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The impact of lifestyle factors, dietary energy restriction and religious fasting on body composition, gastrointestinal hormones and markers of health

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Study Related Publication

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Published Abstracts

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Conferences Presentations

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Poster presentation

Declaration

I, Deema Alogaiel, declare that this thesis is the result of my own work and has not in this, or any other form, been presented to this or any other University in support of an application for any degree other than for which I am now a candidate. In Chapter 2, the literature search, screening process, and quality assessment of studies was conducted independently by Deema Alogaiel and co-author May Alotaibi. The data analysed in chapters 4, 5, and 6 were collected by the Nottingham Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World (PREVIEW) team.

Abbreviations

ADA	American Diabetes Association
ADF	Alternate Day Fasting
ADP	Air Displacement Plethysmography
ADP	adenosine diphosphate
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BAI	Body Adiposity Index
BF%	Body Fat Percentage
BFM	Body Fat Mass
BG	Blood Glucose Concentration
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Blood Pressure
BW	Body Weight
C	Control (non-fasting)
CCK	Cholecystokinin
CI	Confidence Interval
CID	Clinical Investigation Day
CMIA	chemiluminescent microparticle immunoassays
CRP	C-reactive Protein
CT	Computerised Tomography
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DPP	Diabetes prevention program
DXA	Dual Energy X-Ray Absorptiometry
ECG	Electrocardiogram/Electrocardiograph
ECW	Extracellular Water

EPIC	European Prospective Investigation into Cancer and Nutrition
FBC	Full Blood Count
FDP	Finnish Diabetes Prevention Study
FDRs	First Degree Relatives for Diabetic Patients
FFM	Fat-Free Mass
FFQ	Food Frequency Questionnaires
FM	Fat Mass
FPG	fasting plasma glucose
G6P	Glucose-6-phosphate
G6PD	glucose-6-phosphate dehydrogenase
GDP	Gross Domestic Product
GI	Glycaemic Index
GIP	Glucose-Dependent Insulinotropic Polypeptide
GLP-1	Glucagon-like Peptide-1
GP	General Practitioner
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
HbA1c	Haemoglobin A1c
hCG	human Chorionic Gonadotropin
HDL-C	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus.
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPLC	high-performance liquid chromatography
HR	Heart Rate
hs-CRP	High-sensitivity C-reactive protein
HW	Healthy weight
I	Intervention group (fasting)
ICW	Intracellular Water
IDF	International Diabetes Federation
IF	Intermittent Fasting
IFCC	International Federation of Clinical Chemistry

IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IR	insulin resistance
ITT	Intention-to-Treat
LBM	Lean Body Mass
LDH	Lactate Dehydrogenase
LDL-C	Low-Density Lipoprotein Cholesterol
LED	Low-Energy Diet
LFTs	Liver Function Tests
LLM	Leg Lean Mass
LM	Lean Mass
LM-PA	Light and Moderate Physical Activity
MASLD	Metabolic dysfunction-Associated Steatotic Liver Disease
MDH	Malate Dehydrogenase
MRI	Magnetic Resonance Imaging
MVC	Maximum Voluntary Contraction
MVPA	moderate-to-vigorous physical activity
NAD+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
NGSP	National Glycohemoglobin Standardisation Program
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NR	Not Reported
NRES	National Research Ethics Service
OGTT	Oral Glucose Tolerance Test
OW	overweight
PAL	Physical activity log
PODs	Prompts for Optimal Decisions
PREMIT	PREVIEW Behaviour Modification Intervention Toolbox

PREVIEW	Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World
PRIMIS	Primary Care Information Services
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
PYY	Peptide YY
QMC	Queen's Medical Centre
QUICKI	Quantitative Insulin Sensitivity Check Index
RDA	Recommended Dietary Allowance
RIF	Ramadan Intermittent Fasting
RLUs	Relative light units
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of the Mean
SMD	Standardised Mean Difference
T2DM	Type 2 Diabetes Mellitus
TBW	Total Body Water
TFEQ	Three-factor eating questionnaire
TFTs	Thyroid Function Tests
TG	Triglycerides
TRE	Time-Restricted Eating
UK	United Kingdom
US	United States
USD	United States Dollar
VLDL	Very Low-Density Lipoprotein
VLED	Very-Low-Energy Diets
WC	Waist Circumference
WHO	World Health Organisation

WHR	Waist to hip ratio
WMD	Weighted Mean Difference
X	measurements conducted

ABSTRACT

Background

Intermittent fasting and dietary interventions to bring about weight-loss have received considerable attention for their potential metabolic health benefits. However, gaps still exist in understanding their effects on appetite regulation, body composition changes across different age groups, and the methodological challenges in assessing these outcomes. This thesis aimed to: 1. undertake a systematic review to examine the effect of Ramadan intermittent fasting on appetite-regulating hormones in healthy individuals (CHAPTER 3); 2. investigate relationships between lean body mass, physical activity, age, and body composition in overweight individuals with pre-diabetes (CHAPTER 4); 3. explore the impact of lifestyle factors and eating behaviours on body composition changes following an 8-week low-energy diet intervention (CHAPTER 5), and 4. validate commonly used assessment tools for dietary protein intake, body composition, and physical activity against their respective reference standards (CHAPTER 6).

Methods

1. A systematic review and meta-analysis examined 16 studies (n=664 participants) to investigate the effects of Ramadan intermittent fasting on circulating leptin, ghrelin, insulin, gastrin, glucagon-like peptide-1, peptide YY, and cholecystokinin concentrations. 2. A secondary analysis of cross-sectional data from 220 overweight and obese individuals with pre-diabetes (aged 25-70 years) from the Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World (PREVIEW) diabetes risk study to explore relationships between body mass, regional body composition (measured by Dual Energy X-Ray Absorptiometry; DXA), age and physical activity (assessed using accelerometry). 3. A secondary analysis of anthropometric, metabolic, and behavioural outcomes (including eating behaviours measured

through validated questionnaires) from 221 participants (91 males, 130 females) from the PREVIEW cohort, collected before and after an 8-week low-energy diet intervention (810 kcal/day). 4. Dietary protein intake, assessed via 24-hour urinary nitrogen excretion, was compared with self-reported dietary records (n=264); body composition via bioelectrical impedance was compared with data from DXA scans (n=37), and physical activity estimation via the Baecke questionnaire was compared with accelerometry-derived time spent in sedentary and moderate/vigorous activity (MVPA).

Results

1. Ramadan intermittent fasting increased blood ghrelin concentration (3 studies; standardised mean difference (SMD) = 0.31 pg/mL, 95% CI: 0.03 to 0.60), but had no effect on leptin (12 studies; SMD = −0.11 µg/mL, 95% CI: −0.36 to 0.14), insulin (6 studies; SMD = −0.24 µU/mL, 95% CI: −0.54 to 0.02), or gastrin concentrations (3 studies; SMD = 0.23 pg/mL, 95% CI: −0.71 to 0.99), with high heterogeneity observed across leptin studies, suggesting varied individual responses. 2. In individuals with pre-diabetes, lean body mass and leg lean mass decreased with increasing age category in females, with males having greater lean leg mass compared with females across all age groups. Leg lean mass was positively associated with body weight, with physical activity only modifying this relationship in females, though in all participants, no association between leg lean mass and physical activity was observed. 3. The low-energy diet brought about substantial weight loss (median -11.3 kg) in participants, with males experiencing greater reductions in weight, lean mass (-4.5 vs -2.5 kg), and fat mass (-8.8 vs -7.3 kg) compared with females. Higher baseline dietary restraint scores were associated with greater weight loss success ($R=0.276$, $P<0.001$), while higher disinhibition predicted poorer outcomes ($R=-0.173$, $P<0.05$), with these behavioural influences differing between sexes. Baseline physical activity levels did not influence lean mass preservation or fat mass loss

during the period of weight-loss. 4. The 8-week LED intervention resulted in improvements in metabolic parameters, including reduced fasting glucose, insulin, triglycerides, and C-peptide concentrations. Sex-specific responses were observed, with males showing greater reductions in fasting insulin (-25.7 pmol/L difference, $P<0.001$), C-peptide (-211 pmol/L difference, $P<0.001$), and triglycerides (-0.210 mmol/L difference, $P<0.001$) compared with females. Males also demonstrated larger improvements in insulin sensitivity indices (HOMA-IR and QUICKI) than females. 5. Validation studies revealed systematic differences between assessment methods: self-reported dietary protein intake differed from biomarker-derived estimates, with proportional bias between measures emerging during a weight maintenance intervention phase (overall $R=0.394$, $P<0.001$). Body composition assessed by BIA and DXA showed strong agreement between measurements (percentage body fat $R=0.853$, fat mass $R=0.896$, and fat-free mass $R=0.841$) supporting BIA as a practical alternative to DXA. Physical activity assessments demonstrated modest correlations between subjective and objective measures ($R=0.320$ for MVPA) reflecting the different aspects of physical activity being assessed by each tool.

Conclusion

Increased circulating ghrelin, reported during Ramadan fasting, may drive a stimulation of appetite.

Cross-sectional analysis revealed age-related differences in lean mass, with lower lean mass observed among older participants, particularly females. This pattern underscores the importance of women maintaining physical activity as they age to preserve muscle mass. The low-energy diet intervention demonstrated efficacy in reducing body weight and improving metabolic health, with notable sex-specific responses in insulin sensitivity and lipid metabolism, alongside baseline eating behaviours serving as important predictors of weight loss success. The method

validation studies provided methodological insights for future research in nutritional epidemiology and clinical practice.

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Chapter 1 Introduction

1.1 Introduction

The global obesity epidemic continues to escalate at an alarming rate, with recent estimates indicating that over 2.6 billion individuals are now classified as overweight or obese, representing a threefold increase since 1980 [1, 2]. This dramatic rise has turned obesity into a major public health emergency that requires continued attention. Overweight now affects about 43% of the world's adult population, and 16% are living with obesity, with particularly alarming increases noted in low and middle-income countries [1, 2]. Obesity is a primary contributor to the development of many chronic diseases and is the most important modifiable risk factor affecting global health outcomes [2]. Recent research has provided more substantial evidence connecting obesity to a wider variety of severe health conditions, including cardiovascular diseases, different types of cancer, liver disease, and neurodegenerative disorders [3, 4]. Of particular concern is the relationship between obesity and Type 2 Diabetes Mellitus (T2DM), with current projections suggesting that the global diabetic population will reach 783 million by 2045, representing an estimated 12.2% of the global adult population [5].

The economic implications of the obesity epidemic are concerning, with the global economic burden estimated at 2.19% of GDP in 2019, projected to reach about $3 trillion USD annually by 2030, and $4.32 trillion by 2035 (approximately 3% of global GDP) [6]. This figure includes direct healthcare costs, lost productivity, and increased disability-adjusted life years [7]. Low- and middle-income countries face a particularly challenging situation, as they are now dealing with the dual burden of malnutrition and rising obesity rates, which strain their already limited healthcare resources [8, 9]. In response to this growing crisis, international health organisations have established ambitious targets for obesity prevention and control [10]. The World Health Organisation's [2] Global Action Plan aims to reduce obesity prevalence by 2030 through a comprehensive approach combining individual and population-level

interventions [11]. These strategies include better nutritional education, increased promotion of physical activity, implementation of fiscal policies regarding ultra-processed foods, and the creation of supportive built environments [3]. Emerging digital health technologies and precision medicine approaches provide promising new opportunities for obesity prevention and treatment. However, their scalability and cost-effectiveness need further evaluation [12].

1.2 Obesity

1.2.1 Definition and Classification of Overweight and Obesity

Overweight and obesity are complex, chronic conditions linked to excessive or abnormal accumulations of body fat. Adipose tissue is essential for normal physiological functions, including energy regulation, hormone production, and immune system support [13]. However, excess adiposity, especially when centrally distributed, significantly increases health risks [14]. This condition results from adipose tissue expansion through increased fat cell size and number [15]. Overweight and obesity also result from a prolonged period of positive energy balance, where energy intake consistently surpasses energy expenditure [16]. Fat mass can be estimated using several methods, including dual-energy X-ray absorptiometry (DXA), computed tomography (CT), magnetic resonance imaging (MRI), underwater weighing (hydrodensitometry), bioelectrical impedance analysis (BIA), and air displacement plethysmography (ADP). Each of these methods provides assessments of fat and fat-free mass, which can be used to calculate body fat percentage [17, 18]. While there are multiple methods to assess body fatness, body mass index remains the most widely used screening tool for classifying body weight status in adults, primarily due to its simplicity and low cost [19]. Body composition and its assessment are explored in more detail in section 1.3.

Overweight and obesity are generally classified using BMI (**Table1-1**), which is calculated by dividing body weight in kilograms by height in meters squared (kg/m²). Optimal health is linked to maintaining a BMI between 21 and 23 kg/m² [20, 21], whereas the 'healthy' range for adults extends from 18.5 to 24.9 kg/m² [22]. Individuals with a BMI between 25.0 and 29.9 kg/m² are classified as overweight, which increases their risk of developing various health conditions [23]. Those with a BMI of 30 kg/m² or higher are classified as obese, with health risks increasing progressively across obesity classes: Class I (30.0-34.9 kg/m²) carries moderate risk, Class II (35.0-39.9 kg/m²) indicates severe risk, and Class III (≥40 kg/m²) represents very severe risk of comorbidities including cardiovascular disease, T2DM, and premature mortality [24, 25]. The WHO currently uses a unified BMI classification system, although research suggests that ethnic-specific cut-off points may be more appropriate for certain populations. For instance, Asian populations may benefit from lower BMI cut-offs points due to their higher risk of metabolic complications at lower BMI values [26, 27], while Polynesian populations may require higher cut-offs due to their different body composition and fat distribution patterns [28].

Table 1-1: World Health Organisation classification of overweight and obesity in non-Asian adults [29].

Classification	BMI (kg/m^2)
Underweight	<18.5
Healthy Weight	18.5-24.9
Overweight	25.0-29.9
Obesity Class I	30.0-34.9
Obesity Class II	35.0-39.9
Obesity Class III	≥40.0

BMI is a useful screening tool, but it has limitations because it does not directly measure body fat or its distribution [30]. Research indicates that the distribution of adipose tissue, especially visceral or central fat, may

be a stronger predictor of health risks than the body weight to height relationship [31]. Modern classification approaches increasingly incorporate additional measures such as waist circumference, waist-to-hip ratio (WHR), and body composition analysis to provide a more comprehensive evaluation of excess body fat and better determine health risks [32]. Overweight and obesity are complex chronic conditions influenced by multiple factors including genetics, environment, behaviour, and socioeconomic status, rather than simply conditions of excess weight [14, 33].

1.2.2 Global and UK Obesity Pandemic: Prevalence, Trends, and Contributing Factors

The prevalence of obesity continues its alarming upward trajectory worldwide, having nearly tripled between 1975 and 2020 [34]. This global epidemic has severe implications for developed nations, with the United Kingdom (UK) representing a case study of rapid obesity escalation in a high-income country that provides important insights for understanding modern obesogenic environments.

1.2.2.1 Global Context and Trajectory

Globally, obesity prevalence has increased from around 3% of males and 6.6% of females with a BMI ≥30 kg/m² in 1975, to 14.0% of males and 18.5% of females in 2022 [22, 35]. This represents obesity rates that have quadrupled among men and nearly tripled among women, with the absolute number of individuals classified as obese increasing substantially from 1975 to 2022 [36]. Current estimates indicate that 2.1 billion adults and over 340 million children were overweight in 2022, with 890 million adults classified as obese, representing approximately 16% of adults aged 18 years and older worldwide [37]. Current projections indicate that by 2035, nearly 1 in 5 adults and 1 in 10 children will be living with obesity globally [37].

The distribution of the obesity burden varies significantly across regions, with adult obesity rates remaining highest in developed nations such as the United States (US) and Mexico (approximately 40% of adults classified as obese), while Middle Eastern countries also report very high obesity rates in adults: Kuwait (~39%), Saudi Arabia (~38–41%), and Qatar (~37–41%) [3, 22]. In contrast, Asian countries like Japan and South Korea maintain significantly lower rates at 4.3% and 6.2%, respectively [22, 35]. However, several Asian countries are projected to experience rapid increases in obesity rates; expected to reach 12% in South Korea and 18% in China by 2035 [37], suggesting that no region remains immune to this epidemic.

1.2.2.2 The United Kingdom: A Case Study of Developed Nation Challenges

The UK exemplifies the challenges faced by developed nations, ranking among the highest obesity rates in Europe and significantly exceeding the OECD average. UK adult obesity has increased steadily from 23.0% in 2004/05 to 28.0% in 2021/22, with projections indicating continued rises to 29.8% by 2024/25 (**Table 1-2**). This trajectory represents one of the steepest increases among developed nations [38]. Particularly concerning is the increase in severe obesity (BMI ≥40kg/m²), which has nearly doubled from 1.7% to 3.2% between 2004/05 and 2020/21, representing an 88% increase over this period (**Table 1-3**). This trend is significant as individuals with severe obesity face substantially elevated risks for T2DM and demonstrate significantly poorer outcomes in weight management interventions, including higher attrition rates and lower long-term weight loss success compared with those with lower BMI categories [23, 39-41]. The disproportionate growth in severe obesity categories suggests that while overall obesity rates are rising, the severity distribution is becoming increasingly skewed toward the most metabolically dangerous categories [42].

The health consequences of the UK obesity epidemic extend beyond individual impacts to create population-level burdens. As obesity rates increase, there are corresponding rises in related health conditions: T2DM risk increases six-fold in individuals with obesity [43]; hypertension risk increases by 42.5% [44]; and depression shows 55% higher prevalence [45] compared with healthy weight individuals. Importantly, the obesity-depression relationship is bidirectional: while obesity increases depression risk through mechanisms including weight stigma, systemic inflammation, and functional impairment, depression independently predicts obesity development through reduced physical activity, dysregulated eating patterns, and weight-promoting effects of certain antidepressant medications [45]. The economic implications are equally concerning, with the current burden of obesity-related conditions on the NHS amounting to £6.0 billion annually and projected to rise to £9.7 billion by 2050 [46].

Table 1-2: UK Adult Obesity and Overweight Rates (2004-2025).

Year	Total Population		Men		Women	
	Overweight	Obese	Overweight	Obese	Overweight	Obese
2004/05	37.6%	23.0%	43.0%	22.1%	32.8%	23.8%
2008/09	37.4%	24.5%	42.0%	23.8%	32.9%	25.2%
2012/13	37.0%	25.8%	41.7%	25.0%	32.2%	26.5%
2016/17	36.6%	27.0%	42.0%	26.2%	31.2%	27.8%
2020/21	36.3%	28.0%	41.5%	26.9%	31.0%	28.6%
2024/25*	35.5%	29.8%	40.5%	28.0%	30.5%	30.0%

-BMI classification: **Overweight:** BMI 25-29.9 kg/m², **Obese:** BMI ≥ 30 kg/m².
-Data extrapolated from: [47-50].
*2024/25 figures are estimated projections based on current trends, not actual measured data.

Table 1-3: Extreme Obesity Rates (BMI ≥40kg/m²) in the UK Adult Population.

Year	Total Population		Men		Women	
	Prevalence	% Change from 2004/05	Prevalence	% Change from 2004/05	Prevalence	% Change from 2004/05
2004/05	1.7%	N/A	1.1%	N/A	2.1%	N/A
2008/09	2.0%	+0.3%	1.5%	+0.4%	2.5%	+0.4%
2012/13	2.4%	+0.7%	1.8%	+0.7%	3.1%	+1.0%
2016/17	2.9%	+1.2%	2.1%	+1.0%	3.7%	+1.6%
2020/21	3.2%	+1.5%	2.4%	+1.3%	4.0%	+1.9%
2024/25*	3.5%	+1.8%	2.7%	+1.6%	4.3%	+2.2%

-N/A: Not Applicable.
-Data extrapolated from: [47-50].
*2024/25 figures are estimated projections based on current trends, not actual measured data.

1.2.2.3 Demographic Disparities and Population-Specific Risk Patterns

Analysis of UK obesity patterns reveals complex demographic disparities. Obesity demonstrates strong age-related patterns; increasing progressively from 14% among adults aged 16-24 y, to 35% among those aged 55-64 y, and 36% among those aged 65-74 y [47]. These age-related increases coincide with declining physical activity levels, a progressive loss of lean muscle mass (sarcopenia), declining basal metabolic rate, hormonal changes affecting appetite regulation and fat distribution, and reduced insulin sensitivity, all of which predispose an individual to increased adiposity, even with maintained dietary energy intake [51, 52]. Additionally, lifestyle factors such as decreased occupational activity, reduced recreational exercise participation, and changing dietary patterns as people move through the lifespan, compound these biological changes [53]. These changing metabolic profiles create compounding risk factors for chronic disease development; research demonstrates that age-related obesity increases cardiovascular disease risk through mechanisms including increased systemic inflammation, elevated blood pressure, and dyslipidaemia [54].

T2DM risk is particularly elevated, with studies showing that obesity in older adults increases diabetes incidence by 2-3 fold compared with normal-weight peers [55]. Furthermore, the combination of age-related muscle loss and increased adiposity (sarcopenic obesity) creates a particularly high-risk phenotype associated with greater functional decline, increased frailty, and elevated mortality risk compared with either condition alone [56, 57].

Sex differences in overweight and obesity prevalence show nuanced patterns, with overall higher rates in 2021 among men (67% overweight/obese) compared with women (61%), although the distribution varies significantly across age groups. Male prevalence is lowest among those aged 16-24 y (35%) but peaks at 80% among those aged 55-64 y. Among women, prevalence increases from 38% in the youngest group, maintaining relatively high levels of 69% among those aged 55-74 y [47]. These patterns suggest different trajectories of weight gain across the lifespan between sexes, which research attributes to distinct biological, behavioural, and social factors. Men typically demonstrate more consistent weight gain throughout adulthood, driven by declining testosterone levels that reduce muscle mass and metabolic rate, combined with sustained high energy intake patterns and decreased physical activity as occupational demands shift from physical to sedentary work [58, 59]. Women's weight trajectories are more complex, influenced by reproductive events including pregnancy-related weight retention, hormonal contraceptive use, and particularly the menopausal transition, which triggers significant increases in abdominal adiposity due to declining oestrogen levels and altered fat distribution patterns [60, 61]. Additionally, women demonstrate different behavioural responses to weight gain, with higher rates of weight cycling from repeated dieting attempts, which can paradoxically promote long-term weight gain and metabolic dysfunction [62]. Social factors also contribute, with women facing greater appearance-related pressures that may lead to restrictive eating patterns followed by compensatory overeating, while men's weight

gain is often more gradual and less subject to social scrutiny until it reaches clinical significance [63]. These patterns suggest different trajectories of weight gain across the lifespan between sexes, with implications for targeted prevention strategies.

Ethnicity reveals additional complexity, with the highest prevalence of overweight and obesity found among women of Black Caribbean (74%), Pakistani (74%), and Black African (73%) backgrounds. In contrast, the lowest rates were observed among Chinese women (22%) and men (36%) [64]. These disparities are proposed to reflect interactions between genetic predisposition, cultural dietary patterns, socioeconomic factors, and environmental influences that require population-specific intervention approaches to address body weight management. Research demonstrates that genetic factors may contribute to these ethnic differences, with studies showing genetic associations in populations of African descent with variants linked to increased adiposity, though interestingly, African populations typically show less visceral and more subcutaneous fat distribution compared with other ethnic groups at similar BMI levels [65-67]. South Asian populations, including Pakistani communities, show genetic association with increased susceptibility to central adiposity and metabolic dysfunction at lower BMI thresholds, with variants associated with insulin sensitivity and lipid metabolism that may be linked to this phenotype [68, 69]. Conversely, East Asian populations, including Chinese communities, show genetic associations with variants that appear to be linked to lower obesity risk [70]. However, it is important to note that these genetic studies provide associative rather than causative evidence, and the observed ethnic differences in obesity prevalence likely result from complex interactions between genetic predisposition, environmental factors, and cultural practices.

Cultural dietary patterns further compound these genetic predispositions in UK populations. Traditional Caribbean and West African diets, when

adapted to Western food environments, often incorporate high-energy-density processed foods, while maintaining cultural preferences for starchy staples and cooking methods involving added fats [71, 72]. South Asian dietary patterns frequently include high refined carbohydrate intake and cooking practices using substantial amounts of oil, contributing to increased dietary energy density [73]. In contrast, traditional Chinese dietary patterns emphasise vegetables, lean proteins, and cooking methods that require less added fat, though these protective effects may diminish with Western dietary acculturation [74]. Socioeconomic and environmental factors create additional layers of complexity. Ethnic minority communities in the UK often experience higher rates of deprivation, food insecurity, and reside in areas with limited access to affordable healthy foods, factors that independently increase obesity risk [75, 76]. Additionally, cultural attitudes toward body size, with some communities viewing higher body weight as indicative of health and prosperity, may influence weight management behaviours and help-seeking patterns [77].

As alluded to above, perhaps most concerning from a public health equity perspective is that socioeconomic factors significantly impact obesity rates, with adults in the most deprived areas of the UK being 1.75 times more likely to be obese compared with those in the least deprived areas [48]. This gradient suggests that obesity is not merely an individual lifestyle issue but may reflect broader structural inequalities in access to healthy food, safe physical activity environments, and health-promoting resources. Research consistently demonstrates that food access inequalities contribute to socioeconomic obesity disparities. Studies show that deprived neighbourhoods in the UK have fewer supermarkets and grocery stores offering fresh produce, with residents often relying on convenience stores and fast-food outlets that predominantly stock energy-dense, nutrient-poor foods [78, 79]. The 'food desert' phenomenon is particularly pronounced in urban deprived areas, where a single basket of healthy foods can cost up to 25% more than in affluent

areas, creating financial barriers to healthy eating for low-income households [80, 81].

Physical activity environment disparities further compound these inequalities. Deprived communities typically have reduced access to safe recreational facilities, parks, and walking/cycling infrastructure, with studies showing that the most deprived quintile of UK neighbourhoods have 40% fewer sports facilities per capita compared with the least deprived areas [82, 83]. Additionally, safety concerns related to crime, traffic, and poor street lighting significantly limit outdoor physical activity opportunities, particularly affecting women and older adults in these communities [84, 85]. Health-promoting resource inequalities extend beyond food and exercise access. Deprived areas have fewer healthcare facilities offering weight management services, reduced availability of dietitians and nutritionists, and lower rates of health education programs [86]. Educational resources are also more limited, with lower health literacy rates in deprived communities affecting individuals' ability to navigate complex nutritional information and make informed dietary choices [87, 88].

1.2.2.4 Underlying Causes and Risk Factors: A Multifactorial Challenge

The UK obesity epidemic reflects complex interactions between multiple risk factors [89]. Non-modifiable risk factors include genetic predisposition, with studies indicating that 40–70% of BMI variation can be attributed to genetic factors [90], ethnicity-related differences in obesity susceptibility, such as South Asian populations demonstrating increased risk of excess adiposity and metabolic complications at lower BMI thresholds and physiological changes associated with ageing and hormonal transitions [91]. However, the rapid increase in the prevalence of obesity over recent decades clearly indicates that environmental and behavioural factors are important drivers of current trends since genetic

factors cannot account for such rapid population-level changes within a few generations [92].

Surveys reveal concerning patterns in the UK population, with regards to modifiable lifestyle factors shown to influence overweight and obesity. Only 29% of adults aged 19-64 years meet the '5-a-day' fruit and vegetable recommendation, which is significant because inadequate fruit and vegetable consumption is associated with higher energy intake from energy-dense, nutrient-poor foods and reduced dietary fibre intake that typically promotes satiety and weight regulation [93-95], while approximately 25.7% of adults engage in less than 30 minutes of physical activity per week [96]. These dietary and activity patterns interact with biological mechanisms through multiple pathways that promote weight gain. Poor dietary quality, characterised by high intake of processed foods and low fruit and vegetable consumption, leads to increased energy density and reduced meal satiety, promoting energy overconsumption [97]. Simultaneously, sedentary behaviour reduces total daily energy expenditure while also affecting appetite-regulating hormones, including leptin and ghrelin, creating a biological environment that favours positive energy balance [98, 99], where chronic positive energy balance leads to adipose tissue expansion [100].

1.2.2.5 Physical Activity Patterns: A Critical Contributing Factor

Physical inactivity patterns compound the obesity challenge and provide insights into potential targets for intervention. The relationship between physical inactivity and weight gain is well-established through multiple mechanisms. Physical activity accounts for 15-30% of total daily energy expenditure in active individuals, and reductions in activity levels directly contribute to positive energy balance and subsequent weight gain [101]. Beyond energy expenditure, regular physical activity enhances insulin sensitivity, improves fat oxidation capacity, and helps preserve lean

muscle mass during weight loss, all of which are crucial for long-term weight management [102]. However, physical activity interventions alone have shown limited success for bringing about weight loss, with meta-analyses demonstrating that exercise-only interventions typically produce modest weight losses of 1-3 kg over 6-12 months, significantly less than dietary interventions or combined approaches [103, 104]. Data from 2020/21 indicate that only 61.4% of UK adults meet the recommended guideline of 150 minutes of moderate activity per week, while 27.0% are defined as being inactive, as shown in **Table 1-4** [47, 105]. These levels represent modest improvements from historical data but remain inadequate and are likely to compound both the development of overweight and obesity and the risk of developing the chronic diseases that are associated with excess adiposity.

Notable sex disparities exist in physical activity patterns, with 65.3% of men classified as active compared with 57.7% of women [47, 105] (**Table 1-3**). This sex gap in physical activity may partially explain the observed variations in obesity rates and suggests that different approaches may be needed to engage men and women in physical activity promotion due to different barriers, motivations, and preferences between the sexes. Research indicates that women face greater time constraints due to caregiving responsibilities, have higher rates of body image concerns that may discourage gym attendance, and prefer different types of physical activities compared with men [106, 107]. However, the relationship between physical activity and obesity involves complex interactions with metabolic health, body composition, and psychological well-being. Physical activity appears to influence the secretion of appetite-regulating hormones, including leptin, ghrelin, and peptide YY, which can either promote or suppress hunger depending on exercise intensity, duration, and individual factors [98]. Additionally, regular exercise can improve mood and stress management through endorphin release and reduced cortisol levels, which can help prevent emotional eating and stress-related weight gain [108]. Exercise also promotes

favourable changes in body composition by preserving lean muscle mass during weight loss, which helps maintain basal metabolic rate and prevents the metabolic adaptations that typically promote weight regain [109].

The temporal trends in physical activity (**Table 1-4**) show gradual improvements from 2004/05 to 2020/21 in the percentage of those meeting activity guidelines [47, 105], yet this positive trend has been insufficient to counteract the rising obesity epidemic. This disconnect suggests that while promoting greater physical activity is beneficial for health, it may be insufficient as a stand-alone approach to counter the development of overweight and obesity, without addressing the broader obesogenic environment, including dietary factors, built environment, and social determinants of health. This observation is supported by ecological studies demonstrating that countries with increasing physical activity levels can still experience rising obesity rates when dietary changes toward higher energy intake occur simultaneously [110]. The limitations of physical activity alone for weight management are further evidenced by systematic reviews showing that successful long-term weight loss maintenance requires combined interventions addressing both dietary intake and physical activity, along with behavioural modification strategies [111, 112].

Table 1-4: Physical Activity Levels in England Adults (aged 19+ years).

Year	Total Population			Men			Women		
	Active	Fairly Active	Inactive	Active	Fairly Active	Inactive	Active	Fairly Active	Inactive
2004/05	57.2%	12.6%	30.2%	60.8%	12.2%	27.0%	53.7%	13.0%	33.3%
2008/09	59.0%	12.3%	28.7%	63.2%	11.9%	24.9%	55.1%	12.7%	32.2%
2012/13	60.7%	12.2%	27.1%	64.9%	12.0%	23.1%	56.7%	12.4%	30.9%
2016/17	61.8%	12.6%	25.6%	65.8%	11.9%	22.3%	58.2%	13.2%	28.6%

2020/21	61.4%	11.6%	27.0%	65.3%	11.2%	23.5%	57.7%	12.0%	30.3%
2024/25*	64.0%	11.0%	25.0%	67.0%	11.0%	22.0%	61.0%	11.0%	28.0%

- Active: ≥150 minutes of moderate-intensity activity per week. Fairly Active: 30-149 minutes of moderate-intensity activity per week. Inactive: <30 minutes of moderate-intensity activity per week.
- Data collated from: [47, 105].
- * 2024/25 figures are estimated projections based on current trends, not actual measured data.

1.2.2.6 The obesogenic environment

Environmental and socioeconomic factors create what has been termed an 'obesogenic environment' [113], a concept that encompasses the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations. This environment operates across multiple interconnected domains: the physical environment (food availability, portion sizes, urban design), economic environment (food pricing, marketing expenditure), and sociocultural environment (social norms, cultural practices, media influences) [92, 114].

Food marketing represents a powerful component of the obesogenic environment, with the food industry spending approximately £256 million annually on advertising in the UK, with 95% directed toward processed foods high in fat, sugar, and salt [115]. Television advertising of unhealthy foods significantly influences adult consumption patterns, with experimental studies demonstrating that adults exposed to unhealthy food advertisements consume significantly more energy in subsequent meals compared with those exposed to non-food advertising [116]. Digital marketing through social media platforms creates new obesogenic pathways, with systematic review evidence demonstrating that digital marketing of unhealthy commodities significantly influences young people's dietary behaviours, preferences, and consumption patterns through sophisticated targeting mechanisms and interactive engagement strategies [117].

Portion size inflation represents another critical obesogenic factor, with UK Food Standards Agency data demonstrating that average portion sizes for key food categories have increased substantially over recent decades, with some ready meals and restaurant portions now exceeding recommended serving sizes by up to 200% [118]. 'Supersizing' marketing strategies have normalised large portion consumption by positioning bigger meal sizes as 'better economic value' [119]. This portion distortion effect influences consumer expectations and behaviours, with individuals served larger portions consuming approximately 22-29% more food, regardless of hunger levels or satiety cues [120].

Workplace environments also contribute to obesogenic exposure through limited availability of healthy food options, sedentary job requirements, and stress-promoting organisational cultures [121]. Employees in workplaces with comprehensive wellness programs, including healthy cafeteria options and physical activity facilities, have approximately 13% lower obesity rates compared with standard workplace environments [122]. Shift work schedules are thought to compound environmental obesogenic factors through circadian rhythm disruption and limited access to healthy meal options during non-standard work hours [123].

1.2.2.7 Psychological Factors

Psychological factors add additional complexity, as mental health disorders can increase obesity risk through emotional eating and preferences for energy-dense foods high in fat and sugar [124]. Chronic stress represents a psychological contributor through its action via the hypothalamic-pituitary-adrenal axis, resulting in elevated circulating cortisol concentration that promotes central fat deposition, increased appetite for palatable foods, and reduced insulin sensitivity [125]. Cortisol directly stimulates appetite through increased secretion of neuropeptide Y and other hunger-regulating hormones, while promoting preference for 'comfort foods' that temporarily may reduce stress, but contribute to positive energy balance [126]. UK studies demonstrate that individuals

reporting high chronic stress have 23% higher rates of central obesity compared with those with low stress, with this relationship remaining significant after controlling for socioeconomic factors [127].

Anxiety disorders, affecting approximately 8% of adults in the UK, appear to demonstrate independent associations with obesity through proposed mechanisms including increased cortisol production, disrupted eating patterns and reduced physical activity as a consequence of avoidance behaviour [128]. Emotional eating behaviours, where psychological rather than physiological hunger cues drive food consumption, can also contribute to overconsumption of energy-dense foods during times of stress, anxiety, or negative emotions [129].

Sleep disorders, affecting approximately 20% of adults in the UK, are reported to contribute to the development and maintenance of overweight and obesity states through metabolic disruptions involving leptin and ghrelin regulation [130, 131], with short sleep duration increasing obesity risk by 83% for overweight and 57% for obesity categories [132]. Beyond hormonal disruptions, sleep deprivation impairs prefrontal cortex function, reducing cognitive control over food choices and increasing impulsive eating behaviours [133]. Sleep fragmentation, even when total sleep duration is adequate, independently increases obesity risk through repeated cortisol spikes and inflammatory response [134].

Cognitive factors, including executive function deficits and food addiction-like behaviours, create additional psychological barriers to weight management. Approximately 11-25% of individuals with obesity display addictive-like responses to certain foods, characterised by loss of control, preoccupation with food, and continued consumption despite negative consequences [135]. These behaviours appear to involve altered reward processing, with neuroimaging studies showing similar dopamine

dysfunction patterns in food addiction and substance use disorders [136]. Executive function deficits, including reduced working memory and inhibitory control, predict poor weight loss outcomes and increased susceptibility to food cues in the environment, as individuals struggle to regulate intake in response to highly palatable and energy-dense foods [137].

Understanding these psychological pathways is important for developing effective weight-loss interventions, as approaches that fail to address underlying psychological factors show limited long-term success [138]. Integrated interventions that combine behavioural modification, stress management, and cognitive behavioural therapy demonstrate superior outcomes compared with diet and exercise alone, highlighting the importance of addressing the psychological dimensions of obesity alongside physiological factors [139].

1.2.2.8 Eating Behaviours: Dietary Restraint and Disinhibition

Eating behaviours represent complex psychological constructs that significantly influence weight management outcomes and the development and maintenance of obesity. Among the most extensively studied constructs are dietary restraint and dietary disinhibition, which represent opposing behavioural tendencies in relation to food intake regulation [811]. Understanding these eating behaviour patterns is essential for comprehending individual differences in susceptibility to weight gain and responses to weight loss interventions. Dietary restraint refers to the conscious limitation of food intake in order to control body weight or promote weight loss [653]. Individuals exhibiting high dietary restraint typically engage in deliberate cognitive control overeating, implementing rules and restrictions regarding food selection, portion sizes, and eating frequency [832]. This behavioural pattern represents an

attempt to override physiological hunger cues with cognitive control mechanisms. While dietary restraint may initially appear adaptive for weight management, research demonstrates that high levels of restraint paradoxically increase vulnerability to overeating episodes, particularly when self-control resources become depleted or when individuals encounter palatable foods or stressful situations [698]. The restraint theory proposes that chronic dietary restriction creates a state of physiological and psychological deprivation that increases preoccupation with food and heightens reactivity to food cues, thereby increasing the risk of loss of control overeating [833].

Dietary disinhibition, in contrast, describes the tendency to overeat in response to various stimuli, including the presence of palatable foods, emotional states, or social situations [653]. High disinhibition is characterised by opportunistic eating patterns, reduced sensitivity to internal satiety signals, and increased responsiveness to external food cues and environmental eating triggers [811]. Individuals with high disinhibition demonstrate difficulty regulating food intake in obesogenic environments and are more susceptible to portion size effects, food variety effects, and emotional eating patterns [834]. Research consistently demonstrates that disinhibition represents one of the strongest behavioural predictors of weight gain and obesity, with prospective studies showing that baseline disinhibition levels predict future weight change independent of initial body weight [835, 836].

The relationship between restraint and disinhibition is complex and dynamic, with evidence suggesting that rigid dietary restraint may actually promote disinhibited eating patterns through a cycle of restriction and disinhibition [837]. This phenomenon, termed the “restraint-disinhibition cycle,” describes how periods of strict dietary control are followed by episodes of uncontrolled eating when restraint breaks down, leading to guilt, renewed restriction attempts, and perpetuation of the

cycle [698]. The restraint-disinhibition model has important implications for understanding weight cycling and the difficulties many individuals experience in maintaining weight loss following dietary interventions.

Assessment of eating behaviours typically employs validated psychometric instruments, most commonly the Three-Factor Eating Questionnaire (TFEQ), originally developed by Stunkard and Messick [653] and subsequently revised [838]. The TFEQ assesses three distinct dimensions of eating behaviour: cognitive restraint, disinhibition, and hunger. These instruments demonstrate good reliability and validity across diverse populations and have been extensively used in obesity and weight management research [839]. The clinical and research implications of restraint and disinhibition extend to weight loss intervention outcomes. Studies indicate that baseline disinhibition levels predict poorer weight loss outcomes and increased dropout rates in behavioural weight management programmes [41, 840]. Furthermore, changes in eating behaviours during weight loss interventions, particularly reductions in disinhibition and increases in flexible rather than rigid restraint, are associated with greater weight loss success and improved weight maintenance [841]. These findings suggest that addressing maladaptive eating behaviour patterns should constitute an integral component of comprehensive weight management approaches, with interventions designed to promote flexible dietary control strategies while reducing disinhibited eating patterns [842].

1.3 Body Composition

1.3.1 Components of Body Composition

Body composition assessment provides a better understanding of health status than body weight or BMI alone by enabling the quantification of specific tissue compartments and their distribution. This more detailed analysis allows for the identification of adverse body composition profiles,

such as excess visceral adiposity and reduced muscle mass, which are independent risk factors for cardiometabolic diseases [17, 18]. While BMI remains widely used in population studies, its limitations in distinguishing between different tissue types have led to increased emphasis on more detailed body composition analysis [30]. The primary components of body composition include total and regional fat mass, lean mass (LM), and bone mass, each contributing uniquely to physiological function and health outcomes [140, 141].

Fat mass comprises essential and storage fat, with essential fat requirements differing significantly between sexes: women require 8-12% of total body mass as fat, compared with 3-5% for normal physiological function in males [33]. Storage fat accumulates in subcutaneous and visceral compartments, with their relative proportions influencing metabolic risk [142]. Brown adipose tissue represents a specialised form of fat involved in thermogenesis, though it comprises a relatively small proportion of total body fat in adults [143].

Lean mass (LM) includes skeletal muscle tissue, organ tissues, connective tissues, and intracellular and extracellular water [19]. Skeletal muscle, comprising approximately 40% of total body mass in healthy adults, serves as the primary site for glucose disposal and protein metabolism [144]. It accounts for 20-30% of basal metabolic rate and approximately 80% of glucose disposal under insulin-stimulated conditions [145, 146].

Bone mass provides essential structural support and serves as a mineral reservoir with particular relevance in ageing populations [17]. Recent research also reveals that bone tissue functions as an endocrine organ through osteocalcin secretion, which influences glucose metabolism by stimulating pancreatic β-cell proliferation, enhances insulin sensitivity in

skeletal muscle and adipose tissue, and increases adiponectin production from adipocytes [147, 148].

1.3.2 Distribution Patterns

The distribution pattern of body fat has been identified as an important factor in determining health risks associated with overweight and obesity, often proving more significant than total fat mass alone [32]. Two main fat distribution patterns have been identified: android (central) and gynoid (peripheral). Android distribution, characterised by a predominance of upper body and abdominal fat accumulation, is associated with a higher risk of metabolic and cardiovascular disease [149]. This pattern, which is more common in males, is strongly linked to the accumulation of visceral adipose tissue and has a significant association with the development of insulin resistance and T2DM [150]. Furthermore, gynoid distribution, which is characterised by fat accumulation in the hips and thighs, generally presents a lower metabolic risk profile [151]. This pattern, which is more prevalent in females, may provide protective metabolic effects through different adipokine profiles and lower concentration of circulating inflammatory markers [152]. The difference between these distribution patterns extends beyond aesthetic considerations, significantly influencing metabolic disease risk, hormone production, inflammatory profiles, and cardiovascular outcomes [153].

1.3.3 Body Composition Assessment: Methods, Applications, and Considerations

Accurate assessment of body composition is fundamental to understanding obesity-related health risks and monitoring intervention effectiveness, particularly in populations at risk for metabolic diseases. The selection of appropriate body composition assessment methods requires careful consideration of research objectives, population characteristics, and the specific tissue compartments of interest [18]. Population-specific considerations are particularly important as body

composition assessment methods may perform differently across various groups due to differences in body composition patterns, hydration status, and metabolic profiles [154]. In populations with pre-diabetes and T2DM, precise monitoring of body composition changes is crucial as even modest alterations in LM and fat distribution can significantly impact glycaemic control and insulin sensitivity [155, 156]. For instance, reductions of 1 kg of muscle mass are associated with decreased insulin sensitivity [157, 158], while 10-20% reduction in visceral fat can substantially impact glucose metabolism and enhance insulin sensitivity [159]. Therefore, assessment methods must be capable of detecting these clinically relevant changes while considering practical limitations such as cost, accessibility, and patient burden [17, 160].

The following sections examine the principal body composition assessment methods used in the obesity and metabolic health research which form the basis of this thesis. This review, therefore, focuses on the three primary approaches used in the current research project: DXA, BIA, and anthropometric assessments. This overview evaluates each approach's theoretical basis, research applications, and suitability for different study populations, particularly emphasising their utility in pre-diabetes and intervention research. Specific technical procedures and protocols employed in this thesis are detailed in Chapter 3.

1.3.3.1 Dual-Energy X-ray Absorptiometry (DXA)

DXA has emerged as the most widely adopted reference method for body composition research, providing accurate measurements of fat mass, LM, and bone mineral content using a three-compartment model approach [161]. The method’s fundamental principle involves differential X-ray absorption and scatter by different tissue types, enabling tissue discrimination with high reproducibility [162]. DXA offers several key advantages for research applications, including excellent precision for detecting changes over time, regional body composition analysis

capability, and extensive validation against criterion methods [163, 164]. The technique provides valuable information about fat distribution patterns and appendicular LM, making it particularly useful for sarcopenia research and metabolic health studies [165, 166]. However, DXA has important limitations, including the need for specialist facilities, the exposure of the individual to radiation, assumptions about tissue hydration that may vary with age, disease states, and significant weight change [167]. Additionally, accuracy can be influenced by body size, ethnic differences in body composition, and technical factors related to equipment and software variations [162, 168]. Despite these limitations, DXA remains the preferred method for research requiring precise body composition measurements [161]. Given these considerations, DXA represents the optimal choice for studies requiring precise longitudinal assessment of body composition changes, particularly in populations undergoing interventions to bring about metabolic change.

1.3.3.2 Field Methods

Field methods for assessing body composition offer practical alternatives that balance accuracy, accessibility, and ease of use compared with laboratory-based reference methods [17]. These techniques have gained importance in clinical practice and large-scale research studies, where more advanced methods may be impractical or too costly [160, 169]. These fields methods are described in the following sections:

I. Bioelectrical Impedance Analysis (BIA)

BIA measures the body's resistance to an electrical current, based on the principle that different tissues conduct electricity at varying rates depending on their water and electrolyte content [170, 171]. Various BIA technologies are available, including single-frequency, multi-frequency, and bioimpedance spectroscopy, each offering different levels of precision and application suitability [170, 172]. BIA offers significant practical advantages, including portability, rapid measurement, minimal

participant burden, and reasonable accuracy when appropriate population-specific equations are used [170]. The method has been validated against reference methods across diverse populations, though accuracy varies considerably depending on the specific technology and population studied [173, 174]. Key limitations include dependence on hydration status, population-specific validation requirements, and reduced accuracy in individuals with extreme body compositions or altered fluid status [175, 176]. Environmental factors, recent food intake, and exercise can also influence results, requiring standardised measurement conditions for reliable outcomes [170, 177]. These characteristics make BIA a valuable complement to reference methods in large-scale studies where practical constraints limit the use of more sophisticated techniques.

II. Anthropometric Methods

Anthropometric measurements represent traditional yet enduring approaches to body composition assessment, which offer practical advantages in both clinical and field settings despite their inherent limitations. The theoretical foundation rests on the established relationships between external body measurements and internal body composition, though these relationships vary across different populations and body types [178]. These relationships are explained by anatomical principles connecting subcutaneous adipose tissue deposits to total body fatness [179]. While these associations demonstrate statistical significance across diverse populations, the strength and nature of these relationships are influenced by factors such as age, sex, ethnicity, and physical activity status [180]. The distribution of subcutaneous versus visceral adipose tissue introduces additional complexity, as individuals with similar external measurements may exhibit significantly different internal fat distributions [181].

While BMI (calculated from an individual's height and weight) remains widely used due to its simplicity, its significant limitations in differentiating tissue types and assessing health risks have necessitated the development and validation of more sophisticated assessment approaches [30]. For example, BMI cannot distinguish between muscle and fat mass, leading to misclassification of athletes with high muscle mass as overweight or obese despite healthy body fat percentages [182]. Similarly, older adults may maintain normal BMI despite significant muscle loss and increased fat mass, a condition known at its extreme as sarcopenic obesity that is associated with increased risk of metabolic diseases but remains undetected by BMI screening [183]. These limitations are especially problematic in intervention studies where the quality of weight loss, specifically the preservation of LM while reducing fat mass, is often more important for metabolic outcomes than total weight change.

Skinfold thickness measurements involve using calibrated callipers to measure the thickness of a double fold of skin and subcutaneous adipose tissue at standardised anatomical sites [17]. Multiple regression equations have been developed to estimate body density and subsequently body fat percentage, with the seminal work of Durnin and Womersley *et al.* [184] and Jackson and Pollock *et al.* [185] establishing influencing protocols. Validation studies comparing skinfold measurements with reference methods have demonstrated correlation coefficients ranging from 0.70 to 0.84 for body fat percentage estimation [186, 187]. Chambers *et al.* [188] found stronger correlations with DXA-derived body fat percentages in normal-weight adults (R=0.79-0.84) compared with individuals with obesity (R=0.65-0.72), highlighting the influence of adiposity level on measurement accuracy. Similarly, Bacchi *et al.* [189] reported significant differences in measurement bias across ethnic groups, with greater systematic underestimation in Asian populations. Examiner skill significantly influences measurement quality, with intra-observer technical errors ranging from 3% to 10% even among

trained practitioners [190, 191]. The selection of appropriate prediction equations is crucial, as equations developed for specific populations may produce systematic errors when applied to different groups [192]. Furthermore, measurement reliability tends to decrease with increasing adiposity, creating challenges when assessing individuals with obesity [193].

The WHO established specific cut-off points for waist circumference (≥94 cm for men and ≥80 cm for women in Europids) and waist-to-hip ratio (≥0.90 for men and ≥0.85 for women) to identify individuals at increased cardiometabolic risk [194]. These measurements often provide more relevant clinical information than BMI alone [32, 195]. Recent developments in anthropometric assessment include composite indices that combine multiple measurements to improve the estimation of body composition and health risk. The Body Adiposity Index (BAI), calculated from hip circumference and height, was developed by Bergman *et al.* [196] as an alternative to BMI for estimating body fat percentage. The Conicity Index and Body Roundness index have been proposed as measures that may better capture the health implications of body fat distribution [197, 198]. However, validation studies show mixed results, underscoring the necessity for population-specific validation and careful interpretation [199, 200].

The application of anthropometric methods requires careful consideration of population-specific factors that can influence measurement accuracy and interpretation. Age, sex, ethnicity, and physical activity level all affect the relationships between external measurements and internal body composition [201, 202]. Children and adolescents require specialised approaches due to changing body proportions during growth and development [203]. Older adults populations present unique challenges due to changes in fat distribution and muscle mass with ageing [204]. Athletic populations often demonstrate systematic errors when standard

equations are applied, necessitating sport-specific approaches [205]. Despite their limitations, anthropometric measurements continue to play an important role in body composition assessment, particularly in resource-limited settings and large-scale epidemiological studies. When properly executed and interpreted with an understanding of their limitations, these methods provide valuable information about body composition and fat distribution patterns that can inform clinical decision-making and research endeavours [206].

1.4 Physical Activity and Body Composition

1.4.1 The Relationship Between Physical Activity and Body Composition

Physical activity plays an important role in maintaining a healthy body composition and has been established as a key factor in obesity prevention and management. Regular physical activity contributes to energy expenditure, which is a fundamental component of energy balance, and has direct effects on body composition through the preservation and development of lean body mass and the reduction of fat mass [102]. Evidence from cross-sectional studies consistently demonstrates that physically active individuals maintain lower body fat percentages and higher LM compared with their sedentary counterparts, across the lifespan [207]. The relationship between physical activity and body composition is bidirectional. While physical activity influences body composition, body composition also appears to impact physical activity performance and participation [208]. Higher lean body mass is associated with greater strength, power, and functional capacity, which may facilitate engagement in physical activity. Conversely, excess adiposity may lower movement efficiency and increase the physiological cost of activity, which may lead to reduced physical activity participation [209].

1.4.2 Assessment of Physical Activity

Accurate assessment of physical activity is essential for understanding its relationship with body composition and for evaluating intervention effectiveness. Physical activity assessment methods can be broadly categorised as subjective (self-report) and objective measures, each with distinct advantages and limitations.

1.4.2.1 Subjective Assessment Methods

Subjective assessment methods include the use of questionnaires, activity logs, and physical activity diaries. These methods are widely used in epidemiological studies due to their practicality, low cost, and ability to capture contextual information about physical activity [210]. Among the self-report instruments, the Baecke Physical Activity Questionnaire has been extensively utilised in research settings since its development in 1982 [211]. The Baecke questionnaire assesses habitual physical activity across three domains: work-related activity, sports participation, and leisure-time activity (excluding sports). It provides separate indices for each domain, as well as a total activity score [211]. The questionnaire has demonstrated acceptable reliability, with test-retest correlation coefficients ranging from 0.74 to 0.88 for the different indices [212]. However, validation studies comparing the Baecke questionnaire with objective measures have shown variable results, with correlation coefficients ranging from 0.20 to 0.54 when compared with doubly labelled water or accelerometry [213]. It is important to note that self-report measures are susceptible to limitations such as recall bias, social desirability bias, and difficulty in quantifying activity intensity and duration accurately [214]. These limitations are particularly relevant in populations with overweight and obesity, where evidence suggests overestimation of physical activity levels and underestimation of sedentary time compared with objective measures [215].

1.4.2.2 Objective Assessment Methods

Objective assessment methods include accelerometry, pedometry, heart rate monitoring, and combined sensing devices. Among these, accelerometry has emerged as the most commonly used objective method in research settings due to its ability to provide detailed information on physical activity patterns and intensity [216]. Accelerometers are electronic devices that measure acceleration along one, two, or three orthogonal axes, which provide information on movement frequency and duration. While exercise intensity can only be accurately calculated when heart rate (HR) has been simultaneously measured, many accelerometer devices use algorithms to estimate this measure [217]. These devices can capture data over extended periods, but typically 4-7 days are reported to reflect habitual physical activity patterns. Modern accelerometers can also detect body position, allowing for the assessment of sedentary behaviour [218]. Accelerometry data are typically expressed as "counts" per unit time, which can be translated into metrics such as minutes spent in different intensity categories (sedentary, light, moderate, vigorous), total daily activity counts, or steps per day using proprietary algorithms [219]. Accelerometers have demonstrated strong validity against criterion measures such as doubly labelled water for estimating energy expenditure (R=0.70-0.89) and direct observation for measuring time spent in moderate-to-vigorous physical activity (R=0.80-0.90) [220]. However, accelerometry also has limitations. These devices may not accurately capture certain activities such as cycling, swimming, or resistance exercise, and the translation of accelerometer counts to meaningful physiological parameters remains challenging due to variations in data processing methods and cut-points for intensity thresholds [217]. Accuracy errors can also be introduced depending on device placement, with many modern wearables worn around the wrist potentially overestimating activity due to arm movements during non-locomotor activities [221, 222]. Additionally, the higher cost and technical expertise required for data processing limit their use in large-scale epidemiological studies.

1.4.3 Physical Activity, Age, and Lean Body Mass

The age-related decline in physical activity is well-documented across populations and is associated with concomitant reductions in lean body mass [223, 224]. Cross-sectional data indicate that physical activity levels decrease by approximately 7% per decade during adulthood, with more pronounced declines after age 60y [225]. This reduction in physical activity coincides with the age-related decline in lean body mass, commonly termed sarcopenia, which accelerates after the fifth decade of life [226]. Longitudinal studies have demonstrated that higher levels of physical activity, particularly resistance exercise, can attenuate the age-related decline in lean body mass [227]. A meta-analysis of resistance training interventions in older adults showed an average increase in lean body mass of 1.1 kg (95% CI: 0.9-1.2 kg) following resistance training programs [227]. Moreover, the maintenance of regular physical activity throughout adulthood has been associated with 25-50% higher lean body mass in later life compared with sedentary individuals [228].

In intervention studies, the relationship between physical activity and lean body mass is influenced by several factors, including sex, baseline fitness, nutritional status, and hormonal milieu [229]. Men typically exhibit greater absolute increases in LM in response to physical activity interventions, but relative changes are often similar between sexes [230]. Additionally, individuals with higher baseline LM may demonstrate attenuated responses to exercise interventions due to a ceiling effect [227].

1.5 Dietary Assessment and Body Composition

1.5.1 The Role of Diet in Body Composition

Dietary intake plays a fundamental role in determining body composition, by directly influencing energy balance and nutrient provision. While energy balance (dietary energy consumed versus energy expended)

largely determines changes in total body fat mass, the macronutrient composition of the diet can influence the proportion of fat mass to lean body mass [231]. This relationship is particularly important in the context of weight management interventions, where the preservation of lean body mass while reducing fat mass is a primary goal [232]. Among macronutrients, dietary protein has received considerable attention for its effects on body composition. Protein intake exceeding the Recommended Dietary Allowance (RDA) of 0.8 g/kg body weight has been associated with greater preservation and accretion of lean body mass during both weight maintenance and weight loss interventions [233]. A systematic review and meta-analysis of 49 studies found that higher protein intakes (≥1.2 g/kg/day) during energy restriction resulted in greater retention of LM and greater loss of fat mass compared with lower protein intakes [234]. This protein-sparing effect is attributed to increased muscle protein synthesis, reduced muscle protein breakdown, and greater thermogenesis and satiety associated with protein consumption [235].

In individuals with pre-diabetes, dietary composition takes on additional significance due to its impact on glycaemic control and insulin sensitivity. Evidence suggests that diets higher in protein (20-30% of energy) and lower in refined carbohydrates may improve body composition and glycaemic parameters simultaneously [236]. Multiple randomised clinical trials comparing low and high glycaemic index (GI) diets in the treatment of diabetes have found that low GI diets improve glycaemic control in individuals with impaired glucose metabolism [237]. Additionally, the quality of dietary fat intake may also influence body composition, with different fatty acid profiles exhibiting varying effects on fat distribution and metabolic health. Diets rich in monounsaturated and polyunsaturated fatty acids have been associated with lower visceral adiposity and improved insulin sensitivity compared with diets high in saturated and trans fatty acids, even with equivalent total fat intake [238]. Additionally, emerging evidence suggests that dietary patterns such as the

Mediterranean diet may favourably influence body composition independent of their effects on total body weight [239]. Given the complex relationship between dietary intake and body composition, accurately assessing nutritional intake is essential for both research and clinical applications. However, assessing dietary intake involves various methodological challenges, including measurement errors, reporting biases, and day-to-day variability in consumption patterns [240].

1.5.2 Methods of Dietary Assessment

Dietary assessment methods can be broadly categorised as retrospective (recalling past intake) or prospective (recording current intake over a period of time), with each approach offering distinct advantages and limitations. The selection of an appropriate method depends on the specific research question, population characteristics, available resources, and the level of detail required [241].

1.5.2.1 Retrospective Methods

Retrospective dietary assessment methods include 24-hour dietary recalls, food frequency questionnaires (FFQ), and diet history interviews. These methods are based on participants' memory of past food consumption and do not require literacy or a significant participant burden, making them suitable for large-scale epidemiological studies [242]. The 24-hour dietary recall involves a trained interviewer guiding participants through a comprehensive recollection of all foods and beverages consumed during the previous 24 hours. Multiple recalls (typically 2-3) are recommended to account for day-to-day variability and improve the representation of habitual intake [240]. The 24-hour recall method has been widely used in national nutrition surveys and epidemiological studies, benefiting from relatively low participant burden and the ability to capture detailed information about food preparation and eating patterns [241].

Food frequency questionnaires (FFQs) assess habitual intake of specified food items over a defined period (typically the past month or year). FFQs can be tailored to specific populations or research questions, focusing on foods that contribute significantly to nutrients of interest [242]. While FFQs are efficient for ranking individuals according to their relative intake of specific nutrients or food groups, and may enable capture of seasonal variability of nutrient intakes, they may not provide accurate estimates of absolute intake due to limitations in food list comprehensiveness and portion size estimation [241]. Diet history interviews combine elements of 24-hour recalls and FFQ to obtain a comprehensive assessment of usual dietary patterns. This method captures information about meal patterns, food preferences, seasonal variations, and cultural influences on dietary habits [242]. While diet history interviews provide rich contextual data, they require significant time and expertise from interviewers, limiting their practicality for large-scale studies [241].

1.5.2.2 Prospective Methods

Prospective dietary assessment methods include food records (diaries), duplicate portion analysis, and direct observation. These methods do not rely on memory and can provide detailed information about actual consumption patterns, portion sizes, and food preparation techniques [243]. Food records involve participants documenting all foods and beverages consumed in real-time over a specified period, typically ranging from 3 to 7 days. Participants record detailed information about food types, preparation methods, brand names, and portion sizes, often using weighing scales or household measures [243]. Food records are considered one of the most accurate methods for assessing current dietary intake, particularly when foods are weighed rather than estimated. However, this method requires significant participant literacy, motivation, and time commitment, which may limit its applicability in certain

populations [241]. Moreover, the act of recording food intake can influence eating behaviour, a phenomenon known as reactivity bias, where participants modify their consumption patterns to simplify recording or to project more socially desirable eating habits [243]. Research suggests that underreporting of energy intake is common with food records, particularly among individuals with overweight and obesity, where underreporting rates of 20-50% have been observed when compared with doubly labelled water estimates of energy expenditure [244].

Duplicate portion analysis involves participants collecting an identical portion of all foods and beverages consumed for subsequent laboratory analysis. This method provides highly accurate nutrient intake data but is labour-intensive, expensive, and impractical for large-scale studies or extended assessment periods [242]. Direct observation, conducted by trained observers in controlled environments such as school cafeterias or metabolic wards, offers an objective assessment of food intake. However, its application is limited to specific settings and may not reflect typical eating patterns due to the presence of observers [241].

1.5.3 Urinary Nitrogen Excretion and Nitrogen Balance

Assessment of protein intake and protein balance represents a critical component of nutritional evaluation, particularly in the context of weight loss interventions where preservation of lean body mass is a primary concern. Urinary nitrogen excretion serves as an objective biomarker of protein intake and provides the foundation for calculating nitrogen balance, a key indicator of protein adequacy and metabolic status [722].

Nitrogen balance represents the difference between nitrogen intake (primarily from dietary protein) and nitrogen excretion (primarily through urine, with additional losses through faeces, skin, and other routes). The

fundamental principle underlying nitrogen balance assessment is that, under steady-state conditions, dietary protein intake can be estimated from urinary nitrogen excretion, as approximately 80-90% of ingested nitrogen is ultimately excreted in urine [723]. Each gram of protein contains approximately 16% nitrogen; therefore, urinary nitrogen can be converted to protein intake using established conversion factors, with total nitrogen excretion multiplied by 6.25 to estimate protein intake [736].

The gold standard method for assessing protein intake objectively employs 24-hour urinary nitrogen excretion, requiring complete urine collection over a 24-hour period [722]. This method circumvents the limitations associated with self-reported dietary assessment, including reporting bias, memory errors, and the reactive effects of dietary recording [240]. Urinary urea nitrogen accounts for approximately 80-85% of total urinary nitrogen, with the remainder comprising other nitrogenous compounds including creatinine, ammonia, uric acid, and amino acids [723]. Therefore, measurement of urinary urea nitrogen alone can provide a reasonable estimate of total nitrogen excretion, though direct measurement of total urinary nitrogen using the Kjeldahl method or chemiluminescence provides greater accuracy [722].

Nitrogen balance is calculated using the equation: Nitrogen Balance = Nitrogen Intake – (Urinary Nitrogen + Faecal Nitrogen + Obligatory Losses). Nitrogen intake is determined from dietary protein intake (protein intake in grams divided by 6.25), urinary nitrogen is measured from 24-hour urine collections, faecal nitrogen losses are typically estimated at 1-2 g/day or 10-20% of intake, and obligatory losses through skin, hair, nails, and other routes are estimated at approximately 1 g/day [736, 743]. Positive nitrogen balance indicates that nitrogen intake exceeds excretion, suggesting protein accretion and anabolism, while negative nitrogen balance indicates nitrogen losses exceeding intake, suggesting protein catabolism and potential loss of lean tissue [843].

In the context of weight loss interventions, nitrogen balance assessment provides crucial information about the adequacy of protein intake for maintaining lean body mass during energy restriction. Energy restriction typically induces negative nitrogen balance due to increased protein catabolism for gluconeogenesis and energy provision, with the magnitude of negative balance influenced by the degree of energy deficit, protein intake, physical activity level, and individual characteristics [844]. Research demonstrates that higher protein intakes during energy restriction (≥1.2 g/kg/day) can minimise negative nitrogen balance and attenuate lean mass loss compared to lower protein intakes, highlighting the importance of adequate protein provision in weight loss protocols [234, 845].

Urinary nitrogen excretion also serves a critical role in validating self-reported dietary protein intake, addressing a persistent challenge in nutritional research. Validation studies employing urinary nitrogen as an objective biomarker consistently demonstrate that self-reported protein intake frequently underestimates actual intake, with the degree of underreporting varying according to characteristics including body mass index, sex, and social desirability [725, 735]. The agreement between self-reported intake and urinary nitrogen biomarker typically demonstrates correlation coefficients of 0.3-0.6, indicating moderate but imperfect agreement [846]. This relationship can be affected by numerous factors including completeness of urine collection, day-to-day variation in protein intake, renal function, hydration status, and the timing of assessment relative to dietary intake [723]. Understanding these sources of variation and their implications for interpreting dietary assessment data is essential for rigorous nutritional research and appropriate interpretation of diet-disease relationships.

Methodological considerations for urinary nitrogen assessment include ensuring complete 24-hour urine collection, which represents a

significant challenge as incomplete collections introduce substantial measurement error [723]. Collection completeness can be assessed using urinary creatinine excretion or para-aminobenzoic acid recovery methods, though these approaches have their own limitations [767]. Additionally, nitrogen balance calculations assume steady-state conditions, which may not apply during periods of rapid weight change or metabolic adaptation, potentially affecting the accuracy of protein intake estimates derived from urinary nitrogen in dynamic states [743].

1.6 Weight Loss Interventions

1.6.1 Overview of Weight Management Approaches

Weight management interventions encompass a wide range of approaches to reduce or maintain body weight to improve health outcomes. These interventions can be broadly classified as dietary modifications, physical activity programs, behavioural therapies, pharmacological treatments, and surgical procedures, with many comprehensive programs incorporating multiple components [245]. The selection of appropriate interventions depends on several factors, including the degree of excess weight, the presence of comorbidities, individual preferences, available resources, and previous weight loss attempts [246]. For individuals with overweight (BMI 25-29.9 kg/m²) and moderate obesity (BMI 30-34.9 kg/m²), lifestyle interventions focusing on dietary modifications and increased physical activity remain the first-line approach [245]. These interventions typically aim for a 5-10% reduction in initial body weight over 6-12 months, which has been consistently associated with clinically significant improvements in cardiometabolic risk factors, including insulin sensitivity, blood pressure, and lipid profiles [247]. For individuals with more severe obesity (BMI ≥35 kg/m²), particularly those with established comorbidities, more intensive approaches, including very-low-energy diets, pharmacotherapy, or bariatric surgery, may be appropriate [246].

Numerous factors, including intervention intensity, delivery format, duration, and participant characteristics, influence the efficacy of weight management interventions [248]. Programmes that comprehensively address various aspects of weight regulation, provide consistent monitoring and feedback, and include ongoing support for weight maintenance, have demonstrated better outcomes than less intensive methods [249]. However, significant challenges remain in maintaining weight loss over the long term, with many individuals regaining a substantial portion of lost weight within 2-5 years after initial intervention [250]. Recent advances in the understanding of body weight regulation have highlighted the importance of accounting for physiological adaptations to weight loss, including reductions in energy expenditure, alterations in appetite-regulating hormones, and changes in neural responses to food cues, all of which may promote weight regain [251]. This recognition has led to the development of novel intervention approaches focused not only on initial weight loss but also on countering these compensatory mechanisms to support long-term weight maintenance [252].

1.6.2 Low-Energy Diets and Very-Low-Energy Diets

Low-energy diets (LED) and very-low-energy diets (VLED) represent dietary interventions characterised by substantial energy restriction, typically providing 800-1200 kcal/day (3.3-5.0 MJ/day) and <800 kcal/day (<3.3 MJ/day), respectively [253]. These approaches have gained increasing attention in obesity management due to their ability to induce rapid weight loss, particularly in clinical settings where substantial weight reduction is needed to address obesity-related comorbidities or prepare patients for procedures [254]. LED and VLED are typically implemented using nutritionally complete meal replacements, including shakes, soups, and bars, which provide precisely controlled energy and macronutrient content while ensuring adequate micronutrient intake [255]. The

controlled nature of these interventions simplifies dietary adherence by reducing decision-making burden and environmental exposure to high-energy foods [253]. Modern formulations of LED typically provide higher protein content (≥25% of energy or ≥1.2 g/kg ideal body weight) than conventional energy-restricted diets, aiming to preserve lean body mass during rapid weight loss [253].

The efficacy of LED in inducing substantial weight loss has been well-established. A systematic review and meta-analysis of 12 studies found that LED resulted in average weight losses of 9.7 kg over 12 weeks, with adherence rates of 70-80% [256]. The DROPLET study, a randomised clinical trial conducted in primary care settings, showed that participants following an 8-week LED program (810 kcal/day) achieved an average weight loss of 10.7 kg, significantly greater than the 3.1 kg lost in the usual care group [255]. Similar findings were reported in the DiRECT trial, where participants with T2DM lost an average of 10.0 kg after 12 weeks on an 825-850 kcal/day LED, with 86% achieving at least 15 kg weight loss [257].

The impact of LED on body composition has received particular attention due to concerns about the potential loss of lean body mass during rapid weight loss. Research indicates that the proportion of weight lost as lean tissue varies considerably depending on several factors, including the protein content of the diet, concurrent physical activity, baseline body composition, age, and sex [232]. A systematic review by Chaston *et al.* [258] found that rapid weight loss induced by VLED resulted in approximately 25% of weight loss from lean body mass, compared with 17% with moderate energy restriction. However, more recent studies incorporating higher protein content (≥1.2 g/kg/day) and resistance exercise have shown improved preservation of LM, with as little as 10-15% of weight loss coming from lean tissue [259].

Despite their efficacy in inducing rapid weight loss, LED interventions face significant challenges regarding long-term weight maintenance. The transition from a structured meal replacement program to conventional food requires careful planning and support to prevent rapid weight regain [255]. The OPTIWIN study addressed this challenge by implementing a comprehensive lifestyle intervention following the LED phase, focusing on a gradual transition to conventional foods, ongoing meal replacements, and behavioural strategies, including frequent counselling sessions [260]. This approach resulted in maintenance of 10.5% weight loss at 12 months, significantly better than the 2.5% maintained in the standard care group, representing better maintenance outcomes than many previous studies [260]. Additional research by Purcell *et al.* [261] demonstrated that including a structured food reintroduction phase and ongoing intermittent meal replacements after LED completion can help maintain 9.6 kg weight loss at one year.

Recent advances in LED implementation include the development of more flexible approaches, such as intermittent use of meal replacements, partial meal replacement strategies, and the integration of LED within broader lifestyle intervention programs [254]. These adaptations aim to improve long-term adherence while maintaining the benefits of structured energy restriction. Additionally, increasing recognition of the importance of addressing physiological adaptations to weight loss has led to research on complementary strategies, such as pharmacotherapy or specific exercise regimens, to counteract reductions in energy expenditure and increases in appetite that occur during weight loss [252].

1.6.3 Intermittent Fasting Approaches

Intermittent fasting (IF) encompasses a family of dietary regimens that alternate between periods of severely restricted or zero energy intake and periods of *ad libitum* or controlled feeding [262]. These approaches have gained substantial popularity in recent years, both in clinical settings

and among the general public, due to their purported metabolic benefits beyond weight loss and their perceived simplicity compared with continuous energy restriction [263]. Several distinct IF protocols have been studied, including:

- alternate day fasting (ADF), which alternates between "fast days" with minimal energy intake (0-25% of needs) and "feast days" with *ad libitum* consumption.
- the 5:2 diet, consisting of five days of regular eating and two non-consecutive "fast days" with severely restricted intake (500-600 kcal).
- Time-restricted eating (TRE) involves 12-20 hours of daily fasting, with all food consumption occurring within a defined window.
- Periodic fasting, characterised by more extended fasting periods (2-7 days), is implemented less frequently, typically monthly or quarterly.
- Religious fasting, such as Ramadan, where fasting occurs from dawn to sunset for one lunar month.

Each of these approaches differs in its fasting duration, frequency, and degree of energy restriction, potentially leading to distinct physiological responses and practical considerations for implementation [263].

Based on a growing body of evidence, it has been established that IF can effectively help people lose weight. A meta-analysis of 11 randomised controlled trials found that IF approaches produced comparable weight loss to continuous energy restriction, with average reductions of 4-8% of initial body weight over 3-12 months [264]. Similarly, a systematic review by Seimon *et al.* [265] concluded that intermittent energy restriction achieved equivalent weight loss to continuous energy restriction, with no significant differences in dropout rates between approaches.

Furthermore, the effects of IF on body composition have received particular attention, with some studies suggesting potential advantages for LM preservation compared with continuous energy restriction. The meta-analysis by Varady *et al.* [266] found that ADF and TRE resulted in a higher proportion of fat mass loss relative to total weight loss when compared with continuous energy restriction, although the absolute differences were modest. This apparent benefit has been attributed to various mechanisms, including the preservation of resting energy expenditure, enhanced fat oxidation during fasting periods, and potential stimulation of growth hormone secretion, which may promote lipolysis while preserving muscle protein [267].

Beyond weight loss and body composition, IF approaches have demonstrated various metabolic effects that may be partially independent of weight loss. These include improvements in insulin sensitivity, lipid profiles, inflammatory markers, and cellular stress resistance [262]. Time-restricted eating, even without significant weight loss, has been shown to improve glucose tolerance, reduce circulating insulin concentration, and enhance fat oxidation, particularly when the feeding window aligns with circadian rhythms [268]. These findings suggest that the timing of energy intake, in addition to its quantity, may influence metabolic outcomes through mechanisms involving circadian biology and the temporal coordination of metabolic processes [269].

Ramadan fasting represents a unique form of intermittent fasting practised by millions of Muslims worldwide, characterised by abstention from food and drink from dawn to sunset for approximately 29-30 days [270]. This practice differs from other IF protocols in several respects, including its religious and cultural significance, fixed calendar timing, daily repetition for a defined period, and complete abstention from both food and water during daylight hours [270]. The effects of Ramadan fasting on body weight and composition have been extensively studied,

with a meta-analysis of 70 studies showing a modest but significant weight loss of 1.34 kg on average during the month, with greater reductions observed in men compared with women and in individuals with overweight or obesity compared with those with normal weight [271]. Ramadan fasting typically results in modest changes in body composition, with reductions in both fat mass and LM commonly reported [271]. The relative preservation of LM during Ramadan appears to be influenced by protein intake, physical activity patterns, and baseline nutritional status [272]. Studies integrating resistance exercise during Ramadan have shown better maintenance of LM despite overall weight loss, highlighting the importance of physical activity even during religious fasting periods [272].

Despite the growing popularity of IF approaches, several limitations and considerations must be acknowledged. Long-term adherence to IF regimens remains a concern, with some studies reporting higher dropout rates compared with continuous energy restriction, particularly for more restrictive protocols like ADF [273]. Individual responses to IF appear to vary considerably, with factors such as baseline weight, metabolic health, age, sex, and genetic factors potentially influencing outcomes [274]. Additionally, certain populations, including pregnant or breastfeeding women, individuals with diabetes on medication, those with a history of eating disorders, and children or adolescents, may face increased health risks with using IF approaches and require careful medical supervision if these methods are considered [262].

1.6.4 Energy Intake Regulation and the Gut-Brain Axis

The regulation of energy intake represents a highly complex physiological process involving intricate bidirectional communication between the gastrointestinal tract and the central nervous system, collectively termed the gut-brain axis [275]. This sophisticated regulatory system integrates peripheral signals from multiple organs with central

processing in the hypothalamus and brainstem to coordinate food intake, energy expenditure, and metabolic homeostasis [847]. Understanding this system is fundamental to comprehending the biological mechanisms underlying obesity development and the challenges associated with sustained weight loss.

The gut-brain axis operates through multiple complementary signalling pathways, including neural pathways via the vagus nerve, endocrine signals through appetite-regulating hormones, and immune-mediated signals involving inflammatory cytokines [848]. The vagus nerve provides rapid neural feedback from the gastrointestinal tract to the brainstem, conveying information about gastric distension, nutrient composition, and chemical signals from the gut epithelium [849]. This neural pathway enables real-time communication about meal ingestion and contributes to the immediate satiation response that terminates individual eating episodes. Complementing these rapid neural signals, the endocrine arm of the gut-brain axis involves the secretion of numerous peptide hormones from specialised enteroendocrine cells distributed throughout the gastrointestinal tract [850].

Appetite-regulating hormones can be broadly classified as either orexigenic (appetite-stimulating) or anorexigenic (appetite-suppressing) factors [851]. The primary orexigenic hormone is ghrelin, a peptide predominantly synthesised and secreted by specialised cells in the gastric fundus [387]. Ghrelin levels rise pre-prandially in anticipation of meals and decline following food intake, with circulating concentrations exhibiting a reciprocal relationship with body adiposity [852]. Ghrelin exerts its orexigenic effects by binding to growth hormone secretagogue receptors in the arcuate nucleus of the hypothalamus, stimulating the release of neuropeptide Y and agouti-related peptide, which promote hunger and food-seeking behaviours while simultaneously reducing energy expenditure [390].

The anorexigenic hormone system comprises multiple peptides secreted from different regions of the gastrointestinal tract in response to nutrient ingestion. Cholecystokinin (CCK), secreted from duodenal I-cells in response to dietary fats and proteins, represents one of the earliest discovered satiety signals [853]. CCK acts through both vagal afferent pathways and direct effects on hypothalamic neurons to induce meal termination and promote satiation [853]. Peptide YY (PYY), released from L-cells in the distal ileum and colon in proportion to caloric intake, exerts prolonged anorexigenic effects by inhibiting neuropeptide Y neurons in the arcuate nucleus [854]. Glucagon-like peptide-1 (GLP-1), co-secreted with PYY from intestinal L-cells, functions as both an incretin hormone enhancing glucose-dependent insulin secretion and an appetite-suppressing agent that delays gastric emptying and reduces food intake [855].

Beyond gastrointestinal peptides, adipose tissue contributes to energy intake regulation through the secretion of leptin, a hormone whose circulating concentrations reflect total body fat mass [856]. Leptin signals long-term energy status to the hypothalamus, where it inhibits orexigenic pathways and activates anorexigenic pathways, thereby providing feedback regulation of body adiposity [856]. In individuals with obesity, leptin resistance develops, characterised by diminished hypothalamic sensitivity to leptin’s appetite-suppressing effects despite elevated circulating leptin concentrations, contributing to the maintenance of the obese state [857]. The pancreatic hormone insulin similarly provides adiposity signalling to the central nervous system, crossing the blood-brain barrier to inhibit food intake and promote energy expenditure, with insulin resistance in the hypothalamus contributing to impaired energy homeostasis in obesity [858].

The integration of these multiple peripheral signals occurs primarily in the hypothalamic arcuate nucleus, which contains two key neuronal populations: orexigenic neuropeptide Y/agouti-related peptide neurons and anorexigenic pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons [859]. These first-order neurons project to second-order neurons in other hypothalamic nuclei and to brainstem centres, creating a distributed neural network that coordinates appropriate behavioural and metabolic responses to maintain energy balance [860]. This system demonstrates remarkable plasticity, with neuronal responses to satiety signals becoming attenuated in obesity, contributing to the biological resistance to sustained weight loss [861].

Understanding the gut-brain axis has important implications for obesity treatment strategies. The recognition that appetite regulation involves complex, redundant systems that adapt to weight loss helps explain why sustained weight loss proves challenging for most individuals and why pharmacological approaches targeting single hormones often demonstrate limited efficacy [862]. Recent therapeutic developments, including GLP-1 receptor agonists that have demonstrated substantial weight loss efficacy, capitalise on understanding of these pathways, though the full therapeutic potential of targeting the gut-brain axis remains to be realised [324].

1.6.5 Effects of Weight Loss Interventions on Hormonal Regulation of Appetite

Weight loss interventions induce changes in the hormonal regulation of appetite, with implications for both short-term adherence and long-term weight maintenance [251]. These adaptations represent physiological responses to energy deficit and weight loss, serving to protect against further energy depletion by increasing hunger, reducing satiety, and ultimately promoting weight regain [252]. The hormonal regulation of appetite involves complex interactions between peripheral signals from

the gastrointestinal tract, adipose tissue, and pancreas, and central integration in hypothalamic and brainstem nuclei [275]. Key orexigenic (appetite-stimulating) hormones include ghrelin, primarily secreted by the stomach, while anorexigenic (appetite-suppressing) signals include PYY, GLP-1, CCK from the intestine, leptin from adipose tissue, and insulin from the pancreas [276].

Weight loss through energy restriction consistently alters this hormonal milieu in a manner that may promote increased energy intake. A seminal study by Sumithran *et al.* [277] demonstrated that 10% weight loss induced significant increases in circulating ghrelin and decreases in PYY, GLP-1, CCK, insulin, and leptin, with these changes persisting for at least one year despite weight stabilisation. These findings suggest that compensatory hormonal adaptations to weight loss are not transient but may represent long-term biological responses that predispose to weight regain. The magnitude and persistence of these hormonal changes appear to be influenced by several factors, including the rate of weight loss, dietary composition, and level of physical activity [278]. Rapid weight loss induced by VLED typically results in more pronounced changes in appetite hormones compared with gradual weight loss through moderate energy restriction, potentially contributing to the challenges of maintaining weight after intensive interventions [279]. A study by Purcell *et al.* [261] comparing rapid weight loss (12 weeks) with gradual weight loss (36 weeks) found that despite similar total weight reductions, the rapid weight loss group experienced greater increases in ghrelin and reductions in leptin, PYY, and GLP-1 concentration.

The macronutrient composition of weight loss diets may modulate these hormonal responses, with higher protein intake generally associated with more favourable profiles. Acutely, protein intake has been shown to suppress ghrelin secretion more effectively than carbohydrates or fats, while stimulating greater secretion of PYY and GLP-1, potentially

contributing to its established satiating effects [235]. A study by Wycherley *et al.* [280] demonstrated that higher protein intake (33% vs. 22% of energy) during energy restriction resulted in attenuated increases in ghrelin and greater increases in PYY, associated with reduced hunger and higher satiety ratings.

Physical activity in conjunction with dietary intervention may also mitigate unfavourable changes in appetite hormones. Several studies have shown that exercise can acutely suppress ghrelin while increasing PYY and GLP-1, effects that may persist for hours after activity cessation [281]. Moreover, regular physical activity during weight loss appears to improve sensitivity to satiety signals, potentially through mechanisms involving enhanced insulin sensitivity and reduced inflammation [282]. The DIRECT study (which focused on different dietary approaches rather than the diabetes remission protocols developed by Lean *et al.* [257] and Taylor *et al.* [283]) demonstrated that increased physical activity following the weight loss phase was associated with better maintenance of weight loss and improved appetite control, potentially mediated by more favourable appetite hormone profiles [284].

Intermittent fasting approaches may offer unique effects on appetite regulation compared with continuous energy restriction. Some studies suggest that ADF and TRE can lead to adaptations in hunger and fullness perceptions over time, with reduced hunger during fasting periods after several weeks of implementation [285]. These adaptations may involve ghrelin suppression during fasting windows and enhanced postprandial secretion of anorexigenic hormones like PYY and GLP-1 during feeding periods [286]. However, the sustainability of these adaptations and their relevance to long-term weight management remain uncertain.

Ramadan fasting represents a unique form of intermittent fasting that, unlike LED/VLED interventions, is practised for religious rather than weight management purposes and presents a distinct model for studying appetite regulation during intermittent fasting due to its consistent pattern of daytime abstention and night-time feeding for a defined period. The impact of Ramadan fasting on appetite regulation is of particular interest due to the substantial changes in meal timing and frequency. Studies examining appetite-regulating hormones during Ramadan have shown complex and sometimes contradictory results. These findings reflect the heterogeneity in study populations, fasting duration, dietary composition, and timing of measurements [287]. Several studies have observed changes in appetite-regulating hormones during Ramadan, with some reporting alterations in circulating ghrelin, leptin, peptide YY, and glucagon-like peptide-1 concentration that may reflect physiological adaptations to the altered meal patterns [288-290]. Notably, Alzoghaibi *et al.* [291] found increased ghrelin concentrations during Ramadan fasting, particularly before the sunset meal, potentially reflecting anticipatory responses to scheduled feeding.

A systematic review and meta-analysis specifically examining the effects of Ramadan fasting on appetite-regulating hormones would provide valuable insights into these complex relationships. Such analysis could clarify the temporal dynamics of hormone changes throughout the day and across the month, potential moderating factors such as fasting duration and dietary composition, and implications for weight management strategies based on intermittent fasting.

Understanding the hormonal adaptations to weight loss has important implications for intervention design and maintenance strategies. Approaches that attenuate unfavourable hormone changes, such as higher protein diets, regular physical activity, and possibly intermittent fasting, may improve long-term outcomes by reducing the biological drive

toward weight regain [251]. Additionally, the recognition that these adaptations may persist indefinitely after weight loss has led to increasing interest in pharmacological approaches that target specific appetite pathways, such as GLP-1 receptor agonists, which have shown promising results for both weight loss induction and maintenance and are now an established treatment option for weight loss in those with obesity [252]. The UK National Institute for Health and Care Excellence (NICE) guidelines recommend GLP-1 receptor agonists as a treatment option for weight management in adults with obesity when prescribed as part of a multidisciplinary tier 3 weight management service [292].

1.7 Overweight, obesity and diabetes risk

The relationship between obesity and T2DM is so interconnected that it has led to the emergence of the term 'diabesity', emphasising the growing public health challenge where these conditions frequently coexist [293]. In the UK, the systemic effects of obesity-induced metabolic dysfunction manifest as a 6-fold greater risk of developing T2DM [43], compared with healthy weight individuals.

The progression in obesity from healthy glucose regulation to T2DM follows a well-characterised pathway, where excess adiposity drives insulin resistance and progressive β-cell dysfunction [150, 294]. Initially, pancreatic β-cells attempt to compensate for insulin resistance by increasing insulin production, leading to hyperinsulinaemia [295]. However, this compensatory mechanism eventually fails, resulting in decreased insulin production and ultimately, β-cell death [296, 297], and a failure to control blood glucose concentration within a healthy range.

1.7.1 Physical Activity and Body Composition in Overweight and Pre-Diabetic Populations

Individuals with overweight, obesity, and pre-diabetes often exhibit distinct physical activity patterns and body composition profiles compared with metabolically healthy populations. Cross-sectional studies indicate that adults with pre-diabetes engage in approximately 40% less moderate-to-vigorous physical activity than individuals with normal glucose tolerance [298]. Furthermore, individuals with pre-diabetes typically display lower lean body mass relative to total body weight and higher visceral adiposity compared with BMI-matched individuals with healthy glucose metabolism [299]. In individuals with pre-diabetes, the relationship between physical activity and body composition takes on additional significance due to the metabolic implications. Higher LM, particularly skeletal muscle, serves as the primary site for glucose disposal and is associated with improved insulin sensitivity [157]. Physical activity, especially resistance exercise, promotes glucose uptake through both insulin-dependent and insulin-independent pathways, which makes it a valuable intervention for pre-diabetes management [300]. In adults with pre-diabetes, the preservation of lean body mass through physical activity takes on added importance due to its metabolic implications. Higher lean body mass is associated with improved insulin sensitivity and glucose disposal capacity, which may help prevent or delay the progression to T2DM [157]. Emerging evidence suggests that the quality of lean tissue, particularly factors such as intramuscular lipid content and mitochondrial function, may be as important as total LM in determining metabolic health [228].

Physical activity interventions in overweight and pre-diabetic populations have demonstrated significant benefits for body composition and metabolic health. The Diabetes Prevention Program (DPP) showed that a lifestyle intervention emphasising physical activity (150 minutes per week of moderate activity) and modest weight loss (7% of initial body

weight) reduced the incidence of T2DM by 58% over three years compared with a control group [301]. Subsequent analyses of the DPP revealed that changes in physical activity were independently associated with improvements in insulin sensitivity, even after controlling for changes in body weight [302].

Changes in body composition during weight loss have important implications for long-term outcomes. Greater loss of LM during initial weight reduction has been associated with increased risk of weight regain, potentially due to reductions in resting energy expenditure, diminished exercise capacity, and impaired metabolic function [303]. Conversely, interventions that preserve or increase LM while reducing fat mass may promote better weight maintenance through multiple mechanisms, including sustained energy expenditure, enhanced glucose disposal, and improved functional capacity supporting physical activity [304]. Recent studies have explored the specific effects of different physical activity modalities on body composition in pre-diabetic populations. A meta-analysis of 14 randomised controlled trials found that combined aerobic and resistance training resulted in greater improvements in body composition (increased LM, reduced fat mass) and glycaemic control compared with either modality alone [305]. Additionally, emerging evidence suggests that high-intensity interval training may induce comparable or superior improvements in body composition and insulin sensitivity with reduced time commitment compared with traditional continuous aerobic exercise in individuals with pre-diabetes [306]. The STRRIDE-PD study, a randomised controlled trial investigating the effects of different exercise modalities on diabetes prevention, demonstrated that aerobic exercise training significantly improved insulin sensitivity and glucose tolerance in adults with pre-diabetes, with higher volume/moderate intensity exercise producing greater benefits than lower volume/vigorous intensity exercise for reducing diabetes risk [307]. Importantly, the study found that exercise-induced improvements in insulin sensitivity were independent of weight

loss, suggesting that physical activity provides metabolic benefits beyond body composition changes [307]. Baseline data from the LOOK AHEAD study demonstrated positive associations between objectively measured physical activity, lean body mass, and improvements in markers of metabolic health, even after adjusting for confounding factors [308].

1.7.2 Evidence for Intervention: Weight Loss and Diabetes Prevention

Recognising obesity's health consequences provides a strong rationale for intervention, with substantial evidence demonstrating that even modest weight reductions can significantly improve health outcomes and potentially reverse disease processes [309, 310].

The relationship between obesity and diabetes risk demonstrates a clear dose-response relationship, with lifetime diabetes risk increasing dramatically with BMI, rising from 7% to 70% in men and from 12% to 74% in women when BMI increases from less than 18.5 kg/m² to more than 35 kg/m² [311]. This elevated risk, combined with the demographic patterns previously described, creates a convergence of risk factors that demands effective intervention strategies. Successful diabetes prevention strategies must consider not only the magnitude of weight loss required but also the quality of weight loss (preserving LM while reducing adipose tissue), the sustainability of behavioural changes within real-world settings, and the long-term maintenance of both weight loss and metabolic improvements [310, 312]. The evidence for various intervention approaches demonstrates the challenge of sustaining behaviour change and weight maintenance in determining long-term diabetes prevention effectiveness [313]. Of particular relevance to diabetes prevention, the prevalence of obesity in older age groups, combined with the increase in severe obesity categories [47], creates a population at high risk for the development of T2DM [43].

1.7.2.1 Therapeutic Potential of Weight Loss in type 2 diabetes management and remission

Clinical evidence demonstrates that modest weight reductions of 5-10% can significantly reduce T2DM risk and improve glycaemic control in individuals with existing T2DM, regardless of whether individuals remain in the overweight or obese categories [309, 310]. Multiple intervention strategies have shown effectiveness for achieving clinically meaningful weight loss for diabetes prevention and management.

— Lifestyle Intervention Studies

The pioneering Da Qing Diabetes Prevention Study, conducted in China from 1986, was the first randomised controlled trial to demonstrate that lifestyle intervention could prevent T2DM. This landmark study showed that diet and exercise interventions reduced diabetes incidence by 31-46% over 6 years in individuals with impaired glucose tolerance [314]. Remarkably, 23-year follow-up data revealed sustained benefits, with continued reduction in diabetes incidence and cardiovascular mortality, establishing the long-term efficacy of lifestyle intervention [315].

The landmark Diabetes Prevention Program (DPP) subsequently confirmed these findings in a Western population, demonstrating that intensive lifestyle intervention, focused on achieving 7% weight loss and increasing physical activity, reduced diabetes incidence by 58% in high-risk individuals with impaired glucose tolerance over 2.9 years [301]. The Finnish Diabetes Prevention Study (FDP), also implementing an intensive lifestyle (diet and physical activity) counselling program, achieved similar results, with 3.2 kg average weight loss leading to 58% diabetes risk reduction over 3.2 years [316]. UK-based research has confirmed these findings, with the Norfolk Diabetes Prevention Study showing that moderate weight loss through lifestyle intervention could reduce T2DM risk by up to 40-47% in high-risk individuals [317].

Ten-year follow-up of individuals in both the DPP and the FDP showed that although weight regain occurred in the lifestyle intervention groups, on average, they did not return to their pre-intervention weight and retained a lower risk of developing diabetes [318, 319]. Notably, across age categories, the older age group in the DPP showed the smallest weight regain and cumulative frequency of T2DM [318].

— Low Energy Diet Programs

Low-energy diet (LED) approaches have shown efficacy for bringing about weight-loss and T2DM remission. The UK-based Diabetes Remission Clinical Trial (DiRECT) demonstrated that substantial weight loss (≥15kg) through structured low-energy diet programs (825-853 kcal/day for 12-20 weeks**)** could result in diabetes remission for 46% of participants at one year [257]. Participants achieving greater weight loss showed higher remission rates, and those maintaining weight loss for two years were more likely to sustain remission status, highlighting the importance of long-term weight management in controlling T2DM [320, 321]. Detailed body composition analyses from DiRECT revealed that the intervention resulted in reductions in both total body fat and ectopic fat deposition, particularly in the liver and pancreas [283]. Critically, the degree of reduction in pancreatic fat was the strongest predictor of diabetes remission, demonstrating that specific fat depot changes, rather than total weight loss alone, may drive metabolic improvements. However, DiRECT participants showed weight regain over two years following the end of the program, though those maintaining ≥10kg loss sustained high remission rates [321]. Five-year follow-up data revealed further challenges in maintaining long-term benefits, with continued weight regain and declining remission rates, demonstrating the difficulties in sustaining diabetes remission through dietary intervention alone [322].

— Pharmaceutical Interventions

Recent pharmaceutical developments have introduced new weight loss treatment options for diabetes prevention. GLP-1 agonists, such as liraglutide, demonstrated the foundation for this class of pharmaceutical agents, achieving 5.4-8.4% weight loss in clinical trials [323], and establishing a pathway for the development of more potent agents. GLP-1 receptor agonists, particularly semaglutide, have shown exceptional efficacy, with the STEP trials demonstrating 14.9% weight loss over 68 weeks in individuals with overweight or obesity, but without diabetes [324]. In individuals with T2DM, semaglutide achieved 9.6% weight loss alongside significant glycaemic improvements [325]. More recent medications, such as dual GLP-1/GIP receptor agonists like tirzepatide, have shown even greater efficacy, achieving up to 22.5% weight loss in clinical trials [326]. However, a 1-year follow-up of STEP 1 trial participants showed that after discontinuation of GLP-1 agonist medications, approximately 66% of weight was regained [327], and this finding of weight-regain after stopping treatment is supported by a meta-analysis of 8 randomised clinical trials (2372 participants) [328].

— Weight Maintenance Challenges

Despite initial success, weight maintenance remains the primary challenge across all intervention strategies. Systematic reviews indicate that following weight-loss interventions, most individuals regain 30-35% of lost weight within one year, with continued weight regain over subsequent years, highlighting the biological drive toward weight restoration [329]. This weight regain is proposed to reflect underlying physiological adaptations, including persisting reductions in metabolic rate [330], alterations in appetite-regulating hormones (leptin, ghrelin), and increased hunger sensations that can persist for years after weight loss [277, 331].

These adaptations highlight why energy restriction approaches often fail in the long term and underscore the need for comprehensive interventions that address not only initial weight loss but also the physiological adaptations and wider societal factors that promote weight regain. This understanding is particularly relevant for individuals with pre-diabetes, where the goal extends beyond weight loss to include promotion of healthy glucose regulation through the development of sustainable lifestyle modifications that can counteract unfavourable adaptive responses.

In summary, the evidence linking obesity to metabolic dysfunction, combined with the demonstrated efficacy of weight-loss interventions, creates a compelling case for targeted weight management in individuals with pre-diabetes. However, the complexity of maintaining weight loss and the importance of preserving lean body mass during weight reduction suggest that interventions must be carefully designed to optimise both the quantity and quality of weight loss. Understanding the factors that influence successful weight management outcomes, particularly the preservation of metabolically important lean tissue, becomes critical for developing effective diabetes prevention strategies.

1.8 Thesis Rationale

The global diabetes epidemic, related to modifiable lifestyle factors, presents an urgent public health crisis, with projections indicating that 783 million adults will be living with diabetes by 2045 [5]. Within this crisis lies a critical intervention opportunity; the 374 million individuals worldwide with pre-diabetes, who exist in a transitional state where targeted interventions could prevent or delay diabetes progression. Individuals with pre-diabetes represent a unique population characterised by impaired glucose tolerance, insulin resistance, and altered body composition patterns. Many are middle-aged or older, facing the dual challenges of metabolic dysfunction and age-related health

decline. Due to the role of lean body mass in glucose regulation and the importance of physical activity for stimulating glucose disposal, it is of importance that the association and potential interactions between physical activity, age, body mass and LM in pre-diabetic populations is better characterised. This knowledge gap becomes critical when considering that approximately 20-30% of body mass lost during energy restriction typically comes from lean tissue [232]. Understanding what determines LM before weight-loss intervention could inform strategies to optimise body composition outcomes during these diabetes prevention efforts.

Intermittent fasting has emerged as a popular intervention approach for weight management and metabolic health improvement, yet critical knowledge gaps exist regarding the hormonal mechanisms underlying its effects. Appetite-regulating hormones play crucial roles in feeding behaviour and weight regulation, with complex interactions between peripheral signals from the gastrointestinal tract, adipose tissue, and pancreas that influence hunger, satiety, and long-term weight maintenance. Ramadan fasting represents a unique form of intermittent fasting practised by over 1.8 billion Muslims worldwide, characterised by complete daytime abstention from food and water followed by night-time feeding for approximately 30 days. Despite its widespread practice and distinct pattern compared with other intermittent fasting approaches, no systematic review and meta-analysis has examined the effects of Ramadan fasting on appetite-regulating hormones, including ghrelin, leptin, peptide YY, glucagon-like peptide-1, and other key hormones. This represents a gap in the literature that limits understanding of how this specific form of time-restricted eating influences the biological systems that govern appetite regulation.

The validation of assessment methods in the Nottingham PREVIEW cohort was deemed essential for several critical reasons. Firstly, despite

the widespread use of self-reported dietary assessment tools, bioelectrical impedance analysis, and consumer-grade accelerometers in lifestyle intervention research, the validity of these methods can vary substantially across different populations and intervention contexts [725, 863]. Population-specific validation is particularly important because the accuracy of these assessment methods may be influenced by characteristics such as body composition, age, sex, ethnicity, and metabolic status, all of which differ between populations [240, 864]. The PREVIEW cohort comprised individuals with overweight or obesity and pre-diabetes, a specific population in whom assessment tool performance may differ from the general population due to altered metabolic profiles, body composition characteristics, and potential differences in reporting behaviours [865]. Secondly, validation during active intervention periods is crucial because assessment tool performance may change during weight loss and behavioural modification programmes [866]. Factors such as increased nutrition knowledge, heightened awareness of food intake, participant fatigue over extended study periods, social desirability bias related to meeting intervention goals, and physiological changes associated with weight loss may all influence the agreement between self-reported measures and objective criteria [414, 867]. Understanding how the validity of dietary, physical activity, and body composition assessment tools changes across different phases of a lifestyle intervention (baseline, active weight loss, weight maintenance, and potential weight regain) provides essential information for interpreting intervention study results and understanding potential sources of measurement error in longitudinal research [715]. Thirdly, the validation study addresses a methodological gap in the diabetes prevention literature. While numerous studies have investigated dietary interventions, physical activity programmes, and body composition changes in individuals at risk for type 2 diabetes, relatively few have systematically validated the assessment tools used to measure these outcomes within this specific population during active intervention [725]. This gap is significant because accurate measurement of dietary intake, physical activity, and body composition changes is

fundamental to understanding intervention mechanisms, dose-response relationships, and determinants of intervention success [715].

Without validation data, uncertainty exists regarding whether observed relationships between behaviours and outcomes reflect true associations or are confounded by differential measurement error across the range of values or across subgroups [732]. Finally, the availability of objective reference measures within the PREVIEW study (24-hour urinary nitrogen for protein intake, DEXA for body composition, and laboratory-grade accelerometry for physical activity) provided a unique opportunity to conduct rigorous validation analyses that are often not feasible in intervention studies due to cost and participant burden constraints [722, 868]. Capitalising on this existing data to validate commonly used assessment tools not only strengthens the interpretation of the PREVIEW study findings but also provides valuable methodological evidence that can inform future diabetes prevention research and clinical practice. The validation evidence generated can help researchers and clinicians better understand the limitations and appropriate applications of these widely used assessment tools, ultimately contributing to improved measurement quality and more reliable evidence generation in the field of diabetes prevention and weight management [240].

Effective intervention research requires robust measurement tools, yet variability exists regarding how assessment methods for dietary intake, body composition, and physical activity perform during active lifestyle interventions in different cohorts. Understanding the validity and agreement of these commonly used tools is essential for interpreting intervention research and ensuring reliable evidence generation for clinical practice. If these assessment methods demonstrate systematic bias or changing accuracy during intervention periods, due to factors such as altered metabolic status, participant fatigue, or social desirability effects, study conclusions may be compromised, and intervention effects

incorrectly estimated. This methodological uncertainty has profound implications for the field's ability to generate reliable evidence for diabetes prevention strategies.

The PREVIEW study represents an opportunity to address some of these knowledge gaps through secondary analysis of data from one of the most extensive multinational lifestyle intervention trials for diabetes prevention [332]. The study's baseline (pre-intervention) data provides access to a well-characterised cohort of overweight adults with pre-diabetes, with detailed assessments including DXA body composition analysis, objective physical activity measurement via accelerometry, comprehensive dietary assessment, and extensive participant characterisation having been made. This rich dataset enables investigation of research questions that were not addressed in the primary study analyses but are critical for advancing understanding of determinants of health while maximising the value of this substantial research investment.

By elucidating the hormonal responses to intermittent fasting, identifying key determinants of LM preservation, and validating essential assessment tools, this work establishes foundations for more effective, personalised intervention approaches to bring about weight-loss. The insights generated highlight the importance of evidence-based strategies that optimise both weight loss and metabolic health outcomes, addressing a need in the field.

1.9 Thesis Aims and Hypotheses

The over-arching purpose of this thesis was to investigate the impact of Ramadan intermittent fasting on appetite hormones, the determinants of body composition and weight-loss success after an 8-week LED intervention in an overweight population at risk of developing T2DM, and

methodological validation in this cohort. The following specific aims were set in order to address gaps that had been identified in the knowledge **(Table 1-5):**

1. To undertake a systematic review to examine the effect of Ramadan intermittent fasting on gut hormones, including Leptin, Ghrelin, insulin, gastrin, Glucagon-like peptide-1, Peptide YY, and Cholecystokinin in the published literature, in order to better understand the possible effects of time-restricted feeding on appetite regulation hormones (**Chapter 2**).

2. To investigate the relationships between physical activity, body weight and age on leg lean body mass, and body composition across an adult age range, and within biological sex, in overweight individuals with pre-diabetes, testing the hypothesis that lean body mass would be lower with increasing age but higher in those who are more physically active and who are heavier due to greater muscle loading (**Chapter 4**).

3. To explore the impact of lifestyle factors and eating behaviours on body composition changes after 8-weeks' LED in the Nottingham cohort of the Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World (PREVIEW) study, testing the hypotheses that those with higher physical activity pre-intervention will maintain LM better than more sedentary participants, and that those with higher eating disinhibition and hunger scores and lower eating restraint will experience smaller fat mass losses (**Chapter 5**).

4. To validate assessment tools for protein intake, body composition and physical activity in the Nottingham cohort (**Chapter 6**):

   This validation chapter was designed to test several hypotheses regarding the agreement between commonly used assessment methods and their respective reference in the context of a lifestyle intervention trial.

1. Dietary Protein Intake Assessment: It was hypothesised that self-reported dietary protein intake (assessed using 7-day weighed food records) and calculated protein intake derived from 24-hour urinary nitrogen excretion would have a positive correlation. However, it was anticipated that the strength of this relationship would vary across different phases of the intervention, with potential changes in agreement during periods of weight loss/maintenance compared with baseline and phases where there was weight regain, due to factors such as participant fatigue or social desirability bias. It was hypothesised that demographic and anthropometric characteristics (such as sex, age, and body mass index) would significantly modify the relationship between assessment methods. Further, it was hypothesised that proportional bias might be present, i.e. the magnitude of difference between methods would systematically vary across the range of intake levels.

2. Body Composition Assessment: It was hypothesised that BIA would demonstrate strong agreement with DXA to assess body composition parameters, including total body fat mass, fat-free mass, and body fat percentage. High correlations between methods with minimal systematic bias were expected, supporting the use of BIA as a practical alternative to DXA in large-scale intervention studies.

3. Physical Activity Assessment: It was hypothesised that there would be a significant but modest correlation between self-reported physical activity (assessed using the Baecke questionnaire) and objectively measured physical activity (assessed using accelerometry). The correlation was anticipated to be moderate rather than strong, reflecting the different dimensions of physical activity captured by subjective versus objective measurement approaches.

These aims were designed to address knowledge gaps regarding the physiological mechanisms of intermittent fasting on appetite regulation, the determinants of LM maintenance in pre-diabetic populations, factors predicting successful weight loss outcomes, and the validity of commonly used assessment tools in nutritional research.

Table 1-5: Knowledge gaps addressed by thesis studies.

Study	Title	Knowledge gap
Study 1 (Chapter 2)	Effects of Ramadan Intermittent Fasting on Hormones Regulating Appetite in Healthy Individuals: A Systematic Review and Meta-analysis.	Despite growing interest in intermittent fasting, no comprehensive synthesis exists to quantify the effects of Ramadan fasting on appetite-regulating hormones, limiting our understanding of how this specific form of time-restricted eating affects the biological regulation of hunger and satiety.
Study 2 (Chapter 4)	Relationships between Physical Activity, Sex, Age and Body Mass, and Lean Body Mass and Body Composition in an Overweight Cohort with Pre-Diabetes: A Secondary Analysis of the PREVIEW Study Baseline Data.	The relationships between age, sex, physical activity, and lean body mass in overweight individuals with pre-diabetes remain incompletely characterised, limiting the development of targeted intervention strategies for this high-risk population.
Study 3 (Chapter 5)	The Impact of Lifestyle Factors and Eating Behaviours on Body Composition Changes after 8-Weeks' Low-Energy Diet in the Nottingham PREVIEW Cohort.	While low-energy diets effectively reduce total weight, limited evidence exists regarding the specific lifestyle factors and eating behaviours that influence the quality of weight loss, particularly the relative proportion of fat mass versus

		lean mass reduction, which has important implications for metabolic health.
Study 4 (Chapter 6)	Exploring Assessment Methods for determining Dietary Protein Intake, Body Composition, and Physical Activity Measurements used in the Nottingham PREVIEW Cohort.	Research has indicated the value of validating assessment methods in study cohorts to aid data interpretation. Key methods in lifestyle interventions include dietary protein intake, body composition, and physical activity assessment methods, and understanding the strengths and limitations of these tools in those with overweight, obesity, and prediabetes informs both future dietetic practice and research activity.

Chapter 2 Effects of Ramadan Intermittent Fasting on Hormones Regulating Appetite in Healthy Individuals: A Systematic Review and Meta-analysis

This systematic review has been published from this chapter in the journal *Clinical Nutrition* with the corresponding DOI: https://doi.org/10.1016/j.clnu.2025.01.005

2.1 Introduction

Intermittent fasting is a regimen that has emerged over the past few decades as a non-pharmaceutical method for losing weight and improving health [333]. Intermittent fasting can take many forms, with the most common types being time-restricted feeding, alternate-day fasting, twice-a-week fasting, and 24-hour fasting [334]. Ramadan intermittent fasting is a type of time-restricted feeding involving refraining from eating, drinking, and smoking from sunrise to sunset for 12 to 22 hours per day depending on season and geographical region [335-338]. Healthy adult Muslims are expected to fast during the lunar month of Ramadan, which lasts 29-30 days [333]. However, fasting is not recommended for unhealthy adults, pre-pubescent children, and pregnant and lactating women [336].

Ramadan fasting differs significantly from usual daily life, including alterations in meal timing, meal frequency, and diet composition. It can also impact physical activity patterns, daytime sleepiness, and sleep habits [339, 340]. It is likely that these alterations influence appetite and hormonal responses [289, 341]. Several studies demonstrated that Ramadan fasting may affect body composition parameters including body fat mass, fat percentage, hip circumference, waist circumference, and weight control [342-344]. Furthermore, its impact on various health outcomes, such as appetite regulation, remains unclear.

Peripheral tissues, including the intestines and adipocytes, produce and release several hormones that affect brain centres. Furthermore, several peptide hormones found in the gastrointestinal tract also play a role in regulating food intake [345]. Appetite and satiety are controlled primarily by leptin, ghrelin, GLP-1, PYY, and CCK [346]. Leptin, commonly known as the "satiety" hormone and produced by adipose tissue, plays an important role in regulating energy intake and energy balance. It also

modulates satiety by suppressing food intake and stimulating energy expenditure [347, 348].

Ghrelin is another key appetite-regulating hormone, known as a hunger hormone, and functions opposite to leptin. Moreover, ghrelin is produced in the gastrointestinal tract and primarily stimulates hunger in the brain [349]. It acts on the hypothalamus, increasing both hunger and gastric secretions [350]. GLP-1 is a gut peptide hormone secreted mainly from the L cells in the small intestine in response to the nutrients in the gastrointestinal tract [351]. In addition, GLP-1 affects glucose homeostasis by promoting insulin secretion, reducing glucagon secretion, and decreasing gastric motility [352]. GLP-1 affects blood glucose regulation via effects on insulin release and pharmaceutical agonists of GLP-1 receptors are used to reduce blood glucose and control body weight [353]. Another appetite regulator is PYY, which is secreted from L cells of the digestive system, primarily in the ileum and colon in response to food intake [354]. Insulin is a peptide hormone produced by pancreatic beta cells in the islets of Langerhans in response to a rise in blood glucose [355, 356]. Finally, CCK is a short-term satiety hormone released from endocrine cells in the proximal intestine mainly in the duodenum and jejunum [357]. It also stimulates contraction of the gall bladder leading to release of bile into the small intestine that aids fat digestion.

The annual fasting of millions of adult Muslims during Ramadan leads to various lifestyle changes, including changes in meal timings, sleep-wake schedules, sleep duration, light exposure, and exercise, that can impact appetite-regulating hormones [358-360]. This chapter performed a systematic review and meta-analysis to assess the potential effects of intermittent fasting during Ramadan on appetite-regulating hormones.

2.2 Methods

This systematic review was structured according to the guidelines for Systematic Reviews and Meta-Analyses of Preferred Reporting Items (PRISMA based on the PRISMA 2020 checklist to report study findings [361].

2.2.1 Registration of the protocol

The protocol was registered with the International Prospective Register of Systematic Reviews PROSPERO database under registration number: CRD42024506317 (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024506317).

2.2.2 Search strategy

The literature search was performed by two authors (DA and May Alotaibi - MA) through six databases: Cochrane, CINAHL, Google Scholar, EMBASE, PubMed/MEDLINE, and Web of Science for all English-language studies published from any period to the end of March 2024. The search strategy consisted of the following relevant key terms, used separately and in combination: “intermittent fasting”, OR “time-restricted fast”, “alternate day fasting”, OR “Ramadan fasting”, OR “periodic fasting”, OR “Islamic fasting”, OR “intermittent energy restrict*”, OR “recurrent circadian fast”, AND “gastrointestinal hormones”, OR “gut or intestinal or gastrointestinal or enteric hormone”, OR “appetite-regulating hormone”, OR “ghrelin”, OR “glucagon-like-peptide 1”, OR “GLP-1”, OR “glucose-dependent insulinotropic peptide”, OR “GIP”, OR “gastric inhibit polypeptide”, OR “PYY”, OR “Peptide YY”, OR “cholecystokinin”, OR “CCK”.

In addition to key terms, Medical Subject Headings (MeSH) and database-specific controlled vocabulary terms (e.g., Emtree terms in Embase) were systematically incorporated to ensure comprehensive retrieval of relevant literature. The complete search strategy, including both key terms and MeSH/controlled vocabulary terms, is provided in the **Appendix B**.

2.2.3 Screening process

The authors (DA and MA) independently reviewed each study title and abstract during the initial screening phase and excluded those ineligible for inclusion. The search of the databases was completed on 20 March 2024. The selected papers from all databases were exported to the Endnote X9 programme (Clarivate™, JISC Services Ltd., Bristol, UK) and the collected data were shared through Rayyan for the authors (DA and MA) [362]. Then, duplicate papers were deleted from all records through Rayyan software (https://www.rayyan.ai) before the screening [363]. The selected studies were reviewed based on the inclusion and exclusion criteria, and then eligibility was confirmed by the author and her colleague. After assessing selected studies, any disagreement was resolved by discussion with the co-authors (DNL and IAM) until a consensus was reached on any remaining ambiguity through retrieving and reviewing the entire article.

Reference lists of included studies were also checked for relevant studies. The screening phase above was conducted again to ensure that all relevant studies were included in this analysis. All eliminated records are listed on the PRISMA flow chart, and the justification for excluded records was documented. Emails were sent to some corresponding authors who were requested to provide the full text of the publication. Unfortunately, no responses were received.

2.2.4 Inclusion criteria

The inclusion criteria encompassed any type of clinical studies that investigated Ramadan intermittent fasting in participants categorised as normal weight, overweight, and obese. These studies were required to assess the relevant appetite-regulating hormone parameters at a minimum of two distinct time points: prior to the commencement of Ramadan fasting or within the initial days (0 to 7 days) of Ramadan, and subsequently at the conclusion of Ramadan (21 to 30 days). The review included all study designs involving adult participants aged 18 years and older with a BMI ≥18.5 kg/m^2. The review included studies published in the English language that involved human participants across diverse ethnic, demographic, or cultural backgrounds.

2.2.5 Exclusion criteria

Studies on participants with chronic diseases or conditions, such as diabetes, were excluded from the review. In addition, animal studies, those that included pregnant and/or lactating women, clinical investigations on fasting children or athletes, as well as studies that incorporated any dietary or exercise interventions during Ramadan fasting were excluded. Furthermore, studies that did not provide data on appetite-regulating hormones prior to or during the initial days of Ramadan, or at the end of Ramadan, as well as those investigating the impact of Ramadan fasting on alternative outcomes, those with limited access to research data, or those published solely as reports, in conference abstracts, in review articles, or as case reports were excluded from consideration.

2.2.6 PICO Framework

2.2.6.1 Categories of participants

Studies that involved adult participants (aged 18 years or older) who adhered to intermittent fasting practices, specifically during the month of Ramadan as observed in the Muslim tradition.

2.2.6.2 Categories of interventions

All studies that examined Ramadan intermittent fasting, characterised by a fasting duration exceeding 8 hours per day.

2.2.6.3 Categories of comparators

The comparator involved measuring mean hormones at two distinct time points: several days prior to Ramadan or during the initial days of Ramadan (0-7 days), and the end of Ramadan (21-30 days).

2.2.6.4 Categories of outcomes

The outcomes were the measured mean changes in the appetite-regulating hormones among fasting participants during the month of Ramadan. These hormones included leptin, ghrelin, insulin, gastrin, glucagon-like peptide-1, glucose-dependent insulinotropic peptide, peptide YY, and cholecystokinin from baseline and at the end of Ramadan.

2.2.6.5 Categories of studies

All the study designs were included during the search process. No restriction was imposed on the types of studies. Observational studies that examined the effect of Ramadan fasting on appetite-regulating hormones were included during the search process.

2.2.7 Data extraction

Key information regarding article data (first author name, publication date of study, and author contact detail, study design (type of study, statistical method), participant characteristics, sample size, age range, intervention characteristics (duration of fasting per day during Ramadan, and measurement time), outcome measure (appetite-regulating hormones at

baseline and end of Ramadan), and study main finding were extracted by the author (DA). In preparation for analysis, the extracted data was entered into a Microsoft Excel sheet. After data extraction by DA, a second author (MA) reviewed all papers and confirmed the agreement.

2.2.8 Estimating fasting time length

Ramadan fasting duration was estimated using a standardised approach: the daily fasting period was calculated by obtaining location-specific sunrise and sunset times (https://www.timeanddate.com), with an additional 80-minute adjustment to account for the pre-dawn Fajr prayer that marks the start of the fast. This methodology was validated against official Islamic prayer calendars, confirming an average daily fasting duration of approximately 13 hours (787 minutes).

2.2.9 Quality assessment

Two authors (DA and MA) conducted an independent evaluation of the quality of the published papers using the Newcastle-Ottawa Quality Assessment Scale [364]. The quality assessment comprised three sections: selection, comparability, and outcome. The three sections consisting of nine evaluating criteria, which addressed aspects such as sample size, population characteristics, sampling technique, data collection quality, statistical analyses, reporting practices, and the generalisability of the findings. To simplify reporting the appraisal scores range between five and ten, with a score of 5–6 considered a low-quality study, 7–8 moderate quality, and 9–10 high quality. Two independent reviewers assessed the conclusions to ensure consensus and agreement.

The Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) framework was used to evaluate the overall quality

of the evidence [365]. A summary of the quality, certainty, and conclusions regarding the observed changes during Ramadan fasting was derived from the guidelines. The evidence was assessed and categorised into five domains: risk of bias, imprecision, consistency, indirectness, and publication. Consequently, the degree of certainty of the evidence was graded as high, moderate, low, or very low. In addition, it can be used for grading recommendations based on their strength.

2.2.10 Statistical analysis

The outcome values measured during pre- and post-fasting were pooled and represented as means, and standard deviation (SD). In the meta-analysis, the Weighted Mean Difference (WMD) accompanying the 95% confidence interval (CI) was used to calculate the mean difference between measured parameters pre- and post-fasting, based on each study's sample size. For studies that used standard error of the mean instead of SD [366-370], the SD value was calculated based on the following formula: SD = SEM $\times$ $\sqrt{n}$ (where n was the number of participants in each group) [371]. A random-effects model was used to estimate the effect size of intermittent fasting during Ramadan on the appetite-regulating hormones. Consequently, using the random effects model led to the assumption that distribution accurately represents true effect sizes, rather than only one true effect size [372]. The study results were reported in different values; therefore, the standardised mean difference [373] measured from pooled data was calculated using RevMan v5.4 software [374]. The I^2 statistic was used to evaluate heterogeneity and inconsistency between studies, with the values interpreted according to the Cochrane Handbook [375]. Heterogeneity was classified as "not important" for I^2 values between 0-40%, "moderate" for values between 30-60%, "substantial" for values between 50-90%, and "considerable" for values between 75-100%.

2.3 Results

2.3.1 Study selection

Figure 2-1 summarises the search strategy and flow of articles. Of the 375 records initially identified from various databases and additional sources, 113 duplicates were removed, and titles and abstracts screened 262 records. From these, 235 records were excluded for different reasons, leaving 27 full-text articles to be assessed for eligibility. Eleven of these studies were excluded due to specific issues (**details in the Appendix A**), resulting in 16 studies being eligible for inclusion in the final review [289, 344, 366-370, 376-384].

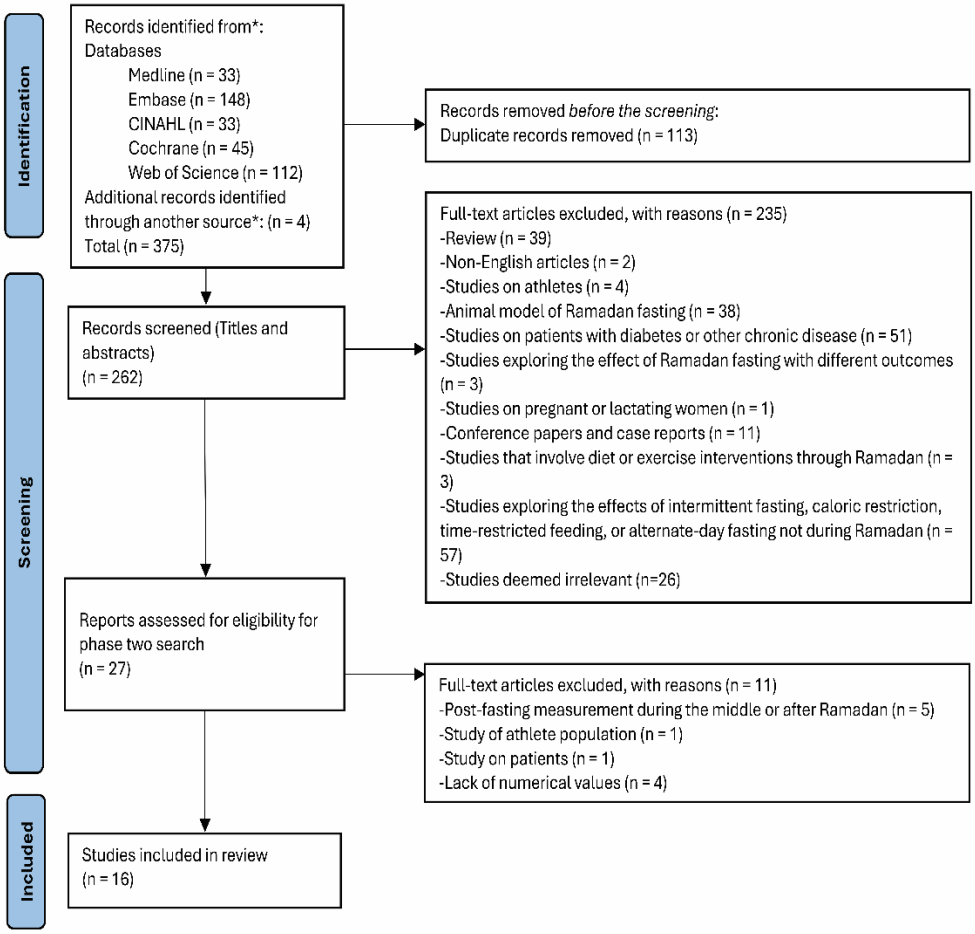


Figure 2-1: PRISMA flow diagram.

2.3.2 Study characteristics

All 16 studies [289, 344, 366-370, 376-384] were published in the English language between 1982 and 2022. The studies were conducted in the United Arab Emirates [289, 344], Tunisia [376], Jordan [377], Germany [378], Pakistan [366], Iran [379, 380], Bahrain [367, 368], Sudan [370], USA [381], Indonesia [382], Algeria [383], Morocco [369], and Yemen [384]. All the studies included in this review implemented a pre-post model to report changes in appetite-regulating hormones before and after fasting. Only one study included a control group of unfasted participants [376]. Sixteen observational studies were identified, including twelve prospective observational reports [289, 344, 366-370, 377, 378, 381-383], three quasi-experimental reports [376, 379, 380], and one cohort study [384]. A brief summary of all included studies is in **Table 2-1.**

Table 2-1: Summary of studies characteristics and reported outcomes in the included studies

Author, year	Country	Study design	Sample size	Sex n (%)	Age, years mean ± SD (range)	Fasting days n	Fasting hours n	BMI (kg/m^2)	Gut hormones	Time of measurement pre-Ramadan fasting	Time of measurement post-Ramadan fasting	Pre-Ramadan (mean ± SD) **	Post-Ramadan (mean ± SD) **	Results
Al-Rawi *et al*. 2020 [289]	United Arab Emirates	Prospective observational study	57	M = 40 (70.2%), F = 17 (29.8%)	38.4± 11.2	F = 23 - 25 M =28 -30	15	25	Ghrelin, leptin	One week before Ramadan	After completing 28 days of fasting at fixed times of the day (11:00 - 13:00)	Ghrelin: 566.0 ± 292.1 pg/mL Leptin: 0.0184 ± 0.0124 µg/mL	Ghrelin: 460 ± 201.8 pg/mL Leptin: 0.016 ± 0.0121 µg/mL	Significantly decreased serum ghrelin and leptin concentrations, (P < 0.001)
Zouhal *et al*. 2020 [376]	Tunisia	Quasi-experimental study*	Total: 30 I = 15 C = 15	M=30 (100%)	24.2±3.6	30	15 - 16	30 -40	Leptin, GLP-1, PYY, CCK, and ghrelin	Just 24 hours before Ramadan	Day after the end of Ramadan at the same time of day (within ~ 1 h, between 8-9)	Leptin: I = 0.01032 ± 0.00226, C = 0.01084 ± 0.00048 µg/mL Ghrelin: I = 908.66 ±	Leptin: I = 0.00993 ± 0.00205, C = 0.01072 ± 0.0007 µg/mL Ghrelin: I = 880.33 ± 135.20, C = 1006.00	Significant decrease in leptin, GLP-1, PYY, and CCK, (P < 0.01). No significant change in ghrelin.

											187.70 , C = 1066.00 ± 190.06 pg/mL GLP-1: I = 0.69± 0.18, C = 0.76 ± 0.15 ng/dL PYY: I = 0.43 ± 0.12, C: 0.49± 0.06 µg /mL CCK: I = 0.44 ± 0.10, C: 0.47 ± 0.12 ng/dL	± 155.04 pg/mL GLP-1: I = 0.66 ± 0.19, C =0.71 ± 0.14 ng/dL PYY: I = 0.39 ± 0.12, C: 0.48 ± 0.06 µg /mL CCK: I = 0.39 ± 0.09, C: 0.52 ± 0.30 ng/dL	
Alzoughool *et al*.	Jordan	Prospective	60	M = 17 (28%), (19 - 24)	NR (assumed to be	15 #	< 30	Leptin	Before the month of Ramadan	In the fourth week	Leptin: 0.0116 ±	Leptin: 0.0086 ±	Significantly decreased

2019 [377]		Observational Study*		F = 43 (72%)		fasted the whole Ramadan)					of Ramadan	0.0077 µg/mL	0.0062 µg /mL	leptin (*P*<0.001)
Faris *et al*. 2019 [344]	United Arab Emirates	Prospective observational study	61	M =23 (37.7%), F =38 (62.3)	36.2 ± 12,5	F = 23 - 25 M = 28 - 30	15	> 25	Insulin, and leptin	One week before Ramadan	Day 28 of fasting (Fourth week of Ramadan) (Between 11:00 and 13;00 after 8-10 h fasting).	Leptin: 0.0022 ± 0.002 µg/mL Insulin: 21 ± 4.3 ng/ml	Leptin: 0.0042 ± 0.003 µg/mL Insulin: 26 ± 5.8 ng/ml	Significantly increased serum leptin (*P*<0.05). No significant change in serum insulin
Vardarli *et al*. 2014 [378]	Germany	Prospective observational study*	19	M = 24 (100%)	36 ± 9	30	15	> 18.5 or < 30	Insulin, and leptin	Day 0, pre-fasting (Sample time between 19:40 to 20:40)	Day 30 of fasting (Sample time between 18:35-19:35)	Leptin: 0.0046 ± 0.0038 µg/mL Insulin: 7.7 ± 4.1 µU/mL	Leptin: 0.0054 ± 0.0031 µg/mL Insulin: 8.3 ± 4.36 µU/mL	There was no significant change in serum insulin or leptin.
Mushtaq *et al*. 2019 [366]	Pakistan	Prospective observational study	Total (110) HW = 30 OW = 20 Obese = 60	M = 55 (50%), F = 55 (50%)	HW = 23.26 ± 0.47, OW = 31.40 ± 1.04 Obese = 32.86 ± 0.90	Males fasted the 30 days of Ramadan whereas females didn't fast through menstruation according to Islamic rule	15	HW = 18.5 - 22.9, OW = 23 - 24.9 Obese= > 25	Leptin	First day of Ramadan	Last week of Ramadan. Note: The blood sample was taken before Iftar after 10-12 h fasting from each participant	Leptin: HW = 0.00491 ± 0.00208, OW = 0.013 ± 0.00581, Obese = 0.0273	Leptin: HW = 0.00428 ± 0.00181, OW= 0.01029 ± 0.00599, Obese = 0.02342 ± 0.0127 µg/mL	A significant decrease in serum of leptin among overweight and obese participants, (*P*<0.05) and (*P*<0.001), respectively.

											3 ± 0.0150 3 µg/mL		
Ganjali *et al*. 2016 [379]	Iran	Quasi-experimental study	Total: 45 HW = 23 Obese = 22	M = 26 (57.8%), F = 19 (42.2%),	37.6 ± 6.9	NR (assumed to be fasted the whole Ramadan)	16	HW = 19 - 25 Obese = > 30	Leptin	Two days before Ramadan from 7:30 to 9:00	Before sunset at the end of Ramadan. <u>Note:</u> Blood samples were taken from all the participants after 10 -12 fasting h.	<u>Leptin:</u> HW = 0.0117 ± 0.0011 6, Obese = 0.026 ± 0.0201 µg/mL	<u>Leptin:</u> HW = 0.0087 ± 0.0108, Obese = 0.0261 ± 0.0253 µg/mL	Leptin was significantly decreased in healthy body weight participants (P=0.001). No significant change in participants with obesity.
Kassab *et al*. 2003 [368]	Bahrain	Prospective observational study*	Total: 44 HW = 26 Obese = 18	F = 44 (100%)	(18 - 45)	NR (assumed to be fasted from 23 to 25 days of Ramadan due to menstruation)	14 #	HW = 22.5 Obese= > 33.1	Leptin and insulin	First day of Ramadan	28 days after the beginning of Ramadan. <u>Note:</u> <u>Fasting venous</u> blood samples were taken from 13:00 - 14:00	<u>Leptin:</u> HW = 9.6 ± 4.08, Obese = 13.5 ± 8.32 µg/mL <u>Insulin</u>: HW = 5.3 ± 1.53 Obese = 7.4 ± 5.09 µU/mL	<u>Leptin:</u> HW = 14.0 ± 5.61, Obese = 20.0 ± 0.04 µg/mL <u>Insulin:</u> HW = 7.4 ± 2.04 Obese = 8.8 ± 5.94 µU/mL	A significant increase in concentration of leptin in both HW and participants with obesity (P<0.05). A significant increase in concentration of insulin in participants with obesity only (P<0.05).

Kassab *et al*. 2004 [367]	Bahrain	Prospective observational study*	46	F = 46 (100%),	22 ± 2	NR (assumed to be fasted from 23 to 25 days of Ramadan due to menstruation)	14#	25.3	Leptin and insulin	First day of Ramadan	28 days after the beginning of Ramadan. Note: Fasting venous blood samples were taken from 13:00-14:00	Insulin: 7.37 ± 10.65 µU/mL Leptin: 10.57 ± 5.56 µg/mL	Insulin: 8.83 ± 8.14 µU/mL Leptin: 14.83±6.85 µg/mL	A significant increase in serum insulin or leptin (P<0.05)
Haghighy *et al*. 2018 [380]	Iran	Quasi-experimental study	Total: 25 HW = 13 Obese = 12	F = 25 (100%),	39.9 ± 8.42	NR (assumed to be fasted from 23 to 25 days of Ramadan due to menstruation)	15 #	HW = 20.31 Obese= > 30	Ghrelin and PYY.	3 days before Ramadan	28 days after the beginning of Ramadan. Note: Blood samples were taken from 15:00 to 16:00 when participants were fasting	Ghrelin: Obese = 119,000 ± 45,050, HW = 147.750 ± 20,710 pg/mL PYY: Obese = 111.20 ± 52, HW = 164.80 ± 32.91 pg/mL	Ghrelin: Obese = 115,600 ± 57,400, HW =142.190 ± 22,530 pg/mL PYY: Obese = 124.60 ± 73.82, HW = 142.10 ± 33.09 pg/mL	No statistically significant change in ghrelin or PYY

Fedail *et al*. 1982 [370]	Sudan	Prospective observational study*	24	F = 4 (16.7%), M = 20 (83.3%)	(21 - 40)	NR (assumed to be fasted the whole Ramadan)	16	NR	Gastrin and insulin	First day of Ramadan	The last day of Ramadan Note: Blood samples were taken first and last day at a similar time in the evening just before the main evening meal	Gastrin: 108.4 ± 85.24 pg/mL Insulin: 2.0 ± 0.15 µU/mL	Gastrin: 119.4 ± 90.63 pg/mL Insulin: 2.0 ± 0.93 µU/mL	No statistically significant change in serum insulin or gastrin.
Nomani *et al*. 2005 [381]	USA	Prospective observational study*	6	M = 6 (100%)	33.5 ± 17.9	NR (assumed to be fasted the whole Ramadan)	11.5	26.5	Gastrin	First day of Ramadan	28 days after the beginning of Ramadan. Note: Blood samples were drawn at 16:00–16:15 approximately one hour before iftar on days 1 and 28 days after 10.5 h of fasting.	Gastrin: 57.3 ± 22.1 pg/mL	Gastrin: 59.3 ± 20.3 pg/mL	No significant change in serum gastrin.
Muhammad *et al*.	Indonesia	Prospective observatio	45	F = 32 (71.11 %), M =	32.8 ± 11.5	Fasting 30 days of Ramadan	13 - 14	28.5	Leptin	Before Ramadan (week 0)	End of Ramadan (week 4)	Leptin: 12.9 ±	Leptin: 10.3 ±	A significant decrease was

2018 [382]		nal cohort study		13 (28.9%)						Note: subjects fasted 8 hrs before blood collection.	Note: blood sample was taken in the afternoon when subjects fasted at least 8 h before blood collection (fasting started at 5 am)	9.7 µg/mL	5.8 µg/mL	reported in serum leptin ($P < 0.05$)
Bogdan *et al*. 2005 [383]	Algeria	Prospective observational study*	10	M = 10 (100%)	34 ± 3.7	NR (assumed to be fasted the whole Ramadan)	15 - 16#	NR	Leptin	One week before Ramadan (Six blood samples drawn: 08.15, 12.15, 16.15, 20.15, 00.15, and 04.15)	23 days of Ramadan (Six blood samples drawn: 08.15, 12.15, 16.15, 20.15, 00.15, and 04.15)	Leptin: 4.19 ± 0.93 µg/mL	Leptin: 3.98 ± 1.26 µg/mL	No significant change in the concentration of leptin.

Iraki *et al*. 1997 [369]	Morocco	Prospective observational study*	7	M = 9 (100%)	25 ± 1.2	NR (assumed to be fasted the whole Ramadan)	15#	NR	Gastrin and insulin	Before Ramadan	24 days of Ramadan Note: blood was taken from 16:30 to 17:30, (Mealtime 18:00)	Gastrin: 64.3 ± 6.09 pg/mL Insulin: 23.9 ± 7.94 µU/mL	Gastrin: 56.5 ± 6.09 pg/mL Insulin: 21.3 ± 6.09 µU/mL	A significant decrease in the concentration of gastrin and insulin in the healthy controls, (P<0.001)
Abdullah *et al*. 2020 [384]	Yemen	Cohort study	Total: 68 C = 31 FDR = 37	M = 100%, Control and FDRs	C = 34.6 ± 4.31 FDR = 34.35 ± 3.83	NR (assumed to be fasted the whole Ramadan)	15#	C = 22.18 ± 3.05 Kg/m^2 FDRs = 24.76 ± 4.37 kg/m^2	Leptin and Insulin	A couple of days before Ramadan	After 3 weeks of Ramadan fasting Note: Blood was collected after an overnight fast of more than 10 h	Insulin: C = 14.31 ± 5.85, FDR = 18.86 ± 6.69 µU/mL Leptin: C = 0.02003 ± 0.0087, FDR: 0.0237 ± 0.0079	Insulin: C = 14.36 ± 5.79, FDR = 21.31 ± 7.98 µU/mL Leptin: C =0.02491 ± 0.00779, FDR: 0.02737 ± 0.00915 µg/mL	A significant increase in serum leptin in FDR and control (P<0.05). RIF Significant increase in serum insulin in FDR (P=0.001), with no effect in control.

1
µg/mL

Abbreviations: I: intervention group (fasting); **C**: control (non-fasting); **NR**: Not Reported; **GLP-1**: glucagon-like peptide-1; **PYY**: tyrosine-tyrosine; **CCK**: cholecystokinin; **M**: Male; **F**: Female; **FDRs**: First Degree Relatives for diabetic patients; **HW**: healthy weight; **OW**: overweight; **RIF**: Ramadan intermitted fasting; **SD**: standard deviation; **n:** number.
* Not reported by study authors.; ** Data transcribed from published original articles; **#**: The duration of fasting hours was not explicitly reported and was, therefore, estimated based on the location and year in which the study was conducted.

2.3.3 Participant characteristics

The 16 studies [289, 344, 366-370, 376-384] included a total of 664 (341 male) participants. In studies that reported sex, three studies included female participants only [367, 368, 380], six studies included only males [369, 376, 378, 381, 383, 384], and seven studies included both males and females [289, 344, 366, 370, 377, 379, 382]. The overall mean (SD) age of the participants was 33.9±10.8 years (age range 18-75 years). The mean fasting length during Ramadan for all included studies was 14.8 hours with an SD of 1.0 ranging between 11.5 and 16 hours per day. In all included studies, the post fasting duration before blood collection varied across studies but generally ranged from 8 to 12 hours. Most blood samples were collected in the early or mid-afternoon period, with specific timing varying across studies: some were collected between 13:00-14:00 hours [367, 368], others between 15:00-16:00 [380], and some between 16:00-18:00 [369]. Two studies [289, 344] standardised collection to 11:00-13:00 after 28 days of fasting. Several studies [366, 378, 379] specifically noted collecting samples approximately one hour before Iftar (the evening meal that breaks the fast), ensuring participants had fasted for 10-12 hours at the time of collection. The pre-Ramadan samples were collected following an overnight fast; however, the exact timing may have varied depending on the study.

2.3.4 Leptin

Leptin concentrations were assessed in 12 studies [289, 344, 366-368, 376-379, 382-384], with a total of 595 participants having pre- and post-fasting leptin concentrations. Al-Rawi *et al*. [289] and Alzoughool *et al*. [377] reported a significant reduction in the leptin concentration at the end of Ramadan when compared with pre-fasting concentrations ($P < 0.001$). Also, Mushtaq *et al*. [366] reported a significant reduction in leptin among participants of both sexes with overweight or obesity following Ramadan fasting, with leptin concentrations significantly lower than those observed prior to fasting ($P < 0.05$, $P < 0.001$, respectively). In contrast, three

studies [344, 367, 368] showed a significant increase in leptin concentration among participants with overweight or obesity at the end of Ramadan ($P < 0.05$), indicating a variable leptin response based on participant characteristics. Also, a study carried out by Vardarli *et al*. [378] found no significant changes in leptin concentration after Ramadan fasting. These findings align with those of Ganjali *et al*. [379] who also reported that there was no significant change in leptin concentrations in participants with obesity, but decreased significantly in participants with normal weight post fasting ($P < 0.01$).

A total of twelve eligible studies with complete data on leptin were included in the meta-analysis [289, 344, 366-368, 376-379, 382-384]. The overall standardised mean difference (SMD) between pre-fasting and post-fasting leptin concentrations was -0.11 µg/mL (95% CI: -0.36 to 0.14, $P = 0.38$, **Figure 2-2**), indicating no significant change in leptin concentrations due to Ramadan fasting. There was a notable degree of heterogeneity among the data ($I^2 = 77\%$).

2.3.5 Ghrelin

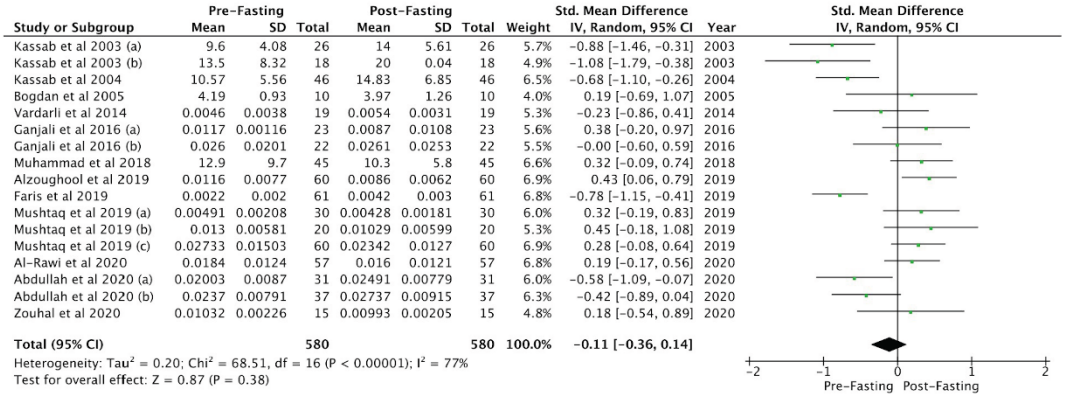
Three studies reported pre- and post-fasting ghrelin concentrations [289, 376, 380] in 112 participants. Al-Rawi *et al*.[289] reported a significant reduction in ghrelin concentration among participants with overweight or obesity, at the end of Ramadan compared with pre-fasting concentrations ($P< 0.001$). Conversely, Zouhal *et al*. [376] and Haghighy *et al*. [380] found no significant changes in ghrelin after Ramadan fasting. A meta-analysis comparing ghrelin concentrations between pre-fasting and post-fasting phases, suggested a significant increase in ghrelin concentrations after Ramadan fasting (SMD 0.31 pg/mL, 95% CI: 0.03 to 0.60, $P = 0.03$, $I^2 = 0\%$, **Figure 2-2**).

2.3.6 Insulin

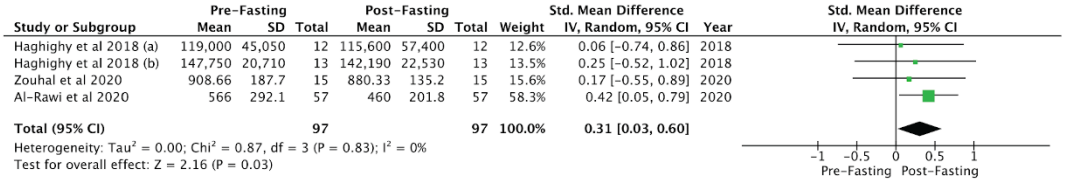
Seven studies reported data on pre- and post-fasting insulin concentrations [344, 367-370, 378, 384]. Two studies [367, 368] reported significant increases in insulin concentrations during the post-fasting compared with pre-fasting concentrations ($P < 0.05$). Three studies [344, 369, 370, 378] reported no statistically significant alterations in insulin concentrations when comparing pre- with post-fasting phases. A meta-analysis indicated no statistically significant difference in insulin concentration before and after fasting (-0.24 µU/mL, 95% CI: -0.54 to 0.02, $P = 0.07$, $I^2 = 43\%$, **Figure 2-2**).

2.3.7 Gastrin

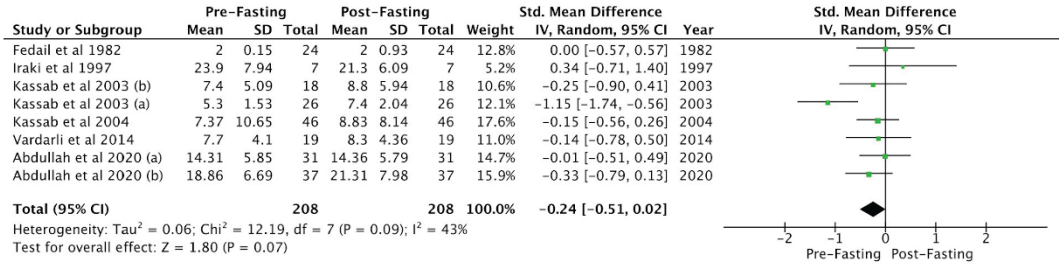
Three studies reported data on the change in gastrin concentrations among 37 participants [369, 370, 381]. Fedail *et al*. [370] found no significant change in gastrin concentrations during Ramadan fasting. A similar finding was reported by Nomani *et al*.[381], demonstrating no differences in gastrin concentration pre-fasting compared with post-fasting. However, Iraki *et al*. [369] showed a significant reduction in plasma gastrin concentration during Ramadan ($P < 0.001$). A meta-analysis of the pooled data from the three studies showed no significant difference in gastrin concentration between pre- and post-fasting phases (SMD 0.23 pg/mL, 95% CI: −0.54 to 0.99, $P = 0.56$, **Figure 2-2**). There was a notable degree of heterogeneity among the data ($I^2 = 51\%$).



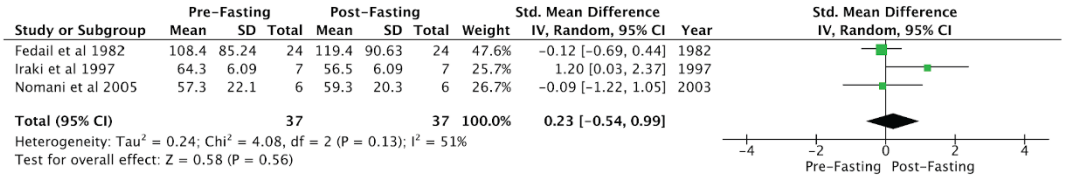
Leptin



Ghrelin



Insulin



Gastrin

Figure 2-2 Meta-analysis of responses of leptin, ghrelin, insulin and gastrin to Ramadan intermittent fasting.

2.3.8 GLP-1

Only one study evaluated the effects of Ramadan fasting on GLP-1 concentrations [376] and reported a significant reduction in GLP-1 after Ramadan fasting ($P < 0.01$) with 15 male participants.

2.3.9 PYY

Two studies evaluated the effects of Ramadan fasting on PYY concentrations [376, 380]. Haghighy *et al*. [380] reported no significant change in PYY during Ramadan fasting in 25 female participants ($P > 0.05$). However, Zouhal *et al*. [376] observed a significant reduction in PYY concentrations at the end of Ramadan ($P < 0.01$) in 15 male participants.

2.3.10 CCK

One study evaluated CCK concentrations pre- and post-Ramadan fasting with 15 male participants and found a significant reduction in CCK concentrations following Ramadan intermittent fasting ($P < 0.01$) [376].

2.3.11 Quality assessment

With regard to the quality of the included studies, three [289, 344, 376] were classified as "high quality" and three [369, 377, 383] were classified as "low quality" as they could not be evaluated in terms of comparability and the selection of the non-exposed cohort. Ten studies [366-368, 370, 378-382, 384] were classified as "moderate quality". **Table 2-2** shows the quality assessment for the included studies. In terms of evidence quality, the certainty regarding the overall values of leptin, ghrelin, insulin, and

gastrin were downgraded to "very low" due to several factors. All the studies included in the meta-analyses were observational studies, most were rated as a "moderate quality", and substantial or/and considerable level of heterogeneity was observed among the studies (**Table 3-2**).

Table 2-2: Summary of the quality appraisal for the included studies using the Newcastle-Ottawa Scale (NOS).

Studies	Selection					Comparability	Outcome			Total
	Representativeness	Sample Size	Nonexposed cohort	Ascertainment of exposure	Outcome of interest defined	Measurement Tool	Outcomes assessment	The Length of follow-up	Adequacy of follow-up	
Al-Rawi *et al.* [289]	*	*	N/A	*	*	**	*	*	*	9
Zouhal *et al.* [376]	N/A	*	*	*	*	**	*	*	*	9
Alzoughool *et al.* [377]	N/A	*	N/A	*	*	N/A	*	*	N/A	5
Faris *et al.* [344]	*	*	N/A	*	*	**	*	*	*	9
Vardarli *et al.* [378]	N/A	*	N/A	*	*	*	*	*	*	7
Mushtaq *et al.* [366]	*	*	N/A	*	*	*	*	*	*	8
Ganjali *et al.* [379]	*	*	N/A	*	*	*	N/A	*	*	7
Kassab *et al.* [368]	N/A	*	N/A	*	*	**	*	*	*	8
Kassab *et al.* [367]	N/A	*	N/A	*	*	**	*	*	*	8
Bogdan *et al.* [383]	N/A	N/A	N/A	*	*	*	*	*	*	6
Haghighy *et al.* [380]	N/A	*	N/A	*	*	*	*	*	*	7
Iraki *et al.* [369]	N/A	N/A	N/A	*	*	**	*	*	N/A	6
Fedail *et al.* [370]	*	*	N/A	*	*	*	N/A	*	*	7
Muhammad *et al.* [382]	*	*	N/A	*	*	**	*	*	N/A	8

Nomani *et al.* [381]	N/A	N/A	N/A	*	*	**	*	*	*	7
Abdullah *et al.* [384]	N/A	*	*	*	*	*	*	*	*	8

5–6 considered a low-quality study
7–8 moderate quality
9–10 high quality

Table 2-3: Summary of the GRADE Assessment of each study included in the Meta-analysis.

Outcomes	№ of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Anticipated absolute effects
			Risk difference with Pre fasting Ramadan
Ghrelin (pg/mL)	97 (3 non-randomised studies)	⊕◯◯◯ Very low [a,b]	SMD (95% CI) **0.31 higher** (0.03 higher to 0.6 higher)
Gastrin (pg/mL)	37 (3 non-randomised studies)	⊕◯◯◯ Very low [a,c,d]	SMD (95% CI) **0.23 higher** (0.54 lower to 0.99 higher)
Insulin (μU/mL)	208 (6 non-randomised studies)	⊕◯◯◯ Very low [a,c,e]	SMD (95% CI) **0.24 lower** (0.51 lower to 0.02 higher)
Leptin (μg/mL)	580 (12 non-randomised studies)	⊕◯◯◯ Very low [a,c,f,g]	SMD (95% CI) **0.11 lower** (0.36 lower to 0.14 higher)

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations

a. The studies included in the analysis were observational.

b. The included studies were rated as having a high risk of bias.

c. Most of the included studies were rated as having a moderate risk of bias.

d. Substantial heterogeneity was observed between the studies (I^2 = 51%).

e. Moderate heterogeneity was observed between the studies (I^2 = 43%).

f. Significant heterogeneity was observed among studies with $P < 0.05$.

g. Substantial heterogeneity was observed between the studies (I^2 = 77%).

2.4 Discussion

This systematic review and meta-analysis investigated the pooled “effect” size of intermittent fasting during Ramadan on the main appetite-regulating hormones. A wide range of studies was included in the analysis, which captured differences in age, sex, geographical area, and fasting duration time. The meta-analysis of 16 studies showed that fasting during Ramadan was associated with an increase in ghrelin concentrations in healthy individuals. However, the analysis revealed a slight decrease in leptin concentrations following Ramadan fasting, although this change was not statistically significant. Among the results of the studies reviewed in this meta-analysis, there is no significant association between fasting and insulin or gastrin hormones. These findings suggest that fasting during Ramadan is associated with only one change in the appetite-regulating hormones profile, potentially impacting appetite regulation and metabolic processes.

The studies in the meta-analyses show inconsistent findings, reflecting moderate to high heterogeneity across studies. While most hormones showed similar response to fasting, the few observed differences suggest that other factors may be involved. The heterogeneity implies that underlying differences in study design (such as the lack of a control group in the majority of studies, differences in measurement timelines, and blood samples collected at different times), or the different socioeconomic status of the study participants might influence the results. Additionally, the length of fasting varies by location due to differences in daylight hours, which may cause varying degrees of rhythm disruption in studies from different regions. This variation in fasting duration could explain the inconsistent leptin responses observed in our meta-analysis.

During Ramadan, many lifestyle changes occur, including meal composition, time of meals [385], sleep/wake schedules, and sleep duration [360], and reduced physical activity [358]. These changes may affect the plasma concentrations of appetite-regulating hormones [358, 386]. It is important to note that the time between the pre-dawn meal (Suhoor), duration of fasting before blood sample, and time of blood collection varied between studies, and this could have influenced the measured hormone concentrations. Other researchers have found significant correlations between sleep and metabolic hormones such as ghrelin and leptin [359, 360]. Particularly, sleep is crucial in regulating ghrelin and leptin, with a lack of sleep leading to an increase in ghrelin and decrease in leptin concentrations [360]. Increased appetite and hunger sensations accompany these changes.

The observed heterogeneity in the results, particularly regarding leptin concentrations, may be partially attributed to the endogenous circadian rhythms of leptin and the varying sampling times across the included studies. For example, the studies that collected samples during the daytime such as the early morning after dawn meal (Suhoor) or early afternoon (7:00-9:00 or 13:00-14:00) reported a significant increase only in leptin concentrations during Ramadan fasting. In contrast, studies in which blood samples were taken in the evening or nighttime reported a significant reduction in leptin concentrations during Ramadan fasting (19:30-20:30 or 00:15). This circadian rhythm is influenced by factors such as dietary patterns, sleep/wake cycles and overall energy balance. It is important to note that individuals may adapt to the changed Ramadan schedule differently and that these adaptations can change throughout the fasting month. Therefore, studies that collect data at various points during Ramadan (e.g., early, middle, or end of Ramadan) may capture different stages of the adaptation process.

Ghrelin is an orexigenic peptide generated by specialised endocrine cells in the stomach [387]. Its concentrations in the bloodstream rise before meals, after periods of food deprivation, and following certain types of weight loss to trigger appetite. Therefore, higher ghrelin concentrations can be anticipated during fasting [388]. The increase in ghrelin concentrations aligns with several studies, such as the work of Akan *et al*. [389], who reported a similar elevation in ghrelin post-Ramadan fasting compared with those before Ramadan. Moreover, another study demonstrated that ghrelin increases not just in response to energy deprivation, and that prolonged fasting raises plasma ghrelin concentrations [390]. However, the present results contradict the findings of Mesci *et al*. [391], who noted lower ghrelin concentrations during Ramadan fasting. On the other hand, Alzoghaibi and colleagues [291] observed no significant change in ghrelin concentrations before and during Ramadan, as all the blood samples were taken at 22:00, 02:00 , 04:00, 06:00, and 11:00. Notably, they found a significant decrease in leptin concentrations at 22:00 during Ramadan. Thus, the results of studies on ghrelin during Ramadan fasting vary. This discrepancy might be attributed to variations in study populations, fasting durations, or differences in dietary composition during non-fasting hours.

Leptin, an appetite-suppressing hormone produced by adipose tissue, opposes the effects of ghrelin. Its concentrations decrease with reduced body fat and food intake, reflecting fat storage size and energy balance changes [392]. Leptin production is mainly controlled by insulin-driven changes in fat cell metabolism [393]. Consequently, variations in insulin concentrations caused by energy restriction during Ramadan might explain the fluctuations in leptin concentrations [394, 395]. Moreover, the timing and distribution of food intake throughout the day influence the daily rhythm of leptin by altering insulin secretion patterns [371]. Leptin concentrations rise more significantly after a regular meal when it is consumed in the evening compared with morning meals, and this

increase is even higher after a longer period of fasting (fasting for 48 hours) [396]. This finding contrasts with the study by Alzoghaibi and colleagues [291], who demonstrated a significant decrease in leptin concentrations at 22:00. This result is inconsistent with the finding of a previous study, which observed no significant change in leptin concentrations during the nighttime [383]. Additionally, Bogdan *et al*. [383] observed a notable shift of about 5 hours in the peak and nadir serum leptin concentrations during the 23rd day of Ramadan fasting. Nevertheless, they did not observe significant changes in the amplitude, or the average 24-hour concentration of leptin compared with measurements taken before Ramadan [383]. The differences in study results may be due to uncontrolled factors like eating habits, and environmental conditions (including light exposure), which could have caused delayed shifts in the observed circadian rhythm. Conversely, a different study found that short-term fasting resulted in a 30% to 66% reduction in leptin concentrations [397]. The discrepancy in study findings could be attributed to regional variations in cultural norms or the extended duration of daytime fasting, which could alter how the body responds.

The mechanisms underlying Ramadan fasting may involve significant alterations in dietary composition, characterised by an increase in carbohydrate-rich and fatty foods, alongside a reduction in protein and vegetable intake. These changes subsequently influence the concentrations of appetite-regulating hormones [398]. Muslims refrain from eating and drinking from dawn to sunset, resulting in notable changes to their meal schedules with specific Ramadan-style dietary patterns [398]. Typically, they have two primary meals: Iftar (after sunset) and Suhoor (before dawn) [399, 400]. This change in eating patterns results in prolonged fasting periods, which affect the secretion of appetite-regulating hormones [376]. Moreover, the diet during Ramadan often shifts towards higher intakes of dietary fat [401], carbohydrates, and sugar-rich food while reducing protein and vegetable intake [398, 402].

This change in macronutrient balance can affect appetite-regulating hormones secretion and metabolism. Despite the common perception of increased food consumption during Iftar and Suhoor, studies have indicated that total energy intake often decreases during Ramadan fasting due to the limited eating window and prolonged fasting periods [403, 404]. Finally, the diet composition changes during Ramadan fasting, characterised by altered meal timing, macronutrient balance, and energy intake, work in concert with modifications in appetite-regulating hormone concentrations.

The present meta-analysis did not show significant changes in insulin concentrations during Ramadan. However, these results should be viewed in light of the broader hormonal and metabolic changes documented in the literature. The lack of significant changes in insulin concentrations in this analysis contrasts with some studies that have found alterations in insulin sensitivity during Ramadan [404]. This discrepancy could be due to the complex mix of factors affecting insulin concentrations, including fasting duration, shifts in meal timing, dietary composition, and frequency during Ramadan, time of blood sample, and physical activity levels can all influence study results [405-407]. It is important to note that while the meta-analysis did not show significant changes, individual responses may vary, and some studies have reported improvements in insulin sensitivity in certain populations. Moreover, some studies have found that Ramadan fasting can improve insulin sensitivity and glucose metabolism, especially in people with metabolic syndrome or type 1 diabetes [406, 408]. However, results vary, another study showed no significant changes in insulin concentrations or insulin sensitivity [407]. While some studies suggest that fasting during Ramadan may improve insulin sensitivity in certain individuals, the findings are inconclusive and depend on individual variations and the methodology of each study.

The finding of no significant change in gastrin concentrations is consistent with the limited existing research on gastrin during Ramadan fasting [381]. This stability in gastrin concentrations indicates that the fasting schedule during Ramadan may not significantly affect gastric acid secretion patterns, even with the changes in meal timing, which is relevant for understanding gastrointestinal health during fasting [381]. Furthermore, the analysis indicates stable insulin and gastrin concentrations during Ramadan despite changes in eating patterns, suggesting the body's adaptability to the altered feeding schedule. However, the accuracy of this stability is highly contingent upon the timing of blood sample collection in relation to the end of the last meal before fasting. This adaptability is further supported by studies that show improvements in lipid profiles and reductions in body weight during Ramadan fasting [409, 410]. Overall, the complex interaction of different physiological adaptations during intermittent fasting needs further study, particularly considering the potential for individual differences and the impact of cultural dietary practices during Ramadan.

The exclusion of participants with diabetes was necessary to isolate the metabolic effects of Ramadan fasting in healthy individuals without the confounding influence of underlying metabolic dysfunction or medication use. However, this exclusion limits the generalisability of findings to the broader population, including those with prediabetes or metabolic syndrome who might benefit from understanding the effects of intermittent fasting. Future studies should consider stratified analyses that include individuals with varying degrees of glucose dysregulation to assess differential responses to Ramadan fasting and inform clinical recommendations for diverse patient populations.

An ideal control group for future Ramadan fasting studies should consist of individuals matched for age, sex, BMI, and baseline metabolic

parameters who maintain their normal eating patterns throughout the same time period. This would control for seasonal variations in diet, physical activity, sleep patterns, and environmental factors unrelated to the fasting intervention. Additionally, a control group of non-fasting individuals from the same cultural background would help distinguish effects specifically attributable to the fasting regimen from broader lifestyle and dietary changes associated with Ramadan observance. Such controlled study designs would significantly strengthen the evidence base and allow for more definitive conclusions about the independent effects of Ramadan intermittent fasting on appetite-regulating hormones.

This systematic review has several limitations. First, the search strategy was restricted to articles published in English, potentially excluding relevant studies in other languages. Moreover, the exclusive inclusion of observational studies may have affected the overall quality of the evidence presented, as those studies are the only studies that fit to the review inclusion criteria. A significant degree of heterogeneity was also noted among the studies. Additionally, several studies that were included lacked clear specifications regarding fasting hours and/or fasting days, which could impact hormone concentrations. These limitations should be considered in future research. Furthermore, many studies did not include a control group or failed to provide data on total energy intake, dietary composition, body weight, or detailed physical activity before and after intermittent fasting during Ramadan. These factors are critical confounders that may influence the interpretation of appetite-regulating hormones concentrations.

This systematic review recommends conducting further well-designed studies to comprehensively evaluate the effects of intermittent fasting during Ramadan on appetite-regulating hormones and metabolic health.

These studies could reveal whether the month-long pattern of daytime fasting and nighttime eating leads to unfavourable changes in hormone regulation or metabolic markers predisposing individuals to adverse long-term outcomes related to insulin sensitivity, glucose regulation, and fat metabolism. Studies should incorporate appropriate control groups for seasonal and lifestyle factors unrelated to fasting, while also considering detailed dietary intakes and meal composition during both fasting and non-fasting periods. Furthermore, studies should assess changes in body weight, body composition, and sleep patterns before, during, and at the end of Ramadan fasting. The diurnal variations in these hormones in response to the altered eating schedule during Ramadan should be evaluated. To facilitate consistent comparisons across studies, standardized measurement techniques and time points should be employed. It is also crucial to include diverse populations to account for variations in fasting duration, climate, and cultural dietary practices. Finally, extended follow-up studies are recommended to explore both the short-term and long-term effects of Ramadan fasting on hormonal regulation and metabolic health.

2.4.1 Conclusion and future perspectives

The findings of these meta-analyses on the potential effects of Ramadan fasting on appetite-regulating hormones revealed a significant increase in ghrelin concentrations, no significant changes in gastrin and insulin concentrations, and a slight non-significant decreased leptin concentrations post-Ramadan fasting. While the meta-analyses focused on ghrelin, leptin, insulin, and gastrin, future well-designed and long-term studies should also examine other relevant hormones, such as cortisol, GLP-1, CCK, and PYY, to provide a more comprehensive picture of the endocrine changes during Ramadan fasting.

Chapter 3 General Methods

In this chapter, study one refers to the relationships between lean body mass and physical activity, age, and body composition in an overweight cohort with Pre-Diabetes, which is reported in Chapter 4, study two refers to the impact of lifestyle factors and eating behaviours on body composition changes after 8-weeks' low-energy diet, which is documented in Chapter 5. It is important to note that I was not involved in any of the original research work described in the following sections. All data collection was conducted by the Nottingham PREVIEW research team. My involvement has been limited to the subsequent analysis of the data obtained after the entire PREVIEW project was completed.

3.1 A brief overview of the PREVIEW study

During the International PREVIEW (Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World) trial, 2300 participants with pre-diabetes were recruited for a 36-month (3 years) intervention. It was a large, randomised, controlled, and multicentre study aimed at investigating how lifestyle interventions, such as diet and physical activity, can help prevent the onset of T2DM in individuals who are overweight or obese and at higher risk of developing T2DM. The trial was conducted in six countries within the European Union, as well as in Australia and New Zealand. The sites involved in this project, included Copenhagen University (Denmark), Maastricht University (the Netherlands), Helsinki University (Finland), University of Nottingham (U.K.), Medical University of Sofia (Bulgaria), Navarra University (Spain), Sydney University (Australia), and Auckland University (New Zealand).

The intervention started with an 8-week weight-loss phase followed by a 34-month weight-maintenance phase. The first phase employed a low-energy diet "LED" intervention to bring about >8% weight loss (see Section 3.5), and the second phase encouraged a lifestyle (diet and exercise) change to help maintain the weight loss (see Section 3.4). Data from the weight-maintenance (second) phase do not form part of this thesis. The Nottingham PREVIEW was conducted at the Queen's Medical Centre (QMC) campus, Life Sciences, Medical School, University of Nottingham, from August 2013 until the end of the study in March 2015. The Nottingham site in the UK aimed to recruit 315 adults aged 19 to 70 but managed to recruit 264 participants within the recruitment window. In this thesis, secondary analysis of data obtained from the Nottingham participants during the first phase of PREVIEW, the international PREVIEW study was registered at ClinicalTrials.gov under NCT01777893.

3.1.1 Ethical considerations

The protocol for Nottingham PREVIEW was approved by the Human Ethics Committees at the UK National Research Ethics Service (NRES) and the East Midlands (Leicester) Ethics Committee; Ethics number 13/EM/0259. All participants gave written consent before any measurements were taken. They were informed that their participation was voluntary and that they could withdraw from the study at any point without needing to give a reason. Participants were also informed that this was an intention-to-treat (ITT) study, meaning all aspects of the study would be analysed. In cases of complete withdrawal, this was documented, and an end-of-trial termination form was completed. Additionally, all data, including source data for analysis, were identified only by a unique participant study code. This ensured that the researcher accessed no personally identifiable information, such as names, dates of birth, contact details, or code logs as part of this PhD programme. The analyses were not expected to yield results that could affect participants' healthcare. Participants were not contacted for further information, and the outcomes were presented using aggregated, group-level data.

3.2 Participants and Recruitment

3.2.1 Process of recruitment and advertising

The recruitment process for Nottingham PREVIEW began in September 2013 and primarily relied on collaboration with primary healthcare providers, such as general practitioner [411] practices and the 'Dietitians in Obesity' networks. Additionally, other methods were employed to broaden outreach, including placing newspaper advertisements, distributing newsletters, publishing articles in local media, and leveraging contacts with national obesity associations to increase the visibility of the study. These combined efforts were designed to ensure a wide range of participants and maximise recruitment. If participants expressed interest in joining the study, they were pre-screened (see Section 3.2.3) and those

who fulfilled initial inclusion criteria, scheduled a convenient appointment for their medical screening at the School of Life Sciences, Medical School, University of Nottingham, located at QMC in Nottingham (see Section 3.2.4).

3.2.2 Study Participant Recruitment

For Nottingham PREVIEW, 3914 adult participants were pre-screened via telephone or email. After pre-screening for eligibility, 979 individuals were invited to be screened, with 264 participants found to be eligible following the screening, and were enrolled in Nottingham PREVIEW, as shown in the following flowchart **Figure 3-1.**

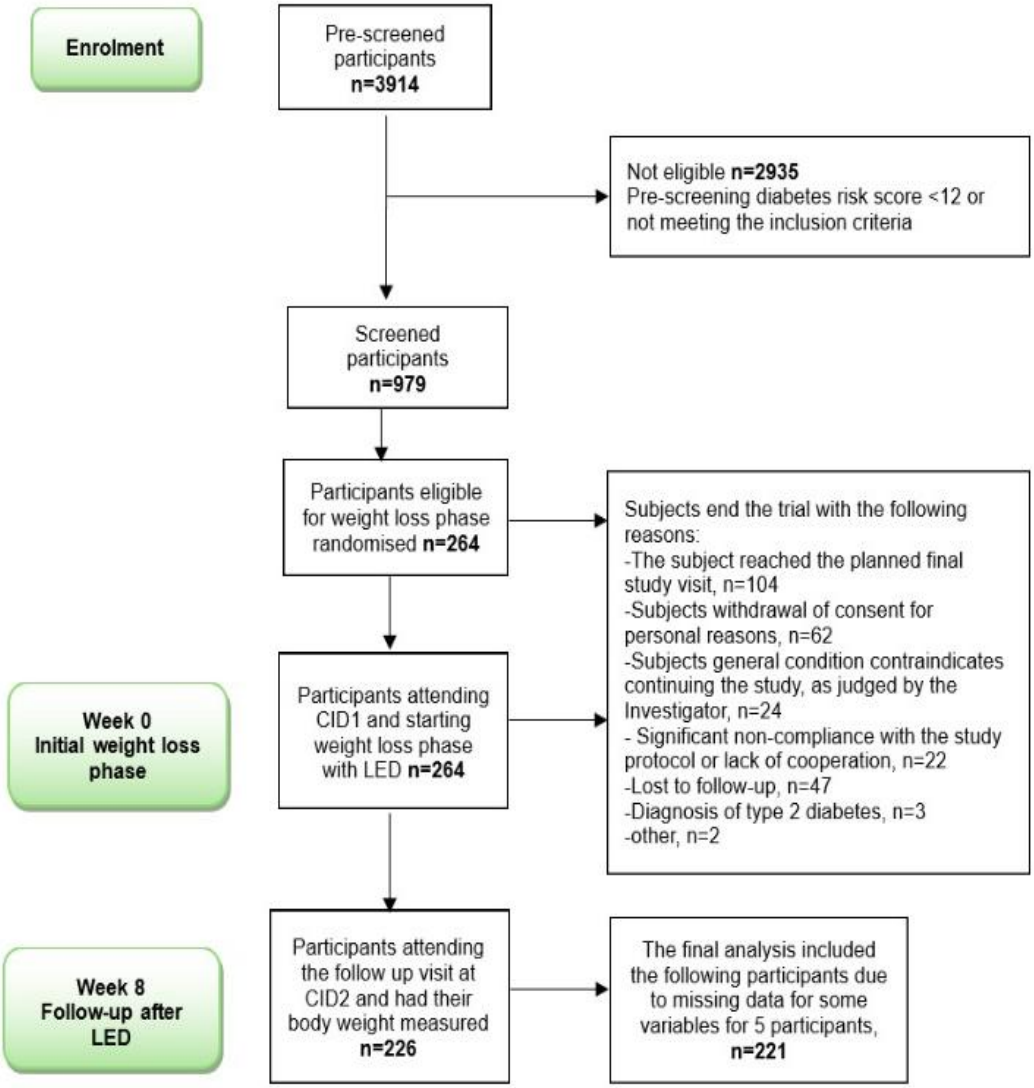


Figure 3-1: Flowchart of the Trial: Pre-screening, screening, initiation of the weight-loss phase, and participant follow-up throughout the study.

3.2.3 Pre-Screening

GP practices in Nottinghamshire were contacted by the research team and provided with project details. Interested practices were recruited, and their patient databases were searched through the PRIMIS system (https://www.nottingham.ac.uk/primis/primis-home.aspx), to identify potentially suitable individuals according to age, BMI, impaired glucose tolerance, and absence of a diabetes diagnosis. GP practices then sent invitation letters and information leaflets to potential participants, who were asked to contact the PREVIEW research office for more information. Researchers conducted pre-screening interviews to explain the study and assess eligibility, including using the Finnish Diabetes Risk Test [412, 413]. Verbal consent was obtained to record these data, and data were securely stored until the end of recruitment to allow follow-up if required, after which all pre-screening data was archived in de-identified format using only a pre-screening ID number.

Eligible individuals, based on pre-screening interviews and a Diabetes Risk Test score above 12 out of 26, were sent a detailed Patient Information Sheet (PIS) [414] and a leaflet explaining the DXA scan. Two weeks later, a follow-up call confirmed receipt and addressed any questions. If interested, participants scheduled their medical screening at the University of Nottingham. Those needing more time or who had not read the PIS were asked to contact the research office later to arrange their screening.

3.2.4 Screening

The screening process was conducted at the School of Life Sciences, Medical School, University of Nottingham, located within the QMC. It included anthropometric measurements and blood screening, in addition

to completion of questionnaires. The anthropometric measurements included standing and seated height, body weight, resting blood pressure [90], and HR. Participants over 55 years old were required to undergo a 12-lead electrocardiogram (ECG). An oral glucose tolerance test (OGTT) was performed to evaluate glycaemic status. This was a simplified two-sample test, with blood samples collected via upper limb venepuncture after 10 hours of fasting and at 120 minutes following consumption of the oral glucose load (75g glucose in 300 ml water) for the analysis of blood glucose concentration [415]. In addition to the standard fasting blood analysis of BG, venous blood was collected prior to the OGTT for the analysis of a full blood count (FBC), thyroid function tests (TFTs), liver function tests (LFTs), urea, and electrolytes. This was done as a precaution to check that participants did not have an underlying medical condition. Collected blood samples were analysed locally at the Nottingham University Hospital QMC Pathology Department, only if the participant was deemed eligible to participate based on the blood glucose results of the OGTT. The screening visit involved a series of questions designed to confirm each individual's eligibility criteria. There were questions about age, ethnicity, medical history, weight changes, and behavioural patterns.

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion Criteria

The study included participants aged 19 to 70 years with a BMI of 25 kg/m² or higher, classifying them as overweight or obese. Participants were required to meet the pre-diabetes criteria set by the WHO and the International Diabetes Federation [5] [416]. Specifically, they had to show either Impaired Fasting Glucose concentration (IFG), indicated by fasting BG between 5.6 and 6.9 mmol/L, or Impaired Glucose Tolerance (IGT), characterised by a two-hour BG of between 7.8 to 11.0 mmol/L after the oral 75-gram OGTT. Glycated haemoglobin (HbA1c) was not considered for eligibility due to the high degree of variability in the assay between

different clinical laboratories in 2013. There were no restrictions on sex or ethnicity for eligibility, and smokers were allowed to participate in the trial as long as they had not changed their smoking habits in the previous month. Additionally, all participants were required to give written informed consent and have a good understanding of spoken and/or written English, as the study materials and sessions were conducted exclusively in English. Finally, participants were required to attend the Clinical Investigation Days (CID) at the School of Life Sciences, Medical School, University of Nottingham.

3.3.2 Exclusion Criteria

Participants were assessed for eligibility through pre-screening interviews and medical questionnaires before recruitment and randomisation, and exclusion criteria are listed in **Table 3-1**. Those with T2DM and normoglycemia were excluded following the screening. Moreover, significant hypertension or a history of uncontrolled hypertension within the past six months and any other significant cardiovascular disease that had occurred within the last six months were included as exclusion criteria.

Table 3-1: Summary of inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Participants were aged ≥18 years and older	Diagnosis with T2DM
They had a BMI of ≥25 kg/m².	Documented major CVD, such as current angina, stroke, or myocardial infarction occurring within the last six months, heart failure, or symptomatic peripheral vascular disease.
They were diagnosed with prediabetes according to WHO/IDF criteria: For IFG, their fasting venous plasma glucose levels were between 5.6 and 6.9 mmol/L, or for IGT; their venous plasma glucose concentration was	Participants with heart failure, or symptomatic peripheral vascular disease. Significant hypertension or uncontrolled hypertension within the past six months (Blood pressure readings with a systolic value >160 mmHg and/or diastolic >100 mmHg, regardless of hypertension treatment

between 7.8 and 11 mmol/L two hours after ingesting a 75 g glucose solution, with fasting glucose levels below 7 mmol/L.	status). If on treatment, no change in medication within the past three months.
Participants read the Participant Information Sheet and signed the Consent Form.	An advanced stage of chronic renal failure
They agreed to participate in the LED and to be randomised into an intervention group.	Severe liver disease, such as cirrhosis (fatty liver disease was permitted).
There was no change in their smoking habits over the past month (smoking was allowed, but no changes were permitted).	Active cancer or in remission within five years of the last treatment.
They committed to attending CID during normal working hours	Active inflammatory bowel disease, chronic pancreatitis, celiac disease, or other disorders that could cause malabsorption.
	Previous bariatric surgery
	Participants with chronic respiratory disease.
	Participants with neurological, musculoskeletal, or other disorders would have unacceptable risk or difficulty complying with the protocol (such as a physical activity program).
	A participant recently underwent surgery until fully recovered.
	Participants with diseases transmitted through blood, such as Hepatitis B and HIV.
	Psychological disorders (such as major depression and bipolar disorder) were excluded.
	Participants were excluded if they had used medications in the past 3 months that could affect BW or glucose metabolism, such as glucocorticoids (except inhaled/topical steroids and bronchodilators), antiepileptic or antidepressant drugs, any weight loss or herbal medications
	Participation in competitive sports
	Self-reported weight change >5 kg within 2 months before baseline.
	On special diets within 2 months before the study starts (e.g. Atkins or vegetarian)
	Severe food intolerances were likely to interfere with the study.
	Overconsumption of alcohol - over 21 standard drinks a week for men or more than 14 drinks for women.
	Drug abuse within the previous 12 months.

	Reported eating disorders.
	Pregnancy or lactation, or intent to become pregnant within the next 36 months.
	Lack of access to a phone or the internet (required for study contact).
	Inadequate ability to understand English.
	Psychological or behavioural issues that could impair protocol compliance.
	Blood donation or transfusion within one month before baseline.
	Laboratory criteria included: venous plasma glucose within eligibility limits, Haemoglobin concentration below local reference values (indicating anaemia), Creatinine levels exceeding 1.5 times the upper limit of the normal reference range, and abnormal ECG, which is required only for participants aged 55-70.
	After the LED phase, excluded participants did not lose 8% of their initial BW at CID2.

Abbreviations: BW; Body weight, **BMI;** Body mass index, **IFG**; impaired fasting glucose, **IGT**; impaired glucose tolerance, **CID**; clinical investigation days, **LED;** Low energy diet, **T2DM;** type 2 diabetes mellitus, **CVD;** cardiovascular disease, **ECG**; Electrocardiograph, **HIV;** Human Immunodeficiency Virus.

3.4 Study Design

The PREVIEW study was conducted as a three-year (156-week) multinational, randomised, controlled clinical intervention trial across eight countries and sites. The PREVIEW study design included two phases, as shown in **Figure 3-2**. First, phase 1 was the 8-week weight loss low-energy diet intervention aimed at achieving a body weight (BW) loss of ≥8%. Participants attended the clinic every two weeks, where their BW was measured, and they participated in group counselling sessions designed to encourage adherence to the diet. This thesis presents data from Phase 1 of the project. Second, phase 2 of the study was a randomised lifestyle intervention lasting 34 months, aimed at maintaining weight loss and preventing weight regain. These data are not included in this thesis.

CIDs were conducted throughout the trial, during which participants underwent anthropometric measurements and laboratory assessments:

CID1 - Baseline (start with weight reduction with LED) pre-LED/ 0 months

CID2 - Post LED/ 2 months (end of weight reduction and start of weight maintenance period)

CID3 – 6 months (these data are not presented in the thesis)

CID4 – 12 months (these data are not presented in the thesis)

CID5 – 18 months (these data are not presented in the thesis)

CID6 – 24 months (these data are not presented in the thesis)

CID 7 - 36 months (these data are not presented in the thesis)

The subsequent sections of this chapter contain details on both CID1 and CID2. **Table 3-2** displays the outcome and assessment time points for data from Phase 1 of PREVIEW.

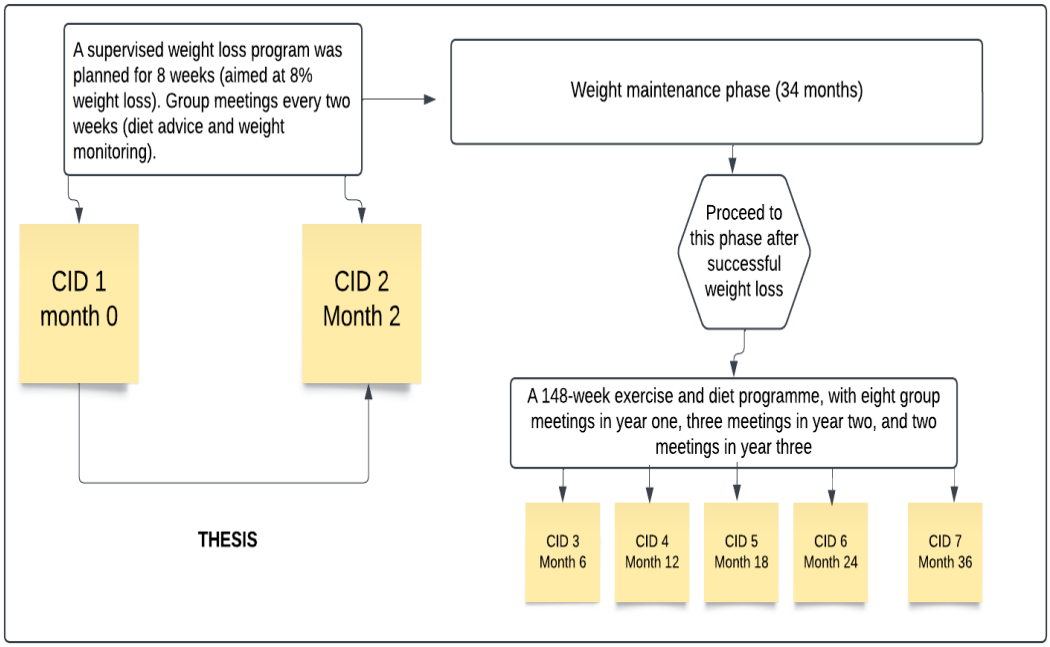


Figure 3-2: PREVIEW Study Design. (**CID**=Clinical Investigation Days)

Table 3-2: PREVIEW Nottingham Study Design.

Data collection	Screening (Pre-recruitment)	Baseline	8-week LED Intervention phase			
Visit	1	2 CID1	3	4	5	6 CID2
Assessment time points (week)		0	2	4	6	8
Age	X					
Sex	X					
ethnicity	X					
Group meeting		X	X	X	X	X
Height		X				
Weight		X	X	X	X	X
Body composition (DXA or BIA)		X				X
Waist, hip and thigh circumferences		X				X
Blood pressure, and heart rate. ECG (55-70 y)		X				X
7-day accelerometry; 7-day PAL, and Baecke questionnaire (work, sport, and leisure score)		X				
PSS score		X				X
PSQI score		X				X
Food diary collection 4-day food record		X				
OGTT (0, 30, 60, 90, 120 min)	0+120 min	X				X
Fasting blood samples		X				X
Urinary nitrogen (g)		X				
4-day food record (dietary protein and energy intake)		X				X
TFEQ (restraint, disinhibition, hunger scores)		X				X

Abbreviations: LED: low-energy diet; **CID:** Clinical Investigation Day; **DXA**: Dual-energy X-ray absorptiometry; **BIA**: Bioelectrical impedance; **ECG:** Electrocardiogram; **PAL:** Physical activity log; **PSS:** Perceived Stress Scale; **PSQI:** Pittsburgh Sleep Quality Index; **OGTT:** Oral glucose tolerance test; **LDL:** low-density lipoproteins; **HDL:** High-density lipoproteins; **TFEQ:** Three-factor eating questionnaire; **X:** measurements conducted.

3.4.1 CID1 (baseline) for eligible participants

After the screening, 264 participants were invited to the CID1 visit for an initial assessment before entering the weight-reduction phase. Prior to this visit, participants were sent a leaflet (Medical Screening Visit) detailing pre-visit requirements (e.g., fasting instructions) and their appointment date and time. The recruited participants were also asked to record all foods and drinks consumed over a 4-day period in a diet diary, complete a 24-hour urine collection, and wear an activity monitor for 8 days before their CID1 (baseline visit).

At the CID1 clinic visit, measurements made included anthropometric measurements (Height, weight, waist, hip, and thigh circumferences). A 20G cannula (Venflon™, Becton Dickinson) was sited antegrade into a superficial vein in the antecubital fossa after prior intradermal administration of ~0.02ml 1% lidocaine. The fasting venous blood sample was then taken from the cannula, via a 3-way tap, for insulin and glucose analysis. Participants were then given 75g of glucose in 300ml of water to consume, and blood samples were collected every 30 minutes for 2 hours. Body composition was then measured via DXA scan (those with BW <120kg) or bioelectrical-impedance, and various questionnaires were completed, all described in detail later in this Chapter. **Table 3-3** shows the parameters measured at CID1.

Following the completion of CID1/Baseline, the Phase 1 intervention began. The LED intervention was explained, and instructions on how to incorporate the meal replacement sachets into their diet were given at an initial LED appointment. A second appointment was scheduled two weeks after meal replacement sachets were distributed. A detailed description of the LED intervention and the 2-weekly visits are provided later in this chapter.

Table 3-3: The parameters measured on CIDs in PREVIEW Nottingham.

Clinical Investigation Days	Parameters measured
Screening	Height, weight BP, HR, ECG (55-70y) 2-sample OGTT including fasting, and 2-hour glucose safety tests, including liver function tests.
CID1 (Baseline) **PRE-LED**	Weight, waist, hip and thigh circumferences. BP, HR Multi-sample OGTT, fasting glucose, fasting insulin, HbA1c, C-peptide, CRP, Lipid profile. Food record diary. Body composition (DXA or BIA).
CID2 (post-LED)	Weight, waist, hip and thigh circumferences. BP, HR Fasting glucose, fasting insulin, HbA1c, C-peptide, CRP, Lipid profile. Body composition (DXA or BIA).

Abbreviations: BP: blood pressure; **HR**: heart rate; **ECG:** electrocardiogram; **OGTT:** oral glucose tolerance test; **HbA1c:** Haemoglobin A1c; **CRP:** C-reactive protein; **DXA**: dual-energy x-ray absorptiometry; **BIA:** Bioelectrical Impedance Analysis; **CID:** Clinical Investigation Day.

3.5 Phase 1- LED intervention

3.5.1 Achieving ≥ 8% Initial Weight Loss with LED

As described in detail in Chapter 5, section 5.2.3. Over the 8-week LED period, participants had group meetings with a dietitian every two weeks (weeks 0, 2, 4 and 6). Each visit lasted approximately 120 minutes and was attended by up to 15 people, including a body weight check to encourage compliance with the diet. The following are the calculations used to calculate each participant's own weight loss goal and total weight loss required to achieve the 8% weight loss goal:

Present weight (_____ kg) × 0.92 = Target weight (_____ kg)

Present weight (_____ kg) − Target weight (_____ kg) = Required weight loss (_____ kg).

3.5.2 Group Support and Education Sessions

As outlined above, group sessions were conducted every two weeks throughout the 8-week weight loss LED phase. During these sessions, participants received dietary counselling in groups of approximately eight

individuals, with an emphasis on maintaining motivation and supporting participants to adhere to the strict diet. Behavioural training was a central component of each session. The group sessions were led by a Registered Dietitian, with additional support staff present to record the participants' weights and issue LED sachets. Each session followed a consistent format, beginning with a 10-minute introduction that included welcome activities, participant introductions, ice-breaking exercises, and an overview of the session's learning outcomes. This was followed by a 20-minute recording and measuring segment where body weight was documented, adverse events were noted, and concomitant medications were recorded. The core of each session was a 30–40-minute educational component featuring presentations and discussions on topics specifically relevant to participants' current stage in the intervention. After this educational segment, a 20-25-minute facilitated group discussion allowed participants to reflect on their personal experiences, address challenges, and share successes. Sessions concluded with a 15-minute resource distribution and summary period during which LED sachets were provided to participants, written resources were distributed, and details about the next meeting were confirmed.

The content of the sessions followed a deliberate progression to support participants through the different stages of the intervention. The Week 2 session focused on explaining the relationship between weight loss, physical activity, and T2DM prevention while also addressing immediate concerns about the LED and managing side effects. Participants were instructed on maintaining hydration and proper medication management while on the LED. By Week 4, the educational focus shifted to how the LED effects body composition and causes weight loss, with guidance on adapting eating routines and physical activity. Participants learned techniques to adapt LED products for greater variety and were introduced to daily "meal planners" to structure their intake. PODs (Prompts for Optimal Decisions) were introduced as motivational tools. As participants

approached the end of the LED phase in Week 6, the sessions prepared them for weaning off the LED after Week 8, educated them on the process of changing lifestyle behaviours, and prepared them for increasing physical activity. Action plans for physical activity were developed during this session. The final session in Week 8 marked a significant transition as participants received their diet and exercise intervention group assignment for the weight maintenance phase. They received detailed explanations of their dietary prescription and meal-based system, comprehensive guidance on weaning off the LED with minimal discomfort and engaged in exercise planning and goal setting for the maintenance phase. This session culminated in formal contracting for commitment to the intervention.

Throughout all sessions, specific behavioural techniques were employed to enhance adherence and motivation, as detailed below. The sessions were designed to accommodate different learning styles through the use of visual aids, group discussions, written resources, and hands-on activities. This comprehensive approach aimed to maintain motivation during the challenging LED phase while gradually preparing participants for the transition to the weight maintenance phase and their randomised diet and exercise interventions.

3.5.3 Behavioural Education Sessions

The PREVIEW behaviour modification intervention toolbox (PREMIT) paradigm was utilised to implement behaviour change in a group setting every two weeks during the LED phase [417]. Staff trained in PREMIT methods delivered the group counselling sessions

PREMIT was specifically designed as a theory-driven, evidence-based toolkit to support lifestyle behaviour modification in the PREVIEW study. According to Kahlert *et al.* [417] PREMIT integrated multiple

psychological models, including the Social Cognitive Theory, the Transtheoretical Model, the Self-Determination Theory, and the Health Action Process Approach. This comprehensive framework enabled a staged approach to behaviour change that aligned with the phases of the PREVIEW intervention.

Social Cognitive Theory [418] contributed the fundamental concept of self-efficacy, which has been consistently identified as a key predictor of successful behaviour change in diabetes prevention programs [419, 420]. Within PREMIT, self-efficacy enhancement strategies included mastery experiences through gradual skill building and vicarious experiences through group-based learning, approaches that have demonstrated effectiveness in prior diabetes prevention research [421, 422].

The Transtheoretical Model [423] informed PREMIT's staged approach to intervention delivery. This model recognises that individuals progress through distinct stages of change: pre-contemplation, contemplation, preparation, action, and maintenance, each requiring tailored strategies. PREVIEW's phased structure aligned with these stages, particularly during the transition from the intensive LED phase (action) to the weight maintenance phase (maintenance), a critical period identified by Greaves *et al.* [424] as requiring specific cognitive and behavioural supports to prevent relapse.

Self-Determination Theory [425] provided the framework for addressing motivation quality through supporting autonomy, competence, and relatedness. These principles were operationalised in PREVIEW through participant choice in activity selection, skill development opportunities, and group-based support, elements that Teixeira *et al.* [426] identified as particularly effective for long-term weight management. The incorporation

of PODs (Prompts for Optimal Decisions) reflects self-determination principles by fostering autonomous decision-making, a strategy that has shown promise in maintaining behavioural changes in similar interventions [427].

The Health Action Process Approach [428] contributed a crucial distinction between motivational processes (goal setting) and volitional processes (goal pursuit and maintenance). This distinction guided PREVIEW's transition from the initial focus on building intention to later emphasis on planning and overcoming barriers. The formal contracting used in week 8 exemplifies the "implementation intentions" concept described by Gollwitzer and Sheeran *et al.* [429], which has demonstrated effectiveness in bridging intention-behaviour gaps across multiple health domains.

This integration of complementary theoretical approaches follows recommendations from systematic reviews by Michie *et al.* [430] and Dombrowski *et al.* [431], which found that interventions combining multiple behaviour change techniques derived from established theories tend to produce more substantial and sustainable results than single-theory approaches. Similar comprehensive theoretical frameworks have been successfully implemented in the Finnish Diabetes Prevention Study [412] and the U.S. Diabetes Prevention Program [432], both of which demonstrated long-term effectiveness in diabetes risk reduction through lifestyle modification. PREMIT's implementation within PREVIEW builds upon these established approaches while tailoring intervention components to the specific challenges of substantial weight loss maintenance following a LED phase, addressing a critical gap identified in previous intensive dietary interventions [433, 434].

During the 8-week LED phase, the behavioural techniques were strategically implemented to address the psychological needs of participants at this stage. In Week 2, a balanced approach of "fear appeal" regarding health risks associated with diabetes, combined with fostering self-efficacy, was employed. Participants were guided to identify past successes they could build upon, creating a foundation for confidence in their ability to adhere to the challenging diet. By Week 4, the behavioural focus expanded to include attribution re-training, helping participants develop positive interpretations of their experiences, particularly when facing difficulties with the LED. Group facilitators were instructed to "promote positive attributions in those who have been struggling" while continuing to foster self-efficacy in those who were "compliant and doing well". In Week 6, as participants prepared for the transition from weight loss to weight maintenance, goal setting was formally introduced. Participants were asked to "identify their own opportunities for increasing physical activity from Week 8" and begin developing action plans. This aligned with the PREMIT approach described by Kahlert *et al.* [417], which emphasised the importance of preparing participants for behavioural transitions between intervention phases. The final session in Week 8 culminated with formal contracting, where participants completed "My PREVIEW Contract" forms as a commitment to the group intervention. This technique emphasised in PREMIT, helped create an explicit commitment to behaviour change goals and strengthened the likelihood of follow-through during the maintenance phase.

Throughout all sessions, the delivery methods were designed to accommodate various learning styles (visual, aural, read/write, and kinaesthetic) to maximise participant engagement and information retention. This multi-modal approach to education delivery was consistent with the PREMIT framework's emphasis on tailoring intervention components to enhance effectiveness.

3.6 Anthropometric Measurements

The time points for CID measurements of anthropometric outcomes are outlined earlier and summarised in **Table 3-2**. The specific procedures for each measurement are detailed below.

3.6.1 Body Weight and Height Measurements

Body weight was measured using a bariatric scale (Marsden, accuracy assured, serial number 21305354, manufactured in China for Marsden by Charder Electronic Co. Ltd.) in a fasting state with an empty bladder, and participants wore only light clothing. Two measurements, accurate to the nearest 0.1 kg, were taken, and the average was calculated. For height measurement, participants removed their shoes and stood with their heels, buttocks, and upper back against a wall-mounted stadiometer, looking directly forward (top of head horizontal). Height was recorded to the nearest 0.5 cm, and the mean of two measurements was calculated.

3.6.2 Waist, Hip, and Thigh Circumference Measurements

Waist, hip, and thigh circumferences were measured with a non-stretch tape to the nearest 0.5 cm while the participant stood. Two measurements were taken, and the average was used. Waist circumference (WC) was measured at the midpoint between the bottom of the rib cage (last floating rib) and the top of the iliac crest, at the end of expiration, as shown in **Figure 3-3**. Hip circumference was measured at the widest part between the hips and buttocks, using the same method as for the waist, as shown in **Figure 3-4**. Mid-thigh circumference was measured on the right side, with the tape placed horizontally around the thigh, halfway between the midpoint of the inguinal crease and the top edge of the patella, as shown in **Figure 3-5**.

Figure 3-3: Measurement of waist circumference [435].

Figure 3-4: Measurement of hip circumference [435]

Figure 3-5: Measurement of the Mid-thigh [435]

3.6.3 Body Composition

Body composition measurements were conducted using either DXA or bio-impedance methods at the David Greenfield Human Physiology Unit Medical School, QMC. All measurements were performed according to the manufacturer's guidelines, with participants in a fasted state and with an empty bladder. The same DXA device (GE Lunar 'Prodigy'; GE Healthcare, Madison, WI, USA) and the manufacturer's enCORE software were used throughout the study, and the machine was calibrated daily as per manufacturer instructions. The analysis followed the standardised PREVIEW multicentre protocol and local quality-control procedures. Before undergoing the DXA scan, women of childbearing age were able to test for pregnancy. Bio-impedance was employed to

estimate body fat percentage for participants who exceeded the DXA machine's weight limit or chose not to undergo an X-ray scan. Body composition assessments were completed for all participants at CID 1 and 2. The key outcomes measured included fat mass in kilograms, body fat percentage, fat-free mass in kilograms, body lean percentage, and bone mineral density (BMD) (DXA only).

In this thesis, lean body mass derived from DXA data was calculated as the sum of fat-free mass and bone mineral content. This variable corresponds to lean soft tissue (LST), as defined in the recent international consensus on standardised body composition terminology by Prado *et al.* [874]. The standardised term "lean mass" is used throughout this thesis to align with nomenclature used in the PREVIEW trial and to facilitate comparison with existing literature, while acknowledging that this refers specifically to LST when DXA-derived. For three participants whose body weight exceeded the manufacturer's limit for DXA scanning (250 kg for GE Lunar DXA), BIA was used to determine body composition. Although DXA-derived LST and BIA-derived lean mass are not methodologically identical, the term "lean mass" has been applied consistently across all participants for whole-body and regional (leg lean mass) assessments to maintain clarity in reporting. This terminology and measurement approach are consistent with those used in the PREVIEW trial, which employed GE Lunar DXA systems across multiple international centres [253, 332]. These studies reported whole-body and regional estimates of fat mass, lean mass, and bone mineral content using comparable methods, allowing direct comparison between the present analyses and PREVIEW outcomes while maintaining alignment with current international consensus on body composition nomenclature.

3.6.3.1 DXA

X-rays are produced when high-speed electrons collide with a tungsten target within a vacuum tube. The energy of the resultant X-ray photons is influenced by the voltage applied across the X-ray tube; for instance, the Lunar Prodigy operates at 76 kV, generating photons with energies typically ranging from 20 to 100 keV [436]. When these X-ray photons interact with the human body, they can undergo one of three processes: transmission, where the photon passes through unaffected; Compton scattering, where the photon collides with loosely bound electrons, resulting in a change in direction and a loss of energy; or absorption, where the photon transfers all its power to a tightly bound electron, ceasing to exist [437]. The combined effects of absorption and scattering contribute to the attenuation of the X-ray beam, which depends on the initial energy of the photons, the mass per unit area of the tissue, and the specific attenuation coefficient of that tissue [438]. Notably, lower energy photons are attenuated more than higher energy photons when passing through the same tissue, and bone exhibits a greater attenuation effect compared with adipose tissue. Each atom and, consequently, each tissue possess a unique 'attenuation coefficient value [439].

In the context of DXA, a filter is utilised to create dual-energy X-rays, effectively separating the spectrum into high and low-energy components, typically peaking at around 40 keV and 70 keV [440]. The fundamental principle underlying DXA acquisition is based on the differential attenuation of these energy levels. A detector measures the attenuation of each energy component, allowing for the calculation of the ratio of low to high energy attenuation, referred to as the R-value. This R-value enables the DXA software to estimate the mass fraction of two tissue components by utilising the mass attenuation coefficients associated with each tissue type [440]. Although the human body comprises various tissues, it can be simplified into dual compartments, such as bone versus non-bone (fat and LM), fat versus non-fat (bone and

lean tissue mass) or lean vs. non-lean (bone and fat). The DXA software analyses these pairings to provide estimates for bone, fat, and LM [441].

— Historical Development and Validation

DXA technology was originally developed in the late 1980s primarily for bone mineral density assessment, but was subsequently adapted for body composition analysis [442]. The technique evolved from single-photon absorptiometry and dual-photon absorptiometry, which used radioactive isotopes as radiation sources. The transition to X-ray-based technology in DXA significantly improved precision and scan speed [443].

Validation studies have compared DXA measurements against reference methods such as underwater weighing, multi-compartment models, and computed tomography. Early validation work by Svendsen *et al.* [444] demonstrated strong correlations between DXA-derived body composition measurements and those obtained from underwater weighing and total body potassium counting. Further validation by Prior *et al.* [445] confirmed DXA's accuracy against the four-compartment model, considered the gold standard for body composition assessment because it separates body mass into fat mass, bone mineral content, total body water, and residual (protein and soft tissue mass). More recent validation studies have continued to support DXA's accuracy while identifying specific limitations [446, 447].

— Strengths and Limitations

DXA offers several advantages as a tool for assessing body composition. Its high precision (reproducibility) is a major strength, with reported coefficients of variation of 1-2% for total body fat measurements [448]. The technique provides regional body composition data, allowing analysis of specific anatomical regions such as trunk, limbs, and android/gynoid distributions [449]. While DXA is often described as

providing a three-compartment analysis (bone, fat, and LM), it's important to note that it cannot directly measure all three components simultaneously in the same position. Instead, DXA distinguishes between bone mass and non-bone mass or, in areas without bone, differentiates between fat and lean tissue. The whole-body composition reported by DXA is an estimation derived from these separate measurements, particularly from non-bony regions [450]. This estimation approach differs from the simpler two-compartment models used in bioelectrical impedance analysis or hydrodensitometry.

Despite these strengths, DXA has important limitations. The technique assumes constant hydration of lean tissue at approximately 73%, but this can vary with age, disease states, and after significant weight loss [167]. This assumption can lead to errors in LM estimation, particularly in clinical populations with altered hydration status [451]. Additionally, DXA's measurement of trunk fat may be less accurate than limb fat due to the heterogeneous tissue composition and greater tissue depth in the trunk region [452].

DXA measurements are also influenced by technical factors, including the specific equipment manufacturer and software version used. The results of cross-calibration studies have shown systematic differences between different DXA systems (e.g., Hologic, Lunar, and Norland), requiring caution when comparing results across different machines [168]. Furthermore, software updates from the same manufacturer can affect absolute values, though relative changes typically remain consistent [162].

— Confounding Factors and Special Populations

Several factors can confound DXA measurements. Excess hydration or dehydration can significantly impact results, with each 5% change in lean tissue hydration potentially altering the estimate of body fat percentage

by 1-2.5% [453]. Recent food consumption can also affect measurements, particularly in trunk regions, due to gastrointestinal contents being inconsistently classified as lean or fat tissue [454]. DXA may be unsuitable or require special consideration for certain patient groups. Pregnant women are typically excluded from DXA scans due to radiation exposure concerns, despite the low dose [455], though it should be noted that pregnant women were already excluded from participating in the PREVIEW study. For individuals with metal implants, the regions containing metal produce artefacts that prevent accurate tissue analysis in those specific areas [456]. Additionally, patients with significant ascites or oedema may have inaccurate body composition estimates due to the altered hydration status of lean tissue [167].

For people with severe obesity, standard DXA scanners present practical challenges. When participants in the PREVIEW study exceeded the scan field dimensions (typically 60-66 cm in width and 193-198 cm in length) or weight capacity (usually 136-159 kg, depending on the scanner model), modified positioning techniques were employed. These included "half-body" scanning, where only one side of the body is scanned and results are doubled, assuming bilateral symmetry [457]. Alternatively, two separate scans were performed with different body portions on the scanning table, and specialised software was used to stitch the images together [458]. These approaches introduce additional measurement errors but allow for approximate assessment in individuals who would otherwise be excluded from DXA analysis. When neither approach was feasible, alternative methods, such as bioelectrical impedance analysis, were employed with appropriate documentation of the methodological difference.

The radiation dose associated with a total body composition scan using DXA is notably low, typically less than 1 μSv, which is comparable to the cosmic radiation exposure from one hour on a transatlantic flight and approximately 100 times less than the radiation dose from a standard

chest X-ray [459]. To ensure the accuracy of DXA measurements, a daily Quality Assurance scan is performed using a phantom block with a known attenuation coefficient, confirming a coefficient of variation [460] for these measurements below 2%. This quality control is essential for confirming the reliability of DXA as a diagnostic tool for assessing bone mineral density and body composition [461].

Figures 3-6 and 3-7 illustrate a representative DXA scan report for body composition analysis for one female participant. These scans provided a detailed analysis of total body composition and manufacturer-defined region-specific measurements for arms, legs, trunks, ribs, spine, and pelvis. The anonymised data collected is described above and analysed to identify patterns in body composition and evaluate changes resulting from the LED interventions (**Chapters 4 and 5**).

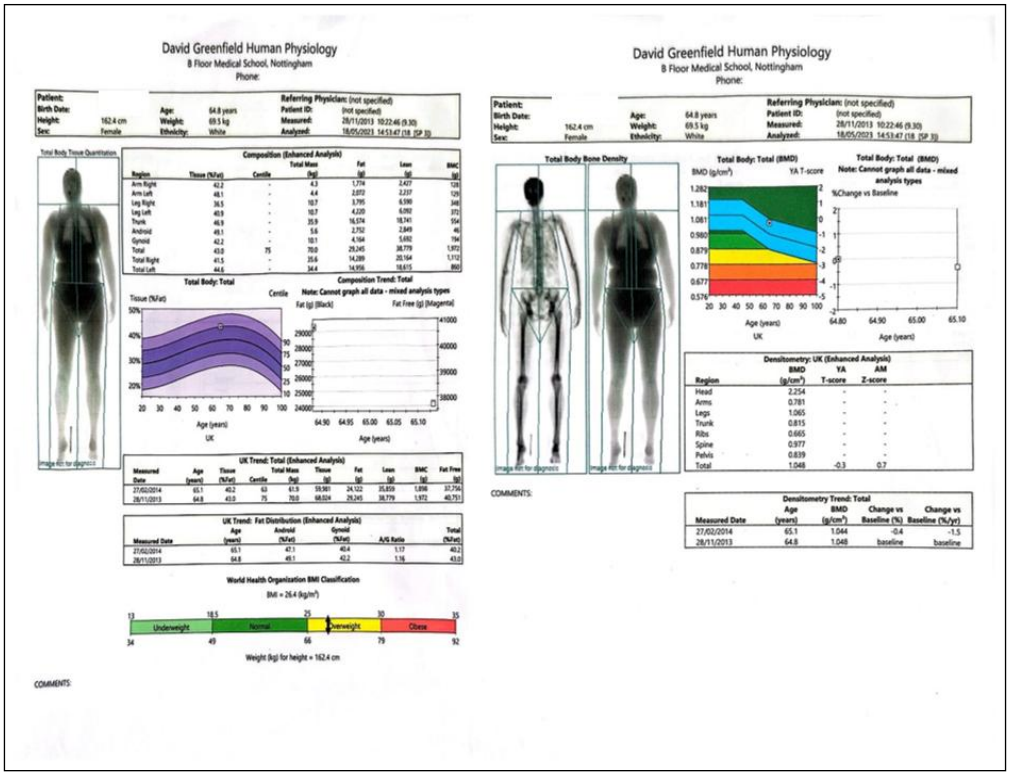


Figure 3-6: DXA Scan Report Showing Body Composition and Bone Mineral Density Analysis.

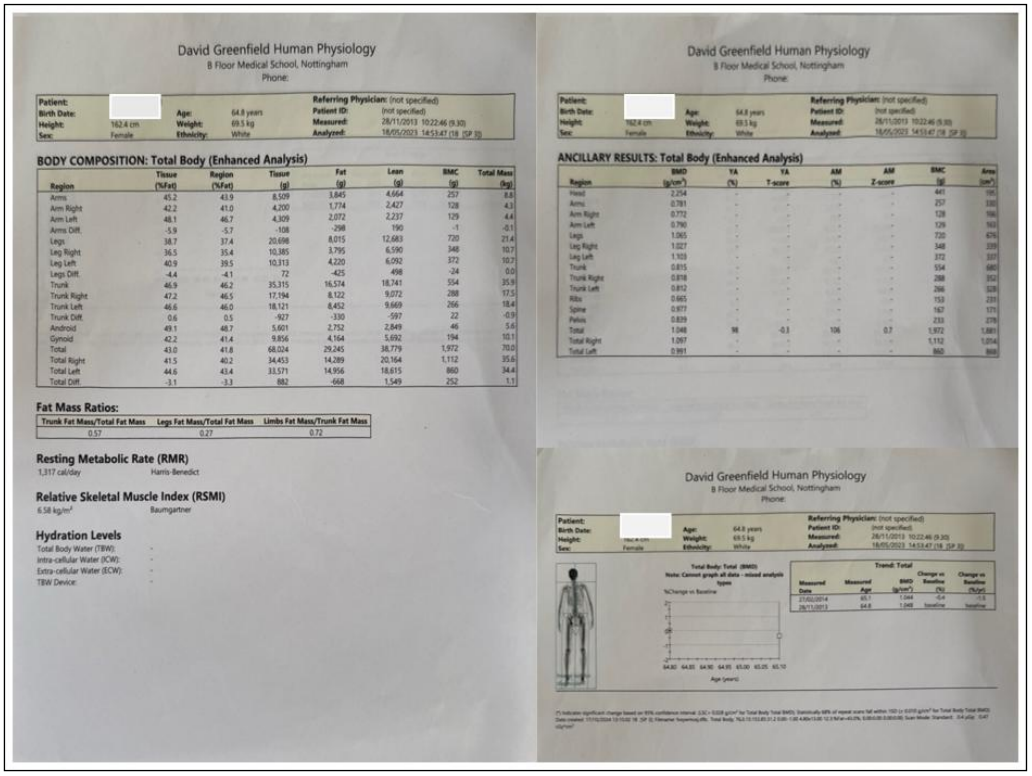


Figure 3-7: Comprehensive DXA Scan Report (for the same participant as in Figure 2) including Body Composition, Fat Mass Ratios, and Bone Mineral Density (BMD) Analysis.

3.6.3.2 Bio-Impedance Analysis (BIA)

The BodyStat QuadScan 4000 (BodyStat Ltd.), a portable battery-powered device, was used for this purpose, as shown in **Figure 3-8**. This multi-frequency bio-impedance analysis unit measures at 5, 50, 100, and 200 kHz, providing a rapid, reliable, non-invasive, and cost-effective means of estimating body composition, including fat-free mass, fat mass, and intra- and extra-cellular fluid.

Figure 3-8: The BodyStat QuadScan 4000.

— Principles and Development

Bio-impedance analysis operates on the principle that different body tissues conduct electrical current to varying degrees due to their physical and chemical composition [462]. Fat-free tissues (muscle, blood, organs) contain high proportions of water and electrolytes, which makes them excellent conductors of electrical current. In contrast, adipose tissue contains minimal water and electrolytes, which causes poor electrical conductivity and higher impedance [170]. BIA technology was developed in the 1960s but gained widespread application in clinical and research settings during the 1980s with the advent of single-frequency devices [463, 464]. As multi-frequency BIA became available in the 1990s, it enabled a more comprehensive measurement of body composition by differentiating between extracellular and intracellular water compartments [172]. This advancement was based on the observation that at low frequencies (≤50 kHz), the electrical current primarily flows through extracellular fluid, while at higher frequencies (≥100 kHz), it penetrates cell membranes and flows through both intracellular and extracellular compartments [465].

— Validation and Algorithmic Development

BIA has been validated against reference methods including hydrodensitometry, DXA, and deuterium dilution. Early validation studies by Lukaski *et al.* [466] and Segal *et al.* [467] established strong correlations (R>0.9) between BIA-derived fat-free mass and hydrodensitometry in healthy adults. More recent validations against four-compartment models have confirmed the accuracy of BIA across diverse populations, though with varying degrees of precision [173, 174].

The algorithms used to convert impedance measurements to body composition estimates are population-specific and typically incorporate variables such as age, sex, height, weight, and ethnicity. The BodyStat QuadScan 4000 (BodyStat Ltd., Douglas, Isle of Man) employs multiple regression equations developed from population-specific reference data. For adults, these predictive equations include:

$$FFM\ (kg) = 0.340 \times (H^2/R) + 15.34 \times (H) + 0.273 \times (W) - 0.127 \times (Age) + 4.56 \times (Sex) - 12.44$$

Where ‘H’ is height in cm, ‘R’ is resistance in ohms, ‘W’ is weight in kg, and ‘Sex’ is coded as 1 for males and 0 for females [468]. For the estimation of total body water (TBW), the device uses the equation:

$$TBW\ (L) = 0.377 \times (H^2/R) + 0.14 \times (W) - 0.08 \times (Age) + 2.9 \times (Sex) + 4.65$$

From this, intracellular water (ICW) and extracellular water (ECW) are derived using multi-frequency measurements, as ICW is primarily reflected in the high-frequency impedance values, while ECW is determined from low-frequency measurements [172].

— Strengths and Limitations

BIA offers several advantages as a method for assessing body composition. It is non-invasive, relatively inexpensive, portable, and requires minimal operator training. The measurements are rapidly obtained (typically under 5 minutes) and have good reproducibility, with reported coefficients of variation of 1-5% for repeated measures [170]. The multi-frequency BIA method also provides information about fluid distribution between intracellular and extracellular compartments, which is useful in assessing hydration status and cellular health [469].

Despite these strengths, BIA has important limitations. The accuracy of BIA measurements is dependent on several assumptions, including consistent tissue hydration at approximately 73% for fat-free mass. A deviation from this standard is likely to introduce significant error due to several factors, including disease conditions, extreme exercise, or dietary factors [175]. The predictive equations are population-specific and may not be accurate when applied to individuals with characteristics different from the reference population. This is particularly relevant for individuals with extreme body composition (very low or high body fat) or abnormal fluid distribution [176].

— Confounding Factors

Multiple factors can confound BIA measurements. Hydration status significantly impacts results, with dehydration leading to overestimation of fat mass and hyperhydration leading to underestimation [177]. It is possible that recent food or fluid intake, particularly within 2-4 hours of measurement, can alter results due to changes in body water distribution and intestinal contents [470]. Body position and limb placement affect fluid distribution; thereby, standardised positioning is essential for reliable measurements [471]. Exercise before measurement affects BIA results through altered blood flow, body temperature, fluid losses, and electrolyte

shifts. Intense exercise should be avoided for at least 8-12 hours before testing [472]. Ambient and skin temperature variations can also influence impedance measurements, with higher temperatures reducing impedance due to vasodilation and increased blood flow [473]. Menstrual cycle phases in women cause fluctuations in total body water and its distribution, potentially altering BIA results by 2-4% across the cycle [175]. Additionally, different BIA devices and their proprietary algorithms may produce varying results for the same individual, which makes it necessary to be cautious when comparing values obtained from different equipment [469].

— Special Populations and Contraindications

BIA may not be suitable for certain patient groups. Individuals who have implanted electronic medical devices such as pacemakers or defibrillators should not undergo BIA due to potential interference with device function, although the risk is minimal with the low currents used [170]. Pregnant women are generally excluded from BIA assessments in research settings due to the altered fluid distribution and lack of validated equations, though the procedure itself is not known to pose any health risks to women during pregnancy [474]. In patients with significant fluid abnormalities, such as those with renal disease, congestive heart failure, or ascites, BIA results may be inaccurate due to non-standard hydration of lean tissue and abnormal fluid distribution [468]. For individuals with extreme obesity (BMI >34 kg/m²), standard BIA equations tend to underestimate fat mass, which requires population-specific equations for accurate assessment [475].

Measurements were taken with participants lying supine for at least 5 minutes, with their limbs slightly abducted from their body. After cleaning the skin with alcohol wipes, four electrodes were placed on the right side of the body: two on the hand (one at the wrist and one on the dorsal surface behind the knuckles) and two on the ipsilateral foot (one at the

ankle and one behind the toes). The device then sent a small, imperceptible electrical current through the body to measure resistance and reactance at the different frequencies. These measurements were then converted to body composition estimates using the manufacturer's proprietary algorithms.

3.6.4 Blood Pressure and Heart Rate Measurements

Systolic (SBP) and diastolic (DBP) blood pressure and HR were measured using a validated automatic device Dynamap™, GE Medical on the right arm after 5-10 minutes of rest. The Dynamap™ device uses oscillometric technology to determine blood pressure non-invasively. This method detects oscillations in arterial wall pressure during cuff deflation, with the point of maximum oscillation corresponding to mean arterial pressure. Systolic and diastolic pressures are then calculated using proprietary algorithms based on characteristic changes in oscillation amplitude. The device also measures HR by detecting the periodic pressure pulses during measurement. Measurements were taken three times with a 1-minute rest between recordings, and the mean value was recorded. Participants were instructed to avoid vigorous physical activity, coffee, and smoking for 12 hours before the measurement. The device was serviced and calibrated annually.

3.7 Metabolic Measurements

As outlined earlier, **Table 3-2** presents the time points for CID measurements of metabolic outcomes. The details of each measurement procedure are provided below. All laboratory analyses were performed at the central laboratory hub in Helsinki, Finland, managed by the National Institute for Health and Welfare. Serum and whole blood samples were initially stored after collection in Nottingham at -80°C in a laboratory at the Medical School, QMC. They were then shipped by air to Finland,

where they were analysed in batches at the central laboratory hub in Helsinki.

3.7.1 Fasting Blood Samples

The fasting blood samples were analysed for concentration of glucose, insulin, HbA1c, C-peptide, C-reactive protein, liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and lipid profiles, including total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides.

3.7.1.1 Glucose Measurement

The enzymatic hexokinase method was used to measure glucose concentration in blood samples using the Architect ci8200 from Abbott Laboratories. This method involves a two-step enzymatic reaction [476, 477]:

1. First, hexokinase catalyses the phosphorylation of glucose with adenosine triphosphate (ATP) to form glucose-6-phosphate (G6P) and adenosine diphosphate (ADP): Glucose + ATP → Glucose-6-phosphate + ADP
2. In the second reaction, glucose-6-phosphate dehydrogenase (G6PD) specifically oxidises G6P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD+) to NADH: Glucose-6-phosphate + NAD+ → 6-phosphogluconate + NADH + H^+

The amount of NADH produced is directly proportional to the glucose concentration in the sample and is measured spectrophotometrically at 340 nm [478]. The hexokinase method is usually considered the reference method for glucose measurement due to its high specificity, precision, and minimal interference from other sugars [479].

3.7.1.2 Insulin and C-peptide Measurements

Insulin and C-peptide concentrations were determined using chemiluminescent microparticle immunoassays (CMIA) on the Architect i2000SR analyser (Abbott Laboratories). The CMIA technology for insulin measurement involves:

1. Anti-insulin antibodies coated on paramagnetic microparticles bind to insulin in the sample.

2. After washing, an acridinium-labelled anti-insulin conjugate is added, which binds to the captured insulin molecules.

3. Following another wash cycle, trigger solutions are added, causing a chemiluminescent reaction that produces light.

4. The light emission is directly proportional to the insulin concentration and is measured in relative light units (RLUs) [480, 481].

The C-peptide assay follows a similar principle but uses specific anti-C-peptide antibodies. These immunoassays demonstrate high sensitivity and specificity with minimal cross-reactivity with proinsulin or insulin analogues, making them suitable for accurate quantification in clinical samples [482].

3.7.1.3 HbA1c Measurement

HbA1c was measured using high-performance liquid chromatography (HPLC) on the TOSOH G8 analyser (Tosoh Bioscience), which is certified by the National Glycohemoglobin Standardisation Program (NGSP). The TOSOH G8 uses cation-exchange HPLC, where:

1. The sample is mixed with a haemolysis solution to lyse red blood cells.

2. The released haemoglobin components are then separated based on their ionic interactions with the negatively charged groups in the column.
3. As different haemoglobin fractions elute at different times, they are detected by measuring absorbance at dual wavelengths (415 nm and 500 nm).
4. The percentage of HbA1c is calculated from the ratio of the HbA1c peak area to the total haemoglobin peak area [483, 484].

This method provides excellent precision and accuracy, with minimal interference from haemoglobin variants, which is critical for the reliable monitoring of glycaemic control [485].

3.7.1.4 High-Sensitivity C-Reactive Protein (hs-CRP) Measurement

High-sensitivity C-reactive protein (hs-CRP) was quantified using an immunoturbidimetric assay on the Architect ci8200 platform. The immunoturbidimetric method operates as follows:

1. Anti-CRP antibodies are added to the sample, forming immune complexes with CRP present in the serum.
2. These immune complexes cause turbidity in the solution, proportional to the CRP concentration.
3. The turbidity is measured by the decrease in light transmitted through the sample at a specific wavelength (typically 340-380 nm) [486, 487].

The high-sensitivity version of this assay uses optimised optics and enhanced signal processing to detect CRP at concentrations as low as 0.1 mg/L, which is necessary for cardiovascular risk assessment [488].

3.7.1.5 Liver Enzymes (ALT and AST) Measurements

For liver enzymes, both ALT and AST were measured using the International Federation of Clinical Chemistry (IFCC) standardised enzymatic method, without pyridoxal phosphate activation, on the Architect ci8200. The enzymatic reactions involved are:

For ALT:

1. ALT catalyses the transfer of an amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate: L-alanine + α-ketoglutarate → pyruvate + L-glutamate

2. Lactate dehydrogenase (LDH) then converts pyruvate to lactate while oxidising NADH to NAD^+: Pyruvate + NADH + H^+ → lactate + NAD^+

The rate of decrease in NADH concentration, measured by the change in absorbance at 340 nm, is directly proportional to ALT activity [489].

For AST:

1. AST catalyses the transfer of an amino group from L-aspartate to α-ketoglutarate, forming oxaloacetate and L-glutamate: L-aspartate + α-ketoglutarate → oxaloacetate + L-glutamate

2. Malate dehydrogenase (MDH) then converts oxaloacetate to malate while oxidising NADH to NAD^+: Oxaloacetate + NADH + H^+ → malate + NAD^+

As with ALT, the rate of NADH consumption is measured spectrophotometrically at 340 nm and is proportional to AST activity [490].

3.7.1.6 Lipid Profile Measurements

The lipid profile components were analysed using the following methods:

1. Total cholesterol was measured by enzymatic colourimetric tests involving:

- Cholesterol esterase hydrolyses cholesterol esters to free cholesterol and fatty acids.
- Cholesterol oxidase then converts free cholesterol to cholest-4-en-3-one and hydrogen peroxide.
- Peroxidase catalyses the reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to form a coloured quinoneimine dye.
- The absorbance of this dye, measured at 500-550 nm, is proportional to the cholesterol concentration [491].

2. Triglycerides were also measured by enzymatic colourimetric tests:

- Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids.
- Glycerol kinase phosphorylates glycerol to glycerol-3-phosphate.
- Glycerol phosphate oxidase oxidises glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide.
- As with cholesterol, peroxidase catalyses the reaction of hydrogen peroxide with chromogenic substrates to form a coloured product.
- The absorbance of this product is proportional to the triglyceride concentration [492].

3. High-density lipoprotein cholesterol (HDL-C) was determined using a direct enzymatic method with selective detergent:

- A specific detergent solubilises only HDL particles, releasing HDL cholesterol.
- Non-HDL lipoproteins (LDL, VLDL, chylomicrons) are rendered non-reactive through the addition of magnesium ions and another specific detergent.
- The released HDL cholesterol is then measured using the same enzymatic reactions as for total cholesterol [493, 494].

4. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula when triglyceride levels were below 4.52 mmol/L: LDL-C = Total cholesterol - HDL-C - (Triglycerides/2.2) [mmol/L]

When triglycerides exceeded this threshold, LDL-C was directly measured using a selective solubilisation method:

- Specific detergents selectively solubilise non-LDL lipoproteins (HDL, VLDL, chylomicrons).
- Cholesterol from these particles is consumed by cholesterol esterase and cholesterol oxidase in a non-colour-forming reaction.
- A second detergent then selectively solubilises LDL, and the LDL cholesterol is measured using the standard enzymatic reaction [495].

Data from fasting blood samples used in this thesis were collected at screening, pre (CID1), and post (CID2) the LED intervention.

3.8 Assessment of Physical Activity Pre-LED

Habitual physical activity before starting the study LED intervention was assessed by 7-day accelerometry recording and required participants to wear a small watch-like device (Actiwatch™, Actigraph, USA; **Figure 3-9)** around their waist for 24 hours a day (excluding when showering, swimming, etc.) over seven full days, including two weekend days. The participants were introduced to wearing and using the accelerometer at screening and returned the device at CID1.

The Actiwatch™ accelerometer functions by measuring physical movements using a piezoelectric sensor that generates an electrical voltage when subjected to mechanical stress as a result of body movement [496]. The sensor detects acceleration in multiple directions, converting kinetic energy from movement into electrical signals. These signals are then filtered, amplified, and digitised at a preset sampling frequency (typically 30-100 Hz) [217]. The resulting data are processed through proprietary algorithms to distinguish between different activity intensities based on movement frequency and amplitude. The device uses epoch-based recording, where activity counts are summed over specified time intervals (usually 15-60 seconds), providing quantifiable measures of physical activity volume and intensity throughout the day [497]. Additionally, the absence of movement for extended periods, along with time stamps, allows for the identification of sedentary behaviour and sleep patterns [216].

The data collected included information on activity levels, sleep, and time spent in bed, as illustrated in the Actigraphy™ output (**Figure 3-10**).

Figure 3-9: Physical Activity Monitor.

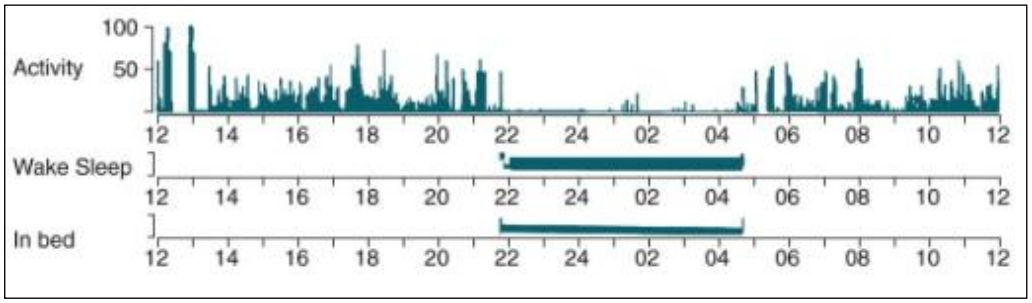


Figure 3-10: Actigraphy Data Showing Physical Activity, Sleep, and Time Spent in Bed Over a 24-Hour Period.

3.9 Nottingham PREVIEW Data Management and Analysis

The PREVIEW study data files provided by the custodians (University of Copenhagen) were collated and uploaded onto a secure University repository, with access restricted to the student and the direct supervisory team. Prior to receiving the data, contact was established with the administrator of the PREVIEW database in Denmark to facilitate the process. As part of the secondary analysis carried out on the thesis, DXA scans archived at Nottingham were reanalysed to determine the fat mass, LM, and bone mineral content of the lower limbs. These additional results were integrated into the PREVIEW data files. The final combined

dataset was interrogated according to the statistical methods described in the statistical analysis section for each chapter.

3.10 Statistical Analysis

A comprehensive statistical analysis is included in each chapter of the thesis. However, several core statistical methods were consistently applied across all studies and are outlined below.

For all studies, data were initially tested for normality using criteria of z-scores of the skewness and kurtosis statistics lying between -1.96 and 1.96. Normally distributed data are presented as mean ± standard deviation (SD), whereas non-parametric data are represented as median [25th-75th percentile] throughout the thesis.

For paired comparisons at single time points (e.g., measurements made pre-LED compared with post-LED), the Wilcoxon Signed Rank Test was used for non-normally distributed data and the paired t-test for normally distributed data. To determine differences between non-paired data (e.g., between males and females), the Mann-Whitney U test was employed for non-normally distributed data and the unpaired t-test for normally distributed data.

When comparing data across multiple sub-groupings (e.g., age categories in Chapter 4), the Kruskal-Wallis Test was used for continuous variables, while the chi-square test was employed for categorical variables such as sex distribution.

The Pearson correlation coefficient (‘R’) was used to investigate associations between variables across all studies.

Linear regression analysis was used across studies to identify predictive variables or statistical modifiers of key outcomes, including lean body mass, lean leg mass, and bone mineral density. Predictors were entered using a stepwise forward selection procedure, in which variables were added sequentially based on their statistical significance (entry criterion $P < 0.05$; removal criterion $P > 0.10$). The pool of candidate predictors was defined a priori from established determinants reported in the PREVIEW literature. Model improvement was assessed by examining the change in adjusted R^2 and the significance of regression coefficients. Data of regression models are presented as the R^2, with significant modifiers of relationships indicated by the R^2 change. When described as “adding a predictor improved the model,” this indicates that inclusion of that variable increased the adjusted R^2 and/or yielded a significant regression coefficient, demonstrating that it explained additional variance beyond the baseline model.

Analyses were performed on a per-protocol basis and included participants who completed the 8-week low-energy diet phase with both baseline and post-intervention measurements available. Participants who withdrew or had missing endpoint data were excluded from the analysis; no imputation of missing data was performed. This approach reflects the physiological efficacy of the intervention among completers rather than the pragmatic effectiveness across all enrolled participants.

All statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) Statistics version 28 (IBM Corp., Armonk, NY, USA). To control for type I error arising from multiple testing, Bonferroni corrections were applied where appropriate. Statistical significance for all analyses was defined using the Bonferroni-adjusted threshold ($P < 0.05$).

Specific analytical approaches unique to individual studies are detailed within the respective chapters.

Chapter 4 Relationships between Lean Body Mass and physical activity, age, and body composition in an overweight cohort with Pre-Diabetes: a secondary analysis of the PREVIEW Study Baseline data.

4.1 Introduction

Skeletal muscle is the largest organ in the human body and accounts for 30 to 40% of body weight [498]. It plays a crucial role in locomotion, posture, temperature regulation, strength, glucose homeostasis, and soft tissue support. In addition, many metabolic processes involved in overall health are regulated by skeletal muscle [499, 500]. Skeletal muscle is the largest protein store in the body, and during times of energy restriction, it serves as an energy reserve and supplies amino acids to critical organs in times of infection or disease [501]. Maintaining muscle mass is based on a dynamic equilibrium between muscle protein synthesis and breakdown, with nutrition and physical activity being external influences on the skeletal muscle [502, 503].

There has been a steady rise in obesity prevalence in older adults in the UK and globally. According to the National Centre for Social Research, there has been an increase in the proportion of medically obese adults in the UK between 1996 and 2010, and by 2025, it is predicted that obesity prevalence in the population will be 47 % in men and 36 % in women [504]. According to the National Centre for Health Statistics, approximately 43 percent of Americans aged 60 years and older were obese in 2017-2018 [505]. Obesity is associated with various chronic diseases, such as non-insulin-dependent diabetes mellitus, cardiovascular disease, osteoarthritis, cancer, stroke, hypertension, and back pain [23, 506-508]. In addition to obesity's adverse effects on health, this condition adversely impacts skeletal muscle and physical performance at all ages, from adolescents to older adults [509-515]. According to these findings, excess body weight and adiposity may negatively affect muscle power and strength as a consequence of a reduction in physical activity.

Several studies have examined the effects of obesity and physical strength in older adults. The incidence of functional decline among older men and women with body mass indexes of more than 30 kg/m^2 is 60% higher than that of older people with healthy-weight [515]. Moreover, a growing ageing population combined with rising obesity rates, will almost certainly result in higher levels of physical disability since physical limitations already increase with age [516]. Research has shown that obesity leads to functional limitations in muscle performance and an increased risk of developing functional disabilities, such as impaired mobility, strength, postural stability, and dynamic balance [517]. In this regard, these findings have significant public health implications.

Regardless of age, individuals with obesity appear to have higher muscle strength than individuals without obesity, suggesting that increased adiposity is associated with increased muscle size and strength due to loading antigravity muscles such as the quadriceps and calf [517]. Several studies have examined lower leg strength and found that individuals with obesity had greater absolute strength than individuals without obesity [509, 510, 513, 518-521]. However, individuals with obesity were found to be weaker when maximum muscular strength was normalized to body mass. Specifically, the consequences of reduced strength relative to body mass in the lower limbs are relevant to older populations, whose functional capacity is typically reduced such as difficulty walking, negotiating stairs, or standing up from a chair or bed [522-524]. In addition, obesity combined with low muscle strength can increase the joint load, which increases the risk of developing osteoarthritis or further exacerbates existing disease, resulting in a reduced quality of life and an increased likelihood of needing joint surgeries e.g. in knee and hip [525, 526].

Several factors affect bone development, including genetics, the environment, and their interactions. Body weight has been shown to be positively correlated with bone density [527] and negatively correlated with bone loss [528]. In addition, these relationships can be influenced by diet, physical activity, and lifestyle choices [528].

Several factors contribute to the relationship between fat mass, muscle mass and bone mass. Firstly, their weight acts as mechanical loading, which induces osteogenesis and prevents bone loss. In addition, fat and muscle directly impact bones [527]. This view is supported by several studies that report that adipose tissue expands in bone marrow with bone loss, suggesting a connection between fat and bone [529, 530]. Leptin and pro-inflammatory cytokines, released from adipocytes, can stimulate osteoclastic bone resorption while suppressing osteoblastic bone formation since adipose tissue acts as an endocrine organ that secretes pro-inflammatory cytokines [531, 532]. Moreover, these researchers noticed that the interaction between muscle and bone was associated with several factors including sex steroids, myokines, muscle-derived progenitor cells, and hormone-signalling pathways [533, 534].

A positive relationship exists between LM and BMD based on studies of body composition [535]. LM, which consists predominantly of skeletal muscle tissue, particularly in the appendicular regions has been shown to have substantial associations with BMD through various physiological mechanisms [536, 537]. It is important to note that body weight plays a fundamental role in determining BMD, primarily due to greater loading of the weight-bearing bones, with studies showing that individuals with higher body weight typically demonstrate higher BMD [538]. A person's body weight is largely the product of their bone mass, fat mass (FM), and LM. There has been considerable controversy regarding the relative contributions of FM and LM components to BMD variation, particularly in

studies that do not adequately control for total body weight. Some studies indicate that LM is more closely linked to BMD than FM, while others indicate FM is more closely related to BMD [539-542]. The latter studies raise an important question about whether the observed relationship between FM and BMD is due to specific properties of adipose tissue or reflects the mechanical loading effect of increased total body weight. Adding to this complexity, other studies have found that both FM and LM are significant predictors of BMD [543-545]. Additional research indicates that the relationship between body composition parameters and BMD may be modulated by demographic factors such as sex, ethnicity, and age. However, further investigation is needed to fully understand these interactions.

Besides being dynamic tissues that change throughout life, bones are also sex dimorphic since males and females have differing hormones. Men's bone length, cortical volume, and outer and inner bone perimeters are significantly greater than women’s [546]. In this regard, LM and FM may affect bones differently based on age and sex. Several studies have shown that LM has a more significant effect on BMD in premenopausal women than FM, and FM has a greater impact on BMD in postmenopausal women than LM [544, 547]. In contrast, other studies have found that only LM is associated with BMD in premenopausal and postmenopausal women [543, 548]. Inconsistent results may result from a high correlation between LM and FM and higher body fat levels in some populations [549, 550].

4.1.1 Aims of the study

This study aimed to investigate the relationship between total body mass and body composition (including regional composition) across an adult age range, and within/between biological sex, in overweight individuals with pre-diabetes (aged 25-70 y). Moreover, it aimed to identify the

aspects of the lifestyle which were associated with a greater lean body mass. The primary hypothesis was that lean body mass would be lower with increasing age category but higher in those who are more physically active and/or heavier (greater muscle loading). Secondary hypotheses were that males would have greater leg lean mass (LLM) compared with females across all age groups, with the age-related decline being more pronounced in females. It was also hypothesised that a positive relationship between body weight and LLM would be modified by physical activity.

4.2 Materials and Methods

4.2.1 Participants

A total of 264 individuals attended the PREVIEW Clinical Investigation Day 1 (baseline data collection pre-intervention; CID1) in Nottingham. Of these, 220 participants (87 males, 133 females) were included in the present analysis. Forty-four participants were excluded due to missing or incomplete data for key variables including DEXA body composition measurements, anthropometric data, or physical activity measurements. Only participants with complete data for all variables relevant to the analyses were included.

4.2.2 Design

This chapter involves the secondary analysis of data, which had been previously collected as part of the PREVIEW intervention [332], using a cross-sectional study design.

4.2.3 Data Collections

Identification of suitable participants began with a thorough review of anonymised PREVIEW Study data files provided by the study coordinating Centre (University of Copenhagen). Data were collated and

uploaded to a secure University repository, with access granted only to the researcher and the direct supervisory team. During this data screening process, the following background data were collected from the files (ID number, age, sex, body weight, height, BMI, waist and hip circumferences, and physical activity measures). The data represent measures collected at the initial screening visit (age, sex), during a 7-day free-living monitoring period (dietary intake and accelerometery) and at CID1 (anthropometry, questionnaires) prior to commencing an 8-week low-energy diet intervention.

DXA scans, archived at Nottingham, were reanalysed by the candidate to determine lower limb lean and fat mass and bone mineral content. The reanalysis methodology is described in detail in this chapter Section 0. These data were added to the PREVIEW data file and interrogated as described in the statistical methods.

4.2.4 Age Categories

Participants were categorised into four age groups (25-39, 40-50, 51-60, and 61-70 years) based on established age-related risk patterns and intervention responses identified in major diabetes prevention trials. This categorisation aligns with evidence from the Diabetes-Prevention-Program-Research-Group *et al.* [421], where different progression rates and intervention effectiveness were observed across age ranges, particularly highlighting distinct patterns in younger adults under 40 compared with older individuals. The categorisation of middle-aged participants into two groups-ages 40-50 and 51-60 highlights a crucial transition period (particularly in women) during which the risk of developing diabetes significantly increases. Studies, such as the Finnish Diabetes Prevention Study by Tuomilehto *et al.* [551], indicate that individuals in these age ranges exhibit varying physiological responses to lifestyle interventions. The oldest age group (61-70 years) was identified based on evidence from both the DPP [421] and Finnish DPS

[551], which demonstrated unique intervention needs and responses in older adults, particularly in relation to physical activity and dietary modifications [552]. This age category corresponds with the American Diabetes Association (ADA) guidelines for risk assessment and intervention based on age [553].

4.2.5 Anthropometry

Physical measurements, including height, weight, and waist circumference, were made by trained researchers. Body weight was measured when the participant was in a fasted state (fasting from midnight the night before) and had an empty bladder. Furthermore, the participants were required to wear light clothing and to remove their shoes during the test. When individuals wore thick clothing in cold weather, they were asked to remove outer clothing before the assessment. Using a weighing scale, two weight measurements were taken to the nearest 0.1 kg, and an average of these two values was used in the analysis.

Height was measured to the nearest 0.5 cm using a portable stadiometer, and the average of the two height assessments was used in the analysis. During this measurement, the participants were required to remove their shoes and have their heels, buttocks, and upper back in contact with the stadiometer. The waist circumference was measured to the nearest 0.5 cm, midway between the xiphoid process and iliac crest, with the participant standing. Two measurements were taken, and the mean was used.

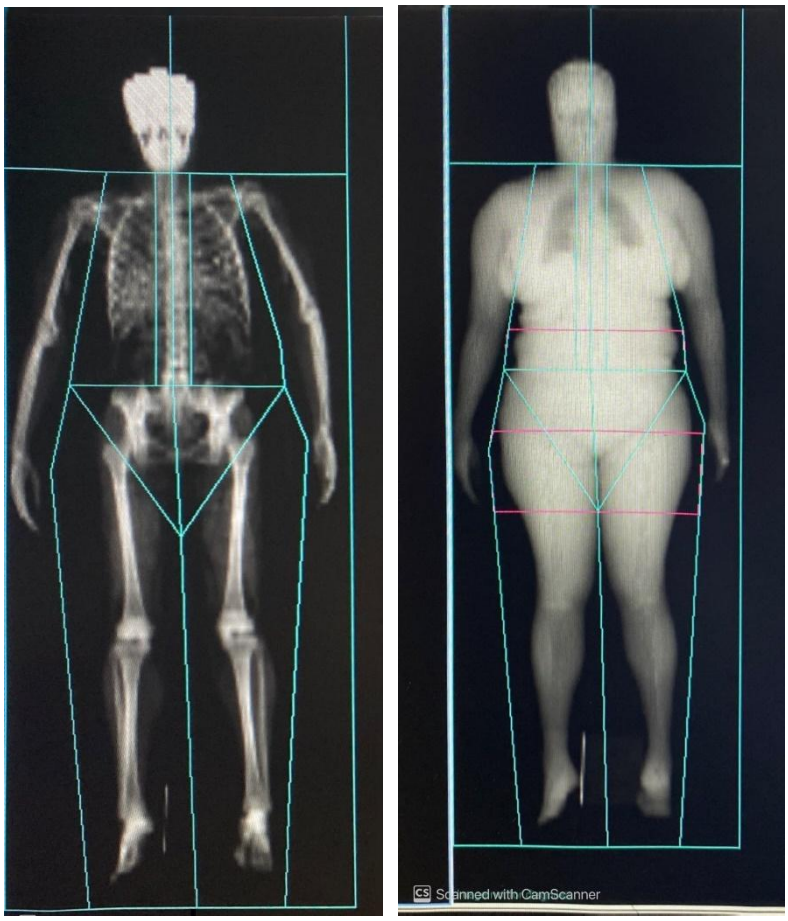
4.2.6 Body composition: Dual Energy X-Ray Absorptiometry (DXA) Scans

Body composition was measured at the David Greenfield Human Physiology Unit (Medical School, QMC) using a DXA scanner (Lunar

Prodigy, GE Medical Systems, Bedford, UK) as shown in **Figure 4-1**. DXA is a technique originally developed to measure bone mineral density for the diagnosis of osteoporosis [554]. However, using this technique, it is also possible to obtain accurate measurements of lean body mass, fat mass, and bone mineral content [555], **Figure 4-2.**

The measurements were performed according to the manufacturer's instructions, and when the participant had an empty bladder. The scanner was calibrated daily following the manufacturer's instructions. The DXA body composition scan was performed on women of reproductive age after they had been tested for pregnancy using a urine human chorionic gonadotropin (hCG) concentration test and been found to not be pregnant.

Figure 4-1: Dual-energy X-ray Absorptiometry Scanner.



Total body and regional assessment of:

- Total Body Fat (kg)
- Total Body Fat %
- Total Body Lean (kg)
- Android Fat %
- Gynoid Fat %
- Leg mass (kg)
- Leg Fat (kg)
- Leg Lean (Kg)
- Leg bone mineral density
- Leg bone mineral content
- BMD
- BMC

Figure 4-2: Copy of Dual-energy X-ray absorptiometry Scan of total body and regional measurements.

4.2.7 Assessment of Physical Activity

Physical activity was assessed by 7-day accelerometery recording across all 24 hours daily. Physical activity was measured with an accelerometer (Actiwatch™, Actigraph, USA). This method required participants to wear a small watch-like device around their waist for 24 hours a day (excluding showering, swimming, etc.) over seven full days, including two weekend days. The participants were introduced to wearing and using the accelerometer at screening and returned the device at CID1.

4.2.8 Statistical analyses

The collected data were subjected to statistical analysis using IBM SPSS (Statistical Package for the Social Sciences) Statistics version 28. Initially, data were checked for normality using criteria of z-scores of the skewness and kurtosis statistics lying between -1.96 and 1.96. Data are represented in tables as the median [25^{th}-75^{th} percentile] and differences in variables, between sex, were assessed using unpaired t-test (data showing normal distribution) or Mann-Whitney-U test (for non-parametric data). The study participants were divided into four different groups based on their age at screening: 25-39 years, 40-50 years, 51-60 years, and 61-70 years. The chi-square test was used for the analysis of sex distribution across age categories, and the difference in continuous variables between age categories was assessed using the Kruskal-Wallis test. Pearson correlation coefficients were used to investigate associations between variables, and regression analysis was then used to identify statistical modifiers of lean body mass, lean leg mass, and bone mineral density.

4.3 Results

4.3.1 Characteristics of the Study Participants

General characteristics of the participants are listed in **Table 4-1.** The median age was not different in males compared with females. Body composition differed between males and females; males were significantly taller and heavier, had greater lean body mass (LBM) and had lower total body fat mass and total body fat percentage than females (each $P<0.001$). Similarly, variables such as android, gynoid, and leg fat mass were higher in females, whereas LLM and BMD were greater in males (each $P<0.001$). Higher android, as well as gynoid fat deposition (when expressed as a percentage of total regional mass) in females, was observed compared with males ($P<0.001$ for both; **Table 4-1).** There

were no significant differences in BMI between the sexes. The ethnic composition of the study cohort was predominantly White Caucasian (n=183, 83.2%), with smaller representations of Black (n=22, 10.0%) and Asian (n=15, 6.8%) participants.

Table 4-1: Participants' characteristics, including anthropometry data, and body composition measured by DXA scan in the different sex groups during pre-LED measurements.

Variable	Males (n=87: 39.5%)	Females (n=133; 60.5%)
	(Median [25th-75th percentile])	
Age (years)	57 [45_63]	51 [43_63]
Height (m)**	1.75 [1.71_1.79]	1.62 [1.58_1.66]
Weight (kg)**	101.6 [93.9_112.2]	92.6 [81.9_105.1]
BMI (kg/m^2)	33.3 [31.2_36.5]	34.9 [31.3_39.5]
Total body fat (kg)**	37.3 [31.4_44.7]	44.4 [38.2_50.4]
Total lean body mass (kg)**	57.5 [51.5_62.1]	41.7 [36.6_46.2]
Total body fat (%) **	38.5 [34.0_41.9]	50.0 [47.1_52.0]
Android fat mass (kg) **	18.8 [14.5_23.6]	24.7 [20.7_29.4]
Android fat (%) **	49.6 [47.0_53.2]	55.8 [53.0_58.5]
Gynoid fat mass (kg) **	15.2 [10.6_18.8]	23.8 [20.1_28.2]
Gynoid fat (%) **	39.7 [33.9_43.1]	53.8 [51.0_56.6]
BMD (g/cm^2) **	1.32 [1.24_1.37]	1.24 [1.19_1.32]
Leg fat mass (kg) **	10.3 [8.7_13.5]	15.5 [12.6_18.0]
Leg lean mass (kg) **	20.7 [19.1_22.5]	15.8 [14.1_17.8]
LM-PA (min/day) *	279 [231_340]	312 [265_383]
Sedentary (min/day) *	625 [569_672]	591 [532_628]

*P<0.05 **P<0.001, **Abbreviations: BMI:** body mass index; **BMD:** bone mineral density; **LM-PA:** Light and Moderate Physical Activity.

The study participants were divided into four different groups based on their age categories **(Table 4-2).** The distribution of the participants

according to age category was 17.7% (25-39 years), 25% (40-50 years), 25.91% (51-60 years), and 31.4% (61-70 years). Using the Kruskal-Wallis test, the analysis showed that body weight, BMI, body fat, android, gynoid, and leg fat mass decreased across the age categories when males and females were combined, while total body and LLM, height, BMD and physical activity were not different across age categories.

Chi square test showed that the analysis of sex distribution across age categories was not different (P=0.217). The Kruskal Wallis H test showed that in females there were significantly different distributions in variables across the age categories such as weight (P<0.001), BMI (P<0.005), body fat (P<0.001), body fat percentage (P<0.05), LBM (P<0.05), BMD (P<0.005), leg fat mass (P<0.001), LLM (P<0.001), android and gynoid fat percentage (P<0.05), except for height. In particular, female cohorts reduced BMI across age categories (**Table 4-3**). Females maintained consistent physical activity levels across age groups and sedentary time. Males showed no significant age-related changes in any anthropometric measures, as seen in **Table 4-4** (P>0.05 in all cases) and did not demonstrate differences in physical activity and sedentary behaviour across age categories.

Table 4-2: Participants' characteristics, including anthropometry data, and body composition measured by DXA scan based on the age categories during pre-LED.

Variable	25-39 y (n= 39) 14M:25F	40-50y (n=55) 16M:39F	51-60y (n=57) 26M:31F	61-70y (n=69) 31M:38F
	(Median [25th-75th percentile])			
Height (m)	1.68 [1.63_1.75]	1.64 [1.59_1.73]	1.64 [1.59_1.77]	1.67 [1.61_1.72]
Weight (kg) *	101.6 [92.6_117.8]	96.1 [88.3_107.4]	94.4 [86.5_109.8]	94.8 [79.7_102.3]
BMI (kg/m^2) *	35.4 [32.4_44.0]	34.0 [31.5_38.9]	34.5 [31.5_38.2]	33.2 [30.0_36.4]
Total body fat (kg) **	45.9 [39.3_54.9]	42.6 [38.7_47.2]	42.2 [34.7_46.6]	37.6 [32.5_47.8]
Total lean body mass (kg)	50.0 [41.7_56.8]	44.9 [39.0_56.0]	47.9 [40.0_57.2]	48.2 [38.0_56.5]
Lean body mass / Body weight *	0.46 [0.43_0.54]	0.46 [0.43_0.53]	0.49 [0.45_0.54]	0.50 [0.45_0.56]
Lean body mass / Hieght^2 (kg/m^2)	17.53 [15.19_19.78]	16.55 [14.91_18.59]	17.43 [15.71_19.41]	17.07 [15.31_18.67]
Total body fat (%) *	49.1 [41.2_51.6]	48.9 [42.1_51.2]	45.7 [38.6_50.3]	44.3 [38.7_49.2]
Android fat mass (kg) *	24.9 [20.9_34.3]	23.6 [20.02_27.7]	22.2 [16.9_27.2]	19.4 [16.5_25.4]
Android fat (%) *	55.7 [52.4_59.3]	55.1 [51.0_57.6]	54.4 [49.0_56.3]	51.8 [49.0_55.7]
Gynoid fat mass (kg) **	23.7 [19.3_30.6]	22.8 [16.02_26.6]	20.7 [14.8_25]	17.6 [13.3_22.8]
Gynoid fat (%) *	52.0 [43.3_56.7]	52.5 [44.9_56.0]	48.8 [39.4_55.3]	46.1 [40.0_52.9]
BMD (g/cm^2)	1.28 [1.22_1.34]	1.30 [1.24_1.36]	1.27 [1.19_1.37]	1.25 [1.20_1.33]
Leg fat mass (kg)**	16.1 [11.8_19.1]	14.4 [10.9_17.3]	12.9 [10.3_16.2]	10.9 [9.1_14.8]
Leg lean mass (kg)	18.9 [16.5_21.5]	17.4 [15.4_20.4]	18.1 [14.9_20.5]	17.7 [14.01_19.6]

LM-PA (min/day)	301 [242_368]	298 [257_394]	302 [255_371]	294 [250_340]
Sedentary (min/day)	583 [549_662]	592 [547_637]	607 [527_655]	602 [549_637]

*P<0.05 **P≤0.001, **Abbreviations: BMI:** body mass index; **BMD:** bone mineral density; **LM-PA:** Light and Moderate Physical Activity.

Table 4-3: Female' characteristics, including anthropometry data, and body composition measured by DXA scan based on the age categories during pre-LED.

Variable	25-39 y (n= 25)	40-50y (n=39)	51-60y (n=31)	61-70y (n=38)
		(Median [25th-75th percentile])		
Height (m)	1.66 [1.61_1.69]	1.61 [1.59_1.65]	1.61 [1.58_1.63]	1.63 [1.58_1.67]
Weight (kg) **	101.6 [92.6_118.5]	93.6 [86_104.8]	88.1 [81.7_95.6]	82.8 [76.3_100.4]
BMI (kg/m^2) *	37.9 [34.4_46.9]	35.3 [32.2_40.4]	33.6 [31.6_38.1]	31.9 [29.2_36.6]
Total body fat (kg) **	50.3 [43.2_62.2]	44.4 [41.7_49.7]	42.7 [37.9_46.9]	39.1 [33.2_49.4]
Total lean body mass (kg)*	45.1 [39.4_53.7]	42.5 [36.5_46]	40.6 [36.2_45.4]	39.5 [36.02_44.7]
Lean body mass/ Body weight	0.43 [0.42_0.46]	0.45 [0.43_0.47]	0.46 [0.42_0.48]	0.46 [0.44_0.49]
Lean body mass/Hieght^2 (kg/m^2)	17.33 [14.77_19.54]	16.29 [14.68_17.99]	15.85 [14.50_17.36]	15.52 [13.89_16.43]
Total body fat (%) *	50.9 [49_53.2]	50.4 [48.7_52.4]	49.7 [46.4_51.9]	48.6 [45.8_50.7]
Android fat mass (kg) **	29.02 [23.02_36.9]	25.2 [22.4_28.6]	24.4 [20.7_27.6]	20.7 [17.3_28.6]
Android fat (%) *	57.7 [52.9_60.1]	56.5 [53.9_58.9]	55.5 [53.4_57.8]	54.6 [50.7_57.9]
Gynoid fat mass (kg) **	27.7 [23.1_34.1]	23.8 [22.5_27.5]	23.5 [19.8_26.9]	21.3 [15.9_27.2]

Gynoid fat (%) *	55.2 [52.3_57.3]	55.5 [51.8_56.3]	54.4 [50.7_58.1]	52.6 [49.1_55.6]
BMD (g/cm^2) *	1.29 [1.21_1.33]	1.29 [1.24_1.34]	1.22 [1.17_1.30]	1.21 [1.16_1.27]
Leg fat mass (kg)**	17.5 [15.6_22.2]	15.5 [13.01_18.3]	14.5 [12.7_17.02]	13.7 [10.3_17.02]
Leg lean mass (kg) **	17.6 [15.7_19.9]	16.5 [14.9_17.8]	15.3 [14.2_17.1]	14.3 [12.9_17.2]
LM-PA (min/day)	314.6 [266.3_380.5]	317.7 [257_402.7]	313.4 [274.8_396.1]	296.9 [256.1_351.1]
Sedentary (min/day)	577.3 [520.04_642.9]	592.1 [541.4_632.5]	596.5 [499.8_629.5]	596.6 [531.3_625.6]

*P<0.05 **P≤0.001, **Abbreviations: BMI:** body mass index; **BMD:** bone mineral density; **LM-PA:** Light and Moderate Physical Activity.

Table 4-4: Male' characteristics, including anthropometry data, and body composition measured by DXA scan, based on the age categories during pre-LED.

Variable	25-39y (n=14)	40-50y (n=16)	51-60y (n=26)	61-70y (n=31)
	(Median [25th-75th percentile])			
Height (m)	1.75 [1.70_1.78]	1.79 [1.74_1.84]	1.77 [1.71_1.80]	1.72 [1.69_1.77]
Weight (kg)	102.8 [91.9_117.5]	103.4 [98.3_109.1]	107.7 [93.3_120.8]	100.1 [91.1_107.8]
BMI (kg/m^2)	32.8 [30.6_38.9]	32.1 [30.6_34.1]	34.7 [31.3_38.9]	33.6 [31.5_36.2]
Total body fat (kg)	41.2 [30.4_46.2]	37.3 [31.6_41.6]	37.8 [31.1_45.9]	36.9 [31.3_42.9]
Total lean body mass (kg)	54.8 [50.2_62.2]	59.4 [53.2_61.9]	57.9 [54.3_65.6]	56.9 [50.5_61.1]
Lean body mass/ Body weight	0.54 [0.51_0.60]	0.56 [0.53_0.59]	0.54 [0.51_0.60]	0.57 [0.52_0.61]
Lean body mass/Hieght^2 (kg/m^2)	18.11 [17.22_20.71]	18.01 [16.34_19.70]	19.20 [17.86_21.02]	18.55 [17.57_20.52]

Total body fat (%)	39.9 [35.3_41.8]	37.7 [34.2_41.5]	37.6 [34.3_42.5]	38.5 [32.8_40.2]
Android fat mass (kg)	22.4 [14.1_24.8]	18.4 [14.9_21.1]	20.4 [14.04_25.7]	18.1 [13.9_21.9]
Android fat (%)	51.2 [46.4_54.6]	48.7 [46.7_54.2]	50 [46.3_53.8]	49.4 [46.5_52.3]
Gynoid fat mass (kg)	16.4 [12.3_20.9]	14.8 [10.8_17.8]	15.4 [10.2_20.8]	14.1 [10.3_17.7]
Gynoid fat (%)	40.7 [37.7_46.8]	39.2 [33.2_41.2]	37.8 [33.7_43.7]	39 [34.3_42.7]
BMD (g/cm^2)	1.28 [1.22_1.35]	1.34 [1.25_1.42]	1.32 [1.25_1.37]	1.32 [1.24_1.39]
Leg fat mass (kg)	10.5 [8.9_14.1]	10.5 [8.8_14.3]	10.4 [8.5_14.4]	9.9 [8.2_10.9]
Leg lean mass (kg)	20.7 [19.2_24.2]	21.8 [19.9_22.5]	20.9 [19.3_23.5]	19.2 [18.4_22.2]
LM-PA (min/day)	252.4 [210.02_324.9]	289.9 [237.2_357.2]	275.8 [222_351.1]	291.6 [240.6_334.6]
Sedentary (min/day)	629.8 [570.7_714.7]	596.8 [572.2_671.04]	631.2 [538.04_683.1]	602.2 [567.4_653.2]

*P<0.05 **P≤0.001, **Abbreviations: BMI:** body mass index; **BMD:** bone mineral density; **LM-PA:** Light and Moderate Physical Activity.

4.3.2 The Relationship between LBM, Body Weight, dietary protein intake and Age, including within biological Sex.

LBM was strongly correlated with body weight in the whole cohort (LBM=0.461 BW + 3.168: R=0.736; P<0.001) and within biological sex (LBM=0.318 BW + 24.398: R=0.636 and LBM=0.376 BW + 7.058: R=0.863 for males and females respectively, both P<0.001). Moreover, LBM positively correlated with dietary protein intake (g) in males and females (R=0.322; P<0.005, and R=0.199; P<0.05 respectively). However, when results were standardised for energy intake (% of total energy from protein), LBM was no longer significantly correlated with protein intake in both males and females (R=0.090; P= 0.409, R=0.054; P=0.539 respectively). It is therefore likely that the higher LBM and

protein intake, reflects body size or biological sex, as daily protein intake was seen to increase with body weight (R=0.278; P<0.001) and males had greater protein intake and stature than females. Indeed, although when LBM was standardised for height^2 a similar pattern of association was seen in males R=0.209; P=0.052, and females R=0.201; P=0.020 for protein intake (g). When LBM was standardised for body weight, the association between LBM and protein intake was no longer significant in males or females (R=0.018; P=0.869, and R=0.095; P=0.276 respectively). The association between LBM and protein composition of the diet reached significance when adjusted for body weight (R=0.202; P<0.05).

In females, as also observed across age categories, there was a decrease in LBM with increasing age (R= -0.314; P<0.001), but this was not observed in males (R=0.048; P=0.657).

4.3.3 The Relationship between Leg Lean Mass and Body Weight, Physical Activity, Age, within Sex.

LLM was strongly correlated with body weight (BW) in both males (LLM = 0.135 BW + 6.722: R= 0.714) and females (LLM = 0.139 BW + 2.995: R=0.854; both P<0.001). In females, the association between LLM and body weight was modified by age, as adding participant age into the multiple regression model, resulted in a significant improvement in the predictive power of the model (ΔR^2 = 0.019; P<0.005).

Similar to whole body LM, LLM decreased with age in females (R= -0.467; P<0.001), but not in males (R= -0.104; P=0.337).

LLM was not associated with time spent in light and moderate physical activity or sedentary time in either sex.

4.3.4 The Relationship between BMD, Body Weight, and Leg Lean Mass between Sex

Correlation analyses showed that there was a significant association between BMD and body weight in males (R=0.341) and females (R=0.483), both P<0.001. Moreover, leg BMD correlated with LLM in males (leg BMD = 0.018 LLM + 1.026: R=0.390 (ΔR^2=0.152)) and females (leg BMD = 0.017 LLM + 0.966: R=0.370 (ΔR^2=0.137); both P<0.001). In females, the regression model was further improved by time spent in light and moderate physical activity (ΔR^2=0.036; P<0.05) and age (ΔR^2=0.047; P<0.01). However, due to the potential for collinearity between variables, the regression models should be interpreted with caution.

Indeed, leg BMD was not associated with time spent in light and moderate physical activity or being sedentary in males and females.

4.3.5 The Relationship between Body Fat Percentage and Physical Activity by Sex or Age

In males, a statistically significant and negative correlation was observed between body fat percentage and the time spent in light and moderate physical activity (R= -0.235; P<0.05). However, this was not observed in females (R=0.041; P=0.637).

There were no significant correlations between body fat percentage and sedentary time in either males or females. Body fat percentage was correlated with age only in females (R= -0.231; P<0.01).

4.3.6 The Relationship between BMI and DXA-derived adiposity measures (whole body fat and regional adiposity).

In the whole cohort, the results showed that BMI was positively related to DXA-derived body fat mass (BFM) (BMI= 1.582 BFM – 13.302; R=0.856) and DXA-derived body fat percentage (BF%) (BMI= 0.599 BF% + 23.879: R=0.457; both P<0.001). The values of BFM and BMI were significantly correlated in males (BFM=0.392.BMI + 19.215: R=0.837) and females (BFM= 0.543 BMI +11.379: R=0.883; both P<0.001). Moreover, DXA BF% and BMI were correlated in males (BF%= 0.7924. BMI +10.795: R=0.657) and females (BF%= 0.3382.BMI + 37.492: R=0.515; both P<0.001).

Table 4-5: Summary of Correlations in Body Composition Analysis

1. Age-Category Patterns			
Research area	**Males**	**Females**	**Statistical Significance**
Body Weight	No significant change	Decreased across age categories	P<0.001 (females)
BMI	No significant change	Decreased across age categories	P<0.005 (females)
Total body fat	No significant change	Decreased across age categories	P<0.001 (females)
Leg lean mass	No significant change	Decreased with age (R=-0.467)	P<0.001 (females)
2. Lean Body Mass (LBM) Relationships			
Variable Pairs	**Males**	**Females**	**Notes**
LBM vs Body weight	R=0.863, P<0.001	R=0.636, P<0.001	Positive correlation in both sexes
LBM vs Age	R=0.048, P=0.657	R=-0.314, P<0.001	Age-related decline only in females
LBM vs Dietary Protein (g)	R=0.322, P<0.005	R=0.199, P<0.05	Positive correlation in both sexes
LBM vs Protein (% energy)	R=0.090, P=0.409	R=0.054, P=0.539	No correlation when adjusted for energy intake
3. Leg Lean Mass (LLM) Relationships			
LLM vs Body Weight	R=0.714, P<0.001	R=0.854, P<0.001	positive correlation in both sexes
LLM vs Age	R=-0.104, P=0.337	R=-0.467, P<0.001	Decline with age only in females
LLM vs Physical Activity	No significant association	No significant association	Neither sex showed a relationship with activity levels
4. Bone Mineral Density (BMD) Relationships			
BMD vs Body Weight	R=0.341, P<0.001	R=0.483, P<0.001	positive correlation in both sexes
Leg BMD vs Leg lean mass	R=0.390, ΔR^2=0.152	R=0.370, ΔR^2=0.137	associations in both sexes
BMD vs Physical Activity	No significant association	ΔR^2=0.036, P<0.05	Activity associated only in females
BMD vs Age	No significant modification	ΔR^2=0.047, P<0.01	Age modified relationship only in females
5. Body Fat Percentage Relationships			
Body fat % vs Physical Activity	R=-0.235, P<0.05	R=0.041, P=0.637	Negative correlation only in males
Body fat % vs Age	No correlation	R=-0.231, P<0.01	Negative correlation only in females
Body fat % vs Sedentary Time	No significant association	No significant association	Neither sex showed the relationship

6. BMI and DXA-derived Adiposity Measures			
Variable Pairs	**Males**	**Females**	**Whole Cohort**
BMI vs Body fat Mass	R=0.837, P<0.001	R=0.883, P<0.001	R=0.856, P<0.001
BMI vs Body fat %	R=0.657, P<0.001	R=0.515, P<0.001	R=0.457, P<0.001

Abbreviations: BMI: body mass index; **LBM**: lean body mass; **LLM:** leg lean mass; **BMD:** bone mineral density.

4.4 Discussion

Age-related muscle mass and strength loss is a major public health concern, with the literature indicating that strength decreases by 3 to 8% per decade from age 30 with an acceleration after age 60 [226]. As a consequence, there can be reduced mobility, increased risk of falls and fractures, and metabolic changes linked to conditions such as T2DM and obesity [226]. Maintaining muscle mass is crucial as we age due to its role in overall health, functional independence, and quality of life [556]. This cross-sectional study examined associations between body composition characteristics and potential modifiers, including age, sex, and physical activity in an overweight population with pre-diabetes. The main finding of the present study, covering a wide age range (25-70 years), was that LLM was most strongly associated with body weight, with time spent in light and moderate physical activity not appearing to have a modifying influence. Additionally, the current study found that LLM decreased with age only in females. Interestingly, Leg BMD correlated most strongly with LLM in both sexes, and in females the regression model was further improved by time spent in light and moderate physical activity and age. As a cross-sectional study, these findings represent associations between variables rather than causal relationships or longitudinal changes.

The body composition analysis across different age groups revealed significant differences in the overall cohort. Body weight, BMI, body fat, and LM were lower in females as the age category increased. However,

previous studies in the general population consistently show that LM declines [557, 558] and total body fat mass tends to increase with age throughout adulthood [559, 560] until extreme old age, when fat mass and LM decreases [561, 562]. However, the present findings showed a different pattern, with females displaying lower fat mass in higher age categories, while males showed no significant age-related differences. This pattern, particularly in females, contrasts with typically observed age-related increases in fat mass in the general population. This difference is likely due to the selection criteria of studying prediabetic individuals. An important potential confounder in interpreting differences among age categories is the likely variation in the duration of prediabetes before study enrolment. The duration of prediabetic status was not able to be determined in participants in the PREVIEW study. However, it may be reasonable to assume that older participants were overweight or obese and insulin-resistant for longer periods than younger participants [563, 564]. Younger participants are more likely to be in the initial stages of metabolic and physiological changes linked to prediabetes, while older participants may have undergone these changes for many years or even decades [563, 565]. This difference in disease progression may be affected by body composition patterns differently across age groups. In addition, prolonged exposure to insulin resistance may affect muscle metabolism and protein synthesis differently than recent onset [566], which could complicate the interpretation of age-related differences in LM. Although this limitation cannot be addressed in the current cross-sectional analysis, it is an important consideration for future longitudinal studies that examine age-related changes in body composition among individuals with prediabetes.

The relationship between prediabetes and body composition in individuals is complex. A ‘pre-diabetes’ diagnosis can develop through multiple pathways, including excess fat mass [567], physical inactivity [568], and age-related insulin resistance [569]. In the young, it is possible that impairments in blood glucose regulation are more commonly

observed with excess adiposity [570], whereas in older age groups, the observations may also be associated with age-related differences in muscle function [571]. The consequence of this would be that the recruited participants in younger age groups would be more likely to have greater adiposity, which is what was observed. This contrast highlights how the present study population may differ from the general aging population. However, these observations in the whole cohort appeared to be predominantly driven by female participants. Analysis of females alone revealed significantly lower values across age categories in weight, BMI, total body fat, android fat mass, and LLM. In contrast, when analysing males alone, body composition was not different across age groups, showing no significant changes in any measured parameters. The strong effect sizes observed in females were substantial enough to influence overall population trends, perhaps due to the unequal sex distribution in the whole cohort and within age group categories.

The findings from the current study support the initial hypothesis that a positive correlation between LLM and body weight would be seen. While differences in muscle protein synthesis have been observed in obesity [572, 573], the hypothesis aligns with the generally established understanding that individuals with higher body weights tend to have greater muscle mass, likely due to increased skeletal muscle being needed to support movement and daily activities [574]. A review of the impact of obesity on skeletal muscle strength and structure [517] presents evidence for this relationship which may be explained by an adaptive response, in which the musculoskeletal system adjusts to increased loading by developing greater muscle mass [574]. A meta-analysis supports this concept, suggesting that resistance training using one's own body weight can be beneficial for muscle gain and maintenance in people with overweight and obesity [575]. These findings also suggest that individuals with higher body weight tend to develop greater LM in their legs as a way to adapt to increased mechanical

loading [576], and that this adaptation can be further enhanced through appropriate resistance training.

While the present findings demonstrate a clear association between body weight and LLM, a deeper examination of the literature reveals a more complex relationship between obesity and muscle function. Research investigating obesity's impact on muscle size and strength has revealed an intriguing paradox in muscle force generation capabilities beyond simple mass accumulation [577]. Studies consistently demonstrate that people with obesity exhibit higher absolute maximum voluntary contraction (MVC) torque and power compared with their normal-weight counterparts [510, 513, 519]. This observation aligns with the theory that excess fat may be associated with increased loading of weight-bearing muscles [578]. This concept was initially validated through experimental work by Bosco *et al.* [579] who documented significant increases in muscle power following three weeks of simulated hypergravity using weighted vests (7-8% body mass). This study offered the same challenge to weight-bearing muscles of the lower limbs as people with obesity do during daily activities but within a shorter time. These strength adaptation mechanisms were attributed to enhanced neural function, specifically increased motor unit firing rates, improved recruitment patterns, and better motor unit synchronisation [579, 580]. However, despite the strong correlation between LLM and body weight found in the present study, contrasting studies indicate that the relationship varies significantly across populations and methodologies. For example, Forbes *et al.* [581] highlighted the relationship between body weight and LM can be complicated, particularly for sedentary populations such as older adults. The author stated that LM has a logarithmic relationship with body weight, indicating that this relationship may vary significantly across different populations and conditions [581], including different age groups [582], or under specific health conditions [583], hormonal influences [584], or can be influenced by dietary interventions [585, 586].

The present study found that the relationship between LLM and body weight in females was different across the age categories such that lean leg mass was lower with advancing age, while males had similar muscle mass across the age categories. It has been reported in several studies that LLM declines over the lifespan differentially between sexes. For instance, a large-scale longitudinal study found that LLM declined with age for both men and women over a 3 year period [556]. However, the rate of decline in the previous study varied between sexes and racial groups. This sex difference has been attributed to hormonal factors; two studies suggest that postmenopausal hormonal changes are associated with greater muscle loss in women [226, 587]. However, the relationship between menopause and muscle loss is not unequivocal, as some studies have not found a significant increase in muscle mass decline after menopause [588], and it may be that changes in lifestyle associated with menopause, such as reduced physical activity [589], may be contributing to these observations. In addition, sex-specific differences in protein metabolism have been observed, with females showing less net protein accretion than males during insulin-resistant conditions [590]. Furthermore, the decline in oestrogen and androgen levels during menopause continues to decrease annually with age, which can negatively impact muscle mass and function [591, 592]. Accordingly, the lower levels of anabolic-acting androgens observed in females between the ages of 60 and 70 are associated with a higher prevalence of muscle loss among these females [593]. Finally, hormonal influences combined with insulin resistance associated with prediabetes may create a physiological environment that accelerates muscle loss in this female population. In contrast, males maintained their LLM across the entire age range studied. This preservation of muscle mass, despite the presence of prediabetes, suggests that androgenic hormones are associated with the maintenance of muscle mass across age categories, even in cases of metabolic dysfunction [594]. This preservation aligns with findings from Szulc *et al.* [595] who demonstrated testosterone's protective effect on muscle maintenance in males. Furthermore, a meta-analysis of 35 studies indicated that the sex-hormonal changes seen with ageing, which

contribute to the acceleration of age-related muscle mass loss, occur more gradually and slowly in males compared with females [593].

The inclusion and exclusion criteria applied in the PREVIEW study are crucial to understanding the unexpected finding that higher physical activity levels did not correlate with increased LLM among participants. The limited range and low levels of physical activity observed in this cohort at the start of the study likely presented a methodological limitation. The statistical models may have been particularly impacted by the narrow activity range, making it difficult to detect meaningful associations between physical activity and LM. Furthermore, in this cohort, physical activity levels may have been insufficient to stimulate muscle growth beyond the chronic loading effect of higher body weight. This interpretation aligns with established dose-response relationships between exercise and muscle adaptations [596], suggesting that a minimum threshold of activity intensity and volume must be reached to stimulate meaningful hypertrophy. In addition, this also aligns with findings from Bann and their colleagues, who observed that while physical activity can increase LM, its effectiveness relies on the intensity and duration of the activity, which PREVIEW participants may not have consistently reached [597]. In addition, obesity-related impairments in muscle protein synthesis have been documented [572, 573].

A well-researched relationship has been demonstrated between muscle mass and BMD, highlighting muscle mass's crucial role in bone health [598]. The maintenance of BMD involves a dynamic process of continuous remodelling, where mechanical stress stimulates bone formation and disuse leads to bone demineralisation [599]. This adaptive response of bone to mechanical loading referred to as Wolff's Law, indicates that bone structure changes to accommodate the forces acting on it [600]. Notably, muscle contractions generate substantial mechanical forces on the skeletal system, which are often more powerful than the

forces generated by body weight alone [601]. Research by Kohrt *et al.* [602] demonstrated that these muscle-generated forces can be ten times greater than gravitational forces acting on bone tissue, emphasizing the crucial interaction between muscular and skeletal systems. These physiological principles suggest a relationship between muscle mass and bone mineral density, prompting an investigation into their association.

The present study found a strong correlation between leg BMD and LLM in both males and females, supporting the idea that muscle mass plays an important role in bone health. This aligns with previous findings that link higher muscle mass with increased BMD, as mechanical loading from muscle contractions promotes bone formation and maintenance [603]. Also, Roelofs et al. (2015) reported a notable relationship between LLM and bone integrity, emphasising muscle mass as a key factor in BMD. Furthermore, research indicates that elite male athletes have significantly higher regional BMD than non-athletes, suggesting that physical activity and muscle use are crucial for preserving bone health [604]. However, in the current study, physical activity only modified the relationship between LLM and BMD in females. The relationship between physical activity, muscle mass, and bone health is more complex than initially theorised. Indeed, previous research suggested enhanced sensitivity to physical loading in women's bone response mechanisms [605], highlighting the importance of sex-specific and age-appropriate approaches in both research and clinical applications. This could include the assessment of physical activity in female bone health screening.

Several studies have revealed that muscle forces place greater mechanical loads on bone than gravitational forces from elevated fat mass alone [606-608]. In addition to direct mechanical stimulation from muscle contractions, muscle tissue also secretes myokines that are associated with bone metabolism directly [609]. Also, maintaining muscle mass plays an important role in maintaining bone health, as LM

generates dynamic loads during muscle contractions, which may be more osteogenic than steady loads generated by elevated fat and consequent body mass. A systematic review of 44 studies suggested that there are higher correlation coefficients between LM and BMD compared with fat mass and BMD, and the impact of LM on BMD was consistently greater in both males and females [610]. The present data also showed that total body LM appears to be a stronger predictor of BMD than fat mass in the whole cohort (R=0.434) compared with BFM (R=0.136). However, in the females, this observation did not hold true as the correlation between BFM and BMD was of a similar magnitude to LBM and BMD (R=0.342 compared with 0.308, respectively). Moreover, Kim *et al.* [548] demonstrated that, after adjusting for LM, fat mass had negative associations with BMD, especially at weight-bearing sites, which could suggest that excessive fat mass may negatively impact bone health. In contrast, among the PREVIEW participants, it was found that after adjusting for LM, fat mass had an independent effect only in females, and this association was positive, not negative. Body weight has been suggested as one of the strongest predictors of BMD [611] and the current study identified a positive relationship between BMD and body weight in both males and females, consistent with findings from previous studies [612-615]. As fat mass provides a greater contribution to total body mass in females, compared with males, this may explain why the relationship between total fat mass and BMD was found in females only.

A strength of the current study is that it used DXA to measure whole body fat and regional adiposity, such as android fat and gynoid fat, as an alternative to standard methods used in epidemiological studies, such as BMI [616, 617]. Prior studies have noted that BMI is an easy-to-measure indicator of adiposity and is a predictor of diabetes [618, 619]. However, BMI is not an appropriate measure in certain patient and participant groups. Indeed, BMI alone may be insufficient for bone health evaluation, as Ho-Pham *et al.* [831] demonstrated that using body composition analysis to understand bone metabolism and skeletal health is a more

comprehensive approach [610]. Furthermore, measurements of regional and total body composition obtained with DXA provide valuable insight into the relationship between soft tissue distribution and BMD [620]. Additional strengths have been identified in this study, including a large dataset and inclusion of male and female participants across a wide age range. Furthermore, regression analysis was adjusted for significant variables that might influence LBM, including time spent in light and moderate physical activity, sedentary time, body weight, age, BMD, and LLM.

Several limitations should be noted in the present study, in particular, the cross-sectional design. Predictive relationships cannot be confirmed by these study designs. Further, re-analysis of data collected for other purposes might not include measures of confounding factors, and measured variables could bias the association between exposure and outcome. An important limitation was the inability to assess the duration of prediabetes in participants. This duration likely varied with age and could have influenced body composition outcomes, particularly in older participants who may have experienced metabolic issues for longer periods. Moreover, the selection criteria of the participants were those with insulin resistance and overweight / obesity. The lack of data from those with normal weight, and the low physical activity levels in the present study cohort, restricts the ability to investigate associations between body composition and physical activity, introduces bias, and reduces the generalisability of the results. In ageing and frailty research, it is important to assess appendicular lean mass to estimate total body muscle mass. In the current analyses, the focus was on the modifiers of leg lean mass and due to errors introduced in values for arm lean mass by using the results of hemiscan in participants with obesity, appendicular lean mass was not included in the analysis plan. However, it is possible that the relationship between lean mass and metabolic markers following weight loss may be better investigated by using appendicular lean mass

rather than total body lean mass in future studies due to the influence of skeletal muscle on glucose regulation. Considering the poor representation of certain ethnicities within the participant cohort of the current study, it is likely that the findings cannot be generalised to the wider UK population.

4.5 Conclusion

In individuals with overweight or obesity and with pre-diabetes, lean body mass and LLM were only lower with increasing age category in females, but in both sexes, a positive association was observed between total body mass and total, plus leg, lean mass. Moreover, the relationship between body weight and LLM was only modified by age in females.

Males did have greater lean leg mass compared with females, with an age-related decline in lean leg mass only being detected in females.

In the current study, no association was observed between LLM and physical activity, which may be attributed to the limited range and low level of activity carried out by the participants.

Chapter 5 The impact of lifestyle factors and eating behaviours on body composition changes after 8-weeks' low-energy diet in the Nottingham PREVIEW cohort

5.1 Introduction

Obesity is a growing public health concern, with rates continuing to rise despite numerous intervention efforts [621]. It appears to be responsible for 80–85% of the risk associated with developing T2DM and is a major contributor to developing cardiovascular disease [622, 623]. Healthy diets and physical activity are the two main lifestyle approaches to preventing and treating obesity. It has been shown that significant weight loss is associated with the remission of T2DM [624-626]. Moreover, substantial weight loss has been associated with a lower risk of developing T2DM, even after the lost weight has been regained [627].

The PREVIEW intervention study (www.previewstudy.com) was a large multinational research project focused on preventing progression to T2DM in people with overweight and obesity and pre-diabetes using a lifestyle modification approach. It employed dietary and physical activity changes in a 3-year intervention, supported by behaviour modification techniques to reinforce lifestyle changes [417]. The study was a multicentre, 2-by-2 factorial, randomised controlled trial. Eligible adult participants first underwent an 8-week low-energy diet "LED" intended to achieve a minimum weight loss of 8%. [332].

LEDs, typically providing 800-1200 kcal per day, have been studied for their potential to achieve rapid weight loss and improve markers of metabolic health [628, 629]. These diets often utilise specially formulated meal replacements to ensure nutritional adequacy, while maintaining a significant energy restriction [630]. However, LEDs remain highly controversial regarding their impact on body composition, particularly fat-free mass (FFM). Early studies indicated that weight loss induced by LEDs could lead to improvements in cardiometabolic risk factors in terms of fat mass reduction [631], but more recent systematic reviews have demonstrated that substantial losses of FFM can occur during LED

interventions [258, 632]. For instance, some reports indicate that losses of FFM can reach up to 25% of total weight lost, even when protein intakes are within recommended levels [303, 633]. The findings of Muller *et al.* [634] support this conclusion, showing that rapid weight loss is associated with significant reductions in skeletal muscle mass, which may compromise long-term metabolic function. This variability in FFM loss during LED interventions establishes the foundation for subsequent research on post-diet strategies, such as exercise, to address the metabolic consequences of rapid weight loss.

Physical activity appears to play a role in influencing an individual's response to weight loss interventions. When combined with severe dietary energy restrictions, physical activity can increase energy deficits and enhance fat loss while helping maintain lean body mass [111]. A systematic review demonstrated that combining an LED with structured exercise results in a weight loss of 1.5 to 2.3 kg more than LED alone throughout 8 to 12 weeks [635]. Moreover, Meta-analyses indicate that combining a low-energy diet with structured resistance training helps maintain 93% of lean body mass, compared with just 80% when following the LED alone [636] Furthermore, this combined approach results in a greater reduction in fat mass, with an average loss of 1.7 kg [635]. This secondary analysis of PREVIEW data aimed to investigate the relationship between habitual physical activity and body composition changes following an 8-week low-energy diet to provide valuable insights into the complex interplay between lifestyle factors and weight loss outcomes.

Research has demonstrated that lifestyle factors and eating behaviours play crucial roles in weight management and body composition, with notable sex differences observed in these relationships [628, 637]. Factors such as physical activity levels, dietary patterns, and psychological aspects of eating can impact an individual's response to

weight loss interventions, often in sex-specific ways [628, 638]. Sex differences in body composition and fat distribution are well-established, with women typically having a higher percentage of body fat and men having a greater proportion of LM (see Chapter 4). Moreover, men tend to have a predominantly android (abdominal) pattern of fat distribution, while women typically have a gynoid [hip/thigh] pattern [639]. Indeed, the distribution of fat is proposed to affect metabolic responses; visceral adipose tissue is more active metabolically and more sensitive to lipolysis than subcutaneous fat [640]. Research has demonstrated that these differences in fat distribution can influence metabolic responses to dietary interventions [639], as well as the effectiveness of exercise prescriptions [641]. Men tend to lose weight more quickly than women when following similar dietary interventions, possibly due to their larger stature, higher metabolic rates and greater muscle mass [642].

Sleep duration and quality have been shown to affect hormonal regulation of appetite and metabolism, potentially impacting weight loss efforts [643]. Inadequate sleep can lead to increased hunger, cravings for high-calorie foods, and alterations in glucose metabolism, all of which may hinder weight loss progress [643]. Interestingly, some studies suggest that women may be more susceptible to weight gain associated with short sleep duration compared with men [644]. Similarly, stress levels can influence eating behaviours and weight management, with sex-specific patterns emerging [645]. Additionally, in some, stress can lead to emotional eating and increased cravings for energy-dense foods, which can hinder adherence to dietary interventions for weight loss [646, 647]. The psychological challenges of following a restrictive diet can be especially pronounced during stressful times, as increased cortisol levels and hormonal changes affect appetite regulation and metabolism, potentially compromising adherence to prescribed energy restrictions [645, 648]. The present study examined sleep patterns alongside habitual physical activity as potential moderators of weight loss outcomes,

recognising that these lifestyle factors often interact to influence energy balance and metabolic regulation during dietary interventions.

5.1.1 Aims of the study

This study examined the impact of an 8-week low-energy diet on an overweight UK population at risk of diabetes, focusing on several main objectives. First, it aimed to determine how baseline lifestyle and characteristics, such as eating behaviours (hunger, disinhibition, and restraint), perceived stress levels, reported sleep patterns, and physical activity, influenced weight loss outcomes following the LED. Second, the study compared the effects of the 8-week LED on metabolic outcomes between males and females, analysing changes in blood-derived indices of fasting insulin resistance, cholesterol profile, and liver enzymes. Additionally, the research explored the potential health benefits of weight loss. It evaluated the ability of the 8-week LED to reduce cardiovascular risk factors, such as HR, blood pressure, and circulating lipids. Lastly, the study aimed to identify modifying factors associated with lean or fat mass loss as a result of the LED intervention

5.2 Materials and Methods

5.2.1 Participants and Recruitment

All participants who attended both the pre-LED (CID1) and post-LED (CID2) assessments and had complete datasets for the key variables being assessed were included in the analysis.

5.2.2 LED Intervention

The PREVIEW study included an initial 8-week weight-loss period using a low-energy diet LED formula aimed at achieving a weight loss of at least 8%. The total energy provided by the LED was 3.4 MJ/d (810 kcal/d), 85 grams of protein, 5 grams of essential fatty acids, and the

vitamins and minerals required per day [649, 650]. The LED was administered using a variety of formula products from the Cambridge Weight Plan Northants, UK, including soups, shakes, and porridges, which were provided to participants free of charge. Participants were instructed to consume four sachets 4 × 40 g daily, with three sachets mixed with 250 mL of skimmed (<0.5% fat) milk or 'light soya' (fat 1.5%) as a plant-based alternative, totalling 750 mL/day, and one sachet mixed with 250 mL of water. Participants with a BMI over 40 kg/m² were encouraged to dissolve all four sachets in milk to meet their protein intake requirements.

The macronutrient composition of the LED was 43.7% of total energy from protein, 41.2% from carbohydrates, and 15.1% from fat, with a relatively low fibre content of 13.3 g/day. For those participants who experienced constipation, psyllium fibre was provided, and they were advised to drink plenty of water to stay hydrated. In addition to the sachets and milk, participants were allowed to consume 375 g of low-starch vegetables, such as tomatoes, cucumbers, and lettuce, each day. Substitutions for these vegetables were not allowed. Throughout the LED intervention, participants attended group sessions at the intervention sites during weeks 2, 4, 6, and 8, where experienced dietitians or counsellors guided the LED.

5.2.3 Anthropometric measurement and body composition

Body weight, height, waist, hip, and thigh measurements were measured at the baseline and post LED as described in Chapter 3, sections 3.6.1 and 3.6.2. Body composition measurements were conducted using either DXA or bio-impedance methods as described in Chapter 3, section 3.6.3.

5.2.4 Assessment of Metabolic Parameters

All outcomes were measured before and after the 8-week intervention during clinical investigation days, which participants attended after fasting for 10-12 hours. Fasting insulin resistance (IR) was assessed using the Homeostasis Model for Assessment (HOMA) and was calculated using the following formula [651]:

$$\text{HOMA-IR} = \frac{\text{fasting plasma glucose (mmol/L)} \times \text{fasting insulin (µIU/mL)}}{22.5}$$

Further to this, the Quantitative Insulin Sensitivity Check Index (QUICKI) was used as an index of whole-body fasting insulin sensitivity and was calculated as follows [652];

$$\frac{1}{[\log \text{insulin (µIU/mL)} + \log \text{glucose (mg/dL)}]}$$

Additional outcomes included changes in fasting plasma glucose (FPG), HbA1c, insulin, C-peptide, total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL-C, triglycerides (TG), C-reactive protein (CRP), and liver enzymes; specifically, ALT and AST.

5.2.5 Biochemical measurements

The samples were collected from participants in the laboratory at the Medical School, QMC. At each CID1 and CID2/ Pre and Post the weight-loss phase, blood samples were taken from the antecubital vein while participants were fasting. Serum, plasma and whole blood samples were initially stored at -80°C in a laboratory at the Medical School, QMC. They were then transported to Finland for central batch analysis at the National Institute for Health and Welfare in Helsinki. The blood specimens were analysed for glucose, insulin, C-peptide, haemoglobin A1c, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, C-reactive

protein, and liver enzymes using the Architect ci8200 integrated system from Abbott Laboratories, Abbott Park, Illinois.

5.2.6 Blood pressure and heart rate measurements

Systolic (SBP) and diastolic (DBP) blood pressure and HR were measured as previously described in Chapter 3, section 3.6.4. Participants were instructed to avoid vigorous physical activity, coffee, and smoking for 12 hours before the measurement. The blood pressure device was serviced and calibrated annually.

5.2.7 The measure of eating behaviour and lifestyle characteristics

Participants were given self-administered questionnaires to assess eating behaviour, sleep and perceived stress, which they completed during the study visits at baseline and after finishing the LED program.

5.2.7.1 Eating behaviour

Eating behaviour was measured using the validated Three-Factor Eating Questionnaire (TFEQ), which consists of 51 questions related to appetite and eating behaviours. The questionnaire generates scores in three areas: 1. restraint, which is cognitive control over how often, how much, and what types of food are consumed; 2. disinhibition, which is the inability to control eating despite being consciously aware of it, and 3. hunger, which is sensitivity of feelings of hunger [653]. The score ranges for restraint, disinhibition, and hunger are 0–21, 0–16, and 0–14, respectively [653]. The questionnaires were returned to a study technician after completion, who immediately reviewed them and asked any clarifying questions, if necessary.

5.2.7.2 Sleeping

Sleep quality was assessed using the validated Pittsburgh Sleep Quality Index (PSQI) at the baseline and after the LED intervention. It evaluates sleep patterns over the past month through 19 self-rated questions. These questions are grouped into seven component scores, each ranging from 0 to 3 points, where a score of 0 indicates no sleep issues and 3 signifies severe difficulty. The component scores are summed to produce a global score ranging from 0 to 21, with 0 representing no difficulties and 21 indicating severe problems across all areas [654].

5.2.7.3 Level of perceived stress

Stress levels were measured using the Perceived Stress Scale [484] at the baseline and after LED intervention. This questionnaire evaluates medium-term perceived stress by assessing emotions and thoughts from the previous month through ten questions. Each question offers five possible responses, with the total score calculated across all items. The total score ranges from 0 to 40, where 0 indicates no perceived stress and 40 indicates the highest level of perceived stress [655].

5.2.8 Assessment of Physical Activity Pre-LED

Physical activity at baseline was assessed using waist-worn accelerometry as described in Chapter 3, section 3.8.

5.2.9 Statistical analyses

Data were initially tested for normality using a criterion of the z-score for skewness and kurtosis statistic being within the range of -1.96 to 1.96. Normally distributed data are described in the text as the mean ± standard deviation (SD) whereas non-parametric data are represented as the median [25th-75th percentile]. For paired comparisons, for example of measurements made pre- LED compared with post-LED, the

Wilcoxon Signed Rank test was used for non-normally distributed data and the paired t-test for normally distributed data.

To determine differences between non-paired data e.g. between males and females, the Mann-Whitney U test was employed for non-normally distributed data and the unpaired t-test for normally distributed data.

The Pearson correlation coefficient was used to investigate associations between variables, and regression analysis was then used to identify predictive variables for BMI and body composition measures.

The significance level for comparisons was indicated by $P<0.05$ throughout.

5.3 Results

The study examined body composition, cardiometabolic, and metabolic parameters before and after 8-week low-energy diet LED intervention. While 226 participants completed both pre- and post-intervention assessments, 5 participants were excluded from the final analysis due to missing key variables, resulting in a final cohort of 221 participants (91 males, 130 females). The trial flowchart is presented in Chapter 3, section 3.2.2, Figure 1. The majority of participants identified as White Caucasian 183 (78.9%), while the rest were Asian 15 (6.5%), Black 22 (9.5%) or Arab 1 (0.4%). A total of 226 participants attended the post-LED, with a dropout rate of 8 weeks of 14.4 %.

5.3.1 Changes in Anthropometric Measurements and Body Composition Following LED Intervention

The LED intervention led to changes in body composition among participants (**Table 5-1**), with total body mass, BMI, FM and LM being

lower post-intervention. BMD showed a strong trend to be lower in all participants, P=0.059. However, this was powered at the level of 12% and a post hoc sample size calculation predicted that 2841 participants would be required to power at the 80% level.

Following the LED, lean body mass decreased more in males than in females (difference in sex medians, -2.0 kg; P<0.001). Moreover, there was a relationship between the lean-body mass pre-intervention and the amount of lean-body mass loss measured post-intervention in the whole cohort (R=0.441; P<0.001), in males (R=0.426; P<0.001) and females (R=0.625; P<0.001). Similarly, FM decreased to a greater extent in males than in females (difference in sex medians, -1.5 kg; P<0.001), with males also experiencing a greater reduction in body fat percentage compared with females (difference in sex medians, -1.0 %; P<0.001).

Regional fat distribution analysis revealed significant reductions in both android and gynoid fat percentages across all participants. Males showed a greater reduction in android fat percentage (difference in sex medians, -3.0 %; P<0.001) and gynoid fat percentage (difference in sex medians, -1.1 %; P<0.001) compared with females. These findings were reflected in the analysis of body circumference measurements, which revealed significant changes in waist, hip, and thigh measurements for all participants following the LED intervention, with waist circumference decreasing more in males than in females (difference in sex medians, -3.8 cm; P<0.001). It is important to note that while all participants showed significant reductions in hip and thigh circumference from their baseline measurements, the magnitude of change between males and females did not differ (P=0.537, P=0.898 respectively).

Table 5-1: The body composition change measured by DXA scan in the different sex groups during pre- and post-LED.

Variable	ALL		Males n=91: 41.2%		Females n=130: 58.8%	
	Median [25th -75th percentile]					
	PRE	Δ	PRE	Δ	PRE	Δ
Body weight (kg)	95.9 [85.4_107.5]	-11.3 [-13.9 _ -9.2] **	101.5 [93.9_115.8]	-13.9 [-16.4 _ -11.5] **	90.3 [80.1_103.5]	-10 [-11.8 _ -8.3] ** ##
BMI (kg/m²)	33.6 [31.2_38.1]	-4.1 [-4.8 _ -3.3] **	33.3 [31.3_36.5]	-4.6 [-5.4 _ -3.8] **	34.4 [31.1 _38.7]	-3.9 [- 4.4 _ -3.3] ** ##
Body Fat mass (kg)	40.8 [34.1_48.5]	-7.65 [-9.68 _ -6.01] **	37.01 [31.1_ 45.0]	-8.8 [-11.01 _ -7.2] **	42.7 [37.8_ 50.3]	-7.3 [-8.8 _ -5.6] ** ##
Body Fat (%)	46.1 [39.9_50.4]	-3.40 [-5.50 _ -1.70] **	38.5 [34.0_42.1]	-4.0 [-6.5 _ -2.4] **	49.9 [46.9_51.9]	-3.0 [-4.6 _ -1.4] ** ##
Body Lean mass (kg)	47 [39.6_56.1]	-3.2 [-5.7 _ -1.7] **	57.5 [51.8_ 61.5]	-4.5 [-6.3 _ -2.6] **	41.2 [36.5_45.4]	-2.5 [-4.7 _ -1.4] ** ##
Body Lean mass (%)	50.7 [46.3_56.9]	2.9 [1.2_5.003] **	58.2 [54.6_62.1]	3.5 [1.8_5.9] **	47.2 [45_49.6]	2.6 [0.9_4.1] ** ##
Android fat (%)	53.4 [49.6_56.8]	-4.6 [-7.8 _ -2.4] **	49.7 [46.9_53.2]	-6.5 [-10.4 _ - 4.1] **	55.6 [53.1_58.4]	-3.5 [-5.8 _ -1.8] ** ##
Gynoid fat (%)	49.8 [40.8_55.0]	-2.7 [-4.3 _ -1.3] **	39.3 [33.9_43.3]	-3.3 [-5.5 _ -1.9] **	53.6 [50.9_56.3]	-2.2 [-3.5 _ -0.8] ** ##

Waist circumference (cm)	109.3[101.3_119.5]	-9.3 [-13.5 _ -5.9] **	115.3 [108.5_123]	-12 [-15.3 _ -7.7] **	104.7 [96.5_ 113.1]	-8.2 [-12 _ -4.3] ** ##
Hip circumference (cm)	116.3 [110.9_ 126.3]	-7.5 [-10.2 _ -4.7] **	115 [109.5_123.3]	-8 [-10 _ -4.7] **	118.7 [111.8_ 129.4]	-7 [-10.5 _ -4.7] **
Thigh circumference (cm)	57 [53_ 61.5]	-3 [-5.5 _ -1] **	56 [52.5_59.5]	-2.8 [-5.5 _ -1] **	57.8 [53.5_62.5]	-3 [-5.5 _ -1] **
BMD	1.3 [1.2_1.3]	-0.007 [-0.030_0.020]	1.3 [1.3_1.4]	-0.005 [-0.024_0.018]	1.2 [1.2_1.3]	-0.008 [-0.036_0.022] ##

Wilcoxon Signed Rank test OR paired t- test: **P≤0.001 'post' compared with 'pre', Mann-Whitney U or unpaired t-test ## P<0.001 compared with males. **Abbreviations: BMI:** body mass index; **BMD:** bone mineral density.

5.3.2 Changes in Cardiometabolic Risk Factors Pre- and Post-LED

This study found changes in cardiometabolic risk factors pre- and post-LED in the whole cohort, as well as separately for males and females, as seen in **Table 5-2**, with males showing a greater reduction in systolic and diastolic blood pressure compared with females (difference in sex medians, -4.1 mm Hg; P<0.05, and -6 mm Hg; P<0.001 respectively. However, HR response to the intervention did not differ between males and females (P=0.368).

Table 5-2: The cardiometabolic risk factors of all the participants during pre- and post-LED.

Variable	ALL		Males n=91: 41.2%		Females n=130: 58.8%	
	Median [25th -75th percentile]					
	PRE	Δ	PRE	Δ	PRE	Δ
Heart rate (%)	72.3 [65.7_80.3]	-6.7 [-12.3 _ -1] **	72 [65_80]	-7.3 [-12 _ -2] **	72.7 [66.3_80.6]	-6.7 [-12.3 _ 0.916] **
Systolic BP (mm Hg)	129.7 [120.3_ 140]	-8.3 [-16.7_0.33] **	131 [124.7_140]	-9.3 [-18.7 _ -4.3] **	127 [118.3_ 139.5]	-5.2 [-14.1 _3.5] ** #
Diastolic BP (mm Hg)	71 [63.7_ 78.8]	-2 [-8.5 _ 4.3] *	76 [70.7_ 80.7]	-5 [-11.3_ 0.00] **	67 [60.7_ 73.4]	1 [-5.1_ 7.8] ##

Wilcoxon Signed Rank test OR paired t-test: *P< 0.05, **P≤0.001 'post' compared with 'pre', Mann-Whitney U or unpaired t-test # P< 0.05, ## P<0.001 compared with males. **Abbreviation: BP:** blood pressure.

5.3.3 Changes in the Metabolic Parameters Following LED Intervention

There were significant improvements in metabolic parameters in blood, including reduced fasting glucose, insulin, TG, and C-peptide concentration in both sexes as a result of the 8-week LED, while changes in parameters (e.g. fasting plasma glucose, HbA1c, total cholesterol, and

LDL) showed varying responses between males and females, as outlined in **Table 5-3**.

The study found no significant difference between sexes in the change in fasting glucose and HbA1c parameters (P=0.089, P=0.383, respectively). However, fasting insulin and C-peptide concentrations decreased to a greater extent in males than in females (difference in sex medians, -25.7 pmol/L; P<0.001 and -211 pmol/l; P<0.001, respectively). These resulted in differences in indices of insulin resistance/sensitivity between males and females. Males had a greater decrease in HOMA-IR and a larger improvement in QUICKI than females (difference in sex medians, -0.98; P<0.001 and 0.015; P<0.001, respectively).

There were no observed sex-specific differences in response to total cholesterol and LDL-C parameters as a result of the intervention (P=0.117, P=0.111, respectively). However, male participants reported a greater reduction in circulating triglyceride concentration than females (difference in sex medians, -0.210 mmol/L; P<0.001).

With regards to liver enzymes, ALT concentration increased as a result of the 8-week LED, with females experiencing a greater rise than males (difference in sex medians, 4 U/L; P<0.05). There were no changes in AST concentration in the whole cohort (P=0.537), but females showed a greater change in AST than males (P<0.05). Serum CRP concentration decreased in the whole cohort, with females having a greater reduction compared with males (difference in sex medians, -0.640 mg/L; P<0.05).

Table 5-3: The metabolic changes in the different sex groups during pre- and post-LED.

Biochemical measurements	ALL		Males n=91: 41.2%		Females n=130: 58.8%	
	Median [25th -75th percentile]					
	PRE	Δ	PRE	Δ	PRE	Δ
Fasting glucose (mmol/L)	5.6 [5.2_6.1]	-0.2 [-0.7_0.2] **	5.6 [5.3_6.2]	-0.3 [-0.8_0.2] **	5.5 [5.2_6.1]	-0.1 [-0.7_0.3] *
Fasting insulin (pmol/L)	97.9 [67.4_136.1]	-36.8 [-64.6_ -12.5] **	109.7 [71.5_156.9]	-52.8 [-77.1_ -25.7] **	86.8 [64.6_118.8]	-27.1 [-50.0_ -8.3] ** ##
HbA1c (mmol/mol)	37 [35_40]	-2 [-4_ -1] **	37 [34_40]	-3 [-4_ -1] **	37.5 [35_40]	-2 [-4 _ -1] **
HOMA-IR	3.4 [2.3_5.02]	-1.3 [-2.7_ -0. 43] **	3.9 [2.6_ 5.9]	-1.9 [-3.1 _ -0.95] **	2.8 [2.2_4.5]	-0.92 [-1.8 _ -0.29] ** ##
QUICKI	0.318 [0.302_0.335]	0.024 [0.009_ 0.041] **	0.312 [0.295_0.331]	0.034 [0.019_0.048] **	0.326 [0.306_0.338]	0.019 [0.006_0.032] ** ##
Total cholesterol (mmol/L)	4.7 [4.1_5.4]	-0.825 [-1.19 _ -0.460] **	4.5 [3.9_5.1]	-0.880 [-1.29 _ -0.600] **	4.9 [4.3_5.6]	-0.780 [-1.15 _ -0.415] **
LDL-cholesterol (mmol/L)	2.8 [2.4_3.5]	-0.545 [-0.820_ -0.222] **	2.7 [2.1_3.2]	-0.560 [-0.875 _ -0.275] **	2.9 [2.5_ 3.7]	-0.480 [-0.785 _ -0.165] **
Triglycerides (mmol/L)	1.3 [0.932_1.7]	-0.290 [-0.617_ -0.022] **	1.4 [1.1_1.9]	-0.430 [-0.870 _ -0.140] **	1.2 [0.9_1.6]	-0.220 [-0.480_0.030] ** ##
ALT (U/L)	19 [13_29]	6 [-1_15] **	23 [14_30]	3 [- 2_12] *	16 [12_26.5]	7 [1_17.50] ** #
AST (U/L)	25 [21_31]	0.00 [-4_5]	27 [23_32]	-1 [-5_3]	24 [20_29]	1 [-3_6] #
C-peptide (pmol/L)	888.5 [693_ 1171.5]	-210.5 [-393.5 _ -75.5] **	1036 [736_1271]	-345 [-524 _ -169] **	838 [673_1075]	-134 [-266.5 _ -38] ** ##

CRP (mg/L)	3.3 [1.4_5.6]	-0.595 [-1.96_0.133] **	2.6 [0.98_4.95]	-0.27 [-1.54_0.35] *	3.7 [2.1_6.5]	-0.910 [-2.02 _ -0.095] ** #

Abbreviations: **HbA1c:** glycated haemoglobin; **HOMA-IR**: homeostatic model assessment of insulin resistance; **QUICKI**: quantitative insulin sensitivity check index; **ALT:** Alanine Transaminase; **AST:** Aspartate Transaminase; **CRP:** C-reactive protein; **LDL-Cholesterol**: low-density lipoprotein.
Wilcoxon Signed Rank test OR paired t-test: * P< 0.05, **P≤0.001 'post' compared with 'pre', Mann-Whitney U or unpaired t-test # P< 0.05, ## P<0.001 compared with males.

5.3.4 Eating Behaviour, Perceived Stress and Sleep Changes Following LED Intervention

Following the LED intervention, participants exhibited changes in eating behaviour, perceived stress and sleep, as measured by TFEQ, Perceived Stress Scale [484], and PSQI (**Table 5-4**).

For the TFEQ, restraint scores in the whole cohort increased, with males showing a larger increase compared with females (difference in sex medians, 2 scores; P<0.05). However, disinhibition and hunger scores decreased, and the response of these variables to the intervention did not differ between males and females (P=0.093 and P=0.115, respectively).

Perceived stress levels and PSQI scores in the whole cohort were reduced after the intervention (the latter indicating an improvement in sleep quality), with the response to the intervention not differing between males and females (P=0.932, and P=0.552, respectively).

Table 5-4: The eating behaviour, perceived stress and sleep quality of all the participants during pre-and post LED

Variable	ALL		Males n=91: 41.2%		Females n=130: 58.8%	
	Median [25th -75th percentile]					
	PRE	Δ	PRE	Δ	PRE	Δ
Restraint	6 [4_9]	3 [1_6] **	5 [3_7]	4 [1_7] **	7 [5_9.3]	2 [1_5] ** #
Disinhibition	9 [6_ 12]	-1 [-3_1] **	9 [6_11]	-1 [-4_ 0.0] **	10 [7_13]	-1 [-3_1] **
Hunger	7 [4_ 10]	-1 [-3_1] **	7 [4_10]	-1 [-3_ 0.0] **	7 [3.5_ 10]	-1 [-2_1] *
Stress	14 [10_ 19]	-1 [-5_2] **	13 [8_ 18.5]	-1 [-5_2] *	14 [11_ 19]	-1.5 [-5_2] *
Sleep	7 [4_ 10]	-1 [-3_ 0.0] **	6.5 [4_9]	-1 [-3_ 0.75] **	7 [5_10]	-1 [-4_0.0] **

Abbreviations: PSS: Perceived Stress Scale; high scores indicate more stress; **PSQI**: Pittsburgh Sleep Quality Index; high scores indicate worse sleep quality.
Wilcoxon Signed Rank test OR paired t-test: * P< 0.05, **P≤0.001 'post' compared with 'pre', Mann-Whitney U or unpaired t-test # P< 0.05, ## P<0.001 compared with males.

5.3.5 Relationship between baseline eating behaviour, perceived stress, sleep quality, Physical Activity, and alterations in BMI and body composition.

In the whole cohort, a positive correlation was observed between baseline (pre-intervention) Restraint (derived from the TFEQ) and the change in BMI (ΔBMI) that occurred as a result of the 8-weeks LED (ΔBMI= 0.083 × Restraint − 4.687: R=0.276; P<0.001), and this relationship was modified by biological sex (R^2 change= 0.046; P=0.001). Pre-intervention Disinhibition negatively correlated with change in BMI (ΔBMI= −0.053 × Disinhibition − 3.619: R= -0.173; P<0.05) and this relationship was also modified by biological sex (R^2 change=0.119; P<0.001). Including Disinhibition, as well as Restraint, in the regression model, improved the power to predict the change in BMI (R^2 change=0.024; P<0.05). There was no association between pre-intervention Hunger reported by the participants and the change in BMI (P=0.133).

There was no association between pre-intervention PSS scores and change in BMI following the intervention (P=0.971). Similarly, no relationship was observed between PSQI scores and ΔBMI (P=0.853), and time spent in low-to-moderate physical activity (LM-PA) also showed no association with ΔBMI (P=0.505).

Regarding body composition, a positive correlation was observed between perceived stress score [484] and change in fat mass (Δ fat mass = 0.076 × PSS − 8.931: R=0.178; P<0.05), suggesting that higher baseline PSS scores were associated with lower loss of fat mass, and this relationship was modified by biological sex (R^2 change=0.072; P<0.001). Finally, there was no association between the time spent in LM-PA, pre-intervention (data presented in Chapter 4, sections 4.3.3, and

4.3.5), and changes in LM and fat mass (R=0.106, P=0.133, R= -0.128, P=0.070, respectively).

5.4 Discussion

The present secondary analysis of the PREVIEW Nottingham dataset examined the effects of an 8-week LED intervention in an overweight population with pre-diabetes. It focused on the relationships between eating behaviour and lifestyle characteristics, metabolic outcomes, and changes in body composition, allowing sex-specific responses to LED interventions and the influence of these factors on weight loss outcomes to be examined. The 8-week LED intervention resulted in reductions in body weight, improvements in metabolic parameters and positive changes in cardiovascular risk, supporting the effectiveness of LED as a therapeutic approach to address overweight / obesity, but with notable sex-specific responses. Males showed greater reductions in body mass, fat mass and LM compared with females, along with greater fasting insulin sensitivity and blood pressure improvements. Importantly, the study found that certain characteristics at baseline, such as dietary restraint and disinhibition, impacted weight loss outcomes, while perceived stress levels showed associations with changes in body composition, with these effects varying by biological sex.

The 8-week LED intervention led to a clinically relevant weight loss of ~12% (median weight reduction of 11.3 kg), exceeding the 10% weight reduction typically proposed as required to bring about meaningful health benefits in obesity management [656]. These findings align with the broader literature on LED interventions, providing strong support for the effectiveness of this approach. A systematic review by Parretti *et al.* [256] of LED interventions across diverse populations found that 8 to 12-week programs consistently achieved weight losses of 10 to 12 kg, closely aligning with the observed results in the Nottingham PREVIEW cohort. Iepsen *et al.* [657] demonstrated similar results in their 8-week LED

intervention for individuals with prediabetes and obesity, achieving a mean weight loss of 12.5 kg. Additional findings from their study reveal metabolic benefits associated with rapid weight loss, which can enhance insulin sensitivity and improve glycaemic control. A large European study using a slightly different protocol achieved similar results, with an average weight loss of 11.0 kg during the 8-week LED phase among overweight and obese adults with prediabetes [658]. The greater weight loss seen in males in the present study is consistent with previous systematic reviews showing greater weight loss in males compared with females is observed during dietary intervention [642]. These sex-based differences in weight loss magnitude may reflect physiological differences in energy expenditure and body composition [659].

Sex-specific differences in body composition changes responding to LEDs were particularly notable in the current study, with males demonstrating greater reductions in both LM and fat mass compared with females. Indeed, the differential loss of LM between males and females was notable and is consistent with the current understanding of body composition changes during rapid weight loss interventions. Cava *et al.* [660] reported that rapid weight loss through energy restriction usually results in loss of both fat and LM, with the proportion varying based on factors such as sex, baseline body composition, and protein intake. The greater loss of LM observed in male participants, including in the current study may be attributed to their higher baseline muscle mass, as individuals with greater initial LM typically experience larger absolute reductions during energy restriction [661]. This was accompanied by sex-specific differences in regional fat distribution in the current study. DXA analysis revealed that males experienced a greater reduction in both android and gynoid fat percentages compared with females. These DXA-derived measurements were supported by anthropometric data, with males showing larger reductions in waist circumference, while changes in hip and thigh circumference were similar between sexes. The DXA measurement of regional fat loss suggests a sex-specific pattern of

adipose tissue reduction that preferentially impacts central adiposity in males. These changes may be important for health due to the strong link between excessive visceral fat and cardiometabolic risk. Ross *et al.* [32] have demonstrated that waist circumference is a vital clinical indicator, independent of BMI, for identifying individuals who are at increased risk for cardiometabolic issues, and Powell-Wiley *et al.* [149] emphasised that central adiposity is positively linked to higher cardiovascular risk, insulin resistance, and inflammatory markers. Interestingly, while changes in waist circumference in the current study differed between sexes, reductions in hip and thigh circumference were similar for both males and females.

The improvements in metabolic parameters observed in the present study, particularly in indices of insulin sensitivity and glycaemic control, align with evidence from other large-scale interventions. For example, Lean *et al.* [321] demonstrated in the DiRECT trial that substantial weight loss through LED significantly reduced HbA1c levels, fasting plasma glucose, and insulin resistance scores in people with T2DM. The present findings were also consistent with the DiRECT trial which reported males having greater reductions in fasting insulin concentration and HOMA-IR. There are several possible mechanisms that could be associated with these differences observed in metabolic responses. First, the greater absolute weight loss achieved by males in the present study (both total fat mass and central adiposity (waist circumference reduction) likely contributed to enhanced metabolic improvements. Research showed that losing approximately 7% of body weight through diet-induced interventions can enhance insulin responsiveness by 43% [662] and greater improvements in insulin sensitivity are linked to larger weight reductions [663]. Moreover, the more pronounced reduction in waist circumference may be particularly relevant for metabolic health improvement, given the established link between visceral adiposity and insulin resistance [640, 664, 665]. These studies have shown that excess visceral adipose tissue is more closely associated with insulin resistance

and metabolic dysfunction than other fat depots. Visceral adipose tissue, in particular, secretes inflammatory mediators that could contribute to insulin resistance [664] and it has been proposed that this link between pro-inflammatory cytokine production and insulin resistance is mediated through increased free fatty acid release, and altered adipokine profiles [640].

The greater weight loss observed in males during the LED intervention warrants fuller consideration of the underlying metabolic mechanisms, particularly in relation to energy requirements and potential implications for nitrogen excretion. Males typically have higher absolute resting metabolic rates than females, with studies demonstrating approximately 23% higher resting metabolic rates in absolute terms [869]. While these differences are largely attributable to greater FFM in males, even after adjustment for body composition differences, males demonstrate approximately 3% higher RMR and consume more energy per kilogram of lean mass (187 kJ/kg FFM versus 170 kJ/kg FFM) compared to females [869, 870]. This suggests inherent metabolic efficiency differences between sexes, with females appearing more efficient at energy conservation, which may represent an evolutionary adaptation to support reproduction.

Given that the LED provided a fixed energy intake of 800 kcal/day, males with their higher baseline energy requirements would have experienced a larger absolute energy deficit compared to females. This greater energy deficit necessitated more substantial mobilisation of endogenous energy stores, contributing to the larger weight loss observed in males. Several mechanisms may explain these sex-specific differences in weight loss response. First, the magnitude of the energy deficit itself differs between sexes when consuming identical caloric intakes. Second, metabolic adaptation during caloric restriction may differ by sex, with research suggesting that activity-related energy expenditure declines more in

females compared to males during prolonged caloric restriction [871]. Third, males' greater baseline FFM means that even if the proportional loss of FFM is similar between sexes, the absolute loss of lean tissue would be greater in males, contributing to larger total weight loss.

The relationship between weight loss magnitude and nitrogen balance during LED interventions is complex. During severe caloric restriction, individuals typically enter negative nitrogen balance as the body mobilises protein stores to meet energy and glucose requirements through gluconeogenesis. Research on very-low-calorie diets has shown that nitrogen excretion initially increases but then diminishes over time, with most individuals maintaining slightly negative nitrogen balance throughout the intervention (mean daily deficits of 1-2 g nitrogen) [872]. The relationship between energy intake and nitrogen balance follows a biphasic pattern, where below a critical minimum energy intake, nitrogen balance remains negative regardless of protein adequacy [873].

Given that males experienced greater weight loss in the current study, it is plausible that they also experienced greater absolute nitrogen losses, particularly if a substantial proportion of weight loss derived from FFM. The correlation between urinary creatinine excretion and nitrogen loss during caloric restriction suggests that nitrogen loss is largely dependent on skeletal muscle breakdown [872]. Therefore, males with greater baseline muscle mass and potentially greater absolute FFM loss would be expected to have higher absolute nitrogen excretion. However, several factors may have moderated this relationship: adequate protein provision in the LED formula (designed to minimise nitrogen losses), potential sex-specific differences in metabolic efficiency of protein utilisation, and the possibility that males preferentially mobilised fat stores due to hormonal differences, particularly higher testosterone levels.

Sex differences in metabolic responses may also reflect the fundamental role of sex hormones in metabolic regulation. Research also shows that sex hormones can affect the patterns of fat deposition and mobilisation during weight loss [639, 666]. The increased fasting insulin sensitivity observed in males in the current study, indicated by greater reductions in HOMA-IR, may be influenced by the role of androgens in glucose metabolism and body composition [667]. They propose that weight loss can lead to increased testosterone levels in males, while higher testosterone levels can simultaneously facilitate further metabolic improvements. This creates a positive feedback loop where initial weight loss may enhance hormonal function, which in turn supports continued metabolic health improvements. Research has shown that testosterone enhances insulin sensitivity measures through multiple mechanisms, including increased muscle glucose uptake, reduced inflammatory cytokine production, and improved body fat distribution [668-670]. For example, as weight loss progresses, changes in testosterone concentrations enhance the preferential mobilisation of abdominal fat by increasing beta-adrenergic sensitivity and improving the lipolytic response in visceral adipose tissue [671]. Oestrogen plays a crucial role in metabolic regulation by suppressing hepatic glucose production and enhancing glucose transporter expression in skeletal muscle [672]. During weight loss, oestrogen levels typically decrease, particularly in females. This occurs because adipose tissue is a significant site of oestrogen production through the aromatisation of androgens, and reduced fat mass leads to decreased overall aromatase activity [673, 674]. Weight loss has been shown to reduce circulating oestrogen concentrations in postmenopausal females [675], and premenopausal females [676], though the magnitude of change can vary based on initial body composition and menopausal status. Changes in oestrogen during weight loss may play a significant role in influencing subcutaneous fat metabolism and affect adipocyte size and function in ways that differ from androgens [677] and these dynamic hormonal changes may contribute to the differing patterns of metabolic improvement seen in female participants in the current study. Combined, these studies showed that

weight loss leads to changes in circulating sex hormone levels, with reduced fat mass decreasing aromatase activity and altering the testosterone-to-oestrogen ratio in both sexes. It is proposed that these hormonal shifts during weight loss may influence regional fat mobilisation patterns and have contributed to the observed sex-specific patterns of fat loss in this study.

Interestingly, while insulin-related parameters exhibited noticeable sex differences, the changes in fasting plasma glucose concentration and HbA1c levels were not different between males and females. This discrepancy between insulin and glucose responses may be attributed to fundamental sex differences in mechanisms of glucose homeostasis as previously suggested by Tramunt *et al.* [678]. This comprehensive review proposed that females and males use different regulatory pathways to achieve similar glycaemic outcomes, with females usually demonstrating better β-cell function and insulin secretion capacity than males [678]. In the context of weight loss, this sexual dimorphism in glucose regulation may explain why both sexes in the current study achieved comparable improvements in glycaemic control despite showing different patterns of change in insulin. Having excess body weight appears to impact insulin sensitivity predominantly through a mechanism of adipose tissue inflammation and ectopic fat deposition. Therefore, weight loss would be expected to improve insulin signalling through reduced inflammatory burden and restoration of normal fat storage patterns, which would result in enhanced glucose uptake regardless of sex-specific differences in β-cell function.

Sex-specific responses extend to other metabolic parameters as well. Both sexes showed improvements in serum total cholesterol and LDL-C concentration, but male participants experienced a greater reduction in triglyceride concentrations. This pattern aligns with research showing that sex hormones affect regional fat distribution and mobilisation during

weight loss [639]. Particularly, testosterone increases the mobilisation of abdominal fat by enhancing beta-adrenergic sensitivity and the lipolytic response in visceral adipose tissue [679], which could explain the greater reductions in visceral fat depots and waist circumference observed in males. However, oestrogen promotes subcutaneous fat accumulation through increased adipogenic differentiation and reduced lipolytic activity in subcutaneous adipose tissue [680]. This could help explain why, in the female participants in the present study, smaller reductions in waist circumference, but proportionally greater decreases in hip circumference was seen, compared with males. The physiological reasons for these differences are complex and involve multiple factors. For example, sex steroids exert depot-specific effects on adipose tissue metabolism by directly influencing adipocyte function [666]. Specifically, testosterone enhances lipolytic enzyme expression and reduces lipogenic enzyme activity in visceral fat, while oestrogen has the opposite effect in subcutaneous fat. During weight loss, these depot-specific effects become particularly important because they influence which fat deposits are preferentially mobilised. Males generally accumulate more fat in visceral deposits, which are more metabolically active and responsive to lipolytic stimuli during energy restriction [681]. This distribution pattern, combined with the enhancement of lipolytic enzyme activity by testosterone, leads to preferential mobilisation of visceral fat during weight loss. Since visceral fat is more directly connected to the hepatic circulation, its mobilisation results in a greater flux of fatty acids to the liver, where they can be more efficiently processed for energy utilisation rather than being repackaged into triglycerides. This mechanistic pathway may explain why male participants showed more pronounced improvements in triglyceride levels. In contrast, oestrogen's role in promoting subcutaneous fat storage through increased adipogenic gene expression and reduced lipolytic enzyme activity in females [680], creates a metabolic environment that affects both fat distribution and mobilisation during weight loss interventions. In the current study, this was reflected in the female participants showing a more consistent pattern of fat loss across different body regions, with smaller reductions

in triglyceride levels compared with males. This aligns with their observed body composition changes, where reductions in both waist and hip circumference were more proportional, suggesting a more evenly distributed pattern of fat loss rather than the preferential visceral fat loss seen in males.

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects approximately 25% of people living with overweight or obesity and prediabetes [682, 683] and elevated circulating concentration of liver enzymes, particularly ALT, is used as a clinical indicator of MASLD, albeit a contentious one [684]. Weight loss interventions through energy restriction can effectively reduce hepatic fat content and improve liver function [685, 686]. The current study revealed alterations in the circulating concentration of liver enzymes after the LED intervention. While AST concentration remained stable in the overall cohort, females showed a different AST response compared with males. The differential response in liver enzymes between sexes included an increase in circulating ALT concentration, which was more pronounced in females compared with males and could suggest sex-specific hepatic adaptation patterns to the energy restriction. The increase in ALT in the current study, particularly in females, contrasts with previous weight loss studies, like the LOOK AHEAD trial, where weight loss was associated with reduced ALT and AST levels [415]. However, Gasteyger *et al.* [687] reported transient ALT elevations, particularly in females, during very low-calorie diet interventions, similar to those observed in the current study. They attributed these changes to increased hepatic metabolic oxidation and oxidative stress [687]. These elevations may also be linked to accelerated protein turnover from gluconeogenesis [688], and the upregulation of gluconeogenic enzymes during rapid weight loss [689]. During energy restriction, the liver undergoes several key metabolic adaptations, including increased fatty acid oxidation to meet energy demands [690], enhanced gluconeogenesis to maintain blood glucose concentration [689], and altered protein metabolism [691], which may

temporarily influence liver enzyme levels as the body adjusts to the reduced energy intake. Rapid weight loss triggers enhanced lipolysis from adipose tissue, leading to increased free fatty acid flux to the liver [692]. The liver upregulates fatty acid oxidation to process these mobilised lipids [693], which can temporarily strain liver function and increase ALT concentration in the blood.

The analysis of cardiometabolic responses showed significant differences in blood pressure regulation between sexes after the LED intervention. Males showed greater reductions in both systolic (-9.3 vs -5.2 mm Hg; $P<0.05$) and diastolic blood pressure (-5 vs +1 mm Hg; $P<0.001$) compared with females. The differential blood pressure response may be related to the greater absolute weight loss in males, which directly reduces blood volume and systemic vascular resistance [245]. Males are reported to have higher baseline sympathetic nervous system activity and weight-loss results in greater reductions in norepinephrine release and vasoconstriction, leading to more pronounced blood pressure decreases compared with females [694]. Additionally, variations in renin-angiotensin system responses to energy restriction may be influenced by sex-specific hormonal differences. Studies suggest that males experience a greater suppression of angiotensin II production during weight loss, leading to increased vasodilation and a more pronounced reduction in blood pressure, and these combined physiological differences have been proposed to explain the observed sex-based variations in blood pressure outcomes after weight-loss [695]. Moreover, oestrogen positively influences blood pressure regulation through increased nitric oxide production and enhanced vasodilation, while also reducing sympathetic activation. However, these protective effects may be temporarily altered during energy restriction as oestrogen levels fluctuate, potentially tempering weight-loss related improvements in blood pressure through other mechanisms [696]. HR responses showed similar reductions between sexes, consistent with findings from large-scale weight loss trials by the

Look-AHEAD-Research-Group *et al.* [697]. They indicated that certain autonomic adaptations, specifically reduced resting HR and decreased cardiac sympathetic tone, occur similarly in both sexes during energy restriction due to shared mechanisms of enhanced parasympathetic activity.

The findings of the current study revealed changes in eating behaviours and lifestyle parameters following the LED intervention, with notable patterns specific to sex, which have also been noted by others. Dietary restraint is defined as the conscious effort to restrict food intake to control body weight [698]. It is an important behavioural factor in weight management that appears to influence daily energy intake. In the current study, the increase in dietary restraint scores was more pronounced in males compared with females, suggesting an interesting deviation from usual patterns because females typically demonstrate higher baseline dietary restraint levels [699]. The more substantial increase in restraint scores in men might suggest that they have a greater capacity for improvement in this domain, given their lower baseline levels. While the current study observed that successful weight loss was associated with increases in dietary restraint, the directionality of this relationship remains complex, as both factors may influence each other [700]. The positive correlation between baseline restraint and changes in BMI aligns with findings from Teixeira *et al.* [701], suggesting that initial restraint levels may help to predict weight loss outcomes. However, Dietrich *et al.* [702] showed that females typically demonstrate greater dietary restraint but also higher emotional eating tendencies compared with males, and the latter could negatively impact compliance to the LED and influence the degree of weight loss achieved. Unfortunately, there is no data on emotional eating measures in the PREVIEW dataset to be able to address this theory.

Interestingly, while both disinhibition and hunger scores decreased to a similar degree in both sexes, the relationship between baseline disinhibition and weight loss outcomes showed sex-specific patterns. The current study found no association between baseline hunger scores and the magnitude of change in BMI. However, the negative correlation between disinhibition and change in BMI was modified by biological sex, suggesting that the impact of disinhibition on weight loss success may differ between males and females. Hansen *et al.* [703] reported contrasting findings in their 8-week LED intervention study. They found higher baseline hunger scores, but not other eating behaviour traits, including disinhibition, were associated with greater weight loss. These conflicting findings highlight the broader inconsistencies found in the literature. Teixeira *et al.* [704] found that baseline eating behaviours were poor predictors of weight loss success in their review of pretreatment predictors. In contrast, Dalle Grave *et al.* [705] demonstrated that eating behaviour traits, such as restraint, disinhibition, and hunger, can all influence weight loss outcomes in dietary interventions, though the relationships are complex and may vary depending on individual psychological characteristics, intervention types and differences across populations. Moreover, Batra *et al*. [830] highlighted that the predictive value of eating behaviour traits can vary depending on the phase of a weight loss intervention. Specifically, higher scores for food cravings or hunger may have a greater impact on success during active weight loss compared with weight maintenance. These contrasting findings highlight the complexity of using baseline behavioural characteristics to help predict weight loss success and suggest that considering sex-specific patterns in behavioural responses may be important to manage successful weight loss with LED interventions.

The observed improvement in perceived stress levels and sleep quality in both sexes indicates that the LED intervention may provide broader benefits beyond weight loss. Lasikiewicz *et al.* [706], demonstrated in their systematic review that higher psychological stress levels reduce

adherence to dietary interventions, leading to poorer weight loss outcomes. The current study revealed that higher baseline PSS scores were associated with lower reductions in fat mass and this relationship was modified by biological sex, with females showing a stronger negative association between baseline perceived stress levels and fat mass loss compared with males. The greater effect of perceived stress on fat mass loss in females could be explained primarily through behavioural mechanisms specific to sex. Females are more likely to engage in stress-induced emotional eating and experience reduced dietary restraint during periods of stress [707], which can decrease adherence to LED interventions. Additionally, physiological stress responses differ by sex. Females show greater cortisol responses to stress [708], which can impair fat mobilisation during caloric restriction through reduced lipolysis and increased fat retention. The interaction between elevated cortisol and oestrogen further inhibits fat mobilisation during energy restriction in females [709]. These sex-specific behavioural and physiological responses to stress could help to explain why higher baseline perceived stress levels were more strongly associated with lower fat mass loss in females during the LED intervention, highlighting the importance of incorporating stress management strategies into weight-loss programs, particularly for female participants.

It was hypothesised that higher levels of physical activity would maintain LM and enhance fat mass loss through increased loading of the muscles involved in ambulation and increased energy expenditure, respectively, as previously demonstrated by Willis *et al.* [710] and Swift *et al.* [207]. However, the analysis revealed no associations between pre-intervention time spent in LM-PA and changes in either LM or fat mass. Although there was a trend suggesting greater fat mass loss with increasing daily LM-PA, the relationship was weak (R^2=0.0165). Factors that contribute to individual differences in FFM preservation during LEDs are still not well understood. It is suggested that exercise type and intensity [711], and baseline body composition [712] play important roles, with physical

activity emerging as a critical factor in FFM preservation during weight loss [711]. However, studies have reported conflicting results regarding the relative contribution of these factors, and their optimal combinations for FFM preservation remain unclear [258]. The lack of strong associations between measures of physical activity and FFM preservation in the current study might be attributed to the relatively narrow range of physical activity levels in our cohort or the possibility that the intensity of physical activity was insufficient to significantly impact body composition changes during the LED intervention.

The findings from this present study have several important implications for both clinical practice and future research in weight management. The observed differences in body composition and metabolic responses between sexes during LED interventions indicate the necessity for more tailored approaches to weight management to promote successful fat mass loss. Males may benefit from specific strategies to preserve lean tissue during rapid weight loss, especially considering the critical role of muscle mass in maintaining metabolic health and physical function. In addition, early assessment of eating behaviours could help identify individuals who might need additional support to promote compliance during LED interventions. Moreover, the current study's findings on perceived stress and their association with changes in fat mass, underline the potential significance of addressing the individual’s broader environment in weight management programs. and suggests that introducing stress management strategies may improve weight loss results, particularly for those with higher baseline stress levels. The notable decrease in blood pressure, especially among male participants, seen in the current study indicates that LED interventions can be effective for individuals affected by hypertension and obesity to manage their blood pressure.

A key strength of this study is that the sample size is relatively large (n=221) with a reasonably balanced sex distribution (41.2% males, 58.8% females), which enabled the analysis of sex-specific responses to the LED intervention. The study had an attrition rate of 14.4%, suggesting good acceptability of the LED intervention among participants at risk of T2DM. The assessment of body composition using DXA scans provided more accurate measurements of fat mass, LM, and bone mineral density changes, compared with other commonly used methods and allowed regional analysis of body composition, which is considered a strength of the study, addressing limitations found in previous work that depended on bioelectrical impedance or anthropometric measures. The measurement of circulating liver enzyme concentration and lipid profiles, alongside glucose and insulin, offers a more comprehensive view of the metabolic effects of rapid weight loss.

However, several limitations should be considered in the present study. At the post-LED visit, indices of insulin sensitivity in the insulin-stimulated state were not assessed. Although HbA1c was measured, this provides an indication of average glycaemia over the previous 2 to 3 months, which may underestimate the impact of weight loss on glucose regulation and does not provide information on the responses of blood glucose and insulin to eating carbohydrate. Therefore, the current study can only comment on changes to limited estimations of insulin sensitivity that resulted from the weight-loss programme. Although repeating the OGTT at CID2 would have provided greater insight into the impact of weight loss on whole-body insulin sensitivity by including a measure in the insulin-stimulated state, to fully understand the impact of weight loss on the sites where changes to insulin sensitivity occurred, one would need to conduct more detailed investigations [713]. Free-living physical activity was assessed only pre- and not during the intervention, which is a key limitation as it assumes that physical activity behaviours at baseline were maintained during the LED period. Furthermore, the lack of association between physical activity and changes in LM or fat mass should,

therefore, be interpreted with caution, as it may reflect the narrow range of physical activity levels in the Nottingham cohort (which primarily consisted of sedentary individuals) or insufficient variability in activity levels rather than a true absence of the relationship. This homogeneity in physical activity levels among participants restricts the ability to draw broader conclusions about how varying levels of physical activity might influence body composition changes during LED interventions. A limitation of the present study is that nitrogen balance was not directly measured through urinary and faecal nitrogen analysis. Such measurements would have provided definitive evidence regarding whether greater weight loss in males was associated with proportionally greater nitrogen losses, or whether sex-specific metabolic adaptations resulted in differential nitrogen sparing. Additionally, serial body composition measurements at multiple timepoints during the LED intervention would have allowed tracking of the temporal relationship between FFM loss and weight loss velocity in males versus females. Future research should incorporate comprehensive nitrogen balance studies with sex-stratified analyses to determine whether standard protein recommendations for LEDs require sex-specific adjustments to optimally preserve FFM, particularly in males who may experience larger absolute energy deficits.

5.5 Conclusion

This study investigated the impacts of an 8-week LED intervention on adults with overweight or obesity at risk of developing diabetes. The intervention resulted in substantial weight loss across the cohort, with males achieving greater reductions than females in both overall weight and body composition measures, suggesting sex-specific responses to the intervention.

There were also improvements in metabolic and cardiovascular health parameters, with both sexes experiencing meaningful improvements in

various metabolic markers, including fasting insulin and lipid profiles, along with reduced HR. However, males showed more substantial improvements in insulin resistance markers and greater reductions in blood pressure and waist circumference compared with females.

Higher baseline dietary restraint was associated with greater weight loss success, while higher disinhibition predicted poorer outcomes. Importantly, these behavioural influences on weight loss differed between males and females. Additionally, greater perceived stress levels reported before the intervention was associated with lower fat mass reduction over the intervention.

Finally, baseline physical activity levels did not spare LM loss or promote fat mass loss over the intervention, though this finding should be considered in the context of the predominantly sedentary study for the Nottingham population.

Chapter 6 Exploring Assessment Methods for determining Dietary Protein Intake, Body Composition, and Physical Activity Measurements used in the Nottingham PREVIEW Cohort.

6.1 Introduction

The accuracy of measurement methods in nutrition and lifestyle intervention studies is fundamental to ensuring research validity and reliability [714]. Researchers' findings may be compromised by systematic measurement errors without reliable and validated assessment tools, which can lead to misinterpretation of intervention effects and limit their translational impact in clinical and public health settings [715]. The choice of measurement method often depends on balancing considerations of accuracy, participant burden, resource availability, and practicality of implementation in the target population [240, 716]. This is particularly important in longitudinal intervention studies like PREVIEW, where small but clinically significant changes must be reliably detected over time to evaluate intervention effectiveness [332]. The purpose of validation studies is to understand the strengths and limitations of different assessment methods, particularly in intervention studies that require precise monitoring, and in this chapter, the focus is on those used to measure dietary intake, body composition, and physical activity levels [715, 717]. Through systematic comparison of different measurement approaches, validation research provides the evidence base needed to select appropriate assessment methods, interpret their outcomes accurately, and understand the magnitude and direction of potential measurement errors [718]. As the field of nutrition and lifestyle research advances with increasingly refined interventions targeting multiple health outcomes, the need for validated measurement methods becomes even more critical to establishing the evidence base for effective prevention and treatment strategies [719].

In dietary assessment, self-reported protein intake frequently differs from actual consumption patterns, presenting a significant challenge in nutrition research [720]. While food diaries and 24-hour dietary recall questionnaires are commonly used tools, their accuracy can be compromised by factors including participant recall bias, portion size

estimation errors, and social desirability bias [721]. Urinary nitrogen excretion has emerged as a validated biomarker for protein intake, providing an objective measure against which self-reported intake can be evaluated [722]. Studies have shown that 24-hour urinary nitrogen collections can reflect dietary protein intake (g per day), using the established conversion factor of 6.25 (nitrogen content of protein being approximately 16%), enabling comparison with reported consumption [723, 724]. However, the method relies on the participants collecting all voided urine in 24 hours. Furthermore, the relationship between self-reported protein intake and urinary nitrogen-derived measurements requires ongoing validation across different populations and study settings [725].

Body composition assessment presents unique challenges in research settings, with various methods differing in their accuracy, accessibility, and cost-effectiveness [160]. DXA is widely considered a gold standard for body composition measurement, offering precise assessments of fat mass, fat-free mass, and bone mineral density [726]. However, its research application is limited by cost, accessibility, participant weight and radiation exposure concerns [161]. BIA provides a more accessible and cost-effective option, though its accuracy can be influenced by factors including hydration status, recent physical activity, posture and body fat distribution [170]. The validation of BIA against DXA measurements is important in research settings that require repeated measurements, as systematic differences between methods could impact study outcomes [727].

Physical activity assessment faces similar challenges in balancing accuracy with practicality [728]. Questionnaires such as the Baecke provide a convenient and cost-effective method for assessing habitual physical activity, particularly in large-scale studies [213]. However, self-reported physical activity may be affected by recall bias, which can result

in an overestimation of activity levels, especially for vigorous-intensity activities [214]. Accelerometry has emerged as an objective measure of physical activity, providing the possibility to collect detailed information about activity patterns, intensity, and duration [217]. Therefore, comparison of questionnaire-based assessments against accelerometry data is important for understanding the reliability of self-reported physical activity measures and their appropriate use in research [729].

The PREVIEW study [332] provides an ideal context for validating these measurement methods. It has been shown that different assessment methods produced varying degrees of agreement in previous validation studies within similar cohorts, highlighting a need for further evaluation in specific populations [730]. Understanding the accuracy and limitations of these measurement techniques is crucial for interpreting study outcomes and informing future research methodology [715].

6.1.1 Aims of the study

The primary aim of the current investigation was to understand the relationship of three commonly used assessment methods against complementary methods in the Nottingham PREVIEW cohort. Specifically, this work aimed to:

- Assess the validity and reliability of dietary protein intake estimated from self-reported dietary records, compared with protein intake calculated from 24-hour urinary nitrogen excretion.
- Assess the agreement between body composition measurements obtained through BIA and the gold standard method of DXA.
- Determine the relationship between physical activity levels assessed using the Baecke questionnaire and objective measurements obtained through accelerometry.

6.2 Materials and Methods

6.2.1 Study Design and Participants

This method validation study was conducted using data generated from the Nottingham PREVIEW cohort, analysing data collected across multiple CIDs. The study included participants who provided measurements for any of the three validation components at any time point.

6.2.2 Dietary Protein Assessment

Dietary protein intake was evaluated using two complementary methods: self-reported dietary records and 24-hour urinary nitrogen collections.

6.2.2.1 Self-reported dietary intake

For dietary assessment, participants completed detailed 4-day food records at several time points throughout the study: baseline (CID1), 26 weeks (CID3), 52 weeks (CID4), 104 weeks (CID6), and 156 weeks (CID7). These records required participants to document all food and drink consumption, including specific details about the time and place of consumption, precise descriptions of consumed items, and portion sizes or amounts. The recording period covered three-week (or work) days and one weekend (or rest) day, with a minimum requirement of two weekdays and one weekend day of acceptable quality for data eligibility.

To ensure accuracy in dietary reporting, participants received comprehensive training on completing food records. While the use of food scales for weighing portions was encouraged, it was not mandatory. Quality control measures included personal submission of food records to a study technician (either a dietitian or equivalent professional), who immediately reviewed the records for completeness and sought clarification where necessary. This direct interaction helped minimise

recording errors and improved data quality. To reduce participant burden, the return of food diaries was coordinated with group meetings or Clinical Investigation Days whenever possible.

All dietary records were analysed using Nutritics Ltd (Dublin, Eire) dietary analysis software, with protein intake calculated using UK and Irish nutrient composition data tables. This standardised approach to dietary analysis ensured consistency in the conversion of food records to nutrient intake data across the study period.

6.2.2.2 24-hour Urinary Nitrogen

For the biomarker validation of protein intake, 24-hour urine collections were obtained at time points corresponding to the dietary records (CID1, CID3, CID4, CID6, and CID7). The methodology was based on the nitrogen balance work of Bingham and Cummings *et al.* [723], who established that approximately 81% of dietary nitrogen is excreted in the urine as total urinary nitrogen, with the remaining nitrogen loss distributed across faeces (10%), skin, and other miscellaneous losses (9%). Since urea represents approximately 85-90% of total urinary nitrogen in healthy adults, urinary urea was used as the primary biomarker for protein intake validation. While this approach captures the majority of urinary nitrogen excretion, it should be noted that small amounts of dietary nitrogen are excreted as non-urea compounds (ammonia, creatinine, uric acid, and other nitrogenous metabolites), which are not captured in this measurement. Participants received detailed instructions (according to a standardised operating procedure) and collection tools to ensure proper sampling. Urine samples were analysed for urea content locally at the Nottingham intervention centre using a colorimetric method (ABX Pentra, Horiba, Montpellier, France). Quality control measures included minimum volume requirements, with collections of less than 0.5 L/day being regarded as incomplete and excluded from analysis. Urinary nitrogen

was determined using the conversion factor of urea × 0.4664, derived from the molecular weights of nitrogen and urea.

Daily protein intake was then calculated using the following formula:

urine calculated protein intake = (urine nitrogen (g) + (body weight (kg) × 0.031)) × 6.25/0.8

Where 0.031 accounts for miscellaneous nitrogen losses relative to body weight, 6.25 is the standard nitrogen-to-protein conversion factor, and dividing by 0.8 adjusts for the 81% urinary nitrogen excretion rate [731].

For this secondary analysis, the relationship between urinary-derived protein intake and dietary records was initially examined using data from all participants and all timepoints. Data collected prior to the intervention (CID1) in all participants was then explored, with age, BMI and sex included as covariates in the regression analysis model to understand whether these modify the relationship between methods. These covariates were chosen because age and sex are known to influence protein metabolism and excretion rates, while BMI may affect the accuracy of dietary reporting and metabolic processes related to protein turnover. Subsequently, data collated at measurement time points during the intervention (in those participants who had complete data sets for all timepoints), was carried out to identify whether the relationship between methods was modified by the stage of the intervention, with each time point analysed separately; CID1 measurements established baseline relationships prior to weight loss, while subsequent time points captured the relationship during the weight maintenance phase.

6.2.3 Physical Activity Assessment

Physical activity was assessed using two methods: the Baecke questionnaire for self-reported physical activity and accelerometry measurements of movement. It is recognised that the two tools measure different aspects and are not directly comparable, but the hypothesis was

that the Baecke score would increase with greater time spent in moderate and vigorous activity and be lower in those having greater sedentary time.

6.2.3.1 Baecke Physical Activity Questionnaire

The Baecke questionnaire, a validated subjective measure of habitual physical activity [211], was administered at week 0 prior to starting study interventions (CID1). This self-administered questionnaire consists of 22 questions designed to evaluate three distinct domains of physical activity: work-related physical activity, sport and exercise participation, and leisure-time physical activity excluding sports. The work index addresses occupational physical and sedentary activities such as sitting, standing, walking, and lifting heavy loads. The sport index evaluates regular sports participation, including the type, intensity, and duration of activities. The leisure index assesses physical and sedentary activities during non-working hours, such as walking, cycling, and television viewing. Responses to each question are scored on a 5-point Likert scale, with separate indices calculated for work, sport, and leisure activities, and with 1 representing the lowest level of activity and 5 representing the highest. The scores for the questions in each domain are averaged to provide a domain score, and the total score is then obtained by summating the work index, sport index and leisure index. The minimum score for the Baecke questionnaire is 3, and the maximum is 15. Quality control measures included personal submission of questionnaires to a study technician who immediately reviewed the responses for completeness and sought clarification where necessary.

6.2.3.2 Accelerometry

For objective physical activity measurement, accelerometry data was collected in the week preceding the first assessment visit; week 0 (CID1), according to the protocol previously described (Chapter 3, Section 3.8). Participants were asked to wear the accelerometer (ActiGraph GT3X) for 7 full days, including 2 weekend days, to create a complete record of their

physical activity patterns during the week and on weekends. The data had to be collected over a minimum of four days, each consisting of a minimum of 10 hours of daytime wearing. Participants were contacted by study staff during each data collection period to address any technical issues. Data quality and participant compliance were monitored using this monitoring method. From the 7-day accelerometry recordings collected at week 0 (CID1), data were processed to calculate the total minutes spent in sedentary, light, moderate, and vigorous physical activity for each valid day. Minutes in each intensity category were averaged across all valid wear days to provide a representative measure of participants' typical activity patterns. For analysis purposes, moderate and vigorous activity minutes were also combined to create a moderate-to-vigorous physical activity (MVPA) metric.

6.2.4 Body Composition Assessment

Body composition measurements were performed using BIA or DXA at CID1, CID2, CID3, CID4, CID6, and CID7, as described in Chapter 3, section 3.6.3. In a subset of participants who were eligible to undertake a DXA scan, a BIA measurement was made immediately after the scan while the participant was still supine on the DXA table. These paired measurements were obtained solely for the purpose of method comparison (validity testing of BIA against DXA as the reference method) in the present chapter; BIA and DXA data were not combined or used interchangeably in any other analyses within this thesis.

6.2.5 Statistical analyses

Statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) Statistics version 28. For each comparison:

Dietary protein: Pearson correlation coefficients were used to investigate associations between reported intake and urinary nitrogen-derived protein intake, with Bland-Altman analysis, with calculation of the mean difference and ±1.96 SD made for agreement assessment. Furthermore, modifiers of the relationship between methods were examined using multiple regression analysis.

Physical activity: Correlation analysis was used to investigate the relationship between Baecke scores and time spent in different activity intensity classifications, obtained from accelerometry data.

Body composition: Comparison of BIA and DXA measurements were made using Pearson correlation coefficients and Bland-Altman plots as described for dietary protein.

The significance level for comparisons is indicated by $P<0.05$ throughout.

For the comparisons between the two assessment methods, normally distributed data are described in the text as the mean ± standard deviation (SD), whereas non-parametric data are represented as the median [25th-75th percentile].

6.3 Results

6.3.1 Validation of Protein Intake Using 24-hour Urinary Nitrogen

The relationship between protein intake calculated from 24-hour urinary nitrogen and self-reported protein intake was examined across all collection time points. A total of 264 participants provided 768 valid 24-hour urine collections and dietary records for analysis.

6.3.1.1 Relationship Between Methods

The data from all-time points, analysed collectively, revealed a positive correlation between calculated protein intake from urinary nitrogen and self-reported protein intake ($R=0.394$, $P<0.001$, and regression equation $y= 0.2951x + 52.021$), as shown in **Figure 6-1**. The overall median difference between methods was -16.54 [-33.88- 2.03] g/day, suggesting the underreporting of protein intake through dietary assessment methods compared with the biomarker approach or the overestimation of protein intake using urinary nitrogen assessment.

Bland-Altman analysis of all data demonstrated a proportional bias across the range of dietary protein intakes (regression equation: $y= -0.4149x + 20.562$, $R= -0.307$, $P<0.001$), with greater discrepancies between methods (greater under-reporting of protein intake in dietary records or greater over-estimation using urinary nitrogen method) as the amount of protein consumed increased (**Figure 6-2**). Furthermore, approximately 5.5% of values lay outside of these limits of agreement.

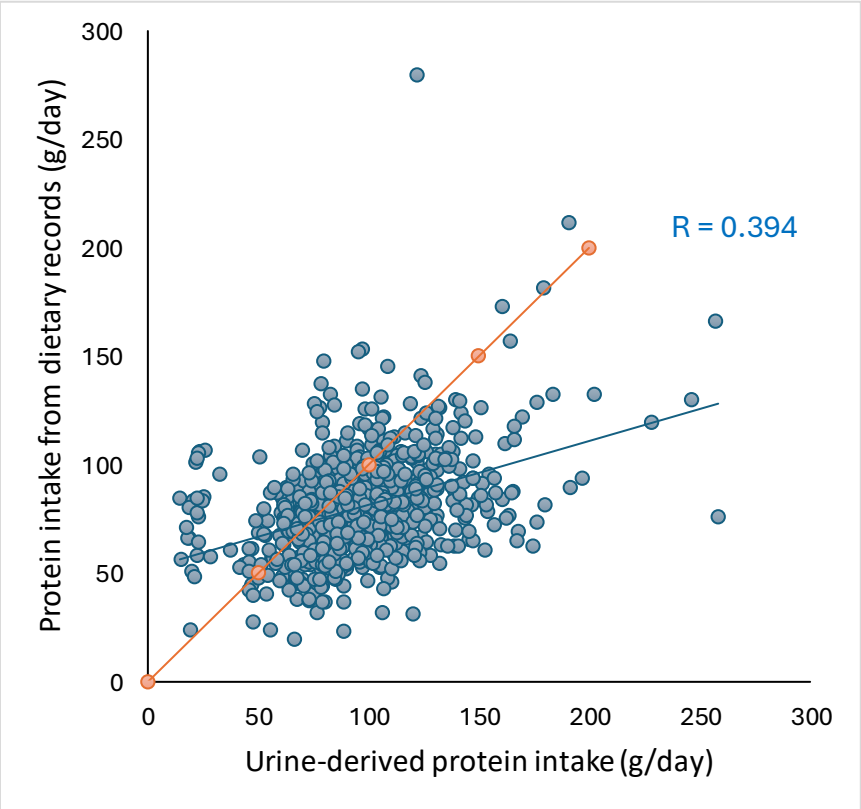


Figure 6-1: Comparison of protein intake estimates from urinary nitrogen excretion and dietary records for all participants' measurement time points. The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.

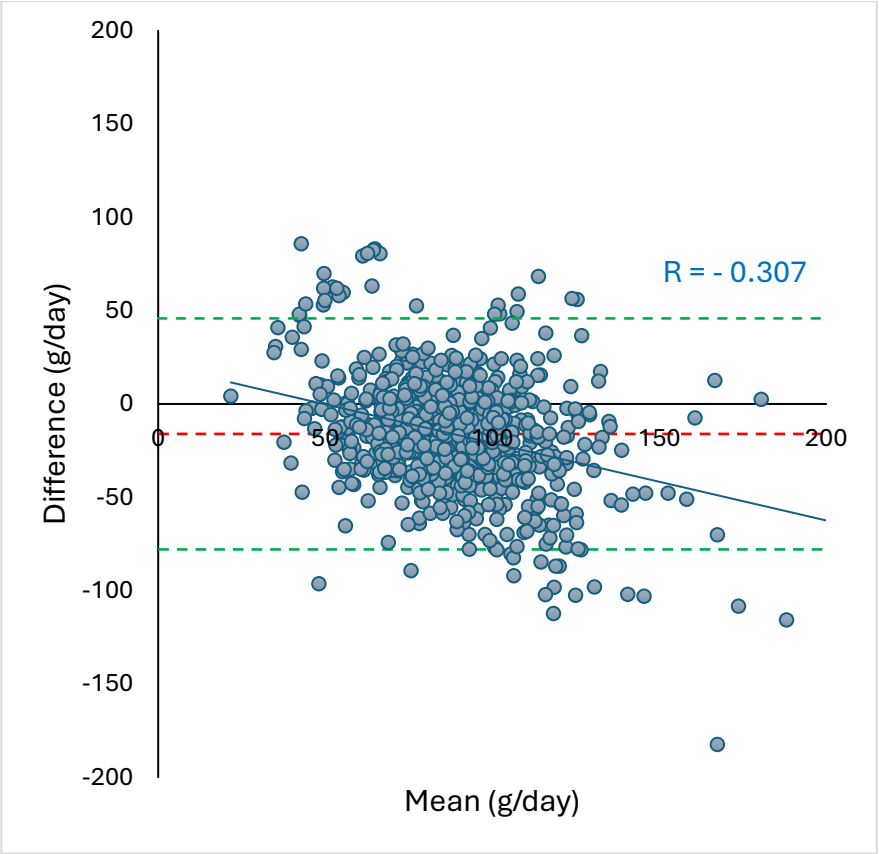


Figure 6-2: Bland-Altman plot showing agreement between protein intake estimated from urinary nitrogen excretion and dietary records, based on all data points. The y-axis represents the difference between methods (Dietary Records - Urinary Nitrogen Estimate), and the x-axis represents the mean of the two measurements ((Dietary Records + Urinary Nitrogen Estimate) ÷ 2) for each participant. The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.1.2 Baseline Measurements (CID1) and modifiers of the relationship between calculated protein intake from urinary nitrogen and self-reported protein intake

At CID1, in all participants (N=264), correlation analysis demonstrated a positive relationship between calculated protein intake from urinary nitrogen and self-reported protein intake (y= 0.5225x + 35.48, R=0.524, P<0.001). The mean difference between the two methods was -9.45 (25.09) g/day **(Figure 6-3).**

The Bland-Altman regression equation indicated that there was no proportional bias at this timepoint, prior to the weight-loss intervention (regression equation: y= -0.0148x - 8.1198, R= -0.014, P=0.836) (**Figure 6-4**), suggesting that the magnitude of difference between methods was consistent across the protein intake range. However, 5.3% of values were outside of the ±1.96 SD limits of agreement.

At this baseline measurement (CID1), sex emerged as a significant modifier, explaining an additional 13.7% of the variance in this relationship (ΔR^2=0.137, P<0.001). BMI also demonstrated a modifying effect, accounting for an additional 3.7% of the variance (ΔR^2=0.037, P<0.001). In contrast, age did not modify this relationship (ΔR^2=0.000, P=0.962).

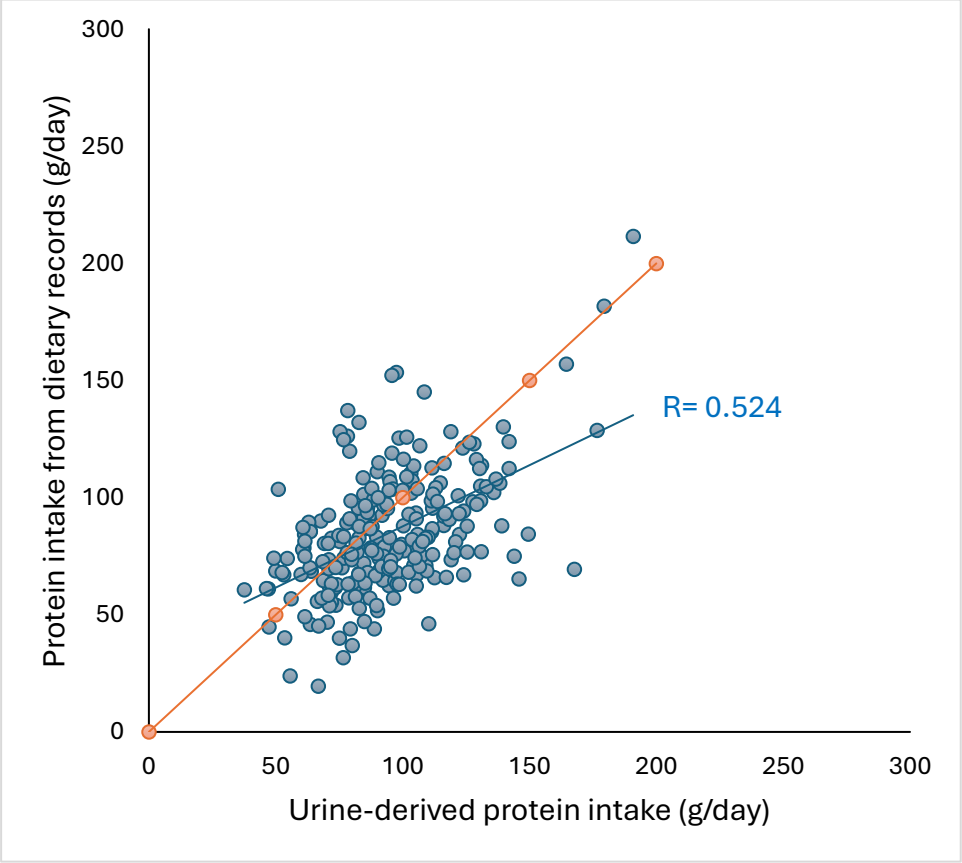


Figure 6-3: Comparison of protein intake estimates from urinary nitrogen excretion and dietary records at baseline for CID1 participants (N=264). The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.

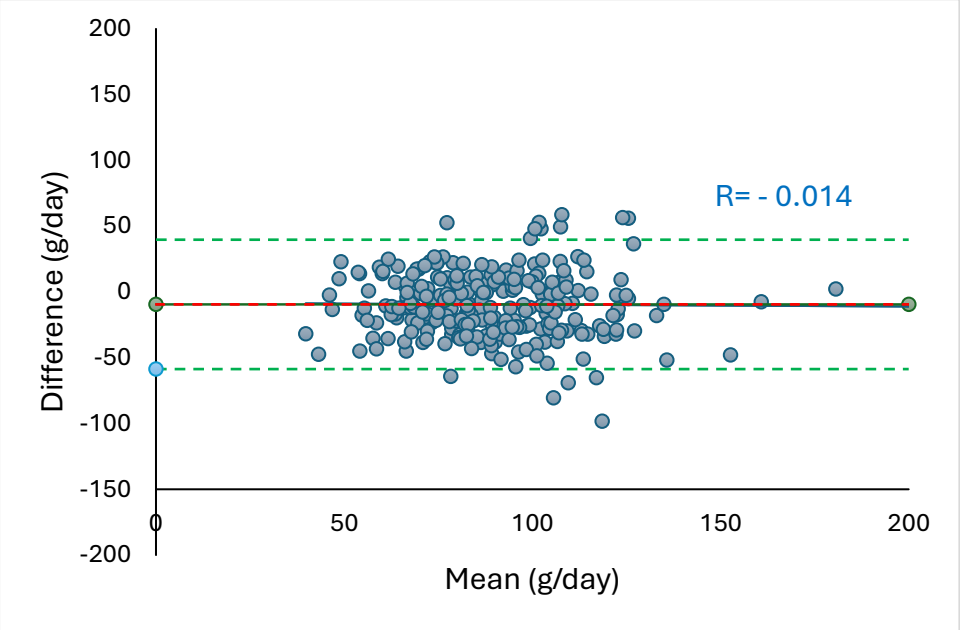


Figure 6-4: Bland–Altman Plot showing agreement between protein intake estimated from urinary nitrogen excretion and dietary records at baseline (CID1), based on data from all participants (N = 264). The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.1.3 Relationship between calculated protein intake from urinary nitrogen and self-reported protein intake according to study phase: baseline (CID1), 6 months (CID3), 12 months (CID4), at 24 months (CID6), and 36 months (CID7), in those who completed the study.

At CID1 (n=71), linear regression analysis demonstrated a positive relationship between calculated protein intake from urinary nitrogen and self-reported protein intake (y= 0.3353x + 51.784, R=0.397, P<0.001), as shown in **Figure 6-5**. The mean difference between the two methods was -12.50 (27.40) g/day.

The Bland-Altman regression equation indicated that there was no proportional bias prior to the weight-loss intervention (regression equation: y= -0.2398x + 9.1921, R= -0.181) (**Figure 6-6**), suggesting that

the magnitude of difference between methods was consistent across the protein intake range.

Following the 8-week LED intervention and 26 months of the weight-maintenance and exercise phase (CID3), the correlation between methods remained significant (R=0.504, P<0.001), with a regression equation of y= 0.406x + 37.891, as shown in **Figure 6-5**. The median difference between the two methods was -24.90 [-36.93_ -6.06] g/day. In contrast to the CID1 data, the Bland-Altman analysis at CID3 revealed a proportional bias (regression equation: y= -0.286x + 2.6416, R= -0.245, P<0.05 (**Figure 6-6**), as seen in the analysis of all data points.

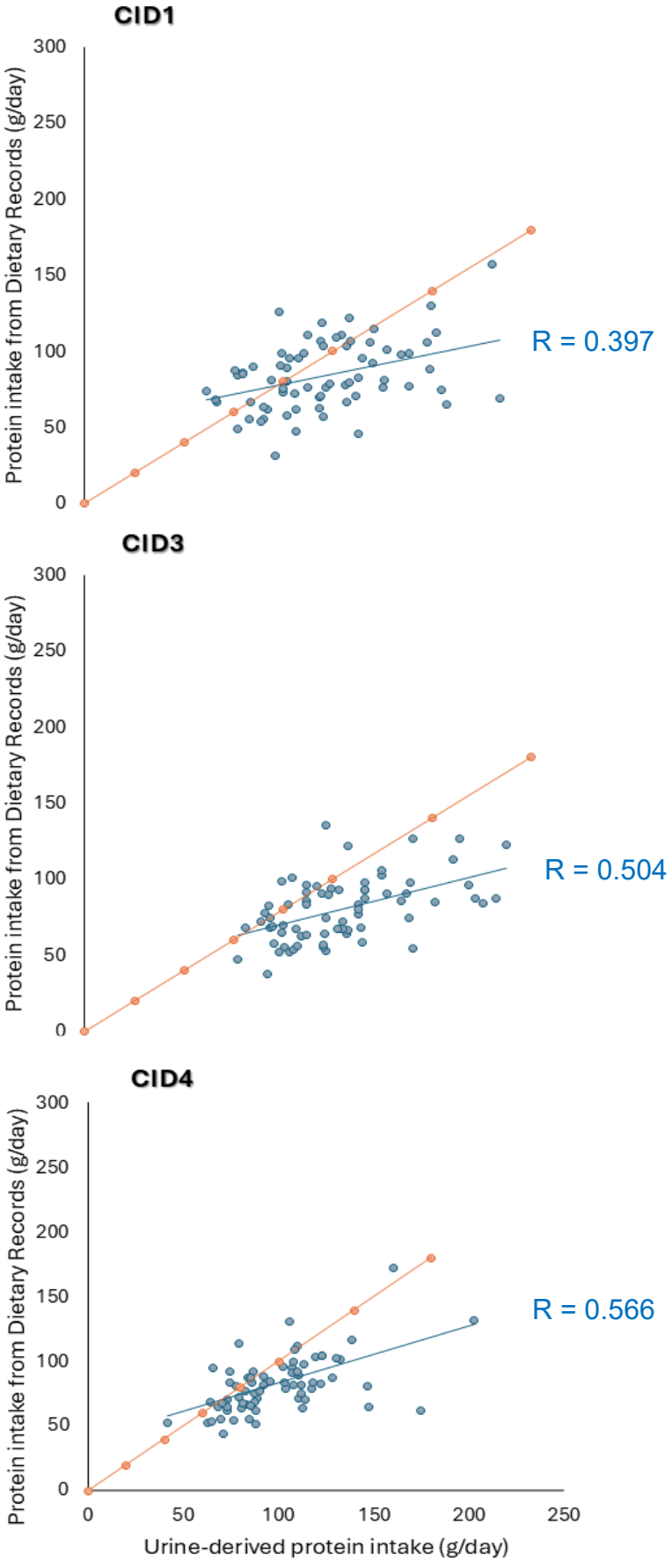
Similar results to CID3 were observed at CID4 (R=0.566, P<0.001), with the regression equation being y= 0.4413x + 38.634 (**Figure 6-5)**. The median difference between methods was -15.28 [-26.54_ -3.21] g/day. The Bland-Altman analysis at this time-point also showed a proportional bias (regression equation: y= -0.3139x + 11.829, R= -0.291, P<0.05) (**Figure 6-6**).

At CID6, the correlation between calculated and reported protein intake was R=0.391 (P<0.001), and the regression equation was y= 0.2901x + 46.872, as shown in **Figure 6-5**. This time point showed a median difference of -28.48 [-41.99_ -9.01] g/day between the two methods, and as seen for other assessment time points during the intervention, the Bland-Altman analysis revealed a proportional bias at this time point (regression equation: y= -0.4202x + 8.4111, R = -0.311, P<0.01) (**Figure 6-6**).

At the final measurement time point (CID7), the correlation was R=0.372 (P=0.001), and the regression equation was y= 0.3979x + 40.498, as shown in **Figure 6-5**. The median difference was -22.78 [-35.21_ -7.51]

g/day. Unlike the previous time points, the Bland-Altman analysis at CID7 did not show a statistically significant proportional bias (regression equation: y= 0.0973x - 30.522, R=0.072, P=0.551) (**Figure 6-6**).

Across the measurement visits, the percentage of values in the Bland-Altman analysis which lay outside the defined limits of agreement ranged from 1.4% (CID7) to 8.5% (CID4).


CID1
Protein intake from Dietary Records (g/day)
300
250
200
150
100
50
0
R = 0.397
CID3
Protein intake from Dietary Records (g/day)
300
250
200
150
100
50
0
R = 0.504
CID4
Protein intake from Dietary Records (g/day)
300
250
200
150
100
50
0
0
50
100
150
200
250
Urine-derived protein intake (g/day)
R = 0.566

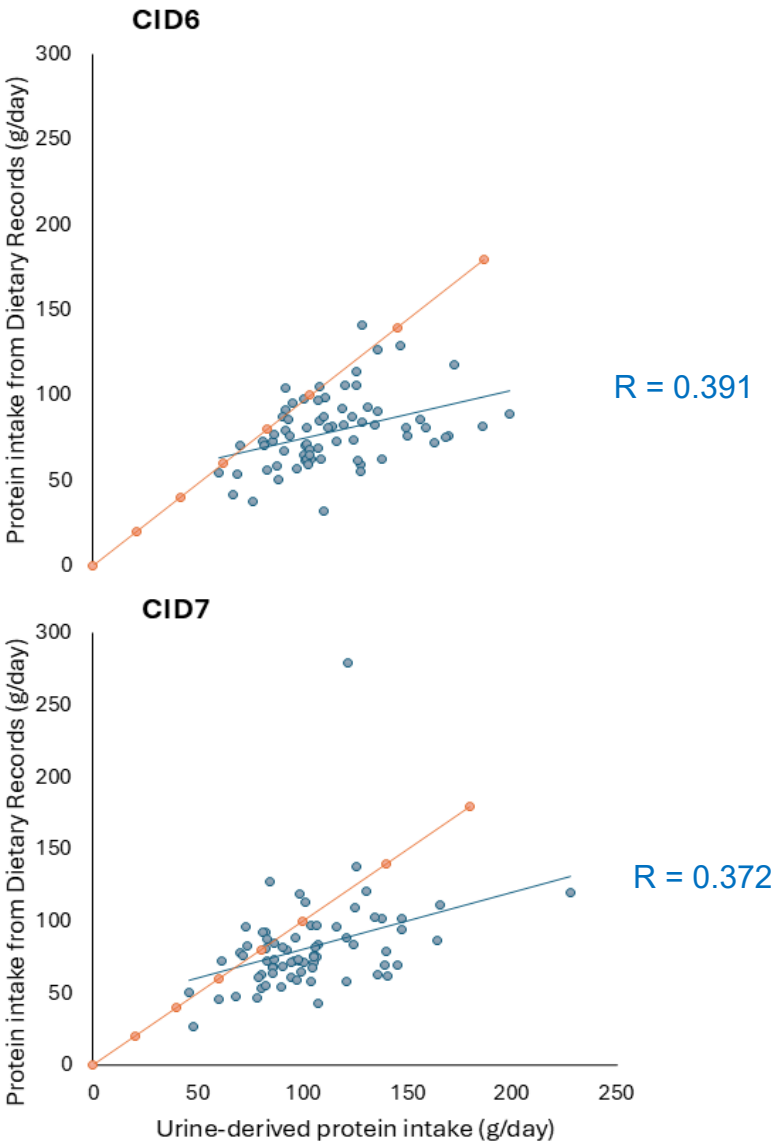
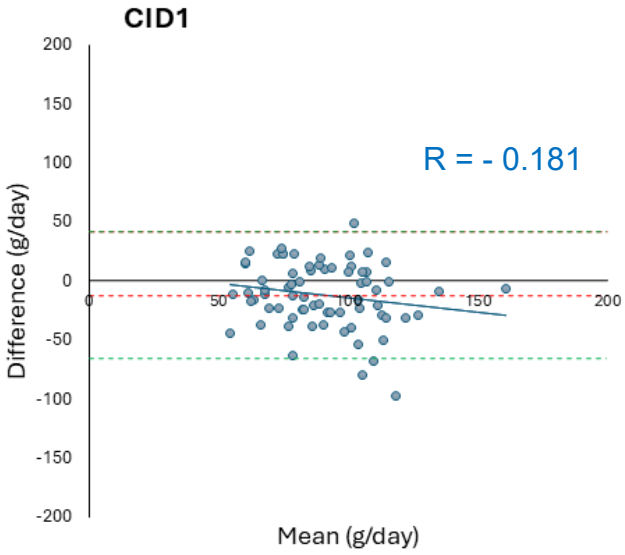
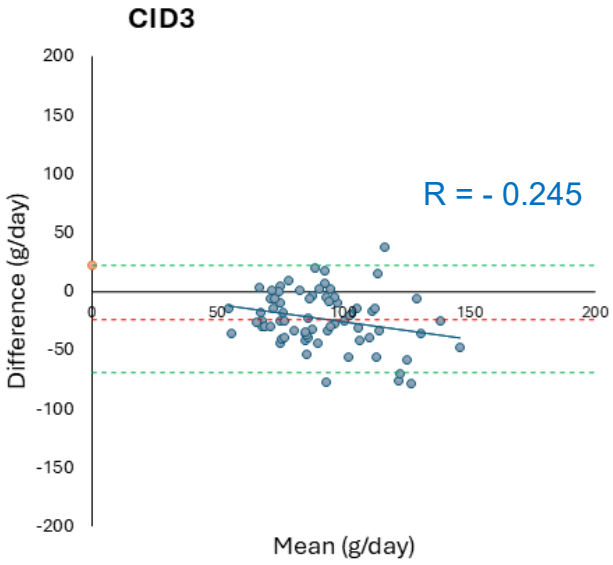
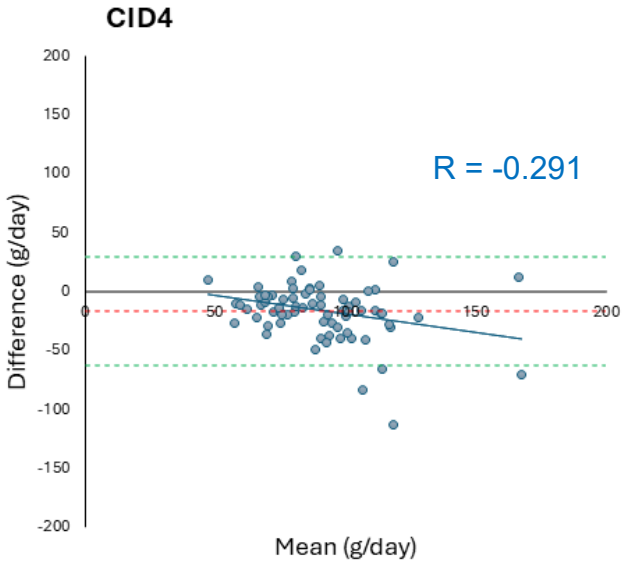


Figure 6-5: Comparison of protein intake estimates from urinary nitrogen excretion and dietary records collected across the different measurement visits (CIDs) in n=71. The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.


CID1
R = - 0.181
Difference (g/day)
Mean (g/day)
200
150
100
50
0
-50
-100
-150
-200
0
50
100
150
200


CID3
R = - 0.245
Difference (g/day)
Mean (g/day)
200
150
100
50
0
-50
-100
-150
-200
0
50
100
150
200


CID4
R = -0.291
Difference (g/day)
Mean (g/day)
200
150
100
50
0
-50
-100
-150
-200
0
50
100
150
200

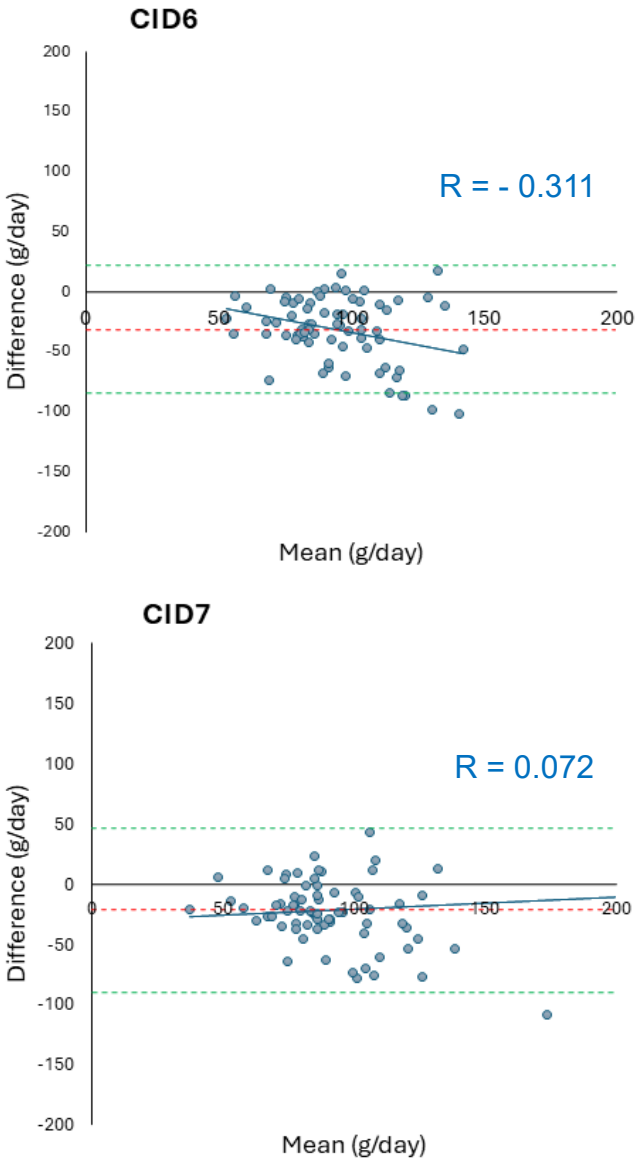


Figure 6-6: Bland-Altman plot from data collected across the different measurement visits (CIDs) in n=71. The difference between protein intake (g/d) calculated from a weighed dietary record (Test method) and protein intake amount (g/d) estimated from 24-h urinary-nitrogen excretion (Reference method) is plotted against their mean. The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.2 Validation between DXA and BIA Measurements

Body composition was assessed using both DXA and BIA in a subset of 37 participants to evaluate the agreement between these methods.

6.3.2.1 Percentage Body Fat

Analysis of the relationship between the measurements made by BIA and DXA for percentage body fat showed a positive correlation (R=0.853, regression equation: y= 0.9494x + 3.7164), as shown in **Figure 6-7**.

The Bland-Altman analysis of percentage body fat (regression equation: y= 0.0892x - 1.691, R=0.160, P=0.231) revealed no proportional bias, i.e. a difference between methods across the range of body fat percentages measured was not detected (**Figure 6-8**). Moreover, 94.6% of values lay within the ±1.96 SD limits of agreement.

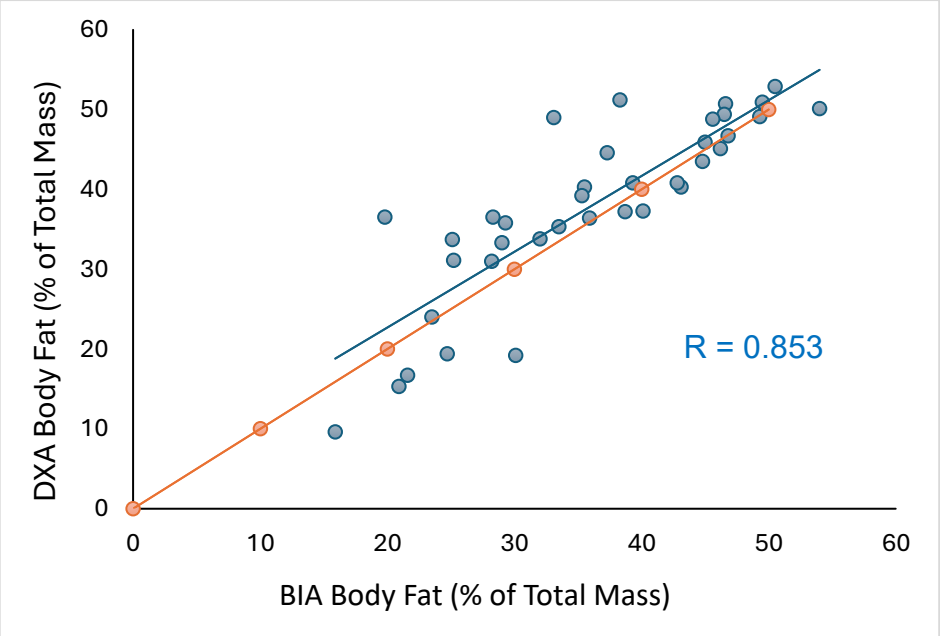


Figure 6-7: Comparison between Dual Energy Absorptiometry (DXA) and bioimpedance (BIA) for percentage body fat in a subset of PREVIEW participants. The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.

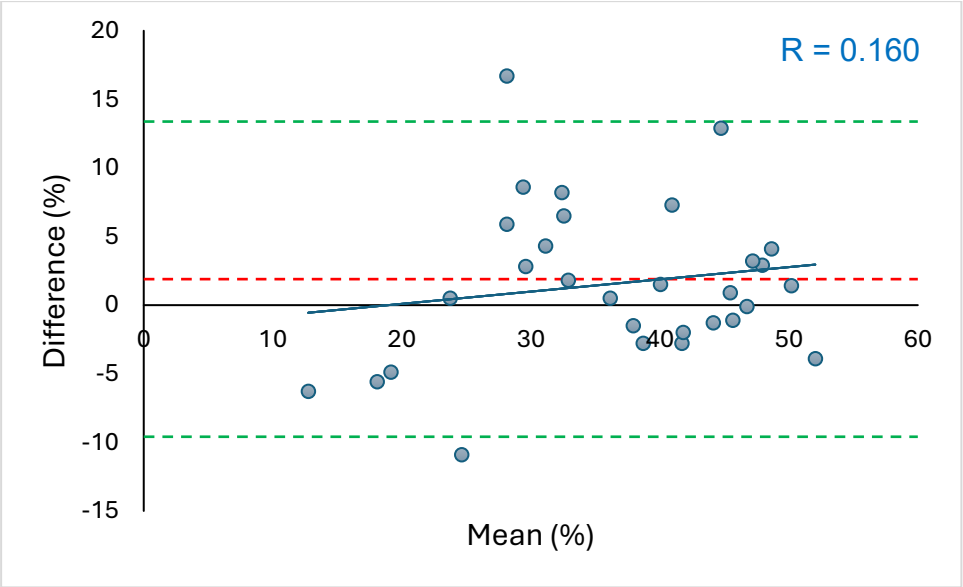


Figure 6-8: Bland-Altman plot depicting the difference between the percentage body fat calculated using bioimpedance (Test method) and the percentage body fat calculated from a Dual Energy Absorptiometry scan (Reference method), plotted against their mean. . The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.2.2 Fat Mass (FM)

Analysis of fat mass again showed a correlation between measurements made by BIA and DXA (R=0.896, regression equation: y= 0.9365x + 2.7301), as shown in **Figure 6-9**. The Bland-Altman analysis for fat mass (regression equation: y= 0.0472x - 0.8411, R=0.1, P=0.555) indicated no proportional bias between methods and 92% of values lying within the limits of agreement (**Figure 6-10**).

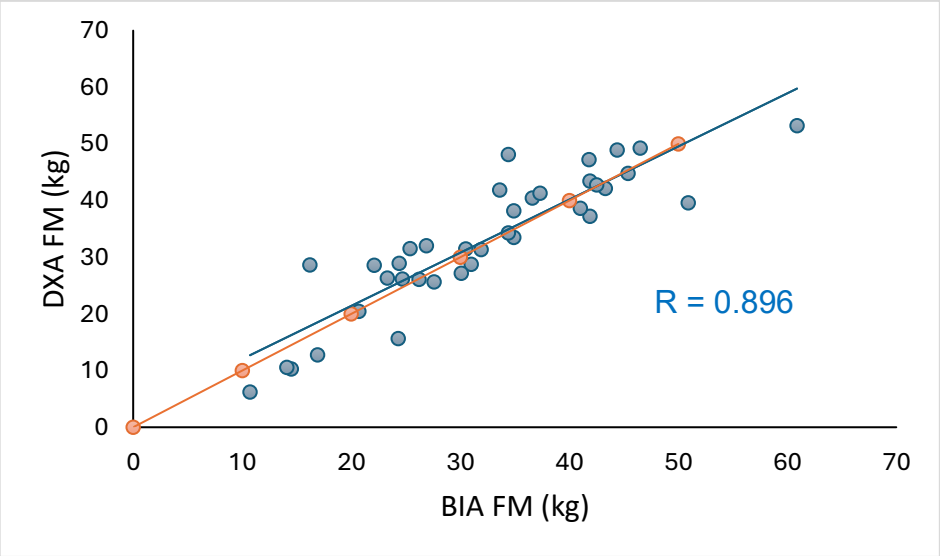


Figure 6-9: Comparison between Dual Energy Absorptiometry (DXA) and bioimpedance (BIA) for total fat mass in a subset of PREVIEW participants. The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.

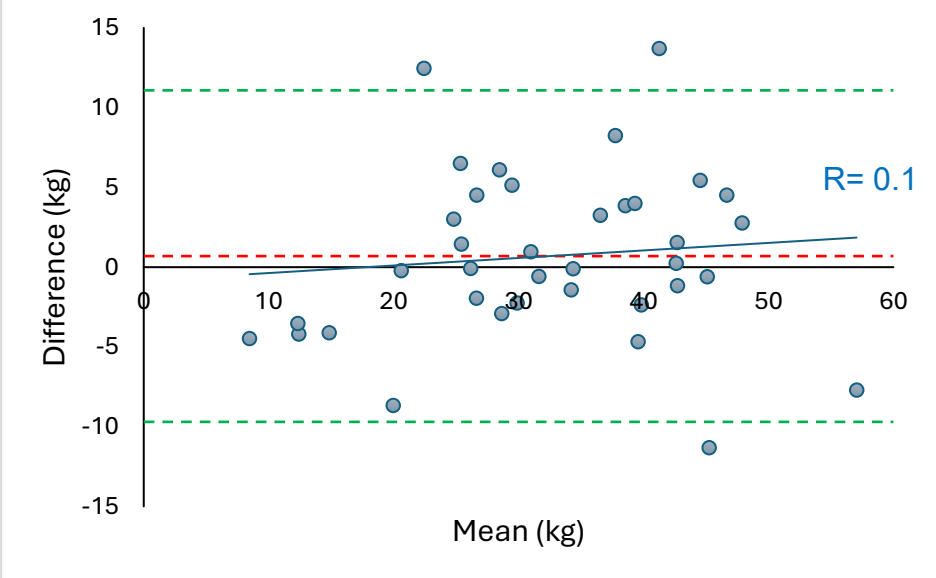


Figure 6-10: Bland-Altman plot depicting the difference between body fat mass calculated using bioimpedance (Test method) and body fat mass calculated from a Dual Energy Absorptiometry scan (Reference method), plotted against their mean. The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.2.3 Fat-Free Mass (FFM)

Analysis of fat-free mass showed a correlation between methods (R=0.841, regression equation: y= 0.7456x + 13.708), as shown in **Figure 6-11**. The Bland-Altman analysis for fat-free mass (regression equation: y= -0.1306x + 6.7493, R=0.218, P=0.195) again did not detect a proportional bias and 92% of values lying within the limits of agreement (**Figure 6-12**).

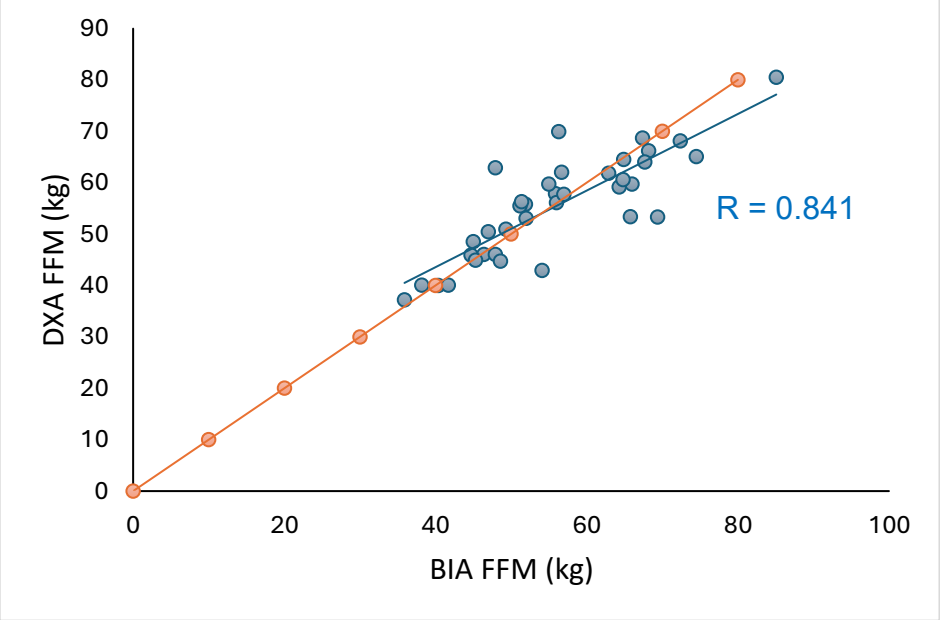


Figure 6-11: Comparison between Dual Energy Absorptiometry (DXA) and bioimpedance (BIA) for total fat-free mass in a subset of PREVIEW participants. The orange line indicates the line of identity, with the blue line indicating the linear regression line for the data.

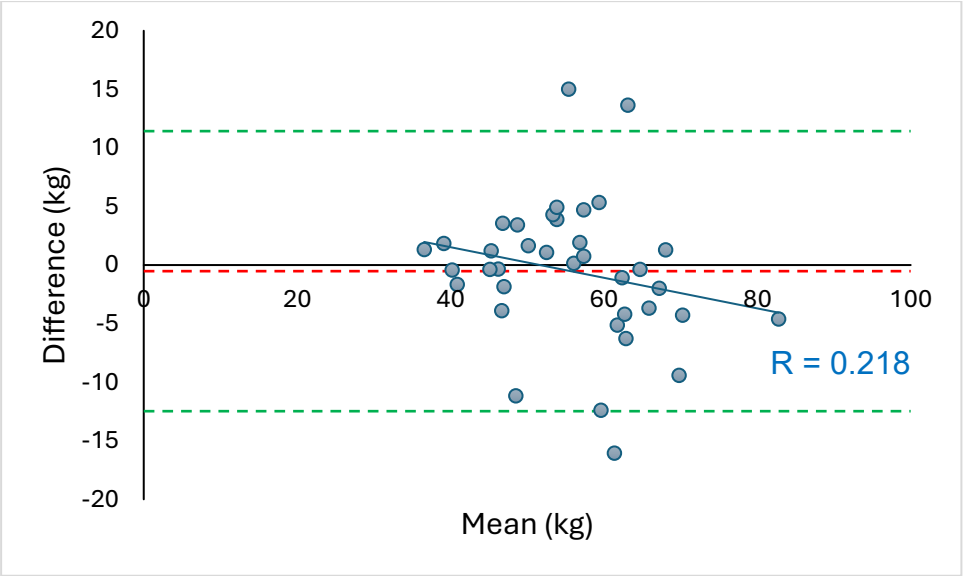


Figure 6-12: Bland-Altman plot depicting the difference between body fat-free mass calculated using bioimpedance (Test method) and body fat-free mass calculated from a Dual Energy Absorptiometry scan (Reference method), plotted against their mean. . The red dashed line shows the mean difference (bias), and the green dashed lines represent the limits of agreement (±1.96 SD).

6.3.3 Relationship between Baecke Physical Activity Questionnaire and Accelerometry

The relationship between self-reported physical activity (Baecke questionnaire) and accelerometry measurements was examined. A positive correlation was observed between time spent in moderate and vigorous activity and Baecke Score (R=0.320, P<0.001), with the linear regression equation being y= 0.0257x + 6.0482, where y represents the Baecke score (**Figure 6-13**). The coefficient of determination (R^2=0.1027) indicated that 10.27% of the variance in Baecke scores could be explained by time spent in moderate and vigorous activity.

For sedentary time, a negative correlation was observed with Baecke scores (R=0.290, P<0.001), with the regression equation: y= -0.005x + 9.295 (**Figure 6-14**). The coefficient of determination (R^2=0.084) indicated that 8.4% of the variance in Baecke scores could be explained by sedentary time.

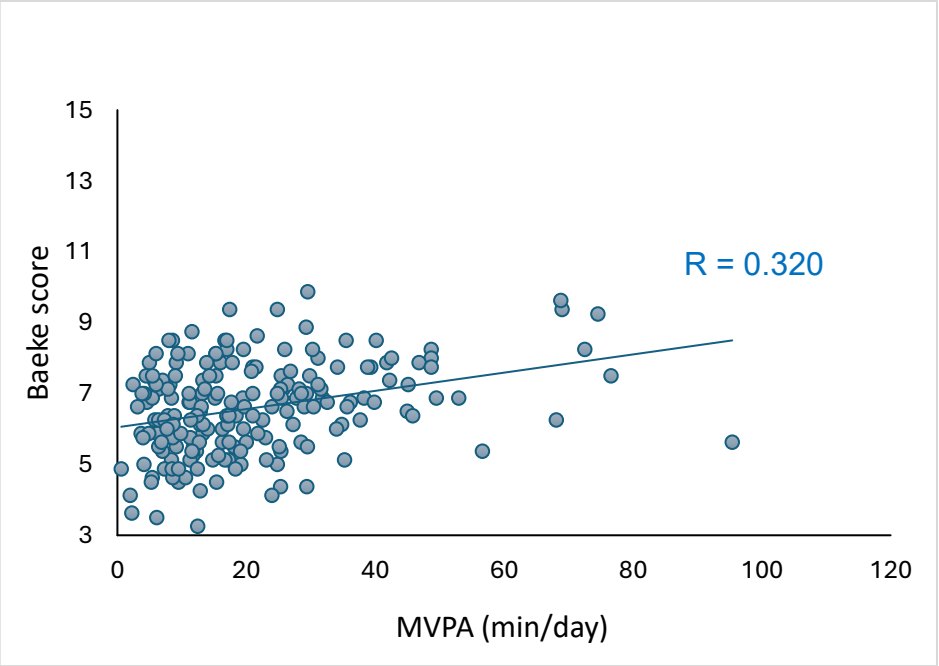


Figure 6-13: Comparison between time per day spent in moderate and physical activity (MVPA), derived from a waist-worn accelerometer and Baecke questionnaire score for participants prior to starting the weight-loss intervention (CID1).

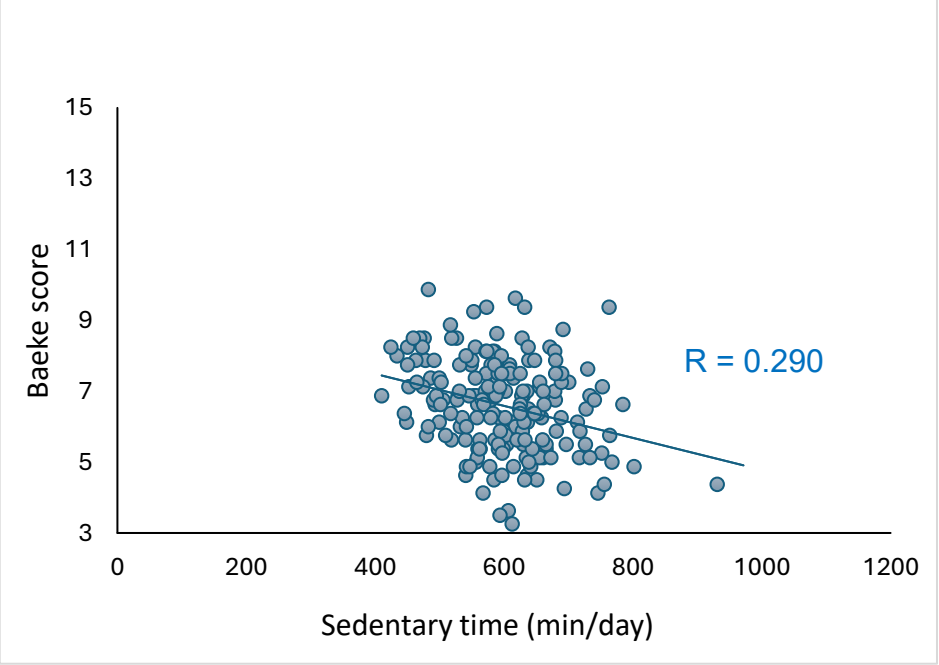


Figure 6-14: Comparison between daily sedentary time derived from a waist-worn accelerometer and Baecke questionnaire score for participants prior to starting the weight-loss intervention (CID1).

6.4 Discussion

This chapter presents findings related to the comparison of three commonly used assessment methods against respective reference methods in the Nottingham PREVIEW cohort. This validation included measurement of dietary protein intake (self-reported dietary records vs. urinary nitrogen excretion), body composition (BIA vs. DXA), and physical activity levels (Baecke questionnaire vs. waist-worn accelerometry). These validation analyses are important for interpreting the findings of nutritional and lifestyle interventions accurately and for understanding the limitations of these assessment methodologies used in research settings [715, 725]. Validation studies comparing self-reported dietary intake against objective biomarkers have demonstrated the importance of understanding measurement error when evaluating the diet-disease relationships [240, 732].

6.4.1 Protein intake estimation by dietary records and assessment of urinary nitrogen excretion.

This study examined the relationship between protein intake derived from 24-hour urinary nitrogen excretion and self-reported dietary records. The data showed a moderate correlation (as defined by Cohen *et al.* [733]), between the two methods, with statistical significance across all measurement time-points. The observed negative median difference between methods suggests potential underreporting in dietary records and/or possible methodological limitations in the urinary nitrogen estimation technique, leading to over-estimation of protein intake. While urinary nitrogen is considered a recovery biomarker for protein intake, several methodological limitations may contribute to measurement error. It should be noted that this study measured urinary urea as a proxy for total urinary nitrogen excretion. In healthy individuals in protein balance, approximately 85-90% of urinary nitrogen is excreted as urea, with the remainder as ammonia, creatinine, and other nitrogenous compounds. However, this distribution can change during periods of protein

catabolism or metabolic stress, where increased protein breakdown may alter the proportion of nitrogen excreted as ammonia relative to urea [734]. While this is unlikely to be a significant issue in the current study population of healthy participants, it represents a potential source of measurement error that should be acknowledged when interpreting urinary nitrogen estimates derived from urea measurements alone. Incomplete 24-hour urine collections represent a significant source of error, with studies demonstrating that collection adherence can substantially affect nitrogen recovery estimates [723, 735]. The standard conversion factors used to estimate protein intake from urinary nitrogen (assuming 16% nitrogen content in dietary protein and 81% recovery in urine) may not apply uniformly across individuals due to variations in protein quality, amino acid composition, digestibility, and individual metabolic differences [736, 737]. Additionally, day-to-day biological variation in nitrogen excretion can be substantial, with coefficients of variation ranging from 15-25% in healthy adults. This intra-individual variability can significantly affect the accuracy of protein intake estimates when based on a single or limited number of 24-hour urine collections [738, 739]. Previous studies have reported an underestimation of protein intake when self-reported dietary assessment methods are compared with biomarker methods. For instance, in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Bingham *et al.* [740] reported underestimation ranging from 11-25%. Similarly, across multiple large cohort studies, Freedman *et al.* [725] found that self-reported protein intake systematically underestimated values measured using recovery biomarkers.

The pattern of agreement between methods, in those with complete data sets, showed consistent linear relationships across assessment time-points. However, the Bland-Altman analyses appeared to show a pattern of proportional bias that varied over time, with no proportional bias at the baseline assessment (CID1), while significant bias emerged during the intervention phase (CID3, CID4, and CID6), before becoming non-

significant again at the final measurement (CID7). This suggests that while the fundamental relationship between methods remained stable, systematic differences between methods may have varied during the intervention period. It is possible that this may reflect variability in the accuracy of the dietary records. In weight loss intervention studies by Lichtman *et al.* [414] and Poslusna *et al.* [244], it was found that underreporting tends to increase at higher levels of dietary intake, especially in intervention settings where dietary changes are emphasised. Subar *et al.* [735] also demonstrated, through biomarker validation, that the degree of underreporting in dietary records increases with greater energy intake, potentially reflecting social desirability bias, where participants report intake closer to prescribed intervention targets rather than actual consumption [741]. However, the variability in method agreement across time-points may also reflect limitations inherent to the urinary nitrogen method itself. Accurate assessment of protein intake from urinary nitrogen depends critically on complete 24-hour urine collection, yet collection completeness remains a significant methodological challenge, with various factors affecting the accuracy of collections [742]. Even small losses in urine volume can substantially underestimate nitrogen excretion and therefore protein intake estimates [738]. Furthermore, physiological factors affecting nitrogen metabolism and excretion, including changes in kidney function, hydration status, and protein utilisation efficiency during dietary interventions, may introduce systematic bias in urinary nitrogen-derived protein intake estimates [743, 744].

Other researchers have observed variations in reporting accuracy in dietary records at different stages of dietary interventions. Dhurandhar *et al.* [715] reported fluctuations in reporting accuracy throughout weight loss interventions, with accuracy particularly affected during active intervention phases. Several factors may explain measurement discrepancies in prolonged dietary studies. These include participant fatigue with detailed dietary recording, the cognitive burden of following

specific dietary guidelines, and potential social desirability bias [745, 746]. The current study identified significant modifying factors in the relationship between dietary protein assessment methods. At baseline, sex explained an additional 13.7% of variance in this relationship, while BMI contributed a smaller but significant 3.7%. The inclusion of these covariates was based on their established influence on protein metabolism and dietary assessment accuracy. Age affects protein turnover rates and kidney function, potentially influencing urinary protein markers [743, 747]. Sex differences exist in muscle mass, metabolic rate, and protein requirements, which could impact the relationship between dietary intake and urinary biomarkers [736]. Finally, BMI was included as a covariate because it may influence both the accuracy of dietary reporting, underreporting being more common at higher BMI [244] and physiological processes that affect protein metabolism and nitrogen excretion. These processes vary with factors such as age, body composition, and metabolic efficiency [743, 744]. These findings may be explained by previous research suggesting that demographic and anthropometric characteristics can influence dietary reporting accuracy [244, 748].

These findings have several important implications for dietary assessment in nutritional research, particularly in intervention studies. The differences between methods highlight the need for caution when interpreting protein intake values based solely on self-reported dietary records. Moreover, the presence of proportional bias observed in the Bland-Altman analyses during certain intervention phases (CID3, CID4 and CID6) of the PREVIEW study, suggests that particular attention should be paid to interpreting self-reported dietary data from participants who consumed higher amounts of protein, as noted by Livingstone and Black *et al.* [748]. The emergence and resolution of proportional bias at different time points underscores the challenges of maintaining accurate dietary assessment during prolonged studies. This has practical implications for study design, suggesting that more frequent validation

assessments might be beneficial during extended interventions. Cade *et al.* [730] recommend incorporating validation studies into the design of nutritional intervention trials to account for potential changes in reporting accuracy over time. As proposed by Freedman *et al.* [749], calibration equations derived from biomarker validations can improve the accuracy of self-reported dietary data. However, the use of urinary nitrogen as a biomarker of protein intake also has significant limitations that may contribute to the observed measurement discrepancies. These limitations include incomplete urine collection errors [722], proportional bias whereby urinary nitrogen may underestimate high protein intake levels and overestimate at low intake levels [722], non-urinary nitrogen losses through skin, sweat, faeces, and hair that are difficult to quantify but can be substantial (estimated at 4-6 g/day) [722, 736], and individual variation in adaptation to different protein intake levels, as some individuals may require several weeks to achieve steady-state nitrogen excretion following dietary changes [747]. Additionally, nitrogen excretion can shift between urine and faeces depending on dietary fibre intake [722].

Overall, these findings highlight the complex measurement challenges inherent in protein intake assessment. While self-reported dietary records remain practical and feasible for large-scale studies, researchers should acknowledge their limitations and consider complementary biomarker assessments when precise quantification of protein intake is required. However, given that both self-reported methods and urinary nitrogen biomarkers have distinct sources of measurement error, discrepancies between these methods cannot definitively establish which approach provides more accurate estimates of true protein intake.

6.4.2 Determination of body composition using bioimpedance and Dual Energy Absorptiometry

The comparison between BIA and DXA for body composition assessment revealed strong agreement between these methods across all measured parameters, indicating that, in the participant group studied, BIA provided measurements closely related to those obtained using the reference standard, DXA. Notably, the Bland-Altman analyses demonstrated no significant proportional bias across the body composition parameters, suggesting that the differences in measurements between BIA and DXA remained consistent across all value ranges. This indicates that BIA delivers representative measurements at a group level, regardless of whether participants have low, moderate, or high levels of adiposity or LM. However, the discrepancy between measurement tools in individuals was, in several cases substantial. For example, for body fat mass, the difference expressed as a percentage of the mean ranged from -49% to 59%. This considerable individual variability suggests that while BIA may be suitable for population-level research and group comparisons, it would not be appropriate for assessing body composition changes in individual participants. Consequently, the use of BIA in clinical settings where individual treatment decisions depend on precise body composition measurements may not be suitable, as the measurement uncertainty could potentially affect treatment planning and monitoring of individual patient progress.

The findings of the present study align with several previous validation studies comparing BIA with DXA for body composition assessment. The high correlation coefficients observed in this study are consistent with those reported by Malavolti *et al.* [750], who found correlations of 0.90 for fat mass and 0.88 for fat-free mass between multi-frequency BIA and DXA in healthy adults. Similarly, Leahy *et al.* [751] reported correlation coefficients of 0.88 for fat mass and 0.76 for fat-free mass when comparing multi-frequency BIA with DXA in an adult population. The

absence of proportional bias in the present study is partially supported by findings from Volgyi *et al.* [727], who reported that BIA measurements showed consistent agreement with DXA across different body composition ranges in women across all BMI categories, though they noted some bias in obese men compared with those with a healthy BMI. Unfortunately, the dataset used in the current analysis was not sufficient to be able to explore this sex difference. While Bosy-Westphal *et al.* [752] demonstrated that modern BIA devices show improved accuracy across a wide range of body compositions compared with older models, the present findings indicate that even multi-frequency BIA technology can exhibit substantial individual measurement errors. Sun *et al.* [173] reported that BIA tends to underestimate fat-free mass in individuals with higher muscle mass, potentially due to differences in muscle density and hydration status. However, the present data did not show a systematic bias in this variable in the Bland-Altman analysis.

The findings of this study have several important implications for the assessment of body composition in clinical and research settings. First, the strong correlations between BIA and DXA across all parameters support the use of BIA as a practical alternative to DXA in settings where DXA may not be feasible due to cost, availability, or radiation exposure concerns, and where population level data are being explored. Second, the absence of proportional bias shows that the measurements provided by BIA are consistent across the range of body composition values seen in the present study population. This is important for longitudinal studies where relative changes in body composition are of interest, as it suggests that BIA may be suitable for tracking changes at the group level regardless of baseline body composition. Third, the systematic differences revealed by the regression equations indicate that while BIA and DXA measurements are strongly correlated at a group level, they are not directly interchangeable. The substantial individual measurement errors observed in this study demonstrate that BIA may be unsuitable to be used to assess body composition at the individual level, particularly in

clinical settings where precise measurements are required for treatment decisions. These differences should be considered when interpreting absolute values or when comparing results across studies using different assessment methods. Fourth, the strong correlation between methods for fat mass suggests that BIA may be most representative for assessing this parameter at the population level. This is valuable information for studies focused primarily on changes in adiposity, such as weight management interventions. Finally, while the present findings support the use of BIA as an alternative to DXA for group-level assessments, they also highlight the importance of method-specific reference ranges and potentially the development of correction equations to improve the accuracy of BIA measurements relative to DXA. As suggested by Kyle *et al.* [170], population-specific equations may enhance the precision of BIA-derived body composition estimates.

6.4.3 Comparison between self-reported physical activity using the Baecke questionnaire and measurements from waist-worn accelerometer

The comparison between self-reported physical activity using the Baecke questionnaire and objective measurements through accelerometry revealed modest relationships between these assessment methods. The analysis showed a significant, but weak (as defined by Cohen *et al.* [733]), positive correlation between Baecke score and accelerometry-measured time spent in moderate-to-vigorous physical activity and a negative correlation with sedentary time. However, the modest coefficient of determination for the relationships between the two measurement approaches suggests that while self-reported physical activity assessment score and objectively measured activity are related, they may capture different aspects of physical activity behaviour.

These results align with previous validation studies comparing self-assessment questionnaires and objective physical activity measurements. The correlation values observed in this study fall within the range typically reported in the accelerometry validation studies of the Baecke questionnaire literature. Philippaerts *et al.* [753] reported a significant correlation coefficient of R=0.47 between Baecke questionnaire and a 4-day movement registration from a tri-axial accelerometer in 40-year-old men. Similarly, Tebar *et al.* [754] found correlations ranging from R=0.34 to 0.38 between Baecke questionnaire and accelerometry in community-dwelling adults with medium to high education levels. These findings suggest that the present study observed correlation of R=0.320 for MVPA falls within the expected validation range for this questionnaire, supporting its comparability to objective measures of physical activity in similar populations.

The low coefficient of determination in the present study reflects the inherent limitations of the Baecke questionnaire's design and scope. In our study, accelerometry data for MVPA explained only 10.27% of the variance in Baecke scores, while sedentary time explained 8.4%. This modest explanatory power is expected given that the Baecke questionnaire was originally developed as a general measure of habitual physical activity rather than as a surrogate for specific activity intensities or sedentary behaviour measurement [211]. The questionnaire comprises three subscales: work activity, sport activity, and leisure-time activity, with sedentary behaviours contributing primarily to the work subscale through occupational sitting questions, while MVPA is captured mainly through the sport and some leisure components [211]. Since sedentary time and MVPA represent only portions of the total Baecke scoring framework, neither would be expected to fully explain the composite score. The negative correlation observed between sedentary time and Baecke scores (R= -0.290) was anticipated, as lower Baecke questionnaire scores indicate greater sedentary behaviour patterns, particularly through the work subscale questions about occupational

sitting [211]. This inverse relationship aligns with the questionnaire's scoring methodology where higher sedentary time would logically correspond to lower activity scores. However, the relatively weak strength of this relationship reflects the limited proportion of the total Baecke score that is derived from sedentary-related questions compared with the broader range of activities assessed. A fundamental consideration in interpreting these correlations is that the Baecke questionnaire was designed to capture a relatively narrow range of structured activities and occupational behaviours, rather than comprehensive daily movement patterns that accelerometry measures [211]. The questionnaire's focus on specific sports participation, work-related activities, and selected leisure pursuits means it may not capture incidental physical activity, light-intensity movements, or the full spectrum of sedentary behaviours that accelerometry records continuously. This methodological difference between self-reported structured activities and objective total movement measurement helps explain why neither MVPA nor sedentary time alone can account for a large proportion of variance in Baecke scores.

Previous validation studies of the Baecke questionnaire have consistently highlighted these limitations. Jacobs *et al.* [755] noted that the questionnaire's strength lies in assessing habitual activity patterns over extended periods rather than providing precise quantification of specific activity intensities. Similarly, Richardson *et al.* [756] emphasised that the Baecke questionnaire was developed primarily for ranking individuals according to their general activity levels rather than serving as a direct measure of energy expenditure or time spent in specific intensity categories. This design purpose explains why the present study correlations, while statistically significant, show only moderate strength. The questionnaire is fulfilling its intended function of general activity assessment rather than serving as a precise proxy for accelerometry-measured behaviours.

A number of important implications can be drawn from this study when measuring physical activity in clinical and research settings. First, data from the Baecke questionnaire and accelerometry are not interchangeable and may be capturing different aspects of physical activity behaviour. While accelerometry provides objective measurement of movement and intensity, questionnaires like the Baecke may incorporate additional dimensions such as perceived exertion, contextual factors, and activities that accelerometers might not accurately capture (such as swimming or cycling). Second, the low coefficients of determination indicate substantial unexplained variation between these methods, suggesting that researchers should be cautious when using self-reported physical activity to indicate actual movement behaviours. This is particularly important in intervention studies where accurate assessment of physical activity changes is crucial for evaluating the effectiveness of the intervention. Third, the weak but significant correlations suggest that while the Baecke questionnaire provides some meaningful information about physical activity patterns, it should ideally be supplemented with objective measurements when precise quantification of activity levels is required. As suggested by Troiano *et al.* [757], a combined approach using both self-report and device-based measures may provide the most comprehensive physical activity assessment. Fourth, the larger correlation coefficient observed for moderate-to-vigorous physical activity compared with sedentary time suggests that the Baecke questionnaire may be more suitable for capturing information about active behaviours rather than sedentary patterns. This aligns with the questionnaire's design focus on work, leisure, and sports activities rather than inactivity. Sjoros *et al.* [758] directly compared Baecke scores and accelerometer data over four weeks, finding moderate correlations with physical activity (R=0.41) and weaker correlation with sedentary time (R= -0.33). They also showed that excluding the Sport Index strengthens associations with activity data, and that the item "After working I am tired" predicted less leisure time activity, highlighting how Baecke may capture the subjective and contextual burden that influences activity patterns. Finally, these findings highlight

the need to carefully interpret physical activity data in research settings, particularly when comparing results across studies using different assessment methodologies. Researchers should acknowledge the limitations of self-reported physical activity data and consider these when drawing conclusions about physical activity levels or relationships with health outcomes.

One of the main strengths of these method comparisons is the comprehensive approach to examine three key assessment methods within the same cohort. The present study provides valuable insights into the methodological considerations for multi-component lifestyle interventions like PREVIEW. Furthermore, the comparison of protein intake assessment methods used data collected in a study with a longitudinal design that allowed researchers to observe method agreement at multiple time points throughout a 36-month intervention period in the same participants. This temporal perspective is relatively rare in validation studies, which often provide only cross-sectional comparisons. This approach allowed for the detection of patterns in method agreement over time, especially regarding the emergence of proportional bias during the intervention phase and its subsequent resolution at the final measurement point in the accuracy of protein intake reporting. The temporary emergence of proportional bias may reflect participants' adaptation to new dietary patterns and reporting requirements during the active intervention phase, with potential contributing factors including learning curves in portion size estimation, intervention-related reporting fatigue, and metabolic adaptations to dietary changes. The resolution of this bias by the final time point suggests stabilisation of both dietary behaviours and reporting accuracy once participants had fully adapted to the intervention protocol. Using a validated reference method for body composition (DXA) strengthens the interpretation of the comparison with BIA. The statistical approach, which combines correlation analyses with Bland-Altman plots and regression analyses, evaluates the strength of the relationship between methods

and the presence of systematic biases. This dual approach is recommended in method comparison studies but is not consistently implemented [759-761]. The identification of significant modifying factors in the protein intake validation adds another layer of methodological insight. The finding that sex explained an additional 13.7% of variance in the relationship between methods, while BMI contributed 3.7%, provides important information for interpreting differences between dietary assessment approaches. Finally, the protein intake validation employed three complementary analytical approaches: 1. a comprehensive analysis including all available data (768 valid urine collections from 264 participants across all CIDs), 2. baseline-only analysis (n=264 participants at CID1), and 3. longitudinal complete-case analysis (n=71 participants who completed all five measurement points from CID1 to CID7). The baseline-only analysis provides truly independent observations from 264 individuals, offering robust statistical power without clustering effects. In contrast, both the comprehensive analysis (768 observations from 264 participants across multiple time points) and longitudinal analysis (355 observations from 71 participants across five time points) represent repeated measurements from the same participants, meaning observations are not entirely independent due to within-subject correlations. This clustering effect should be considered when interpreting the strength of correlations in these repeated-measures analyses, as multiple measurements from the same participants may influence correlation coefficients compared with the independent baseline-only observations. While the sample sizes for the body composition (n=37) and physical activity validations were more modest, they were comparable to similar validation studies in the literature [750, 762].

While there are several strengths to consider, it is also important to acknowledge some limitations. First, the study population consisted of participants with prediabetes enrolled in an intervention trial, which limits the generalisability of the present findings to other populations.

Participants in intervention studies may have different reporting behaviours than the general population, particularly given their awareness of study goals and the potential for social desirability bias during dietary reporting. Second, while the sample size for protein intake validation was large, the smaller sample for the body composition comparison (n=37) may limit the statistical power for detecting subtle differences between BIA and DXA. This is particularly relevant for the subgroup analyses and assessment of proportional bias across different body composition ranges, where the data from the few individuals with high values for measurement variables may influence the regression line to a greater extent. Third, the protein intake validation assumed that participants were in nitrogen balance, which may not have held true at all time points, particularly during the active weight loss phase. Nitrogen balance is fundamentally dependent on energy balance, and different study phases represented different energy states. During negative energy balance, the body mobilises protein from lean tissue to meet energy demands, leading to increased urinary nitrogen excretion that overestimates dietary protein intake [763, 764]. At baseline (CID1), participants were presumably in approximate energy balance, making urinary nitrogen a valid biomarker. However, during active weight loss at 6 and 12 months (CID3 and CID4), negative energy balance would have disrupted this relationship through endogenous protein catabolism. By 36 months (CID7), participants who maintained weight loss had likely achieved a new equilibrium, re-establishing nitrogen balance. This temporal pattern provides a physiological explanation for why proportional bias emerged during the intervention phase but resolved at the final assessment, reflecting not only adaptation to dietary reporting but also fundamental changes in nitrogen metabolism driven by shifts in energy balance. Future validation studies in weight loss interventions should consider stratifying analyses by intervention phase or incorporating measures of energy balance status when interpreting discrepancies between dietary assessment and biomarker methods. The modest positive correlation between protein intake from dietary records and urine-derived protein intake across all timepoints suggests

reasonable agreement but also indicates that other factors may have influenced protein metabolism during the intervention. The emergence of proportional bias during the intervention phase (CID3, CID4, and CID6) but not at baseline (CID1) or final assessment (CID7) suggests that intervention-related factors may have affected the relationship between methods. Factors such as fluctuations in body weight, changes in protein metabolism, and nitrogen retention or loss prior to assessments may have contributed to the variability observed in the agreement between methods over time, as nitrogen balance can be significantly altered during periods of energy restriction and metabolic adaptation [763, 764]. However, the time intervals between protein intake and weight assessments (baseline, 6, 12, 24, and 36 months) may have failed to capture the impact of dynamic changes in nitrogen balance that occur during periods of rapid weight change, when short-term fluctuations in protein metabolism and nitrogen retention can significantly affect urinary nitrogen excretion [722, 765]. During the early weight loss phase of the study, shorter collection intervals might have enabled the impact of weight trajectory on the relationship between urinary nitrogen excretion and actual protein consumption. Fourth, the present study did not assess day-to-day variability in the biomarker measures, which could have provided insights into the reliability of these reference methods themselves. Previous research has demonstrated that single biomarker measurements show considerable within-person variation, with urinary nitrogen requiring multiple days of collection to reliably estimate habitual protein intake [722, 766]. Single biomarker measurements may not fully capture the habitual patterns they aim to assess, as demonstrated by studies showing that 3-7 days of urine collection are needed to achieve acceptable precision for nitrogen excretion [723, 767]. Finally, it should be noted that each assessment method was examined against its respective reference. For body composition, BIA was compared with the gold-standard reference method (DXA). However, for the assessment of dietary protein intake, comparing dietary records and urinary nitrogen presents inherent methodological challenges, as both methods have recognised limitations. Dietary records are prone to underreporting, with

protein intake typically underestimated by 10% to 20%, and in some individuals particularly those who are overweight or obese, the underestimation may exceed 30% [240, 722, 745]. Urinary nitrogen, while considered a recovery biomarker, also relies on the assumption of nitrogen balance and complete 24-hour urine collections, which may not always be achieved in free-living settings. These limitations make it difficult to determine which method may be under- or over-reporting protein intake. Similarly, for physical activity assessment, the comparison between accelerometry and the Baecke questionnaire represents a relationship between methods measuring different activity constructs rather than a true validation, as accelerometry captures movement patterns while questionnaires assess perceived activity levels across different domains [214, 215]. Without access to component scores from the Baecke questionnaire, it was not possible to determine whether sedentary-related questions correlated with measured sedentary time or whether activity-related scores corresponded to time spent in moderate-to-vigorous physical activity, representing a significant limitation in interpreting the physical activity comparison. Such interactions could provide additional context for interpreting the validation results.

6.5 Conclusion

This validation study provides insights into methodological considerations for assessing dietary protein intake, body composition, and physical activity in lifestyle intervention research. By comparing commonly used assessment methods within the Nottingham PREVIEW cohort, this study has identified patterns of agreement, systematic biases, and temporal variability in relationships that may have implications for both research and clinical practice.

This piece of work demonstrates that self-reported dietary protein intake differed from biomarker-derived estimates, with the magnitude and pattern of this discrepancy varying across the intervention timeline. The varying energy balance states across different study phases from

baseline equilibrium through active weight loss to weight maintenance likely contributed to the temporal patterns observed in method agreement, with the emergence and resolution of proportional bias reflecting both participants' adaptation and fundamental physiological changes in nitrogen metabolism driven by shifts in energy balance. The identification of significant modifying factors, particularly sex and BMI, provides important considerations for interpreting differences between dietary assessment approaches.

BIA showed strong agreement with dual-energy X-ray absorptiometry for body composition assessment, with high correlations and no significant proportional bias across all parameters, but discrepancies in agreement at the individual level. These findings support using BIA as a practical alternative to DXA in settings where the latter may not be feasible, while acknowledging some systematic differences between methods that should be considered when interpreting absolute values.

For physical activity assessment, the modest correlations between the Baecke questionnaire and accelerometry reflect the multi-dimensional nature of physical activity and the complementary information provided by subjective and objective methods. The limited shared variance between these approaches underscores the value of combining assessment methods when a comprehensive evaluation of physical activity patterns is required.

Overall, this study reinforces the importance of method validation in lifestyle intervention research and provides specific guidance for researchers using these assessment tools in similar populations and study designs. The findings highlight the importance of considering participants' energy balance status when validating dietary assessment

methods in weight loss interventions, as the validity of urinary nitrogen as a biomarker is compromised during periods of energy imbalance.

Chapter 7 GENERAL DISCUSSION AND CONCLUSION

7.1 Overview of Thesis Research

Intermittent fasting and dietary interventions to bring about weight-loss have received considerable attention for their potential metabolic health benefits. However, gaps still exist in understanding their effects on appetite regulation, body composition changes across different age groups, and the methodological challenges in assessing these outcomes. Therefore, the overarching purpose of this thesis was to explore the physiological effects of a period of dietary energy restriction. A series of specific aims was set, which addressed identified gaps in knowledge in this area. The extent to which each aim has been achieved will be addressed, broader conclusions drawn, and potential applications to public health strategy and clinical practice considered. Finally, an indication of potential future work will be outlined in this chapter.

This thesis presents a broad investigation through a systematic review and three interconnected secondary analyses of the Nottingham cohort of the PREVIEW diabetes risk study: the review explored the effects of Ramadan intermittent fasting on appetite-regulating hormones (**Chapter 2**), followed by the series of secondary analyses examining, in individuals with overweight / obesity and prediabetes, the determinants of body composition (**Chapter 4**), the impact of lifestyle factors on body composition changes during a weight loss intervention (**Chapter 5**), and validating assessment methodologies used during the research (**Chapter 6**). **Chapter 4** comprised a cross-sectional analysis which investigated relationships between physical activity, age, biological sex and body composition, particularly lean body mass, in overweight individuals with pre-diabetes; **Chapter 5** explored how biological sex, age, lifestyle factors (physical activity, stress) and eating behaviours impacted body composition changes following an 8-week LED ; and **Chapter 6** validated commonly used assessment methods against their respective references for dietary protein intake, body composition, and physical activity. Collectively, these studies contribute to the understanding of body

composition assessment, nutritional interventions, and physical activity measurement in individuals with or at risk for metabolic disorders. This discussion chapter synthesises the key findings across all studies, examines their clinical and practical implications, acknowledges methodological considerations, and identifies directions for future research.

7.2 Synthesis of Key Findings

7.2.1 Effects of Ramadan Intermittent Fasting on Appetite-Regulating Hormones and Connections to Weight Management

The systematic review and meta-analysis in **Chapter 2** aimed to examine how Ramadan intermittent fasting, practised by millions globally, influences appetite-regulating hormones during this unique form of time-restricted eating in order to better understand the possible effects of time-restricted feeding on appetite-regulating hormones. This investigation aligns with growing interest in intermittent fasting approaches for weight management and diabetes prevention and management, with recent systematic reviews demonstrating that time-restricted eating can improve glycaemic control and insulin sensitivity in individuals with prediabetes and T2DM [286, 768, 769]. However, the long-term success of weight management interventions remains challenging, with many individuals experiencing difficulty maintaining weight loss due to increased subjective appetite and food cravings following initial weight reduction [98, 770]. This phenomenon of weight regain after successful weight loss affects the majority of individuals who lose weight, regardless of the intervention method employed [434, 656]. Limited research has examined whether the modest weight losses observed during Ramadan are maintained following the conclusion of the fasting period, though the available studies suggest that post-Ramadan weight regain commonly occurs, with individuals typically returning to baseline weight within 2-5

weeks of resuming normal eating patterns [271, 771, 772]. The mechanisms underlying these changes in appetite perception and subsequent weight regain may be partially explained by alterations in appetite-regulating hormones, which play crucial roles in signalling hunger, satiety, and energy balance [277, 331]. Understanding the mechanistic underpinnings of these effects through assessment of appetite hormones represents a critical knowledge gap in translating intermittent fasting research into clinical practice.

7.2.1.1 Ramadan Fasting as a Model for Prolonged Time-Restricted Eating

Ramadan fasting represents a unique natural experiment in prolonged time-restricted eating, involving complete abstinence from food and fluid during daylight hours for approximately 30 days. This pattern differs substantially from laboratory-based intermittent fasting studies, which typically involve shorter fasting periods (12-16 hours) and often allow water consumption [773, 774]. The distinct hormonal profile observed in the current systematic review, characterised by selective ghrelin elevation without corresponding leptin suppression, differs markedly from the coordinated appetite hormone responses typically reported in other time-restricted eating modalities. For example, early time-restricted feeding protocols generally show decreases in both ghrelin and leptin [775], while alternate-day fasting demonstrates increases in ghrelin accompanied by proportional reductions in leptin [776]. This suggests that Ramadan fasting may influence appetite regulation through distinct mechanisms not captured by shorter or less restrictive intermittent fasting protocols.

The ecological validity of Ramadan fasting research has been proposed as providing insights into the real-world implementation of prolonged intermittent fasting [777]. However, the unique hormonal signature identified in this review (**Chapter 2**) challenges this assumption, as the

appetite hormone responses observed during Ramadan fasting appear to be specific to this particular combination of prolonged fasting duration, complete fluid restriction, and shifted meal timing. Evidence supporting the ecological validity claim remains limited, as direct comparisons between Ramadan fasting and secular time-restricted eating interventions of similar duration and intensity are scarce in the literature.

However, several characteristics of Ramadan fasting limit direct translation to clinical interventions. The combination of extended fasting with complete fluid restriction may produce distinct physiological responses compared with other intermittent fasting protocols, while the cultural and religious context provides motivation and social support structures typically absent in clinical settings [778, 779]. A critical methodological consideration when comparing Ramadan fasting with other intermittent fasting protocols is the timing of blood sampling relative to the last time food was consumed. During Ramadan, blood samples are typically collected after 12-16 hours of fasting (depending on season and location), which represents a substantially longer post-prandial interval compared with most other intermittent fasting studies, where sampling often occurs within 2-8 hours of feeding. This extended fasting duration may account for some of the distinct appetite hormone profiles observed during Ramadan, as the temporal dynamics of ghrelin, leptin, and other appetite-regulating hormones follow circadian patterns that are influenced by both meal timing and fasting duration [780]. Given the distinct appetite hormone profile observed in this review (**Chapter 2**), Ramadan fasting may be better viewed as a unique model of prolonged religious fasting rather than a generalisable framework for secular time-restricted eating interventions. Future research should prioritise standardising blood sampling protocols across different intermittent fasting modalities and investigating the time course of appetite hormone changes throughout extended fasting periods to better understand the mechanistic differences between various fasting approaches.

7.2.1.2 Distinct Hormonal Signature and Mechanistic Implications

The selective increase in circulating ghrelin without corresponding changes in serum leptin, insulin, or gastrin represents a distinctive hormonal signature that differs markedly from responses observed during continuous caloric restriction. Traditional weight loss interventions typically produce coordinated changes in appetite-regulating hormones, with increases in ghrelin accompanied by decreases in leptin, creating a hormonal milieu that promotes hunger and energy conservation [277, 781]. The isolated ghrelin response during Ramadan fasting represents an interesting phenomenon that warrants further investigation, though the underlying mechanisms remain unclear from the current observational evidence. The circadian entrainment of ghrelin secretion, shifted by altered meal timing during Ramadan, aligns with emerging research showing that meal timing can influence hormonal rhythms independent of total energy intake [775, 782]. However, the clinical significance of these temporal shifts in appetite hormones remains to be established through controlled experimental studies.

This interpretation requires careful consideration of whether the absence of leptin reduction reflects a unique mechanism of time-restricted eating or simply results from the modest weight loss typically observed during Ramadan fasting. The magnitude of weight loss appears to be a critical factor, as leptin concentrations in the blood are closely correlated with fat mass changes. Studies examining other intermittent fasting regimens with comparable modest weight loss outcomes (typically 2-4 kg) provide mixed findings; some report similar preservation of leptin levels with modest weight reductions [783, 784], while others demonstrate that leptin decreases proportionally to weight loss regardless of the fasting approach [785, 786]. This suggests that the magnitude of weight loss, rather than the fasting pattern itself, may be the primary determinant of leptin responses.

Without controlled studies that manipulate these variables independently, such as comparing different intermittent fasting protocols with matched weight loss outcomes or examining hormone responses during weight-matched interventions of varying duration and fasting intensity, it remains unclear whether the observed hormonal pattern represents a distinct mechanistic pathway or reflects the modest energy deficit achieved during Ramadan fasting. Future experimental research is needed to determine the relative contributions of fasting pattern, weight loss magnitude, and intervention duration to these appetite hormone responses.

7.2.1.4 Clinical Implications for Appetite Regulation in Time-Restricted Eating

The appetite hormone findings contribute to proposing the mechanisms underlying the effectiveness of different time-restricted eating approaches for appetite regulation. While the current review focused solely on appetite hormones during Ramadan fasting and cannot make claims about metabolic outcomes, the distinct hormonal profile observed suggests that different time-restricted eating protocols may influence appetite regulation through varied mechanisms. Other time-restricted eating modalities have shown promise for improving metabolic markers alongside appetite regulation. Early time-restricted eating protocols have demonstrated improvements in insulin sensitivity and glucose tolerance even without significant weight loss [782], while various time-restricted eating approaches have been associated with enhanced glucose regulation in overweight individuals [268]. These metabolic benefits appear to occur alongside changes in appetite-regulating hormones, perhaps suggesting potential mechanistic links between meal timing, hormonal responses, and metabolic health.

The circadian aspects of time-restricted eating may be particularly relevant for understanding these combined effects on appetite and

metabolism. Research demonstrates that meal timing can influence both appetite hormones and glucose regulation, independent of total caloric intake, with structured eating patterns potentially providing benefits beyond simple energy restriction [780, 787]. Future research examining the relationship between appetite hormone responses and metabolic outcomes across different time-restricted eating protocols could provide valuable insights for optimising these interventions.

7.2.2 Body Composition Dynamics Across Age and Sex

In **Chapter 4**, it was hypothesised that lean body mass would decrease with age but be higher in those who were more physically active and who were heavier, due to greater muscle loading. Similarly, in **Chapter 5**, it was proposed that those with higher physical activity pre-intervention would maintain LM better than more sedentary participants. These hypotheses were grounded in the established understanding of age-related muscle mass decline and sex differences in body composition among adults with metabolic dysfunction [788, 789]. The analysis revealed important sex-specific differences in how body composition is related to ageing in individuals with overweight or obesity and pre-diabetes, findings that contribute to understanding muscle mass preservation in middle-aged adults at risk for T2DM.

A key finding was that lean body mass and LLM were only lower with increasing age category in females. This sex-specific pattern aligns with research showing that women experience more pronounced changes in body composition during middle age, particularly related to the menopausal transition, with studies documenting 10-15% muscle mass losses over 5-7 years in perimenopausal women [790, 791]. The mechanistic basis for this sex difference in the reduction of lean tissue mass with age may involve oestrogen decline during menopause in females, which significantly affects muscle protein synthesis pathways and glucose uptake in skeletal muscle [584, 672, 788]. Additionally,

activity levels have been shown to decline at menopause and beyond, which may contribute to this observed age-related muscle mass decline in females [790]. However, in the cross-sectional analysis in **Chapter 4**, we did not observe a reduction in physical activity measures or an increase in sedentary time with increasing age, which supports this finding.

In both sexes, a positive association was observed between total body mass and total, plus leg, lean mass, confirming the mechanical loading hypothesis that greater body weight provides a protective stimulus for muscle maintenance [792]. This was further supported by the positive association observed between the amount of fat mass lost as a result of the LED intervention and the decline in LLM (**Chapter 5**). Additionally, **Chapter 5** demonstrated that individuals with greater baseline LM experienced proportionally greater muscle loss during the intervention, a pattern consistent with other unloading studies, such as immobilisation and bedrest research, where weight-bearing muscles show greater absolute losses when mechanical stimulus is reduced [793-795].

The relationship between fat mass and LM during weight loss has been extensively studied, with the widely cited "one-fourth rule" suggesting that approximately 25% of weight loss consists of fat-free mass under typical conditions [796]. However, this relationship is highly variable and influenced by multiple factors, including initial body composition, the rate of weight loss, the magnitude of the energy deficit, and individual characteristics [797]. The findings from **Chapter 5** show that greater fat mass loss was directly related to greater LM loss, and provide longitudinal support for the cross-sectional associations observed and align with the mechanical loading hypothesis, whereby the reduction in mechanical stress on muscles during fat loss contributes to LM decline, particularly in weight-bearing muscle groups such as the legs [796].

Beyond mechanical factors, adipose tissue functions as an active endocrine organ, secreting adipokines such as leptin and adiponectin that can influence muscle protein synthesis and breakdown [57]. During periods of energy restriction, the loss of fat mass is typically accompanied by reductions in circulating leptin, which may contribute to decreased muscle protein synthesis and increased protein breakdown, potentially contributing to the observed relationship between fat loss and LM decline demonstrated in **Chapter 5**.

Several factors can modify the fat mass-LM relationship during weight loss interventions. Age has been proposed as a critical modifier, with older adults experiencing disproportionately greater LM loss relative to fat mass loss compared with younger individuals [711]. Cross-sectional analysis in **Chapter 4** revealed that lean body mass and LLM decreased with increasing age category, but this age-related decline was only observed in females, with no significant age-related changes observed in males. However, the LED intervention study did not specifically analyse whether the magnitude of age-related differences in fat-to-LM loss ratios during weight loss limited the ability to fully confirm this established relationship in this cohort. Sex differences also influence this relationship, with women typically demonstrating better preservation of LM relative to fat mass loss than men during equivalent energy deficits [258]. In the LED cohort, women demonstrated superior preservation of LM relative to fat mass loss compared with men, supporting this established sex difference in body composition responses to energy restriction. Additionally, the rate and magnitude of weight loss can significantly impact body composition changes, with more rapid weight loss generally associated with greater LM losses [711]. Mathematical models have demonstrated that the proportion of fat-free mass lost increases with greater energy deficits and shorter intervention durations [797]. As demonstrated in **Chapters 4 and 5**, the sex-specific differences observed in both the cross-sectional analysis and LED intervention data could explain the individual variability observed in body composition responses

to the LED intervention and highlight the importance of considering patient-specific characteristics when predicting LM preservation during weight loss.

Interestingly, the relationship between body weight and LLM was only modified by physical activity in females. This sex-specific interaction suggests that males and females may have different sensitivity thresholds to the muscle-preserving effects of physical activity. Males typically possess greater absolute muscle mass [498, 798] and may require higher activity levels to detect additional benefits beyond the baseline mechanical stimulus provided by their larger body weight [799]. The relatively narrow range of physical activity measured in this cohort may have been insufficient to detect activity-related benefits in males, whereas females showed measurable responses within this same activity range. This finding has methodological implications for future studies, perhaps suggesting that physical activity assessment and intervention thresholds may need to be sex-specific to optimise muscle preservation outcomes. This represents a critical research gap, as the sex-specific patterns observed in the current study suggest that optimal intervention strategies may differ between males and females.

These findings are particularly relevant given the established relationship between muscle mass and diabetes risk. Recent research demonstrates that lower skeletal muscle mass is associated with higher odds of prevalent diabetes, particularly in younger adults, independent of body fat distribution [157, 800]. Studies in populations with pre-diabetes and diabetes show that muscle mass preservation is critical for maintaining insulin sensitivity and glucose metabolism, as skeletal muscle accounts for approximately 75% of glucose uptake during insulin-stimulated conditions [156, 566]. The sex-specific muscle mass decline observed in our pre-diabetic cohort could influence diabetes progression.

While both sexes showed the hypothesised positive correlation between LLM and body weight (reflecting adaptation to mechanical loading), the influence of age and likely hormonal factors created distinct patterns between sexes that support the need for tailored intervention strategies. Research has demonstrated that physical activity interventions, particularly resistance training, can effectively preserve LM during weight loss interventions. For example, Chomentowski *et al.* [801] demonstrated that combining resistance exercise with energy restriction resulted in significantly better preservation of LM compared with energy restriction alone in older adults. Similarly, Villareal *et al.* [802] found that a combined aerobic and resistance exercise program during weight loss maintained muscle mass and strength in obese older adults, with benefits observed in both sexes. However, few studies have directly compared the effectiveness of these strategies between males and females during weight loss interventions. The limited available evidence suggests potential sex differences in response to exercise interventions, with some studies indicating that women may show greater relative improvements in muscle quality with resistance training, while men typically demonstrate larger absolute gains in muscle mass [799, 803]. This represents a critical research gap, as the sex-specific patterns observed in the current study suggest that optimal intervention strategies may differ between males and females.

These findings highlight the critical importance of implementing sex-specific approaches to muscle preservation in pre-diabetic populations, particularly for women approaching menopause who may be at highest risk for accelerated muscle loss and progression to type 2 diabetes. As previously indicated, future research should investigate whether resistance training protocols need to be tailored differently for males and females to optimise LM preservation during energy restriction to promote metabolic improvements, with evidence suggesting a "window of opportunity" for muscle preservation interventions during the perimenopausal period [791]. Such research could inform the

development of personalised intervention strategies that account for both mechanical and hormonal factors influencing muscle preservation during weight loss.

7.2.3 Low-Energy Diet Interventions and the Complex Interplay of Biological and Psychological Factors

The examination of an 8-week LED intervention in **Chapter 5** provides interesting insights into the multifaceted nature of weight loss success, revealing how biological sex, psychological characteristics, and stress interact to influence intervention outcomes. Rather than simply documenting weight loss responses, these findings illuminate fundamental questions about personalised approaches to metabolic health interventions and challenge assumptions about the effectiveness of universal interventions.

7.2.3.1 Reconceptualising Sex Differences in Metabolic Interventions

The pronounced sex differences observed in both body composition changes and metabolic improvements during LED intervention extend beyond simple differences in the magnitude of weight loss. While previous research has documented that men typically lose more weight than women during dietary interventions, the present findings suggest that sex differences in metabolic responsiveness may be more fundamental than previously recognised [642, 804].

This pattern aligns with findings from the Diabetes Prevention Program, where men achieved greater initial weight loss than women following lifestyle interventions [805]. DXA analysis revealed that males experienced a greater reduction in both android and gynoid fat percentages compared with females, with larger reductions in waist

circumference but similar changes in hip and thigh circumference. These findings are consistent with the established understanding that men preferentially lose visceral adipose tissue during energy restriction, while women show more resistance to central fat loss [666, 806].

The greater metabolic improvements observed in the males, including more substantial improvements in insulin resistance markers (HOMA-IR and QUICKI) and greater reductions in blood pressure, appeared to be largely attributable to the greater magnitude of both absolute weight loss and percentage weight loss achieved by males compared with females, with males losing 13.9 kg (13.7% of baseline weight) versus 10.0 kg (11.1% of baseline weight) in females. This dose-response relationship between weight loss magnitude and metabolic benefit is well-established in the literature [807, 808]. However, the consistent pattern of enhanced male responses across multiple metabolic parameters suggests that sex-specific physiological factors may contribute beyond simple weight loss magnitude. Research indicates that men may experience greater improvements in insulin sensitivity per unit of weight lost compared with women, potentially due to differences in adipose tissue distribution and hormonal profiles [809].

7.2.3.2 Psychological Determinants of Weight Loss Success

It was initially hypothesised that individuals with higher eating disinhibition and hunger scores and lower eating restraint (as measured using the Three-factor eating questionnaire) would experience smaller fat mass losses during the LED intervention. The investigation of eating behaviours revealed significant associations between baseline psychological factors and weight loss outcomes, adding to the substantial literature demonstrating the importance of cognitive and behavioural factors in weight management success [810]. Higher baseline dietary restraint was positively associated with changes in BMI, while higher

disinhibition was negatively correlated with changes in BMI. These findings confirm my initial hypothesis and align with previous research using the Three-Factor Eating Questionnaire, where dietary restraint has consistently been associated with better weight loss outcomes, while disinhibition predicts weight regain and poorer long-term weight-maintenance success [811, 812]. However, in the Nottingham PREVIEW cohort, higher reported hunger scores did not appear to influence the degree of weight lost over the intervention period.

The sex-specific modification of these relationships observed represents an important finding. While the Three-Factor Eating Questionnaire has been extensively validated across populations, few studies have examined whether the predictive validity of eating behaviours differs between men and women in the context of structured dietary interventions. This suggests that the psychological mechanisms underlying successful energy restriction may operate differently between sexes, warranting further investigation. This also emphasises that interventions focusing solely on dietary prescription without addressing psychological factors such as disinhibition and hunger may not achieve optimal results. Therefore, integrating strategies to improve eating behaviours with traditional dietary guidance could enhance treatment outcomes.

7.2.3.3 Role of Stress in Weight Loss Outcomes

The observed relationship between perceived stress levels and fat mass reduction, with higher baseline stress scores associated with lower fat mass loss, particularly in females, contributes to the emerging understanding of stress as a barrier to successful weight management and confirms the initial study hypothesis [647, 813]. This finding is particularly relevant in the context of pre-diabetes, where psychological stress is both a risk factor for diabetes development and a common barrier to lifestyle modification success [814, 815].

The stress-eating pathway has been extensively documented, with chronic stress leading to elevated cortisol levels, increased appetite for palatable foods, and preferential abdominal fat storage [125, 645]. The present findings suggest that these stress-related mechanisms may be particularly pronounced in women, consistent with research showing greater cortisol reactivity and stress-eating responses in females compared with males [816, 817]. This has important implications for the design of weight loss interventions, suggesting that stress management components may be particularly beneficial for female participants.

7.2.4 Methodological Insights and Their Impact on Interpretation

The method comparison studies in **Chapter 6** provide context for interpreting the findings across **Chapters 4 and 5** and highlight broader methodological considerations for pre-diabetes research. Rather than simply confirming measurement accuracy, these comparisons reveal systematic patterns of measurement error that have important implications for understanding the true relationships observed in the PREVIEW study analyses.

7.2.4.1 Dietary Protein Intake Assessment

It was hypothesised that self-reported dietary protein intake and calculated protein intake derived from 24-hour urinary nitrogen excretion would have a positive correlation, which was indeed confirmed with the PREVIEW dataset. However, it was anticipated that the strength of this relationship would vary across different phases of the intervention, with potential changes in agreement during periods of weight loss/maintenance compared with baseline and phases where there was weight regain, due to factors such as participant fatigue or social desirability bias. While the strength of the relationship did not appear to differ across the PREVIEW study, the difference between the two

measures did become greater as protein intake increased. The potential systematic under-reporting of protein intake during the study weight-maintenance intervention provides an important perspective on interpreting findings from the wider PREVIEW study. The documented tendency for individuals to under-report protein intake during periods of dietary focus suggests that the relationship between dietary composition and body composition changes may be stronger than apparent from self-reported data. This finding aligns with emerging research suggesting that measurement error in dietary assessment substantially attenuates observed diet-health relationships, potentially explaining why some nutritional interventions show modest effects despite strong biological plausibility [725, 818]. However, the lack of a systematic bias between the dietary records and the urinary biomarker method at the pre-intervention assessment suggests that the protein intake used in chapter 4 to examine the relationship between protein intake and LM was not subject to error due to under-reporting at higher intakes.

It was further hypothesised that demographic and anthropometric characteristics (such as sex, age, and body mass index) would significantly modify the relationship between protein intake assessment methods. The relationship was modified by sex and BMI, but not by age. These findings are consistent with previous validation studies of dietary assessment methods. Research comparing urinary nitrogen excretion with dietary records has shown that women tend to under-report protein intake more than men, particularly when BMI is higher [738, 819]. Studies have consistently reported greater discrepancies between self-reported protein intake and urinary nitrogen biomarkers in individuals with higher BMI, attributed to increased under-reporting of protein-rich foods among overweight and obese participants [720, 820]. The sex difference in protein reporting accuracy may reflect differential dietary restraint behaviours and social desirability bias regarding protein-dense foods, particularly meat consumption [821].

These demographic modifiers of protein reporting accuracy have important implications for interpreting the protein-LM relationships observed in **Chapter 4**. Since the analysis of baseline data in **Chapter 6** showed no systematic bias between methods across the cohort at pre-intervention, the observed associations between protein intake and LM preservation described in **Chapter 4** are likely valid. However, the sex and BMI differences in protein reporting accuracy suggest that future analyses examining protein-body composition relationships should consider these demographic factors as potential effect modifiers. The stronger tendency for protein under-reporting in women with higher BMI could potentially lead to underestimation of true protein-LM associations in these subgroups, though this would not have affected the baseline relationships examined in **Chapter 4**, where systematic bias was not detected.

In conclusion, while self-reported dietary assessment remains practical for routine use, the documented biases should be considered when interpreting results, particularly during weight loss interventions where protein intake may be significantly underestimated.

7.2.4.2 Physical Activity Assessment

The moderate correlations between physical activity assessment methods (**Chapter 6**) provide context for interpreting the absence of significant relationships between physical activity and body composition in both the cross-sectional analysis (**Chapter 4**) and the LED intervention (**Chapter 5**). The validation work suggests that neither questionnaire nor accelerometry alone captures the full spectrum of physical activity behaviours relevant to muscle mass maintenance in pre-diabetic populations.

This measurement limitation may explain why the hypothesised protective effect of physical activity on age-related muscle loss was only detectable in females (**Chapter 4**), despite biological evidence supporting physical activity's role in muscle preservation across both sexes [226, 822]. The finding suggests that future research should consider composite physical activity measures or focus on specific types of activity (e.g., resistance training) that may be more strongly related to body composition outcomes but poorly captured by general activity questionnaires. However, the range of time that participants spent in low, moderate and vigorous activity (measured by accelerometry) was narrow, as the PREVIEW cohort was not physically active, and this would also influence the ability of statistical models to be able to detect associations between measures.

7.2.4.3 Body Composition Assessment

In **Chapter 6**, it was hypothesised that BIA would demonstrate strong agreement with DXA to assess body composition parameters, including total body fat mass, fat-free mass, and body fat percentage. High correlations between methods with minimal systematic bias were expected, supporting the use of BIA as a practical alternative to DXA in large-scale intervention studies. The body composition validation demonstrated that BIA provides sufficiently accurate measurements for detecting the sex differences in age-related muscle changes (**Chapter 4**) and intervention responses (**Chapter 5**) observed in the PREVIEW cohort.

However, the individual-level discrepancies observed between BIA and DXA have important implications regarding the use of BIA in assessing body composition at an individual level. It could introduce greater variability into a data set and compromise the ability to detect small changes, potentially explaining inconsistencies in the literature regarding sex differences in the relationship of body composition changes to

metabolic health [806]. In the PREVIEW Nottingham cohort, there were only three individuals (0.3%) who were unsuitable to have a DXA scan and in whom body composition was assessed using BIA. Findings from the comparison study do not suggest that the use of BIA in these individuals would have impacted findings in **Chapters 4 and 5**.

Therefore, while DXA remains the gold standard for body composition assessment, BIA can provide acceptable estimates of total body composition, but in routine clinical practice (despite strong correlations for body fat percentage and fat mass), clinicians should be aware of its limitations for regional body composition, particularly in individuals with higher levels of adiposity and the potential for error in the assessment.

7.2.4.4 Implications for Intervention Design and Monitoring

The validation findings collectively suggest that comprehensive assessment strategies may be more important than relying on single "best" methods. The combination of self-reported dietary assessment with periodic biomarker validation could improve intervention monitoring. Using both questionnaires and objective physical activity measures might better capture the full range of behaviours relevant to metabolic health outcomes.

The sex differences in intervention responses observed in **Chapter 5** were detected using DXA measurements. However, validation studies suggest that these differences may be attenuated when using more accessible clinical tools, such as BIA. This creates a translation challenge: while research demonstrates important sex-specific patterns, these may be more difficult to detect and monitor in routine clinical practice. Healthcare providers should be aware that modest but clinically

meaningful differences may exist even when not readily apparent using standard assessment methods.

The systematic biases and method-specific limitations observed suggest that apparent null findings in the literature may sometimes reflect measurement limitations introducing greater variability into the dataset, rather than a true absence of relationships. This is particularly relevant for understanding inconsistencies in research on the relationships between physical activity, dietary composition, and body composition in metabolic health studies, where differing measurement methods are employed.

7.3 Clinical and Practical Implications

7.3.1 For Pre-diabetes Management and Prevention

Based on the findings of this thesis, several important implications can be drawn for optimising health, pre-diabetes management and prevention strategies. The observed sex differences in age-related muscle mass maintenance reveal that women, especially those in the age groups where many will experience peri and post menopause, face particular risks for muscle loss. Evidence from the cross-sectional analysis (**Chapter 4**) showed a female-specific age-related decline, and although analysis of the Nottingham PREVIEW dataset did not detect an association of protein intake or physical activity level on lean body mass, evidence from the wider literature suggests that targeted interventions and structured resistance exercise programs may be essential for this population [823, 824].

7.3.2 For Weight Loss Interventions

The present findings regarding how physical activity and eating behaviours influence responses to low-energy diets have implications for

the design of weight loss interventions. Despite the absence of significant associations between baseline physical activity and changes in LM or fat mass, physical activity remains an essential component of maintaining overall health and weight management. It is possible that physical activity may contribute to other favourable outcomes, such as improved metabolic health or psychological readiness for dietary change, which were not directly captured in this study. Future research may help clarify whether the timing or type of physical activity (e.g., resistance training vs. aerobic) has a differential impact on body composition in the context of energy-restricted diets.

Time-restricted eating patterns like those during Ramadan appear to induce different hormonal responses compared with continuous energy restriction. This raises the possibility that some individuals might achieve better outcomes and adherence with intermittent fasting approaches, while others might respond more favourably to continuous restriction. Personalisation of energy restriction patterns based on individual preferences and physiological responses could improve long-term outcomes [286, 825]. Additionally, the relationship between eating behaviours and weight loss outcomes resulting from the PREVIEW LED intervention highlights the importance of comprehensive behavioural assessments before prescribing weight loss interventions. Individuals with higher disinhibition and hunger scores may benefit from additional behavioural support or modified dietary approaches that specifically address these challenges, such as higher protein intake [235, 826] to improve satiety or more frequent, smaller meals to manage hunger [827, 828].

7.3.3 For Body Composition Assessment

The validation of assessment methods against reference standards provides practical guidance for selecting appropriate measurement tools in both research and clinical settings. While DXA remains the gold

standard for precise body composition assessment, the findings suggest acceptable applications for more accessible methods like BIA under specific conditions (such as standardised hydration status, fasting state, and consistent measurement protocols). The BIA method provides a reasonable compromise between accessibility and accuracy for total body composition assessment in clinical practice. However, clinicians should be cautious when interpreting regional estimates, particularly in individuals with higher adiposity. The systematic biases observed in the validation study could potentially be addressed through population-specific correction factors, improving the utility of these more accessible methods in routine care.

For physical activity assessment, the comparison of the Baecke questionnaire with accelerometry highlights the limitations of self-reported measures. While accelerometry provides objective measurement of movement and eliminates recall bias, it has limitations including inability to capture certain activities (such as swimming, cycling, or resistance training), potential for non-wear time, and challenges in accurately classifying activity intensity across different movement patterns [217, 497]. Self-reported questionnaires, though subject to recall bias and social desirability bias, can capture a broader range of activities and provide contextual information about activity types that accelerometry may miss [214]. In clinical settings where precise activity monitoring is important, such as in exercise prescription for pre-diabetic individuals, the choice between objective measures like accelerometry, and self-report questionnaires should consider the specific research question, available resources, and the complementary information each method provides. A combined approach using both methods may provide the most comprehensive assessment, with accelerometry capturing habitual movement patterns and questionnaires providing activity-specific details [829].

7.4 Strengths of the Research Approach

A strength of the work carried out for this thesis is its comprehensive approach to examining body composition and related factors in individuals with pre-diabetes. By conducting interconnected studies within the same cohort (PREVIEW), this research was able to explore complementary aspects in the relationships between body composition, physical activity, and nutritional assessment while ensuring that comparisons were made within a consistent population sample. Using an integrated approach allows for more reliable cross-study comparisons and synthesis than would not be possible with separate study populations. The body composition analysis using DXA in **Chapter 4** allowed for a detailed examination of regional fat distribution and LM, providing more precise information than would be possible with simpler anthropometric measures. This assessment revealed important sex-specific and age-related patterns in body composition that have implications for healthy ageing and metabolic health. The inclusion of both sexes across a wide age range (25-70 years) in the PREVIEW study allowed for exploration of sex and age interactions with body composition and intervention responses. This inclusive approach improves the generalisability of findings compared with more narrowly focused studies and has revealed important sex-specific patterns that might otherwise have been missed.

The validation component in **Chapter 6** represents another methodological strength. By directly comparing commonly used assessment methods against commonly used alternative methods within the same population, this study gained valuable insights into measurement comparability and limitations applicable to the studied population. The analysis of protein intake assessment across longitudinal study timepoints provided insights into how measurement accuracy can be modified by participation in a lifestyle intervention. Finally, the combination of cross-sectional, observational analysis (**Chapter 4**),

intervention assessment (**Chapter 5**), method comparison work (**Chapter 6**), and systematic review methodology (**Chapter 2**) demonstrates a methodological scope which provides complementary perspectives on the research questions. This multi-method approach strengthens the overall conclusions by addressing limitations inherent to any single research methodology.

7.5 Limitations Across Studies

Several limitations should be acknowledged when interpreting the findings across these studies. First, the cross-sectional design of **Chapter 4** limits causal inferences regarding the relationships between lean body mass, physical activity, and age. While the study observed significant associations, longitudinal studies with relevant interventions would be necessary to confirm developmental trajectories and causal relationships. Second, the selection criteria of the PREVIEW study participants (people with overweight or obesity and pre-diabetes) restrict the generalisability of findings to this specific population. The lack of normal-weight healthy comparators limits the ability to identify whether the observed relationships are specific to pre-diabetic physiology or would apply more broadly. In **Chapter 5**, a key limitation was that free-living physical activity was assessed only pre-intervention and not during the LED period. This made the assumption that physical activity behaviours at baseline were maintained during the intervention, which may not have been the case. The lack of association between physical activity and changes in LM or fat mass should therefore be interpreted with caution, as it may be that pre-intervention activity was not maintained during the LED or reflect the narrow range of physical activity levels in the cohort rather than a true absence of relationship.

Another important limitation across studies was the inability to assess the duration of metabolic impairment (pre-diabetes) in participants. There may have been a variation in this duration with age, which might have

influenced outcomes after the period of weight-loss, especially in older participants who may have experienced metabolic issues for longer periods. Without this information, it is difficult to disentangle the effects of ageing and adiposity from those of prolonged metabolic dysfunction. Finally, the ethnic homogeneity of the PREVIEW cohort limits the generalisability of findings across more diverse populations. Considering the poor representation of certain ethnicities within the participant cohort, the findings cannot be generalised to the broader global population, where pre-diabetes prevalence varies substantially across ethnic groups.

7.6 Recommendations for Future Research

Several avenues for future research emerge from the findings and limitations of this thesis. First, comparative studies directly contrasting intermittent fasting modalities (as studied in the systematic review) with continuous energy restriction (as used in **Chapter 5**) in pre-diabetic populations would provide valuable insights into the optimal dietary approach for this group at high-risk of developing non-communicable diseases. Such studies should comprehensively assess both body composition changes and metabolic parameters, including direct measurement of circulating appetite hormones across both approaches, to better understand how these different dietary strategies affect physiological responses. Second, investigation of the mechanisms underlying the sex-specific responses observed in the LED intervention is warranted. There is potential for future research to investigate the physiological mechanisms associated with sex differences in response to weight-loss, possibly including hormonal assessments to determine whether sex hormones modify the response to dietary interventions. Third, prospective studies examining how addressing baseline eating behaviours (such as restraint and disinhibition) influence long-term success with different dietary approaches would be valuable. Moreover, whether these psychological factors remain predictive of weight-loss or weight-maintenance success over longer time periods and across

different intervention types would also be of interest. Fourth, the development of correction factors for self-reported dietary intake based on weight trajectory could improve nutritional assessment accuracy, which would make it possible to monitor progress more precisely and adjust interventions accordingly. Finally, people with prediabetes may benefit from intervention studies that aim to preserve LM in light of the muscle loss observed in **Chapter 5**. Menopausal status should be considered in these interventions, since hormonal changes may affect sex-specific patterns of muscle maintenance.

7.7 Conclusion

This thesis has provided insights into body composition dynamics, assessment methodologies, and intervention responses in individuals with pre-diabetes. The findings reveal important sex-specific patterns in age-related muscle mass maintenance, compare commonly used assessment methods against alternative methods, and identify key modifiers of body composition changes during weight loss interventions.

Overall, these studies help to advance the understanding of how body composition is influenced by age, sex, physical activity, and dietary interventions in pre-diabetic populations. The observed sex differences in age-related muscle maintenance highlight the need for approaches to preserve muscle mass in women as they age. The method comparison work provides practical guidance for selecting appropriate assessment methods in both research and clinical settings, acknowledging the trade-offs between precision and accessibility.

The findings regarding eating behaviours and physical activity as modifiers of weight loss responses suggest opportunities for optimising intervention strategies through better pre-intervention assessment and preparation. Additionally, the insights from the systematic review on

Ramadan fasting suggest that time-restricted eating patterns may offer an alternative approach to energy restriction with distinct hormonal effects compared with continuous restriction.

These contributions also have implications for clinical practice, research methodology, and intervention design for pre-diabetes management. By enhancing understanding of the complex interplay between body composition, physical activity, and dietary interventions, more effective, personalised approaches could be developed to prevent diabetes progression and improve metabolic health in this high-risk population. Future research should build on these findings to further refine the understanding of the mechanisms behind these relationships and to develop targeted intervention strategies that account for individual characteristics and needs.

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Appendix

Appendix A: Table of the excluded studies with the reason for the exclusion:

Author/ Year	Title	Reason of exclusion
Akan et al. 2023	The effect of Ramadan fasting on mental health and some hormonal levels in healthy males. The Egyptian Journal of Neurology, Psychiatry and Neurosurgery 2023; 59:20 https://doi.org/10.1186/s41983-023-00623-9	• Post-fasting measurement during the middle of Ramadan.
Ajabnoor et al. 2014	Health impact of fasting in Saudi Arabia during Ramadan: association with disturbed circadian rhythm and metabolic and sleeping patterns. PloS one 2014;9(830): p.e96500 https://doi.org/10.1371/journal.pone.0096500	• Post-fasting measurement during the second week of Ramadan.
Alzoghaibi et al. 2014	Diurnal Intermittent Fasting during Ramadan: The Effects on Leptin and Ghrelin Levels. PloS one 2014; *9*(3): p.e92214 https://doi.org/10.1371/journal.pone.0092214	• Post-fasting measurement during the second week of Ramadan.
TELCİ ÇAKLILI et al. 2017	Differences in leptin, ghrelin, and glucagon-like peptide-1 levels between religious fasting and normal fasting. Turkish Journal of Medical Sciences 2017; *47*[90] https://doi.org/10.3906/sag-1603-32	• Post-fasting measurement after Ramadan.
Hasan et al. 2022	Ramadan intermittent fasting is associated with changes in circulating proprotein convertase subtilisin/kexin Type 9 (PCSK9) in metabolically healthy obese subjects. Medicina 2022; 58[90]:p.503 https://doi.org/10.3390/medicina58040503	• Lack of numerical values

Haghighi et al. 2019	Effects of fasting on glucagon-like peptide-1 hormone (GLP-1), and lipid profile indices in obese and thin women. International Journal of Pediatric 2019;7(3):9095-9102. https://doi.org/10.22038/ijp.2018.36085.3147	• Lack of numerical values
Alharbi et al. 2017	Brief report: Ramadan as a model of intermittent fasting: Effects on body composition, metabolic parameters, gut hormones and appetite in adults with and without type 2 diabetes mellitus. Obesity Medicine 2017; 6:15-17. https://doi.org/10.1016/j.obmed.2017.03.002	• Lack of numerical values
Abdulsada et al. 2021	The effect of four-week intermittent fasting from dawn to sunset on circulating brain-derived neurotrophic factor levels in subjects with metabolic syndrome and healthy subjects. Metabolism Open 2021; 9: p.100070. https://doi.org/10.1016/j.metop.2020.100070	• Study design
Mindikoglu et al. 2020	Intermittent fasting from dawn to sunset for four consecutive weeks induces anticancer serum proteome response and improves metabolic syndrome. Scientific Reports 2020;10(831):p.18341. https://doi.org/10.1038/s41598-020-73767-w	• Study design
Mesci et al. 2012	Effect of intended fasting on serum leptin, adiponectin and ghrelin levels. Pakistan Journal of Medical Sciences 2012;28(830):909-912. www.pjms.com.pk	• Post-fasting measurement after Ramadan.
Abedelmalek et al. 2015	Does Ramadan fasting affect acylated ghrelin and growth hormone concentrations during short-term maximal exercise in the afternoon? Biological Rhythm Research 2015; 46(830): pp.691-701. https://doi.org/10.1080/09291016.2015.1048949	• Study of athlete population.

Appendix B: Detailed search strategy

Database:

Ovid MEDLINE <1946 to March 5, 2024>

#	Query	Results from 5 March 2024
1	exp Intermittent Fasting/	169
2	(intermittent adj3 fasting).mp.	1668
3	("time restricted" adj3 (eat* or feed* or fast*)).mp.	870
4	("alternate day fasting*" or (ramadan adj3 fasting*) or "periodic fasting*" or "islamic fasting*").mp.	1574
5	("intermittent energy restrict*" or "recurrent circadian fast*").mp.	127
6	1 or 2 or 3 or 4 or 5	3452
7	exp Gastrointestinal Hormones/	86124
8	((gut or intestinal or gastrointestinal or enteric) adj2 hormone*).mp.	12445
9	("appetite regulating hormone*" or ghrelin or "glucagon-like peptide 1" or "GLP-1" or "glucose dependent insulinotropic peptide" or GIP or "gastric inhibitory polypeptide" or Pyy or "peptide yy" or cholecystokinin or CCK).mp.	76903
10	7 or 8 or 9	135057
11	6 and 10	88
12	ramadan.mp.	1868
13	exp Fasting/	39059
14	12 and 13	1154
15	10 and 14	21
16	11 or 15	92
17	limit 16 to humans	60
18	limit 17 to "all adult (19 plus years)"	33

Database:

Ovid EMBASE <1974 to March 06, 2024>

#	Query	Results from 6 March 2024
1	exp intermittent fasting/	2075
2	(intermittent adj3 fasting).mp.	2375
3	("time restricted" adj3 (eat* or feed* or fast*)).mp.	1188
4	("alternate day fasting*" or (ramadan adj3 fasting*) or "periodic fasting*" or "islamic fasting*" or "muslim fasting*" or "religious fasting*").mp.	2465
5	("intermittent energy restrict*" or "recurrent circadian fast*").mp.	176
6	1 or 2 or 3 or 4 or 5	5205
7	((gut or intestinal or gastrointestinal or enteric) adj2 hormone*).mp.	10743
8	("appetite regulating hormone*" or ghrelin or "glucagon-like peptide 1" or "GLP-1" or "glucose dependent insulinotropic peptide" or GIP or "gastric inhibitory polypeptide" or Pyy or "peptide yy" or cholecystokinin or CCK).mp.	122167
9	ramadan.mp.	2718
10	exp Fasting/	28208
11	exp ramadan Fasting/	800
12	9 and 10	997
13	1 or 2 or 3 or 4 or 5 or 11 or 12	5248
14	exp gastrointestinal hormone/	171679
15	(gastrin* or VIP or "vasoactive intestin* peptide*" or secretin* or leptin*).mp.	197621
16	7 or 8 or 14 or 15	357863
17	13 and 16	435
18	limit 17 to human	316
19	limit 18 to (adult <18 to 64 years> or aged <65+ years>)	148

Database:

Cochrane Library search <1996 to March 12, 2024>

#	Query	Results from 12 March 2024
1	MeSH descriptor: [Intermittent Fasting] explode all trees	17
2	(intermittent NEAR/3 fasting):ti,ab,kw	415
3	("time restricted" NEAR/3 (eat* or feed* or fast*)):ti,ab,kw	410
4	(“alternate day” NEXT fasting*):ti,ab,kw	86
5	(Ramadan NEAR/3 fasting*):ti,ab,kw	154
6	(periodic NEXT fasting*):ti,ab,kw	13
7	(Islamic NEAR/3 fasting*):ti,ab,kw	4
8	(“intermittent energy” NEXT restrict*):ti,ab,kw	110
9	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8	1028
10	MeSH descriptor: [Gastrointestinal Hormones] explode all trees	4932
11	((gut or intestinal or gastrointestinal or enteric) NEAR/2 hormone*):ti,ab,kw	1403
12	(“appetite regulating” NEXT hormone*):ti,ab,kw	151
13	(ghrelin or "glucagon-like peptide 1" or GLP-1 or "glucose-dependent insulinotropic peptide" or GIP or "gastric inhibitory polypeptide" or PYY or "peptide YY" or cholecystokinin or CCK):ti,ab,kw	9318
14	#10 OR #11 OR #12 OR #13	11577
15	#9 and #14	45
16	(Ramadan):ti,ab,kw	242
17	MeSH descriptor: [Fasting] explode all trees	4443
18	#16 and #17	76
19	#14 and #18	4
20	#15 or #19	45

Database:

CINAHL(Ebsco) <1937 to March 15, 2024>

S	Query	Results from 1**5** March 2024
1	(MH "Intermittent Fasting")	193
2	intermittent N3 fasting	653
3	"time restricted" N3 (eat* or feed* or fast*)	321
4	"alternate day fasting*" or "periodic fasting*" or "Islamic fasting*"	121
5	ramadan N3 fasting*	559
6	“intermittent energy restrict*”	55
7	“recurrent circadian fast*”	1
8	S1 or S2 or S3 or S4 or S5 or S6 or S7	1438
9	(MH "Gastrointestinal Hormones+")	3231
10	(gut or intestinal or gastrointestinal or enteric) N2 hormone*	1564
11	“appetite regulating hormone*” or ghrelin or “glucagon-like peptide 1” or GLP-1 or “glucose dependent insulinotropic peptide” or GIP or “gastric inhibitory polypeptide” or PYY or “peptide YY” or cholecystokinin or CCK	7615
12	S9 OR S10 OR S11	8788
13	S8 AND S12	30
14	Ramadan	950
15	(MH "Fasting+")	8347
16	S14 AND S15	678
17	S12 AND S16	5
18	S13 OR S17	33

Database

Web of Science <1900 to 17 March,2024>

#	Query	Results from 1**7** March 2024
1	ALL=(Intermittent fasting)	5644

2	(TI=(Intermittent Near/3 fasting)) OR AB=(Intermittent Near/3 fasting)	1872
3	(TI=("time restricted" Near/3 (eat* or feed* or fast*))) OR AB=("time restricted" Near/3 (eat* or feed* or fast*))	880
4	(TI=("alternate day fasting*" or (Ramadan Near/3 fasting*) or "periodic fasting*" or "Islamic fasting*")) OR AB=("alternate day fasting*" or (Ramadan N3 fasting*) or "periodic fasting*" or "Islamic fasting*")	1357
5	(TI=("intermittent energy restrict*" or "recurrent circadian fast*")) OR AB=("intermittent energy restrict*" or "recurrent circadian fast*")	113
6	#5 OR #4 OR #3 OR #2 OR #1	7336
7	ALL=(Gastrointestinal Hormones)	11290
8	(TI=((gut or intestinal or gastrointestinal or enteric) Near/2 hormone*)) OR AB=((gut or intestinal or gastrointestinal or enteric) Near/2 hormone*)	6289
9	(TI=("appetite regulating hormone*" or ghrelin or "glucagon-like peptide 1" or "GLP-1" or "glucose dependent insulinotropic peptide" or GIP or "gastric inhibitory polypeptide" or PYY or "peptide YY" or cholecystokinin or CCK)) OR AB=("appetite regulating hormone*" or ghrelin or "glucagon-like peptide 1" or "GLP-1" or "glucose dependent insulinotropic peptide" or GIP or "gastric inhibitory polypeptide" or PYY or "peptide YY" or cholecystokinin or CCK)	78876
10	#7 OR #8 OR #9	88776
11	#6 AND #10	102
12	(TI=(Ramadan)) OR AB=(Ramadan)	2621
13	ALL=("fasting")	129627
14	ALL=(fasting)	1176963
15	#12 AND #13	1766
16	#10 AND #15	25
17	#11 OR #16	112