat (kg)	17.19±4.12	16.88±3.82	10.09±1.03	9.51±0.95	14.91±3.35	15.13±3.19	9.71±1.86	9.18±1.79
VO_{2PEAK} (L/min)	3.05±0.30	2.91±0.41	2.63±0.68	2.32±0.63	2.47±0.40	2.61±0.46	3.13±0.31	3.02±0.31
VO_{2PEAK} (ml/kg/min)	35.86±6.33	33.89±4.22	40.72±7.23	36.04±6.68	31.26±5.64	32.99±5.69	49.11±3.26	47.46±5.02
RMR (kcal/d)	1721±375	1758±319	1412±300	1473±228	1539±275	1642±284	1383±135	1505±216
RER (VCO_2/VO_2)	0.76±0.05	0.78±0.06	0.76±0.05	0.82±0.09	0.78±0.07	0.82±0.07	0.81±0.07	0.81±0.11

Table 4.6. Physiological characteristics pre and post intervention of responders and non-responders, separated into Ov/Ob and lean groups. Data are presented as means ± SD. * $P < 0.1$ for differences between pre and post intervention.

	Ov/Ob responders		Lean responders		Ov/Ob non-responders		Lean non-responders	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HR (bpm)								
Rest	79.83±11.13	74.83±10.7*	69.00±19.4	67.4.0±14.94*	63.83±4.96	70.00±4.69	67.5.0±4.32	67.83±5.98
25 w	128.38±11.24	121.25±10.55*	121.40±23.91	111.20±18.98*	107.13±8.96	114.40±10.93	110.29±11.74	113.43±11.70
50 w	136.38±9.87	125.50±12.11*	131.80±20.46	117.60±17.59*	114.87±9.19	122.73±12.98	117.14±13.02	119.57±14.06
70 w	144.25±9.41	134.75±12.23*	146.60±16.32	133.00±13.29*	124.87±10.34	131.53±14.40	128.57±14.54	131.86±16.53
90 w	154.13±9.43	143.75±12.51*	156.00±13.64	144.60±10.45*	136.00±12.37	142.67±14.88	141.29±15.03	143.43±19.36
110 w	161.25±11.82	151.63±11.69*	165.40±13.50	155.40±9.13*	146.80±13.05	152.93±15.65	151.00±14.58	154.86±19.07
130 w	168.75±11.59	161.25±11.51*	173.60±11.06	165.80±9.44*	156.57±12.05	160.50±15.60	162.57±13.99	163.71±17.70

Table 4.7. Heart rate (HR) during each watt (w) stage of $\dot{V}O_{2PEAK}$ assessment. Data are presented as means ± SD. * $P < 0.05$ for differences pre and post intervention.

Metabolic measures

As stated above, no significant changes in $\dot{V}O_{2PEAK}$ were seen as a result of completing the 8 week exercise intervention and no significant differences were seen between the responders or non-responders either; this was the case for both absolute and relative $\dot{V}O_{2PEAK}$ (Table 4.5, all *P*'s > 0.05). RMR and RER did not reveal any significant differences either but resting heart rate did prove to be significantly different, as responders demonstrated a significant reduction in HR post training and non-responders showed no change, this alteration was also repeated in all stages of $\dot{V}O_{2PEAK}$ assessment from 25 watts through to 130 watts (Table 4.6, all *P*'s < 0.05).

Blood parameters

Blood analyses were also compared between responders and non-responders and no differences were found in cholesterol, glucose, insulin, HOMA 2 results or leptin (All P's > 0.05), this was also taking into account fasting and postprandial levels of parameters. However, analysis of fasting amylin levels did reveal significant differences between groups (Fig. 4.4, *P* = 0.034), it seems that Ov/Ob participants reduced amylin levels and lean participants increased, as previously described but when comparing responders against non-responders, Ov/Ob non-responders decreased more so than Ov/Ob responders and lean responders increased in contrast to lean non-responders which decreased post intervention.

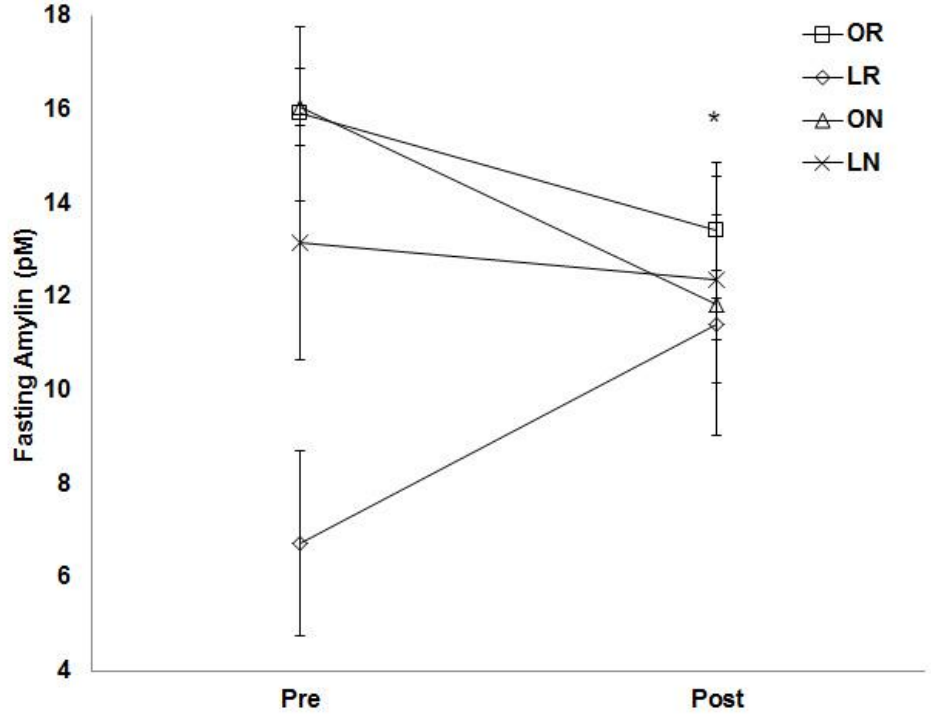

Fig. 4.4. Plasma amylin concentrations after an overnight fast in Ov/Ob responders (OR), lean responders (LR), Ov/Ob non-responders (ON) and lean non-responders (LN). Data are presented as means ± SEM. * $P < 0.05$ for significant interaction between response to exercise and time.

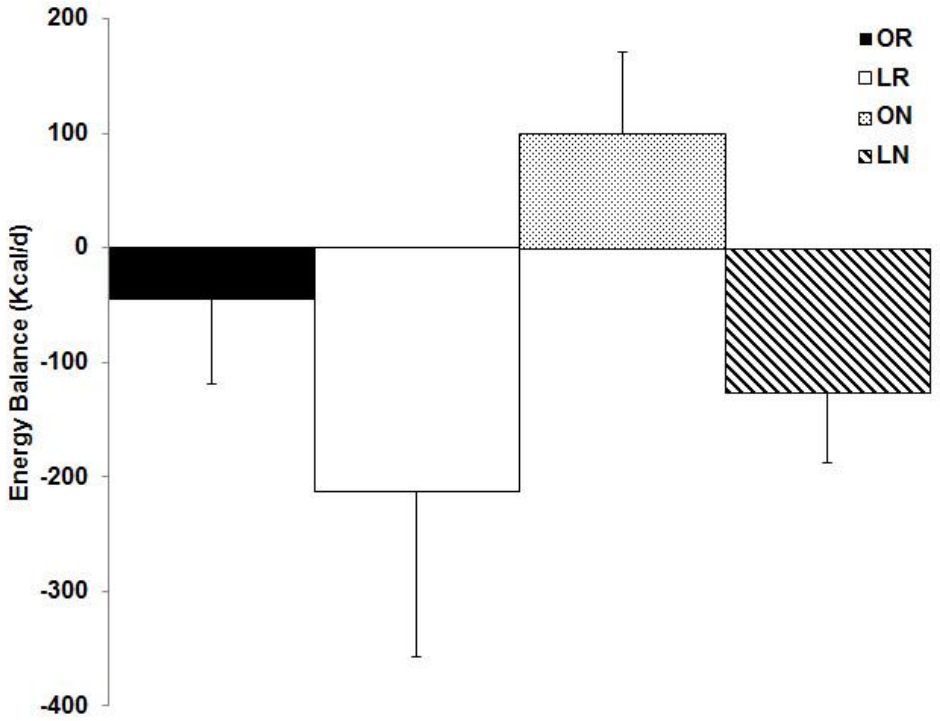

Fig. 4.5. Energy Balance (kcal/d) during the 8 week training intervention, calculated from DEXA scans, for Ov/Ob responders (OR), lean responders (LR), Ov/Ob non-responders (ON) and lean non-responders (LN). Data are presented as means ± SEM.

Energy intake, expenditure and macronutrient composition

Energy intake, expenditure, balance and macronutrient composition after the 8 week training intervention were compared between groups (Ov/Ob responders, lean responders, Ov/Ob non-responders and lean responders) by one-way ANOVA. No differences were seen between responders and non-responders for total energy expenditure or energy intake (All P's > 0.05), however a significant trend was seen for energy balance (Fig. 4.5, $P = 0.063$), although not significant lean responders showed a greater negative energy balance than the lean non responders, Ov/Ob also displayed a lesser negative energy balance and the Ov/Ob non responders were the only group to show a positive energy balance. Analysis of macronutrient composition showed no significant differences in percentage protein, carbohydrate, sugar or fat intake (All P's > 0.05).

Discussion

The main findings of the current study are that an 8 week exercise training intervention, with ad libitum energy intake, did not induce weight or fat loss in Ov/Ob individuals, independent of exercise intensity. This is all the more meaningful as participants were not looking to lose weight and they were informed we were not looking to induce weight loss either. This is the first study to investigate the effects of chronic exercise on ad libitum energy intake in this manner, in an effort to better understand the compensatory mechanisms regulating energy balance. Our results suggest that body weight is part of a homeostatic mechanism closely regulated around a set point, which may exist in both lean and Ov/Ob populations but simply Ov/Ob individuals' set point is upregulated. The significant reduction of amylin in Ov/Ob participants indicates that it may play a key role in regulating energy balance of these individuals. Although, no weight loss was observed post intervention, metabolic alterations to exercise were seen. Additionally, some individuals demonstrated far greater response to exercise than others and how these differ and why is discussed.

The main aim of this study was to investigate the effects of an 8 week exercise intervention with ad libitum energy intake, on the regulation of energy balance in lean and Ov/Ob individuals and to analyse the potential mechanisms which may prevent weight or fat loss from increased energy expenditure through exercise. As stated above the training intervention at either intensity did not result in any weight or fat loss of Ov/Ob participants even though the on average participants expended 3220.53 ± 1167.62 kcal through exercise during the intervention, this should have been sufficient enough to bring about weight loss if participants kept to the same habitual diet as before training. As this was not the case it is evident that a compensatory mechanism promoting energy intake post exercise must exist in these individuals. No weight loss was achieved in lean participants either however, a significant reduction in percentage body fat was observed, suggesting that a compensatory mechanism regulating body weight exists in these participants also but this is inherently different from that of Ov/Ob individuals (Lustig 2006). In support of this, energy intake was seen to be lower in these individuals resulting in a significantly different energy balance also and this is likely responsible for the significant reduction in percentage body fat. In addition, the ability for the lean individuals to reduce body fat as a result

of the increases in physical activity may be a consequence of differences in absolute intensity. Despite the intensity being matched between groups through percentage of VO_{2PEAK} due to the large differences in body weight, and body fat limiting their cardio-respiratory capacity, the lean participants were able to work at a higher absolute intensity enabling them to produce an exercise induced adaptation and drop in percentage body fat. As previously demonstrated long term high intensity exercise intervention combining ad libitum energy intake have been able to elicit greater changes in fat mass than lower intensity interventions (Tremblay, Drapeau 1999, Trapp et al. 2008).Finally, in line with previous investigations no significant alterations were identified in macronutrient composition following the 8 week training intervention (Martins, Robertson & Morgan 2008, Donnelly et al. 2003, Tremblay, Drapeau 1999). No significant changes were reported in carbohydrate, sugar, fat or protein intake of either of the lean or Ov/Ob participants, no differences were seen between groups either.

Analysis of blood samples, more specifically parameters controlling glucose homeostasis were largely unaffected by the exercise intervention, this is unsurprising as the intervention did not alter macronutrient composition in either lean or Ov/Ob individuals. However, Ov/Ob participants were seen to have significantly higher fasting insulin levels and from HOMA 2 comparisons, scores of insulin resistance and beta cell function were significantly higher, with insulin sensitivity reportedly lower in these participants also, demonstrating a movement towards impaired glucose homeostasis in this population as previously well documented (Olefsky, Reaven & Farquhar 1974, Ferrannini et al. 1997).

A further observation from the blood samples of the present study was the expected and significantly greater plasma leptin levels of the Ov/Ob subjects. The greater prevalence of high leptin concentrations in obesity is well established in the previous literature (Hellstrom et al. 2004, Hagobian et al. 2009). Additionally, also as previously seen no significant alterations were observed post intervention in any of the exercise groups (Martins, Robertson & Morgan 2008, Christiansen et al. 2010, Perusse et al. 1997). This was still the case even though participants in the lean exercise groups saw a reduction in percentage body fat, indicating that changes in both are not synonymous in lean participants at least. Moreover, in the present study

significant correlations were observed between insulin resistance and leptin (r = 0.621) as previously described (Lustig 2006) indicating a potential leptin resistance mechanism which may prevent weight/fat loss in obesity and support a theory of body weight set point regulation. However, more research is needed into leptin resistance to greater understand these pathways (Kalra 2008).

In the present study, both fasting and postprandial amylin concentrations were significantly reduced post training in Ov/Ob individuals, compared to no alteration in lean participants. This is the first study to document a significant reduction in both fasting and postprandial amylin concentrations after a long term exercise intervention. Previously, a significant reduction in amylin concentrations has been seen after a 14 day diet and exercise intervention (Izadpanah et al. 2012) but mechanisms behind the reduction were not discussed and hence still very little is known about the effect of exercise on amylin levels. However, weight lowering effects in obese individuals have been associated with the amylin-analogue pramlintide, after 6 weeks of administration before meals in obese subjects; participants reduced body weight, daily food intake, portion size and binge eating tendencies (Smith et al. 2007). These findings suggest that a reduction in amylin would result in increased energy intake, as seen in the current study in the Ov/Ob participants, and that amylin may be part of a homeostatic mechanism protecting against a negative energy balance and therefore weight loss in obese individuals. It is possible that Ov/Ob participants in a hyperinsulinaemia and hyperleptinaemia state may have developed an over-reliance on amylin for satiety signalling, in turn increasing energy intake after exercise and overcompensating for an exercise induced energy deficit.

In addition to the lack of weight loss after the 8 week exercise intervention, no exercise adaptations were observed in $\dot{V}O_{2PEAK,}$ RMR or RER. Although, at the beginning of the exercise intervention there were also some underlying differences in characteristics of lean and Ov/Ob individuals, lean participants had a significantly higher relative $\dot{V}O_{2PEAK}$ and lower RMR than their Ov/Ob counterparts but this was much as expected and as previously stated neither was altered by the intervention. From the data collected during the $\dot{V}O_{2PEAK}$ measurement, it seems that the lean high intensity group had the biggest response to exercise as a significant reduction in HR

was seen for each of the workloads prescribed. This group also showed the greatest changes in RPE, suggesting that their exercise tolerance was improved by the 8 week training intervention. This seems to support the earlier discussions that the lean participants were able to achieve the highest absolute intensity during training and therefore demonstrated the largest training adaptations. Moreover, these findings also offer some support for previous research which state that high intensity exercise interventions are more effective than the low intensity equivalent (Trapp et al. 2008, Tremblay, Simoneau & Bouchard 1994). Energy expenditure during low intensity exercise was another factor measured during the $\dot{V}O_{2PEAK}$ assessment that seemed to reveal some possible adaptations to the exercise intervention. Following the alterations seen in chapter 3 of the Ov/Ob participants during the 25 watts stage, energy expenditure was significantly lower in Ov/Ob participants prior to the 8 week training programme but post training no significant difference were seen when compared to the lean participants. This finding strengthens the argument in chapter 3, that Ov/Ob individuals tend to rely more heavily on anaerobic metabolism during low intensity exercise (Hunter et al. 2006). Furthermore, it also indicates that an exercise intervention can alter this metabolic condition and improve the oxidative capacity of obese individuals, this most likely achieved through improved lactate metabolism by promoting monocarboxylate transporter activity (Dubouchaud et al. 2000) .

From the exercise data of the present study is it evident that participants varied substantially in their response to exercise, and there is a need to try and further understand why some individuals seem to respond to exercise more so than others (Booth, Laye 2010). A large number of factors have been identified to vary in responsiveness to exercise (Bouchard, Rankinen 2001) and exactly why these differ is likely largely due to genetic factors, as much as 48% (Bouchard et al. 1999). In the present study, participants were separated between those that demonstrated clear training adaptations and those that did not. Heart rate (HR) as previously seen to vary adaptive responses to exercise (An et al. 2003), was chosen as a mediator and participants who illustrated an improvement in this parameter during $\dot{V}O_{2PEAK}$ assessment were grouped and referred to as responders, those that did not are referred to as non-responders. When grouped the responders showed a significant reduction in heart at all stages of the $\dot{V}O_{2PEAK}$ test between 25 and 130 watts post intervention,

resting heart rate was also significantly reduced. Responders and non-responders were also split into lean and Ov/Ob groups (Ov/Ob responders (n = 8), lean responders (n = 5), Ov/Ob non-responders (n = 14) and lean non-responders (n = 7)), signifying that response to exercise seems to be independent of body type. From anthropometric data responders displayed a trend towards a decrease in BMI and body weight although this was not significant; non-responders showed no significant differences post intervention. This was in conjunction with a significant difference in energy balance, both lean and Ov/Ob responders displayed a negative energy balance which tended to be lower than lean and Ov/Ob non-responders. Finally, when comparing amylin concentrations, lean responders displayed the lowest amylin levels and increased after the intervention compared to the Ov/Ob non-responders which showed the highest levels and decreased the most. Lean-non responders reduced but to a lesser degree and Ov/Ob responders decreased but also to a lesser extent. In summary, responders showed a trend towards greater weight loss and a more negative energy balance; moreover, responders demonstrated reduced effects of exercise on amylin concentrations. These findings seem to suggest that some individuals, irrespective of body weight or fat mass, are able to respond to exercise and achieve weight loss, this may be because they have not developed the postulated leptin resistance hypothesised earlier, meaning they do not develop the over reliance on amylin for satiety signalling. Without an accurate measure of leptin resistance it is not possible to test this assumption, highlighting the need for further research in this area.

To conclude, the present study provides further evidence that a body weight set point may exist in lean and obese individuals but the regulation of this set point may be controlled by different mechanisms in both populations. It is apparent that in obesity, leptin resistance may lead to the promotion of amylin as a key regulator in a homeostatic mechanism that protects against any potential weight or fat loss through an increase in exercise induced energy expenditure. However, even though weight loss is not achieved in this population there are still positive metabolic alterations associated with exercise in obese participants. Finally, it is suggested that some individuals may be more susceptible to these training adaptations than others, and this may also influence a leptin-amylin mechanism regulating body weight.

CHAPTER V

GENERAL DISCUSSION

Summary of the main findings

The aim of chapter 2 was to investigate the associated metabolic alterations of high glucose availability on skeletal muscle; both in vivo and in vitro studies were designed to analyse the periodic and chronic effects. A 4 week sugar-sweetened beverage (SSB) intervention was prescribed to healthy, lightly active, normal weight individuals, with very little or no history of previous soft drink consumption to analyse the in vivo effects of periodic high glucose availability and to analyse the chronic effects in vitro, primary human muscle cell cultures were exposed to hyperglycaemia for 7 days and compared to control conditions. Western blotting and real time reverse-transcriptase polymerase chain reaction (RT-PCR) were conducted to analyse protein and gene expression of several metabolic markers. It was hypothesised that both in vivo and in vitro conditions would display elevated glycolytic and lipogenic activity, reduced oxidative activity and increased glucose sensing mechanisms, in response to the high glucose availability.

From the results of chapter 2, the impact of high glucose availability on metabolism is clear to see. After only 4 weeks of exposure to high periodic glucose levels through SSB consumption, otherwise young healthy normal weight individuals with low physical activity demonstrated considerable alterations in metabolism of skeletal muscle comparable to those found in muscle cell cultures under hyperglycaemic conditions and type 2 diabetes mellitus patients. Our in vivo study showed that after only 4 weeks of SSB consumption on top of their normal habitual diet, fasting glucose and insulin levels were heightened, reducing insulin sensitivity. Moreover, fat mass was increased along with an altered substrate preference towards increased carbohydrate oxidation during fasting conditions, all demonstrating considerable changes to glucose homeostasis, metabolism and possible shifts in glycogen storage. These are among the more preliminary findings and further in depth analysis of fundamental changes to metabolic markers and glucose signalling systems on a protein and gene expression level were seen.

Both our in vivo and in vitro studies demonstrated a shift towards increased glycolytic activity and reduced oxidative activity similar to that found in type 2 diabetes mellitus patients (He, Kelley 2004, Simoneau, Kelley 1997), after the high glucose availability

interventions. In addition, it is hypothesised that this alteration in metabolic phenotype may be influenced by changes to glucose dependent signalling and it is suggested that the MondoA-TXNIP relationship may play a key role in this adaptation. In both the in vitro and in vivo studies an increase in MondoA expression was observed and in the in vitro cell cultures TXNIP expression was also increased but this wasn't seen under in vivo conditions. As TXNIP was not significantly altered in vivo it is possible that the SSB intervention was not long enough to promote MondoA activity sufficiently to bring about significant change to TXNIP expression but with a longer period of SSB consumption this is likely to change.

The fact that the findings from in vivo study are comparable to those from the in vitro study, demonstrates the potency of high glucose availability in skeletal muscle. Cells in the in vitro study were under similar conditions to those reported postprandially in type 2 diabetes mellitus patients (Matsuda, DeFronzo 1999, Monnier et al. 2006) for 7 days and under in vivo conditions participants were given on average 2 bottle of soft drink per day for 4 weeks, this was similar to average consumption in the UK (BSDA 2011). The results are all the more alarming as the participants were young healthy individuals, not overweight or obese and with very little previous history of SSB consumption, highlighting just how damaging the effects of SSBs can be and the significant role they play in the development of obesity and type 2 diabetes mellitus.

In chapter 3, 4 weeks moderate intensity exercise training was used to investigate the effects of chronic exercise with *ad libitum* energy intake on body composition, metabolism and mechanisms regulating energy balance. Both sedentary lean and Ov/Ob women, naïve to the true purpose of the study and no desire to lose weight underwent various assessments to measure the above factors. It was anticipated that Ov/Ob participants would respond to the increase in energy expenditure from exercise by adapting energy intake and therefore compensating for any possible weight loss from exercise induced energy expenditure. Furthermore, it was expected the lean exercise group would not display this mechanism and significant differences in the regulatory hormones and metabolic data of lean and Ov/Ob participants would offer further support for this argument.

The main outcomes from chapter 3 seem to suggest that exercise with ad libitum energy intake does not seem to induce weight or fat loss in Ov/Ob individuals. Importantly, as stated previously participants were not made aware that the true aim of the study was to investigate the effects of exercise on energy balance regulation, so that participants would not try to consciously alter their diet in anyway. However, even though Ov/Ob participants did not alter their body weight or fat mass, lean exercisers did show a trend towards reduced body fat and increased lean mass after the 4 week exercise intervention. These findings seem to suggest that both lean and Ov/Ob individuals have a body weight set point that is tightly regulated and not influenced by an increase in energy expenditure through physical activity. The fact that lean individuals were able to respond to exercise with altered body composition may be the explained by their ability to exercise at a higher absolute intensity, promoting more pronounced physiological adaptations to exercise. Furthermore, it is suggested that the lack of weight loss can be explained by an over-compensatory mechanism present in non-exercisers, however the trend towards a more tightly coupled energy intake and expenditure later in the exercise intervention does show that exercise can improve eating sensitivity of individuals with previously low levels of physical activity (Martins, Robertson & Morgan 2008, Martins, Truby & Morgan 2007). Additionally, a further explanation for the differences seen here in lean and Ov/Ob individuals may be the observed differences in protein intake. It is suggested that the lean participants reported greater consumption of protein intake may have led to their increase in lean mass and subsequent reduction in percentage body fat. Finally, significant differences were seen in the leptin concentrations of the two different body types as expected (Hellstrom et al. 2004, Hagobian et al. 2009) and it is implied that this difference may be important in regulating a body weight set point (Farooqi et al. 2001).

Importantly, a further finding of the 4 week exercise intervention in chapter 3 is the shift in anaerobic metabolism of the Ov/Ob participants during low intensity exercise towards aerobic metabolism at the same intensity post intervention. This was seen in the normalisation of energy expenditure in these individuals towards those of their lean counterparts. It is postulated that this exercise induced metabolic alteration is associated with improved lactate metabolism resulting in increased oxidative capacity of skeletal muscle and hence improved efficiency (Dubouchaud et al. 2000).

The 8 week exercise intervention in chapter 4 was an expansion on the findings from chapter 3 and the 4 week exercise intervention. The intervention still kept the ad libitum design and participants were still naïve to the true purpose of the study but the intervention and data collection was more overreaching, building on the observations of the 4 week intervention. Not only was the intervention twice as long but greater analysis was carried out on the blood samples, collected pre and post intervention, including the addition of a test meal and postprandial sampling. In addition, the effect of both high and low intensity exercise was investigated and greater control of heart rate and energy expenditure was upheld, with the use of a heart rate telemetric monitoring system. Finally, resting metabolic rate was also measured to gain a more complete view of the participants' metabolic profiles. Similar to chapter 3 it was hypothesised that Ov/Ob participants would display compensatory tendencies in response to the exercise induced energy expenditure, adapting energy and macronutrient intake, thus preventing any weight or fat loss. Importantly, this was expected to be facilitated by a reduction in amylin concentrations, explained by reduced insulin and leptin sensitivity in this population. This was not anticipated in the lean participants with normal metabolic function, because of this it was expected lean participants would demonstrate a stronger response to exercise.

To summarise the findings of the 8 week training intervention, no significant weight loss was observed as per the 4 week intervention. Neither Ov/Ob nor lean participants reported a reduction in body weight; however lean participants did show a significant decrease in percentage body fat. This finding reaffirms the notion that with an increase in exercise induced energy expenditure, Ov/Ob individuals demonstrate a tendency to over-compensate this deficit with increased energy intake, negating any possible weight or fat loss and possibly even promote weight gain. On the contrary lean individuals do not display the same over-compensation to the increased energy expenditure and there energy intake is unchanged. As previously described in relation to the 4 week exercise study, it is hypothesised that the lean participants were able to display a reduction in body fat through the ability to exercise at a higher absolute intensity.

As greater analysis of blood samples was carried in the 8 week exercise intervention it was possible to further investigate and identify possible regulators and relationships that may control a body weight set point or homeostatic mechanism, preventing or protecting against weight/fat loss in obese individuals. Following 8 weeks exercise training a significant drop in amylin concentrations was observed in the Ov/Ob participants, compared to no change in the lean subjects. In light of this finding, it was proposed that Ov/Ob individuals, who also displayed heightened insulin resistance and hyperleptinemia, have developed an overreliance on amylin for satiety signalling. Moreover, in conjunction with the inability to achieve an absolute intensity high enough to induce adaptive responses to exercise, OvOb individuals with reduced amylin secretion, increase energy intake after exercise and prevent any possible weight or fat loss.

Furthermore, it's not all bad news as adaptive responses to exercise were seen in line with the 4 week exercise study, the Ov/Ob individuals in the 8 week exercise study also displayed a positive movement away from a preference to rely on anaerobic metabolism at low exercise intensity pre-intervention, towards more anaerobic metabolism post-intervention. It was suggested, that this metabolic alteration was as a result of improved lactate metabolism, facilitating an improvement of the oxidative capacity of skeletal muscle (Dubouchaud et al. 2000).

In addition, following the 8 week exercise intervention, the inter-individual variability in response to exercise was investigated. As previously described individuals seem to vary greatly in their response to exercise (Bouchard et al. 1999) and in this study participants were separated based on their heart rate response, which individuals have previously been identified to vary in magnitude of response (An et al. 2003). After separation into responders and non-responders, it was revealed that those who clearly demonstrated adaptations, also tended to lose more weight, maintain a negative energy balance, and avoid the maladaptive decrease in amylin concentrations. Suggesting these individuals do not develop the hypothesised leptin resistance, and overreliance on amylin signal, meaning they do not overcompensate for increased exercise induced energy expenditure and can lose weight.

Strengths and weaknesses of the research design

The major strength of the studies in chapter 2 are their generalizability, the conditions are similar to those seen in a 'real world' situation. Although, energy intake was not controlled and may be considered a confounding factor, participants were asked to keep to their normal habitual diet and diet records were kept to see how the SSB intervention may influence this. Furthermore, glucose load was prescribed based on average consumption values, as reported by the British soft drinks association, making the findings generalizable to a large proportion of the UK population or western society. This study was also the first to attempt to measure MondoA responses in vivo and the first to demonstrate that MondoA protein and gene expression can be influenced by chronic hyperglycaemia in vitro and further elevated by periodic high glucose availability in vivo.

One of the main strengths of the exercise interventions in chapter 3 and 4 is also the more realistic approach to the design of the intervention. Many exercise studies in the past has employed very demanding high intensity exercise interventions, in a lab based environment with a large time commitment. In contrast, these studies were undertaken in a gym environment with a group circuit training based intervention, 3 days a week, for an hour. This is more typical and achievable for a subject that is not used to taking part in physical activity and hence, the results are more generalizable to what an individual may be able to undertake in a free living environment. A further strength of the study design was the naivety of the participants to the true nature of experiments; this meant participants were unlikely to modify their energy intake purposefully and the sensitivity of data was improved. Finally, both of these studies are the first of their kind to investigate the differing responses of Ov/Ob and lean individuals to chronic exercise with ad libitum energy intake, and the 8 week exercise intervention was the first study to identify amylin's key role in mediating weight loss through exercise.

A possible limitation of the in vivo study into SSB consumption may be the choice of SSB itself, participants in this study were prescribed a glucose syrup based soft drink and therefore careful consideration must be taken when generalising the findings to all

soft drinks. More frequently high fructose corn syrups are used to sweeten soft drinks and fructose may vary in its effects on metabolism. Sample size may be considered a further limitation of this study, eleven subjects were recruited and took part in the study, but only ten participants were able to provide a blood sample and muscle biopsy post intervention. It is possible that the findings in this sample may not be representative of the population, participants were chosen to take part in the study after completing a questionnaire based on their soft drink habits and it was difficult to find participants with a very little previous history of SSB consumption that met the inclusion criteria.

One of the limitations of the 4 week exercise study was the lack of an accurate resting metabolic rate (RMR) measurement. In this study resting energy expenditure was estimated based on the indirect calorimetry of the 3 minutes of rest before $\dot{V}O_{2PEAK}$ assessment. However, this limitation was reconciled in the 8 week exercise study, resting metabolic rate was measured following previously published guidelines (Compher et al. 2006). A limitation of the 8 week study may be the use of the telemetric heart rate monitoring system in estimating energy expenditure. High variability in heart rate during exercise has previously been demonstrated (Elia, Stratton & Stubbs 2003) but for an intervention of this nature and size, more accurate means of measurement would not be justifiable. A further limitation of both of these studies is the use of self-reported diet diaries in analysing energy intake. Previous literature demonstrates that the mere introduction of dietary intake methodology can alter dietary habits (Bingham 1991, Stallone et al. 1997) and obese individuals have previously been known to under report food intake by as much as 30 % (Lichtman et al. 1992). However taking these considerations into account there is realistic alternative to replace this measure in this study design.

Recommendations for future research

Taking into account the findings of chapter 2 and the possible limitations to the study design, larger and broader studies need to be conducted to further develop the understanding of the role MondoA and TXNIP play in the regulation of metabolic adaptations to high glucose availability. Future studies would be well placed to

investigate the counterbalancing effects of exercise in protecting against the effects of high glucose availability.

Outcomes from both exercise studies seem to agree that Ov/Ob individuals counterbalance the increased energy expenditure from physical exercise interventions with increased energy intake, when no emphasis is placed on adjusting dietary intake. It has been suggested that amylin may play an important role in regulating this homeostatic mechanism. As already discussed this is the first study to observe this finding and therefore more research is required to investigate this mechanism and reaffirm these findings. Additionally, the current study was carried out using only female participants; logically further research needs to be carried out to investigate whether the mechanisms suggested here are gender specific. Previous literature has led to the suggestion that exercise training programs may be less effective with female populations, than with their lean counterparts (Donnelly et al. 2003, Potteiger et al. 2003). Men and women have also previously been shown to vary leptin concentrations in response to exercise (Hickey et al. 1997).

Furthermore, exercise modality is an important consideration in prescribing exercise, in this thesis circuit training was chosen as it was assumed this would be the most effective group based training intervention and combined both resistance and aerobic elements of exercise. To date there is conflicting research investigating the most appropriate training modality to induce weight loss and bring about training adaptations in the Ov/Ob. Prior literature employing an 8 month intervention comparing aerobic training (AT), resistance training (RT) and combined aerobic and resistance training (AT/RT), demonstrated reduced energy intake and body mass in all groups but the combined approach also increased lean mass (Bales et al. 2012). Similarly, a further study also observed significantly reduced body mass, BMI and body fat in AT and AT/RT after 12 months but the AT/RT group saw an additional increase in lean mass also (Carnier et al. 2013). In a shorter duration study of 3 months, a combined approach to training yielded significantly greater improvements in body mass, fat mass and cardiorespiratory fitness, more so than AT or RT alone (Ho et al. 2012). Greater responses to combined training have also been seen in participants with metabolic syndrome (Dutheil et al. 2013) and insulin resistance (Davidson et al. 2009). In contrast, no differences have also been reported when

comparing modalities after a 48 week training intervention (Wadden et al. 1998). Wadden et al. (1998) observed no differences in AT, RT or AT/RT, only a smaller reduction in REE of the AT group compared to RT. Further studies only comparing AT and RT have also measured no differences in response, however exercise capacity was still improved in both cases (Sale et al. 1995, Sarsan et al. 2006). Consequently it has been argued that because an AT/RT intervention did not produce significantly greater reductions in body mass or fat mass, with the additional time commitments, AT alone may be the optimal modality for many where lean mass is not of a concern (Willis et al. 2012). Interestingly, the findings from this thesis clearly demonstrate that lean individuals were able to achieve greater adaptive response to exercise than their Ov/Ob counterparts; possibly through an ability to exercise at a greater absolute intensity. Future exercise studies with obese individuals would likely benefit more from combined AT/RT training which may increase lean mass and stimulate greater metabolic alterations, preventing the maladaptive leptin-amylin response and facilitating weight loss.

Finally, in these studies, there was a considerable pressure and lack of funding which has limited the scope of these investigations. Future research would benefit from larger scale studies, with greater and more representative sampling which would improve the generalizability of findings. Taking into account all of the studies employed in this thesis, subsequent studies would inevitably need to investigate the effectiveness of diet and exercise interventions. A randomised pre-test post-test design with control, diet, and diet-exercise groups would be a logical progression. Pre and post-test measures would involve both blood and muscle biopsy sampling, to analyse both of the key mechanisms put forth in this thesis, controlling metabolic alterations of skeletal muscle to high glucose availability and response to exercise.

Conclusions

From the overwhelming evidence provided in chapter 2, the contribution of SSBs to the current obesity and type 2 diabetes epidemic is all too apparent. Through the constant exposure of periodic high glucose availability for just 4 weeks, otherwise lean healthy individuals with low physical activity and very little previous usage of SSB consumption demonstrated a clear alteration in metabolic phenotype and

substrate preference which could lead to shifts in metabolism of skeletal muscle similar to those in type 2 diabetes mellitus. The fact that it was possible to observe these changes in this population highlights the potency of regular SSB consumption and as the findings were comparable to those seen in primary muscle cell cultures under chronic hyperglycaemic conditions, the role of chronic and periodic high glucose availability in skeletal muscle contributing to obesity is undeniable. It is hypothesised that increases in MondoA and TXNIP contribute to the development of this metabolic alteration and movement towards a pre-diabetic state and this is where future treatment of type 2 diabetes mellitus might lead.

Additionally, in combating obesity, many health professionals look to and recommend promoting physical activity in obese populations and this is what led to the exercise intervention studies in chapter 3 and 4. In order to truly test the effectiveness of exercise as a cure for obesity, a more ecologically valid methodological approach was employed, with ad libitum energy intake and a more realistic and typical exercise programme that previously sedentary individuals would be capable to sustain. From these exercise interventions it was concluded that Ov/Ob individuals are not capable to achieve the desired weight or fat loss normally associated with exercise. Instead, Ov/Ob subjects respond to exercise by increasing their energy intake and over-compensating the induced energy deficit, resulting in no weight loss and possibly even weight gain. This is not to say that obese populations consciously alter their intake but alternatively, through an already impaired metabolism, likely caused by a poor diet high in refined carbohydrate and sugars, they have developed a metabolic phenotype controlled by leptin resistance and maladaptive amylin satiety mechanism, where they are incapable to achieve weight loss through exercise alone.

The previous statements may not show exercise prescription, for obese populations, in a positive light but this does not mean it should not be recommended in this population, as there are still positive outcomes through promoting physical activity in the obese. Even without weight loss Ov/Ob participants in both exercise interventions demonstrated positive training adaptations, in the form of improved oxidative capacity of skeletal muscle. Bearing this in mind and taking into account all of the considerations outlined above, moving forward in the treatment of obesity it is clear that an exercise only or a one size fits all approach is not the answer. A more multi-

dimensional approach is necessary and in order to reduce many of the associated lifestyle disease markers, both diet and levels of physical activity must be addressed in the obese. Recommendations for future intervention studies must address this and combine a diet low in refined carbohydrates and sugars, with high intensity interval training and resistance exercise.

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APPENDICES

Appendix A - Participant information sheets

A.I – Chapter 2

A.II – Chapter 3

A.III – Chapter 4

Appendix B - Screening questionnaires

B.I – Chapter 2

B.II – Chapter 3 & 4

Appendix C - Exercise training

C.I – Example circuit training session

APPENDIX A.1

Participant information sheet for sugar-sweetened beverage study

Study Title: *Effects of a 4 week sugar-sweetened beverage (SSB) intervention on psychophysiological factors*

You are being invited to take part in a research study. This information sheet is designed to help you decide whether or not you would like to participate.

Please ask at any time if you feel anything is unclear or contact:
Francesco Sartor *pep421@bangor.ac.uk* *tel:* 01248 388254

What is the background of the study?
With this study we want to look at the effects of sugar-sweetened beverage (SSB) consumption on several systems of the human body. We will analyse how the metabolism is affected, whether taste is affected, whether there is an effect on mental task performance.

Do I have to take part?
It is up to you to decide whether or not to take part. If you 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 free to withdraw at any time and without giving a reason.

If you are a student this will not affect the marks you receive, the outcome of your period of study at SSHES or your standing with your supervisor, other staff or with the School. After completion of this study your expenses and time commitment will be reimbursed by £100.

So that the results of this study will answer our research question we need to be sure that the people who take part have similar sort of physiques. Because of this we have a strict list of who can and cannot take part in the study. We will ask you a list of questions to make sure that you have the sort of physique we are looking for.

What will happen to me if I take part?
SUMMARY
First of all we will ask you to keep a diet diary where you will write precisely what you eat and drink for two weeks. Then several tests will be carried out. These tests will include a muscle biopsy, a blood sample, a taste test and a mental task. They are described in detail below. Once you have done all these tests you will start taking the SSB supplement. You will be asked to keep taking the supplements every day for 4 weeks. During this period you will be asked to keep a diet diary to make sure that your eating habits stay the same. The amount of drink you will be asked to take each day will depend on your weight. If your body weight is around 70 kg (11 stones) you

will be asked to drink two one bottles (380ml a bottle) of Lucozade a day, if your weight is around 90 kg (14 stones) you will be asked to drink three bottles of Lucozade per day, if your body weight is greater than 90 kg (more than 14 stones) we will give you three bottles and a half per day. When the four week period has finished we will repeat all the tests we did at the beginning. Finally, you will be asked to keep a diet diary for the two weeks following the end of the study.

Details of Study
The details of the study are set out in the flow chart.

DIET DIARY
We will give you a Food Record Sheet (FRS) where you should describe with accuracy and precision your daily food and beverage intake. You will be asked to keep a record of your diet before, during and after the supplementation. The FRS will include 7 days food record. Those 7 days will be randomly chosen over a period of two weeks concerning the pre-tests and post-tests periods. A 14 day food record (two FRS) will be used for the 4 weeks of the supplementation period. How to describe foods and drinks will be fully explained in the Food Record Form.

Tests: Day 1 (55 minutes)

MENTAL TASK
We will ask you to undergo a computerised way of measuring the speed with which you respond to the pictures of different objects. This will allow us to asses your reaction times and whether they might be changed by drinking SSBs. This test requires about 10 minutes at the computer.

TASTE TEST
You will be asked to refrain from 1) drinking alcohol the day before this test, 2) caffeine on the day of testing. In this test we are looking at how you respond to sweet and salty tastes. We will prepare sweet or salty solutions at different strengths. You will be asked to wash your mouth with each solution for about 5 seconds, spit the solution out and then rate how strong and how pleasant the taste is. We will tell you whether the solution is meant to be sweet or salty, but we will not tell you anything about its concentration. Between each taste you should rinse your mouth with a glass of demineralised water for 20 seconds. The total time of this test will be around 30 minutes.

DEXA SCAN
A whole body low radiation (DXA) scan will be performed to assess how much bone, fat and muscle mass you have. For this procedure you need to wear shorts, socks, underwear (no bra) and t-shirt. The scan will last 15 minutes. This procedure is painless, and it is not more than lying down on a bed. Although there are small risks involved in radiation exposure, the amount of exposure during a DXA scan is minimal

and is lower than the amount of background radiation that we are exposed to during the course of two weeks in North Wales.

Tests: Day 2 (1h 10 min)
You will be asked to come to our labs in a fasting state (without eating anything since the night before).

RESPIRATORY QUOTIENT
You will be asked to lie on a bed for about 30 minutes while we will measure how much oxygen you uptake and carbon dioxide you produce. We will use a breath by breath gas analyser which does not involve you inhaling gas of any sort. The analyser just collects the air that you normally breathe in and out. This test is necessary to find out more details about your metabolism.

BLOOD SAMPLING
We will take only two blood samples, from a vein of your forearm. A small amount (8 ml) of blood will be collected (0.2% of the total blood volume). We will use sterile needles to draw the blood samples. It is very important that you have fasted overnight. The first blood sample will be used to measure the fasting glucose and insulin levels in your blood. Afterwards, we will give you a very sweet drink (75 g sugar). One hour after you drank it we will take the second and last blood sample. This will give us precious information about your metabolic response to a sugar load.

NEEDLE MUSCLE BIOPSY
After one hour you drank the sweet drink, mention above, we shall take one small piece of muscle from one of your thighs. The procedure to remove the specimens will be performed by Dr Verena Matschke, Rheumatology research fellow, from SSHES experienced in taking muscle specimens. First of all the skin of the thigh is numbed with local anaesthetic and then a special sterile needle is inserted in to the muscle to take the sample. This is done twice. The procedure is usually painless. At the most, it will feel as you have been given a light punch in the thigh. No more than 40 mg of muscle tissue will be taken. There are usually no side effects but sometimes a bruise may form in the muscle. If this happens, the leg muscle will feel a bit stiff and sore for the next few days. It is possible that infection could be introduced. This is most unusual because the procedure is done under sterile conditions. If it does occur you will be given an antibiotic which should resolve things quickly. This procedure takes about 30 minutes. If any problems do arise you can contact us (details below).

Experiment
4 WEEKS SSB SUPPLEMENTATION
Once you have completed the baseline measures, we will provide a SSB supplementation on top of your habitual diet. It will be important that you do not change your usual diet. The supplementation will consist of two bottles (380ml a bottle) of Lucozade if your body weight is around 70 kg (11 stones) or three bottles (1

litre) of Lucozade if your weight is around 90 kg (14 stones) and three bottles and if you body weight is greater than 90 kg (more than 14 stones) per day. {Lucozade contains only 0.012 % caffeine. You will not be consuming more than about 92 mg of caffeine/day, which means much less than a cup of coffee (100-240 mg per cup)}.
During the whole duration of the supplementation we randomly will ask you to collect a sample of urine for us. This will help us to control your adherence to the supplementation.
NB Moreover, we advice on having a good dental care throughout the supplementation period, for this reason we suggest cleaning well your mouth and teeth after each meal and Lucozade ingestion.

Post-tests in the lab
At the end of the supplementation period all the tests will be repeated in order to find differences between baseline and post supplementation.

Timetable
Including the two weeks of baseline where you will be asked to keep only a diet diary of what you eat, this study lasts 6 weeks. For 4 weeks what you are asked to do is only to keep a diet diary and drink the SSBs provided.

Possible risks of taking part:
You might experience some discomfort associated with the muscle biopsy. Muscle biopsy might cause some bleeding. We will give you a special plaster which reduces the risk of bruises. Although, we have never seen this to occur, there is a slight possibility of infection related to the muscle biopsy. In this case antibiotic should resolve things quickly. You also might experience discomfort during blood sampling. The blood sampling might be stressful for people who do not like needles or seeing blood. However, one blood sample is often as stressful as being stung by a thorn of a rose. High consumption of SSBs may provoke slight weight gain (0.5 kg; 1.1 pounds in 4 weeks). All these effects are reversible when the supplementation is stopped and when a normal diet and physical activity are reintroduced.

Possible benefits of taking part:
An economic benefit of £100 will be provided in case you carry out the study. By taking part in this study you will have the possibility to find out valuable information about your health.
Your participation in this study will provide us with valuable insights into how the human body and mind adapts to drinking SSBs. It will add to our understanding of why some people develop common conditions like diabetes and obesity which are major health problems in this day and age.

Confidentiality
All information which is collected about you during the course of the research will be kept strictly confidential. Any information which leaves the School will have your

name and address removed so that you cannot be recognised from it. It will not be possible to identify you in any report or publication of the study.

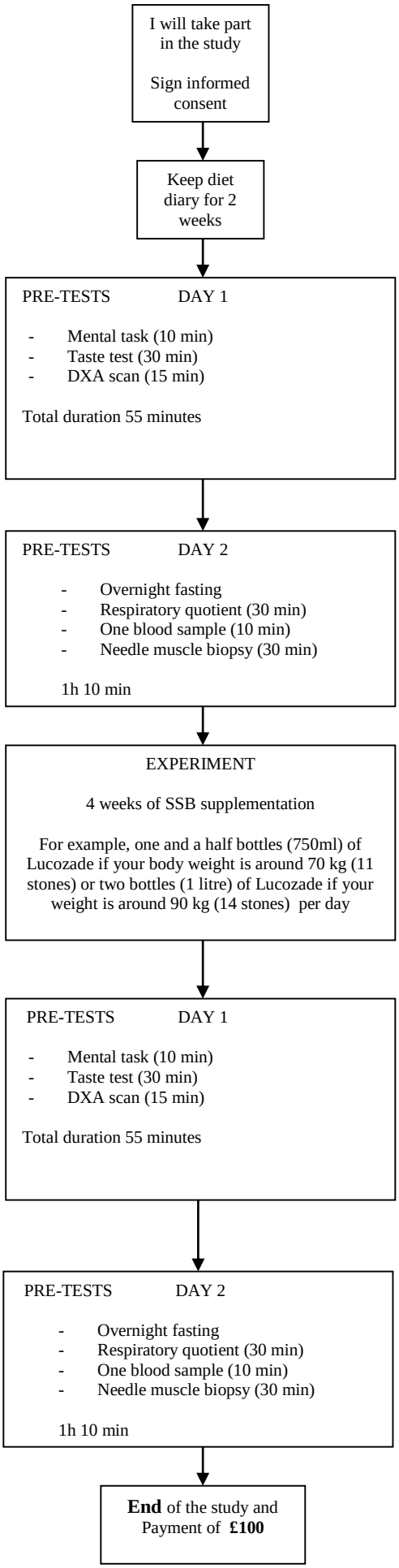
Who is organising or funding the research?
This study has been organised by Mr Francesco Sartor and Dr Hans-Peter Kubis from the SSHES of Bangor University, in collaboration with Dr David Markland from SSHES and Dr Verena Matschke, Rheumatology research fellow, from SSHES too, and in collaboration with Dr John Parkinson from the School of Psychology of Bangor University and Prof Lucy Donaldson from the Department of Physiology and Psychopharmacology Unit of the University of Bristol.

Who has reviewed the study?
This study has been approved by the SSHES Ethics Committee.

Any Questions?
Please ask us if you have any questions.

Principal investigator: Francesco Sartor, pep421@bangor.ac.uk , (01248) 388 254.
Chief investigator: Dr Hans-Peter Kubis, pes203@bangor.ac.uk, (01248) 388 261.

For any complaints please contact Prof Michael Khan m.khan@bangor.ac.uk, (01248) 38 8275


I will take part in the study
Sign informed consent
Keep diet diary for 2 weeks
PRE-TESTS DAY 1
- Mental task (10 min)
- Taste test (30 min)
- DXA scan (15 min)
Total duration 55 minutes
PRE-TESTS DAY 2
- Overnight fasting
- Respiratory quotient (30 min)
- One blood sample (10 min)
- Needle muscle biopsy (30 min)
1h 10 min
EXPERIMENT
4 weeks of SSB supplementation
For example, one and a half bottles (750ml) of Lucozade if your body weight is around 70 kg (11 stones) or two bottles (1 litre) of Lucozade if your weight is around 90 kg (14 stones) per day
PRE-TESTS DAY 1
- Mental task (10 min)
- Taste test (30 min)
- DXA scan (15 min)
Total duration 55 minutes
PRE-TESTS DAY 2
- Overnight fasting
- Respiratory quotient (30 min)
- One blood sample (10 min)
- Needle muscle biopsy (30 min)
1h 10 min
End of the study and Payment of £100

APPENDIX A.II

Participant information sheet for 4 week study

1. **Title**

   The effect of a 4 week training programme on metabolism and cognitive reactions (Reaction Time) in lean and overweight inactive women.

2. **What is the purpose of the study?**

   The aim of this study is to find out the effects of 4 weeks of circuit training on metabolism and cognitive reactions (reaction time) in lean inactive women. This study will hopefully bring a greater knowledge of the effect exercise has on metabolism and cognitive reactions (reaction time) in inactive lean and overweight women.

3. **Why have I been chosen?**

   You have been chosen because of the information you gave us in the pre-screening questionnaire matches that required for participation in this study.

4. **Do I have to take part?**

   Your taking part in this study is entirely voluntary. It is up to you to decide whether or not to take part. If you 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 reason.

5. **What do I have to do?**

   As a participant, you will be asked to complete a food diary 3 days a week for 8-weeks.

   Pre-testing will consist of a cognitive (reaction time) test, a body composition analysis (DEXA scan), a resting capillary blood sample from the earlobe, and maximal exercise ($\dot{V}O_2max$) test. These tests in total will take between 60 and 90 minutes.

   Half of the participants will be asked to take part in a 4 week circuit training intervention, working at 70% individual $\dot{V}O_2max$ 3 times a week for one hour and continue to complete food diaries on the same 3 days each week for 8-weeks. The other half of the participants will only complete the food diaries.

After those 4 weeks all participants will re-take the cognitive (reaction time) test, DEXA scan and $\dot{V}O_2max$. The results will be compared with the initial test results.

Although your results will be used within the final report, the information you provide will remain anonymous. Results, therefore, will not be attributed to a specific individual.

6. Benefits of taking part?

You will complete two $\dot{V}O_2max$ tests, this fitness assessment cost £200-250 if assessed privately. Your body composition will be assessed using a DEXA scan. This is the most accurate measurement of body fat, muscle and bone mass currently available and is not available as a measure of body composition to the general public.

Food diaries are routinely used to assess current dietary habits and can identify any areas for improvement. A 7-day diet analysis can cost around £40.

Participants taking part in circuit training will receive 3 supervised training sessions each week for 4-weeks. These sessions can cost £2.50 - £5 at local leisure centres.

7. Who is organising the Study?

This project is being organised by Kirstie Tew and Kholoud Alabduljader at the School of Sport Health and Exercise Science, Bangor University.

8. Contacts for further information

Kirstie Tew (MSc student)	pepa14@bangor.ac.uk	07849229997
Kholoud Alabduljader (MSc student)	elpaab@bangor.ac.uk	07759893217
Supervised by:		
Dr. Hans-Peter Kubis (Senior lecturer)	pes203@bangor.ac.uk	(01248) 388261
Dr. David Markland (Senior lecturer)	d.a.markland@bangor.ac.uk	(01248) 383487

APPENDIX A.III

Participant information sheet for 8 week study

Study Title: Effects of exercise intensity on substrate utilisation and cognitive function.

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and take time to decide whether you wish to take part, or not.

Please contact the researchers if anything is unclear or you would like more information.

Matthew Jackson pepa2f@bangor.ac.uk Tel: 01248388254

Fardin Fatahi pepc09@bangor.ac.uk Tel: 01248388254

What is the background of the study?

The main aim of this study is to investigate the effects of exercise intensity on substrate utilisation and cognitive function in inactive women, with a range in BMI. Specifically, we will analyse how exercise of high and low intensity affects the body's metabolism and how quick you can recognise visual cues and perform simple cognitive tasks. Substrate utilisation is the composition of fuel we use, both when exercising and at rest, this fuel can be derived from either carbohydrate, protein or fat and the amount of each can alter based on;- energy requirements, energy balance, dietary composition, body composition and aerobic capacity. It is known that regular exercise is beneficial for cardiovascular fitness and enhances cognitive performance. The effects of exercise on substrate utilisation may be one of the underlying mechanisms responsible for these beneficial outcomes. An 8 week exercise intervention will be used to test these assumptions. A further aim of the study is to investigate the influence of different exercise intensities on cognitive function e.g. reaction times in a task combining pictures and words in a computer based test.

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 free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

Summary

You will be asked to keep a diet diary for one week before the study begins, in which you will need to write down precisely and accurately what you have had to eat and drink each day. After this the first bout of testing will take place;- these tests will include a dual-energy x-ray absorptiometry (DEXA) scan to assess your body composition, a VO2 max test to measure your aerobic/endurance fitness, analysis of resting metabolic rate, blood sampling before and after a test meal, and a cognitive function task. The tests will be completed over three visits to the lab. Each of these tests are discussed in greater depth below. Once the preliminary testing has been carried out the exercise intervention begins. You will be asked to attend exercise sessions 3 times a week for an hour or so on each occasion. These exercise classes will be conducted in a group format, and exercise will be done in a circuit training fashion. The exercise programme will last for 8 weeks in total, during this time you will also need to fill out a diet diary for two days of each week. Once the 8 week training period is finished, a second bout of testing will take place, in exactly the same format as the first with all the same tests. You will then be asked to fill out a diet diary once again as before. In the case, you are in the control group; you will carry out all testing before and after the training period but you will not take part in the exercise programme.

Diet Diary

We will give you a Food Record Sheet (FRS) where you should describe with accuracy and precision your daily food and beverage intake. You will be asked to keep a record of your diet for 7 days pre-intervention, 7 days post-intervention, and 2 days per week during the intervention. The 2 days of each week during the intervention randomly chosen. How to describe foods and drinks will be fully explained in the Food Record Sheet. The analysis of your food composition is necessary to exclude influence of certain foodstuff on measures we take. It is fine to eat whatever you wish, but be sure to note everything that you eat on your food record sheet.

Cognitive Function Task

You will be asked to undergo a computerised test of the speed with which you respond to pictures of different objects. This will allow us to assess your cognitive function and whether it is affected by exercise. The test will last for approximately 20 minutes.

DEXA Scan

A whole body low radiation (DEXA) scan will be performed to assess how much bone, fat and muscle mass you have. For this procedure you need to wear shorts, socks, underwear (no bra) and t-shirt. The scan will last 15 minutes. This procedure is painless, and it is not more than lying down on a bed. Although there are small risks involved in radiation exposure, the amount of exposure during a DEXA scan is minimal and is lower than the amount of background radiation that we are exposed to during the course of two weeks outside in North Wales.

VO2 Max Assessment

VO2 max is a measure of your aerobic capacity and it is achieved with an incremental exercise test. You will begin by cycling at a fixed speed and a low resistance, and with every minute of the test the resistance will be increased slightly so the exercise becomes harder. The resistance will be increased until you have reached maximal exertion and can carry on no longer. During the test will be monitoring your heart rate, oxygen uptake and carbon dioxide production, The latter two will be measured with a breath by breath gas analyser, which collects the air you breath in and out. This is necessary so we can gain an accurate measure of your cardiovascular fitness. The test will normally last no longer than 30 minutes.

Resting Metabolic rate

You will be asked to arrive at the laboratory in an overnight fasted state and then to lie on a bed for about 30 minutes. As with the VO2 max test we will measure your oxygen uptake and carbon dioxide production with a breath by breath gas analyser. This test is necessary to find out more about your metabolism.

Blood Sampling and Test Meal

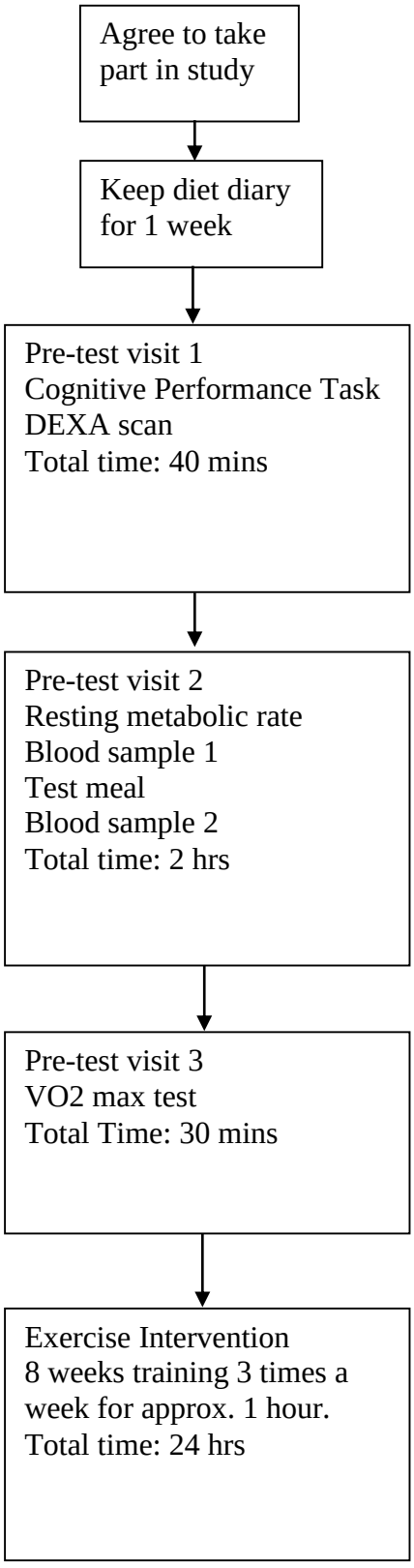
We will take two blood samples (i.e. pre and post-meal) from a vein of your forearm, on two separate occasions (i.e. pre and post exercise intervention). A small amount (8 ml) of blood will be collected (0.2% of the total blood volume). We will use sterile needles to draw the blood samples. It is very important that you have fasted overnight. Afterwards, we will give you a test meal which will be in the form a milk shake. Then one hour after the test meal we will take the second blood sample. The blood samples will be use to measure hormone levels in your blood, which will give us important information about how exercise alters the way foodstuff is consumed in your body.

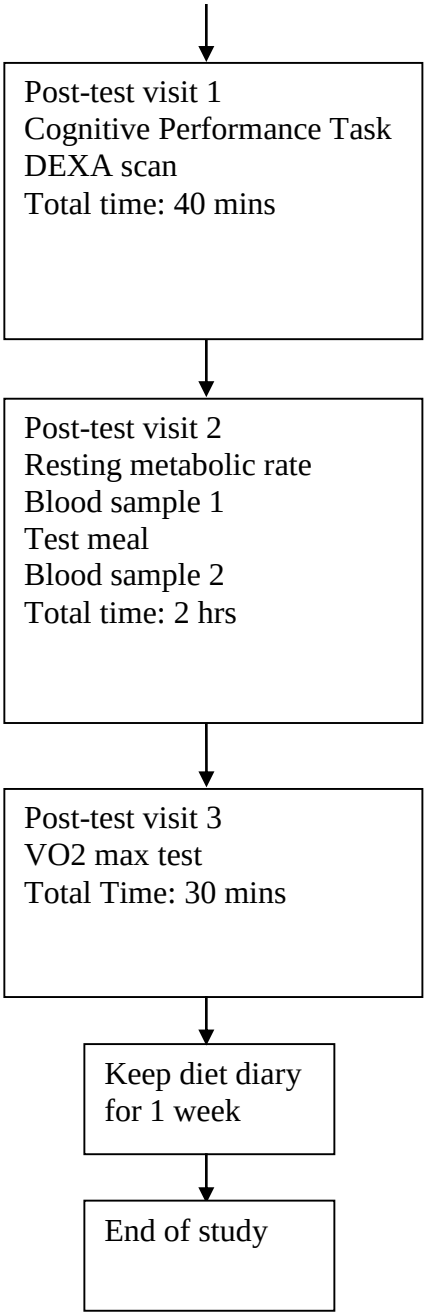
Exercise Intervention

Once you have completed all the baseline measures, we will begin the exercise intervention. As stated above, the exercise programme will last for 8 weeks and you will be asked to attend 3 exercise sessions per week, each exercise session will last for an hour or so and participants will train as a group. There will be 3 groups in this

study, a high intensity exercise group, a low intensity exercise group and a control. Each participant will be randomly allocated to one of these groups for the entire duration of the study. Exercise sessions will be in a circuit training format, this is where a series of exercise stations are completed one after another until a whole circuit is completed. During exercise sessions you will also be required to where chest belts so that we can monitor your heart rate. Finally, exercise sessions will take place at Normal Site Sports Hall, you will need to make your own way there.

Timeline




Post-test visit 1
Cognitive Performance Task
DEXA scan
Total time: 40 mins
Post-test visit 2
Resting metabolic rate
Blood sample 1
Test meal
Blood sample 2
Total time: 2 hrs
Post-test visit 3
VO2 max test
Total Time: 30 mins
Keep diet diary
for 1 week
End of study

What are the possible disadvantages and risks of taking part?

You may experience some discomfort during blood sampling. The blood sampling might be stressful for people who do not like needles or seeing blood. You may also find the VO2 max testing somewhat uncomfortable as you are required to exercise up to maximal exertion. There is a relatively large time commitment for this study, pre-testing and post-testing will last for just over 3 hours each and you will need to exercise for approximately 3 hours per week for 8 weeks.

What are the possible benefits of taking part?

By taking part in this study you have the opportunity to join in a comprehensive 8 week exercise intervention with a team of sport scientists who are passionate about the benefits of exercise to health and who have many years of combined experience in fitness training. You have a chance of reducing the possible risks of lifestyle related diseases associated with low physical activity and improving your overall health. Other benefits of exercise include improved cardiovascular fitness and metabolism, and possibly cognitive performance. Your participation in this study will also provide us with valuable insights into how the human body and mind adapts to exercise and will add to our understanding of how exercise positively influences health. In addition to the above, upon completion of the study you will be eligible for a free pair of trainers of your choice up to the value of £100 (these will be chosen from wiggle.co.uk) as a reimbursement for your effort and time commitment.

Confidentiality

All information which is collected about you during the course of the research will be kept strictly confidential. Any data which is to leave the school, for possible publications or reports, will have your name and any other personal information removed so that you cannot be recognised from it. It will not be possible to identify you in any of these reports or publications.

Who is organising or funding the research?

This study has been organised by Matthew Jackson (PhD student), Fardin Fatahi (PhD student), Dr Hans-Peter Kubis (Senior lecturer) and Dr David Markland (Senior lecturer) from the School of Sport, Health and Exercise Sciences (SSHES) of Bangor University.

Who has reviewed the study?

This study has been reviewed by the SSHES Ethics Committee and North Wales Research Ethics Committee – West.

Feedback and conduct of research

SSHES is always keen to hear the views of research participants about their experience. If you would like to give feedback, please ask your researcher to provide you with Form 6 – Participant Feedback Form – from the Ethics Guidelines Handbook. Completion of this form is optional and can be done anonymously. The completed form should be returned to Dr Andrew Lemmey, Chair, SSHES Ethics Committee, SSHES, Bangor University, Bangor LL57 2PZ. All information will be treated in a strictly confidential manner.

Complaints

If during the course of this study you feel the need to complain about how the research has been carried out, for any reason, then please contact the Head of School, Dr Tim Woodman, SSHES, Bangor University, Bangor LL57 2PZ.

Any questions?

Please ask us if you have any questions. Here are the contact details of all investigators in this study:

Matthew Jackson (PhD student) pepa2f@bangor.ac.uk Tel: 01248 38 8254
Fardin Fatahi (PhD student) pepc09@bangor.ac.uk Tel: 01248 38 8254
Dr Hans-Peter Kubis (Senior Lecturer) pes203@bangor.ac.uk Tel: 01248 38 8261
Dr David Markland (Senior Lecturer) d.a.markland@bangor.ac.uk Tel: 01248 38 3487

APPENDIX B.I

Lifestyle questionnaire for sugar-sweetened beverage study

Please take a few minutes to read the following introduction:

This questionnaire has been designed to recruit new participants for our nutrition related Research Study. Once you have filled in this questionnaire we will be able to see whether you are eligible as a possible participant. In case you are eligible we would like to get in touch with you. So please leave contact details at the end of this questionnaire. It is up to you to decide whether or not to take part. If you decide to take part you will be given an information sheet to keep and be asked to sign a consent from. If you decide to take part you are free to withdraw at any time and without giving a reason. This will not affect the marks you receive, the outcome of your period of study at SSHES or your standing with your supervisor, other staff or with the School. The outcome of this questionnaire will be held confidential by the researcher.

In case you complete the entire study you will be reimbursed with £ 100.

Please ask at any time if you feel anything is unclear or contact:
Francesco Sartor *pep421@bangor.ac.uk* *tel:* 01248 388254

How many cigarettes do you smoke per day?

1) none
2) less than a pack
3) a pack
4) more than a pack

How much physically active are you?

1) physically inactive (little or no exercise)
2) lightly active (light exercise or sports 1-3 days a week)
3) moderately active (moderate exercise or sports 3-5 days a week)
4) very active (hard exercise or sports 6-7 days a week)

How many steps do you think you walk on average per day?

1) less than 5000
2) between 5000 and 7000
3) between 7000 and 10000
4) more than 10000

How often do you drink soft drinks?

1. more than 4 pints (or 2 litre) a week
2. 1 to 2 pints a week
3. less then 1 pint (or a can) a week
4. none

How much water do you drink per day?

1) about 4 pints (about 2 litres)
2) more than 4 pints (more than 2 litres)
3) less than 4 pint
4) less than 1 pint

How many times do you eat fish per week?

1) More than 3
2) 2-3
3) 1
4) 0

How much alcohol do you drink per week?
NB (500 ml, a can of lager 4% alcohol is 2 units; 160 ml, a glass of wine 12.5% alcohol is 2 units; 125 ml, party cocktail 40% is 5 units)

1) more than 30 units
2) 10 to 30 units
3) 2 to 10 units
4) less then 2 or none

What sport/s do you practice?

1 ……
2 ……
3 ……

Do you drink sports drinks, if yes how often?

1) once in a while
2) once a week
3) more than once a week
4) every day

Do you eat vegetables, if yes how often?

1) every day
2) once in a while
3) more than once a week
4) once a week

If you train: how long does it take on average each training session?

…….min

When you go upstairs do you use the lift?

1) always
2) 50%-50%
3) I prefer the stairs (70%-30%)
4) never

Do you eat red meat? How often?

1) every day
2) never
3) once or twice a week
4) every other week

Do you eat potatoes and/or pasta? How often?

1) never
2) every day
3) every day twice a day
4) sometimes

How much fruit do you eat?

1) none
2) one piece a day
3) 1 to 3 pieces a day
4) more than 3 pieces a day

How often do you drink fruit juices?

1) more than 4 pints (or 2 litre) a week
2) 1 to 2 pints a week
3) less then 1 pint (or a can) a week
4) none

Please, if interested remember to leave a contact telephone/mobile number or e-mail address:

THANK YOU

APPENDIX B.II
Pre-screening questionnaire for exercise studies

Name of Participant: ……..…… Date of Birth: .....

Height: ……..
Email:……...

Weight: ..... Contact No:…….

Nationality:……..

HEALTH:

1. Are you in good health? YES ☐ NO ☐

   If no, please explain:

2. Have you suffered from a serious illness or accident? YES ☐ NO ☐

   If yes, please provide details:

3. Do you suffer or have you ever suffered from:

	YES	NO
Asthma		
Bronchitis		
Diabetes		
Epilepsy		
Heart Condition		
High Blood Pressure		
Bone or Joint Problem		

4. Are you currently taking medication? YES ☐ NO ☐

   If yes, please provide details:

5. Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? YES NO

   If yes, please provide details: ☐ ☐

6. Do you have any allergies? YES NO

   If yes, please provide details: ☐ ☐

7. Menstruation:
   a. Please indicate your current menstrual status:

      ☐ No menstruation

      ☐ Irregular menstruation

      ☐ Regular menstruation

   b. How many days since your last menstruation? .....

   c. Do you currently take an oral contraceptive? YES NO

      ☐ ☐

PHYSICAL ACTIVITY:

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< once per month		2 times per week	
once per month		3-4 times per week	
2 times per month		> 5 times per week	
1 times per week			

NUTRITION:

1. How would you describe your current diet:

   Westernised ☐

   Traditional ☐

2. Are you currently:

   Vegetarian YES ☐ NO ☐

   Vegan YES ☐ NO ☐

3. Are you currently attempting to lose weight? YES ☐ NO ☐

Have you, or are you presently taking part in any other laboratory experiment?

YES ☐ NO ☐

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature ……. Date ……..
Participant

Signature ……. Date ……..
Experimenter

APPENDIX C.I
Example circuit training session

WARM-UP (10mins):

Moving in a circle around the sports hall, progress through:

- Walk
- Mobilisation while walking
    - Arm swings forwards
    - Arm swings backwards
    - Pass the rugby ball body rotation
    - "Opening the gate" legs
    - "Closing the gate" legs
- Jog
- High knees
- Butt kicks
- Jog

SPRINTS:

Put the exercisers into 2 equal sized teams for races. During the race, those not running should do the exercise in brackets.

1. Shuttle runs (star jumps)
2. Side shuffle (squats)

CIRCUIT:

- 12 stations in a circuit around the sports hall
    - Squat jumps
    - Leg raises
    - Triceps dips
    - Toe touches
    - Abdominal bike
    - Press-ups
    - Step-ups
    - Dorsal raises
    - Star jumps
    - Ladder climb
    - Lunges
    - Plank

- Circuit 1: 45-seconds exercise/15-seconds rest
- Circuit 2: 30-seconds exercise/15-seconds rest
- 3-minutes rest between circuit 1 and 2.

COOL-DOWN (5-10minutes):
Moving in a circle around the sports hall, progress through:

- Jog
- Walk
- Mobilisation while walking
    - Arm swings forwards
    - Arm swings backwards
    - Pass the rugby ball body rotation
    - “Opening the gate” legs
    - “Closing the gate” legs
- Stretches
    - Neck
    - Cross-chest shoulder stretch
    - Triceps stretch
    - Side stretch
    - Quadriceps
    - Hamstrings
    - Glutes