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An examination into the effects of very-low calorie diets and glucagon-like peptide-1 receptor agonists, individually or in combination, on skeletal muscle metabolism and glycaemic outcomes in individuals with type 2 diabetes living with obesity

Doctor of Philosophy (PhD) Thesis

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List of Abbreviations

1RM	1-repetition maximum
3MH	3-methylhistidine
Ach	Acetylcholine
ADP	Air-displacement plethysmography
AIRg	Acute insulin response to glucose
ANOVA	Analysis of variance
APE	Atom percent excess
ATP	Adenosine triphosphate
AUC	Area under curve
BCF	Beta-cell function
BIA	Bio-impedance analysis
BMI	Body mass index
CI	Confidence interval
COMB	Combined VLCD and Semaglutide group
CSA	Cross-sectional area
CS_I	Calculated insulin sensitivity index
D_2O	Deuterium oxide
D3MH	Deuterium-3-(methyl)-3-methylhistidine
DPP4	Dipeptidyl peptidase-4
DXA	Dual-energy X-ray absorptiometry
EAA	Essential amino acids

EGP	Endogenous glucose production
EH clamp	Euglycaemic hyperinsulinaemic clamp
ELISA	Enzyme-linked immunosorbent assay
FM	Fat mass
FFM	Fat-free mass
GDP	Gross Domestic Product
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP1RA	Glucagon-like peptide-1 receptor agonist
GLUT	Glucose transporters
HbA1c	Glycated haemoglobin
HOMA	Homeostatic model of assessment
HOMA-IR	Homeostatic model of assessment insulin resistance index
IMCL	Intramyocellular lipid
IQR	Interquartile range
IR	Insulin resistance
IRMS	Isotope ratio mass spectrometer
IS	Insulin sensitivity
IVGTT	Intravenous glucose tolerance test
kcal	Kilocalories
LBM	Lean body mass
LCD	Low calorie diet
MASLD	Metabolic dysfunction-associated steatotic liver disease

MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
mTORC1	Mammalian target of rapamycin complex 1
MTT	Meal tolerance test
MVC	Maximal voluntary contraction
NDM	Individuals without T2D
NMJ	Neuromuscular junction
NOS	Newcastle-Ottawa Scale
OECD	Organisation for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
OHS	Obesity hypoventilation syndrome
OSA	Obstructive sleep apnoea
PI3K	Phosphatidylinositol 3-kinase
PIS	Participant Information Sheet
pV1	Preliminary visit
QUICKI	Quantitative insulin sensitivity check index
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RCT	Randomised controlled trials
ROB2	Cochrane Risk of Bias 2 tool
RYGB	Roux-en-y gastric bypass
SD	Standard deviation
SE	Standard error of the mean

SEM	Semaglutide-only group
SGLT2	Sodium-glucose cotransporter-2
SMM	Skeletal muscle mass
T2D	Type 2 diabetes
TBW	Total body weight
UPP	Ubiquitin-proteasomal pathway
USD	United States Dollars
UK	United Kingdom
V1	Visit 1
V2	Visit 2
V3	Visit 3
VL	Vastus lateralis
VLCD	Very-low calorie diet
WMD	Weighted mean difference

Publications

O. Anyiam, B. Phillips, K. Quinn, D. Wilkinson, K. Smith, P. Atherton, I. Idris; "**Metabolic effects of very-low calorie diet, Semaglutide, or combination of the two, in individuals with type 2 diabetes mellitus**"; Clinical Nutrition, 2024, https://www.sciencedirect.com/science/article/pii/S0261561424002267

O. Anyiam, I. Idris; "**Differential metabolic responses to very-low calorie diets in individuals with, and without type 2 diabetes: A review**"; [Manuscript submitted to Expert Review of Endocrinology & Metabolism for publication]

O. Anyiam, A. Ardavani, R. Abdul Rashid, A. Panesar, I. Idris; "**Do Glucagon-Like Peptide-1 Receptor Agonists Preserve Muscle Mass in Individuals with, and without, Type 2 Diabetes: A Systematic Review and Meta-Analysis**"; [Manuscript submitted to Obesity Reviews for publication]

O. Anyiam, R. Abdul Rashid, A. Bhatti, S. Khan-Madni, O. Ogunyemi, A. Ardavani, I. Idris; "**A Systematic Review and Meta-Analysis of the Effect of Caloric Restriction on Skeletal Muscle Mass in Individuals with, and without, Type 2 Diabetes**"; [Manuscript submitted to Nutrients for publication]

Abstract

Type 2 diabetes (T2D) and obesity are major issues of modern healthcare. Both are increasing in prevalence around the world and responsible for considerable expenditure in global health systems. On a patient level, individuals with both conditions suffer significantly from the consequences of associated complications, leading to increased levels of mortality and disability. Weight loss interventions are crucial for the management of both conditions, and two such interventions are very-low calorie diets (VLCD) and glucagon-like peptide-1 receptor agonists (GLP1RAs). They are both recommended for the treatment of T2D, and a role in weight management is also recognised.

However, the apparent loss of skeletal muscle mass (MM) associated with these, and other weight loss interventions is increasingly attracting interest. This is particularly relevant given that individuals with T2D and obesity exhibit increased rates of age-related loss of MM, predisposing them to greater risk of frailty and disability. Despite these concerns, there still remains inconsistency and uncertainty regarding the true impact of these interventions on MM, and methods of mitigating these effects remain elusive. Furthermore, some evidence suggests a potential muscle-preserving effect of GLP1RAs, which could be particularly attractive given its already well-recognised weight and glycaemic effects.

This body of work aimed to perform a detailed examination of the effects of these two weight loss interventions on muscle and clinical outcomes. In addition, a novel

approach of combining the two interventions was explored, in order to determine whether this could elicit additive benefits on these outcomes.

Two meta-analyses were performed, providing a systematic review and quantitative synthesis of existing literature around the effects of these interventions on MM. The results of the initial meta-analysis suggested that GLP1RAs did not induce significant MM loss in individuals with T2D, but did in individuals without T2D. Notably, this differential effect is likely due to the doses used in the two populations. It was also found that less than 20% of total weight loss was attributable to MM. The second meta-analysis supported previous assertions that VLCD induce significant MM reductions, which comprise approximately 25% of total weight loss. However, this proportion was similar between individuals with and without T2D, providing reassurance that T2D does not aggravate MM loss during caloric restriction interventions.

The first empirical study outlined changes in MM in response to an 800 kilocalorie per day VLCD, the GLP1RA Semaglutide and a combination of the two, and related these to muscle protein synthesis (MPS) and breakdown (MPB). MPS significantly reduced in all groups, in a pattern consistent with the corresponding MM losses. Notably, Semaglutide reduced MPS to a lower extent than VLCD. Combination of the two interventions elicited numerically lower reductions in MM and MPS than VLCD alone, however these were non-significant. MPB was not significantly different between the groups, however the lack of a baseline measurement severely limited the conclusions that could be drawn from this.

The second empirical study assessed the impact of these three interventions on clinical outcomes relevant to individuals with T2D, in order to put the above findings into context. As expected, all interventions stimulated significant weight loss and improvement in overall glycaemic control. VLCD and the combination elicited greater weight and FM reductions than Semaglutide. Similar outcomes were observed between the VLCD and combination groups, except for beta-cell function (BCF) which improved to a significantly greater extent with the combination. This is particularly relevant as a larger magnitude of BCF amelioration is associated with more robust long-term diabetes remission.

Thus, the findings of the empirical studies suggest that combining VLCD and a GLP1RA may confer beneficial effects on both muscle-related and glycaemic outcomes, and as such requires further investigation. The findings of the meta-analyses were supported by the empirical mechanistic data. MM loss during weight loss interventions appears to be primarily mediated by changes in MPS, therefore approaches should be targeted at ameliorating this (such as exercise or increased protein intake). However, a synergistic role of MPB in this process cannot be ruled out and both of these topics present exciting avenues for ongoing research.

1. General Introduction

1.1. Obesity

1.1.1. Definition and Prevalence

Historically obesity has been defined simply as a body mass index (BMI) greater than 30kg.m^{-2}. However, over the last three decades, there has been increasing recognition of obesity as a condition with its own unique set of pathological processes, which ultimately result in a constellation of debilitating conditions (Jung, 1997; Allison et al., 2008; Bray, Kim and Wilding, 2017). Various definitions exist regarding the condition of obesity, and there is even debate about whether such a condition can be defined (Luli et al., 2023). Nonetheless, the most commonly stated definition of obesity is as described by the World Health Organization – "a chronic complex disease defined by excessive fat deposits that can impair health" (World Health Organization, 2024). In comparison, overweight is defined as "a condition of excessive fat deposits" (World Health Organization, 2024), and is characterised by a BMI greater than 25kg.m^{-2}.

Over a similar time-period, our population has observed a significant shift in overweight and obesity prevalence. Whilst overweight and obesity have historically affected a minority of the population, in more recent times, overweight has become the predominant state in most western countries (OECD, 2017; Temple and Conklin, 2019). Overweight now affects over 70% of individuals in countries such as the USA, Mexico and Chile (OECD, 2019). In the UK, 63.8% of the population is classified as overweight, with 25.9% with a BMI above 30kg.m^{-2} (House of Commons Library, 2019). Thus, a quarter of people in the UK live with obesity, a figure that has almost doubled since 1993 (House of Commons Library, 2019). Most concerningly, this rising

trend of overweight and obesity prevalence exists across the globe, regardless of national socioeconomic status (Murray et al., 2020).

1.1.2. Obesity-related mortality and morbidity

This significant rise in overweight and obesity prevalence, has predictably led to a corresponding increase in obesity-related complications, such as type 2 diabetes (T2D) (Public Health England, 2014), metabolic dysfunction-associated steatotic liver disease (MASLD) (Godoy-Matos, Silva Júnior and Valerio, 2020; Chan et al., 2023), obstructive sleep apnoea (OSA) (J. F. Garvey et al., 2015) & obesity hypoventilation syndrome (OHS) (Macavei et al., 2013), and osteoarthritis (Zheng and Chen, 2015). Additionally, obesity increases the risk of developing many other conditions such as cardiovascular disease, chronic kidney disease, and multiple cancers (R. B. Kumar et al., 2021).

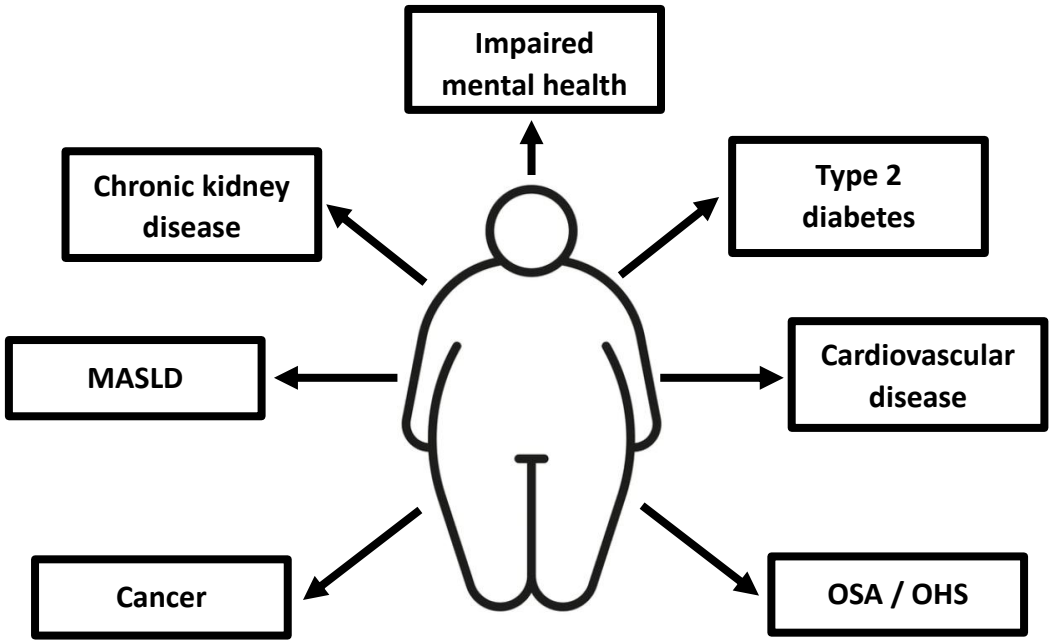

Figure 1.1.2.1. Diagram illustrating medical comorbidities strongly associated with obesity

Mortality among individuals living with obesity is also significantly higher than those without (Calle et al., 1999). In Organisation for Economic Co-operation and

Development (OECD) countries, overweight and obesity is estimated to cause 3 million premature deaths per year (OECD, 2019). A population-based cohort study in the UK demonstrated that every extra 5kg.m^{-2} rise in BMI confers an extra 20% increase in all-cause mortality (Bhaskaran et al., 2018). This BMI-associated increase in mortality persists when considering communicable diseases, non-communicable diseases, and injuries/external causes, and even in non-smokers (Bhaskaran et al., 2018). This highlights the fact that this risk is due not just to the presence of obesity-related comorbidities, but also an element of physical disability, which impairs an individual's ability to survive the impact of non-related health insults.

Accordingly, overweight and obesity also predispose to higher levels of disability, both physical and psychological. Individuals living with obesity are more prone to impaired mobility requiring use of aids, as well as frank disability limiting their ability to leave their homes (Vincent, Vincent and Lamb, 2010). Overweight and obesity is associated with higher rates of mental health conditions, such as depression and anxiety (Onyike et al., 2003), which may also contribute to the propagation of excess weight through the influence poor mental health can have on eating behaviours (Avila et al., 2015).

1.1.3. The economic consequences of obesity

Consequently, epidemiological studies suggest that individuals living with obesity are more likely to have more days off work due to illness, take early retirement, receive disability pensions (Y. C. Wang et al., 2011) and experience work-based discrimination (Giel et al., 2010). Furthermore, obesity and its related conditions result in a significant proportion of global health expenditure. The OECD estimates that the 52

countries spend 425 billion United States Dollars (USD) per year treating overweight, obesity, and their associated conditions (OECD, 2019). This represents approximately 8.4% of total health spending on average across the countries (OECD, 2019), equating to approximately £20 billion in the UK, based on the latest health expenditure figures (Office for National Statistics, 2023).

However, the costs to global economies are not just limited to healthcare expenditure. Individuals living with obesity experience lower levels of employment, increased absenteeism, and higher rates of early retirement (OECD, 2019). This combination has an impact on national economic productivity, with a reported 3.3% reduction in national gross domestic product (GDP) estimated across OECD countries (OECD, 2019). It is therefore calculated that each individual in OECD nation states pays an extra 359 USD per year in taxation, due to the effects of overweight and obesity on national economies (OECD, 2019).

1.1.4. Pathogenesis of overweight and obesity

Traditionally, overweight and obesity has been considered the result of an imbalance between energy intake and energy expenditure (R. B. Kumar et al., 2021), with an excess of the former being driven by easy access to energy-dense food products, and changes in occupational practices and transport methods fuelling the reduction in the latter. However, the wealth of studies stimulated by interest in methods to curb the seemingly relentless rise in obesity prevalence has vastly improved understanding of the complex physiological pathways that underpin appetite regulation, and therefore development of obesity. Thus, overweight and obesity is now thought to arise primarily due to an abnormal and excessive accumulation of adipose tissue, along

with the inappropriate defence of the resultant excessively elevated fat mass level (Guyenet and Schwartz, 2012). This abnormal accumulation and maintenance of fat mass has several aspects which can be divided into genetic, epigenetic and environmental factors.

Between 40-70% of weight is heritable, based on the results of twin and family studies (Riveros-McKay et al., 2019; Mahmoud, Kimonis and Butler, 2022). An exploration of the response of groups of twins to chronic overfeeding highlighted this, demonstrating large differences in weight gain from 4.3kg to 13.3kg between twin groups, with significantly more variance between twin-pairs than within (Bouchard et al., 1990). Similar results were observed when exposing twin groups to chronic calorie deficit, leading to the conclusion that individual's responses to chronic over (or under) feeding are largely related to genetic factors (Bouchard and Tremblay, 1997).

Many hundreds of candidate genes have been identified as having a potential association with obesity, and research into this is rapidly advancing (Loos & Yeo, 2022). Several forms of obesity have been identified that result from malfunction of specific genes encoding proteins, receptors and signalling pathways located within the hypothalamus, where energy homeostasis and satiety are primarily regulated (R. B. Kumar et al., 2021). These are commonly described as syndromic, in the presence of concurrent neurodevelopmental or multisystem sequelae (e.g. Prader Willi Syndrome), or monogenic, in which there is a lack of these additional features (e.g. isolated leptin deficiency) (Huvenne et al., 2016). Specific treatments exist for some of these conditions, through either replacement of the deficient hormone or agonism

of the melanocortin-4 receptor (Loos and Yeo, 2022). However, these are responsible for a tiny proportion of obesity cases, with polygenic forms accounting for the overwhelming majority (Mahmoud, Kimonis and Butler, 2022).

Although genetic factors are, to a large extent, associated with obesity, this does not explain the rapid rise in obesity rates observed over the last 30 years. Whilst identification of obesity-related genes has become more widely available, human genetics have not altered significantly enough to account for the large changes in body weight has occurred within a generation (Albuquerque et al., 2017). In contrast, the environment in which we live has changed dramatically over recent years and is thought to be a primary driver for the current weight trends. Most pertinently, food availability has increased dramatically, as has the energy density of food products (Sadeghirad et al., 2016). Food manufacturing companies invest significant amounts of money into increasing the palatability of their products, which can alter our natural hormonal responses to food (Guyenet and Schwartz, 2012). This results in increased desire for similar items, predisposing to the development of abnormal eating behaviours, at the most extreme end of the spectrum (Guyenet and Schwartz, 2012).

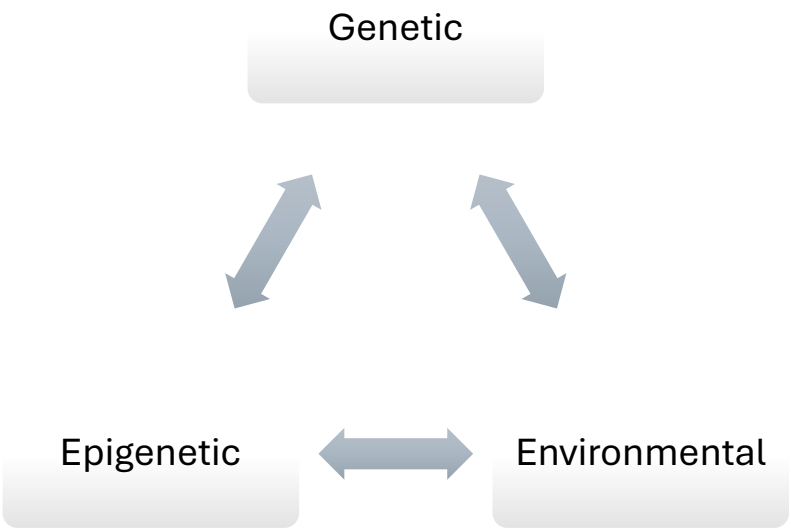

Figure 1.1.4.1. Main factors involved in the pathogenesis of obesity. Each group of factors interact in a complicated manner, synergistically driving development and maintenance of obesity

In addition, certain environmental factors can disrupt physiological pathways, leading to abnormal fat accumulation, thus predisposing to obesity. Poor sleeping practices and high levels of stress are strongly related to increased insulin resistance, increased appetite and abdominal fat accumulation (Beccuti and Pannain, 2011; van der Valk, Savas and van Rossum, 2018). Medication use can also directly influence weight, either via insulin-mediated pathways or through modulation of other hormones (Wharton et al., 2018). Moreover, environmental factors can interact with genetic function, via epigenetic changes that can also be attributed to weight gain (Bray et al., 2016). For example, modifications to DNA material have been demonstrated in response to high-fat diets (Jacobsen et al., 2012), physical activity (Rönn et al., 2013) and bariatric surgery (Benton et al., 2015). Thus, obesity arises from a complex milieux of factors that interact in a complicated manner (Figure 1.1.4.1), exerting powerful effects on energy homeostasis, fat accumulation and distribution, and overall weight control.

1.1.5. Management of overweight and obesity

Management of individuals living with overweight or obesity has traditionally focussed on advice to reduce food intake and increase physical activity levels – also known as the "eat less, do more" approach (Tremblett et al., 2023). However, improved understanding of the underlying mechanisms driving obesity has led to a realisation that relying on individual self-motivation is not always appropriate (Jebb and Aveyard, 2023). The majority of individuals living with overweight or obesity would have previously attempted to lose weight prior to seeking specialist weight management assistance, with varying degrees of success (Piernas, Aveyard and Jebb, 2016). Unfortunately, maintenance of weight loss is often the challenge, due to the abnormal physiological mechanisms that aim to counteract the weight change, and return body weight to pre-weight loss levels (Hall and Kahan, 2018). Thus, weight management is difficult, requires a multi-modal approach, and interventions are constantly being developed with new research investigating novel approaches emerging frequently.

Weight management can be grouped into three categories: lifestyle, pharmacological, and surgical. In addition to this, due to the well-recognised co-existence of mental health / psychological issues, specific therapies tackling these are often included into weight management plans. There is currently an increasing emphasis on individualised weight management plans, focussed on identifying potential causes of an individual's condition and therefore the most appropriate method/s required for their management (Hall and Kahan, 2018).

Lifestyle interventions range from simple dietetic advice to severe caloric restriction programmes through meal replacement. Dietetic advice promotes behavioural change that can be continued long-term, in order to help people lost weight persistently. Caloric restriction programmes such as low calorie diets (LCDs) or very-low calorie diets (VLCD) are effective for induction of significant weight loss, particularly in the short term (Franz et al., 2007). The former usually involves assisting individuals to cook normal food within a target calorie amount (up to 1200 kilocalories), whilst VLCD typically requires total meal replacement, where the majority of an individual's diet is comprised of specially manufactured products. This enables calorie intake to be severely restricted (less than 800 kilocalories), whilst still ingesting all of the required micronutrients and macronutrients to remain healthy and prevent complications.

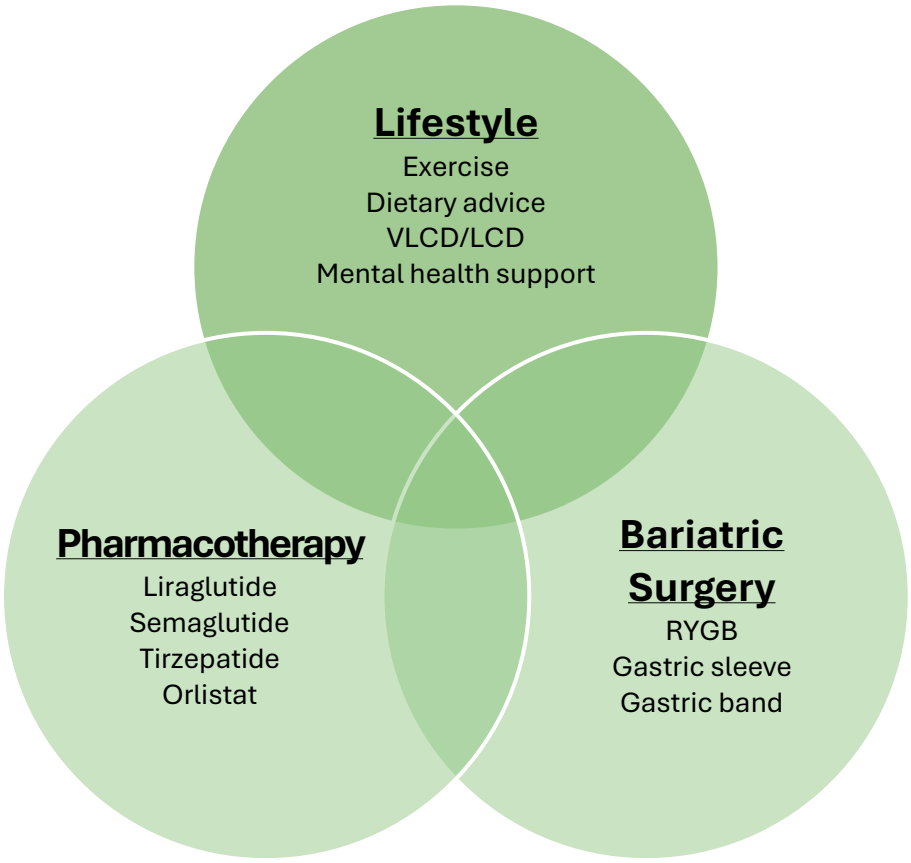

Figure 1.1.5.1. Current treatment options for managing obesity

Significant effort and resource has been invested into the development of pharmacological therapies that can help manage weight. The first iteration of medications for weight loss had modest efficacy, however adverse events were significant (Rodgers, Tschöp and Wilding, 2012). More recently, the discovery of the glucagon-like peptide 1 (GLP-1) hormone and its powerful anorexogenic properties have led to the development of GLP-1 receptor agonists (GLP1RA) for the treatment of obesity. Two GLP1RAs – Liraglutide and Semaglutide – are currently licensed for the treatment of obesity and have attracted significant interest due to their efficacy and relatively mild side effects (Updike et al., 2021). However, widespread adoption of these medications is inhibited by stock issues not meeting the global demand, and their relatively high cost.

It is widely recognised that the most definitive intervention for weight management with the most optimal long-term efficacy is bariatric surgery (Sjöström et al., 2004, 2007). Bariatric surgical procedures reduce gastric capacity, through varying methods, in order to impose restriction on food intake, thus resulting in weight loss. However, the efficacy of bariatric surgery is not purely the result of mechanically restricting gastric size, and several other underlying mechanisms have been postulated. These include induction of a relatively malabsorptive state, early stimulation of satiety hormone secretion, changes in bile acid physiology, alterations in gut microbiome composition and function, and modulation of taste sensation (Pucci & Batterham, 2019). The relative contribution of these mechanisms remain incompletely understood, however it is likely that they all interact to varying degrees resulting in an overall reduction in energy intake.

Multiple types of procedures exist, however gastric sleeve and roux-en-y gastric bypass (RYGB) are the most common (Arterburn et al., 2020). Large-scale long-term studies report similar outcomes between the two (Arterburn et al., 2020; Biter et al., 2024), thus choice of procedure often comes down to both surgeon and patient preference. Insights from metabolic studies investigating hormonal responses to bariatric surgery have shed light on the physiology of obesity, and informed the development of weight reducing pharmacotherapy (Waseem et al., 2007).

In the UK, weight management follows a tiered approach, as depicted by Welbourn et al. (2016). Tiers 1 and 2 are community delivered lifestyle programmes which can be accessed through self-referral or via primary care practitioners. Tier 3 is the first level of specialist weight management, involving specialist dieticians, nutritionists, physicians and psychologists trained in obesity management. Surgical weight management is delivered in Tier 4 with varying levels of input from other weight management professionals. Thus, management of weight is multi-modal, and the choice of intervention is entirely dependent on an individual's prevailing physical condition and circumstances.

Despite these management options, obesity continues to pose a significant challenge to health systems worldwide. The next section will introduce one of the most well-known obesity related conditions, T2D.

1.2. Type 2 diabetes

1.2.1. Definition and Prevalence

T2D is a complex disorder of glucose metabolism characterised by insulin resistance (IR) creating an increased demand on pancreatic beta cells to secrete greater amounts of insulin (DeFronzo, Bonadonna and Ferrannini, 1992). This increasing demand leads to the progressive destruction of these cells, eventually resulting in chronic hyperglycaemia – the hallmark of manifest T2D (Chatterjee and Scobie, 2002). The ensuing chronic hyperglycaemia causes extensive microvascular damage leading to a myriad of complications including retinopathy, neuropathy, nephropathy, peripheral vascular disease and cardiovascular disease (Kovacic et al., 2014). Cardiovascular disease is the largest cause of death in individuals with T2D (Einarson et al., 2018), and consequently primary prevention of this is the main focus of diabetes management (Reboldi et al., 2011; de Vries et al., 2012).

Similar to obesity, T2D is also rising in prevalence around the world. Over half a billion adults are diagnosed with T2D, and this is projected to increase to over three-quarters of a billion by 2045 (Sun et al., 2022). Its prevalence is increasing in all continents of the world with the most rapid increases observed in African and Middle-Eastern countries (International Diabetes Federation, 2021). Latest figures from the Diabetes UK charity reveals that there are approximately 4 million people in the UK living with T2D (Diabetes UK, 2023). This has increased from just under 3 million in 2013 (Diabetes UK, 2014a) and is set to continue rising through the rest of this decade (Public Health England, 2014). Thus, by the end of the decade, it is

expected that almost 9% of the UK population will be diagnosed with T2D (Public Health England, 2014).

The trends observed with T2D are highly related to the rising prevalence of overweight and obesity. These conditions confer a 3-fold and 7-fold increased risk of developing T2D, respectively (Abdullah et al., 2010), however, this increase has been quoted to be up to 12-fold in other population-based studies (Wilding, 2014). Ninety percent of individuals diagnosed with T2D also live with concurrent overweight or obesity (Public Health England, 2014). Furthermore, BMI correlates with IR (Ludvik et al., 1995), a key factor in the pathogenesis of T2D. This emphasises the close association that exists between T2D and excess weight, and highlights the key role weight management could have in controlling, and preventing T2D.

1.2.2. Economic impact

Over 950 billion USD is spent on managing diabetes and its complication across the world (Sun et al., 2022). The amount spent per person varies by region however from 112 USD in South-East Asia to over 8000 USD in North America (Sun et al., 2022). In the UK, the cost of treating diabetes and complications has risen from approximately £10 billion in 2014 (Diabetes UK, 2014b), to approximately £14 billion (Diabetes.co.uk: the global diabetes community, 2023). Thus, the UK healthcare system spends between £2000 - £3500 per person on treating diabetes (Diabetes.co.uk: the global diabetes community, 2023; H. I. Wang et al., 2022). Notably, the majority of this expenditure is on complications of diabetes, with cardiovascular disease comprising almost half of this (Diabetes UK, 2014b). In addition, as with overweight and obesity, diabetes leads to reduced economic

activity, which was estimated to reduce the economic output of the United States of America by over 100 billion USD, equating to a 6.6% reduction in GDP (Parker et al., 2024). Although these figures describe the impact of all forms of diabetes mellitus, T2D comprises 90% of diabetes diagnoses (Diabetes UK, 2014a), and therefore constitutes the majority of this spending. Thus, T2D has a significant economic impact and reducing the impact of the condition on individuals could translate into benefits in a nation's economic state.

1.2.3. Pathogenesis of T2D

T2D results from a state of insulin deficiency, resulting from the inability of the pancreas to secrete adequate levels of insulin to maintain normoglycaemia (DeFronzo, Bonadonna and Ferrannini, 1992). Insulin is a crucial hormone for normal homeostasis and severe insulin deficiency (as observed in type 1 diabetes) can be fatal, if left untreated. The actions of insulin are as follows (Dimitriadis et al., 2011):

- Inhibition of glycogen breakdown and gluconeogenesis in the liver and muscle cells
- Increase rate of glucose uptake & metabolism, and glycogenesis in muscle and adipose tissue
- Increased triglyceride uptake and reduced rate of lipolysis in adipose tissue
- Simulation of fatty acid synthesis in tissues (from glucose metabolism)
- Increase synthesis of cholesterol and very-low-density lipoprotein in hepatocytes
- Increase protein synthesis, primarily in muscle (also adipose tissue and liver)

Insulin is synthesised and secreted from pancreatic beta-cells, a process which is intricately connected to, and regulated by, plasma glucose concentrations. Under

fasted (postabsorptive) conditions, no food digestion is occurring and the liver, via gluconeogenesis, provides the main source of glucose for other tissues to metabolise. Accordingly, plasma insulin levels are low and its primary role in this setting is to interact with the liver to regulate this process and maintain normoglycaemia (Gale and Anderson, 2012). In the postprandial state, insulin secretion increases dramatically in response to rising plasma glucose levels in the portal circulation, resulting from nutrient digestion in the gut (Defronzo et al., 1985). In this situation, insulin action becomes focussed on muscle and adipose tissue, stimulating increased uptake of glucose, increased glycogenesis, as well as promoting protein synthesis and inhibiting lipolysis (in favour of glucose metabolism) in myocytes and adipocytes, respectively (Dimitriadis et al., 2011). At these elevated levels, insulin also acts on hepatocytes to inhibit gluconeogenesis and glycogenolysis (Defronzo et al., 1985), and stimulate cholesterol synthesis (Uehara et al., 2023). These actions occur synergistically with the overall aim to maintain normal blood glucose concentrations in response to nutrient ingestion, and under normal circumstances this process is remarkably effective (Gale and Anderson, 2012). A summary of these actions is depicted in Figure 1.2.3.1.

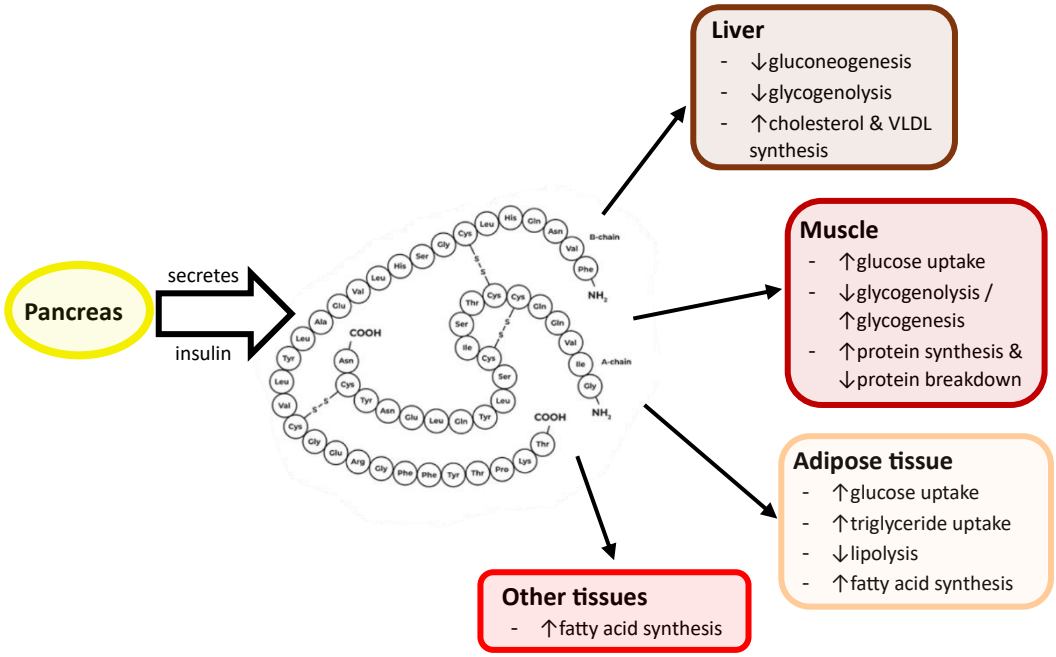

Figure 1.2.3.1. Diagram of insulin actions around the human body

In the context of T2D, multiple aspects of this process become dysregulated, ultimately resulting in chronic hyperglycaemia, and the associated sequelae of this (Cerf, 2013). It is widely recognised that skeletal muscle resistance to insulin is a key driver for the development of T2D (Chatterjee and Scobie, 2002; Defronzo and Tripathy, 2009). As detailed above, insulin action in the postprandial state is primarily focussed on skeletal muscle, which is the largest insulin-sensing organ of the body, and responsible for approximately 80% of whole-body glucose uptake under clamp conditions (Thiebaud et al., 1982). Thus, even mild disturbance of this process would result in significant consequences to overall glucose homeostasis.

Excess weight induces IR in skeletal muscle (Ludvik et al., 1995) in a process that appears to be mediated by elevated fatty acid concentrations, which is associated with impaired insulin signalling (Fujimoto, 2000). There is also a significant genetic component as expression of several genes has been linked to various pathogenic factors, including impaired insulin signalling, abnormal fatty acid & triglyceride

metabolism (leading to increased diacylglycerol and ceramide formation), and reduced insulin receptor number & function (Klein et al., 2022). Reduced levels of physical activity also contributes to this process, and is independently linked to the development of skeletal muscle IR (Balkau et al., 2008; Bird and Hawley, 2017). Thus, it is likely that the genetic factors increase the predisposition to development of IR in skeletal muscle, and the presence of elevated fatty concentrations, in addition to reduced physical activity, alcohol and other factors associated with increased BMI and excess energy intake, result in the IR becoming manifest.

Similar processes have also been observed in the liver, thus giving rise to the opinion that hepatic IR is a major factor driving development of T2D (Taylor, 2008). The association between excess fatty acids and hepatic IR is well-described (Samuel and Shulman, 2012). Fatty acids are rapidly absorbed by hepatocytes and under normal circumstances, these are metabolised for energy, converted into glucose or stored as intrahepatic triglyceride (Sears and Perry, 2015). However, by-products of this metabolic process including diacylglycerol and ceramides are known to disrupt insulin signalling pathways and receptor function, leading to hepatic IR (Sears and Perry, 2015). Similar to muscle, genetic abnormalities can influence this process by impairing triglyceride metabolism, leading to increased preference for diacylglycerol and ceramide synthesis, and a consequent predisposition to developing hepatic IR (Klein et al., 2022). Furthermore, increased plasma glucose and insulin levels resulting from IR in skeletal muscle results in increased de novo lipogenesis, causing higher intrahepatic fatty acid levels, further driving the development of hepatic IR (Samuel and Shulman, 2012). The resultant increase in triacylglycerol release by hepatocytes

into systemic circulation, further contributes to skeletal muscle IR, creating a vicious cycle of progressive IR.

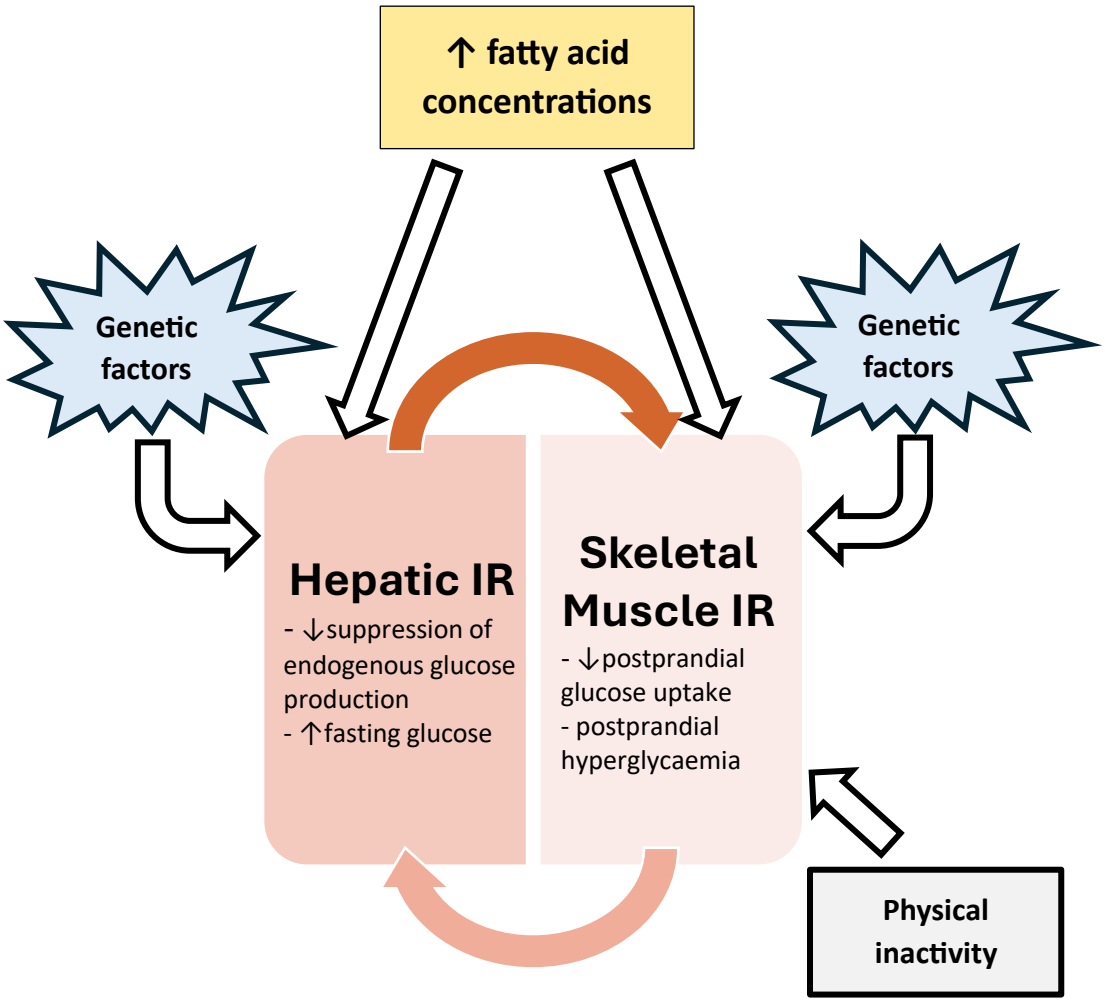

Figure 1.2.3.2. Summary of the various factors influencing development of IR

Thus, it appears development of both hepatic and skeletal muscle IR are relevant to the development of T2D, and are closely interrelated with the presence of one driving the progression of the other (Figure 1.2.3.2). Moreover, it can be suggested that the predominant type of IR varies between individuals, and is strongly influenced by the presence of certain genetic factors.

It is important to note, however, that not all individuals with IR develop T2D (Tatsumi et al., 2015). Additionally, 10% of individuals with T2D do not have an elevated BMI above 25kg.m^{-2}, and over 85% of individuals with an elevated BMI never develop T2D

(Public Health England, 2014). This is relevant because under normal circumstances, IR is matched by a compensatory increase in insulin secretion from pancreatic beta-cells (Cavaghan, Ehrmann and Polonsky, 2000). If the compensatory hyperinsulinaemia is adequate then normoglycaemia is maintained, which is the case in the majority of individuals living with overweight or obesity.

Thus, it is the concurrent presence of impaired beta-cell function (BCF) that ultimately results in clinically manifest T2D (Taylor, 2013). The mechanisms underlying impaired BCF are poorly described, but a clear association between pancreatic fat content and BCF has been observed (Taylor, 2013). Furthermore, reduction in pancreatic fat content has been demonstrated to underlie diet-induced amelioration of BCF, further crystallising the link between the two (Lim et al., 2011). As with IR, there are genetic factors that predispose to impaired BCF. These are heavily influenced by ethnicity, providing some explanation for the increased risk of T2D development at lower BMI levels in certain ethnic groups.

1.2.4. Management of T2D

For several decades, management of T2D was mainly focussed on the control of hyperglycaemia (Mudaliar, 2023). Thus, insulin replacement therapy and medications that stimulate insulin secretion were the mainstay of T2D management, along with the biguanide Metformin, which has insulin-sensitizing effects. However, publication of long-term population-based studies demonstrating continued progression of beta-cell failure despite optimal management with these agents (UK Prospective Diabetes Study (UKPDS) Group, 1998) guided a shift away from this glucocentric focus. Furthermore, the higher risk of mortality from cardiovascular causes highlighted in

the ACCORD study precipitated a greater focus on prevention of cardiovascular disease – the largest cause of death among individuals with T2D (Diabetes UK, 2014b). These observations have informed the development of several agents approved for use in T2D management, each with their own selection of advantages and considerations. In addition to this, control of blood pressure, lipids, alcohol intake and cessation of smoking are essential components of T2D management (NICE, 2015a) as they are all key contributors to the development of cardiovascular and other non-communicable diseases.

More recently, an argument has been made for weight management as a primary consideration for treating T2D (W. T. Garvey, Umpierrez, et al., 2022; Lingvay et al., 2022; Wilding, 2014). The majority of individuals with T2D live with overweight and obesity, and weight loss has been consistently demonstrated to result in improved glycaemic control, regardless of the underlying method (Gummesson et al., 2017; R. R. Wing et al., 2011). Moreover, weight loss carries the additional benefit of improving risk factors associated with cardiovascular disease (W. T. Garvey, Umpierrez, et al., 2022; The Look AHEAD Research Group, 2016), which is particularly important due to the high-risk nature of individuals with T2D. Thus, considerable attention has been focussed on developing interventions that promote weight loss in the context of T2D. At present, there are three classes of medication that are approved for management of T2D and have proven weight loss benefits.

Sodium-glucose cotransporter-2 (SGLT2) inhibitors promote glucose excretion from the kidneys, thus resulting in reduced plasma glucose (Thomas and Cherney, 2018). An unintended consequence of this is weight loss, and this class of medication has

also been robustly associated with reductions in cardiovascular disease, likely resulting from increased diuresis (Verma and McMurray, 2018). GLP1RAs also promote weight loss by stimulating satiety, and reducing gastric emptying, alongside their insulin secretory actions, resulting in significant weight loss and improvements in overall glycaemia (J. E. Campbell & Drucker, 2013; Drucker, 1998). Most recently, unimolecular agonists of multiple appetite regulating hormone receptors have been developed and studied in early-phase clinical trials (Alexiadou, Anyiam and Tan, 2019). The most successful, Tirzepatide, is an agonist of both GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) receptors, and has been approved for management of T2D in the UK (NICE, 2023b).

Another group of interventions that has attracted recent attention in line with the focus on weight loss is caloric restriction, particularly VLCD (Sellahewa et al., 2017). A series of large-scale studies have demonstrated the ability of caloric restriction programmes to be delivered at a population level to induce diabetes remission (Lean et al., 2018, 2019; Taheri et al., 2020). Thus, such programmes have also been incorporated into guidelines for management of T2D, which now have a significant focus on weight management, and cardiovascular protection (M. J. Davies et al., 2022). In the following sections, VLCD and GLP1RA will be described in more detail as interventions for treating T2D and obesity.

1.3. Very-low calorie diets

1.3.1. Introduction to VLCD

The VLCD is a dietary intervention that is utilised in a variety of settings to help stimulate significant weight loss over a short period of time. Daily energy intake is restricted to less than 800 kilocalories (kcal), thus it is described as severe caloric restriction. The extent of weight loss achieved depends on the level of caloric restriction imposed, as well as the length of the intervention (Kloecker et al., 2019).

VLCD were particularly popular between 1970-1990, when they were mainly used in commercial weight loss programmes, and were (Tsai and Wadden, 2006). However, concerns started to develop regarding their safety due to observed associations with cardiac abnormalities, occasionally leading to fatalities (Wadden, Stunkard and Brownell, 1983). These were felt to be likely resulting from nutrient (particularly protein) deficiencies that arose from imposing such marked caloric deficits (Wadden, Stunkard and Brownell, 1983). Consequently, modern VLCD products are manufactured in a way that ensures adequate levels of essential macronutrients are provided, leading to a very positive safety profile associated with their use (Saris, 2001).

Several studies have been performed to investigate the efficacy of VLCD in a variety of settings. Short-term weight loss of approximately 16% has been reported in individuals living with obesity (Tsai and Wadden, 2006). In the context of T2D, similar weight losses have been observed with one meta-analysis reporting a mean weight loss of 13.2kg across 17 studies (Sellahewa et al., 2017). Another meta-analysis investigated differences in weight loss outcomes between individuals with and

without T2D and found no significant difference, however this was limited to only four studies (Leslie et al., 2017). VLCD has also been shown to be effective in individuals with fatty liver disease, with one systematic review highlighting improvements in multiple parameters associated with the condition (Herrington et al., 2022). Children and adolescents also appear to benefit from VLCD interventions (Andela et al., 2019), although significant concerns exist regarding the safety and tolerability of these interventions in such populations (Gow et al., 2021; Hoare et al., 2021). Additionally, VLCD has been demonstrated to improve physical function in individuals that suffer with osteoarthritis (Webb, Osmotherly and Baines, 2021), and disease activity in sufferers of psoriatic arthritis (Klingberg et al., 2019). Thus, VLCD can be an effective weight loss option in a range of populations and can potentially precipitate improvements in the symptoms of certain conditions.

1.3.2. VLCD and glycaemic outcomes

The impact of VLCD-based interventions on glycaemic parameters in the context of individuals with T2D has been studied extensively in the literature. As far back as the early 1990s, Wing *et al*. demonstrated beneficial effects on fasting glucose and glycated haemoglobin after 8 weeks of a 1680 kilojoule (≈400 kcal) per day VLCD (R. R. Wing et al., 1991). Other studies conducted in the same decade also reported significant improvements in both fasting and peak postprandial plasma glucose concentrations (Kelley et al., 1993; Rotella et al., 1994). Another study by Wing *et al*. later in the decade highlighted the relative benefits of VLCD over a less restrictive LCD (R. R. Wing, Blair, Bononi, et al., 1994). They subjected two groups to either 400 kilocalories or 1000 kilocalories per day and assessed changes in fasting glucose after

achieving 11% weight loss. Although, the LCD group required a longer duration to achieve the target weight loss, fasting glucose reduced significantly more in the VLCD group despite achieving identical weight loss (R. R. Wing, Blair, Bononi, et al., 1994). They concluded that the VLCD caused greater improvement in insulin sensitivity (IS), possibly related to the greater composition of protein in these types of diets and the associated reductions in postprandial plasma glucose elevations. Thus, even at this time, the potential positive effect of VLCD on the glycaemic control of T2D was being realised.

More recently, there has been a significant attention placed in the ability of VLCD to induce diabetes remission, provoked by observations of bariatric surgical outcomes in individuals with T2D (Taylor, 2013). Various authors have reported resolution of fasting glucose levels to the normal range, less than a week after performing bariatric surgery (Pories et al., 1995; Guidone et al., 2006). This dramatic and rapid improvement in blood glucose levels had never been observed with any other intervention involving T2D, and attracted significant intrigue into discovering the underlying mechanisms (Taylor, 2013). In addition to this, bariatric procedures were also demonstrating the ability to induce long-term remission of T2D (Sjöström et al., 2004; Arterburn et al., 2013). These insights challenged the prevailing opinion of T2D as a relentless condition that progressed despite optimal management, and shifted the focus of its management going forwards.

Various theories have been postulated to provide explanations for these observations, implicating alterations in the physiology of gut hormone secretion, bile acid activity within the digestive system, and enteric microbiome interaction with

nutrients prior to absorption into the enteric circulation (Pérez-Pevida et al., 2019). The role of the caloric restriction imposed on individuals post-operatively was another potential mechanism highlighted by many authors (Taylor, 2013), re-igniting the interest in VLCD-based interventions for T2D. Several authors have explored similarities between physiological changes following bariatric surgery and caloric restriction with varied results. While Jackness et al. and Steven et al. reported similar outcomes between the two interventions (Jackness et al., 2013; S Steven et al., 2016), other authors demonstrated differences in response, using this to highlight the presence of other factors involved in the physiological response to bariatric surgery (Pournaras et al., 2010; Plum et al., 2011). Although these disagreements regarding the precise mechanisms underlying diabetes remission following bariatric procedures exist, there is a general acceptance of a role for caloric restriction (Dirksen et al., 2012).

This interest in VLCD led to numerous studies investigating the potential of VLCD to produce long-term diabetes remission. In 2011, Lim et al. were able to demonstrate diabetes remission following an 8-week VLCD, and related this to significant reductions in pancreatic and hepatic fat content, as well as improvements in pancreatic BCF and hepatic IS (Lim et al., 2011). Glycated haemoglobin (HbA1c) and fasting glucose reduced from 57 to 42mmol/mol and 9.2 to 5.7mmol/l, respectively, highlighting the potential for VLCD to not just improve glycaemic control, but normalise it (Lim et al., 2011). Other authors subsequently performed similar short-term studies and were also able to demonstrate normalisation of glycaemic control (Rothberg et al., 2014; Steven and Taylor, 2015; Lips et al., 2016), adding further weight to the potential of VLCD as a therapy for inducing diabetes remission.

Eventually, a study by Steven et al. became the first to outline the potential of VLCD to induce long-term diabetes remission (Sarah Steven et al., 2016). They subjected participants to 8 weeks of VLCD, then followed them up for a further 6 months following VLCD cessation, during which time participants received individualised advice regarding diet and physical activity. Both HbA1c and weight improved significantly following VLCD, and then remained stable for the following 6 months, and provided a basis for VLCD as a tool for inducing long-term diabetes remission (Sarah Steven et al., 2016).

With the rising evidence base regarding VLCD-induced diabetes remission, it became necessary to determine whether VLCD-based interventions could be delivered in a population-based setting, and in a cost-effective manner. The primary care-led weight management for remission of type 2 diabetes (DiRECT) study was the first study to determine this, in which participants were recruited from primary care practices and provided with a clinician delivered programme across multiple sites (Lean et al., 2018). The programme provided a total diet replacement of 825-853 kcal per day, for 3-5 months, and then follow-up with structured dietary and physical activity support, similar to that described by Steven et al. above (Sarah Steven et al., 2016). After the 12-month intervention, 46% of individuals achieved diabetes remission, and the likelihood of remission was 20-fold greater in the intervention group than the controls (Lean et al., 2018).

In the same year, a similar study was conducted in individuals without T2D to demonstrate the feasibility of delivering a programme based on VLCD principles in a community-based setting (Astbury et al., 2018). Significant improvements were

reported in weight and markers of cardiometabolic disease in the intervention group, when compared to controls (Astbury et al., 2018). Furthermore, although, this study was not exclusively restricted to individuals with T2D, improvements in HbA1c were significantly greater in the intervention group (Astbury et al., 2018). Further studies exploring the efficacy of community based caloric restriction programmes for inducing diabetes remission have since been conducted, confirming the ability to deliver these programmes in this setting (Bynoe et al., 2019; Taheri et al., 2020; Marples et al., 2022).

Consequently, the UK National health Service now recommends a caloric restriction intervention based on VLCD principles for individuals that meet certain criteria (Diabetes UK, 2018; NHS England, 2020). Its structure is similar to the intervention described in the DiRECT study (Lean et al., 2018), therefore at 825-853 kcal per day it does not strictly fall under the definition of a VLCD (Ahmad and Alfaris, 2018). However, it is delivered by total meal replacement and the physiological effects are identical to those observed with VLCD (Saris, 2001; Leslie et al., 2017). Caloric restriction programmes are now suggested in guidelines for managing T2D as an additional option for promoting weight loss (M. J. Davies et al., 2022).

1.3.3. Pitfalls of VLCD

Despite the observed improvements in many health parameters which are not limited to individuals with T2D, widespread adoption of VLCD in other conditions has not occurred. This is due to a number of significant and well-recognised potential drawbacks of VLCD interventions (Baker et al., 2009a; Tsai & Wadden, 2006). Arguably, the most significant concern is regarding weight regain, and the associated

deterioration or relapse of previously resolved conditions. Weight regain following VLCD cessation is a common observation, limiting their long-term efficacy. One particular meta-analysis reported a mean weight loss of approximately 18kg after 6 months, however this had reduced to 6kg by 3 years, representing a regain of 66% of original weight loss (Franz et al., 2007). Another review highlighted that some studies reported over 100% weight regain, suggesting that participants completed the follow-up heavier than prior to commencing the intervention (Saris, 2001). Studies have been conducted to determine the presence of physiological predictors of weight regain and identify methods to mitigate these, however these have largely proved unsuccessful (Nymo et al., 2019; Martins et al., 2023). However, other studies assessing the impact of adjuvant interventions such as exercise or GLP1RAs on weight loss maintenance have demonstrated promising results (Fogelholm et al., 2000; Borg et al., 2002; Lundgren et al., 2021).

In the context of T2D, weight regain causes deterioration in glycaemic control, and threatens maintenance of diabetes remission. Multiple studies report deterioration in glycaemic control associated with weight regain (Dhindsa et al., 2003; Snel, Sleddering, et al., 2012), with one study outlining a return to pre-intervention glycaemic control, despite participants maintaining a proportion of the weight lost (R. R. Wing, Blair, Marcus, et al., 1994). There is very limited evidence regarding the risk of deterioration in glycaemic control more than 1 year following VLCD-based programmes. However, two- and five-year follow up reports of outcomes from the DiRECT study have reported a gradual reduction in number of participants maintaining diabetes remission, with only 13% managing to maintain remission by 5 years (Lean et al., 2019, 2024).

Another significant concern regarding VLCD is the associated loss of muscle mass (Donnelly, Jakicic and Gunderson, 1991). One review highlights that approximately 25% of total weight loss as a result of VLCD may be from skeletal muscle (Willoughby, Hewlings and Kalman, 2018). This is particularly relevant in individuals with T2D who experience accelerated reductions in muscle mass (MM), strength and quality (Nishitani et al., 2011; S. W. Park et al., 2009; S. W. Park, Goodpaster, Strotmeyer, de Rekeneire, et al., 2006). Skeletal muscle is crucial for normal glucose homeostasis and lower skeletal MM correlates negatively with glycaemic control in individuals with T2D (Bower et al., 2017a). Thus, promotion of further losses may exacerbate future worsening of glycaemic control. In summary, despite the potential benefits of VLCD outlined above, concerns exist regarding their use in T2D. These concerns necessitate further exploration into the mechanisms underlying these deleterious effects, which will enable identification of possible strategies to mitigate their impact.

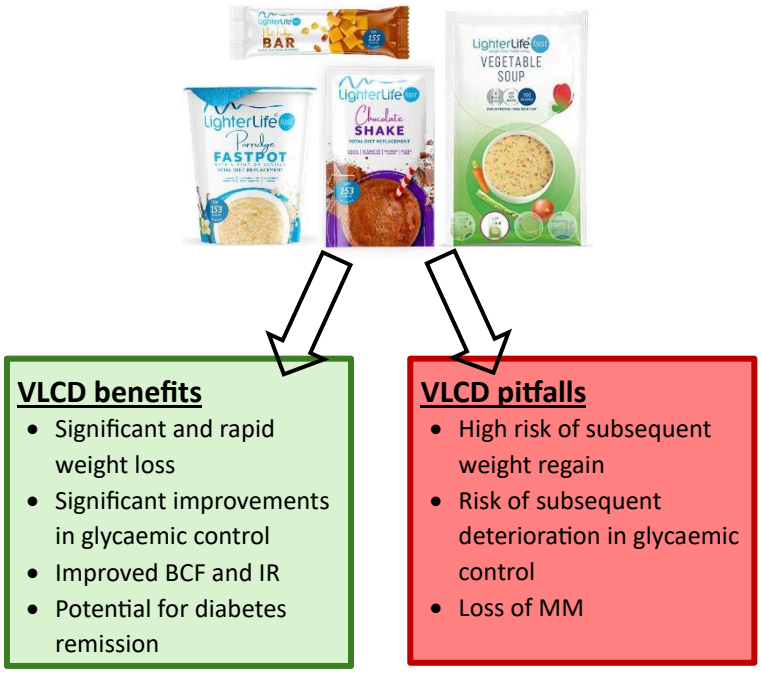

Figure 1.3.1. Summary of the advantages and disadvantages of VLCD in individuals with T2D

1.4. A narrative review of the glucometabolic effects of VLCD

In order to appreciate the benefits of VLCD in T2D (and therefore the requirement to mitigate against the potential negative effects), an appreciation of the impact of VLCD on the main pathological drivers underlying T2D is necessary. As previously outlined, T2D is characterised by the presence of IR and impaired pancreatic BCF, ultimately resulting in chronic fasting and postprandial hyperglycaemia. As IR and impaired BCF play a crucial role in the pathogenesis of T2D, diabetes remission is primarily dependent on resolution of these parameters (Klein et al., 2022; Taylor, 2013).

It is important to note that individuals without T2D are also prone to IR (Abbasi et al., 2002; Ludvik et al., 1995), which in itself is independently associated with poor health outcomes (Ausk et al., 2010; Gast et al., 2012). Therefore, for any individual living with obesity, interventions that improve IR and BCF are highly desirable, irrespective of diabetes status.

Although the effects of VLCD on IR and BCF has been extensively studies, there exists considerable inconsistency regarding the effect of VLCD on IR and BCF. Whilst one group have repeatedly reported improvements in BCF with VLCD (Lim et al., 2011; Steven, Hollingsworth, Al-Mrabeh, et al., 2016; Taylor, 2013; Taylor et al., 2018), this is not supported by several other authors that report no significant alterations (Fukuda et al., 1989; Steven, Hollingsworth, Small, et al., 2016; Svendsen et al., 2012). Similarly, when examining effects of VLCD on IR, significant improvement is reported by some authors (Bakker et al., 2015; Jazet et al., 2008; Snel, Gastaldelli, et al., 2012), but not others (Adam et al., 2005; Berggren et al., 2017; Herzog et al., 2020).

With the incorporation of caloric restriction programmes in weight management guidelines (Markovic et al., 2022), and the consequent increase in use of VLCD-based interventions, these conflicting findings pose a challenge to healthcare professionals involved in the management of individuals living with overweight, obesity and T2D. A clear understanding of the effects of VLCD-based interventions on BCF and IR will inform decisions around maximising their outcomes, facilitate identification of individuals that will benefit most from them, and enable provision of appropriate advice to individuals undergoing these interventions.

Therefore, this narrative review discusses evidence related to VLCD-induced effects on BCF and IR, with a view to providing much-needed clarity on this topic. This may be beneficial to healthcare professionals involved in the use of VLCD in the clinical management of individuals living with overweight and obesity, with and without T2D.

1.4.1. Methods of assessing BCF and IR

In order to critically discuss this topic, knowledge of the available methods for measurement of BCF and IR, as well as their benefits and pitfalls, is advisable. Several methods are utilised for assessing VLCD-induced alterations in these parameters, with varying levels of complexity and user-friendliness. The most commonly employed methods are briefly outlined in Table 1.4.1.1 to provide context to the remainder of the review.

Table 1.4.1.1. Common methods for quantifying BCF & IR	
Clamp techniques	**Hyperglycaemic clamp** – This is the gold standard method for measuring beta cell function (Cersosimo et al., 2014). Glucose is infused to elevate plasma concentrations above baseline with measurement of plasma insulin over a specified period (typically 2 hours) (DeFronzo et al., 1979). Both the initial surge of insulin secretion in response to rising blood glucose (known as the first phase response), and the slower onset more gradual second phase response can be directly visualised. The first phase response is particularly relevant as this is absent in individuals with T2D (Brunzell et al., 1976; Cerasi et al., 1972), and its restoration is associated with diabetes remission (Lim et al., 2011). Thus, the first phase response provides an accurate estimation of beta cell functional recovery. An insulin secretagogue can also be infused at the end of the hyperglycaemic clamp, which stimulates maximal insulin secretion (Cersosimo et al., 2014), enabling assessment of maximal beta cell capacity. **Euglycaemic-hyperinsulinaemic (EH) clamp** – Similarly, the EH clamp is the gold standard tool for assessing IR (Gastaldelli, 2022). This technique involves continuous infusion of insulin, along with co-infusion of glucose solution to maintain euglycaemia. At steady state, the rate of glucose infusion required to achieve this is equivalent to glucose uptake into peripheral tissues, termed "glucose disposal" (DeFronzo et al., 1979). This permits determination of peripheral IR, such that a higher rate of glucose infusion denotes lower level of insulin resistance (DeFronzo et al., 1979). The infusion of isotopic tracers prior to commencing the EH clamp also enables measurement of hepatic endogenous glucose production (EGP) (Gastaldelli, 2022), providing a measure of hepatic IR. This can be combined with a calculation of the extent to which EGP is suppressed by insulin to provide a more comprehensive assessment of IR. Both clamp techniques provide multiple measures of BCF and IR, and are very reproducible (Cersosimo et al., 2014). However, appropriate technical expertise are required to perform these procedures accurately, and they are very resource and time intensive.

Intravenous glucose tolerance test (IVGTT)	The IVGTT assesses both BCF and IR through a single test (Hannon et al., 2018). An intravenous bolus of glucose is infused over a short period of time (2-3 minutes) with plasma glucose and insulin concentrations measured at multiple timepoints following this (Bingley et al., 1992). Due to this rapid entry of glucose into systemic circulation, first and second phase insulin responses can be directly observed, providing a comprehensive assessment of BCF. Exogenous insulin can also be infused during the test in order to increase the accuracy of IR measurements, via minimal modelling calculations (Muniyappa et al., 2008). However, results obtained from IVGTT are less reproducible than the clamp techniques, particularly in individuals with more advanced T2D (Hannon et al., 2018).
Oral glucose tolerance test (OGTT) & meal tolerance test (MTT)	The OGTT and MTT (also known as the mixed-meal tolerance test) are often described as the most physiological tests of the body's ability to respond to ingested glucose (Muniyappa et al., 2008). The OGTT procedure involves ingestion of a specified amount of glucose, with subsequent measurement of plasma glucose and insulin levels, for up to 4 hours. In the MTT, a standardised "meal" is ingested which contains all macronutrients, and can be liquid, semi-solid or solid. This gives it an advantage over the OGTT because it is a more precise simulation of human energy consumption. Both tests enable observation of the incretin response, which is a critical aspect of the physiological response to nutrient intake, and is bypassed by the above techniques. The response of other gut hormones to energy intake can also be determined. Various calculations can be utilised for assessment of IR, which correlate well with the euglycaemic hyperinsulinaemic clamp (Otten et al., 2014). However, as glucose absorption via the enteral route is slow, along with significant interindividual variations in nutrient digestion and gut motility, it is not possible to accurately quantify first and second phase insulin responses (Hannon et al., 2018). Despite this, the disposition index can be utilised to provide an estimate of overall BCF in both types of test (Cersosimo et al., 2014; Hannon et al., 2018).
Fasting measures	Indices of BCF and IR can also be derived from measurement of fasting glucose and insulin, although they are predominantly used for the latter. The advantage of these indices is their simplicity, permitting their use in clinical settings also. Various indices are available, the most common being the homeostatic model of assessment (HOMA), although the QUICKI index has recently been deemed to correlate best with

	the EH clamp (Otten et al., 2014). As these measures rely on fasting glucose, they most closely reflect hepatic IR (Muniyappa et al., 2008), as fasting plasma glucose concentration is entirely dependent upon insulin-induced suppression of hepatic EGP. Despite this, they have been shown to correlate reasonably well with gold standard techniques (Matthews et al., 1985), although they are usually used in combination with other techniques to provide a more comprehensive assessment of these parameters.

1.4.2. The effect of VLCD on BCF

1.4.2.1. Individuals with T2D

Studies exploring the effect of VLCD on BCF have been conducted since the 1970s. In 1979, Savage et al. compared the metabolic responses of 10 individuals with T2D and 10 nondiabetic individuals to 4 weeks of 500 kcal VLCD (Savage et al., 1979). IVGTT was used to assess BCF and demonstrated a significant increase in acute insulin response to glucose (AIRg – a measure of first phase response). A year later, Stanik et al. also reported improved BCF in a cohort of 7 individuals with T2D undergoing a 600 kcal VLCD for 4 – 12 weeks to induce 11kg weight loss (Stanik & Marcus, 1980). This study measured BCF with a combined OGTT and intravenous tolbutamide approach which is not a common method of BCF assessment, however was still able to demonstrate significant increase in insulin secretion, despite low participant numbers (Stanik & Marcus, 1980).

However, these early observations were not replicated by several subsequent studies over the following two decades. Three studies from different groups evaluating BCF using glucagon tests (measuring insulin or C-peptide response to intravenous glucagon bolus) showed no significant improvement following VLCD (Fukuda et al., 1989; Laakso et al., 1988; Rotella et al., 1994). All three studies used more restrictive VLCD regimes than the studies outlined above, albeit for shorted periods of time (ranging between two and four weeks). Two of the studies were limited by low numbers, however, this was not the case for Rotella et al. (Rotella et al., 1994). Importantly, glucagon tests may not be the most sensitive method of assessing changes in BCF, particularly in response to short-term interventions, and like the

intravenous tolbutamide approach, are less commonly utilized in more current literature.

An article published in the same year as Rotella et al., used IVGTT and appeared to show an improvement in AIRg, in line with the findings of Savage et al. fifteen years earlier, although this was not tested statistically (R. R. Wing, Blair, Bononi, et al., 1994). Subsequently, in the early 2000s, Jazet et al. failed to demonstrate a significant improvement in AIRg following a 30-day VLCD despite also measuring BCF with IVGTT. Importantly, the baseline assessments were performed 2 days after participants commenced the VLCD, which may have led to a blunting of the observed effect, given the relative short-term of the intervention. Later, in 2011, a study comparing changes in glycaemic outcomes between roux-en-y gastric bypass (RYGB) surgery and 800 kilocalorie VLCD, did report significant increase in AIRg in both the VLCD and surgical groups (Plum et al., 2011). This study utilised frequently sampled IVGTT for assessment of BCF, enabling a more precise calculation of the first phase response. Of note, the length of the VLCD intervention in this study was greater than most of the earlier studies mentioned above.

Until this point, there had been no consistent reports of improvement in BCF following VLCD in individuals with T2D. However, in the same year the seminal study published by Lim et al. resulted in a significant shift in the understanding of VLCD in the context of T2D management (Lim et al., 2011). Using hyperglycaemic clamps with additional arginine stimulation, the authors observed that VLCD may not only improve BCF, but restore it to levels recorded in healthy controls (Lim et al., 2011). This introduced the prospect of induction of diabetes remission with VLCD, and

contrasted with the prevailing understanding of T2D being an unrelenting disease of progressive beta cell dysfunction (UK Prospective Diabetes Study (UKPDS) Group, 1995). Furthermore, they provided a physiological basis for this, relating the observed resolution of BCF to reductions in pancreatic fat, which supported the twin-cycle hypothesis proposed as a mechanism for the pathogenesis of T2D (Taylor, 2013).

This group continued to publish several articles in an effort to characterise these initial findings further. In 2016, two studies reported conflicting results, despite using similar techniques. In a study exploring the mechanisms underlying RYGB-induced diabetes remission, first phase response and maximal secretion did not significantly change in either the VLCD or RYGB cohorts (Steven, Hollingsworth, Small, et al., 2016). However, a second study did demonstrate improvement in first phase response, although again there was no change in maximal secretion (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). Both studies used approximately 700 kcal VLCD, with hyperglycaemic clamp plus arginine stimulation. However, it is likely the former study was affected by limited participant numbers, with only 9 in each group (compared to 29 in the latter study) as a numerical increase in response was documented (Steven, Hollingsworth, Small, et al., 2016). Furthermore, the study measures were assessed after one week of VLCD, and the initial Lim et al. study showed that significant improvement in BCF did not occur until 8 weeks of VLCD intervention (Lim et al., 2011).

This second study also introduced the concept of VLCD “responders” (individuals that achieve fasting glucose < 7 mmol/l) and “nonresponders”. Notably, although BCF improved significantly in both groups, the nonresponders had markedly lower

baseline BCF compared to responders, and the improvement was of a significantly smaller magnitude (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). The responders were younger, and had shorter duration of T2D diagnosis as well as better baseline glycaemic control (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). These findings suggested that the capacity for beta cells to recover normal functioning depends on the initial level of impairment, which appeared to be associated with several pre-existing clinical characteristics.

This theory was further tested in a subsequent study, in which a subgroup of participants from a community-based VLCD programme (M. E. Lean et al., 2018) had more detailed metabolic testing performed (Taylor et al., 2018). On this occasion, "responders" were defined as having HbA1c < 6.5%, in addition to normalised fasting glucose. Using similar techniques, the authors described significant improvement in first phase response in the responder group, however in this case there was no change in the nonresponders (Taylor et al., 2018). It is important to note that the nonresponder group in this study appeared to have greater impairment in BCF than those in the previous study, which may explain the lack of response. Also, the intervention diet was 825-854 kcal, compared to 700 kcal in the previous study, and this modest increase in daily calorie intake may have attenuated the observed effect on BCF. Nonetheless, this study provided further evidence supporting the notion that the likelihood of long-term diabetes remission may be dependent on baseline BCF.

Outside of this group, Malandrucco et al. studied a cohort of 14 individuals with morbid obesity and well-controlled T2D using a 400 kcal VLCD for 1 week (Malandrucco et al., 2012). Despite the short study duration, they demonstrated an

improvement in BCF using hyperglycaemic clamps. This observation was at odds with the findings of Lim et al. (2011) and Steven et al. (2016) who showed no significant change at 1 week. It is possible that the extreme caloric restriction imposed by Malandrucco et al. was able to induce enough of a dramatic shift in metabolism to cause improvement in BCF within this short time period. This may also explain the enhanced BCF noted in the studies by Savage et al. (1979) and Stanik & Marcus (1980) despite the relatively short intervention lengths. Furthermore, the participants in this study had a relatively short duration of T2D diagnosis and good baseline glycaemic control, again supporting the assertions of Steven et al. (2016).

Subsequent studies assessing the effect of short term VLCD on BCF have outlined similar findings, both using IVGTT as the method of measurement. Liu et al. reported improved AIRg after only 9 days of VLCD in a cohort of 20 individuals with T2D, although the extent of caloric restriction was undisclosed (Liu et al., 2015). Interestingly, this study included lean individuals and found no significant difference in change in AIRg between lean (BMI < 24kg.m^{-2}) and overweight (BMI ≥ 24kg.m^{-2}) participants. Thus, VLCD has the potential to restore BCF in lean individuals with T2D, as well as individuals with excess weight. Jackness et al. also recorded improved AIRg after only 3 weeks of a 500 kcal VLCD, and found no significant difference between this and the improvements observed in a parallel group of participants that underwent RYGB (Jackness et al., 2013). Another study assessing BCF changes following matched weight loss from VLCD or RYGB used an index derived from insulin and glucose ratios from a liquid MTT and EH clamp (Yoshino et al., 2020). Similar to Jackness et al., they observed significant improvement in BCF to the same extent as the RYGB group, although the VLCD group required 50% more time to achieve the

desired weight loss (Yoshino et al., 2020). Both studies concluded that the improvements in BCF were due to the weight loss induced by caloric restriction, rather than any metabolic changes specific to surgery.

1.4.2.2. Individuals without T2D

As BCF is typically normal in individuals without T2D, it is unsurprising that few studies have examined the effects of VLCD in this population. The early studies by Savage et al. and Fukuda et al. included nondiabetic cohorts, with contrasting results (Fukuda et al., 1989; Savage et al., 1979). Whilst the Savage et al. did demonstrate improved BCF in the nondiabetic group, Fukuda et al. did not, although the measures employed in the latter study are not commonly used now. Svendsen et al. administered a 500 – 600 kcal VLCD for 8 weeks to a group of 10 women with BMI > 30kg.m^{-2} and conducted IVGTTs, as well as EH clamps and OGTTs. They found no significant change in AIRg, or disposition index. However, a different study was able to demonstrate a significant improvement in MTT-derived disposition index following an 800 kcal VLCD for 8 weeks, although there was no change in other markers of BCF (S. B. K. Jensen et al., 2023). This was a large study involving 195 participants, who were subsequently randomised into 4 groups to assess different methods of weight maintenance following VLCD-induced weight loss.

1.4.2.3. Summary

In all studies that assessed BCF in response to long-term (≥8 week) VLCD, BCF improved significantly, regardless of whether hyperglycaemic clamps or IVGTT were used for BCF assessment. Short-term VLCD may also improve BCF, particularly with more extreme caloric restriction and the greater degree of weight loss related to this.

It is important to be aware of the duration of T2D diagnosis, the age of participants and baseline glycaemic control as these appear to influence the capacity of beta cells to recover their function in response to caloric restriction. In all studies using glucagon tests, no significant changes were noted, suggesting that the test may lack the sensitivity required to detect BCF alterations, particularly in response to short-term VLCD interventions. An acknowledgement of the type of test chosen to assess BCF is crucial when assessing BCF response to VLCD interventions.

Limited evidence suggests that BCF can also improve in lean individuals, as well as those with excess weight. This concept requires further investigation as approximately 10% of individuals with T2D have a BMI within the “normal” range (Public Health England, 2014) and this evidence suggests that VLCD may also be beneficial for them too. Few studies report the effect of VLCD on BCF in individuals without T2D, with two reporting some improvement and two others reporting no change. However, BCF typically is not impaired in this cohort, highlighting the importance of noting the diabetes status of participants when reviewing studies on this topic.

Figure 1.4.2.1. Summary Box
Factors increasing the likelihood of BCF improvement:
VLCD duration > 8 weeks
Increased level of caloric restriction (≤600 kcal per day)
Shorter duration of diabetes diagnosis
Lower age
Better baseline glycaemic control

1.4.3. The effect of VLCD on IR

1.4.3.1. Individuals with T2D

As with BCF, multiple studies exploring the impact of VLCD on IR in people with T2D have been conducted over the previous five decades, with various inconsistencies throughout. The two aforementioned studies by Savage et al. (1979) and Stanik & Marcus (1980) both reported improved insulin sensitivity, in addition to the amelioration of BCF. In contrast to this, a study conducted in 1987 measuring IR in 9 Pima Indians with T2D showed no change following a 5-week 617 kcal VLCD (Zawadzki et al., 1987). The VLCD interventions in these studies were relatively short-term and each study used methods that provide an estimation of whole-body IR. Although the first two studies used glucose tolerance test techniques (Savage et al., 1979; Stanik & Marcus, 1980), a modified clamp technique was employed in the latter study (Zawadzki et al., 1987), which may account for the differences in their findings.

Within the same decade, two studies conducted by Henry et al. performed EH clamps before and after VLCD to assess changes in tissue-specific IR (Henry et al., 1985, 1986). In the first study, a severely restrictive 330 kcal VLCD was conducted for 40 days resulting in a profound reduction in hepatic EGP (Henry et al., 1985). Moreover, this reduction was shown to be significant by 10 days of VLCD, with further reductions thereafter. This study also reported improved glucose and insulin responses to MTT. The VLCD regimen in the second study was slightly less restrictive allowing 330-600 kcal per day, but was continued over a longer duration of up to 380 days (Henry et al., 1986). Again, significant reduction in hepatic EGP was noted, and

glucose responses to OGTT and MTT improved. Both studies reported a high correlation between hepatic EGP and fasting plasma glucose, emphasising the close association between hepatic IR and fasting hyperglycaemia.

These findings were further supported by Laakso et al. who in the same decade, also showed that a 500 kcal VLCD over 12 days reduced basal hepatic EGP (Laakso et al., 1988). Of interest, they included a group of individuals on insulin, who also experienced a significant reduction in EGP. Furthermore, clamp-derived peripheral glucose disposal also increased in both groups, albeit to a significantly greater extent in the VLCD group. Kelley et al. also demonstrated both reduced basal EGP and improved peripheral glucose disposal in response to VLCD (Kelley et al., 1993). Although this study's intervention period was much longer, serial measures were collected, and improvements were observed in both parameters within the initial week of caloric restriction (Kelley et al., 1993).

A subsequent study by Jazet et al. demonstrated that improvements in hepatic IR can be visible within as little as two days of VLCD (Jazet et al., 2005). In a cohort of 12 insulin-treated individuals with T2D, 450 kcal VLCD for 2 days significantly reduced basal EGP (Jazet et al., 2005). Whilst they did not report any change in insulin-mediated suppression of EGP or glucose disposal over this time period, a follow-up study in which 10 participants underwent repeat testing following 50% excess weight loss did observe a significant improvement in both of these parameters (Jazet et al., 2008). This finding implied that the various aspects of IR may respond to caloric restriction at differing rates, which could provide some explanation to the contradictory findings observed in the literature in relation to related to VLCD-

induced alterations in IR. Notably, muscle tissue was also obtained in this study, allowing the authors to describe significant negative correlations between intramyocellular lipid (IMCL) content and glucose disposal, implicating a potential role of IMCL in peripheral IR pathophysiology (Jazet et al., 2008).

Similar temporal trends in IR changes were reported by Lim et al. who performed serial EH clamps in a cohort of 11 individuals with T2D (Lim et al., 2011). They observed significantly reduced EGP within a week of VLCD initiation, as well as increased suppression of EGP by insulin. Furthermore, both of these indices continued to improve such that by 8 weeks there was no significant difference between the intervention cohort and a group of healthy controls. Interestingly, glucose disposal did not change significantly, despite the rather restrictive 600 kcal VLCD and the resultant average weight loss of approximately 15kg (although there was an increase in glucose metabolic clearance rate).

Various other studies have reported no change in specific peripheral IR. A similar study conducted by the group mentioned above divided participants into responders (who achieved normal fasting blood glucose levels) and nonresponders (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). They did not observe significant improvements in glucose disposal in either cohort, but did notice significant improvement in hepatic IR, even in the nonresponders. Additionally, Lara-Castro et al. also reported no significant improvement in glucose disposal in their cohort of 7 individuals with T2D, although this was after only 6 days of VLCD (Lara-Castro et al., 2008). Interestingly, when these results of both intervention groups were combined with those from the other cohort of nondiabetics, they did observe a significant

improvement in glucose disposal. Furthermore, IMCL content was also examined in this study, with significant reductions in both cohorts and negative correlation to glucose disposal highlighted, in line with the findings of Jazet et al. (2008).

The studies by Laakso et al. (1988) and Kelley et al. (1993) however are not unique in their demonstration of improved clamp-derived peripheral IR in individuals with T2D. More recent studies have also supported these findings, including a study investigating the effects of adjuvant exercise in individuals with insulin dependent T2D undergoing VLCD. Both intervention groups exhibited an increased glucose disposal rate, to similar degrees (Snel, Gastaldelli, et al., 2012). Likewise, a study by Yoshino et al. also reported significantly improved glucose disposal in response to VLCD (Yoshino et al., 2020). This study also assessed insulin mediated suppression of adipose tissue lipolysis and reported a significant increase in this too, suggesting improved adipose tissue IR (Yoshino et al., 2020). In these two studies, the VLCD intervention was of a longer duration than the studies that reported no improvement in glucose disposal. Thus, it can be postulated that changes in peripheral IR are more likely to occur over the longer term and may require a prolonged period of caloric restriction to ensure they consistently become manifest.

Studies employing other methods of measuring IR reveal similar trends to those observed in clamp-based studies. HOMA indices rely upon fasting measures of glucose and insulin, which is dependent on hepatic EGP and its responsiveness to prevailing insulin concentrations. Several studies assessing the HOMA insulin resistance index (HOMA-IR) have demonstrated an improvement in this measure following VLCD in individuals with T2D (Gulsin et al., 2020; Jackness et al., 2013; Lara-

Castro et al., 2008; Liu et al., 2015; Snel et al., 2011). These studies vary in intervention duration from 6 days to 4 months, with a wide range of caloric restriction, suggesting that HOMA-IR improves early and can occur with any level of calorie restriction.

As described previously, measures derived from IVGTT, OGTT and MTT provide an estimation of whole-body IR, formed from a composite of both hepatic and peripheral IR. In addition to the studies discussed previously (Savage et al., 1979; Stanik & Marcus, 1980), several other authors have reported similar findings over a range of intervention durations. Wing et al. reported significant improvement in IVGTT-derived IR in response to a 400 kcal VLCD (R. R. Wing, Blair, Bononi, et al., 1994). Intriguingly, this improvement was greater than that observed with a 1000 kcal per day diet, despite matched weight loss (over a longer period). The authors concluded that changes in IR are more associated with the degree of caloric restriction, rather than the amount of weight loss, providing additional insight into the discrepancies in changes in IR between differing studies.

In a more recent evaluation of a community-delivered 8-week VLCD programme using MTTs, the authors again observed significant improvement in post-prandial glucose levels following the intervention (Bynoe et al., 2019). Moreover, significant improvement in Matsuda index (a measure of insulin resistance derived from OGTT / MTT) were reported following only 3 weeks of a 600 kilocalorie per day VLCD (Lips et al., 2014). Postprandial blood glucose levels are reliant upon both insulin-stimulated glucose uptake into skeletal muscle *and* suppression of hepatic glucose production. Therefore, it is possible that these relatively early changes in IR measures derived

from oral nutrient stimulus may be accounted for by the early and profound improvement in hepatic glucose production, as well as modest improvements in peripheral insulin resistance that are beginning to occur.

In individuals with pre-diabetes, similar trends can be found. A large study of 2020 participants assessing the ability of VLCD to induce significant weight loss and prevent progression to manifest T2D used HOMA-IR as the primary outcome (P. Christensen et al., 2018). They reported a highly significant improvement in HOMA-IR in this population, finding no significant difference when analysing males and females separately. A smaller study based on similar principles performed OGTTs before and after 8 weeks of VLCD prior to randomising participants into two different weight maintenance approaches (Drummen et al., 2018). Again, they observed a significant reduction in HOMA-IR and improvement in Matsuda index, although notably the follow-up assessments were performed after 4 months of weight maintenance, not immediately upon VLCD cessation (P. Christensen et al., 2018).

1.4.3.2. Individuals without T2D

Although nondiabetic individuals characteristically maintain normal blood glucose levels, IR can exist in this population, especially with increasing levels of excess adiposity (Tatsumi et al., 2015). IR contributes to the onset of T2D (Kahn, 2003; Kahn et al., 2006), so reducing this in nondiabetic individuals is essential to preventing of this and other related comorbidities.

In an interesting study exploring ethnic differences between response to VLCD, individuals of South Asian and European heritage were subjected to 450 kcal VLCD for 8 days (Bakker et al., 2015). EH clamps were performed at baseline and follow-up,

and similar to the studies in individuals with T2D above, they observed a significant reduction in EGP and the resulting calculated hepatic IR (Bakker et al., 2015). They also reported the compelling finding of increased hepatic IR at baseline in the South Asian group, concluding that to some extent this may account for the widely acknowledged increased risk of T2D in this ethnic group. There was also no significant change in glucose disposal, in line with other studies utilising short-term VLCD interventions in individuals with T2D (Jazet et al., 2005; Lim et al., 2011).

A similar lack of effect on glucose disposal was reported in a series of studies examining the impact of VLCD on adipose tissue in nondiabetic women with obesity (Koppo et al., 2012; Siklova-Vitkova et al., 2009). Both studies administered an 800 kcal VLCD for one month and reported no significant change in glucose disposal. Of note, the study by Koppo et al. reported significant increase in glucose disposal following a further 2 months of low calorie diet (600 kcal less than calculated daily requirements), providing further evidence that changes in peripheral insulin sensitivity require prolonged periods of caloric restriction to occur (Koppo et al., 2012). This assertion is further supported by a study that utilised a 400 kcal per day VLCD until 10% weight loss was achieved (Bryson et al., 1996). This study required a longer intervention period to induce this degree of weight loss, and consequently an improvement in glucose disposal was demonstrated.

Responses of MTT-derived measures of IR also follow the trend observed in individuals with T2D. Rayner et al. performed MTT assessments after 1 and 8 weeks of 800 kcal VLCD (Rayner et al., 2018). HOMA-IR significantly reduced within the first week, but significant increase in Matsuda index occurred only after 8 weeks of the

intervention, again highlighting the discordance between the time-course of changes in hepatic and peripheral IR. Three further studies report improved Matsuda index following 8 weeks of VLCD in nondiabetic individuals, one of which included 281 participants and utilised OGTT assessments, rather than MTT (Iepsen et al., 2016; S. B. K. Jensen et al., 2023; Meyer et al., 2018).

Several studies assessing VLCD effects on IR indices based on fasting measures have been performed in individuals without T2D. Srámková et al. reported improvement in HOMA-IR and QUICKI within 2 days of commencing an 800 kcal VLCD (Srámková et al., 2016), in line with the studies already highlighted (Lara-Castro et al., 2008; Rayner et al., 2018). Two reports of VLCD administered in a hospital setting to a mixed cohort of individuals with and without T2D originating from the same group demonstrated reduced HOMA-IR after 3 weeks (Ožvald et al., 2021, 2022). Multiple other studies have reported improved HOMA-IR in nondiabetic individuals over more prolonged periods also (Hausmann et al., 2019; Isbell et al., 2010; Sayda et al., 2022; Scragg et al., 2020; Svendsen et al., 2012).

However, not all studies report significant reduction in HOMA-IR. A study conducted by Adam et al. administered a 600 kcal VLCD for 6 weeks and did not observe any significant change between baseline and follow-up, despite fasting glucose being significantly reduced (Adam et al., 2005). The authors attribute this to the modest mean weight loss of 6kg achieved by participants. This is less than that observed in other studies with a similar degree of caloric restriction (Lips et al., 2014; Sayda et al., 2022), and suggests a degree of participant non-compliance with the study

intervention. Moreover, the participants started with a relatively low baseline BMI and HOMA-IR, which may have affected the response to weight loss.

1.4.3.3. Summary

IR changes in response to VLCD depend on the method being used and the component of IR that is being measured. Studies assessing hepatic IR consistently show improvements, from as early as two days of VLCD initiation. Peripheral IR tends to require longer term caloric restriction before significant changes become evident, although this is not always the case. Post-prandial methods that reflect whole-body IR (incorporating both hepatic and peripheral components) show positive changes within a few weeks. This is likely primarily resulting from increased suppression of EGP – as observed in the study by Yoshino et al. (2020) – rather than early amelioration of peripheral IR. Changes in IR are similar between individuals with and without T2D, which is not unexpected considering the close association between excess weight and elevated IR that exists irrespective of prevailing glycaemic control (Ludvik et al., 1995).

Figure 1.4.3.1. Summary Box 2	
Hepatic IR	Improves rapidly, within days of starting VLCD
Peripheral IR	Improves more gradually Can improve rapidly but this is inconsistent
Whole-body IR	Improvements within weeks of starting VLCD Likely initially driven by changes in hepatic IR

1.4.4. Conclusions

The seemingly inextricable rise in prevalence of obesity and T2D poses a significant challenge to health systems around the world, making weight loss interventions that also improve glycaemic control highly desirable. Thus, interest in VLCD is warranted, especially given the well documented potential to induce diabetes remission. Impaired BCF and IR are hallmark features of T2D, and IR is also strongly associated with obesity in individuals without T2D. Therefore, an understanding of the effects of VLCD on these parameters is important particularly when the use of VLCD is becoming more widespread for managing weight in individuals with, and without T2D.

The reviewed evidence broadly implies that VLCD exerts a positive effect on BCF and IR, although the time-course of these changes differs between the various parameters. Hepatic IR appears to be very sensitive to extreme caloric restriction with improvements observed within days of VLCD initiation (Henry et al., 1985; Jazet et al., 2005; Laakso et al., 1988). Thus, resolution of hepatic IR is possible with short-term VLCD, stimulating discernible improvements in glycaemic control, particularly fasting glucose. However, intervention durations of approximately 3 months or greater seem necessary to induce consistent improvements in peripheral IR. Similarly, significant improvements in BCF tend to occur following approximately 8 weeks of VLCD, although factors related to the individual's baseline characteristics may also influence beta cells' capacity for recovery. These changes are summarised in Figure 1.4.4.1.

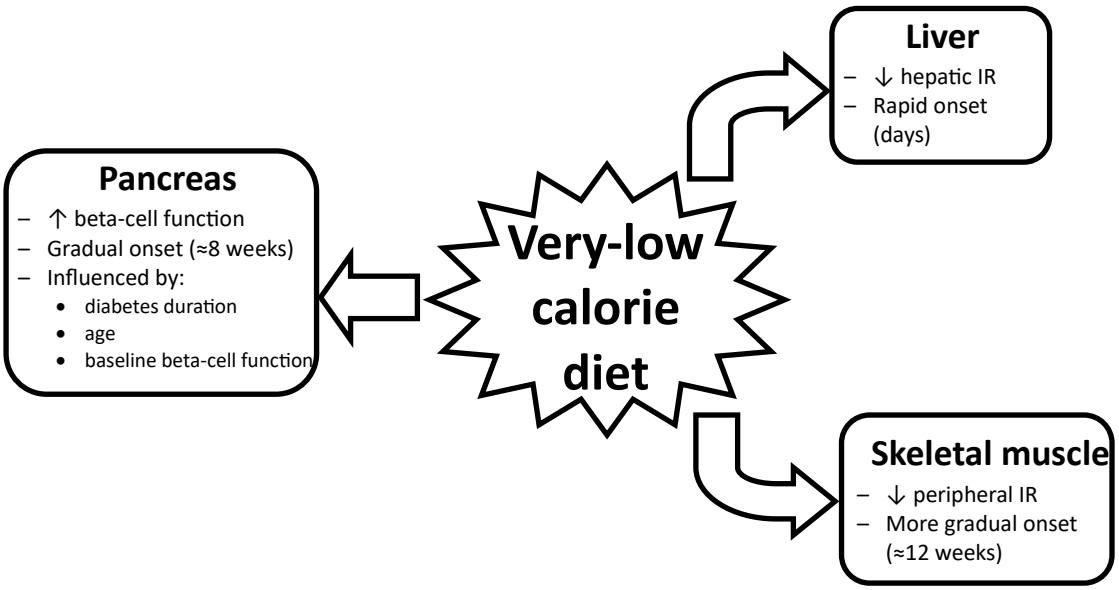

Figure 1.4.4.1. Diagrammatic representation of changes to beta-cell function & insulin resistance induced by very-low calorie diets Hepatic insulin resistance reduces rapidly upon caloric restriction, whilst changes at the peripheral level occur over the longer term, likely due to differences in the mechanisms underlying development of insulin resistance between hepatocytes and myocytes. Improvements in beta-cell function also tend to occur gradually, although the potential for beta-cell recovery is dependent on certain clinical characteristics.

This differential temporal response to caloric restriction between hepatic IR and peripheral IR has been highlighted by other authors (Klein et al., 2022; Taylor, 2008), and is also observed following bariatric surgery (Dirksen et al., 2012). Mechanisms underlying this phenomenon are unclear but likely reflect differences in glucose metabolism and the underlying pathogenesis of IR in these tissues. Hepatic IR and EGP are closely associated with liver fat content (Gastaldelli et al., 2007; London et al., 2021) and fat accumulation in the liver is very responsive to changes in dietary intake. One study reported that just 3 weeks of carbohydrate overfeeding resulted in a 27% increase in liver fat, with a concurrent 28% rise in plasma alanine aminotransferase (Sevastianova et al., 2012). Accordingly, liver fat has been shown to reduce by approximately 30% within 1 week of VLCD, resulting in normalisation of hepatic EGP, and consequently IR (Lim et al., 2011). These studies highlight the liver's

sensitivity to dietary changes, particularly in the context of extreme caloric restriction during a VLCD.

It must be acknowledged that the 30% reduction in liver fat observed in the aforementioned study does not fully account for the immediate amelioration of hepatic EGP. Whilst liver fat content continued to decrease for the remaining 7 weeks of the intervention, EGP remained stable (Lim et al., 2011), suggesting the presence of another contributory factor. It is widely recognised that free fatty acids (FFA) stimulate hepatic gluconeogenesis (Lam et al., 2003), and inhibition of FFA production leads to reduced gluconeogenesis (Puhakainen & Yki-Jarvinen, 1993). VLCD typically contains a much lower fat content that western diets, and therefore causes a dramatic reduction in digested FFAs absorbed into hepatic circulation immediately following initiation of the diet. It is possible this directly leads to a consequential reduction in gluconeogenesis, an assertion that is strongly supported by a study in which this was observed following a 3-day VLCD in rats (R. J. Perry et al., 2018). Taken together, the improvement in hepatic IR appears to be associated with reductions in liver fat and hepatic gluconeogenesis, both of which occur rapidly following onset of VLCD.

Peripheral IR primarily reflects the inability of skeletal muscle to adequately increase its uptake of glucose in the post-prandial state (Defronzo & Tripathy, 2009). Although the underlying pathogenesis of peripheral IR is not completely understood, excess IMCL, reduced insulin receptor activity and impaired intracellular insulin signalling pathways have been implicated (Defronzo & Tripathy, 2009; Klein et al., 2022). Similar to liver fat, IMCL content also reduces significantly within days of commencing VLCD

(Lara-Castro et al., 2008), however this study did not observe an associated improvement in peripheral IR. Furthermore, VLCD-induced alterations in insulin receptor number and activity were not found to correlate with changes in peripheral IR either (Rotella et al., 1994).

Several studies have reported reduced peripheral IR and associated enhanced insulin signalling following VLCD (Jazet et al., 2008; Y.-B. Kim et al., 2003; Snel, Gastaldelli, et al., 2012), although these VLCD interventions were of a longer duration, lasting between 16 and 24 weeks. Studies assessing acute effects of VLCD on insulin signalling are scarce, however a rodent study assessing the effects of a 3-week low-fat diet on insulin signalling found no significant change, despite significant weight reduction (Young Jung et al., 2013). These findings suggest that changes in insulin signalling may be a key factor driving VLCD-stimulated amelioration of peripheral IR in myocytes. Additionally, this process may require a longer period to occur than the rapid metabolic changes occurring in hepatocytes. Whilst this may provide some insight into the protracted nature of changes in peripheral IR compared to hepatic IR, detailed metabolic studies examining this phenomenon are required to characterise the mechanisms underlying this further.

As outlined above, VLCD-induced improvement in BCF also tends to require more prolonged caloric restriction. Pancreatic fat is detrimental to BCF, and similar to hepatic fat, reduces in response to VLCD (Lim et al., 2011; Taylor et al., 2018), albeit at a slower rate (Lim et al., 2011). Hyperglycaemia in conjunction further disrupts BCF, and it has been suggested that rapid improvements in hepatic IR and thus fasting hyperglycaemia, permits the development of an environment in which beta cells can

recover their function (Taylor, 2013). This recovery is likely to be a gradual process and, along with the slower rate of pancreatic fat reduction, may explain the more gradual nature of BCF improvement. Some evidence suggests this process can be accelerated by more intense caloric restriction (Jackness et al., 2013; Liu et al., 2015), possibly due to the greater magnitude of hepatic IR resolution induced by this. Certain characteristics such as an individual's age, duration of diabetes diagnosis and baseline glycaemic control also affect the ability of BCF to improve (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). This likely reflects the extent of underlying beta cell damage and de-differentiation, which determines their restorative capacity (Taylor, 2013).

It is important to note that the potential mechanisms outlined above describing differences in response of these parameters to VLCD are purely speculative. Further research into these observed trends is required, as this may facilitate identification of adjuvant interventions that accelerate the improvement in certain parameters. For example, use of concurrent exercise programmes may enhance improvement in peripheral IR, resulting in greater reductions in glycaemic control induced by VLCD. Furthermore, evidence describing the impact of VLCD on BCF in individuals without T2D is very limited, thus more studies are also required in this area. Although impaired BCF is not typical in this group, VLCD can reduce progression to T2D (P. Christensen et al., 2018), thus modest improvement in baseline BCF within the "normal" range, could be a contributing factor.

VLCD exhibits several metabolic effects in individuals with, and without T2D. As VLCD becomes more embedded within guidelines for management of T2D and obesity, a

clear understanding of these metabolic effects is necessary. Although further research into this area is required, the conclusions from this narrative review will provide context to subsequent chapters of this thesis. Furthermore, it should enable health care professionals involved in prescription of VLCD interventions to make more appropriate decisions regarding their use, and provide more detailed advice on their anticipated effects to patients.

1.5. Glucagon-like peptide-1 receptor agonists

1.5.1. Physiological actions and effects of the GLP-1 hormone

GLP1RAs are compounds that agonise the receptor of the GLP-1 hormone. GLP-1 is an incretin hormone (Kreymann et al., 1987), termed due to its ability to stimulate additional insulin secretion in response to oral glucose ingestion, compared to what would be observed with intravenous glucose infusion (Creutzfeldt, 1979). Along with glucose-dependent insulinotropic polypeptide (GIP), the two hormones contribute to this effect in equal measure (Vilsbøll et al., 2003).

GLP-1 is secreted by enteroendocrine L cells, primarily in the ileum and colon (Drucker, 2005), and its secretion is stimulated by the presence of all macronutrients, not just glucose (Steinert et al., 2017). It is formed from post-translational processing of proglucagon, yielding GLP-1, glucagon-like peptide-2, oxyntomodulin and glicentin (Baggio and Drucker, 2007). GLP-1 is secreted in two forms GLP-$1_{7\text{-}37}$ and GLP-$1_{7\text{-}36amide}$ which are equally as potent (Drucker, 2005), with the latter being the predominant form (Steinert et al., 2017).

The primary glucometabolic effect of GLP-1 is stimulation of insulin secretion from pancreatic beta cells, and inhibition of glucagon secretion from alpha cells (Nauck and Meier, 2018). Both of these actions ultimately result in reduction of blood glucose levels following food ingestion. Furthermore, these actions are glucose-dependent, and cease to occur once blood glucose levels normalise (Drucker, 2005). In addition to this, GLP-1 hormone exhibits powerful effects on stimulation of satiety and slowing of gastric emptying (Flint et al., 1998; Shah and Vella, 2014). This occurs via GLP-1 receptors expressed in the brain, particularly the appetite regulating areas of the

hypothalamus (Van Bloemendaal et al., 2014), and within the stomach (Shah and Vella, 2014). The combination of these effects inhibits food ingestion, and assists in the mechanisms regulating energy intake. The insulin secretory, glucose-lowering effects, combined with the reduction in energy intake makes agonism of the GLP-1 receptor particularly attractive for management of T2D, especially with the large proportion of patients also living with overweight or obesity.

However, the physiological effects of GLP-1 are not limited to glucose homeostasis and appetite regulation. GLP-1 receptors are ubiquitously expressed in multiple tissues of the body. In particular, cardiac, vascular, neuronal, hepatic and skeletal muscle tissues are also heavily populated with these receptors, leading to a broad range of effects in the individual tissues (Baggio and Drucker, 2007). Reports of GLP-1 increasing heart rate are common in literature (Robinson et al., 2013; Lorenz et al., 2017), although this has not been associated with an increase in cardiac arrhythmias (Wu et al., 2022). GLP1RAs have been associated with improved endothelial function in individuals with T2D (Menghini et al., 2023). Moreover, GLP-1 infusion has demonstrated cardioprotective effects in experimental models of acute ischaemic injury or heart failure (Baggio and Drucker, 2007), and in a small pilot clinical study (Nikolaidis et al., 2004). Its role in hepatic tissues is not fully described, however there have been suggestions of stimulation of improved glucose clearance and suppression of hepatic glucose production (Baggio and Drucker, 2007). These actions would work in partnership with the pancreatic effects to reduce blood glucose levels in the postprandial state.

Of particular interest is the expression of GLP-1 receptors in skeletal muscle tissue, and the associated roles within this. Again, this remains poorly understood, but increases in glucose incorporation into glycogen, glycogen synthase activity and insulin-stimulated glucose uptake have been observed in the presence of GLP-1 (Abdulla et al., 2014). Similar effects have also been demonstrated in experimental adipose tissue models suggesting that the glucoregulatory role of GLP-1 is not limited to the pancreas, liver and stomach (Abdulla et al., 2014).

Furthermore, it has been suggested that GLP-1 may have a role in modulating muscle protein synthesis (MPS). One study performed continuous intravenous infusions of GLP-1 hormone in healthy older men, demonstrating increased MPS and microvascular perfusion in the postprandial state, when compared to controls (Abdulla et al., 2020). In addition, another study cultured myotubules from a rodent model of muscular atrophy and were able to demonstrate amelioration in muscle wastage, through suppression of atrophic and enhancement of myogenic factors (Y. Hong et al., 2019). Both studies demonstrate a potential role in regulating muscle growth in both healthy and diseased states, which again would be very attractive to individuals with T2D, given the accelerated age-related muscle loss observed in this population (S. W. Park et al., 2009).

Thus, GLP-1 hormone has a wide array of effects across multiple tissues in the body (Figure 1.5.1.1), several of which would be beneficial for individuals with T2D. It is therefore little surprise that the last 3-4 decades has experienced significant investment into manufacturing medications that agonise this hormone, thus exploiting its effects for the treatment of T2D. One initial challenge to this aim was

the short half-life of GLP-1 hormone, which is rapidly degraded by endo-peptidase dipeptidyl peptidase-4 (DPP4) within 1-2 minutes (Alexiadou, Anyiam and Tan, 2019). Thus, production of a medication with similar structure to GLP-1 would suffer a similar fate, requiring multiple doses a day to maintain plasma steady state. Consequently, molecular alterations were required to protect the compound from these effects, whilst still maintaining its ability to agonise the GLP-1 receptor (Knudsen and Lau, 2019). Additionally, the effects of GLP-1 hormone are attenuated in individuals with T2D, a finding that has been implicated in the development of the condition (Nauck et al., 1986).

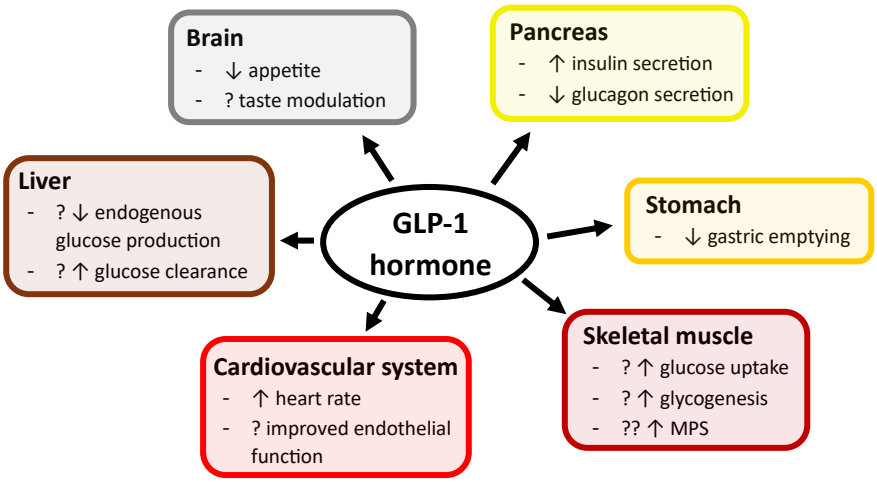

Figure 1.5.1.1. Summary of the widespread actions of GLP-1 hormone in the human body

1.5.2. History and efficacy of GLP1RAs

The first GLP1RA to be developed was Exenatide, which is structurally identical to Exendin-4, a naturally occurring GLP1RA isolated from the saliva of the Gila Monster lizard *Heloderma suspectum* (Troke, Tan and Bloom, 2014). This almost accidental discovery was timely as despite it only sharing 53% of the structure of the native human GLP-1 hormone, it was a potent agonist of the GLP-1 receptor and resistant to degradation by DPP4 (Troke, Tan and Bloom, 2014). This prolonged its half-life to the

extent where twice daily injection was possible (Robles and Singh-Franco, 2009), and the medication received approval for the treatment of T2D in 2005.

Initial studies assessing the efficacy of Exenatide were positive, reporting significant HbA1c reductions of approximately 0.9 and 0.8 percentage-points in individuals inadequately controlled on a sulphonylurea (Buse et al., 2004), and both metformin and sulphonylurea (Kendall et al., 2005), respectively. Furthermore, in both studies, participants experienced modest weight reductions, confirming the suspicion that the appetite-reducing effects of GLP-1 could translate to significant weight loss.

Subsequent studies comparing the effect of exenatide to insulin demonstrated similar improvements in glycaemic control between the two interventions (Barnett et al., 2007; Nauck et al., 2007). Moreover, exenatide again induced weight loss, whilst the insulin-treated groups in comparison experienced weight gain (Barnett et al., 2007; Nauck et al., 2007). A meta-analysis performed 2 years later provided further evidence of the ability of Exenatide to stimulate significant improvements in glycaemic control, similar to that achieved with insulin, with the additive benefit of weight loss (Norris et al., 2009).

Following this, research continued into GLP1RAs that were less prone to deactivation by DPP4, which eventually led to the development of the once-daily GLP1RA Liraglutide. Through binding of the active site to albumin, Liraglutide is able to evade degradation by DPP4, as well as filtration by the kidney, resulting in a markedly increased half-life (Knudsen and Lau, 2019). Liraglutide became approved for use in T2D in 2009 following very positive results from the LEAD series of phase 3 clinical trials. These studies demonstrated that Liraglutide could stimulate significant

improvements in glycaemic control and weight, in a variety of populations with T2D (Garber et al., 2009; Marre et al., 2009; Nauck et al., 2009; Zinman et al., 2009). Furthermore, it was found to be superior for glycaemic control versus insulin glargine (Russell-Jones et al., 2009), and precipitated significantly greater weight loss and improvements in HbA1c than Exenatide (Buse et al., 2009). These findings were of great interest to clinicians managing T2D and Liraglutide rapidly became the most popular GLP1RA.

Following the success of the LEAD series of studies, the LEADER study was the first large-scale study of GLP1RAs assessing cardiovascular outcomes (Steven P Marso et al., 2016). In this study of almost 10,000 participants with T2D, Liraglutide reduced the risk of the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke by 13% (Steven P Marso et al., 2016). Furthermore, death from all causes reduced by 15% (Steven P Marso et al., 2016), making Liraglutide one of few medications to show cardiovascular and mortality benefit in individuals with T2D.

Furthermore, the powerful appetite suppressant effects of Liraglutide did not go unnoticed and the SCALE study series was subsequently developed to explore the potential benefits of Liraglutide doses up to 3.0mg to help individuals without T2D lose weight. Again, these were largely positive, reporting 8% and 6% weight loss in individuals living with obesity, and individuals with T2D, respectively (M. J. Davies et al., 2015; Pi-Sunyer et al., 2015). This eventually led to Liraglutide becoming the first GLP1RA to be approved for weight management in individuals living with obesity.

Other once daily formulations of GLP1RA were investigated with varying success. One such was Lixisenatide which was also approved for clinical use and available around the same time as Liraglutide. However, this was found to be less efficacious in subsequent head-to-head studies (Nauck et al., 2016). Nonetheless, ongoing research into albumin binding and other molecular alterations led to the development of several GLP1RAs with dramatically extended half-lives (Knudsen and Lau, 2019). These compounds were able to be administered once-weekly, which was a much more attractive concept for patients.

Of the four that were eventually approved, Semaglutide is by far the most successful. The SUSTAIN series of phase 3 studies outlined the superior efficacy of Semaglutide (Ahrén et al., 2017; Aroda et al., 2017; Sorli et al., 2017; Ahmann et al., 2018), including over Liraglutide (Capehorn et al., 2020). This coupled with the less frequent administration made it a very attractive choice for patients. Furthermore, similar to Liraglutide it was also shown to confer cardiovascular benefit with a 26% reduction in the risk of cardiovascular death, nonfatal myocardial infarction or nonfatal stroke (Steven P. Marso et al., 2016).

As with Liraglutide, there were also attempts to exploit the satiating effects of Semaglutide, and a higher-dose formulation was eventually found to be effective for inducing weight loss in individuals living with obesity. Consistent observations of double-digit percentage weight loss (W. T. Garvey, Batterham, et al., 2022; D. Rubino et al., 2021; D. M. Rubino et al., 2022; Wadden et al., 2021; Wilding et al., 2021) led to this formulation of Semaglutide being approved for use in this population. Unlike Semaglutide, the other approved once-weekly GLP1RA formulations – Exenatide ER,

Dulaglutide and Albiglutide – were only approved for management of T2D, and due to their lesser efficacy (Madsbad, 2016; Sfairopoulos et al., 2018) did not enjoy similar popularity. Dulaglutide is currently used in specific situations, whilst Albiglutide has been removed from the market altogether (Lyseng-Williamson, 2019).

1.5.3. GLP1RA effects on BCF and IR

As previously outlined, T2D is characterised by the presence of both IR and impaired BCF, albeit to varying degrees in individuals with the condition. Thus, studies examining the effects of GLP1RAs on these parameters have been performed, albeit to a lesser degree than VLCD interventions. One early study investigating exendin-4 assessed the insulin response when the compound was infused during a hyperglycaemic clamp (Egan, Clocquet and Elahi, 2002). Exendin-4 stimulated a 4- to 5-fold increase in insulin secretion, providing initial evidence of a BCF-promoting effect (Egan, Clocquet and Elahi, 2002).

The ability of Exenatide treatment to improve BCF was subsequently confirmed by studies that performed clamps, with comparisons made between this, Rosiglitazone (Defronzo et al., 2010), and insulin (Bunck et al., 2009). Both showed improved BCF, and Defronzo et al. were also able to demonstrate improvement in clamp-derived IR (Defronzo et al., 2010). Notably, Bunck et al. proceeded to report that following 3 years of treatment with Exenatide, and a subsequent 4 weeks off the medication, BCF was still significantly improved compared to insulin glargine, postulating a beneficial effect on beta-cell health (Bunck et al., 2011).

A short-term study of Liraglutide for 1 week was able to demonstrate improved first-phase insulin response and reduced endogenous glucose production, primarily as a

result of reduced glycogenolysis (Degn et al., 2004). The latter finding was in keeping with the known effects of GLP-1 hormone on hepatocytes (Baggio and Drucker, 2007), and this study provided evidence that these effects occur within a week of commencing the medication. This improvement in BCF was also demonstrated over the long-term with Liraglutide treatment over twelve and fourteen weeks (Vilsbøll et al., 2008; Anholm et al., 2017). Interestingly, despite significant weight loss, insulin sensitivity did not improve in either study. This was at odds with Jinnouchi et al. who were able to demonstrate significant improvements in insulin sensitivity after only 4 weeks (Jinnouchi et al., 2015). Furthermore, in contrast to the observations with Exenatide, improvements in BCF observed with Liraglutide were not present after stopping the medication for 3 months (RISE Consortium, 2019).

Few studies have assessed the impact of Semaglutide on BCF and IR. Kapitza et al. reported improved first- and second-phase response to intravenous glucose following chronic Semaglutide treatment (Kapitza et al., 2017a). Although the authors did not provide an assessment of IR, they did not observe any significant change in fasting insulin, despite significant weight loss (Kapitza et al., 2017a). These BCF changes were subsequently replicated in a study using a 2-stage hyperglycaemic clamp following 20 weeks of Semaglutide (Ingersen et al., 2023). Interestingly, adjuvant exercise resulted in further increases in BCF, suggesting a synergistic effect between the two interventions (Ingersen et al., 2023). Again, no specific comment was made regarding IR.

Figure 1.5.3.1. Summary of GLP1RA effects on BCF and IR	
BCF	Significant improvement in first-phase response
Hepatic IR	Likely early improvement – possibly related to direct effects on hepatocytes
Peripheral IR	Possible improvements but results inconsistent

1.5.4. Summary

In summary, GLP1RAs are a class of medication that has been successfully used for the treatment of T2D for almost 20 years. They induce improvements in glycaemic control, with accompanying reduction in weight, making them a very attractive choice for T2D management. Their remarkable weight-reducing effects has led to two GLP1RA medications becoming approved for management of obesity. Although evidence is limited, GLP1RAs have persistently been demonstrated to result in improved BCF, with augmentation of both first and second-phase insulin response reported. The effects of GLP1RA on IR however are inconsistent and require further investigation.

Due to the widespread distribution of GLP-1 hormone receptors, it is likely the effects of GLP1RA may extend beyond improving glycaemic control and weight, although this remains largely unexplored. One particular area of interest is the effect of GLP1RA on skeletal muscle. One particular study suggests that the GLP-1 hormone may stimulate MPS. Thus, a potential muscle-preserving effect of GLP1RA has been postulated by some authors (Cetrone et al., 2014; X. Zhang et al., 2021) and presents an intriguing concept that requires further exploration. The next section will describe the structure and function of skeletal muscle, before discussing the impact of different scenarios on normal muscle homeostasis.

1.6. Overview of skeletal muscle and function

1.6.1. Introduction to skeletal muscle

Skeletal muscle is the largest organ in the human body (Pedersen, 2013). Approximately 40% of total body mass is skeletal muscle (Rasmussen and Phillips, 2003), although this can vary significantly between individuals. Between 50-75% of all body proteins are stored in skeletal muscle (Rasmussen and Phillips, 2003), although protein only comprises 20% of skeletal muscle weight. The remaining 80% consists of water (75%) and other substances including fat, carbohydrates, inorganic salts and minerals (Frontera and Ochala, 2015).

The most widely recognised function of skeletal muscle is to produce locomotion, enabling movement, maintenance of posture, communication and various other associated functions that are critical to human survival. However, skeletal muscle also has several less well-known functions that contribute to normal human body homeostasis. It is the body's store of amino acids, which are essential for the normal function of other organs such as the brain and heart, as well as for gluconeogenesis in the liver during periods of fasting (Spargo, Pratt and Daniel, 1979). Along with adipose and hepatic tissues, it forms part of the storage system for other nutrients (especially glucose and lipids) that are digested and absorbed into systemic circulation following food ingestion (J. Jensen et al., 2011; Morales et al., 2017). It is also responsible for the production of heat to maintain core temperature in cold conditions (Periasamy, Herrera and Reis, 2017; Townsend et al., 2023). Finally, it secretes its own metabolic substances – known as myokines – which regulate the metabolic activities of other tissues in the body (Pedersen, 2013). Thus, skeletal

muscle is a key part of many metabolic functions in the body, and slight disturbances in skeletal muscle function can result in wide ranging effects, with detrimental consequences to overall health.

Skeletal muscle is one of two types of striated muscle, the other being cardiac muscle. Cardiac muscle contracts autonomously from early in life, so is therefore self-stimulating, non-fatiguing and requires a constant steady energy supply (Mukund and Subramaniam, 2020). In contrast, skeletal muscle is innervated and contracts on voluntary stimulation. It has a high energy requirement, and exhibits fatigue, particularly in larger muscle groups (Mukund and Subramaniam, 2020). Therefore, despite sharing a similar appearance with cardiac muscle, skeletal muscle has its own unique physiology, with marked differences in how individual functions are implemented.

The basic component of skeletal muscle is the muscle cell, also known as a myofiber. Myofibers are bundled together into fascicles, and each skeletal muscle contains multiple fascicles (Dave, Shook and Varacallo, 2024). Individual myofibers can span the entire length of a muscle, with multiple nuclei and mitochondria interspersed throughout (Dave, Shook and Varacallo, 2024). Each myofiber is surrounded by a layer of cell membrane, known as the sarcolemma, which interacts with the surrounding extracellular structures (Frontera and Ochala, 2015). Located outside myofibers, within the surrounding network of connective tissue, are satellite cells. These are stem cells that retain the ability to proliferate and differentiate into new myofibers and contribute to growth and repair of existing myofibers (Frontera and Ochala, 2015). Skeletal muscle is heavily vascularised with dense networks of blood

vessels and capillaries surrounding the myofibers to enable adequate transport of nutrients and oxygen to them, as well as removing waste products of metabolism (Dave, Shook and Varacallo, 2024).

Internally, myofibers contain thousands of myofibrils which span the entire length of the muscle cell, and are tightly arranged into bundles to provide structural integrity (N. M. Luis & Schnorrer, 2021). Myofibrils in turn contain thousands of myofilaments, of which there are two types – “thick” and “thin” filaments (Frontera and Ochala, 2015). Muscle contraction results from active sliding of thin filament over thick filaments, and the consequent shortening of myocellular myofibrils. Thick filaments contain myosin, while thin filaments are primarily composed of actin (Greising et al., 2012). Other contractile proteins such as troponin, tropomyosin and titin are also integral to the myofibrillar function (Lin, Song and Sadayappan, 2017). Myofilaments are structurally organised into subunits called sarcomeres, which are repeated along the length of a myofibril, and form the basic unit of force generation (N. M. Luis & Schnorrer, 2021).

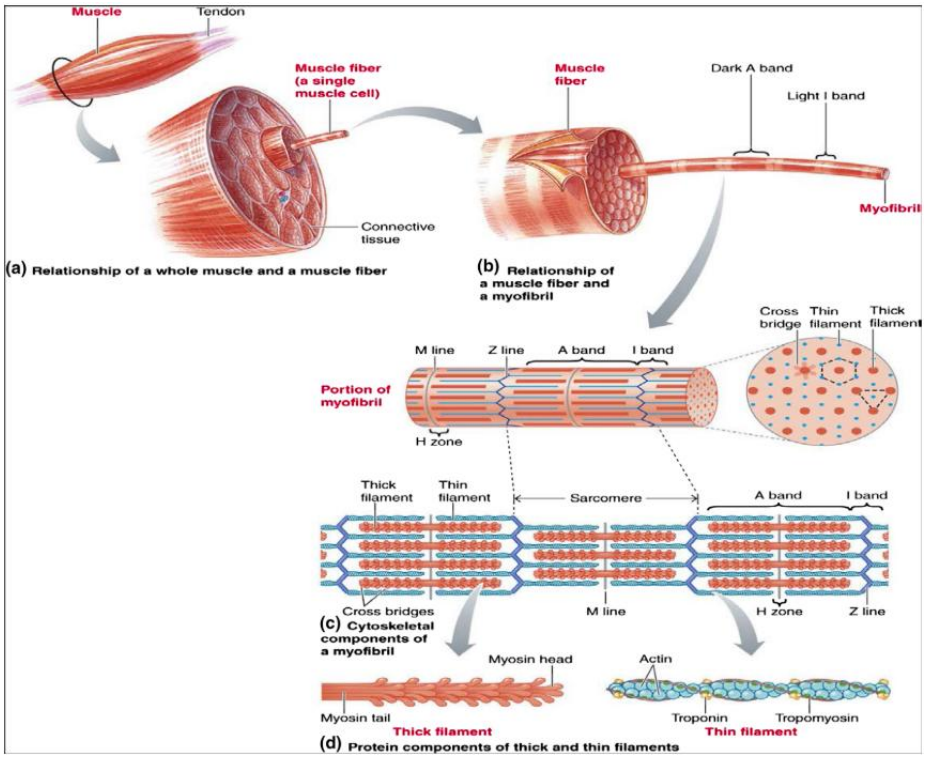

Figure 1.6.1.1. Diagram providing an overview of skeletal muscle structure. Taken from Frontera & Ochala, 2015, original in Sherwood 2010 (Sherwood, 2010)

1.6.2. Brief overview of glucose metabolism in skeletal muscle

As outlined above, one of the many key functions of skeletal muscle in humans is regulation of glucose control. Skeletal muscle absorbs over 60% of glucose released into systemic circulation following food ingestion, which accounts for approximately 85% of insulin-mediated glucose disposal in this context (Abdulla et al., 2014). Under hyperinsulinaemic clamp conditions, skeletal muscle was responsible for approximately 90% of whole-body glucose disposal (Defronzo et al., 1985). Thus, skeletal muscle plays a crucial role in the uptake and storage of postprandial glucose, and insulin is the major mediator of this process (Sylow et al., 2021).

In order to perform this function, insulin induces multiple metabolic processes, which occur simultaneously in a synergistic fashion (Sylow et al., 2021). Initially, the rise in systemic insulin concentrations following food ingestion acts to promote vasodilation

of the skeletal muscle vasculature, thereby increasing blood flow and crucially, nutrient delivery (Hulett, Scalzo and Reusch, 2022). This process is mediated by nitric oxide which is released by interaction of insulin with its receptor on endothelial cell, causing smooth muscle relaxation (Zeng and Quon, 1996; Zeng et al., 2000).

The following step in skeletal muscle postprandial glucose metabolism is stimulation of glucose uptake. Glucose uptake into skeletal muscle occurs through glucose transporters (GLUT) located in the sarcolemma. Under fasted conditions, glucose uptake is restricted, due to the relative absence of GLUTs in myofiber plasma membranes (Merz and Thurmond, 2020). The appearance of insulin in skeletal muscle vasculature activates the insulin receptor leading to tyrosine phosphorylation of the insulin receptor substrate triggering a series of downstream events mediated by phosphatidylinositol 3-kinase (PI3K) activation (Carnagarin, Dharmarajan and Dass, 2015). These complex intracellular processes ultimately culminate in the translocation of intracellular GLUT4 receptors onto the sarcolemma, significantly increasing the flow of glucose into myofibers (Merz and Thurmond, 2020). Thus, insulin-induced expression of the GLUT4 transporters in the sarcolemma is the rate-limiting step governing the rate of glucose uptake into skeletal muscle (Jaldin-Fincati et al., 2017).

Further to the two processes outlined above, insulin also promotes glycogen synthesis via activation of the enzyme glycogen synthase by PI3K (Sylow et al., 2021), enabling intracellular storage of glucose for use during fasting. In addition to this, insulin also stimulates uptake of fatty acids for storage of lipids, as well as amino acids via processes which will be described subsequently in this chapter. Therefore, food

intake stimulates increase blood flow to skeletal muscle, increased myofiber uptake of glucose via GLUT4 translocation into sarcolemma, and storage of glucose through glycogenesis, as well as other nutrients. These processes are all controlled by insulin, highlighting its vital role in the postprandial metabolic response of skeletal muscle, and the potentially significant impact impaired insulin action could have on overall glucose control.

1.6.3. Skeletal muscle contraction

Aside from regulating glucose in the postprandial state, arguably the most important function of skeletal muscle in humans is induction of locomotion. Movement of the human body is enabled by contraction of muscles against the skeletal structures they are connected to. Contraction occurs through stimulation of myofibers by motor neurones, which occurs via the neuromuscular junction (NMJ). Whilst motor neurones can innervation hundreds of myofibers, each myofiber typically contains only one NMJ (Rodríguez Cruz et al., 2020), based in an area of the skeletal muscle known as the motor point.

The process of myofiber contraction in response to a nervous stimulus is comprehensively summarised by Mukund & Subramaniam (Mukund and Subramaniam, 2020). Briefly, upon excitation of the motor neurone, voltage-gated calcium channels open allowing inflow of calcium into the distal (pre-synaptic) membrane of the motor neurone. This triggers fusion of vesicles containing acetylcholine (Ach) with the pre-synaptic membrane, releasing this into the synaptic cleft. Ach then binds to receptors located on the myofiber sarcolemma located within the NMJ, stimulating a cascade of events, ultimately culminating in the rapid influx of

positively charged ions (mainly sodium) located within the synaptic space, into the junctional sarcoplasm. This causes depolarisation and generation of an action potential within the myofiber. This depolarisation is rapidly distributed throughout the myofiber through a network of T-tubules which also penetrate into its centre, enabling uniform contraction throughout the myofiber. As one motor neurone innervates multiple myofibers, the result is a coordinated contraction, which is essential for the production of functional movements of limbs and digits.

The generation of a myofiber contraction following an action potential is known as “excitation contraction coupling” (Mukund and Subramaniam, 2020). The depolarisation of the myofiber stimulates the opening of voltage-gated calcium channels within the membrane of the T-tubules. Each T-tubule forms a triad structure with two terminal ends of the sarcoplasmic reticulum (terminal cisternae) in which large amounts of calcium are stored. The opening of the voltage-gated channels permits an influx of calcium into the sarcoplasm of the myofiber, within which the myofibrils are located. Exposure of the myofibrils to high calcium concentrations results in exposure of the myosin binding sites located on the actin filaments, due to binding of calcium to troponin-C. These sites are blocked by tropomyosin in the rested state, however the presence of calcium-activated troponin-C stimulates a conformational change in tropomyosin, removing it from the myosin. This enables binding of myosin to actin filaments, which effectively “pulls” actin filaments closer together. In the presence of energy provided by adenosine triphosphate (ATP), this process recurs in a cyclical fashion, resulting in myosin “crawling” along the length of the actin filament and the generation of a muscular contraction (Mukund and Subramaniam, 2020).

1.6.4. Skeletal muscle growth and breakdown

Growth of muscle occurs primarily through growth of individual myofibers. Myofibrillar protein synthesis leads to the creation of new contractile proteins – actin, myosin, troponin, tropomyosin and titin (Balagopal, 1998) – which are then arranged into a sarcomere (Jorgenson, Phillips and Hornberger, 2020). A newly synthesised sarcomere can either be added to existing myofibrils extending their length, or integrated into newly developing myofibrils (Jorgenson, Phillips and Hornberger, 2020). Satellite cells also contribute to this process by fusing to the myofiber leading to the addition of new myonuclei or less commonly, differentiation into new myofibers (Frontera and Ochala, 2015).

Muscle breakdown is thought to occur via three distinct mechanisms working simultaneously to varying degrees depending on the situation (Combaret, Taillandier and Attaix, 2001; Tipton, Hamilton and Gallagher, 2018). The ubiquitin-proteasomal pathway (UPP) is the most widely studied pathway and appears to be the predominant mechanism in humans (Kitajima, Yoshioka and Suzuki, 2020). This involves the addition of ubiquitin to proteins that are targeted for degradation. Ubiquitin initially requires activation by the E1 enzyme, which is an ATP-dependent process (Attaix et al., 2005). This activated ubiquitin is then transferred onto a lysine group on the target protein by E3 ubiquitin ligase (Tipton, Hamilton and Gallagher, 2018). Once four ubiquitin molecules are present on a protein, it is recognised by a proteosome which results in its degradation (Kitajima, Yoshioka and Suzuki, 2020). Calpain Ca^{2+}-dependent cysteine proteases and autophagy are the other proteolytic processes, with the former primarily utilised to break sarcomeres into their

component parts, and the latter involved in degradation of membrane-bound proteins (Tipton, Hamilton and Gallagher, 2018). Ubiquitin activity and expression is increased in the presence of low insulin and increased glucocorticoid concentrations, and is inhibited by hyperinsulinaemia (Combaret, Taillandier and Attaix, 2001).

Both MPS and MPB are essential for maintenance of healthy skeletal muscle. MPS is critical for the hypertrophic response associated with exercise. However, MPB is also necessary in this process, allowing myofibers to adapt through destruction of damaged or unnecessary functional proteins, which enables the development of newer, more appropriate ones. Thus, maintenance of a steady skeletal muscle mass requires a balance of both processes (Wilkinson, Piasecki and Atherton, 2018), via the mechanisms described above. Furthermore, both actions play a key role in regulation of whole-body homeostasis, and are critical mediators of the muscle response to various disease states.

1.6.5. MPS and MPB in response to nutrition and exercise

During normal fasted conditions, no glucose is being absorbed from the gut, and therefore alternative substrates must be utilised to ensure tissues can continue to function adequately. Skeletal muscle and adipose tissue are the largest sites of amino acid and fatty acid storage (Attaix et al., 2005; Frayn, Arner and Yki-Järvinen, 2006), respectively, and therefore are the main sources of these substrates during fasting. Accordingly, MPB increases in the fasted state, in order to release amino acids into systemic circulation for hepatic gluconeogenesis to maintain normoglycaemia (Kettelhut, Wing and Goldberg, 1988), or direct metabolism by various other tissues (S. S. Wing & Goldberg, 1993). This process is mediated by the relatively high

glucocorticoid and low insulin levels present in the fasted state (Kettelhut, Wing and Goldberg, 1988), stimulating the three processes of protein degradation listed above. In addition, to maintain the availability of amino acids for release into circulation, MPS is suppressed during fasting (Rennie et al., 1982).

Upon food ingestion, plasma nutrient concentrations increase, rapidly followed by a consequent rise in insulin. This provokes suppression of MPB to approximately 50% of its activity in the fasted state (Wilkes et al., 2009). Alongside this, there is a corresponding increase in MPS which rises 3-fold from fasting levels and peaks between 45-90 minutes after feeding (Atherton et al., 2010). Thereafter, MPS reduces to fasting levels, even in the continued presence of nutrients (Atherton et al., 2010). This phenomenon is termed “muscle full” which describes a state in which the muscle is unable to continue MPS, despite the continued presence of stimulatory nutrients (Atherton et al., 2010).

The MPS process appears to be primarily stimulated by the presence of amino acids, particularly essential amino acids (EAA) (Atherton and Smith, 2012). The branched chain amino acid leucine has been noted to be particularly potent in this role (Atherton and Smith, 2012). Insulin has historically been identified as a key regulator of MPS stimulation (Gelfand and Barrett, 1987; Bennet et al., 1990). However, more recent evidence suggests that maximal MPS can occur at fasting levels of insulin, with no additive effect on MPS with higher insulin concentrations (Greenhaff et al., 2008). Regardless of the peripheral stimulus, the final common process leading to MPS is activation of the mammalian target of rapamycin complex 1 (mTORC1) which acts on

translational and transcription factors that drive the process of MPS (Schiaffino et al., 2021).

Thus, in a steady state without any additional stimuli, MPS and MPB exist in equilibrium (Figure 1.6.5.1) with post-prandial MPS being employed to replenish muscular protein losses experienced due to MPB during fasting (Wackerhage and Rennie, 2006). However, various situations exist where this equilibrium is disrupted, leading either to net gain, or net losses of skeletal muscle.

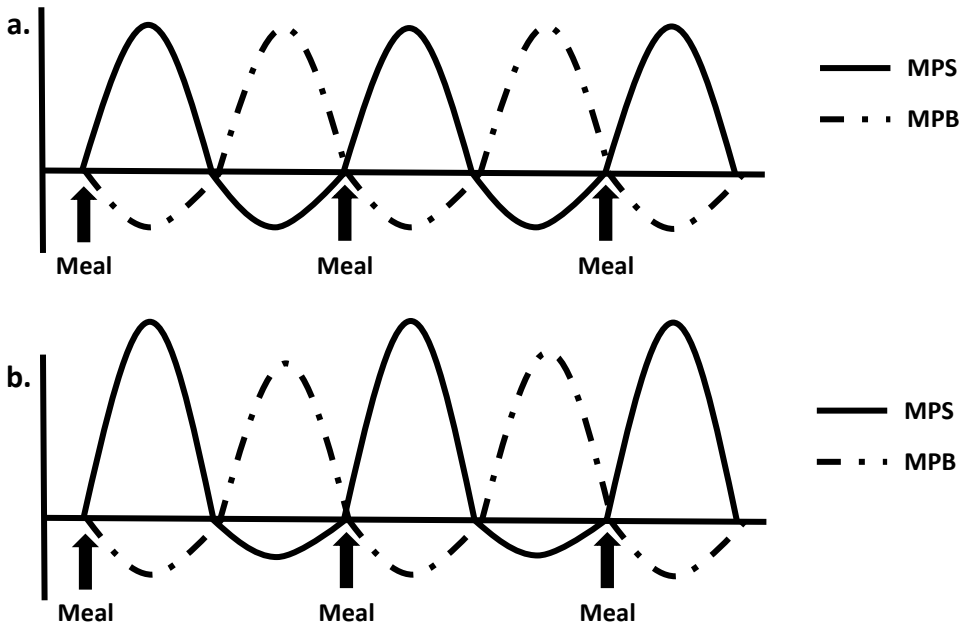

Figure 1.6.5.1. Overview of MPS and MPB in a) normal and b) post-exercise situations . Under normal circumstances, postprandial increases in MPS match the increased MPB during fasting, therefore MM remains stable. In response to exercise, postprandial MPS increases to a greater extent, along with a rise in MPS during fasting. Note, MPB also increases following exercise, however this increase is less than the increase in overall MPS. Thus, there is net accretion of muscle protein, resulting in muscle growth.

One such situation is increased physical activity, which is a well-known stimulus for skeletal muscle growth. Resistance exercise, aerobic exercise and high-intensity interval training are the main modalities of exercise, and each has been demonstrated to cause significant increases in muscle size and mass (Schoenfeld et

al., 2015, 2017; Hughes, Ellefsen and Baar, 2018; Callahan et al., 2021; Khodadadi et al., 2023). Resistance exercise appears to be the most potent stimulator of muscle growth (Atherton and Smith, 2012), and has therefore received the most attention when exploring physiological responses to training. Indeed, increased MPS has been demonstrated following both short-term (K. E. Bell et al., 2015), and long-term resistance exercise (Brook et al., 2015).

The bulk of exercise induced MPS occurs in the post-prandial state, where digested nutrients are available for absorption. Again, similarly to the non-exercised state, the presence of EAAs plays a key role in mediating this process (B. E. Phillips et al., 2012). Intriguingly, the initial rate of increase in MPS following a single bout of resistance exercise is similar to that observed in the non-exercised state (Atherton & Smith, 2012; B. E. Phillips et al., 2012). However, resistance exercises leads to prolongation of the muscle full effect, enabling a greater amount of MPS to occur following each meal (Atherton and Smith, 2012). Furthermore, this sensitising of the muscle to greater MPS can persist for over 48 hours following each exercise session, depending on the training status of the individual (Damas et al., 2015). Thus, with repeated resistance training, there is a continuous sensitisation of muscle to elicit greater MPS with each meal, thus leading to consistently elevated post-prandial MPS and muscle growth (Brook, Wilkinson, Smith, et al., 2016).

It is important to note that MPB also increases with resistance exercise (V. Kumar et al., 2009), although this is likely the result of myofiber damage sustained during the exercise, requiring myofibril remodelling and repair. However, under conditions of adequate nutrient (EAA in particular) supply, the increase in MPS outweighs the

increase in MPB, resulting in a positive net protein balance, and thus, hypertrophy. Inadequate nutritional supply may explain the observation of reduced myofiber size following resistance exercise (Stec et al., 2016), emphasising the critical importance of adequate nutrition to enable skeletal muscle growth to occur.

The underlying mechanisms that stimulate MPS in response to exercise are poorly understood. As with MPS induced by EAA, the mTORC1 pathway appears to be a critical mediator in this (Brook, Wilkinson, Smith, et al., 2016). A process of mechanotransduction appears to be integral in converting the forces experienced by the myofibers into a series of signals that ultimately leads to activation of mTORC1 (Hornberger, 2011). There are two multiprotein complexes that have been described which may mediate this process, dystrophin-glycoprotein complex and integrin-adhesin complex (Schiaffino et al., 2021). These have been shown to interact with the extracellular matrix and the intracellular cytoskeleton, enabling detection of mechanical forces outside and within the myofibers. These may interact directly with mTORC1, however no final pathway linking these complexes has yet been identified (Schiaffino et al., 2021).

Nevertheless, the ultimate consequence of exercise-induced increases in MPS is growth of the muscle, primarily through myofiber hypertrophy (Jorgenson, Phillips and Hornberger, 2020). At a microscopic level, the exact process of this has not been fully elucidated but is likely to include hypertrophy of myofibrils (through production of new myofilaments, or extension of existing ones), along with hyperplasia (Jorgenson, Phillips and Hornberger, 2020). These processes possibly occur in a cyclical fashion leading to an increase in both the size and number of myofibrils,

thereby resulting in hypertrophy of the myofiber (Jorgenson, Phillips and Hornberger, 2020).

1.6.6. Skeletal muscle and ageing

Whilst exercise is a condition that stimulates a net positive balance between MPS and MPB, thus promoting muscle growth, there are multiple conditions in which the opposite occurs. The most commonly studied of these is ageing. It is widely appreciated that older individuals demonstrate lower muscle size and physical strength compared to their younger counterparts (Frontera et al., 2000). From the middle age, skeletal muscle mass reduces with every year of life at a rate of approximately 0.47% per year in men and 0.37% per year in women (Mitchell et al., 2012). Alarmingly, this rate increases to 0.80-0.98% per year in men and 0.64-0.70% per year in women after 75 years of age (Mitchell et al., 2012). Furthermore, strength decreases at a 2 to 5-fold faster rate (Mitchell et al., 2012). This unrelenting process is known as sarcopenia, which is defined by the International Working Group on Sarcopenia as “the age-associated loss of skeletal muscle mass and function” (Fielding et al., 2011). Notably, the definition also states that increased fat mass (FM) can also occur in conjunction with loss of MM within the process of sarcopenia (Fielding et al., 2011).

Sarcopenia eventually leads to frailty, which describes a state of increased vulnerability and heightened risk of developing disability from minor everyday stressors (Morley et al., 2005; Xue, 2011). Accordingly, ongoing sarcopenia and the development of frailty increases the risk of recurrent falls, bone fractures, disability, and death (Metter et al., 2002; Ensrud et al., 2008, 2009; Reinders et al., 2016). As

the general age of our population rises, a consequent increase in frailty prevalence has also been reported (Walsh et al., 2023). This has stimulated significant interest in identifying interventions that can perturb the processes that underlie sarcopenia.

The mechanisms mediating the development of sarcopenia are complex and incompletely understood. However, ultimately, there is a chronic discordance between MPS and MPB, such that the amount of muscle growth within postprandial states does not sufficiently replenish the intramuscular protein lost during preceding episodes of MPB during fasting (Wilkinson, Piasecki and Atherton, 2018). One central mechanism appears to be related to a reduction in the ability of nutrients and exercise to stimulate MPS (Wilkinson, Piasecki and Atherton, 2018; Deane et al., 2021). Several studies have robustly demonstrated reduced MPS response in older adults to amino acid ingestion (Moore et al., 2015; Wall et al., 2015), and exercise (Durham et al., 2010; Brook, Wilkinson, Mitchell, et al., 2016), when compared to young individuals. Notably, this effect only appears to be significant in the context of post-prandial MPS, and there is a general consensus that fasting MPS is not affected significantly by ageing (Fry and Rasmussen, 2011; Deane et al., 2021).

The effect of ageing on MPB is less well defined, but may also play a role in sarcopenia. One study has reported a decrease in insulin-induced inhibition of MPB in older muscle compared to young (Wilkes et al., 2009), and another identified an upregulation of genes associated with MPB in older women following resistance training (Raue et al., 2007). Thus, sarcopenia involves a combination of reduced ability to stimulate MPS and inhibit MPB postprandially, thereby resulting in a

persistent negative protein balance, leading to chronic reductions in skeletal muscle mass over time.

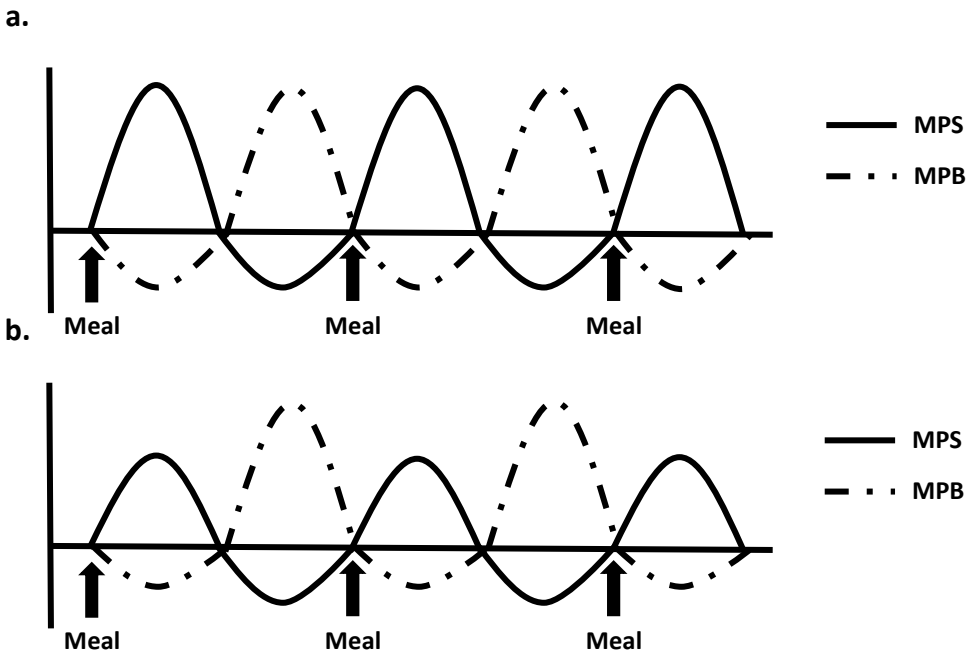

Figure 1.6.6.1. Observed changes in MPS with ageing (b) compared to normal (a) Ageing is associated with reduced post-prandial MPS, which therefore does not replenish muscle loss to MPB during fasting. Additionally, reduced postprandial inhibition of MPB may occur. These effects ultimately result in chronic gradual reduction in MM.

Furthermore, this may be aggravated by reduced physical activity, which is common in older individuals. Indeed, two weeks of reduced step count led to significant reductions in MPS, as well as increased IR (Breen et al., 2013). Thus, the reduced activity observed with sarcopenia may exacerbated to process, further fuelling the progression of sarcopenia.

1.6.7. Skeletal muscle in the context of T2D and obesity

It has become apparent that the presence of T2D exacerbates the sarcopenia process in ageing adults. Large population-based studies have demonstrated a higher rate of MM loss, and reduction in strength and quality in individuals with T2D (S. W. Park et al., 2009; S. W. Park, Goodpaster, Strotmeyer, de Rekeneire, et al., 2006).

Furthermore, this reduction in muscle quality was most severe in individuals with longer duration of T2D and poorer glycaemic control (S. W. Park, Goodpaster, Strotmeyer, de Rekeneire, et al., 2006). Accordingly, T2D is associated with a 2 to 3-fold greater risk of disability (Kalyani et al., 2010).

As with the effects of ageing, the relationship between T2D and increased sarcopenia is incompletely understood. However, a central role of IR has been postulated, which is relevant given the close association between IR and T2D (Defronzo and Tripathy, 2009). Indeed, age-related sarcopenia has been associated with resistance to the insulin-mediated postprandial reduction in MPB (Wilkes et al., 2009). Additionally, reduced MPS response to insulin during a HE clamp has been reported in older individuals (Rasmussen et al., 2006).

Reduced insulin-mediated MPS has been demonstrated in individuals with T2D (Bennet et al., 1991; J. Murphy et al., 2015), with one study showing a high correlation between glucose disposal during a HE clamp and whole-body anabolic response (Morais, Jacob and Chevalier, 2018). Another study also demonstrated that 2 weeks of reduced physical activity in individuals with prediabetes significantly reduced IS and MPS, and this did not recover with return to normal activity (McGlory et al., 2018). This was in contrast to younger normoglycaemic individuals in which both parameters did recover (McGlory et al., 2018). Thus, IR is likely to play a key role in the exacerbation of sarcopenia, in addition to its crucial role in the development of chronic hyperglycaemia and manifest T2D.

Considering that the vast majority of individuals with T2D have a BMI > 25kg.m^{-2} (Public Health England, 2014) and the significant and robust correlation between

increasing BMI and IR (Esteghamati et al., 2008; Tatsumi et al., 2015), it is prudent to also consider the impact of overweight and obesity on MM. Indeed, obesity can also independently drive sarcopenia (Roubenoff, 2004; Donini et al., 2022), with one study suggesting that the presence of obesity confers a 1.5-fold increased risk of frailty (Monteil et al., 2020). The presence of increased FM alongside concurrent sarcopenia is known as sarcopenic obesity (Donini et al., 2022) and has been associated with a 2-3 fold higher risk of all-cause mortality, compared to individuals with sarcopenia only (Benz et al., 2024). With the increased risk of frailty and mortality, there are concerns that sarcopenic obesity could become a significant challenge for health systems across the globe (Roubenoff, 2004).

Similar to observations in individuals with T2D, IR has been identified as a key modulator of the process driving sarcopenic obesity (S. H. Hong & Choi, 2020). Various studies have reported reduced MPS associated with increased IR in individuals living with obesity but without T2D (Smeuninx et al., 2017; Morais, Jacob and Chevalier, 2018). One study demonstrated that this impairment was similar individuals with obesity, regardless of the presence of concurrent T2D (J. Murphy et al., 2015). Furthermore, reduction in insulin-mediated inhibition of MPB has been reported in individuals with obesity (Guillet et al., 2009). Various other mechanisms have been proposed to contribute to sarcopenia in the context of obesity, either by driving IR, or by independently disrupting protein balance.

Chronic low-grade inflammation is another such contributory mechanism that has been postulated. Both obesity and T2D represent chronic inflammatory states characterised by the presence of elevated levels of pro-inflammatory cytokines (S. H.

Hong & Choi, 2020; B. D. Perry et al., 2016). Inflammation in muscle exacerbates IR and promotes a catabolic state leading to stimulation of MPB and consequent muscle atrophy (Crossland et al., 2019; B. D. Perry et al., 2016). Related to this is the accumulation of IMCL, which occurs in both obesity and T2D (Bassil and Gougeon, 2013; Crossland et al., 2019). IMCL content is strongly associated with IR (Machann et al., 2004; Crossland et al., 2019) and reduced muscle strength (Hilton et al., 2008). IMCL may also contribute to the pro-inflammatory state in a similar way to adipose tissue, which has been shown to drive the release of pro-inflammatory cytokines when ectopically deposited in other organs (S. H. Hong & Choi, 2020).

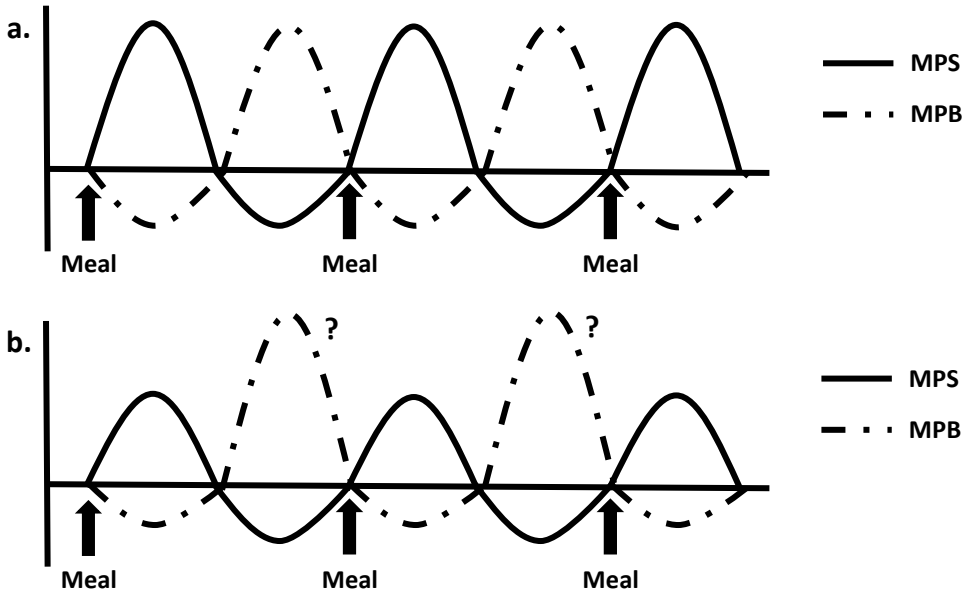

Figure 1.6.7.1. Potential changes in MPS and MPB associated with T2D & obesity (b) compared to normal (a). In addition to the changes described during ageing, T2D & obesity may also provoke increased fasting MPB due to the chronic inflammatory state that exists with these conditions. This further exacerbates the mismatch between MPS and MPB, resulting in accelerated MM loss.

Given the high level of interrelatedness between overweight / obesity and T2D, it is likely that the mechanisms underlying sarcopenia in both conditions share common pathways. Although there still remains significant gaps in knowledge in the processes

underlying this phenomenon, the mechanisms described above are likely to play a key role, either individually, or in an interconnected manner, acting synergistically to drive the onward progression of sarcopenia and it deleterious effects.

1.6.8. Skeletal muscle response to calorie deficit and weight loss

As described in previous sections, weight loss is an essential aspect of management in the context of both obesity and T2D. However, the increased presence of sarcopenia, and frailty arising from these conditions mandates an appreciation of the potential negative effects weight loss could have on MM. Weight loss from dietary interventions in individuals with obesity has been reported to induce a loss of approximately 2-10% of MM (Cava, Yeat and Mittendorfer, 2017). Concerningly, significant weight loss may also lead to reduced muscle strength (B. Kim et al., 2017; Zibellini et al., 2016), predisposing to frailty.

Bariatric surgery is the only intervention that has proven effects of long-term weight loss in individuals with obesity, with or without concurrent T2D. A recent meta-analysis of body composition changes following bariatric surgery reported an approximately 10kg reduction in fat-free mass (FFM) (Haghighat et al., 2021). Intriguingly, due to the greater relative loss of FM (approximately 25kg), there was a consequent post-weight loss increase in FFM proportion of total weight in the order of 10 percentage points (Haghighat et al., 2021). Another meta-analysis noted similar losses of FFM, highlighting consistent loss through the course of the initial post-operative year (Nuijten et al., 2022).

The impact of weight loss of MPS has been scarcely studied. Three studies have demonstrated reduction in MPS following short-term caloric restriction (Pasiakos et al., 2013; C. H. Murphy et al., 2015; Hector et al., 2015), whilst two other studies have demonstrated contrasting results (W. W. Campbell et al., 2009; Villareal et al., 2012). One notable difference between these studies is that the caloric restriction intervention was continued over much longer time periods (3 months +) than the former studies which ranged from 2 to 4 weeks. This suggests that there may be a compensatory increase in MPS following longer-term caloric restriction, to counteract the initial losses observed in the early stages of the intervention.

Additionally, one author postulated that improvements in IS associated with weight loss may explain the increased post-prandial MPS observed by their cohort (W. W. Campbell et al., 2009). Intriguingly, in the studies that demonstrated reduced MPS following caloric restriction, this reduction was attenuated by increased protein intake (Pasiakos et al., 2013; Hector et al., 2015), or resistance exercise (C. H. Murphy et al., 2015). Unfortunately, none of these studies examined the effects of such interventions on MPB. However, it has been postulated that MPB increases significantly in response to the caloric restriction (Cava, Yeat and Mittendorfer, 2017), and may explain the loss of MM, even in situations where MPS has increased (Villareal et al., 2012).

Therefore, there still remains a large number of unknowns regarding the impact of weight loss interventions on MM. As weight loss remains a critical aspect of the management of obesity and T2D, an increased understanding of the impact of these interventions on a global and molecular level remains of crucial importance.

1.7. Summary

Over the last few decades, we have observed a dramatic increase in the prevalence of obesity and T2D. Current projections suggest that these conditions could become major threats to the health and well-being of several million individuals around the world. The economic impact of both conditions and their associated sequelae is set to significantly challenges global health systems, and interventions that manage both conditions are urgently required. Weight loss is effective for this purpose, and to that end, GLP1RAs and VLCD have emerged as measures that are capable of inducing significant weight loss, as well as improvement in multiple pathological processes associated with both obesity and T2D.

The well-described benefits of weight loss using GLP1RAs and VLCD are somewhat outweighed by concerns regarding their effects on MM. As discussed, individuals with obesity and T2D are predisposed to higher rates of sarcopenia and frailty, with an associated resultant increase in mortality. Weight loss is associated with loss of MM, therefore the potential of contributing to low MM state using interventions designed to improve the health of individuals with both conditions is highly counterproductive. The specific effects of GLP1RAs and VLCD on MM are incompletely understood, and as such require urgent clarification. Furthermore, the metabolic mechanisms underlying these changes is a relatively under-studied area, and could provide insight into potential methods of mitigating these negative effects.

Furthermore, there exists some evidence that GLP1RAs may have a protective effect of muscle in the context of weight loss. Greater understanding of this is necessary, particularly in an environment where the popularity of this class of medication is

growing as a rapid rate. Finally, this potential muscle-preserving effect of GLP1RAs raises the potential of combination therapy with VLCD as a method of attenuating the muscle loss associated with caloric restriction, whilst also enhancing the metabolic effects, particularly in the context of T2D. This is an entirely novel area of research with no previous studies investigating the effects of this combination in this context. The subsequent chapters will describe a series of investigations performed to provide a detailed exploration of the discussed areas, and address some of these gaps identified in the established literature.

2. General Methods

2.1. Chapter Summary

This chapter describes a study designed to address some of the gaps in the literature highlighted in the previous chapter. It was a single-centre interventional physiological study conducted entirely at the Centre of Metabolism, Ageing, and Physiology, University of Nottingham. It was designed to assess the effect of the GLP1RA Semaglutide and VLCD on measures of muscle metabolism and function, and clinical outcomes related to T2D and obesity. Additionally, the effect of combining the two interventions was explored on these outcomes, with comparisons against the interventions in isolation. Thus, the study comprised of three interventional groups with comparisons made between all groups.

Initially, the study protocol is outlined in detail with full description of the methods employed to obtain the data required to address the research questions. Following this is a discussion of the specific investigations utilised in the study, and the rationale for the choices made in this regard. This will include consideration of the strengths and limitations of the chosen investigations, which will provide context to the results reported in the subsequent chapters.

2.2. Study Outline

2.2.1. Study Aim

The aim of this study was to determine the effect of VLCD and GLP1RA on a) skeletal muscle protein turnover, b) skeletal muscle mass & function, and c) BCF & IS, in the context of weight loss in individuals with T2D. These parameters were assessed using the GLP1RA Semaglutide (Novo Nordisk), which is also used for the management of T2D and obesity, and an 800 kcal per day VLCD. They were also compared to a group receiving both interventions to determine whether GLP-1 agonism can attenuate the effects of severe caloric restriction, whilst also inducing additive benefits on clinical outcomes.

2.2.2. Primary Objective

The primary objective of this study was to determine the effect of Semaglutide on skeletal muscle protein turnover (MPS and MPB) in individuals with T2D undergoing VLCD-induced weight loss

2.2.3. Secondary Objectives

The secondary objectives of this study were as follows:

1. To determine the effect of VLCD and Semaglutide in isolation on skeletal muscle protein turnover in individuals with T2D
2. To investigate how Semaglutide influences skeletal muscle mass and function in individuals with T2D undergoing VLCD, compared to the individual interventions
3. To explore changes in BCF and IS related to combined VLCD and Semaglutide, compared to the individual interventions

2.2.4. Study Hypotheses

I hypothesise that:

a) Participants on combined Semaglutide and VLCD will demonstrate increased MPS compared to those on VLCD alone, despite comparable weight loss. MPB will be similar, thus overall protein balance will be increased in individuals on the combination

b) Participants on Semaglutide alone will exhibit increased MPS compared to the other groups, and compared to their baseline

c) Combined Semaglutide and VLCD will lead to greater skeletal muscle mass and improved skeletal muscular function, when compared to the VLCD in isolation

d) BCF and IS will improve to a greater extent in participants on the combination of interventions, than in participants on the individual interventions

2.2.5. Study Outcomes

The primary outcomes of this study were MPS and MPB. The following secondary outcomes have also been obtained in the study:

- Skeletal muscle mass – determined from dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical) scanning and stable isotopic techniques
- Vastus lateralis size and structure – using muscle ultrasonography
- Total body weight
- FM – derived from DXA scanning
- BCF – determined from IVGTT

- Whole-body IS – determined from IVGTT and HOMA-IR

2.2.6. Recruitment

The primary routes of recruitment were via general practice, community diabetes clinics and secondary care clinics. Clinicians involved in the care of individuals with T2D were informed about the study via email and word-of-mouth. They were asked to identify individuals that may meet the criteria for treatment with Semaglutide or VLCD in routine practice, as this meant they met the inclusion criteria for this study. They then obtained the prospective participant's consent to pass their details to a member of the research team. Upon receipt of these details, the individual was contacted and a Participant Information Sheet (PIS) was forwarded.

In addition to the above, advertisements containing details of the study were displayed in waiting rooms of primary care, community and secondary care clinics for individuals to notice of their own accord. Advertisements were also placed in local print media and on-line social media. Details of the research team were included in the literature for individuals to directly make contact if they were interested. Any prospective participants identified via this route were forwarded a participant information sheet (PIS) to read in their own time. A final route of recruitment was a registry of potential participants collated from previous studies in our research department. This contained the details of individuals that have registered their interest in research participation, and provided their consent to be contacted in future. This registry was consulted for potentially suitable individuals and again, any prospective participants were contacted and sent a study PIS if they showed interest.

Upon receipt of a PIS, individuals were advised to read the document fully and then reply if they remained interested in involvement in the study. They were then invited to a virtual or face-to-face meeting during which all aspects pertaining to participation in the study were discussed and a medical history was obtained. With this information, the individual's eligibility for the study was scrutinised and if no exclusion criteria were identified, they were invited to a face-to-face screening appointment. In this appointment, a physical examination and blood tests were performed, and consent to participate in the study was sought. All participants were recruited between September 2021 and March 2023.

2.2.7. Eligibility Criteria

The inclusion criteria for the study were as follows:

- Adults between the age of 18-75 years
- Confirmed Type 2 Diabetes Mellitus (HbA1c >48mmol/mol (6.5%))
- Body mass index > 27kg·m^{-2}
- Eligible for VLCD, Semaglutide (or both), within routine practice
- Ability to provide informed consent

Participants were excluded from the study if any of the following criteria were present:

- BMI > 50kg·m^{-2}
- Current pregnancy or breastfeeding, or intention to fall pregnant within the next 3 months
- Uncontrolled hypertension (blood pressure >200/120mmHg)
- Current treatment with insulin

- Current or recent use of GLP-1 agonists
- Previous adverse reaction to a GLP-1 agonist
- Current or recent involvement in a VLCD programme (within the last 12 months)
- History of >5% weight loss within the preceding 12 months
- Ingestion of exogenous deuterium oxide (D_2O) within the preceding 12 months
- History of clinically significant:
  - cardiovascular disease
  - cerebrovascular disease
  - respiratory disease
  - neurological disorders
  - musculoskeletal problems
- History of malignancy undergoing current treatment or palliation
- History of any medical condition contraindicating the use of GLP1RA medication
- Any other medical condition deemed by the investigators to preclude inclusion into the study

Participants taking insulin were excluded due to the known positive effects of insulin therapy on MM in individuals with T2D (Bouchi et al., 2017; Gougeon et al., 1997). Thus, their inclusion could have introduced an important source of uncertainty to any observed results. In addition, there are reports of an elevated risk of hypoglycaemia associated with VLCD use in insulin-treated patients (Baker et al., 2009b; van Wyk &

Daniels, 2016). This risk is also elevated in individuals taking sulphonylurea medication (Baker et al., 2009b; van Wyk & Daniels, 2016), thus these were stopped for two weeks prior to inclusion in the study. This approach could not be adopted for insulin-treated individuals due to the risk of precipitating diabetic ketoacidosis upon sudden cessation of insulin. All other medications were continued during the study.

2.2.8. Randomisation

Following determination of eligibility and receipt of informed consent, participants were formally recruited into the study. Immediately prior to the first study visit, participants were randomised to their intervention group. The were randomised in a 1:1:1 ratio to the following groups:

- Semaglutide-only group
- VLCD-only group
- Combined Semaglutide plus VLCD group

Randomisation was performed using the "Sealed Envelope" online programme (https://www.sealedenvelope.com/), in which a random order of allocation to groups is generated according to certain pre-defined parameters. The allocation sequence was generated by a study supervisor that was not in contact with participants or involved in conducting study visits, and was only revealed for each new participant upon recruitment. No stratification for specific characteristics was utilised during the randomisation process. The study was conducted in an open-label fashion, as due to the nature of the study interventions blinding of participants or investigators was not possible.

2.2.9. Study Schedule

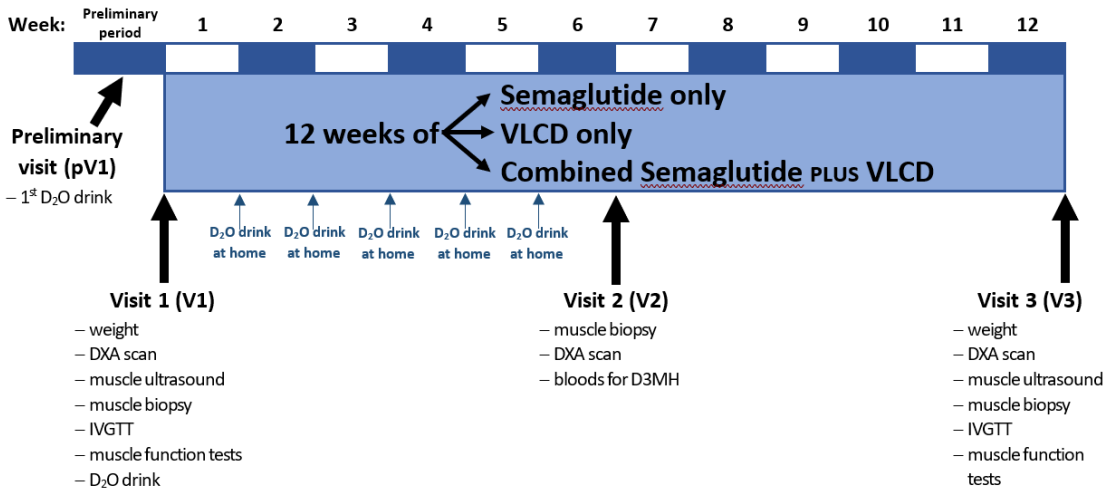

Figure 2.2.9.1. Schematic of the overall study schedule

2.2.9.1. Preliminary Visit (pV1)

Following randomisation, participants attended for the preliminary study visit, which took place between three and seven days before Visit 1 (V1). This was a short visit where participants were provided with doses of the stable isotope tracer D_2O to ingest before the initial study visit, which was used for assessment of MPS.

Blood and saliva samples were collected from participants prior to ingestion of these tracers. Due to the risk of nausea and dizziness following ingestion of large amounts of D_2O, the doses were divided into 50ml aliquots which participants drank 1 hour apart. Two hours following ingestion of the D_2O solution, a second saliva sample was required to determine peak D_2O enrichment of their body's fluid compartment. The first aliquot of D_2O was consumed in the research department, while ingestion of subsequent aliquots and production of the second saliva sample was performed in their own home. Participants were instructed to freeze their saliva sample in a -20 degree freezer until their next study visit.

In addition to ingestion of the isotope tracer, participants were provided with equipment to measure baseline physical activity levels, and a diet diary for assessment of baseline energy intake. Physical activity was assessed using Fitbit devices (Google, California USA) which obtained measures of step count, daily distance travelled, and total daily energy expenditure. The energy intake assessment could be performed either via paper diet diary, or using an app designed for this purpose (Nutracheck, UK), depending on the individual's preference. The diary or instructions to access the app were emailed to participants at least four days prior to the first study visit, in order to enable collection of four full days of diet history.

2.2.9.2. Baseline Visit (V1)

Prior to this visit, participants were requested to fast from midnight the night before, from which point they were only allowed to consume plain water. On the day of V1, participants attended the clinical research facility between 0830 and 0900. Their body weight was measured, before proceeding to a DXA scan, performed in a dedicated room by a member of the research group trained in using this equipment.

Following this, participants were laid onto a clinical bed and ultrasound scans of the right thigh muscles will be performed to assess skeletal muscle architecture or the vastus lateralis. Next, a single muscle biopsy was obtained from the this muscle using the conchotome technique (Dietrichson et al., 1987), under local anaesthetic. After obtaining muscle samples, an intravenous cannula was inserted into the participant's arm and initial blood tests obtained. The participant then underwent an IVGTT, using an intravenous bolus of 20% glucose of 0.5g/kg body weight, up to a maximum of 35g (Bingley et al., 1992). Blood samples for glucose and insulin were collected at 2, 4, 6,

8, 10, 20, 30, 40, 50, 60, 70, 80 & 90 minutes following the glucose bolus. After completion of the IVGTT, participants received a light lunch. The schematic below provides a visual representation of the first part of the study visit.

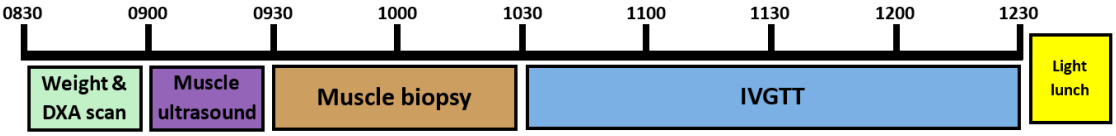

Figure 2.2.9.2. Visual schematic of the first half of V1.

After the light lunch, assessment of knee extensor maximal voluntary contraction (MVC) and handgrip strength was performed to provide a measure of gross skeletal muscle function. This concluded the study measures for the visit.

Prior to leaving, participants received verbal instructions and written information regarding their allocated interventions. In the Semaglutide group, the pen devices were provided and participants were guided through administration of their initial dose, using the paperwork supplied by the manufacturer. Participants in the VLCD group were given all of the meal replacement items for the subsequent six weeks, and taken through the diet plan in detail. Participants in the Combined group went through both of these procedures.

Additionally, all participants were supplied with further doses of D_2O solution and a dose of deuterium-3-(methyl)-3-methylhistidine (D3MH) to store at home. The D_2O required weekly top-up in order to maintain stable serum levels of enrichment for the 6-week period in which MPS was being measured. The amount required was calculated according to established protocols. Furthermore, participants were required to collect saliva samples before, and two hours after, every dose of D_2O. The dose of D3MH required ingestion 24 hours before the next study visit, thus

participants were advised to store the solution in the freezer until then. Participants were provided with clear instructions regarding the storage of the drinks and the required timings of their consumption.

2.2.9.3. Intervention Period

Following collection of baseline study measures, participants commenced their assigned interventions. The Semaglutide group commenced the medication at a dose of 0.25mg once a week, which was then up titrated at two-weekly intervals, if tolerated. Thus, participants were on the maximum 1mg dose from week 5 until the end of the intervention period. If intolerable side effects developed then dose escalation was delayed, and in the most severe cases of side effects, participant continuation in the study was halted, leading to their exclusion. Participants in this group were provided dietary advice focussed on reducing portion sizes, avoiding food with high-fat content, keeping well hydrated and awareness of satiety. They were contacted at least weekly through the initial six weeks to guide them through dose alterations, and monitor for emergence of side effects, particularly around the dose alteration periods.

The VLCD group was asked to commence a total meal replacement supplied by LighterLife UK Limited products, with a choice of soups, milkshakes, porridge or nutritional bars. These food packs provided approximately 600 kcal of energy per day and 100% of the recommended daily allowance of vitamins and minerals. This enabled a 200 kcal allowance for participants to consume fruits or vegetables within the daily limit of 800 kcal. Thus, participants were advised that they could consume up to two portions of fruit or vegetables per day and provided with examples of what

would comprise a portion (such as a large carrot, or 3 sticks of celery). As with the Semaglutide group, VLCD participants were guided through the process closely, especially in the initial weeks, and were contacted regularly for support.

Participants in the Combined group received both Semaglutide and VLCD as described above and received the same level of monitoring as the other groups. Adverse event data for all groups were recorded and analysed at regular intervals to determine emergence of concerning trends. All participants will be required to monitor their physical activity and dietary intake during the intervention period, in a similar to manner to the process described above. Physical activity measurements using the Fitbit device will be required again during week 6 of the intervention period and participants will keep the device with them for the duration of the study. Dietary intake will also need to be recorded during week 6 of the intervention period.

2.2.9.4. Visit 2 (V2)

V2 occurred six weeks into the 12-week interventional period. As described above, 24 hours prior to V2, participants were required to ingest the D3MH solution they had received at V1. In addition, participants completed a second cycle of physical activity and dietary intake monitoring in four days preceding V2.

On the day of V2, an intravenous cannula was inserted upon arrival, allowing a series of blood tests to be collected for measurement of plasma levels of D3MH. The blood tests were collected every 40 minutes for 4 hours commencing precisely 24 hours after the ingestion of the D3MH solution. During this 4-hour period, a DXA scan and a single muscle biopsy were also performed. After the 4-hour blood testing period,

participants were given a light lunch and their instructions for the remaining 6 weeks of the study.

2.2.9.5. Visit 3 (V3)

At the end of the 12-week intervention period, participants attended the research facility for their final follow-up visit. This followed a similar format to V1, with the exception that at the end of this visit participants were asked to return any equipment provided.

2.2.10. Compliance monitoring

As previously outlined, participants were contacted regularly to prompt them to perform all of the required study procedures, as well as identify adverse effects and address any potential concerns. These contacts were also an opportunity to determine participants' compliance with the interventions. Participants in the Semaglutide and Combined groups were instructed to document the date and amount of each dose of Semaglutide they administered. All participants were advised to record their weights each week and these were reviewed during the participant contact events. Evidence suggestive of non-compliance, such as plateauing weight or lack of Semaglutide administration was discussed directly with the participants, in order to identify any issues and potential solutions, if appropriate. Participants were advised during screening that they would be removed from the study if they:

- missed ≥2 consecutive doses of Semaglutide or >25% of expected doses through the duration of the study

  or

- were unable to adhere to the limits of the total meal replacement for ≥4 consecutive days or ≥25% of the duration of the study

However, no participants were excluded due to non-compliance.

2.2.11. Ethical considerations

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (World Medical Association, 2013) and received ethical approval from the East of England, Essex Research Ethics Committee (reference number 21/EE/0163). All participants provided fully informed consent to participate in this study, and ongoing consent to continue was sought at each study visit.

2.3. Specific outcomes and rationale for choice of measurement

2.3.1. Stable isotopic techniques for measuring muscle metabolism

Determination of MPS and MPB was a central aspect of this work. As such, an appropriate method of measuring these processes was required to ensure that accurate results were obtained. Muscle is biologically active, with new proteins being synthesised and existing ones broken down constantly. Therefore, a method of measuring this in vivo requires the ability to distinguish newly formed and recently broken down protein from pre-existing tissue.

Stable isotopes of common elements in biological tissues have become widely used for this purpose. Stable isotopes are species of an element that have the same chemical properties but differ in molecular weight due to alterations in the number of neutrons contained within the atomic nucleus (Wilkinson et al., 2017). These weight differences can be recognised using specialist equipment such as a mass spectrometer. This permits calculation of the ratio between the isotope of interest (known as the tracer) and the commonly occurring element (tracee) in a compound, with changes in this ratio providing insight into the metabolic process in question. As opposed to radioisotopes, stable isotopes are not radioactive enabling their safe use in humans (Wilkinson et al., 2017). Furthermore, they largely do not significantly affect biological processes, therefore leading to a low incidence of adverse effects (Davies, 2020).

Stable isotopes have been safely used to assess various aspects of human physiology for several decades (Roberts et al., 1986; Jones, Scanu and Schoeller, 1988; Leitch and Jones, 1991). Historically, muscle metabolism was examined using infusions of amino acids labelled with stable isotopes of carbon (^{13}C) or nitrogen (^{15}N) measured via gas chromatography-mass spectrometry (Millward and Smith, 2019). Through serial collection of muscle tissue during infusion of isotopically-labelled compounds, the varying tracer:tracee ratios reflect the rate of incorporation of these compounds into muscle, providing a measure of MPS (Rennie, Smith and Watt, 1994). MPS can also be measured by calculating the rate of disappearance of the labelled compounds from systemic vasculature, although with the limitation of the inability to guarantee that all of the removed compound was used exclusively in the metabolic process being measured (Rennie, Smith and Watt, 1994).

Whilst the isotopically-labelled infusion method is robustly validated and currently utilised in a variety of studies (Church, Ferrando and Wolfe, 2024; Dideriksen et al., 2024; Pinckaers et al., 2024), it has several limitations. Firstly, it requires intravenous infusions for a prolonged period, thus necessitating participants to be confined to a bed within the research department throughout the experimental protocol. Additionally, multiple biopsies are taken within a short period of time, which can be off-putting for participants. Most relevant to this study, this method can only measure MPS over period of a few hours, and thus can only reflect acute changes in MPS over this time (Wilkinson et al., 2017). As this study is assessing changes in MPS in response to a dietary intervention, this method would not be appropriate for achieving this aim.

The development of the D_2O (also known as “heavy water”) method represented a significant shift in the measurement of muscle physiology. The significant advantage of D_2O lies in its ability to be administered as a drink which can be ingested in participants’ home. Additionally, it equilibrates rapidly with the body’s water compartments, enabling its incorporation in various metabolic processes, including MPS (Miller et al., 2020). At low doses, it is extremely safe with minimal incidence of adverse effects (Davies, 2020). Thus, this method can be used to safely assess MPS over days or weeks, making it the ideal method for this study.

D_2O solution is ingested prior to the intervention, with regular top-up doses to maintain enrichment of the labelled compound in the body (replacing the need for constant infusion). The rate of deuterium incorporation into muscle amino acids (alanine in particular) can be determined by obtaining muscle samples, providing an estimation of MPS. This method has been validated in studies showing similar findings to those observed using labelled tracer infusions (Wilkinson et al., 2014, 2015). One previous study by our research department utilised this method to determine MPS over 6 weeks in response to unilateral resistance training (Brook et al., 2015). Notably, serial changes in MPS measured by this method matched observed changes in vastus lateralis thickness, fibre length and pennation angle (Brook et al., 2015), providing further validation of this method over a 6-week period.

In addition, blood enrichment can provide a valid measure of baseline muscle enrichment of tracer (Heys et al., 1990; S. M. Phillips et al., 2011), eliminating the requirement for a baseline muscle sample. Whilst the use of such an approach in the

context of D_2O is novel, this dramatically reduced the burden on our participants, which we felt would improve the ability to recruit to the study.

Using similar methodology, MPB was determined using labelled 3-methylhistidine (3MH). Endogenous 3MH is generated from the breakdown of muscle proteins and has been used as a marker of muscle proteolysis for some time (Sheffield-Moore et al., 2014). Our department has recently developed a protocol that enables estimation of MPB using serial measurements of isotopically labelled 3MH following ingestion of an oral solution. This again performs comparably to more established infusion methods (Cegielski et al., 2021, 2022). Thus, with the well-being of our participants in mind, we opted for this method.

2.3.2. DXA scanning

DXA is a form of scanning that is commonly used in both clinical and research contexts. DXA utilises X-ray energy at two intensities and measures the attenuation of both as they pass through the subject being scanned (Laskey, 1996). Comparison of the attenuation of these two energy frequencies permits calculation of FM and FFM / lean body mass (LBM) compartments of the body, enabling measurement of body composition (Laskey, 1996). DXA has been validated for use of body composition assessment (Haarbo et al., 1991; Lohman et al., 2000), and is relatively easy to perform, albeit with prior technical training in the functioning of individual machines. It is important to note that DXA utilises X-rays, exposing participants to ionising radiation. However, this dose is minimal and similar to the normal background radiation experienced during one day at sea level (Shepherd et al., 2017).

Other methods of body composition measurement include magnetic resonance imaging (MRI) techniques, bio-impedance analysis (BIA), and air-displacement plethysmography (ADP). BIA measures the impedance of small electrical currents passed through the participants' body and uses several assumptions to calculate body composition. Its main limitation lies in the accuracy of these assumptions in individuals with large variations in body size and phenotype (Liao et al., 2020), whilst DXA is less reliant on such assumptions (Kohrt, 1995). ADP also requires mathematical calculations to determine body composition, which leaves it vulnerable to similar limitations as BIA, particularly at the extremes of weight (Lowry and Tomiyama, 2015).

MRI is an accurate method of determining body composition over the range of body sizes, and can provide more precise data regarding different compartments of the body, including visceral fat (Ross et al., 2000). However, it is much more expensive, requires a higher level of operator expertise, and takes longer to perform. Additionally, the enclosed environment during an MRI scan can leave participants feeling claustrophobic. Given our resource limitations, lack of expertise, and higher likelihood of claustrophobia in our participants that live with obesity, MRI was not a feasible option. Thus, DXA was chosen as the method of measuring body composition in this study.

Participants received three DXA scans during the course of this study. The total dose of radiation received by participants was assessed by the Clinical Radiation Expert for our department and was estimated at 0.4 – 0.8µSv, which is equivalent to a few hours of background radiation and equated to a negligible risk of developing fatal cancer.

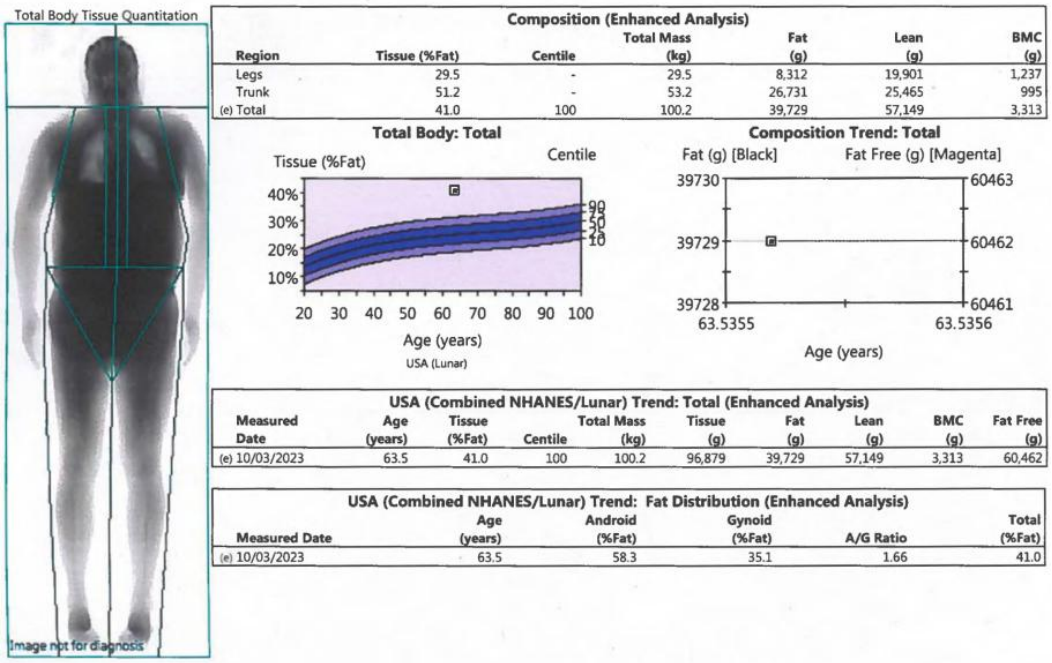

Figure 2.3.2. Example DXA report showing demarcated regions of interest (left) and the display of regional and whole-body fat and lean body mass (top right)

2.3.3. Muscle ultrasonography

In addition to DXA scanning, ultrasonography of the vastus lateralis (VL) will be performed. While DXA will provide a measure of whole-body muscle mass and fat mass, ultrasonography provides much more detailed analysis of specific muscle size and structure. The VL is the muscle in which MPS will be assessed, therefore an understanding of the changes in the characteristics of this muscle was necessary to provide context to the observed alterations in MPS.

Ultrasonography is quick, non-invasive and easy to perform (Betz et al., 2021), and has been shown to be reliable (Reeves, Maganaris and Narici, 2004; English, Fisher and Thoirs, 2012). MRI can also be used for the purpose of analysing changes in the structure of specific muscles, however it has significant limitations, as previously mentioned. Furthermore, ultrasound derived measures have been shown to correlate well with MRI assessments (Reeves, Maganaris and Narici, 2004; Betz et al., 2021).

Muscle thickness and cross-sectional area (CSA) were measures that were derived from the ultrasound scans, which both correlate significantly with results of strength assessments within tested muscle (Maughan, Watson and Weir, 1983; Strasser et al., 2013).

Figure 2.3.3.1. Example images obtained from VL ultrasonography used to assess a) CSA and b) muscle thickness. Yellow lines are the marks used for analysis.

2.3.4. Strength assessment

In addition to the ultrasonographic assessment of vastus lateralis structure, muscle strength was also assessed using MVC or knee extension. The VL is one of the muscles involved in knee extension, thus this provides a direct examination of the functional capability of the muscle, in addition to the aforementioned metabolic and structural measures. From a clinical perspective, alterations in strength are particularly relevant as it is muscle weakness that predisposes individuals to frailty and disability. Thus, strength assessment provides an idea of the clinical relevance of any observed changes in muscle size.

MVC was assessed in an isometric dynamometer, with the leg fixed in a 90 degree flexion. The participant was asked to contract this leg as hard as possible against a force transducer, which simultaneously measures the force generated and displays this on a connected monitor. This is in contrast to 1-repetition maximum (1RM) testing which involves repeated attempts at moving a selected weight which increases following each successful attempt. Both measures correlate well with each other (Portilla-Cueto et al., 2022) and MVC has been shown to relate well with measures of muscle size (Lanza et al., 2022). The advantage of MVC is it is very quick, as opposed to 1RM that could require multiple attempts to achieve the true value.

The disadvantage is that MVC requires specialist equipment, including a force transducer which are not widely available. As a force transducer is available within our research department, MVC was chosen as the method of strength assessment.

Of note, MVC was determined in the left leg, which was contralateral to the site of the biopsy. It was recognised that the prior muscle biopsy could significantly impact the resultant MVC if measured on the same leg. Therefore, in order to obtain accurate measurements of muscle strength, it was decided to test the contralateral leg, with the assumption that the changes in muscle mass would be equal in both legs, as would any corresponding alterations in strength.

2.3.5. Assessment of BCF and IS

Along with the metabolic and functional muscle outcomes listed above, it was important to assess the glycaemic impacts of the studied interventions. This was particularly relevant in the group receiving combined VLCD with Semaglutide as this is an entirely novel approach in individuals with T2D. To that end, BCF and IS were determined in addition to HbA1c, which provided a measure of overall glycaemic control.

The gold standard for measuring both parameters are clamp techniques, with the HE clamp used to assess IS, and the hyperglycaemic clamp employed for BCF (Hannon et al., 2018; Gastaldelli, 2022). These techniques provide highly accurate and reproduceable estimations of these parameters, however require specialist equipment, significant expertise and are therefore expensive to perform, particularly in the case of the HE clamp (Cersosimo et al., 2014). Additionally, this type of clamp is

associated with the potential risk of hypoglycaemia, due to the use of high-dose insulin infusions.

The IVGTT can measure both IS and BCF in the same test (Muniyappa et al., 2008), making it attractive for studies with multiple metabolic outcomes. It is less time and resource intensive than the clamp techniques (Muniyappa et al., 2008), although some expertise is required to ensure it is conducted correctly. It correlates well with the gold standard clamp techniques for ascertaining both parameters (Saad et al., 1994; Rakotoambinina et al., 1997; Tura et al., 2010).

Methods assessing metabolic response to oral ingestion of nutrients (such as OGTT and MTT) were other options. However, the slow and high-variability in nutrient absorption prevents direct visualisation of the initial phase of insulin secretion (the first-phase response). This is an important marker of BCF as it is typically lost in individuals with T2D (Davies et al., 1994), and its restoration is thought to be crucial for the attainment of robust diabetes remission (Lim et al., 2011). As IVGTT enables direct visualisation of the first-phase response, in conjunction with a measurement of insulin sensitivity, this was the most appropriate choice for assessing glycaemic metabolic response to the interventions.

3. The effects of GLP1RA on muscle mass – a systematic review and meta-analysis

3.1. Chapter Synopsis

Background: GLP1RAs are used for the management of both T2D and obesity. These medications induce significant weight loss, however concerns have been raised regarding the associated effects on MM. On the other hand, various studies have suggested a potentially muscle preserving effect of GLP-1 hormone and empirical data regarding body composition during GLP1RA interventions are inconsistent. This systematic review and meta-analysis was conducted to clarify the effects of GLP1RAs on MM in individuals living with overweight or obesity, with and without T2D.

Methods: Comprehensive search of Medline, Pubmed, EMBASE, CINAHL, the Cochrane Central Register of Controlled Trials (CENTRAL) and Google Scholar was performed. Studies involving cohorts with a mean age over 40 years and mean body mass index over 25kg.m^{-2} were included. The primary outcome was any measure of MM, whilst FM and total body weight were included as secondary outcomes.

Results: Thirty-eight publications, involving 1735 participants, were included in the review. Separate meta-analyses were performed for studies involving participants with T2D and individuals without T2D (non-DM). In individuals with T2D, GLP1RAs induced a non-significant reduction in MM (-0.71kg, 95% CI: -1.61, 0.14, $p = 0.10$), despite significantly reducing FM (-3.18kg, 95% CI: -4.09, -2.28, $p < 0.0001$). In the non-DM analysis, a significant reduction in MM was observed (-1.41kg, 95% CI: -2.12, -0.71, $p = 0.0001$), however this was significantly less than the reduction in FM (-6.02kg, 95% CI: -7.53, -4.50, $p < 0.0001$). In both populations, the proportion of total weight loss arising from MM was less than 20%.

Conclusions: These findings suggest that GLP1RAs do promote preservation of MM whilst inducing weight loss, particularly in individuals with T2D. Although significant reductions in MM were noted in individuals without T2D, this is likely due to increased weight loss elicited by the higher doses of GLP1RAs used in this population. Reassuringly, the proportion of weight loss attributable to MM was minimal (less than 20%) in both populations.

3.2. Introduction

As highlighted in Chapter 1, the association between T2D and excess weight is well described, with approximately 85-90% of individuals with T2D classified as either overweight or obese (Public Health England, 2014). Weight loss is widely recognised to lead to improved glycaemic control (Wilding, 2014), and reduced incidence of complications (Gregg et al., 2024; The Look AHEAD Research Group, 2016). Consequently, interventions that improve glycaemic control, whilst also inducing significant weight loss, are highly sought after (Lingvay et al., 2022).

GLP1RAs are widely used for the management of T2D. Amelioration of hyperglycaemia occurs via stimulation of insulin secretion and suppression of glucagon release from pancreatic cells (Hedrington et al., 2018), along with improved BCF (Kapitza et al., 2017a; Vilsbøll et al., 2008). In addition, GLP1RAs act on enteric receptors to slow gastric emptying, as well as centrally to modulate satiety centres in the human brain (Sfairopoulos et al., 2018), the latter induced reductions in appetite and food intake, resulting in weight loss (Blundell et al., 2017).

This constellation of effects has made GLP1RAs highly attractive interventions for the management of T2D, and currently a range of medications in this class are indicated for treating T2D with concurrent obesity (M. J. Davies et al., 2022). In addition, following the positive results of the SCALE (Pi-Sunyer et al., 2015; Wadden et al., 2013, 2020) and STEP (W. T. Garvey, Batterham, et al., 2022; D. Rubino et al., 2021; Wadden et al., 2021; Wilding et al., 2021) series of studies, two GLP1RAs – Liraglutide and Semaglutide – have recently become approved for weight management in individuals living with obesity.

The potential of significant MM loss induced by GLP1RAs and other weight loss interventions however, cannot be ignored. MM loss has been reported to be as high as 40% of the total amount of weight lost (Buntz, 2024; Ida et al., 2020). Concerningly, weight regain is thought to almost entirely consist of fat (Editorial, 2023). Thus, recurrent cycles of weight loss and regain may result in body composition changes that contribute to ongoing difficulties maintaining weight lost. This could put affected individuals at increased risk of sarcopenic obesity and long-term adverse metabolic health (Editorial, 2023).

The age-related loss of MM in individuals with T2D is accelerated compared to those without the condition (S. W. Park et al., 2009), and obesity itself is strongly associated with poor muscle quality and function (Cava et al., 2017). To that end, preservation of MM is of utmost importance in individuals living with obesity, particularly those with concurrent T2D, and must be given due consideration when choosing interventions that promote weight loss in these populations (Cava et al., 2017; Ida et al., 2020).

As such, a detailed understanding of the effects of GLP1RAs on MM is warranted, in individuals with T2D, as well as those without the condition. Several studies have assessed the impact of GLP1RAs on measures of MM, with varied results. One review highlights this, reporting 4-60% of total weight loss arising from MM (Sargeant et al., 2019). Another more recent review suggests GLP1RA treatment may have a protective effect on MM in individuals with T2D (X. Zhang et al., 2021). Intriguingly, a number of epidemiological and pre-clinical studies, including from our group, have reported positive benefits of GLP-1 on muscle mass and metabolism (Abdulla et al., 2020; Bouchi et al., 2018; Y. Hong et al., 2019).

Despite the presence of these studies, the impact of GLP1RAs on MM in the context of clinical practice remains unclear. No meta-analysis has quantitatively examined the effect of GLP1RA-induced weight loss on MM in people with or without T2D. Thus, this systematic review and meta-analysis was performed to fill this gap in the literature by assessing the effect GLP1RAs on measures of MM, relative to changes in FM and total body weight (TBW) in individuals with, and without T2D.

3.3. Methods

This systematic review was conducted in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidance. The review protocol is registered in the International Prospective Register of Systematic Reviews, registration number CRD42023489617.

The overall aim of the review was to examine absolute changes in measures of MM among individuals with overweight (BMI ≥ 25kg.m^{-2}), with and without concurrent T2D, relative to changes in FM and TBW. Absolute changes were used, rather than comparisons with other interventions or placebo, as MM alterations often associate with changes in weight. As similar weight loss cannot be expected between GLP1RAs and other interventions, a relevant comparison on MM changes cannot be performed. Thus, we opted to assess absolute changes, and relate these to changes observed in FM and TBW.

3.3.1. Search strategy and study selection criteria

A comprehensive electronic search was performed on 15th November 2023 for all studies published until that date in the Medline, EMBASE, PubMed, CINAHL and Cochrane Central Register of Controlled Trials (CENTRAL) databases. Google Scholar was also searched for identification of grey area literature relevant to the review topic on the same date. The choice of search terms was informed by a scoping review performed during the development of the review protocol. The general terms used in the search are listed below, and were adapted to each individual database, with mapping to MeSH or EMTREE terms wherever possible and appropriate (see Appendix II for an example).

- Population: overweight OR diabet* OR obes*
- Intervention: albiglutide OR lixisenatide OR dulaglutide OR exen* OR semaglutide OR liraglutide OR GLP1RA OR glucagon like peptide 1 receptor agonist
- Outcomes: muscle mass OR fat mass OR fat free mass OR lean body mass OR body composition

The Boolean command "AND" was used to combine the sets of terms for the final search. Examples of the search strategy can be found in the Appendices.

Studies inclusion criteria were:

1. full article written in English and published in a peer-reviewed journal
2. population mean body mass index (BMI) ≥25kg.m^{-2}
3. population mean age ≥40 years
4. at least one intervention group treated with any GLP1RA for ≥6 weeks without any concurrent intervention
5. reporting an outcome related to MM – for example LBM, FFM, skeletal muscle mass (SMM)

FM and TBW were secondary outcomes, thus reporting of data related to these outcomes was not essential for inclusion of the study. Both randomised controlled trials (RCT) and non-RCT studies were included, with the essential requirement that at least one intervention group was treated with a GLP1RA, without any concurrent intervention. Studies involving individuals diagnosed with type 1 diabetes, or pregnant, neonatal, paediatric or adolescent populations were excluded.

After removal of duplicates, two reviewers independently screened the titles and abstracts of the studies identified from the primary search. Disagreements regarding inclusion or rejection at this stage were discussed, with involvement of a third reviewer in cases where consensus could not be achieved. Inter-observer agreement rate was 91%, with Cohen's K 0.44, suggesting moderate concordance. Articles that addressed the research question but did not meet the specific inclusion criteria were selected as secondary sources. The reference lists of these secondary sources were manually reviewed for identification of additional potentially relevant studies. Following completion of screening, full articles from both primary and secondary searches were assessed for eligibility concurrently by two reviewers with disagreements resolved as above.

3.3.2. Data collection and validity assessment

Data collection was performed by a single independent reviewer and assembled into a data extraction spreadsheet (Microsoft Excel), which was cross-checked by another reviewer for accuracy. Pre- and post-intervention measures of the primary outcome (e.g. LBM, FFM, SMM) and the secondary outcomes (FM and TBW, if available) were extracted from each included article. In addition to this, the following information regarding each study was obtained: GLP1RA medication(s) dose(s) and frequency, intervention duration, mean cohort age and BMI, participant gender, diabetes status, study authors, publication date, study design, comparator intervention and dose (if applicable), and main study outcomes.

In publications where more than one intervention group met the criteria for inclusion in the analysis, each group was recorded independently. Where data were not

available in a form that was amenable to meta-analysis, corresponding authors were contacted requesting additional data. If no reply was received despite multiple contact attempts, study results were included in the narrative synthesis only.

The method of bias assessment employed was dependent on the study design. For RCTs, the Cochrane Risk of Bias 2 (ROB2) tool was utilised (J. Higgins, Savović, et al., 2023; J. P. T. Higgins et al., 2011), whilst the relevant Joanna Briggs Institute Critical Appraisal Checklist was implemented for non-RCTs (JBI, 2024). Bias assessments were performed by two reviewers, and disagreements were resolved by a third. RCTs received a “low”, “some concerns” or “high risk of bias” rating based on five bias domains (randomization process, deviation from intended intervention, missing outcome data, measurement of the outcome, and selection of the reported results). Non-RCTs received a global rating of quality based on responses to a checklist of questions, which was decided upon collaboration of the two reviewers.

3.3.3. Statistical Analysis

StataSE version 18 (StataCorp, Texas, USA) was used to perform meta-analysis of available data. Pre- and post-intervention means and standard deviations (SD) from each study / intervention group were used to generate a weighted mean difference (WMD) for the outcomes. All measures were reported in kilograms, therefore enabling use of WMD as the effect-size measure. The random effects Hedges model was selected as we anticipated a potential of significant heterogeneity due to the different medications and doses, and likely variations in population characteristics.

It was initially intended to perform subgroup analysis according to diabetes status, due to the expected heterogeneity between these two populations. However, due to

the large number of eligible articles identified, a post-hoc decision was made to perform two separate analyses of studies involving participants with T2D and studies involving individuals without T2D. The analysis was divided in this manner as different doses of GLP1RA are utilised for management of T2D and obesity without T2D. Thus, this approach would remove a potentially major source of heterogeneity from the resulting quantitative synthesis. Included studies that involved participants with prediabetes used GLP1RA doses associated with T2D management, thus were incorporated within the T2D analysis.

Forest plots were generated for each analysis and sub-grouped by GLP1RA. In order to provide context to any changes in measures of MM, forest plots with these measures were presented alongside the corresponding forest plot for FM and TBW. Therefore, two groups of paired forest plots were generated (MM:FM, and MM:TBW), only including data from studies that reported both measures. Test for subgroup was performed when two or more data points were present.

Where studies only reported standard error, SD was calculated in line with Cochrane guidance (J. Higgins, Li, et al., 2023). In situations where only a pre- or post-intervention measure and change value have been reported, and the required information was not made available upon request, the missing measure was calculated using the change value, and the SD was assumed to be the same as the available measure. Sensitivity analysis was performed to examine whether this influenced the overall results by analysing the result obtained when only studies containing both pre- and post-intervention data were included. Sensitivity analysis was also performed to determine the effect of including non-RCTs. Finally, as there

are multiple surrogate measures of MM, as well as multiple methods used to determine body composition, these were also subject to sensitivity analysis to provide more certainty to the robustness of our results.

Study heterogeneity was assessed using I^2 and Tau statistics (interpreted in accordance with Cochrane guidance), as well as visual examination of the generated forest plots. Evidence of homogeneity was assessed using the H^2 and test for Θ_i. Evidence of publication bias was examined through visual inspection of funnel plots and performance of the Egger test. Statistical significance was defined as $p < 0.05$ and data are presented as WMD with 95% confidence intervals.

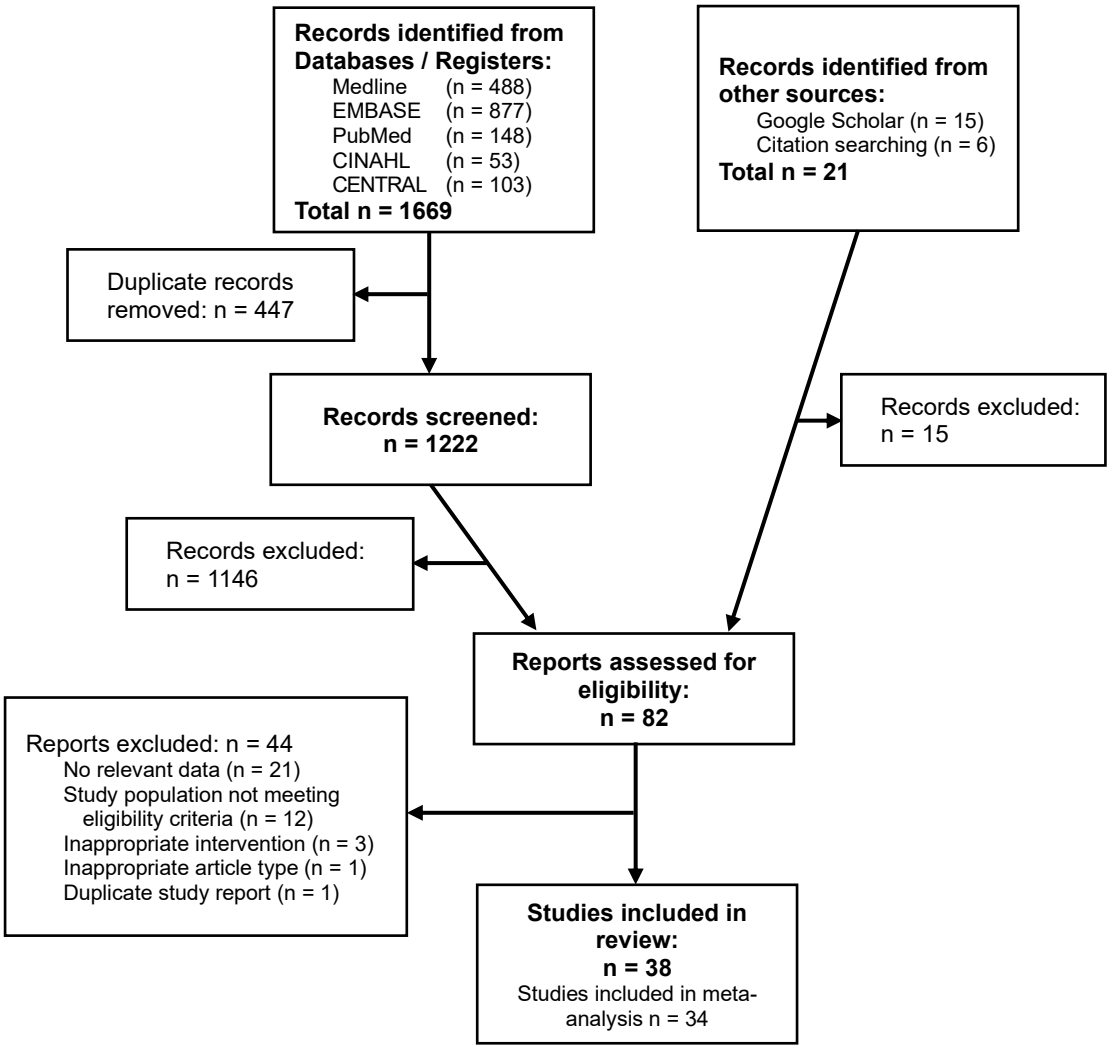

Figure 3.4.1. Flow diagram of study selection

Study	Study design	GLP1RA & dose	Duration	Sample size & gender (no. of females)	Diabetes status	Age – mean (SD)	Baseline BMI – mean (SD)	Method of body composition assessment	Outcomes of interest measured	Study primary outcome/s
Van Eyk et al., 2019	RCT	Liraglutide, 1.8mg od	26 weeks	22 (14)	T2D	55 (11)	30.4 (3.8)	BIA	LBM, TBW	Cardiac function
Díaz-Soto et al., 2014	Non-RCT (SG)	Liraglutide, 1.8mg od	14 weeks	55 (27)	T2D	61 (11)	33.9 (5.7)	BIA	FFM% / FM% / BMI	Undisclosed
Yabe et al., 2022	RCT	Dulaglutide, 0.75mg ow	52 weeks	19 (3)	T2D	58.6 (7.5)	27.5 (3.5)	BIA	LBM / FM / TBW	Medication pharmacodynamics
C. J. Li et al., 2014	Non-RCT (SG)	Liraglutide, 1.2mg od	12 weeks	31 (15)	T2D	48.5 (11.4)	31.7 (3.6)	DXA	LBM / FM / TBW	Change in cardiac natiuretic peptides
Stefanakis et al., 2023	Non-RCT (MG)	Liraglutide, 3mg od	24 weeks	9 (3)	Non-DM	44.6 (7.4)	37.9 (4.6)	BIA	FFM / FM / TBW	Postprandial C-peptide & proglucagon-derived peptides
J. S. Park et al., 2021	Non-RCT (SG)	Liraglutide, 3mg od	Variable, 74 days average	169 (98)	Mixed	41.5 (9.6)	30.8 (3.5)	BIA	SMM / FM / TBW	Undisclosed
Agcakaya et al., 2023	Non-RCT (SG, retrospective)	Exenatide, 10mcg bd	6 months	50 (46)	T2D	56.3 (9.7)	35.3 (7.6)	BIA	FFM% / FM% / TBW	Weight loss & body composition
Seko et al., 2017	Non-RCT (SG, retrospective)	Dulaglutide, 0.75mg ow	12 weeks	5 (undisclosed)	T2D	66.8 (2.7)	28.2 (1.2)	BIA	SMM / FM / TBW	Change in liver function & fibrosis markers
Freitas & Rodrigues, 2023a	Cohort study	Liraglutide, 3mg od	6 months	24 (all female)	Mixed	46 (9)	36.3 (3.9)	BIA	SMM / FM / TBW	Undisclosed
Freitas & Rodrigues, 2023b	Cohort study	Liraglutide, 3mg od	6 months	33 (all male)	Mixed	42 (11)	38.3 (5.8)	BIA	SMM / FM / TBW	Undisclosed
Silver et al., 2023	RCT	Liraglutide, 1.8mg od	14 weeks	44 (31)	Pre-DM	49.8 (10.1)	38.6 (6.1)	DXA	LBM / FM / TBW	Appetite, food intake & fat distribution
Yin et al., 2018	RCT	Exenatide, 10mcg bd	16 weeks	19 (7)	T2D	47.6 (10.9)	28.1 (2.2)	DXA	LBM / FM / TBW	HbA1c
Neeland et al., 2021	RCT	Liraglutide, 3mg od	40 weeks	73 (67)	Non-DM	49.6 (9.8)	37.2 (6.0)	MRI based measure	Lean tissue / adipose tissue / TBW	Visceral adipose tissue
Feng et al., 2019	RCT	Liraglutide, 1.8mg od	24 weeks	29 (8)	T2D	46.8 (9.7)	28.1 (3.2)	DXA	LBM / FM / TBW	Weight
Blundell et al., 2017	Crossover RCT	Semaglutide, 1mg ow	12 weeks	28 (18)	Non-DM	42 (range 21-70)	33.8 (range 30.5-42.8)	ADP	LBM / FM / TBW	Change in energy intake
Gibbons et al., 2021	Crossover RCT	Semaglutide, 14mg od	12 weeks	13 (2)	T2D	58.2 (no SD)	30.8 (no SD)	ADP	LBM / FM / TBW	Postprandial glucose metabolism
Hong et al., 2016	Non-RCT (SG)	Exenatide, 10mcg bd	12 weeks	32 (undisclosed)	T2D	49.0 (11.2)	32.9 (4.7)	BIA	SMM / FM / TBW	Undisclosed
De Luis et al., 2015a	Non-RCT (MG)	Liraglutide, 1.8mg od	14 weeks	51 (26)	T2D	60.5 (9.2)	34.9 (6.6)	BIA	FFM / FM / TBW	Body weight

De Luis et al., 2015b	Non-RCT (MG)	Liraglutide, 1.8mg od	14 weeks	39 (18)	T2D	61.2 (10.1)	33.1 (4.9)	BIA	FFM / FM / TBW	Body weight
Bunck et al., 2010	RCT	Exenatide, 20mcg tds	52 weeks	29 (8)	T2D	58.4 (7.5)	30.9 (3.8)	DXA	LBM / FM / TBW	Beta-cell function
Kadouh et al., 2020	RCT	Liraglutide, 3mg od	16 weeks	17 (16)	Non-DM	42 (IQR 32-51)	37.2 (IQR 33.6-41.0)	DXA	LBM / FM% / TBW	Undisclosed
McCrimmon et al., 2020	RCT	Semaglutide, 1mg ow	52 weeks	88 (undisclosed)	T2D	57.8 (9.9)	32.6 (6.4)	DXA	LBM / FM / TBW	Body composition
Lundgren et al., 2021	RCT	Liraglutide, 1mg od	52 weeks	41 (23)	Non-DM	43 (12)	32.7 (3.1)	DXA	LBM / FM / TBW	Body weight
Perna et al., 2016	Non-RCT (SG)	Liraglutide, 3mg od	24 weeks	9 (3)	T2D	68.2 (3.9)	32.4 (4.9)	DXA	FFM / FM / TBW	Body composition
Ishii et al., 2019	Non-RCT (SG)	Liraglutide, 0.9mg od	24 weeks	9 (5)	T2D	44.7 (12.2)	37.4 (6.4)	DXA	SMI / FI / BMI	Body composition
Santini et al., 2023	Non-RCT (SG)	Liraglutide, 3mg od	10 months	36 (7)	Non-DM	43.6 (11.6)	40.8 (5.7)	DXA	LBM / FM / TBW	Body weight
Wilding et al., 2021	RCT	Semaglutide, 2.4mg ow	68 weeks	95 (72)	Non-DM	50 (12)	34.8 (3.6)	DXA	LBM / FM	Percentage weight change
Volpe, Lisco, Racaniello, et al., 2022	Non-RCT (SG)	Semaglutide, 0.5mg ow	26 weeks	40 (19)	T2D	64.9 (10.8)	38.8 (7.7)	BIA	SMM / FMI / TBW	Body composition & weight
Volpe, Lisco, Fanelli, et al., 2022	Non-RCT (SG)	Semaglutide, 1mg ow	52 weeks	48 (22)	T2D	57.7 (8.4)	38.8 (8.3)	BIA	SMI / FMI / TBW	Liver function and appearance
Volpe et al., 2023	Non-RCT (SG)	Semaglutide, 7mg od	26 weeks	32 (18)	T2D	66.3 (8.5)	28.2 (3.3)	BIA	FFM / FM / TBW	Body composition
Uchiyama et al., 2023	Non-RCT (SG)	Semaglutide, 14mg od	24 weeks	25 (11)	T2D	54.1 (13.5)	29.3 (3.4)	BIA	LBM / FM / BMI	Body composition
Iepsen et al., 2018	Non-RCT (MG)	Liraglutide, 3mg od	16 weeks	28 (12)	Non-DM	42.8 (10.2)	36.8 (4.8)	DXA	LBM / FM / TBW	Body weight
De Luis et al., 2014a	Non-RCT (MG)	Liraglutide, 1.8mg od	14 weeks	51 (26)	T2D	48.9 (16.3)	34.1 (6.9)	BIA	FFM / FM / TBW	Body weight
De Luis et al., 2014b	Non-RCT (MG)	Liraglutide, 1.8mg od	14 weeks	35 (16)	T2D	47.3 (16.4)	31.8 (5.1)	BIA	FFM / FM / TBW	Body weight
Astrup et al., 2012a	RCT	Liraglutide, 1.2mg od	20 weeks	15 (undisclosed)	Non-DM	47.2 (9.7)	34.8 (2.6)	DXA	LBM / FM	Weight change
Astrup et al., 2012b	RCT	Liraglutide, 1.8mg od	20 weeks	13 (undisclosed)	Non-DM	45.5 (10.9)	35.0 (2.6)	DXA	LBM / FM	Weight change
Astrup et al., 2012c	RCT	Liraglutide, 2.4mg od	20 weeks	15 (undisclosed)	Non-DM	45.0 (11.1)	35.0 (2.6)	DXA	LBM / FM	Weight change
Astrup et al., 2012d	RCT	Liraglutide, 3.0mg od	20 weeks	15 (undisclosed)	Non-DM	45.9 (10.7)	34.8 (2.8)	DXA	LBM / FM	Weight change
Harder et al., 2004	RCT	Liraglutide, 0.6mg od	8 weeks	21 (10)	T2D	59.9 (11.0)	36.8 (4.6)	DXA	LBM / FM / TBW	Undisclosed

Ozeki et al., 2022	Non-RCT (SG, retrospective)	Semaglutide – dose disclosed	3 months	13 (undisclosed)	T2D	52.0 (6.9)	35.9 (6.1)	BIA	SMM / FM / TBW	Body composition
Heise et al., 2023	RCT	Semaglutide, 1mg ow	28 weeks	44 (10)	T2D	63.7 (5.9)	30.8 (3.8)	ADP	FFM / FM / TBW	Beta-cell function & insulin sensitivity
Rondanelli et al., 2016	Non-RCT (SG)	Liraglutide, 3mg od	6 months	28 (12)	T2D	58.8 (9.3)	34.1 (5.5)	DXA	FFM / FM / TBW	Body composition
Jendle et al., 2009a	RCT	Liraglutide, 1.2mg od	52 weeks	23 (11)	T2D	55 (11)	Undisclosed	DXA	LBM / FM / TBW	Body composition
Jendle et al., 2009b	RCT	Liraglutide, 1.8mg od	52 weeks	20 (10)	T2D	54 (9)	Undisclosed	DXA	LBM / FM / TBW	Body composition
Timofte et al., 2015a	Non-RCT (MG)	Exenatide – dose undisclosed	6 months	112 (45)	T2D	58.7 (11.4) / 58.1 (12.1)	37.3 (IQR 33.2-42.4)	BIA	FFM / FM / TBW	Undisclosed
Timofte et al., 2015b	Non-RCT (MG)	Liraglutide – dose undisclosed	6 months	98 (36)	T2D	58.5 (12.6) / 57.5 (11.4)	36.8 (IQR 33.1-41.2)	BIA	FFM / FM / TBW	Undisclosed

Table 3.4.1. Characteristics of studies included in the review. RCT – randomised controlled trial, Non-DM – without diabetes, Pre-DM – prediabetic, SG – single group, MG – multiple group, IQR – interquartile range, BIA – bioimpedance analysis, DXA – dual-energy x-ray absorptiometry, MRI – magnetic resonance imaging, ADP – air displacement plethysmography, SMM – skeletal muscle mass

3.4. Results

3.4.1. Literature search and study characteristics

Initial search of the five databases identified 1222 unique citations, following removal of duplicates. Google Scholar search yielded 15 relevant citations, and manual reviewing of secondary sources provided a further six citations. The full texts of 82 publications were reviewed, of which 44 were excluded at this stage. Thus, 38 studies were included in this systematic review and their characteristics are presented in Table 3.4.1. Four studies did not contain data amenable to meta-analysis, therefore 34 were included in the quantitative synthesis. An overview of the entire study selection process is outlined in Figure 3.4.1.

Of the 38 studies included, 17 were RCTs (including two crossover RCTs), seven were non-randomised multiple-group studies, and 14 were single-cohort studies. The 38 studies included a total of 1735 participants, although the number in each study ranged from five to 169. Thirty-five of the studies reported mixed-gender cohorts, and one study segregated males and females into separate groups. In two studies the gender of participants was not disclosed. Six studies contained more than one intervention group that fulfilled the inclusion criteria, and the data were presented as separate cohorts. Five of the six studies contained two eligible cohorts, and one study contained four. In total, 46 eligible cohorts were included in the review, 41 of which were incorporated into the quantitative synthesis.

Twenty-seven studies included individuals with either T2D or pre-DM, and 24 studies were included in the meta-analysis. Eleven studies containing 15 cohorts were included in the review of studies involving participants without T2D, with ten studies

(14 cohorts) included in the “non-DM” meta-analysis. Of these studies, two had populations that included a mixture of individuals with and without T2D. However, in both cases, the majority of participants were not diagnosed with T2D, the mean baseline HbA1c was in the normal range, and the dose of GLP1RA used is more commonly associated with non-DM weight management. Thus, both studies were included in the non-DM analysis.

In the T2D analysis, four studies investigated Exenatide, two studies investigated Dulaglutide, twelve investigated Liraglutide and eight investigated Semaglutide. One study investigated both Liraglutide and Exenatide in two distinct intervention groups. In the non-DM analysis, only Liraglutide (nine studies) and Semaglutide (two studies) were investigated. Of the studies included in the meta-analysis, 33 reported a measure of MM alongside FM (23 T2D, 10 non-DM), while 29 reported a measure of MM with TBW (22 T2D, 7 non-DM).

3.4.2. Individuals with T2D

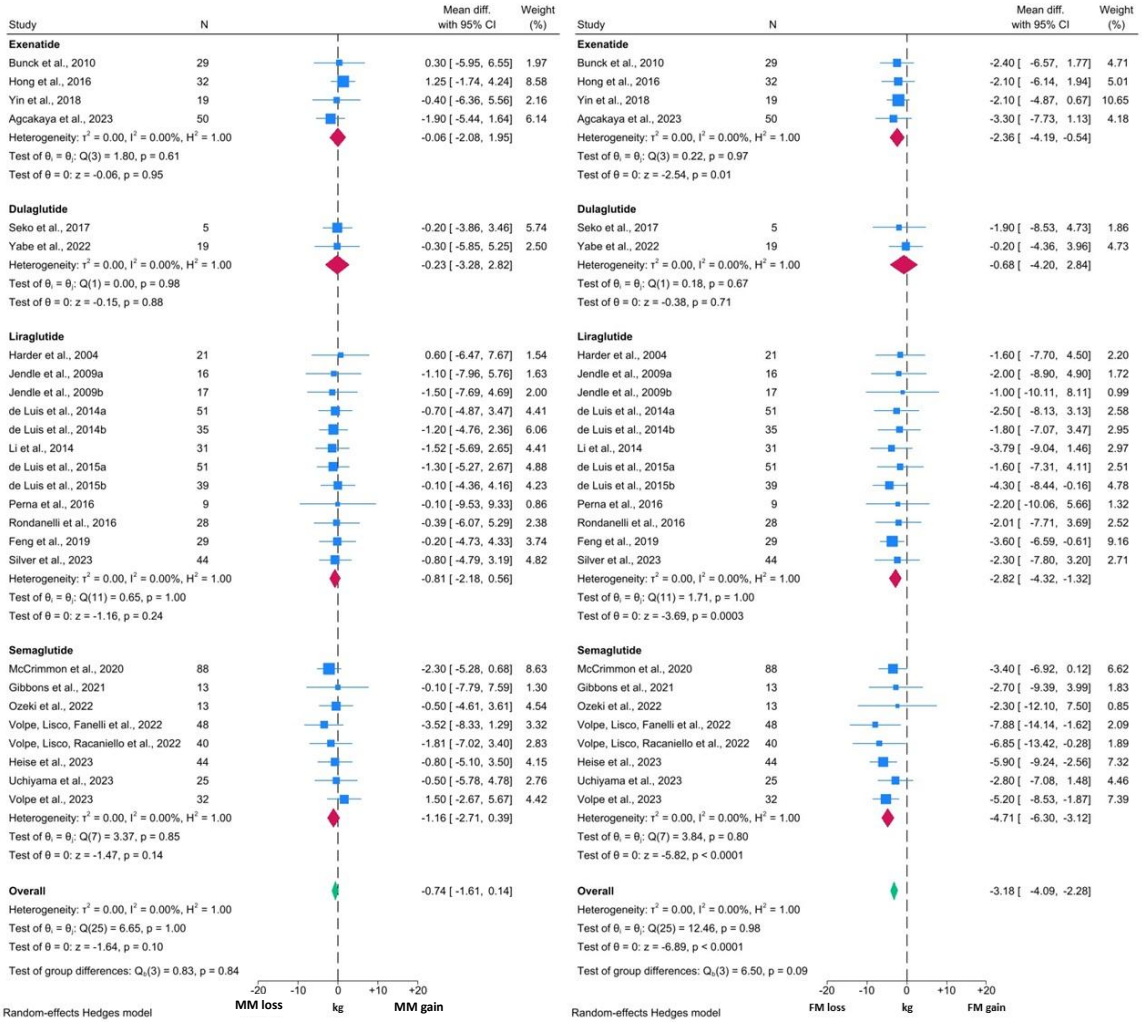

Figure 3.4.2.1. Forest plots showing meta-analysis results of studies reporting both MM measures and FM in individuals with T2D

The 24 studies included in the T2D meta-analysis contained a total of 27 cohorts and 850 participants. Figure 3.4.2.1 presents the comparisons between mean change from baseline in MM and FM for each included cohort, sub-grouped by administered GLP1RA. There was a significant reduction in FM with a WMD of -3.18kg (95% CI: -4.09, -2.28; $p < 0.0001$). In comparison, the WMD for MM was -0.71kg (95% CI: -1.61, 0.14; $p = 0.10$). Thus, despite a significant reduction in FM, a non-significant reduction in MM was observed. This lack of significant change in MM was consistent across the four GLP1RAs that were included in the subgroup analysis. Three out of the four

GLP1RAs (Exenatide, Liraglutide and Semaglutide) elicited significant loss of FM, whilst no significant reduction in FM was observed with Dulaglutide.

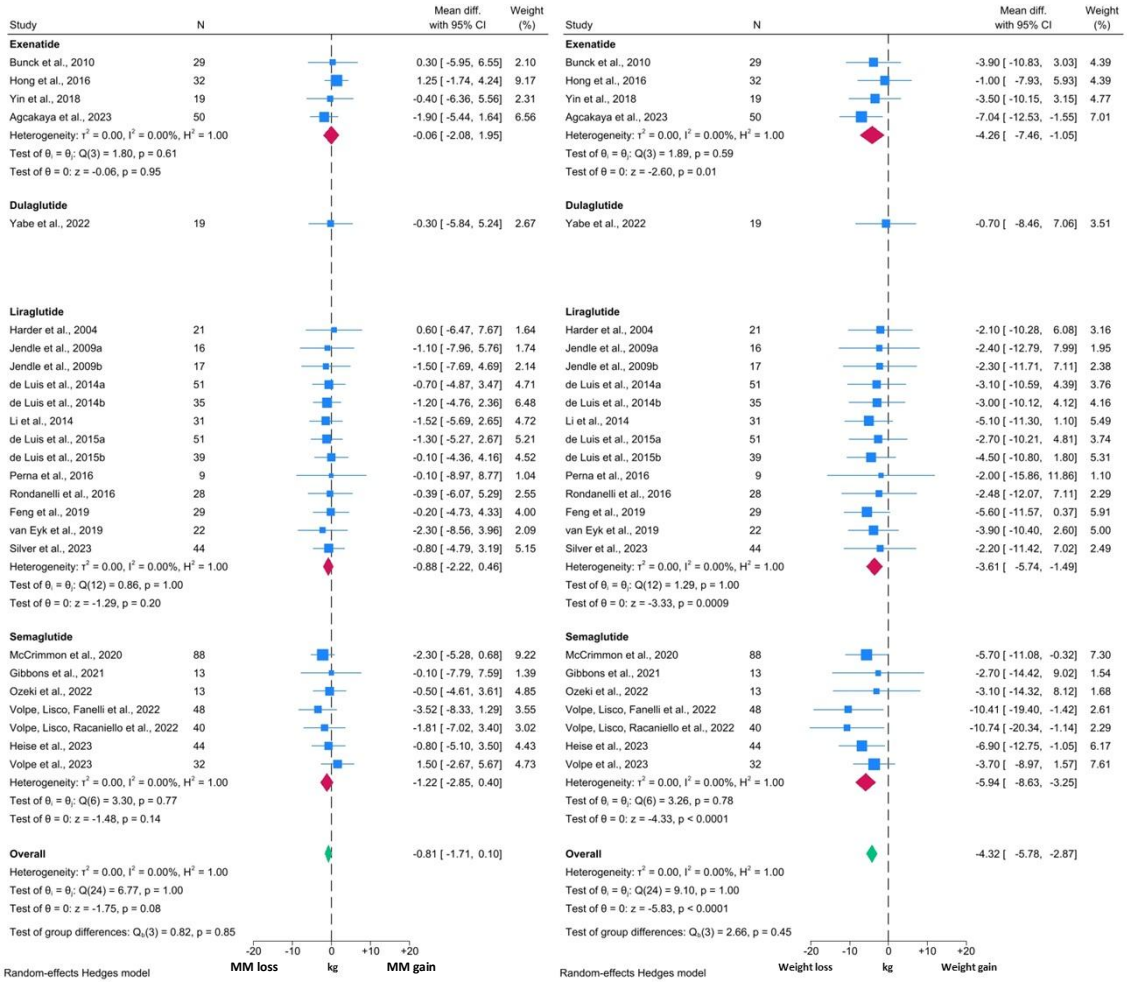

Figure 3.4.2.2. Forest plots showing meta-analysis results of studies reporting both MM measures and TBW in individuals with T2D

The comparison between changes in MM and TBW revealed similar findings (Figure 3.4.2.2). The change in MM from baseline was -0.81kg (95% CI: -1.71, 0.10; p = 0.08) which again was not significant. Total body weight reduced significantly by 4.32kg (95% CI: -5.78, -2.87; p < 0.0001). Thus, MM constituted approximately 18.8% of total weight loss in these studies. The reduction in TBW was significant with Exenatide, Liraglutide and Semaglutide. Only one study investigating Dulaglutide was included in this analysis and the TBW changes were non-significant.

I^2 values across all analyses within these two comparisons were 0.00% suggesting the presence of minimal statistical heterogeneity. However, visual inspection of the forest plots identified likely clinical heterogeneity, as represented by a relative excess in 95% CI range. Further, homogeneity assessments were equivocal for all subgroups ($p > 0.05$). In both MM forest plots, some studies reported numerical increases in MM, with others reporting reductions. Additionally, whilst all studies reported numerical decreases in FM and TBW, there were variations in the degree of observed reduction. In both cases, this appears to be primarily related to the methodological characteristics of each individual study. For example, the two studies published in 2022 by Volpe et al. (Volpe, Lisco, Fanelli, et al., 2022; Volpe, Lisco, Racaniello, et al., 2022) which reported the greatest reduction in FM and TBW, used Semaglutide as the intervention GLP1RA. On the other hand, Yabe et al. (Yabe et al., 2022) investigated Dulaglutide, which is known be less effective for weight loss (Chubb et al., 2021; Viljoen et al., 2019). Additionally, duration of the intervention may have introduced further heterogeneity. The intervention duration in the study by Harder et al. (Harder et al., 2004) was eight weeks, whilst Feng et al. (Feng et al., 2019) administered Liraglutide for 24 weeks, resulting in greater weight loss. Notably, the association between study duration and TBW/FM changes was not always consistent, as the intervention length in the study by Jendle et al. (Jendle et al., 2009) was 52 weeks, which reported similar changes to Harder et al. (Harder et al., 2004).

Three studies could not be included in the meta-analysis, due to lack of amenable data. Kadouh *et al*. reported a non-significant 1.3kg reduction in LBM in the context of a significant 5.8kg weight loss and 2 percentage-point reduction in FM percentage (Kadouh et al., 2020). Timofte *et al*. outlined the effects of both Exenatide and

Liraglutide on body composition of their cohorts (Timofte et al., 2015). In contrast to Kadouh *et al*., this study reported significant reductions in FFM, along with decreased FM and BMI, in both GLP1RA groups (Timofte et al., 2015). This study observed a numerically greater loss of FM than FFM, however there was no comment on the significance of this difference (Timofte et al., 2015). Finally, Diaz-Soto *et al*. reported changes in FM and FFM percentage, with a significant decrease in FM percentage and a corresponding increase in FFM percentage observed following 14 weeks of Liraglutide treatment (Díaz-Soto et al., 2014).

Taken together, these findings suggest that GLP1RAs, when used in the context of managing T2D, induce significant reductions in body weight and FM and a non-significant reduction in LBM. LBM constituted under 20% of the total weight reduction, resulting in a consequent increase in LBM percentage post-intervention.

3.4.3. Individuals without T2D

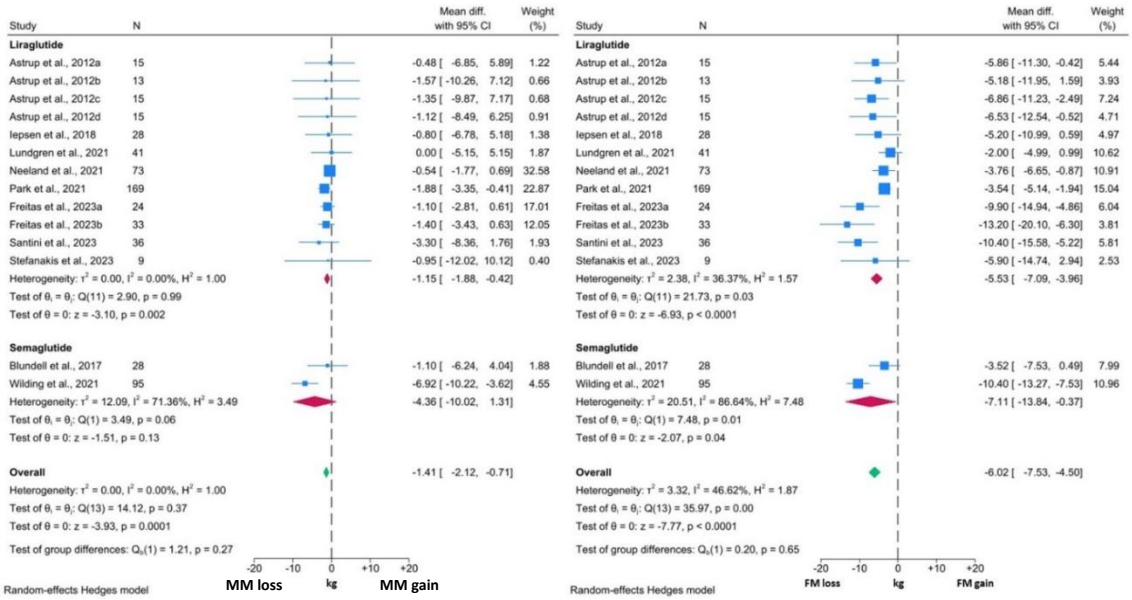

Figure 3.4.3.1. Forest plots showing meta-analysis results of studies reporting both MM measures and FM in individuals without T2D

Ten studies were included in the non-DM meta-analysis, which contained 14 intervention cohorts, and 594 participants. The forest plots for the comparison between MM and FM are presented in Figure 3.4.3.1. Quantitative analysis revealed a significant reduction in both MM and FM among these studies. FM reduced by 6.02kg (95% CI: -7.53, -4.50; $p < 0.0001$), whilst MM reduced by -1.41kg (95% CI: -2.12, -0.71; $p = 0.0001$). Therefore, the reduction of FM was significantly greater than the MM reduction observed in these studies. Only two GLP1RAs were evaluated in this analysis, and the loss of FM was significant across both. Only Liraglutide demonstrated significant reduction in MM, whilst a non-significant reduction was observed with Semaglutide. Notably, only two studies investigated body composition changes with Semaglutide, with substantial heterogeneity between the reported effects.

This heterogeneity is likely due to methodological differences between the studies with Blundell et al. administering Semaglutide 1mg for 12 weeks (Blundell et al., 2017) and Wilding et al. investigating Semaglutide 2.4mg for 68 weeks (Wilding et al., 2021). This was supported by the elevated I^2 values in MM and FM analysis for Semaglutide. Furthermore, FM analysis in the Liraglutide subgroup exhibited moderate to substantial heterogeneity, with evidence of limited homogeneity also observed ($p < 0.05$). The widest variation in FM change was between Freitas et al. (Freitas & Rodrigues, 2023) and the studies by Neeland et al. and Lundgren et al. (Lundgren et al., 2021; Neeland et al., 2021). This was despite very similar methodologies using Liraglutide 3mg for 6 – 12 months. Thus, differences in the clinical features of the populations in these studies are likely to account for the heterogeneity noted in this analysis. Interestingly, this heterogeneity was not reflected in the LBM analysis, suggesting consistency among LBM changes, despite large differences in FM alterations.

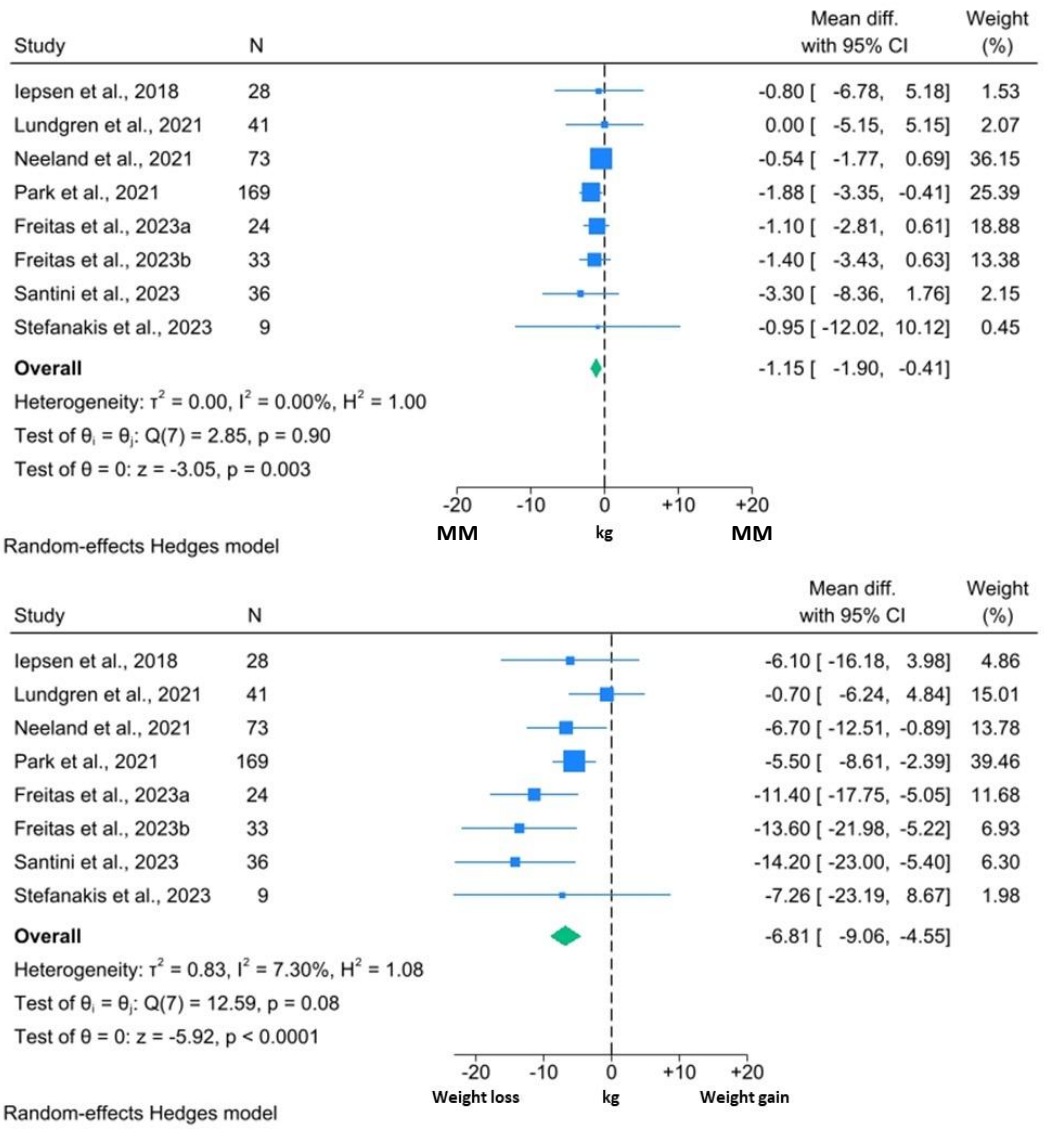

Figure 3.4.3.2. Forest plots showing meta-analysis results of studies reporting both MM measures and TBW in non-DM individuals. Liraglutide was the only GLP1RA assessed in this analysis

Comparisons between changes in MM and TBW were only possible in studies investigating the GLP1RA Liraglutide and these are outlined in Figure 3.4.3.2. Again, both TBW and MM reduced significantly, with Liraglutide inducing a 6.81kg weight reduction (95% CI: -9.06, -4.55; $p < 0.0001$) and a corresponding 1.15kg reduction in measures of MM (95% CI: -1.90, -0.41; $p = 0.003$). Thus, MM comprised of 16.9% of total weight loss experienced by individuals without T2D. The I^2 for the MM forest plot was 0.00%, whilst the corresponding value for TBW was 7.30% suggesting

heterogeneity might not be important. Visual inspection of the forest plot showed variation in weight reduction, which again is likely reflective of clinical differences between the study populations.

One study by Ishii et al. was unable to be included in the meta-analysis (Ishii et al., 2019). This study administered Liraglutide 0.9mg once daily for 24 weeks, and calculated indices of SMM and FM relative to height (Ishii et al., 2019). The authors reported significant reduction in fat index and BMI however, this was associated with a non-significant reduction in skeletal muscle index (Ishii et al., 2019).

Overall, contrary to the results observed in the T2D analysis, GLP1RAs induced significant reductions in MM in individuals without T2D. However, this again was significantly less than the reduction in FM. Comparison between changes in MM and TBW was limited to studies investigating Liraglutide, however similar to the T2D analysis, MM comprised a small proportion of total weight loss in the included studies.

3.4.4. Sensitivity analysis

To assess the impact of including studies that did not report both pre-intervention and post-intervention measures on the results, we performed sensitivity analysis, including only studies that contained a full data set. The results are presented in Appendix III, and were consistent with those from the main analysis, suggesting that inclusion of these studies did not have a significant effect on the observed results.

Secondly, we examined the impact of including non-RCTs by performing analysis including only RCT studies. For the T2D studies, the results were consistent with the main analysis. For non-DM studies, the findings of the MM:FM comparison was again

consistent with the main analysis. This was not the case with the MM:TBW comparison in which, contrary to the main analysis findings, there was no significant change in either parameter. Notably, only two studies were eligible for inclusion in this sensitivity analysis, and their results were markedly heterogenous. The study by Lungren *et al.* (Lundgren et al., 2021) in particular reported minimal changes in LBM and weight. This non-significant change in weight is inconsistent with established literature of GLP1RAs in individuals without T2DM, thus the skewing of this sensitivity analysis by this study accounts for the observed discrepancy.

We also performed sensitivity analysis to determine whether the method of measuring body composition and the measure of MM reported by studies may have influenced the results. Regardless of the three methods of body composition analysis chosen, the changes in MM and FM were consistent with those of the main findings (Table S3). Similarly, whether FFM, LBM or SMM were reported as surrogate measures of MM, the findings were similar in T2D and non-DM studies (Table S4). Thus, these two factors did not significantly influence the results obtained.

3.4.5. Risk of Bias assessments

Quality assessments for RCTs were performed using the Cochrane ROB2 tool and are displayed in Appendix IV. Five of the 15 standard RCT studies were rated as “low” ROB. The remaining 10 studies were classified as having “some concerns”. This is primarily due to concerns noted in domain 5, arising from lack of information expressing a pre-specified plan for analysing body composition outcomes. Two studies did not explicitly state whether body composition assessors were blinded to the allocation of participants, and therefore were rated “some concerns” in domain 4.

Finally, one study had a concern related to domain 1 caused by inability to confirm if the allocation sequence was concealed.

The two crossover RCTs were assessed with the ROB2 tool specific for this type of study. Both studies were rated with “some concerns” in domains 1 and 5, arising from lack of information regarding the performance and concealment of the randomised allocation sequence, and uncertainty about a pre-specified plan for outcome analysis.

The quality of the 14 non-RCT studies was assessed using checklists developed by the Joanna Briggs Institute, which enable an overall judgement of study quality and are also displayed in Appendix IV. Eight of the studies exhibited good quality. The remaining studies exhibited methodological constraints reducing their quality status. Considering global assessment of each study, one (J. Y. Hong et al., 2016) received “no” or “unclear” answers to five out of 10 questions, and therefore was identified as reduced study quality based on this model of assessment (64).

Of the seven multiple-group non-RCT studies, four received a “yes” answer to all relevant questions, suggesting good quality. The remaining three produced “no” answers in at least one question, raising concerns regarding their quality. Nonetheless, all studies received “yes” answers to the majority of questions and therefore overall quality of the included studies was considered acceptable.

3.4.6. Publication bias assessment

Funnel plots and Egger test was performed for T2D and non-DM studies separately. Both funnel plots demonstrated good symmetry around the mean value implying a lack of publication bias (Appendix V). In agreement with this, the Egger test was non-

significant (T2D $p = 0.95$, non-DM $p = 0.48$), providing further evidence of a lack of publication bias within our analysis.

3.5. Discussion

This systematic review and meta-analysis has demonstrated novel findings with regard to the impact of GLP1RAs on MM, dependent on glycaemic status. In individuals with T2D, GLP1RAs induce a non-significant reduction in MM, despite corresponding significant reductions in TBW and FM. In individuals without T2D, a significant reduction in MM was observed, however this was to a significantly lower extent than the associated FM reduction in these individuals. Furthermore, our statistical analysis of both populations revealed that MM comprised less than 20% of the total observed weight loss in the included studies.

T2D is associated with accelerated age-related loss of MM (S. W. Park et al., 2009; S. W. Park, Goodpaster, Strotmeyer, De Rekeneire, et al., 2006), whilst the cycles of weight loss and weight regain among individuals living with obesity predisposes to sarcopenic obesity (Roubenoff, 2004). Both of these pathological states significantly increase the risk of disability and death (Benz et al., 2024; Simpson et al., 2019). Weight loss interventions are a critical aspect of managing T2D and obesity, however concerns exist regarding the associated loss of MM (Cava et al., 2017). Whilst measures such as adjuvant resistance training or protein supplementation have emerged as potential methods of ameliorating MM reduction in this context, interventions that induce weight loss while preserving MM remain highly sought after (Editorial, 2023).

Several authors have hinted at the potential ability of GLP1RAs to preserve MM during weight loss in individuals with T2D (Cetrone et al., 2014; Ma & Chen, 2021; X. Zhang et al., 2021). The authors of a narrative review on this topic concluded that

GLP1RAs and SGLT2-inhibitors (another group of diabetes medications) were associated with reductions in LBM (Sargeant et al., 2019). However, they also highlight that as LBM losses were of a lower magnitude to those observed in FM, participants had a more favourable body composition post-intervention (Sargeant et al., 2019). A network meta-analysis of multiple agents used for T2D management suggested that GLP1RAs, particularly Semaglutide, were associated with loss of FFM, and stressed the importance of considering this when prescribing these medications (Ida et al., 2020).

These findings add objective, systematic evidence to the proposition that GLP1RAs may act to preserve MM in individuals with T2D in the context of weight loss. The present systematic review and meta-analysis is the only one to assess this quantitatively, across a range of GLP1RAs and various study types. This was felt to be essential to ensure the broadest range of information was considered. Sensitivity analysis revealed that similar findings would have been derived if only RCTs were included, adding further strength to the conclusions.

In non-DM studies, while the loss of MM was significant, the mean 1.15kg loss was a small proportion of the total 6.81kg weight loss observed across those studies. This contrasts with bariatric surgery, where a meta-analysis has reported a 9.74kg reduction in LBM in the context of 25.65kg FM loss (Haghighat et al., 2021). This would equate to a greater proportion of total weight loss, although it is important to note that the studies in the analyses performed were not matched. Meanwhile, a systematic review of FFM losses with caloric restriction interventions estimated that 25% of total weight lost was derived from FFM, although this was not quantitively

assessed, and total weight loss from these interventions was not reported (Weinheimer et al., 2010). In a study performed by this research group group, MM accounted for almost 40% of total weight lost following a VLCD intervention in overweight individuals without T2D (Aziz et al., 2022).

Thus, these findings suggest a lower MM proportion of total weight loss with respect to GLP1RAs, compared to bariatric surgery and dietary interventions. This observation implies the presence of a direct muscle-preserving effect specific to GLP1RAs. A detailed exploration of the mechanisms underlying this effect is not within the scope of this review. However, this assertion is supported by the widespread expression of GLP-1 hormone receptors in skeletal muscle cells (Luque et al., 2005). Moreover, this group has also recently reported that myofibrillar MPS under postprandial conditions increased in the presence of GLP-1 hormone infusion, along with a consequent rise in plasma insulin concentrations (Abdulla et al., 2020).

It could be speculated that this effect is mediated by insulin, given that GLP-1 stimulates insulin secretion (Holst et al., 1987), and insulin has been associated with increased MPS (Bassil & Gougeon, 2013; Morais et al., 2018). However, a previous meta-analysis performed by colleagues of mine concluded that insulin does not significantly increase MPS, but does suppress MPB, with an associated increase in net protein balance (Abdulla et al., 2016). Thus, GLP-1 hormone could modulate MPB via insulin-dependent mechanisms, rather than MPS. Furthermore, in a rodent study, the GLP1RA exendin-4 was found to ameliorate muscle wasting and recover MM through direct suppression of atrophic factors and enhancement of myogenic factors (Y. Hong et al., 2019). Taken together, GLP1RAs appear to inhibit MPB directly or indirectly via

insulin, and appear to also stimulate MPS via insulin-independent pathways, consequently leading to an overall protective effect on MM.

The significant loss of FM along with weight loss was not unsurprising and has been observed in previous reviews (Sargeant et al., 2019). A meta-analysis quantitatively demonstrated significant reductions in both visceral and subcutaneous adipose tissue when compared to placebo in individuals with T2D (Duan et al., 2022). Contrastingly, another meta-analysis failed to demonstrate significant reductions in FM with Liraglutide (Aladel et al., 2023). However, only two studies were included in the quantitative analysis, which did not demonstrate significant weight loss, limiting the conclusions that could be drawn (Aladel et al., 2023). The weight loss reported by the present review was comparable to that observed with GLP1RAs in the context of T2D (X. Li et al., 2018; Norris et al., 2009; Ostawal et al., 2016) and obesity (Mehta et al., 2017).

3.5.1. Limitations

This meta-analysis was limited by the lack of placebo-controlled RCTs exploring this issue, necessitating the inclusion of data from non-RCT studies and calculation of pre-post intervention changes. Sensitivity analysis suggested that inclusion of non-RCT did not significantly alter the obtained results. As body composition analysis is usually automated, it is unlikely that lack of randomisation or blinding would have introduced significant methodological bias to the findings.

Mild to moderate heterogeneity was detected within some analyses, which has likely arisen from differences in intervention doses, intervention durations, and clinical characteristics of participants in the included studies. Finally, this review did not

include the exploration of potential mechanisms underlying GLP1RA induced preservation of MM. Therefore, further placebo-controlled studies assessing body composition with similar methodologies would enable the performance of a more rigorous review, as well as further studies examining mechanisms of GLP1RA action on muscle metabolism.

3.5.2. Conclusions

In conclusion, this meta-analysis demonstrated that when used in individuals with T2D, GLP1RAs do not induce a significant reduction in MM, despite significant reductions in weight and fat mass. In individuals with obesity, a significant reduction in MM was observed, however this was significantly less than the corresponding reduction in fat mass. In both cases, MM constituted less than 20% of total weight lost, which appears to be less than the amount reported from bariatric surgery and dietary interventions. The reduction in weight in these studies was comparable to that observed in other studies of GLP1RAs, suggesting our findings are robust. This information is pertinent to clinicians involved in the management of T2D and obesity, however, further studies are required to qualify the reported findings.

4. The effects of caloric restriction interventions on muscle mass – a systematic review and meta-analysis

4.1. Chapter Synopsis

Background: Severe caloric restriction interventions (such as VLCD and LCD) are effective for inducing significant weight loss and remission of T2D. However, suggestions of associated significant MM loss create apprehension regarding their widespread use. This systematic review and meta-analysis aimed to provide a quantitative assessment of the effect of caloric restriction interventions on measures of MM in individuals with, or without, T2D.

Methods: EMBASE, Medline, Pubmed, CINAHL, CENTRAL and Google Scholar were systematically searched for studies involving caloric restriction interventions up to 900 kcal per day reporting any measure of MM, in addition to FM or weight. Studies including populations with a mean age ≥ 40 years and mean BMI ≥ 25kg.m^{-2} were included.

Results: Forty-nine studies were eligible for inclusion, involving 4785 participants. Individuals with T2D experienced significant reductions in MM (WMD -2.88kg, 95% CI: -3.54, -2.22; $p < 0.0001$), although this was significantly less than the reduction in FM (WMD -7.62kg, 95% CI: -10.87, -4.37; $p < 0.0001$). Similarly, MM reduced significantly in individuals without T2D (WMD -2.77kg, 95% CI: -3.29, -2.24; $p < 0.0001$), and again this change was significantly lower than the observed change in FM (WMD -6.91kg, 95% CI: -7.41, -6.41; $p < 0.0001$). MM constituted approximately 25.5% of overall weight loss in individuals with T2D, and 27.5% in individuals without T2D. Subgroup analysis paradoxically revealed greater weight and FM reductions with less restrictive interventions.

Discussion: The results of this review suggests that caloric restriction interventions up to 900 kcal per day are associated with significant reduction in MM, albeit in the context of significantly greater reduction in FM. Furthermore, MM constituted approximately a quarter of the total weight lost. Finally, these data support the use of less restrictive interventions which appear to be more beneficial for weight and FM loss.

4.2. Introduction

Caloric restriction interventions such as VLCD and LCD lead to significant weight loss and improved outcomes in a variety of clinical contexts (Andersen et al., 2022; Bosy-Westphal et al., 2010; Ein et al., 2019; Gudbergsen et al., 2021; Klingberg et al., 2019; Price & Sumithran, 2022; Weinstock et al., 1998; Wolf et al., 2019). As previously noted, VLCD has received particular interest recently as a strategy for managing T2D. Several mechanistic studies have revealed the potential for VLCD to induce diabetes remission (Jackness et al., 2013; Lim et al., 2011; Malandrucco et al., 2012; Taylor et al., 2018). This concept was later confirmed in a large-scale population-based study in which an 825-853 kcal per day intervention induced remission in 46% of participants (M. E. Lean et al., 2018). Although this would not strictly be described as a VLCD, these remarkable results resulted in the UK NHS endorsing the use of a similar programme for management of T2D in England (Diabetes UK, 2018).

Very recently, an analysis of the real-world application of the programme has provided further evidence of its efficacy to induce diabetes remission albeit with a lower rate of success than demonstrated in the research context (Valabhji et al., 2024). These findings have firmly validated the role of caloric restriction interventions in T2D management, and their use is likely to become more commonplace in the future.

However, as with GLP1RA interventions, a potentially considerable drawback of caloric restriction is the associated loss of skeletal MM (Cava et al., 2017). Again, this is particularly pertinent to individuals with T2D due to the accelerated age-related loss of skeletal muscle linked to the condition (S. W. Park et al., 2009). Furthermore,

given the major role that skeletal muscle plays in the regulation of glucose metabolism, excessive losses could harbour deleterious effects on this process. Indeed, lower skeletal MM has been associated with worse glycaemic control (Bower et al., 2017a).

Given the increasing use of caloric restriction interventions for T2D management, a full awareness of the exact effects of caloric restriction on MM is urgently required. Whilst reviews of this topic exist which have suggested significant loss of MM following VLCD (Ardavani et al., 2021; Chaston et al., 2007), these have been narrative in nature with no quantitative synthesis or statistical analysis of the available evidence. Moreover, no review has addressed this topic specifically in individuals with T2D, despite the unique risks associated with MM loss in this population.

This systematic review and meta-analysis was therefore undertaken to examine the effects of interventions involving caloric restriction up to 900 kcal on measures of MM in individuals with T2D. An analysis of these effects in individuals without T2D was also included to provide some comparison and determine whether T2D confers a greater risk of excessive MM loss.

4.3. Methods

This systematic review was conducted in accordance with PRISMA guidance. The methodology and search strategy were outlined in a protocol prior to commencing the review, and published in the International Prospective Register of Systematic Reviews, registration number CRD42024542940.

4.3.1. Search strategy

A systematic search was performed for relevant studies published on the EMBASE, Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials (CENTRAL) databases. The searches were performed on the 9th May 2024 and any article published from inception until that date meeting the eligibility criteria was included. A search of Google Scholar was also performed on the 10th May 2024 to examine for any relevant grey area literature, and any eligible articles identified within the first 10 pages of results were also included.

The following combination of terms was utilised for the overall search strategy: [(obes*) OR (diabet*) OR (overweight)] AND [(low calorie diet) OR (low energy diet) OR (hypocaloric diet) OR (meal replacement) OR (total diet replacement)] AND [(body composition) OR (lean body mass) OR (muscle mass) OR (fat free mass) OR (fat mass)]. These terms were adjusted for each specific database if required, and in the case of EMBASE and Medline, terms were mapped to EMTREE or MeSH subject headings where appropriate. Examples of the search strategy used in specific databases can be found in the Appendix VII.

4.3.2. Study eligibility criteria

Studies were included if they were full articles of an interventional design published in a peer-reviewed journal, involving a VLCD, or LCD up to 900 kcal per day, for at least 4 weeks. Both RCTs and non-randomised studies were eligible for inclusion. We decided to include LCD interventions up to 900kcal as the UK NHS programme uses an 825-853kcal intervention, thus extending the calorie limit makes the findings more relevant to clinical practice. Furthermore, a meta-analysis comparing strict VLCD to low calorie liquid formula diets >800kcal/day observed no significant difference between the amount of weight loss in individuals with T2D (Leslie et al., 2017). The meta-analyses were sub-grouped by caloric restriction, in order to identify and account for any potential heterogeneity that could have been introduced by inclusion of these studies. For the remainder of this review, all included interventions will be described as VLCD.

Included studies were required to report a measure of MM, e.g. FFM, LBM or SMM. FM and weight were listed as secondary outcomes, and data regarding these measures were extracted, although it was not a requirement of studies to report them. At least one experimental group in which VLCD was the only intervention was necessary, and study groups were only included if the mean age was ≥ 40 years and mean BMI ≥ 25kg.m^{-2}. Studies were excluded if they investigated individuals with type 1 diabetes, or pregnant, neonatal, paediatric and adolescent populations. Very-low calorie ketogenic diet interventions, or if the VLCD occurred alongside a concurrent formal exercise programme, these studies were also excluded. Finally, case reports,

conference proceedings or articles published in a non-English language were not eligible for inclusion.

4.3.3. Study selection

Following conduction of searches and removal of duplicates, the titles and abstracts of the results were independently screened by four reviewers. Any non-interventional article that broadly met the inclusion criteria (such as a review) was identified as a secondary source. The reference lists of secondary sources were then evaluated by another reviewer in order to identify further potentially eligible studies. The full texts of all citations from the initial and secondary searches were obtained and independently assessed for eligibility by the same authors, and all articles deemed to meet the stated criteria were included.

4.3.4. Data extraction

Required data from all included studies were extracted and collated into a single spreadsheet (Microsoft Excel). In studies containing more than one intervention group that could be included in the review, data were obtained for each cohort. Information regarding the study type, intervention duration, daily caloric restriction and method of body composition assessment were collected for included studies. Additionally, participant number and characteristics (gender, mean age, mean BMI & diabetes status) were extracted for each included intervention cohort.

For the primary and secondary outcomes, both pre-intervention and post-intervention measures were recorded. If either of these values were not immediately available from the article or any published supplementary materials, corresponding authors were contacted to request the missing data. If despite this the data were not

obtained, then the results of these studies were discussed in the narrative synthesis only. Alternatively, if the data were presented graphically within the article, Plot Digitizer online software (https://plotdigitizer.com/) was used to provide an estimate of the missing values, in accordance with Cochrane guidance (T. Li et al., 2023).

4.3.5. Quality assessment

Risk of bias was assessed using the Cochrane ROB2 tool for RCTs (J. Higgins, Savović, et al., 2023; J. P. T. Higgins et al., 2011). Each study was attributed a rating of "low", "some concerns" or "high risk of bias" based on appraisal of five bias domains (J. P. T. Higgins et al., 2011).

In the case of single-group or multiple-group non-randomised interventional studies, the Newcastle-Ottawa Scale (NOS) was utilised which provides an evaluation of overall study quality based on the responses to eight questions divided into three domains (Wells et al., 2021). Stars were allocated depending on the responses to these questions and a study was deemed "high quality" if it received 7-9 stars, "fair quality" if 4-6 stars, and any study receiving less than 4 stars was rated as "low quality" overall. For single-group studies, as two questions were not relevant to these type of studies, the criteria for each rating was altered as thus: "high quality" 6-8 stars, "fair quality" 3-5 stars, "low quality" <3 stars.

In an analysis where 10 or more studies were included, the presence of publication bias was assessed via generation of a funnel plot for visual inspection of the distribution of results, and performance of Egger's regression test.

4.3.6. Statistical analysis

Statistical analysis was performed using the StataSE version 18.5 (StataCorp, Texas, USA) software package. The WMD was calculated using the pre-intervention and post-intervention mean and SD from each included cohort. Calculations were performed using the random effects Hedges model, as a potential of significant heterogeneity was anticipated due to variations in caloric restriction and population characteristics. Statistical significance was defined as $p < 0.05$ and data are presented as WMD with 95% CI.

In cases where standard error of the mean (SE) or 95% CI was the reported variance value in the article, SD was calculated from these as advised by Cochrane guidance (J. Higgins, Li, et al., 2023). In the situation where only a pre- or post-intervention measure was reported alongside the mean change in the outcome, the required information was requested. If this was not received then the missing measure was calculated using available data, and the corresponding SD was assumed to be equal to that of the reported measure. Sensitivity analysis was performed to determine whether inclusion of these studies and their calculated values significantly altered the observed effect.

Studies were divided depending on the glycaemic status of the intervention groups with separate analysis performed for studies including individuals with T2D (including prediabetes) and those involving individuals without T2D. This enabled comparison of the effect of VLCD on MM between these two populations to establish if differential effects exist. In cases where the cohort was mixed the predominant status of the

participants was established, and the study was included in the corresponding analysis.

Forest plots were generated for each set of populations, with the analysis of individuals without T2D (NDM analysis) further divided into subgroups based on the level of caloric restriction. Subgroups were tested for significant differences if ≥2 data points were present. Each forest plot of change in MM measures was presented alongside the corresponding forest plot for FM or weight changes, in order to enable direct comparisons between the outcome measures, providing greater context to the results.

In addition to the sensitivity analysis described above, additional sensitivity analysis was performed to determine if the reported measure of MM, or the method of assessing body composition significantly affected the observed findings. Study heterogeneity was assessed with visual examination of the generated forest plots, alongside I^2 and Tau statistics (interpreted in accordance with Cochrane guidance).

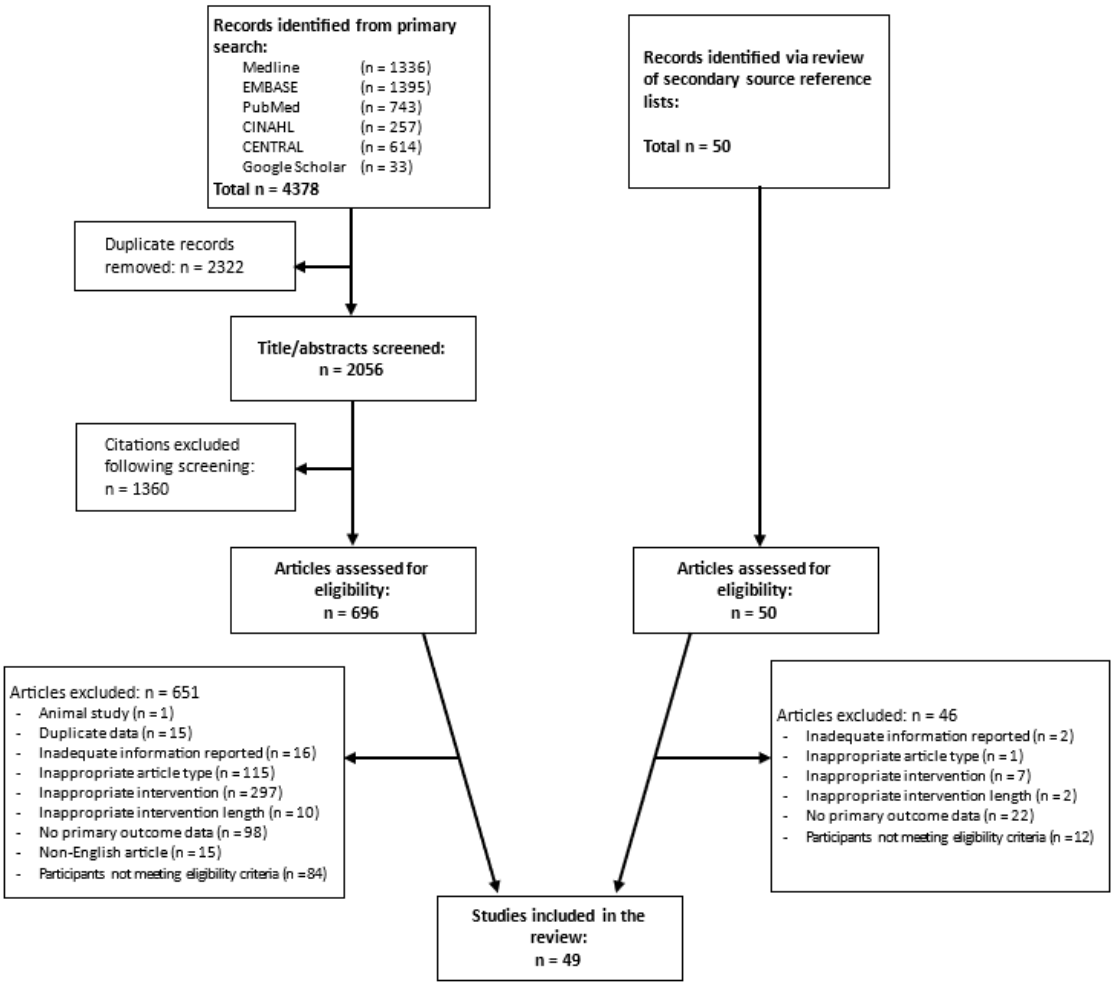

Figure 4.4.1.1. Study selection process overview

4.4. Results

4.4.1. Study characteristics

An overview of the study selection process is summarised in Figure 4.4.1.1. Primary search of the databases and Google Scholar yielded a total of 4378 citations, which was reduced to 2056 following removal of duplications. Screening of titles and abstracts of these citations resulted in exclusion of 1360, thus 696 full texts were reviewed to determine eligibility for inclusion. In addition, 50 potentially eligible citations were identified from secondary source review, and the full texts of these articles were also assessed. Of the 746 articles that were reviewed, 49 were deemed to have met the inclusion criteria and were therefore included in the review. Of this number, 43 were included in the quantitative synthesis, as five did not report data in a format that was amenable to this and one did not explicitly state the daily caloric limit imposed upon participants. The details of the 49 studies included in the review are outlined in Table 4.4.1.1.

Thirty-two articles reported the outcomes of single-group interventional studies, forming the vast majority of the included studies. The remaining studies comprised of 10 RCTs and 7 multiple-group non-randomised interventional studies. Several studies were RCTs by design, however all participants followed an initial VLCD period prior to randomisation to a subsequent intervention. As all participants acted as one intervention group during the period of interest, these were classified as single-group interventional studies for the purposes of this review. Eight studies contained more than one cohort that was eligible for inclusion in the review, with each cohort being

analysed as a separate entity. Thus, 51 eligible cohorts were included in the meta-analysis.

A total of 4785 participants were involved in the studies included in this review. The caloric restriction ranged from 430kcal to 900kcal per day, and the intervention length ranged from four to 16 weeks. In one study the intervention was a 33% caloric restriction which was described as a VLCD. As the specific daily calorie intake limit could not be quantified, this study was not included in the meta-analysis and was therefore reported in the narrative synthesis only. Nine studies involved participants with T2D or prediabetes, whilst the remaining 40 included individuals without T2D. Six study cohorts involving 2451 participants were included in the T2D meta-analysis, while 45 cohorts involving 2110 participants were included in the NDM meta-analysis.

Study	Study design	Daily caloric restriction	Intervention Duration	Sample size (number of females)	Diabetes status	Age mean (SD)	Baseline BMI mean (SD)	Method of body composition assessment	Outcomes of interest measured
Krotkiewski et al., 1990	SGCS	544 kcal	4 weeks	32(32)	NDM	40.1 (2.92)*	36.9 (1.2)*	Potassium estimation	LBM, FM, weight
Van Dale, Beckers et al., 1990a	MGCS	3.0 MJ	4 weeks	6 (all female)	NDM	41 (2.4)*	33.2 (1.0)*	Body density calculation	FM, FFM, weight
Van Dale, Beckers et al., 1990b	MGCS	3.0 MJ	4 weeks	6 (all female)	NDM	41 (1.9)*	33.5 (0.8)*	Body density calculation	FM, FFM, weight
van Dale, Schrijver & Saris, 1990	MGCS	3.0 MJ	5 weeks	15 (all male)	NDM	97.8 (4.8)*	32.0 (2.0)*	Hydrostatic weighing	FM, FFM, weight
Hoie et al., 1993	SGCS	430 kcal	8 weeks	127 (82)	NDM	41.2 (range 18-72)	33.2 (range 25-51)	Infra-red technology	LBM, FM, weight
Carella et al., 1997	SGCS	800 kcal	12 weeks	37 (24)	NDM	42 (8.2)	Female – 37 (6) Male – 43 (4)	BIA	FM, FFM, weight
Borg et al., 2002	SGCS	500 kcal	2 months	90 (all men)	NDM	42.6 (4.6)	32.9 (2.6)	Hydrostatic weighing	FM, FFM, weight
Tomlinson et al., 2004	SGCS	559 kcal (male) / 425 kcal (female)	10 weeks	12 (6)	NDM	49 (range 23-58)	35.9 (0.9)	DXA	LBM, FM, weight
Westerterp-Plantenga et al., 2004a	SGCS	2.1 MJ	4 weeks	148 (gender undisclosed)	NDM	43.8 (10.1)	29.3 (2.5)	Deuterium dilution	FM, FFM, weight
Westerterp-Plantenga et al., 2004b	SGCS	2.1 MJ	4 weeks	148 (gender undisclosed)	NDM	44.5 (10.5)	29.7 (2.6)	Deuterium dilution	FM, FFM, weight
Christensen et al., 2005	RCT	3.4 MJ	8 weeks	96 (35)	NDM	62.6 (11.1)	35.9 (5.1)	BIA	LBM, FM, FFM
Packianathan et al., 2005	SGCS	900 kcal	16 weeks	150 (all female)	NDM	48.5 (8.25)	36.1 (5.62)	ADP	LBM, FM, weight
Vogels & Westerterp-Plantenga, 2005	SGCS	2.1 MJ	6 weeks	133 (gender undisclosed)	NDM	48.1 (9.5)	31.1 (3.7)	Deuterium dilution	FFM, FM, weight
Adam et al., 2006	SGCS	2540 kJ	6 weeks	32(23)	NDM	44 (9)	30.1 (2.6)	Deuterium dilution	FFM, weight
Luscombe et al., 2006	SGCS	3300 kJ	8 weeks	11 (5)	NDM	50 (3)*	34.0 (1.7)*	DXA	LBM, FM, weight
Diepvens et al., 2007a	RCT	2.1 MJ	6 weeks	50 (all female)	NDM	40.3 (9.7)	28.9 (1.7)	Deuterium dilution	FM, FFM, weight
Diepvens et al., 2007b	RCT	2.1 MJ	6 weeks	50 (all female)	NDM	41.2 (9.3)	28.5 (2.2)	Deuterium dilution	FM, FFM, weight
Vogels et al., 2007a	SGCS	2.1 MJ	6 weeks	13 (gender undisclosed)	NDM	48.1 (9.5)	33.7 (4.7)	Deuterium dilution	FFM, FM, weight
Vogels et al., 2007b	SGCS	2.1 MJ	6 weeks	90 (gender undisclosed)	NDM	48.1 (9.5)	30.5 (3.5)	Deuterium dilution	FFM, FM, weight
Belza et al., 2009)	SGCS	812 kcal	8 weeks	33 (17)	NDM	43.0 (10.5)	34.0 (3.1)	BIA	FFM, FM, weight
Claessens et al., 2009	RCT	500 kcal	5 – 6 weeks	48 (18)	NDM	46.0 (2.2) / 45.4 (2.2) / 44.9 (2.0)*	32.4 (1.2) / 32.9 (1.6) / 33.4 (1.0)*	Hydrostatic weighing	FFM, FM, weight,
Hursel & Westerterp-Plantenga, 2009a	RCT	501 kcal	4 weeks	20 (11)	NDM	44 (2)	29.6 (2.1)	Deuterium dilution	FFM, FM, weight
Hursel & Westerterp-Plantenga, 2009b	RCT	501 kcal	4 weeks	20 (11)	NDM	44 (2)	29.5 (2.0)	Deuterium dilution	FFM, FM, weight
Hursel & Westerterp-Plantenga, 2009c	RCT	501 kcal	4 weeks	20 (11)	NDM	44 (2)	29.6 (2.1)	Deuterium dilution	FFM, FM, weight
Hursel & Westerterp-Plantenga, 2009d	RCT	501 kcal	4 weeks	20 (11)	NDM	44 (2)	29.5 (1.9)	Deuterium dilution	FFM, FM, weight
Uusi-Rasi et al., 2010	SGCS	778 kcal	3 months	62 (gender undisclosed)	NDM	42.1 (3.7)	33.3 (3.3)	DXA	FFM, FM, weight
Lim et al., 2011	SGCS	600 kcal	8 weeks	11 (2)	T2D	49.5 (2.5)*	33.6 (1.2)*	ADP	FFM, FM, weight
Munro & Garg, 2011	SGCS	717 kcal	4 weeks	14 (3)	NDM	42 (2)	33.04 (3.17)	BIA	FFM, FM, weight
Rolland et al., 2011	RCT	550 kcal	9 months	14 (gender undisclosed)	NDM	41.9 (6.5)	46.7 (9.0)	BIA	FFM, FM, weight
Schulte et al., 2012	SGCS	800 kcal	12 weeks	23 (15)	NDM	42.8 (2.6)*	44.1 (1.1)*	BIA	LBM, FM, weight

Munro & Garg, 2013	RCT	717 kcal	4 weeks	19 (15)	NDM	47.11 (2.05)*	33.70 (0.83)*	BIA	FFM, FM, weight
Nordstrand et al., 2013	MGCS	900 kcal	7 weeks	91 (57)	NDM	42.3 (9.6)	45.7 (5.5)	BIA	SMM, FM, weight
Soenen et al., 2013	RCT	33% CR	6 weeks	36 (24)	NDM	44 (4)	32 (0.5)	ADP	FFM, FM, weight
Iepsen et al., 2016	SGCS	800 kcal	8 weeks	20 (gender undisclosed)	NDM	43 (9.6)	29.1 (1.9)	DXA	LBM, FM, weight
Ng Tang Fui et al., 2016	RCT	640 kcal	10 weeks	51 (gender undisclosed)	NDM	52.8 (IQR 47.6-60.1)	37.3 (IQR 34.7-41.6)	DXA	LBM, FM, weight
Tam et al., 2016	MGCS	800 kcal	8 weeks	13 (gender undisclosed)	Mixed (majority NDM)	45 (5)	48.8 (3.2)	DXA	FFM, FM, weight
Vink et al., 2016	RCT	500 kcal	5 weeks	28 (15)	NDM	50.7 (1.5)*	31.0 (0.4)*	ADP	FFM, FM, weight
Bucci et al., 2015	SGCS	550 kcal	6 weeks	18 (12)	NDM	42 (6)	34.0 (3.9)	BIA	Muscle mass, FM, weight
Vink et al., 2017	RCT	500 kcal	5 weeks	27 (15)	NDM	50.8 (1.5)*	30.8 (0.4)*	ADP	FFM, FM, weight
Christensen et al., 2018	RCT	810 kcal	8 weeks	2020 (1504)	Prediabetes	51.6 (11.6)	35.4 (6.6)	DXA	FFM, FM, weight
Nymo et al., 2018	SGCS	670 kcal (male) / 550 kcal (female)	8 weeks	31 (13)	NDM	43 (10)	36.7 (4.5)	ADP	FFM, FM, weight
Liljensøe et al., 2021	RCT	810 kcal	8 weeks	38 (27)	Mixed (majority NDM)	65 (range 46-81)	31.6 (range 30.6 – 32.6)	DXA	LBM, FM, weight
Lundgren et al., 2021	SGCS	800 kcal	8 weeks	195 (124)	NDM	42 (12)	37.0 (2.9)	DXA	LBM, FM, weight
Brown et al., 2020	RCT	800-820 kcal	12 weeks	45 (25)	T2D	58.5 (IQR 50.1 – 64.2)	36.6 (5.1)	BIA	LBM, FM, weight
Scragg et al., 2020	SGCS	800 kcal/day	8 weeks	30 (12)	Mixed (majority T2D)	55 (11)	42 (8)	BIA	SMM, FM, weight
Behary et al., 2019	RCT	800 kcal	4 weeks	22 (12)	T2D	47.0 (10.2)	39.1 (4.3)	BIA	Muscle mass, FM, weight
Camps et al., 2019	SGCS	500 kcal	8 weeks	148 (109)	NDM	41 (9)	31.9 (3.0)	ADP	FFM, FM, weight
Näätänen et al., 2021a	RCT	600 kcal	7 weeks	42 (30)	NDM	49.6 (9.5)	34 (2.3)	BIA	FFM, FM, weight
Näätänen et al., 2021b	RCT	600 kcal	7 weeks	40 (31)	NDM	49.1 (9.1)	34.3 (2.7)	BIA	FFM, FM, weight
Ivan et al., 2022	RCT	800 kcal	30 days	40 (20)	T2D	51.83 (1.8)	32.64 (0.8)	BIA	FFM, FM, weight
Jian et al., 2022	MGCS	810 kcal	8 weeks	211 (156)	NDM	54 (95% CI 53-55)	34.1 (95% CI 33.3-34.9)	BIA	FFM, FM, weight
Marples et al., 2022	SGCS	825-853 kcal	12 weeks	35 (15)	T2D	50.4 (10.5)	34.4 (4.4)	BIA	FFM, FM, weight
Sayda et al., 2022	SGCS	800 kcal	6 weeks	10 (all male)	NDM	45.9 (8.3)	32.2 (4)	DXA	LBM, FM, weight
Athithan et al., 2023a	MGCS	810 kcal	12 weeks	15 (4)	T2D	52.5 (5.1)	37.2 (6.8)	DXA	LBM, FM, weight
Athithan et al., 2023b	MGCS	810 kcal	12 weeks	12 (6)	T2D	46.6 (7.0)	36.0 (4.9)	DXA	LBM, FM, weight
Aukan et al., 2023	MGCS	750 kcal	10 weeks	15 (10)	Mixed (majority NDM)	45.5 (2.6)*	39.7 (0.9)*	ADP	FFM, FM, weight
Martins et al., 2023	SGCS	550-660 kcal	8 weeks	70 (41)	NDM	44 (9)	36.3 (4.4)	ADP	FFM, FM, weight
Näätänen et al., 2023a	RCT	600 kcal	7 weeks	51 (36)	NDM	50.5 (9.2)	34.0 (2.4)	BIA	FFM, FM, weight
Näätänen et al., 2023b	RCT	600 kcal	7 weeks	28 (22)	NDM	48.3 (8.6)	34.6 (2.8)	BIA	FFM, FM, weight

Table 4.4.1.1. Details of studies included in caloric restriction muscle mass meta-analysis. RCT – randomised controlled trial, SGCS – single group interventional studies , MGCS – multiple group interventional studies (non-randomised), IQR – interquartile range, BIA – bioimpedance analysis, DXA – dual-energy x-ray absorptiometry, ADP – air displacement plethysmography. * denotes SE

4.4.2. T2D analysis

The results of the T2D meta-analysis are presented graphically in Figures 4.4.2.1 and 4.4.2.2. All but one study (Lim et al., 2011) involved interventions with daily calorie limits between 800-900 kcal and therefore subgroup analysis by caloric restriction was not performed.

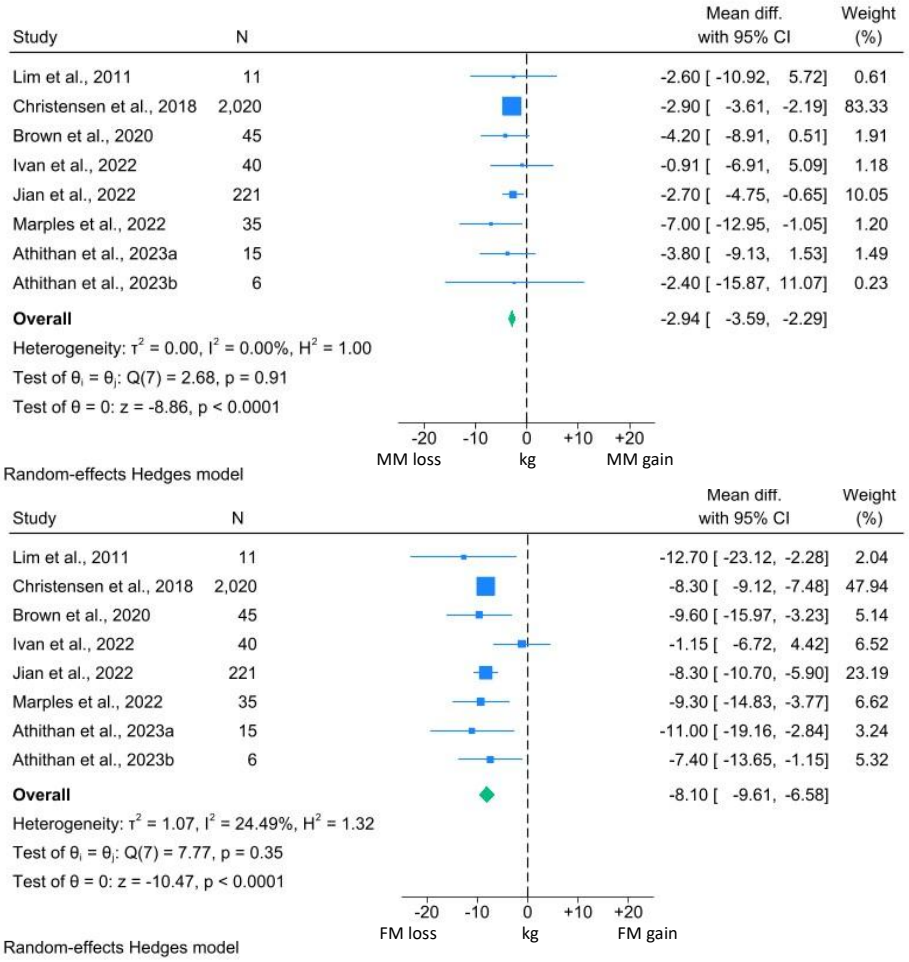

Figure 4.4.2.1. Forest plots showing comparison between change in MM and FM in individuals with T2D

Among the included studies, the interventions induced a significant 2.94kg reduction in MM (95% CI: -3.59, -2.29; $p < 0.0001$). However, this was significantly less than the 8.10kg reduction in FM (95% CI: -9.61, -6.58; $p < 0.0001$). A similar reduction in MM was observed in the studies reporting both MM and weight changes. MM reduced by

2.89kg (95% CI: -3.52, -2.26; p < 0.0001) in the context of a 11.32kg (95% CI: -12.43, -10.22; p < 0.0001) weight loss. Thus, 25.5% of the weight loss experienced by the participants in these studies comprised of MM. Of note, one included study examined changes in body composition within two ethnic groups and did not report any significant differences between them (Marples et al., 2022).

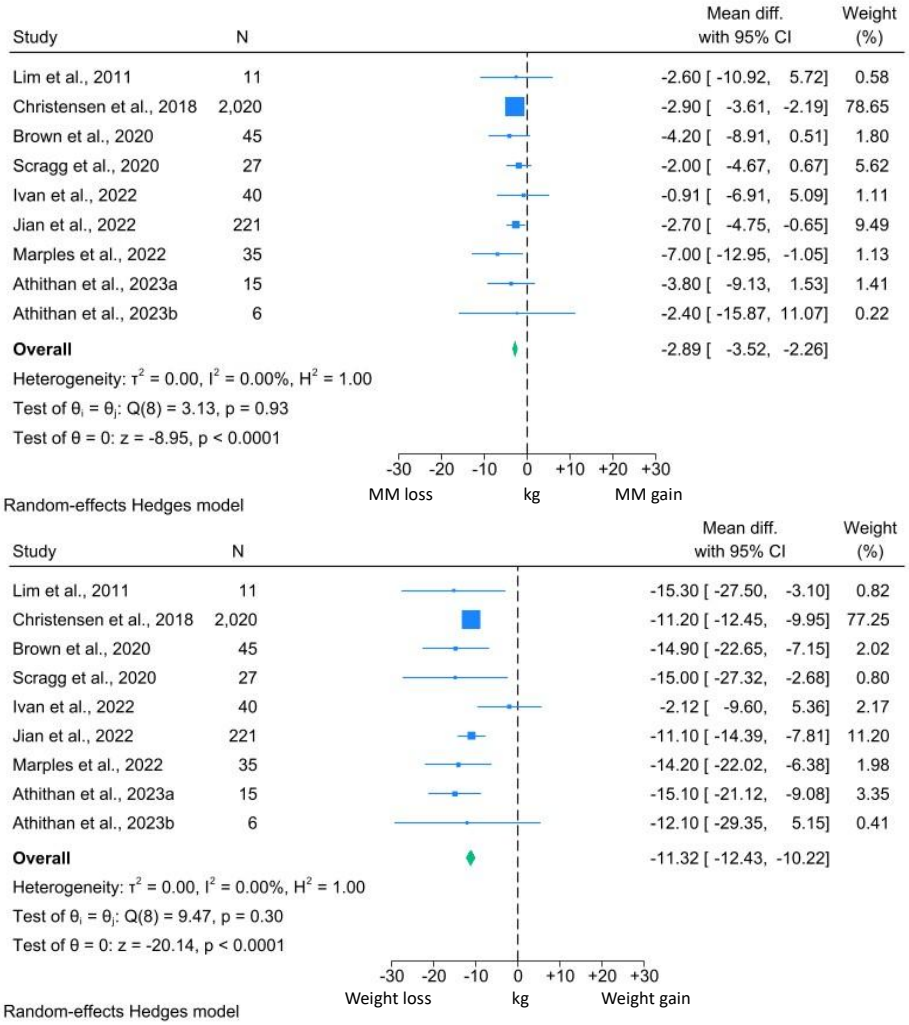

Figure 4.4.2.2. Forest plots showing comparison between change in MM and weight in individuals with T2D

I^2 values in the weight and both MM forest plots were 0.00% suggesting minimal heterogeneity within these data sets. However, I^2 values of 24.5% was suggestive of significant heterogeneity within the FM analysis. From visual inspection of the forest

plots, it can be appreciated that while all studies reported reductions in FM, the reductions in the study by Ivan et al. (Ivan et al., 2022) were uncharacteristically low for both FM and weight. Review of the methodology employed by this study revealed that participants were allowed to independently prepare their intervention diets, rather than total diet replacement more typically used in VLCD. Thus, the minimal weight and FM reduction may reflect issues with compliance to this study's intervention, which may explain the observed heterogeneity.

One study was not amenable for inclusion in the quantitative synthesis but reported a significant 2.9kg reduction in MM, in the context of 8.2kg weight loss over 4 weeks of an 800 kcal per day VLCD (Behary et al., 2019). Of interest, the study by Athithan et al. reported changes in LBM and FM percentage, with participants grouped according to ethnicity (Athithan et al., 2023). LBM percentage significantly increased following the 12-week intervention in both cohorts, alongside significant reductions in FM percentage. Furthermore, there were no significant differences between the changes in each cohort, suggesting that changes in body composition are consistent across ethnic groups (Athithan et al., 2023).

In summary, the studies included in this analysis suggest that significant loss of MM occurs with VLCD in individuals with T2D. However, these losses are significantly less than the reduction in FM and comprise approximately 25% of the overall weight loss, although there is some notable variation in this. As the extent of MM reduction is significantly lower than the corresponding amount of FM, MM percentage experiences a corresponding increase, and this finding appears to be consistent across different ethnicities.

4.4.3. NDM analysis

The forest plots generated from the NDM analyses are presented in Figures 4.4.3.1 and 4.4.3.2. As observed in the T2D analysis, MM reduced significantly by 2.77kg (95% CI: -3.29, -2.24; $p < 0.0001$) and this reduction was significantly lower than that observed in FM (WMD -6.91kg, 95% CI: -7.41, -6.41; $p < 0.0001$). This analysis was sub-grouped by level of caloric restriction with 20 cohorts included in the 400-600 kcal per day group, 15 cohorts included in the 600-800 kcal per day group and the remaining 7 cohorts in the 800-900 kcal per day group. No significant differences were observed between the MM changes in any subgroup (test for group differences $p = 0.50$). However, the test for group differences was significant in the FM analysis ($p = 0.01$), with the 400-600 kcal per day subgroup demonstrating lower reductions in FM (WMD -6.32kg, 95% CI: -7.62, -5.01) than the other subgroups (600-800 kcal WMD -8.78kg, 95% CI: -9.83, -7.74; 800-900 kcal WMD -7.18kg, 95% CI: -8.26, -6.11).

In the comparison with weight loss, VLCD induced a -2.73kg (95% CI: -3.26, -2.29; $p < 0.0001$) change in MM, alongside a -9.92kg (95% CI: -10.67, -9.16; $p < 0.0001$) change in weight (Figure 5). Thus, 27.5% of the total weight lost was attributable to MM, which was similar to the figure observed in the T2D analysis. Overall, similar weight loss and reductions in MM and FM were observed across both T2D and NDM analyses. Again, there was no significant difference in MM changes between the different levels of caloric restriction (test for group differences $p = 0.69$). However, in contrast weight loss did differ significantly between the subgroups (test for group differences $p < 0.05$). The lowest weight reductions were again noted in the 400-600 kcal per day subgroup (WMD -8.62kg, 95% CI: -10.24, -6.99), compared to -11.60kg

(95% CI: -13.25, -9.96) with 600-800 kcal per day and -11.52kg (95% CI: -13.25, -9.78) with 800-900 kcal per day interventions.

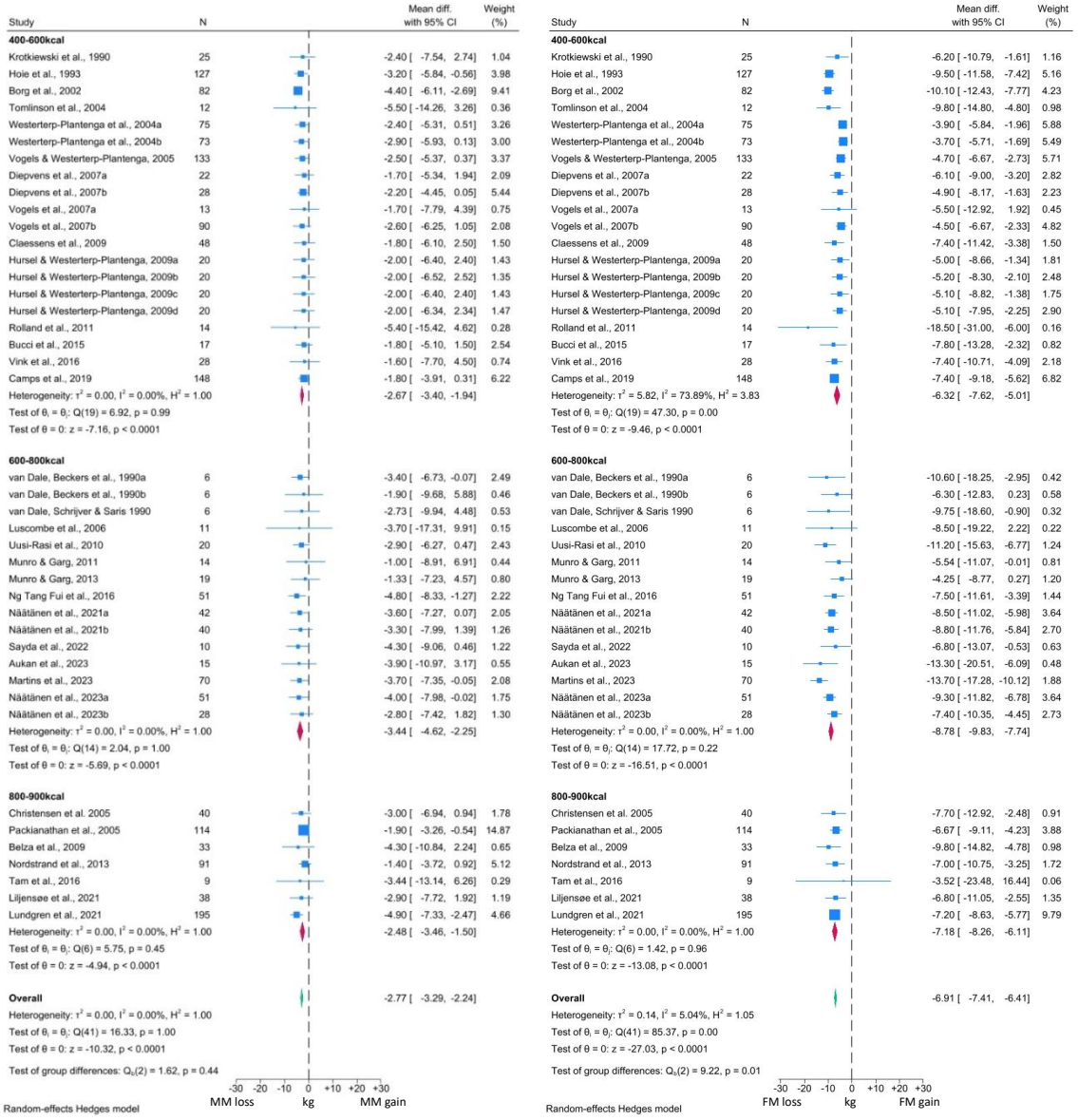

Figure 4.4.3.1. Forest plots showing comparison between change in MM and FM in individuals without T2D

Visual inspection of both MM forest plots and analysis of I^2 values again suggested minimal heterogeneity in MM changes across the included studies. However, the presence of significant heterogeneity was detected among the FM and weight measures, albeit confined to the 400-600 kcal subgroup in both cases. More detailed inspection revealed that the study by Rolland et al. (Rolland et al., 2011) was likely to

be the primary driver of this heterogeneity, due to the greater degree of weight and FM loss reported by this study. Notably, this study subjected participants to three months of 550 kcal per day VLCD, which was longer than any other study in this subgroup. Thus, this methodological difference is the likely reason for the observed heterogeneity. There was also some variation in response noted upon visual inspection of the forest plots, which are likely to arise from clinical differences between the intervention populations of each study cohort.

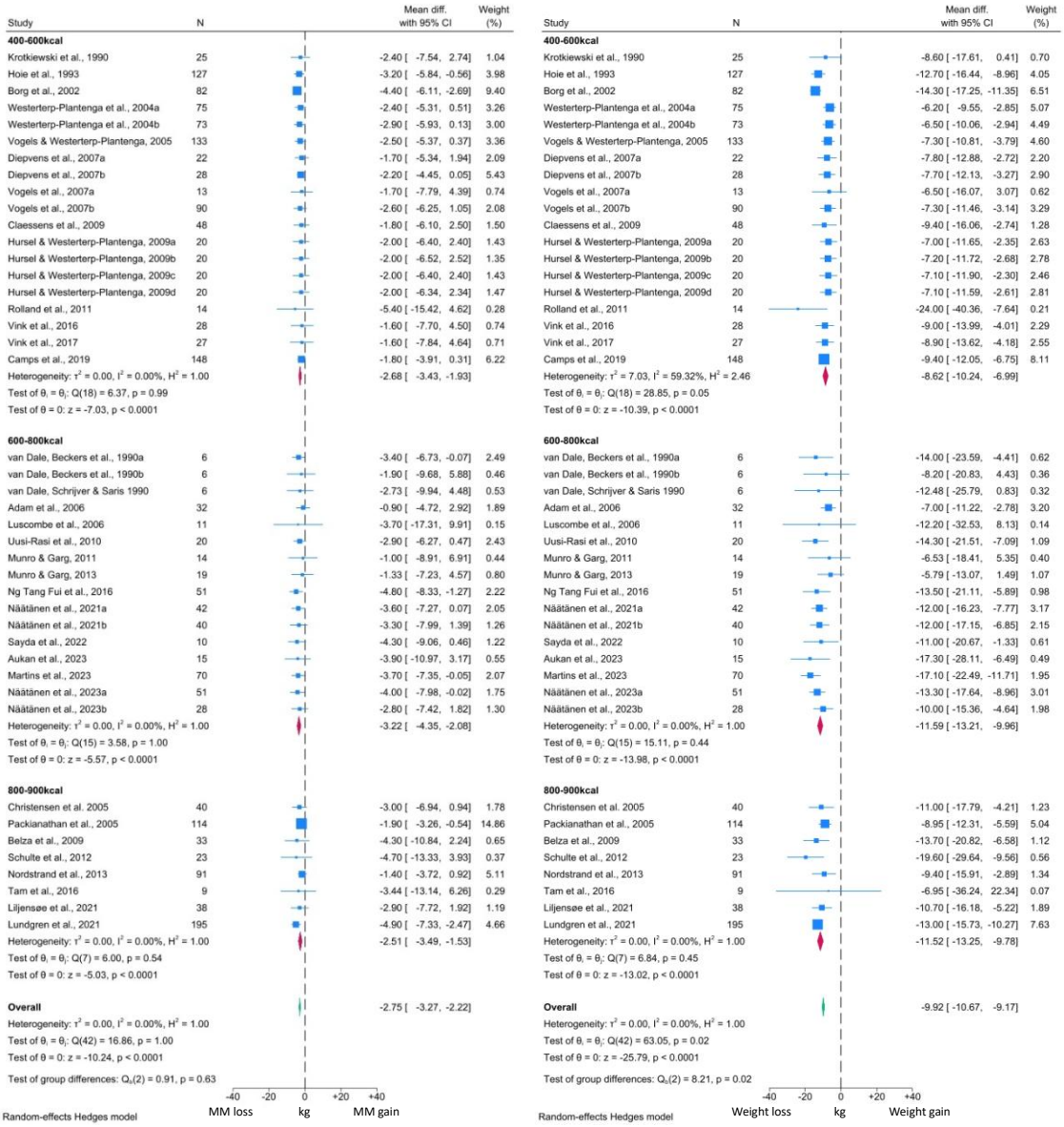

Figure 4.4.3.2. Forest plots showing comparison between change in MM and weight in individuals without T2D

Four studies were not included in the meta-analysis. In keeping with the findings of the meta-analysis, Soenen et al. reported significant reductions in both FFM and FM following 6 weeks of 33% caloric restriction, associated with significant weight loss (Soenen et al., 2013). Similar findings were observed by Nymo et al. in response to 8 weeks of VLCD in 31 individuals (Nymo et al., 2018). Carella et al. also reported numerical reductions in FFM following 12 weeks of VLCD, however there was no comment on the significance of these reductions, and there was no associated report of FM or weight change (Carella et al., 1997). Finally, Iepsen et al. reported changes in LBM and FM percentage in response to 8 weeks of an 810kcal per day intervention (Iepsen et al., 2016). Similar to the findings of Athithan et al. (2023) in individuals with T2D, Iepsen et al. observed significant reduction in FM percentage, with a consequent significant rise in LBM percentage (Iepsen et al., 2016). Thus, the findings of these four studies appear to be consistent with the results of this analysis.

4.4.4. Sensitivity analysis

In order to determine whether inclusion of studies with incomplete data had a significant effect on the review results, sensitivity analysis was performed. Repeat analysis of the data was performed excluding studies which required estimation of post-intervention mean and SD, or those which required estimation of the outcomes from graphs within the published article. Seven studies included in the meta-analysis did not report the full data required (R. Christensen et al., 2005; Marples et al., 2022; Ng Tang Fui et al., 2016; Nordstrand et al., 2013; Tam et al., 2016; van Dale et al., 1990; Vink et al., 2016). As reported in Appendix VII removal of these studies did not significantly alter the observed effects.

Additionally, sensitivity analysis was performed to determine whether the method of body composition assessment used in each study, or the measure of MM reported by studies could have had a significant interaction with the overall result. Neither of these factors demonstrated any differences in the results with any method or measure (test for group differences, $p = 0.33$ and $p = 0.32$, respectively).

4.4.5. Study quality assessment

All but two of the single-group interventional studies received six stars using the NOS. These two studies (Adam et al., 2006; Marples et al., 2022) experienced a high number of participants dropping out of the study with no clear attempt to account for the dropouts and any potential bias this may have introduced into their results. Nevertheless, all studies received a general “high quality” rating overall. Similarly, all multiple-group cohort studies received 8 stars, corresponding to a “high quality” overall rating.

Four out of the 10 included RCTs received an overall low risk of bias rating using the Cochrane ROB2 tool. Five RCTs (R. Christensen et al., 2005; Liljensøe et al., 2021; Soenen et al., 2013; Vink et al., 2016, 2017) were deemed to have some concerns, primarily due to lack of information regarding whether the allocation sequence was concealed until participant recruitment and assignment to their respective intervention. Additionally, in two studies (Liljensøe et al., 2021; Vink et al., 2017), there were concerns noted regarding potential deviations from the intended intervention protocol. One study (Rolland et al., 2011) was rated high risk of bias, due to the presence of significant differences in the baseline characteristics between the

intervention groups, raising the suspicion of an issue with the randomisation process. The quality assessments are displayed in Appendix IX

4.4.6. Publication bias

Less than 10 studies were included in the T2D analysis, therefore publication bias was only assessed within the studies included in the NDM analysis. No evidence of publication bias was detected using either assessment. The funnel plot (Appendix X) demonstrated good symmetry around the mean value and the calculated Egger regression test was non-significant ($p = 0.78$).

4.5. Discussion

VLCD-based interventions are increasing in popularity and an 825-853 kcal per day meal replacement programme is currently recommended for the treatment of T2D in the UK (NHS England, 2020; NICE, 2015b). However, concerns regarding potential significant loss of MM associated with VLCD and similar interventions (Alhamdan et al., 2016; Donnelly et al., 1991; Kreitzman, 1989; National Task Force on the Prevention and Treatment of Obesity, 1993) in clinical populations persists. Despite other comprehensive reviews on this topic (Ardavani et al., 2021; Chaston et al., 2007; Janssen et al., 2023), no quantitative synthesis of the available evidence specific to VLCD-induced changes has been conducted. Furthermore, the effect of VLCD-based interventions in individuals with T2D specifically has never been determined, despite this population being most likely to receive these interventions as part of their routine care

This systematic review and meta-analysis showed that interventions restricting daily energy intake to 900 kcal or less cause significant reductions in MM, in both individuals with T2D, and those without T2D. Expectedly, significant weight loss occurred, and our results demonstrated that approximately 25.5% and 27.5% of the total weight loss was attributable to loss of MM among individuals with and without T2D, respectively. In keeping with this, there was a significantly greater absolute reduction in FM than MM in our included studies. This would result in a significant increase in relative MM percentage following the intervention, which was corroborated by some of the studies discussed in the narrative synthesis (Athithan et

al., 2023; Iepsen et al., 2016). Perhaps most notably, the demonstrated reductions in MM were similar, regardless of the glycaemic status of the included participants.

This lattermost point is particularly relevant given the aforementioned liquid energy diet programme ongoing in the UK. Following positive results in the recently published real-world evaluation of the programme (Valabhji et al., 2024), it is likely that this will continue for the medium to long-term. However, the increased rate of age-related muscle loss associated with T2D (S. W. Park, Goodpaster, Strotmeyer, de Rekeneire, et al., 2006), understandably causes significant apprehension regarding the use of these types of intervention in this population (Sellahewa et al., 2017). The findings of this review suggest that although VLCD-based interventions do cause significant reduction in MM, the concurrent presence of T2D does not appear to further exacerbate this. This is a novel finding and may provide reassurance to clinicians involved in the management of individuals with T2D.

The proportion of weight loss attributable to changes in MM was remarkably similar to the 25% figure reported by Willoughby et al. (Willoughby et al., 2018) suggesting that these findings are robust. Chaston et al. also observed similar proportions when examining the FFM proportion of weight loss across multiple weight loss interventions (Chaston et al., 2007). This compares favourably to the 30-33% proportion of total weight loss observed within the first 3 months following bariatric surgery reported in a recent meta-analysis (Nuijten et al., 2022). It is however important to note that the concurrent weight loss observed over this time-period was not reported, and associations between the proportion of MM loss and rate of weight reduction have been suggested (Chaston et al., 2007).

Although caloric restriction regimes between 800 and 900 kcal per day are not strictly considered as VLCD, the UK NHS programme employs an 825-853 kcal per day caloric restriction, thus inclusion of these studies made these findings more clinically relevant. Intriguingly, no significant difference in the weight loss, and associated change in body composition, was observed between the 800-900 kcal per day and 600-800 kcal per day subgroups. This is in line with the findings of Leslie et al. (Leslie et al., 2017), providing further weight to the argument against the use of unnecessarily restrictive VLCD interventions.

Furthermore, these results appeared to suggest a significant weight loss benefit associated with the higher calorie limit studies, compared to the 400-600 kcal per day interventions. Notably, similar assertions have been made in guidance published over 30 years ago regarding the use of VLCD for treatment of obesity (National Task Force on the Prevention and Treatment of Obesity, 1993). Whilst this appears counterintuitive, this is likely to reflect improved compliance as well as greater ability to sustain the intervention for longer (National Task Force on the Prevention and Treatment of Obesity, 1993). Indeed, the interventions in the most restrictive subgroup were largely between 4-6 weeks' duration, whilst those of the other two subgroups tended to be conducted over 7 weeks or more. Despite the increased weight loss, MM change did not significantly differ, providing further benefit to the use of less restrictive caloric restriction regimes.

Although this review has demonstrated significant reductions in MM associated with VLCD, it is not possible to relate these to changes in muscle strength, or other clinically important outcomes such as disability or frailty. Evidence regarding the

effect of VLCD-induced MM loss on muscle strength is lacking, particularly in the context of T2D. However, one meta-analysis has suggested that VLCD causes significant reductions in knee extensor strength, although there was no concurrent reduction in hand-grip strength (Zibellini et al., 2016). The authors also highlighted a potential relationship between changes in MM and these measures of muscle strength (Zibellini et al., 2016). Thus, in light of these findings, the associated changes in muscle strength following VLCD use in T2D requires further investigation, particularly since individuals with T2D are already at risk of lower muscle strength and greater disability (Kalyani et al., 2010; Nishitani et al., 2011).

In relation to this, cessation of VLCD interventions is typically followed by significant weight regain (Franz et al., 2007; Saris, 2001). It has been suggested that the majority of the weight regained is FM, which could subsequently result in a net reduction in MM, despite weight returning to baseline (Editorial, 2023). This could eventually stimulate deleterious effects on overall metabolic health (Editorial, 2023). In contrast with these assertions, several studies included in this review reported post-VLCD changes in body composition and largely demonstrated that increases in both FM and MM occur (Lundgren et al., 2021; Martins et al., 2023; Vink et al., 2016, 2017; Vogels & Westerterp-Plantenga, 2007). Notably, none of these studies were conducted in individuals with T2D who are known to exhibit increased levels of ectopic fat deposition (Sattar & Gill, 2014), thus may be prone to greater accumulation of fat during post-VLCD weight regain. This represents another area that requires further review.

4.5.1. Limitations

One major limitation of this review was the lack of high-quality RCTs investigating the effect of VLCD on body composition. It is important to recognise that the nature of VLCD interventions generates difficulties in blinding of participants and investigators to the allocated intervention. However, RCTs can still be conducted to investigate the efficacy of this intervention despite these challenges. The majority of articles included in this review were of single-cohort interventional study design. These are inherently prone to bias, although body composition is typically measured via automated processes, which may limit the level of bias that can be introduced into the results of such studies. The initial scoping review conducted during development of the protocol revealed a scarcity of RCTs, thus including all types of studies was necessary to provide some insight into this topic. Conduction of more high-quality RCTs will enable the performance of a much more robust systematic review to address this question.

Additionally, a few studies reported incomplete data, precluding their inclusion in the meta-analysis. Where possible, missing data were calculated from the available data, which contributed to the overall findings. Of note, the sensitivity analysis revealed that exclusion of such studies from the meta-analysis did not significantly alter the results. Significant heterogeneity was detected in some of the analyses performed, primarily resulting from methodological differences between the included studies, along with some likely clinical differences within the studied intervention populations. Finally, as previously mentioned, this review did not examine changes in

strength or risk of disability, which is crucial to understanding the effect of these observed changes to the lives of individuals undergoing these interventions.

4.5.2. Conclusions

In conclusion, this systematic review and meta-analysis has demonstrated that individuals with T2D, VLCD stimulate significant reductions in MM, which comprises approximately 25.5% of the total weight lost, similar to that observed in individuals without T2D. The presence of T2D therefore does not exacerbate the loss of MM during these interventions. Greater weight loss was observed with the less restrictive interventions, without significant changes in MM loss, suggesting that 800-900 kcal per day is adequate for maximising weight loss. Further studies are required to assess the potential impact of this MM loss on strength, physical function and long-term weight maintenance / regain, particularly in individuals with T2D in which evidence is severely lacking. The findings of this review should be of value to clinicians involved in the care of individuals with T2D when making decisions regarding the use of caloric restriction interventions for their management.

5. Study investigating the impact of VLCD, GLP1RA, or combination of the two, on muscle metabolism in individuals with T2D

5.1. Chapter Synopsis

Background: A stable balance between postprandial MPS and fasting MPB is critical to the maintenance of stable MM. Situations such as ageing and disuse result in net loss of MM, resulting from an imbalance between these two processes. Weight loss interventions (such as VLCD and GLP1RA) are also associated with loss of MM, however the mechanisms underlying these losses are not fully understood. Additionally, emerging evidence suggests that GLP1RA may exhibit protective effects over muscle. This study was conducted to increase the understanding of VLCD and GLP1RA induced effects on MPS and MPB, whilst also assessing whether their use in combination may confer additive effects.

Methods: Thirty-one individuals with T2D (age 18-75 years, BMI 27-50kg.m^{-2}) were recruited to the study and randomly assigned to receive Semaglutide (SEM), 800 kcal per day VLCD (VLCD), or both in combination (COMB) for 12 weeks. MPS was measured at baseline and over the initial 6 weeks of the intervention using the oral D_2O method. MPB was assessed at 6 weeks. Food intake and physical activity were also determined at baseline and 6 weeks, while body weight and body composition (via DXA) were additionally measured at 12 weeks.

Results: Significant weight loss occurred in all three groups, with associated significant reductions in LBM and FM. In accordance with the LBM changes, MPS also reduced significantly in all groups, compared to baseline (SEM: 1.22 ± 0.05%/day to 0.79 ± 0.04%/day, $p < 0.0001$; VLCD 1.33 ± 0.08%/day to 0.70 ± 0.05%/day, $p < 0.0001$; COMB 1.24 ± 0.06%/day to 0.71 ± 0.04%/day, $p < 0.0001$). The reduction in MPS was significantly less in SEM than VLCD (-0.44 ± 0.04%/day vs -0.63 ± 0.05%/day,

$p < 0.05$), in keeping with the changes in LBM (SEM -1.7 ± 0.4kg vs VLCD -4.3 ± 0.5kg, $p < 0.01$). Furthermore, change in MPS correlated significantly with change in LBM and weight. MPB did not differ significantly between the groups. There were no significant differences in protein intake or physical activity levels between the groups. Intriguingly, at 12 weeks whilst ongoing weight and FM reductions were observed, LBM remained stable in all groups.

Conclusions: These findings suggest that the reduction in MM associated with weight loss interventions is primarily driven by reductions in MPS. Semaglutide reduced MPS to a lesser extent that VLCD, providing a physiological basis for the potential muscle preserving effects previously suggested. Unfortunately, the study was significantly limited by the lack of a baseline MPB measure, thus a comprehensive assessment of the contribution could not be performed. The lack of change in LBM in the latter 6 weeks of the study, despite ongoing weight and FM losses suggests the presence of counterregulatory mechanisms preventing further MM loss and requires further investigation.

5.2. Introduction

Maintenance of a stable mass of skeletal muscle requires a tightly controlled balance between MPS and MPB. Whilst both processes are constantly occurring MPS typically exceeds MPB in the postprandial state, and the opposite occurs during periods of fasting (Atherton and Smith, 2012). When the postprandial excess in MPS equals the surplus MPB during fasting, overall skeletal MM remains constant (Atherton and Smith, 2012), representing the situation in healthy weight-stable individuals. The precise mechanisms underlying the tight regulation of stable MM remain to be fully elucidated, although it has been postulated that postprandial MPS occurs primarily to replace muscle protein lost for use as substrates for the metabolism of other tissues during fasting (Rasmussen and Phillips, 2003).

Any factor that affects either of these processes can disrupt this existing dynamic equilibrium, and eventually result in long term changes to MM. In the context of exercise, this disruption can be associated with desirable effects, with an increase in MM characteristically observed with physical activity programmes, particularly resistance training (Hughes, Ellefsen and Baar, 2018). Resistance training is well known to be a potent stimulator of postprandial MPS (Wackerhage and Rennie, 2006), and this potentiation effect can endure for up to 72 hours following the exercise bout (Miller et al., 2005). Although MPB also increases following exercise (V. Kumar et al., 2009), the increase in MPS is greater, resulting in a net gain of intramuscular protein (also known as a positive net balance). Repeated bouts of exercise maintain this potentiation of postprandial MPS, and successive periods of

positive net balance following meals ultimately results in gradual accretion of muscle proteins, and thus an increase in MM (Damas et al., 2015).

Contrastingly, several states exist that induce opposing effects of exercise, resulting in a negative net balance, and thus muscle loss. Sarcopenia (age-related muscle loss) is the most obvious and extensively studied example, typically occurring from approximately the fourth decade of life (Mitchell et al., 2012). Whilst exercise induces an exaggeration of postprandial MPS during subsequent meals, this response is blunted in older individuals (Trommelen, Betz and van Loon, 2019; Deane et al., 2021). This reduction in postprandial MPS response with ageing not only occurs following exercise (Wall et al., 2015), but also after normal feeding (Cuthbertson et al., 2005; Moore et al., 2015). Chronic attenuation of MPS has also been demonstrated in older individuals during a long-term exercise intervention (Brook, Wilkinson, Mitchell, et al., 2016), qualifying the findings of the aforementioned acute studies.

In addition to the blunted MPS response, blunted post-prandial inhibition of MPB may also be implicated. This was demonstrated by Wilkes et al. (Wilkes et al., 2009), who observed a significant attenuation of MPB inhibition in response to postprandial levels of insulin under euglycaemic isoaminoacidaemic clamp conditions. This ultimately results in elevated postprandial MPB, which acts to further compound the effect of the observed reductions in postprandial MPS. Notably, it has consistently been shown that fasting MPS and MPB rates are unaffected by ageing (Cuthbertson et al., 2005; Wilkes et al., 2009; Breen and Phillips, 2011; Moore et al., 2015; Wall et

al., 2015), highlighting that ageing exclusively disrupts postprandial net protein balance.

States of muscle disuse have also been extensively studied, due to the recognised contribution of physical inactivity to age-related muscle loss, and the potential effect of disuse in driving muscle loss in critical illness scenarios. Disuse can lead to dramatic reductions in MM, estimated at approximately 0.5-0.6% of total MM per day (Wall, Dirks and Van Loon, 2013). Chronic experimental studies investigating the effect of disuse on MPS have shown significant reductions in overall MPS through the immobilised period (Kortebein et al., 2007; Shad et al., 2019; Brook et al., 2022).

Intriguingly, acute MPS responses to disuse reveal that fasting MPS (de Boer et al., 2007; Glover et al., 2008; Wall et al., 2016), as well as postprandial MPS (Glover et al., 2008) is reduced, contradicting the observations in age-related muscle loss. An increase in fasting MPB has also been suggested, although this remains controversial (Rudrappa et al., 2016). Thus, muscle loss can result from disruption in net protein balance in the fasted or postprandial states, or both. Furthermore, this disruption can arise from either reduced MPS, or increased MPB, although there is more robust evidence for the former in both age-related and disuse-induced muscle loss.

Individuals with T2D experience accelerated rates of age-related muscle loss (S. W. Park et al., 2009), resulting in lower MM (Bower et al., 2017a) and muscle strength (Nishitani et al., 2011) than normoglycaemic individuals. This suggests a exacerbation of the chronic negative net protein balance that exists with natural ageing. Few studies have examined the underlying myocellular metabolic mechanisms contributing to this, with contrasting results.

Early work by Bennet et al. compared protein turnover in young individuals with insulin-dependent diabetes with matched individuals without diabetes, and demonstrated blunted MPS response to high dose amino acid and insulin (Bennet et al., 1991). Similar findings were subsequently reported by two studies published by another group using similar techniques in older individuals (J. Murphy et al., 2015; Morais, Jacob and Chevalier, 2018). However, several studies have not been able to appreciate a significant reduction in MPS between individuals with and without T2D, during fasted (J. A. Bell et al., 2006; Halvatsiotis et al., 2002) and postprandial (Manders et al., 2008; Kouw et al., 2015) conditions.

Furthermore, in a study by Bell et al., individuals with T2D recorded a significantly increased fasted MPB when compared to the control group (J. A. Bell et al., 2006). A study in individuals with or without T2D receiving haemodialysis also noted significant differences in the rate of MPB, which was 85% higher in the T2D cohort than the matched normoglycaemic controls (Pupim et al., 2005). They also were unable to identify any differences in MPS during their experiments which were performed under fasted conditions (Pupim et al., 2005).

It is also important to recognise potential interaction of obesity with these changes observed in individuals with T2D. The vast majority of individuals with T2D live with excess weight (Public Health England, 2014), characterised by a body mass index (BMI) greater than 25kg.m^{-2}. Excess body weight, in particular excess body fat, can exacerbate age-related muscle loss (Benz et al., 2024), and obesity is associated with increased risk of frailty and disability (Monteil et al., 2020).

Indeed, both reduced MPS (Smeuninx et al., 2017) and increased MPB (Guillet et al., 2009) have been associated in obesity without concurrent T2D. One well-designed study examined both MPB and MPS in individuals with obesity and T2D, BMI and age-matched individuals without T2D, and a control group of age-matched lean individuals without T2D, with each measure assessed under fasted and postprandial conditions (J. Murphy et al., 2015). They reported no significant difference in fasted MPS or MPB between any of the groups. However, whilst significant postprandial reductions in MPB occurred in all groups, MPS only increased significantly in the control group. Furthermore, postprandial net balance was numerically lower than controls in both obese groups, with significance only in the T2D group (J. Murphy et al., 2015).

Taken together, inconsistencies exist regarding the molecular mechanisms underpinning the accelerated sarcopenia associated with T2D. Both reduced MPS and increased MPB have been observed, and are therefore likely to both contribute to some extent. The presence of concurrent obesity, common in individuals with T2D, appears to contribute to these processes, although there is possibly an effect of T2D on MM loss that is independent of weight-related factors. Either way, preservation of muscle loss is critical for individuals with T2D and excess weight, especially given the increased risk of disability and frailty associated with both conditions (Benz et al., 2024; S. W. Park, Goodpaster, Strotmeyer, De Rekeneire, et al., 2006).

Weight loss is an essential strategy for the management of T2D and obesity. It results in improved glycaemic control (W. T. Garvey, Umpierrez, et al., 2022; Lingvay et al., 2022) and reduced morbidity from obesity-related conditions (Magkos et al., 2016;

Horn, Almandoz and Look, 2022). The very-low calorie diet (VLCD) is a form of caloric restriction intervention, due its ability to stimulate rapid weight loss (Tsai and Wadden, 2006) and improvements in glycaemic control (Sellahewa et al., 2017), leading to diabetes remission (Das, 2019). Caloric restriction interventions are recommended for the treatment of T2D in the UK NHS (NHS England, 2020), and for achieving rapid weight loss in individuals without T2D in certain situations (Markovic et al., 2022). However, as outlined in Chapter 4, and noted by other authors (Donnelly, Jakicic and Gunderson, 1991; Chaston, Dixon and O'brien, 2007), these interventions are associated with significant losses of MM. Given the aforementioned accelerated sarcopenia associated with T2D and obesity, this could be detrimental in the long term.

GLP1RAs are another weight loss intervention that have been receiving increased attention over the last two decades. They are recommended for the management of both T2D (NICE, 2015b) and obesity (NICE, 2014, 2023a), due to their profound effects on glycaemic control (Sfairopoulos et al., 2018; Lyseng-Williamson, 2019) and weight loss (Madsbad, 2016). Some evidence suggests GLP1RAs could preserve skeletal MM despite weight loss (X. Zhang et al., 2021), and the results of the meta-analysis reported in Chapter 3 support this. However, as previously noted there are contradicting suggestions of significant loss of MM occurring with GLP1RA administration (Sargeant et al., 2019).

Surprisingly, despite these concerns data regarding the specific effects of VLCD and GLP1RAs on MPS and MPB are extremely limited. It is not known whether the loss of MM induced by VLCD (and potentially from GLP1RAs) result from reduced MPS or

increased MPB. This lack of understanding limits the ability to identify methods of mitigating these losses during such interventions, inhibiting the potential to improve the overall outcomes. Therefore, an exploration of the mechanisms underlying these processes is necessary, particularly in individuals with T2D.

This study aimed to shed light on this gap in the literature by investigating the impact of VLCD and a GLP1RA on muscle related outcomes, including MPS and MPB, in individuals with T2D. Furthermore, given the identified potential of GLP1RAs to preserve MM, the effect of combined VLCD and GLP1RA on these outcomes was also assessed, in order to explore a potentially novel approach for attenuating VLCD-induced MM loss.

5.3. Methods

5.3.1. Participant selection, recruitment, randomisation and study procedures

Participant recruitment and eligibility criteria have been described in Chapter 2. Briefly, adults aged between 18-75 years that had been diagnosed with T2D and a BMI between 27-50kg.m^{-2} were recruited into the study. Participants administering exogenous insulin for diabetes management were excluded, due to the risk of hypoglycaemia associated with VLCD in these individuals, and the potential stimulatory effect of exogenous insulin on MPS (R. Gougeon et al., 1998). Participants were recruited from primary and secondary care settings, and once deemed eligible for inclusion in the study, were randomised to receive:

a. the GLP1RA Semaglutide
b. an 800 kcal per day VLCD
c. or combination of both Semaglutide and VLCD

Participants followed the study schedule previously described and therefore received their allocated intervention for 12 weeks. Between 3-7 days prior to commencing the intervention, participants attended the clinical research facility for a preliminary visit (pV1) in order to enable measurement of baseline food intake and physical activity, and obtain baseline plasma and saliva samples. Additionally, the first doses of stable isotope tracers were administered for measurement of MPS. Participants then proceeded to undergo visit 1 (V1) immediately before commencing the interventions, and underwent visit 2 (V2) and visit 3 (V3) after 6 and 12 weeks, respectively.

Participant follow-up and compliance assessment was conducted as previously described.

5.3.2. Study outcomes

The outcomes assessed and reported in this study are as follows:

- MPS at baseline and 6 weeks
- MPB at 6 weeks
- Food intake and physical activity at baseline and 6 weeks
- Weight and LBM changes at 6 and 12 weeks, compared to baseline
- Vastus lateralis (VL) structural changes at 12 weeks, compared to baseline
- Measures of site-specific and global muscle strength at 12 weeks, compared to baseline

Stable isotopic techniques were employed to provide accurate estimations of MPS and MPB. Rate of deuterium incorporation into muscle following oral D_2O solution ingestion enable measurement of MPS. MPB was determined by calculating the rate of D3MH decay in plasma following oral ingestion of a single dose of D3MH solution the preceding day. Tissue enrichment of deuterium was measured by mass spectrometry. Due to the high complexity of the sample processing procedures, and the delicacy of the spectrometer instruments, mass spectrometry was performed by my colleagues Dr. Hannah Crossland and Dr. Paula Scaife, under the supervision of Professor Ken Smith and Dr. Daniel Wilkinson. All other procedures pertaining to this study were performed by me.

5.3.2.1. MPS estimation

The use of D_2O for measurement of MPS has been robustly validated (Wilkinson et al., 2015) and widely cited in publications assessing MPS in health, disease and in response to interventions (Scalzo et al., 2014; Brook et al., 2015; Brook, Wilkinson, Smith, et al., 2016; Camera et al., 2017; Stead et al., 2020; Aziz et al., 2022). This method was selected due to its ability to measure MPS over a period of days to weeks. In addition, it imposes a relatively lower burden on participants, when compared to other techniques which require prolonged periods of multiple infusions.

Initially a baseline plasma sample was collected, enabling calculation of baseline enrichment of deuterium among plasma proteins. This equates to baseline muscle deuterium enrichment, and avoided the need for a baseline muscle biopsy. Following this, a loading dose of D_2O solution was administered to participants. Due to previous anecdotal experience within our research group of significant nausea associated with the initial D_2O dose, the dose was administered in several 50ml aliquots, each spaced 1 hour apart. The first aliquot was administered during pV1 and participants consumed the remaining aliquots at home with a written schedule provided to ensure the aliquots were ingested at the correct time.

At V1, the first muscle biopsy was performed to obtain a fresh muscle sample. This sample was subsequently analysed for deuterium enrichment of muscle alanine allowing comparison between baseline plasma enrichment to determine pre-intervention MPS. The weekly top-up doses of D_2O solution were administered to maintain stable body enrichment of deuterium over the following 6 weeks. Saliva samples were obtained immediately before, and two hours after each D_2O dose to

measure trough and peak enrichment levels, respectively. At V2, a second muscle specimen was obtained for determination of deuterium enrichment, and the difference in enrichment between this and the V1 muscle specimen provided a measure of MPS during the 6 weeks of the intervention.

In order to determine body water deuterium enrichment levels, 0.1µl of each saliva sample was injected into a high-temperature conversion elemental analyser connected to an isotope ratio mass spectrometer (IRMS). The samples were analysed against a standard curve of samples with known D_2O enrichment. Following high-temperature conversion into hydrogen gas the ratio of deuterium:hydrogen was determined. Atom percentage was subsequently determined using a standard formula (Wilkinson et al., 2014) and then converted to an atom percent excess (APE) value corrected for background enrichment.

Analysis of muscle samples for deuterium enrichment of alanine began with homogenization of the muscle tissue in a pre-specified buffer mixed with a protease inhibitor cocktail. The homogenate was then rotated for 10 minutes before centrifugation to enable removal of the supernatant. This pellet was then solubilized in sodium hydroxide and centrifuged again to separate the myofibrillar and collagen fractions. The supernatant was then extracted and myofibrillar proteins were precipitated using perchloric acid, before washing with ethanol to remove any remaining non-myofibrillar proteins.

This purified collection of myofibrillar proteins were broken down into their constituent amino acids by overnight incubation with hydrochloric acid in a Dowex H^+ resin slurry. They were then washed with ammonia to release them from the resin,

and evaporated to dryness. Subsequently, the amino acids were derivatized into their n-methoxycarbonyl methyl esters and then extracted into 100µl of chloroform. Finally, the samples were analysed using gas chromatography-pyrolysis-isotope ratio mass spectrometry (GC-Pyr-IRMS) alongside a standard curve of known L-alanine enrichment. Atom percentage was calculated and converted to APE using the same process as with salivary enrichment described above.

The D_2O enrichment of plasma alanine was also used to determine baseline MPS. Initially, 200µl plasma was mixed with 100% ethanol to precipitate large plasma proteins. Following centrifugation, the supernatant was then evaporated to dryness before addition of hydrochloric acid to enable removal of the lipid fraction. The remaining amino acids were then derivatized as above and then analysed using GC-Pyr-IRMS with a standard curve of known L-alanine concentrations.

Once APE was determined for the plasma, saliva and muscle samples, MPS was calculated using the following equation (Wilkinson et al., 2014):

$$FSR = [(APE_{Ala})] / [(APE_{P}) \times t] \times 100$$

APE_{Ala} is calculated as the difference in alanine enrichment between the two muscle samples (or the muscle and plasma samples in the case of baseline FSR). APE_P is the mean precursor enrichment over the period between the analysis timepoints (t). FSR is then reported as %/day.

5.3.2.2. MPB measurement

Due to concerns regarding the risk of exposing participants to an excessive burden of research activities, and the potential implications this could have on recruitment, it

was decided that MPB would only be assessed once at 6 weeks into the intervention. It was assumed that MPB would be similar between the three groups at baseline, therefore differences detected at this timepoint would highlight changes induced by the interventions. Furthermore, previous literature exploring protein balance changes during weight loss interventions suggested a more significant effect on MPS, than MPB (C. H. Murphy et al., 2015; Hector et al., 2018), thus making this the primary focus of the study given the aforementioned limitations.

MPB was determined by obtaining plasma samples at 40-minute intervals for a period of 4 hours. This occurred 24 hours after ingesting a solution of 3MH labelled with deuterium (D3MH). Plasma samples were subsequently analysed by GC-Pyr-IRMS using a similar process to that described above for alanine analysis. The rate of dilution of deuterium-labelled 3MH with non-labelled (endogenous) 3MH enabled calculation of endogenous MPB. This method has also been confirmed to be robust when employed for this purpose (Sheffield-Moore et al., 2014; Crossland et al., 2020; Cegielski et al., 2021).

5.3.2.3. Assessment of muscle structure

VL muscle structure was examined using B-mode ultrasonography while participants were laid in the supine position. The belly of the VL was identified by measuring its entire length and noting the midpoint of this line. Muscle CSA was determined along the mid-line using panoramic image rendering between the medial and lateral borders. Muscle thickness was also measured along the longitudinal axis of the muscle by aligning the ultrasound probe along the fascicle plane at the VL midpoint. At least three images of each parameter were saved for subsequent analysis which

was performed using Image J software (National Institute of Health, Maryland, USA). As this study was focussed on the effects of the interventions on whole-body skeletal muscle protein turnover, mass and function, rather than microcellular structure, histological analysis was not performed.

5.3.2.4. Other outcomes

Body weight was measured on the same set of digital scales which were calibrated for accuracy regularly. Lean body mass was determined using DXA scanning, which produced an automated report of body composition measures. Participants were instructed to self-report food intake prior to commencing the intervention and at 6 weeks, prior to attending for V2. They were given the choice of using a digital application (Nutracheck), or a paper diet diary. The digital diary calculated macronutrient composition of the recorded food and provided an automatically generated report of daily energy and macronutrient intake. For analysis of the paper diaries, recorded food items were manually transcribed into analysis software (Nutritics, Dublin, Ireland) which calculated the required data. Physical activity was assessed by monitoring step count, distance travelled and calculated active energy expenditure using FitBits (Google, California, USA) worn by the participants for up to 4 days.

5.3.2.5. Statistical analysis

Data are presented as mean ± SE – or median (IQR) if non normally distributed – and significance was determined as $p < 0.05$. Normality of data was tested by visual inspection of the generated frequency histogram, and statistically with the Kolmogorov-Smirnov test. Differences between pre-intervention and post-

intervention measures for each outcome in the three groups were assessed for significance using two-way analysis of variance (ANOVA). For some outcomes, the absolute change was elucidated, and one-way ANOVA (or a non-parametric alternative if required) was employed to assess for between-group differences. Correlation between two outcomes was assessed by calculating the Pearson r correlation coefficient (unless otherwise stated), again $p < 0.05$ was determined to be significant. All analyses were performed in GraphPad Prism (La Jolla, USA).

5.4. Results

5.4.1. Participant characteristics

Overall, 44 individuals were screened for inclusion in the study and 32 were deemed eligible for recruitment into the study. Following randomisation, 11 were allocated into the Semaglutide only (SEM) and combined VLCD + Semaglutide (COMB) groups, with 10 allocated to received VLCD only (VLCD). One participant in SEM developed significant intolerance to the medication after the first and second doses, rendering her unable to continue with the study and she was thus excluded. Another participant in COMB completed the majority of the intervention period, however developed COVID-19 infection just before V3 was due. Therefore, he was unable to complete the study visit, however as he completed V2, his data has been included in some analyses. Baseline values of all recruited participants are presented in Table 5.4.1.1.

	SEM (n = 11)	**VLCD** (n = 10)	**COMB** (n = 11)	
Gender	5M / 6F	7M / 3F	5M / 6F	
Age (years)	62.4 ± 3.0	51.6 ± 4.2	56.8 ± 2.6	ns
BMI (kg.m^{-2})	36.1 ± 1.6	39.9 ± 2.0	37.0 ± 2.0	ns
HbA1c (mmol/mol)	62.0 (54.0 – 64.0)	55.0 (48.5 – 73.3)	56.0 (48.0 – 70.0)	ns
LBM (kg)	54.6 ± 3.1	64.6 ± 2.6	55.7 ± 3.4	ns
LBM %age	54.0 ± 2.2	54.1 ± 2.0	53.8 ± 2.1	ns

Table 5.4.1.1. Baseline characteristics of all included participants. Data reported as mean ± SE or median (IQR). Significant differences assessed using one-way ANOVA or Kruskal-Wallis tests

Two participants were on prescribed anticoagulant medication for pre-existing medical conditions, so were unable to undergo muscle biopsies due to the risk of excessive bleeding. Additionally, one participant developed significant adverse symptoms during her first biopsy and it was felt inappropriate to continue with this,

or perform future biopsies. Consequently, MPS data were not available for these participants, resulting in 28 participants (SEM 9, VLCD 9, COMB 10) being included in the MPS analysis.

5.4.2. Baseline to 6-week weight and body composition changes

As highlighted previously only one participant dropped out between V1 and V2, thus 31 participants are included in this analysis. Table 5.4.2.1 summarises the mean body weight, body composition, food intake and physical activity measurements at baseline and 6 weeks in each group.

	SEM (n = 10)			**VLCD** (n = 10)			**COMB** (n = 11)		
	Baseline	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**
Weight (kg)	103.2 ± 4.1	99.1 ± 4.1	<0.001	121.6 ± 5.9	112.3 ± 6.6	<0.0001	105.2 ± 6.9	96.6 ± 6.4	<0.0001
BMI (kg.m^{-2})	35.0 ± 1.6	33.7 ± 1.7	<0.001	39.6 ± 2.0	36.6 ± 2.2	<0.0001	36.4 ± 1.9	33.4 ± 1.8	<0.0001
LBM (kg)	55.9 ± 3.1	54.1 ± 3.1	<0.01	64.6 ± 2.6	60.4 ± 2.6	<0.0001	55.7 ± 3.4	53.0 ± 3.2	<0.0001
FM (kg)	43.5 ± 3.2	41.4 ± 3.3	<0.05	52.3 ± 4.2	47.2 ± 5.0	<0.0001	46.0 ± 4.6	40.5 ± 4.3	<0.0001

Table 5.4.2.1. Mean anthropometric, energy intake and physical activity measures before and after the intervention period, in each group. Significant differences assessed using two-way ANOVA

Body weight reduced significantly in all three groups in the first 6 weeks of the intervention period. Semaglutide reduced body weight by 4.0 ± 0.5kg, whilst participants in the VLCD and COMB groups experienced mean weight loss of 9.2 ± 1.1kg and 8.6 ± 0.9kg respectively. Thus, VLCD and COMB stimulated significantly greater weight loss than SEM (SEM v VLCD $p < 0.001$, SEM v COMB $p < 0.01$). This is depicted graphically in Figure 5.4.2.1. BMI also followed this trend with significantly

greater reduction in VLCD (3.0 ± 0.4 kg.m^{-2}) and COMB (3.0 ± 0.3 kg.m^{-2}) than SEM (1.3 ± 0.1 kg.m^{-2}, p < 0.001 v both).

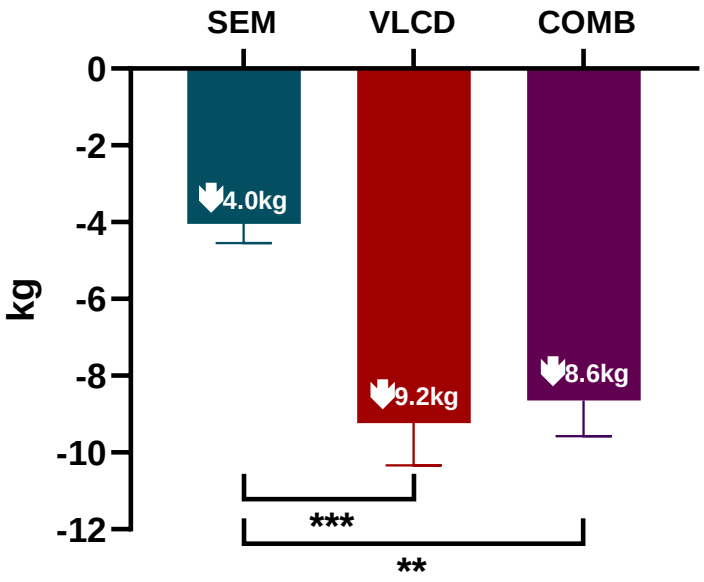

Figure 5.4.2.1. Mean weight loss in each group. Significant differences assessed with one-way ANOVA. ** p < 0.01, *** p < 0.001

In keeping with this, all groups experienced significant reductions in both LBM and FM. LBM reduced by 1.7 ± 0.4kg in SEM, 4.3 ± 0.5kg in VLCD and 2.7 ± 0.5kg in COMB (see Figure 5.4.2.2). The reduction in VLCD was significantly greater than that elicited by SEM (p < 0.01), however whilst COMB experienced a numerically greater LBM reduction, this did not reach significance (p = 0.066).

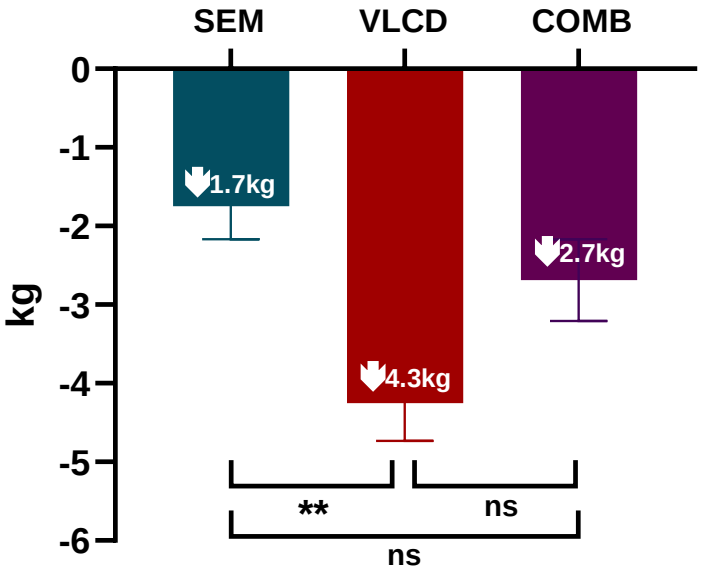

Figure 5.4.2.2. Mean change in LBM in each group. Significant differences assessed with one-way ANOVA. ** p < 0.01

FM reductions in these participants (Figure 5.4.2.3) followed a similar pattern to weight loss, such that similar reductions were observed in VLCD and COMB, which were both significantly greater than SEM (SEM v VLCD $p < 0.05$, SEM v COMB $p < 0.01$).

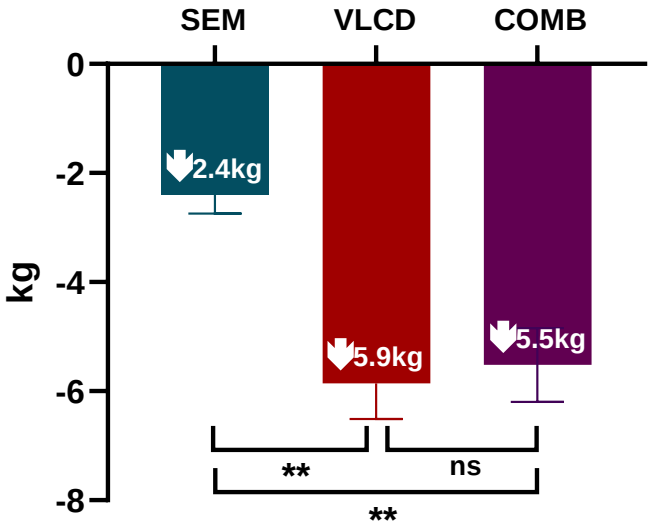

Figure 5.4.2.3. Mean change in FM in each group. Significant differences assessed with one-way ANOVA. ** p < 0.01

5.4.3. Assessment of energy intake and physical activity

Dietary analysis revealed expected reductions in energy intake (Table 5.4.3.1), which suggested good compliance with the interventions in all groups.

	SEM (n = 10)			**VLCD** (n = 10)			**COMB** (n = 11)		
	Baseline	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**
Total Energy Intake (kcal)	1666.6 ± 246.4	1130.0 ± 101.3	<0.01	1893.4 ± 149.5	717.9 ± 35.4	<0.0001	1787.3 ± 156.8	636.7 ± 35.4	<0.0001
Protein intake (g)	78.8 ± 13.9	51.8 ± 5.0	<0.05	85.4 ± 7.3	57.4 ± 3.3	<0.01	80.8 ± 8.5	47.6 ± 3.4	<0.01
Fat intake (g)	67.5 ± 12.4	45.2 ± 6.3	<0.05	71.4 ± 7.8	19.8 ± 0.9	<0.0001	69.1 ± 6.7	17.7 ± 1.3	<0.0001
Carbohydrate intake (g)	168.9 ± 23.3	121.1 ± 11.7	<0.05	196.2 ± 14.9	70.8 ± 3.7	<0.0001	201.7 ± 23.3	69.0 ± 3.9	<0.0001
Calculated energy expenditure (kcal)	2783.4 ± 172.0	2543.6 ± 183.1	0.38	3342.7 ± 247.1	3187.7 ± 223.4	0.72	2929.5 ± 205.7	2759.3 ± 178.3	0.62
Total daily steps	6651 ± 1024	5628 ± 1087	0.41	6031 ± 1632	6927 ± 1201	0.52	6144 ± 841	5414 ± 776	0.64
Daily distance travelled (km)	4.8 ± 0.8	4.0 ± 0.8	0.41	4.4 ± 1.2	5.2 ± 0.9	0.41	4.4 ± 0.6	3.9 ± 0.5	0.61

Table 5.4.3.1. Mean energy intake and physical activity measures before and after the intervention period, in each group. Significant differences assessed using two-way ANOVA

Total energy intake measured at 6 weeks was 717.9 ± 35.4 kcal and 636.7 ± 35.4 kcal in VLCD and COMB, respectively, which was well within the 800 kilocalorie per day limit (Figure 5.3.3.1a). Participants in SEM ingested a mean 1130.0 ± 101.3 kcal per day which was also significantly lower than their baseline energy intake.

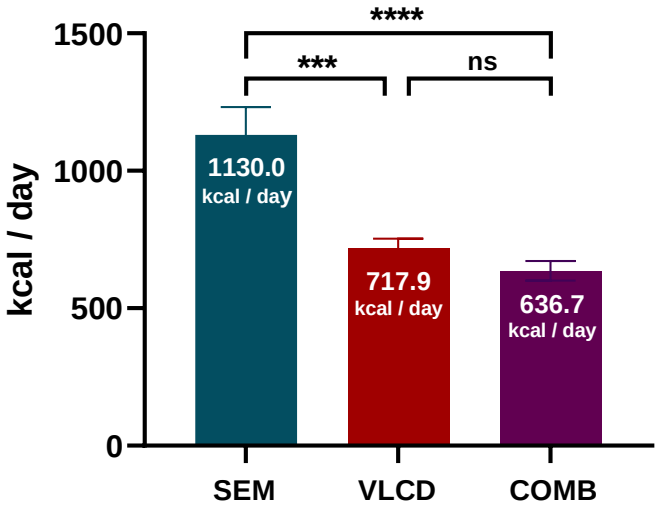

Figure 5.4.3.1. Mean total energy intake at 6 weeks. Significant differences assessed with one-way ANOVA. *** p < 0.001, **** p < 0.0001

Protein intake also significantly reduced in all three groups, however in contrast with total energy intake, peri-intervention protein intake (Figure 5.4.3.2) was not significantly different between the groups (SEM 52.1 ± 4.5g, VLCD 57.1 ± 3.0g, COMB 47.8 ± 3.1g). In contrast, fat intake was significantly lower in VLCD (19.7 ± 0.8g) and COMB (17.7 ± 1.2g) than SEM (44.7 ± 5.7g, p < 0.05 v both).

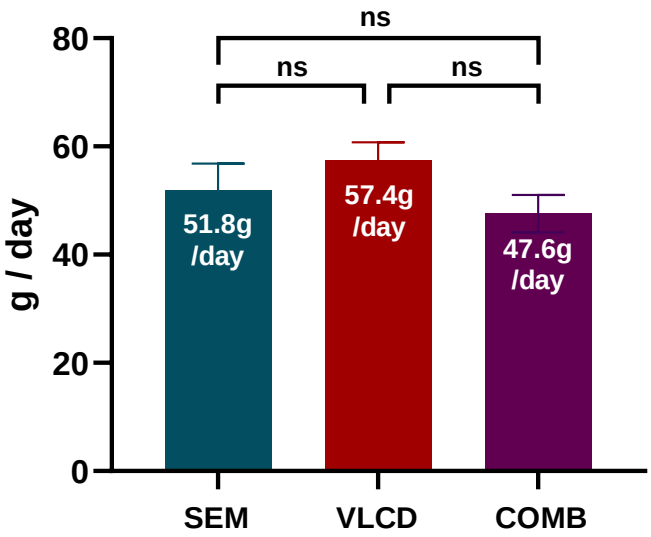

Figure 5.4.3.2. Mean protein intake at 6 weeks. Significant differences assessed with one-way ANOVA

In order to ensure that any changes in weight and body composition were not due to alterations in levels of physical activity, participants were advised to maintain their habitual levels of activity through the study. Measures of physical activity were assessed through daily step count, distance travelled and calculated energy expenditure at baseline and 6 weeks. Analysis revealed no significant changes between these timepoints in any of these measures in the three groups (Figure 5.4.3.3), highlighting adequate compliance of participants with the advice they received. Furthermore, there were no significant between-group differences in any measure at both baseline and 6 weeks.

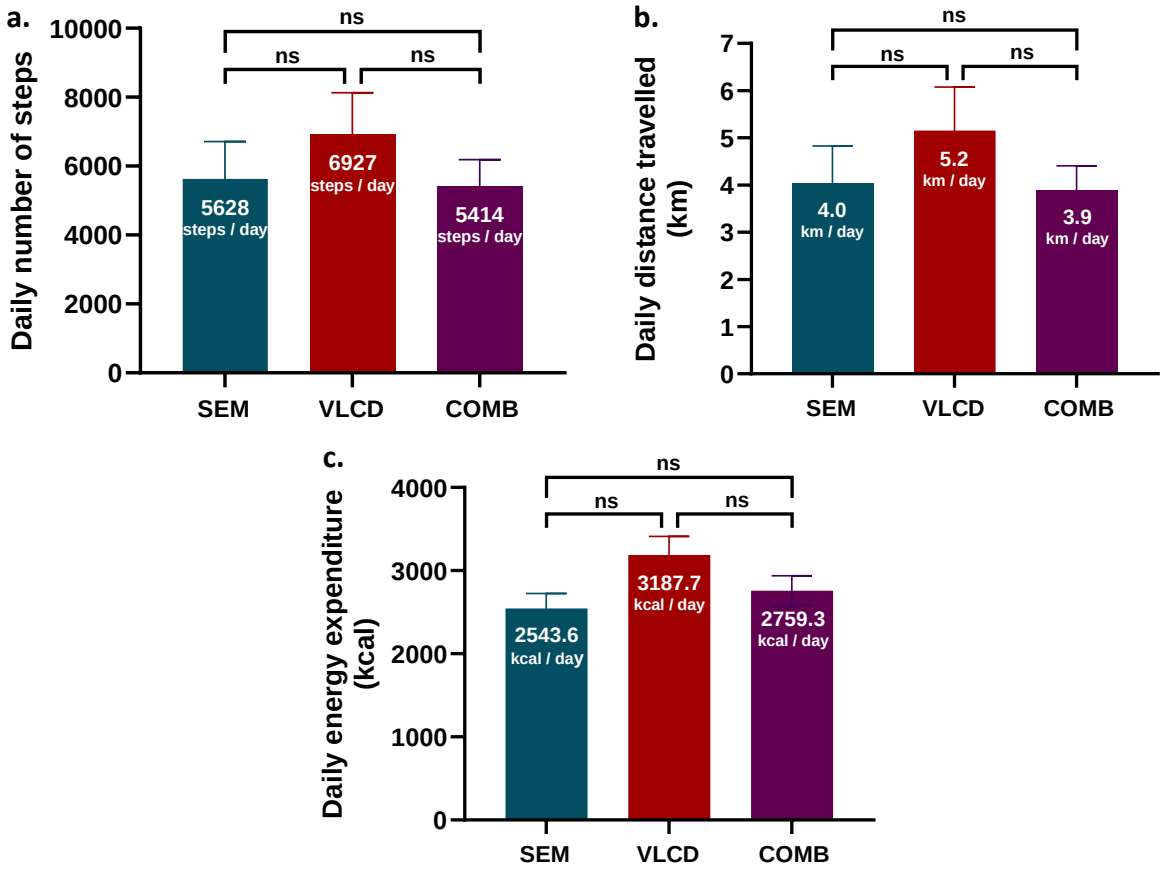

Figure 5.4.3.3. Graphical representations of mean 6-week physical activity measures in each group; a) daily step count, b) daily distance travelled, c) total daily calculated energy expenditure. Significance assessed with one-way ANOVA

5.4.4. MPS and MPB

Twenty-eight participants underwent MPS measurements which were performed over 4-7 days at baseline, and then during the first 6 weeks of the intervention. The results are summarised in Table 5.4.4.1.

	Baseline	0-6 weeks	Change
SEM (n=9)	1.22 ± 0.05	0.79 ± 0.04****	-0.44 ± 0.04
VLCD (n=9)	1.33 ± 0.08	0.70 ± 0.05****	-0.63 ± 0.05$^{\#}$
COMB (n=10)	1.24 ± 0.06	0.71 ± 0.04****	-0.53 ± 0.06

Table 5.4.4.1. Baseline, 0-6 week and change in MPS in each group (% per day). Significant difference between baseline and 0-6 weeks assessed with two-way ANOVA. One-way ANOVA utilised to examine differences between the changes observed in each group. **** p < 0.0001 v baseline, $^{\#}$ p < 0.05 v SEM

Reductions in MPS were observed in all intervention groups with a high level of statistical significance (p < 0.0001 for all groups).

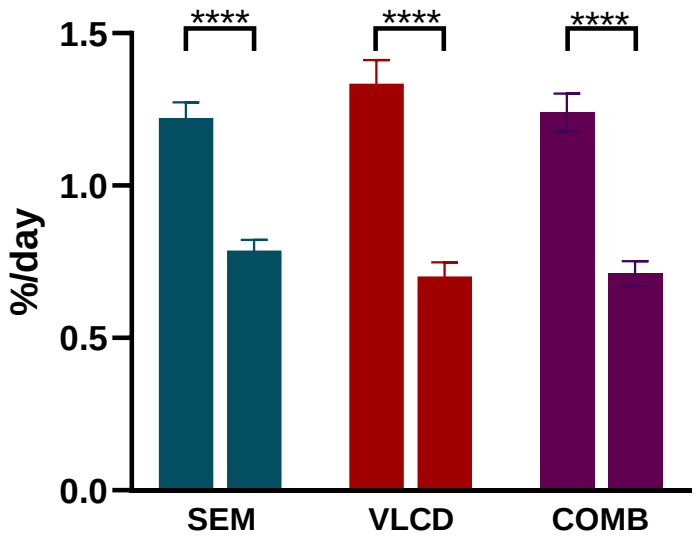

Figure 5.4.4.1. Baseline and 6-week MPS in each group. Significance between the timepoints assessed with two-way ANOVA. **** p < 0.0001

However, the VLCD group experienced a 0.63 percentage-point reduction in MPS, which was significantly greater than the 0.44 percentage-point reduction in SEM (p < 0.05). The reduction observed by COMB was not significantly different to either group. These changes are depicted graphically in Figure 5.4.4.2.

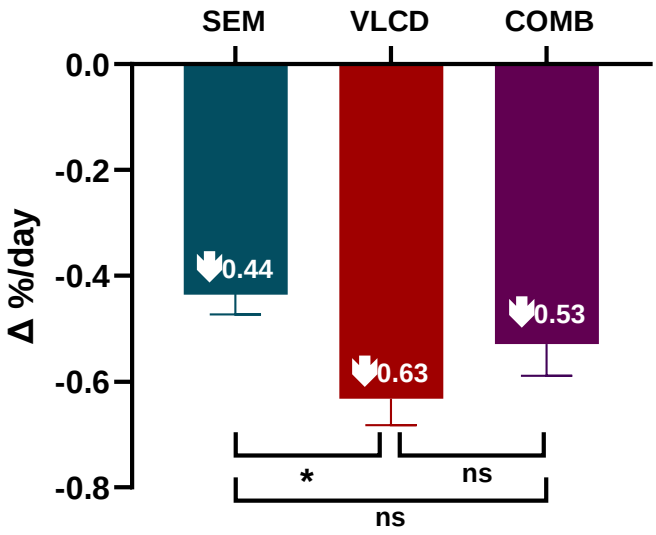

Figure 5.4.4.2. Reductions in MPS between baseline and 6 weeks. Significant differences assessed with one-way ANOVA. * p < 0.05

Due to resource and technical limitations MPB was only determined at one timepoint during the intervention. Thirty-one participants underwent MPB measurement (distributed among the groups as described in Table 5.4.2.1) and the results are represented graphically in Figure 5.4.3.2. Median MPB was 0.015 (IQR 0.007 – 0.027) in SEM, 0.032 (IQR 0.021 – 0.048) in VLCD and 0.024 (IQR 0.014 – 0.029). There were no significant differences in MPB between any of the groups (p = 0.10 assessed by Kruskal-Wallis test).

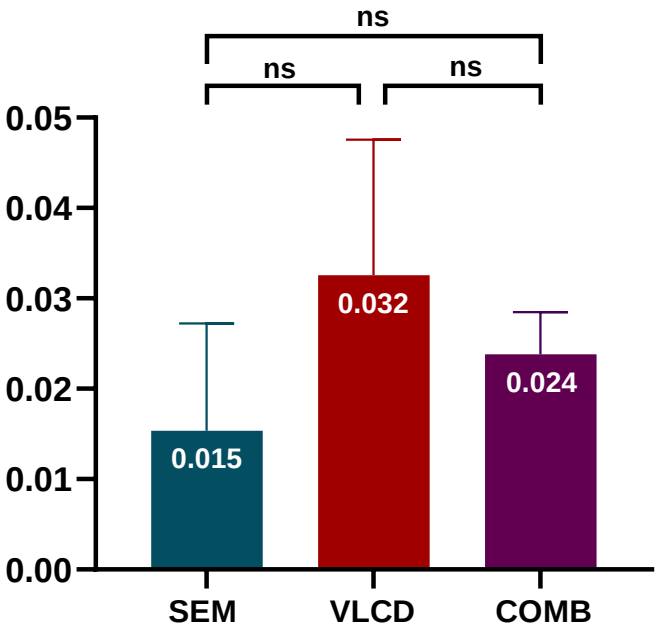

Figure 5.4.4.3. Median change in MPB in each group. Significance assessed with Kruskal-Wallis test

In order to explore for any potential associations between MPS, MPB, and changes in LBM and overall body weight, correlation analysis was performed using data from all participants pooled together. MPS at 6 weeks correlated significantly with change in body weight ($r^2 = 0.40$, $p < 0.001$), but not with change in LBM ($r^2 = 0.12$, $p = 0.07$). These results are depicted below in Figure 5.4.4.4.

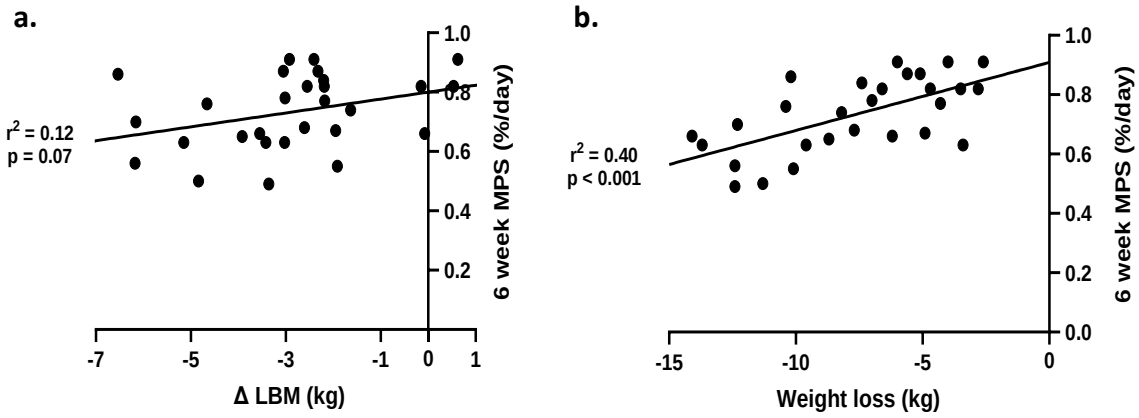

Figure 5.4.4.4. Correlation analysis between a) 6 week MPS and change in LBM, b) 6 week MPS and change in body weight

However, when change in MPS between baseline and 0-6 weeks was assessed against change in LBM, the association was significant ($r^2 = 0.16$, $p < 0.05$).

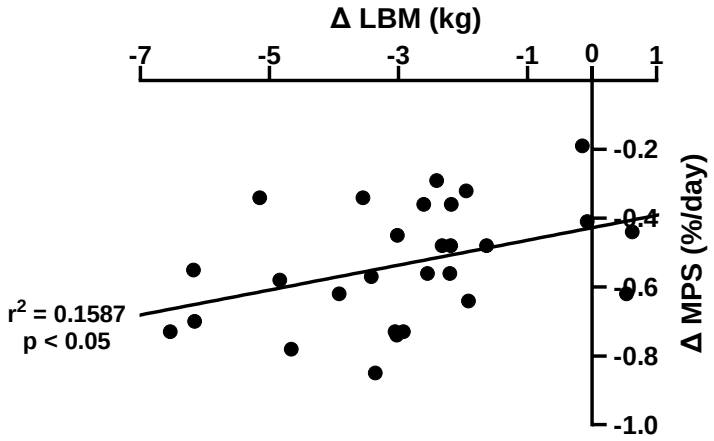

Figure 5.4.4.5 Correlation analysis between change in MPS and change in LBM

Similar to 6-week MPS, change in MPS also correlated significantly with change in body weight ($r^2 = 0.17$, $p < 0.05$).

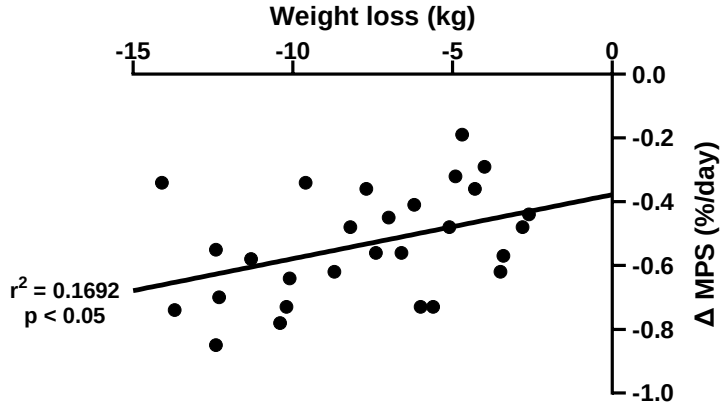

Figure 5.4.4.6. Correlation analysis between change in MPS and weight loss

Thus, a greater reduction in MPS was significantly associated with enhanced LBM loss (as shown in Figure 5.4.4.5) and increased overall weight loss (Figure 5.4.4.6), whilst higher 6-week MPS was significantly associated with higher weight loss only.

Correlation analysis of MPB with weight and LBM change revealed no significant correlations (Figure 5.4.4.7). However, there appeared to be a potential relationship between higher MPB and a greater degree of LBM loss (r^2 = 0.122, p = 0.081).

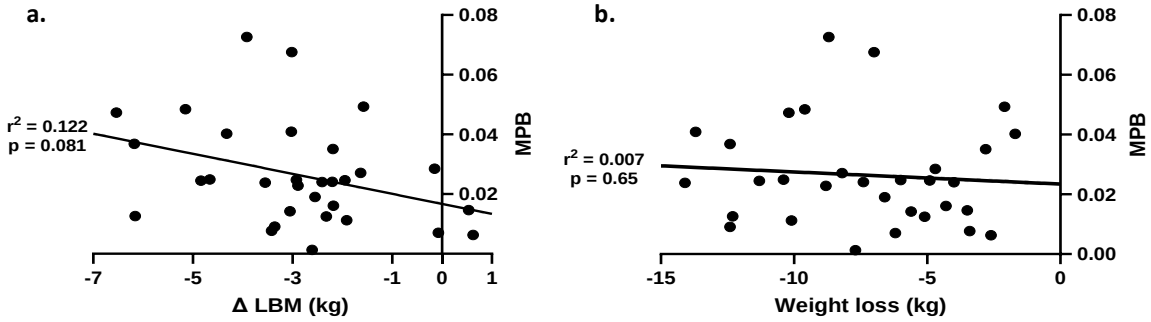

Figure 5.4.4.7. Correlation analysis between a) MPB and change in LBM, and b) MPB and change in body weight. Analysis performed with Spearman correlation test

5.4.5. Baseline to 12-week anthropometric, structural and functional changes

Thirty participants were able to complete the entire 12-week intervention, and were distributed equally between the three groups. Results of the measures taken at baseline and 12 weeks are summarised in Table 5.4.5.1 below.

	SEM (n = 10)			**VLCD** (n = 10)			**COMB** (n = 10)		
	Baseline	**12 weeks**	**p**	**Baseline**	**12 weeks**	**p**	**Baseline**	**12 weeks**	**p**
Weight (kg)	103.2 ± 4.1	97.1 ± 4.0	<0.0001	121.6 ± 5.9	108.4 ± 7.0	<0.0001	104.5 ± 7.5	91.0 ± 6.6	<0.0001
LBM (kg)	55.9 ± 3.1	54.2 ± 2.8	0.07	64.6 ± 2.6	60.9 ± 2.7	<0.0001	51.5 ± 2.2	48.2 ± 2.3	<0.0001
FM (kg)	43.5 ± 3.2	39.5 ± 3.3	<0.0001	52.3 ± 4.2	43.3 ± 5.1	<0.0001	44.6 ± 4.9	35.6 ± 4.6	<0.0001
VL thickness (cm)	2.02 ± 0.14	1.97 ± 0.13	0.85	2.21 ± 0.11	1.85 ± 0.14	<0.0001	1.93 ± 0.06	1.69 ± 0.10	<0.01
VL CSA (cm^2)	22.60 ± 1.51	20.18 ± 1.13	<0.05	24.16 ± 0.98	21.74 ± 1.22	<0.05	21.18 ± 1.08	18.09 ± 0.84	<0.01
MVC (Newtons)	336.6 ± 39.6	298.1 ± 31.0	0.47	446.6 ± 56.8	381.8 ± 21.7	0.09	290.5 ± 28.3	261.1 ± 29.1	0.68
Handgrip strength (Newtons)	29.4 ± 3.4	29.7 ± 3.7	0.98	37.6 ± 2.9	36.7 ± 3.1	0.70	31.6 ± 3.6	31.1 ± 3.3	0.94

Table 5.4.5.1. Mean anthropometric, muscle architectural and muscle strength measures before and after the intervention period, in each group. Significant differences assessed using two-way ANOVA

Weight reduced significantly in all three groups over the 12-week intervention period as outlined in Table 5.4.5.1 and Figure 5.4.5.1. As previously highlighted, there was a significant reduction in weight during the first 6 weeks, and in the case of VLCD and COMB, further significant reductions occurred between weeks 6 and 12 (VLCD: 112.3 ± 6.6kg to 108.4 ± 7.0kg, $p < 0.01$, COMB: 95.8 ± 6.9kg to 91.0 ± 6.6kg, $p < 0.001$). However, SEM did not observe any further significant weight reduction after week 6 (99.1 ± 4.1kg to 97.1 ± 4.0kg, $p = 0.54$).

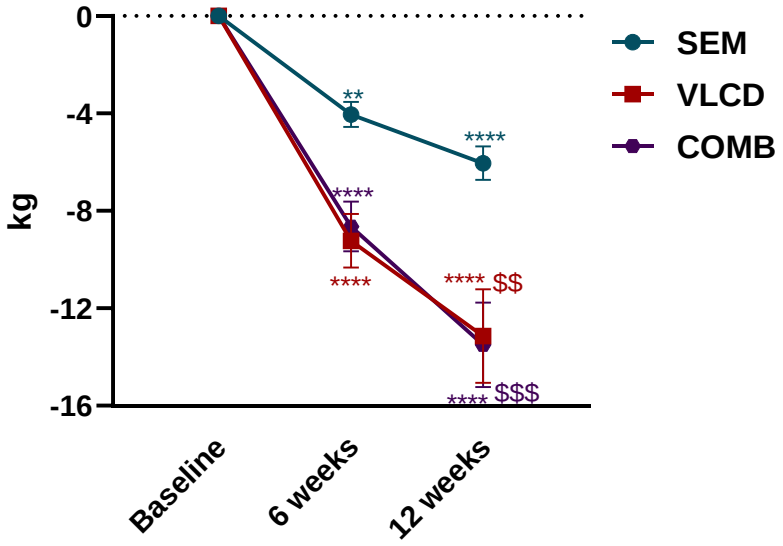

Figure 5.4.5.1. Graphical representation of changes in weight through the intervention period. Significance assessed with two-way ANOVA. ** p < 0.01 v baseline, **** p < 0.0001 v baseline, $$ p < 0.01 v 6 weeks, $$$ p < 0.001 v 6 weeks

FM (Figure 5.4.5.2) followed a similar trajectory with significant reductions elicited by all three interventions over 12 weeks, which was sustained in the early and later phase of the intervention period in VLCD and COMB, but not in SEM.

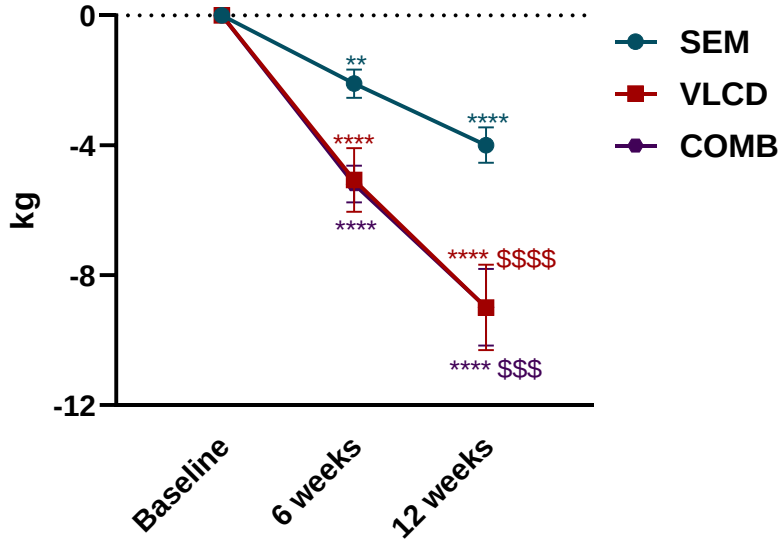

Figure 5.4.5.2. Graphical representation of changes in FM through the intervention period. Significance assessed with two-way ANOVA. ** p < 0.01 v baseline, **** p < 0.0001 v baseline, $$$ p < 0.001 v 6 weeks, $$$$ p < 0.0001 v 6 weeks

As shown in Figure 5.4.5.3, despite significantly reducing in all three groups during the first 6 weeks, reductions in LBM were non-significant in SEM at 12 weeks (55.9 ± 3.1kg to 54.2 ± 2.8kg, p = 0.07). Furthermore, no group observed any further significant reduction in LBM between weeks 6 and 12, with numerical increases noted in the SEM and VLCD groups (SEM: 54.1 ± 3.1kg to 54.2 ± 2.8kg, p > 0.99, VLCD: 60.4 ± 2.6kg to 60.9 ± 2.7kg, p = 0.99, COMB: 49.0 ± 2.3kg to 48.2 ± 2.3kg, p = 0.90). Figure 5.4.4.1 shows these described changes in weight and LBM.

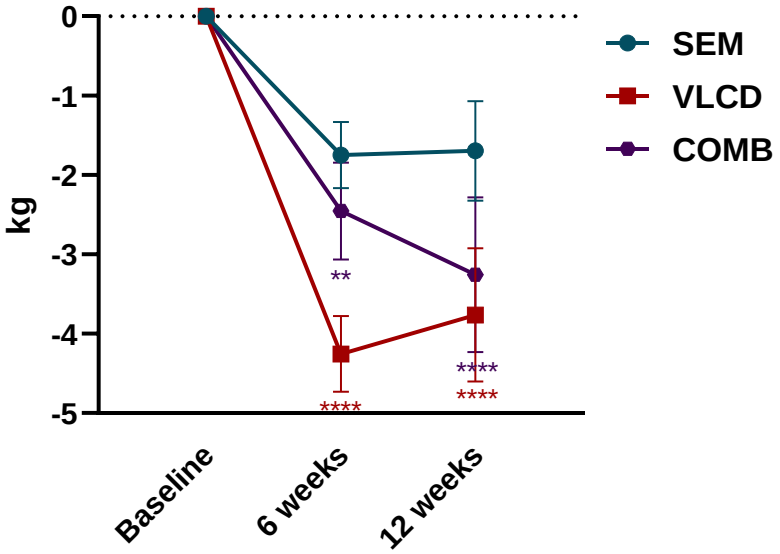

Figure 5.4.5.3. Graphical representation of changes in LBM through the intervention period. Significance assessed with two-way ANOVA. ** p < 0.01 v baseline, **** p < 0.0001 v baseline

Ultrasound assessment of right VL structure revealed significant reductions in thickness and CSA in both VLCD and COMB (see Table 5.4.5.1). Inconsistent results were observed in SEM, as CSA significantly reduced (22.60 ± 1.51cm^2 to 20.18 ± 1.13cm^2, p < 0.05), without significant change in VL thickness (2.02 ± 0.14cm to 1.97 ± 0.13cm, p = 0.85). Consequently, the change in VL thickness elicited by SEM was significantly lower than by VLCD (p < 0.05). There was no significant difference

between COMB and either group. Figure 5.4.5.4 displays these changes in VL CSA and thickness.

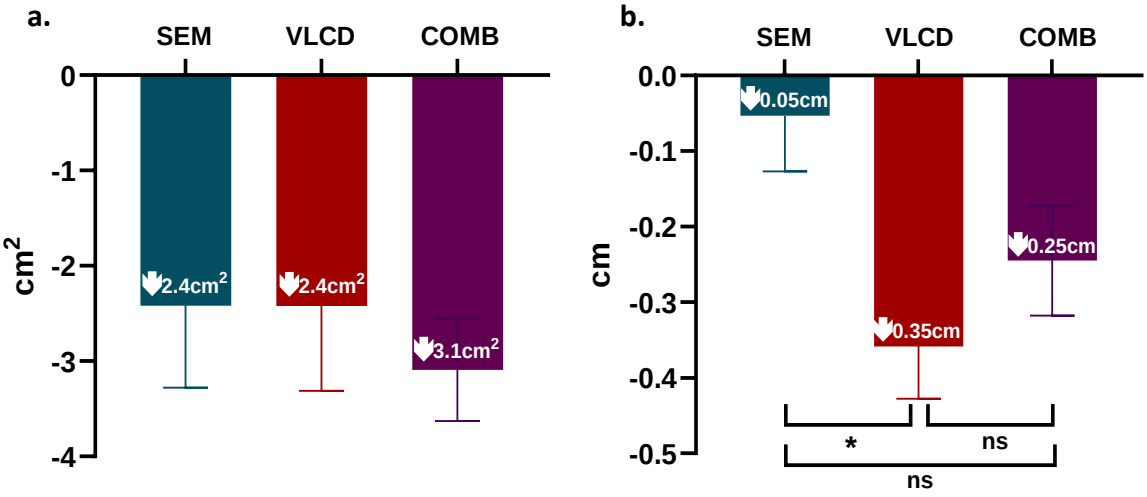

Figure 5.4.5.4. Changes in a) VL CSA and b) VL thickness in each group. Significance assessed using one-way ANOVA. * p < 0.05

MVC of right thigh extension did not change significantly in any group and dominant handgrip strength also did not change significantly (Figure 5.4.5.5).

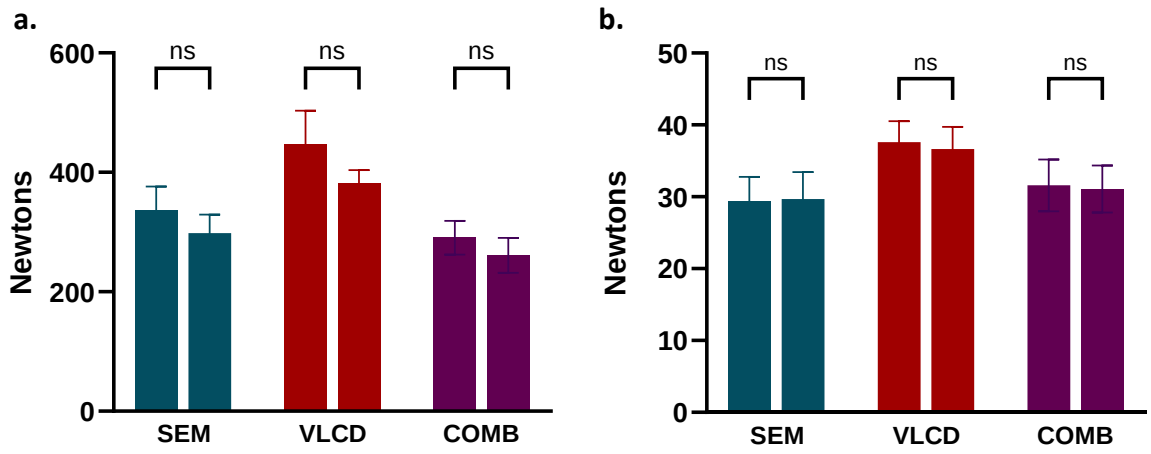

Figure 5.4.5.5. Mean a) MVC of knee extension and b) dominant handgrip strength at baseline and follow-up in each group. Significance assessed using two-way ANOVA

5.5. Discussion

This study was conducted to assess the skeletal muscle response to different weight loss interventions in individuals with T2D and obesity. Unsurprisingly, following 12 weeks of the GLP1RA Semaglutide, an 800 kilocalorie per day VLCD, or a combination of the two, significant weight loss occurred. However, despite this, Semaglutide did not induce significant reduction in LBM over the 12-week intervention period. Furthermore, in the two groups that did experience significant reduction in LBM, the majority of this reduction occurred in the first 6 weeks, with no significant further reductions in LBM during the second half of the intervention period. Indeed, VLCD appeared to induce a numerical increase in LBM from weeks 6 to 12 of the intervention, although this was non-significant. These non-significant changes in LBM occurred in the presence of continued reductions in FM and weight, suggesting that weight loss in the latter half of the intervention period was primarily driven by changes in FM.

Over the initial 6 weeks when MPS and MPB were assessed, all groups experienced significant reductions in weight, FM and LBM. VLCD and combined VLCD with Semaglutide induced greater weight and FM losses than Semaglutide in isolation, however only VLCD induced greater loss of LBM than Semaglutide. MPS reflected these changes, reducing from baseline significantly in all three groups, with significantly greater reduction experienced by the participants in VLCD than that induced by Semaglutide. MPB was not significantly different between the three groups, suggesting that the changes in LBM were primarily due to changes in MPS. However, the MPB assessment was considerably limited by the lack of a baseline

measurement. Finally, change in MPS from baseline significantly correlated with change in LBM, while 6-week MPS did not, suggesting that the change in MPS was more associated with LBM losses, rather than the absolute MPS during the intervention. Intriguingly, both 6-week MPS and change in MPS from baseline were significantly correlated with weight loss over the initial 6 weeks.

To my knowledge, this is the first study to assess the effect of weight loss interventions indicated for use in individuals with T2D on MPS and MPB. In addition, no study has examined the effects of combined VLCD and Semaglutide use on any outcomes in individuals with T2D. Furthermore, studies reporting serial body composition measurements during VLCD and Semaglutide interventions are very limited. Thus, the findings of this study are entirely novel and outline several avenues for further research.

Excess weight is a major contributor to the development of T2D (Carey et al., 1996; Kahn, Hull and Utzschneider. Kristina M., 2006; Abdullah et al., 2010) and weight loss is a critical aspect of managing the condition (W. T. Garvey, Umpierrez, et al., 2022; Lingvay et al., 2022). Consequently, weight loss interventions are recommended for the treatment of T2D in many international guidelines (M. J. Davies et al., 2022; NICE, 2015b). However, T2D is also associated with accelerated age-related muscle loss (S. W. Park et al., 2009; S. W. Park, Goodpaster, Strotmeyer, De Rekeneire, et al., 2006), leading to higher rates of frailty and disability in sufferers of the condition (Kalyani et al., 2010). The mechanisms underlying this phenomenon are incompletely understood but an attenuated MPS response to nutritional intake (J. Murphy et al.,

2015; Morais, Jacob and Chevalier, 2018), and a chronic increase in MPB (J. A. Bell et al., 2006; Pupim et al., 2005) have both been implicated.

VLCD and GLP1RAs are important options for the management of T2D, especially in individuals with concurrent obesity. However, concerns have been expressed regarding significant loss of MM with both types of intervention (Donnelly, Jakicic and Gunderson, 1991; Alhamdan et al., 2016; Editorial, 2023; Buntz, 2024). An understanding of the metabolic processes underlying this loss of MM is essential for identification of potential methods to attenuate this, hereby maximising the efficacy of these interventions.

Limited evidence exists regarding the effect of VLCD on MPS and MPB. One study evaluated muscle metabolism using the [^{15}N]glycine method in individuals living with obesity divided by glycaemic status, following four weeks of 460 kilocalorie per day VLCD (R. Gougeon et al., 1998). They reported significantly reduced MPS in both individuals with T2D and those without, ultimately leading to overall negative net protein balance (R. Gougeon et al., 1998). Another study subjected individuals without T2D to 5 days of 800 kcal per day VLCD, however metabolic studies were only performed after the intervention as the aim was to test response to different doses of whey protein (Larsen et al., 2023).

Similarly, studies assessing the response of muscle metabolism to GLP1RA treatment are lacking. One study assessing the acute MPS response to infusion of GLP-1 demonstrated stimulated MPS in the presence of this hormone (Abdulla et al., 2020). However, this was also performed in individuals without T2D, thus the effect of GLP-1 hormone on muscle of individuals with this condition remains elusive.

Although not VLCD, multiple studies have reported the effects of hypocaloric diets on muscle metabolism, although these have overwhelmingly utilised intravenous isotope tracer techniques measuring acute MPS and MPB response. In keeping with the findings of Gougeon et al. (R. Gougeon et al., 1998), attenuated MPS has also been demonstrated following non-VLCD weight loss (Pasiakos et al., 2013; Areta et al., 2014; Hector et al., 2015), although some have reported contrasting findings (Villareal et al., 2012; Colleluori et al., 2019). MPB elevations induced by dietary restriction have also been suggested, however this has either been observed in animal models (S. S. Wing & Goldberg, 1993) or during acute starvation in healthy normal weight individuals (Giesecke et al., 1989).

In the present study, VLCD induced significant reductions in MPS, in line with observations from previous studies assessing weight loss secondary to dietary restriction outlined above. Notably, Semaglutide also induced reduction in MPS, which is a novel finding. However, the reduction in MPS with Semaglutide was to a lower extent than that elicited by VLCD. This difference occurred despite similar levels of protein intake and physical activity, suggesting that these factors did not have a potentially confounding influence on the observed results. Therefore, these findings could imply a specific stimulatory effect on MPS, mediated by GLP-1 agonism, as suggested by Abdulla et al. (Abdulla et al., 2020).

It is also important to note that change in MPS correlated significantly with degree of weight loss, which is related to level of caloric restriction. Thus, the greater reduction in MPS induced by VLCD may be in part related to the lower caloric intake in this group. However, in disagreement with this assertion, MPS in the combination group

did not reduce to a significantly greater extent than in the Semaglutide group, despite significantly lower energy intake and greater weight loss. This therefore also supports the theory of a muscle preserving effect of GLP1RAs. This requires extensive further investigation, ideally with studies inducing matched weight loss between individuals undergoing GLP1RA or dietary interventions.

This study did not demonstrate any significant differences between MPB in each group. As only one timepoint was assessed, changes in MPB could not be appreciated, and therefore further study into the changes in MPB following weight loss interventions are required. However, the MPS changes observed in each group matched the changes in LBM between baseline and 6 weeks, suggesting that reduced MPS, rather than elevated MPB was largely responsible for the loss of muscle mass. This assertion is supported by a study using similar oral D_2O techniques to quantify chronic MPS over a period of energy restriction, and an infusion of ^{15}N-phenylalanine to measure MPB (Hector et al., 2018). The authors reported reduced MPS over 10 days of a 40% energy restricted diet, with no associated increase in MPB (Hector et al., 2018). Thus, similar changes may have occurred in response to the three interventions tested in this study, although this will required confirmation in much more detailed studies.

Analysis of the serial body composition measures revealed interesting results. LBM significantly reduced with all three interventions during the first 6 weeks, however no further significant reductions occurred during the subsequent 6 weeks, despite ongoing reductions in weight and FM. These results are consistent with the observations of Gougeon et al. who reported an acute drop in nitrogen balance

within the first 2 days following VLCD initiation, with a subsequent gradual increase in back to baseline levels over the subsequent 3 weeks (R. Gougeon et al., 1998). Furthermore, a meta-analysis of MM changes following bariatric surgery revealed that the majority of these changes occurred within the first 3 post-operative months, with sequential reductions in MM loss subsequent to this (Nuijten et al., 2022). Thus, loss of MM appears to be particularly pronounced in the early stages of weight loss, regardless of the intervention, with subsequent stabilisation of the underlying processes.

Notably, despite the recorded reductions in LBM, particularly with VLCD and the combination of VLCD plus Semaglutide, no corresponding changes in muscle strength were observed. This was the case with knee extensor MVC – in which VL thickness, CSA and MPS were reduced – and with hand grip strength as a marker of upper limb strength. These findings in the presence of reduced LBM are suggestive of increased muscle quality resulting from weight loss, which has been observed previously (Cava, Yeat and Mittendorfer, 2017). Individuals with T2D have excessive levels of intramuscular lipid (Yu et al., 2022), which in turn reduces muscle strength and function (Bittel et al., 2015). Significant weight loss decreases IMCL (Jazet et al., 2008; Lara-Castro et al., 2008). Therefore, it can be postulated that the loss of ectopic myocellular lipid generates improvements in muscle strength that mitigate against the potentially deleterious effects of reduced MPS and overall muscle size. However, as histological analysis was not performed, it is not possible to examine this hypothesis, which is a limitation of this work.

The discordance between the results of VL thickness and VL CSA in the Semaglutide group is noteworthy. Whilst VL thickness did not change significantly in this group, in keeping with LBM, a significant reduction in CSA was observed. Muscle thickness has been found to be a more reliable and reproducible measure of muscle size (Betz et al., 2021; Strasser et al., 2013). Moreover, discordance between CSA and muscle strength in trained athletes has also been described (Maughan et al., 1983), which along with the results of the present study, suggests that muscle CSA may not be the most accurate measure of muscle size in this setting.

5.5.1. Limitations

As with all research, the present study had a number of limitations that need to be addressed. As mentioned previously, the major limitation was the lack of a baseline measurement of MPB. This was primarily to ensure that the burden of research activities placed upon participants did not inhibit recruitment into the study. Additionally, review of the literature suggested that MPS was implicated in dietary-induced MM loss to a greater extent so it was decided that this should be the main focus of the study.

Another major limitation was the inability to measure MPS over the entire 12-week period. Six weeks is the maximum amount of time that MPS has been measured using the oral D_2O method. Administering D_2O for further time risks the potential of achieving maximal incorporation, as well as deuterium recycling, hindering the ability to interpret the results effectively. Therefore, novel approaches need to be developed to enable MPS measurement over longer time periods. An alternative option would be to measure MPS in two specific time periods through the 12-week intervention,

which enables measurement of early and late MPS. This would reduce the risks of encountering the aforementioned issues, and provide insight into possible mechanisms underlying the observed differences in rates of MM loss during a prolonged period of caloric restriction.

The study was also limited by low participant numbers. This may have resulted in non-significant results occurring where alterations may in fact have occurred, particularly in the Semaglutide group where changes in some parameters were close to statistical significance. Additionally, the Semaglutide dose was escalated every 2 weeks which is not the standard initiation schedule used in clinical practice. This escalation regime was used in order to enable exposure to the full intervention dose of Semaglutide during the 6-week MPS measurement period. Finally, as previously mentioned, histological analysis of the myocellular changes in response to the interventions would have provided further understanding of the observed changes in whole-body MM.

5.5.2. Conclusions and further work

In summary, this study has demonstrated that 6 weeks of VLCD, Semaglutide or combination of the two led to reductions in LBM which appear to have been primarily driven by reductions in MPS. VLCD caused greater reductions in MPS, and thus LBM, compared to Semaglutide, whilst the combination was not significantly different from either. MPB was not significantly different between the three groups, however this was only measured at one timepoint during the intervention. Change in MPS correlated significantly with changes in LBM, adding further validation to the

assertion that changes in MPS drive reductions in LBM associated with weight loss interventions.

Intriguingly, no further alterations in LBM occurred in any group for the subsequent 6 weeks, despite ongoing weight and FM reductions with VLCD and the combination. Furthermore, although significant loss of LBM occurred over the entire 12 weeks in these two groups, this reassuringly did not translate into reductions in strength. These findings provide important novel insights into the effects of two widely used interventions in the management of T2D on MM, and highlight several avenues for potential further work.

In particular, the potential muscle mass preserving effect of GLP1RA needs closer investigation. This effect has been suggested by several authors (Abdulla et al., 2020; Editorial, 2023) and this study adds to a range of studies showing non-significant reductions in MM during GLP1RA-induced weight loss in individuals with T2D (Harder et al., 2004; Rondanelli et al., 2016; Gibbons et al., 2021; Volpe et al., 2023), as well as the findings of the meta-analysis detailed in Chapter 3. A crucial aspect would be to determine whether MPS is relatively preserved when compared to non-GLP1RA induced weight loss. Due to the correlation between MPS and weight change identified in the present results, weight loss could be a confounding factor in MPS changes, therefore this would need to be matched between both groups.

Another important aspect to explore is the early changes in MPS during weight loss interventions. The greatest loss of LBM occurred during the first 6 weeks of this study and the reduction in MPS replicated this. However, this measured MPS was a composite of the initial 6 weeks, and the majority of the change in MPS could have

occurred during the initial week of energy restriction, as suggested by the findings of Gougeon et al. (R. Gougeon et al., 1998). This could be investigated using multiple biopsies during the period of D_2O administration to provide serial measurements of MPS, as performed by Brook et al. (Brook et al., 2015). Additionally, a measure of MPS during the late stages of a weight loss intervention when MM loss appears to stabilise would be useful to develop an understanding of the counter-regulatory mechanisms underlying this.

Similarly, serial measures of MPB would be extremely useful, particularly if linked with serial measures of MPS. Although this and previous studies have not identified any changes in MPB, this may be because MPB changes predominate in the early stages of energy restriction, and are therefore missed when being measured at the end of an intervention spanning multiple weeks. Histological analysis would also provide useful information on changes in myocellular fat, especially if the lack of change in MPB is replicated. Finally, use of these techniques could also be expanded to determine whether it is primarily changes in MPS or MPB (or both) which are responsible for the differential rate of age-related muscle loss between individuals with T2D, and those without.

6. Study investigating the impact of VLCD, GLP1RA, or combination of the two, on gluco-metabolic outcomes in individuals with T2D

6.1. Chapter Synopsis

Background: VLCD and the GLP1RA Semaglutide are recommended for the management of T2D. They both induce significant weight loss and improved glycaemic control in these individuals however, no direct comparison of the two interventions has previously occurred. Furthermore, combination of the two interventions presents a novel approach with the potential for significant benefits in individuals with T2D. This study was therefore conducted to explore the comparative short-term effects of these interventions individually, and in combination, on clinical outcomes including weight and body composition, as well as glucometabolic parameters (BCF and IS).

Methods: Thirty individuals with T2D (age 18-75 years, BMI 27-50kg.m^{-2}) were randomly assigned to receive Semaglutide (SEM), 800 kcal per day VLCD (VLCD), or both in combination (COMB) for 12 weeks. Measurement of weight, body composition and HbA1c were performed at baseline and post-intervention. Diet diaries were utilised to assess compliance. IVGTTs were also performed with insulin first phase response providing a marker of BCF, and IS assessed using HOMA-IR and a calculated insulin sensitivity index (CS_I).

Results: Significantly greater reductions in body weight and FM were observed in VLCD and COMB, than SEM ($p < 0.01$ v both). VLCD and COMB resulted in a 5.4 and 7 percentage-point greater weight loss than SEM, respectively. HbA1c and fasting glucose reduced significantly in all groups. Fasting insulin and HOMA-IR improved significantly in VLCD and COMB only, whilst CS_I only significantly improved in VLCD, with an unexpected significant deterioration observed in SEM. Insulin first phase

response during IVGTT increased in SEM and COMB, with COMB experiencing a 9-fold greater increase than VLCD which was statistically significant ($p < 0.01$).

Conclusion: VLCD elicited greater short-term losses of weight and FM than Semaglutide. Adding VLCD to Semaglutide stimulated further weight loss than Semaglutide alone. The combination did not yield any additive effects on weight and body composition above VLCD alone, but did provoke greater improvements in BCF. Thus, VLCD is more effective for short-term weight loss than Semaglutide, and combination of the two interventions warrants further exploration as a novel approach to T2D management.

6.2. Introduction

As previously noted, strategies to induce weight loss play a crucial role in effective T2D care and form an essential component of T2D management guidelines (M. J. Davies et al., 2022). VLCD interventions are used to successfully reduce weight in individuals with T2D, with associated improvements in glycaemic control (Sellahewa et al., 2017). VLCD carries the additional advantage of the potential to induce diabetes remission. An important mechanism underlying VLCD-induced T2D remission is restoration of pancreatic beta-cell dysfunction, specifically the first phase of insulin secretion (Taylor, 2013). This is absent in individuals with T2D (DeFronzo et al., 1992), and its recovery is the primary feature driving remission of T2D (Steven, Hollingsworth, Al-Mrabeh, et al., 2016).

Additionally, strong associations have been highlighted between increased FM and both impaired glycaemic control (Bower et al., 2017b) and IR (M. Zhang et al., 2015). In accordance with this, greater reductions in glycated haemoglobin (HbA1c) result from larger loss of FM (Igarashi et al., 2023). VLCD interventions cause significant reductions in FM (Sayda et al., 2022) and, as noted in Chapters 4 and 5, FM comprises the majority of VLCD-induced weight loss. This reduction in FM, and the resulting improvement in IR, also contributes to the remission of T2D observed in individuals undergoing VLCD.

The GLP1RA Semaglutide has also demonstrated favourable weight loss outcomes in individuals with T2D. Two independent meta-analyses of Semaglutide in T2D have reported mean weight loss of approximately 3.9 and 4.9kg (Hu et al., 2023; S. Wang et al., 2024). Improvements in glycaemic control also occur, with absolute reductions

in HbA1c between 1.5-1.8% typically described (Gomez-Peralta & Abreu, 2019). The glucoregulatory effects of Semaglutide arise from its enhancement of post-prandial insulin secretion, as well as suppression of glucagon (Drucker, 2005). Thus, improved BCF has been reported by Kapitza et al. who outlined an increase in insulin first-phase response following 12 weeks of Semaglutide treatment (Kapitza et al., 2017b).

Similar to VLCD, weight loss induced by Semaglutide is predominantly from FM, as shown in Chapter 5 and postulated by Sargeant et al. (Sargeant et al., 2019). Furthermore, the results of Chapter 3, as well as reports by other authors (Uchiyama et al., 2023; Volpe et al., 2023), suggest that Semaglutide may preserve MM in individuals with T2D, despite stimulating significant weight loss.

Thus, both VLCD and Semaglutide induce significant weight loss, seemingly with a preference for FM, whilst also showing favourable improvements in glycaemic control and insulin first phase response. However, studies that directly compare the effect of these interventions on these parameters are not available.

Furthermore, the results of previous chapters suggest a potential MM preserving effect of GLP1RA during weight loss, which may mitigate against the losses incurred by VLCD. Thus, combination of the two interventions may provide a novel approach to managing this issue. It was therefore prudent to perform an examination of the clinical, and glucometabolic effects of this approach, in individuals with T2D, particularly as use of these interventions in combination has never been investigated.

To that end, this study was therefore conducted to explore the effects of VLCD and Semaglutide in isolation, and in combination, on weight, overall glycaemic control, body composition, and in particular, BCF and IS.

6.3. Methods

6.3.1. Study outline

The specific aim of this study was to determine the effects of short term VLCD, Semaglutide, and a combination of the two, on body weight, body composition, overall glycaemic control, and physiological parameters related to glucose metabolism (BCF and IS). Participants were recruited as previously described in Section 2.2. Individuals with T2D aged 18-75 years with a BMI between 27-50kg.m^{-2} were recruited from primary and secondary care settings and randomly allocated to one of three groups:

d. Semaglutide (SEM)
e. VLCD (VLCD)
f. combination of both Semaglutide and VLCD (COMB)

As previously described, participants received the interventions for 12 weeks, and all medications were continued except sulphonylureas which were stopped two weeks prior. Baseline measures were obtained during V1, with follow-up measures taken at V3. The exception to this was food intake diaries which were obtained prior to V1, and repeated mid-way through the interventional period, at approximately week 6.

Participants were monitored extensively through the intervention period, particularly during the initial 6 weeks, and around the Semaglutide dose escalation periods. This was necessary as Semaglutide is associated with potential adverse effects, and combination with VLCD is a novel concept that has never been reported in individuals with T2D. Any adverse effects were recorded and reported along with the results.

Compliance was also assessed when monitoring participants, with the potential of exclusion from the study if compliance targets were not met. No participants were excluded from the study on this basis.

6.3.2. Study outcomes

Data regarding the following outcomes were collected in this study:

- Body weight
- Body composition
- Food intake and macronutrient composition
- Overall glycaemic control – assessed by glycated haemoglobin (HbA1c) and plasma fasting glucose
- BCF – determined from 1st phase insulin response during IVGTT
- Insulin sensitivity – using HOMA-IR and calculated insulin sensitivity index (CS_I)

6.3.2.1. BCF assessment

BCF was assessed using from the IVGTT. The initial surge of insulin secretion following glucose administration (i.e. the first phase response) is an accurate measure of BCF (Chatterjee & Scobie, 2002). It is typically absent in individuals with T2D (M. J. Davies et al., 1994; Vilsbøll et al., 2002), and its restoration is indicative of T2D remission (Lim et al., 2011; Taylor, 2013). The derived AIRg provides a surrogate marker of first phase response (Cersosimo et al., 2014). As previously explained in Section 2.3.5, whilst AIRg obtained from a hyperglycaemic clamp represents the gold standard methodology of estimating first phase response, the IVGTT has been deemed a

reliable method of obtaining this measure which correlates well with the clamp (Korytkowski et al., 1995; Rakotoambinina et al., 1997).

Immediately following intravenous infusion of glucose, insulin secretion occurs, leading to a rapid increase in plasma insulin levels. AIRg was derived from the increase in insulin concentrations above the basal plasma insulin level over the first 10 minutes following glucose administration (Cersosimo et al., 2014; Hannon et al., 2018). The trapezoidal rule was utilised to calculate the area-under-curve (AUC) of insulin concentrations over this time period, providing a measure of AIRg. This method has been employed to demonstrate improved BCF in response to both VLCD (Jackness et al., 2013; Liu et al., 2015) and GLP1RA medications (Anholm et al., 2017; Kapitza et al., 2017b). Figure 6.3.2.1 depicts typical glucose and insulin response to IVGTT.

6.3.2.2. Measurement of IS measures

The main strength of the IVGTT lies in the ability to reliably measure BCF and IS from the same test. Traditionally, IS is evaluated using minimal model analysis over a 3-hour IVGTT (Pacini & Bergman, 1986). However, more recently, a more simple calculated measure of IS (CS_I) was described permitting performance of a significantly shorter IVGTT over 1 hour. This has been validated against calculations using the minimal model, as well as indices derived from the gold standard HE clamp (Tura et al., 2010). Additionally, it has successfully been used to demonstrate changes in IS by various authors (Arpón et al., 2019; Gillen et al., 2016; Mora-Rodriguez et al., 2020).

The calculation relies upon the plasma glucose and insulin concentrations between 10 and 50 minutes of the IVGTT. The following formula provides the CS_I value:

$$CS_I = \alpha \frac{K_G}{\Delta AUC_{INS} / T}$$

α is a constant (0.604), K_G is the slope of log glucose (over 10-50 minutes), ΔAUC_{INS} is the AUC of suprabasal insulin concentrations between 10 and 50 minutes, and T is the time interval (40 minutes).

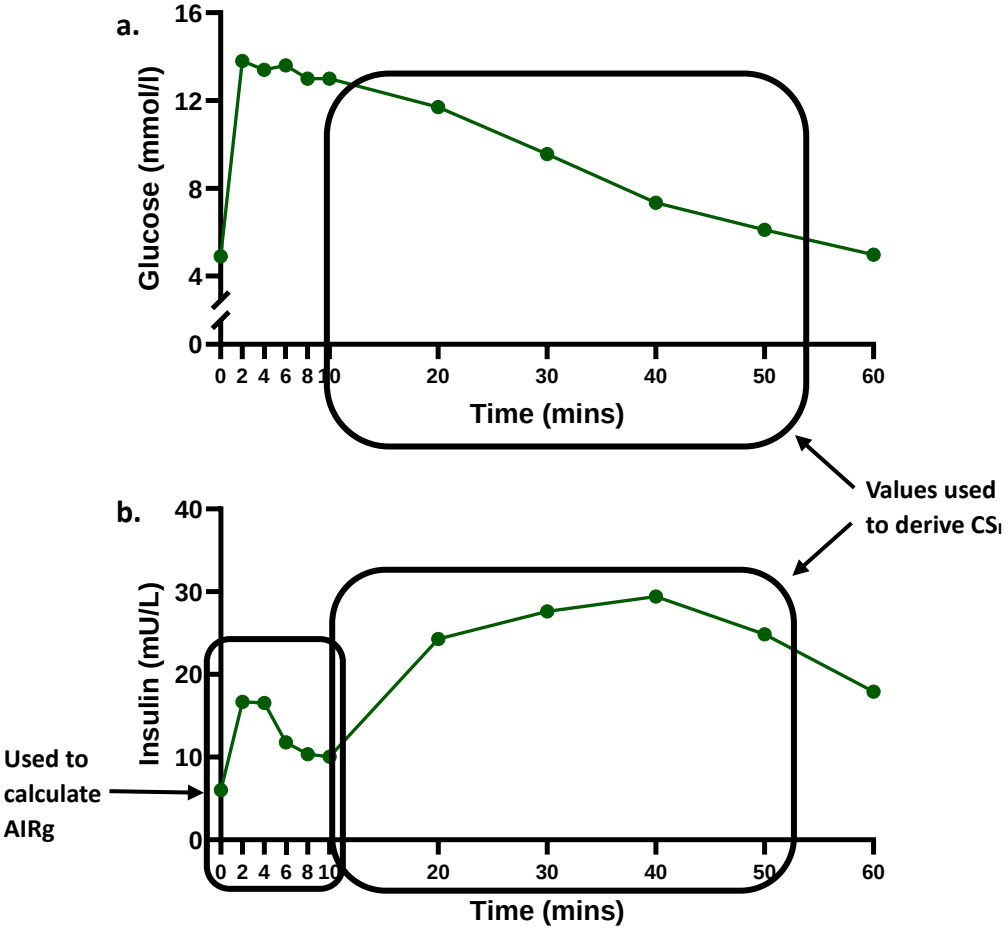

Figure 6.3.2.1. Typical a) glucose, and b) insulin response to IVGTT with annotations outlining measures used to determine AIRg and CS_I.

In addition to CS_I, HOMA-IR was also calculated as another surrogate marker of IS. HOMA-IR is primarily a measure of hepatic IS (Muniyappa et al., 2008) whilst CSI reflects whole-body (mainly skeletal muscle) IS. Thus, estimation of HOMA-IR provides a more comprehensive assessment of overall IS. HOMA-IR was calculated

using the “HOMA2” Calculator software (Radcliffe Department of Medicine, 2023), which performs the calculation from fasting glucose and insulin concentrations.

6.3.2.3. Plasma insulin concentrations

Insulin concentrations were determined using an ultrasensitive Enzyme Linked Immunosorbent Assay (ELISA) kit (Mercodia, Sweden). The process for performing the ELISA was as follows:

1. Pipette 25µl of calibrators, control samples and test samples into the supplied 96-well plate
2. Add 100µl enzyme conjugate solution to each well
3. Incubate the 96-well plate on a plate shaker for 1 hour at room temperature. The plate shaker was set at 800 revolutions per minute
4. After incubation, wash the plate by adding 350µl wash buffer solution and discarding into a sink. Tap firmly against absorbent paper to remove excess liquid. Perform this step a total of six times
5. Following washing, add 200µl Substrate TMB to each well
6. Incubate for 30 minutes at room temperature
7. Add 50µl stop solution to each well and mix well by placing on a plate shaker for approximately five seconds
8. Insert into a microplate reading machine and read absorbance from each well at an optical density of 450nm

Test sample concentrations were calculated from a standard curve generated from the calibrators containing known concentrations of insulin. The kit’s upper limit of

detection was 20mU/l, therefore the samples required a 1:15 dilution to ensure all sample results fell within the detection range.

6.3.2.3. Other outcomes

Body weight was measured via one single set of scales which was kept in the same site within the research department, and received regular calibrations to maintain its accuracy. Body composition was measured using a DXA scanner (see Section 2.3.2). Participants were scanned in loose clothing, laid flat in the scanner and advised to be as still as possible. Food intake data were obtained as previously described in Section 5.3.2.4. HbA1c was quantified by the local hospital biochemistry laboratory in an automated analyser machine. Plasma glucose was determined from immediate analysis in a YSI glucose analyser machine (YSI Incorporated, USA) located within our research department.

6.3.2.4. Sample and statistical analysis

As this was an exploratory pilot study, no sample size calculation was conducted. Statistical analysis was performed using GraphPad Prism (GraphPad, USA). Data are presented as mean ± SE and significance defined as $p < 0.05$. Statistical comparisons between pre-intervention and post-intervention measurements within groups were determined using two-way ANOVA with post-hoc Sidak's testing. One-way ANOVA with post-hoc Tukey's testing was utilised for comparisons of changes in variables between individual groups (or the Kurskal-Wallis test in cases of non-parametric data).

6.4. Results

6.4.1. Participant characteristics

As previously outlined in section 5.4.2, a total of 32 participants were successfully recruited into the study, including 15 females. Two participants dropped out of the study before the final study visit. One participant from COMB dropped out of the study due to development of COVID-19 infection at the time of V3, which meant that this visit could not proceed and data regarding the post-intervention outcomes could not be collected. Another participant from SEM developed intolerable side effects at the lowest dose of Semaglutide, therefore could not proceed any further. Thus, 30 participants completed the full study protocol and were divided equally between the three groups. The characteristics of the completing participants are outlined in Table 6.4.1.1. Baseline age, weight, HbA1c, duration of diabetes and body composition were not significantly different.

	SEM (n = 10)	**VLCD** (n = 10)	**COMB** (n = 10)	
Gender	5M / 5F	7M / 3F	4M / 6F	
Age (years)	61.1 ± 3.0	51.6 ± 4.2	57.0 ± 2.8	ns
BMI (kg.m^{-2})	35.7 ± 1.6	39.9 ± 2.0	37.3 ± 2.2	ns
HbA1c (mmol/mol)	60.0 (53.0 – 64.3)	55.0 (48.5 – 73.3)	59.0 (48.8 – 73.0)	ns
Diabetes duration (years)	10.3 (3.9 – 19.3)	5.0 (2.4 – 9.8)	10.0 (4.8 – 12.8)	ns
FM (kg)	43.5 ± 3.2	52.3 ± 4.2	46.9 ± 4.9	ns
FM %age	42.4 ± 2.4	43.1 ± 2.2	44.5 ± 2.1	ns
LBM (kg)	55.9 ± 3.1	64.6 ± 2.6	54.1 ± 3.3	ns
LBM %age	54.7 ± 2.3	54.1 ± 2.0	52.8 ± 2.0	ns

Table 6.4.1.1. Baseline characteristics of all participants included in these analyses. Data reported as mean ± SE or median (IQR). Significant differences assessed using one-way ANOVA or Kruskal-Wallis tests

6.4.2. Body weight and composition

Pre- and post-intervention measures are summarised in Table 6.4.2.1. Total body weight significantly reduced in all groups (Figure 6.4.2.1). Participants in SEM experienced weight reduction from 103.2 ± 4.1kg to 97.1 ± 4.0kg ($p < 0.01$), whilst in VLCD the corresponding weight change was 121.6 ± 5.9kg to 108.4 ± 7.0kg ($p < 0.0001$). COMB elicited a reduction in weight from 104.5 ± 7.5kg to 91.0 ± 6.6kg ($p<0.0001$).

	SEM			VLCD			COMB		
	Baseline	**12 weeks**	**p**	**Baseline**	**12 weeks**	**p**	**Baseline**	**12 weeks**	**p**
Weight (kg)	103.2 ± 4.1	97.1 ± 4.0	<0.01	121.6 ± 5.9	108.4 ± 7.0	<0.0001	104.5 ± 7.5	91.0 ± 6.6	<0.0001
BMI (kg.m^{-2})	35.0 ± 1.6	33.0 ± 1.6	<0.01	39.6 ± 2.0	35.3 ± 2.3	<0.0001	36.6 ± 2.0	31.9 ± 1.9	<0.0001
FM (kg)	43.5 ± 3.2	39.5 ± 3.3	<0.01	52.3 ± 4.2	43.3 ± 5.1	<0.0001	44.6 ± 4.9	35.6 ± 4.6	<0.0001
FM %age	42.4 ± 2.4	40.7 ± 2.5	<0.05	43.1 ± 2.2	39.3 ± 2.7	<0.0001	44.4 ± 2.3	40.2 ± 2.8	<0.0001
LBM (kg)	55.9 ± 3.1	54.2 ± 2.8	0.13	65.6 ± 2.5	60.9 ± 2.7	<0.001	51.5 ± 2.2	48.2 ± 2.3	<0.01
LBM %age	54.7 ± 2.3	56.3 ± 2.4	<0.05	54.1 ± 2.0	57.5 ± 2.5	<0.0001	52.8 ± 2.2	56.7 ± 2.6	<0.0001

Table 6.4.2.1. Pre- and post-intervention body weight and composition measures. Significant differences assessed using two-way ANOVA

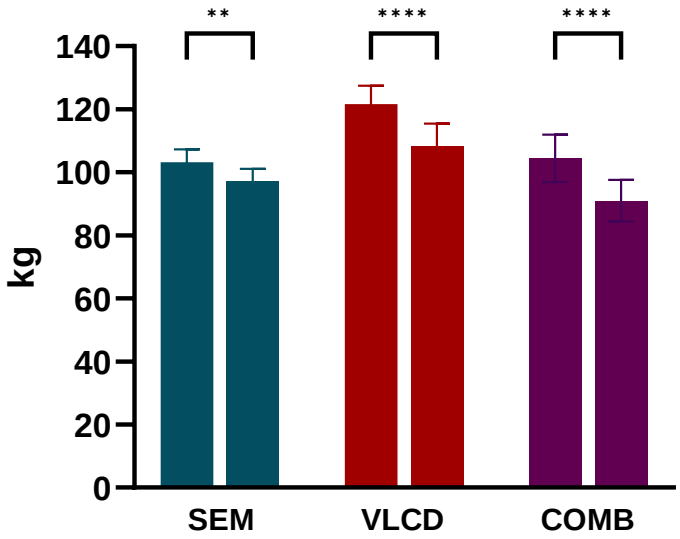

Figure 6.4.2.1. Graph displaying mean weight at baseline and follow-up in each group. Significance assessed with two-way ANOVA. ** p < 0.01, **** p < 0.0001

As shown in Figure 6.4.2.2, absolute weight reductions in VLCD and COMB were similar with no significant differences between the groups (13.1 ± 1.9kg vs 13.5 ± 1.7kg, respectively, p = 0.99). However, these were both significantly greater than the 6.0 ± 0.7kg reduction observed in SEM (p < 0.01 v both groups).

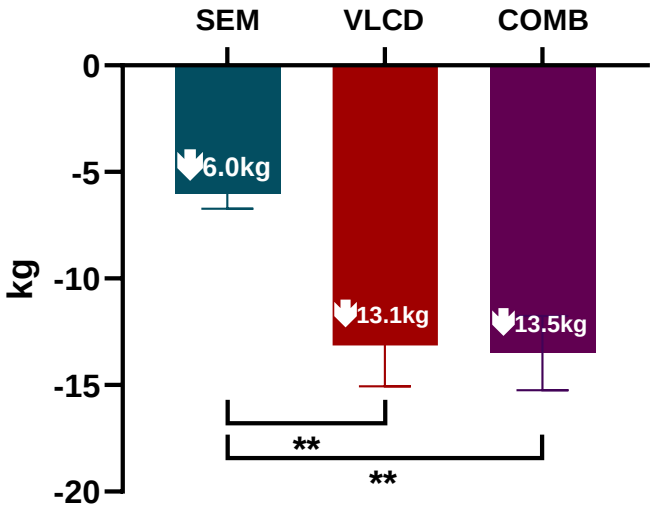

Figure 6.4.2.2. Mean total weight loss in each group. Significance assessed with one-way ANOVA. ** p < 0.01

Similarly, VLCD and COMB demonstrated significantly greater reductions in percentage weight loss and BMI than SEM ($p < 0.05$ SEM vs VLCD, $p < 0.01$ SEM vs COMB). VLCD and COMB observed an additional 5.4 and 7 percentage-point reduction in weight compared to SEM, respectively (Figure 6.4.2.3a). This corresponded to an additional 2.3 $kg.m^{-2}$ and 2.7 $kg.m^{-2}$ reduction in BMI, respectively (Figure 6.4.2.3b). There was no significant difference between the percentage weight loss or change in BMI between VLCD and COMB ($p = 0.70$ and $p = 0.84$, respectively).

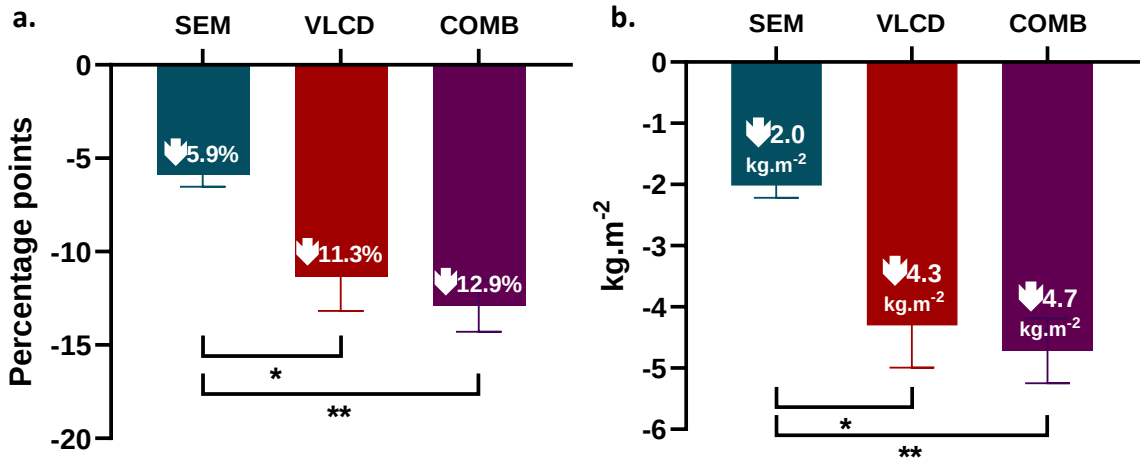

Figure 6.4.2.3. Graphs depicting a) mean percentage weight loss and b) mean BMI reductions in each group. Significance assessed with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$

FM also reduced significantly in all groups (SEM 43.5 ± 3.2kg to 39.5 ± 3.3kg, $p < 0.01$; VLCD 52.3 ± 4.2kg to 43.3 ± 5.1kg, $p < 0.0001$; COMB 44.6 ± 4.9kg to 35.6 ± 4.6kg, $p < 0.0001$). FM reductions were identical between VLCD and COMB (-9.0 ± 1.3kg and -9.0 ± 1.2kg, respectively). The SEM group demonstrated a significantly lower reduction in FM, which was less than half of that observed in the other two intervention groups (-4.0 ± 0.5kg, $p < 0.01$ vs both).

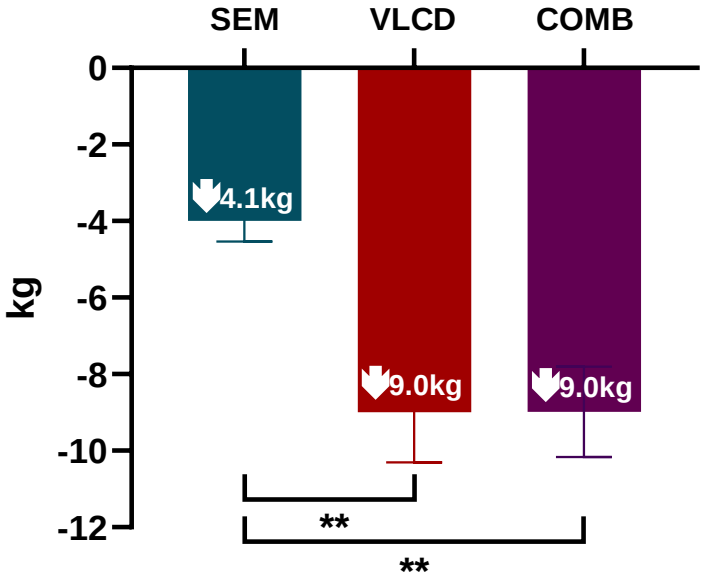

Figure 6.4.2.4. Mean change in FM in each group. Significance assessed with one-way ANOVA. ** p < 0.01

Unlike FM, LBM reduced to a similar extent in the three groups with no significant between-group differences (data reported in section 5.4.5). In all groups, the reduction in FM was greater than the loss of LBM, resulting in an increase in relative LBM percentage compared to baseline (Figure 6.4.2.5).

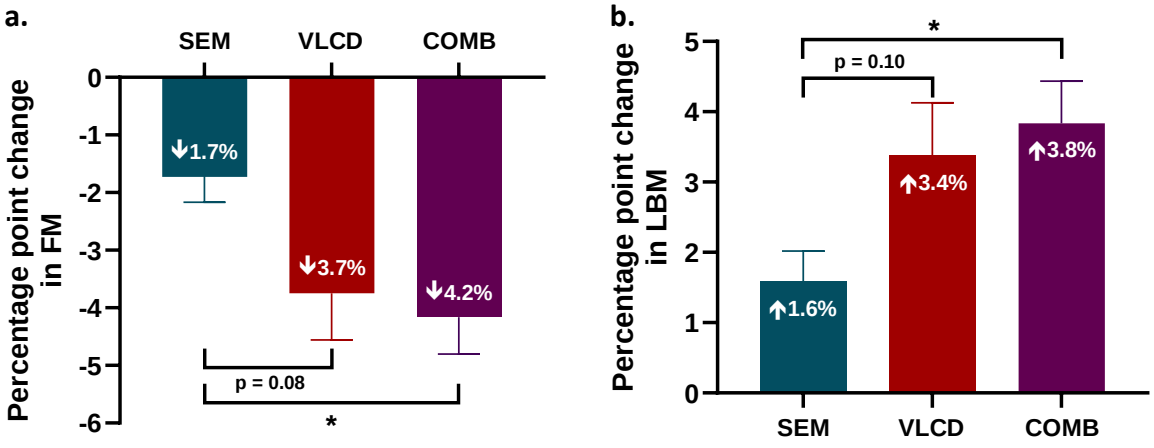

Figure 6.4.2.5. Mean change in a) FM percentage, and b) LBM percentage of total body weight in each group. Significance assessed with one-way ANOVA. * p < 0.05

6.4.3. Diet diary analysis

Analysis of food intake and macronutrient composition was performed at baseline and 6 weeks into the intervention period, the results are outline in Table 6.4.3.1. There was no significant difference between mean daily energy intake at baseline (SEM 1603.9 ± 229.1 kcal / day, VLCD 1859.1 ± 138.0 kcal / day, COMB 1762.6 ± 168.7 kcal / day). Semaglutide treatment significantly reduced daily energy intake to 1123.9 ± 90.8 kcal ($p < 0.05$). Significant reductions were also observed in the VLCD and COMB groups to 712.5 ± 32.1 kcal ($p < 0.0001$) and 626.1 ± 33.7 kcal ($p < 0.0001$), respectively. These follow-up energy intake values suggested good compliance with the VLCD programme in both groups.

	SEM			VLCD			COMB		
	Baseline	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**	**Baseline**	**6 weeks**	**p**
Total energy intake (kcal)	1603.9 ± 229.1	1123.9 ± 90.8	<0.05	1859.1 ± 138.0	712.5 ± 32.1	<0.0001	1762.6 ± 168.7	626.1 ± 33.7	<0.0001
Fat intake (g)	65.7 ± 11.2	44.7 ± 5.7	<0.05	74.1 ± 7.5	19.7 ± 0.8	<0.0001	68.8 ± 6.8	17.5 ± 1.3	<0.0001
Fat proportion of total energy intake (%)	36.9 ± 3.6	34.9 ± 2.4	0.48	36.0 ± 3.4	25.3 ± 0.6	<0.001	35.3 ± 1.9	25.0 ± 1.0	<0.01
Protein intake (g)	75.9 ± 12.8	52.1 ± 4.5	<0.05	83.6 ± 6.8	57.1 ± 3.0	<0.01	81.4 ± 8.3	47.2 ± 3.4	<0.0001
Protein proportion of total energy intake (%)	19.0 ± 1.4	18.6 ± 1.2	0.77	18.0 ± 0.7	31.7 ± 1.0	<0.0001	18.9 ± 1.3	30.0 ± 1.1	<0.0001
Carbohydrate intake (g)	161.9 ± 22.0	118.0 ± 11.0	0.055	187.4 ± 16.0	70.0 ± 3.4	<0.0001	193.7 ± 26.1	66.8 ± 3.7	<0.0001
Carbohydrate proportion of total energy intake (%)	42.1 ± 3.0	44.1 ± 3.4	0.41	40.5 ± 2.4	39.4 ± 1.3	0.64	43.2 ± 3.0	43.2 ± 1.9	0.99

Table 6.4.3.1. Pre- and post-intervention energy intake and macronutrient composition. Significant differences assessed using two-way ANOVA

In accordance with the changes in body weight, the reductions in daily energy intake were also significantly smaller in SEM than the VLCD and COMB groups (Figure 6.4.3.1).

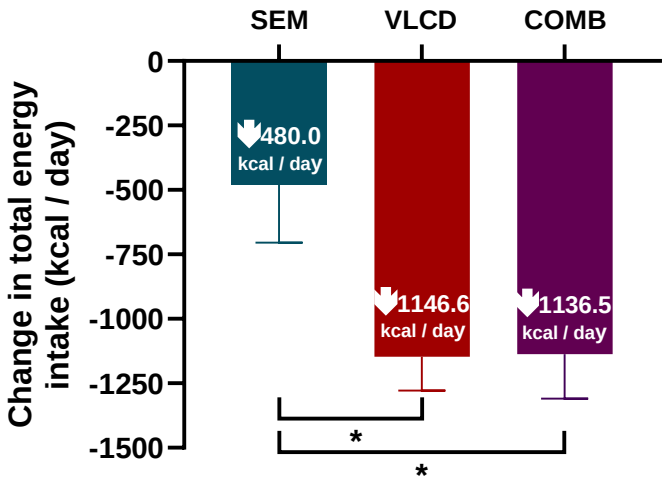

Figure 6.4.3.1. Mean change in total daily energy intake. Significance assessed with one-way ANOVA. * $p < 0.05$

Macronutrient content of ingested food was also calculated. Absolute mean intake of protein and fat reduced significantly in all groups. Carbohydrate intake also reduced significantly in VLCD and COMB with a borderline non-significant reduction in SEM ($p=0.055$). In a similar pattern to daily energy intake, the reduction in fat intake was significantly greater in VLCD and COMB than in SEM, mirroring the changes in weight and FM (Figure 6.4.3.2).

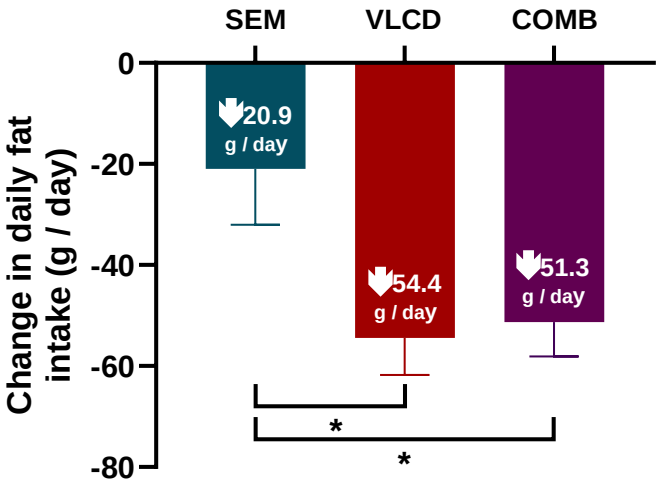

Figure 6.4.3.2. Mean change in daily fat intake. Significance assessed with one-way ANOVA. * p < 0.05

Conversely, reduction in protein intake was similar across the three groups (Figure 6.4.3.3a). The reduction in carbohydrate intake was greater in VLCD and COMB than SEM, although this difference was only significant with COMB (Figure 6.4.3.3b).

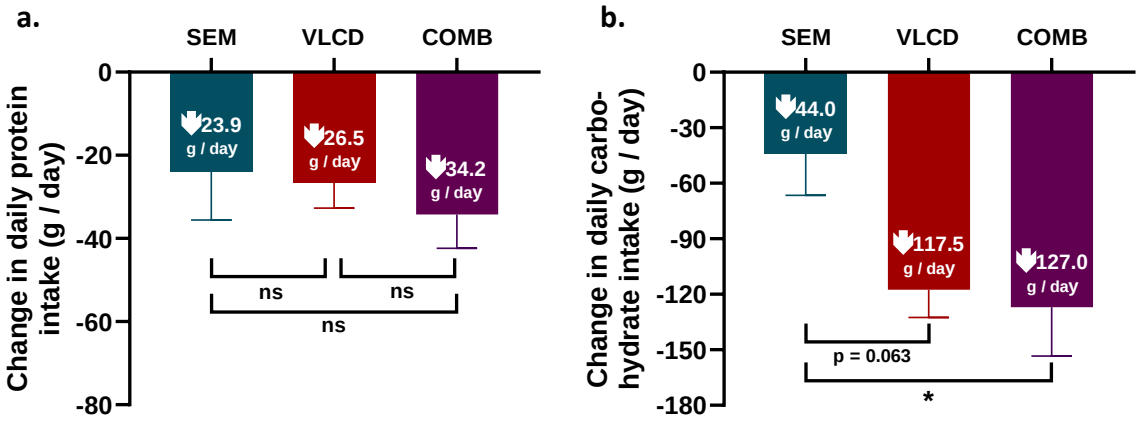

Figure 6.4.3.3. Mean change in a) daily protein and b) daily carbohydrate intake. Significance assessed with one-way ANOVA. * p < 0.05

Notably, further analysis of the macronutrient proportions revealed unequal alterations in protein and fat intake, leading to marked shifts in the VLCD and COMB group (Figure 6.4.3.4). In VLCD, the proportion of daily energy intake from protein increased significantly from 18.0 ± 0.7% to 31.7 ± 1.0% ($p < 0.0001$), while dietary fat content reduced significantly from 36.0 ± 3.4% to 25.3 ± 0.6% ($p < 0.001$). Similar

alterations were observed in the COMB group (protein: 18.9 ± 1.3% to 30.0 ± 1.1%, $p < 0.0001$; fat: 35.3 ± 1.9% to 25.0 ± 1.0%, $p < 0.01$). No such changes occurred in SEM in which macronutrient composition during the intervention was not significantly different from baseline, despite the observed reduction in overall energy intake (Figure 6.4.3.4a).

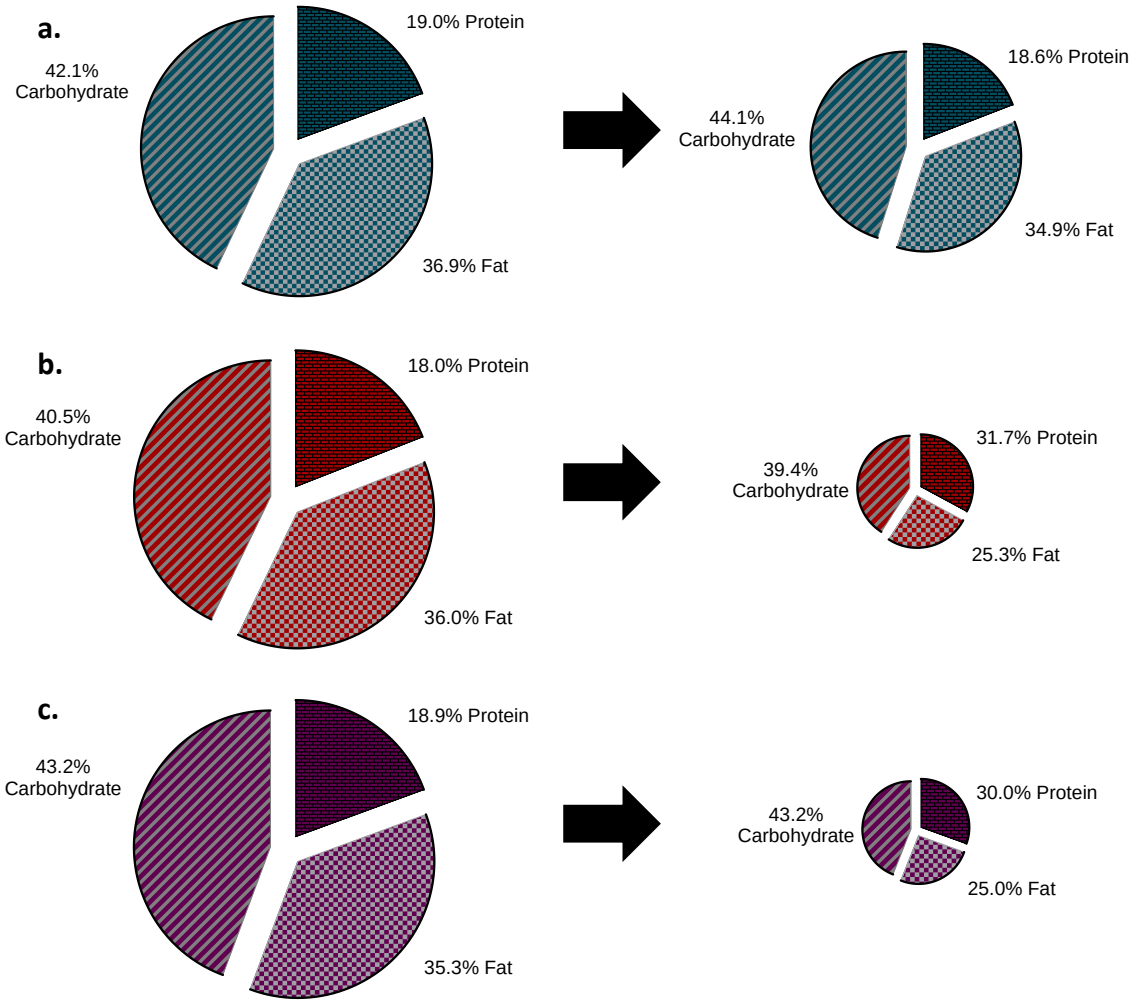

Figure 6.4.3.4. Graphical representations of the change in macronutrient composition in a) SEM, b) VLCD, and c) COMB. The relative size of the circles is representative of the total daily energy intake.

Consequently, the protein proportion in the food ingested by participants was significantly higher in VLCD and COMB, than SEM ($p < 0.0001$ vs both), and the inverse was the case regarding proportionate fat content ($p < 0.05$ vs both).

Carbohydrate proportion of total energy intake did not change significantly in any group.

6.4.4. Glycaemic measures

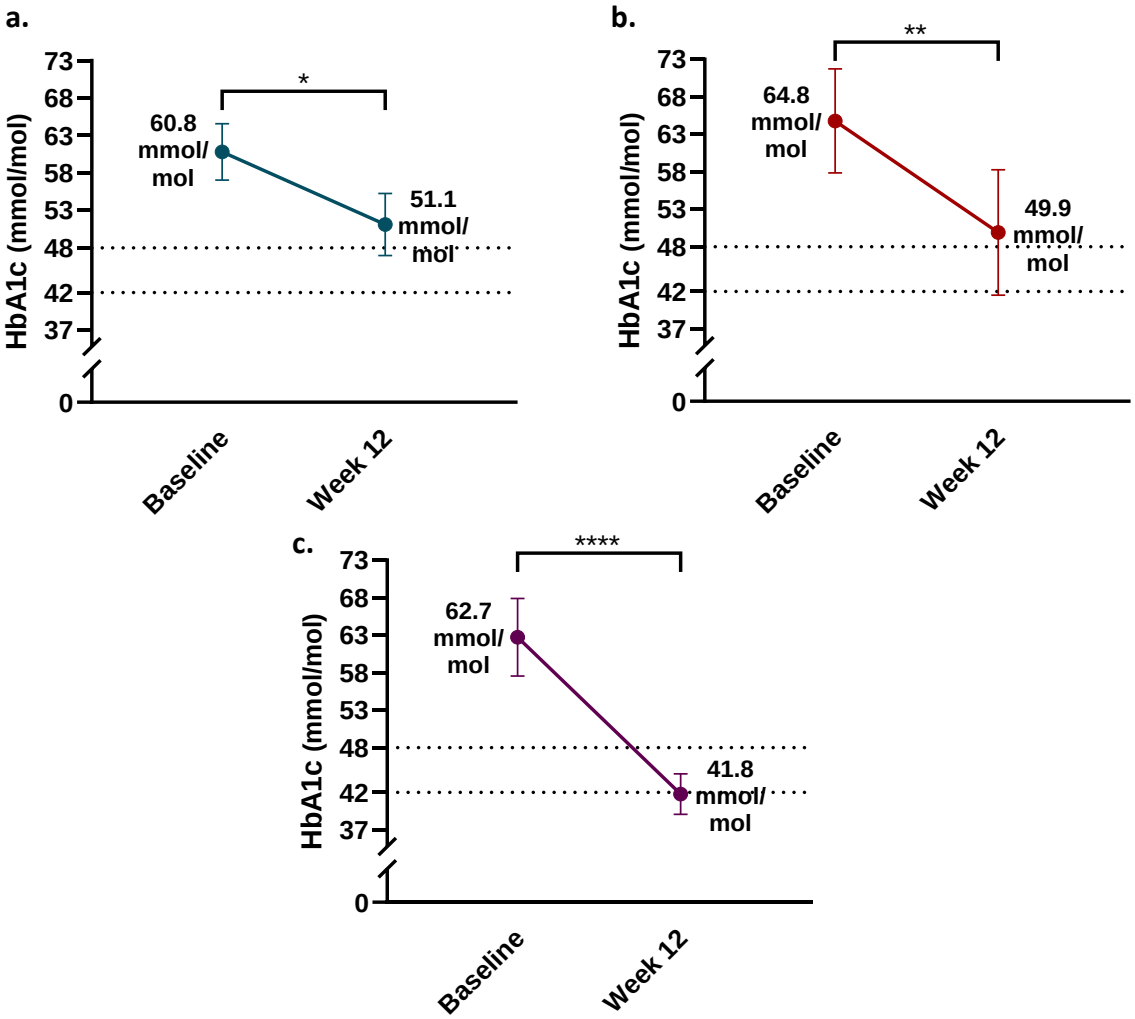

Figure 6.4.4.1. Mean HbA1c at baseline and follow-up in a) SEM, b) VLCD, and c) COMB. Significance assessed with two-way ANOVA. * p < 0.05, ** p < 0.01, **** p < 0.0001

As shown in Figure 6.4.4.1, HbA1c significantly reduced in all three groups. COMB observed a numerically greater reduction in HbA1c compared to the other groups (Figure 6.4.4.2), however this was not statistically significant (p = 0.08 vs SEM, p = 0.46 vs VLCD).

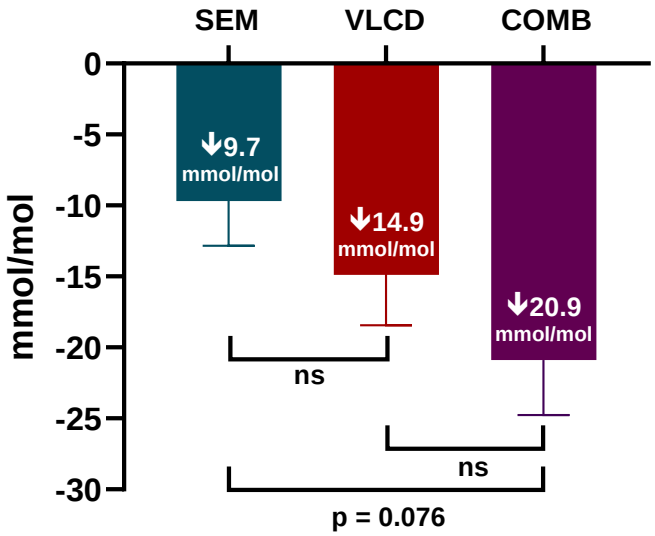

Figure 6.4.4.2. Mean change in HbA1c in each group. Significance assessed with one-way ANOVA

Fasting glucose also reduced significantly in all groups (Figure 6.4.4.3), with no significant between-group differences in the magnitude of reduction.

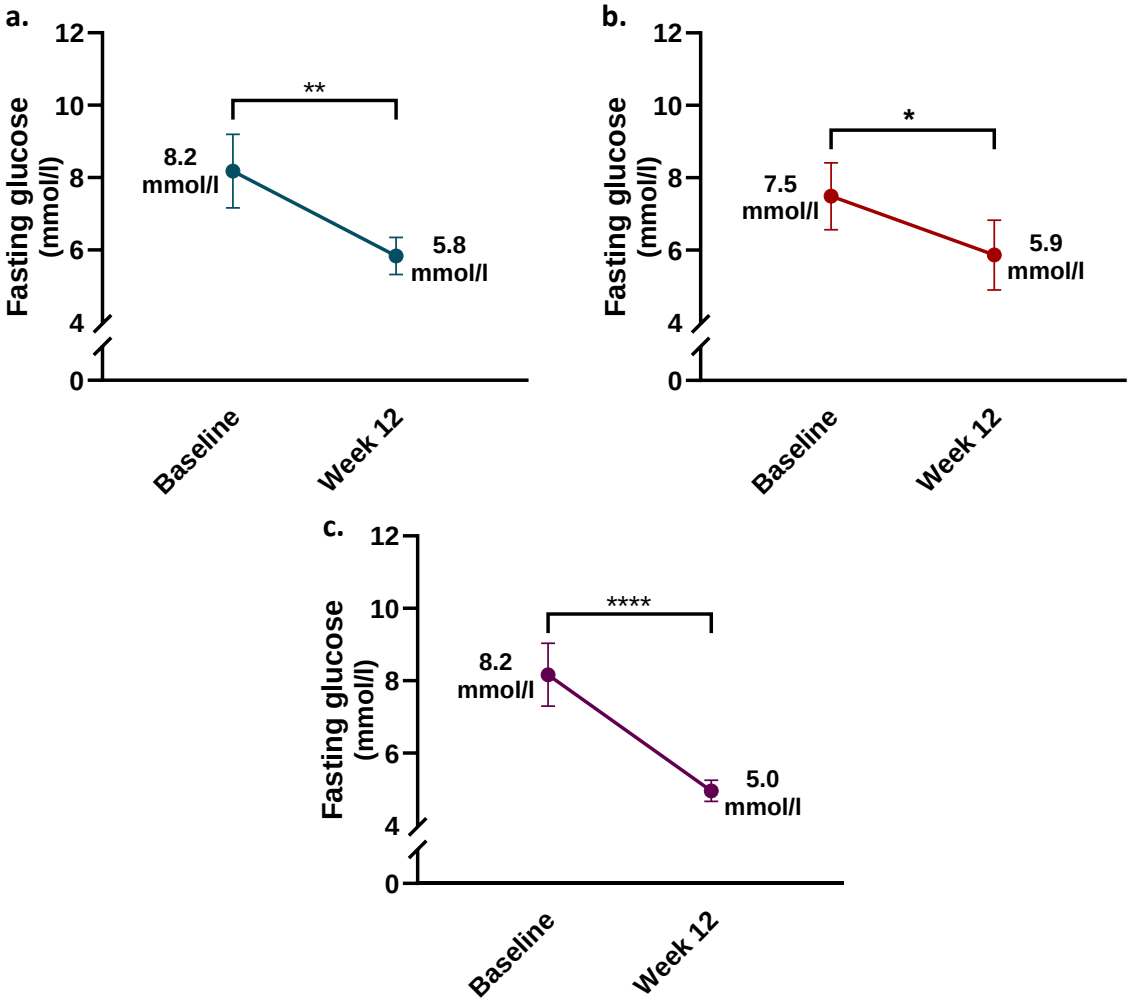

Figure 6.4.4.3. Mean fasting glucose at baseline and follow-up in a) SEM, b) VLCD, and c) COMB. Significance assessed with two-way ANOVA. * p < 0.05, ** p < 0.01, **** p < 0.0001

Fasting insulin reduced significantly in VLCD (16.8±2.1mmol/l to 9.2±2.2mmol/l, p<0.01) and COMB (17.4±3.0mmol/l to 8.6±1.1mmol/l, p<0.001), whilst in SEM there was a slight but non-significant increase (11.3±1.5mmol/l to 12.1±1.9mmol/l, p=0.96).

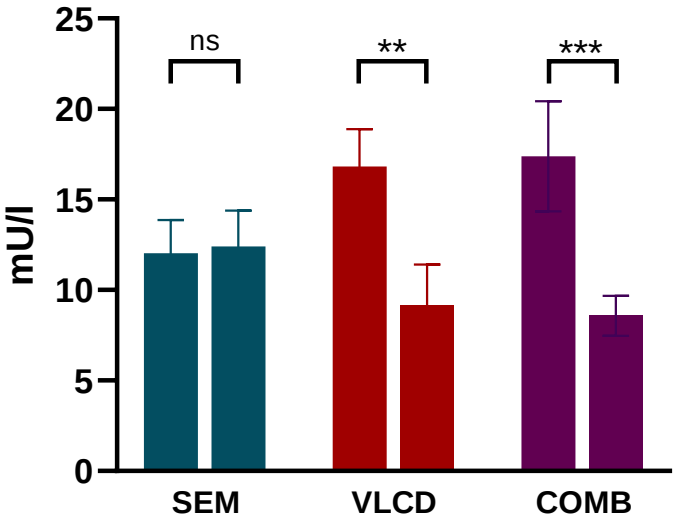

Figure 6.4.4.4. Mean fasting insulin at baseline and follow-up in each group. Significance assessed with two-way ANOVA. ** p < 0.01, *** p < 0.001

First phase response, as determined by the AIRg was used as a marker of BCF in this study. At baseline, each group demonstrated a minimal first-phase response, as expected for a cohort of individuals with T2D.

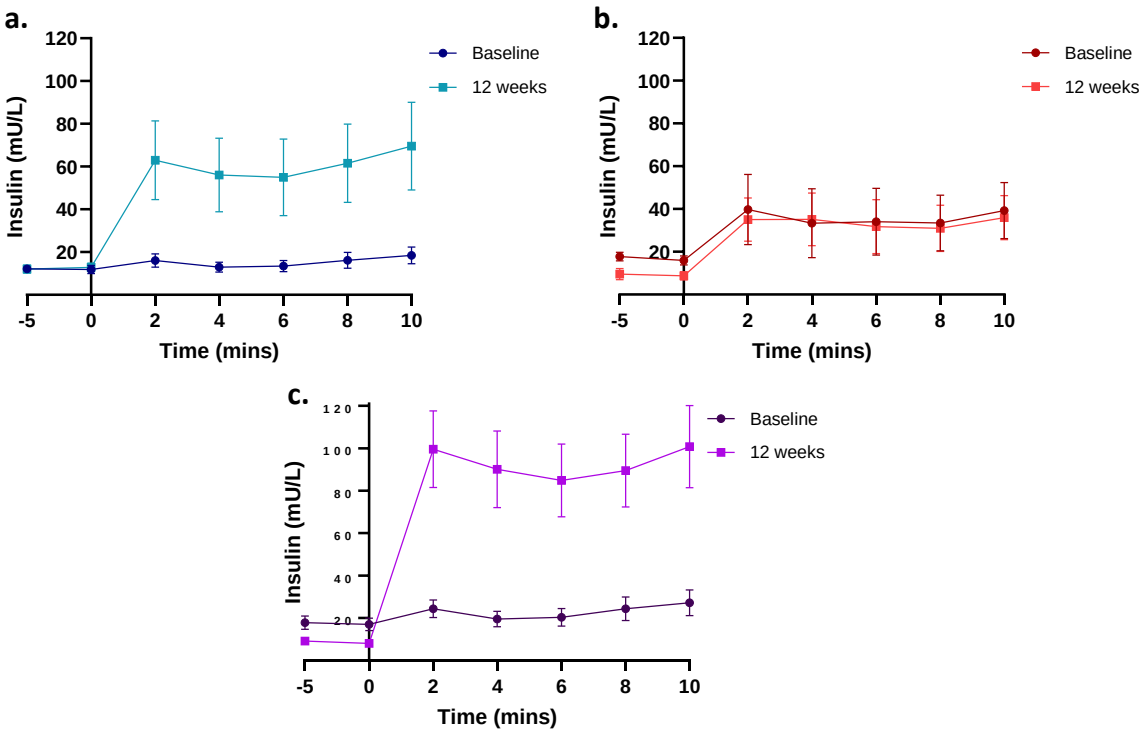

Figure 6.4.4.5. Acute insulin response at baseline and follow-up in a) SEM, b) VLCD, and c) COMB.

However, as demonstrated in Figures 6.4.4.5 and 6.4.4.6, following the 12-week intervention AIRg increased significantly in SEM (52.3 ± 17.0μU.ml^{-1}.min to 431.1 ± 155.6 μU.ml^{-1}.min, $p < 0.05$) and COMB (83.1 ± 23.7 μU.ml^{-1}.min to 753.7 ± 150.0 μU.ml^{-1}.min, $p < 0.0001$). There was a minimal increase in VLCD, which was non-significant (199.9 ± 122.3 μU.ml^{-1}.min to 234.2 ± 78.2 μU.ml^{-1}.min, $p = 0.99$).

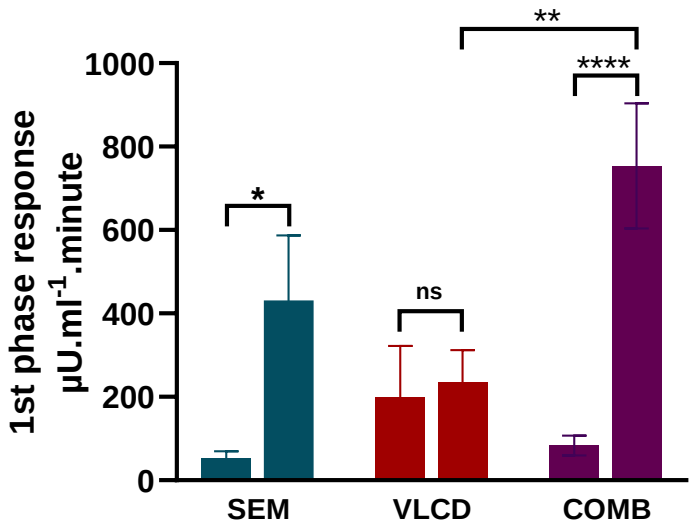

Figure 6.4.4.6. Mean AIRg at baseline and follow-up in each group. Significance assessed with two-way ANOVA. * p < 0.05, ** p < 0.01, ** p < 0.0001

In the case of COMB, this equated to a 9-fold increase in AIRg, such that the follow-up AIRg was significantly higher than the corresponding value in the VLCD group. Furthermore, the magnitude of change was significantly greater than the change observed in VLCD ($p < 0.01$). This difference in magnitude of change between the groups is depicted graphically below in Figure 6.4.4.7.

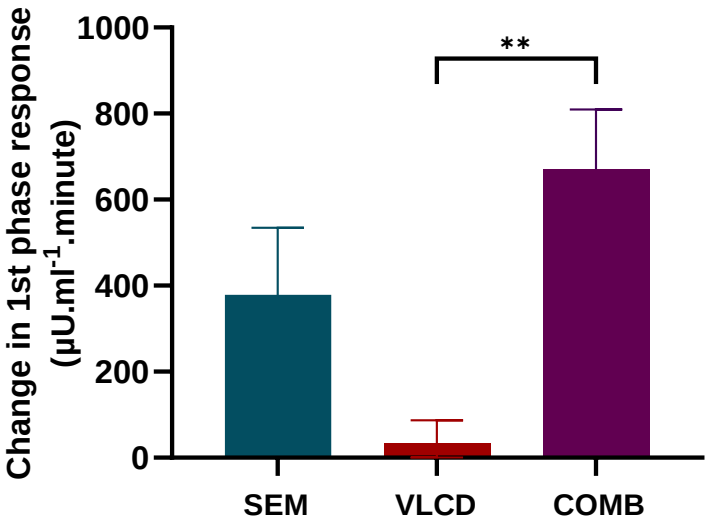

Figure 6.4.4.7. Mean change in AIRg in each group. Significance assessed with one-way ANOVA. ** p < 0.01

With respect to IS, HOMA2-IR significantly reduced in VLCD (0.34 ± 0.04 to 0.18 ± 0.05, p < 0.01) and COMB (0.36 ± 0.06 to 0.16 ± 0.02, p < 0.0001), with no significant change in SEM (0.24 ± 0.03 to 0.23 ± 0.04, p = 1.00).

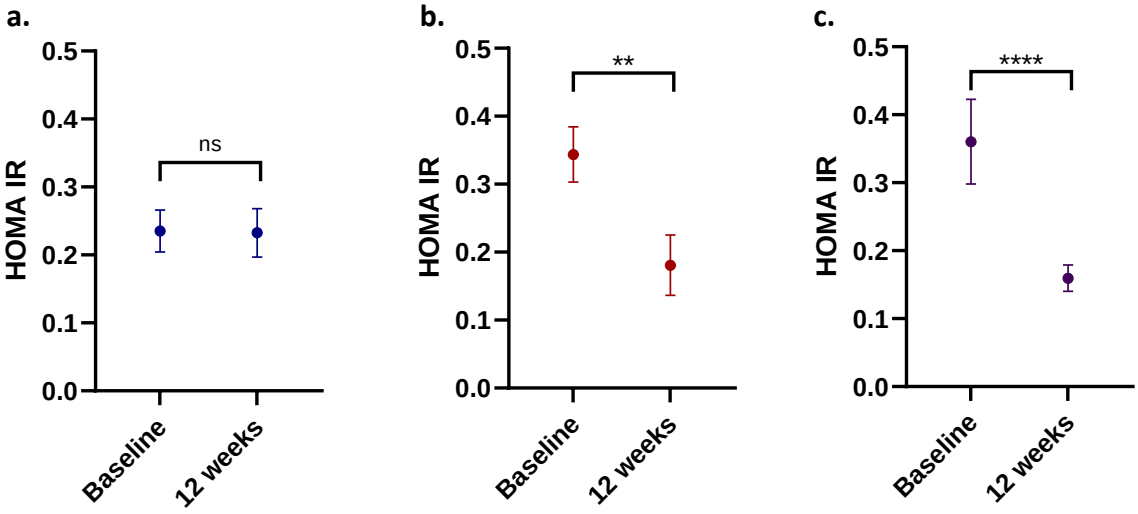

Figure 6.4.4.8. Baseline and follow-up HOMA-IR in a) SEM, b) VLCD, and c) COMB. Significance assessed with two-way ANOVA. ** p < 0.01, **** p < 0.0001

However, assessment of IS using CS_I revealed different results from those described above (Figure 6.4.4.8). Whilst a significant increase was observed in VLCD in keeping with the HOMA-IR analysis (0.64 ± 0.11 to 0.99 ± 0.17, p < 0.05), a significant

decrease in CS_I was observed in SEM (1.06 ± 0.34 to 0.64 ± 0.13, $p < 0.05$). COMB induced a numerical but non-significant reduction in CS_I, also in disagreement with the corresponding HOMA-IR results.

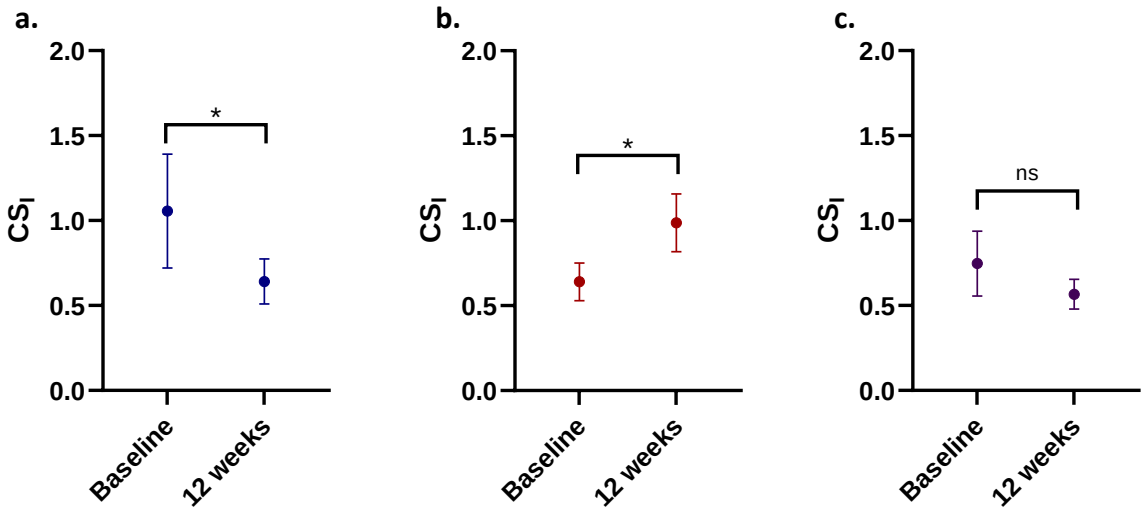

Figure 6.4.4.8. Baseline and follow-up CSI in a) SEM, b) VLCD, and c) COMB. Significance assessed with two-way ANOVA. * $p < 0.05$

6.4.5. Adverse events

Given that this study was exploring an entirely novel approach to T2D management, an assessment of adverse events experienced by participants was felt to be essential. The most common adverse effect induced by SEM was nausea which was experienced by five (45.4%) participants. The majority of these cases were mild, with only one participant developing severe nausea. This occurred on the lowest dose of Semaglutide, resulting in the decision to cease her involvement in the study. Another participant was unable to tolerate the maximum dose, despite slowing dose increments to 4-weekly, and therefore remained on 0.5mg for the study duration. Participants in SEM also experienced constipation (36.4%), mild abdominal discomfort (27.3%), abdominal bloating (27.3%) and lethargy (27.3%).

In VLCD the most common adverse effect was constipation which was experienced by four (40%) participants. In COMB, seven (63.6%) participants reported constipation, with one participant requiring medication to manage this. Four (36.3%) experienced mild abdominal discomfort and three (36.3%) experienced mild nausea or headache. Six participants developed COVID-19 infection during the intervention period, with prompt recovery. Unfortunately, one participant in COMB was unable to complete their follow-up visit due to this and was therefore excluded from the study. No participants required emergency or unplanned admission to hospital.

6.5. Discussion

Considerable attention has focused on the ability of VLCD to induce weight loss and diabetes remission, as well as the weight reducing effects of Semaglutide. However, there is no comparison of the relative short-term efficacy of these interventions on clinical outcomes such as body weight, body composition and glycaemic control. Additionally, no previous study has explored the effects of VLCD and Semaglutide in combination on these parameters. The potential MM-preserving effects of GLP1RAs make the idea of combined treatment with VLCD an exciting prospect, and thus an understanding of the clinical effects of this approach was warranted.

Several findings can be highlighted from the results of this study. Firstly, in these participants over 12 weeks, VLCD resulted in greater weight reduction than Semaglutide, which was primarily driven by enhanced FM losses. VLCD elicited a corresponding improvement in IS, which did not occur with Semaglutide, however this did not translate to differences in amelioration of overall glycaemic control between the two interventions. Secondly, addition of VLCD enhanced the weight loss observed with Semaglutide by 7 percentage points. This was also driven by greater FM losses, mirroring the findings in the VLCD-only group. Furthermore, addition of VLCD to Semaglutide showed a potential trend towards a greater improvement in glycaemic control. Finally, adjuvant administration of Semaglutide alongside VLCD did not provoke any significant improvements in weight loss or body composition changes compared to VLCD-alone. However, the combination did stimulate a greater increase in insulin first phase response than the minimal change observed with VLCD.

The fact that VLCD stimulated greater short-term weight loss than Semaglutide was unsurprising and is in keeping with results of studies investigating short term weight outcomes of the two interventions indirectly (Blundell et al., 2017; Brown et al., 2020). However, to my knowledge no study has directly compared the effect of these intervention in individuals with T2D. The extra weight loss exhibited by the VLCD group appears to be driven primarily by greater FM losses. Dietary analysis highlighted a significantly greater reduction in fat intake with VLCD, which is likely to account for this. VLCD was associated with significant reduction in fat proportion of total energy intake, while Semaglutide did not elicit any change in macronutrient composition. This has been previously observed (Blundell et al., 2017), and suggests that the prescriptive nature of VLCD may provide an additional benefit of limiting fat intake, therefore stimulating a resultant increase in FM loss. Greater FM losses are associated with more substantial reductions in HbA1c (Igarashi et al., 2023), making this finding particularly pertinent to individuals with T2D.

VLCD also elicited significant improvements in both measures of IS, whilst Semaglutide did not, despite the weight loss observed in these participants. Notably, lack of change in IS has also been reported by studies using IVGTT with minimal modelling calculations in response to another GLP1RA, Lirgalutide (Anholm et al., 2017; Vilsbøll et al., 2008). Weight loss is typically associated with reduction in fasting insulin (Silvestre et al., 2018), however this did not occur in our Semaglutide cohort. Indeed, in another study assessing Semaglutide effects on glycaemic parameters, fasting insulin increased despite significant weight loss (Kapitza et al., 2017a).

The direct insulin stimulatory effect of GLP1RAs may explain these observations, resulting in artificial elevations in insulin concentrations. This could explain the lack of change in IS, as both measures are reliant upon endogenous plasma insulin concentrations. In agreement with this, GLP1RAs have shown significant improvement in IS when assessed via the gold standard HE clamp (Bunck et al., 2011; Jinnouchi et al., 2015). Taken together, it appears that fasting methods of assessing IS (such as HOMA-IR) and measures derived from IVGTT may not be optimal strategies for measuring this in the context of GLP1RAs.

The lack of significant change in BCF in the VLCD group was unexpected. Previous studies have outlined the ability of VLCD interventions to improve BCF assessed by first phase response during IVGTT (Jackness et al., 2013; Plum et al., 2011). This finding may be explained by the relatively long duration of diabetes diagnosis in our study population. Previous studies suggest that a shorter duration of diabetes is associated with a greater chance of diabetes remission (Steven, Hollingsworth, Al-Mrabeh, et al., 2016). Accordingly, the UK NHS programme only accepts patients that received their T2D diagnosis within the previous 6 years (NHS England, 2020). Each group's mean duration of T2D diagnosis was greater than 6 years, suggesting that our participants' beta-cells had less capacity for recovery. Intriguingly, despite this, Semaglutide and combination of VLCD with Semaglutide stimulated significant improvement in first-phase response. This implies that the stimulatory effect of Semaglutide persists, even in individuals with longer duration T2D.

The significantly greater BCF induced by the combination in comparison to VLCD alone was the only difference demonstrated between these two groups. This finding

could be of particular relevance to individuals with T2D as a recent report outlining the 5-year follow-up outcomes of the DiRECT study (M. E. Lean et al., 2018) upon which the NHS caloric restriction programme is based, suggested that the majority of participants eventually experienced relapse of T2D (M. E. Lean et al., 2024). Previous mechanistic studies have reported that the extent of insulin first-phase response restoration may a major determinant of long-term T2D remission (Steven, Hollingsworth, Al-Mrabeh, et al., 2016; Taylor, 2013). Thus, greater improvement in BCF following VLCD-based interventions could result in more robust long-term remission. The observed potentiation of first phase response with combined VLCD and Semaglutide demonstrated in the present study is an entirely novel finding, and requires further investigation in the clinical context.

In contrast, the comparison between the combination approach and Semaglutide-alone revealed several differences, outlining potential additive benefits of adjuvant VLCD use alongside Semaglutide, at least in the initial 12 weeks of initiation. Recently, a formulation of Semaglutide has been approved for weight management in individuals living with obesity, following publication of a series of large clinical studies reporting up to 18% mean weight loss in this context (D. Rubino et al., 2021; Wadden et al., 2021; Wilding et al., 2021). Intriguingly, our findings suggest that addition of VLCD during the first 12 weeks of Semaglutide dose escalation could provoke additional weight loss of approximately 7-percentage points.

Larger magnitudes of weight loss are more effective for managing obesity-related comorbidities (Horn et al., 2022), thus this approach could be particularly beneficial in individuals with multi-morbidity related to weight. Adjuvant VLCD alongside

Semaglutide also stimulated greater improvements in FM and IS, which could be relevant in individuals at high risk of T2D, or those with associated metabolic syndrome. Importantly, the present study only lasted 12 weeks, so the long-term effects of this cannot be extrapolated without a critical investigation of this.

6.5.1. Study Limitations

There are several important limitations to this study. Firstly, this was an exploratory study with low participant numbers limiting the generalisability of the results. Secondly, the rapid dose escalation of Semaglutide used in our study is not commonly used in clinical practice, and likely led to a greater degree of weight loss than observed in other short-term clinical studies (Blundell et al., 2017). Notably, the adverse event profile observed from the Semaglutide group was comparable to studies using the standard dose escalation (Wilding et al., 2021). There were numerically more adverse events in the combination group, which is to be expected in the context of two interventions being used simultaneously. This will require assessment in larger scale studies to determine the significance of this and whether this outweighs the potential benefits of this approach. Thirdly, whilst the IVGTT is a valid method of assessing BCF and IS (Bingley et al., 1992; Cersosimo et al., 2014), it is not the gold-standard. HOMA-IR and CS_I do not appear to be reliable when assessing IS changes in response to GLP1RA interventions. Thus, further long-term studies involving larger participant numbers, standard dose escalation, longer-term follow-up and clamp techniques would be necessary to qualify the reported findings.

6.5.2. Conclusions

Despite the limitations, this study has provided some insight into a relatively under-explored area of T2D management, and highlighted several potential avenues for further investigation. Our findings suggest that when short-term weight loss is required (for example, prior to surgery), VLCD is more favourable than Semaglutide, with the additional benefits of FM reduction and amelioration of IR. Combining Semaglutide with VLCD appears to stimulate greater improvement in BCF, and the consequences of this in the context of inducing diabetes remission requires deeper exploration. Moreover, an evaluation of the long-term efficacy of concurrent VLCD during the initial stages of Semaglutide treatment is warranted, given the potential additive effects on weight, FM and IR suggested by this study. The additional weight loss may be particularly relevant to individuals living with multiple weight-related comorbidities. Finally, a full assessment of the adverse effect profile and cost-effectiveness of the combination approach is necessary before it can be considered for widespread adoption in the clinical setting.

7. Overall Conclusions

This work was undertaken to improve understanding of a topic that is very relevant to modern medicine. Over the last few decades, obesity and T2D have rapidly emerged as major threats to healthcare systems across the world. Accordingly, novel interventions to tackle these critical issues are being developed at pace, with various candidates already being utilised. VLCD and GLP1RAs have both become embedded within guidelines for managing both conditions (M. J. Davies et al., 2022; NICE, 2015b). With the recent adoption of the NHS Path to Remission Programme (NHS England, 2020) and the approval of Semaglutide for treatment of T2D and obesity, these interventions will become more widely utilised in clinical practice for years to come.

On the other hand, frailty and disability related to sarcopenia are equally significant issues, particularly with the ageing population associated with modern society (Dodds & Sayer, 2015). A considerable amount of attention has focussed on understanding normal muscle physiology and the mechanisms underlying age-related muscle loss. Alongside this arose an awareness around the potentially substantial risks of exacerbating the process of age-related muscle loss (Cava et al., 2017). Furthermore, given that individuals with T2D and obesity already experience accelerations in this process (S. W. Park et al., 2009), they are a group that are at particularly high risk of frailty and disability (Kalyani et al., 2010).

The potential loss of MM associated with weight loss interventions has been extensively cited (Janssen et al., 2023; Kalaitzoglou et al., 2019; Kreitzman, 1989; Sargeant et al., 2019), however uncertainties regarding the true extent of these changes were still apparent. Additionally, whilst GLP1RAs have been identified as

potentially muscle-sparing, again this had yet to be confirmed (Buntz, 2024). Furthermore, empirical data regarding the physiological effects of these interventions on muscle when used in individuals with T2D and obesity were scarce, or non-existent. And finally, the potential benefits of using these interventions in combination had never been explored. This work therefore addressed several gaps identified in the literature, and the overall findings are discussed below.

7.1. The effects of VLCD and GLP1RA on overall muscle mass

Alongside the generation of empirical data to explore these gaps outlined above, I felt it was necessary to review the existing literature and synthesise the pre-existing data that were already available.

With regards to severe caloric restriction interventions (including VLCD), the results of the meta-analysis outlined in Chapter 4 clearly demonstrate significant loss of MM associated with these interventions. This was the case for both individuals with T2D and those without. Approximately one quarter of the weight lost was comprised of MM, which is similar to values previously quoted in literature (Willoughby et al., 2018). Crucially, the amount of MM lost, and the proportion of weight loss attributable to MM was no worse in individuals with T2D. This implies that despite the accelerated MM loss associated with T2D, there does not appear to be a direct interaction between T2D and diet-induced MM reductions. This should be reassuring to clinicians like me who manage individuals with T2D and recommend participation in caloric restriction interventions such as the NHS-endorsed programme.

One other intriguing finding of this meta-analysis was that less restrictive interventions appear to stimulate greater weight loss and FM loss than more

restrictive (< 600 kcal) ones. This is in keeping with the observations of others (Leslie et al., 2017; National Task Force on the Prevention and Treatment of Obesity, 1993), providing support for the decision to limit daily energy intake to 825-853 kcal in the NHS programme. Furthermore, this extra FM and weight loss was not linked with the disadvantage of greater MM loss, which was not significantly different between the subgroups.

In contrast, the meta-analysis detailed in Chapter 3 concluded that GLP1RAs induce a non-significant reduction of MM in individuals with T2D. Although the possibility of GLP1RAs preserving MM has previously been postulated (Ma & Chen, 2021), this is the first review using statistical methodology that provides evidence supporting this assertion. Individuals without T2D did observe significant loss of MM, however this was in the context of a larger degree of weight loss. This was to be expected as doses of GLP1RA used for managing obesity are higher than those used for T2D. Perhaps most intriguingly, the proportion of weight lost consisting of MM was approximately 16-17%. This compares favourably to the 25% figure noted with VLCD, and the 33% reported following bariatric surgery (Nuijten et al., 2022). This adds further support to the notion that GLP1RAs have a muscle-preserving effect.

Both meta-analyses revealed novel findings and enabled some clarity to be achieved on two topics in which inconsistencies existed. Furthermore, they provided a context to the results of the subsequent empirical studies.

7.2. Both interventions cause early significant reductions in MM

The results of the conducted empirical study were in keeping with the meta-analysis findings. Whilst 12 weeks of VLCD induced significant MM loss, the changes with Semaglutide were non-significant. Notably, when measured at 6 weeks, all groups had lost significant MM, however no further changes occurred following this. This is an important finding as it suggests that changes in MM may occur early in the course of weight loss, but then eventually stabilise. Evidence supporting this exists, with a meta-analysis demonstrating diminishing losses of MM as time progressed following bariatric surgery (Nuijten et al., 2022). This requires further assessment as one could infer that methods of mitigating muscle loss during weight reduction interventions may only be required for the first few weeks following intervention commencement. It also suggests that the mechanistic alterations responsible for muscle loss associated with these interventions are dynamic in nature. Thus, it is likely that different physiological processes are occurring at specific time periods during the intervention (Figure 7.2.1). Further, it remains unknown whether this early MM loss is due to reduction in intramuscular fat or breakdown of functional contractile proteins.

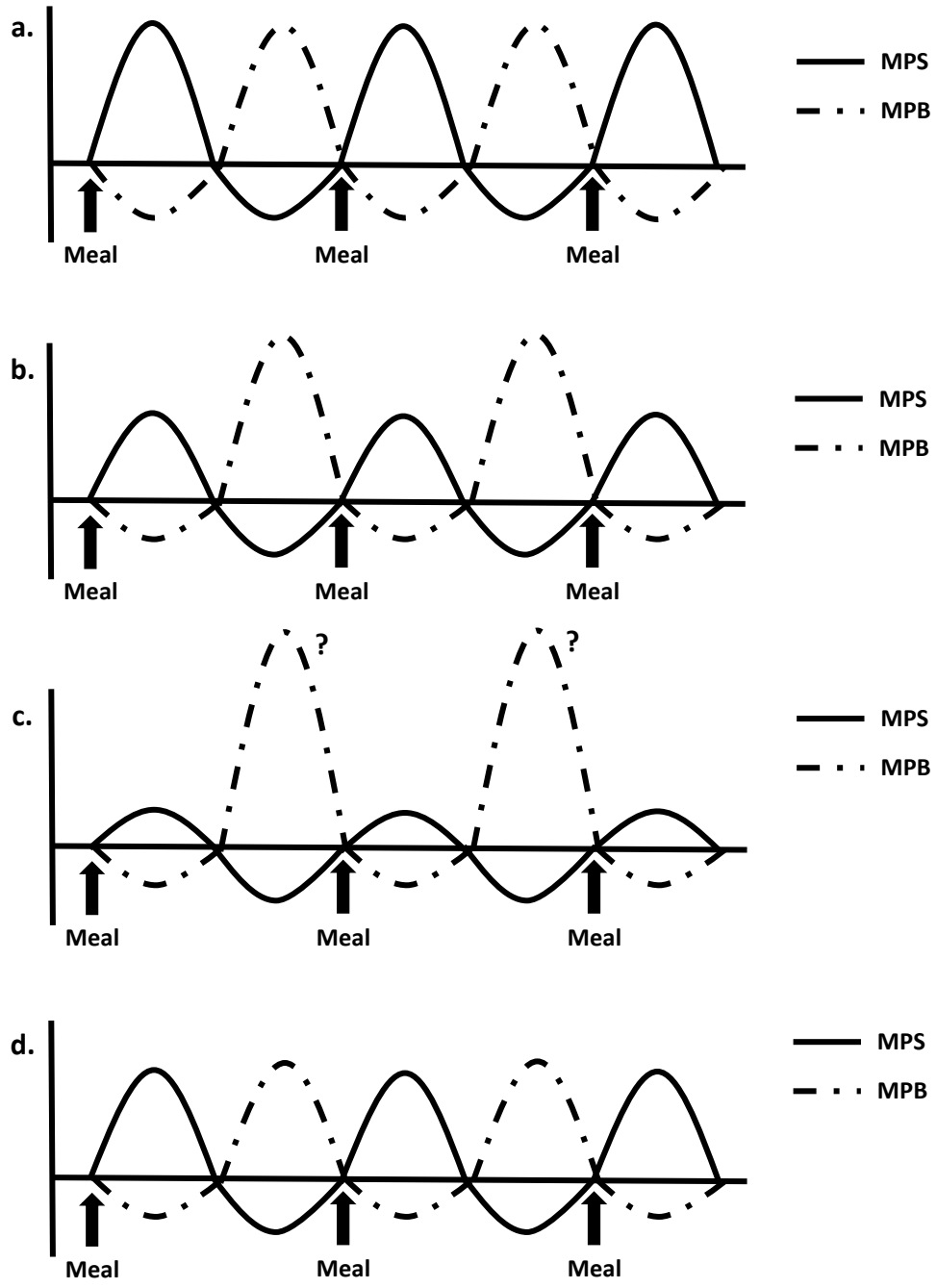

Figure 7.2.1. Diagram showing postulated muscle metabolism changes at early (c) and late (d) stages of caloric restriction compared to healthy individuals (a) and individuals with T2D / obesity (b). Compared to baseline, caloric restriction initially induces a profound reduction in MPS, and may also stimulate a marked increase in MPB, leading to rapid losses of MM. However, over time these processes stabilise resulting in maintenance of MM, despite ongoing weight and FM loss.

7.3. MM loss during weight reduction interventions appear to be primarily mediated by MPS changes

Over the 6-week period of MPS measurement, a significant reduction compared to baseline was observed in all three groups. Furthermore, changes in MPS mirrored the changes in LBM, with a significantly smaller changed noted with Semaglutide, than with VLCD, in both analyses. In addition, change in MPS correlated significantly with change in LBM, adding further support to the above findings. Unfortunately, it was not possible to measure MPB at two timepoints. This was a major limitation as it impeded the ability to fully assess whether changes in MPB could have contributed to the changes in LBM. However, other authors have made similar observations with regard to MPS (Hector et al., 2018; Janssen et al., 2023), and these findings add more evidence to the notion that reduced MPS is primarily responsible for changes in MM during caloric restriction. It remains to be seen whether MPB contributes to this process, and this represents a very intriguing possible area for further work.

The changes in MPS induced by Semaglutide are of particular interest. In accordance with LBM, MPS also reduced over the first 6 weeks, however the extent of this was significantly lower than that stimulated by VLCD. One could deduce that this was the result of a muscle-preserving effect of the GLP1RA. However, these results should be interpreted with caution as the Semaglutide group also experienced significantly less weight loss. This was also associated with a lower level of caloric restriction, with this group ingesting approximately 1100 kcal, compared to 700 kcal in the VLCD group. Thus, the lower caloric restriction and consequent weight loss could to some extent explain the lower reduction in MPS. Indeed, change in MPS also correlated

significantly with weight loss, and an association between weight change and MM loss has previously been reported. This also likely explains the meta-analysis findings of non-significant MM change in individuals with T2D, but significant reduction in individuals without T2D.

Thus, in order to comprehensively examine whether GLP1RAs do truly exert a muscle-preserving effect, comparisons between two groups with matched weight loss will be required.

7.4. The combined approach may lead to MM preservation

One of the most novel aspects of this work was the exploration into combining both VLCD and Semaglutide together. Unfortunately, no significant differences were noted between the combined group and the VLCD group with regard to changes in LBM and MPS. However, there were numerical reductions in both compared to VLCD, which occurred in the presence of similar weight loss. Additionally, there was a trend towards significantly lower loss of MM in the combined group ($p = 0.066$). Thus, although non-significant, with greater participant numbers a potential difference may be established. Therefore, this concept requires further study to determine whether the combination possibly can provide a method of mitigating MM losses during VLCD.

7.5. The combined approach confers some significant clinical benefits, despite inconclusive effects on muscle metabolism

In contrast to the muscle metabolism outcomes, Chapter 6 outlined some significant clinical and glucometabolic outcomes from combining VLCD and Semaglutide, over the individual interventions alone. The combination elicited greater weight loss and FM reduction, than Semaglutide, whilst also being able to induce significant

reduction in IS (assessed by HOMA-IR). There was also a trend towards a more significant reduction in HbA1c than Semaglutide, which would be an important finding for individuals with T2D.

In comparison to VLCD, the combination did not yield improvements in any outcome, apart from BCF. The combination demonstrated a considerable increase in AIRg, whilst no significant change was observed with VLCD. This is important for two reasons. Firstly, greater increases in BCF are associated with more robust long-term diabetes remission (Steven, Hollingsworth, Al-Mrabeh, et al., 2016; Taylor, 2013), therefore it can be postulated that the combination could improve long-term remission rates. Secondly, the participants involved in this study had a long (> 6 year) mean duration of diabetes diagnosis. The VLCD was unable to induce an increase in AIRg, which is likely to be related to this. However, the fact that the combined approach was able to overcome this inability to improve BCF was unexpected. This suggests that this approach may be capable of restoring BCF to a clinically important level, regardless of the duration of diabetes. This again is a novel finding that requires further exploration.

Previous studies have assessed the effects of combining dietary interventions with GLP1RAs and have been able to demonstrate ongoing weight loss following cessation of the diet (Capristo et al., 2018; Wadden et al., 2021). Thus, there is the possibility that addition of VLCD to the first 12 weeks of Semaglutide could enhance the long-term weight loss. The combination induced a 7-percentage-point additional weight loss over 12 weeks, when compared to Semaglutide. Given that the higher dose formulation of Semaglutide has been associated with 16% weight loss, addition of

VLCD during the initial dose escalation period could induce weight loss in excess of 20%. The enhanced weight loss without greater LBM loss will also be of reassurance, given the aforementioned concerns regarding T2D and MM.

7.6. Future work

In summary, this work has produced several novel findings that would be of interest to clinicians managing individuals with T2D and obesity, as well as scientists interested in the effects of interventions on skeletal muscle. As a consequence, there are multiple lines of enquiry that arise from the findings that warrant further investigation:

- The effects of weight loss interventions on MPB still remain largely unknown and require examination. It would also be prudent to examine serial changes in MPB as early changes have been previously suggested (Giesecke et al., 1989; S. S. Wing & Goldberg, 1993) and only performing measurements late into an intervention risks missing them.
- Similarly, serial measurements of MPS in conjunction with the above should provide some insight into the time-dependent changes in LBM observed with weight loss interventions.
- Further assessment of the effect of GLP1RA on MPS during weight loss is required, given the suggested muscle preserving effects noted in this work. This would ideally involve assessing individuals administered GLP1RA in comparison to individuals that are not, with matched weight loss between the groups.
- The combination of VLCD with Semaglutide warrants assessment in a clinical context to determine the clinical efficacy and cost-effectiveness of such an

approach. These findings suggest individuals with T2D could benefit significantly from combining these interventions, which are both approved for T2D management. The potential muscle preserving effect also requires assessment in this context and is a particularly exciting avenue to pursue.

- Ultimately, the identification of an optimal method to prevent loss of skeletal muscle during weight loss interventions remains elusive. Adjuvant exercise interventions have been postulated by various authors with promising results (Areta et al., 2014; W. W. Campbell et al., 2009; C. H. Murphy et al., 2018). However, my findings suggest that a more targeted approach may be required. For example, exercise may only be required during the early stages on an intervention, allowing for withdrawal once MM levels have stabilised. Thus, this ultimate aim must remain the main focus of any future work that is conducted.

7.7. Concluding remarks

While significant progress has been made in the management of T2D and obesity over recent years, these conditions still remain significant challenges to the health of millions of people. Weight loss interventions are of significant benefit in these conditions, however their negative effects cannot be ignored. The understanding of muscle physiology has increased dramatically recently, although the search for optimal strategies for mitigating the negative effects of weight loss interventions continues. However, it is my firm belief that with further work and increased knowledge, such strategies will be identified, and it is my sincere hope that this work will contribute to their eventual discovery.

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Appendices

Appendix I: GLP1RA Effect on MM Meta-analysis PICOS table

Domain	Specification	Justification
Population	Inclusion • Overweight or obese individuals (mean BMI ≥ 25kgm^2) • Individuals **with or without** type 2 diabetes (mixed cohorts can also be included) • Mean age ≥40 years Exclusion: Individuals with type 1 diabetes, pregnant, neonatal, paediatric or adolescent participants, cohorts with a mean age <40 years or mean BMI < 25kg/m^2.	GLP1RAs are primarily used for the treatment of individuals with an elevated BMI (>25kg/m^2) with T2D, and without T2D. Scoping search suggest some studies include mixed cohorts and these will be included (if appropriate) and analysed as a separate subgroup if necessary. Age-related decline in LBM becomes apparent from age 40 onwards, therefore this review will mainly focus on this population of individuals who are most at risk of the effects of accelerated sarcopenia.
Intervention	Inclusion • Any GLP-1 receptor agonist (**subcutaneous and oral**) • Intervention period of ≥6 weeks • Can be given with any co-therapy (except insulin) • Must include at least one group in which a GLP-1 receptor agonist is the only study intervention Exclusion: Any co-therapy with trial-specific insulin use; any GLP-1 receptor/other receptor co-agonist; intervention period <6 weeks.	Interventions needs to last long enough to ensure significant weight loss has occurred. Scoping search suggests that this is from 6 weeks onwards. Insulin co-therapy has been excluded as insulin is a well-known promoter of LBM retention (as well as weight gain). Co-agonists also have wide-ranging physiological effects that are yet to be fully defined, and are not within the scope of this review.
Comparison	Any comparator. Studies with no comparator can also be included.	Scoping search demonstrated that very few comparator studies have been conducted reporting body composition outcomes. Furthermore, the range of comparators is wide, therefore cannot provide a consistent basis for comparison. Therefore, this review will outline absolute changes in LBM, compared to TM and FM.
Outcomes	Change in the following parameters, based on pre- and post-intervention measurements Primary Outcomes (article MUST report this outcome to be included for selection): • Lean body mass (LBM) – can also include that report other surrogate measures of LBM (e.g. LBM percentage, fat-free mass, skeletal muscle mass) Secondary outcomes: • Fat mass (FM) • Total mass (TM)	Change in LBM is the outcome measure of choice, however change in FM and TM are required to provide context. Surrogate measures of LBM will be included as this review is examining the pre- to post-intervention change, which we expected will be the same, regardless of the measure reported. Sensitivity analysis will be conducted to examine this assumption formally.

Study Design	Inclusion: All types of studies reported in a full peer-reviewed article. English language only. Exclusion: Abstract-only articles, case reports or conference proceedings	Only full studies will be included to ensure full data are available, and enough information is presented to perform valid risk of bias assessment. We also envisage this will increase the overall quality of the synthesised evidence.
Supplementary X | Summary of the utilised PICOS framework with justifications.		

Appendix II: GLP1RA Effect on MM Meta-analysis example search strategy

Database:
Embase <1974 to 2023 November 14>

#	Query	Results from 15 Nov 2023
1	obes*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	750,904
2	exp obesity/	672,765
3	exp diabetic obesity/	4,728
4	exp diabetes mellitus/	1,240,805
5	diabet*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	1,473,875
6	overweight.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	137,173
7	1 or 2 or 3 or 4 or 5 or 6	2,023,063
8	exp glucagon like peptide 1 receptor agonist/	49,574
9	liraglutide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	13,505
10	semaglutide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	4,302
11	dulaglutide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	2,994
12	lixisenatide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	2,393
13	albiglutide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	1,453
14	exenatide.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	4,623
15	8 or 9 or 10 or 11 or 12 or 13 or 14	50,039
16	lean body mass.ti.	986
17	lean body mass.ti,ab.	11,962
18	fat mass.ti,ab.	39,020
19	fat free mass.ti,ab.	12,764
20	muscle mass.ti,ab.	37,467
21	body composition.ti,ab.	63,784
22	16 or 17 or 18 or 19 or 20 or 21	122,358
23	7 and 15 and 22	877

Appendix III: GLP1RA Effect on MM Meta-analysis sensitivity analyses

Sensitivity analysis – results from studies reporting full data set only

		Weighted Mean Difference	95% confidence interval	p-value
T2DM LBM:FM comparison	**LBM**	-0.58kg	-1.59 to 0.44	0.27
	FM	-3.12kg	-4.23 to -2.02	<0.0001
T2DM LBM:Weight comparison	**LBM**	-0.62kg	-1.70 to 0.46	0.26
	Weight	-4.27kg	-6.08 to -2.46	<0.0001
Non-DM LBM:FM comparison	**LBM**	-1.31kg	-2.51 to -0.12	0.03
	FM	-7.80kg	-10.55 to -5.04	<0.0001
Non-DM LBM:Weight comparison	**LBM**	-1.33kg	-2.55 to -0.10	0.03
	Weight	-11.38kg	-15.29 to -7.48	<0.0001

Sensitivity analysis – results from RCT studies only

		Weighted Mean Difference	95% confidence interval	p-value
T2DM LBM:FM comparison	**LBM**	-0.95kg	-2.45 to 0.54	0.21
	FM	-2.85kg	-4.14 to -1.55	<0.0001
T2DM LBM:Weight comparison	**LBM**	-1.02kg	-2.48 to 0.43	0.17
	Weight	-4.23kg	-6.27 to -2.19	<0.0001
Non-DM LBM:FM comparison	**LBM**	-1.24kg	-2.32 to -0.16	0.02
	FM	-5.45kg	-7.01 to -3.89	<0.0001
Non-DM LBM:Weight comparison	**LBM**	-0.51kg	-1.71 to 0.69	0.40
	Weight	-3.63kg	-9.51 to 2.24	0.23

Table S3: Sensitivity analysis – result by method of body composition assessment

			Weighted Mean Difference	95% confidence interval	p-value
T2DM	LBM	ADP	-0.63kg	-4.39 to 3.12	0.74
		BIA	-0.56kg	-1.69 to 0.57	0.33
		DXA	-1.06kg	-2.56 to 0.44	0.16
			Test of group differences		**0.87**
	FM	ADP	-5.26kg	-8.25 to -2.27	0.0006
		BIA	-3.26kg	-4.60 to -1.91	<0.0001
		DXA	-2.69kg	-4.03 to -1.35	<0.0001
			Test of group differences		**0.30**
Non-DM	LBM	BIA	-1.51kg	-2.48 to -0.54	0.002
		DXA	-3.22kg	-5.16 to -1.28	0.001
			Test of group differences		**0.12**
	FM	BIA	-7.27kg	-11.09 to -3.44	0.0002
		DXA	-6.60kg	-8.11 to -4.89	<0.0001
			Test of group differences		**0.75**

Table S4: Sensitivity analysis – result by measure of SM mass

		Weighted Mean Difference	95% confidence interval	p-value
T2DM	FFM	-0.99kg	-2.32 to 0.34	0.14
	LBM	-1.00kg	-2.43 to 0.43	0.17
	SMM	-0.39kg	-1.63 to 2.41	0.71
		Test of group differences		**0.48**
Non-DM	LBM	-2.96kg	-4.77 to -1.15	0.001
	SMM	-1.51kg	-2.49 to -0.54	0.002
		Test of group differences		**0.17**

Appendix IV: GLP1RA Effect on MM Meta-analysis risk of bias assessments

Figure S1: Risk of bias assessment results for RCT studies

	Risk of bias domains					
Study	**D1**	**D2**	**D3**	**D4**	**D5**	**Overall**
Harder *et al.*, 2004	+	+	+	+	!	!
Jendle *et al.*, 2009	+	+	+	+	!	!
Bunck *et al.*, 2010	!	+	+	+	!	!
Astrup *et al.*, 2012	+	+	+	+	!	!
Yin *et al.*, 2018	+	+	+	!	+	!
Feng *et al.*, 2019	+	+	+	!	!	!
van Eyk *et al.*, 2019	+	+	+	+	+	+
Kadouh *et al.*, 2020	+	+	+	+	!	!
McCrimmon *et al.*, 2020	+	+	+	+	+	+
Lundgren *et al.*, 2021	+	+	+	+	+	+
Neeland *et al.*, 2021	+	+	+	+	+	+
Wilding *et al.*, 2021	+	+	+	+	+	+
Yabe *et al.*, 2022	+	+	+	+	!	!
Heise *et al.*, 2023	+	+	+	+	!	!
Silver *et al.*, 2023	+	+	+	+	!	!

Figure S2: Risk of bias assessment results for Crossover RCT studies

	Risk of bias domains						
Study	**D1**	**DS**	**D2**	**D3**	**D4**	**D5**	**Overall**
Blundell *et al.*, 2017	!	+	+	+	+	!	!
Gibbons *et al.*, 2021	!	+	+	+	+	!	!

Risk of bias assessments for single group non-RCT studies

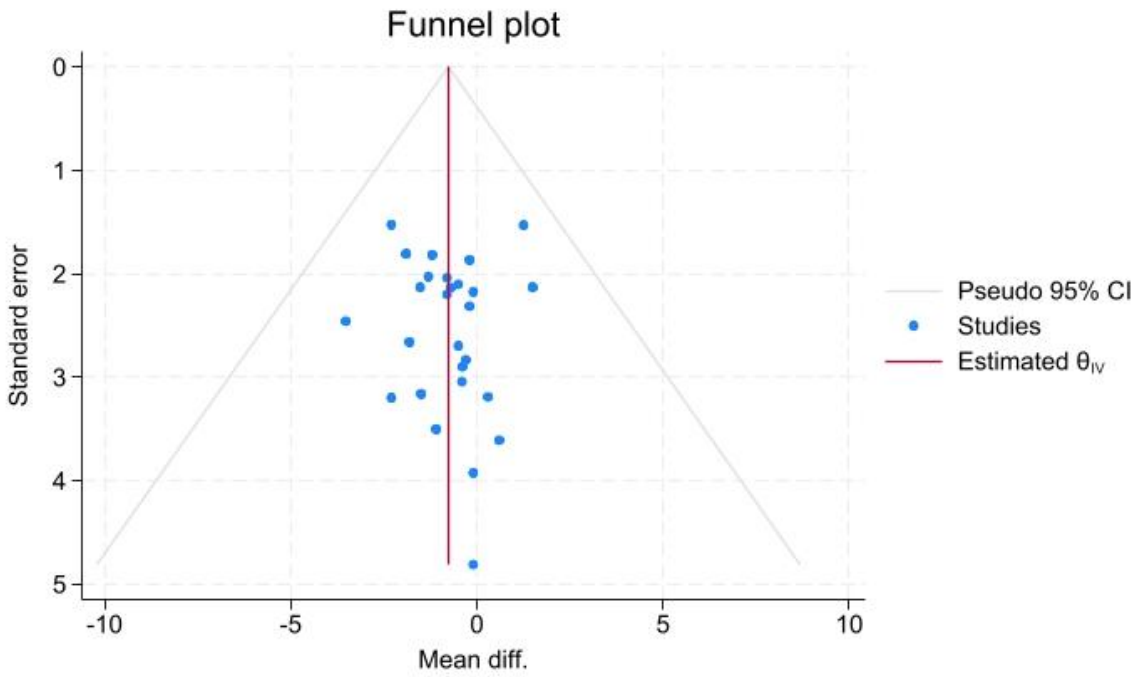
	Diaz-Soto *et al.*, 2013	Li *et al.*, 2014	Hong *et al.*, 2016	Perna *et al.*, 2016	Rondanelli *et al.*, 2016	Seko *et al.*, 2017	Ishii *et al.*, 2019	Park *et al.*, 2021	Ozeki *et al.*, 2022	Volpe, Lisco, Fanelli *et al.*, 2022	Volpe, Lisco, Racaniello *et al.*, 2022	Santini *et al.*, 2023	Uchiyama *et al.*, 2023	Volpe *et al.*, 2023
Were there clear criteria for inclusion in the case series?	N	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Was the condition measured in a standard, reliable way for all participants included in the case series?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were valid methods used for identification of the condition for all participants included in the case series?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Did the case series have consecutive inclusion of participants?	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y	Y	U	Y
Did the case series have complete inclusion of participants?	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y	Y	U	Y
Was there clear reporting of the demographics of the participants in the study?	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Was there clear reporting of clinical information of the participants?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were the outcomes or follow up results of cases clearly reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Was statistical analysis appropriate?	Y	Y	Y	N	Y	U	Y	Y	Y	Y	Y	Y	Y	Y

Risk of bias assessment for multiple group non-RCT studies

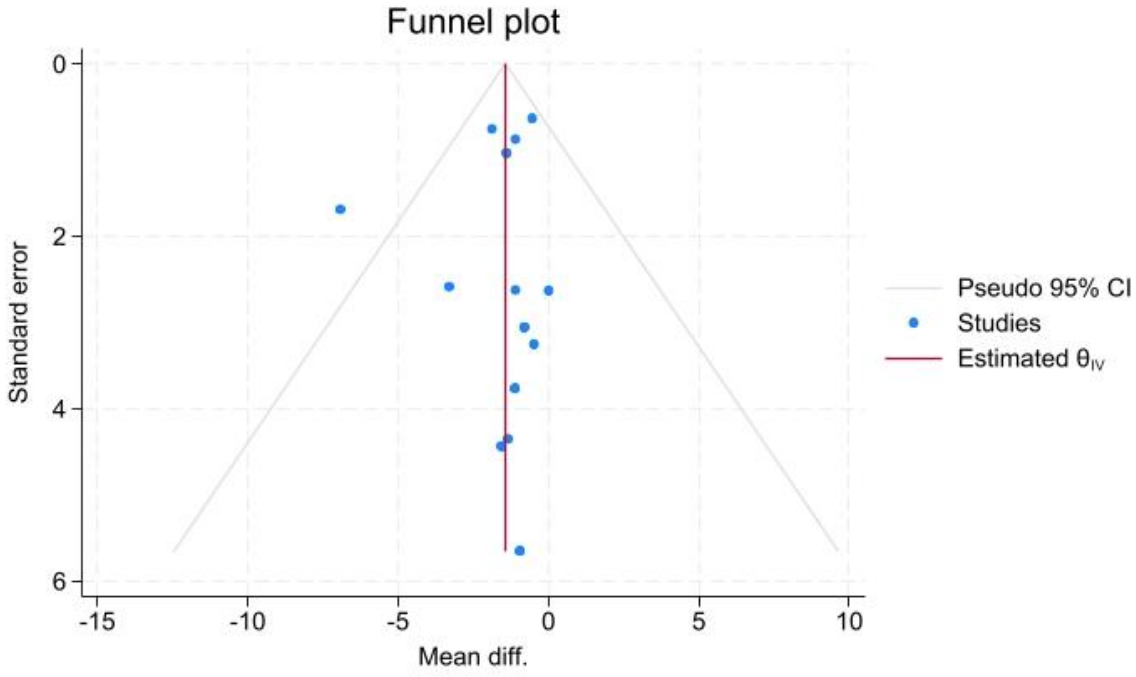
	de Luis et al., 2014	de Luis et al., 2015	Timofte et al., 2015	Iepsen et al., 2018	Agcakaya et al. 2023	Frietas et al., 2023	Stefanakis et al., 2023
Were the two groups similar and recruited from the same population?	Y	Y	Y	N/A	Y	Y	Y
Were the exposures measured similarly to assign people to both exposed and unexposed groups?	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Was the exposure measured in a valid and reliable way?	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Were confounding factors identified?	Y	Y	N	Y	Y	Y	Y
Were strategies to deal with confounding factors stated?	N	Y	N	Y	Y	Y	Y
Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Were the outcomes measured in a valid and reliable way?	Y	Y	Y	Y	Y	Y	Y
Was the follow up time reported and sufficient to be long enough for outcomes to occur?	Y	Y	Y	Y	Y	Y	Y
Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	Y	Y	Y	Y	Y	Y	Y
Were strategies to address incomplete follow up utilized?	N/A	N/A	N/A	N/A	N	N/A	N/A
Was statistical analysis appropriate?	Y	Y	Y	Y	Y	Y	Y

Appendix V: GLP1RA Effect on MM Meta-analysis publication bias assessment

Funnel plot of T2DM studies reporting measures of MM



Funnel plot of non-DM studies reporting measures of MM



Appendix VI: VLCD Effects on MM Meta-analysis PICOS table

Domain	Specification	Justification
Population	Inclusion • Overweight or obese individuals (mean BMI ≥ 25kgm^2) • Mean age ≥40 years • Individuals **with or without** type 2 diabetes (mixed cohorts can also be included) Exclusion: Studies involving individuals with type 1 diabetes, pregnant / neonatal / paediatric / adolescent participants, or cohorts with a mean age <40 years / mean BMI < 25kg/m^2.	Caloric restriction interventions primarily used for the treatment of individuals with an elevated BMI (>25kg/m^2) with T2D. However, also used for individuals without T2D in specific situations. Scoping search suggest some studies include mixed cohorts and these will be included (if appropriate) and analysed as a separate subgroup if necessary. Age-related decline in muscle mass occurs from age 40 onwards, therefore this is the population that are most at risk of the effects of accelerated sarcopenia.
Intervention	Inclusion • Any dietary intervention (VLCD or LCD) up to 900 kilocalories per day • Intervention period of ≥4 weeks • Must include at least one group in which a VLCD/LCD is the only study intervention Exclusion: Dietary interventions with a concurrent exercise intervention, very-low calorie ketogenic diets (VLKD/VLCKD), study intervention period <4 weeks.	Dietary interventions up to 900kcal/day are included as the approved NHS programme is 825-850kcal/day and the physiological effects are similar to "true" (<800kcal/day) VLCDs. Studies will be sub-grouped by level of caloric restriction to determine differences in weight loss and muscle mass changes. Interventions needs to last long enough to ensure significant weight loss has occurred. Scoping search suggests that this is from 4 weeks onwards. Exercise is a well-known promoter of muscle growth and therefore interventions with concurrent exercise will confound the findings. Ketogenic interventions typically have higher protein content, therefore will likely introduce extra heterogeneity to the results. This review is focussed on non-ketogenic interventions, so these are excluded.
Comparison	Any comparator. Studies with no comparator can also be included.	Scoping search demonstrated that very few comparator studies have been conducted reporting body composition outcomes. Furthermore, the range of comparators is wide, therefore cannot provide a consistent basis for comparison. Therefore, this review will outline absolute changes in muscle mass, compared to FM and TM.
Outcomes	Change in the following parameters, based on pre- and post-intervention measurements Primary Outcomes (article MUST report this outcome to be included for selection): • Any measure of muscle mass – includes LBM, FFM, SMM (can also include percentages) Secondary outcomes: • Fat mass (FM) • Total mass (TM) • Baseline HbA1c	Change in muscle mass is the outcome measure of choice, however change in FM and TM are required to provide context. Multiple surrogate measures of muscle mass exist so all will be included. This review is examining pre- to post-intervention change, so we expect this will be the same, regardless of the measure reported. Sensitivity analysis will be conducted to examine this assumption formally. Baseline HbA1c data will also be collected to assess for an association between this and MM changes.
Study Design	Inclusion: All types of studies reported in a full peer-reviewed article. English language only. Exclusion: Abstract-only articles, case reports or conference proceedings	Only full studies will be included to ensure full data are available, and enough information is presented to perform valid risk of bias assessment. We also envisage this will increase the overall quality of the synthesised evidence.

Supplementary X | Summary of the utilised PICOS framework with justifications.

Appendix VII: VLCD Effects on MM Meta-analysis example search strategy

#	Query	Results from 9 May 2024
1	exp Overweight/	695,875
2	obes*.ti,ab.	586,213
3	overweight.ti,ab.	138,517
4	exp Diabetes Mellitus/	1,263,120
5	diabet*.ti,ab.	1,224,509
6	exp Body Composition/	132,938
7	lean body mass.mp.	12,449
8	muscle mass.mp.	55,211
9	fat free mass.mp.	15,175
10	fat mass.mp.	47,355
11	fat distribution.mp.	14,831
12	exp Caloric Restriction/	19,950
13	low calorie diet.mp.	4,651
14	low energy diet.mp.	917
15	hypocaloric diet.mp.	1,718
16	meal replacement.mp.	864
17	total diet replacement.mp.	104
18	1 or 2 or 3 or 4 or 5	2,057,400
19	6 or 7 or 8 or 9 or 10 or 11	202,871
20	12 or 13 or 14 or 15 or 16 or 17	26,511
21	18 and 19 and 20	3,308
22	exp obesity/	695,875
23	exp diabetic obesity/	4,916
24	exp diabetes mellitus/	1,263,120
25	obes*.mp.	775,976
26	diabet*.mp.	1,523,756
27	overweight.mp.	141,454
28	22 or 23 or 24 or 25 or 26 or 27	2,093,041
29	exp body composition/	132,938
30	body composition.mp.	97,590
31	exp lean body weight/	20,793
32	lean body mass.mp.	12,449
33	muscle mass.mp.	55,211
34	fat free mass.mp.	15,175
35	fat mass.mp.	47,355
36	fat distribution.mp.	14,831
37	29 or 30 or 31 or 32 or 33 or 34 or 35 or 36	216,059

38	exp low calorie diet/	2,602
39	low calorie diet.mp.	4,651
40	low energy diet.mp.	917
41	total diet replacement.mp.	104
42	meal replacement.mp.	864
43	38 or 39 or 40 or 41 or 42	6,253
44	28 and 37 and 43	1,395

Appendix VIII: VLCD Effects on MM Meta-analysis sensitivity analyses

Sensitivity analysis – results from studies reporting full data set only

T2D analysis		Weighted Mean Difference	95% confidence interval	p-value
MM v FM comparison	MM	-2.89kg	-3.55 to -2.24	<0.0001
	FM	-7.92kg	-9.92 to -5.91	<0.0001
MM v Weight comparison	MM	-2.84kg	-3.48 to -2.21	<0.0001
	Weight	-11.27kg	-12.38 to -10.15	<0.0001

NDM analysis		Weighted Mean Difference	95% confidence interval	p-value
MM v FM comparison	MM	-2.80kg	-3.36 to -2.24	<0.0001
	FM	-6.97kg	-7.61 to -6.33	<0.0001
MM v Weight comparison	MM	-2.76kg	-3.32 to -2.20	<0.0001
	Weight	-9.89kg	-10.67 to -9.11	<0.0001

Sensitivity analysis – result by measure of MM

	Number of cohorts	Weighted Mean Difference	95% confidence interval	p-value
FFM	36	-2.80kg	-3.27 to -2.33	<0.0001
LBM	12	-3.03kg	-3.94 to -2.12	<0.0001
SMM	3	-1.69kg	-3.24 to -0.14	0.03
	Test of group differences			**0.33**

Sensitivity analysis – result by method of body composition assessment

	Number of cohorts	Weighted Mean Difference	95% confidence interval	p-value
Air displacement plethysmography	7	-2.06kg	-3.10 to -1.02	<0.0001
Bioimpedance analysis	15	-2.50kg	-3.56 to -1.43	<0.0001
Dual-energy X-ray absorptiometry	10	-3.11kg	-3.72 to -2.49	<0.0001
Deuterium dilution	12	-2.18kg	-3.19 to -1.18	<0.0001
Underwater weighing	3	-3.98kg	-5.54 to -2.43	<0.0001
	Test of group differences			**0.32**

Appendix IX: VLCD Effects on MM Meta-analysis quality assessments

Risk of bias assessment results for RCT studies

	Risk of bias domains					
Study	**D1**	**D2**	**D3**	**D4**	**D5**	**Overall**
Rolland *et al*., 2011	-	+	+	+	+	-
Vink *et al*., 2016	!	+	+	+	+	!
Christensen *et al*. 2005	!	+	+	+	+	!
Ng Tang Fui *et al*., 2016	+	+	+	+	+	+
Liljensøe *et al*., 2021	+	!	+	+	+	!
Brown *et al*., 2020	+	+	+	+	+	+
Behary *et al*., 2019	+	+	+	+	+	+
Vink *et al*., 2017	!	!	+	+	+	!
Soenen *et al*., 2013	!	+	+	+	+	!
Munro & Garg, 2013	+	+	+	+	+	+

Quality assessments for single group interventional studies

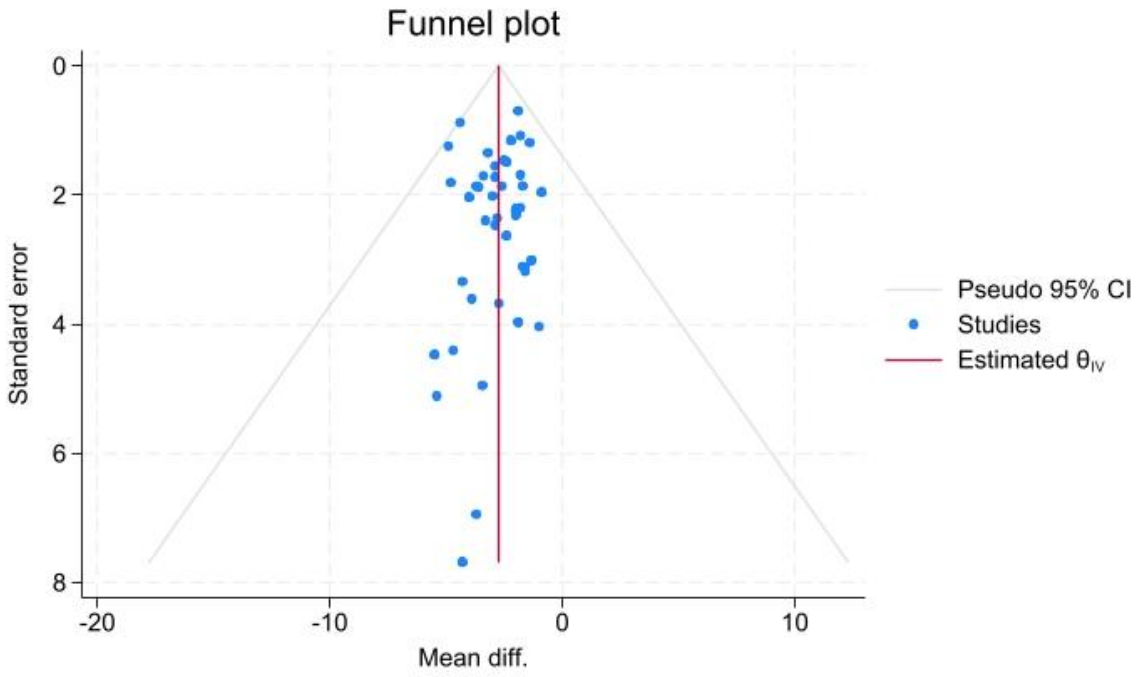
	Borg et al., 2002	Bucci et al., 2015	Westerterp -Plantenga et al., 2004	Carella et al., 1997	Vogels et al., 2007	Nymo et al., 2018	Munro & Garg., 2011	Tomlinson et al., 2004	Hursel & Westerterp, 2009	Claessens et al., 2009	Uusi-Rasi et al., 2010	Lundgren et al., 2021	Iepsen et al., 2016
Selection													
1. Representativeness of the exposed cohort	a	a	a	a	a	a	a	a	a	a	a	a	a
2. Selection of the non exposed cohort													
3. Ascertainment of exposure	a	a	a	a	a	a	a	a	a	a	a	a	a
4. Demonstration that outcome of interest was not present at start of study	a	a	a	a	a	a	a	a	a	a	a	a	a
Comparability													
1. Comparability of cohorts on the basis of the design or analysis													
Outcome													
1. Assessment of outcome	a	a	a	a	a	a	a	a	a	a	a	a	a
2. Was follow-up long enough for outcomes to occur	a	a	a	a	a	a	a	a	a	a	a	a	a
3. Adequacy of follow up of cohorts	a	b	b	b	b	b	b	a	b	b	b	b	a
Number of stars	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**

	Luscombe et al., 2006	Martins et al., 2023	Camps et al., 2019	Sayda et al., 2022	Vogels & Westerterp, 2005	Belza et al., 2009	Näätänen et al., 2023	Adam et al., 2006	Näätänen et al., 2021	Schulte et al., 2012	Packianathan et al., 2005	Krotkiewski et al., 1990	Hoie et al., 1993
Selection													
1. Representativeness of the exposed cohort	a	a	a	a	a	a	a	a	a	a	a	a	a
2. Selection of the non exposed cohort													
3. Ascertainment of exposure	a	a	a	a	a	a	a	a	a	a	a	a	a
4. Demonstration that outcome of interest was not present at start of study	a	a	a	a	a	a	a	a	a	a	a	a	a
Comparability													
1. Comparability of cohorts on the basis of the design or analysis													
Outcome													
1. Assessment of outcome	a	a	a	a	a	a	a	a	a	a	a	a	a
2. Was follow-up long enough for outcomes to occur	a	a	a	a	a	a	a	a	a	a	a	a	a
3. Adequacy of follow up of cohorts	b	a	a	a	a	b	b	c	a	a	b	b	b
Number of stars	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**

	Scragg et al., 2020	Jian et al., 2022	Diepviens et al., 2007	Christensen et al., 2018	Marples et al., 2022	Lim et al., 2011
Selection						
1. Representativeness of the exposed cohort	b	b	a	b	b	b
2. Selection of the non exposed cohort						
3. Ascertainment of exposure	a	a	a	a	a	a
4. Demonstration that outcome of interest was not present at start of study	a	a	a	a	a	a
Comparability						
1. Comparability of cohorts on the basis of the design or analysis						
Outcome						
1. Assessment of outcome	a	a	a	a	a	a
2. Was follow-up long enough for outcomes to occur	a	a	a	a	a	a
3. Adequacy of follow up of cohorts	b	a	a	b	c	b
Number of stars	**6**	**6**	**6**	**6**	**5**	**6**

Quality assessments for multiple-group non-RCT studies

	Tam et al., 2016	Aukan et al., 2023	Athithan et al., 2023	van Dale et al., 1990a	Ivan et al., 2022	Nordstrand et al., 2013	van Dale et al., 1990b
Selection							
1. Representativeness of the exposed cohort	b	b	a	a	a	b	a
2. Selection of the non exposed cohort	a	a	a	a	a	a	a
3. Ascertainment of exposure	a	a	a	a	a	a	a
4. Demonstration that outcome of interest was not present at start of study	a	a	a	a	a	a	a
Comparability							
1. Comparability of cohorts on the basis of the design or analysis	a	a	a	a	a	a	a
Outcome							
1. Assessment of outcome	a	a	a	a	a	a	a
2. Was follow-up long enough for outcomes to occur	a	a	a	a	a	a	a
3. Adequacy of follow up of cohorts	b	b	b	a	a	b	b
Number of stars	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Appendix X: VLCD Effects on MM Meta-analysis publication bias assessment

Funnel plot of studies included in NDM analysis