Effect of early versus late time-restricted eating on glycaemic control, measured by continuous glucose monitoring, in adults at risk for type 2 diabetes.

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Effect of early versus late time-restricted eating on glycaemic control, measured by continuous glucose monitoring, in adults at risk for type 2 diabetes

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Abbreviations

Akt substrate of 160 kDa	AS160
Area under the curve	AUC
Atherogenic lipoprotein particles	Non-HDL
Baseline before early time restricted eating	beTRE
Baseline before late time restricted eating	blTRE
Body mass index	BMI
c-Jun N-terminal kinases	JNK
Carbohydrates	CHO
Cholesterol ratio	Chol/HDL
Coefficient of variation	CV
Continuous Glucose Monitors	CGM
Continuous overall net glycaemic action	CONGA
Coronary artery disease	CAD
Delayed time restricted eating	dTRE
Diabetes Remission Clinical trial	DiReCT
Early time restricted eating	eTRE
Fasting plasma glucose	FPG
Forkhead box protein O1	FOXO1
Free fatty acids	FFA
Glucose transporter type 4	GLUT4
Glycaemic variability	GV
Glycated haemoglobin	HbA1c
Glycogen synthase kinase 3	GSK3
High blood glycaemic index	HBGI
High density lipoprotein	HDL
Homeostatic Model Assessment for Insulin Resistance	HOMA-IR
Impaired glucose tolerance	IGT
Insulin receptor substrate 2	IRS2

Insulin receptor substrates	IRS
Insulin resistance	IR
Interleukin 6	IL-6
Intermittent fasting	IF
International Diabetes Federation	IDF
Late time restricted eating	lTRE
Low blood glycaemic index	LBGI
Low calorie diet	LCD
Low density lipoprotein	LDL
Mammalian target of rapamycin	mTOR
Mammalian target of rapamycin complex 1	mTORC1
Mean absolute glucose	MAG
Mean amplitude of glycaemic excursions	MAGE
Mean value index of glycaemic variability	MVALUE IGV
Mid-day time restricted eating	mTRE
National Health Service	NHS
Oral glucose tolerance test	OGTT
Phosphatase and tensin homolog	PTEN
Phosphoinositide 3-kinases	PI3K
Physical activity	PA
Protein kinase B	AKT
Protein Tyrosine Phosphatase 1B	PTP1B
Renin-angiotensin system inhibitors	RASI
Resting energy expenditure	REE
Self-monitoring of blood glucose	SMBG
Suppressor of cytokine signalling	SOCS
Time restricted eating	TRE
Tumour Necrosis Factor alpha	TNF-a
Type 2 diabetes	T2D
Very low-calorie diet	VLCD
World Health Organisation	WHO

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1. Abstract

Aim: To investigate via the use of continuous glucose monitors (CGM) the acute effect of time-restricted eating (TRE) in early (eTRE; 8:00-16:00 hrs) versus late (lTRE; 12:00-20:00 hrs) conditions on inter-day glycaemic variability (GV) in adults at risk for type 2 diabetes (T2D). **Methods**: Eight sedentary individuals (five female/ three male; 51±6 years; body mass index (BMI) 28.0±2.9 kg/m^2; Glycated haemoglobin (HbA1c) 37.9±3.3 mmol/mol) with a habitual food intake distribution >14 hrs/day, participated in a randomised-crossover control TRE study. Protocol compared eTRE to lTRE as well as non-TRE to TRE periods, with each phase lasting 3-days. TRE conditions utilised were isocaloric and eucaloric, and energy intake was calculated via Mifflin-St Jeor equation. Meals provided within the TRE condition consisted of 60% CHO, 25% protein, and 16% fat. CGM was used to assess markers of GV, including but not exclusive to mean absolute glucose (MAG), coefficient of variation (CV), and mean amplitude of glycaemic excursions (MAGE). A 2 factor repeated measures analysis of variance was used (2 conditions [eTRE vs lTRE] x 2 time-points [baseline vs TRE]) for statistical testing. **Results**: Following TRE, a significant reduction, was observed within MAG by 0.4 mmol/l (95% CI 0.1 to 0.8; 0.041), MAGE by 0.4 mmol/l (95% CI 0.1 to 0.8; *P*= 0.024) and CV by 2.5 mmol/l (0.6, 4.4) p values 0.041, 0.024, 0.016 respectively (i.e., baseline to TRE). There were no significant changes in physical activity (PA) or diet (total caloric intake, CHO and fat), however protein did significantly differ from baseline to TRE, 21.8g (baseline before eTRE; beTRE) and 23.2g (baseline before lTRE; blTRE) less in comparison to TRE (150g). **Conclusion**: In the absence of calorie restriction, TRE improved some markers of inter-day GV. This data indicates a possible therapeutic role of TRE in adults at risk for T2D. **Further direction**: This small study of short-term TRE provided an understanding for possible effects exhibited by an 8-hour TRE protocol on glycaemic markers. Future studies should determine whether the significant effects are still experienced in the long-term via a longer intervention period. Additionally, a larger, more varied cohort should be utilised analysing various ages, ethnicities, and BMI groups due to the low generalisability of the few current studies available.

2. Introduction

2.1. Obesity

Obesity is a rapidly increasing worldwide disorder defined by the World Health Organisation (WHO) (Shimizu, 2023) as an abnormal or excessive fat accumulation that is detrimental to health. If current trends persist, one in three people will be obese by 2034 and one in ten will develop T2D (NHS, 2020). In the UK, it leads to economic problems due to the substantial burden it places on the National Health Service (NHS). In 2018/19, it was reported that two thirds of adults (63%) in the UK, were either overweight or obese (Office for Health Improvement & Disparities, 2023). This statistic occurred before the recent Covid-19 pandemic where activity levels of the population were low for a prolonged period (Wunsch et al., 2022). Data obtained from NHS Health Survey for England, (2020) noted that 64% of adults (individuals aged 16 and over) were classed as overweight (including obese) and even when corrected height and weight is taken in account, 26% of adults were classified as obese. Obesity is associated with higher rates of death in relation to its associated comorbidities such as dyslipidaemia, hypertension, forms of cancer, T2D and heart failure (Scully et al., 2020). Obesity is known to induce hypertension, which once they coincide are both known causes of 40% of the leading causes of deaths in 2019; these are ischemic heart disease, stroke, kidney disease and T2D (WHO, 2020). Worsening diabetes is associated with poor glycaemic control, uncontrolled hypertension and obesity (Shukla and Tripathy, 2022).

A common characteristic associated with obesity is insulin resistance (IR) within peripheral tissues, such as skeletal muscle, adipose tissue and liver. IR leads to an increased demand of insulin to maintain normal concentration of glucose in the blood, this triggers pancreatic βcell adaptation by increasing both β-cell mass and function (Cerf, 2013). This form of compensatory response leads to an increased rate of insulin secretion, and basal insulin concentration, resulting in the development of hyperinsulinemia. Hyperinsulinemia is a condition where there are higher levels of insulin circulating which overtime leads to metabolic dysregulations observed in obesity and T2D (Chiasson and Rabasa-Lhoret, 2004)

(Shanik et al., 2008). Even though obesity is closely linked with T2D, so much so it has led to the connotation ‘diabesity' (Leitner et al., 2017), the disease can also develop within nonobese individuals (Vaag and Lund, 2007).

2.2. Impaired Glucose Tolerance (IGT)

IGT is the inability of insulin to fulfil its role, thereby an increased amount of glucose within the bloodstream, which is linked to higher levels of visceral fat (Kumar et al., 2018). Insulin is a hormone that is responsible for instructing respiring cells to uptake glucose from the bloodstream as an energy source as well as the conversion of blood glucose into glycogen in the liver and skeletal muscle cells (Rachdaoui, 2020). The WHO defines IGT being present if an individual has a blood glucose greater than or equal to 7.8 mmol/L but less than 11.1 mmol/L after completion of a 2-hour oral glucose tolerance test (OGTT). IGT possesses no obvious symptoms, thus many people may possess the condition and be unaware of it. Diabetes UK has estimated that 13.2 million people in the UK has IGT, where these individuals have an increased risk of developing T2D (Diabetes UK, 2023a).

2.3. Type 2 Diabetes

T2D, formally known as insulin dependent diabetes, is a disorder where the body ineffectively uses insulin usually due to an individual having a higher amount of body fat than a healthy individual (Loke, 2022). Diabetes costs NHS approximately £10 billion each year, with its associated complications costing around £8 billion to treat, with one in six patients in hospital is classed as diabetic (Whicher et al., 2020). Moreover, Diabetes UK has stated that the number of diabetic cases within the UK has doubled within the last 15 years (Diabetes UK, 2021).

Type 2 diabetic cases are the most prominent form of diabetes, being responsible for approximately 90% of all diabetic cases. The prevalence of diabetic cases globally has quadrupled from 108 million in 1980 to 422 million in 2014 (WHO, 2022). Diabetes is regarded as one of the most common chronic diseases within the UK, where its prevalence is

increasing (NICE, 2020). In 2019, it has been reported that 3.8 million people are currently living with diabetes in UK (Diabetes UK, 2019b). Furthermore, Diabetic cases within the UK are only going to keep growing with an estimated 5.3 million by 2025 (Diabetes UK, 2019a), even though evidence proves that T2D is largely preventable disease (Diabetes UK, 2023b).

2.4. Dietary intervention as a therapeutic approach

Therapeutic techniques which can be implemented simultaneously to improve glycaemic control include PA and dietary modifications, this improves IGT leading to these individuals being the target group for primary prevention techniques (Franz et al., 2015; Pan et al., 1997; Mackerras, 2003; Herman et al., 2005).

Within type 2 diabetics, worsening glycaemic control has been associated with being overweight and obesity (Boye et al., 2021). Improvements within glycaemic control would reduce the conversion rate from prediabetes (non-diabetic hyperglycaemia) into T2D (Vijan et al., 2005). TRE is a form of intermittent fasting (IF) which creates an extended fasting period by only allowing for caloric intake to occur during a 6- to 10-hour window (Regmi and Heilbronn, 2020). TRE has been reported to improve glycaemic control via improvements made in regards to IR, insulin sensitivity, and glucose tolerance (Sutton et al., 2018; Jamshed et al., 2019; Kesztyüs et al., 2019; Cienfuegos et al., 2020; Jones et al., 2020; Parr et al., 2020). This topic is discussed further within section “3.6. Time restricted eating”.

3. Literature Review

3.1. Commonly referred to definitions

Various disorders are referred to throughout this report, definitions for these disorders obtained from WHO are detailed within table 1.

Table 1 *Commonly referred to definitions alongside their clinical classifications, mentioned throughout literature review obtained from various WHO sources.*

Disorder	**WHO Definition**	**Clinical classification**
Obesity (Shimizu, 2023)	"Abnormal or excessive fat accumulation that presents a risk to health"	Overweight — BMI of 25-29.9 kg/m^2 Obesity l — BMI of 30-34.9 kg/m^2 Obesity ll — BMI of 35-39.9 kg/m^2 Obesity lll — BMI of 40 kg/m^2 or more
Metabolic syndrome (WHO, 2011)	"A pathologic condition characterised by the following; abdominal obesity, IR, hypertension, and hyperlipidaemia."	Glucose intolerance, IGT (IGT) or T2D, and/or IR, alongside two or more of the components listed below: Raised arterial pressure, (≥140/90 mmHg) Raised plasma triglyceride (≥1.8 mmol/L) and/or low levels of high density lipoprotein (HDL) (<1.9 mmol/L in men and <2.2 mmol/L in women) Central obesity, i.e. waist/hip ratio >0.9 in men

		and >0.85 in women and/or BMI >30 kg/m^2 Microalbuminuria, i.e., urinary albumin excretion rate ≥ 20 µgm/minute or albumin/creatine ratio ≥ 30 µgm/mg.
IGT (Loke, 2022)	"Intermediate conditions in the transition between normality and diabetes. People diagnosed with IGT are at high risk of progressing to T2D, although this is not inevitable."	Fasting plasma glucose (FPG) of 6.1-6.9 mmol/L
Prediabetes (WHO, 2006)	"A state of intermediate hyperglycaemia using two specific parameters, impaired fasting glucose and IGT after ingestion of 75g of oral glucose load or a combination of the two based on a 2-hour OGTT. "	FPG of 6.1-6.9 mmol/L and IGT defined as 2-hour plasma glucose of 7.8-11.0 mmol/L after ingesting a 75 g of oral glucose load or a combination of both techniques following a 2hour OGTT.
T2D (WHO, 2011)	"Metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of CHO, fat and protein metabolism resulting from defects in	FPG test: >7.0 mmol/L OGTT: ≥11.0 mmol/L HbA1c: ≥6.5%

	insulin secretion, insulin action, or both.”	
HbA1c (WHO, 2011)	An “unusual” haemoglobin in patients with diabetes” used as an objective measure of glycaemic control.	HbA1c test Prediabetes: 5.7%–6.4% Diabetic: ≥6.5%

3.2. Metabolic Regulation of glucose

3.2.1. Normal metabolic regulation of glucose

Both the type of food and the amount of food ingested by an individual possess an influence on the decline hunger sensations and an increase in satiety. Food passes to the colon within one to two hours after consumption, where it is fermented by the microbiota (Livovsky et al., 2020). There are various organs involved in the metabolism of glucose; nervous system, pancreas, liver, the gastrointestinal tract, and adipocytes all of which participate towards the breakdown of glucose in various ways.

The nervous system instructs the pancreas, the organ responsible to produce insulin, via both autonomic functions, sympathetic and parasympathetic innervation. The autonomic nervous system regulates involuntary physiological processes via innervation of various metabolic organs (Waxenbaum et al., 2022). With regards to T2D, skeletal muscle is responsible for insulin-stimulated glucose uptake following consumption of a meal following autonomic activation (Han et al., 2016). Dysfunction of the autonomic nervous system due to IR is one of the significant complications associated with T2D (Sucharita et al., 2011). The autonomic nervous system influences islet hormone secretion which manages plasma glucose homeostasis via instructing the pancreas by autonomic nerve stimulation and neurotransmitters which trigger either more or less insulin to be released in response to glucose levels within the bloodstream (Esguerra et al., 2018; AhrØn, 2000).

The liver controls glycogenesis and gluconeogenesis which leads to the storage of excess glucose into glycogen or the release of glycogen from hepatic cells and skeletal muscle (Jensen et al., 2011). After the ingestion of food, the rise in blood glucose leads to a rise in insulin from the pancreas, this leads to glucose being stored as glycogen. As plasma glucose is stored, there is a lower concentration of glucose in the blood, the liver recognises this decline and triggers the release of glucose into the bloodstream.

The gastrointestinal tract releases appropriate hormones in response to ingestion. The hormones released within this system participate in various processes such as appetite regulation, glucose production/ removal and gastric emptying (Chaudhri et al., 2006). Adipocytes are cells which form adipose tissue, which secrete adipokines which then regulate insulin release and insulin gene expression (Rabe et al., 2008).

Motility of the small intestine plays an important role towards the management of satiety and appetite as well as glycaemic control due to the lower gastrointestinal tract possessing the ability to influence the microbiota, microorganisms which occupy the gut (Valdes et al., 2018). This process allows for selective pressures from either the host or environment to protect the gut from injury and maintain homeostasis (Thursby and Juge, 2017). The gut microbiota is thought to be involved in various physiological functions related to energy and substrate metabolism via cross-organ signalling, therefore it may lead to the development of certain metabolic diseases such as obesity and T2D aetiology (Müller et al., 2018).

Circulating plasma glucose needs to be transported over a membrane for it to be utilised within cells. To be transported across a cell membrane into the cytoplasm of the cell, glucose enters via protein carrier molecule on the cell membrane utilising a process known as facilitative diffusion. However, glucose does not pass through the cell membrane of a cell readily due to its high molecular weight and it being relatively insoluble in the phospholipid bilayer of the cell membrane (Shigenobu and McNamee, 2012). Once glucose is in the cell, it is phosphorylated in order for it to be utilised for the release of energy via glycolysis and then the citric acid cycle to form ATP or stored as glycogen (Chaudhry and Varacallo, 2022).

a. Diurnal patterns of glucose metabolism

Diurnal patterns in energy metabolism are linked the 24-hour rhythm, known as circadian rhythm, which controls various molecular, physiological, and behavioural processes. In addition, glucose homeostasis also follows diurnal variation due to various oscillating levels throughout a 24-hour period (Segers and Depoortere, 2021; Mancilla et al., 2020). High blood glucose occurs after meal ingestion even within individuals who possess a normal glucose tolerance, due to glucose exhibiting variations throughout a 24hour period (Bowen and Reeves, 1967). After ingestion of a meal, postprandial glucose levels increase where skeletal muscle uptakes around 80% of circulating blood glucose from the bloodstream, allowing normoglycaemia to be maintained in a healthy range (Mancilla et al., 2020). Levels of insulin sensitivity and glucose tolerance have been observed to be lower during the evening then in the morning, this is due to glucose levels peaking at 'circadian night' (Mason et al., 2020). Glucose tolerance pattern was examined utilising various techniques in the 20th century via OGTT, which is the current gold standard for diagnosing diabetes (Phillips, 2012).

3.2.2. Impaired metabolic regulation

An increased level of BMI and total adiposity are both positively correlated with progression of cardiometabolic disease risk (Goossens, 2017). This is achieved via overnutrition which results in chronic overexposure of nutrients such as glucose fatty acids and amino acids towards pancreatic β-cell, promoting the development of T2D (Poitout et al., 2010).

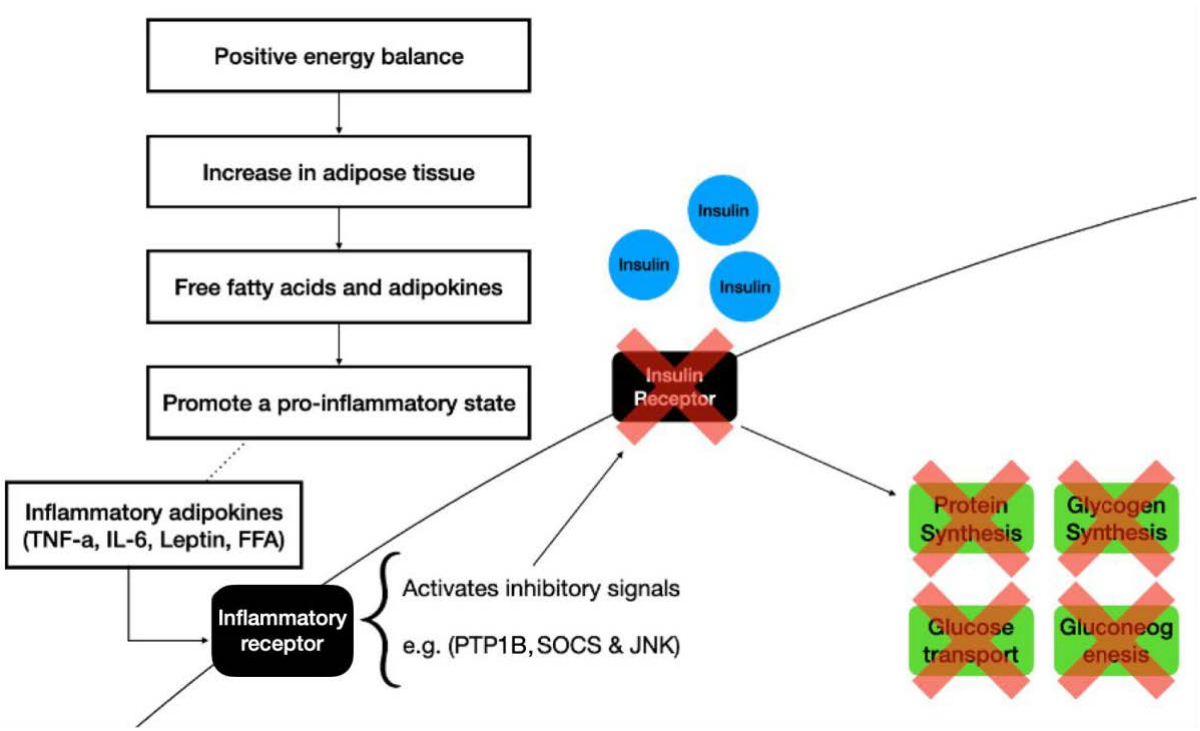

Figure 1 *Display of the pathway taken following a positive energy balance (energy intake exceeds energy expended) which results in IR. The dotted line signifies secretion of adipokines due to the pro-inflammatory state achieved. Figure inspired by Boden, (2011) and Kwon and Pessin, (2013). Tumour Necrosis Factor alpha (TNF-a); Interleukin 6 (IL-6); Free fatty acids (FFA); Protein Tyrosine Phosphatase 1B (PTP1B); Suppressor of cytokine signalling (SOCS); c-Jun N-terminal kinases (JNK).*

Effects exhibited following a positive energy balance is depicted in figure 1, and the steps displayed are discussed below. Obesity occurs due to a positive energy balance; where energy intake exceeds energy expenditure and therefore surpassing the 'ideal daily intake of calories' proposed by the NHS of 2000 calories for a woman and 2500 calories for a man (NHS, 2019). This leads to an expanded adipose tissue mass which is associated with an increased level of weight gain. Obesity leads to a dysregulated adipokine secretion pathway from adipose tissue occurring which promotes low-level inflammation due to a shift in immune profile that impairs insulin sensitivity. The proinflammatory molecules produced from this low-grade inflammation bind to their specific receptors and lead to the activation of inhibitory signals (e.g., protein tyrosine phosphatase 1B (PTP1B), suppressor of cytokine signalling (SOCS)/ c-Jun N-terminal kinases (JNK)) which negatively effects insulin receptors.

This action impairs the sensing and transduction pathway which is crucial for insulin detection at its receptor, resulting in an increased level of plasma glucose associated with T2D. Increased plasma glucose caused by insulin not being detected by its receptor and the signal transduction pathway associated with glucose uptake not occurring and therefore glucose channels aren't present on the target cell membrane and glucose cannot be transported (Kwon and Pessin, 2013; Boden, 2011).

a. Impaired metabolic mechanisms

Mammalian target of rapamycin (mTOR): Protein synthesis

Mammalian target of rapamycin complex 1 (mTORC1) is a pathway responsible for monitoring cell proliferation and growth otherwise known as autophagy which is a cellular process responsible for clearing long-lived proteins and dysfunctional organelles. mTOR is also responsible for activating the integrated stress response due to exposure to stressors which results in restoration of glucose homeostasis (Manoogian et al., 2022). T2D is associated with obesity and overnutrition due to a prolonged intake of high energy diet which results in excess dietary proteins being converted into circulating amino acids that are responsible for over activation of mTORC1. Chronic overactivation of mTORC1 is a known inhibitor of autophagy, which typically serves as a protective mechanism towards pancreatic β cells in attempt to manage β cell survival during the progression to T2D (Guillén and Benito, 2018). During chronic hyperactivation of mTORC1, cells becoming more prone to undergoing apoptosis due to cellular stressors, allowing for the progression of pre-diabetes to T2D to occur. If chronic hyperactivation remains this leads to pancreatic β cell death, compromising compensatory insulin section (Ali et al., 2017).

Hyperactivation of mTORC1 signals via insulin receptor substrates (IRS) for the regulation of insulin and growth factors. If this hyperactivation is prolonged due to consumption of a high energy diet, IRS function is inhibited resulting in it being incapable of glucose transfer due to IRS not transporting glucose transporters to the cell surface. Glucose transporters are not brought to the cell surface via IRS due to it becoming resistant to responding to insulin

signals, otherwise known as IR (Ali et al., 2017). Communications occur between the liver and brain via the vagal nerve to white adipose tissue via sympathetic nerves. This communication triggers a decrease in lipoprotein lipases and an increase in triglycerides in the bloodstream resulting in hyperlipidaemia and hypertriglyceridemia. These disorders cause IR developing as well as an increased production of hepatic glucose which is converted into fatty acids and lipid deposits (Jia et al., 2014).

In addition to this, skeletal muscle uptake of circulating plasma glucose is impaired due to high activation of mTORC1. Within skeletal muscle of obese and high energy intake individuals, ribosomal S6 kinase 1-mediated feedback inhibition of insulin signals leads to a decreased glucose uptake (Yarahmadi et al., 2021). mTORC1, when activated, normally promotes phosphorylation of SK61, allowing for vital cellular processes including transcription, translation, protein synthesis, lipid synthesis, cell growth and metabolism to occur (Laplante and Sabatini, 2012).

(Velingkaar et al., 2021) detailed how mTORC1 activity in the liver is under circadian clock influence and activity of mTORC1 is highly sensitive to the feeding-fasting cycle. This is due to it being a nutrient sensor which is influenced by insulin and an important regulator of liver metabolism. mTOR rhythms can be adjusted to 12-hour periods in response to metabolic adaptations and the frequency of meal consumption. Postprandial increases of glucose, lipids and amino acids alter circadian rhythm due to mTOR and Sirtuin 1 (Schuppelius et al., 2021). Jamshed et al., (2019) noted that eTRE resulted in an increased rate of mTOR expression during the evening. This is due to mTOR being stimulated by insulin, protein and various growth factors which drive protein synthesis. Jamshed et al., (2019) concluded that the increase in mTOR levels experienced during the evening mirrored the increase in fasting insulin. Moro et al., (2016) utilised a delayed 8-hour TRE protocol between the times of 1pm and 8pm and noted a significant decrease in insulin-like growth factor 1 which is an activator for IGF-1/ protein kinase B (AKT)/mTOR pathway. However, it is important to note that this decrease was observed within resistance-trained male athletes, who are not at risk for developing T2D and was obtained in a small, male only cohort.

Phosphoinositide 3-kinases (PI3K)/ AKT pathway

PI3K/Akt intracellular pathway is an important regulator of cell cycles; it is associated with cellular quiescence, proliferation, cancers and monitoring the longevity of cellular lifespans. The pathway is activated by PI3K phosphorylating Akt which is required for maintaining glucose homeostasis (Bathina and Das, 2018). Downregulation/ knockdown of Akt leads to a decreased level of insulin signalling which affects many processes resulting in IR developing. Knockout or knockdown Akt results in a reduced rate of insulin-induced glucose uptake from circulation (Huang et al., 2018; Miao et al., 2022).

Saini et al., (2022) utilised an 8-hour TRE protocol where 2083 participants selected their own food consumption window. The intervention noticed statistically significant downregulation of the miRNA associated with a phosphatase known as phosphatase and tensin homolog (PTEN). PTEN is known to negatively impact the PI3K-AKT signalling pathway, promoting cell survival over cell growth.

Akt substrate of 160 kDa (AS160): Glucose transport

Akt is responsible for phosphorylating AS160 which induces glucose transporter type 4 (GLUT4) translocation. Glucose transport via GLUT4 transporters to the plasma membrane allows for skeletal muscle cells to uptake circulating glucose from the bloodstream following insulin and Akt stimulation (Stöckli et al., 2011; Batista et al., 2021). Due to GLUT4 being dependent on Akt phosphorylating AS160, reductions in insulin stimulated Akt activation leads to a decrease in glucose uptake within skeletal muscle, which results in hyperglycaemia and IR (Jaiswal et al., 2019).

Forkhead box protein O1 (FOXO1): Gluconeogenesis

FOXO1 is a protein associated with pathogenesis of T2D, by inducing β-cell survival or death, apoptosis, insulin secretion and peripheral IR. Upregulation of FOXO1 displayed that it possesses a significant role in the development of IR due to IGT and insulin tolerance being exhibited within skeletal muscle. Suggesting that FoxO1

promotes both IR and muscle atrophy as a result of it being upregulated. This is due to FOXO1 upregulating phosphoenolpyruvate carboxykinases and Glucose 6phosphatase expression which promotes IR (Li et al., 2021). The impaired insulin tolerance decreases the rate of glycogen synthesis and increases gluconeogenesis, the process of converting non-CHO-based substrates into glucose. In a mice model, FOXO1 knock in depicted the development of both obesity and glucose tolerance due to food intake increasing, a decrease in energy expended and impaired insulin secretion and hypertriglyceridemia (Kim et al., 2012).

Sirtuin 1 is a post-translational regulator that plays a role in modulating inflammation (Yang et al., 2022) which can be activated by time-restricted feeding or IF (McCarty, 2022). FOXO1 is modulated by SIRT1 activity where deacetylase activity of SIRT1 may lead to a reduction in FOXO1 activity meaning glycolysis is increased (Sin et al., 2015).

Glycogen synthase kinase 3 (GSK3): Glycogen synthesis

Akt mediates glucose uptake form the circulation via phosphorylation in order to inhibit GSK3 behaviours increasing glycogen synthase activity promoting glycogen synthesis (Bathina and Das, 2018). Due to downregulation of AKT, a decreased rate of GSK3 phosphorylation occurs and as a direct effect of this glycogen synthesis is suppressed. Circulating glucose remains in the blood stream because it is not being converted into glycogen within skeletal muscle, inducing hyperglycaemia (Huang et al., 2018).

3.2.3. Ectopic fat distribution

Negative health impacts arise from being diagnosed with metabolic syndrome, which is a medical disorder linked with diabetes, hypertension and obesity leads to a greater risk of developing life-threatening cardiovascular complications such as coronary heart disease, strokes, and blood vessel related conditions (Kassi et al., 2011; Huang, 2009). Obesity is a

major factor behind transitioning from normoglycemia to impaired glucose and eventually to T2D due to an increased BMI.

Excessive body fat content does not always lead to metabolic disruption and evidence indicates that the distribution of bodily fat more accurately predicts metabolic risk (Tchernof and Després, 2013). This is due to caloric overloads which leads to fat accumulation in ectopic tissues and metabolically active organs such as the liver, skeletal muscle, pancreas, and heart as well as visceral adipose depots (Longo et al., 2019; Saad et al., 2022). Lack of PA and poor dietary habits results in the expansion of adipose tissue, particularly in visceral adipose tissue depots. IR often occurs as a result of fat accumulation in visceral regions, which alongside other risk factors results in metabolic syndrome developing, which is a reversable risk factor of T2D (Després et al., 2008; Chait and den Hartigh, 2020). Visceral adipose tissue forms when fat stores occur within intra-abdominal regions which drains through the portal vein from the digestive tract towards the liver (Mittal, 2019). Increased levels of visceral adipose tissue are associated with an increased risk of developing T2D (Agrawal et al., 2023) due to visceral adipose fat being associated with a reduction in insulin sensitivity (Mtintsilana et al., 2019). This is due to visceral adipose fat acting as the source of FFA which reach the liver and accumulate. Accumulation of this form of fat leads to dysfunction in the liver's mitochondria leading to oxidative stress (Dimitrijevic-Sreckovic et al., 2022).

3.2.4. Metabolic syndrome

Metabolic syndrome is an umbrella term for the presence of multiple interrelated risk factors for disorders such as T2D and cardiovascular disease. These risk factors are detailed in Section 3.1 "Commonly referred to definitions" in table 1, section "Metabolic syndrome". Individuals diagnosed with metabolic syndrome display a five-fold increase of developing T2D and double the risk of cardiovascular disease (Alberti et al., 2009; Sperling et al., 2015).

i. Metabolic syndrome and waist circumference

The International Diabetes Federation's (IDF) definition of metabolic syndrome defines an indirect method of measuring central adiposity as waist circumference unless BMI is greater than 30 (Zhu et al., 2020). If BMI is greater than 30, then central adiposity can be assumed.

Table 2 *Ethnic specific values for waist circumference provided by 'The IDF consensus worldwide definition of the Metabolic Syndrome' (IDF, 2006) to determine central adiposity. This table applies to individuals who possess a BMI<30, otherwise central adiposity is assumed.*

Country/ethnic group	**Gender**	**Waist circumference**
Europids	Male	≥ 94cm
	Female	≥ 80cm
South Asians	Male	≥ 90cm
	Female	≥ 80cm
Chinese	Male	≥ 90cm
	Female	≥ 80cm
Japanese	Male	≥ 90cm
	Female	≥ 80cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available.	
Sub-Saharan Africans	Use European data until more specific data are available.	
Eastern Mediterranean and Middle East populations	Use European data until more specific data are available.	

The various waist circumference values experienced in different ethnic groups are featured in table 2, these values depict the link between waist circumference and the development of metabolic syndrome. In addition to a waist circumference greater than or equal to the value in table, an individual must also have one of the following: raised triglycerides (1.7 mmol/L), reduced HDL cholesterol (<1.03 mmol/L in males or <1.29 in females), raised blood pressure (systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg) or raised FPG (≥5.6 mmol/L) (Alberti et al., 2006).

Measuring waist circumference is a method utilised to measure abdominal fat accumulation. It is a better method than BMI, which ignores any other reason for increased weight such as muscle mass. An increased waist circumference isn't accurate alone when predicting visceral fat. It is noted by Després et al., (2008) that measuring levels of fasting triglyceride may also prove useful when trying to distinguish adipose tissue from excess visceral (adipose tissue stored in a person's abdominal cavity) or ectopic (adipose tissue stored in sites such as liver, skeletal muscle, heart, and pancreas) fat. In human TRE trials, it has been noted that a reduction in waist circumference can be achieved irrespective of vast nutritional change and food quality/quantity (Wilkinson et al., 2020; Kesztyüs et al., 2019).

Ii. Metabolic syndrome and IR

Diet induced obesity is a disorder commonly associated with metabolic syndrome, resulting in a loss of insulin function. This loss of function spans from the lack of transportation of glucose across the membrane of the cell due to insulin being unable to bind with its receptor, thus IR predisposes T2D (Taylor, 2012).

A signalling pathway known as the Akt pathway plays an important role in normal metabolism maintenance, therefore an imbalance in this leads to obesity and T2D development (Huang et al., 2018). The Akt pathway is responsible for the critical cellular process, glucose homeostasis, specifically it controls the movement of plasma glucose into either skeletal muscle or adipose tissue.

In a state of IR because of overeating, there is an increased amount of hepatic glucose released. This disrupts the GLUT4 transporter, which is an insulin-responding glucose transporter, leading to a reduction in the amount of glucose uptake from circulation (Czech, 2017). This raise in blood glucose levels then stimulates islet β-cell resulting in the secretion of more insulin, eventually leading to hyperinsulinemia. It has been reported by Czech., (2017) that hyperinsulinemia may account for the disruption experienced in obese individuals which results in IR. High circulatory insulin levels, under both fasting and fed states has been

examined, where a conclusion was drawn that it plays an important role towards metabolic dysregulations which are commonly associated with obesity.

iii. Metabolic syndrome, hypertension, and blood pressure.

Managing blood pressure is an important tactic issued when trying to treat an individual who currently have metabolic syndrome. Due to the coexistence of hypertension and metabolic syndrome, end-organ damage becomes more prevalent meaning blood pressure control within hypertensive people is vital (Katsimardou et al., 2020). One of the therapeutic drugs prescribed to combat high blood pressure is an angiotensin II receptor blocker known as irbesartan, where renin-angiotensin system inhibitors (RASI) are regarded as the most beneficial type of drugs for treating hypertension. Utilisation of RASI produced significant reductions in blood pressure, HDL levels, TG levels, waist circumference and fasting glucose (Kintscher et al., 2007) and systolic/ diastolic blood pressure (Ezequiel et al., 2013) following a 24-hour treatment period. According to the AHA guidelines, an individual can be declared as hypertensive if they have a systolic blood pressure of ≥130 mmHg and/ or a diastolic blood pressure of ≥80 mmHg (Flack and Adekola, 2020).

However, Zimlichman., (2014) has suggested that lowering blood pressure alone is not enough to treat individuals with metabolic syndrome. An approach focusing on decreasing the progression of atherosclerosis via decreasing age-associated arterial stiffening via either breaking down collagen cross-links or preventing formation of the cross-links may prove to be useful when treating metabolic syndrome in the future. These potential future approaches focus on decreasing the progression of atherosclerosis, preventing these age-related arterial structural changes from occurring.

3.2.5. Risk factors interactions

i. Obesity and TRE

Altering the time of day and hours to which an obese individual can consume food may prove to be beneficial.

In a 12-week randomized clinical trial conducted by Lowe et al., (2020) which utilised a cohort of individuals with a BMI between 30-40 kg/m^2, allowing for the classification as clinically obese. The TRE intervention possessed a food consumption window from 12pm until 8pm, and for their control, they instructed the participants to consume three structured meals per day. These structured meals that the participants were asked to consume were no different to their normal day to day meals, due to there being no recommended calorie or macronutrient restrictions in terms of intake. The participants were merely told to eat during certain times. This study also provided no fixed snack windows, allowing their participants to eat snacks at any time to attempt to prevent any cravings affecting their next specified meal window. For the TRE intervention, the participants were given no exact mealtimes to adhere to. Participants were informed that they could eat whatever they want between the times of 12pm-8pm, giving the individuals in the TRE intervention more freedom than the control group. The control cohort were told what time of day that they could consume their meals in rigid time periods (breakfast: 7am-11am, lunch: 11am-3pm, dinner: 4pm-10pm). Energy intake in both the control and TRE cohort was not predetermined and participants ate ad libitum, the study provided no recommendations towards caloric/ macronutrient intake or towards PA. It was determined that no significant differences occurred with regards to estimated energy intake in both the TRE intervention and control groups. The primary outcome for this study focussed more towards whether there was any weight loss experienced as a result of TRE intervention, it was noted that there was a significant within-group decrease in weight in the TRE cohort from their baseline. A secondary outcome assessed was HbA1c, which did not significantly differ between pre-condition and post-condition.

Another randomized control trial focusing on the effects of TRE in an obese cohort was Peeke et al., (2021) who altered the diet of 60 men and women for a period of 8-weeks.
These participants were randomized into either the ‘active comparator’, opposed to a placebo or TRE which utilised a 14-hour metabolic fast, differing to the last study mentioned due to both cohorts conforming to time restrictions which dictated when they could eat, and

the diet prescribed. Both TRE interventions were told to comply with a calorie and macronutrients-controlled diet, participate in an exercise program, and they were provided with weekly counselling support sessions. The primary outcome of this was to observe whether there was a change in body weight from baseline to week 8. Each participant was told to comply with either a 10-hour TRE intervention (14-hour fasting window) or a 12-hour TRE which served as an active control, where an approximate 500–1000 kcal/day deficit would occur. week 8 it was noted that a change in body weight was statistically significant in both the 10- and 12-hour TRE interventions, where a more severe change in body weight can be visualised in the 10-hour TRE intervention, the changes towards body weight is depicted in figure 2. Figure 2 depicts a reduction in body weight in the 10-hour TRE, however this was not significant.

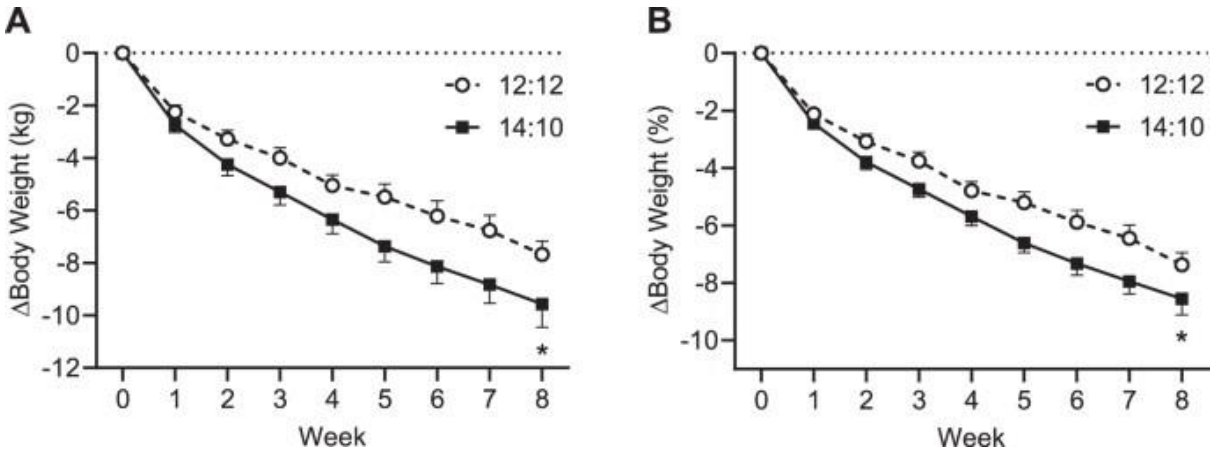

Figure 2 *A graphical depiction of weight changes which occurred as a result of completing either a 12:12 diet (12-hour food consumption window and 12-hour fasting window) against a prolonged fasting diet 14:10 (10-hour food consumption window and a 14-hour fasting window). A steeper decline was observed in the 14:10 cohort which displayed a stronger decline in body weight, however this was not significant. (A) Change in body weight. (B) Change in percent body weight. Data are mean ± SE for the completer population (n = 30). *P < 0.05. Figure is from (Peeke et al., 2021).*

Another study by Allison et al., (2021) detailed how a delayed eating schedule is associated with an increased risk of both obesity and metabolic dysfunction in humans. They issued their healthy to overweight participants, who had a BMI of 19–27 kg/m^2, an 8-week eating

schedule where they were told to consume food either early in the day (8am-7pm) or delayed (12pm-11pm). It was noted that the earlier eating schedule had many benefits including weight loss, improved IR, fat oxidation as well as benefiting cardiometabolic health, all of which would be classed as beneficial to the obese individuals. Interestingly within this study ghrelin, leptin, and glucose were not affected by either eating schedules, it was concluded that the delayed eating an individual my partake in may have adverse effects on both body weight and metabolic measurements other than caloric intake due to caloric intake being similar in both conditions. An increased respiratory quotient was exhibited in the delayed eating condition, which is associated with a low level of fat oxidation and high CHO oxidation. This decreased ability to oxidise fatty acids following an overnight fast contributes towards weight gain which is associated with metabolic syndrome and T2D. With regards to trunk-to-leg fat ratio, the delayed eating condition did not demonstrate as much of a reduction as the daytime protocol. An increased trunk-to-leg fat ratio was reported to display a positive correlation with triglycerides, total cholesterol, and systolic blood pressure, whilst possessing a negative correlation with HDL. Additionally, a high-trunk to-leg fat ratio is also associated with an increased diabetes risk, allowing for possible conclusions to be drawn that eating earlier may lead to a decreased risk of T2D developing.

ii. Pathogenesis of T2D and Obesity

T2D and obesity both share numerous contributors which result in both disorders occurring. They share common pathways which lead to the development of IR, oxidative stress and prothrombotic/ proinflammatory patterns. Diet induced obesity achieved because of overeating leads to metabolic disruption, resulting in ectopic fat accumulation surrounding the organs, leading to organ damage overtime. This dysregulation eventually results in the induction of metabolic disorders such as IR, prediabetes, T2D and cardiovascular diseases (La Sala and Pontiroli, 2020). T2D is caused by IR succeeded by a reduction in B-cell insulin secreting capacity (Wiegand et al., 2005).

iii. Insulin Resistance

i. Pathophysiology

IR is defined as the body's response to insulin becoming desensitised, this leads to a decline in plasma glucose uptake from insulin responsive organs (Kahn et al., 2006; Czech, 2017). IR progresses into T2D when there is dysfunction in pancreatic β-cell and impairments associated with pancreatic insulin release (Goyal and Jialal, 2022) leading to glucose homeostasis not being maintained, resulting in hyperglycaemia (Galicia-Garcia et al., 2020). Compensatory hyperinsulinemia associated with IR also advances hyperglucagonemia, a process where hepatic glucose output increases worsening hyperglycaemia (Thomas et al., 2019). IR promotes weight gain due to compensatory hyperinsulinemia this results in worsening IR and metabolic disorders resulting from a prior hyperinsulinemia (Barber et al., 2021).

ii. Relationship between TRE and IR

Many studies have linked TRE and IR with various outcomes depending on the protocol utilised. Che et al., (2021) utilised a 10-hour protocol where food was consumed between the hours of 8am-6pm. This protocol was complied with for 12-weeks where improved plasma glucose and insulin sensitivity was observed. Sutton et al., (2018) conducted a trial which utilised a shorter food consumption time window of 6-hours where the third and final meal was consumed by 3pm, thus they utilised an eTRE protocol. The protocol has been reported to improve both insulin sensitivity and β-cell responsiveness to insulin. This study was conducted in eight prediabetic men, the small sample size utilised may mean that a type II error was experienced where a false positive could have been achieved. The study protocol also needs to be replicated in women to allow for the results to be representative of the population. Jones et al., (2020) also reported on improvements in insulin sensitivity via OGTT and the Matsuda index following a 8-hour eTRE 2 week protocol.

Cienfuegos et al., (2020) utilised a 4-hour and 6-hour TRE protocol versus a control group, they noted a decrease in IR via homeostatic model assessment for insulin resistance (HOMA-IR) following an 8-week protocol between both TRE conditions and the control group.

However various studies have also shown no effect observed between TRE and IR, where a common theme is clear in these studies, protocol utilised was either an lTRE condition or participant self-selected food consumption windows. Andriessen et al., (2022) utilised a 10-hour protocol where the last meal must be consumed by 6pm, however this protocol was much shorter lasting 3-weeks. This study reported on improved levels of glucose homeostasis however they observed no changes in insulin sensitivity or hepatic glycogen output. (Carlson et al., 2007) utilised a protocol where participants consumed either 1 or 3 meals per day between the times of 5pm and 9pm. Fasting insulin levels were not affected by meal-frequency, meaning no significant effect on improving IR was observed. Gabel et al., (2018) completed an 8-hour TRE intervention where participants were allowed to eat ad libitum between the hours of 10am and 6pm. Both levels of fasting insulin and HOMA-IR did not significantly differ from the controls utilised after complying with the protocol for 12 weeks. Wilkinson et al., (2020) utilised a self-selected 10-hour TRE protocol where trends towards improvements in fasting glucose and insulin were observed, however the values obtained were not statistically significant.

To conclude, the studies compiled above imply that utilising eTRE protocol with specified eating hours may improve fasting glucose/ insulin levels resulting in improved IR.

3.2.6. Impaired β-Cell function

A common characteristic associated with obesity is IR in peripheral tissues in areas such as skeletal muscle, adipose tissue and liver. This leads to an increased demand of insulin which triggers pancreatic β-cell adaptation to maintain normoglycaemia via releasing sufficient amount of insulin by increasing both β-cell mass and function. This form of compensatory response leads to an increased rate of insulin secretion, resulting in the development of

hyperinsulinemia. Hyperinsulinemia is a condition where there are higher levels of insulin circulating which overtime leads to metabolic dysregulations observed in obesity and T2D (Chiasson and Rabasa-Lhoret, 2004; Shanik et al., 2008). Even though obesity is closely linked with T2D, so much so it has led to the connotation 'diabesity' being coined (Leitner et al., 2017). The disease can also develop in non-obese individuals (Vaag and Lund, 2007).

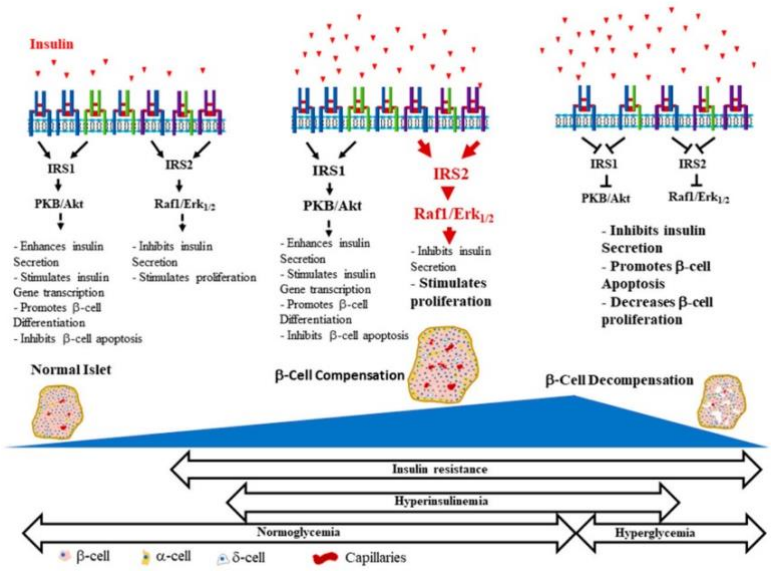

Figure 3 *A schematic from Rachdaoui, (2020) detailing both β-Cell life and death due to the development of obesity-mediated T2D. Depicted in is the events which take place during the β-Cell lifespan, demonstrating the disruption caused to insulin receptor substrate 2 (IRS2) pathway which results in β-Cell decompensation and eventually IR.*

Maintenance of β-cell mass is achieved via β-cell proliferation and inhibition of apoptosis. Figure 3 displays the essential role provided by both insulin and IGF-1 towards their specific signalling pathways. For normal β-cell function to occur, communications between β-cells and endothelial cells are essential. Impairment of these communications result in pancreatic β-cells not being able to maintain normoglycaemia. β-cell function and mass are depicted in figure 3 and the damaging effects of their dysregulation. Overexpression of insulin may lead to negative autocrine actions on β-cells due to insulin receptor substrate 2 (IRS2) pathway (Rachdaoui, 2020). The insulin signalling molecule IRS2 possesses an important role towards antiapoptotic effects exhibited by insulin. Various study discuss that in IRS2 deficient mice, that there was an increased rate of β-cell apoptosis and an increased level of T2D

development (Kubota et al., 2000; Ren et al., 2014, Withers et al., 1998). In essence, insulin promotes β-cell proliferation resulting in β-cell compensation in attempt to combat insulin-resistance during prediabetes (Beith et al., 2008; Mezza et al., 2019). This leads to the development of hyperinsulinemia due to β-cell adapting their size and number in order to enhance their insulin secretory capacity (Cerf, 2013). This hyperinsulinemia then contributes towards β-cell decompensation via negative impacts their function and mass. This inhibition of function results in β-cell decompensation leading to the development of T2D (Wiegand et al., 2005).

i. Hepatic insulin sensitivity (resistance)

Pathophysiology associated with hyperglycemia in already established T2D is caused by hepatic IR. This theory has been displayed in published studies which focused on 'moderate' calorie restrictions in a T2D cohort. in these studies there was a trend visualised with regards to a decrease in the amount of fat stored in hepatic cells which has led to improvements in both hepatic insulin sensitivity and fasting blood glucose. This study concluded that within poorly controlled T2D, moderate weight loss normalises fasting hyperglycemia via the mobilisation of intrahepatic lipids which leads to the reversal of hepatic IR (Petersen et al., 2005; Welton et al., 2020). Of recent, it has been noted that restricting the number of calories consumed achieves prolonged levels of normal insulin secretion due to the amount of fat in pancreatic cells decreasing (Lim et al., 2011).

3.3. Complications of prediabetes and T2D

Corresponding complications of prediabetes and T2D have proportionally increased in accordance with the increase of cases (Feldman et al., 2019). Once classified as type 2 diabetic, individuals have a significant risk of developing cardiovascular related diseases (Martín Timon et al., 2014), with individuals exhibiting a two-to-four-fold increase towards developing cardiovascular related morbidities and mortality when compared to non-diabetic individuals (Hudspeth, 2018).

3.3.1. Microvascular complications

Good glycaemic management of blood glucose levels reduces the risk of microvascular complications developing (Benhalima et al., 2011). Microvascular complications may be present at diagnosis or in undiagnosed individuals (Valencia and Florez, 2017), due to such complications arising as a result of longstanding or uncontrolled diseases. These complications vary, affecting many places in the body such as retinopathy (damage to the retina), nephropathy (deterioration of kidney function) and peripheral neuropathy (peripheral nerve damage) (Chawla et al., 2016; Zimmerman, 2016). Progression of these complications result in potential loss of visual, renal, and neurologic functions, where if they progress due to uncontrolled or untreated T2D, they have the possibility to result in irreversible damage and in some cases even premature death due to cerebrovascular and cardiovascular events (Kalofoutis et al., 2007; Forrester et al., 2020).

Retinopathy occurs due to T2D where irreversible, vision-threatening damage to the retina of the eye occurs (Kashim et al., 2018). This eventually progresses to blindness, which is the most common and serious ocular complication (Shukla and Tripathy, 2022).

Diabetic nephropathy is a major cause of end-stage renal disease, which is caused by hyperfiltration and albuminuria during early phases succeeded by a progressive decline in renal function. Mortality in individuals with diabetic kidney disease is approximately 30 times higher than diabetic individuals who do not have nephropathy (Sagoo and Gnudi, 2020). Peripheral neuropathy is an untreatable condition where there is a loss of sensory function in lower extremities. It can be characterised by significant morbidity and is also associated with an increased level of pain and decreased quality of life (Feldman et al., 2019). This is due to the possibility that the condition has towards effecting the longer peripheral nerves in the hands, feet, and arms, leading to lower limb denervation. 15– 20% of diabetic individuals have an increased risk of foot ulceration occurring as well as a 15-fold increased risk of lower limb amputation when compared to non-diabetic individual (Cole and Florez, 2020). The prevalence of diabetic neuropathy in diabetic adults is thought to be between 6-51% depending on multiple factors; age, diabetes duration, level of glucose control, and whether an individual suffers from type 1 or T2D (Hicks and Selvin, 2019).

3.3.2. Macrovascular complications

Type 2 diabetic individuals have an association with significant macrovascular complications occurring. These include coronary artery disease (CAD), peripheral arterial disease and cerebrovascular diseases including stroke (Chiong and Evans-Molina, 2013).

CAD is a disease affecting blood vessels which supply the heart muscle with oxygen (Shahjehan and Bhutta, 2023), where the risk for developing CAD is exacerbated by an individual who are currently classed as T2D (van Zuydam et al., 2020). Clinical outcomes obtained from individuals who have been diagnosed with CAD and T2D display poor improvements, even when medications and other interventions are implemented (Naito and Kasai, 2015). Peripheral artery disease develops due to a build-up of fatty deposits, otherwise known as atherosclerotic plaques, on the walls of blood vessels which leads to a decreased arterial diameter (Soyoye et al., 2021). It is a disease which is commonly associated with T2D due to individuals being prone to higher levels of cholesterol and an increased risk of heart disease (Tang et al., 2018). Pathologic states such as hyperglycaemia and IR result in the development and progression of peripheral artery disease due to the overproduction of reactive oxygen species which results in endothelial dysfunction and inflammation (Paneni et al., 2013; Thiruvoipati et al., 2015).

Development of prediabetes and T2D are risk factors for stroke, double the risk of a stroke occurring. Strokes account for approximately 20% of deaths in type 1 and T2D individuals combined (Boehme et al., 2017), approximately 50% in individuals who have T2D (Ma et al., 2022). Uncontrolled diabetes caused by untreated hyperglycaemia increases a T2D individuals' risk of suffering from both ischemic and haemorrhagic strokes. This is due to T2D leading to an increased level of arterial stiffness and decreased level arterial elasticity. This is due to T2D leading to an increased inflammatory response which leads to the progression of atherosclerotic plaques (Chen et al., 2016).

3.4. Therapeutic targets

3.4.1. Drug treatment

To assure the most effective management of T2D is achieved, pharmacologic therapies are advised to occur alongside lifestyle modifications in order for individuals to meet target glycaemic ranges (Ganesan et al., 2022). Drug treatment therapies for T2D range from insulin secretagogues, metformin, thiazolidinediones, alpha glucosidase inhibitors, incretin mimetics, amylin antagonists and sodium-glucose co-transporter-2 inhibitors. Each of these drug treatment therapies possess a different action exhibited on certain areas of the body to improve negative effects exhibited by T2D individuals (Padhi et al., 2020).

Due to drug therapies having a short half-life, this requires the T2D individual to ingest a frequent dosage of the drug leading to potential side effects occurring resulting in therapy ineffectiveness (Padhi et al., 2020).

3.4.2. Exercise, reduction in body weight and Diet

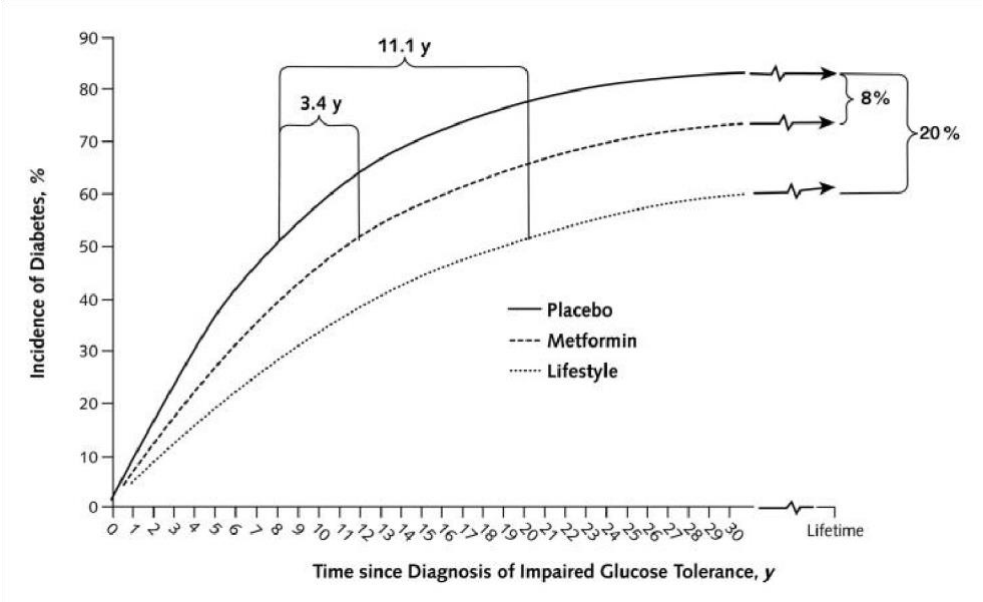

Figure 4 *A visual representation of incidence of T2D via intervention groups based on analysis calculated by 3 years of data obtained by the Diabetes Prevention Program. Three*

groups were assessed: placebo (no intervention), lifestyle intervention and metformin (Herman, 2015).

Figure 4 from Herman, (2015) depicts how if detected early and treated via lifestyle interventions or metformin administration, pre-diabetes can be prevented progressing into T2D. When compared to the control placebo intervention, the lifestyle intervention group had a 20% reduced risk of developing T2D and delayed the onset of T2D by approximately 11 years across an individual's lifetime. Lifestyle intervention reduced T2D incidence rate more successfully than drug intervention (metformin) by 12%, delaying onset of T2D by 7.7 years at 50% incidence.

Current recommendations implemented to reduce body weight are forms of dietary modifications and increasing PA levels in order to improve IGT (Franz et al., 2015; Pan et al., 1997; Mackerras, 2003; Herman et al., 2005). Implementing both techniques simultaneously improves glycaemic control, due to control becoming worse within obese adults, and reduces the conversion rate of impaired glucose into T2D in addition to reducing cardiovascular disease risk (Vijan et al., 2005).

3.4.3. Relationship between T2D and PA

A method of combating this increase in body weight would be increasing energy expenditure via exercise, surpassing the amount of energy gained via food consumption. This study does not utilise a change in PA, however in addition to TRE, PA may prove useful with regards to preventing weight regain via maintaining energy balance.

It has been reported that after 24 months of intervention, 36% sustained their level of remission (Lean, 2019). Moreover, 15% of the obese individuals focused on will continue to lose weight in the long term (Ayyad and Andersen, 2000), allowing for a conclusion to be drawn that another strategy may be required to be implemented alongside a very-low calorie diet (VLCD). Exercise intervention would be required to create such an environment where

respiring cells have a greater requirement for energy, thus becoming more sensitive to insulin leading to an uptake from the blood.

Due to this research being a relatively novel study, there are not any published studies to directly compare. However, studies such as van Aggel-Leijssen et al., (2002) as well as Achten and Jeukendrup, (2004) have concluded that lower levels of energy expenditure (40% VO2 Max) would be enough to increase overall fat metabolism. Fatty acid metabolism is important to prevent weight gain due to it preventing the excess secretion of insulin, which is the hormone responsible for creating the fatty acid stores in adipose tissue.

Diet and PA are the two main principal lifestyle intervention targets for intervention (Pfeiffer and Klein, 2014). Techniques utilised for intervention include consumption of a healthy diet, increasing PA and reducing body weight as these factors have been noted to lower T2D incidence when compared to a control group within Lindström et al., (2003) and Molitch et al., (2003) by 58%. In addition to this, meta-analyses conducted by Chen et al., (2022) stated that lifestyle interventions displayed a similar effect on reducing incidence of T2D across various ethnic groups, via improving glycaemic outcomes, PA, energy intake and anthropometric measures (BMI and waist circumference). However, in these various ethnic groups, different levels of weight loss were observed. Chen et al., (2022) noted that European, South Asian, East and Southeast Asian, Middle Eastern ethnicities responded better to short term weight loss interventions less than a year, producing a significant weight loss. Meta-analyses conducted displayed that lifestyle interventions which implement a higher number of sessions were significantly associated with a greater reduction in body weight and T2D incidence.

3.4.4. Diet interventions

Reducing of approximately 500 calories per day, also known as a ‘hypocaloric diets’ leads to a modest amount of weight being lost alongside significant reductions in fasting glucose and HOMA-IR (Katsarou et al., 2021). Approximately 0.5kg could be lost via this technique per

week, however this is usually short lived. Individuals utilising this form of diet would eventually experience hormonal adaptations, implemented in attempt to resist losing weight (Maclean et al., 2011).

Weight regain can be predicted by improved insulin sensitivity which leads to an enhanced response to glucose load where CHO are utilised for energy production (Maclean et al., 2011). This is due to biological responses acting to restore depleted energy reserves, thus meaning that weight loss strategies must implement a technique which control this metabolic influence to allow for long-term successful weight loss (Li et al., 2022). Reductions in metabolic improvements occur despite reductions in body weight still occurring, due to insulin sensitivity age-related decline in obese individuals. TRE only requires restrictions for specific, predetermined days of the week, meaning adherence with this protocol may be more practical (Heymsfield et al., 2007).

a. T2D and a VLCD

Complying with a VLCD of approximately 800 calories a day may prove to be difficult due to its effects on the individual's social life, whereas compliance with a VLCD instead of a lowcalorie diet proves to be more effective. Moriconi et al., (2021) observed the effects of different diets and their effects on decreasing the amount of diabetic medication required. They reported that individuals who complied with just a low-calorie diet had to increase the amount of anti-diabetic medications whereas in their VLCD group, 26.6% of their patients stopped requiring anti-diabetic medications, and 73.3% were taking only one medication. A randomised clinical trial which ran for 4 months stated that both weight loss and a reduction in waist circumference was achieved when the individual undertakes a VLCD, additionally demonstrating a decline in Hb1Ac thus an improved level of glycemic control when compared to the control group (Goday et al., 2016).

However, the issue with weight loss from a VLCD alone arises after 12 months as weight regain would occur, providing evidence supporting the notion that holistic lifestyle modifications must occur to assure that long-term weight loss is achieved (Kakoschke et al.,

2021). Purcell et al., (2014) demonstrated that 2 years after a diet-induced weight-loss investigation was completed, the individuals within this study regained approximately 70% of the weight they lost. A randomised clinical trial which ran for 4 months stated that both weight loss and a reduction in waist circumference was achieved when the individual undertakes a VLCD, additionally demonstrating a decline in Hb1Ac thus an improved level of glycaemic control when compared to the control group (Goday et al., 2016).

i. Compliance with a VLCD may achieve optimal glycaemic control.

Previously it has been reported that compliance with a VLCD is a highly effective intervention in regards to achieving optimal glycaemic control. A study conducted by Umphonsathien et al., (2022) noted that in a randomised control trial which contained 40 participants, the levels of both HbA1c and IR index decreased. These levels were determined after completing an OGTT at baseline, weeks 2, 10 and 20. This study provides evidence supporting the notion that compliance with a VLCD for a relatively short period of time improves T2D individuals' quality of life and it achieves this via achieving glycaemic control which may result in diabetic remission. However, diabetic remission was only achieved in 8 of the 40 participants, meaning only 20% achieved diabetic remission after 20 weeks of VLCD compliance. This study noted that utilising an intermittent VLCD rather than a continuous one may serve as an easier option for obese patients, due to themselves perhaps finding it difficult to adhere to continuous VLCD to maintain weight loss. In addition to this, an important fact was raised that there is currently no standard definition of intermittent caloric restriction or VLCD, meaning that it may be difficult to compare all the various studies focusing on intermittent VLCD/ continuous VLCD due to the differences in populations, study duration and the various forms of VLCD.

However, compliance with VLCD where participants were having to consume ~600kcal per day would not be sustainable long-term, due to a lower amount of CHO consumed. This may lead to an increased level of fatigue, which could reduce the drive to complete required PA. Moreover, compliance could be jeopardised due to low caloric intake and CHO having to be removed (Gow et al., 2016).

3.4.5. Reversal of T2D via diet

a. Entering remission

T2D may be able to enter remission after completing a VLCD/ LDC where they must adhere to either a calorie restriction or liquid meal replacement diet (Lean, 2019; Taheri et al., 2020).

Lim et al., (2011) conducted an 8-week VLCD intervention study on 11 individuals, where following successful completion of the VLCD diet participants returned to normal eating with healthy portion sizes and information about healthy eating. A fifth of the originally recruited 15 participants failed to comply with the diet protocol for 8-weeks. In addition to this, Steven et al., (2016) noted that after 6 months of returning back to normal eating following an 8-week liquid only VLCD diet, only 43% of individuals-maintained remission. Increasing the VLCD window from the commonly used 8-week protocol to 16-20 weeks still observed similar effects (Lean, 2019). 25% of those who achieved remission following completion of the 12-month VLCD regained a significant amount of weight following a further 12-months. Additionally, these individuals relapsed at 24 months, meaning they were again regarded as T2D. Lean et al., (2019) utilised a cluster randomised control trial known as Diabetes Remission Clinical trial (DiReCT), which aimed to assess a possible link between intensive weight management and T2D remission. Xin et al., (2019) disclosed the one-year cost per participant from the DiReCT study protocol. The intervention group (VLCD group) displayed significantly lower costs per participant due to antidiabetic drugs, antihypertensive drugs and GP practice visits due to a reduced requirement for routine resources. However, the control group cost on average £1067 less than the intervention group due to them not requiring intervention delivery. In the intervention group, only 46% of the 149 people who participated did achieve remission by the end of the 12-month intervention. On top of this, after 24-month only 36% managed to maintain this remission. Sustained remission was achieved via sustained weight loss (Lean et al., 2019), therefore further studies must be

completed examining how this method could transpire into everyday life, allowing for long-term results and compliance. Umphonsathien et al., (2022) conducted a VLCD study where participants consumed 600kcal/day for 10 days across the course of 2 weeks to assess compliance. If participants achieved 90% compliance, they would then complete 8 weeks of VLCD. Caloric intake varied over the course of the 8 weeks, increasing initially from 800kcal/day to 1500kcal/day in week 12. It was concluded that at both 8 and 12 weeks, remission rate was 79% however at 12-months remission rate dropped to approximately 30%.

Many studies have assessed the effects of a low-calorie diet (LCD), where participants typically consume 1000-1500kcal, in T2D individuals (Bhatt et al., 2017; Sarathi et al., 2017). Sarathi et al., (2017) utilised a protocol with 3-month, 1 year and 2 year check-ups where remission rates were as follows: 24 participants (75%) at month 3, 24 participants (75%) after a year and 22 (68.75%) after 2 years. Bhatt et al., (2017) used a much shorter protocol which only lasted 12-weeks. This study detailed that many participants stopped taking their antidiabetic medications and a significant decrease was reported in HbA1c, where their HbA1c recording was now below a diabetic range for six participants. In addition to this, in the cohort utilised postprandial glucose and fasting glucose levels also significantly reduced. Gregg et al., (2012) conducted an ancillary study on a 4-year randomised control trial where participants consumed a total caloric intake of 1200-1800 kcal/day. This was achieved via reducing the amount of total and saturated fat consumed. It was concluded that participants who achieved complete remission, defined as transitioning from diabetic to nondiabetic level of glycaemia without the use of medication, was more common in the intensive lifestyle intervention group, which utilised both LCD and PA, than the diabetes support and education control group across the length of the study.

In summary, the studies mentioned above have proven that VLCD/LCD protocols allow for a method in which T2D individuals can enter remission, however this remission may be short-lived in the long term and another method such as must be implemented.

3.4.6. Acceptability long term

The original application submitted at the beginning of my Master of Research focussed on implementing a behavioural intervention targeting PA in attempt to sustain weight loss following completion of a VLCD. Sustaining weight loss would potentially allow T2D individuals who achieved remission because of completing the initial VLCD program which involved participants to consume ~800kcal/day via low-calorie foods such as shakes and soups, to sustain this remission via exercise interventions. This intervention aimed to increase PA levels to maintain weight loss following VLCD. As mentioned above in section "3.5.5.a. Entering remission", compliance with a VLCD within studies which lasted 12-24 months was low (Lean et al., 2019; Umphonsathien et al., 2022). Anecdotal reports from NHS England pilot sites have suggested that only ~50% of people referred to low calorie diets beginning the programme.

3.4.7. Prevention in prediabetic individuals

i. Testing for T2D

As reported by ADA, there are 4 testing methods utilised to allow for the diagnosis of T2D and pre-diabetes, where for prediabetes the methods for conducting these tests are the same (American Diabetes Association, 2014).

These four methods are as follows:

1. HbA1c test: Laboratory test analysing the levels of HbA1c (glucose bound to haemoglobin molecules).
2. FPG test: The participant would be fasted of food and drink, apart from water (at least 8 hours prior to test completion)
3. OGTT: Participants would be asked to consume 75g glucose syrup before, where following this a 2-hour plasma glucose examination would occur.
4. Random plasma glucose test: If an individual displays a level of ≥11.1 mmol/L, this is regarded as hyperglycaemic or undergoing hyperglycaemic crisis. (Goyal and Jialal, 2022)

The values which are used for diagnosis are detailed below in table 3.

Table 3 *Adapted from ADA guidelines, 'Classification, and diagnosis of diabetes' detailing the various tests for T2D and their diagnoses (American Diabetes Association, 2015).*

Diagnosis	***HbA1c (percent)***	***FPG (mmol/L)***	***OGTT (mmol/L)***	***Random plasma glucose test (mmol/L)***
Normal	below 5.7	<5.5	7.7 or below	
Prediabetes	5.7 to 6.4	5.6 to 6.9	7.8 to 11.0	
Diabetes	6.5 or above	7.0 or above	11.1 or above	11.1 or above

The OGTT has been considered the 'gold standard' test for diagnosing T2D since it was created in 1997, due to its counterparts the 'FPG' and 2-hour plasma glucose test' not possessing cut off values in youth (Brar, 2019). Brar (2019) also mentions the benefits that the OGTT have over its counterparts. Examples include how OGTT provides a view towards β-cell function, both fasting and prandial (during dinner or lunch), towards IR, which is a disorder present in an age demographic not suitable for the other tests (obese youths). In addition to this, Brar (2019) mention how the OGTT can distinguish between the forms of resistance displayed in a T2D person, who could be suffering from either hepatic IR or postprandial resistance.

3.5. Improvements in IGT- CGM and GV

Current recommendations implemented to reduce body weight are forms of dietary modifications and increasing PA levels in order to improve IGT (Franz et al., 2015; Pan et al., 1997; Mackerras, 2003; Herman et al., 2005). Reductions of the number of individuals progressing towards T2D from IGT occurs if glucose tolerance is improved. Obese individuals have a level of IR where cellular actions imposed by insulin such as inhibiting glucose output from the liver/ skeletal muscle cells aren't detected by insulin receptors. In addition, insulin cannot adequately instruct glucose uptake into adipose tissue/ muscle cells (Lambadiari et

al., 2015). In healthy individuals, ingestion of a meal still results in short term hyperglycaemia. However, unlike in T2D individuals, this increase is easily rectified by the pancreas due to its ability to release insulin to respond to this increase in plasma glucose. A T2D individual's plasma glucose levels would remain elevated rather than returning to baseline levels by the 2-hour mark, demonstrating a need for intervention (Altuntaş, 2019). This is due to type 2 diabetic individuals possessing an impaired level of glycaemic control as a result of IR, which prevents blood glucose homeostasis from occurring (Schwingshackl et al., 2011).

Glycaemic control is achieved via the diabetic individual maintaining euglycemic (within the normal range 3.9-10 mmol/L) blood glucose levels. This is achieved due to the individual implementing strategies to avoid hyper/hypoglycaemic events (Perlmuter et al., 2008). To allow for proper management of T2D, continuous monitoring of glucose levels must be monitored constantly, in order for behaviours to be altered to stay within the normal healthy range (Funtanilla et al., 2019). Janapala et al., (2019) conducted a systematic review evaluating the use of CGM over self-monitoring of blood glucose (SMBG). They concluded that utilising a CGM over SMBG to monitor T2D produces beneficial effects due to significant reductions in HbA1c occurring. CGM devices measure glucose levels in interstitial fluid every minute providing a stored mean every 15 minutes (Blum, 2018). SMBG prior to the development of CGM required a diabetic individual to prick their finger several times a day. This method may lead to undesirable issues occurring, including pain associated with the finger prick needle, infections occurring because of the skin barrier being breached and loss of sensitivity in the fingertip due to scaring and callus formation (Holzer et al., 2022). These issues were resolved via the development of a CGM which allows for an individual to obtain a value for their plasma glucose level via a device which possesses a sensor in subcutaneous tissue which obtains readings from interstitial fluid (Olczuk and Priefer, 2018). To stay within a healthy range, individuals would rectify their blood glucose appropriately. If blood glucose is lower than the desired level due to various reasons (e.g. missing a meal, taking too much insulin, exercising more than normal) consuming either glucose tablets or sugary drinks/ food should occur (Carlson et al., 2017). If blood glucose is too high due to illness stress, consuming too much food or not providing enough insulin, methods such as exercising, following a meal plan, and taking medication when instructed should be utilised (Centers for Disease Control, 2022).

GV are fluctuations in blood glucose levels, determined by the value which deviates from the mean to maintain glucose homeostasis over a given time period (Martinez et al., 2021). This time period varies, ranging from within a 24-hour period, within a week or long-term (Zhou et al., 2020). Assessing GV via the use of a CGM utilises glucose measurements taken every minute, where readings are received from interstitial fluid and stored every 15minutes (Blum, 2018). Hall et al., (2018) detailed the differing levels of glucose variability expressed in healthy individuals, detailing magnitude, and degree of variability. Severity of glucose variability is classified by establishing how much glucose concentration amplitudes deviate from the mean. It was noted in this study that utilisation of CGM allowed for a comprehensive metric of determining glycaemic state to be determined when compared to existing measurement techniques. Examples of this include a gold standard assessment technique, HbA1c measurement, which provides a singular value depicting average plasma glucose levels. This measurement does not consider glucose variability (fluctuations) which CGM does display (Umpierrez and Kovatchev, 2018).

CGM is a relatively new technique for evaluating glucose homeostasis in a clinical setting (Li et al., 2022). CGM devices have moved beyond solely providing a value for glucose via providing real-time data alongside predictive glycaemic data (Miller, 2020). CGM devices now have the ability to detect trends and can review GV over its wear, providing values for time spent above and below range (Yapanis et al., 2022). Time spent within healthy/ undesirable ranges has been stated by Yapanis et al., (2022) to be the most consistent measure obtained from CGM, supporting its use in a clinical setting. This is because CGM devices possess the ability to effectively reduce the amount of time spent within a hypo/hyperglycaemic range as it can detect and alert the user (Miller, 2020).

CGM recordings have the capability to show progression from hypo/hyperglycaemia to prediabetes then T2D (Barua et al., 2021). Individuals in a study undertaken by Tuomilehto et al., (2001) with IGT tended to be obese and inactive, which allowed for the conclusion to be drawn that a dose-response relationship between these factors must be corrected in order to reduce the risk of T2D development. A limitation of this study is that it was not a crossover design, participants were randomised to either control or intervention groups

potentially resulting in the study not being representative of the general population due to a disproportionate distribution of biological sex and ethnicities.

3.6. Time Restricted Eating

3.6.1. Defining the various forms of IF

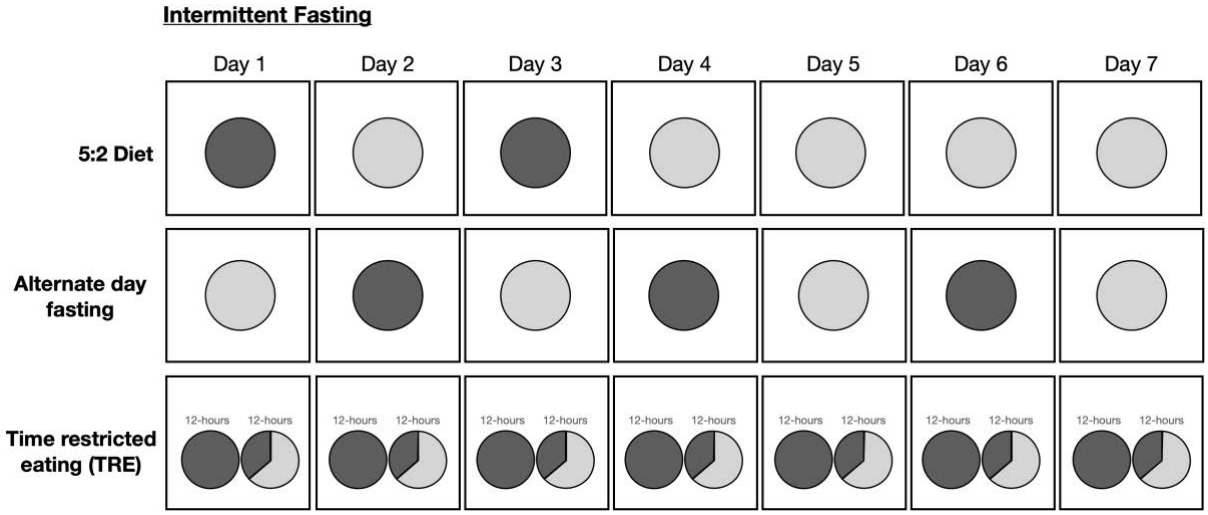

Figure 5 *A schematic comparing the various forms of IF protocols. Fasting windows are depicted in dark grey, caloric intake varies depending on which protocol is being completed during this phase, and food consumption windows are depicted in light grey. 5:2 diet involves two 24-hour fasting windows per week. Alternate day fasting involves fasting for 24-hours each alternate day. Time restricted eating (TRE) involves a period of fasting and eating during a 24-hour period.*

Figure 5 depicts the various forms of IF and possible times which they could take place. IF is defined as a dietary intervention where an individual fasts for an extended period of time followed by a period of consuming their typical or prescribed diet (Zhang et al., 2022). The 5:2 Diet is a form of IF where a fast day occurs twice a week on non-consecutive days (Gabel et al., 2018). Alternate day fasting protocols require more fasting periods than the 5:2 diet involving a 24-hour fasting window on alternate days (Park et al., 2020). During the fasting window, individuals may consume anywhere between zero to 25% of their caloric

requirements depending on the protocol (Welton et al., 2020). TRE protocol requires a fixed time feeding window followed by an extended fasting period (Gabel et al., 2019). The hours to which an individual would fast varies, depending on protocol implemented, ranging from 12-21 hours (i.e. 8/9-hours feeding window to 16/15-hours fasting) (McAllister et al., 2020). Anton et al., (2019) reported on TRE protocols exhibiting similar biological pathways to calorie restriction protocols.

Fasting techniques allow the body to utilise alternative, secondary metabolic phases which are less dependent on glucose and rely more on utilising ketone body carbon sources as their substrates (Wang et al., 2022). Circulating levels of ketone were increased in persons with T2D. One reason for increased ketone body usage in the diabetic ketolysis yields more energy available to synthesize ATP than fatty acid oxidation (Kolb et al., 2021). During fasting, or a low CHO diet, the levels of circulating insulin are decreased to promote lipolysis, a process where fat becomes primary energy source (McCarthy et al., 2021). Hepatic cells regulate metabolism under these conditions, where ketone bodies are generated via βoxidation of FFA, this process occurs alongside gluconeogenesis. Increased levels of plasma ketone bodies occur as a result of there not being enough CHO being consumed via diet (Dhillon and Gupta, 2022).

Zhang et al., (2022) discussed whether continuous calorie restriction or IF is superior with regards to weight loss, however they built on this by noting that IF is superior in terms of reducing body mass to improve glucose and lipid metabolism. These benefits reduce the risk associated with T2D, cardiovascular diseases and stroke from developing. IF achieves this by improving glucose homeostasis via autophagy. TRE promotes expression of glycolytic genes which inhibits gluconeogenesis which promotes glucose uptake in peripheral tissue (Martinez-Lopez et al., 2017). The TRE pattern that was utilised in this study follows an 8-hour eating window followed by a 16-hour fast. This aims to limit food intake over a 24-hour period as well as calorie-dense drinks that may generally be consumed through the day as participants were only allowed to consume low calorie drinks such as tea, coffee, or water.

3.6.2. Concept of time of day i.e., Circadian Rhythm

a. Circadian regulation

The time of day and the duration of food consumption are important factors to consider when undertaking a diet due to the role which it plays with regards to metabolic dysfunction/ regulation. This natural process is known as circadian rhythm, an internal process which is ruled by the sleep-wake cycle which repeats every 24-hours (Adafer et al., 2020).

Circadian regulation may lead to obesity due to alterations in leptin and ghrelin. If an individual is sleep deprived, this will lead to an increased amount of ghrelin, a hormone which increases an individual's drive to eat, and a decreased amount of leptin, a hormone which inhibits hunger (Rácz et al., 2019). Circadian rhythm is a process which aligns the daynight cycle with the fasting-feeding cycle, instructing metabolic processes to coincide with feeding cycles. These metabolic disturbances may be caused by either excess food intake leading to a nutrient's imbalance or associated disorders such as obesity, T2D, metabolic syndrome or hypertension. These disorders may lead to an alteration in circadian rhythm due to counterregulatory hormones known as cortisol, glucagon, adrenaline and growth hormone all play a role in influencing nocturnal fluctuations of glucose (Vieira et al., 2014).

The antagonistic effects exhibited on insulin by glucagon, which triggers insulin secretion from pancreatic cells known as islet β-cell (Song et al., 2017) and adrenaline, promotes glycogenolysis in both the liver and skeletal muscle (Verberne et al., 2016), due to the need to coordinate a rapid response to hypoglycaemia. This is due to individuals with T2D displaying an impaired ability to secrete stored glucagon, adrenaline secretion becomes the primary response to coordinate a counter-regulatory response to hypoglycaemia. Whilst effects imposed by cortisol and growth hormone possess a delayed release which displays a slower onset but experience a prolonged duration effect of several hours (Smith and Lager, 1989).

Circadian disruption exhibits detrimental effects on human health, due to it leading to the development of cardiovascular and metabolic pathologies (Hernández-García et al., 2020).

This is due to various factors such as an increased rate of fasting glucose, postprandial glucose, inadequate pancreatic β-cell response, and reductions in postprandial insulin levels (Reutrakul and Knutson, 2015). Moreover, it has been demonstrated to increase likelihood of certain cancers, mood disorders, neurodegenerative disorders and leads to dysregulation of the immune system (Nassan and Videnovic, 2022; Lin and Farkas, 2018; Walker et al., 2020).

Early morning requirements for insulin occur between 5 a.m. and 9 a.m. (Bolli and Gerich, 1984) and are required twice as much as daytime demand throughout the day (12 p.m.-4p.m). This supports the dawn phenomenon theory of an abrupt increase in insulin and plasma glucose levels being required prior to hypoglycaemia occurring (Trümper et al., 1995). Insulin sensitivity reduces during sleep, due to both growth hormone and cortisol levels increasing. This ensures that blood glucose levels are kept within a normal range. Both hormones achieve this via two methods; switching from the use of glucose as a reactant in respiring muscle cells to non-esterified FFA oxidation or controlling the influence of glucose production from both the kidney and liver (Dimitriadis et al., 2021). Nocturnal hyperglycaemia is visible in T2D due to an increased rate of gluconeogenesis via hyperglucagonemia, the process of transforming non-CHO precursors into glucose molecules (Zhang et al., 2018). An imbalance between glucagon and cortisol may trigger nocturnal hyperglucagonemia due to there not being a rise in cortisol levels but excessive glucagon secretion (Basu et al., 2020).

Insulin-antagonistic hormones (glucagon, adrenaline, cortisol, and growth hormone) lead to fluctuating levels of glucose in overweight, obese and insulin-resistant individuals. Within Lundqvist et al., (2021) there was an independent association observed between an increased BMI and a lower cortisol response. Within Campbell et al., (1985), it was noted that a nocturnal surge in growth hormone leads to an individual who have insulin dependent diabetes mellitus to experience what is known as the dawn phenomenon. This phenomenon occurs inducing sudden hypoglycaemia when there is a spike in either plasma glucose or insulin requirements between the time of 5-9am, before hypoglycaemia occurs (Bolli et al., 1984). The dawn phenomenon is associated with overall poor glycaemic control, where achieving control of the sudden increase associated with this effect may improve overall glucose control in type 2 diabetics (Li et al., 2020).

b. Dawn Phenomenon: Glucose homeostasis

The dawn phenomenon occurs in the evening where glucose tolerance appears to be less effective due to insulin sensitivity and pancreatic β-cell function declining (Van Cauter et al., 1997). Pancreatic β-cell function declining occurs due to the inability for the cells to produce enough insulin to meet metabolic requirements (Skyler et al., 2017). Type 2 diabetics experience a nocturnal raise in blood glucose during sleep whilst possessing a decreased level of insulin sensitivity (Radziuk and Pye, 2006). Basu et al., (2000) reported that type 2 diabetic individuals have a muscle glucose uptake impairment as well as an impaired hepatic glucose uptake ability which both contribute towards hyperglycaemia. This is due to adipose tissue being deposited in hepatic and muscle cells which inhibits their function. As the adipose tissue continues to accumulate in these areas, IR worsens and becomes more prominent (Skyler et al., 2017). Non-diabetic individuals experience a peak of both glucose and insulin levels during habitual sleep, this supports the notion that circadian rhythm influences the behaviour of glucose (Van Cauter et al., 1997).

Plasma glucose concentrations vary across 24-hour circadian rhythm, where daily rises occur due to either a decreased rate of glucose uptake or an increased rate of glucose output (Kalsbeek et al., 2014). Singh et al., (2020) noted in their circadian energy restriction investigation that eating in the evening makes an individual more susceptible to obesity, central adiposity, and increased levels of FPG, as well as an increased HbA1c recording. These before-mentioned indicators may lead to the development of metabolic syndrome. Moreover, Singh et al., (2020) detailed how early morning eating would decrease HbA1c as well as systolic blood pressure, which provides reasoning towards this earlier food consumption window resulting in a protective mechanism against the development of metabolic syndrome occurring.

Carlson et al., (2007) noted that TRE only improves metabolic health when the food consumption window is matched with earlier hours. Situating the eating window in the evening has been associated with a significant increase in body weight, fat mass, as well as glycaemic levels, resulting in a higher risk of developing obesity and IR.

TRE studies have been completed assessing both prediabetic and diabetic individuals, who are classed as having IGT. These individuals displayed lower insulin levels as well as improved insulin sensitivity after following a 8-hour TRE intervention (Sutton et al., 2018; Jamshed et al., 2019; Kesztyüs et al., 2019). Jamshed et al., (2019) also noted improvements in 24-hour glucose levels in their 14-week eTRE (7am-3pm) intervention cohort. When compared against an extended eating window (7am-9pm), Parr et al., (2020) reported that short-term 5-day TRE (10am-5pm) resulted in improved nocturnal glycaemic control in their cohort of overweight/ obese men. Additionally, Hutchison et al., (2019) noted an effect of treatment on mean fasting glucose measured by CGM, stating that eTRE significantly reduced mean fasting glucose when compared to baseline. This suggests that eTRE may exhibit improvements with regards to better glycaemic control (via reducing HbA1c, fasting blood glucose and post prandial glucose) and insulin sensitivity.

3.6.3. Critical evaluation of studies in a systematic way

It has been reported that undertaking a fasting period of >12 hours improves health outcomes due to a shift in metabolic behaviours occurring. Sutton et al., (2018) noted that extending the evening fasting period via consuming food earlier in the day increases insulin sensitivity as well as improving blood lipid profiles. Hutchison et al., (2019) reported that even when eating commenced at 8 am and 12 pm, both methods reduced postprandial plasma glucose as well as fasting triglycerides and both time conditions demonstrated no significant difference between one another.

In addition to this, these benefits were witnessed without altering energy intake or expenditure. Clayton et al., (2020) supported this notion by reporting that TRE patterns which extend overnight fasting improves glycaemic control. Delaying the consumption of breakfast via either consuming the meal later in the day or omitting it completely has been noted to unintentionally reduce energy intake (Min et al., 2011). In addition to this, Hutchison et al., (2019) reported that even when eating commenced at 8 am and 12 pm, both methods reduced postprandial plasma glucose as well as fasting triglycerides and both time conditions demonstrated no significant difference between one another. The current

methods implemented to study TRE utilise an overnight fast to study the effect of meal timings. However, the effects of evening and morning fasting towards energy balance is unclear (Clayton et al., 2020). Clayton et al., (2020) also noted that overnight fasting appears to be the most efficient fasting method to improve glycaemic control, however morning and evening fasting is underreported and their effects on energy balance remain unclear.

3.6.4. Review of TRE studies

Restricting mealtime consumption has emerged as a promising dietary technique which allows for management of metabolic disorders and obesity (Pellegrini et al., 2020). Metabolic homeostasis is regulated by circadian rhythm, which is the underlying basis of TRE (Moon et al., 2020). The initial concept of TRE arose form studies which examined possible effects of food consumption timings on the circadian rhythms on rodents (Kohsaka et al., 2007; Hatori et al., 2012). This progressed into clinical trials, where studies differ in terms of duration of the food consumption window, meal frequency, daily energy distribution, and meal regularity.

A three-week TRE trial conducted by Andriessen et al., (2022) utilised a randomised cross over design to assess fourteen adults with T2D via a 10-hour TRE protocol against a control. The food consumption window instructed for participants to follow involved the final meal being completed no later than 6pm. Significant improvements occurred within 24-hour glucose homeostasis alongside a significant decrease in fasting glucose. In this cohort, TRE exhibited positive effects on the cohort via significantly increasing the amount of time spend within a normoglycaemia range. Hepatic glycogen content was similar between both the TRE and control cohort, and insulin-induced non-oxidative glucose disposal was significant increased with TRE. An improved rate of insulin-induced non-oxidative glucose disposal is an effect exhibited when a T2D individual partakes in exercise (Yokoyama et al., 2008).

In this analysis, it was concluded that this 10-hour TRE protocol improved overall 24hour glucose homeostasis assessed via CGM in adults with T2D and did not affect insulin sensitivity and hepatic glycogen output. Limitations of this study include an unintentional

weight loss experienced during the TRE intervention, which could explain any alterations in glucose homeostasis experienced. In addition, a study conducted by Sutton et al., (2018) displayed an improved insulin sensitivity experienced within their TRE cohort which differed from the results obtained in this current study. Sutton et al., utilised an earlier consumption time of the final meal (3pm rather than 6pm) resulting in a longer fasting window, which may be the reason for an improvement in insulin sensitivity not occurring in Andriessen et al., (2022).

The cohort utilised within this study consisted of adults both dependent on glucose lowering medications and not dependent on such medication. Utilising a cohort of this nature may have produced less of an effect within the TRE intervention due to it displaying similar effects towards the same metabolic pathways. However, utilising a cohort not on this form of medication would not have been representative of the target T2D population. This study had a low sample size consisting of only fourteen adults which reduces the power of the study and increases the margin of error due to endpoints gained as a result likely being underpowered (Andriessen et al., 2022).

Lowe et al., (2020) assessed 116 men and women who were classed as either overweight or obesity with regards to weight loss as well as other metabolic parameters. An 8-hour TRE was utilised running from 12pm to 8pm for 12-weeks. Participants were randomised to either the TRE condition or the control where the control group was instructed to eat 3 structured meals per day where snacking between meals was permitted. Energy intake was obtained via self-reports of energy and macronutrients submitted by the participants who were advised to eat ad libitum. No significant within-group or between-group differences exhibited with regards to fasting glucose, fasting insulin, HOMA-IR, HbA1c, triglycerides, total cholesterol, low density lipoprotein (LDL) or HDL levels. A significant weight loss in the TRE intervention group, there was no difference between groups. Limitations include a lack of self-reported diet diaries displaying measures of energy intake/ macronutrients which prevents proper analysis of typical diet and analysis of a possible significant changes between typical diet and TRE intervention occurring. They concluded that there may have been possible alterations of protein intake due to a loss of appendicular lean mass within their TRE intervention group.

Lowe et al., (2020) concluded that further studies should be completed, specifically assessing any possible differences experienced utilising eTRE vs lTRE.

Xie et al., (2022) conducted a 5-week randomised-control trial assessing whether TRE effects on healthy volunteers without obesity. The primary outcome assessed was whether there were any changes in IR between groups and this was achieved via HOMA-IR. Participants were randomised into either eTRE (food consumption window 6am to 3pm), mid-day TRE (mTRE) (food consumption window 11am to 8pm), or the control group (eat ad libitum) and researchers assessing the various outcome measures did not know what group the participants were randomised to. It was determined that eTRE exhibited improvements with regards to insulin sensitivity but mTRE did not. In addition to this, eTRE reduced FPG alongside body mass and adiposity. Limitations associated with this study included it not utilising a cross over design, even though the study recruited 90 participants, only 30 participated in each condition (control/ eTRE/ mTRE).

Each group having a small sample size means that results may not be representative of the whole population alongside a possibility for reductions in the power of the study and increases in margin of error. Energy intake was estimated via pictures of the meals which participants consumed, which is not a very accurate method for measuring calories compared to food diaries. The trial utilised more women than men, with the cohort being 78% female, gender differences may have occurred, and the results may not be representative of the wider population. Participants were instructed to consume their meals within an 8-hour period to allow for mealtime consumption to be assessed, however specific timings and the duration of mealtimes varied between each group which may have affected the results. Prior to participating in the study, participants would have experienced different fasting periods before testing which may have influenced the results due to a major change being implemented.

Sutton et al., (2018) utilised a 6-hour TRE protocol spanning from 10am to 6pm to assess its effects on insulin sensitivity, blood pressure and oxidative stress of eight prediabetic men. Prediabetic status was proven utilising a HbA1c assessment. Energy intake was calculated to each participants unique energy requirements where each meal provided approximately 33%

of daily caloric needs. Participants were fed enough food throughout the study to maintain their weight via the use of an equation provided by Redman et al., (2009). Both feeding window were complied with for 5 weeks, where the TRE protocol required participants to consume their final meal before 3pm allowing for an 18-hour fast and the control group followed a 12-hour feeding period before both groups crossed over. A 3-hour OGTT was completed during the morning at baseline and post-intervention for both arms of the study. It was noted that eTRE did not significantly affect fasting glucose levels or any glucose levels obtained throughout the 3-hour OGTT and mean glucose levels did not change between the control and intervention group. However, TRE did demonstrate improvements of their participants insulin profile due to an improved level of β-cell responsiveness and a decreased IR measurement by 3-hour incremental area under the curve (AUC) ratio.

Limitations of this trial include it possessing a small, male only sample which may mean results are not representative of the wider population and results obtained as a result of the trial may be underpowered. Further work imposed by the research team included the study being repeated in a larger cohort which includes women also. The prolonged fast exhibited during the trial was not matched prior to testing beginning, this may have led to insulin sensitivity being underestimated and explain why the trial noted increased levels of both triglycerides and total cholesterol. This study protocol did not measure 24-hour glucose levels therefore it cannot be determined whether eTRE directly influenced alterations in glucose levels. In addition to this, not measuring blood pressure levels across a 24-hour period may lead to an overestimation of eTRE's effects on blood pressure as it was only measured in the morning. This trial concluded that further work is required with regards to optimal length and timings of food consumption windows being implemented.

Jamshed et al., (2022) examined the effectiveness of eTRE in obese adults for weight loss, fat loss, and cardiometabolic health over the course of 14-weeks. 90 people, 18 males and 72 females, were recruited and randomised to either an 8-hour eTRE (7am-3pm) or control group, where the eTRE condition adhered to the diet 6 days out of the week. Both intervention methods received weight-loss treatment via energy restriction where the eTRE energy restriction group was more effective method for losing weight, did not affect body fat, and improving diastolic blood pressure. The eTRE intervention utilised in this study did not

note any improvements in postprandial or 24-hour glucose, insulin sensitivity or fasting insulin levels, stating that eTRE was no more effective than the control. Limitations associated with this study includes timings of meal consumption not being the only independent variable being assessed, diet was also modified via the use of a weight-loss program via energy restriction being implemented. This program utilised both a hypocaloric diet (500kcal less than their resting energy expenditure) alongside 75-150 minutes per week. It was noted that this study did not measure glycaemic endpoints in the postprandial state which is a more responsive method to dietary intervention. There was also a larger variability in fasting insulin relative to the values obtained in their previous trial (Sutton et al., 2018).

Parr et al., (2020) assessed 11 sedentary males assigning them to either an 8-hour TRE protocol or an extended feeding intervention where both protocols lasted 5-days each. The TRE protocol ran from 10am to 5pm and the extended feeding protocol ran from 7am to 9pm, both diet protocols were isoenergetic. They suggested that delaying breakfast creates a longer fasting window which improves markers of glycaemic control in individuals. 24-hour and postprandial metabolism was assessed, where it was reported that total 24hour glucose AUC was lower within TRE when compared to extended feeding. This improved 24-hour glycaemic control was viewed when analysing nocturnal glucose as no difference was observed during waking hours. Reductions in nocturnal glucose has been linked with a 4-day eTRE (food consumption window between 8am-3pm) protocol conducted by Jamshed et al., (2019) where it was determined that 24-hour glucose AUC was lower when compared to a control. Similar to Sutton et al., (2018) this protocol utilised a small, male only cohort which may mean results are not representative of the wider population and results obtained following study completion may be underpowered. The diet utilised in this study had moderately high levels of fat, where participants consumed 50% fat, 30% CHO and 20% protein. There wasn't a pre-study investigation assessing levels of insulin sensitivity prior alongside a structured extended feeding group where no snacking or ad libitum intake was allowed, meaning the extended feeding group wasn't following their typical diet.

An eTRE protocol utilised in Jamshed et al., (2019) required participants to eat between the times of 8am to 2pm, compared against a control which participants consumed their food

between 8 am and 8 pm, assessed cardiometabolic risk factors, hormones, and gene expression of whole blood cells. Participants underwent both the TRE and control condition, with both intervention group lasting 4-days. When compared to the control group, eTRE intervention led to a significant decrease in mean 24-hour glucose and glycaemic excursions alongside alterations in cortisol and other circadian clock gene expression. Fasted blood samples obtained in the morning displayed increased levels of ketones and cholesterol whereas in the evening increased expression of mTOR and brain derived neurotropic factor occurred.

In summary, eTRE improved 24-hour glucose levels alongside alterations of lipid metabolism and expression of circadian clock genes. The sample size utilised in this protocol consisted of only eleven overweight individuals, where data received from this study may be underpowered due to the sample size being too small. Outcomes received from this study should be re-tested with a larger cohort. The TRE intervention utilised was relatively short-lived, only lasting 4-days. Because of this, metabolic adaptations may not have had sufficient time to occur. Single blood samples were obtained in a fasted state at 8pm on day 3 and 7:30am on day. Measurements taken at multiple timepoints across a 24hour period may provide a better display any possible changes in serum analytes and gene expression.

In Hutchison et al., (2019), fifteen men were fitted with a CGM during baseline and both intervention periods. The 9-hour TRE protocols utilised were eTRE, where participants were instructed to consume food between 8am and 5pm, as well as lTRE, where the food consumption windows were 12pm to 9pm. Participants consumed their habitual diet within the TRE interventions, habitual diet was assessed 1 week prior to intervention randomisation during free-living periods as well as during a week wash-out period before beginning the alternate TRE intervention. Intervention duration occurred for 7-days, where it was reported that no statistical difference in the mean 24-hour blood glucose, continuous overall net glycaemic action (CONGA), MAGE, or mean of daily differences between conditions were observed. Mean glucose levels 3-hours before the first meal was consumed was reduced in the eTRE and lTRE protocols when compared against their respective baselines. It was noted that there was a significant result obtained with regards to effect of treatment with regards to mean fasting glucose via CGM.

Limitations of this study include the small cohort utilised, as this may lead to underpowered endpoints occurring in secondary outcomes such as the glucose profiles analysed by CGM. In addition, the results cannot be applied towards women, those of a normal weight or towards individuals with established T2D due to these specific groups not being present in the cohort examined. Participants were told to consume their regular dietary habits across both conditions, meaning energy intake was not standardised which may have influenced any significant results experienced. To better assess TRE interventions, the study should be completed in a larger cohort and over a longer period.

Ramanathan et al., (2022) conducted a study on Nile grass rats and assessed the effects of various light cycles against a control for 6 weeks. 28 male and female Nile grass rats were randomised to either an ad libitum group (n=10), light cycle (8am–2pm) (n=8), or second half of the light cycle (2pm–8pm) (n=10). Energy intake consisted of a high-fat diet where 60% kcal fat (lard and soybean oil), 20% kcal protein, and 20% kcal CHO were consumed. Measurements of glycaemic control and glucose levels were obtained via a glucometer and insulin and triglyceride levels were obtained from harvested plasma. eTRE lead to a reduced rate in weight gain and improvement in metabolic function (glycaemic control and fasting insulin levels). Limitations included the fact that not all animals were fasted for the same time period at the time of sacrifice. In addition to this, the high-fat ad-libitum cohort undertook a 10-hour fast whereas the other TRE protocols used different fasting lengths depending on the protocol they were assigned to. The light cycle cohort had the longest fasting window prior to sacrifice (18 hours) whereas the cohort which followed the second half of the light cycle was sacrificed after only 12 hours of fasting. This means that food consumption windows were not the only change, the extended fasting window also altered.

Jones et al., (2020) conducted a 2-week trial utilising sixteen healthy males assessing both whole-body and skeletal muscle insulin as well as anabolic sensitivity. The cohort utilised was young and had a healthy BMI, the mean age was 23 years, and the average BMI was 24.0. A 1-week baseline assessment occurred before the 2-week intervention where participants were assigned to either an eTRE condition, who ate ad libitum, or a control group which was calorie restricted. In the eTRE condition, food consumption could only occur between the

window of 8am to 4pm (8-hour TRE protocol) and the energy restriction utilised in the control group possessed macronutrient composition of 45% CHO, 35% fat, and 20% protein.

Both the control and eTRE cohort witnessed an increase in circulating glucose and insulin concentrations in response to ingestion of the CHO and protein drink. A significant interaction effect was also recognised with regards to circulating glucose where values increasing post-control and decreasing post-eTRF compared with their baseline recordings. It was determined that improvements in whole-body insulin were observed and an increased rate of postprandial skeletal muscle nutrient uptake following a 2-week eTRE intervention. They concluded that eTRE could potentially be utilised as an alternative to calorie restrictions.

Limitations of this study include the utilisation of healthy men which means potential gender differences may have not been observed with regards to intervention and therefore the study cannot be applied to the wider population. The duration of the protocol was short-lived due to the intervention itself only occurring for 2-weeks, utilisation of a longer TRE intervention period would allow for any potential long-term metabolic changes to occur. The energy-matched diet utilised by the control group used a short term (1-week) self-reported energy intake data to prescribe the energy matched diet.

The vast majority of TRE studies possess limitations. First of which tends to be the small sample sizes utilised (n<30), which creates a high chance of bias via possible results gained being underpowered. The other present limitation is that most studies deploy a short intervention duration, ranging from 5-days (Parr et al., 2020) to 14-weeks (Jamshed et al., 2022) which means that analysis of any potential long-term benefits towards T2D evoked by TRE cannot be determined until a long-term study is completed.

3.7. Study aims and hypothesis.

3.7.1. Aim

The aim of this study is to investigate via the use of CGM the acute (3-day) effect of TRE in early (eTRE; 8 am-4pm) versus late (lTRE; 12pm-8pm) conditions on inter-day GV in adults at risk for T2D.
Secondary aim: Assessing whether TRE has any benefits towards glucose control in the absence of weight loss.

3.7.2. Hypothesis

eTRE would improve GV more than lTRE in accordance with circadian rhythm.

4. Methodology

4.1. Ethics and consent

This research conformed to the *Declaration of Helsinki* and was ethically approved via the Faculty of Science and Engineering, Manchester Metropolitan University board (EthOS reference 44884). Full ethical procedures (i.e., informed consent, right to withdraw etc.) were adhered to throughout the duration of this study. Participants were provided with approved written study information (i.e., participant information sheet; appendix [A]) and verbally briefed on what the study would entail before obtaining signed, written consent.

4.2. Inclusion and exclusion criteria

Participants were male or female, aged between 45-60 years and classed as clinically overweight (BMI above 25 kg/m^2) or clinically obese (BMI ≥30 kg/m^2) per NHS BMI calculations. Participants were required to have a typical eating pattern over the entire day (eating/drinking for greater than 12 hours per day). This factor was assessed during screening by asking participants to disclose their usual eating habits.

The exclusion criteria for this research included any of the following: individuals currently classed as type 1 or type 2 diabetic (HbA1c > 48 mmol/mol), individuals enrolled in another

lifestyle intervention or clinical research trial, have a history of substance of abuse, be pregnant or considering pregnancy, suffer from known cancer, history of myocardial infarction during the previous 6 months, have learning difficulties or have been diagnosed with an eating disorder.

4.3. Recruitment

An approved recruitment poster (appendix [B]) advertising the study was displayed in university buildings and other public places (notice boards in shops), shared on social media and also emailed to university staff. There was also an element of word-of-mouth from participants to potential participants. Interested individuals were sent the approved participant information sheet, once familiarised any queries were addressed before further discussions regarding eligibility and suitability. Participants must have been able to attend repeat visits. If suitable, an initial screening visit was arranged.

4.4. Participants

A total of nine participants gave informed consent to participate. Eight sedentary individuals (5F/3M; 51±6 years; BMI 28.0±2.9 m/kg^2; HbA1c 37.9±3.3 mmol/mol) with a habitual food intake distribution >14 hrs/day, participated in a randomised crossover study. Screening, recruitment, retention and completion study numbers and details are provided in figure 6. All glycaemic data is available for 8 individuals however, due to Covid-19 related issues, biochemistry data is missing for one timepoint of one participant, thus only baseline and eTRE data is presented for this individual.

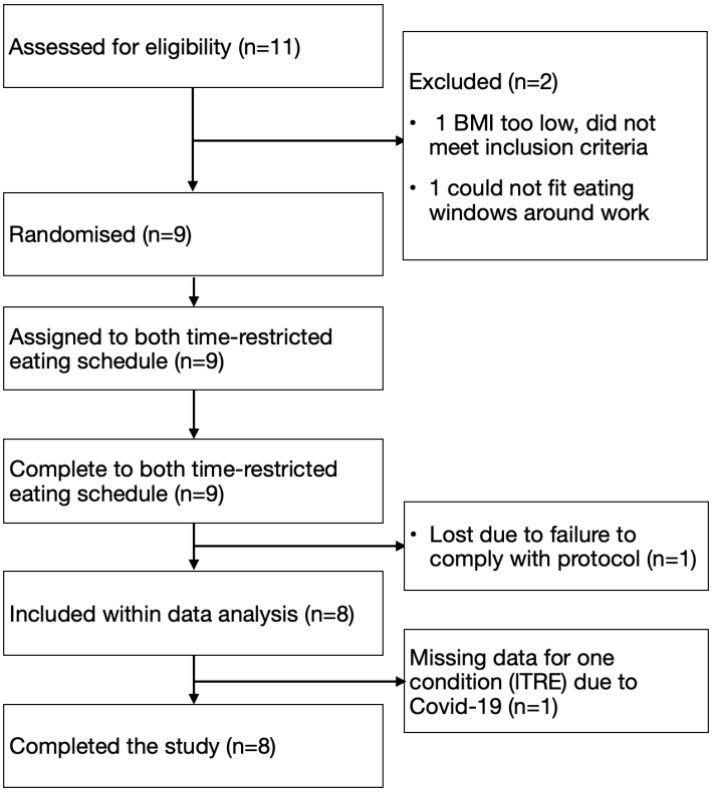

Figure 6 *Flowchart detailing participant screening, recruitment, retention, and completion. A total of 11 individuals registered their interest via email or word of mouth, however only nine participants met the inclusion criteria and could be included in the intervention. Eight individuals were included in data analysis as an individual was lost due to failure to comply with the protocol.*

4.5. Study design

A randomised cross-over design was employed to examine the effects of 3 days of eTRE (8 am to 4 pm) versus 3 days of lTRE (12 pm to 8 pm), demonstrated in figure 7(A). Specific meal timings for eTRE were breakfast 8am, snack 1 at 10am, lunch at 12 pm, snack 2 at 2 pm and dinner at 4 pm. The specific meal timings for the lTRE cohort were breakfast 12 pm, 'snack 1' at 1 pm, lunch at 4 pm, 'snack 2' at 6 pm and dinner at 8 pm Meal timings were selected to align with diurnal circadian rhythm patterns, assessing an 8-hour period at either end of the daytime cycle whilst also allowing for a prolonged period of fast.

Timings implemented during the eTRE condition prevented the consumption of an evening meal, whereas the lTRE condition prevented participants from consuming their first meal of the day until 12 pm (Heilbronn and Regmi, 2020).

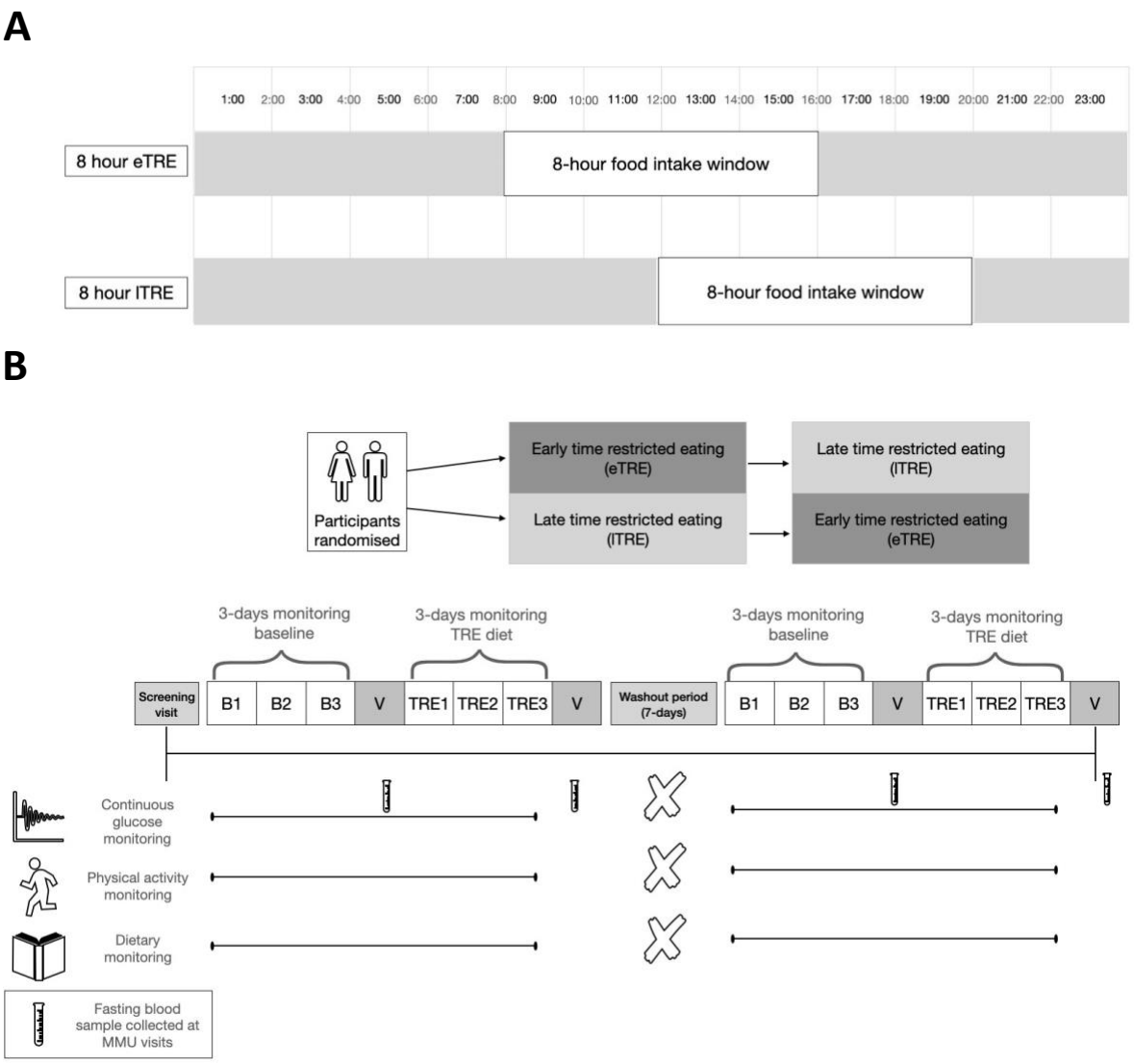

Figure 7 *Schematic (A) illustrating the timings of time restricted eating (TRE) windows, food consumption would either begin at 8am (eTRE) or 12pm (lTRE) and (B) overview of assessment measures utilised demonstrating a typical length of time a participant would partake in this study. Early time restricted eating (eTRE); late time restricted eating (lTRE); Baseline day 1/2/3 (B1/2/3); Time restricted eating day 1/2/3 (TRE1/2/3); laboratory visit day (V); Manchester Metropolitan University; (MMU).*

Randomisation to determine which dietary conditions (eTRE/lTRE) occurred first was generated before the commencement of the study via computer software (Randomiser.org).

In figure 7(B), what was expected of the participants is visually depicted. At the screening visit, once the inclusion criteria were met and consent was obtained, a CGM device was placed on the arm 24 to 36 hours before the start of the experimental protocol to allow for the device to stabilise before it records experimental data. The experimental protocol begins requesting participants to complete a 3-day habitual baseline diet which reflects typical eating habits to allow for a period of standardisation pre-intervention where data is recorded following this 3-day habitual diet baseline. Food consumed during the baseline diets was recorded via the completion of a food diary where the time of food ingestion, the food ingested, and the amount (grams/portion) was noted. Participants then attended a laboratory visit where fasted baseline biochemical recordings were obtained.

Following this visit, a 3-day TRE condition commenced where participants were instructed to consume pre-determined diets (displayed in table 4) at the timings required for the selected TRE intervention. Participants noted the exact time that they consumed the meals on a mealtime ingestion form provided, this allowed for easier detection of when the food was ingested once CGM raw data analysis begun. A 7-day washout period then commenced to remove any influence that the TRE condition may have exhibited on their physiological values. Participants were not monitored during this phase. Following this, the second baseline phase occurred where participants were instructed to consume the same food items consumed during their first baseline. The second 3-day TRE condition would then commence, with the alternate TRE condition occurring.

4.6. Dietary provision

Participants were provided with their food to consume during both 3-day TRE condition phases. Food which was consumed during the TRE portion of the trial was selected and ordered, where any missing items were purchased outside of university hours. A Mifflin St Jeor equation (Mifflin et al., 1990) was utilised which allowed for a caloric and macronutrients figure to be obtained based on the participants' age (years), height (cm),

weight (kg), and gender. Resulting in an approximate number of calories that the participants body would burn to maintain basic life-sustaining functions, otherwise known as basal metabolic rate, to be calculated (Frankenfield et al., 2005). A weighed food diary was implemented, providing participants with a personalised meal plan.

Resting energy expenditure (REE) (males) = 10 x weight (kg) + 6.25 x height (cm) - 5 x age (y) + 5

REE (females) = 10 x weight (kg) + 6.25 x height (cm) - 5 x age (y) - 161.

Total daily energy expenditure= (REE) x 1.4

Any allergies or food preferences were accommodated for via meal adjustments and alternative foods, which substituted to ensure that calory targets were met. These substitutions are depicted in brackets in table 4. Food alternatives were chosen based on them possessing similar macronutrients to the original item. Participants (n=3) consumed the same foods for each TRE condition.

Table 4 *Dietary intervention provided to participants during the TRE intervention. Substitutions (n=3) due to allergies, intolerances, or dislike are displayed in brackets.*

Day 1	Day 2	Day 3
Breakfast	**Breakfast**	**Breakfast**
Eggs Wholemeal bread Blueberries Semi-skimmed milk	Eggs Wholemeal bread Blueberries Semi-skimmed milk	Eggs Wholemeal bread Blueberries Semi-skimmed milk
Snack 1	**Snack 1**	**Snack 1**
Banana *(orange juice)* Raisin nut mix *(Graze BBQ crunch)*	Banana *(orange juice)* Raisin nut mix *(Graze BBQ crunch)*	Banana *(orange juice)* Raisin nut mix *(Graze BBQ crunch)*
Lunch	**Lunch**	**Lunch**
Apple *(nectarine)* Wholemeal bread Ham Mature cheddar cheese Creamy tomato soup	Apple *(nectarine)* Wholemeal bread Ham Mature cheddar cheese Creamy tomato soup	Apple *(nectarine)* Wholemeal bread Ham Mature cheddar cheese Creamy tomato soup
Snack 2	**Snack 2**	**Snack 2**
Greek yoghurt 0% fat Crunchy Muesli- Summer fruit	Greek yoghurt 0% fat Crunchy Muesli- Summer fruit	Greek yoghurt 0% fat Crunchy Muesli- Summer fruit
Dinner	**Dinner**	**Dinner**
White pasta Tuna chunks Tomato Sauce Mixed Salad Rice Custard *(Muller light strawberry yoghurt)*	White pasta Tuna chunks Tomato Sauce Mixed Salad Rice Custard *(Muller light strawberry yoghurt)*	White pasta Tuna chunks Tomato Sauce Mixed Salad Rice Custard *(Muller light strawberry yoghurt)*

4.7. Experimental measures

Participants visited the Institute of Sport at Manchester Metropolitan University to be assessed. All visits were standardised and took place in the morning to account for circadian variation. Participants visited the laboratory at approximately the same time of day for each

visit, where possible, and were instructed to not having consumed food for >8 hours prior to laboratory visits.

4.8. Assessment measures

4.8.1. Continuous Glucose Monitoring

Application of a Freestyle Libre 2 system CGM (Freestyle Libre 2, Abbott Laboratories Limited, Illinois, United States) occurred before the baseline recordings and during the dietary phases. The device was applied by a member of the research team and placed on the back of the upper arm, where the filament in the needle can access subcutaneous tissue. It was applied at the screening visit as well as another laboratory visit before the second baseline occurs. The sensor on the back of the arm has a lifespan of 14 days so it required changing before the second phase began.

This wireless device monitored fluctuations in plasma glucose levels in the interstitial fluid during 24-hour period. This differs from blood glucose monitoring via a finger prick technique as it does not require finger prick calibration (Jelinek et al., 2010). The device records data by communicating via Bluetooth from the individuals' phones to a data management system called Libreview, where the research team can view the reports obtained. Values obtained in the report displayed levels of plasma glucose at 15-minute increments throughout a 24-hour period to allow for a glucose profile to be determined. The values obtained from the CGM supplied values detailing time spent within the ideal glucose ranges (3.9 to 10 mmol/L), the number of nocturnal events with glucose levels <3.1 mmol/L in 7 hours, and time spent with glucose levels >13.0 mmol/L.

The CGM data obtained was then analysed against the following measurements: time spent within the normal glucose range (3.9-10 mmol/L), time spent above 7mmol/L (indicating hyperglycaemia), MAGE and time above (>10 mmol/L)/ below range (<3.9 mmol/L). MAGE indicates the degree to which plasma glucose exceeds the 24-hour mean calculated by one standard deviation, it indicates the index of GV (Akasaka et al., 2017). Various calculations

were completed to measure GV from CGM data using a simple interface known as EasyGV Version 9.0.R2. These calculations include M-value (Schlichtkrull et al., 1965), MAGE (Service et al., 1970a), high blood glycaemic index (HBGI)/ low blood glycaemic index (LBGI) (B. P. Kovatchev et al., 2003), CONGA (McDonnell et al., 2005) and MAG (Hermanides et al., 2010).

4.8.2. Dietary analysis

Following the completion of 3-day food diaries for both baselines and TRE conditions, analysis of usual habitual diet occurred utilising a software called Nutritics (Nutritics, 2022). Analysis of food diaries allowed for total energy intake and macronutrients to be determined. Macronutrient components obtained detail the number of CHO, proteins and fats consumed in grams from their 3-day baseline diets during typical eating. The diet used in each of the TRE conditions was isocaloric (having approximately the same calorific value each day) and eucaloric (the number of calories consumed via food is approximately the same as the number expended) (Saidi et al., 2021).

This study utilised individuals who currently weren't partaking in a dietary intervention or lifestyle study. The study calculated metabolic energy requirements via utilising the Mifflin St Jeor equation (Mifflin et al., 1990), meaning there was no calorie restriction and participants should maintain body weight. Participants self-verified that they weren't following a diet prior to starting this study allowing for meal frequency to be the only major change in diet during the study. The diet prescribed met energy requirements via utilising the Mifflin St Jeor equation, meaning there was no energy restriction. Participants ingested their daily caloric amounts by consuming 5 meals per day: breakfast (20%), lunch (30%), dinner (30%) and x2 snacks (10%). The distribution of relative energy intake was calculated and is in line with NHS recommendations. Habitual caloric dietary intake will be analysed to assess whether there was a statistical difference between the number of calories consumed during baseline and the value calculated to consume during either TRE phase.

4.8.3. Anthropometrics

Height was measured whilst participants were standing upright, with their back and head straight so that their Frankfurt plane was horizontal, to the nearest 0.5cm using a stadiometer (Holtain Harpenden Stadiometer, Holtain Limited, Wales). Weight was measured utilising digital scales (Seca 807 Digital personal scale with extra-flat dimensions, Seca GmbH & Co. KG, Germany); this provided a measurement of total body mass to allow for the individual's BMI to be calculated (mass (kg) / height (m^2)).

Waist circumference measurements at the umbilicus and hip circumference measurements at the greater trochanter were obtained provided three repeated measurements, waist to hip ratio (waist circumference (cm) / hip circumference (cm)) was calculated from this. After a period of 5 minutes rest, blood pressure (mmHg) and resting heart rate (BPM) were determined from an average of three repeated measures using an automated blood pressure monitor and an Intelli wrap cuff (Omron M6 comfort blood pressure monitor, Omron Corporation, Japan).

4.8.4. Biochemical measurements

Biological tests included a fasting glucose/ lactate test, and a lipid profile test were run. Lipid profile tests allow for 6 forms of blood lipid markers to be obtained (total cholesterol, triglycerides, HDL, LDL, Chol:HDL and Atherogenic lipoprotein particles (non-HDL))

A capillary blood sample was taken via a finger prick lancet (Accu-chek Safe-T-Pro Plus, Roche Diabetes Care Limited, Switzerland). Blood extracted from this sample was utilised in a fasting glucose and lactate analyser (Biosen C-Line clinic, EKF Diagnostics, Germany), and a lipid panel test (Cobas b 101, Lipid panel, Roche Diagnostics, Switzerland). Analysis was performed to determine whether there were any changes in fasting glucose, fasting lactate, triglycerides, HDL, LDL, total cholesterol, non-HDL and cholesterol ratio (Chol/HDL) as a result of altering mealtime ingestion.

4.8.5. PA monitoring

GeneActiv tri-axial PA monitors were worn on the wrist by participants for a minimum of seven consecutive days per condition in accordance with the assessment time points (baseline, first condition and then baseline and the second condition). The monitors allowed for an in-depth understanding of step count and PA levels. Monitors detect a change in velocity, meaning the directional speed of an individual. This was utilised to determine participants energy expenditure levels, sedentary behaviours different PA intensities (light, moderate and vigorous) as well as the amount of time for which they sleep. Participants are informed to keep the monitor on their chosen wrist throughout the seven days unless they're exposed to water for above 30 minutes (i.e. bathing). The device was set up with the ability to record data for a period of 12 days at 60Hz.

4.9. Statistical Analysis

Raw CGM and PA data received from participants was cleaned initially, which required screening the data manually and removing any days that the participant was not under assessment and identifying the timepoints of interest. With regards to CGM data, a compliance screening occurred where >70% (Battelino et al., 2019) of the 15-minute interval data was required, approximately 15.3% of the data per participant was imputed utilising computer software (EasyGV). PA data required exported raw data files to be converted into a useable data file which was inserted into a statistical computing program R Studio (RStudio, Boston MA, 2020). Validated cut points were used to identify PA behaviours (sedentary, light, and moderate-vigorous activity levels) (Esliger et al., 2011). PA monitor wear time was 97.9% during the baseline before eTRE (beTRE) (n=6), 99.5% in eTRE (n=6), 97.0% within baseline before lTRE (blTRE) (n=5) and 98.1% within lTRE (n=6). Dietary data received from participants was manually entered into a dietary analysis software Nutritics version 5.84 (Nutritics, Dublin, 2023), where no habitual dietary information was missing.

Statistical analysis was performed utilising SPSS version 27.0 (SPSS, Inc, Chicago IL, 2020) utilising Shapiro-Wilks test for normality, as well as assessing skewness and kurtosis. Repeated measures analysis of variance (ANOVA) follows, assessing the four conditions utilised within this protocol (eTRE versus lTRE against baseline and matched diet). Values obtained were then further assessed via post hoc tests with Bonferroni corrections to locate

the possibility of a statistical difference between condition and time. Data presented as mean ± SD or (95% CI). P<0.05 is deemed the statistical significance level.

5. Results

5.1. Patient characteristics

Grouped participant characteristics (n=8) obtained from the first chronological laboratory visit following a 3-day habitual dietary assessment are tabulated (table 5). Overall, participants had higher than average values for total cholesterol (mmol/L), LDL cholesterol (mmol/L), and triglycerides (mmol/L).

Table 5 *Grouped patient demographic table at initial baseline (n=8). Clinical, biochemical, and PA characteristics of study are detailed below. Participants were categorised as overweight or obese meaning subsequently they are at risk of developing prediabetes. Mean, ranges and standard deviation are displayed within the table.*

	Participants	***Ranges***	***Standard deviation***
N	8		-
	Anthropometrics		
Age (average years)	51	(45-60)	0.08
Biological sex	3 M 5 F		-
Overweight BMI (n=5) (kg/m^2)	26.9	(25.1-29.5)	-
Obese BMI (n=3) (kg/m^2)	31.4	(30.8-32.1)	-
Systolic blood pressure (mmHg)	118	(103-132)	10.29
Diastolic blood pressure (mmHg)	81	(74-95)	8.80
Heart rate (BPM)	69	(61-77)	6.16

	Fasting blood biochemistry		
FPG (mmol/mol)	4.87	(4.21-6.04)	0.49
Fasting plasma lactate (mmol/mol)	1.72	(0.7-5.93)	1.63
HbA1c, (mmol/mol)	36.3	(30.8-39.0)	2.71
Total cholesterol (mmol/L)	5.25	(4.08-5.88)	0.71
LDL Cholesterol (mmol/L)	3.05	(1.72-3.89)	0.72
HDL Cholesterol (mmol/L)	1.39	(0.93-1.79)	0.25
Triglycerides (mmol/L)	1.78	(1.26-2.88)	0.53
Chol/HDL	3.99	(3.2-6.5)	1.02
Non-HDL (mmol/L)	3.86	(2.82-5.08)	0.74
	Physical Activity		
Steps (day)	11117	(2677-16642)	4588
Sedentary (mins/day)	601	(514-732)	87
Light (mins/day)	197	(34-417)	177
Moderate-vigorous (mins/day)	372	(31-785)	302

Fasting plasma glucose (FPG); Glycated haemoglobin (HbA1c); low density lipoprotein (LDL); high density lipoprotein (HDL); cholesterol ratio (Chol/HDL); Atherogenic lipoprotein particles (non-HDL).

Grouped participant characteristics visible in table 5 displays anthropometric and fasting blood biochemical values, and PA data obtained from the first laboratory visit, after completion of the first baseline condition.

5.2. Dietary analysis

Macronutrient data was obtained from the participants initial habitual intake which occurred pre-TRE intervention. Mean and ±SD of macronutrient energy values acquired were 205±59g CHO, 81±32g protein, and 75±35g fat.

Total energy consumption (*P*=0.517), CHO (*P*=0.129), and fat (*P*=0.432) were not significantly different between either baseline period or TRE condition. A significant main effect for protein approached significance (*P*=0.055); the pairwise comparison shown a higher amount of protein consumption (Figure 8, panel C) during TRE when compared to baseline, however this difference was not significant (*P*=0.082).

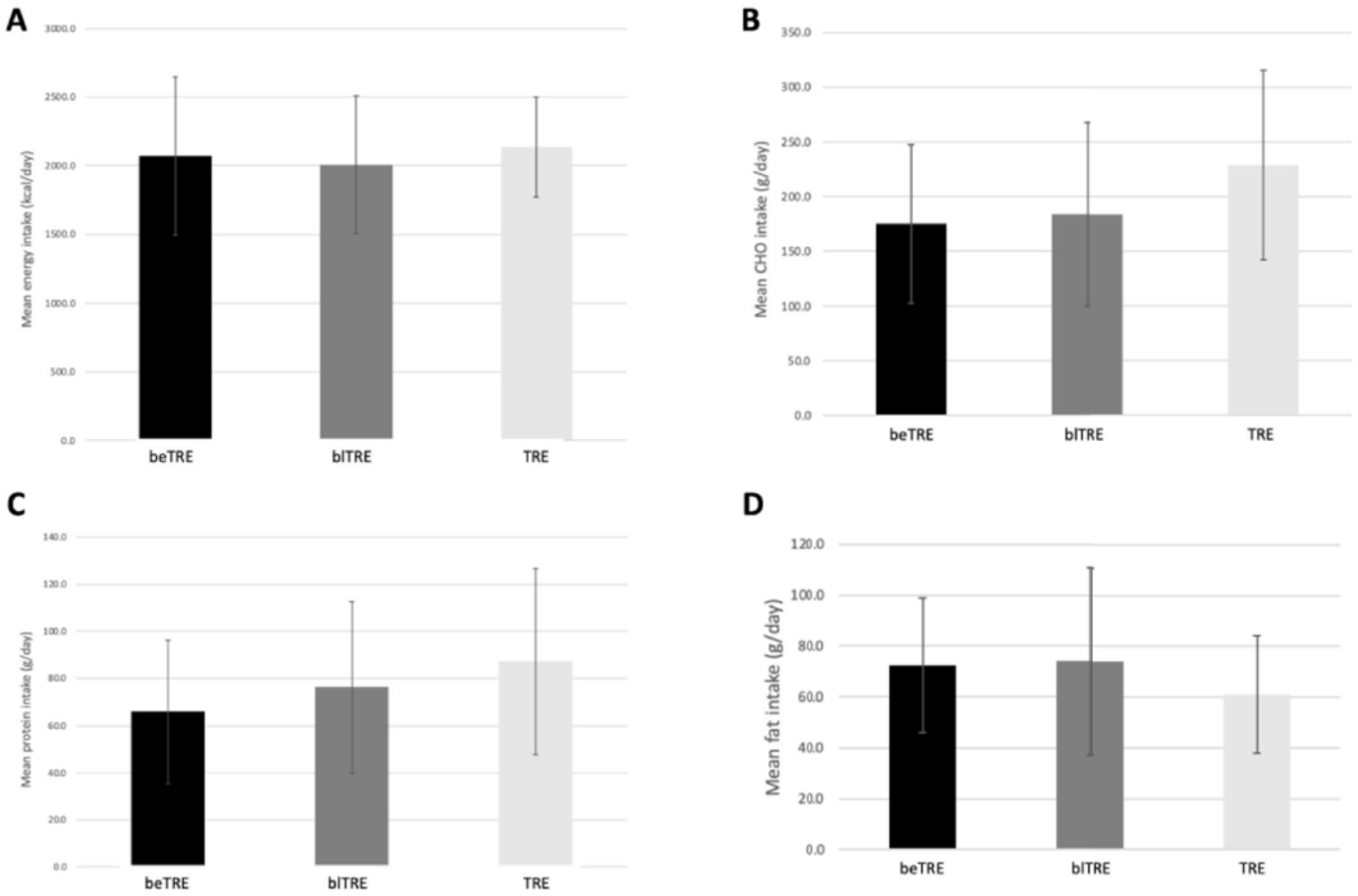

Figure 8 Mean daily energy intake (A), carbohydrate [CHO] (B), protein (C) and fat (D) as measured by dietary report during baseline early (beTRE; indicated in black), baseline late (blTRE; indicated in dark grey) and time-restricted eating (TRE; indicated in light grey). Values are displayed as means with standard deviations.

5.3. PA monitor analysis

As shown in Table 6, average daily steps, time spent completing sedentary, light, moderate and vigorous activity was not significantly different between baseline and TRE intervention (time) and there was no observed effect of eTRE versus lTRE (condition).

Table 6 Average physical activity levels obtained from baseline (beTRE and blTRE) and TRE condition (eTRE and lTRE) measured by accelerometer (GeneActiv) to report average daily steps, alongside the duration of activity in intensities in sedentary, light, moderate and vigorous for each 3-day period. Mean value, 95% confidence intervals (lower bound, upper bound) and *P*-values obtained are listed below.

	Early		***Late***		***ANOVA (P value)***		
	Baseline	**TRE**	**Baseline**	**TRE**	**Condition**	**Time**	**Interaction**
Step count (steps/day)	10873 (9392, 12353)	9423 (5719, 13127)	10784 (3735, 17833)	8302 (4373, 12231)	0.459	0.361	0.766
Sedentary (mins/day)	571 (442, 699)	573 (383, 763)	561 (418, 705)	580 (519, 642)	0.988	0.812	0.637
Light (mins/day)	305 (214, 396)	292 (132, 452)	324 (76, 572)	353 (106, 600)	0.413	0.783	0.369
Moderate (mins/day)	141 (111, 171)	150 (52, 247)	113 (-16, 242)	105 (47, 163)	0.190	0.987	0.738
Vigorous (mins/day)	5 (-7, 16)	1 (-1, 3)	5 (-3, 13)	2 (-2, 6)	0.661	0.217	0.948

5.4. Blood biochemistry analysis

Blood biochemical markers (displayed within Table 7) did not significantly change from pre- to post-TRE (time) and there was no observed effect of early versus late TRE (condition).

Table 7 *Mean biochemical blood values obtained for each condition examined. Mean value, 95% confidence intervals (lower bound, upper bound) and p-values obtained are listed below.*

	Early		***Late***		***ANOVA***		
	Baseline	**TRE**	**Baseline**	**TRE**	**Condition**	**Time**	**Interaction**
Plasma TG (mmol/L)	1.4 (1.3, 1.6)	1.6 (1.3, 1.6)	1.8 (1.0, 2.6)	1.6 (1.1, 2.1)	0.265	0.896	0.232
Cholesterol (mmol/L)	5.3 (4.6, 6.1)	5.4 (4.7, 6.0)	5.5 (5.2, 5.9)	5.6 (5.2, 6.1)	0.315	0.754	0.849
HDL (mmol/L)	1.4 (1.1, 1.7)	1.5 (1.2, 1.7)	1.5 (1.2, 1.7)	1.5 (1.2, 1.8)	0.849	0.756	0.139
LDL (mmol/L)	3.2 (2.5, 3.9)	3.3 (2.6, 3.9)	3.2 (2.8, 3.7)	3.5 (2.9, 4.0)	0.509	0.214	0.408
Chol/HDL	4.0 (3.1, 4.7)	3.9 (2.7, 5.1)	4.0 (2.9, 5.1)	3.9 (3.1, 4.7)	0.973	0.224	0.736
Non-HDL (mmol/L)	3.9 (3.2, 4.7)	4.0 (3.2, 4.7)	4.1 (3.6, 4.6)	4.2 (3.5, 4.8)	0.380	0.672	0.683
Glucose (mmol/L)	4.6 (4.3, 4.9)	4.5 (4.2, 4.7)	4.7 (4.1, 5.4)	4.8 (4.5, 5.0)	0.210	0.775	0.694

**p<0.05; triglycerides (TG): high density lipoprotein (HDL); low density lipoprotein (LDL); cholesterol ratio (Chol/HDL); Atherogenic lipoprotein particles (non-HDL).*

5.5. Continuous glucose monitors

A significant reduction, depicted within table 8, was produced within MAG by 0.4 mmol/L

(95% CI 0.1 to 0.8; 0.041), MAGE by 0.4 mmol/L (95% CI 0.1 to 0.8; *P*= 0.024) and CV by 2.5 % (0.6, 4.4) experienced between baseline and TRE intervention (time). No significant effect of condition (eTRE vs lTRE) was observed (*P*>0.05). A graphical display of the changes exhibited by MAGE and MAG from both the baseline and TRE intervention has been depicted within Figure 9.

Table 8 *Table depicting each output received from CGM software assessed via a mixed-measures ANOVA in regard to the effect of condition (eTRE vs lTRE) and time (baseline vs TRE). Mean values, 95% confidence intervals (lower bound, upper bound) and p-values obtained are listed below.*

	EARLY		LATE		ANOVA		
	Baseline	**TRE**	**Baseline**	**TRE**	**Condition**	**Time**	**Interaction**
MEAN GLUCOSE (MMOL/L)	5.8 (5.2, 6.3)	5.6 (5.1, 6.1)	5.7 (5.4, 6.1)	5.5 (5.2, 5.8)	0.684	0.086	0.579
MAGE (MMOL/L)	2.4 (2.0, 2.7)	1.9 (1.5, 2.2)	2.3 (1.9, 2.7)	1.9 (1.5, 2.2)	0.744	0.024*	0.732
MAG (MMOL/L)	3.6 (2.8, 4.3)	2.9 (2.5, 4.3)	3.5 (2.9, 4.2)	2.8 (2.3, 3.3)	0.713	0.041*	0.809
CONGA (MMOL/L)	5.0 (4.5, 5.6)	5.0 (4.5, 5.4)	5.0 (4.6, 5.4)	4.9 (4.6, 5.2)	0.723	0.463	0.761
LBGI	1.5 (0.5, 2.5)	1.4 (0.5, 2.3)	1.4 (0.7, 2.2)	1.4 (0.6, 2.1)	0.923	0.847	0.945
HBGI	0.6 (0.4, 0.9)	0.4 (0.2, 0.6)	0.5 (0.3, 0.7)	0.4 (0.2, 0.7)	0.780	0.125	0.125
MVALUE IGV	1.7 (0.2,3.3)	1.6 (0.3, 3.0)	1.6 (0.4, 2.9)	1.7 (0.5, 2.9)	0.953	0.950	0.857
CV (%)	14.4 (12.0, 16.8)	12.0 (10.2, 13.4)	14.3 (12.0, 16.7)	12.0 (10.1, 13.9)	0.947	0.016*	0.845

**p<0.05; Mean amplitude of glycaemic excursions (MAGE); mean absolute glucose (MAG); Continuous overall net glycaemic action (CONGA); Low blood glycaemic index (LBGI); High blood glycaemic index (HBGI); mean value index of glycaemic variability (MVALUE IGV); coefficient of variation (CV)*

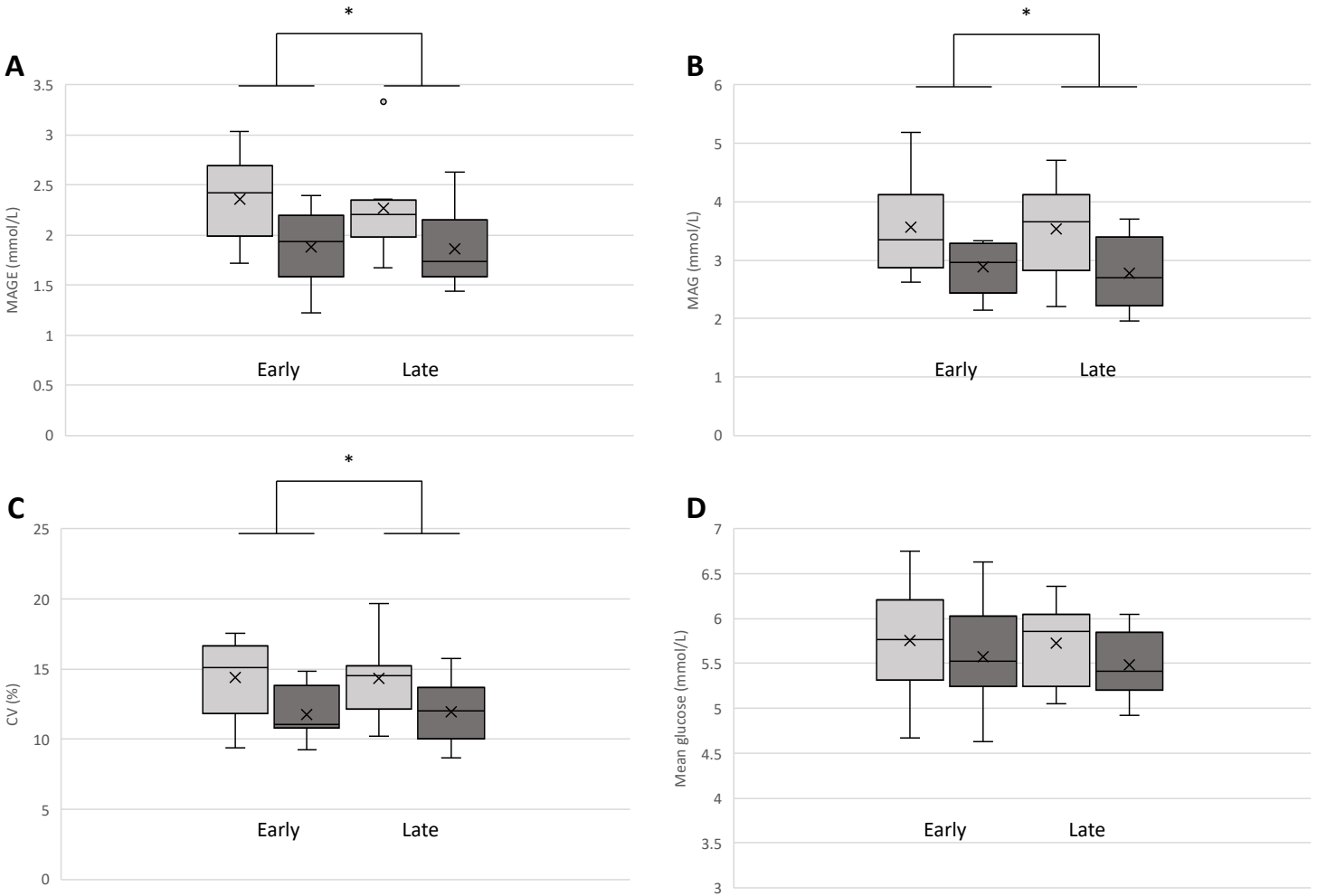

Figure 9 *Changes within MAGE (A), MAG (B), CV (C) and mean glucose (D) from baseline condition (light grey) compared to their corresponding early or late 8-hour TRE intervention (dark grey). *P<0.05 main effect of time (baseline vs TRE).*

6. Discussion

The results of this study show that TRE improved inter-day GV, in the absence of calorie restriction. This study showed a possible therapeutic role of TRE in adults who are at risk for T2D. Assessment of GV and any potential differences exhibited by eTRE or lTRE was the aim of this research. The hypothesis proposed within this research study stated that the eTRE intervention would be the more beneficial intervention towards GV due to published theories detailing the effects of circadian rhythm, however the evidence gathered from this study did not support this.

6.1. CGM Data

Ingested calories are proposed to be more efficiently used during the morning than later in the day (Ruddick-Collins et al., 2018). This data shows no benefit to consumption earlier in the day (8:00-16:00) but reducing food intake to an 8-hour window hat altering the time of 8-hour window improved overall glycaemic excursions throughout the day. It has been reported that a later eating schedule is associated with worsened glycaemic control (St-Onge et al., 2017), greater 24-hour glucose (Hatamoto et al., 2023), IGT (Bandín et al., 2015), and postprandial hyperglycaemia (Papakonstantinou et al., 2022) all of which are factors that contribute towards the development of T2D. This study found no significant difference of GV when comparing eTRE and lTRE; however, it was not significantly powered.

Sutton et al., (2018) employed a 5-week randomised-control cross over design. It was determined that eTRE (6-hour feeding window, where dinner must be consumed before 15:00) did not affect mean glucose during 3-hour OGTT. However, eTRE did significantly lower mean insulin levels in comparison to control schedule (12-hour feeding window) and insulin resistance between both conditions. Participants self-selected a time to start eating breakfast, this time was between 6:30am and 8:30am where lunch and dinner were timed accordingly. Participants were provided with their meals by study staff and were fed

enough to maintain their weight. Food intake occurred on a meal-by-meal basis, meaning that across the two arms any potential errors due to confounding effects from food intake or meal frequency were avoided; with the only difference between the two cohorts being the timing of meal consumption.

Participants maintained a consistent PA and sleeping pattern throughout the study. The results of this well controlled study show that eTRE did not affect postprandial glucose levels, but it did reduce postprandial insulin levels suggesting increased insulin sensitivity. Indeed, the methodology employed by Sutton et al., (2018) was longer in duration to that of the present research and perhaps the benefits of TRE were cumulative over the weeks but our observed short-term improvements in GV reported are indicative of improved insulin sensitivity. During prediabetes, hepatic and respiring cells have a reduced rate of response to insulin, which leads to a requirement for the pancreas to release more insulin in attempt to respond to the glucose levels in the blood (Cerf et al., 2013; Petersen and Shulman., 2018).

In agreement with Sutton et al. (2028) the data obtained from the 3-day TRE intervention employed within this study also shown no significant difference for mean blood glucose between both the baseline and TRE conditions. Additionally, both studies did not match the fasting duration windows prior to conducting the conditions, as participants who were currently conducting the eTRE protocol possessed a much longer window of overnight fast prior to laboratory assessments. This is an issue due to acute fasting triggering insulin resistance and generally worsening overall β cell responsiveness, even after only a short window of around 24-hours. This is intensified partially via elevation of triglycerides and/or free fatty acids from lipolysis (Antoni et al., 2016).

During the lTRE intervention, evening meals were consumed at a late time (8pm) where a CHO-rich meal (pasta) would be consumed. Consuming meals in the evening (10:30pm) has been reported to produce a greater increase in post prandial glucose and elevate HbA1c levels when compared to a 7pm meal consumed with the control

group (Sato et al., 2011). Late meal consumption has also been linked with IGT due to a disruption towards circadian rhythm which may result in metabolic consequences (Lopez-Minguez et al., 2019). Additionally, late mealtime consumption increases risk of obesity, T2D and metabolic syndrome more than if the same meals were consumed earlier in the day (Haldar et al., 2020; Arora and Taheri, 2015; Yu et al., 2015; Reutrakul et al., 2013). Additionally, poorer glucose tolerance has been associated with late night meal consumption timings, even when identical meals were consumed alongside equally implemented fasting windows (Morgan et al., 1999; Gupta et al., 2017).

Hutchison et al., (2019) conducted a 7-day randomized crossover trial which detailed how their lTRE intervention (12 pm to 9 pm), where the first meal of the day was consumed at 12 pm, produced on average a ~21% higher glucose incremental AUC when compared to eTRE condition (8 am to 5 pm).

Like the study conducted within this piece of research, Hutchinson et al., (2019) instructed participants to maintain their usual lifestyles, including sleep patterns. However, where the two studies differed was their approach to meal consumption. Hutchinson et al., (2019) consume their habitual diet within the specified TRE slots during each condition, whereas the study we controlled diet allowing for the number of calories consumed via food is approximately the same as the number expended.

Hutchinson et al., (2019) also conducted a rather short-term, highly controlled intervention, which may not be translatable long-term (beyond 7-days) or in a free-living environment. The study conducted within this research project employed an even shorter intervention window of 3-days, however it did assess the cohort within a free-living environment via the use of a CGM to obtain the primary outcome.

These findings were obtained from a male-only cohort, meaning that their results cannot be directly extended to women. Moreover, their cohort consisted of overweight individuals who had no prior diagnosis of T2D meaning that their findings may not be applicable to those with a healthy BMI, or those with already established metabolic

disturbances, such as T2D. The cohort observed within this research allowed for both males and females to be assessed, meaning any potential gender differences to be observed.

Another limitation of Hutchinson et al., (2019) which was addressed within this piece of research was the lack of standardisation in regard to the food intake plan during the TRE conditions. Participants were instructed to consume their habitual diet within the specified TRE condition which may result in meaning their results may lack accuracy as they were obtained in a free-living cohort where participants provided a brief description of the food which was consumed. Within this research project, food ingested during the TRE intervention window was predetermined, allowing for accurate macronutrient values to be obtained alongside standardisation across both TRE conditions. This was achieved by the exact same food product being purchased for both TRE intervention windows.

The eligibility criteria which were put in place between both studies excluded those who perform shift work alongside including those who habitually consume meals across the entire day. These categories of individuals are the cohort who would be affected by a disrupted diurnal variation who would therefore most at risk for complications associated e.g., T2D, where neither of the studies could apply their results to this specific cohort.

Additionally, Hutchinson et al., (2019) study design only implemented a singular baseline observation window, meaning that the participants were not permitted to wear a CGM in the week leading up to the second TRE condition. Furthermore, any potential differences experienced before the second TRE condition were not analysed, meaning that any potential differences experienced before this condition begun were not reported on and therefore could not be analysed. The study completed during this research assessed two baseline conditions against their respective TRE conditions, and no significant differences occurred between both baselines.

Within Hutchinson et al., (2019) there was no statistically significant effect produced by either mealtime or TRE towards fasting glucose or fasting insulin measured by incremental AUC. Due to lTRE producing higher incremental AUC values which reflects entire glucose excursion (Sakaguchi et al., 2016) following a short-term 7-day TRE intervention, the hypothesis that lTRE would evoke worse GV when compared to eTRE was proposed, reflecting diurnal variations in circadian rhythm (Carroll and Nestel, 1973; Zimmet et al., 1974).

Sakaguchi et al., (2016) utilised a 75g OGTT to obtain their index of glucose intolerance in preference to the HbA1c testing and fasting plasma glucose test used within this studies design. OGTT is a more accurate method utilised to observe the effects of glucose. This method is currently the gold standard for the diagnosis of diabetes, which our protocol did not utilise.

The cohort utilised within Sakaguchi et al., (2016) was large, consisting of 520 individuals, which differs to a limitation of our study which possessed a small cohort of 8 individuals. This may lead to in the results obtained in Sakaguchi et al., (2016) being more accurate and applicable to the general population than our research. However, irrespective of the large cohort obtained, a single group (fasting glucose) was a lot smaller, with it having only 8 individuals within it which may have led to potential inaccuracies within this cohort.

Sakaguchi et al., (2016) received their data from various health associations, therefore the environment in which their data was received from could not have been controlled within their cohort. Within this research study a more controlled environment was utilised, participants were fed enough food to maintain their weight, participants were instructed when they could consume their meals during the TRE conditions, meaning that across any potential errors due to confounding effects of meal frequency were minimised, and participants were instructed to maintain a consistent PA and sleeping pattern throughout the study (measured by PA monitor throughout the study).

Thus, the study expected eTRE to produce greater improvements towards GV, and that lTRE would not evoke these improvements. However, no statistical significance was observed as a result of condition (eTRE vs lTRE), conversely values produced significant changes in response to time (baseline vs TRE intervention) which are discussed below.

6.1.1. MAGE

This study showed a significant decrease in MAGE in response to 3-day TRE intervention obtained via CGM. MAGE displayed a mean decrease of 0.48 mmol/L between eTRE baseline and eTRE intervention, and 0.40 mmol/L between lTRE baseline and lTRE intervention.

MAGE is an important measure of metabolic control alongside increased risk for the development of diabetes complications (Rizzo et al., 2010), where higher MAGE values being a characteristic of glucose instability (Service et al., 1987; Service et al., 1970b) as well as being present within obese individuals (Cooper et al., 2022). MAGE is regarded as the gold standard for assessing short-term within-day GV, which is an important component when analysing overall glycaemic control (Vergès et al., 2022). MAGE is highly correlated with indicators of oxidative stress such as 24-h urinary 8isoprostane F2α, where increased oxidative stress is also associated with increased HbA1C within T2D (Liu et al., 2022). High MAGE has also been reported by Akasaka et al., (2017) to have higher incidence rate of secondary cardiac events, higher recordings is present within individuals who have recently had a myocardial infarction as well as being associated with coronary stenosis.

The decrease in MAGE obtained from this 3-day TRE intervention as a result of time (baseline vs TRE condition) at present has only been discussed within one other study. Jamshed et al., (2019) reported on the possible link between eTRE evoking a statistically significant decrease on 24-hour mean glucose by 0.2 mmol/L and MAGE by

0.67 mmol/L within their 4-day TRE intervention group when compared to the control. Within Jamshed et al., (2019) only eTRE was assessed, therefore further investigation towards the possible effects of MAGE in response to lTRE intervention was required.

However, the notion that MAGE is reduced as a result of TRE was not supported by Hutchison et al., (2019) who noted no statistical difference within mean 24-hour blood glucose concentration and MAGE within both eTRE and lTRE treatment groups. Additionally, a 3-day randomised cross over trial conducted by Nakamura et al., (2021) analysed two different evening mealtime consumption windows, early at 6pm and a late at 9pm. As a result of this, it was noted that the early dinner cohort displayed a more significant decrease of MAGE than what was experienced within the later evening meal group. Furthermore, in a TRE protocol conducted by Naguib et al., (2022) they reported on there being no significant change in MAGE over the study period between the 8-hour TRE condition and the 12-hour control.

Naguib et al., (2022) had a cohort of 8 individuals who were at risk of developing prediabetes due to advanced age (45-60 years, average 51 years) and participants being classed as either overweight or obese. Naguib et al., (2022) utilised a 12-week protocol to assess a much younger cohort (14-18 years, average 16 years) who were obese.

Observing a younger cohort in relation to T2D may serve to provide useful analyses due to it possessing the potential for the effects of longer diabetes duration to be examined (Nanayakkara et al., 2020). However, similar to the study conducted during this research project, the study timeframe utilised within Naguib et al., (2022) wasn't longitudinal and therefore the potential benefits of long-term analysis of this cohort would not be observed. Additionally, this cohort would not be representative of the general population due to a narrow age range being utilised, a limitation which this study experienced to a different degree. The age range included within this research project was 45-60 years, as being ≥45 years old is a risk factor for developing prediabetes.

Participants within Naguib et al., (2022) self-selected their 8-hour TRE protocol, where all participants selected an afternoon/evening eating window during the TRE intervention phase. This factor may have contributed to the nonsignificant findings regarding MAGE between the 8-hour TRE condition compared to the 12-hour control group. Additionally, the protocol was only conducted for 5-days per week, over the course of the 12-week protocol and participants self-administered the CGM device where some participants experienced very low glucose levels due to the device being administered incorrectly, via too much pressure.

Naguib et al., (2022) allowed the cohort who took part in their research to self-selected their eating window resulting in their findings being not as standardised as those implemented within this study, as we instructed people to eat at a set-time each day. Having participants consume food at the same time each day in each condition employs a more standardised process, where more accurate comparisons can be constructed from the date points collected due to meal-time ingestion being a fixed variable for each participant.

Kajiyama et al., (2018) conducted a mealtime investigation on young healthy women consumed an evening meal at 6pm, 9pm, or consumed a divided evening meal (where half of the meal was consumed at 6pm and the other half was consumed later that day at 9pm). Kajiyama et al., (2018) observed a significant reduction of MAGE by 0.57 mmol/L within the divided evening meal group when compared to the 9pm evening meal condition, which was reflected by an increased incremental glucose peak and postprandial hyperglycaemia experienced within the 9pm evening meal group of their cohort. Additionally, MAGE was reportedly higher within the 9pm evening meal cohort than that of the 6pm evening meal cohort after such a short intervention (5-days), however no statistical difference was observed.

The cohort was comprised of young, healthy women who did not currently possess a level of IGT. Therefore, this data could not be applied to individuals with metabolic

disorders, such as T2D, and towards males which is not representative of the general population. The cohort utilised within this research possessed an increased BMI, allowing for them to be classified as overweight or obese, alongside their age being between 45-60 years and them being physically inactive. These are all risk factors for developing prediabetes, which is caused by blood glucose levels being higher than desired.

Imai et al., (2017) conducted a mealtime investigation on type 2 diabetic patients where participants also consumed an evening meal at 6pm, 9pm, or consumed a divided evening meal (where half of the meal was consumed at 6pm and the other half was consumed later on that day at 9pm). Imai et al., (2017) observed a significant reduction of MAGE by 1.66 mmol/L within the divided evening meal group when compared to the 9pm evening meal condition over the course of the 5-day intervention.

No significant difference in MAGE was expressed between eating an earlier evening meal at 6pm when compared to a later evening meal consumed at 9pm (Imai et al., 2017; Kajiyama et al., 2018). This was also demonstrated within this study where no significant difference regarding MAGE was experienced as a result of eTRE vs lTRE.

Imai et al., (2018) examined the effects of a different snack window (consumed at 12:30pm or 3:30pm) within a day where breakfast was eaten at 8am, lunch was eaten at 12pm and the evening meal was consumed at 7pm. Within their type 2 diabetic cohort, consuming the 12:30pm snack soon after lunch resulted in a 95.9 g intake of CHO within that hour, whereas consumption of biscuits alone at 3:30pm amounted to the consumption of only 13.5 g of CHO within that hour. MAGE was reported to be 1.71 mmol/L lower within the 3:30pm snack window, as CHO was consumed gradually over the course of the day. Therefore, even though a larger volume of CHO was consumed at a later time, this did not negatively affect MAGE, it led to significant improvements due to mealtime consumptions being spread out. This finding supported the result achieved within this study, which was that eating later in the day (lTRE) was not as

detrimental towards GV, specifically in this instance MAGE, as what was proposed by the hypothesis.

Hatamoto et al., (2023) assessed 8 young males (20.9±0.8 years) in two conditions for 9-days within an early and late TRE eating schedule. Participants completed a 7-day free living TRE intervention then on day 8 to 9 participated within a 24-hour experiment window. During the eTRE, participants were instructed to consume 3 meals; breakfast between 7:30am–08:30am; lunch between 12:00–1:00pm; and dinner between 7pm–8pm whereas during the lTRE protocol participants consumed their 3 meals of breakfast between 12pm–1pm; lunch, 4:30pm–5:30pm; and dinner, 11pm–12am. Following the 7-day TRE intervention, participants underwent a 3-day experiment within an environmental chamber for each TRE intervention. A CGM device was placed on each participant on the morning of day 6 or 7, ready for analysis within the 24-hour experiment window. GV measurements obtained included SD and MAGE as well as AUC for post prandial glucose levels, where none of which differed significantly between the two conditions.

Even though Hatamoto et al., (2023) utilised a 7-day TRE intervention, which was longer than the intervention implemented within this study, the CGM device only recorded for a 24-hour period from 8 am on day 8 until 8 am on day 9 following completion of both the eTRE and lTRE condition. Only recording data for a singular day which may not reflect any possible abnormalities experienced the 7-day TRE protocol and thus may not provide an accurate glucose profile. Additionally, glucose data was not obtained within a free-living condition like the protocol utilised within the work conducted for this study, therefore it might not reflect what is experienced in an everyday environment. This TRE intervention showed a decrease in MAGE because of condition (baseline vs TRE) after only 3-days of TRE, where participants wore their monitor throughout the duration of the study in a free-living environment.

Within this study, no significant effect of condition (eTRE vs lTRE) was exhibited, which also occurred within Hatamoto et al., (2023). Due to there being no CGM data available

within Hatamoto et al., (2023) prior to the 7-day TRE, no comparison between TRE to a baseline condition could be constructed. Therefore, the true effects of eTRE and lTRE via a CGM may not be fully examined within Hatamoto et al., (2023) due to both conditions not being compared to a control group and CGM monitoring only occurring over a singular 24-hour period. This limitation was addressed within this research due to the participant being instructed to wear the CGM throughout the 3-day intervention periods alongside their respective controls.

6.1.2. MAG

This study showed a significant decrease in MAG in response to 3-day TRE intervention obtained via CGM. MAG displayed a mean decrease of 0.67 mmol/L between eTRE baseline and eTRE intervention, and 0.77 mmol/L between lTRE baseline and lTRE intervention. This decrease did not significantly differ between condition (eTRE vs lTRE).

The calculation completed to obtain MAG involves taking values of absolute changes in glucose and dividing these values by the time over which measurements were taken (DeVries, 2013). This measurement allows for the visualisation of glucose fluctuations to be identified, as participants may have the same mean glucose value and standard deviation but have different glycaemic profiles. Figure 10 provides a visual representation of the point discussed above; participant 2 displays a higher glucose variability due to a higher frequency of oscillation. MAG reports on the duration of time which blood glucose fluctuations occur (Kovatchev and Cobelli, 2016). Thus, measuring MAG as an outcome is important MAG values of an unstable diabetic would be higher than those of a stable diabetic, due to the unstable diabetic displaying a greater number of oscillations, stable diabetics still have a higher MAG than those with normoglycaemia (Service et al., 1970a). DeVries., (2013) noted than to assess GV, MAG and CV are both useful tools.

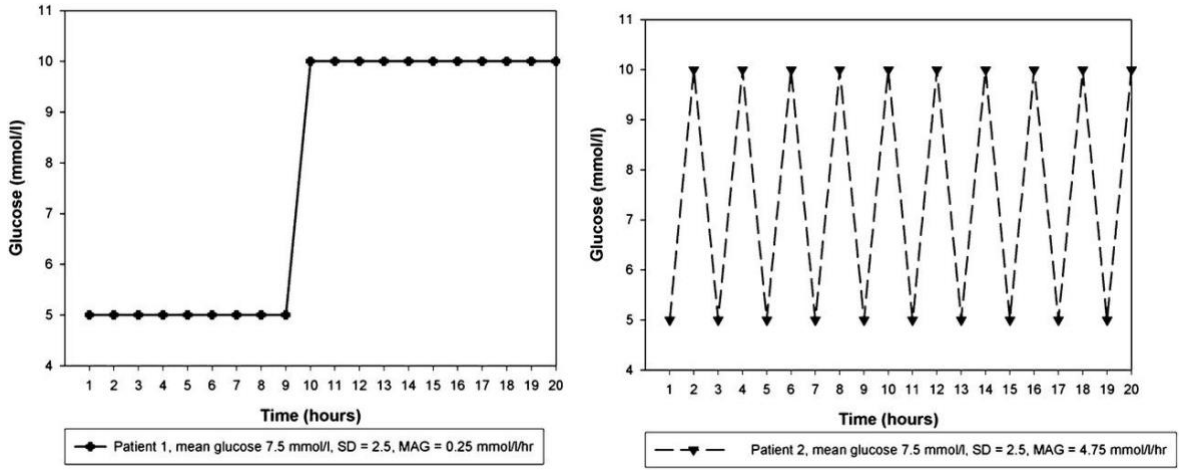

Figure 10 *Visual representation of MAG and how it is a useful tool to determine glycaemic variability. Patient 1 displays a different pattern of variability than participant 2, where participant 2 depicts a higher glucose variability due to a higher frequency of oscillation (MAG) (Hermanides et al., 2010).*

Various TRE intervention studies have assessed mean glucose as an outcome (Jamshed et al., 2019; Ravussin et al., 2019; Jones et al. 2020), however this may not be as accurate as MAG due to it not detailing frequency of glucose oscillations throughout this 24-hour period. Mean 24-hour glucose reflects an average value for GV, Jones et al., (2020) described how they experienced no change in mean 24-hour glucose after 2-weeks of eTRE whilst Jamshed et al., (2019) experienced a decrease within their mean 24-h glucose recordings as a result of 4-day eTRE. This lower number obtained reflects a lower frequency of high glucose periods. Andriessen et al., (2022) conducted a 10-hour TRE protocol where they also experienced a decrease in 24-hour glucose levels in free-living adults with T2D. Hutchison, Regmi et al., (2019) did measure a similar measure to MAG, mean 24-hour blood glucose, by CGM where it was reported that there was no significant difference in regard to treatment (baseline, eTRE and lTRE) experienced. This was not experienced within this study, which reported on a significant decrease occurring between baseline and TRE intervention.

Within this study, lower frequency of glucose fluctuations during the TRE intervention when compared to the baseline condition were observed. However, it is important to

mention that due to the differences diets between baseline and TRE intervention days, this cannot be solely attributed to the TRE per se, but the fact that food intake was prescribed and different in calories and content compared to baseline recording.

At present, there has not been another TRE study which has assessed MAG via CGM as an outcome, therefore this measurement requires further investigation within short- and long-term interventions.

6.1.3. CV

This study showed a decrease in CV in response to 3-day TRE intervention obtained via CGM. CV displayed a mean decrease of 2.63% between eTRE baseline and eTRE intervention, and 2.37% between lTRE baseline and lTRE intervention. This decrease did not significantly differ between condition (eTRE vs lTRE).

CV is a measurement technique which assesses the magnitude of GV relative to mean blood glucose (Umpierrez and Kovatchev, 2018), which is calculated easily whilst being independent of the mean glucose concentration (Mo et al., 2021). CV is a useful technique for analysing the glycaemic fluctuations in diabetics, where individuals can be deemed stable or unstable depending on whether their calculated %CV exceeds the calculated maximum CV. Stable glycaemic fluctuations occur when %CV≤ maximum CV and unstable glycaemic fluctuations occurs when %CV ≥ maximum CV (Nwadiugwu et al., 2021).

Within this 3-day TRE intervention CV significantly reduced, however this contrasts with previous evidence within a study conducted by Chow et al., (2020), where participants were randomised to either a non-TRE intervention group (n=9) or TRE intervention group (n=11), where 20 participants out of the 22 completed the 12-week protocol. Chow et al., (2020), reported no significant difference occurring with regards to CV

because of intervention when comparing TRE to an unrestricted control. It is important to note that Chow et al., (2020) mentioned that within the TRE intervention group following a 2-hour OGTT, the results suggested prediabetes (7.9 mmol/l). Naguib et al., (2022) also noted within their lTRE cohort that there was no significant change in the GV as measured by CV between groups.

Chow et al., (2020) did not possess a cross-over design, instead they randomised participants to either TRE condtion or non-TRE condition, where participants self-selected their food consumption windows to result in 'ad libitum' intake. Self-selecting a food consumption window resulted in the inability to address whether there was an effect of TRE timing (eTRE vs lTRE), which is something our study could determine. Both study protocols implemented for this piece of research and Chow et al., (2020) stated within their inclusion criteria's an element of prolonged eating, this results in the possibility of their findings to be tenuous due to them being less applicable to humans who possess a shorter and/or irregular eating window.

Chow et al.,(2020) stratified their conditions (TRE vs non-TRE) by sex (male/female) as well as age (<45 vs ≥45 years old), where participants once randomised did not participate within the other trial condition. Within our study we utilised a randomised-control cross over design which allowed for each participant to experience each condition which allowed us to assess cause and effect of condition (non-TRE vs TRE) and time (eTREvs lTRE). Thus, removing inter-subject variability between groups as well as reducing the effect of covariates which would have been experienced within Chow et al., (2020) (Lim and In, 2021).

Naguib et al., (2022) possessed a small sample size, similar to what was obtained within this research, resulting in findings not being able to be generalised to different populations and settings. However, even though similar sized cohorts were obtained, Naguib et al., (2022) conducted their study over a much longer time frame of 12-

weeks meaning that their results may exhibit whether there was a long-term effect evoked.

This study experienced a lower magnitude of GV relative to mean blood glucose during the TRE intervention when compared to the baseline condition. Few studies have assessed the relationship between CV via CGM with regards to TRE, therefore this area warrants further investigation within short- and long-term interventions.

6.1.4. Statistically insignificant CGM results

Following the completion of the eight-hour TRE protocol, no significant differences in mean glucose, CONGA, LBGI, HBGI, and MVALUE IGV were observed. In line with the results achieved from this study, Hutchison et al., (2019) noted no statistical differences within mean 24-hour blood glucose concentrations and CONGA. However, they did note a significant decrease in mean fasting glucose within the eTRE protocol but not in the lTRE when compared with baseline. Naguib et al., (2022) also discussed how they experienced no significant changes within their lTRE protocol towards GV measured CV, MAGE, and glucose AUC which opposed two of the significant changes achieved within this protocol.

Whilst the results obtained expressed a significant change (MAGE, MAG and CV), they are classed as subclinical changes.

A clinical study conducted by Thomsen et al., (2020) assessed the effects of a CHO reduced high-protein diet against a conventional diet for 48-hours per intervention within type 2 diabetic individuals. Significant decreases occurred in MAGE, MAG and CV, uniformly reducing within all 16 participants assessed. MAGE significantly reduced by 1.9 mmol/L compared to the 0.4 mmol/L decrease within this study, MAG 0.5 mmol/L, and CV by 7% which was similar to what was experienced within this study, specifically a comparable mean decrease in MAGE of 0.44 mmol/L, mean decrease in

MAG of 0.72 mmol/L and a mean decrease in CV of 2.5% was noted after this TRE dietary intervention. The results here show small and significant improvements towards certain GV markers (MAGE, MAG, and CV) after a short dietary intervention which although subclinical, have the potential therapeutic role in the longer term.

Unlike the cohort within this study, Thomsen et al., (2020) utilised a cohort who were currently diagnosed with T2D which was metformin-treated. This would make the results obtained more applicable to those who may benefit from implementing a TRE diet, due to them already being insulin resistance and being currently regarded as type 2 diabetic. However, due to a small cohort and homogeneity of the population, the cohort was majority male who possessed good glycaemic control, this limits the generalisability of the findings if compared to a larger, more heterogeneous populations who are diagnosed with T2D.

This study is not a definitive way of proving that TRE is beneficial in the general population, it does however show signs that within this controlled study design TRE can have an impact on the outcomes assessed. However to date, there has not been a long-term study conducted to support this conclusion, therefore long-term testing of TRE dietary technique is required to assess possible long-term effects. The cohort utilised in this study were not prediabetic, however they were at risk of developing prediabetes due to advanced age alongside a BMI ≥25, which categorises individuals as overweight or obese. Additionally, five out of the eight participants assessed measured higher levels of cholesterol (≥5 mmol/L) and LDL (>3 mmol/L), three out of eight had higher than desired levels of triglycerides (>1.7 mmol/L) and one out of eight of the participants had lower than desired HDL (<1.0 mmol/L men, <1.2 mmol/L women) (Heart UK, 2023) at the initial visit. This results in individuals being more at risk for developing metabolic syndrome and T2D (Adiels et al., 2008; Denimal et al., 2023).

6.2. Blood biochemistry

Following the completion of the 3-day TRE protocols, no significant differences in plasma triglycerides, cholesterol, HDL, LDL, Chol/HDL, non-HDL, and fasting glucose were observed as a result of time (baseline vs intervention) or condition (eTRE vs lTRE).

A study completed by Hutchison et al., (2019) utilised a cross-over design protocol in which participants completed both a 7-day eTRE and a 7-day delayed-TRE (dTRE) condition. The study required participants to complete two 9-hour TRE interventions, where the eTRE condition consumed their food between the times of 8am to 5pm and the dTRE condition consumed their food between the times of 12pm-9pm. The study reported on a significant difference within fasting triglycerides occurring in response to TRE, stating that there was no significant difference experienced between the eTRE and dTRE interventions, alongside a significant reduction of mean fasting glucose within the eTRE condition. A significant reduction in fasting triglycerides was experienced in response to TRE within Hutchison et al., (2019), whereas this did not occur within the TRE protocol utilised within this study.

Differences in results obtained between Hutchison et al., (2019) and this study conducted could be explained by Hutchison et al., (2019) only utilising a small cohort of 15 participants, all of which were male meaning any potential gender differences were not observed. Furthermore, Hutchison et al., (2019) employed a longer eating window for a longer period of 7-days using only an obese cohort.

Tinsley et al., (2019) utilised an 8-hour TRE intervention for 4-weeks on healthy, resistance trained young (18-30 years) females. Participants were randomised to either a control group, who were instructed to consume breakfast soon after waking and consumed the rest of their food at self-selected intervals throughout the day, or a TRE condition who consumed their food between the times of 12pm to 8pm. Those randomised to the TRE condition were placed into either a TRE intervention without supplementation or a TRE intervention with β-hydroxy β-methylbutyrate supplementation. In line with what was experienced within the 3-day TRE protocol utilised within this protocol, there was no significant changes experienced within this

4-week intervention study between the control and intervention groups within this study with regards to HDL, LDL, plasma triglycerides, and cholesterol.

Sutton et al., (2018) utilised a 6-hour eTRE protocol which required participants to consume their final meal before 3 p.m. against a control group which utilised a 12-hour feeding period for 5 weeks, participants later crossed over to the other schedule. Similar to this study, levels of HDL and LDL were unaffected by the eTRE protocol utilised however the statistically insignificant results experienced within this protocol with regards to plasma triglycerides and cholesterol was not mirrored within Sutton et al., (2018). They noted a significant increase of fasting triglycerides and total cholesterol during the morning within the 5-week eTRE intervention group.

Protocols which implemented an 8-week TRE protocol included Cienfuegos et al., (2020) and Moro et al., (2016). Cienfuegos et al., (2020) compared a 4-hour TRE protocol, where food was consumed between the times of 3pm-7pm, against 6-hour protocol, which had a food consumption window of 1pm-7pm against a control group which had no time restrictions with regards to energy intake. Moro et al., (2016) assessed 34 resistance trained males, who were randomly assigned to either a TRE condition or a control group. TRE subjects consumed their food between the times of 1pm. Cienfuegos et al., (2020) reported outcomes similar to those achieved within this research, stating that no significant change was reported with regards to HDL, LDL or plasma triglycerides by the end of the 8-week intervention. Similar to the protocol utilised within this study, Moro et al., (2016) utilised an 8-hour TRE intervention, however their study took place over a longer time period of 8-weeks compared to the 3-day intervention of this study. Moro et al., (2016) also reported on experiencing no significant change within HDL, LDL and cholesterol in line with what was observed within this study.

12-week TRE interventions completed by Gabel et al., (2018), Chow et al., (2020) and Wilkinson et al., (2020) produced varied results from their 8 to 10-hour TRE protocols. Gabel et al., (2018) utilised an 8-hour protocol, where their TRE condition ate ad

libitum between the hours of 10am to 6pm. Within this study they noted no significant difference within HDL, LDL, plasma triglycerides, cholesterol, and fasting glucose, which was also experienced within this studies 8-hour TRE protocol. Similarly, the 8hour TRE protocol utilised by Chow et al., (2020) produced the same non-significant results within HDL, LDL, plasma triglycerides, and fasting glucose within their study which allowed participants to self-select their 8-hour food consumption window. In line with Chow et al., (2020), Wilkinson et al., (2020) conducted their TRE intervention over 12-weeks, however they utilised a longer TRE protocol which allowed participants to consume their food within a self-selected 10-hour consumption window. They experienced a statistically significant decrease in total cholesterol, LDL, and non-HDL, but no statistical difference with regards to HDL. Wilkinson et al., (2020) may have experienced these significant reductions in certain lipids due to the utilisation of a longer TRE intervention in terms of length of intervention and TRE timings than the one utilised within this study, alongside a larger cohort of 19 individuals who were already diagnosed with metabolic syndrome before taking part within their study.

6.3. Dietary analysis

Following the completion of the 8-hour TRE protocols utilised within this study, no significant differences in caloric intake, CHO, and fat were observed. However, protein did statistically differ between baseline and TRE intervention. Even though baseline diet significantly differed in terms of protein intake, the diet used within each of the TRE conditions (eTRE/lTRE) were the same, with total energy intake and macro components matched per meal.

The diet prescribed for consumption during the TRE intervention used a Mifflin-St Jeor equation to calculate appropriate caloric intake and macronutrients, displaying the number of calories burnt by the body during a non-active period. The macronutrient values obtained for this study were derived from 'The Eatwell Guide' published by the NHS (NHS, 2022).

This study design used a eucaloric diet based on the nutritious macronutrient values calculated in terms of carbohydrate, fat, and protein, where the same items were purchased in each arm of the intervention. A 1.4 factor was applied to the Mifflin St Jeor equation to account for the additional energy required to carry out basic tasks (walking, cooking, thinking, moving etc.) in the sedentary cohort.

The Mifflin-St Jeor values was validated via data received from food diary records. Food diary records possess well documented limitations and are prone to underreporting (Dhurandhar et al., 2014; Jonnalagadda et al., 2000; do Nascimento et al., 2020). This may be due to individuals under-report their food intakes during this form of reporting (Macdiarmid and Blundell, 1998; Kagawa & Hills, 2020; Moran et al., 2018). Despite this, Cancello et al., (2018) discussed how the Mifflin-St Jeor equation performed best within an obese cohort that had with ≥3 comorbidities alongside patients with type 2 diabetes (Marra et al., 2017).

Mifflin-St Jeor is regarded as one of the most accurate predictor equations of resting energy expenditure (Frankenfield, 2013; Thom et al., 2020) which had the ability to predict within 10% of the actual resting metabolic rate value within nonobese and obese individuals (Frankenfield et al., 2005). When compared to the current gold standard, indirect calorimetry, it was noted by (Maury-Sintjago et al., 2022) that the Mifflin-St Jeor equation displayed no statistically significant difference. The Mifflin-St Jeor equation was established from indirect calorimetry (Deng and Scott, 2019), and is regarded as one of the most accurate predictors of resting metabolic rate (Thom et al., 2020). The Mifflin-St Jeor equation provides the most consistent accuracy across BMI categories due to it being developed from a large sample which included individuals of a healthy weight, overweight and obese BMI (Thom et al., 2020).

Mifflin St Jeor has been reported to significantly overestimate resting metabolic rate within certain ethnicities, however this overestimation does not occur within Caucasian individuals (Reneau et al., 2019). Hassan et al., (2013) noted that the Mifflin-St. Jeor equation calculated the closest estimate resting energy expenditure obtained from

indirect calorimetry via predicting the resting energy expenditure of participants who were a normal weight or overweight.

However, the accuracy of Mifflin-St Jeor has been disputed with some studies stating that it provides underestimate of resting energy expenditure (Grassi et al., 2020) where Amirkalali et al., (2008) concluded that underestimates of REE occurred within females whilst males experienced an overestimate. These errors may be due to the racial origin of the population utilised within the study which derived this equation being unknown, leading to a possible conclusion that this equation is not appropriate for certain racial/ ethnic groups (Deng and Scott, 2019; Aliasgharzadeh et al., 2015).

Other TRE protocols which have utilised energy intake calculations which required the participants body weight, height, and age to determine daily energy intake (Bao et al., 2022; Isenmann et al., 2021). Both studies used a Harris-Benedict equation. Within the Bao et al., (2022) study, they conducted a 5.5-hour isocaloric diet and compared it against an 11-hour control schedule. Each participant was confined to a metabolic chamber and consumed a controlled-nutrient diet which consisted of 55% CHO, 30% fat, and 15% protein, where no significant difference was observed in regard to total energy, CHO, fat, and protein intake between the TRE and control group. Bao et al., (2022) reported that there was no statistical difference observed as a result of mealtime ingestion of three meals, however they did note that TRE could result in a negative energy balance occurring due to an increased rate of fecal and urine energy excretion which may explain weight loss experienced within other TRE studies. TRE was reported to have improved the glycaemic profiles and metabolic flexibility of participants. The improvements of glycaemic profiles were thought to be due to 24hour glucose levels reducing due to the attenuation of postprandial glycaemic profiles, however postprandial insulin also reduced because of TRE meaning that this reduction was unlikely to be the cause of the hypoglycaemic effects. The overall study designed only spanned 3-days, where the intervention only took place for 24-hours on the second day after acclimatisation, with the last day being a recovery day. Considering this, the conclusion that metabolic changes may have occurred after only

24-hours is doubtful as metabolic adaptions have been reported to take numerous weeks to months to occur (Hall, 2018; Ross and Leveritt, 2001).

Similar to Bao et al., (2022), Isenmann et al., (2021) utilised Harris-Benedict formula to calculate basal metabolic rate allowing for approximation of total daily energy intake to be obtained. The primary outcomes assessed were anthropometric parameters; body weight, lean body mass, fat mass, BMI, and waist and hip circumference and did not involve analysis of glucose concentrations or blood lipids to support the positive effects of the nutritional strategy. Utilising these biomarkers would have strengthened the results obtained.

6.3.1. Protein alteration

Within this study on average individuals consumed 21.8g (beTRE) and 23.2g (blTRE) of less protein per day during baseline measurements compared to 105g which was consumed during the TRE intervention. This significant increase within the TRE may have resulted in the significant GV outcomes being obtained. Due to this alteration, it cannot be determined whether TRE or increased protein intake was the cause of the significant reductions of MAGE and MAG.

The diet utilised within the TRE protocol did not match the habitual diet assessed during the baseline. Participants consumed different amounts compared to baseline of total calories consumed and each macronutrient assessed however there was not a significant difference. However, there was a significant difference with regards to the amount of protein, which increased during TRE intervention. The Mifflin-St Jeor equation, which calculated the total daily calories and macronutrients consumed during the TRE intervention, computed the resting metabolic rate for each participant within the study. The difference between the baseline protein and the protein consumed during the TRE intervention may potentially be reflective of how the

habitual protein which participants consumed, calculated by the Mifflin St Jeor equation (Mifflin et al., 1990), is not what it should be.

TRE studies which reported on an alteration in protein intake during the TRE phase include Moro et al., (2016) and Lowe et al., (2020). Moro et al., (2016) utilised an 8week, 8-hour lTRE protocol where participants consumed meals at 1pm 4pm and 8pm compared against a non-diet cohort who consumed their food at 8am, 1pm and 8pm. Tinsley et al., (2019) noted that Moro et al., (2016) matched kilocalories and macronutrient distribution, however they prescribed a higher protein intake in both the TRE and control group. Moro et al., (2016) reported that no significant change occurred in regard to total cholesterol, HDL and LDL but a significant decrease in triglycerides in the TRE condition. This significant change may have occurred due to Moro et al., (2016) utilising a longer intervention length (8-weeks) in a larger, resistance-trained, male cohort (n=34) who were younger (29.21 ± 3.8 years) than the cohort used within this study conducted. Similarly, Lowe et al., (2020) within their 12week intervention have noted that a decrease in protein intake may have occurred during the TRE intervention, stating that this may have led to loss of appendicular lean mass within participants, concluding that possible effects of protein intake should be studied further.

It has been reported that high protein intake within T2D could augment prandial insulin secretion which may lead to an improvement of glycaemic control (Promintzer and Krebs, 2006). In addition to this, Nesti et al., (2019) discussed that premeal ingestion of a non-CHO macronutrients (protein or fat) reduces postprandial hyperglycaemia within those at risk of T2D as well as those currently classified as T2D. Studies which have assessed the effects of consumption of whey protein before a meal experienced enhanced β-Cell function, reduced insulin clearance (Smith et al., 2023) as well as reductions in hyperglycaemia in people with T2D (Smith et al., 2022).

Consumption of a high protein diet has been reported to result in a greater MAGE value (Smart et al., 2013), where glucose excursions have been reported to increase in

response to high protein meals within type 1 diabetic youths. Liu et al., (2022) is currently conducting a protocol which evaluates any possible effects exhibited on GV within prediabetic individuals in response to high-protein vs. high-fat snacks. They hypothesised that high protein snacks would exhibit a better effect on GV, than a high fat snack, due to high protein diets being associated with improved glucose metabolism, β-cell function and satiety (Ortinau et al., 2014; Tricò et al., 2016; Glynn et al., 2022). A diet which is high in protein has been associated with significant reductions in MAGE (Thomsen et al., 2020; Tettamanzi et al., 2021). Similar effects of high protein diets have been reported with regards to MAG, where significant reductions were viewed within (Thomsen et al., 2020). In summary, the studies discussed above suggest that high-protein diets may have beneficial effects with regards to glycaemic control in T2D individuals.

6.4. Physical activity

Following the completion of the 3-day TRE protocols, average daily steps, time spent completing sedentary, light, and moderate-vigorous activity did not significantly differ as a result of time (baseline vs intervention) or condition (eTRE vs lTRE).

This protocol utilised the device GeneActiv tri-axial PA monitors to provide an estimate of energy expenditure whilst assessing whether there were any changes in PA levels pre- / post- intervention. This measure was a control measure, as participants were instructed to maintain levels of PA between both arms of the intervention (Chow et al. 2020; Gabel et al., 2020; Jones et al. 2020; Wilkinson et al., 2020). Thus, any potential changes evoked by TRE would likely be related to the modified meal timings. Measurements obtained from the accelerometer assessed both compliance and PA levels, due to wear time being measured by the device. TRE intervention did not significantly alter average daily steps, time spent completing sedentary, light, and moderate-vigorous activity obtained during baseline to TRE (time) as well as no

observed effect of early versus late TRE (condition). PA alterations could be a potential confounder, which is why it was controlled for within this protocol.

Parr et al., (2023) employed a similar technique where a tri-axial PA monitor was utilised and participants were instructed to not alter PA. They noted no changes in PA patterns between both the habitual period and 9-hour TRE intervention. Similarly, Parr et al., (2022) also utilised an accelerometer known as ActiGraph accelerometer, where PA levels were consistent between the habitual 'lead-in' period and the 8-hour TRE intervention period. (Santos–Báez et al., 2022) measured PA via a the ActiGraph-GT3X on their non-dominant wrist during both 2-week assessment periods, they noted that it would be unlikely that participants would increase their PA levels in response to TRE intervention. Hutchison et al., (2019) observed no significant change within PA levels as a result of their 9-hour eTRE (8 am to 5 pm) and lTRE (12 pm–9 pm) intervention. Energy expenditure, total number of steps, as well as time spent sleeping was measured via a SenseWear armband. Andriessen et al., 2022 noted that as a result of their three week, 10-hour protocol that there was no change with regards to energy expenditure in response to their protocol. An 8-hour TRE study which did not control for PA due to the protocol being conducted during the height of the COVID-19 pandemic was Naguib et al., (2022), who noted that this considerable limitation may have impacted the glycaemic profiles obtained in this cohort.

The previously mentioned studies concur with the logic implemented within this protocol to control against PA changes. This removes the chance of PA alterations potentially influencing glycaemic outcomes.

Previous studies have discussed an effect of TRE (mainly lTRE) on physical activity energy expenditure. Examples of this include Templeman et al., (2021) who within their randomised control trial utilised a different form of IF dietary intervention known as alternate day fasting. They assessed 12 healthy individuals across a 3-week period placing these individuals into one of two arms. One arm instructed participants to enter a 24-hour fast on the first day, followed by a 150% increase in energy intake. The

other matched their energy intake on both days, possessing a 75% energy intake across both days. They concluded that both groups maintained similar levels of PA energy expenditure throughout the weight loss period.

Betts et al., (2014) conducted a 6-week randomised controlled trial assessing daily breakfast consumption against an extended morning fast where the individuals could consume food at 12pm. They observed a significant difference between groups where with the breakfast group completed more light intensity activity during the morning than the extended morning fast group. The breakfast cohort displayed significantly higher rate of PA thermogenesis which is thought to be due to the differences in energy expenditure in regard to light-intensity activities completed during the morning.

It was concluded that daily breakfast consumption is causally linked to a higher rate of PA thermogenesis, with greater overall dietary energy intake but no change in resting metabolism. Additionally, they noted that breakfast consumption maintained more stable glycaemic level in the afternoon and evening when compared to fasting.

The cohort utilised was completed in a free-living environment, assessing habitual diet and displayed no significant difference between the various exercise intensities. However, it is important to note that the study is regarded as an acute study, due to the dietary intervention only lasting 3-days. Additionally, this study did not match energy expenditure to energy intake, meaning bodyweight may not be maintained which may influence the results.

6.5. Strengths and Limitations

6.5.1. Strengths

Strengths of this study include the utilisation of both males and females who were between the age of 45-60 years and possessed a of BMI≥25. These attributes mean that the individuals assessed have a higher risk for developing prediabetes, therefore it was useful to assess this cohort. Utilising a FreeStyle Libre 2 device allows for 24-hours worth of data to be obtained which is useful when assessing GV over the course of an intervention. Taking measurements via this method is better than results taken at limited occurrences (i.e. once or twice a day) as glucose varies throughout a 24-hour period. TRE research to date has largely ignored what food is consumed (i.e., macronutrient composition and energy density), allowing people to consume ad libitum. This study addressed this issue by analysing both baseline and prescribed TRE diet. Seven of the individuals who successfully completed the intervention were Caucasian, for which the Mifflin-St Jeor equation is able to accurately calculate resting metabolic rate (Reneau et al., 2019).

6.5.2. Limitations

However, the generalisability of the results is limited by the small sample size utilised which may have led to a results obtained having a higher margin of error and reductions in power of the study. Additionally, the cohort was largely limited to a single ethnicity with seven individuals being Caucasian.

Issues which arose when gathering or analysing data include myself contracting COVID19 which resulted in visits where I would have obtained secondary outcomes (blood biochemistry). Moreover, some CGM data had to be imputed due to participants not uploading their results to the data management software LibreView.

Confounding variables which could not be controlled include participants not consuming the same diet for each baseline as instructed alongside different foods being consumed during the TRE intervention window due to allergies or disliking the

available foods. Once set up, there was no way of checking whether the fitness watches were recording until reviewing the data, which led to data not being obtained for both intervention periods for some participants (n=2). Due to work commitments, traffic, or other life events (anniversaries/ birthdays), some participants experienced irregular visit times or longer washout windows.

6.6. Further direction

The use of a short-term TRE intervention model provided an understanding for possible short-term effects exhibited by an 8-hour TRE protocol on glycaemic markers. Future studies should determine whether the significant effects are still experienced in the long-term via a longer intervention period. Furthermore, a larger, more varied cohort should be utilised analysing various ages, ethnicities, and BMI groups due to the low generalisability of the few current studies available. Therefore, more studies are required in order for the effects of TRE in T2D people to be determined.

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8. Appendix

Appendix [A]: Participant information sheet:

Participant Information Sheet

Understanding blood glucose responses to eating

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You are being invited to take part in a research study. Before you decide whether or not you wish to take part it is important that you understand why the research is being done and what it will involve. Please read this information carefully and discuss it with others if you wish. Please do not hesitate to contact us if anything is unclear, or if you require more information. Take time to decide whether or not you wish to take part. Details about the conduct of the study are also explained which will help you to decide whether or not you wish to take part.

What is the purpose of this study?

Dietary lifestyle interventions are one proposed strategy in obese populations to assist in weight loss and improve metabolic health, in particular the control of blood sugar. An emerging strategy is time-restricted eating (TRE). This protocol prescribes to consume all daily calories within a fixed daily time window, followed by a prolonged period of fasting over a total 24 hour period. Currently, some research points at metabolic benefits when an 8 hour window to consume food is followed by 16 hours of fasting. The rationale for implementing an 8 hour feeding window comes from recent interest in how circadian rhythms (24-h light-dark cycle) tightly regulate metabolism. Previous research has shown benefits of TRE, however whether the 8 hour eating window yields improved metabolic health when applied early (i.e. 8:00 till 16:00) or late until later (i.e. 12:00 till 20:00) is still unclear. In addition, it is unknown whether these shown improvements are the result of an adaptive and cumulative effect of multiple days of TRE or can already be observed acutely. Thus, the aim of this study is

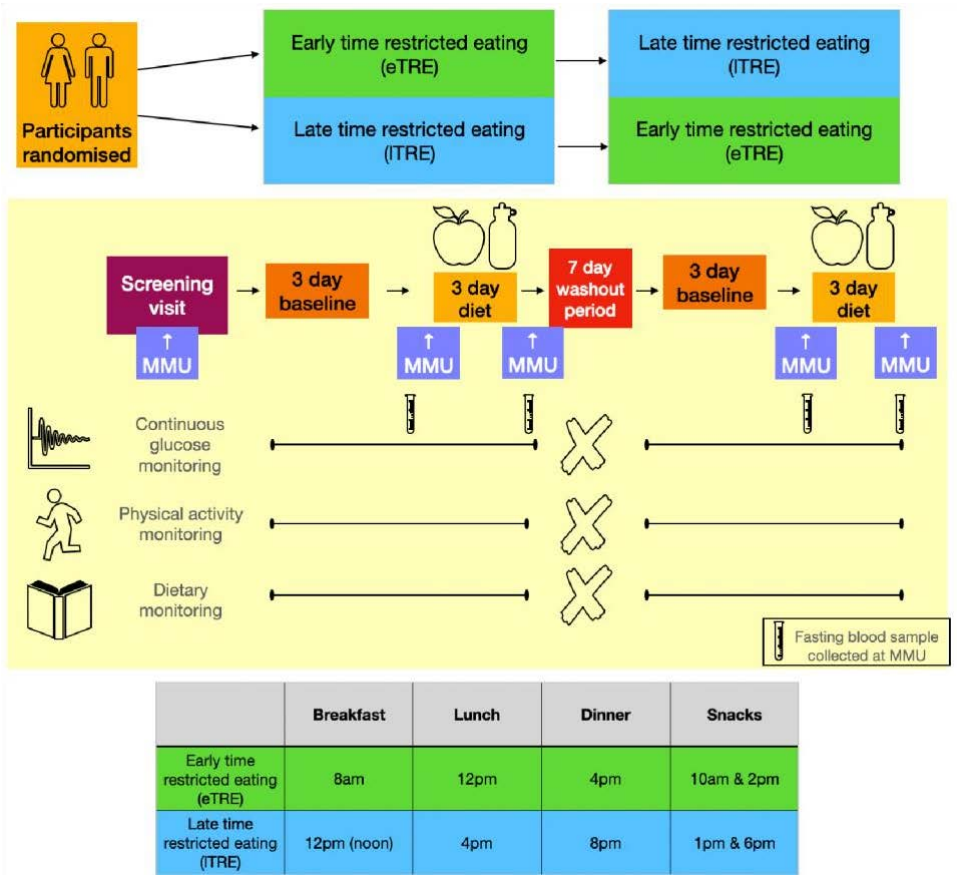
to assess the effects of three days of both early or late time-restricted eating on 24 hour blood sugar levels.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to do so, you will be asked to sign a consent form. However, you will still be free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

We will contact you by phone or email to answer any questions you may have about the study. We will then ask you some questions about your medical history to check whether you can participate. You will not be able to participate in the study if you are a smoker, have type 1 or type 2 diabetes, are pregnant, alcohol consumption >14 units weekly, have food allergies or intolerances to any foods consumed in this study, have identified disturbed eating patterns or diagnosed eating disorders, recent major body weight change (+/- 3 kg in the last month), are a shift-worker, have any medical conditions or if you are taking medications that will affect the measurements in the study. If you are a suitable participant, we will invite you to attend the University research facilities on 5 separate occasions, lasting between 30 and 45 minutes per visit.



	Breakfast	Lunch	Dinner	Snacks
Early time restricted eating (eTRE)	8am	12pm	4pm	10am & 2pm
Late time restricted eating (lTRE)	12pm (noon)	4pm	8pm	1pm & 6pm

Figure 1: Explanation of study protocol, timing of measurements and timing of meals.

What will I have to do?

Visit 1 (Screening)

After contact via email or phone, you will be invited into the laboratory for a screening and familiarization trial. This visit will happen at a time that is suitable for you. At this visit you will be invited to ask any questions or raise any concerns before agreeing to take part in the study. Your participation will then depend on the satisfactory completion of a medical and exercise history questionnaire. If you are considered eligible and agree to take part you will sign an informed consent form.

You will then have your height, weight and waist circumference taken as well your body

composition. You will be explained how to wear and use a continuous glucose monitoring device and an accelerometer. Next, we will place an accelerometer on your hip or wrist (your preference) which will measure your normal physical activity levels for three days. In these three days, we will also ask you to keep a dietary diary and record all the food and times of food consumption. After the fourth day, you can take off the accelerometer and take the device and dietary diary with you to your next visit. At the end of the screening, we will schedule the next appointment for the start of the trial.

Visit 2

At least 4 days later (after wearing the accelerometer), you will attend the lab for the start of your trial. Attendance will be between 24 - 36 h before the start of the experimental protocol and has to happen in a fasted state (not having consumed food for several hours). We will collect a fasted blood sample upon arrival. The reason for the 24 - 36 h prior to the start is to place the continuous glucose monitor and let it stabilise before it records experimental data. We will provide you with the food that you will consume over the three days and explain when you are expected to consume the meals and snacks. You will undergo both conditions (early time-restricted feeding and late time-restricted), but the order will be based on randomisation. We will also place an accelerometer to measure physical activity levels over the three days and we will ask that you keep a dietary diary to indicate the timing when you consumed your meals and snacks. Throughout the three day intervention period, we will also send you automated text messages at appropriate times to remind you to consume your next meal or snack. Visit 2 should take approximately 30-45 minutes.

Visit 3

The morning after the three-day intervention, we will ask you to come to the lab in a fasted state. We will collect a blood sample and you will hand in the dietary diary,

accelerometer and we will remove the continuous glucose monitor device. We will also schedule the next 3-day trial which is at least a week (7 days) later. Visit 3 will take approximately 30-45 minutes.

Visit 4 and 5

Visits 4 and 5 are identical to visit 2 and visit 3, except that you will be asked to follow the opposite eating pattern over the 3-day trial. Remember that the order of 3-day trial conditions (early or late) is randomised.

What are the possible benefits of taking part?

During the screening, we will measure your weight, height, body composition and measure some blood markers. This will give you insight in your physique and health and learn about your body. Additionally, we will provide you with the groceries needed to follow a predetermined diet on the two trials, which totals 6 days worth of food.

Will my participation involve any physical discomfort?

Yes, there are some possibilities that you experience physical discomfort. During the time-restricted eating trials, you will be asked to strictly adhere to food consumption at predetermined times of the day. This means that during the fasting phase you might experience a feeling of having an empty stomach. You are allowed to consume water without restrictions, that might help to suppress hunger feelings. We have designed the diet to mimic normal eating patterns and provide two snacks moments between the three main meals to reduce cravings.

Secondly, on the experimental days you will have to wear a continuous glucose monitor for 24 hours. A fine sensor will be inserted into the skin that will feel like a small prick. In extreme cases, an infection might develop around the site of insertion. An experienced researcher will handle the procedure of insertion. Lastly, on four (4) occasions, we will take a blood sample (15 mL). Thus a total of 90 mL of blood will be collected throughout

the study. For comparison: during a blood donation 500ml blood is taken in one day. You may experience some mild discomfort when blood samples are being taken. This is not a painful procedure, but you will feel a slight prick when the needle is inserted and you may have some very light, small bruising following, which is a completely normal response. However, all samples will be collected by appropriately trained personnel (certified phlebotomist/IV cannulation) to minimize this possibility. This will be discussed at the outset of the study and if you do not wish to take part on this basis then that is your choice.

What will happen if I feel unwell during the diet section of the study?

If you feel unwell at any point during the diet portion of the study, you may return to your normal diet and contact a member of the study team (emails at end of information sheet), you will then be withdrawn from the trial.

What will happen if anything goes wrong?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you wish to make a complaint you can contact the principal investigator Kelly Bowden Davies whose details are on the front page on this information sheet.

Will my taking part in this study be kept confidential?

Yes. All information that is collected about you during the course of this research will be

kept strictly confidential. You will be provided with a unique code so that any

information collected will be unidentifiable to yourself.

What will happen to the study results?

The overall results of the study may be presented at scientific meetings or published in a scientific journal. You will not be identified in any of these presentations or publications.

We will be happy to discuss the results with you when the study is completed and will let you know where you can obtain a copy of the published results.

Contact for further information

If you have any further questions, then please contact either Abigail Walker abigail.walker2@stu.mmu.ac.uk or Dr Kelly Bowden Davies K.Bowden.Davies@mmu.ac.uk

Thank you for having taken the time to read this information sheet and your interest in the study. If you do decide to take part in the study, you will be given a copy of the information sheets and a signed consent form for you to keep.

Appendix [B]: Approved recruitment poster

Research volunteers needed for eating study

Aim of the research:
To understand if the time we eat and drink alters blood sugar control.

Who can take part?
- Men and women,
- aged 45 – 60 years,
- typically eating over the entire day,
- low levels of physical activity.

What would I need to do?
- Track food and drink intake
- Track blood sugars using continuous glucose monitoring system inserted into your arm

- Change the time you eat and drink for 3 days on two occasions (all food will be provided)
- Attend the research facility at Manchester Met on 5 separate occasions (visits would last approximately 30 minutes)

What are the benefits of taking part?
- Understand more about your health including diet, physical activity and diabetes
- Travel expenses will be reimbursed and all food provided

Interested to find out more?
Contact a member of the research team at
K.Bowden.Davies@mmu.ac.uk

Manchester Metropolitan University