Lancaster University
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Determining the Dose-Response Relationship between Exercise and Glycaemic Control and Examining Exercise as a Treatment for Type 2 Diabetes.

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Declaration

All of the data presented within this thesis were collected, analysed and presented by myself unless otherwise stated below.

In section 3.1.9, Medical Doctors from Lancaster Royal Infirmary inserted retrograde cannulas and withdrew the blood samples from participants for analysis. In section 3.2.4, Miss Kate Rattley acted as a second independent researcher to screen and assess the eligibility of trials to be included in the meta-analysis.

I declare that all of the data presented is my own work unless stated otherwise and this thesis was constructed by myself. Appropriate referencing has been used for all the published literature referred to within this thesis. None of the data presented within this thesis has previously been submitted for assessment towards a higher degree.

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Glossary

Abbreviation	Meaning
Kcal	Calorie
HbA1c	Glycosylated Haemoglobin
B	Beta
NHS	National Health Service
BMI	Body Mass Index
PKC	Protein Kinase C
AKT/PKB	Protein Kinase B
ATP	Adenosine triphosphate
K^+	Potassium
GLUT4	Glucose Transporter 4
Ca^{2+}	Calcium
TCA cycle	Tricarboxylic Acid Cycle
IAAP	Islet Amyloid Peptide
HR	Heart Rate
AMPK	AMP-Activated Protein Kinase
mRNA	Messenger Ribonucleic Acid
IL-1β	Interleukin-1 Beta
INSR	Insulin Receptor
A	Alpha
IRS	Insulin Receptor Substrate
MAPK	Mitogen Activated Protein Kinase
PI3K	Phosphoinositide 3-Kinase
PTB domain	Phosphotyrosine Binding Domain
PIP_3	Phosphatidylinositol Triphosphate
PIP_2	Phosphatidylinositol Biphosphate
PDK	Pyruvate Dehydrogenase Kinase
GIP	Glucose-dependent Insulinotropic Polypeptide
GLP-1	Glucagon-like Peptide 1
GIPR	Glucose-dependent Insulinotropic Polypeptide Receptor
cAMP	Cyclic Adenosine Monophosphate
LPL	Low-density Lipoprotein
HDL	High-density Lipoprotein
FFA	Free Fatty Acids
MAG	Monoacylglycerol
VLDL	Very Low-density Lipoprotein
IDL	Intermediate Density Lipoprotein
TNF	Tumour Necrosis Factor
PTPs	Protein Tyrosine Phosphatases
OGTT	Oral Glucose Tolerance Test
CPET	Cardiopulmonary Exercise Testing
RER	Respiratory Exchange Ratio
ECG	Echocardiogram
PPE	Personal Protective Equipment

SMD	Standardised Mean Difference
SD	Standard Deviation
PEDro	Physiotherapy Evidence Database
RCT	Randomised Control Trial
RPM	Revolutions per Minute
RPE	Rate of Perceived Exertion
ROS	Reactive Oxygen Species
AGE	Advanced Glycation End Products
DAG	Diacylglycerol
NO	Nitrogen Oxide
TLR	Toll-like Receptors
THL	Tetrahydrolipstatin
EGTA	Ethylene Glycol Tetraacetic Acid
CV	Coefficient of Variation
µl	Microlitre
ACSM	American College of Sports Medicine
VO_2	Oxygen Consumption
FOXO	Forkhead Box Transcription Factors

1. Abstract

Objectives

It is well known that exercise contributes to the beneficial regulation of glycaemic control. However, the dose-response relationship between exercise and glycaemic control is currently unclear. This study therefore aimed to identify the dose-response relationship between exercise and glycaemic control.

Methods

A clinical trial was carried out where participants were required to carry out four doses of exercise (0, 175, 350 and 700 kcal) to investigate the impact of each dose of exercise on glycaemic control. Due to the Covid-19 pandemic this clinical trial is still ongoing.

A meta-analysis and meta-regression were carried out to investigate the effects of exercise dose on glycaemic control. Volume and intensity were used as modifiers in the meta-regression to analyse their association.

Results

The clinical trial is still in its early stages due to the Covid-19 pandemic, but research so far has suggested that 350 kcal of exercise could improve glycaemic control 24hrs post-exercise when compared to no exercise.

The meta-analysis shows that an exercise programmes improve glycaemic control and BMI in participants with type 2 diabetes. The meta-regression shows no statistically significant associations between the two variables volume and intensity, however, there could be a potential trend between volume and HbA1c.

Conclusions

The meta-analysis and meta-regression show that exercise positively benefits glycaemic control. The volume of exercise may be important when investigating the optimal dose of exercise required to treat type 2 diabetes as an association between

higher volumes and improvements in glycaemic control was indicated by the meta-regression.

2. Introduction

2.1. What is type two diabetes?

Type 2 diabetes occurs when cells no longer respond properly to insulin (loss of insulin sensitivity) and this is accompanied by inadequate secretion of insulin to compensate as the disease progresses and β cells fail (Colberg et al., 2010). It occurs in response to chronic hyperglycaemia which results in insulin resistance and eventual pancreatic β cell dysfunction, disrupting the overall metabolic homeostasis in the body (Hameed et al., 2015). These two factors, insulin resistance at target tissues and impaired insulin secretion from β cells, are the main features of type 2 diabetes (Hameed et al., 2015). Type 2 diabetes results from a combination of genetic and environmental factors but genetic factors are unable to account for the rapid increase in recent years (Tuomi et al., 2014). The genes specific to an individual may determine how susceptible they are to the modifiable environmental factors (Dietrich et al., 2019). An individual's environment, for example, physical activity, diet, and stress levels, are likely to either counteract or enhance certain genes individuals have which may predispose them to a higher risk of type 2 diabetes (Dietrich et al., 2019). Therefore, it is thought that environmental factors are more important when investigating this global epidemic (Tuomi et al., 2014).

2.2. Prevalence of type two diabetes-

The number of people with diabetes has more than doubled in the last 20 years (Zimmet et al., 2014) and type 2 diabetes accounts for around 90% of all diagnosed diabetes cases (Saeedi et al., 2019). In 2019, 9.3% of the adult population were diagnosed with diabetes (Saeedi et al., 2019). Those with the disease are at risk of multiple pathologies. They experience a two to three-fold increase in the risk of heart attacks and strokes (Sarwar et al., 2010). Diabetes is one of the leading causes of

kidney failure (Collins et al., 2015) and 2.6% of global blindness occurs in those with the disease (Bourne et al., 2013). It is predicted that, due to the ageing population, by 2038 there will be 20% more cases than there was in 2000 (Bagust et al., 2002) and in 2017, diabetes contributed to 1.8% of global gross domestic product (Bommer et al., 2017). Diabetes will pose a major clinical and financial challenge to the NHS with the annual cost hospital services for those with diabetes estimated to be around £3bn in 2019 (Stedman et al., 2020). Due to the ageing population, the economic burden of this cost is likely to be unfairly and disproportionately supported by the decreasing economically productive age groups (Bagust et al., 2002).

2.3. Risk factors

There are both modifiable and non-modifiable risk factors of diabetes; non-modifiable risk factors including age, genetics and ethnicity (Bellou et al., 2018). It is the modifiable risk factors which primarily contribute to the pathogenesis of the disease. These include body mass index (BMI), physical inactivity, poor nutrition, hypertension, smoking and alcohol use (Bi et al., 2012). Physical inactivity and poor nutrition help contribute to higher BMI, and it is the higher dietary glycaemic load and trans-fat intake which is associated with this that contributes to increased diabetes risk (Kyrou et al., 2020). Three hours of TV viewing per day has detrimental effects on overall health and considerably increases the risk of type 2 diabetes (Patterson et al., 2018), and those who participate in recommended levels of moderate exercise reduce their risk of developing diabetes by 26% (Mayor, 2016). Although moderate consumption of alcohol has been shown to decrease risk of diabetes, heavy drinkers have an increased risk (Bi et al., 2012). Type 2 diabetes risk is also 3.27 times higher in individuals that smoke (Campagna et al., 2019). Nicotine exposure from smoking has also been shown to cause beta-cell dysfunction and apoptosis (Bi et al., 2012). Certain psychosocial factors also show evidence of increasing the risk of diabetes, this includes depression, increased stress and lower social support (Deshpande et

al., 2008). Research has found that those who have a history of a major depressive disorder experience a two-fold increase in the risk of diabetes (Arroyo et al., 2004).

2.4. Pathophysiology of diabetes

The feedback system between the β cell and insulin sensitive tissues tightly regulates glucose homeostasis (Scheen, 2003). In healthy individuals, β cells are stimulated to release insulin so that insulin can then mediate the uptake of glucose, amino acids, and fatty acids into tissues (Kahn et al., 2014). A large majority of people suffering from type 2 diabetes are obese, with central visceral adiposity (Scheen, 2003) and there is an inverse linear relationship between BMI and the age at which type 2 diabetes is diagnosed (Galicia-Garcia et al., 2020). The adipose tissue in obese individuals helps to promote insulin resistance (Galicia-Garcia et al., 2020). This is due to various inflammatory mechanisms, including an increase in free fatty acid release and adipokine deregulation (Galicia-Garcia et al., 2020). When insulin resistance is present, the β cells must increase the concentration of insulin released to maintain glucose uptake (Kahn et al., 2014) (Figure.2). Glucotoxicity, lipotoxicity and glucolipotoxicity are also present in obese individuals, and all of these factors help induce metabolic and oxidative stress which damages β cells (Galicia-Garcia et al., 2020). Glucotoxicity is caused by an excess of glucose which has detrimental effects on cells (Kaiser et al., 2003), lipotoxicity results from lipid accumulation in non-adipose tissue (Engin, 2017) and glucolipotoxicity is the combination of both which results extremely harmful effects on cells and tissues (Weir, 2020). β cell dysfunction means that β cells cannot sustain the increased insulin output needed to maintain glucose homeostasis (Kahn et al., 2014). This causes plasma glucose levels to rise and the magnitude of β cell dysfunction correlates to the degree of elevation in glucose concentration (Kahn et al., 2014). β-cell deficit and β-cell apoptosis further develop in those with type 2 diabetes as the disease progresses,

and along with insulin resistance and impaired insulin secretion, this disrupts the homeostasis in which insulin communicates with cells (Scheen, 2003).

2.5. Metabolism in healthy individuals

2.5.1. Glucose stimulated insulin secretion

Insulin, along with glucagon, is a key regulator of glucose homeostasis, and the intracellular network of insulin secretion is multifactorial. High concentrations of plasma glucose initiate an adenosine triphosphate (ATP)-sensitive potassium (K^+) channel-dependent mechanism which causes insulin to be initially secreted (Komatsu et al., 2013). A K^+-ATP channel-independent mechanism then gradually takes over. Insulin is secreted by β-cells in the islets of Langerhans of the endocrine pancreas (Kulkarni, 2004). When plasma glucose concentrations increase, glucose enters cells through the glucose transporter 4 (GLUT-4) (Navale & Paranjape, 2016). Once in the cytoplasm, glucokinase phosphorylates glucose to glucose-6-phosphate (Karwi et al., 2020). Glucose-6-phosphate then enters the glycolytic pathway to produce pyruvate (Ahmed, 2016). This pathway subsequently produces ATP. Pyruvate dehydrogenase and pyruvate carboxylase metabolise pyruvate and transfer it to the mitochondria for the tricarboxylic acid cycle to take place (Figure 1) (Komatsu et al., 2013). The transfer of pyruvate is also associated with the efflux of tricarboxylic cycle intermediates (Komatsu et al., 2013).

Glycolysis causes the concentration of ATP to increase, this results in ATP-sensitive K^+ channels closing and membrane depolarisation occurring (Ahmed, 2016). L-type voltage-dependent calcium (Ca^{2+}) ion channels open, due to the change in membrane voltage, causing an influx of Ca^{2+} and therefore an elevation of free cytosolic Ca^{2+} concentration (Komatsu et al., 2013). It is this Ca^{2+} influx which stimulates the rapid exocytosis of insulin from granule stores near the cell membrane (Trexler & Taraska, 2017). The magnitude of this first phase secretory response is dependent on the size of the granules (Ahmed, 2016). Insulin secretion is only

sustained if it is further stimulated by metabolizable secretagogues, and other granule stores deeper within the β cell must be utilised (Ahmed, 2016; Komatsu et al., 2013). ‘Fusion and replenishment’ is a way the biphasic response of insulin can be viewed because readily releasable granules of insulin near the cell membrane are replenished by reserve pools (Figure 1) (Komatsu et al., 2013). It is thought that the time dependent increase of insulin in response to high glucose concentration is triggered by a number of metabolic stimuli, such as mitochondrial intermediates, including amino acids (Komatsu et al., 2013).

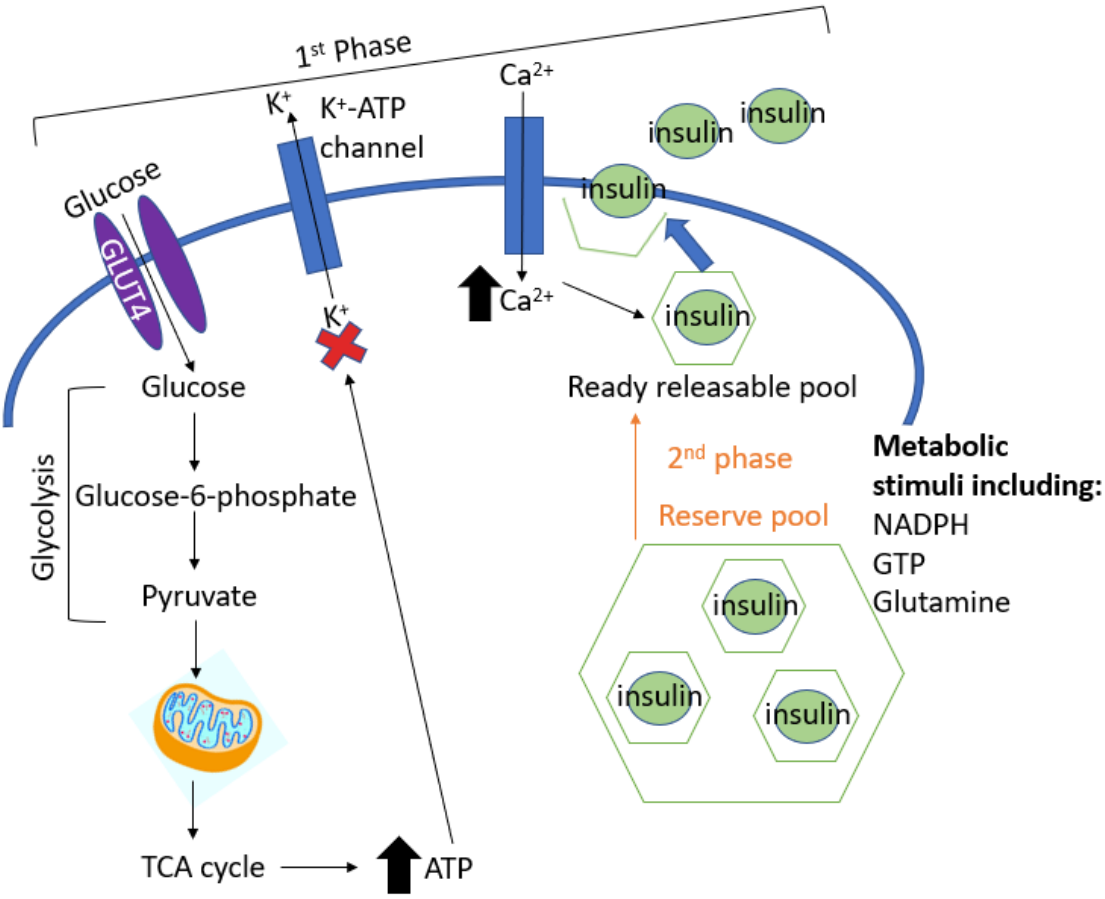


Figure 1. Glucose stimulated insulin secretion.

A high concentration of glucose within the plasma activates ATP-K^+ channels on the plasma membrane of β cells and this initiates insulin secretion at the first stage (Ahmed, 2016). *Glucose enters the cell via GLUT 4 and the glycolytic pathway begins to eventually produce pyruvate. Increases in ATP due to glycolysis and the TCA cycle close ATP-K^+ sensitive channels which results in membrane depolarisation* (Ahmed, 2016). *This depolarisation causes Ca^{2+} channels to open and rapid influx of Ca^{2+} occurs. This increase in concentration of Ca^{2+} activates the rapid exocytosis of insulin from a readily releasable pool of granules* (Ahmed, 2016). *The 2nd stage allows insulin secretion to be sustained by a reserve pool* (Ahmed, 2016). *[Redrawn and modified from:* (la Vega-Monroy & Fernandez-Meji, 2011)*]*

When high glucose concentrations are sustained, both proinsulin biosynthesis and islet amyloid polypeptide (IAAP) concentrations increase (Röckl et al., 2008). This causes an increase in the production of reactive oxygen species caused by the

accumulation of misfolded insulin and IAAP (Röckl et al., 2008). These factors contribute to the alteration of endoplasmic reticulum Ca^{2+} mobilisation (Röckl et al., 2008). They also increase proapoptotic signalling and degradation of proinsulin mRNA due to the activation of the apoptotic protein response pathway induced through endoplasmic reticulum stress (Röckl et al., 2008). Interleukin-1 beta (IL-1β) release is also induced; this recruits macrophages contributing to immune dysregulation and increases local islet inflammation (Asano, 2014). The literature states that these factors all help contribute to the disruption of islet integrity, impair cell-to-cell communication, and subsequently, contribute to the worsening of hyperglycaemia (Galicia-Garcia et al., 2020).

It is the increasing dysfunction of beta cells which allows impaired glucose tolerance (a fasting blood glucose concentration of 6.1mmol/L and above (NICE, 2017)) to progress to type 2 diabetes (a fasting blood glucose concentration of 7.0mmol/L and above (NICE, 2017)) (Kahn et al., 2014). A range of environmental factors contribute to this progression, age, nutrition, and a sedentary lifestyle, are just a few examples. Beyond the age of 30, the human pancreas is unable to keep renewing β cells and therefore loss of beta-cells, such as due to glucolipotoxicity, explains why there is an increased risk of type 2 diabetes with age (Kahn et al., 2014).

However, even though type 2 diabetes was previously thought to only occur in the middle aged and elderly, the onset of the disease is now becoming even more prevalent in those under the age of 30 years old (Lin et al., 2020). This is thought to be due to increases in sedentary lifestyles and the adoption of western diets resulting in higher rates of obesity in the younger population (Alberti et al., 2004). Central visceral adiposity associated with obesity is an important determinant of hyperinsulinemia which occurs during the early stages in the development of type 2 diabetes (Alberti et al., 2004; Martín-Timón et al., 2014). It is therefore the industrialisation and globalisation of society inducing these changes in lifestyle which

have contributed to obesity and type 2 diabetes becoming more prevalent in the younger generation (Alberti et al., 2004). If preventative measures and effective treatments aren't put in place, then this will have significant impacts on workforce and healthcare systems when individuals are burdened with the effects of the disease (Alberti et al., 2004). Understanding the doses of exercise which help regulate glycaemic control and provide effective prevention or treatment for type 2 diabetes is therefore likely to have significant beneficial impacts on society.

2.5.2. The effect of insulin on target tissues

Insulin binds to specific receptors on the plasma membrane of target cells to initiate an anabolic response and due to changes in nutrient concentrations, a signalling pathway is activated (Petersen & Shulman, 2018). Insulin can cause diverse physiological responses depending on the cell type it binds to and this variation in response is likely to underpin the pathogenesis of insulin resistance (Haeusler et al., 2018). The insulin receptor (INSR) is a heterotetrametric receptor tyrosine kinase with two extracellular α subunits which bind insulin and two β subunits which span the membrane, each subunit containing a tyrosine kinase domain (Petersen & Shulman, 2018). The two α binding sites have negative cooperativity so only one insulin molecule binds at a time (Petersen & Shulman, 2018).

When insulin binds to the receptor it induces a conformational change in the membrane spanning β-subunit (Haeusler et al., 2018). This causes the cis-autoinhibition in the kinase activation loop to be released allowing trans-autophosphorylation of the tyrosine's in the activation loop (Petersen & Shulman, 2018). Further tyrosine phosphorylation occurs in the juxtamembrane region and this is important for the recruitment of substrates (Petersen & Shulman, 2018).

Mitogenic and metabolic signals can both be produced from the activation of the insulin receptor due to the activation of several phosphotyrosine binding proteins

(Petersen & Shulman, 2018). Most metabolic effects of insulin result from activation of the phosphoinositide 3-kinase (PI3K) pathway, connected exclusively by the phosphotyrosine binding protein- insulin receptor substrate (IRS) (Skyler, 2020). This allows the conversion of the tyrosine phosphorylation signal into a lipid kinase signal (Haeusler et al., 2018). The mitogen activated protein kinase (MAPK) pathway is involved in the regulation of gene expression and involves both IRS and Shc (Petersen & Shulman, 2018). Mitogenesis and differentiation are controlled by a combination of these pathways (Skyler, 2020).

In the metabolic pathway, IRS proteins have specific phosphotyrosine binding (PTB) domains and NH_2-terminal homology which assist the proteins in targeting INSRs when activated (Petersen & Shulman, 2018). The PTB domain of IRS binds to $pTyr^{972}$ of INSR and this allows INSR to phosphorylate a number of tyrosine residues on IRS (Petersen & Shulman, 2018). Once this phosphorylation has occurred, the PI3K pathway is initiated. The p85 or p55 regulatory subunits of PI3K bind to IRS which activates the p110 catalytic subunit of the heterodimer (Petersen & Shulman, 2018), (Skyler, 2020). The catalytic subunit then catalyses the production of phosphatidylinositol-3,4,5-triphosphate (PIP_3) from phosphatidylinositol-3,4,5-biphosphate (PIP_2) (Haeusler et al., 2018; Petersen & Shulman, 2018). The generation and accumulation of PIP_3 helps to localise downstream signalling effectors by recruiting phosphoinositide-dependent protein kinases (PDK) (Skyler, 2020). This activates 3 isoforms of AKT/protein kinase B (PKB) which can activate four downstream substrates critical to metabolism (Skyler, 2020). These are mTOR-involved in the regulation of lipid and protein synthesis, Glycogen synthase kinase 3 (GSK3)-involved in the regulation of glycogen synthesis, Forkhead Family Box O (FOXO) transcription factors- involved in glucose production vs utilisation and regulation of gluconeogenic and adipogenic genes, and AS160/TBC1D1-involved in regulating glucose transport (Figure 2) (Haeusler et al., 2018; Skyler, 2020).

Following food consumption there is an increased risk of postprandial hyperglycaemia and glucose disposal into muscle plays a major part in reducing this risk. The glucose transporter GLUT4 is stored in vesicles close to the plasma membrane (Skyler, 2020). It is the PI3K/PDK/AKT insulin signalling pathway which phosphorylates the AS160/TBC1D1 substrates and initiates the translocation of the GLUT4 containing vesicles to the plasma membrane (Figure 2) (Skyler, 2020).

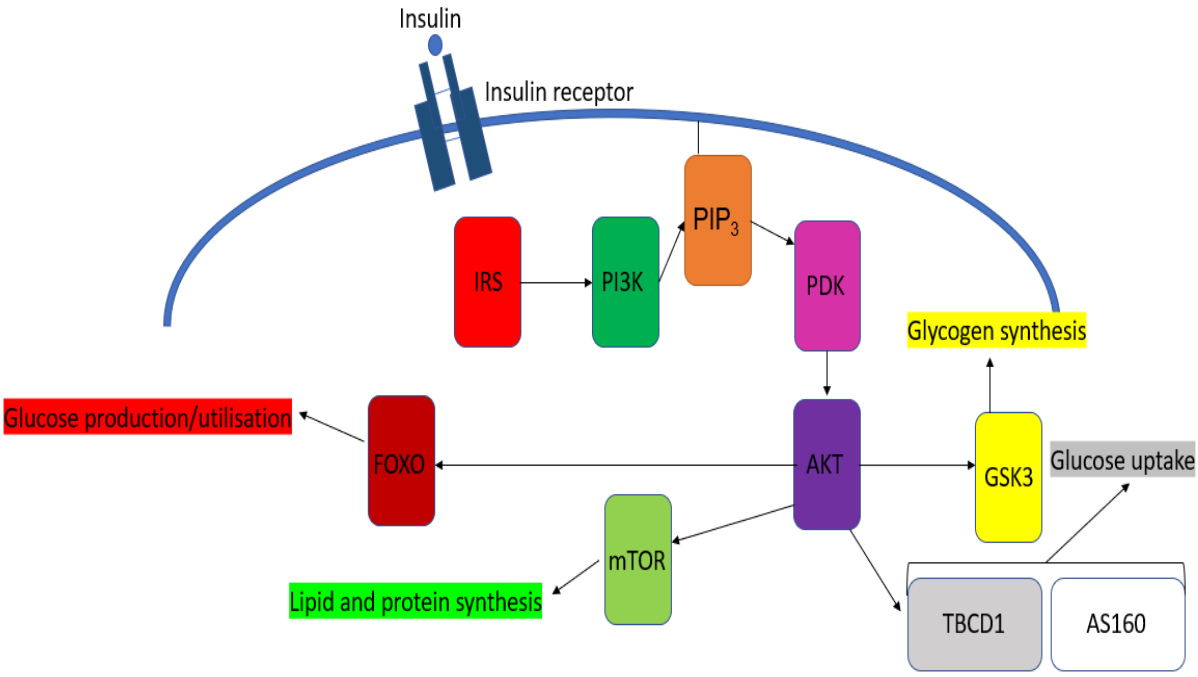


Figure 2. Insulin signalling and its downstream effectors.

Insulin binds to its receptor, INSR, which causes tyrosine phosphorylation and a conformational change of the receptor (Petersen & Shulman, 2018)*. This activates IRS which converts the tyrosine phosphorylation signal into a lipid kinase signal via recruitment of PI3K* (Haeusler et al., 2018)*. This enzyme catalyses the production of PIP_3 which ultimately activates AKT* (Petersen & Shulman, 2018)*. Once activated ATK phosphorylates the substrates, FOXO, mTOR, GSK3 and TBCD1/AS160 via their Ser/Thr residues which activates a range of mechanisms involved in glucose uptake, utilisation and production, glycogen synthesis, and lipid and protein synthesis* (Haeusler et al., 2018)*.* [Diagram redrawn and modified with information from: (Haeusler et al., 2018)]

2.5.3. The role of incretins in glucose homeostasis

The incretin hormones are released from enteroendocrine cells into the blood after a meal is consumed. After oral glucose intake, they are responsible for 50% of the total secretion of insulin (Kim & Egan, 2008). The incretin hormones consist of glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), both of which exhibit glucose dependent insulinotropic activity (Kim & Egan, 2008). This means the effect they have on the pancreas brings about a three-fold amplification of

glucose induced insulin release and the hormones alone do not cause hypoglycaemic episodes (Ahmed, 2016).

GIP is synthesised and released from K cells present in the jejunum and duodenum of the small intestine, and food containing glucose or fat stimulates its release (Ahmed, 2016). GIP binds to its specific receptor (GIPR) on β cells (Kim & Egan, 2008). This binding is positively coupled to increases in intracellular cyclic adenosine monophosphate (cAMP) and Ca^{2+} levels which mediate a rise in insulin secretion (W. Kim & Egan, 2008), (Ahmed, 2016). GIP also has other roles, it is involved in fat metabolism at adipocytes, helps to moderate fatty acid synthesis, enhances the insulin simulated conversion of fatty acids into triglycerides and promotes beta-cell proliferation and cell survival (Kim & Egan, 2008).

GLP-1 is a product of the post-translational cleavage of the proglucagon gene (Kim & Egan, 2008) and is one of the most potent insulin secretagogues (Ahmed, 2016). It is released from L cells in the mucosa of the ileum and colon (Ahmed, 2016). It exists in multiple forms; 80% of the biologically active circulating GLP-1 is the COOH-terminally amidated form- GLP-1 (7-36) and the glycine extended form- GLP-1 (7-37) is found in much lower concentrations (Ørskov et al., 1986). In a similar way to GIP, it binds to its specific receptor on β cells and this binding is coupled to increases in cAMP and Ca^{2+} concentrations which cause an increase in insulin (Ahmed, 2016). GLP-1 is also involved in gastric emptying, inhibits glucagon secretion and slows the rate of endogenous glucose production (Kim & Egan, 2008). Treatment of those with type 2 diabetes with GLP-1 has been found to be successful in normalising blood glucose levels, improve beta-cell function and can help to restore first-phase insulin secretion (Kim & Egan, 2008).

2.5.4. Secretagogues and insulin secretion

Insulin secretagogues can be endogenous biological agents, exogenous compounds from plants or artificially synthesised pharmacological agents (Hemmingsen et al., 2016). They stimulate the release of insulin and are therefore commonly used as a pharmacological treatment for diabetes. There are two main classes; sulphonylureas and meglitinide analogues (Hemmingsen et al., 2016). Their major primary action is causing the closure of ATP sensitive K^+ channels (Hemmingsen et al., 2016). This coincides with the stimulation of Ca^{2+} influx into islet cells and therefore contributes to the increased secretion of insulin. Although secretagogues are a common treatment for type 2 diabetes to stimulate the release of insulin, research has found that their use might further ameliorate the problem of β cell dysfunction associated with the disease and therefore cause the worsening of the problems caused by type 2 diabetes in the longer term (Russo et al., 2014). Using exercise as a treatment to regulate glycaemic control could therefore be an extremely beneficial alternative to individuals with the disease.

2.5.5. Lipid metabolism in healthy individuals

After ingestion of a meal containing fat, the exogenous lipoprotein pathway is initiated. It begins with the incorporation of triglycerides into chylomicrons in the intestine, and they are released into circulation via the thoracic duct (Klop et al., 2013), (Feingold, 2000). Once the chylomicrons reach muscle and adipose tissue, they utilise lipoprotein lipase (LPL) as a docking station (Feingold, 2000). The triglycerides are metabolised by LPL into free fatty acids (FFA) and 2-monoacylglycerols (MAG) (Klop et al., 2013). These molecules can then be taken up by both passive diffusion and specific transporters into enterocytes, and results in chylomicron remnants being formed (Feingold, 2000). LPL is strongly expressed in tissues which require larger amounts of FFA for energy production.

The endogenous lipoprotein pathway begins in the liver via the synthesis of triglyceride-rich very low-density lipoproteins (VLDL). VLDL and chylomicron synthesis is very similar but VLDL uses APO B100 as the structural protein whereas chylomicrons use APO B48 (Feingold, 2000; Klop et al., 2013). VLDL also uses LPL as a docking station for the metabolism of triglycerides for uptake into cells (Klop et al., 2013). This forms intermediate-density lipoprotein (IDL) which is further metabolised into low-density lipoprotein (LDL) (Feingold, 2000). LDL is enriched with cholesterol, carrying the majority of cholesterol in circulation which therefore makes it extremely pro-atherogenic (Feingold, 2000).

High density lipoprotein (HDL) plays a major role in reverse cholesterol transport (Feingold, 2000). These particles acquire cholesterol and phospholipids after their efflux from cells. This occurs via the transporter ABCA1 which allows the formation of mature HDL (Feingold, 2000). Once the cholesterol and phospholipids have been acquired by HDL, cholesterol becomes esterified (Klop et al., 2013). Transfer to the liver then occurs either directly or indirectly via interaction with hepatic SR-BI or via transfer of cholesterol to VLDL/LDL by cholesterylester-transfer protein respectively (Klop et al., 2013). During the transfer of cholesterol esters to VLDL/LDL, HDL gains triglycerides in a direct exchange (Klop et al., 2013). Due to its role in reverse cholesterol transport, HDL is anti-atherogenic and also has other benefits such as an anti-inflammatory, anti-apoptotic and antioxidant properties (Krauss, 2004).

2.6. Metabolism in those with type 2 diabetes

2.6.1. Insulin resistance

Insulin resistance occurs when the biological effect of insulin on cells is lower than what is required to maintain glucose homeostasis (Ormazabal et al., 2018). The impact of insulin resistance varies depending on the physiological function of the tissues which interact with it and how much they rely on insulin to control their metabolic processes (Ormazabal et al., 2018), (Sowers & Frohlich, 2004). Insulin

resistance has major impacts on skeletal muscle, adipocytes, and liver tissues as they rely heavily on insulin to regulate their high metabolic demand (Ormazabal et al., 2018). Skeletal muscle is responsible for around 75-80% of glucose uptake and therefore the presence of insulin resistance within these cells has a huge impact on increasing hyperglycaemia (DeFronzo & Tripathy, 2009). Higher concentrations of insulin are required to elicit a normal response when insulin resistance is present (Ormazabal et al., 2018) (Figure 3). In skeletal muscle, hyperinsulinemia within the plasma, which occurs as a response to reduced insulin sensitivity, further reduces insulin sensitivity within these cells (DeFronzo & Tripathy, 2009). It therefore has a negative impact rather than the intended benefit of maintaining glucose uptake (DeFronzo & Tripathy, 2009). Research has shown that even hyperinsulinemia induced in healthy individuals for 72-96hrs, contributed to a significant reduction in insulin-stimulated glucose disposal by around 30-40% in skeletal muscle (impaired nonoxidative glucose disposal) due to increasing the insulin resistance of the tissue (DeFronzo & Tripathy, 2009). This demonstrates that compensatory hyperinsulinemia aims to counteract some of the effects of insulin resistance, but can also ameliorate the effects of insulin resistance in skeletal muscle (DeFronzo & Tripathy, 2009). As diabetes progresses, β cells fail due to prolonged hyperglycaemia and insulin production is therefore reduced (Bar-Tana, 2020) (Figure 3). A range of risk factors contribute to an individual developing insulin resistance, including obesity and a sedentary lifestyle (Ormazabal et al., 2018).

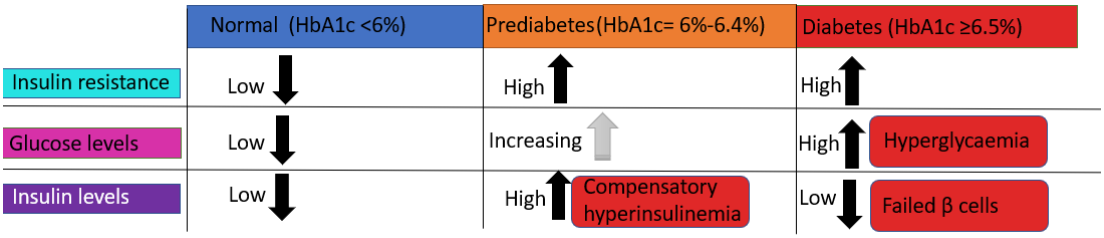


Figure 3. Insulin and Glucose trends as type 2 diabetes progresses.

In normal individuals, the fasting plasma glucose is <6.1mmol/L and HbA1c concentrations <6%, with little insulin resistance present (NICE, 2017). *In individuals with prediabetes, glucose concentration increases between 6.1-6.9mmol/L and HbA1c concentrations between 6%-6.4%* (NICE, 2017). *As soon as insulin resistance increases and insulin sensitivity decreases, insulin concentration increases to try and compensate for the lack of sensitivity* (Bar-Tana, 2020). *Glucose concentrations increase in individuals with type 2 diabetes, and they have glucose concentrations >6.9mmol/L and HbA1c ≥6.5%* (NICE, 2017). *[Own figure with information from:* (Bar-Tana, 2020)*]*

Obesity is associated with an increase in fatty acids which contribute to lipid accumulation (Ormazabal et al., 2018; Saini, 2010). One theory which links obesity to the development of insulin resistance is that an increased concentration of fatty acids could lead to an activation of an atypical protein kinase cascade (PKC) (Dresner et al., 1999). This would contribute to the inhibition of insulin signalling and therefore reduce insulin-stimulated glucose uptake, particularly in skeletal muscles (Figure 4) (Dresner et al., 1999; Ormazabal et al., 2018). The activation of PKC causes the serine phosphorylation of insulin receptor subtrate-1 (IRS-1) labelling it for ubiquitination and degradation (Figure 5) (Lasram et al., 2014; Saini, 2010). Recent evidence suggests that the serine phosphorylation of IRS proteins reduces the ability of the IRS proteins to attract PI3-kinase (Saini, 2010). This minimises the activation of this molecule and decreases IRS-1 tyrosine phosphorylation, further impairing signalling to downstream effectors (Ormazabal et al., 2018).

These signalling defects caused by obesity may also be altered by the increased concentrations of several protein tyrosine phosphatases (PTPs), such as PTB1B and LAR (Kahn & Flier, 2000). These proteins propagate tyrosyl phosphorylation events which dephosphorylate and contribute to the termination of signalling activity (Kahn & Flier, 2000). There is evidence that the expression and activity of at least three PTPs is increased in those who are obese. Research has specifically shown that LAR and PTB1B activity increases, contributing to the dephosphorylation of the insulin receptor (Kahn & Flier, 2000).

Research also suggests that those who are obese experience an increased expression of the p85-α subunit of PI3-kinase (Saini, 2010). This alters the balance between the PI3-kinase subunits which significantly impacts the ability of insulin to stimulate the p85-p110 heterodimer to associate effectively with IRS-1 (Saini, 2010). This is another factor which contributes to the reduction of PI3-kinase insulin signalling and therefore reduces insulin sensitivity.

These alterations in insulin signalling occur in both adipose and muscle cells, but some of the molecular defects may be tissue specific (Kahn & Flier, 2000). There is evidence that in adipose tissue, IRS-1 expression is significantly reduced which decreases the availability of IRS-1 for PI3K to use as a docking protein (Kahn & Flier, 2000). Whereas, in skeletal muscle, the availability of IRS-1 remains normal but the actual association of IRS with PI3K is significantly altered (Figure 4) (Kahn & Flier, 2000). Both of these factors reduce insulin sensitivity.

The location of fat deposits in those who are obese has been found to influence the risk of insulin resistance. Central fat deposits are more strongly linked to developing insulin resistance (Kahn & Flier, 2000). One suggestion as to why this is the case is due to intra-abdominal adipocytes being more lipolytically active. This is likely to contribute to increases in free fatty acid levels and this could inhibit insulin clearance, although the exact mechanisms are currently uncertain (Kahn & Flier, 2000). A high

fat diet and an increase in the supply of fatty acids, increases the concentrations of the bioactive lipids: diacylglycerols (DAGs) and ceramides (Sokolowska & Blachnio-Zabielska, 2019). The excess lipid concentration due to a high fat diet is initially stored in adipose tissue, and this could be one of the reasons why a high amount of central visceral adiposity is strongly linked to type 2 diabetes (Sokolowska & Blachnio-Zabielska, 2019). Once adipose tissue has reached capacity, the lipids start to be stored in non-adipose tissue, such as skeletal muscle and the liver, causing organ dysfunction through lipotoxicity (Sokolowska & Blachnio-Zabielska, 2019).

Research has shown DAGs and ceramides, may also play a major role in hepatic insulin resistance (Petersen & Shulman, 2017). The liver is responsible for 90% of glucose production and therefore a lack of glucose regulation, due to the decreased insulin sensitivity of the tissue, has major implications on the development and progression of type 2 diabetes (Petersen & Shulman, 2017). DAGs are thought to play a mechanistic role by activating protein kinase C (PKC) which then phosphorylates the insulin receptor, inhibiting insulin receptor tyrosine kinase activity (Petersen & Shulman, 2017). This inhibits overall hepatocellular insulin signalling, preventing downstream signalling and regulation from occurring (Petersen & Shulman, 2017).

Ceramides accumulate due to an increased availability of fatty acids (Sokolowska & Blachnio-Zabielska, 2019). The mechanisms of how ceramides are associated with hepatic insulin resistance are currently under debate (Petersen & Shulman, 2017). It is thought that ceramides either impair the translocation of or inactivate protein kinase B (AKT) so it can no longer participate in the insulin pathway (Petersen & Shulman, 2017). Some research suggests that ceramides activate PKC which prevents the translocation of AKT to the plasma membrane (Salinas et al., 2000). Other research suggests ceramides might activate protein phosphatase 2A which causes the dephosphorylation and deactivation of AKT (Zhou et al., 1998).

Evidence has also shown that ceramides increase the activation of proinflammatory cytokines, promote hepatic lipid accumulation and contribute to inducing the apoptotic pathway of β cells (Sokolowska & Blachnio-Zabielska, 2019). All of these factors contribute to hepatic insulin resistance and even though the exact mechanisms aren't well understood, it is clear that ceramides are associated with insulin resistance. These two bioactive lipids, diacylglycerols and ceramides, are therefore an example of two major players in the development of type 2 diabetes.

Reduced glucose transport into cells is another major factor which contributes to the effects of increased insulin resistance experienced by those with type 2 diabetes. Evidence shows that in adipocytes, GLUT4 is downregulated which impairs insulin stimulated glucose uptake (Kahn & Flier, 2000). Adipose tissue produces adipokines which can alter glucose concentrations (Saini, 2010). Specifically, TNF-α is released which contributes to this down regulation of GLUT4 in adipose tissue and increases insulin resistance (Saini, 2010). This is different to skeletal muscle cells, where GLUT4 expression remains normal but defective transport is present instead (Kahn & Flier, 2000). This is related to the impaired translocation, docking or fusion of GLUT 4 vesicles to the specific plasma membrane (Kahn & Flier, 2000).

JNK, PKC and IKK are three major kinases which cause the serine phosphorylation of IRS and its subsequent ubiquitination and degradation (Figure 4) (Lasram et al., 2014). They are activated by hyperinsulinemia, hyperglycaemia, and dyslipidaemia, all of which are features of type 2 diabetes. Hyperglycaemia results in the production of both AGEs and ROS (Lasram et al., 2014). AGEs interact with the AGE receptor which can either result in the direct inhibition of IRS or activate the three major kinases to phosphorylate IRS (Lasram et al., 2014). ROS contribute to the overall REDOX disturbance within the cell and contributes to the activation of the three kinases (Lasram et al., 2014). As discussed above, an increase in fatty acids, due to dyslipidaemia, can activate PKC directly or indirectly via DAG and ceramides to

contribute to the phosphorylation of IRS (Lasram et al., 2014). All of these factors contribute to increasing insulin resistance in those with type 2 diabetes as downstream insulin signalling is unable to be activated when ubiquitination and degradation of IRS prevents the pathway from being maintained (Figure 4). Activation of JNK and IKK by these factors also initiates signalling pathways which activate the transcription factors API and NF- κB (Lasram et al., 2014). These transcription factors cause the increased transcription of genes which contribute to reduced insulin sensitivity (Figure 4) (Lasram et al., 2014).

All of the factors discussed above, contribute to the reduced insulin sensitivity and increased insulin resistance individuals experience in the development of type 2 diabetes. The variety and range of tissues impacted by insulin resistance, and the effects this has on the body, is one of the reasons individuals with type 2 diabetes experience an increased risk of multimorbidity (Sowers & Frohlich, 2004).

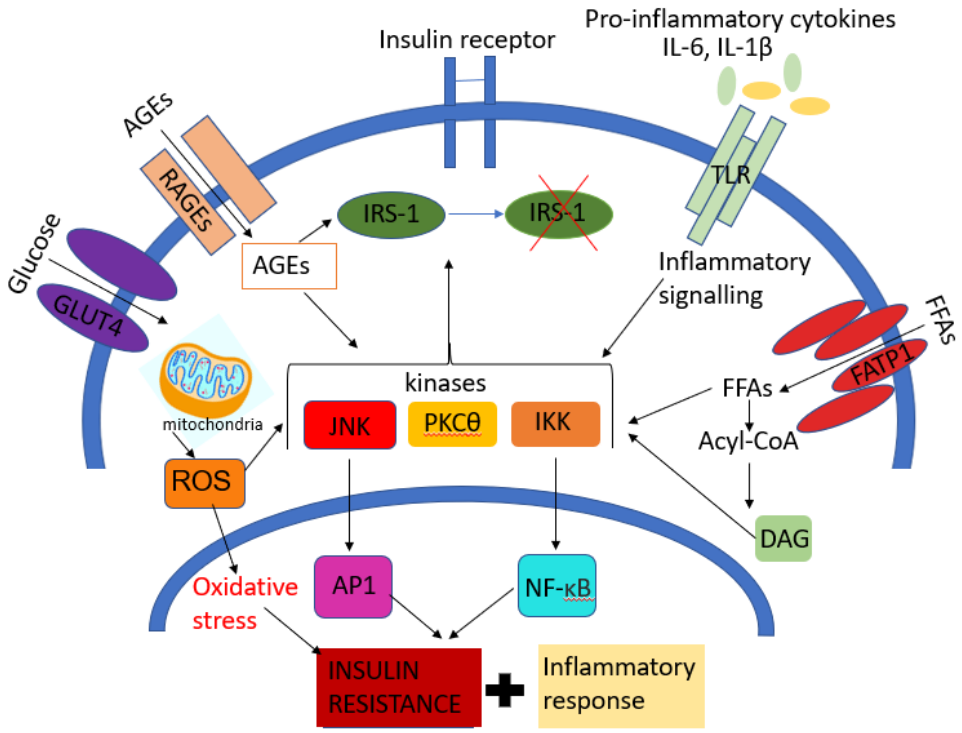


Figure 4. The cellular and molecular mechanisms of insulin resistance.

JNK, PKCθ and IKK are important kinases which play significant roles in the metabolic responses within the cell. They contribute to the serine phosphorylation of IRS-1 which leads to its subsequent degradation, preventing downstream signalling (Lasram et al., 2014). *Free fatty acids and pro-inflammatory cytokines, such as IL-6 and IL-1β contribute to the activation of the kinases. Hyperglycaemia increases AGE and ROS concentrations. AGEs interact with the AGE receptor and this can directly inhibit IRS-1* (Lasram et al., 2014). *ROS contributes to the disturbance of redox status in the cell contributing to insulin resistance* (Lasram et al., 2014). [Redrawn and modified from (Lasram et al., 2014)]

2.6.2. Measures of glucose concentrations and diabetes diagnosis

Glycated haemoglobin (HbA1c) is the gold standard measure for both the diagnosis and prognosis of diabetes by the American Diabetes Association (Sherwani et al., 2016). Glycation occurs when glucose binds to the N-terminal of haemoglobin in a non-enzymatic irreversible reaction (Sherwani et al., 2016). HbA1c is therefore a reliable measure of the cumulative glucose history of the individual for the previous 2-

3months due to the half-life of a red blood cell being around 12 weeks (Sherwani et al., 2016). Increases in HbA1c are directly proportional to increases in blood glucose concentrations and can also act as a prognosis predictor due to its correlation with long term risk (Sherwani et al., 2016). If HbA1c measurements are unable to be taken, then fasting blood glucose concentrations can act as a secondary measure. Fasting blood glucose, however, only shows blood glucose concentrations at a specific point in time, therefore isn't reliable for indicating a longer-term trend and can be impacted by day-to-day changes such as stress (Ghazanfari et al., 2010).

The oral glucose tolerance test (OGTT) is another method of diagnosing diabetes and examining blood glucose concentrations (Jagannathan et al., 2020). It measures blood glucose concentrations for a 2hr period after a glucose load (Jagannathan et al., 2020). It helps to determine how quickly glucose can be cleared from the blood and it can therefore be used as an indirect measure of insulin resistance and β-cell function (Jagannathan et al., 2020). However, this test is time consuming for the patient and also sensitive to day-to-day variations such as exercise and stress (Jagannathan et al., 2020).

2.6.3. Type 2 Diabetes and Lipid Metabolism

Obesity greatly alters lipid metabolism, resulting in dyslipidaemia. Those with obesity typically have increased fasting and post-prandial triglycerides, and an increase in small dense LDL along with reductions in HDL-C (Klop et al., 2013). Obesity induces a reduction in mRNA expression levels of LPL resulting in reduced LPL activity in adipose tissue. There is therefore increased competition between chylomicrons and VLDL for the lipolysis of triglycerides (Klop et al., 2013). This causes a reduction in the clearance of triglyceride-rich lipoproteins, and along with the increased hepatic production of VLDL particles, results in increased serum triglyceride levels (Repas, 2011). HDL-C levels are also typically lower in those with obesity which means the

transfer of cholesterol to VLDL/LDL in return for triglycerides is unable to occur (Feingold, 2000), further increasing serum triglyceride levels (Repas, 2011). The triglycerides present on LDL are then hydrolysed by hepatic lipase which produces small dense LDL (Repas, 2011).

A combination of obesity and insulin resistance causes an increased efflux of fatty acids from adipose tissue and impairs the uptake of free fatty acids into skeletal muscle (Krauss, 2004). This increases the delivery of fatty acids to the liver (Krauss, 2004). The increased concentration of fatty acids is then utilised in the synthesis of triglycerides (Repas, 2011). An abundance of triglycerides prevents Apo B-100 from being degraded which results in the increased formation and secretion of VLDL (Repas, 2011). The increased secretion of VLDL contributes to the increased concentration of serum triglycerides (Repas, 2011).

Another factor which increases triglyceride levels in those with type 2 diabetes, is the reduced activity of lipoprotein lipase. A lack of insulin-induced stimulation of lipoprotein lipase reduces the degradation of triglycerides and increases Apo-C-III levels which the further inhibits lipoprotein lipase (Repas, 2011). A meta-analysis found that a 1mmol/L increase in serum triglyceride, increases coronary disease risk by 32% in men and 76% in women (Hokanson & Austin, 1996). The increase in serum triglycerides is just one of the factors that contributes to an increased risk of cardiovascular diseases in those with type 2 diabetes.

Another factor that increases serum triglyceride levels is the proinflammatory state induced by obesity due to an increase in macrophages that infiltrate adipose tissue and the resultant increase in pro-inflammatory cytokines, TNF, and IL-1 (Repas, 2011). Inflammation is known to be a major player in the development of atherosclerosis which promotes thrombosis, both of which increase the risk of developing cardiovascular disease (Meerarani et al., 2006). TNF and IL-1 help stimulate the lipolysis of triglycerides in adipose tissue, contributing to the increased

circulation and delivery of fatty acids to the liver (Repas, 2011). This results in the increased production and secretion of VLDL (Yuan et al., 2007). These cytokines also reduce the expression of lipoprotein lipase, reducing the degradation of triglycerides within circulation (Repas, 2011; Yuan et al., 2007), further contributing to the increased concentration of triglycerides. It is thought that triglycerides are very pro-atherogenic and may contribute to the increased formation of arterial-wall foam cells, increasing the risk of cardiovascular disease in those with type 2 diabetes (Yuan et al., 2007).

HDL metabolism is also effected by these pro-inflammatory cytokines which decreases reverse cholesterol transport and contributes to cholesterol accumulation increasing atherosclerosis (Repas, 2011). There is a direct correlation between reduced HDL-C levels and increased cardiovascular disease risk (Rader & Hovingh, 2014). HDL have a range of beneficial effects including direct cardioprotective effects, increasing cellular cholesterol efflux, and direct antioxidative and anti-inflammatory properties (Krauss, 2004). Those with type 2 diabetes, have reduced HDL concentrations and therefore experience a reduction in these benefits, increasing cardiovascular disease risk.

Whether individuals with type 2 diabetes experience increased concentrations of LDL, which also contribute to the increased risk of cardiovascular diseases, is currently under debate. However, evidence shows that those with type 2 diabetes are more likely to have LDL with increased density and reduced size which results in the particles becoming more atherogenic (Krauss, 2004). These LDL have a reduced affinity for LDL receptors, they enter the subendothelial space much more easily, they can bind strongly to arterial wall proteoglycans and the particles experience increased susceptibility to oxidative modifications (Repas, 2011), (Krauss, 2004). The oxidative modifications increase the likelihood of uptake of LDL into macrophages

(Repas, 2011). All of these factors of small dense LDL contribute to increased arterial wall damage, increasing the risk of cardiovascular disease.

2.7.Common morbidities

Diabetes unfortunately effects range of different organ systems within the body, and it is this which causes the multiple morbidities experienced by sufferers. They experience a much higher prevalence of microvascular complications including neuropathy, nephropathy, and retinopathy, but also experience macrovascular complications, such as cardiovascular disease, stroke and peripheral vascular disease (Deshpande et al., 2008). 10,000 new cases of blindness result from diabetic retinopathy every year which makes it one of the most common microvascular complications (Deshpande et al., 2008). It is the morbidities associated with diabetes which increase the mortality rate. Cardiovascular disease, for example, causes 65% of deaths in all people with diabetes (Deshpande et al., 2008). People with type 2 diabetes are also 15 times more likely to have a lower leg amputation (Deshpande et al., 2008) due to peripheral neuropathy and peripheral arterial disease. Therefore, effective treatments, such as lifestyle interventions, are needed to reduce the symptoms, expenses, life expectancy and quality of life of those with type 2 diabetes.

2.8.Cardiovascular disease

Cardiovascular disease is a serious morbidity associated with insulin resistance and type 2 diabetes, as it accounts for the majority of deaths in individuals with type 2 diabetes (Ormazabal et al., 2018). The risk of developing cardiovascular disease increases two to eight-fold in those with type 2 diabetes (Martín-Timón et al., 2014). It is thought that hyperglycaemia and insulin resistance are the main protagonists (Ormazabal et al., 2018). Research has suggested that the risk of cardiovascular disease increases by around 18% with only a 1 unit increase in glycosylated haemoglobin (HbA1c) (Selvin et al., 2004). There are two main factors which cause

cardiovascular disease, these are atheroma formation, and ventricular hypertrophy (Feingold, 2000). Hyperinsulinemia occurs as a result of insulin resistance and this excess insulin upregulates the cluster differentiation protein 36, an important free fatty acid transporter (Su & Abumrad, 2009). This increases the intracellular free fatty acid concentration of cardiomyocytes and contributes to the heart becoming saturated in a fatty acid and glucose environment (Kolwicz et al., 2013). As the severity of insulin resistance increases, the heart's ability to utilise free fatty acids decreases (Grynberg & Demaison, 1996). This results in intramyocardial lipid accumulation which negatively impacts the functioning of the heart through apoptosis, impaired mitochondrial function, cardiac hypertrophy, and contractile dysfunction (Goldberg et al., 2012). All of these factors contribute to increasing the risk of cardiomyopathy and heart failure.

An increase in the amount of visceral fat mass due chronic caloric excess associated with obesity contributes to the hypertrophy of individual adipocytes (Longo et al., 2019). This results in the increased release of chemotactic factors including monocyte chemoattractant protein-1 (MCP-1) and TNF-α (Ormazabal et al., 2018). MCP-1 is involved in the migration and differentiation of monocytes into macrophages within visceral adipose tissue (Ormazabal et al., 2018). The macrophages then secrete TNF-α which initiates a chain of events, involving triglyceride biosynthesis and adipocyte storage, resulting in an increase in plasma triglyceride concentrations (Guilherme et al., 2008). It is then hypothesised that this results in ectopic lipid deposition in extra-adipose tissue (Ormazabal et al., 2018). In the heart, this increases the volume of epicardial adipose tissue which is associated with a range of negative impacts, including an increase in the mass within both ventricles, resulting in ventricular hypertrophy and therefore contractile dysfunction (Fitzgibbons & Czech, 2014).

Hyperglycaemia and obesity are thought to significantly contribute to an increased risk of atheroma formation. Advanced glycation end (AGE) products, which are non-enzymatic glycation products of lipids and proteins, are produced due to the presence of hyperglycaemia (Nowotny et al., 2015). These molecules damage the integrity and structure of the endothelium by accumulating in vessel walls, increasing an individual's risk of developing atherosclerosis (Nowotny et al., 2015). AGE products also play a part in a range of diabetic complications, such as retinopathy and neuropathy and also contribute to the development of insulin resistance (Figure 5) (Nowotny et al., 2015).

Pro-inflammatory and pro-coagulant factors also increase in the presence of hyperglycaemia. This includes increasing the ability of leukocyte cells to associate to endothelial cells via adhesion (Kahn & Flier, 2000). This is another factor which induces apoptosis and endothelial damage (Kahn & Flier, 2000). Pro-coagulant factor PAI-1 expression increases (Sowers & Frohlich, 2004). This molecule enhances platelet aggregation which is evidenced in insulin resistant states (Sowers & Frohlich, 2004). Endothelin 1 secretion is also elevated when insulin resistance is present in an individual (Sowers & Frohlich, 2004). This is a vasoconstrictor and competes with nitric oxide (NO) (Sowers & Frohlich, 2004). NO regulates endothelial dependent relaxation in the large arteries and is also beneficial in inhibiting platelet aggregation (Sowers & Frohlich, 2004). An increase in endothelin 1 secretion therefore contributes to increasing the risk of atherosclerosis and therefore cardiovascular disease as a result. The range of impacts insulin resistance has on the heart contributes to our understanding of why cardiovascular disease risk increases so significantly in individuals with type 2 diabetes.

2.9. Exercise training as a treatment for type 2 diabetes

2.9.1. Acute effects

Insulin and exercise are the two factors which reduce plasma glucose concentrations and enhance glucose homeostasis (Colberg et al., 2010). When at rest the body requires insulin-dependent mechanisms to control glucose homeostasis (Asano, 2014). Insulin signalling requires the activation of phosphatidylinositol 3-kinase, phosphorylation of the insulin receptor and the insulin receptor substrate (Röckl et al., 2008). Exercise doesn't require any of these pathways and therefore insulin resistant tissue will still take up glucose during exercise (Sylow et al., 2017). There are three key steps which allow exercise to increase glucose uptake by up to 50%, these are delivery, transport across the membrane and oxidation causing intracellular influx (Sylow et al., 2017). Delivery of glucose to active muscle cells is increased due to a rise in blood flow. This is associated with an increase in capillary recruitment which subsequently increases the surface area for the influx of glucose and oxygen (Sylow et al., 2017). Oxygen consumption is coupled to blood flow and this ensures sufficient glucose reaches active muscle tissue (Sylow et al., 2017).

Exercise also causes the translocation of the GLUT4 transporter to the muscle cell membrane. There have been a number of signalling pathways implicated in this process, including the use of the proteins: AMP-activated protein kinase (AMPK), Ca^{2+}/calmodulin-dependent protein kinases and protein kinase C (Röckl et al., 2008). During muscle contraction, AMPK activity increases significantly (Asano, 2014) and evidence has suggested that there is a positive correlation between AMPK activity and glucose uptake (Röckl et al., 2008). It is thought that AMPK is a major sensor of intracellular energy status and helps balance cellular energy supply and demand through regulation of anabolic and catabolic pathways (Kjøbsted et al., 2018).

Intracellular Ca^{2+} concentrations increase significantly during muscle contraction (Röckl et al., 2008). Ca^{2+}/calmodulin-dependent protein kinases which regulate this

increase have also play a critical role in glucose transport via GLUT4 into the contracting muscle cells (Röckl et al., 2008). Evidence has also found that the GLUT4 target genes are upregulated due the increases in Ca^{2+} (Chin, 2005). The increased translocation of GLUT4 (Figure 5), allows the permeability of the muscle cells to increase in regards to glucose influx. Once glucose enters the cell, it is used in either aerobic or anaerobic mechanisms, depending on the intensity and volume of exercise (Hargreaves & Spriet, 2020). The enzyme hexokinase phosphorylates glucose to produce glucose-6-phosphate (Sylow et al., 2017). The production of glucose-6-phosphate preserves the diffusion gradient for facilitated diffusion to occur and also traps glucose within the cell (Sylow et al., 2017).

All of these factors induced during exercise allow glucose to be up taken into muscle cells, reducing the concentration of plasma glucose and therefore helping to reduce hyperglycaemia (Asano, 2014). This therefore means exercise can be used as a non-pharmacological method to regulate glucose homeostasis in those with type 2 diabetes. Although the immediate metabolic effects of exercise are significant, they begin to fade in around 24 hours (Kirwan et al., 2017). Regular exercise therefore needs to be maintained in order to provide more sustained improvements in glycaemic control.

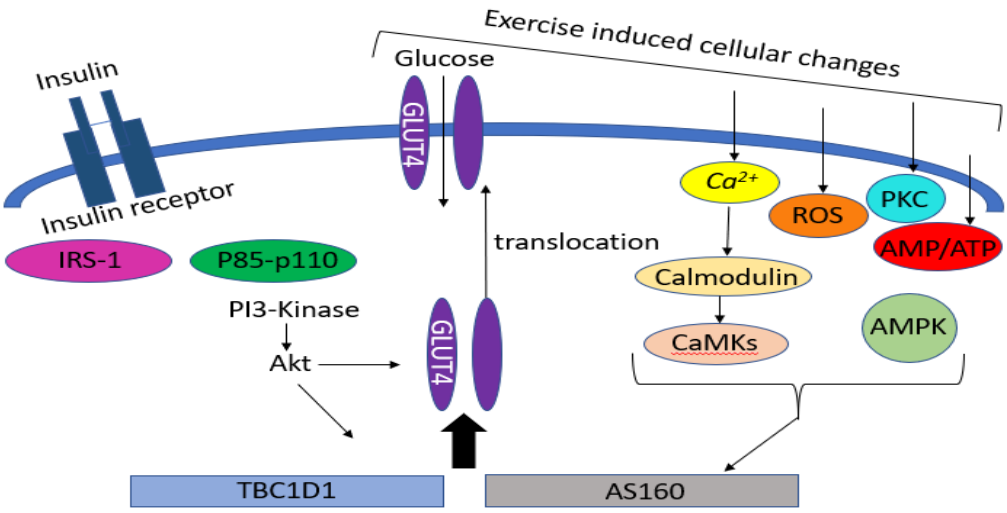


Figure 5. Exercise and insulin stimulated GLUT 4 translocation.

Exercise increases the translocation of GLUT4 to the skeletal muscle membrane and induces muscle contraction which increases intracellular calcium (Ca^{2+}) content (Stanford & Goodyear, 2014)*. Both the signalling pathways of exercise and insulin stimulated glucose uptake converge at two molecules; TBC1D1 and AS160 which regulate the translocation of GLUT4* (Stanford & Goodyear, 2014)*. All of these factors and signalling pathways contribute to the increased uptake of glucose in response to exercise via the increased translocation of GLUT 4 to the plasma membrane. [Figure redrawn and modified from* (Stanford & Goodyear, 2014)*]*

2.9.2. Chronic effects

Evidence has shown that chronic exercise training can improve body composition by increasing energy expenditure. This therefore reduces visceral fat, if calorie intake is kept constant or reduced, resulting in a reduced burden on heart and lungs (O'Hagan et al., 2013). Regular exercise causes muscle growth, increasing muscle mass (Pearson, 1990). This increases the uptake of glucose as skeletal muscle is responsible for 75-80% of glucose uptake and glucose can also then be utilised quicker to produce energy for contracting muscles (DeFronzo & Tripathy, 2009). Chronic exercise also has benefits on insulin resistance and glycosylated haemoglobin levels. Even as little as one week of exercise training can improve

insulin sensitivity in type 2 diabetes patients (O'Hagan et al., 2013). Regular training increases both the expression and activity of proteins involved in insulin signalling pathways (Colberg et al., 2010), therefore improving tissue sensitivity. One clinical trial has found that insulin sensitivity improves by around 25-35% after just 6 weeks of regular physical activity (Koivisto & Yki-Järvinen, 1986).

Those with type 2 diabetes have increased concentrations of plasma glucose and lipids (Colberg et al., 2010). Regular exercise gradually consumes both glucose and lipids to help counteract the negative effects of high concentrations of both types of molecules (Yang et al., 2019). This helps to alleviate the glucotoxicity and lipotoxicity experienced by pancreatic beta cells, reducing inflammation and oxidative stress on tissues (Yang et al., 2019). This allows for the recovery of damaged beta cells and acts to protect functional islets (Yang et al., 2019), improving or at least preventing the worsening of endocrine function (Yang et al., 2019). Researchers have found that 12-16 weeks of aerobic exercise in type 2 diabetics, significantly improves β cell function (Yang et al., 2019).

Those with type 2 diabetes typically have increased levels of both triglycerides and LDL and low levels of HDL cholesterol (Yang et al., 2019). All these factors contribute to an increased risk of cardiovascular disease (Krauss, 2004). Chronic exercise increases the concentration of catecholamines (Yang et al., 2019) and increases the activity of lipoprotein lipases (Wang & Xu, 2017). This results in lipids being hydrolysed into fatty acids which can then be utilised by mitochondria within cells (Yang et al., 2019). This helps to reduce the risk of cardiovascular disease which is one of the most significant comorbidities associated with type 2 diabetes.

Cardiovascular complications of type 2 diabetes are thought to be caused by both metabolic dysregulation and chronic inflammation (Karstoft & Pedersen, 2016). IL-1β is proinflammatory mediator of β-cell damage and therefore a direct cause of β-cell dysfunction (Karstoft & Pedersen, 2016). The phosphorylation dysfunction of insulin

signal transduction protein is caused by macrophage accumulation and chemotaxis which are also key indicators of inflammation (Yang et al., 2019). As chronic exercise reduces the accumulation of visceral fat, which is more inflamed than subcutaneous fat, it provides anti-inflammatory effects (Karstoft & Pedersen, 2016). It also helps to inhibit the accumulation and aggregation of macrophages, and reduces the production of cytokines, all of which contribute to inflammation (Yang et al., 2019). Levels of the major proinflammatory molecule, C-reactive protein is also significantly reduced when exercise is maintained (Kirwan et al., 2017). This therefore means that chronic exercise helps to maintain functional insulin signalling and reduces the risk of cardiovascular complications in those with type 2 diabetes.

Exercise has benefits on the immune system (da Silveira et al., 2021) which helps to reduce the risk of disease and infection in those with type 2 diabetes. It also helps to improve the psychological state, including reducing rates of anxiety and depression in sufferers (Yang et al., 2019).

2.10. Understanding the effects of different doses of exercise on glycaemic control

Exercise interventions are often recommended for the improvement of (general) physical and mental health. For example, the American Diabetes Association suggests that individuals with type 2 diabetes should undertake 150 mins of moderate-vigorous exercise at least 3 times per week (Colberg et al., 2010). This, however, doesn't consider intraindividual heterogeneity and differences in physiological and functional capacity. Therefore, understanding the dose-response relationship of exercise in improving glycaemic control in those with type 2 diabetes would hopefully maximise the efficiency and effectiveness of exercise programmes (Mann et al., 2013). It is well known that regular physical activity helps improve glycaemic control, however, the dose (frequency, intensity, duration) required to achieve an effective response is currently unknown (Savikj & Zierath, 2020)F.

There is contradictory evidence as to what dose of exercise might be most effective. For example, a recent systematic review (Moxley & Bugaieski, 2019), stated that the majority of studies found improvements in HbA1c irrespective of exercise intensity. Whereas a clinical trial found that, despite high intensity interval training (HIIT) being 45% lower in training volume than a continuous endurance protocol, individuals participating experienced similar or even better improvements in glycaemic control (Winding et al., 2018). This specific research therefore suggests that high intensity exercise could be a more time-efficient treatment (Winding et al., 2018). There are also similar debates around the optimal frequency and volume of exercise sessions within a programme. A meta-analysis investigated the association of volume of exercise with improvements in glycaemic control (Umpierre et al., 2011). Eighteen studies were included within the meta-analysis and the analysis indicated that aerobic exercise ≥150 minutes/week was associated with a reduction in HbA1c of 0.89%, whereas ≤150 minutes/week was only associated with a 0.36% reduction in HbA1c (Umpierre et al., 2011). Similar research also discovered that moderate (55%) and high (79%) intensity exercise resulted in similar reductions (Umpierre et al., 2013). However, this poses the question as to whether it is the lower volume of high intensity interval training that is the limiting factor contributing to the lack of improvement in glycaemic control seen in this research (Umpierre et al., 2013). Another randomised control trial investigating the relationship of exercise volume indicated that there was a significant association between an increase in the volume of exercise and a subsequent decrease in HbA1c concentrations (Nicolucci et al., 2012).

Another factor to consider when determining the optimal dose of exercise to be included within an exercise programme for those with type 2 diabetes is the likelihood of individuals to engage and adhere to the programme. One specific clinical trial investigated the effects of energy matched continuous low to moderate intensity

exercise compared to moderate to high intensity continuous aerobic exercise (Hansen et al., 2009). This research found equal effects in both groups in reducing HbA1c concentrations (Hansen et al., 2009). This could indicate that calorised dose of exercise may be more useful in determining benefits of exercise on glycaemic control. This research also indicates that it might be beneficial to concentrate on giving low to moderate intensity exercise programmes to sedentary individuals (Hansen et al., 2009). Research has discovered that there is an inverse relationship between exercise intensity and the likelihood that individuals adhere to the programme (Perri et al., 2002). This means there is typically higher drop-out rates in higher intensity programmes (Perri et al., 2002). This could suggest that programmes with a higher frequency or volume of exercise may be more influential within an exercise programme.

Another factor to consider when designing an individual's exercise programme is whether they are likely to be supervised. Supervised exercise is associated with significantly greater reductions in HbA1c concentrations (Umpierre et al., 2013). If supervision isn't possible, an understanding of the minimal dose of exercise an individual needs to undertake in order to effectively improve glycaemic control would be even more important in improving the likelihood that an induvial adheres to the programme.

It seems to be that there is a general consensus within the literature that a small dose of physical activity is better than nothing, but more physical activity is also better than a small dose (Dubé et al., 2012). Significant improvements in glycaemic control and insulin sensitivity have been associated with an exercise dose of ~400 kcal which is only 40%-50% of suggested guidelines (Dubé et al., 2012). An increase in low-intensity daily activity such as going for 15-minute walk significantly improves glucose homeostasis after the consumption of a meal (Pahra et al., 2017). Whereas a 45-minute session of moderate intensity exercise significantly reduces

hyperglycaemia for an entire 24-hour period (Van Dijk, Venema, et al., 2013). This therefore shows that low-intensity exercise is beneficial but moderate-intensity exercise for longer durations becomes increasingly beneficial. Understanding the dose-response relationship between exercise and glycaemic control, with the dose of exercise being defined by frequency, intensity, and duration of exercise, will hopefully provide an estimation of the responses specific doses of exercise can cause. An understanding of this relationship will improve the personalisation and individualisation of exercise programmes so that they are better suited to an individual's physiological and functional capacity whilst still contributing to the improvement of glycaemic control.

2.11. Importance of the research

Type 2 diabetes is slowly becoming an epidemic as obesity levels continue to increase and this will place a huge clinical and financial burden on society (Bagust et al., 2002). Blood glucose-lowering drugs to treat diabetes currently cost the NHS £1bn annually (Stedman et al., 2019) and have significant side effects to patients who have to rely upon them (Palmer et al., 2016). There are also a number of morbidities associated with diabetes and it is these diseases which have the biggest burden on the health care services. Indirect costs of diabetes due to morbidities are predicted to reach around £22.8bn by 2035 (Hex et al., 2012). It is well understood in the literature that exercise can help improve glycaemic control (Colberg et al., 2010; Shambrook et al., 2018). Research has also shown that the beneficial effects of exercise on glycaemic control in those with type 2 diabetes can be experienced up to 24-48 hours post exercise (Van Dijk, Manders, et al., 2013). Exercise also has a range of beneficial impacts, such lowering BMI and improving lipid profiles, and this can help reduce the risk of individuals developing morbidities associated with the disease (Colberg et al., 2010). This therefore suggests that exercise could be used as a treatment to improve

glycaemic control and also improve the health outcomes of individuals with type 2 diabetes.

However, the specific dose of exercise which would provide optimal treatment for individuals with the disease is currently unknown (Mann et al., 2013). A better understanding of the dose-response relationship between exercise and glycaemic control would help identify suitable minimum doses of exercise for individuals. The aim of this research was to determine the minimum dose of exercise that would provide appropriate glycaemic control by plotting a dose-response curve. If the minimum dose of exercise needed to treat type 2 diabetes could be determined when the clinical trial progresses, then exercise could be used as a reliable treatment with individuals becoming more motivated to exercise. Understanding the dose-response relationship of exercise and glycaemic control would help in reducing the costs of the disease, reducing the reliance upon pharmacological treatments, and reducing the burden the morbidities of the disease place on health care services. This research, if successful, could therefore have significant beneficial impacts on society.

2.12. Aims of the research

One aim of this research was to determine the dose-response relationship between exercise and glycaemic control. This was be done by providing individuals with different doses of exercise (measured in calories) and analysing their glycaemic control 24 hrs after exercise. The results were used to produce a dose-response curve which will hopefully help determine the minimum doses of exercise required to provide effective glycaemic control in those with type 2 diabetes. Due to the research being postponed in the Covid-19 pandemic, a secondary aim was investigated. The aim of this research was to carry out a systematic review, including a meta-analysis and meta-regression, to examine the effect of different intensities and volumes of exercise within exercise programmes on glycaemic control. This will hopefully help

develop our understanding about the optimal doses of exercise that should be included within personalised exercise programmes used to treat type 2 diabetes. Each research aim will help improve our understanding of the dose of exercise which is required to provide effective glycaemic control in individuals with type 2 diabetes and therefore help improve the effectiveness of using exercise as a treatment.

3. Clinical trial

3.1. Methodology

3.1.1. Overview

The aim of the study was to investigate the optimal dose-response relationship between exercise and glycaemic control to assist in improving exercise adherence and use as a treatment in those with type 2 diabetes. Participants took part in four different bouts of cycling expending 0 kcal, 175 kcal, 350 kcal and 700 kcal. The protocol was based around the finding that ~70 mins of exercise expending ~350 kcal at 50% $\dot{V}O_2$ max improved insulin sensitivity by around 35% the following day but the minimal dose of exercise required to provide glycaemic control is currently unknown (Newsom et al., 2013).

The protocol included exercise expending half as many calories (175 kcal), as well as twice as many (700 kcal) to help in plotting the dose-response curve. This will allow the dose-response relationship between exercise and glycaemic control to be accurately modelled. From this, the minimal dose of exercise required for glycaemic control can be determined.

This was a registered interventional clinical trial (NCT04129268) that began in October 2020. The trial was based between the Human Performance Laboratory (HPL) at Lancaster University and the Royal Lancaster Infirmary, Lancaster. In accordance with the Declaration of Helsinki (Version 2013) ethical principles (World

Medical Association, 2013), ethical documentation was submitted and approved by the NHS research ethics committee (REC Ref: 19/NW/0066) and participant recruitment began.

3.1.2. Participant recruitment

The study aimed to recruit three groups of overweight (BMI ≥ 25kg/m^2) males. The first two cohorts were healthy individuals split into two age groups (n=9) of 18-30 years and 40-65 years. The third cohort (n=9) were those with a diagnosis of type 2 diabetes aged between 18-65 years. The testing only took place on males due to the protective effects of oestrogen and a lower number of women with type 2 diabetes (Iorga et al., 2017). Unfortunately, due to the Covid-19 pandemic, testing could only safely be carried out on healthy male individuals aged 18-30 years with a BMI <25 kg/m^2, who were at a lower risk of serious implications of the virus. This level of testing could only begin after ethical approval was reapproved at the end of May 2021.

Participants were recruited via word of mouth, advertisements on social media and via posters placed in the local area. Members of the public who expressed interest were contacted via a phone call to have the protocol explained, making them aware of the process, and the inclusion and exclusion criteria was discussed to ensure they were suitable candidates. Participants were excluded from the trial if; they were outside the age ranges stated above, if candidate felt they were unable to perform the physical activity within the protocol, or if they had any of the following diseases: cardiovascular disease, peripheral or cerebral vascular disease, muscle disorders, renal disorders, liver disease or neurological disease. They were also excluded if they took any medication which may effect glycaemic control such as corticosteroids or biguanides (Korayem, 2017). After this discussion, participants were emailed a

copy of the participant information sheet and given a date for an in-person screening to take place.

Below is a schematic of the protocol the participants followed (Figure 6). The first stage was for participants to take part in an in-person screening where a family history questionnaire was filled out, anthropometric measurements were recorded, and it was ensured that participants were fully comfortable with and understood the protocol before appropriate consent was given.

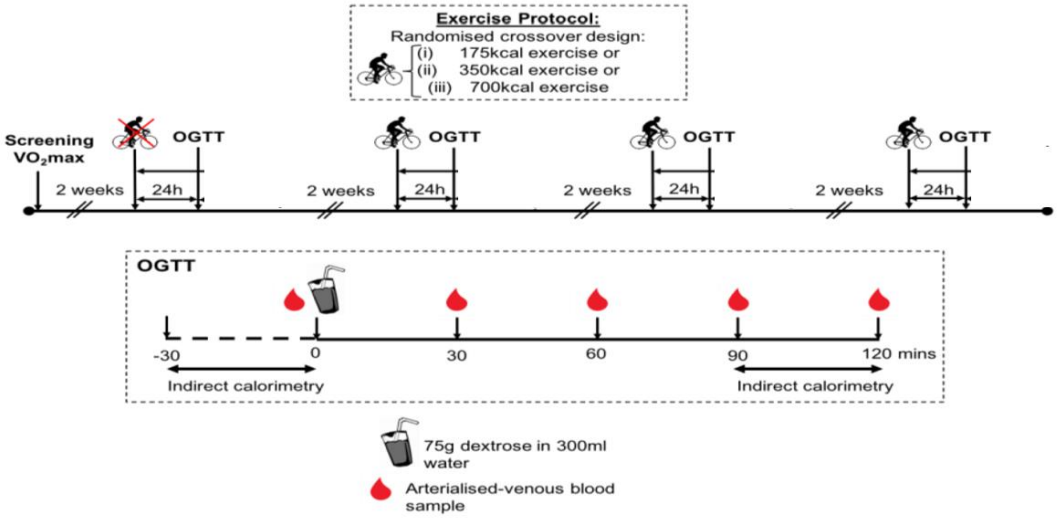


Figure 6. A schematic of the protocol.

The protocol involved an initial screening visit, followed by four exercise visits (0, 175, 350 and 700kCal). 24 hours after each exercise visit, an oral glucose tolerance test (OGTT) took place to identify the effects of each exercise visit on glycaemic control.

3.1.3. Participant medical screening

To further assess whether those interested were suitable, they filled in a range of health questionnaires, such as family history, if any of the results from this meant the participant would be put at risk during the protocol then they were excluded from

participating. A copy of the health questionnaire based on the American College of Sports Medicine (ACSM) readiness to exercise criteria is given in the appendices (Figure 17) (Riebe et al., 2015). Health status was also assessed by the following procedures. This involved an ECG carried out by a Cardiochek device (SECA, CT6i, Birmingham, UK) connected to a mobile device (AliveCor, Kardia Mobile, New York City, USA) to ensure atrial fibrillation wasn't present. Blood pressure was measured using an automated blood pressure monitor (Omron Healthcare, Version M2, Milton Keynes, UK) to ensure levels were within a safe range between 90/60 mmHg and 140/90 mmHg (NHS. 2019). Finger-tip venous blood samples were taken according to the protocol detailed in section 3.1.4 and were analysed by the Biosen Analyser (EKF Diagnostics, Biosen, UK) to measure fasting blood glucose and lactate concentrations.

Anthropometric measurements where then taken. First height and weight were measured using a stadiometer (SECA, Version 213, Birmingham, UK) and weighing scales (SECA, Version 813, Birmingham, UK) respectively. This data was then used to calculate BMI using the following formula:

$$BMI = \frac{weight\ (kg)}{(height\ (m))^2} \quad \text{(NHS, 2018)}$$

This could identify if participants were classed as overweight (BMI ≥ 25-30kg/m^2). Finally, various body composition data was collected using bioelectrical impedance scales (TANITA, DC-430 MA P, Manchester, UK), including percentage body fat, percentage muscle volume and basal metabolic rate (BMR).

All these data, including health status, questionnaire response and anthropometric measurements were reviewed by one of the research team's medical doctors to ensure it was safe for the volunteer to participate. If approval was given for participation, the protocol was explained once more to the participant to ensure they were fully informed, they had the opportunity to ask any questions, and written

informed consent was obtained. The participant was then assigned a code to ensure confidentiality was maintained throughout the protocol and all data was kept in a password protected and encrypted Excel (Microsoft, Redmond, USA) file.

3.1.4. Finger-tip blood sample

The member of the research team carrying out the sample always wore appropriate PPE and followed the health and safety protocols of the laboratory. On their non-dominant hand, the participant's fingertip where the sample was to be taken was first sanitised with an antiseptic wipe and was given the chance to air dry. A lancet (Accu-check Safe-T-Pro Uno Lancet, Roche Diabetes Care, West Sussex, UK) was then used to puncture the skin with one quick deliberate stroke. The first drop of blood was wiped away due to potential contamination of that sample. The blood was then collected within a capillary tube to be analysed by the Biosen Analyser (EKF Diagnostics, Biosen, UK). Once the sample was successfully taken, pressure was applied to the wound to prevent further bleeding and a plaster was place on the finger.

3.1.5. Submaximal exercise test

To predict $\dot{V}O_2$ max, the Ekblom-Bak et al (2012) sub-maximal protocol was used (Schultz et al., 2020). Participants were instructed not to perform any heavy/prolonged physical activity 48 hours prior to experimentation. Once participants arrived at the lab, they were fitted with a heart rate monitor (Polar H10, Warwick, UK) around their thorax. The test was conducted on a Corvical CPET cycle ergometer (Lode, Groningen, Netherlands). For the 8 minutes of the submaximal test, participants were advised to pedal at 60 revolutions per minute (RPM). The ergometer was set at a fixed rate of 30 watts (W) for the first 4 minutes of the test. For the remaining 4 minutes of the test, the ergometer was set at an individualised work rate. This work rate varied between 60 and 200 W depending on the individual

and the work rate they perceived to be 13-15 (high exertion) on the Borg rating of perceived exertion (RPE) (Borg, 1982). Average heart rate (HR) was recorded for the final minute of the individualised work rate stage of testing; HR recordings were taken at 3:15, 3:30, 3:45 and 4:00 minutes and then averaged.

This data was then used to predict $\dot{V}O_2$ max using the Ekblom-Bak prediction equation (Schultz et al., 2020).

$$\ln(\dot{V}O_2\ \max) = 2.04900 - 0.00858 \times age - 0.90742 \times \left(\frac{\Delta HR}{\Delta PO}\right) + 0.00178 \times \Delta PO - 0.00290 \times HR\ at\ initial\ work\ rate$$

Where ΔHR is the difference between the initial HR and the individualised work rate average HR, and ΔPO is the difference in work rate (watts) between the two stages. $\dot{V}O_2$ max can then be estimated using the natural logarithm of the value calculated by the equation.

3.1.6. Exercise visits

The protocol required participants to attend the lab for four exercise visits to complete 0, 175, 350 and 700 kcal of exercise on the cycle ergometer. The four exercise visits were randomised, and each separated by a 2-week break before the next visit to allow participants to recover from the oral glucose tolerance test (OGTT) and ensure any effects from the previous exercise visit weren't still present.

The cardiopulmonary exercise testing (CPET) equipment (Cortex Metalyzer 3B, Leipzig, Germany) uses breath by breath technology to determine the respiratory exchange ratio (RER) and $\dot{V}O_2$ of participants (Chrif et al., 2018). CPET involves an

incremental exercise protocol to measure oxygen uptake ($\dot{V}O_2$) and it is the maximum oxygen uptake of an individual which determines their $\dot{V}O_2$ max (Chrif et al., 2018). To improve the ability of individuals with type 2 diabetes completing the programme we used a submaximal CPET test to predict $\dot{V}O_2$ max. $\dot{V}O_2$ max helps give an indication of an individual's exercise and functional capacity (Albouaini et al., 2007). The respiratory exchange ratio (RER) gives an estimation of metabolic events and the fuel sources that an individual relies upon at certain stages of exercise by measuring a ratio of carbon dioxide output and oxygen uptake (Albouaini et al., 2007). This provides the data needed to calculate the time of exercise required to burn the specific number of calories and the formula which was used to calculate this is stated below.

$$Time\ (minutes) = \left(\frac{Kcal\ to\ be\ burned}{\dot{V}O^2 \text{value}\left[\frac{\text{L}}{\text{min}}\right]}\right) \times RER_{calorific\ equivalent}$$

The $\dot{V}O_2$ and RER values were recorded manually every 30 seconds between 3 and 10 minutes at the start of the protocol and the average was taken. The RER calorific equivalent can then be found from the values in the table below (Table 1).

Table 1. Table of values used to determine RER calorific equivalent.

Calorific values for the ratio of oxygen and carbon dioxide from the respiratory exchange ratio and the energy contribution from the proportion of carbohydrates and fats consumed. [Table retrieved from: (Carpenter, 1933)]

Non-protein respiratory quotient.	Calories per liter of O_2.[1]		Calories per liter of CO_2.[2]		Proportion of calories from—	
	Number.	Logarithm.	Number.	Logarithm.	Carbohydrate.[3]	Fat.[4]
					Per cent	*Per cent*
0.70	4.686	0.67080	6.694	0.82569	0.0	100.0
.71	4.690	67117	6.606	81994	1.4	98.6
.72	4.702	67228	6.531	81498	4.8	95.2
.73	4.714	67339	6.458	81010	8.2	91.8
.74	4.727	67459	6.388	80536	11.6	88.4
.75	4.739	67569	6.319	80065	15.0	85.0
.76	4.752	67688	6.253	79609	18.4	81.6
.77	4.764	67797	6.187	79148	21.8	78.2
.78	4.776	67906	6.123	78696	25.2	74.8
.79	4.789	68024	6.062	78262	28.6	71.4
.80	4.801	68133	6.001	77822	32.0	68.0
.81	4.813	68242	5.942	77393	35.4	64.6
.82	4.825	68350	5.884	76967	38.8	61.2
.83	4.838	68467	5.829	76559	42.2	57.8
.84	4.850	68574	5.774	76148	45.6	54.4
.85	4.863	68690	5.721	75747	49.0	51.0
.86	4.875	68797	5.669	75351	52.4	47.6
.87	4.887	68904	5.617	74950	55.8	44.2
.88	4.900	69020	5.568	74570	59.2	40.8
.89	4.912	69126	5.519	74186	62.6	37.4
.90	4.924	69232	5.471	73807	66.0	34.0
.91	4.936	69338	5.424	73432	69.4	30.6
.92	4.948	69443	5.378	73062	72.8	27.2
.93	4.960	69548	5.333	72697	76.2	23.8
.94	4.973	69662	5.290	72346	79.6	20.4
.95	4.985	69767	5.247	71991	83.0	17.0
.96	4.997	69871	5.205	71642	86.4	13.6
.97	5.010	69984	5.165	71307	89.8	10.2
.98	5.022	70088	5.124	70961	93.2	6.8
.99	5.034	70191	5.085	70629	96.6	3.4
1.00	5.047	70303	5.047	70303	100.0	0.0

These values were then input into the equation to determine the length of time participants needed to cycle for to burn the specific number of calories. All participants cycled at the speed corresponding to 60% of their $\dot{V}O_2$ max, which was calculated in the sub-maximal exercise test.

3.1.7. Physical activity monitoring

Participants were fitted with an exercise tracker, a GENE-Activ tri-axial accelerometer (Active Insights, Kimbolton, UK), for 72 hours post exercise visit. Evidence has

shown that the GENE-Activ accelerometer has excellent technical reliability and its use in a range of other diabetes related studies has confirmed its validity for use within this trial (Bell et al., 2015; Cassidy et al., 2018; Esliger et al., 2011). This was set up with their height, weight and age within the lab and was programmed to automatically record data as soon as the visit was complete. The purpose of the exercise tracker was to monitor their energy expenditure, ensure they were not exceeding their designated energy expenditure for three days post visit and provide evidence of their typical day-to-day activities. Evidence shows three days is sufficient to be an accurate and reliable predictor of weekly activity levels (Dillon et al., 2016).

3.1.8. Dietary analysis

Participants were also required to record everything they ate in a food diary for the day of the exercise visit (the day before the OGTT), including the control meal, and for 3 days after the Oral Glucose Tolerance Test (OGTT) visit. This was to assess the effects their food consumption could be having on their blood glucose levels which may effect and help explain results collected. After every exercise visit, participants were given the same standardised meal (Sainsbury's spinach and ricotta tortellini); 736 kcal, carbohydrate=50.3 g, fat=11.6 g, and protein=13.2 g to consume that same evening. Participants consumed the meal between 6.00 and 8.00pm after which, they were only allowed to consume water until after the OGTT was completed.

3.1.9. Oral Glucose tolerance test

The morning following the exercise visit, the participants attended the lab in a 12-hour fasted state for an Oral Glucose Tolerance Test (OGTT). They were seated in a semi-supine position on a bed (Plinth 2000, Plinth Medical, Suffolk, UK) and could use their mobile device to keep them occupied. Firstly, a finger-tip blood sample was taken following the protocol in section 3.1.4, and blood glucose was measured using a Biosen Analyser (EKF Diagnostics, Biosen, Cardiff, UK). A blood glucose value of

≤5.56 mmol/L indicates that the participant was sufficiently fasted (Moebus et al., 2011). They had a retrograde venous cannula inserted into the dorsal surface of their non-dominant arm by a qualified member of the research team (Figure 7) (Brooks et al., 1989; Copeland et al., 1992). It is inserted in the opposite direction to an anterograde cannula and therefore allows the collection of distal blood samples where arterial venous shunting is more likely to occur (McGuire et al., 1976). The participants arm was then placed in a heated box (52°C) for 15 minutes to allow for arterialisation of the blood (Brooks et al., 1989). This acts as a less invasive alternative to arterial catheterisation but still allows accurate metabolic measurements to be collected (McGuire et al., 1976). A dextrose drink was created by dissolving 75 g of dextrose (Bulk Powders, Colchester, UK) in 300 ml of water. A fasted blood sample was taken from the cannula before consuming the drink. A timer was started and then blood samples (6 ml) were taken at 30, 60, 90 and 120 minutes to assess the effect of the glucose drink on glycaemic control.

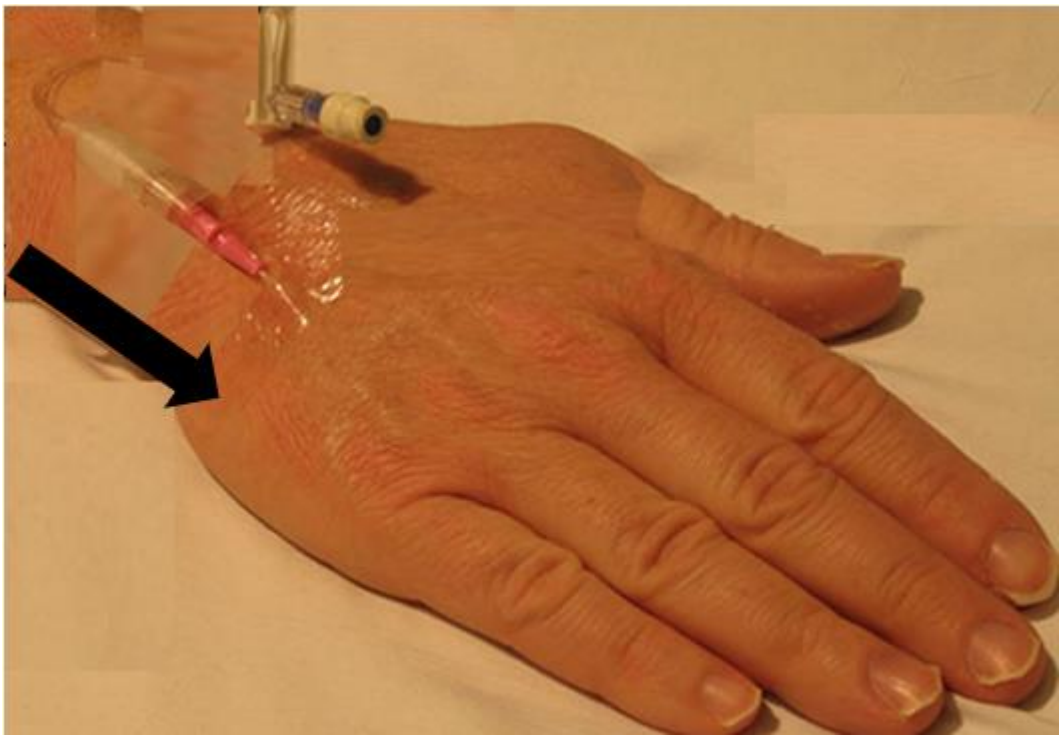


Figure 7. A retrograde venous cannula inserted into the dorsal surface of an individual's hand.

A retrograde cannula is inserted in the opposite direction, towards the fingertips, to an anterograde cannula. The hand is then placed in a heated box and this allows bloods to be taken, where arterial venous shunting is more likely to occur, so that accurate metabolic measurements can be taken in a research setting (McGuire et al., 1976). [Figure adapted from (Schumacher & Leonard, 2014)]

From each blood sample taken, blood glucose, free fatty acids (FFA), plasma and insulin were measured. To do this, 10 ul of blood was taken for blood glucose to be analysed by the Biosen analyser. Then, at each time point the sample was split into 2 vacutainers (VACUETTE, Greiner-Bio One, Gloucestershire, UK), one green (sodium heparin containing 30 µl EGTA-glutathionine which act as preservatives, improving the effective storage of red blood cells (Kucherenko & Bernhardt, 2015; Van'T Erve et al., 2014)) and one gold (serum). The green vacutainer was spun at 1800 RCF for 10 minutes in a 4°C centrifuge (Eppendorf, 5702R, Stevenage UK). The supernatant was split into two microfuge tubes, one labelled FFA and the other labelled plasma.

The tube labelled FFA also had 5 µl tetrahydrolipstatin (THL) – a lipase inhibitor added to prevent degradation of the fatty acids (Hadvary et al., 1991; Krebs et al., 2000). The blood in the gold vacutainer was allowed to clot for 15 minutes at room temperature before centrifugation. The gold vacutainer was then spun at 1800 RCF for 10 minutes in a 4°C centrifuge (Eppendorf, 5702R, Stevenage, UK). The supernatant was transferred to a microfuge tube labelled insulin. This generated a total of 15 microfuge tubes which were frozen at -80°C in the university laboratories to be further analysed.

3.1.10. Glucose response curves

Glucose response curves were constructed from the blood glucose concentrations, recorded at each time point by the Biosen analyser, using the computer software GraphPad Prism (GraphPad Software, Prism, California, USA). The shape of a glucose response curve can help determine the effect of the dose exercise on glycaemic control (Kaga et al., 2020). The area under the curve was calculated using the trapezoidal method within GraphPad Prism to allow for comparison between overall glucose concentrations during the OGTT after different energy expenditures (Allison et al., 1995).

3.1.11. Coefficient of variation

The coefficient of variation (CV) was calculated for the Biosen analyser and the Tanita scales to determine the variation in measurements. This allowed us to determine the accuracy of the equipment and therefore the precision of results (Reed et al., 2002). Multiple measurements of the same conditions were taken, and the following equation was used to calculate the accuracy of each measurement taken with each of these devices (Reed et al., 2002):

$$Coefficient\ of\ variation = \frac{standard\ deviation}{mean}$$

Due to the Covid-19 pandemic, human testing was unable to place as it was deemed unsafe for both participants and the research team. This meant the clinical trial was postponed until May 2021 when ethical approval was given for 18-25 year old, healthy participants to take part in the protocol. To mitigate the impact this had on my MSc by research, during the period which human testing was unable to take place, I undertook a systematic review, meta-analyses and meta-regressions on a topic similar to the clinical trial. Below are the methods used to carry out these analyses.

3.2. Clinical trial - Results

Due to the Covid-19 pandemic, human research was postponed to ensure the safety of participants and the research team. Government lockdown and tier-system restrictions prevented any laboratory access between the 14th October 2020 and 8th March 2021. Ethical approval was given on the 6th May 2021 to carry out the research on young healthy participants (18–25 year olds) who were at a lower risk of the serious implications of Covid-19. The research is in its early stages and therefore minimal data collection has occurred thus far. Four participants are currently enrolled and have started the protocol.

3.2.1. Coefficient of variance

Firstly, coefficient of variance (CV) calculations were carried out on a range of measurements taken within the protocol to ensure the equipment used provided accurate data. Repeat measures of BMI, weight, muscle mass, fat mass, blood glucose concentrations and blood lactate concentrations were recorded under the same conditions using one participant.

Table 2. Coefficient of variation (CV) values for a range of protocol measures.

Values were calculated as a percentage for BMI, Body mass, Fat mass, Muscle mass, Blood glucose and Blood lactate.

	CV (%)
BMI (kg/m^2)	0.22
Body mass (kg)	0.19
Fat mass (kg)	0.79
Muscle mass (kg)	0.1
Blood glucose (mmol/L)	4.18
Blood lactate (mmol/L)	3.97

The four measures recorded on the Tanita Scales (BMI, Weight, Fat Mass, Muscle Mass) all gave coefficient of variation values <1 (0.22%, 0.19%, 0.79% and 0.1% respectively) (Table 2). The two measures recorded by the Biosen (blood glucose and lactate) both gave coefficient of variation values of <5 (4.18% and 3.97% respectively).

3.2.2. OGTT results

Three participants completed one exercise visit and OGTT, and one participant completed two exercise visits (no exercise and 350 kcal).

Glucose concentrations were recorded every 30 minutes for a 2hr period and the recordings at each time point have been plotted to create glucose response curves (Figure 8). Figure 15a shows the response after no exercise and an area under the curve value of 739.9 mmol/L/120mins was calculated. Figure 15b shows the response after 350 kcal of exercise and an area under the curve value of 621.6 mmol/L/120 minutes was calculated. The overall shapes of both curves are relatively similar, showing a similar overall response during the OGTT. Glucose concentration

peaks in the first 30 minutes after consumption of the dextrose drink, with the peak being around 8 mmol/L for 0 kcal energy expenditure (Figure 8a) and 7 mmol/L for 350 kcal energy expenditure (Figure 8b). The glucose concentration then slowly decreases. For 350 kcal of exercise, the reduction in blood glucose concentration appears to plateau quicker at 90mins, whereas for 0 kcal energy expenditure, the glucose concentration still appears to be decreasing at the 120-minute time point.

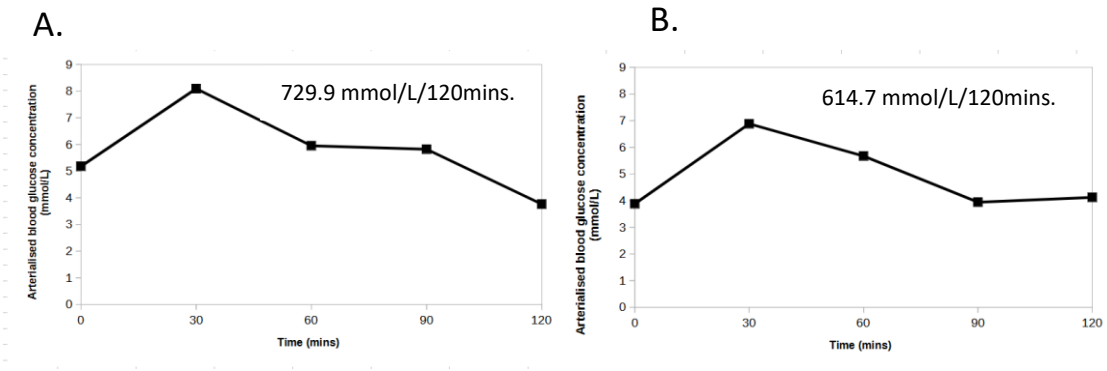


Figure 8. Glucose Response Curves. A. OGTT results after 0kcal energy expenditure. B. OGTT results after 350kcal energy expenditure.

*These graphs plot glucose concentrations throughout OGTTs after exercise visits expending 0 kcal (control) and 350 kcal of energy . **(A)** the glucose response after 0 kcal of exercise. **(B)** the glucose response after 175 kcal of exercise. Area under the curve (AUC) values calculated using the trapezoidal method within GraphPad Prism, the average value of AUC glucose (AUC mmol/L/120mins) is presented.*

3.3. The Clinical Trial - Discussion

The minimal data collected from the clinical trial was used to create glucose response curves of the 2hr OGTT glucose concentrations after two doses of exercise, 0 and 350 kcal.

3.3.1. Glucose response curves

The area under the curve (AUC) of the glucose response curves was calculated to determine the total change in glucose concentrations during the OGTT and to allow comparison of the two different energy expenditures of exercise. For no exercise prior to the OGTT, the AUC was 739.9 mmol/L/120mins. This decreased for 350 kcal of exercise 24 hours prior to the OGTT to an AUC of 621.6 mmol/L/120mins. These results had an extremely small sample size and therefore the reliability of them is unlikely to be high. However, these results support evidence from similar research carried out previously. For example, one clinical trial investigated the effects of 10 days of exercise in obese men (Angelopoulos et al., 2002). They concluded that the area under the curve of the results from an OGTT significantly differed between the exercise and control group from 944.6 mmol/L/120mins in the control group to 884.4 mmol/L/120mins in the exercise intervention group (Angelopoulos et al., 2002). Another clinical trial investigated the effects of three levels of exercise activity on glucose response via an OGTT (Simper et al., 2020). They discovered similar results that exercise decreased the AUC, but they also found that the higher intensity and volume of exercise had the biggest decreases in the AUC (Simper et al., 2020). These results also give more evidence to suggest that the meta-regression using intensity and volume as moderators, may be more significant if a higher sample size and a larger range of exercise programmes were utilised within the analysis.

However, there is some conflicting evidence within the literature as to whether any amount of exercise will result in a reduction in the AUC after an OGTT. One clinical trial found no significant difference between the AUC of the exercise intervention and the control (Potteiger et al., 2002). This could be due to the prescription and dose of exercise given to participants. More clarification could be given on this matter if the clinical trial continues to progress and data is collected on a higher sample size with all four doses of exercise (0, 175, 350, 700 kcal) being analysed.

The general shape of the glucose response curves (Figure 8) are similar to each other with glucose rising to a peak and then gradually beginning to decrease again. When compared to the typical shape of a glucose response curve in healthy individuals, the general shape of both curves is very similar (Arslanian et al., 2019), which suggests the participants are healthy in terms of glycaemic control. The two curves, of both doses of exercise (0 and 350 kcal), peak within the first 30 minutes of the OGTT. Evidence has shown that if glucose peaks after 30 minutes in an OGTT it is potentially indicative of prediabetes (Chung et al., 2017), further supporting the idea that the participants are healthy in regards to glycaemic control.

The value at which glucose concentration peaks, decreases from 8.09 mmol/L in the OGTT after 0 kcal of exercise to 6.88 mmol/L in the OGTT after 350 kcal of exercise. This supports the idea that the acute effects of exercise on improving glycaemic control can still be experienced at least 24 hrs post-exercise (Van Dijk, Manders, et al., 2013). Evidence has shown that acute exercise increases the signalling of non-insulin dependent glucose signalling pathways and also increases the translocation of GLUT4 to the plasma membrane to ultimately increase glucose uptake (Röckl et al., 2008). The lower peak after 350 kcal of exercise may indicate that these changes are still present 24 hours post exercise. Therefore, this allows glucose uptake into cells to occur at a faster rate in the participants that undertake exercise prior to the OGTT which results in the lower peak plasma glucose concentrations.

Interestingly, the glucose concentration at the 2-hour time point is lower following 0kcal energy expenditure (Figure 8a). For 0 kcal energy expenditure, the final glucose concentration is 3.76 mmol/L whereas for 350 kcal energy expenditure, the final glucose concentration is 4.12 mmol/L. This contradicts other research, which suggests in OGTTs after a bout of exercise, the final glucose concentration measurement is significantly lower than the final measure taken after no exercise (Angelopoulos et al., 2002). However, the OGTT results after 350 kcal are shown to

either plateau or slowly begin to peak again after the 90-minute time point (Figure 8b) so this could provide an explanation for the higher glucose concentration. Research has been carried out into identifying the shapes and the impacts of both monophasic and biphasic curves (Tschritter et al., 2003). Monophasic curves have one peak and then a gradual decrease of glucose concentration whereas biphasic curves have an initial peak and decrease, and then the glucose concentration begins to increase again (Figure 9) (Tschritter et al., 2003). This could suggest that the 0 kcal glucose response curve is monophasic due to the peak and then decrease in glucose concentration. The 350 kcal glucose response curve could potentially be biphasic as there is an initial peak and decrease and then the glucose concentration could be gradually starting to increase after 90 mins. Evidence has also shown that biphasic curves are more indicative of improved insulin sensitivity (Kim et al., 2016; Tschritter et al., 2003). Therefore, this could suggest that insulin sensitivity has improved due to the 350 kcal of exercise prior to the OGTT, subsequently improving glycaemic control.

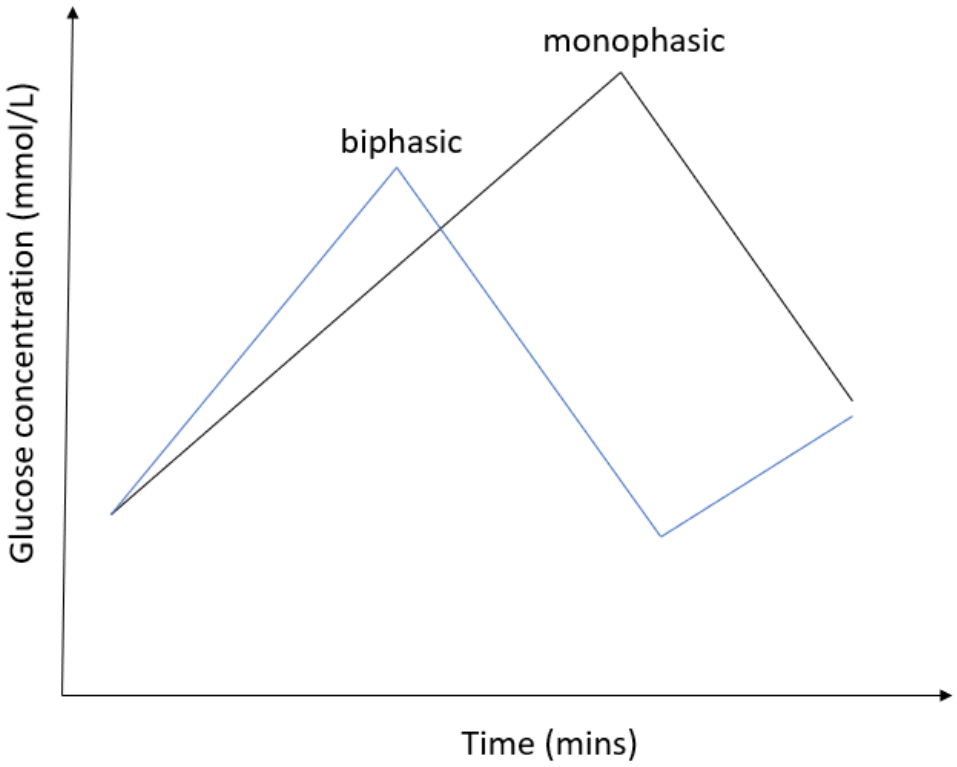


Figure 9. Different shapes of glucose response curves after an OGTT.

A monophasic curve has a peak plasma glucose concentration and then a steady decline. A biphasic curve has an initial peak, plasma blood glucose concentrations begin to decline and then concentrations begin to increase again. [information to create figure taken from:(de Andrade Mesquita et al., 2018)*]*

3.3.2. Coefficients of variation

Coefficient of variation (CV) statistics are calculated to determine the reliability of the testing equipment used as this provides confidence that changes within the data are accurate and not just due to variation within the measurements taken using the equipment (Reed et al., 2002). To investigate this, coefficient of variation values were calculated for blood glucose and lactate concentrations recorded by the Biosen device, and also for BMI, weight, fat mass and muscle mass recorded by the bioimpedance scales (Tanita scales). All of the CV values for the measurements taken by the Tanita scales were less than 1%. This suggests that a

less than 1% change in the data is due to chance rather than the variables investigated (Reed et al., 2002). Therefore, if change is observed in the data higher than 1%, then it can be assumed that the data is reliable and indicative of change due to the intervention (Reed et al., 2002). The accuracy of the calculated CV values from the Tanita scales is supported by CV values calculated from the scales on other young healthy individuals (Pietrobelli et al., 2004)(Langer et al., 2016).

The blood glucose and lactate concentrations determined using the Biosen had a higher percentage of variation than the Tanita scales but the CV values for both glucose and lactate measurements was still less than 5%. This is indicative of relatively low variation in the data due to the reliability of the equipment (Ospina & Marmolejo-Ramos, 2019) and suggests changes within data collected from this device greater than 5% are likely to be due to the interventions in place.

3.3.3. Overall summary of the findings

These results show that there could be an association in increased doses of exercise improving glycaemic control. However, due to the incomplete data set and low sample size, assumptions can only be made with support from existing literature. The coefficient of variation calculations demonstrate that, when more data is collected, the data is likely to be accurate and reliable due to the extremely small likelihood that differences in results are due to chance, meaning the intervention is causing the effect.

3.3.4. Limitations

One limitation of the research was with difficulties in retrograde cannulation, which prevented successful blood draws. This was typically due to the cannula being inserted near the vein wall, or due to insufficient pressure preventing blow flow out of the cannula (Cadamuro et al., 2015). Some of the blood taken from participants also

haemolysed which meant red blood cells had burst and the contents were released into the plasma and therefore it is likely that inaccurate data was recorded (Cadamuro et al., 2015). Once identified, changes were put in place to try and mitigate these issues such as readjusting the cannula after a test blood draw and using shorter connective kits which connect the cannula to a collection syringe.

Another limitation of the research was in reducing subject bias within the protocol. It was difficult to blind participants to the dose of exercise they were completing as higher doses of exercise took a longer time to complete. It was also difficult to blind the research team as to which dose of exercise the random generator had generated for the participant to complete. This is because the investigator had to carry out calculations to determine the time required to exercise for that specific number of calories to be burnt, and they also had to set up the equipment to ensure the correct dose was completed. One way to reduce subject bias would be to not inform the participant of the dose of exercise they will be completing prior to them starting the exercise. Another way to reduce subject bias would be to get another member of the research team that isn't collecting or analysing the results to randomise the dose of exercise and carry out the calculations to determine the length of time participants need to cycle for.

As none of the participants were able to fully complete the protocol, the laboratory analyses on free fatty acid and insulin concentrations within the bloods taken for the OGTTs were not analysed. An understanding of FFA and insulin concentrations would be useful in determining the optimal doses of exercise required for participants to use an exercise programme for treatment. FFA concentrations have found to be significantly increased in obese individuals and individuals with type 2 diabetes (Boden, 1999). This increase can be detrimental to the health of individuals, increasing both the risk of cardiovascular disease and the level of insulin resistance (Boden, 1999). Evidence suggests that both FFA concentrations and insulin sensitivity

can be improved through an exercise programme (Mika et al., 2019) and improvements in these factors could therefore contribute to improved glycaemic control in individuals with type 2 diabetes. FFA and insulin concentrations are therefore important factors to measure within this research.

Finally, ensuring participants adhere to the requirements in the protocol relies mainly on trust. This includes participants not exercising for 72 hours prior to the visit, not consuming alcohol 48 hours prior, and only consuming the control meal 12 hours prior to the OGTT. All of these factors are likely to interfere with the data collected during the protocol. The use of an exercise tracker to measure their activity for a period before and after the exercise visit was one method put in place to monitor their activity levels. Blood glucose samples were taken before we began the OGTT to ensure the participant was suitably fasted. These measurements helped to ensure the glycaemic control measured during the OGTT was only in response to the dextrose drink and that changes seen in the OGTT were only due to the dose of exercise prescribed.

3.3.5. Conclusions

At the current stage, the study has been unable to successfully achieve its aims. From the data collected, the minimal dose of exercise to improve glycaemic control was unable to be determined. The results collected imply, with support from the current literature, that 350kcal of exercise has beneficial impacts on improving glycaemic control in young healthy individuals which is also likely to have similar benefits, if not greater benefits, on individuals with type 2 diabetes (Bird & Hawley, 2017). Due the importance of the research, discussed in section 2.11, the clinical trial will be encouraged to continue. This will allow the aims to be achieved and give a better understanding of the optimal dose and minimum dose of exercise required to treat type 2 diabetes.

3.3.6. Challenges of the research

The Covid-19 pandemic had a major impact on this clinical trial. This is evidenced by the extremely small sample size and incomplete research protocol. Research was postponed until May 2021 as it was deemed unsafe due to the high risk it posed to both participants and the research team. Recruitment of young healthy participants was finally approved by the ethics committee and therefore recruitment began in May 2021. During the period which research was unable to take place, the systematic review, meta-analyses, and meta-regressions were completed.

Recruitment after this point still proved to be difficult as individuals were cautious about attending the lab, even with Covid-19 safety precautions in place. There was also a number of individuals that had to postpone participating in the protocol due to having to isolate, as Covid-19 Delta variant cases began to rise in the area (Office for National Statistics, 2021). The number of people testing positive for Covid-19 in the north west increased from 0.45% on the 30th May 21 to 2.06% on the 7th August 21 (Office for National Statistics, 2021) and therefore, just after ethical approval had been given to begin testing at the start of May 21, the progression of the trial was impacted again by Covid-19. Four participants were initially recruited, three of which were university students who were no longer available out of term time from the beginning of July onwards. Recruitment was also made more difficult due to the reliance of the protocol on medics to cannulate volunteers who were extremely busy due to the high cases of Covid-19 in the area. Therefore, there were a number of weeks where no medics were available, and research could not take place.

4. Meta-analysis

4.1. Methods

4.1.1. Aims and objectives

The benefits of exercise for type 2 diabetes are well documented. However, the precise volume and intensity of exercise required to improve glycaemic control is unknown. Exercise is effective in helping to regulate glycaemic control in those with type 2 diabetes, however, a meta-analysis hasn't been completed since 2012 to analyse the impact of the dose of exercise on glycaemic control (Umpierre et al., 2013). The exclusion criteria for this meta-analysis narrowed down the papers retrieved from the search further, compared to the meta-analysis previously completed, and only included aerobic exercise programmes. This was done with the goal of minimising heterogeneity in the results. The aim of these meta-analyses and meta-regressions was to determine the impact of volume and intensity of exercise on glycaemic control in people with type 2 diabetes. This was carried out by conducting a meta-analysis and then meta-regression of studies examining the impact of volume and intensity on HbA1c concentrations in those with type 2 diabetes, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021).

4.1.2. Eligibility criteria

This systematic review and meta-analysis were conducted in accordance with PRISMA guidelines (Page et al., 2021). Studies that met the following criteria were included in this meta-analysis: (1) published as a full-text manuscript; (2) not a review; (3) participants had type 2 diabetes (age >17 years); (4) studies were required to employ an intervention design and include a supervised aerobic exercise training period of ≥12 weeks. Additionally, descriptive data (e.g., sample size, mean, and standard deviation) were required to be reported. Where this was not possible, details were requested from authors. The exclusion criteria was created to be more specific than the previous meta-

analysis carried out (Umpierre et al., 2013). This meant, for example, only including aerobic exercise protocols and excluding protocols where individuals were taking alternative glucose lowering medication, such as metformin and corticosteroids. The primary aim was to investigate whether HbA1c was effected by aerobic exercise training and therefore we only included studies that measured HbA1c. Where an investigation took multiple measures such as BMI, fasting blood glucose, total cholesterol, triglycerides, LDL and HDL, we included them as separate datasets.

HbA1c was chosen as the primary measure as it is now recommended as the standard of care for testing diabetes (Sherwani et al., 2016). HbA1c provides a reliable estimate of the patient's blood glucose concentrations over a 3-month period as this is the average half-life of a red blood cell (Sherwani et al., 2016). Training programmes selected were therefore ≥12 weeks to ensure exercise could have an impact on and provide an accurate measurement of HbA1c concentrations. Ensuring that programmes were supervised increases the likelihood that participants successfully complete all the exercise training as stated in the methodology of the research paper.

4.1.3. Information sources

The following databases were identified and searched: MEDLINE (accessed by PubMed), Cochrane Central Register of Controlled Trials, Embase, and Web of Science. The literature search was completed up until 15th December 2020 and there was no start date. The search was performed within all fields and terms were "exercise AND glycaemic control", "Type 2 Diabetes AND exercise", and "exercise AND glycaemic control AND Type 2 diabetes AND randomised control trial". The detailed PubMed search is given in the appendices as an example (Figure 18).

4.1.4. Study selection

The eligibility assessment was conducted by myself in an unblinded and standardised manner and was confirmed by a second independent researcher. Once each database search was completed and manuscripts were sourced, all studies were downloaded into a single reference list with duplicates removed. Titles and abstracts were then screened for eligibility and full texts were only retrieved for studies with HbA1c and a supervised exercise intervention incorporated. All studies retrieved as full texts were then thoroughly assessed using the complete eligibility criteria by myself and a second independent researcher confirming inclusion and exclusion.

Additionally, retrieved full-text articles were classified using a PEDro (Physiotherapy Evidence Database) scale (Herbert et al., 2000). The PEDro scale is widely used to assess the internal validity of results and whether the level of statistical analysis allows the results to be interpretable (Albanese et al., 2020; de Morton, 2009). The score is rated out of 10 and depends on 11 different criteria, 1 (eligibility criteria) of which isn't included in the score due to assessing the external rather than internal validity of the trial. The criteria includes; an eligibility criteria, random allocation, allocation concealment, similar starting baselines of participants, blinding of subjects, blinding of those administering the protocol, blinding of those taking measurements, measurements obtained from ≥85% of participants, ensuring subjects received treatment and control as allocated, between group statistical analysis for at least one outcome, and providing point measures and measures of variability for at least one outcome (Herbert et al., 2000). PEDro scores are provided in section 4, table 2.

4.1.5. Data collection process

Data were extracted for pre- and post-exercise HbA1c concentrations primarily. Then pre- and post- exercise BMI, fasting blood glucose, triglyceride, total cholesterol, HDL and LDL concentrations were extracted from papers which included these values.

Information on the number of participants, age of participants, trial period, number of exercise sessions each week and the length of each session was also recorded. In cases of missing data, authors were contacted via email and asked to provide necessary information. If no response was received, means and standard deviations (SDs) were estimated from figures using computer software (Image J, Maryland, USA). There were four requests sent for additional data and a two-week time frame for replies allowed for a response. No responses were received.

The data for each variable was converted into the same units and if values were reported as median (range) then they were converted to mean ± SD using the following equations:

$$\text{Mean} = \frac{a+2m+b}{4}$$

Where a = lowest value, m = median and b = highest value

$$\text{SD}^2 = \frac{1}{12}\left(\frac{(a-2m+b)^2}{4} + (b-a)^2\right)$$

Where a = lowest value, m = median and b = highest value

The difference between pre- and post- exercise means and standard deviations was calculated ready for the meta-analysis to be carried out. If the change in standard deviation wasn't available, it was calculated from the pre- and post- standard deviations using the following formula:

$$SD_{change} = \sqrt{SD^2{}_{baseline} + SD^2{}_{final} - (2\ x\ Corr\ x\ SD_{baseline} x\ SD_{final})}$$

Information, including changes in control mean, control standard deviation, control group sample size and exercise mean, exercise standard deviation and exercise group sample size for each variable, were imported into a spreadsheet within Jamovi (The Jamovi project (2021), *Jamovi* (Version 1.6) [Computer Software]) for the analysis to

take place. This software is specifically designed for meta-analyses. A random effects model of the meta-analysis with a tau^2 estimator: maximum likelihood was calculated for each variable; HbA1c, fasting blood glucose, BMI, total cholesterol, triglycerides, HDL, and LDL. This software was used to produce forest plots to present the data and calculate standard mean differences (SMD) for each variable and the overall SMD of the meta-analysis. Funnel plots were created, and Egger's regression values were calculated to assess the asymmetry of the results. The funnel plots plot the SMD (treatment effect) for individual studies on the horizontal access and the standard error on the vertical axis and this was then used to examine how effect sizes differed with the sample sizes, measured by the standard error of the studies (Song et al., 2002). Egger's regression values were calculated within Jamovi to statistically examine whether asymmetry was present within the funnel plot (Sterne et al., 2000). Visual examination of the funnel plots and the statistical analysis using Egger's regression can help identify whether any publication or associated bias is present within the data (Sterne et al., 2000).

4.1.6. Meta-Regression

Also using Jamovi, meta-regressions were carried out to try and establish if there was a dose-response relationship between intensity and volume on improvement in HbA1c i.e., if improvements in HbA1c were greater in higher intensity exercise or longer exercise interventions. To determine this, the intensity of the exercise sessions was calculated as a percentage of maximum exercise capacity. This measurement was chosen as the intensity stated in the research papers were expressed in different formats. Volume of exercise was calculated as the total dose of exercise undertaken throughout the programme and was calculated from the length of the exercise session, number of exercise sessions per week and total length of programme.

Random effects meta-regressions (continuous covariates) were then carried out to explore the association of volume and intensity of exercise with changes in HbA1c concentrations. Maximum exercise capacity (intensity) was input as a moderator into the analysis. A separate meta-regression was then produced using volume as a moderator to assess the effect of volume on HbA1c. A final meta-regression was produced which combined volume and intensity together to use as a moderator within the analysis. Meta-regression analyses were carried out in Jamovi and R (The R foundation (2021), *R (version 4.1.0)* [computer software] retrieved from https://cran.r-project.org), using the SMD estimates of HbA1c and the two exercise programme variables- intensity and volume. Scatter bubble plots were constructed graphically within R to display proportional weights of different trials. The code written by myself within R to carry out the analysis and create the bubble plots is presented below. The meta function was used to generate the initial meta-analysis. The metareg function of the Metafor package then used the meta-analysis and the covariate (moderator) as the input (Harrer et al., 2021). Bubble plots were then created using the function bubble to show the predicted regression slope with the y axis representing the SMD of HbA1c and the x axis representing the moderator, the size of the bubble depends on the weight of each study (Harrer et al., 2021). R^2_* values were also calculated for each regression analysis run to examine the amount of variability within the effect size of HbA1c which can be accounted for by the model (each of the moderators) (Hamilton et al., 2015). It was calculated using the equation below (Harrer et al., 2021).

$$R^2 *= 1 - \frac{\tau^2\ unexplained}{\tau^2(total)}$$

$$where\ \tau^2 = heterogeneity$$

Code written to carry out meta-regression analysis and create bubble plots in R:

```
file.choose()

data1<-read.csv("C:\\Users\\lizzi\\OneDrive\\Desktop\\hba1c for metafor.csv", header=TRUE)

attach(data1)

str(data1)

library(metafor)

library(meta)

meta1<-metacont(mean.e=ex.mean, sd.e=ex.sd, n.e=ex.sample.size, mean.c=ï..control.mean, sd.c=control.SD, n.c=control.sample.size, studlab=author, data=data1, sm="SMD")

meta2<-metareg(meta1, ~intensity)

bubble(meta2, studlab=data1$author, xlim=c(0.4, 0.8), ylim=c(-8.5, 2), main="bubbleplot")

meta3<-metareg(meta1, ~volume)

bubble(meta3, studlab=data1$author, xlim=c(-30, 125), ylim=c(-8.5, 2), main="bubbleplot")

meta4<-metareg(meta6, ~volumexintenisty)

bubble(meta4, studlab=data1$author, xlim=c(-30, 100), ylim=c(-8.5, 2), main="bubbleplot")
```

4.1.7. Data items

Heterogeneity was quantified with the I^2 statistic and through the analysis of the funnel plots created. An I^2 value of 25% may be interpreted as low, 50% as moderate and

75% as high between study heterogeneity (Higgins et al., 2003). A random-effect meta-analysis was carried out on aerobic exercise protocols. Data extracted from each study included: study sample size, intervention/control group descriptions, study design, analysis method, and outcome data. Outliers were excluded if they had a studentised residual larger than the 100 x (1 - 0.05/(2 X k))th percentile of a standard normal distribution (Ranganai, 2016). Methodological quality was assessed using the modified 0-10 PEDro scale (de Morton, 2009). The primary outcome variables were defined as HbA1c pre- and post-intervention. Standardised mean differences (SMD) were retrieved for inclusion into the meta-analysis. Further analyses were performed based on research design as a means of investigating heterogeneous results. Random effects meta-regressions (continuous covariates) were carried out to explore the association of volume (total volume) and intensity of exercise (maximum exercise capacity) with changes in HbA1c concentrations. Scatter bubble plots were constructed graphically to display proportional weights of different trials and R^{2}_{*} values were calculated.

4.2. Results

This research consisted of both a systematic review including a meta-analysis with meta-regression and a clinical trial to investigate the effects of different doses of exercise on glycaemic control and to help examine exercise as a treatment. The clinical trial was postponed due to the Covid-19 pandemic until May 2021 and is therefore in the early stages of research where human testing on young healthy participants has only just begun.

4.2.1. Meta-analysis and meta-regression

Both a meta-analysis and meta-regression were carried out to investigate the effects of volume and intenstiy of exercise programmes on HbA1c concentrations and ultimately glycaemic control. In the meta-regression, intensity and volume of exercise were used as modifiers to identify the specific effects of these factors on HbA1c concentrations.

4.2.2. Study selection

After the initial database search, 5364 records were identified (Figure 10). Once duplicates were removed, 3775 titles and abstracts were screened for inclusion, resulting in 190 studies being retrieved as full text and assessed for eligibility. Of those, 174 were excluded and 20 articles remained, and due to missing details 16 studies were used in the final quantitative synthesis. To assess publication bias, funnel plots were computed and Egger's regression value was calculated to statistically analyse the asymmetry of the plots (Song et al., 2002; Sterne et al., 2000).

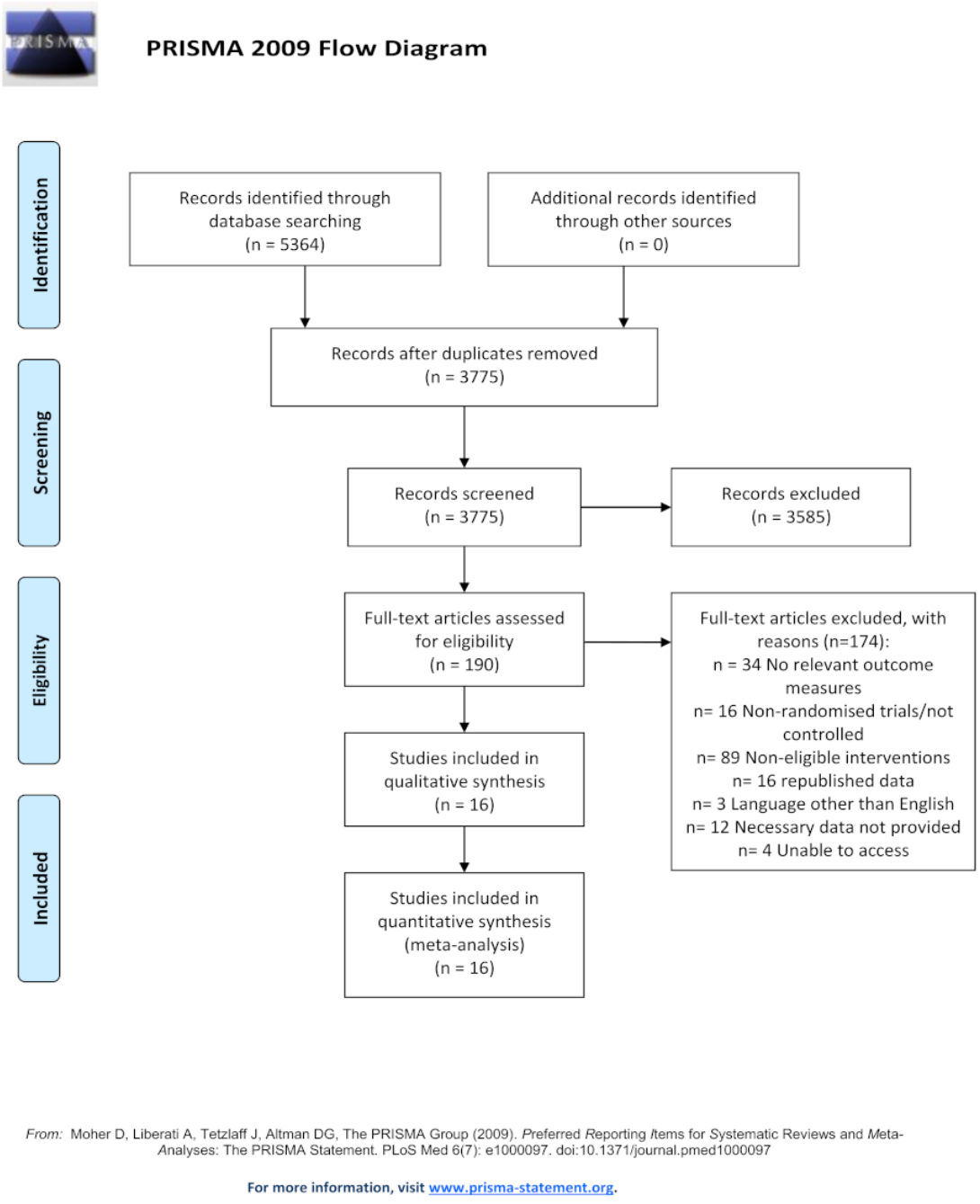


Figure 10. PRISMA flow diagram of studies included in the systematic review.

Studies were identified through database searching and then compiled into a spreadsheet. Duplicates were removed and articles were screened via title and abstracts. Full texts were then assessed, and this resulted in 16 studies being included in the quantitative synthesis. [Figure template retrieved from: (Page et al., 2021)]

4.2.3. Trial setting and participants

The exercise programme settings included university health or research departments, community-based exercise facilities and large public hospitals. The number of participants in each clinical trial ranged from n = 19 to n = 251 (Belli et al., 2011; Sigal et al., 2007). One trial included only female participants (Belli et al., 2011) and one trial included only male participants (Yan et al., 2014). All trials were carried out on adults and the age of participants ranged from 30-75 years. Baseline HbA1c (%) ranged from an average of 6.7% (Backx et al., 2011) to 8.8% (Yan et al., 2014) for all trials included. The intensity of the exercise programmes (as % total exercise capacity) ranged from 50% (Ku et al., 2010) to 75% (Balducci et al., 2010; Jorge et al., 2011) and the total volume of exercise for the programme ranged from 9 hours (20-37.5 min 3 d/week for 16 weeks) (Alvarez et al., 2016) to 390 hours (50 mins 3-5 d/week for 39 weeks) (Sénéchal et al., 2013).

4.2.4. Interventions

A description of the exercise programmes included is given (Table 3). The duration of interventions ranged from 12 weeks to 52 weeks, with a median of 12 weeks. The studies mainly included aerobic exercise in the form of cycling, jogging, and walking. 75% of the studies included had three exercise sessions per week, two interventions had only two sessions per week (Emerenziani et al., 2015)(Balducci et al., 2010) and two interventions had up to five sessions per week (Ku et al., 2010; Sénéchal et al., 2013). Each exercise session lasted between 12 minutes and 1 hour, with most sessions lasting between 30 minutes and 1 hour. All interventions were supervised.

Table 3. Description of included studies and data sets.

Descriptions of studies included in the meta-analyses and meta-regressions. Details of the exercise interventions included within the study programme, design method, outcome measures recorded, number of participants and the PEDro score of the trial. MEC= maximum exercise capacity, RCT = randomised control trial; HbA1c = Glycated Haemoglobin, BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein.

Reference	Exercise Intervention	Design method	Outcome measures	Participants	PEDro score	Findings
(Sénéchal et al., 2013)	Aerobic exercise 150mins per week at 65% MEC	RCT	HbA1c	Control=41, EX=72 Age=30-75 BMI <48kg/m^2	7/10	Reduction in central adiposity and increase in fitness contributed to greater reductions in HbA1c
(Mitranun et al., 2014)	Aerobic exercise for 105mins per week at 58% MEC.	RCT	HbA1c, FBG, total cholesterol, triglycerides, LDL, HDL, BMI	Control=15, EX=14 Age=50-70 BMI=29.55kg/m^2	6/10	Interval training confers greater advantage than continuous training for glycaemic control
(Yavari et al., 2012)	Aerobic exercise 120min per week, at 65% $\dot{V}O_2$ MEC.	RCT	HbA1c, BMI, total cholesterol, FBG	Control=20, EX=20 Age=33-69 BMI <43kg.m^2	6/10	Aerobic exercise reduces HbA1c and triglyceride concentrations
(Maria et al., 2008)	Averaged at 120mins per week and 62.5% MEC.	RCT	HbA1c	Control=17, EX=14 Age= 40-65 BMI=25-36kg/m^2	6/10	Aerobic exercise caused no significant decrease in HbA1c concentration
(Belli et al., 2011)	90 mins per week at 65% MEC. Walking at ventilatory threshold.	RCT	HbA1c, total cholesterol, triglyceride, LDL, HDL, BMI	Control= 10, EX=9 Age=60-75 BMI=30.05kg/m^2	7/10	Overground walking at ventilatory threshold improves long term glycaemic control
(Emerenziani et al., 2015)	100 mins per week at 53% MEC.	RCT	HbA1c, total cholesterol, LDL, HDL, BMI	Control= 15, EX=15 Age= 39-70 BMI= 34.6kg/m^2	5/10	Exercise training improved maximal exercise capacity and glycaemic control

(Sigal et al., 2007)	Averaged at 100min per week, 60% MEC.	RCT	HbA1c, total cholesterol, triglyceride, LDL, HDL, BMI	Control=63, EX=60 Age= 40-75 BMI=35kg/m^2	7/10	Hba1c reduced after aerobic exercise but highest improvement with combined exercise
(Alvarez et al., 2016)	Interval training averaging at 97.5 mins per week and 58% MEC	RCT	HbA1c, FBG, total cholesterol, triglycerides, LDL, HDL, BMI	Control=10, EX= 13 Age=40-65 BMI= 30.5kg/m^2	6/10	Improvements in HbA1c greater after high intensity interval training than control
(Ku et al., 2010)	120mins exercise per week at ~50% maximal exercise capacity	RCT	HbA1c, BMI	Control=16, EX=15 Age=18-65	6/10	Aerobic exercise improves insulin sensitivity and Hba1c concentration
(Balducci et al., 2010)	120 mins per week, 75% maximal exercise capacity.	RCT	HbA1c, total cholesterol, triglyceride, LDL, HDL, BMI	Control=20, EX=20 Age=50-70	7/10	Exercise improves HbA1c but higher intensity does not provide additional benefits
(Yavari et al., 2010)	195mins per week at 65% maximal exercise capacity	RCT	HbA1c, FBG, BMI	Control=30, EX=35 Age=44-69 BMI <43	4/10	Aerobic exercise improves HbA1c but combined exercise has greater positive changes
(Way et al., 2020)	135 mins per week at 60% maximal exercise capacity	RCT	HbA1c, FBG, total cholesterol, LDL, HDL, BMI	Control=11, EX=12 Age=40-75years BMI=36.1kg/m^2	7/10	HbA1c concentration reduces and cardiovascular health improves after low volume HIIT
(Jorge et al., 2011)	180 mins per week, 75% maximal exercise capacity (lactate threshold)	RCT	HbA1c, FBG, total cholesterol, triglyceride, HDL, BMI	Control=12, EX=12 Age=40-70years BMI=25-40kg/m^2	4/10	Exercise improves glycaemic control
(Backx et al., 2011)	180mins per week at 56% maximal exercise capacity.	RCT	HbA1c, FBG, total cholesterol, triglycerides, LDL, HDL, BMI	Control=9, EX=10 Age= 50-75 BMI=30.0kg/m^2	5/10	Exercise programme improved HbA1c concentration through enhanced β cell function
(Middlebrooke et al., 2006)	90 mins per week at 65% maximal	RCT	HbA1c, FBG, total cholesterol,	Control=30, EX=22 Age=40-70	7/10	No significant difference between

	exercise capacity.		triglycerides, LDL, HDL, BMI	BMI=30.3kg/m^2		exercise and control group HbA1c concentration
(Yan et al., 2014)	135 mins per week averaging at 62.5% maximal exercise capacity.	RCT	HbA1c, BMI	Control=10, EX=31 Age=50-65 BMI=27.1kg/m^2	6/10	Exercise improved HbA1c in already active T2DM patients.

4.2.5. Synthesis of results

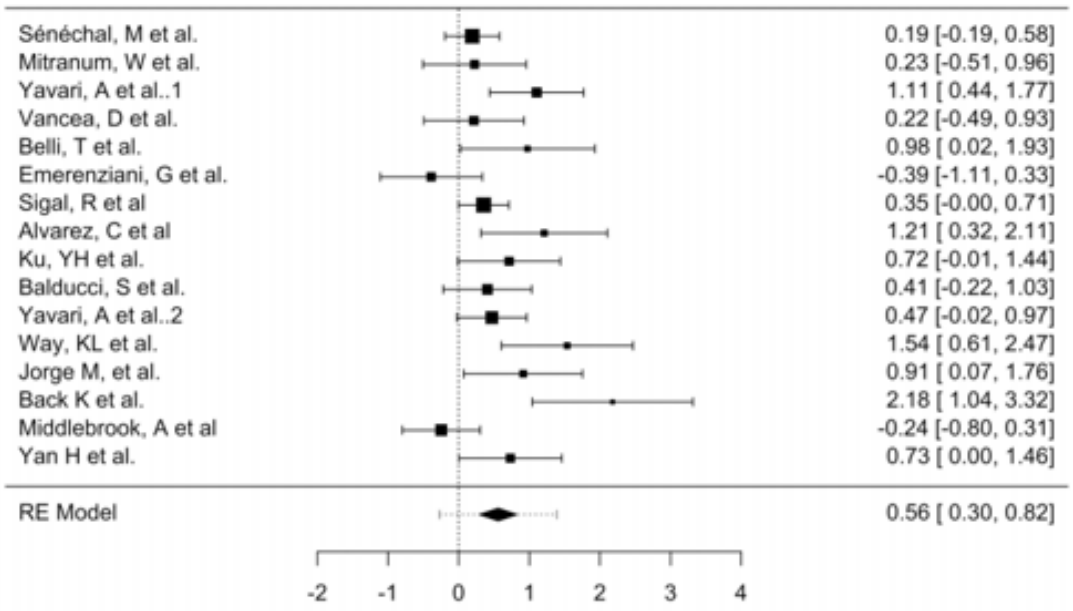
Of the 16 studies included, all were randomised control trials (RCTs). Where a study had multiple conditions, they were treated separately.

4.2.6. Glycaemic control

HbA1c reflects a cumulative history of an individual's blood glucose levels during the previous 2-3 months, due to the half-life of a red blood cell being around 12 weeks (Sherwani et al., 2016). This meta-analysis investigated changes in HbA1c (as the gold-standard measure) in response to regular supervised aerobic exercise to determine the impact on glycaemic control. All 16 trials provided data on HbA1c. The individual mean difference of pre-post changes in HbA1c in control and aerobic groups, respectively, ranged from -1.2% to 1.1% (median = -0.09%) and -1.3% to 0.1% (median = -0.51%). Standardised mean difference (SMD) of within-group change in HbA1c between control and aerobic groups in each of the trials ranged from -0.39 to 2.18, with 88% of trials showing a positive change towards reducing HbA1c. The pooled summary estimate of standardised mean difference of within-group change in HbA1c between control and aerobic groups was 0.56 (95% CI 0.3 – 0.82) with p <0.001 (Figure 11), favouring aerobic exercise over control for reducing HbA1c concentration. There was considerable heterogeneity in this meta-analysis (I^2= 61.05%, p <0.001)

and the funnel plot shows potential asymmetry (Figure 11b) with Egger's regression value supporting this (Egger's regression value = 3.21, p <0.05).

a.



b.

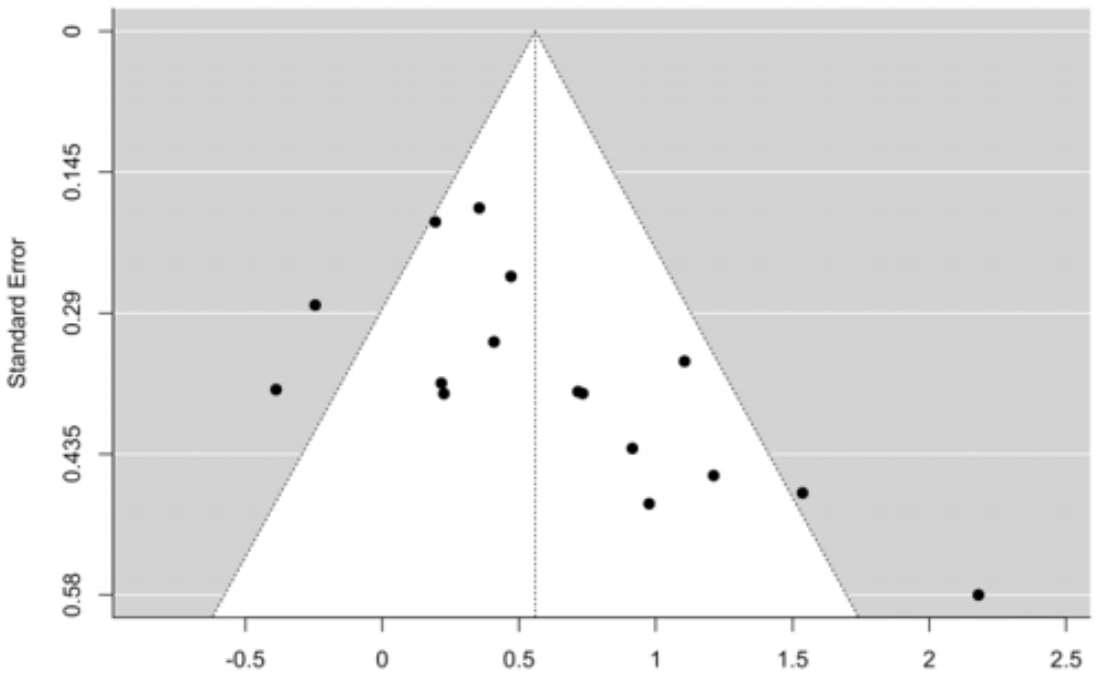
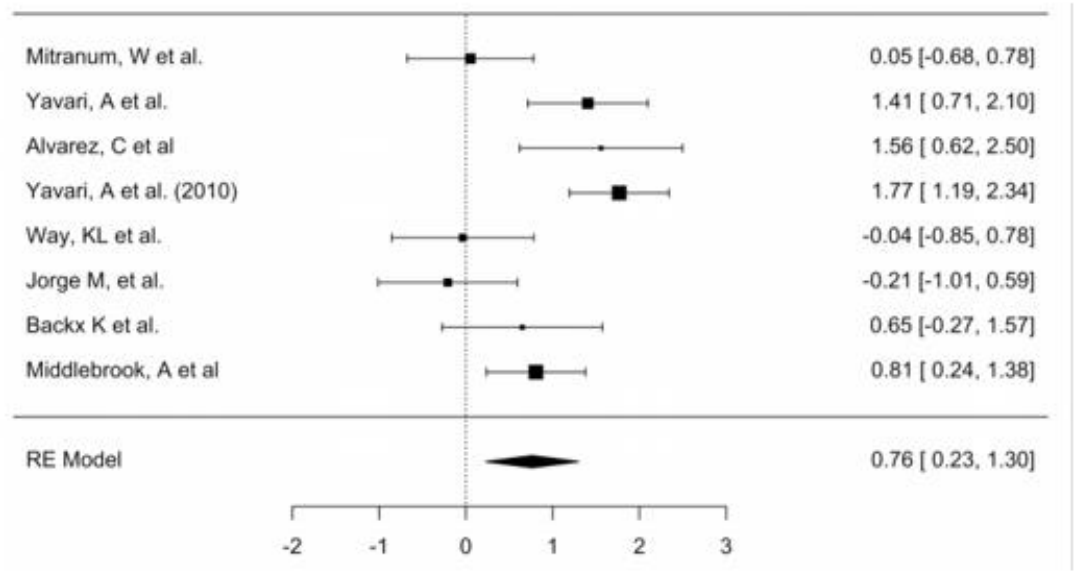


Figure 11. Meta-analysis of HbA1c.

(a) *Forest plot comparing the effects of exercise on HbA1c concentrations (SMD). A positive value represents a bigger difference in the SMD between exercise and control group, indicating that exercise reduces HbA1c.* ***(b)*** *Funnel plot of studies evaluating the effect of exercise on HbA1c.*

Fasting blood glucose measures the plasma glucose concentration after a period without food consumption (Bouma et al., 1999). This helps to examine the change in the exact glucose concentration between the start and end of the exercise programme (Bouma et al., 1999). Although this may not be representative of the average long-term change in glycaemic control like HbA1c, it was used in this meta-analysis as a second line of evidence to support the results indicated by HbA1c (Bouma et al., 1999). Eight trials recorded fasting blood glucose concentrations and one trial was removed due to being a large outlier in the data set (Alvarez et al., 2016). Therefore, seven trials were included in this meta-analysis. The individual mean difference of pre-post changes in fasting blood glucose in control and aerobic groups, respectively, ranged from -1.35 mmol/L to 0.6 mmol/L (median = 0.5 mmol/L) and -1.53 mmol/L to 0.15 mmol/L (median= -0.99 mmol/L). SMD of within-group change in fasting blood glucose between control and aerobic groups in each of the trials ranged from -0.21 to 1.77, with 75% of trials showing a positive change towards reducing fasting blood glucose. The pooled summary estimate of the standardised mean difference of within-group change in fasting blood glucose between control and aerobic groups was 0.76 (95% CI 0.23 – 1.3) with $p < 0.05$ (Figure 12), showing a significant reduction in fasting blood glucose with exercise programmes. There was considerable heterogeneity in this meta-analysis (I^2= 76.67%, p <0.001). Examination of the funnel plot (Figure 12b) and Egger's regression value (Egger's regression value = -0.72, p >0.05) suggests no asymmetry is present.

a.



b.

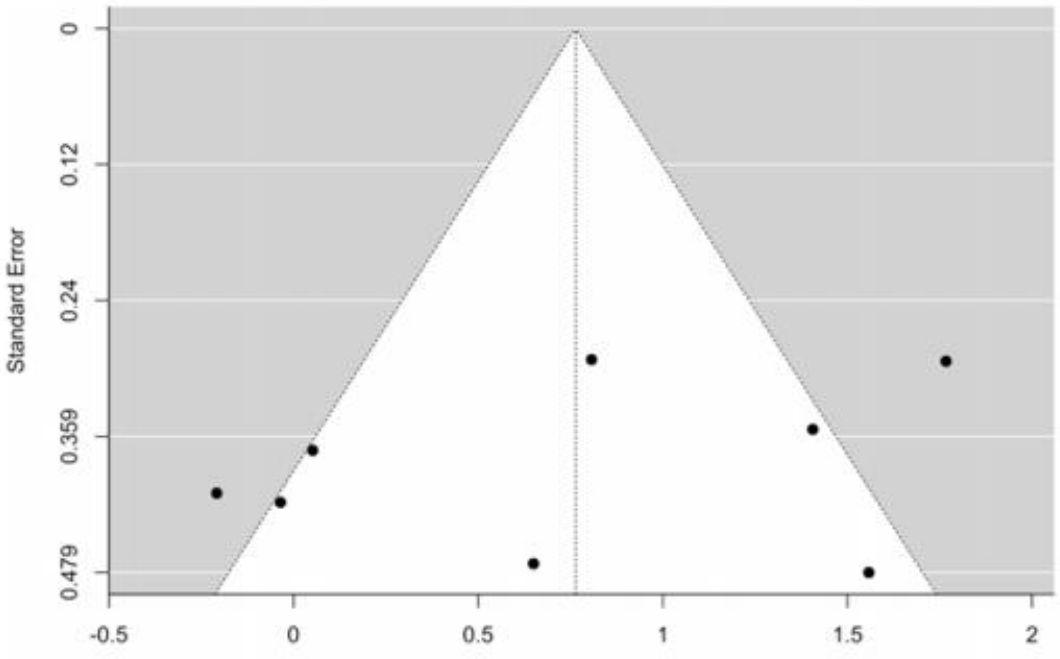


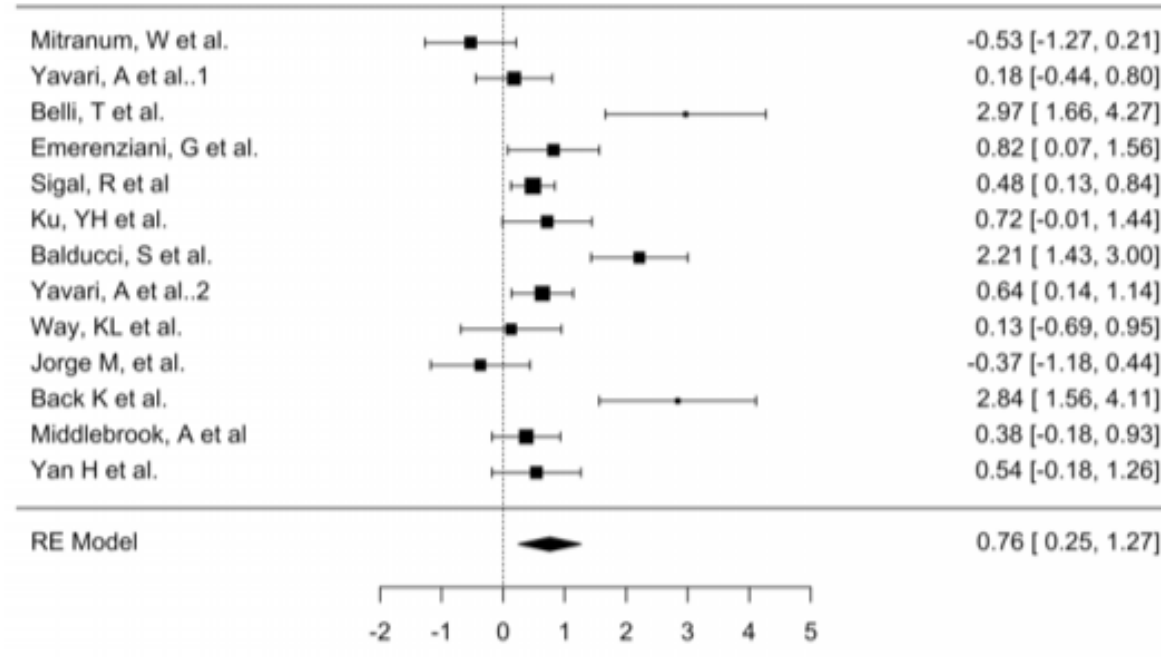
Figure 12. Meta-analysis of fasting blood glucose.

(a). *Forest plot comparing the effects of exercise on fasting blood glucose concentrations (SMD). A positive value represents a bigger difference in the SMD between exercise and control group, indicating that exercise reduces fasting blood glucose. (**b**). Funnel plot of studies evaluating the effect of exercise on fasting blood glucose.*

4.2.7. Body Mass Index

Improvements in glycaemic control are often associated with weight loss and decreases in BMI but there's some debate as to whether they are directly correlated (Franz, 2007). 13 trials recorded the change in BMI after the exercise programme and this meta-analysis was therefore carried out on 13 trials. The individual mean difference of pre-post changes in BMI in control and aerobic groups, respectively, ranged from -0.7 kg/m^2 to 0.4 kg/m^2 (median = -0.1 kg/m^2) and -2.2 kg/m^2 to 0.21 kg/m^2 (median = -0.8 kg/m^2). SMD of within-group change in BMI between control and aerobic groups in each of the trials ranged from -0.53 to 2.97, with 85% of studies showing a positive value in respect to reducing BMI. The pooled summary estimate of the standardised mean difference of within-group change in BMI between control and aerobic groups was 0.76 (95% CI 0.25 – 1.27) with p <0.05, and therefore the exercise programmes significantly reduced BMI (Figure 13). There was considerable heterogeneity in this meta-analysis (I^2= 86.35 %, p <0.001) and asymmetry was present after examination of the funnel plot (Figure 13b) and calculation of Egger's regression value (Egger's regression value = 2.878, p <0.05).

a.



b.

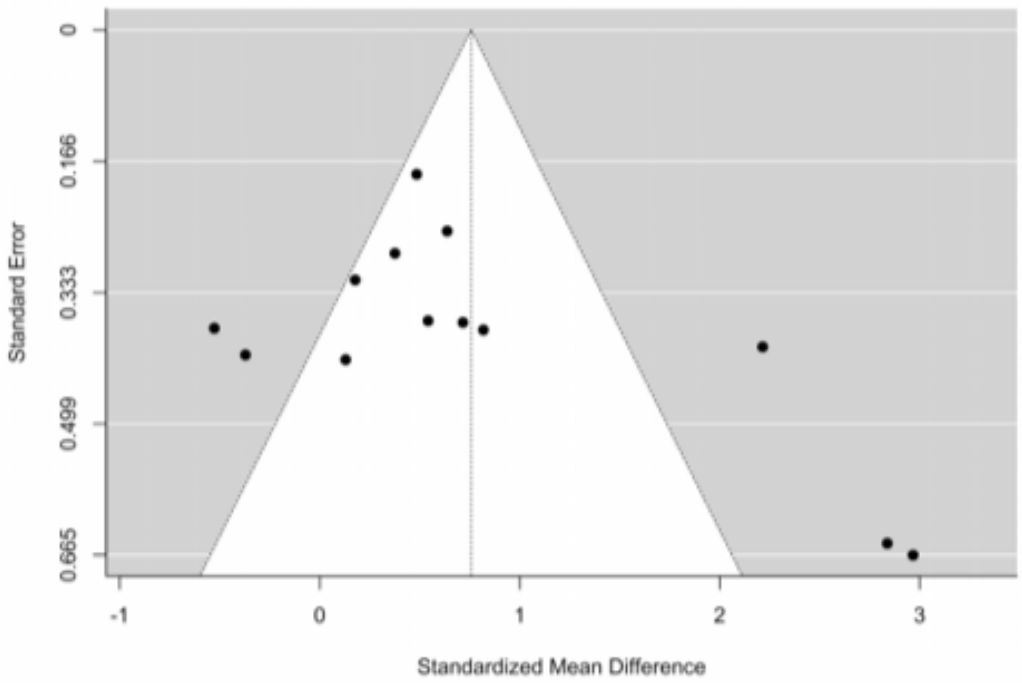


Figure 13. Meta-analysis of BMI.

*Forest plot comparing the effects of exercise on BMI concentrations (SMD). A positive value represents a bigger difference in the SMD between exercise and control group, indicating that exercise reduces BMI. (**b**) Funnel plot of studies evaluating the effect of exercise on BMI.*

4.2.8. Lipid Profiles- Triglycerides

This meta-analysis analysed the effect supervised aerobic exercise programmes have on triglyceride concentrations in those with type 2 diabetes to investigate how significantly exercise can be used as a treatment. Eight trials recorded triglyceride concentrations and one trial was removed due to being a large outlier (Balducci et al., 2010). This meta-analysis was therefore carried out on seven trials. The individual mean difference of pre-post changes in triglyceride concentration in control and aerobic groups, respectively, ranged from -0.58 mmol/L to 0.2 mmol/L (median = -0.05 mmol/L) and -0.48 mmol/L to 0 mmol/L (median = -0.16mmol/L). SMD of within-group change in triglycerides between control and aerobic groups in each of the trials ranged from -0.33 to 0.84, with 86% of studies showing a positive result in reducing triglyceride concentration. The pooled summary estimate of the standardised mean difference of within-group change in triglycerides between control and aerobic groups was 0.15 (95% CI -0.08 – 0.38) with p >0.05 (Figure 14). Thus, the exercise programmes did not significantly reduce triglyceride concentrations. There was no heterogeneity in the meta-analysis (I^2= 0%, p >0.05) and no asymmetry was found after examination of the funnel plot (Figure 14b) and calculation of Egger's regression value (Egger's regression value = 0.386, p >0.05).

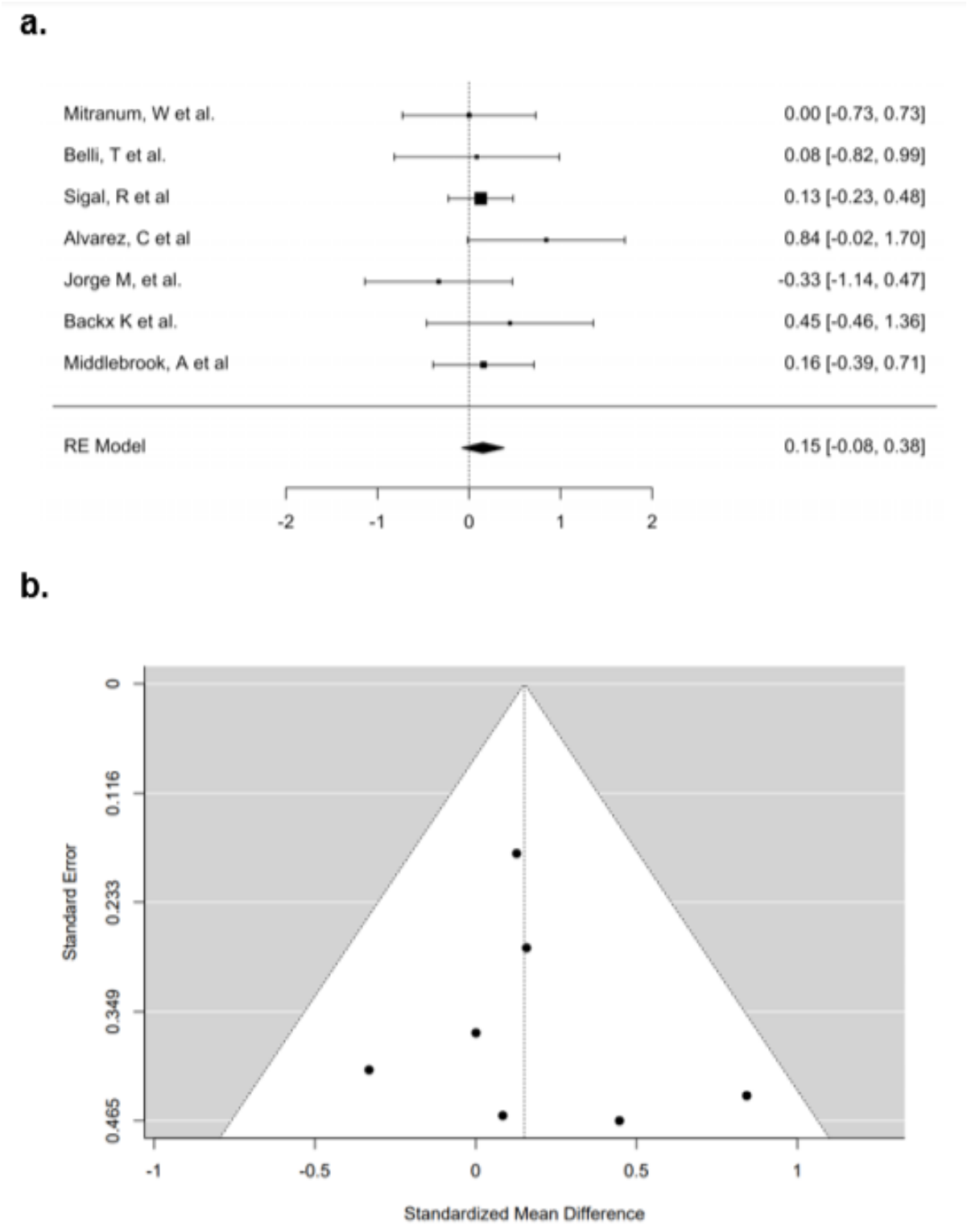


Figure 14. Meta-analysis of triglyceride concentrations.

*(**a**) Forest plot comparing the effects of exercise on triglyceride concentrations (SMD). A positive value represents a bigger difference in the SMD between exercise and control group, indicating that exercise reduces triglycerides. (**b**) Funnel plot of studies evaluating the effect of exercise on triglycerides.*

4.2.9. Lipid Profiles- Total Cholesterol

Total cholesterol is analysed within this meta-analysis to investigate whether the concentration in individuals with type 2 diabetes changes after a period of supervised aerobic exercise. 11 trials reported total cholesterol concentration and three trials were removed due to being large outliers (Alvarez et al., 2016; Balducci et al., 2010; Yavari et al., 2010). This meta-analysis was therefore carried out on eight trials. The individual mean difference of pre-post changes in total cholesterol concentration in control and aerobic groups, respectively, ranged from -0.35 mmol/L to 0.33 mmol/L (median = 0.05 mmol/L) and -0.7 mmol/L to 0.16 mmol/L (median = -0.11 mmol/L). SMD of within-group change in total cholesterol between control and aerobic groups in each of the trials ranged from -0.39 to 0.95. The pooled summary estimate of the standardised mean difference of within-group change in total cholesterol between control and aerobic groups was 0.09 (95% CI -0.13 – 0.31) with p >0.05, and therefore the exercise programmes did not significantly reduce total cholesterol (Figure 15a). There was no substantial heterogeneity in this meta-analysis (I^2= 0%, p >0.05) and examination of the funnel plot and Egger's regression value (Egger's regression value = 0.742, p >0.05) indicate that asymmetry wasn't present within the results.

4.2.10. Lipid Profiles- High Density Lipoprotein

This meta-analysis investigates whether a period of supervised aerobic exercise has beneficial effects on increasing HDL and reducing the risk of cardiovascular disease. 10 trials recorded high density lipoprotein (HDL) concentrations and one trial was removed due to being a large outlier. This meta-analysis was therefore carried out on nine trials. The individual mean difference of pre-post changes in HDL concentration in control and aerobic groups, respectively, ranged from -0.06 mmol/L to 0.2 mmol/L (median = 0.02 mmol/L) and -0.15 mmol/L to 0.5

mmol/L (median = 0.07 mmol/L). SMD in within-group change in HDL between control and aerobic groups in each of the trials ranged from -0.38 to 0.47. The pooled summary estimate of the standardised mean difference of within-group change in HDL between control and aerobic groups was -0.05 (95% CI -0.25 – 0.16) with p >0.05, and therefore the exercise programmes did not significantly change HDL concentrations (Figure15b). There was considerable heterogeneity in this meta-analysis (I^2= 83.08%, p < 0.001). After examination of the funnel plot produced and Egger's regression value (Egger's regression value = -0.123, p >0.05) no asymmetry was found to be present within the results.

4.2.11. Lipid Profiles- Low Density Lipoprotein

LDL is investigated within this meta-analysis to examine whether a programme of aerobic exercise reduces the increased concentrations of LDL resultant of obesity and therefore type 2 diabetes as a secondary component (Lazarte & Hegele, 2020). Nine trials recorded low density lipoprotein concentrations and therefore this meta-analysis included nine trials. The individual mean difference of pre-post changes in LDL concentration in control and aerobic groups, respectively, ranged from -0.83 mmol/L to 0.09 mmol/L (median = -0.06 mmol/L) and -1.3 mmol/L to 0.35 mmol/L (median = -0.125 mmol/L). SMD of within-group change in LDL between control and aerobic groups in each of the trials ranged from -2.01 to 1.93. The pooled summary estimate of the standardised mean difference of within-group change in LDL between control and aerobic groups was 0.12 (95% CI -0.55 – 0.79) with p >0.05, and therefore the exercise programmes did not significantly change LDL (Figure 15c). There was considerable heterogeneity in this meta-analysis (I^2= 88.31%, p <0.001). After examination of the funnel plot and Egger's regression value (Egger's regression value = 0.055, p >0.05) no asymmetry was found within the results.

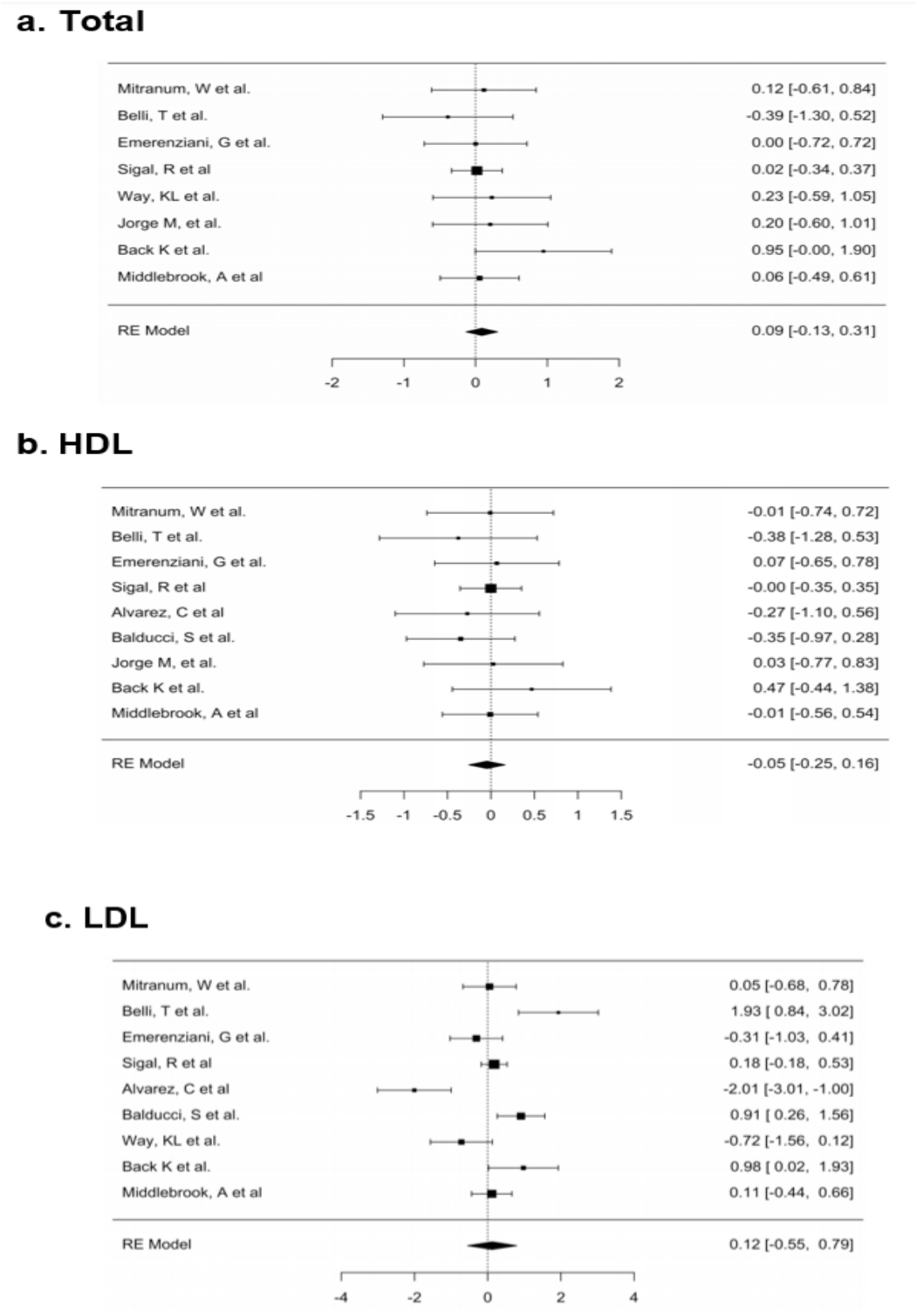


Figure 15. Meta-analysis of total cholesterol, HDL, and LDL concentrations.

(a) *Forest plot comparing the effects of exercise on total cholesterol concentrations (SMD).* ***(b)*** *Forest plot comparing the effects of exercise on HDL concentrations (SMD).* ***(c)*** *Forest plot comparing the effects of exercise on LDL concentrations (SMD).*

4.2.12. Meta-Regression

Meta-regression analyses are useful for understanding the effect of modifiers, such as variables within exercise programmes, on changes within outcomes measures within the trials. Meta-regression analyses were carried out to explore the association of volume and intensity with changes in HbA1c concentrations. This was to understand whether there was a relationship between increases in intensity and decreases in HbA1c, increases in volume and decreases in HbA1c, and a combination of intensity and volume and the impact this has on HbA1c. All these analyses were weighted by the inverse of the variance of each observation and scatter bubble plots were constructed to graphically display proportional weights of different trials. Intensity, volume, and volume x intensity had no significant relationship with changes in HbA1c (p = 0.96, 0.116 and 0.602, respectively) (Figure 16) and the results showed moderate heterogeneity (I^2 = 60.92%, 58.63% and 60.93%). The R^{2}_{*} values, showing how much variability within HbA1c could be accounted for by the moderators, were 1.25%, 25.45% and 3.13% for intensity, volume and volume x intensity respectively.

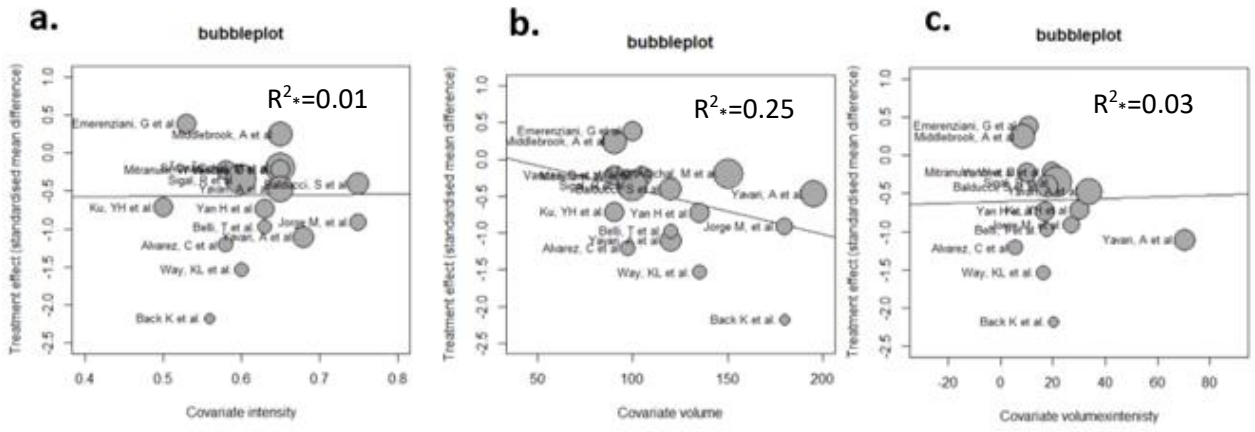


Figure 16. Meta-regression of (a) intensity, (b) volume, and (c) intensity x volume on HbA1c.

Bubble plots showing the association of (a) intensity, (b) volume and (c) intensity x volume of exercise on HbA1c concentrations after a programme of exercise. The values are plot in relation to the moderators on the x axis and the SMD of HbA1c on the y axis.

4.3. Discussion- Meta-analysis and meta-regression

4.3.1. Glycaemic control

HbA1c significantly decreased in those with type 2 diabetes after ≥12 weeks of supervised exercise. This meta-analysis showed that 87.5% of exercise interventions reduced HbA1c concentrations. The overall HbA1c SMD of the aerobic exercise interventions was 0.56 (95% CI 0.3 – 0.82) which indicates that the exercise programme is around 0.5 times more effective in decreasing HbA1c concentrations than the control group (Faraone, 2008). Exercise programmes of ≥12 weeks help regulate glycaemic control and are therefore likely to be beneficial in treating the disease and helping to reduce the risk of comorbidities. Contracting muscles require larger amounts of glucose and delivery to muscle cells is increased due to a rise in blood flow (Sylow et al., 2017). Capillary recruitment is upregulated which increases the surface area for glucose uptake into cells (Sylow et al., 2017). The improvement in glucose uptake could be mediated through two pathways. Exercise does not require PI3K to prompt GLUT-4 translocation and thus, insulin resistant tissue will still take up glucose during exercise (Sylow et al., 2017). Intracellular Ca^{2+} concentrations increase significantly within contracting muscles (Röckl et al., 2008), along with ROS and PKC concentrations increasing (Stanford & Goodyear, 2014). These molecules are able to activate a range of signalling cascades (Stanford & Goodyear, 2014). Evidence shows Ca^{2+} increases the expression of GLUT4 genes, it does this via increased Ca^{2+}/calmodulin signalling. AS160-phospho- Akt substrate site and TBC1D1 phosphorylation occur as a result of this signalling (Stanford & Goodyear, 2014), increasing the availability of GLUT4 at the surface (Chin, 2005). The increased translocation of GLUT4 allows glucose transport to increase. The cell then utilises this glucose to produce energy for muscle contraction which maintains the diffusion gradient for glucose influx into the cell (Sylow et al., 2017). All of these

factors contribute to reduced plasma glucose concentrations which will subsequently reduce concentrations of HbA1c. This demonstrates an understanding of the mechanisms involved in how exercise lowers the high blood glucose concentrations associated with type 2 diabetes, and evidence for this is provided by the reductions in HbA1c seen in this meta-analysis.

If the exercise programme is completed regularly and maintained, for example for 12 weeks or more, then the body begins to adapt to become better equipped to deal with exercise (Colberg et al., 2010). The expression and activity of proteins involved in the insulin signalling pathway increases which improves insulin sensitivity (Colberg et al., 2010). Evidence has shown that even periods of exercise of only 6 weeks improve insulin sensitivity (Koivisto & Yki-Järvinen, 1986) and therefore, this could provide another explanation for how exercise contributes to reductions in HbA1c as seen in this meta-analysis.

Adiposity is strongly linked to insulin resistance, and therefore weight loss induced by the exercise programme (indicated through a reduction in BMI) may increase insulin sensitivity. Regular exercise utilises the higher concentrations of glucose and lipids in those who are overweight and have type 2 diabetes (Yang et al., 2019). This helps reduce the glucotoxicity and lipotoxicity that cells are exposed to, helping to improve insulin sensitivity (Yang et al., 2019). This allows insulin to work more effectively in providing glycaemic control and could be an additional explanation for the reduction in HbA1c.

One of the major consequences' individuals with type 2 diabetes experience due high blood glucose concentrations is both macro- and micro- vascular complications (Fowler, 2011). Around 20% of people in the UK with diabetes experience a macrovascular complication, and these complications account for 59% of deaths in those individuals (Turner, 1998). Improvements in glycaemic control have been shown to significantly reduce the risk of morbidities and improve the outcomes of those with

type 2 diabetes (Schinner, 2009). The evidence provided in this meta-analysis, that exercise significantly reduces HbA1c, shows that exercise can be used as a treatment of type 2 diabetes and also a preventative measure against other morbidities. This aligns well with other research which have also concluded that exercise can be used as a preventative measure by improving glycaemic control (Colberg et al., 2010; De Lade et al., 2016; Larose et al., 2011; Najafipour et al., 2017).

Metformin is one of the most common drugs used to treat type 2 diabetes and it is usually given as an initial treatment to help regulate HbA1c concentrations (American Diabetes Association, 2017). However, it is associated with side effects, including gastrointestinal intolerance, vitamin B12 deficiency and the worsening of the effects of nephropathy (American Diabetes Association, 2017). It is also unlikely to prevent the progression of the disease and therefore after a few years, it is likely to be used in combination with other pharmacological therapies, including insulin and secretagogues, both of which are associated with their own side effects (American Diabetes Association, 2017). Using exercise as an alternative has a range of benefits, both acutely and chronically, improving blood glucose control, improving insulin sensitivity, reducing cardiovascular disease risk, and ultimately reducing the risk of mortality, accompanied by minimal side effects (Colberg et al., 2010). Therefore, understanding the dose-response to exercise and the minimal amount of exercise required to provide improved glycaemic control so it can be used as an effective treatment will be extremely beneficial in reducing the reliance on pharmacological treatments, reducing the risk of morbidities and the cost of medication, ultimately reducing the overall burden on healthcare systems.

Fasting blood glucose also significantly changed after an exercise programme of ≥12 weeks with 75% of studies included in the meta-analysis demonstrating a lowering of fasting blood glucose. It is likely to be statistically significant as it shows blood glucose concentrations as a reflection of the momentary situation pre- and post-

exercise programme and this demonstrates blood glucose concentrations reduced after the exercise programme (Bouma et al., 1999). However, it is an acute measurement parameter and is therefore only indicative of glucose concentrations at the exact point of measurement, rather than an average change throughout like HbA1c (Bouma et al., 1999). The standard of care for monitoring diabetes, recommended by the American Diabetes Association, is HbA1c due to its direct correlation with average plasma glucose concentrations (Sherwani et al., 2016). The 75% of studies that showed a decrease in blood glucose concentrations help to support the evidence provided by HbA1c, that exercise programmes improve glycaemic control in those with type 2 diabetes.

4.3.2. HbA1c and BMI association

The BMI of participants significantly decreased by 0.6 kg/m^2 after the exercise programme of ≥12 weeks. People with type 2 diabetes typically have a higher BMI, including increased visceral adiposity (Eckel et al., 2011). The average BMI of participants at the beginning of the trials included in this meta-analysis was 30.1 kg/m^2 and 80% of studies included showed a significant decrease in BMI after the exercise programme by 2.1% on average. The overall BMI SMD of the aerobic exercise interventions was 0.76 (95% CI 0.25 – 1.27), which suggests that the exercise programmes significantly decrease BMI by a magnitude of 0.76 times more than the no exercise control group. In those with type 2 diabetes, even small reductions (around 5%) in body mass have shown to have significant impacts on improving glycaemic control (Wilding, 2014). For example, a 0.1% reduction in HbA1c has been found with each 1 kg of body mass lost in patients with the disease (Gummesson et al., 2017). Improvements in BMI have also been associated with reducing the risk of mortality from comorbidities, such as cardiovascular disease (Wilding, 2014). Weight loss has

been associated with a 25% reduction in overall mortality risk in those with type 2 diabetes, and a 28% reduction in the risk of mortality from cardiovascular disease (CVD) after a 12-year prospective analysis study on 4970 overweight individuals with the disease (Williamson et al., 2000). Evidence suggests weight loss by increased energy output can reduce lipid profiles, including total cholesterol, HDL, LDL and triglycerides, in overweight individuals at high risk of CVD, such as those with type 2 diabetes (Ryan & Yockey, 2017). Evidence for significant improvements in cholesterol concentrations has been found when approximately 7% weight loss is maintained (Ryan & Yockey, 2017). This meta-analysis therefore provides further evidence that exercise programmes of ≥12 weeks, which have beneficial impacts on weight loss, can also reduce the risk of comorbidities and secondary complications associated with the disease.

4.3.3. Lipid profiles

Out of all the lipid profiles analysed, none changed significantly at the p <0.05 level, with SMD also showing little change. This suggests that these exercise programmes did not have a beneficial impact on total cholesterol, triglyceride, HDL, and LDL concentrations. There is variation in the evidence from other meta-analyses as to whether exercise programmes significantly improve lipid profiles (Kelley & Kelley, 2007; Yoo & Lee, 2005). One meta-analysis found that aerobic exercise only significantly changed LDL concentrations and non-significant changes were found in total cholesterol, triglyceride, and HDL concentrations (Kelley & Kelley, 2007). Total cholesterol, HDL, and LDL all significantly changed in another meta-analysis (Yoo & Lee, 2005) and this was supported by evidence from another meta-analysis showing positive changes in both LDL and HDL (Hayashino et al., 2012). The variation in the results of all these meta-analyses could be due to differences in exclusion criteria. For example, two of the meta-analyses included both aerobic and resistance exercise

(Hayashino et al., 2012; Yoo & Lee, 2005), whereas this meta-analysis and one other only included aerobic exercise trials (Kelley & Kelley, 2007).

Evidence has found that exercise programmes reduce the concentration of small dense lipid particles and increase the concentration of large, less dense particles which is most significantly seen in LDL (Wang & Xu, 2017). This is likely to reduce the risk of cardiovascular disease as larger, less dense particles are less atherosclerotic (Wang & Xu, 2017). Therefore, beneficial changes might be found in the structure and composition of lipid particles rather than overall concentrations (Kelley & Kelley, 2007).

Other factors which might contribute to the lack of significance, at the $p < 0.05$ level, in these meta-analyses are the variations in programme length, and volume, frequency and intensity of exercise prescribed to participants within the trials included. From the analysis, HDL and LDL show a large amount of heterogeneity ($I^2 = 83.08\%$ and 88.31% respectively) which could further support the idea that the variation within programme prescription might impact the significance seen in these results (Biau et al., 2008). Total cholesterol and triglyceride concentrations show low heterogeneity ($I^2 = 0\%$) which implies that all studies have the same effect.

Evidence has found that total energy expenditure and intensity are key factors in determining the impact an exercise programme has on lipid profiles (S. Mann et al., 2014). Longer programmes at high intensity have been shown to reduce total cholesterol and increase HDL cholesterol concentrations (Dunn et al., 1997) and evidence for this is seen within some of the trials included within this meta-analysis. For example, one trial (Yavari et al., 2010) carried out a protocol for 52 weeks, with 3 sessions per week at an average intensity of 67.5%. The SMD for this trial was 1.72 providing evidence that this longer and higher intensity programme reduced total cholesterol concentrations when compared with the control group. A shorter programme but at a higher intensity has been suggested to reduce triglyceride concentrations and increase HDL concentrations (LeMura et al., 2000), and therefore

demonstrates the conflict within the literature as to what dose of exercise is optimal for individuals in regards to lipid profiles. The most significant improvements are seen in high volume and high intensity programmes (Kraus et al., 2002).

The intensity of the exercise programmes included in this meta-analysis varies from 50-75% of total exercise capacity, and the volume of exercise within the programme varies between 9 hours and 390 hours in total, ranging from 12 mins 3x per week for 16 weeks (Alvarez et al., 2016) to 50 mins 3x per week for 40 weeks (Sénéchal et al., 2013). HDL, LDL, BMI, fasting blood glucose and HbA1c meta-analyses all demonstrate substantial statistical heterogeneity, and this means there is variation within the analysis that isn't due to chance. This is likely due to differences within the protocols of each trial, including differences in intensity, volume, and type of aerobic exercise. One way in which this heterogeneity could be investigated is by carrying out a meta-regression using volume and intensity as modifiers and this was investigated next.

4.3.4. Association of HbA1c with Intensity and Volume

A meta-regression was carried out on HbA1c using intensity and volume as moderators to investigate the effects these variables could have on glycaemic control. When used as separate moderators, intensity, and volume were not significantly associated with HbA1c concentrations (p = 0.96 and 0.116, respectively). However, both variables also have moderate heterogeneity within the results (I^2 = 60.92% and 58.63%). This could be due to variations within exercise programmes. Confidence in the conclusions could be improved by having a bigger sample size available to use within the meta-regression. For the effects of exercise programmes to be better investigated within the meta-regression, trials need to investigate a bigger range of intensities. The trials included only range in intensity from 50-75% with a big proportion of exercise programmes ranging between intensities of 60-70%, this is therefore

unlikely to give the true association of intensity with HbA1c. A meta-analysis investigating the difference between high intensity interval training (HIIT) and moderate intensity interval training (MIIT) found that HIIT at 85-95% max HR showed a 0.37% greater reduction in HbA1c than MIIT (Liu et al., 2019). Further supporting the idea that exercise intensity in this meta-regression might be too low to show an association.

However, the literature is conflicting as to whether increases in intensity have a significant effect on decreasing HbA1c concentrations. For example, one clinical trial investigated the effects of exercise programmes with the same energy expenditure but different intensities of exercise (Hansen et al., 2009). They found that both groups (low-moderate intensity and moderate-high intensity) had equal effects in reducing HbA1c concentrations (Hansen et al., 2009). Another clinical trial supports this idea as moderate and high intensity interval exercise resulted in non-significant differences in reductions in HbA1c (Ahmad, 2019). This poses the question as to whether it is the lower volume of high intensity exercise that acts as the limiting factor because high intensity exercise can't be maintained for long periods (Hansen et al., 2009). A meta-regression was therefore carried out to investigate volume as a moderator.

The volume of exercise was a non-significant moderator of HbA1c, $p = 0.116$ and $R^2_* = 25.45\%$ which shows that 25% of the variation within the effect size of HbA1c was accounted for by volume (Harrer et al., 2021). Even though the results weren't statistically significant when the *p* value is taken into account, there is a trend within the data due to $R^2_* = 25.45\%$ and this could be because the analysis was too underpowered to show an effect (Suresh & Chandrashekara, 2012). A power value of 27% was calculated in the computer software Jamovi. This suggests that there's only a 27% chance that this meta-regression would correctly report a statistically significant result (Suresh & Chandrashekara, 2012). There could therefore be a potential association between increasing volume and improvements in glycaemic control but a lack of studies available to be included within the analysis could mean the analysis

was too underpowered to show significance (Suresh & Chandrashekara, 2012). More confident conclusions could be made if a larger number of trials with a larger sample size were investigated. A previous meta-analysis, even though it has a slightly different exclusion criteria, found from 18 studies that aerobic exercise ≥150mins/week was associated with greater reductions in HbA1c (Umpierre et al., 2011). This supports the suggestion that higher volumes might be significantly correlated to decreases in HbA1c and this should be an area for further research.

A meta-regression was carried out combining volume and intensity as a moderator to investigate the association with HbA1c. The results of this meta-regression were also non-significant, likely due to the reasons stated above.

4.3.5. Limitations

While the literature assessment was comprehensive, it is possible that studies may have been missed from the analysis. There was significant heterogeneity in a range of measures included, this indicates variation in the data which could be caused by both clinical diversity or methodological diversity. The exercise programmes vary between trials and therefore this could account for clinical diversity. Methodological diversity is shown by the varying PEDro scores (Table 2). However, the inclusion and exclusion criteria used for these meta-analyses was created with providing sufficient homogeneity between studies in mind. Whilst random effects modelling was also chosen to mitigate this effect, this remains a limitation. Some analyses included have achieved statistical significance, however the change to be considered clinically relevant remains to be fully determined.

Publication bias could also be a limitation of these meta-analyses. Both the meta-analyses of HbA1c and BMI showed asymmetry after visual analysis of the funnel plots and statistical analysis with Egger's regression values. This indicates that 'small-study effects' could be present due to bias, such as publication bias, and heterogeneity within

the studies, contributing to smaller studies presenting larger treatment effects (Song et al., 2002; Sterne et al., 2000). There are a range of reasons why this might have occurred.

Significant results are more likely to be cited within the literature, especially in the area of medical research and if the language is in English (Devito & Goldacre, 2019; Sterne et al., 2000). This encourages and biases editors towards publishing research with significant findings, and researchers are less likely to submit research with non-significant findings for publication (Devito & Goldacre, 2019). This results in an inability to accurately analyse the true range of data and therefore it becomes difficult to make confident conclusions in the findings (Devito & Goldacre, 2019). An attempt to mitigate and analyse the impacts of publication bias was made within these meta-analyses by producing funnel plots and calculating Egger's regression values to identify any asymmetry within the data (Song et al., 2002). Another method which could be used within meta-analyses to take into account publication bias is *p*-uniform calculations, but this can overestimate effect sizes in analyses with considerable heterogeneity and therefore should be used with caution (McShane et al., 2016). An inclusion of a range of studies published in different languages could also potentially reduce the bias in meta-analyses but this relies on access to individuals who are able to translate articles in other languages (Sterne et al., 2000).

Open Science is growing in popularity and this could be extremely beneficial in reducing publication bias within systematic reviews, meta-analyses and meta-regressions (Allen & Mehler, 2019). It requires the preregistration of methodology, analyses and hypotheses to be made publicly accessible prior to data collection, and aims to increase the scientific validity of research and also the chance of null findings being published (Allen & Mehler, 2019). It would therefore allow a more accurate representation of data to be analysed within meta-analyses.

4.3.6. Conclusion

Although there is substantial evidence indicating the benefits of aerobic exercise as a treatment for those with type 2 diabetes, there is still insufficient evidence on the exact intensity, volume and duration of exercise required to provide optimal glycaemic control. This analysis suggested that increases in intensity didn't have greater benefits on glycaemic control, but there was a substantial association (R^2*=25%) between increases in volume and improvements in glycaemic control. Investigation into the optimal volume of exercise to treat type 2 diabetes could therefore be an interesting and extremely beneficial area of further research. This systematic review and meta-analysis provide evidence that short-term interventions begin to show improvements in glycaemic control, but long-term interventions are likely to be required to provide significant beneficial changes in lipid profiles. Therefore, future long-term studies are needed to provide accurate conclusions on the minimum duration, intensity and volume of aerobic exercise needed to treat type 2 diabetes.

Future research

To further investigate the impact of volume and intensity of exercise on glycaemic control in individuals with type 2 diabetes, clinical trials need to be carried out on a larger range of volumes and intensities of exercise. This will allow another meta-regression to be carried out with a higher study sample size and a wider range of results to be analysed. This will give a clearer understanding as to whether there are associations with volume of exercise in particular, but also intensity of exercise, and glycaemic control. Another idea for future research related to findings from the meta-analysis, would be to analyse the lipid profiles of individuals who take part in exercise programmes for a long period (>12 months). This would allow conclusions to be made as to whether exercise has to be maintained for longer periods for beneficial improvements in lipid profiles to be experienced. There is also conflict within the

literature as to what form of exercise will provide the best improvements in glycaemic control and treatment for individuals with type 2 diabetes. A future meta-regression could therefore compare the responses of different types of exercise, including aerobic, resistance, a combination of both, on glycaemic control to give further clarification of which would be most beneficial.

The clinical trial will hopefully continue now that Covid-19 restrictions have eased, and vaccinations have successfully rolled out. This will allow the sample size to increase and data to be collected across the full range of energy expenditures of exercise. A dose-response relationship between exercise and glycaemic control will then be determined which, if successful, will allow the minimum dose of exercise required to treat type 2 diabetes to be better understood. Continuous glucose monitor (CGM) data, and data on insulin and free fatty acids would also be encouraged to be collected within the protocol to ensure the range of beneficial effects of exercise as a treatment can be understood. If individuals understand the range of benefits of exercise and also, the minimal dose of exercise they need to complete for it to provide effective glycaemic control, then they will be more likely to participate in exercise and hopefully maintain this within the long term.

In addition to this clinical trial, it would also be useful to investigate the impacts of a multi-modal treatment plan including nutrition, well-being, and educational advice. This would help us better investigate the combination of methods to treat type 2 diabetes which would be most beneficial. One potential idea would be to carry out a retrospective study comparing the treatment regimes put in place at different GP/community services, especially now social prescribing is increasing in capacity.

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Appendices

Screening Questionnaire

Name: _____ **Study Code** [ ]

Risk Factors		**Risk Factor**	**No Risk Factor**
Q1. **Age** _____years	Male Female	≥45 ≥55	<45 <55
Q2. Are you a current **smoker**? *If yes, how many cigarettes do you smoker per day?* _____ If no, have you ever smoked? *If yes, how long has it been since you quit?* _____		Yes Yes	No No
Q3. **Family history:** Have any parents or siblings had a heart attack, bypass surgery, angioplasty or sudden death* prior to 55 years (male relatives) or 65 years (female relatives)? *If yes, please circle what they had.*		Yes	No
Q4. What is your **ethnicity**? *Please provide here* _____			
Q5. **Sport (if applicable):** _____			
Q6. **Physical activity:** In the past year, have you engaged in a regular strength training programme including the front and back squat?		Yes	No
Q7. **Body Size:** Weight _____ kg Height _____ cm **Body Fat %**	Males Females	≥20 ≥30	

*Advise to visit GP Question highlighted in **blue** will be completed after measurement taken

Personal History of Disease			
Q10. Heart disease		Yes	No
Q11. Peripheral vascular disease		Yes	No
Q12. Cerebrovascular disease (e.g. stroke)		Yes	No
Q13. Asthma		Yes	No
Q14. Chronic obstructive pulmonary disease		Yes	No
Q15. Diabetes mellitus	Type 1	Yes	No
	Type 2	Yes	No
Q16. Thyroid disorder		Yes	No
Q17. Renal (kidney) disease		Yes	No
Q18. Liver disease		Yes	No
Q19. Musculoskeletal conditions	Osteoarthritis	Yes	No
	Rheumatoid arthritis	Yes	No
	Osteoporosis	Yes	No
Q20. Any other condition? Please provide details _____ _____		Yes	No
Q20. Do you have an injury that may be worsened with exercise? *If so, please provide details* _____ _____		Yes	No
Q21. Are you taking any prescribed or non-prescribed medications/drugs? *If so, please provide details* _____ _____		Yes	No

Additional Questions		
Q9. Have you had any **alcohol** to drink in the last 24 hours? *If yes,* how many units? _____ units how long ago? _____ hours	Yes	No
Q10. Is there a chance you could be pregnant?	Yes	No

Figure 17. Screening Questionnaire.

A number of questions asked to participants based on personal and family history, and lifestyle habits, used to determine whether participants were at a low risk and safe to participate. Questions based on the American College of Sports Medicine (ACSM) readiness to exercise criteria.

PubMed search terms

("glycemic control"[MeSH Terms] OR ("glycemic"[All Fields] AND "control"[All Fields]) OR "glycemic control"[All Fields] OR ("glycaemic"[All Fields] AND "control"[All Fields]) OR "glycaemic control"[All Fields]) AND ("diabetes mellitus, type 2"[MeSH Terms] OR "type 2 diabetes mellitus"[All Fields] OR "type 2 diabetes"[All Fields]) AND ("exercise"[MeSH Terms] OR "exercise"[All Fields] OR "exercises"[All Fields] OR "exercise therapy"[MeSH Terms] OR ("exercise"[All Fields] AND "therapy"[All Fields]) OR "exercise therapy"[All Fields] OR "exercise s"[All Fields] OR "exercised"[All Fields] OR "exerciser"[All Fields] OR "exercisers"[All Fields] OR "exercising"[All Fields]) AND (("random allocation"[MeSH Terms] OR ("random"[All Fields] AND "allocation"[All Fields]) OR "random allocation"[All Fields] OR "random"[All Fields] OR "randomization"[All Fields] OR "randomized"[All Fields] OR "randomisation"[All Fields] OR "randomisations"[All Fields] OR "randomise"[All Fields] OR "randomised"[All Fields] OR "randomising"[All Fields] OR "randomizations"[All Fields] OR "randomize"[All Fields] OR "randomizes"[All Fields] OR "randomizing"[All Fields] OR "randomness"[All Fields] OR "randoms"[All Fields]) AND ("controling"[All Fields] OR "controllability"[All Fields] OR "controllable"[All Fields] OR "controllably"[All Fields] OR "controller"[All Fields] OR "controller s"[All Fields] OR "controllers"[All Fields] OR "controlling"[All Fields] OR "controls"[All Fields] OR "prevention and control"[MeSH Subheading] OR ("prevention"[All Fields] AND "control"[All Fields]) OR "prevention and control"[All Fields] OR "control"[All Fields] OR "control groups"[MeSH Terms] OR ("control"[All Fields] AND "groups"[All Fields]) OR "control groups"[All Fields]) AND ("clinical trials as topic"[MeSH Terms] OR ("clinical"[All Fields] AND "trials"[All Fields] AND "topic"[All Fields]) OR "clinical trials as topic"[All Fields] OR "trial"[All Fields] OR "trial s"[All Fields] OR "trialed"[All Fields] OR "trialing"[All Fields] OR "trials"[All Fields]))

Translations

glycaemic control: "glycemic control"[MeSH Terms] OR ("glycemic"[All Fields] AND "control"[All Fields]) OR "glycemic control"[All Fields] OR ("glycaemic"[All Fields] AND "control"[All Fields]) OR "glycaemic control"[All Fields]

type 2 diabetes: "diabetes mellitus, type 2"[MeSH Terms] OR "type 2 diabetes mellitus"[All Fields] OR "type 2 diabetes"[All Fields]

exercise: "exercise"[MeSH Terms] OR "exercise"[All Fields] OR "exercises"[All Fields] OR "exercise therapy"[MeSH Terms] OR ("exercise"[All Fields] AND "therapy"[All Fields]) OR "exercise therapy"[All Fields] OR "exercise's"[All Fields] OR "exercised"[All Fields] OR "exerciser"[All Fields] OR "exercisers"[All Fields] OR "exercising"[All Fields]

randomised: "random allocation"[MeSH Terms] OR ("random"[All Fields] AND "allocation"[All Fields]) OR "random allocation"[All Fields] OR "random"[All Fields] OR "randomization"[All Fields] OR "randomized"[All Fields] OR "randomisation"[All Fields] OR "randomisations"[All Fields] OR "randomise"[All Fields] OR "randomised"[All Fields] OR "randomising"[All Fields] OR "randomizations"[All Fields] OR "randomize"[All Fields] OR

"randomizes"[All Fields] OR "randomizing"[All Fields] OR "randomness"[All Fields] OR "randoms"[All Fields]
control: "controling"[All Fields] OR "controllability"[All Fields] OR "controllable"[All Fields] OR "controllably"[All Fields] OR "controller"[All Fields] OR "controller's"[All Fields] OR "controllers"[All Fields] OR "controlling"[All Fields] OR "controls"[All Fields] OR "prevention and control"[Subheading] OR ("prevention"[All Fields] AND "control"[All Fields]) OR "prevention and control"[All Fields] OR "control"[All Fields] OR "control groups"[MeSH Terms] OR ("control"[All Fields] AND "groups"[All Fields]) OR "control groups"[All Fields]
trial: "clinical trials as topic"[MeSH Terms] OR ("clinical"[All Fields] AND "trials"[All Fields] AND "topic"[All Fields]) OR "clinical trials as topic"[All Fields] OR "trial"[All Fields] OR "trial's"[All Fields] OR "trialed"[All Fields] OR "trialing"[All Fields] OR "trials"[All Fields]

Figure 18. PubMed Search terms used in the meta-analysis.