Obesity Related-Energy Expenditure & Peripheral Neuropathy in Obesity and Type 1 Diabetes

Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor in Philosophy by

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STATEMENT OF ORIGINALITY

I declare that this entire thesis was composed by me and that the work contained therein is my own, except where explicitly stated otherwise in the manuscript or text. The work within this thesis has not been submitted for any other degree or professional qualification.

ABSTRACT

BACKGROUND: Decreased resting energy expenditure (REE), activity energy expenditure, diet-induced thermogenesis, or a combination of all these components, plays an important role in the development of obesity. The positive energy balance and higher respiratory quotient (RQ) from the indirect calorimetry (IC), a proxy for substrate oxidation, subsequently predicts weight gain. The aim was (i) to evaluate the effectiveness of providing IC-guided dietary intervention (INT) versus standard of care (SC) during weight loss intervention in obesity; (ii) to evaluate if the baseline RQ or change in RQ correlates with the observed weight loss. Obesity is an independently associated risk factor for peripheral neuropathy, but its contribution in the development of peripheral neuropathy remains unclear. The aim was (iii) to evaluate the prevalence of obesity-mediated peripheral neuropathy compared to primarily hyperglycaemia-driven neuropathy in type 1 diabetes (T1D); (iv) to investigate the role of corneal confocal microscopy (CCM) in detecting diabetic peripheral neuropathy (DPN) in diabetic neuropathic foot ulcer (DFU) in T1D.

METHODS: A randomized controlled trial was performed to evaluate INT vs SC in people with obesity. A systematic review was performed to evaluate current literature to address the question "Does baseline RQ or change in RQ during weight intervention correlate with observed weight loss?" A prospective cross-sectional study was performed to characterize neuropathy phenotype and compare corneal nerve morphology using CCM between people with T1D versus obesity. CCM and other measures of neuropathy were utilized to explore the large and small nerve fibre deficits between people with T1D-DPN and T1D-DFU.

RESULTS: The systematic review (ten RCTs included) demonstrated that baseline or change in RQ during weight loss intervention does not correlate with weight loss. IC-guided (INT) group had greater weight loss ($P<0.001$), reduced fat-mass ($p<0.001$), decreased REE ($p=0.016$) and decreased RQ ($p=0.018$) compared to SC. Greater centripetal adiposity was associated with peripheral neuropathy with 33% prevalence in obesity, compared with 50% prevalence in T1D. However, the RQ did not correlate with small and large fibre deficits in peripheral neuropathy. Greater small and large nerve fibre deficits were observed in a progressive manner from T1D to T1D-DPN to

T1D-DFU. For the diagnosis of DFU in T1D, ROC analysis showed that corneal nerve fibre density (CNFD) (AUC of 0.92; SN 0.88; SP 0.72) and corneal nerve branch density (CNBD) (AUC 0.85; SN 0.88; SP 0.72) has high sensitivity and specificity.

CONCLUSIONS: Providing EE information improved compliance to dietary intervention. Substrate oxidation alters in response to macronutrient diet composition and degree of energy restriction. Greater centripetal adiposity leads to microvascular dysfunction resulting in peripheral neuropathy. CCM is a useful biomarker to detect small nerve fibre degeneration in people with obesity and T1D, with high diagnostic sensitivity and specificity to detect DFU in T1D.

PUBLICATIONS AND CONFERENCES

Publications directly related to the results presented in this thesis

Lim JZM, Burgess J, Ooi CG, Ponirakis G, Malik RA, Wilding JPH, Alam U. The Peripheral Neuropathy Prevalence and Characteristics Are Comparable in People with Obesity and Long-Duration Type 1 Diabetes.
Adv Ther. 2022 Sep;39(9):4218-4229. doi: 10.1007/s12325-022-02208-z. Epub 2022 Jul 22. PMID: 35867275; PMCID: PMC9402741.

Lim J, Alam U, Cuthbertson D, *et al*
Design of a randomised controlled trial: does indirect calorimetry energy information influence weight loss in obesity?
BMJ Open 2021;**11:**e044519. doi: 10.1136/bmjopen-2020-044519

CONFERENCE PRESENTATION

International Congress on Obesity (ICO) 18-22nd October 2022 (Melbourne, Australia)

- **Oral Presentation**
  A randomized controlled trial to evaluate the effectiveness of providing 'real-time' energy expenditure insights with indirect calorimetry (ECAL) during a weight loss intervention.
  Jonathan Zhang Ming Lim, Jamie Burgess, Daniel Cuthbertson, Uazman Alam, John Wilding

- **Poster Presentation**
  Does the respiratory quotient from indirect calorimetry predict the change in body weight during diet + exercise weight loss interventions in overweight and obesity? A systematic review
  Jonathan Zhang Ming Lim, Jamie Burgess, Daniel Cuthbertson, Uazman Alam, John Wilding

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To my loving wife Natasha and daughter Grace, for truly believing in me, for their unconditional love and patience in supporting me in pursuit of academic excellence. To my parents and siblings who have inspired me to strive for excellence and to believe in myself during seasons of doubt and failure. I thank God for providing me with the best possible support, to see this PhD journey come to fruition.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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CONTRIBUTION

This section is to confirm that, I, Jonathan Zhang Ming Lim, the author of this thesis, was actively involved and had a significant contribution in all chapters/ studies presented and discussed in this thesis. Briefly, I recruited all the fifty participants with obesity in the 'ECAL study' for Weight Management. I was involved in the research proposal, research ethics submission, design, and protocol write-up & submission, and was the sub-investigator for the 'ECAL' randomized controlled trial. I was involved in the consent of all the subjects, measurement of Indirect Calorimetry, anthropometric assessments, questionnaires and performed the analysis of the dataset, after extracting the data with consent of the sponsor University of Liverpool. I performed all the statistical analysis and presented the results of the study in the manuscript attached.

For Chapter 2, I proposed the research question, registered the systematic review, and performed the full literature search to address the research hypothesis. I collated the full text articles and compiled the extracted data and information from the relevant studies included. I compiled all the tables with information contained herein and have written up the entire manuscript.

For Chapters 4 to Chapter 6, I consented and recruited majority of the participants with type 1 diabetes, peripheral neuropathy, and neuropathic foot ulcers from the specialist diabetes clinics. I performed peripheral neuropathy assessments and screening questionnaires, nerve conduction studies and corneal confocal microscopy in all the participants I consented in the cross-sectional studies.

For all the studies, I performed all the statistical analyses in this thesis with knowledge gained through support of the Biostatistician in the University of Liverpool. Finally, I have written all the chapters of this thesis which have been reviewed by my supervisors, Professor John Wilding and Dr Uazman Alam.

LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITIONS
BMI	Body mass index
CCM	Corneal confocal microscopy
CNBD	Corneal nerve branch density
CNFD	Corneal nerve fibre density
CNFL	Corneal nerve fibre length
CST	Cold thermal sensation threshold
DPN	Diabetic peripheral neuropathy
DFU	Diabetic foot ulcer
EE	Energy Expenditure
FM	Fat-mass
FFM	Fat-free mass
FEO_2	fraction of oxygen inspired
$FECO_2$	fraction of carbon dioxide expired
HbA1c	Glycated haemoglobin
IC	Indirect calorimeter
IGT	Impaired glucose tolerance
INT	Intervention group
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile range
MetS	Metabolic syndrome
NCS	Nerve conduction studies
NSP	Neuropathy symptom profile
NDS	Neuropathy disability score
QST	Quantitative sensory testing
REE	Resting Energy Expenditure
RQ	Respiratory Quotient
SC	Standard of care group (control)
SD	Standard deviation
SFN	Small fibre neuropathy
SNAP	Sural Nerve Amplitude
SNCV	Sural Nerve Conduction Velocity
SWMS	Specialist weight management service
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
VAS	Visual analogue score
VCO2	Volume of carbon dioxide
VO2	Volume of oxygen
VPT	Vibration perception threshold
WC	Waist circumference
WST	Warm thermal sensation threshold

Table of Contents

1 Chapter I – Introduction

Obesity is a global health burden with increasing prevalence over the last two decades. It is estimated that by 2030, the prevalence of obesity could rise from 35% in developing countries to 48.9%, out of which 25-30% will consist of people with severe obesity (1). The World Health Organization (WHO) (2) reported that up to 39% adults are overweight, out of which 13% consisted of people with obesity. A national survey in the UK confirmed that the prevalence of obesity among the adolescent and youth have increased from 21.0% (2019-2020) to 25.5% (2020-2021) (3). Global, regional, and national prevalence of obesity has exponentially increased to levels where it is a global public health concern (4). Over the three decades from 1980 to 2013, the prevalence of people with overweight (BMI of ≥ 25 kg/m^2) and obesity (BMI ≥ 30kg/m2) increased from 29% to 37% and 30 to 38% in men and women respectively. In England, up to 60% of men and women and more than a quarter of children aged 2-15 years are currently living with overweight or obesity (5). Obesity substantially increases the risk of metabolic disorders (including type 2 diabetes (T2D) and fatty liver disease), musculoskeletal disorders (osteoarthritis), respiratory issues (obstructive sleep apnoea), neurological impairment (Alzheimer's disease), microvascular impairment (neuropathy), depression, and certain types of malignancy (breast, colon, ovarian, prostate, liver, and kidney). Obesity is associated with impairment in quality of life, loss of job opportunities, reduced self-esteem, and social disadvantages.

The WHO have escalated the global concern of obesity and made it a priority to reduce the obesity-related burden to health and societies as well as reversing the increase in global prevalence as part of the 'Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020'(2). Current health recommendations and government obesity policies in the UK are based upon encouraging individual & personal responsibility to limit food intake from energy-rich foods and to increase the consumption of more healthy foods (increase in vegetable and fruit portions) as well as encouraging regular physical activity (150 minutes per week for adults). The policy of interventions has been designed in a way that reliant upon the premise that obesity is simply "an imbalance of energy intake and calories expended". Hence, the initial formulated plan and recommendation from the health commission policies were to educate the public based on 'eat less, exercise more'. However, there are multiple

challenges to support the person with obesity at an individual, regional, and national level.

1.1 Obesity as a Chronic Disease

Obesity is one of the priority missions within the WHO 'Global Action Plan for Prevention and Control of Non-Communicable Diseases 2013-2020' (6). The European Association for the Study of Obesity (EASO) (7), and the American Medical Association (AMA) (8) have strong position statements that classifies obesity as a 'non-communicable disease'. Obesity as a chronic disease has a major impact on the socio-economic factors due to two-fold increased risk of cardiovascular disease (9, 10), increased risk of cerebrovascular accidents, hypertension, gastrointestinal and colorectal malignancies, and obstructive sleep apnoea (11), and increased prevalence of anxiety and depression. Chronic surplus of energy balance leads to overspill of energy stores causing adipose tissue hypertrophy and increased adiposity in internal organs. Obesity is a major risk factor for cardiovascular disease, metabolic disorders, colorectal and gynaecological malignancies (12). People with obesity have two-fold greater risk of developing coronary heart disease (odds ratio 1.88) compared to the general population and more than two-fold greater risk of developing type 2 diabetes (odds ratio 2.74) and hypertension (odds ratio 2.54) (12).

The Organization for Economic Cooperation and Development (OECD) reported that estimated healthcare and economic cost of obesity (13) in OECD countries amount to 8.4% of their total health budget (approximately ~311 billion USD per year). Complications of obesity accounts for about 70% of the overall treatment cost including management of type 2 diabetes mellitus (T2D), and up to 23% cardiovascular diseases and 9% of obesity-related cancers (13) (breast, ovarian, prostate, liver, kidney and colorectal malignancies) (14). Obesogenic factors due to environmental influence, social determinants, cultural influences, and food consumer habits each play a role in contributing to obesity, but there is no clear 'one size fits all' strategy on the optimal management of obesity.

Numerous countries have not been successful in reversing the current obesity pandemic (15). The major challenges for supporting the ongoing lifestyle intervention

is integrating and promoting positive behaviour change, providing health access and education for individuals to link with the local health services across the different sectors (16). At the individual level, the current recommendations for weight loss aimed at dietary restriction and increasing exercise have been far from the expected actual weight loss in the long-term (17-19). Strategies such as behaviour modifications at the individual and organizational level in the UK over the last three decades have been largely ineffective due to the lack of supporting educational and health policies, agriculture policies, environment, food processing, marketing and health education (20).

1.2 Current Challenges to Managing Obesity within UK Tier-based Services

The Public Health England survey from 2019-2020 attributed over 1 million hospital admissions per year attributed directly or indirectly to obesity. This figure represents a 17% increase in hospital admission rates compared to the figures in 2018-2019 (21) (summarized data in **Figure 1.1**). The UK National Institutes of Health categorically classified obesity into categories of overweight (BMI of 25-29.9 kg/m2), Class I (BMI 30-34.9 kg/m2), Class II (BMI 35-39.9 kg/m2) and Class III (severe obesity with BMI >40kg/m2) (22). The UK has a four-tiered pathway for obesity services: (Illustrated in **Figure 1.2**).

i. Tier 1 refers to universal obesity prevention services
ii. Tier 2 covers community-based lifestyle weight management services
iii. Tier 3 is specialist obesity services (provided by a multidisciplinary team)
iv. Tier 4 involves bariatric surgery (23)

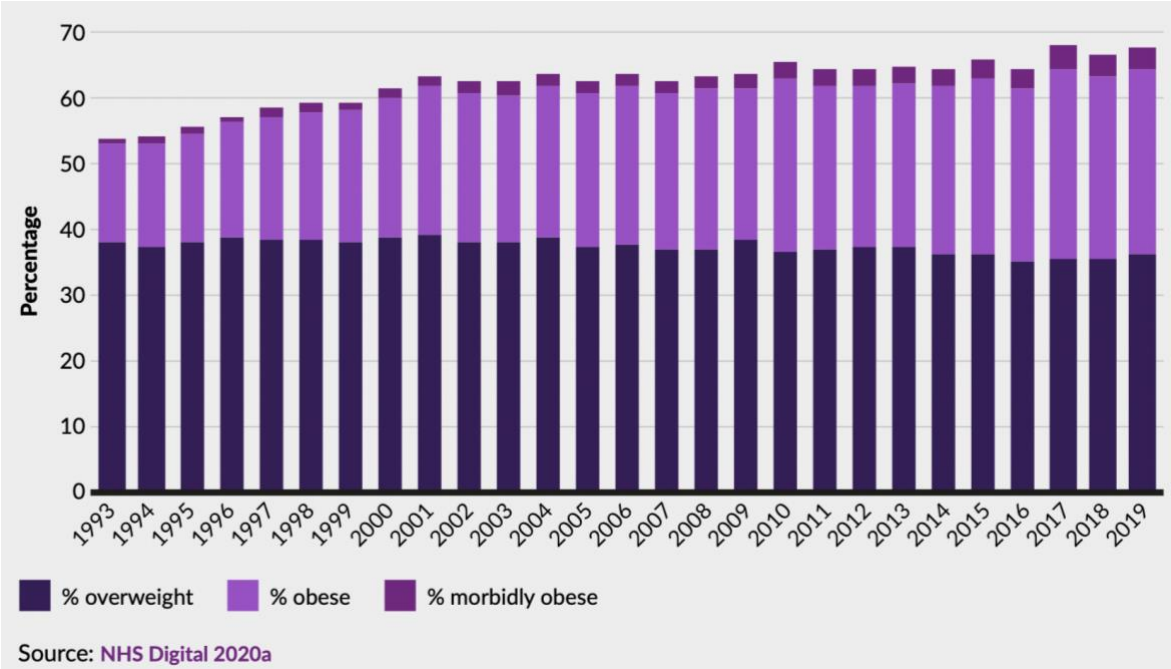
The current challenges to manage obesity as a chronic disease involves a multi approach strategies involving the integrated healthcare systems. In the UK, The 4-tier model is primary accepted with the aim of integrating community and secondary-care services through combined lifestyle intervention and, where necessary, additional supportive medical therapies such as pharmacotherapy and surgical treatments with bariatric surgery (24). Despite the available care pathways and clinical guidelines supported by evidence-based healthcare, there remains no single group willing to take overall responsibility for obesity care. There is a huge need for further improvement

in education and prevention, relapse prevention and long-term strategies of behavioural and weight management support.

The CG43 NICE guidelines (CG189) in 2014 recommends that patients with BMI ≥30 kg/m2, with metabolic disorders (Type 2 diabetes) onset within 10 years should be considered for assessment of bariatric surgery. Whether the patient does proceed to bariatric surgery or not depends on the BMI at time of assessment in Tier 4. The combined Tier 3 / Tier 4 services have existed with the potential to provide support through intensive non-surgical MDT management. However, in many geographical areas across the UK, lack of funding and availability of tier 3 services despite the recommendation by Public Health England led to variability in access, and service provision of specialist obesity management (25).

The metabolic change that comes from energy deficit expressed on the tissue adaptation varies in every individual. Variability in genetic diversity and epigenetic factors influence how the homeostatic system functions in weight regulation (26). Adaptations to body weight is highly variable, which may partly explain why some individuals are more susceptible to an obesogenic environment (27). Summary of the common factors that predisposes the individual to obesity is displayed in **Figure 1.3**.

Figure 1.1 Percent of Adults Classified as 'Overweight', 'Obese' and 'Morbidly Obese' in England, 1993 to 2019. Figure adapted from NHS Digital 2020 (5) (Accessed on June 2022)



Overweight and obesity are defined using the BMI classification (kg/m^2). People who are overweight have a BMI of 25-29.9; people who have obesity have a BMI of 30-39.9; and people who have severe obesity have a BMI >40.

Figure 1.2 The Tier Structure of Weight Management, adapted from the Original NHS Rotherham Healthy Weight Commissioning Framework (2008), which has become the current accepted model in the NHS. Adapted from: (28) (Accessed on June-2022)

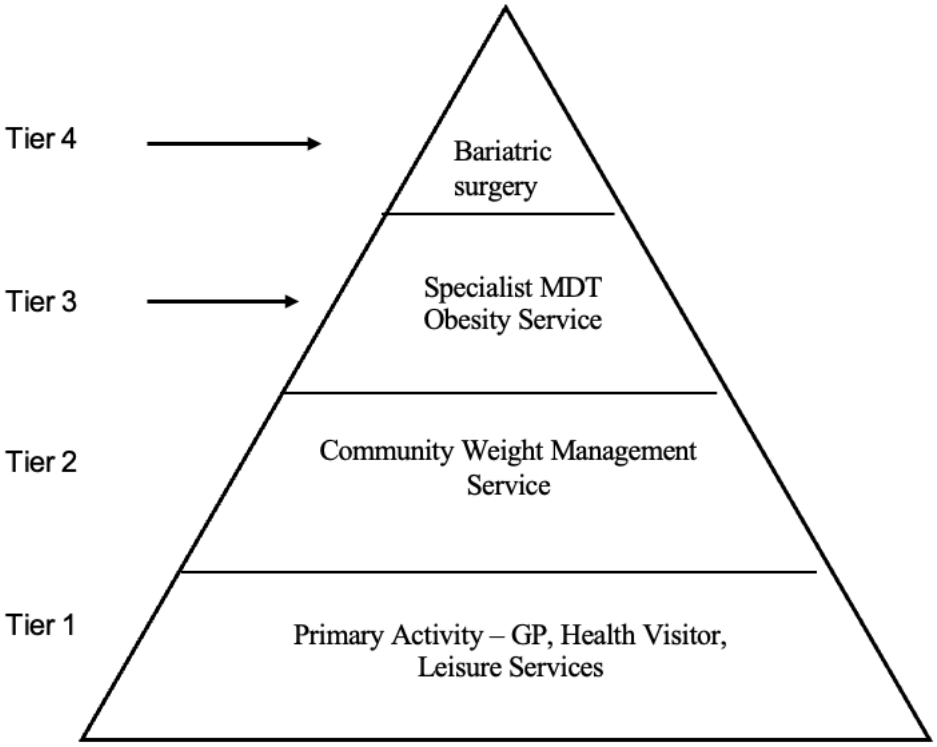
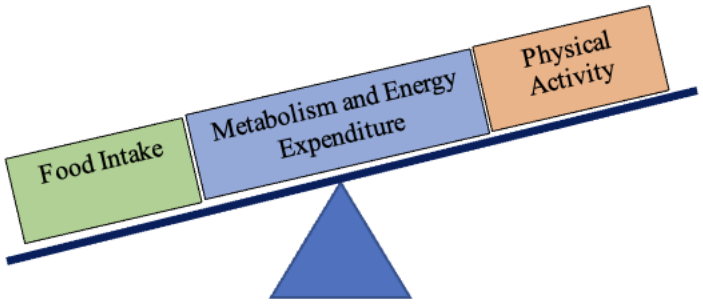


Figure 1.3 Factors that influence chronic positive energy surplus, predisposing to obesity. Weight gain factors consists of combination of increased energy intake, low physical activity, and reduced energy expenditure.



High Energy Intake	Low Energy Expenditure	Physical Inactivity
• Overeating behaviour trait • Socio-cultural • Lack of awareness • Peer Pressure • Uncontrolled Hedonic Eating • Hunger drive • Emotional eating • Snacking • Lack of sleep • Medications	• Ageing • Sex • Genetic and epigenetics • Neuroendorine factors • Prandial thermogenesis • Brown fat • Sarcopenia • Gut microbiota • Medications	• Socio-cultural • Behavioural norm • Physical challenge • Chronic Fatigue • Fibromyalgia & muscle pain • Chronic Joint Pain • Low fitness level • Emotional barriers • Workplace & sedentary lifestyle • Medications

1.3 Complexity of Energy Balance

The principle of weight gain is based on a simple energy balance equation: energy intake exceeds energy expenditure. Over time a new 'energy balance' state is achieved which is often observed when individuals maintaining the similar caloric deficit would stop losing weight. The energy requirement of the body is dynamic and fluctuates according to the exposed environment. Most of the change and physiological adaptations may start from very small changes, but if maintained over time would influence the potential for weight gain / loss (29).

To initiate and maintain weight loss, the energy intake must be consistently lower than the total daily energy requirements, and remain below the set threshold, to prevent further weight regain. Conversely, in conditions of chronic energy surplus, environmental influence, cultural and societal factors affect the 'norm' of eating habits and health behaviours. Health behaviours are closely linked to familial and obesogenic environmental influence traits. In such obesogenic environments, sustained energy deficit would prove remarkably challenging. Hence the simple equation can help deduce the rapid flux and change in energy stores.

"Change in Energy Stores = Energy Intake – Energy Expenditure"

However, this direct equation is over simplified because it does not account for the increase in energy expenditure with increasing weight (30). Conversely, it does not consider that energy restriction induces adaptive changes causing decrease in EE during weight loss (31-33). In the Minnesota Experiment, where severe calorie restriction of 1900kcal/day in normal weight male participants by for 24 weeks, the new energy balance was at <50% of maintenance requirement at the beginning of the intervention (34). Decrease in the Total Energy expenditure (TEE) was explained by decreased maintenance costs of a reduced body mass, decrease in processing cost of the reduced food ingestion, and reduced energy costs to maintain a smaller body (34). About 11% of the decrease in TEE was determined by adaptive change in REE (35) and the 35% reduction in TEE was due to adaptive change in activity EE (35-37). The proposed valid dynamic equation involves:

"Change of energy stores = rate of energy intake – rate of energy expenditure"

The time-dependent change in energy stores is crucial determinant especially in the context of fat-free mass (FFM) and body composition on REE (38, 39).

1.4 Determinants of Total Daily Energy Expenditure

The main determinants of body energy balance depend on the total daily energy expenditure (TDEE). TDEE can be divided to three components:

i. resting energy expenditure (REE)
ii. energy expenditure for processing of ingested food or thermic effect of food (TEF) and
iii. activity-induced energy expenditure (AEE)

1.4.1 Resting Energy Expenditure

Resting energy expenditure (REE) contributes to between 50-70% for energy expenditure to support metabolism whilst awake and alert at rest. Several factors contribute towards the determinants of REE including body weight, gender, ethnicity, age, body composition and the interdependent genetic and familial traits. The REE was observed to be relatively lower in people with obesity as compared to the healthy lean individuals (40, 41).

In a longitudinal study by Piaggi et al (42), 24-hr energy expenditure (EE) adjusted for gender, age, and overall fat-free mass (FFM) was inversely correlated with weight change and the rate of the body weight change (ρ= -0.158, P=0.007). In a large cohort study in the ageing population in Baltimore, subjects with lower than normal or relatively reduced 24-hr EE were more prone to gain weight in the subsequent 2-5 year follow up (43). Another large cohort study (44) in Italy demonstrated similar findings that reduced REE, when adjusted for fat-free mass (FFM), was associated with weight gain. Reduced 24-h EE adjusted for body composition, age and gender correlated with rate of change in body weight (45) over a two-year period (r=-0.39. P<0.001). There is a strong influence of genetic predisposition and familial traits in individuals with

reduced or lower than normal 24-hr EE (45). Indeed, within the Pima Indians, family membership was the strongest determinant for reduced 24-hr REE (46).

Conversely, there are conflicting findings that demonstrated no correlation between reduced REE and body weight gain. In the Baltimore Longitudinal study in overweight adults there was no association between the relatively low 24-hr REE and body weight gain (47). The lack of association between REE and body weight in studies may be due to lack of adjustment for FFM and body composition (47). Weinsier et al (48) also reported no association between relatively lower REE with the subsequent body weight change. To further understand this relationship between EE and regulation of body weight we discuss the principles of energy balance.

1.4.2 Thermic Effect of Food

The measurement of heat energy released (or exuded or exchanged during active metabolism) called the Thermic Effect of food (TEF). TEF is composed of the EE associated with the digestion process and absorption into the circulation and energy storage. TEF also culminates the EE above the expected requirements for digestion. Usually TEF accounts for approximately 5-15% of the TDEE during energy balance conditions. TEF is increased by larger meal portion sizes and greater intake of carbohydrate and protein (instead of dietary fat) (49).

1.4.3 Activity Energy Expenditure / Non-Exercise Activity Thermogenesis

The final component is the EE from movement or the *activity thermogenesis. This activity* energy expenditure *(AEE)* includes energy requirements during the non-volitional activities, which is commonly classified as spontaneous physical activity (SPA) and during non-exercise activity thermogenesis (NEAT) (50). The energy cost of maintaining posture, fidgeting, washing the dishes, are considered the energy expended during well-structured or defined exercise (volitional activities or exercise activity thermogenesis (EAT). Activity thermogenesis component accounts for 20-30% of TDEE. In people with obesity who are sedentary, without exercise-activity related thermogenesis, their entire activity-related energy expenditure comprises

almost entirely of NEAT(50). Low level of NEAT is strongly associated with obesity based on longitudinal data (51).

1.5 Factors that Influence Energy Balance

The energy intake provides the necessary amount of energy for maintaining the requirements of all body cells, whole-body homeostasis, and growth. The energy balance is the relationship between the metabolizable energy and the TDEE (52-54). The remaining metabolizable energy considered as 'excess' after meeting all the basal tissue requirements, will be stored predominantly as fatty tissue or adiposity (*retained energy*). The excess energy store, usually referred as '*positive energy balance*' (energy intake > energy expenditure), may implicate to retain that surplus energy as body stores. On the contrary, if the metabolizable energy is not sufficient to meet all the energy requirements or demands, usually referred to as '*negative energy balance*', the body will consume its own energy depots. Energy intake is regulated by internal body mechanisms to meet the energy requirements of the basal respiration (55).

The energy balance is an important factor which consist of an equilibrium between energy intake versus energy expenditure, influenced by environmental factors (Food availability, reduced physical activity levels, socio-economic influences, cultural factors) and linked to metabolic predisposition which may predict body weight. (Full information summarized in **Figure 1.3**). These metabolic drivers are of huge significance because of the impact of evaluating the 'energy balance' homeostasis in a dynamic association between the regulation of the person's energy intake and energy expenditure. Sustained energy deficit (reduced energy intake), even with 1-2% deficit from daily energy intake could lead to sustained long-term changes in body weight, in the magnitude of 15-20kg (approximately 10-15% of body weight) (56). However, the weight loss benefits are not often achieved by most people struggling with obesity due to various factors influencing their health choices, eating habits, motivation levels and access to continuous support for weight management.

1.5.1 Energy Balance during weight maintenance

During weight maintenance, there are limited fluctuations in energy intake vs energy expenditure. However, the energy intake and the correlation with body weight change is often disproportionate and different (40), particularly where there is a marked difference between self-reported energy intake versus actual (observed or directly supervised) energy intake (57, 58). Some studies have suggested that people with obesity reported only about 50—70% of their total daily energy intake compared with 80-100% for lean counterparts (59-61). The time dependent factor as a 'rate of change' in energy introduces an element of the effect of change in energy storage (especially lean body mass) on the energy expenditure equation (62). Any minor increment in the energy intake or reduced thermic effect of food will not lead to massive change or large increases in body weight over several years based on this equation. During the positive energy balance, the increase in energy intake would lead to an increase in energy expenditure which will counteract the increase in energy intake. Hence, the individual will revert to a state of energy balance, with greater energy intake, higher energy expenditure and higher energy stores. Positive energy balance achieved will occur through fluctuations in food intake over few hours and several days to fulfil the energy needs.

1.5.2 The Fat Macronutrient Balance Equations

Body fat stores are large and body fat intake has no influence on fat oxidation (63, 64). Daily fat intake represents <1% of total energy stored as fat. Body weight change is associated with the energy balance and fat balance influenced by the daily fluctuations in positive or negative energy balances (65). Ingestion of a mixed meal is followed by an increase in carbohydrate oxidation and a decrease in fat oxidation and the addition of extra fat does not alter that mix of nutrient oxidation (65, 66). The amount of total body fat exerts a small, but significant influence on the fat oxidation in the state of the energy balance (46). These studies have provided the scientific basis of energy balance which changes and fluctuates frequently and never achieves equilibrium or steady state except in conditions where energy intake and physical activity is constant over a set period. We discuss the role and practical applicability of use of non-invasive modalities to determine the EE required and the ratio of fat versus carbohydrate oxidation indicated by measured RQ from the indirect calorimetry (IC).

1.6 Indirect Calorimetry

Utilising indirect calorimeter (IC), combined with urinary nitrogen excretion rates (67), whole-body substrate oxidation can be measured. Fasting respiratory quotient (RQ), an index of nutrient utilization provides an indication of fat utilization a metabolic substrate during the fasting state. Substrate oxidation may be influenced by the state of energy balance and the diet composition. Increase in energy intake and reduced EE in overweight and obesity leads to a chronic surplus of energy, stored predominantly as body fat. Impaired capacity to mobilize or oxidize energy stores is associated with chronic energy surplus in obesity. During energy deficit, there are physiological adaptations which normally mobilizes tissue oxidation and switches from carbohydrate towards increased oxidation of body fat (68), in persistent state of negative energy balance, typically reflected by a lower RQ (69, 70). Despite a controlled environment to achieve energy balance state, where the energy intake matches the energy expenditure, it is not uncommon to observe up to 300 kcal variations between energy intake and EE when measured using the whole room calorimetry (69, 70). The metabolic inflexibility to switch from carbohydrate to fat substrate oxidation, or dysfunction in adipose tissue, would lead to impaired utilization of energy stores.

The IC method allows for indirect measurement of heat production and with technological advancements is available as a portable bedside system. The development of the open circuit mask system pioneered by Muller and Franz in 1952, instead of the Douglas Bag method (1911) has allowed increased application in the context of measuring REE and has wider application in the field of human metabolism. Weir (71) proposed a method to accurately calculate EE using whole body-calorimetry completely based upon measured gas quantities of oxygen consumed and carbon dioxide released. Variations exist amongst the numerous established predictive equations including the Harris-Benedict, Schofield, Mifflin-St Jeor and WHO equations, effectively offering a quick assessment but running the risk of excessive restriction or further weight gain (72, 73). Studies on REE change with regards to weight loss pose further uncertainty as to whether REE decreases to a greater extent than predicted for the loss of body mass with weight loss (42, 74).

The IC has been validated for accuracy of measurement of REE (75) through multiple studies based on the calorimeter airflow rate and the change in fractional O_2 concentration inspired and CO_2 expired (76). Ratio of gas exchange of CO_2 expired against O_2 consumed during a defined period is the respiratory quotient (RQ). RQ values are indicative of substrate oxidation (fat vs carbohydrate) metabolism at the time of measurement (75).

Evidence base for use of energy expenditure (EE) information and its impact on weight loss via behaviour modification is currently limited (75, 77, 78). Previous research has established the validity and accuracy of REE measurement using the ECAL indirect calorimeter (Metabolic Health Solutions) as compared to GEM and DeltaTrac metabolic carts (79). The Deltatrac Metabolic Cart was considered the reference standard from evidence of validation studies which compared it to the Douglas bag method (80). There is a gap in the knowledge of the applicability of the utility of information from indirect calorimetry in the context of weight management in people with obesity.

1.6.1 Respiratory Quotient Indicative of Energy Balance

In conditions of energy surplus, the positive energy intake increases the RQ due to the rise in carbohydrate oxidation and decrease in fat oxidation (81). Conversely, a negative energy balance causes a decrease in RQ due to the suppression of glucose or carbohydrate oxidation and an increase in fatty acid oxidation (82). When a meal is ingested, this is an acute rise in energy intake leading to a positive energy balance, and even meals which are high in fat and very low carbohydrate and protein content increase glucose oxidation (81).

During the resting post-absorptive phase, energy balance becomes negative and RQ decreases due to inhibition of glucose oxidation and stimulation of lipolysis and increased free fatty acid (FFA) oxidation (81, 83). The same relationship between energy balance and RQ applies when viewed over 24 h (84). During indirect calorimetry measurements in the respiratory chamber, a direct and positive relationship between the 24 h RQ and 24 h energy balance is reported (85, 86). When The macronutrient composition of diet is set and fixed and exactly matched to the

subject's energy requirements, the metabolism will eventually oxidize macronutrients in the same proportions as the diet intake (87).

Within controlled experimental environments, metabolically healthy individuals would require approximately 72 hours to achieve a state of macronutrient energy balance. In a state of macronutrient balance, the RQ derived from metabolic equation would be equivalent to the food quotient (FQ) (88). However, such metabolic changes in real-world uncontrolled conditions are more unpredictable, such that daily variation in energy intake as well as macronutrient diet composition would influence the state of energy balance (88).

1.7 Weight Management Strategies

1.7.1 Dietary Intervention for Weight Loss

Dietary intervention for weight management have been a huge topic of contention due to lack of consistency and reliability on the impact of weight loss in the long term. Evidence from the meta-analysis of dietary intervention programs confirm that caloric restriction primarily is the mainstay of weight loss intervention, closely followed by alteration in the macronutrient diet composition (89). In contrast, several popular diets including Atkins, Weight Watchers diet results in moderate weight loss with similar magnitude of long-term weight loss at 1 year (90). However, several critical questions remain unanswered. There remains lack of objective methods to establish accurate means of reporting food consumption, and dietary intake. Complex factors shape and influence diets especially for weight management. The type of macronutrient within diet and timing of the meals are the key components of weight-management strategies, are crucial evidence-based dietary interventions for weight loss. Reduced energy intake and dietary macronutrient consumption remains the cornerstone of weight loss intervention and prevention of weight regain.

The success of weight maintenance is reliant upon sustainable lifestyle interventions through restriction of energy intake with a balanced diet, and /or concomitant increase in energy expenditure. The current evidence suggests that short-term 'extreme' caloric restriction, may have short-term weight loss benefits, but majority struggle with weight

rebound (91, 92). Very-low-calorie diets (400-800 kcals/day) is not recommended for participants with obesity due to inability to maintain VLCD diet and the risk of weight rebound (93). Hence, the current evidence-based recommendation is to recommend sustainable and consistent moderate caloric restriction to prevent this 'weight cycling' phenomenon (93, 94). More evidence is supportive of early interventions to support behaviour change and improve engagement in individuals who successfully lose weight in the early stages, by adhering to intensive programmes, including the use of meal replacement formula diets (91).

1.7.2 Exercise and Physical Activity for Weight Loss

The "*Eat less, or exercise more*?" premise remains controversial because of the prevailing evidence that exercise as a sole intervention, is not as effective on weight loss, although associated with only minor weight loss benefit. The main benefit of exercise intervention (both aerobic and resistance exercise) on weight loss is observed in combination with energy restriction. Physical exercise and increased activity level have a modest role in maintaining weight loss and long-term improvement in gaining lean muscle mass (95). Further, persistent conscious 'willpower' or individual effort are required to achieve successful weight loss and prevent weight regain in conditions of constant availability and food abundance. However, it is a common misconception that the lack of willpower, or motivation is contributing to the failure of weight interventions. Hedonic influences and hunger, satiety sensors centrally are derived from the unconscious brain, affected by the food availability in ways that overwhelm the individual's ability to resist the sensory stimulus and trigger affected by the food environment. Therefore, common methods of caloric restriction per se, may trigger an increase hunger drive, and interventions fail because the 'willpower' influence alone is ineffective over the stronger potent influence from hedonic food behaviours. Given the 'state of mind battle' between the regulation of willpower versus the hedonic drive and temptation from the food environment, the weight interventions are should not be limited to willpower centred individually focussed approaches alone, but rather a shift of perspective to tackle wider collective population-based issues.

1.8 Metabolic Phenotype Predisposing Development of Peripheral Neuropathy

The prevalence of obesity is rapidly increasing globally. Metabolic impairment associated with obesity has been strongly linked to peripheral neuropathy independent of the hyperglycaemia (96). Peripheral neuropathy is highly prevalent, particular in the elderly population with studies confirming the prevalence of polyneuropathy in 5.5% (definite) up to 9.4% (probable and definite neuropathy) in Netherlands (97, 98). Peripheral neuropathy is a major cause of reduced quality of life and disability due to sensory impairment, pain, gait disturbance, increased falls, foot ulceration and limb amputation (99). Obesity is the second greatest metabolic risk factor contributing to increased risk of peripheral neuropathy (100, 101). Recently the EURODIAB study demonstrated that the body mass index (BMI), hypertension, and dyslipidaemia had increased risk, comparable to hyperglycaemia-related cause of peripheral neuropathy (102).

Associated metabolic and cardiovascular risk factors, in particular obesity (103) and hypertriglyceridemia (104) have been associated with development of diabetic peripheral neuropathy (DPN). Further, the Anglo-Danish-Dutch study of Intensive Treatment of Diabetes in Primary Care (ADDITION) confirmed that abdominal obesity independently predicted peripheral neuropathy in people who were newly diagnosed with type 2 diabetes (T2D) (105). The development of insulin resistance, which is pathognomonic to T2D, include metabolic impairment and inflexibility (impaired or reduced glucose utilization). Increasing evidence have demonstrated that intensive glycaemic control has little impact on the development of DPN in people with T2D (106-108). Despite substantial glycaemic control, there have been no definite positive prevention studies other that the associated metabolic risk factor modifications which lead to DPN.

Several biochemical factors link obesity to peripheral neuropathy (109). A growing literature links obesity and metabolic syndrome (MetS) to neuropathy (110). Similar associations were found between patients with metabolic syndrome and diabetes, more specifically features of abdominal obesity and dyslipidaemia (110). Increased risk of sensory polyneuropathy has been reported in patients with MetS and prediabetes (111,

112). Decreased compound muscle action potential amplitude of tibial and peroneal nerves and decreased sensory action potential amplitude (113) has been reported in obesity, independent of glycaemic status. The impairment in metabolism and impaired glucose regulation preceding development of diabetes (110), may lead to symptoms and signs related to peripheral sensory neuropathy in people with obesity. We intend to evaluate and identify the phenotypical traits and the clinical characteristics of people with obesity without diabetes as compared to those with primarily hyperglycaemia driven neuropathy in people with T1D. Improved understanding of the obesity-phenotype manifesting with signs and symptoms of peripheral neuropathy may help early identification and prevent foot complications associated with peripheral neuropathy.

1.8.1 Definition of Peripheral neuropathy

The Toronto Consensus proposed definition for diabetic sensory peripheral neuropathy (DPN) as a 'symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and micro vessel alterations as a result of chronic hyperglycaemia exposure (diabetes) and cardiovascular risk covariates' (114). The abnormality of nerve conduction testing is the first objective quantitative indication of peripheral neuropathy. The use of nerve conduction testing has been recommended as an early and reliable indicator of the occurrence of peripheral neuropathy (115).

Some longitudinal studies have suggested painful neuropathic symptoms develop with worsening sensory loss which in practice may include symptoms of 'symmetrical, prickling, deep aching, sharp, like a numbness, burning, electric shock and hyperalgesia with allodynia upon clinical examination (116).

1.8.2 Phenotype of Distal Symmetric Peripheral Neuropathy

The term symmetric distal peripheral neuropathy (DSP) typically refers to 'damage to peripheral nerve, extending from the cell body (dorsal root ganglion or anterior horn cell) to the cell projection itself, with its myelin sheath and axonal projection being involved' (117). The length-dependent neuropathy typically described as 'stocking' or stocking-glove' distribution may affect and manifest as a sensory loss. Large fibres

(type A-beta somatic fibres) have a function for joint position sense, vibration, and touch/protective sensation (118). Classically, neuropathy affects the large nerve fibres complain of the sensory deficit of numbness, tingling and aching discomfort (119). The large fibre involvement particularly in features of longstanding neuropathy can lead to loss of protective sensation on the feet, leading to absent sensory symptoms, placing the person at significant risk of foot ulceration (119). The use of nerve conduction testing has been recommended as an early and reliable indicator of the occurrence of peripheral neuropathy (115).

In addition, some people with diabetic peripheral neuropathy (DPN) nerve damage involving small-diameter thinly myelinated A-delta fibres, which function to conduct cold sensation and mechanical pain information and the unmyelinated C somatic nerve fibres, which carry thermal mechanical and chemical pain signals ('small fibres') (119). Damage to small fibres typically manifest as reduced thermal and pain sensation with relative sparing of vibration and proprioceptive sensation (119). Neuropathic pain is particularly common, such as burning stinging, pins and needles and aching(119).

1.8.3 Corneal Confocal Microscopy to evaluate Corneal Nerve Fibre Damage

Corneal Confocal Microscopy (CCM) is a novel non-invasive reproducible ophthalmic screening and diagnostic technique that allows the researcher to quantify and evaluate and detect early changes in small fibre degeneration in corneal nerve morphology in people with diabetes (120) and associated forms of peripheral neuropathies (121, 122). CCM has been utilized to determine and morphologically quantify the unmyelinated C fibres on the surface of the cornea (119, 123). CCM allows in vivo visualization of the depth of the corneal layers including the epithelium, stromal keratocytes and the endothelium layer (124). Multiple studies have demonstrated the utility of CCM to evaluate the intraepidermal nerve fibre density and morphology in people with diabetic neuropathy, fibromyalgia (125) and amyloid neuropathy, which predicts the development of diabetic neuropathy (126). Moreover, CCM have been associated with evaluating nerve fibre loss in associated degenerative conditions in multiple sclerosis and Parkinson's disease (127, 128). There is limited data on the natural history and progression of peripheral neuropathy in people with obesity without diabetes. We

hypothesise that the CCM has good diagnostic and prognostic capability as a surrogate marker to evaluate systemic small fibre corneal nerve damage in people with severe obesity.

Figure 1.4 Corneal Confocal Microscope. HRT3 Heidelberg, Germany. Images obtained with the consent of the participant.

1.8.4 Small Nerve Fibre Neuropathy in Diabetic Neuropathic Foot Ulcer

Up to one-third of patients with diabetes suffer with neuropathic pain due to painful DPN (129-132). Numerous screening modalities for DPN have been proposed to including the value of rapid, reliable and simple sensory tests such as the Semmes-Weinstein monofilament (133) and the 128-hertz tuning fork (134, 135). The Semmes-Weinstein 10g monofilament tool is valuable in the diagnosis of DPN, but there are other validated tools capable of evaluating or quantifying the progressive nerve degeneration and regeneration in DPN, and subsequent neuropathic foot ulceration (136, 137). In the absence of macrovascular issues and occlusive arterial disease, a neuropathic foot with palpable pulses may imply small fibre neuropathy (SFN) as a causative factor in the development of an ulcer (138). Features of small fibre nerve injury were associated with delayed (superficial) wound healing as determined through decreased intra-epidermal nerve fibre density (IENFD) (139, 140). Small unmyelinated nerve fibres (C-fibre) in cutaneous layer are most susceptible to microvascular dysfunction. Nerve injury and regeneration have been demonstrated in people with T2D and impaired glucose tolerance (141). Corneal confocal microscopy (CCM) has emerged as a new diagnostic and screening modality with reasonably high sensitivity and specificity to assess DPN, especially of the small fibres (142, 143). However, there remains little data on CCM in the patients with neuropathic foot ulcers in T1D. Accurate quantification of neuropathy using validated diagnostic biomarkers is critical to identify those at high risk of developing DFU and further understand the pathophysiological changes and adaptations from DPN to neuropathic DFU (144).

There remains a lack of clinical research that specifically evaluates the association between small nerve fibres specifically in the context of progression of DPN and the subsequent changes in patients who develop DFU in people with T1D. Current evidence from studies on neuropathic foot ulcers predominantly involves people with impaired glucose regulation (prediabetes) and T2D. Small-fibre neuropathies (SFN) may present as a spectrum with patients being symptomatic yet escape the detection by the standard tests (145). Degeneration of small fibres may be missed based upon standard electrophysiological tests, and SFN may be present even in individuals who may exhibit normal motor power, preserved reflexes, and normal electrophysiology. Structural degeneration to small nerve fibres, reduced intraepidermal nerve fibre length and increased regeneration and axonal nerve inflammation have been reported in patients with painful DPN (146). In two smaller cross-sectional studies, greater corneal nerve fibre deficits in the central corneal region (146, 147) and inferior whorl were observed in patients with painful compared to painless DPN (148). The aim is to further evaluate the occurrence of small fibre damage in people with neuropathic DFU in T1D and compare that with DPN, to understand if small fibre neuropathy contributes towards the neuropathic foot ulceration in T1D.

2 CHAPTER II - Respiratory Quotient and Weight Change during Diet Restriction & Exercise

Contribution: Jonathan Lim contributed to the conception and design of the systematic review, Registration of the study question and design on PROSPERO, reviewed and extracted the full text articles. He has written up the findings from the systematic review and writing of this chapter.

Submission for Publication – pending submission to peer reviewed journal

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2.1 Abstract

Background: The respiratory quotient (RQ) from indirect calorimetry (IC) is representative of substrate oxidation during energy balance. Higher baseline RQ was associated with weight gain during low-calorie diet restriction, and likewise, individuals with a lower RQ had a better response towards weight loss during dietary restriction.

Aim: To systematically review the evidence to address the question: In adults with overweight and obesity undergoing weight loss intervention with diet and/or exercise, does the measured RQ during (1) baseline measurement or during (2) acute energy deficit significantly correlate with the observed weight loss?

Methods: We searched in PUBMED (last search in on June 1, 2022), EMBASE, the Cochrane Central Register of Controlled trials CENTRAL, PsychInfo and CINAHL for reports that measured RQ and weight loss response to diet and/or exercise or in combination in adults with overweight and obesity. Only randomized controlled trial study design was considered, and risk of bias was assessed with a checklist from RoB version 2 tool. From the 2,438 records identified, ten remained for the analysis (five diet intervention, three exercise intervention, two combined diet & exercise intervention for weight loss).

Results: Our main findings were that: (a) RQ decreased in response to diet intervention, and combined diet & exercise intervention; (b) Baseline RQ did not correlate with observed weight loss; (c) Greater RQ decrease (during negative energy balance) did not correlate with observed weight loss (follow-up duration of 8-24 weeks).

Discussion: While most people with overweight and obesity may not have lower-than-expected RQ, the data from included studies suggested an increased (over-) reliance on carbohydrate oxidation, thus, rendering them "metabolically inflexible", a state which could be reversed through improved compliance with diet and/or exercise.

Systematic Review Registration: PROSPERO [CRD42021272071]

Obesity is recognised by the World Obesity Federation as a 'chronic relapsing disease' and numerous professional healthcare organizations have prioritized the need to tackle obesity pandemic globally (149-151). Lifestyle based weight intervention strategies have proven effective for weight loss (152), however, there remains is an unmet need to ascertain why certain people are less responsive to attempts at weight loss (153, 154). Majority of people with overweight and obesity attempting weight loss intervention, do not attain their target weight, and of those individuals who do lose weight, many experience weight regain (155, 156). The all-too-common report of the repeated weight loss and weight regain (weight cycling phenomenon) eventually leading to weight regain to more than initial body weight (157). Many people discover and report that the amount of desired weight loss is far from the expected despite adhering to recommended energy deficit (33).

2.2 High Degree of Variability in Weight Loss Response during Energy-Restriction

In response to energy deficit from low-calorie diet (LCD) (up to 1,000 kcal/day total energy intake), Goele et al (158) reported that the discrepancy between the actual (only 44% of predicted) and predicted weight loss may be accounted for by the lack of compliance to the LCD diet. Similarly, Heymsfield et al (159) reviewed the weight interventions from LCD, based on sixteen intervention studies with weight loss ranging from 1.7 kg to 8.1 kg (ranging from 11-45% of the weight loss predicted value). The observed variability in the actual versus predicted weight loss despite the calculated energy-restriction may be explained by principle of adaptive thermogenesis (159). Adaptive thermogenesis is observed where the decrease in REE is greater than would be expected based on the decrease of fat-free mass (FFM) per kg body weight (160-162) in the context of sustained energy restriction.

Anderson et al (163) performed a meta-analysis that included 29 studies consisting of long-term follow-up studies on the efficacy of weight maintenance in individuals who have lost 10-30% (ranging from 6.1kg to 41.6kg) of initial body weight through diet and exercise interventions lasting >1 year (range 1-5 year follow-up). In the meta-analysis (163), it was observed that within the cohort of individuals, more than half of initial weight lost was regained after two-years of follow-up, and greater than >80% of the weight regained after five-years. Despite the initial success of weight loss, the change in body composition (decreased FFM) and adaptive thermogenesis in response to sustained energy restriction and weight loss, may have led to the discrepancy between the actual versus predicted weight loss. Majority of the evidence points towards the issue of lack of compliance in energy restriction, hence there is a tendency for weight regain. There are possible components of metabolic adaptation that have led to this unsurprising weight regain. This chapter explores the potential physiological changes in terms of markers of substrate oxidation (i.e. RQ based on gaseous exchange from indirect calorimetry) and its association with body weight change.

2.3 Respiratory Quotient Indicative of Substrate Oxidation

Respiration involves the process of utilization or breakdown of substrate to release energy. The process of glycolysis is simplified in Figure 2.1 (164). The uptake of oxygen is measured by a respirometer directly at the tissue level through the volume of inspired oxygen. The respiratory quotient (RQ), also known as the respiratory exchange ratio, is defined by the volume of carbon dioxide (V_{CO2}) released over the volume of oxygen (V_{O2}) absorbed during respiration i.e.

RQ = V_{CO2} released / V_{O2} absorbed

Carbohydrates and fatty acids are the main substrate during feeding and fasting cycles in human metabolism (165-167). Intrinsic factors which may influence RQ measure include defects in mitochondrial function, adipose tissue toxicity, state of hyperinsulinemia, and endoplasmic reticulum stress (168). High RQ (~1.0) is indicative of low-fat, high carbohydrate oxidation. Carbohydrates have a 6-carbon chain and metabolize via glycolysis to form 2 pyruvate substrates, releasing CO_2 as a by-product when converting to acetyl CoA. CO_2 is also a by-product in the Krebs cycle when 2 carbon acetyl CoA reacts with a 4-carbon citrate, making a total of 3 CO_2 in each metabolizing cascade. Carbohydrate metabolism is simplified as follows:

- $C_6H_{12}O_2 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy (ATP)
- RQ = 6 CO_2 / 6 O_2 = 1.0

In comparison the RQ of fat oxidation is 0.7. The equation for fat metabolism is simplified as follows:

- $C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16CO_2 + 16\ H_2O$ + Energy
- RQ = 16 CO_2 / 23 O_2 = 0.7

Fatty acids include 12, 16, 18, 20 or 22 carbon molecules, hence undergo B-oxidation to form acetyl Co-A which does not generate carbon dioxide. Therefore, when using fat over carbohydrates as a form of metabolic fuel, less CO_2 is generated for every molecule of oxygen consumed. Hence, the RQ of fat oxidation is closer to 0.7.

2.4 Metabolic Inflexibility Influences Response to Energy Restriction during Negative Energy Balance

Metabolic flexibility is defined by the ability of the active metabolic tissue to respond or adapt according to the changes in metabolic or energy demand as well as the prevailing conditions or activity (169). Metabolic flexibility in human physiology and

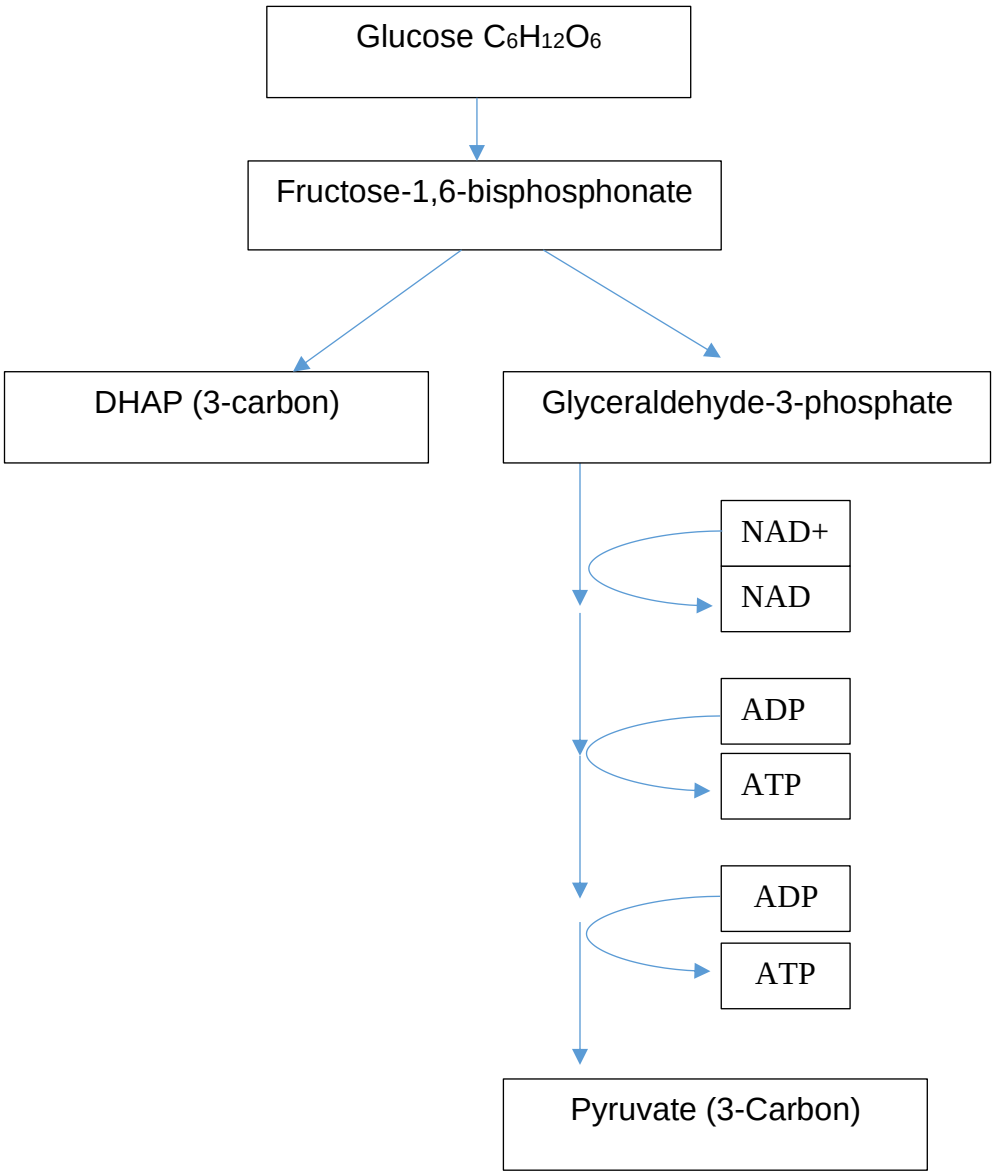
weight regulation refers to the ability to respond to the metabolic state to switch between glucose and fatty acids during the transition between the fed and fasting state. Increasing evidence suggests the role of skeletal muscle and adipose tissue in determining the metabolic flexibility in humans. Regardless of the skeletal or adipose tissue content in obesity, there is impairment at the level of cellular and organelle processes, most pertinently in the mitochondria, in terms of the inability to switch from carbohydrate (glucose) metabolism to fat oxidation (170). In healthy transition from the fasted to fed state, shift in substrate oxidation occurs from predominantly fatty acid metabolism to more reliance on glucose oxidation in skeletal muscle (171).

Given the thermic effect of food may only contribute to <10% of the energy expenditure (49), the shift in substrate oxidation serves to efficiently utilize the energy sources based on the content or macronutrient composition in the diet consumed. The primary drive of shift in substrate oxidation is to switch to anabolic process whereby energy can be effectively stored in skeletal muscle, adipose and liver tissue (172). Insulin secretion in response to a prandial meal is the primary driver of switch in substrate oxidation. Insulin is responsible for the process of glycolysis and release of energy in the form of ATP (summarized in **Figure 2.1**).

In conditions of insulin resistance (in obesity and type 2 diabetes) and metabolic impairment, the impaired mitochondrial fatty acid oxidation in and excess accumulation of lipid metabolites in skeletal muscle and liver tissue contributes toward metabolic inflexibility (173). In obesity, persistent surplus of energy stores, precipitated by reduced activity energy expenditure precipitates a state of persistent influx of metabolic fuel that leads to accumulation of reactive oxygen species, reducing equivalents in the mitochondria and increase in mitochondrial membrane

potential (174). This reactive inflammation causes deleterious protein modification, cell injury, the imbalance between mitochondrial energy delivery and is partly responsible for why some individuals with obesity are deemed metabolically inefficient (14, 175). Excessive influx of ROS (176) combined with a state of insulin resistance and impaired glucose uptake, leads to reactive inflammation causing deleterious protein modification, cell injury, the imbalance between mitochondrial energy delivery and oxidative stress (177). Mitochondrial dysfunction leads to impairment of oxidation (respiration) of macronutrient and influences energy expenditure (178). Hence, it is postulated that impaired capacity to oxidize dietary fat is a metabolic determinant of weight change (179).

Figure 2.1. The simplified process of Glycolysis. Adapted from Website; https://www.khanacademy.org/science/biology/cellular-respiration-and-fermentation/glycolysis/a/glycolysis



DHAP – Dihydroxyacetone phosphate; Glyceraldehyde-3-phosphate; NADH; NAD+

Energy-requiring Phase:

- Phosphate is transferred to Glucose to make Glucose-6-phosphate.
  Glucose-6-phosphate is converted to isomer fructose-6-phosphate.
  Phosphate group is transferred from ATP to produce fructose-1,6-bisphosphate.
- Fructose-1,6-bisphosphate splits into DHAP and glyceraldehyde-3-phosphate.
  Only Glyceraldehyde-3-phosphate can directly progress to glycolysis

Energy-releasing Phase:

- Glyceraldehyde-3-phosphate is oxidized and 2) NAD+ is reduced to NADH and H+
  Exergonic energy released used to phosphorylate forming 1,3-bisphosphoglycerate
- 1,3-bisphosphoglycerate donates phosphate to ADP, making ATP and change to 3-phosphoglycerate.
- 3-phosphoglycerate is converted to isomer 2-phosphoglycerate – becoming phosphoenolpyruvate (PEP)
- PEP donates its phosphate group to ADP, making a second molecule of ATP.

2.5 Longitudinal Data evaluating Baseline Respiratory Quotient and Observed Weight Change

Shook et al. (77) reported that high baseline RQ was predictive of weight gain and fat mass in overweight (BMI 25.6±3.8 kg/m^2) young adults (age 27.6±3.8 years) observed over 12 months. Participants with the top tertile baseline RQ (mean RQ=0.841±0.032) not only maintained the relatively higher RQ levels at the 6- and 12-months, but also had larger gains in body weight and fat after 12 months as compared to individuals in the lower tertiles (77). Several other studies have indicated that high RQ measured during the fasting state (47, 180), or over 24 hours (46) was a significant predictor of long-term weight gain, independent of the REE (46, 47, 180). Further, high RQ in non-insulin treated individuals with type 2 diabetes was associated with weight gain and greater body mass index (BMI) (181, 182). After one year, more than half of the participants (7/13 patients) with higher RQ>1.0 gained more than 3kg, while only 5/32 oral-hypoglycaemic treated patients with diabetes gained more than 3kg ($p<0.05$) (181). Thus, RQ is suggested as a useful tool to predict weight gain (181). There remains an unanswered clinical question of whether there is an impact of the measured RQ using indirect calorimetry on the body weight change during weight loss interventions in overweight and obesity.

2.6 Aim

This systematic review will assess the available evidence to answer the question: In adults with overweight and obesity undergoing a weight loss intervention with diet and/or exercise, does the measured RQ (using IC) during (1) baseline measurement or during (2) acute energy deficit significantly correlates with the observed weight loss.

2.7 Methods

This review was registered online on PROSPERO (2021: systematic review number CRD42021272071). This review has been conducted according to the PRISMA statement for reporting systematic reviews and meta-analyses (183). The full PRISMA flow diagram is shown in **Figure 2.2**

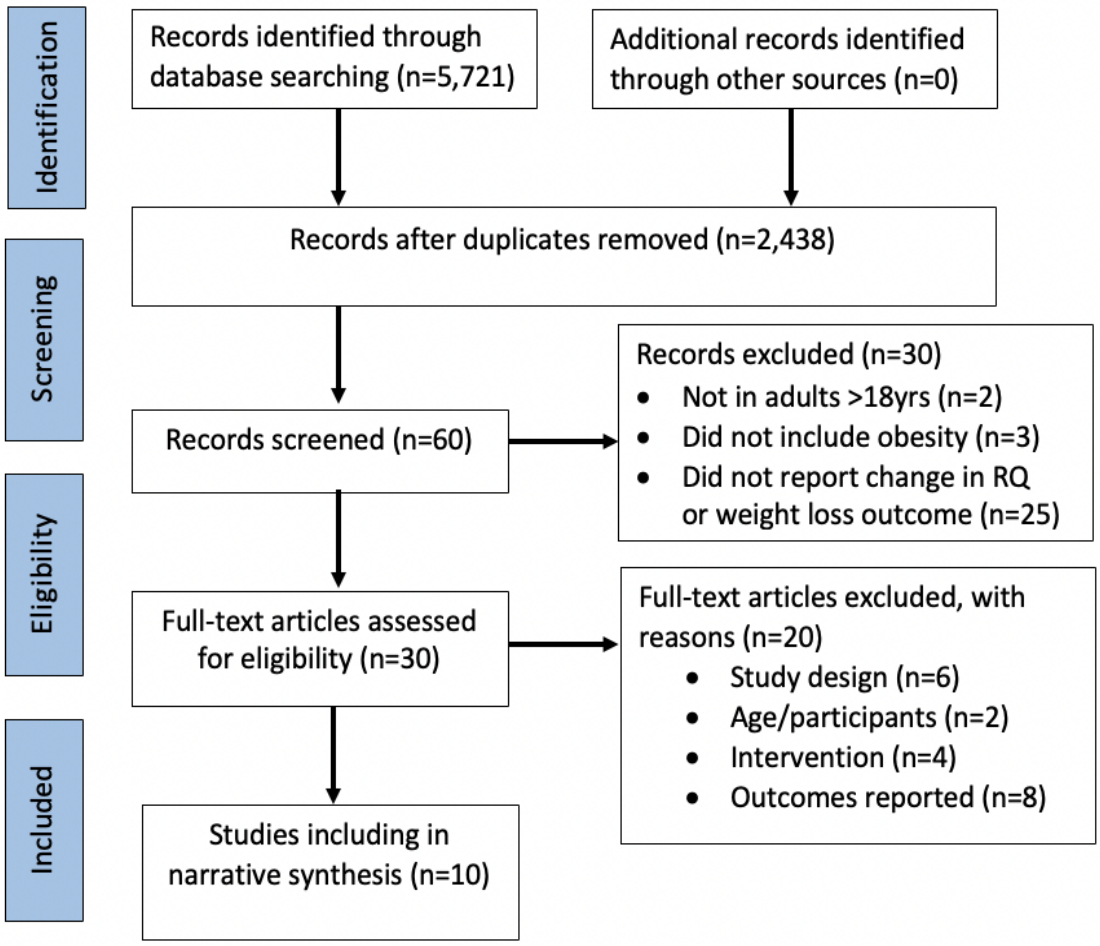


Figure 2.2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) study flow diagram describing the process of study selection. Reviews or preclinical studies (defined as not providing clinical outcome data) and abstracts were excluded. Full-text articles that provided no outcome of interest were also excluded.

2.7.1 Search Strategy

The systematic review was conducted according to the following steps: (1) formulation of the review question (2) inclusion criteria for the participants: people who are overweight and /or living with obesity (3) formulating the inclusion and exclusion criteria based on the patients, intervention, comparator, outcomes (PICO) approach, (4) formulation of a search strategy for identification of relevant intervention studies that included RQ or respiratory exchange ratio and specified participants who underwent a period of observation or weight loss intervention with follow-up data on change in body weight after the determined period. (5) the analysis of the data through systematic review.

The electronic search consisted of eligible literature published from 1980 to October 2021 from PubMed, Central Cochrane Library, and OVID (Medline and EMBASE), Psych INFO and CINAHL for grey literature. The PRISMA flow diagram of the full text extraction from our search is summarized in **Figure 2.3**.

2.7.2 Study Selection

Titles and abstracts of studies were screened independently by two investigators (J.L. and J.B.) based on the inclusion and exclusion criteria. Studies were included if they (1) recruited adults (aged ≥18 years) classified as overweight or obese (people with a BMI≥25 and ≥30kg/m^2, respectively), (2) was a randomized controlled trial (3) weight loss intervention through either dietary restriction (i.e. consists of a minimum 30% energy restriction from the total daily energy requirements) and/or increased physical

activity or exercise (4) specified the change in body weight (in kg) before and after the intervention, (5) reported the use of indirect calorimetry with RQ results before and after the intervention. Articles with of overlapping participants were also screened and considered independent of the "parent" study. A record number was assigned to each included study. Any disagreements were overcome by consensus and arbitration by the senior author. A full text review was then undertaken. Of the 2,438 included, full text review was undertaken for 30 articles. Ten randomized controlled trials were eligible for inclusion.

2.7.3 Data Extraction

Data was extracted by two independent investigators (J.L. and J.B.) author and year, country. Study design, length of intervention, sample size, patient's characteristics, intervention details, comparator features and outcomes (weight loss, free fat mass, fat mass, RQ). For all included studies, mean ± standard deviation (SD) or standard error of mean (SEM) or median and interquartile range (IQR) were used for data extracted.

2.7.4 Quality Appraisal

The quality of all included studies was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system. The GRADE ranks as follows: not serious, serious, and very serious. The GRADE level of evidence was determined independently by two authors (JL and JB) and consensus was achieved by discussion.

2.7.5 Assessment of Risk of Bias

The eligible studies were evaluated for the risk of bias using the revised Cochrane risk of bias tool, as described in Chapter 8, in the Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (version August 2019) (184). The Cochrane risk of bias tool comprises of the following five domains: 1) randomisation process [sequence generation & allocation concealment; 2) deviations from intended interventions; 3) missing outcome data; 4) measurement of the outcome; and 5) selection of the results. The risk of bias was evaluated using algorithms that depend on the answers to the questions in each domain. As a result, each domain was assigned 1 of 3 levels (*high risk, low risk* and *some concerns*). The risk of bias in the included studies was assessed by combining the results across the domain responses. The two reviewers (JL and JB) independently assessed the risk of bias in each article. If there were differences in the evaluation between the two reviewers, they were discussed and resolved.

2.7.6 Data Analysis

We sub-categorized the included studies based on the type of weight intervention (diet, exercise, or combination of diet + exercise). From the selected studies, the change in RQ (directionality + magnitude of change between baseline and post-intervention RQ) was compared against the change in body weight (in absolute terms, kg) pre- and post-intervention. In studies which did not report the absolute value of the RQ, we have included the data where subjects were divided into ‘subgroups’ of high vs low RQ evaluated against the ‘response towards weight loss intervention’ in terms of decrease in body weight.

2.8 Results

2.8.1 Study Selection

The Figure 2.1a shows the PRISMA Flow diagram. From the 5,721 records identified through database searching, 2,438 articles were discovered after the duplicates were removed. Of those, 87 records were screened that met the initial eligibility criteria. The full-text articles (n=30) were extracted, after which twenty articles were excluded (reasons detailed in PRISMA diagram in Figure 2.1). Studies which had been excluded were mainly due to the study design, type of participants included, inadequate specifics regarding weight intervention and lack of consistency in the reported outcomes (weight change and RQ change) were excluded. Finally, the remaining ten randomized controlled studies were included.

The criteria in our search methodology to identify the most relevant studies was as follows: First, we included only reports that applied weight loss intervention and reported the measured RQ from an indirect calorimeter (at baseline and post-intervention); Second, we included only reports with designs that were longitudinal study, cohort study or randomized-controlled trials; Third, we included only reports with participants with overweight and/or obesity (BMI >25kg/m^2) who underwent lifestyle-based weight loss intervention only (i.e. diet restriction, or exercise, or combination of both diet + exercise). After applying these criteria, ten reports remained for the analysis.

2.8.2 Study characteristics

All the included studies (n=10) were randomized controlled trials (RCT). Altogether there were 661 participants with overweight or obesity who underwent Indirect Calorimetry RQ measurements included in this systematic review. The complete summary of the study characteristics is shown in Table 2.1. The majority (n=4) of the studies were from the USA, followed by Europe (n=3), with the remainder from Australia (n=1), Israel (n=1) and Brazil (n=1). All ten studies used the indirect calorimetry method to determine RQ measurement. Eight studies performed the indirect calorimetry measurement during a resting and an overnight fasted metabolic state. Two studies measured RQ during a fasted, post-exercise training phase.

Ninety-five percent of the included participants (628/661) were in the obese category with BMI ≥30kg/m2 and only five percent (32/661) were in the overweight category (BMI 25-30 kg/m2). Average reported BMI ranged from 27.5 to 34.1 kg/m^2, and average age of participants included within the studies ranged from 33-54 years. There were more female participants (n=435/661, 66%) overall in the included studies.

Table 2.1a, b and c. Characteristics of studies included in the systematic review. [A -Dietary Intervention only]

Author, (Ref.), Year, country	Design	Sample size (N)	Age, mean±SD, years	Gender	BMI mean±SD, kg/m^2	Duration weeks	Intervention vs control (Energy restriction in kcal/d) (Total Daily Energy Intake (EI) in kcal/d)
Bogardus *et al.*, (185) 1981, USA	RCT	Total (8)	CC:25±1.4 CR:29±1.9	8 F	31±2.5	8	**Energy-restricted CHO-containing group (CC)** (35% protein, 29% fat, 36% CHO) Total Daily EI: 830 kcal/d vs **Energy-restricted CHO-restricted group (CR)** (35% protein, 64% fat, 1% CHO) Total Daily EI: 830 kcal/d
Goldensh-luger *et al.*, (186) 2021, Israel	RCT	Total (159) LC (80) vs LF (79)	47.7	136 M 23 F	31.1±4.1	24	**Mediterranean /Low CHO diet:** Energy Restriction: 775±1599 kcal/d Total Daily EI: Female: 1500 kcal/d Male: 1800 kcal/d vs **Low Fat Diet:** Energy Restriction: 933±1072 kcal/d Total Daily EI:

							Female: 1500kcal/d Male: 1800kcal/d
Luscombe *et al.*, (187) 2003, Australia	RCT	Total (36) LP:19 HP:17	LP: 53±2 HP: 55±2	10 M 26 F	34.1±0.7	12	**Low protein Diet (LP):** Energy Restriction: Not reported (15% protein, 55% CHO) Total Daily EI: 1553±45 kcal/day for 12WK, then 1958±63 kcal/day for 4WK **High protein Diet (HP):** Energy Restriction: Not reported (30% protein, 40% CHO) Total Daily EI: 1518±34 kcal/d for 12WK; then 1927±89kcal/d for 4WK
Piccolo *et al.*, (188) 2015, USA. (secondary analysis of primary RCT)	RCT	Total (44) HR:22 LR:22	32.9±9.2	12 M 32 F	HR 32.5 (2.9) LR 32.4 (2.5)	12	**In high-responder Group (HR):** Energy Restriction: *pre-intervention:* 514 kcal/d *post-intervention:* 500 kcal/d Total Daily EI: *pre-intervention:* 2555±454 kcal/d *Post-intervention:* 2058±454 kcal/d **In low-responder Group (LR):** Energy Restriction: *pre-intervention:* 506 kcal/d *Post-intervention:* 498 kcal/d Total Daily EI: *pre-intervention:* 2651±406 kcal/d

							Post-intervention: 2166±406 kcal/d
Rubini *et al., (189)* 2015, USA	RCT	Total (32)	MD 44.7±13.9 KD 51.4±12.4	32 F	MD 27.5±2.8 KD 29.3±2.8	8	**Ketogenic-Diet (KD)** (27% protein, 34% CHO, 38% fat) Total daily EI: (t0-t20 days): 848 kcal/d (t20-t40 days): 938 kcal/d (t40-t60 days): 1400 kcal/d **Mediterranean-Diet (MD)** Total Daily EI (t0-t20 days): 1200 kcal/d (t20-t40 days): 1400 kcal/d (t40-t60 days): 1400 kcal/d

Table 2.1a, b and c. Characteristics of studies included in the systematic review. [B – Exercise intervention only]

Author, (Ref.), Year, country	Design	Sample size (N)	Age mean±SD, years	Gender	BMI mean±SD, kg/m^2	Duration, weeks	Intervention vs control (Energy Deficit via Exercise in kcal/d) (pre- and post-intervention RMR)
Amaro-Gahete *et al. (190)*, 2021, Spain	RCT	Total (12) Control (6) Exercise (6)	Control 43.7±6.1 Exercise 41.3±4.4	12 M	Control: 32.5±3.0 Exercise 32.1±3.6	12	**Control:** *pre-intervention* V_{O2}max 20.4±4.2 ml/kg/min *post-intervention* V_{O2}max 24.1±3.6 ml/kg/min *pre-intervention RMR* 1904±101 kcal/d *post-intervention RMR 2094*±181 kcal/d **Intervention (Exercise):** *pre-intervention* V_{O2}max 20.4±4.2 ml/kg/min *post-intervention* V_{O2}max 24.1±3.6 ml/kg/min *pre-intervention RMR* 1962±246 kcal/d *post-intervention RMR* 1960± kcal/d
Botero *et al.,(191)* 2014, Brazil	RCT	Total (32) Low intensity (17) High intensity (15)	35±2	32 F	31.97±3.13	12	**Low training intensity:** Cycling training at low intensity (50% VO2max) *pre-intervention* VO2max 21.3±3.6 ml/kg/min *post-intervention* VO2max 24.7±3.5 ml/kg/min *pre-intervention BMR* 1568±120 kcal/d *post-intervention BMR* 1554±120 kcal/d **High training intensity:** Cycling training at highest intensity (80% VO2max)

							pre-intervention VO2max 20.4±4.2 ml/kg/min *post-intervention* VO2max 24.1±3.6 ml/kg/min *pre-intervention RMR* 1572±89 kcal/d *post-intervention RMR* 1553±85 kcal/d
Fearnbach *et al.*, (192) 2020, USA (secondary analysis)	RCT	Total (110)	49±12	31 M 79 F	31.5±4.7	24	1) Low-Exercise training intensity 65-85% V_{O2}max (equivalent of 8 kcal/kg body weight/week) Energy deficit of 700 kcal/week or **100kcal/day** 2) High-Exercise training intensity 65-85% V_{O2}max (equivalent of 20 kcal/kg body weight/week) Energy deficit of 1760 kcal/week or **251kcal/day** 3) Non-exercise healthy living control

Table 2.1 a, b and c. Characteristics of studies included in the systematic review. [C -Combined Diet + Exercise Intervention]

Author, (Ref.), Year, country	Design	Sample size (N)	Age mean±SD, years	Gender	BMI mean±SD, kg/m^2	Duration, weeks	Intervention vs control (Amount of energy restriction in kcal/d) (Total Daily Energy Intake (EI) in kcal/d)
Sartor *et al., (193)* 2010, United Kingdom	RCT	Total (19)	D: 37±10 D+E: 41±14	5 M 14 F	D: 32±4 DE: 32±3	2	**Diet only (D):** 30% energy restriction of daily energy intake (1600 kcal/d). Total Daily EI: *Baseline:* **2317±581 kcal/d;** *CHO-reduced diet:* **1662±316kcal/d** **Diet + Exercise (D+E):** 30% energy restriction of daily energy intake (1800 kcal/d). Total Daily EI: *Baseline:* **2363±452 kcal/d;** *CHO-reduced diet:* **1886±345 kcal/d** *PLUS* Exercise intensity 90±0.5% of V_{O2max}
Svendsen *et al.*, (194) 1996, Sweden	RCT	Total (121) Diet only (D) (51) Diet &Exercise (DE) (49) Control (C) (21)	53.8±2.5	121 F	29.7±3.1	12	**Control:** Amount of energy restriction: 14±80 *kcal/d* *Baseline energy intake:* **1879±45 kcal/d** Total Daily EI: **1865 kcal/d** **Diet only:** Amount of energy restriction: 785±71 *kcal/d* *Baseline* EI*:* **1879±45 kcal/d** Total Daily EI: **1094 kcal/d** **Diet + Exercise:** Amount of energy restriction:

							842±83 *kcal/d* *Baseline* EI*:* **1879±45 kcal/d** Total Daily EI: **1037 kcal/d** **+** *Aerobic exercise >70% V_{O2}max and resistance weight training*

Table 2.2a, b, and c: Characteristics of treatment and outcome measures of the included studies. [A - Dietary Intervention Only]

Author, Year (Ref.)	INT vs control	RQ Method RQ Outcome measures Mean± SD	Weight Outcome Mean ±SD (kg)	Summary
Bogardus 1981, (185)	CHO-containing (CC) vs CHO-restricted (CR)	Douglas Bag. Post-exercise RQ during heavier workloads and light-exercise **Light exercise + CC:** Baseline RQ 0.78±0.06 RQ WK1 0.80±0.06 RQ WK6 0.76±0.04 Overall (↔) unchanged RQ vs **Light exercise + CR:** Baseline RQ 0.83±0.05 RQ WK1 0.84±0.05 RQ WK6 0.86±0.7 Overall (↔) unchanged RQ **Heavy exercise + CC:** Baseline RQ 0.87±0.05 RQ WK1 0.90±0.07 RQ WK6 0.91±0.04 Overall (↑) increase in RQ	CR group: ↓ weight 8.4±0.5 CC group: ↓weight 7.4±1.2 After 1WK, CR Group: ↓ weight 4.6±0.5% CC Group: ↓weight 3.4±0.2% (P<0.05). significant ↓weight in CR vs CC (P<0.05) at 6WK; but significant weight loss not maintained after 6WK.	Measured RQ increased (↑) post-exercise in heavy-workload, but unchanged (↔) during light-exercise. Greater reliance on glucose oxidation during acute heavy-workload exercise. No significant association between change in RQ and body weight (NS)

		vs **Heavy exercise + CR:** Baseline RQ 1.00±0.02 RQ WK1 0.96±0.06 RQ WK6 0.93±0.07 Overall (↓) in RQ		
Golden-shluger 2021, (186)	Med. Low-CHO (LC) vs Low-fat diet (LF)	Indirect calorimetry (QUARK RMR-COSMED)) at baseline and 6-months Resting state RQ. At baseline, RQ similar LF: 0.80±0.04; LC 0.81±0.05, p=NS). After 6-months, ↓ RQ in LC only but no change in RQ (↔) in LF group. Mean change: RQ ↓ by 0.022±0.007 (LC) RQ ↑ 0.002±0.008 (LF) Mean difference in RQ: -0.024, p=0.005). Greater reduction ↓ RQ in LC diet compared to LF group. ↓ RQ observed in LC group, but no change in RQ (↔) in LF group.	LF: Weight: 90.6±14.6 LC: Weight 92.4±14.2 Mean difference -1.8 [-6.3-2.7] (p=0.43)	**Higher baseline RQ (↑)** associated with: ↑ visceral adipose tissue ↑ hepatic fat ↑ homeostatic insulin resistance Change in RQ measure: **Decrease in fasting RQ** (↓) corresponds with the metabolic characteristics of participants. Association between RQ and body weight, BMI, or body fat mass: Not significant (NS)

Luscombe 2003, (187)	HP vs LP diet	IC Deltatrac Mean fasting RQ increased (↑) from 0.78±0.007 at baseline to 0.82±0.008 at WK16 (P<0.001). Fasting RQ: LP diet: WK0, fasting RQ 0.77±0.009 WK16, fasting RQ 0.83±0.010 Change in RQ: 0.052±0.010 HP diet: WK0, fasting RQ 0.80±0.012 WK16, fasting RQ 0.82±0.013 Change in RQ: 0.020±0.011 Combined LP + HP groups WK0 fasting RQ 0.78±0.007 WK16, fasting RQ 0.82±0.008 Change in RQ: 0.04±0.008 Increase ↑ in fasting RQ from baseline to WK16 in LP > HP group (p=0.02).	(↓) decrease weight: 7.9±0.6 (↓) decrease body fat: 6.8±0.5 (↓) decrease abdomen fat: 0.8±0.1 (↓) decrease total lean mass: 1.5±0.3 Decrease in total energy intake and not the protein-to-carbohydrate ratio of the diet was the determinant for weight loss.	Fasting RQ (↔) unchanged in response to mild to moderate energy restriction (diet) only. Association between RQ and weight change: Not formally assessed (N/A)

		After WK16, mean fasting RQ increased from 0.78±0.007 (WK0) to 0.82±0.008 (WK16)		
Piccolo 2015, (188)	Energy restricted diet	Metabolic cart (TrueMax 2400) RQ measured during resting state. Low-responder (LR) group: Pre: 0.86±0.06 Post: 0.86±0.05 High-responder (HR) group: Pre: 0.81±0.03 Post: 0.82±0.03	High-weight loss responder vs Low-weight loss responder LR: weight (pre: 91.4±11 kg) weight (post: 88.7±11 kg) HR: *Weight* (pre: 91.8±12 kg) weight (post: 82.5±12 kg) [Decrease in body weight: LR: -2.7±1.6 (-2.95%) HR: -9.4±1.8 (-10.2%)]	Decrease (↓) RQ was observed in HR vs LR. ↓RQ correlated with greater ↓ weight loss during energy-restricted phase. Decrease or Lower (↓) fasting RQ during energy deficit was associated with elevated (↑) fatty-acids. Association between RQ and weight change: **positive association** **(**Clinically significant)
Rubini 2015, (189)	MD vs KD	RQ measured by Indirect calorimetry Vmax ® Encore 29 System. RQ measuring during resting state Decrease ↓ RQ ($p<0.05$) (at t_{20} and t_{40}) KD group, but not in the MD group. RQ was maintained at lower	MD: t_0 weight 77.2±9.8 kg, BMI 27.5±2.8kg/m^2; t_{20} weight 74.4±10.0 kg t_{40} weight 72.5±9.6 kg t_{2m} weight 72.1±10.7 kg	Decrease ↓ RQ ($p<0.05$) (at t_{20} and t_{40}) KD group, but not in the MD group. Greater percentage of fat loss reported in KD vs the MD diet after 2 months.

		levels after 40 days (t_{40}), when subjects no more in ketosis. RQ continued to decrease on (t_{40}), returned to the baseline as measured in 2 months (t_{2m}).	KD: Baseline weight 82±12.4 kg, BMI 29.3±2.8 kg/m^2. t_0 weight 82.0±12.4 kg, BMI 27.5±2.8 kg/m^2; t_{20} weight 77.8±12.0 kg t_{40} weight 74.8±11.7 kg t_{2m} weight 73.5±12.6 kg Most significant body weight loss between (t_0) and (t_{20}) -8.4 kg (KD) and -5.1 kg (MD) at t_{2m} Overall, body weight decreased by -10.2% in KD and -6.6% in MD group respectively.	Decrease RQ (↓) reflects increased (↑) fat oxidation during KD. Association between RQ and weight change: Not formally assessed (N/A)

Table 2.2 a, b and c. Characteristics of treatment and outcome measures of the included studies. [B – Exercise Intervention Only]

Author, Year (Ref.)	**INT vs Control**	**RQ Method** **RQ Outcome measures Mean± SD**	**Weight Outcome Mean ±SD (kg)**	**Summary**
Amaro-Gahete 2021, (190)	Exercise (E) vs Control (C)	IC Jaeger MasterScreen CPX. RQ measured during resting fasted state. Exercise (E): Baseline: 0.831±0.194 Control (C): Baseline: 0.867±0.172 After 12 weeks: E: 0.824±0.188 vs C: 0.861±0.173 **↓RQ not significant** [RQ of −0.04 (95% CI = −1.17, 1.09)] **↔ Resting fat oxidation** at baseline and after 12 weeks (C) **↔ Resting carbohydrate oxidation** at baseline and after 12 weeks (C) **↔ Resting fat oxidation** At baseline and after 12 weeks (E)	↓ weight, ↓BMI ↓FM in exercise intervention group vs control (P<0.049) After 12 weeks, ↓weight (-2.08±1.33 kg) (Exercise) ↑Weight (2.13±0.88 kg) (Control) ↓weight -1.23 kg (95% CI = 0.01, 2.45)	↔ RQ from baseline to 12WK in exercise + control group. ↔ Fat/CHO oxidation from baseline to 12WK in exercise + control group. ↓Body Weight significant in exercise intervention vs Control. Association between RQ and weight change: No significant association

Botero 2014, (191)	Low vs high-intensity Exercise	IC (VO2000 Aerosport) during post-exercise state. High-intensity exercise workload corresponding to Ventilatory Threshold (high RQ) vs Low-intensity exercise training (low RQ)	**Low Intensity exercise:** Weight (baseline: 83.5±8.7 kg) (post-int: 81.0±8.9 kg); [Weight loss -3%] (P<0.05) Body fat% (pre: 41.0±4.5%) (post: 40.2±4.8%) (P<0.05) **High-intensity exercise:** baseline weight: 83.4±12.2 kg) (Post: 81.4±11.9 kg) [Weight loss -2.4%] (P<0.05) Body fat% (pre: 41.2±4.7% (Post: 41.1±4.7%) (P=NS) In high-intensity exercise: Decrease (↓) FFM Decrease (↓) REE	High-intensity exercise: greater decrease (↓) BMI as compared to low-intensity exercise. ↓weight in both aerobic exercise groups was significant but no difference between the groups. Association between RQ and weight change: No significant association
Fearnbach 2020, (192)	Low vs high-intensity Exercise vs control	IC -ParvoMedics True Max 2400 Metabolic Measurement Cart RQ measured during exercise. Decrease (↓) RQ from baseline to WK12. RQ stabilized through weeks 12-24, regardless of weight compensation (time P<0.001).	Weight loss outcomes are conflated with the intervention group outcome. Categorically grouped into non-compensators and compensators based on percent	Decrease (↓) RQ from baseline to week 12 across all three exercise workloads. The decrease in RQ is maintained between week 12 to 24. Change in RQ during exercise was not associated with the % expected weight loss (P>0.22)

		Across all three groups, ↓ in Max RQ -0.03 (control), RQ -0.05 (compensators), RQ -0.03 (non-compensators) ↓RQ (adjusted for workload): RQ/%Max METs decreased ↓ from baseline to WK12 (P<0.001). ↓RQ continue to decrease from baseline to WK24 (P<0.05), with no main effect on weight compensation (P=0.43).	of expected weight loss (%EWL) achieved. Predictors of %expected weight loss analysed in response to changes in VO2 peak and RQ with training using %EWL as dependent variable in multiple linear regression.	Association between change in RQ and body weight: NS

Characteristics of treatment and outcome measures of the included studies

Table 2.2C - Combined Diet + Exercise Intervention only

Author, Year (Ref.)	INT vs Control	RQ Method RQ Outcome measures Mean± SD	Weight Outcome Mean ±SD (kg)	Summary
Sartor 2010, (193)	Diet vs Diet + Exercise	IC ZAN 600 CPET RQ measured during rest + overnight fast. Decrease (↓) fasting RQ in both D and D+E. D+E: fasting RQ (pre: 0.91±0.06) fasting RQ (post: 0.88±0.06) vs D: fasting RQ (pre: 0.92±0.07) fasting RQ (post: 0.86±0.07; p=0.002).	DE: baseline 91±15 D: baseline 91±18 kg WK2 DE: 90±16kg D: 89±18 kg (-2.2%) (P<0.05))	Decrease (↓) in fasting RQ more prominent in studies which combined diet + exercise as compared to diet only. No significant correlation between change in RQ and change in body fat mass: .
Svendsen 1996, (194)	Diet vs Diet + Exercise vs Control (C)	IC MedGraphics CPE 2000. Mean RQ during submaximal exercise workload. RQ (↓) decreased (in both groups by 2-4% (P<0.01). Baseline RQ: 0.86±0.06; decreased (↓) in D+E (-0.039±0.06) and D (-0.017±0.09), compared with control	Baseline: (all participants) Weight 78.0±0.8kg WK12 Post-INT: Change in weight WK12: C: +0.5±0.4kg D: -9.5±0.4kg	Decreased (↓) RQ during submaximal exercise workload. No significant correlation between change in RQ and change in body fat mass. No statistically significant correlation bet submaximal RQ during exercise testing and weight or BMI. RQ was not correlated

		group (+0.042±0.007) (P<0.002). No significant difference in change in RQ between D vs D+E.	D+E: -10.3±0.4kg; ANOVA, p<0.001 (Overall body weight decreased by 13.2%)	with body fatness (FM), or lean body mass, or muscle fibre composition.

2.8.3 Quality Appraisal

The quality of the included studies was evaluated for inconsistencies and imprecisions using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system.

Table 2.3 Quality Appraisal of studies using the GRADE system.

Outcomes	Number of studies	Study Design	Risk of Bias	Inconsistency	Indirectness	Imprecision
Body Weight	10	Randomised trials	No serious risk of bias	No serious inconsistency	No serious indirectness	No serious imprecision
Respiratory Quotient	10	Randomized trials	No serious risk of bias	Serious	Serious	Serious

2.8.4 Assessment of Risk of Bias

The ROB2 Risk of Bias assessment tool was utilized to evaluate the risk of bias of all the ten RCTs included within this systematic review. Three studies (3/10) had been considered ‘low risk of bias’. Majority of the studies (7/10, 70%) had a ‘some concerns’ owing to the domains concerning measurement of outcome bias and attrition bias. All 10 studies had low risk of bias in the randomization process and in selecting the reported result.

Table 2.4 Quality assessment for all the include studies.

	D1	D2	D3	D4	D5	Overall
Amaro-Gahete 2021	+	+	+	S	+	S
Bogardus 1981	+	S	+	S	+	S
Botero 2014	+	S	+	S	+	S
Fearnbach 2020	+	+	S	S	S	S
Goldenshluger 2021	+	+	+	S	+	S
Luscombe 2003	+	+	+	S	+	+
Piccolo 2015	+	+	+	S	+	+
Rubini 2015	+	+	+	S	+	+
Sartor 2010	+	+	+	S	+	S
Svendsen 1996	+	+	+	S	+	S

Table Legend:

+ Low risk

S Some concerns

- High Risk

D1 Randomization process

D2 Deviations from the intended interventions

D3 Missing outcome data

D4 Measurement of the outcome data

D5 Selection of the reported result

2.8.5 Characteristics of included Dietary Intervention Studies

Seven studies involved a randomized controlled trial design involving dietary intervention. As prespecified in the PROSPERO search criteria, the dietary intervention of the included studies must consist of at least a 30% energy restriction based on the calculated/estimated total daily energy intake of the participant(s). The full list of the dietary intervention and details from each study was summarized in **Table 2.2**. Three RCTs (185, 189, 194) involved low-calorie diet of 830-1,037 kcal/day for 8-12 weeks; whilst another the three RCTs (186-188) involved restriction to 1,200-1,600 kcal/day for 12-24 weeks and one RCT (193) involved 1,600 kcal/day for only 2 weeks. Amongst the seven RCTs, two studies investigated the difference between diet restriction as compared to diet restriction plus increased exercise (193, 194). The full summary of average weight loss (SD) was summarized in **Table 2.5a, b, and c**.

2.8.6 Characteristics of included Exercise/ Combined Diet and Exercise Intervention Studies

The respective characteristics of the exercise intervention and the combined diet + exercise intervention in all the included RCTs were summarized in **Table 2.2**. Botero et al (191) compared different intensity of aerobic exercise (high vs low intensity) over 12 weeks with an overall average weight reduction of -2 kg (2.2% body weight). Amaro-Gahete et al (190) compared effects of aerobic exercise training over 12 weeks, with reported decrease of -4.2kg. Sartor et al (193) demonstrated similar weight loss effects between diet only (-2kg) versus diet + exercise (-1kg) but a greater reduction in central adiposity 1.1% body fat in the diet + exercise group when compared to the diet only group. Svendsen et al (194) compared a control group versus diet versus diet

+ exercise with corresponding weight loss of -0.5kg, -9.5kg and -10.3kg respectively. Overall, the evidence from these ten RCTs suggests that combined diet + exercise intervention over the short-term (up to 12 weeks) resulted in greater magnitude of weight loss compared to diet-only interventions.

TABLE 2.5A: Weight Loss Outcomes in Dietary Only Interventions

Author (Ref.)	Total Daily Energy Intake (kcal/day)	Duration of Intervention (weeks)	Mean Change in body weight (kg)
Bogardus (185)	830	1 6	-3.4 to -4.6 -7.4 to -8.4
Rubini (189)	848	8	-5.1 (MD); -8.5 (KD)
Piccolo (188)	1000	12	-2.7 (PR); -9.7 (HR)
Luscombe (187)	1500	12	-7.9
Sartor (193)	1600	2	-2.0

Table abbreviations:

MD – Mediterranean Diet

KD – Ketogenic Diet

PR – poor-weight loss responder

HR – high-weight loss responder

Table 2.5B: Weight Loss Outcomes in Exercise Intervention only OR combined Diet and Exercise Weight Interventions

Author, (Ref.)	Exercise Description	Duration of intervention (weeks)	Mean Change in Body weight (kg)
Amaro-Gahete (190)	Aerobic exercise (60-70% V_{O2} max)	12	-4.21
Fearnbach (192)	Aerobic exercise (-100 kcal/day) vs (-251 kcal/day) vs Control	24	not specified
Botero (191)	Aerobic exercise	12	-2.0 to -2.5
Svendsen (194)	D vs D+E vs C	12	-0.5 (C) -9.5 (D) -10.3 (D+E)
Sartor (193)	D vs D+E	2	-1.0 (D) -2.0 (D+E)

Table abbreviations:

C- Control

D- Diet only

D+E – Diet + exercise only intervention

2.8.7 Change in RQ during Diet Intervention

Piccolo et al (188) evaluated results of secondary analysis from an RCT evaluating 12-week energy restriction (-500 kcal/day) diet and sub-categorized participants into high vs low weight loss outcomes. High weight loss outcomes demonstrated lower fasting RQ at baseline and post-intervention when compared against the poor weight loss outcomes ($P<0.05$). The low fasting RQ was reported despite the indifference in resting energy expenditure (REE) and energy intake. Further, participants who spent more time engaging in low to moderate intensity physical activity had lower RQ as compared to those who were more sedentary.

Rubini et al (189) evaluated the effects of very-low carbohydrate ketogenic-diet (KD) vs Mediterranean-diet (MD). During the acute energy-restriction phase (2 weeks), there was significantly greater decrease in RQ in KD as compared to MD, due to greater caloric restriction. The decrease in RQ correlated with greater reduction in body weight and fat mass (FM), particularly in the KD group where participants underwent 'very low-calorie diet' (830 kcal/day). During the weight maintenance phase (2 months), RQ remained lower in the participants on KD as compared to MD. Isoenergetic exchange of fat and carbohydrate, being an adaptation towards high fat, no carbohydrate intake, typical of KD diet, caused a shift in RQ towards fat oxidation. Given the participants were on 'isocaloric' weight maintenance phase, there were no significant difference in the change in body weight after 2-month follow-up.

Goldenshluger et al (186) compared the effects of hypocaloric low-fat (LF) vs the Mediterranean/low carbohydrate (LC) diet. LC group demonstrated greater reduction in fasting RQ as compared to LF group. The decrease in fasting RQ corresponds with the metabolic characteristics of the participants. Higher baseline fasting RQ was associated with increased visceral adipose tissue, higher hepatic fat, and increased homeostatic insulin resistance. Multivariate linear regression model (RQ increase/ decrease) adjusted for confounders (age, gender, diet group, baseline RQ and 6-month change in BMI) was performed. Based on the model, there was no significant association between RQ and body weight, BMI, or body fat mass (186).

Sartor et al. (195) demonstrated a decrease in fasting RQ (over 2 weeks) with corresponding moderate weight loss. However, the decrease in RQ was suggested to be related to low-carbohydrate availability. There was no significant correlation

between the decrease in RQ and change in body fat mass, during the short-term follow up of 2 weeks.

Svendsen et al (194) evaluated effect of an energy-restrictive high protein diet with or without exercise. Overall, fasting RQ decreased during sub-maximal exercise workload. The decrease in RQ during the sub-maximal exercise workload was not associated with change in fat mass or body composition. No association was found between RQ measured during sub-maximal exercise weight and subsequent change in body weight.

Luscombe et al. (187) evaluated impact of high-protein (HP) versus low-protein (LP) diet. After 16 weeks, there was an increase in fasting RQ from baseline in both the LP (0.77±0.009 to 0.83±0.010) and the HP (0.80±0.012 to 0.82±0.013) (time-by-diet effect, P=0.02) groups. The greater increase in fasting RQ (12 weeks) on LP diet, probably reflects the greater carbohydrate content of the LP diet used in study i.e. 55% vs 40% CHO content. Diet composition had a significant effect on fasting RQ. Luscombe et al. (187) also measured post-prandial RQ. Greater increase in post-prandial RQ was observed in LP as compared to HP group. The magnitude of increase in postprandial RQ was, however, smaller than expected. Similarly, diet composition had a significant effect on post-prandial RQ (187).

In summary, five out of the seven RCTs (186, 188, 189, 193, 194) involving diet restriction for participants with overweight and obesity during 'negative energy-deficit' phase of weight loss demonstrated a decrease in fasting RQ when measured during overnight, fasted, and resting state. Two out of the seven RCTs (185, 187)

reported unchanged RQ in response to energy-restriction. In terms of the association between the decrease in RQ and weight loss: four RCTs (185, 186, 193, 194) reported 'no association' between the decrease in RQ with magnitude of weight loss, one RCT (188) reported 'positive association' between decrease in RQ and weight loss, and two remaining RCTs (187, 189) 'did not formally evaluate' the association between RQ and weight loss.

2.8.8 Change in RQ during Combined Diet + Exercise Intervention

Bogardus et al. (185) evaluated whether there were changes to metabolic fuel use or capacity for strenuous exercise after a carbohydrate-containing (CC) vs carbohydrate-restricted (CR) hypocaloric diet (830 kcal/d). During the 6-week carbohydrate-restricted diet, there was reduction in muscle glycogen stores and reduction in capacity for endurance exercise. There was no significant difference in RQ measured during loaded pedalling between the CC and CR group. When RQ was measured during the exercise-phase, the RQ was greater (increased) in both CC and CR group during the heavy exercise workloads ($P<0.002$), but not during the light-exercise unloaded pedalling challenge. The mean body fat loss was equivalent between both groups after a modest weight loss averaging at 7.4±1.2kg (CR) vs 8.4±0.5kg after 6 months. No association was found between change in RQ and body weight loss.

2.8.9 Change in RQ during Exercise Intervention

In this systematic review, we included three RCTs which evaluated the effect of exercise-based weight loss interventions (190-192) in people with overweight and obesity. Of the three RCTs, two RCTs (191, 192) measured RQ whilst the subject is performing the aerobic exercise (different exercise intensities) and one RCT (190) measured RQ when subject is in a rested state 24-48 hours after completing the

exercise intervention. Fearnbach et al. (192) performed a secondary analysis of 110 participants with obesity to evaluate effects of exercise on body composition and effects of exercise on RQ measured during sub-maximal exercise training. Participants who underwent the highest intensity exercise workload over time had the greatest decrease in RQ (at 12 weeks) measured during sub-maximal exercise. However, the change in RQ with training was not associated with percentage expected weight loss ($P>0.22$).

Amaro et al. (190) compared the effects of aerobic and resistance exercise at sub-maximal exercise intensity (60-70% heart rate reserve) up to 180 minutes/week for 12 weeks against with regular activity (without exercise training). Participants completed 86% of the exercise sessions over 12 weeks. Despite the greater weight loss reported in the exercise versus the control group, there was no significant change in the RQ from baseline to week 12 in both the intervention (exercise) and control groups. The minor decrease in RQ in response to exercise training did not correlate with the expected weight loss (190).

Botero et al. (191) compared low-intensity training vs high-intensity exercise training (corresponding to ventilatory threshold) and measured RQ during the post-exercise phase. The two groups were compared in accordance with aerobic exercise i.e. cycling at intensity corresponding to low RQ versus the intensity of exercise corresponding to high RQ (at the ventilatory threshold) in adults with obesity. There was no actual RQ value reported. There was no significant difference in weight loss between the group with greater exercise intensity (corresponding to high RQ equal to ventilatory threshold) compared to the lower exercise intensity. The low-intensity exercise

training (corresponding to lower RQ) was associated with decreased triglycerides ($P<0.001$) and reduced fat-free mass ($P<0.001$) as compared to high-intensity exercise (corresponding to high RQ).

In summary, fasting RQ measured during a resting state 24-h after exercise did not change at baseline to post-intervention (12 weeks) despite a moderate decrease in weight (-2.08±1.33 kg) after 12 weeks. The intensity of exercise (comparing exercise corresponding to high RQ vs exercise corresponding to low RQ), was associated with decrease in triglycerides and reduced FFM, but there was no significant difference in weight loss. Overall, there was insufficient evidence to evaluate the association between decrease in RQ in response to exercise against the change in body weight in people with overweight and obesity.

2.9 Discussion

The main findings of this systematic review were that: (a) RQ decreased in response to diet intervention, and combined diet & exercise intervention; (b) Baseline RQ did not correlate with observed weight loss; (c) Greater RQ decrease (during negative energy balance) did not correlate with observed weight loss (follow-up duration of 8-24 weeks). During negative energy balance (acute energy deficit from diet +/- exercise intervention), the increase in utilization of endogenous fat (lipolysis) is reflected by the decrease in RQ. Current evidence suggests the fall in RQ may also indicate the state of energy balance and energy stores in the body. However, based on the included studies, there was a lack of correlation between the ‘less-than-expected’ decrease in RQ with the observed magnitude of weight loss during dietary and/or increased exercise in weight loss intervention in people with overweight and obesity.

During energy restriction, the increase in lipolysis and decrease in the oxidation of carbohydrate are necessary to maintain steady circulating glucose levels, both of which depend upon carbohydrate availability (196, 197).

Greater overall decrease in RQ was reported in studies that involved 'no carbohydrate/ ketogenic' or low-carbohydrate diet (198, 199). Macronutrient composition of the diet is more closely related to the change in the RQ than the amount of weight loss (65). Impaired fat oxidation in obesity may be associated with a relatively higher fasting RQ (198). Conversely, lower RQ measured in a resting state was associated with reduced serum fatty acid levels (200, 201). The acute effects of diet restriction reduce the carbohydrate availability which in turn leads to decrease in RQ. The duration of this decrease in RQ is relatively short, with one study of a very-low calorie diet (VLCD) intervention, showing RQ reverts to usual baseline within 1 week of stopping the diet intervention (202). In studies involving VLCD diet (800-1000 kcal/day), significant caloric restriction enables more efficient fatty acid oxidation, and improved insulin sensitivity in the short term (203). Profound caloric restriction reduces liver fat, due to hepatic lipolysis and reduced ectopic triglyceride deposition (204), leading to significant decrease in RQ over 2-4 weeks (202, 205, 206), consistent with our findings.

2.9.1 Contrasting Evidence from Longitudinal Data

In contrast to our findings, certain longitudinal studies reported a direct correlation between RQ and rate of body weight gain over time, when adjusted for fat-free mass and REE ($r=0.89$, $P<0.01$) (207). Zurlo et al (46) reported that the 24-h RQ measured in Pima Indians with obesity in a real-world longitudinal study showed higher 24-h

RQ (90th percentile) independent of 24-h energy expenditure were almost 2.5 times higher risk of gaining >5 kg body weight than those with the lower 24-h RQ (10th percentile). Toubro et al (208) reported that substrate oxidation rates determined by 24-h RQ correlated with the familial traits after adjusting for age, sex, physical activity levels and 24-h energy balance.

In studies on chronic overfeeding, higher 24-h RQ was an independent predictor of *ad libitum* food intake (209), when adjusting for the REE and FFM. Previous studies reported that 24h-carbohydrate oxidation during energy balanced metabolic state in a respiratory chamber correlated with subsequent 3-day *ad libitum* food intake (210). The rate of weight loss was also inversely related to baseline RQ (46, 206). The higher RQ was strongly influenced by the familial resemblance in macronutrient diet composition (208). The correlation between 24-h RQ measurement and familial macronutrient intake, was increased after adjustment for 24h energy balance, and age and gender demographics (208). Differences in fat utilization strongly influences the post-absorptive RQ (86), however no marked changes in body weight occurred in association with alterations in post-absorptive baseline RQ.

In the fasting state, a higher RQ has been associated with higher weight gain and fat storage (182, 211). Reduced capacity for fat oxidation or impaired metabolic flexibility may contribute towards greater adiposity and could explain why people with obesity have limited exercise capacity (212). There was an increase in RQ following weight loss in people with previous obesity (48). The shift in substrate oxidation to favour carbohydrate utilization in the low-fat diet group may be related to the individual responses to insulin regulation and the reduction in availability of body fat (186). In

multivariate model to evaluate the effect of RQ increase or RQ decrease on body fat composition whilst adjusting for age, sex, baseline RQ, diet and changes in BMI revealed that decrease in superficial subcutaneous adipose tissue and pancreatic fat was associated with the decrease in RQ (186). However, there was no association between the decrease in RQ on deep subcutaneous adipose tissue, visceral adipose tissue, or hepatic fat (186).

2.9.2 Carbohydrate Availability Influences Substrate Oxidation

Higher RQ during fasted conditions was reported in participants who consumed carbohydrate in the hour (s) before beginning exercise (213-215). Suppression of fat oxidation is apparent over a wide range of exercise intensities. The peak rate of fat oxidation is observed during moderate exercise intensities (45 – 60% of VO2 max) accounting for factors including gender, fitness level, intraindividual capacity of VO2 max and dietary consumption. During greater intensity of exercise (80-90% of VO2 max), there was downregulation of fat oxidation (216, 217).

Lipids are the substrate that is predominantly responsible for the energy supply during periods of submaximal exercise (218, 219). Subcutaneous adipose tissue, intramuscular medium-chain triacylglycerols (MCTs), cholesterol and ingestion of dietary fat all contributes towards fatty acid oxidation (216). Dietary interventions are most effective when reduced carbohydrate intake, hours before evaluating fat oxidation. The timing of the carbohydrate intake ingested (in hours before the exercise, from start of exercise, or at any point during exercise) result in a marked reduction in fatty acid oxidation (220).

Greater fat oxidation from rest to moderate exercise intensities is the result of increased fatty acid availability. The increased lipolysis and reduction in re-esterification of fatty acid had close association with the intensity of exercise. Decrease in lipolysis in combination with three-fold increase in fatty acid release from TAG hydrolysis resulted in six-fold increase in the availability of fatty acid for oxidation (217). The rate of appearance of glycerol as an indicator or biomarker of whole-body lipolysis (221). Plasma fatty acid concentrations are unchanged or decrease when exercise intensity is increased from moderate to high (213, 217). Reduced Fatty acid availability could be a factor suggested to cause lower fat oxidation rates at high intensities. Increased fatty acid availability resulted in greater increase in fat oxidation, up to 27% greater fat oxidation, as compared to control (saline infusion), indicating that the lowered availability of fatty acid is a rate limiting factor towards fat oxidation (217).

2.9.3 Future Research

We postulate that future studies on the change in RQ on the propensity for weight gain/ loss in obesity must account for the energy requirements (222), energy stores and body fat composition (205) and the preferential standardization of macronutrient intake (223, 224). It is crucial to recognise there will be substantial variation in responses due to genetic variation, macronutrient energy intake, sex, fitness, and physical activity levels in response to energy restriction and/or exercise. Another limitation is that there is possibility of sub-optimal dietary adherence, thus inadequately maintained energy restriction, confounding interpretation of energy expenditure data. Hence, future studies should account for measures of active lean tissue mass and fat mass to provide adjusted comparisons of RQ measure before, during and after a period of weight loss intervention. Other methods such as doubly labelled water (225), could be applied to

evaluate substrate oxidation and energy expenditure alongside indirect calorimetry measurements.

2.9.4 Limitations

A limitation of this systematic review is the substantial heterogeneity within the studies included, indicating inconsistent effect across studies. Heterogeneity in the studies to some degree would be expected given the various series of intervention designs, baseline characteristics of the participants, and comparator diets. In all the RCTs selected, there was 'some concerns' in at least one domain of the risk of bias assessment, specifically in terms of measurement of the outcome data and attrition bias.

The end outcome was change in RQ from baseline to end of intervention. Measurement of RQ using indirect calorimetry in a 30-minute steady state differs from RQ measured in metabolic chamber over 24 hours or during sub-maximal exercise. In all the included studies, the protocols for RQ measurement have been consistently described. However, many factors contribute to variability including environment (temperature, ambient lighting, environmental stimulus), adherence and consistency of determining steady resting state prior to RQ measurement, and genetic predisposition (affecting individual variation of resting metabolic rate) may contribute to large intra-and inter-individual variability when measured under different experimental protocols and conditions (31, 226). In addition, our search was limited to English language publications, did not include other potential databases, or a search of grey literature, which may be reduced or limited the included number of clinical trials.

The majority of the RCTs were from short to moderate term follow up <1 year duration and predominantly dietary intervention trials, given large-scale long-term trials of this nature may not be plausible.

The evidence from the included studies consists of short-to-moderate follow up (8-24 weeks duration). Given the short duration of intervention, the full effect of the diet intervention and/or exercise on weight loss in the 'free-living' conditions may not be entirely consistent due to lack of reported data on dietary adherence. Given the high variability in indirect calorimetry methods to consistently measure and determine RQ in resting metabolic state, the actual measurement of RQ data may be too speculative to draw any strong conclusions. Nevertheless, there are findings from certain studies (188) which have reported the positive associations between decrease in RQ and weight loss that is worth considering.

This systematic review focussed only on RCTs in overweight and obese participants who underwent dietary intervention and/or exercise, thus excluding studies in 'normal weight, lean adults' with presumably normal metabolic flexibility. We have excluded studies which involved conditions of metabolic impairment such as type 2 diabetes and metabolic syndrome. We have also excluded *in vitro* studies because of the difficulty in determining the indirect calorimetry measured in that model, but many of these studies have been described previously.

2.10 Conclusion

In conclusion, this systematic review demonstrates no significant association between change in measured RQ and propensity for weight loss during diet and/or exercise intervention in people with overweight and obesity. The decrease in RQ during dietary

restriction is strongly suggestive of increased lipolysis and utilization of energy stores in adipose tissue. There remains a paucity of high-quality evidence to evaluate the metabolic switch from carbohydrate to fat oxidation through diet and/or exercise during weight loss phase of obesity treatment, to address the substantial variation in energy balance. The data clearly shows that importance of diet and/or exercise in obesity treatment beyond weight loss *per se*. There is need for more accurate and consistent methods to capture the energy expenditure data (RQ) in a reproducible and reliable manner. Despite the lack of correlation between the 'less-than-expected' decrease in RQ with the observed magnitude of weight loss, this may suggest that individuals with overweight and obesity have metabolic inflexibility, a state which could be reversed through improved compliance with diet and/or exercise.

3. CHAPTER III - Methods

Contribution: Jonathan Lim contributed to the design of the study and made a major contribution to the recruitment of subjects, indirect calorimetry assessments, anthropometric and biochemical assessments, all statistical analyses and writing of this chapter.

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Jonathan Lim, Uazman Alam, Daniel Cuthbertson, John Wilding. Design of a randomised controlled trial: Does Indirect Calorimetry Energy Information Influence Weight Loss in Obesity? BMJ Open. 2021 Mar 24;11(3):e044519. Doi: 10.1136/bmjopen-2020-044519. PMID: 33762240; PMCID: PMC7993246.

3.1 Abstract

Introduction: Respiratory quotient (RQ) provides an indication of the relative balance of carbohydrate and fat oxidation. RQ could serve as an early biomarker of negative energy balance during weight loss. Restriction of energy intake relative to total daily energy requirements produces a negative energy balance which can lead to a fall in RQ, accompanied by a decrease in resting energy expenditure (REE). However, the net change in body weight does not usually match predicted weight change due to intraindividual metabolic adaptations.

Aim: To determine the effectiveness of utilising EE information from indirect calorimetry (IC) during weight loss intervention in obesity.

Methods and analysis: We will undertake an assessor- blinded, parallel-group randomised controlled trial of 105 adults with obesity randomised in 1:1 ratio to receive either standard weight management care (SC) or IC-guided EE information (INT) during a 24-week multicomponent weight management programme.

Primary outcome: The difference in magnitude of weight loss between INT and SC group at 24 weeks. **Secondary outcomes**: change in RQ, REE. Generalised linear mixed models (intention to treat) will assess outcomes for treatment (INT vs SC), time (baseline, 24 weeks) and the treatment-by-time interaction. This will be the first study to evaluate impact of utilising measured REE and RQ on the lifestyle-based intensive intervention programme.

Ethics and dissemination: Ethics approval was obtained from the Health Research Authority and the North-West Research Ethics Committee (18/NW/0645). Results from this trial will be disseminated through publication in peer- reviewed journals, national and international presentations. Trial registration numbers NCT03638895; UoL001379

3.2 Introduction

The prevalence of obesity has reached pandemic levels both worldwide and, in the UK, having more than doubled globally over the last three decades (227). Reducing the obesity-related health burden as well as tackling the obesity pandemic is core to WHO 'Global Action Plan for Prevention and Control of Non-Communicable Diseases 2013-2020' (6). The obesogenic environment is a consequence of industrialisation of labour and food systems, leading to over-consumption of energy-dense, nutrient-poor foods combined with an increasingly sedentary lifestyle (228). Behaviour-centred weight loss through intensive lifestyle modifications has proven effective in the short-term in attaining modest but clinically significant weight loss (156, 229). However, excessive energy intake over prolonged period leads to inability to maintain the consistent negative energy deficit required to achieve weight loss. Individuals seeking weight loss encounter much difficulty due to increased hunger and a disproportionate reduction in energy expenditure (EE) driven by neuro-hormonal adaptations (18). Consequently, maintenance of weight loss is difficult, and weight regain common (156, 230).

Practical methods to better engage with people using information, self-directed education and promote effective weight loss are continually evolving amongst specialist weight management programmes. The ECAL indirect calorimeter (IC) (Metabolic Health Solutions Pty Ltd, Perth, Australia) is an open-circuit portable device that measures fractional volume exchange in oxygen consumed (VO_2) and carbon dioxide expired (VCO_2) within a small mixing chamber(79). The ECAL IC uses a proprietary mouthpiece and nose clip. O_2 measurements were calculated with a galvanic fuel cell oxygen sensor and CO_2 measurements were obtained using a novel

LED non-dispersive IR sensor. VO_2 and VCO_2 gas exchange measurements were derived from physiological methods based on Weir's equation(167). Data generated from breath-by-breath exchange during a steady volumetric flow whilst in an energy-balanced resting state, generates the resting energy expenditure (REE) and respiratory quotient (RQ). ECAL IC has been compared against other IC including the GEM and DeltaTrac. Kennedy et al. (79) reported that measures of VO2, RMR, RQ, carbohydrate oxidation, and fat oxidation showed greatest variation on the ECAL IC as compared against Deltatrac (as the standard reference device). The mean difference in RMR measures collected on ECAL and Deltatrac showed wide limits of agreement (lower 95% limit of agreement was -2562 kJ/d and upper 95% limit of agreement was 3480 kJ/d). A greater proportional bias was observed between measured RMR of ECAL against Deltatrac suggesting that at higher RMR the difference between the two devices was greater but was acceptable for repeat RMR measures between individuals. In terms of repeatability of measures of ECAL IC (intra-machine variability), Kennedy et al. reported that no significant differences were found between repeated measures of VCO2, RMR, RQ and substrate oxidation measures. However, greatest bias occurred within the ECAL with a mean difference of 475 ± 1083 kJ/d and wide limits of agreement (-2641 and 1691 kJ/d). Comparing between devices, coefficient of variance was 4 (±5.3)% on the Deltatrac, 4.9 (±4.5)% on the GEM and 11.2 (±12.1)% on the ECAL (79). Deltatrac was previously considered the reference standard after validation studies demonstrated low bias and good precision in comparison to Douglas bag method, however the Deltatrac is now discontinued from commercial sale (80). The RQ is the index of VCO_2 against VO_2 which is expressed as a ratio. Typically, metabolism consisting solely of fat or lipids generates an RQ of 0.70 - 0.75. The approximate RQ of mixed metabolism of fat and carbohydrate is 0.80. Likewise,

protein metabolism generates approximate RQ of 0.80. Metabolism consisting solely of carbohydrate produces RQ of 1.0.

Longitudinal prospective studies reported the association between low RQ (<0.75) with weight loss and weight loss maintenance (46, 65, 85, 205). Numerous epidemiological and experimental studies further support this correlation that a lower RQ predicts weight loss (46, 47, 231, 232). Goris et al. reported post-absorptive RQ was strongly related to change in body mass ($r=0.57$; $P=0.0001$), but not BMI, fat mass or fat-free mass (233). Conversely, adjusting for variables of sex, ethnicity, familial predisposition, those with higher RQ were associated with weight gain or struggled to maintain the loss (47, 205). In the Baltimore Longitudinal study in Aging, Seidell et al. reported that high fasting RQ was a weak but significant predictor of weight gain (47). In previously overweight and obese subjects, high RQ predicted an increase in body fat mass independent of energy balance, circulating insulin and insulin sensitivity (180, 182). Marra et al. reported baseline RQ in non-obese women was a significant predictor of body weight ($P<0.05$) after a 6-year follow-up period (180). Hainer *et al.* reported that people with obesity on very low calorie diet with high RQ regained weight at the 2-year follow up (234). Based on the current evidence in literature, RQ serves as a valuable indicator of substrate oxidation during an energy-balanced state. If substrate oxidation was examined in a positive energy balance state, i.e. energy intake > expenditure, typically carbohydrate oxidation increases and fat oxidation decreases (235). Conversely, if measured in a negative energy balance state i.e. energy intake < expenditure, carbohydrate oxidation decreases and fat oxidation increases (236). The predominant factors that influences the accuracy of RQ measure of substrate oxidation is determined by body composition, energy intake and EE (237).

Behavioural weight loss programs classically involve decreased calorie intake, increased EE and use behavioural strategies such as goal setting and self-monitoring. This is the first study that has been specifically designed to investigate the utilisation of energy expenditure information (REE and RQ) from the ECAL IC on the change in body weight. We postulate use of individualised EE information generated from the portable ECAL IC will result in greater weight change behaviour modification.

This paper describes the protocol and statistical analysis plan for the randomised controlled trial of EE from ECAL IC in a Multicomponent Weight Management Service (NCT03638895). This RCT incorporates the use of EE information from the ECAL IC versus standard care (SC) in participants with obesity and severe obesity attending a secondary care-based specialist weight management service (SWMS).

3.3 Objectives

3.3.1 Primary objective

The primary aim of the study is to determine the effectiveness of providing EE information from indirect calorimetry to influence the outcome of weight loss in patients with obesity without diabetes receiving dietary restriction, exercise, and behaviour modification therapy.

3.3.2 Secondary objectives

The secondary objectives are to compare the health outcomes in terms of:

i. association of weight change with the change in measured respiratory quotient
ii. association of weight change with change in measured resting energy expenditure

3.4 Methods and Analysis

3.4.1 Study Design

Prospective Parallel-Group Randomized Controlled Assessor Blinded Study

The study protocol version 1.3 (Date: 28/08/2020) has been approved by the Health Research Authority (HRA) and the Northwest Research Ethics Committee (REC 18/NW/0645) and is prospectively registered with Clinical Trials Registry (NCT03638895). The design, conduct and reporting of this study has been approved and sponsored by the University of Liverpool / Liverpool Joint Research Office.

3.4.2 Patient and Public Involvement

Patients were involved in the design and conduct of this research. During the feasibility stage, priority of the research question, choice of outcome measures, and methods of recruitment were informed by discussions with patients through two focus group sessions. Members of the Tier 3 specialist weight management service at Liverpool University Hospitals NHS Trust also identified this research as being a priority area for clinicians and patients undergoing weight management intervention. Once the trial has been published, participants will be informed of the results through the local trust website and will be sent details of the results in a study newsletter suitable for a layperson audience.

3.4.3 Trial Oversight and Governance

The study sponsor is the University of Liverpool. The study is managed and overseen by the University of Liverpool / Liverpool Joint Research Office. The study is registered at the http://www.clinicaltrials.gov (NCT03638895) before the enrolment of the first participant and monitored by the University of Liverpool / Liverpool Joint Research Office. Safety of the participants will be monitored by the University of

Liverpool / Liverpool Joint Research Office. Decision for any interim data analysis and stopping guidelines will be assessed and validated through the University of Liverpool/ Liverpool Joint Research Office.

3.4.4 Participants

3.4.4.1 Eligibility Criteria

To determine eligibility, the participants must fulfil all the eligibility criteria (see **Table 3.1**). Criteria were designed to ensure that participants were able to safely engage with the weight management programme.

Table 3.1: Inclusion and exclusion criteria from ECAL study protocol

Inclusion criteria
• Man or woman, 18 to 70 years of age • BMI ≥30 kg/ m^2 to ≤ 60 kg/m^2 at screening visit • Stable weight (change of < 5% within 12 weeks before screening based on medical history) • Subjects are in the investigator's opinion, well-motivated, capable, and willing to learn how to undergo indirect calorimetry testing, as required for study • Willing and able to adhere to the prohibitions and restrictions specified within this protocol
Exclusion criteria
• Taking weight loss medication within 12 weeks prior to randomisation • Previous or planned bariatric surgery • History of type 1, type 2 diabetes mellitus, diabetic ketoacidosis, or diabetes secondary to pancreatitis • Has a HbA1c of ≥ 6.5% (or ≥ 48 mmol/mol) • History of obesity with a known secondary cause (Cushing's syndrome) • Oral corticosteroid use (except in the short-term use of a 7-10-day course) • Ongoing, inadequately controlled thyroid disorder defined as thyroid-stimulating hormone >6mIU/litre or <0.4 mIU/litre • History of malignancy within 3 years before screening (or diagnosis of malignancy within this period) • eGFR ≤30ml/min/1.73m^2 on serum testing • Alanine aminotransferase level is >2.0 times the upper limit of normal or total bilirubin is >1.5 times the upper limit of normal at screening • Other major illness likely to preclude participation in the trial • History of glucagonoma • A myocardial infarction, unstable angina, revascularization procedure (stent or bypass graft surgery) or cerebrovascular accident within 12 weeks before screening • Heart Failure NYHA Class III-IV

• End-stage Chronic Obstructive Pulmonary Disease (COPD)
Withdrawal Criteria
• Terminal illness or loss of capacity during participation in clinical trial • In the opinion of the investigator is unsafe for continuation in study for medical, safety, regulatory or other reasons • Loss to follow-up • Female participants with a positive pregnancy test

3.4.5 Recruitment

Recruitment of participants will take place from February 2019 to February 2022 in Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom. Participants will be recruited through current referrals to Aintree LOSS community-based weight management service, face-to-face clinic encounters, group-based education sessions, electronic database of participants attending the Tier 3 specialist weight management service, and notices in the hospital. Participants with BMIs ≥ 30 kg/m^2 will be identified from referral lists and weight management clinics. Participants will receive a study letter which briefly explains study aims and advises that researchers will be in contact within 2 weeks to provide further details of the study. If participants do not respond to study advertising within 6 months of commencement of recruitment, there will be a further broadening of recruitment sites to include local weight management services subject to prior ethical approval. All individuals identified will be given a patient information leaflet and required to provide written informed consent prior to enrolment. People interested in participating will be met by researchers at the study site to obtain consent and complete the baseline questionnaires.

3.4.6 Randomisation, allocation concealment and sequence generation

Data collected from the screening visit will be utilised to assign participants to groups based on sex and BMI in the process of randomisation by minimisation. This process will ensure the baseline characteristics are balanced between the treatment groups and have been utilised as a reasonable method for randomisation for small clinical trials to reduce bias. Participants will be randomised at an individual level by an independent statistician without contact with participants during the trial. Using a computer-based random number-producing algorithm, allocation sequence within will be generated to allow equal ratio of 1:1 to either the intervention or control group. Complete separation will be achieved between the statistician who generated the randomisation sequence and those involved in assessing participants and performing data entry.

It will not be possible to 'mask' researchers or participants to group allocation. However, those responsible for analysis will be masked to group allocation. A research assistant who will not be involved in the enrolment, assessment or allocation of participants will pack and sequence pre-packed envelopes with the group allocation. Only after the study investigator/ sub-investigator reviews the eligibility based on health records, laboratory results and concomitant medications, and confirms eligibility to proceed, will the envelope be opened, and details of the study group will be revealed to the participant. If the participant is randomised into the intervention (INT) group, layperson client printouts and summary will then be provided with their program resources.

3.4.7 Sample Size Calculation

The sample size calculation was based upon retrospective dataset from the SWMS (238), with a derived standard deviation of 4kg. Thus, 42 participants in each group will give our study 80% power to detect a difference in weight change between groups of 3kg at the 5% significance level using a two-sided test. A between-group difference of 3kg was chosen as this is outside the range of normal weight fluctuation and is sufficient to sustain clinically meaningful weight loss (229). Accounting for attrition rate of 20%, the total target recruitment is 105 participants.

3.4.8 Study Intervention

The intervention (INT) group participants will receive EE information generated from ECAL IC which will encompass the REE and RQ delivered in the form of a layperson client report containing summary of recommendations to help improve understanding about metabolic health. During the study visit, these results will serve as a reference tool for the dietitian when formulating a dietary plan based on the measured REE to determine total daily EE (TDEE). Further, the RQ data will also be used as an indicator of substrate oxidation to deliver key messages on carbohydrate vs. fat oxidation and to facilitate decision making in energy-restricted dietary recommendations. The clinician will explain the measured REE (supposedly more accurate than predicted REE) in the context of weight loss and make recommendations on dietary plan energy restrictions based on measured REE. Food diaries will be provided to study participants to support and check compliance and used to record compliance with recommendation. The threshold of compliance with study intervention is set at those who complete at least 5 out of the 9 study visits from baseline to final visit with completion of the IC test. The energy requirements of each participant in the INT group will be calculated using the measured REE and the self-reported physical activity captured via the International

Physical Activity Questionnaire (IPAQ). The recommended energy restriction for weight loss will be up to 30% less than total daily energy requirement to achieve the intended weight loss. The individualized weight loss plans will be modified at study visits based on the REE and RQ information and the weight loss plans adapted if more energy restriction is required. The dietitians will utilise standardized diet checklists to assist with monitoring dietary compliance and cross-check with the self-completed food diaries. We have set the threshold of compliance with dietary recommendations at 50% compliance.

In comparison, the participants in the standard care (SC) or control group will receive usual care delivered via multidisciplinary weight management intervention including dietary intervention, recommendations of physical activity and behaviour modification. Dietary restrictions will be estimated based upon the Harris-Benedict equation for total daily energy requirements, with a recommended 30% energy deficit from total daily energy requirement. Typically, suggested meal plans range from approximately 1,200 to 2,500 kcal per day. Dietetic advice will be based upon the Association of UK dieticians (BDA)(239) recommendation on daily intake of carbohydrates (40-60%), protein (20-30%), and fat (20-30%). Participants will be encouraged not to have prolonged episodes of fasting, but to maintain up to three regular portioned balanced meals/day, while limiting snacks and reinforcing portion control. Dietitian will provide the participant with a food diary and offer them self-monitoring tools to monitor diet using MyFitnessPal^{TM} Mobile App (www.myfitnesspal.com) for at least three days per week. Up to 150 minutes of physical activity/week will be recommended. Participants will be encouraged to formulate personalised dietary restriction and achievable activity goals each week

during each study visit. To make a comparison of indirect measurements of REE and RQ between groups, the participants receiving SC will also undergo IC measurement, but without receiving the EE information. For full details on assessments during study visits, refer to **Table 3.2.**

Table 3.2: Assessments and procedures during ECAL study visits.

Procedure	**SCR**	**W0**	**W1**	**W2**	**W4**	**W8**	**W12**	**W16**	**W20**	**W24**
Visit window(days)	**±7**		**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Informed consent	X									
Inclusion exclusion criteria	X	X								
Randomisation criteria and randomisation		X								
Medical History	X									
Physical examination	X									
Indirect calorimetry		X	X	X	X	X	X	X	X	X
Full blood count	X									
Renal profile	X									
Lipid profile		X								X
Liver function test	X									X
Thyroid function	X									
GLP1, GIP, PYY		X								X

Fasting glucose		X								X
HbA1c	X									X
Urine pregnancy test	X									
Height	X									
Weight	X	X	X	X	X	X	X	X	X	X
Waist, hip, thigh circumference		X		X	X	X	X	X	X	X
FFM, FM		X		X	X	X	X	X	X	X
Blood pressure	X	X			X	X	X	X	X	X
Diet counselling		X			X	X	X	X	X	X
Food Diary		X	X	X	X	X	X	X	X	X
Compliance check			X	X	X	X	X	X	X	X
IPAQ		X				X		X		X
Concomitant medication check	X	X	X	X	X	X	X	X	X	X
AE reporting		X			X	X	X	X	X	X

3.5 Study Outcomes

3.5.1 Primary Outcome

The primary outcome is the difference in change of weight, in absolute value (kg), between participants in the INT group (EE information plus SC) versus the SC group at baseline and 24-weeks after randomisation.

3.5.2 Secondary Measurements and Outcomes

Anthropometrics, weight change, body composition (fat mass, fat-free mass), obesity-associated comorbidities, changes in REE, RQ and substrate (fat and glucose) oxidation based on IC, will be measured, and determined throughout the study.

3.6 Data Collection

The following section outlines the data and biochemical evaluations being collected during the test periods (see **Table 3.2** for a summary).

3.6.1 Anthropometry

Assessment of anthropometric data will be obtained at screening, baseline, week 4, 8, 12, 16, 20 and 24 visits. Body weight will be measured and recorded to the nearest 100g following an overnight fast and will be captured twice on each occasion. The weighing scale used will be the same scale which will be calibrated and used throughout the study. Height will be measured twice to the nearest 1 mm with the average value taken and recorded from a stadio-meter at the screening visit. BMI will be determined as weight/height squared (kg/m^2). Body composition will be determined using the two-electrode leg-to-leg bio-impedance analyser machine (Tanita TBF-

300MA, Tanita Corporation, Tokyo, Japan) and the average value calculated. Participants will wear a light gown, and all external metal objects will be removed prior to measurement. Total body fat mass (%, kg), total body lean mass (%, kg) will be obtained. Waist circumference will be measured to the nearest 1 mm, using a measuring tape at the mid-point between the lower costal border and the iliac crest with the average of 3 measurements taken. In addition to the baseline anthropometric measurements and end of weight loss intervention (week 24), weight will also be recorded during dietetic visits and counselling to provide feedback to participants in both groups. Regular weight monitoring allows the study clinicians and participants to assist with weight monitoring, associated with improved success and compliance to weight loss intervention.

3.6.2 Biochemical measures

At screening, baseline, and end of study (week 24) visits, fasting (>8hours) venous blood samples will be collected by study clinician. Collected blood samples will be centrifuged (at 4°C, 4000 rpm, 10 min) to separate plasma or serum and stored at -80°C for subsequent analysis.

3.6.3 ECAL IC Protocol

Prior to each IC measurement, all participants will receive detailed preparatory oral and printed information. Participants will be asked to abstain from food, alcohol, and any calorie-containing beverages for at least 8 hours and refrain from vigorous physical activity for at least 12 hours prior to the measurement. Participants will arrive between 10 – 12 hours after an overnight fast before undergoing the IC test. Participants will be asked to confirm their fasting duration and will be reminded to

follow preparatory procedures prior to next visit. The ECAL indirect calorimeter (Metabolic Health Solutions Pty Ltd, Australia) used will be calibrated using the 5% carbon dioxide to calibrate gas flow, as per manufacturer specifications. Anthropometric data will be entered into the EC Health software linked up to the ECAL IC. Participant will be lying comfortably in a supine position and elevated at a 45-degrees angle. After 15 minutes, a nose clip will be applied over the nostrils and participant will be instructed to breathe through a single-use mouthpiece, ensuring a tight seal over it (as illustrated in **Figure 3.1)**. The test instructor will observe the volumetric flow and ensure that a steady volume and rate of breathing is maintained for between 8 to 10 minutes. A successful test will be defined as a steady-state achieved with a minimum of 5 consecutive minutes with less than 10% coefficient of variation in FEO_2 and $FECO_2$ (240). The instructor will stop the indirect calorimetry measurement when this steady state has been achieved. Should any interruptions occur or failing to achieve a steady state, a repeat testing will be performed.

Figure 3.1 ECAL™ Indirect Calorimeter: Nose clip applied over the nostrils. Participants lying in supine position whilst breathing through the single-use mouthpiece. Image provided and authorised for use by Metabolic Health Solutions, UK

3.6.4 Client Report

A client report will be generated using the EC Health software when the IC measurement is complete. A series of questions related to their time-specific metabolism will be completed and a client report generated for participant and

clinician in the intervention group only. An example of client report can be found in Supplementary Material, Appendix.

3.6.5 Resting Energy Expenditure

All participants including the SC and INT group will undergo ECAL IC after an overnight fast at baseline, week 2, 4, 8, 12, 16, 20 and 24 visits, using a mouthpiece whilst in a supine and rested state. The ECAL IC will be calibrated as described within the ECAL IC protocol section. All ECAL IC testing will be conducted in the morning in a temperature-neutral environment with participants lying in a comfortable supine position. During the measurement duration of between 8-10 minutes, participants will be asked to remain as relaxed as possible without falling asleep and instructed not to speak or fidget. The VO_2 and VCO_2 will be continuously measured for 8-10 minutes. After discarding the first 2 min of data, REE will be calculated as the lowest consecutive 5 min average value, provided that the coefficient of variation within that 5 min interval is <10%. The ECAL IC will calculate the REE based on the Weir equation (71). This information will be utilized by the dietitian to formulate a dietary plan and make recommendations of an energy-restricted diet for participants randomised to the INT group. In contrast, participants and dietitians providing care within the SC group will not receive this information but dietary estimate of total daily EE will be based upon the Harris-Benedict predictive EE equation.

3.6.6 Respiratory Quotient

Similarly, the ECAL IC testing conducted at baseline, week 2, 4, 8, 12, 16, 20 and 24 visits, will generate an RQ value alongside the REE. The IC will analyse the respiratory gas exchange of the participants to determine VO2 and VCO2 and the ratio of VCO2 to VO2 (VCO2/VO2), will determine the RQ. RQ will be utilised as an indicator of measurement adequacy and of substrate oxidation. Based on the gas

exchange dataset, the ECAL IC will generate information on carbohydrate oxidation (%) and fat oxidation (%). This information will be utilized by the dietitian to formulate a dietary plan and make recommendations of an energy-restricted diet for participants randomised to the INT group.

3.6.7 Dietary Analysis

Participants will be provided instructions on completing the 3-day food diary. Participants will be provided with cups, spoons, or utilise a food scale to accurately determine food portion sizes. Participants will be asked to record estimate volumes and portion sizes. Issues regarding estimating portion sizes and measuring foods will be discussed. Participants will be informed to record two weekdays and one weekend day before their next study visit and were told to provide exact brand names where possible. Participants will be encouraged to select days when their normal routine was least likely to be disrupted and were instructed to record meals eaten away from the home to the best of their ability. All food diaries will be checked by the study dietitians for missing data or obvious errors in recording. Adherence to the energy-restricted dietary recommendations (30% below total daily EE) will be assessed using the food diary 3 x 24-hour dietary recalls together with the dietician at study visit (baseline, week 4, 8, 12, 16, 20, and 24). The threshold of compliance to recommended dietary restriction based on self-reported food diary will be set at >80%. All participants will be asked to return the food diary and provided a reminder to bring along the subsequent food diary at the following visit.

3.6.8 Activity Energy Expenditure

Physical activity will be evaluated based upon the self-reported International Physical Activity Questionnaire (IPAQ) long form (241) at baseline, week 12 and week 24. Participants will be asked to record the duration and frequency of mild-, moderate- and vigorous-intensity activity levels within the past 7 days. The hours spent in sleep and mild-, moderate- and vigorous-activity were multiplied by respective metabolic equivalents (METs), summed, and finally expressed as total MET-h/week. The data on physical activity from self-reported IPAQ captured will be used to calculate energy recommendation for weight loss.

3.7 Data management

All study participants will be given a unique identifier which will be utilized to identify their electronic and paper-trail data and biochemical lab samples. The database will be stored on a password-protected computer, accessible to the study researchers only, containing participant identifiers and their associated unique identifier. Paper based data will be stored securely at the Research & Development Centre, Clinical Sciences Building, University Hospital Aintree, Liverpool, United Kingdom for 15 years, after which it may be destroyed. Case report forms will be utilised to compile and collect data at all study visits. Before analysis, data will be compared between files to ensure accuracy of transcription. Biological samples including blood tests will be stored in a secure swipe card access secured, -80°C freezer, with a temperature logbook and alarm system that alerts a staff member should temperature rise above a predetermined set range. Every sample stored in the freezer will be recorded in the logbook of biological samples. Samples will be stored for up to 15 years from collection date and disposed of accordingly after that time.

3.8 Data Monitoring

The study will not have a formal data monitoring committee as adverse events of treatments are well known within weight loss interventions within a specialist healthcare setting. Any unexpected serious adverse events or outcomes (such as incapacitation or death) will be discussed by the trial management committee (identical to the authors of this protocol). Furthermore, the trial management committee will monitor recruitment, treatment and attrition rates and any concerns related to the study.

3.8.1 Protocol deviations

Deviations from proposed study protocol will be communicated to the study sponsor at University of Liverpool / Liverpool Joint Research Office and the Research & Development Unit based at University Hospital Aintree, Liverpool.

3.8.2 Adverse events

Adverse events will be captured onto the case report forms and will be reported to the University of Liverpool / Liverpool Joint Research Office and the Research & Development Unit based at University Hospital Aintree, Liverpool. Adverse events that lead to participant withdrawals will be reported in any future publications. We do not intend to formally analyse adverse events.

3.9 Statistical Analysis Plan

Statistical analysis will be performed using IBM SPSS Statistics for Windows V.24.0 or later. All variables will be checked for plausibility and missing values. Outcome data will be presented as mean (SD) for continuous variables and counts (percentages)

for categorical variables. The characteristics of completers will be tested using independent *t*-tests for between group analyses and paired t-test for within group analyses. A non-parametric counterpart will be used if data are not normally distributed. Chi-squared (χ^2) tests will be utilised for categorical variables. The primary analysis will utilise linear mixed models to assess impact of intervention (EE information plus SC vs. SC alone) on the net change in body weight (baseline, week 24), in absolute terms (kg) between both groups. The covariates age, sex and BMI will also be included in the primary analysis model. Both intention-to-treat (ITT) and per-protocol analyses (for those who achieve a minimum of 5 out of the 9 study visits with completion of IC tests as defined in the 'study intervention') will be completed. Where main effects are identified, Bonferroni post hoc tests will be performed to identify significant differences between means (a significant p-value set at <0.05). While the ITT analysis will be the main analysis, the per-protocol analysis will allow us to determine that the true efficacy of the intervention for participants who strictly adhered to the protocol.

The secondary analyses will include change of body composition (body fat %, fat-free mass), change in REE, change in RQ with time (treated as categorical with levels at baseline, 4, 8, 12 and 24 weeks), and treatment-by-time interaction. We did not undertake formal sample size calculation for secondary analysis as the study was powered to the primary endpoint only. As a result, this will provide data as exploratory analysis only. Type I error will be modified by utilizing Bonferroni adjustment or the equivalent non-parametric test. Age, body mass index, fat-free mass, obesity-related comorbidities (King's Obesity Staging Criteria), quality of life outcomes and glycaemic variability will be examined to determine whether they contribute

significantly to the models. If a covariate is significant, a term will be added to the model to adjust for the effects and two-way interactions with time and treatment will also be examined. If these interactions are also significant, they will be similarly adjusted for in the model using analysis of covariance (ANCOVA).

3.9.1 Data access

There are no contractual agreements that require the data from this trial to be shared.

3.10 Ethics & Dissemination

Ethics approval was obtained from the Health Research Authority and the Northwest Research Ethics Committee (18/NW/0645) and is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP). Participants will also receive a copy of their individual results including anthropometric data, body weight change and results of EE and RQ from ECAL IC tests. They will also be provided with a lay summary of overall results of the study. Findings from this study will be disseminated at relevant scientific conferences and the data published in research manuscripts in peer-reviewed journals.

3.11 Discussion

The study goal is to assess the impact of utilising REE and RQ from IC as an adjunctive management for weight loss. The role of utilising EE information to influence behaviour has yet to be evaluated within a real-world pragmatic RCT. Further, the results will help advance existing evidence about the relationship between RQ, REE and change in body weight.

Several experimental studies evaluating change in REE as a response to overfeeding (i.e. 40-70% increase from baseline energy requirements) suggested that short-term (2-8 weeks) overfeeding produces less weight gain than expected from the controlled increase in intake, due to greater increase in REE (242-246). Such compensatory increase in energy intake led to weight increases, of which 55-67% constitutes fat mass gain (242, 243, 247, 248). Conversely, underfeeding studies reveal that in short-term, intentional weight loss leads to decrease in EE beyond predicted values(44, 249, 250). Decrease in REE is disproportionate to weight measured per kilogram against FFM after weight loss. The regression analysis of change in REE against the change in FFM reveals that decline in REE tended to be greater than accounted for by loss of FFM. Such over-compensatory metabolic changes act to oppose further weight change (251).

Variability in substrate oxidation between individuals may be an inherent variability that contributes towards weight regain and fat storage. Variability of macronutrient composition intake between participants may influence the measured RQ and influence the results associated with fat vs. carbohydrate oxidation(252). Several studies have suggested macronutrient alterations could also influence appetite-regulation (253, 254). Further, individual differences in circulating insulin levels or sensitivity may confound the reported associations between RQ, REE and fat mass(65, 255, 256). We have taken this into account and have therefore excluded patients with diabetes mellitus or metabolic disorders.

IC use has been validated through several detailed experimental in vitro and in vivo studies, demonstrating accuracy of measuring EE when compared to the mass spectrometry and Douglas Bag method (257). Given that previous studies on other

commercial metabolic monitors showed variations in gas exchange rates (258), leading to secondary metabolic results variability, participants will be invited to strictly adhere to the protocol requirements and all measurements performed under controlled and steady-state experimental setting.

3.11.1 Strengths

There are several strengths to the study methodology, particularly the randomized controlled trial design. This will be the first RCT to assess whether providing EE and RQ from ECAL IC will influence the outcome of weight loss intervention when integrated with communication between the clinician and the patient. The study is powered statistically to evaluate weight change within the primary analysis model. The study has the potential to expand our understanding of how EE information can be integrated into communication and behaviour modification aspects of weight management clinics, while providing assessment of detailed secondary outcome measures including but not limited to body composition, EE, substrate oxidation, quality of life outcome measures. Further, the secondary analysis provides a comparison between the measured REE from IC with that of predicted REE from Harris-Benedict equation which serves to enhance our understanding of accuracy and practicality of use of ECAL IC to help determine weight change in obesity. One of the challenges with any weight management study is to maintain high levels of motivation and engagement. We have been successful in recruiting large number of participants for similar weight loss intervention trials and plan to provide support for participants with routine appointments with the dietician and multidisciplinary weight management team.

3.11.2 Limitations

Whilst the ECAL IC is purpose-built as a portable, convenient and practical device designed to capture EE information in users over a relatively brief time period, ECAL IC provides a wider degree of variation and greater limits of acceptability in the repeatability of the REE, RQ and substrate oxidation measures during steady state when the device is compared against other IC machines using Deltatrac as the standard reference method (79). In addition, the measured REE will be influenced by age, sex, body size and variation in individual physical activity levels (259, 260). Under strict controlled research lab environments with overnight fasting, the EE information obtained are accurate within the time constraints of those settings (steady state & fasting) only, but may not reflect the day-to-day fluctuations in the assessment of energy cost of participants due to energy cost of physical activity, change in energy intake and macronutrient dietary composition (261). The utility of IPAQ as a self-reported indicator to determine physical activity level in people with obesity showed a weaker correlation, and tended to overestimate activity levels when compared against reference standards using an accelerometer or pedometer (262). A recent systematic review reported that increased exercise and activity levels, including short-term moderate- to high-intensity exercise training was associated with only modest changes in body composition (263). The reliance on bioelectrical impedance analysis to capture data on body composition may result in a wide degree of variation in capturing data on fat-mass and FFM when compared against the use of body densitometry.

3.12 Conclusion

This randomized controlled trial was designed to evaluate the impact of providing IC-guided dietary intervention versus SC over the 24-week duration on the effectiveness of weight loss outcomes. This 6-month RCT will provide practical feedback on

acceptability and applicability of utilizing the portable ECAL IC to facilitate weight loss strategies as part of the multicomponent weight loss intervention. This study will serve as a feasibility study to assess the efficacy of incorporating strategies for weight loss using EE and RQ as basis of dietetic advice and provide recommendations which will inform the design of future weight management trials.

4. CHAPTER IV – Efficacy of Energy Expenditure Information from Indirect Calorimetry on Weight Outcomes

Contribution: Jonathan Lim contributed to the conception and design of the study and made a major contribution to the recruitment of subjects, indirect calorimetry assessments, anthropometric and biochemical assessments, all statistical analyses and writing of this chapter.

To be submitted for publication

4.1 Abstract

Background: Indirect calorimetry (IC) provides non-invasive measurements of energy expenditure (EE) including the resting metabolic rate (REE) and substrate oxidation rates (via a respiratory quotient (RQ) derived from gas exchange). During a dietary intervention, with energy restriction, IC provides patient feedback on evolving changes in REE and RQ. This takes account of the dynamic, non-linear, relationship between weight change, body composition and EE and may improve intervention compliance, and hence, enhance weight loss.

Objective: To determine the effectiveness of utilising EE information from indirect calorimetry (IC) during weight loss intervention in obesity.

Design and Methods: An assessor-blinded, parallel-group randomised controlled trial. Participants were all counselled on a dietary restriction with a calorie deficit and randomised to 1) **Standard-of-care (SC)** with caloric deficit based on calculated total daily energy expenditure (TDEE) from standard equations *vs.* 2) **IC-guided care intervention (INT)**: with caloric deficit based on EE information provided by a patient-facing, portable IC device.

Results: Fifty participants (age 46±10y, BMI 42 ± 3 kg/m^2, body fat 48±2%, female n=41, 82%) were assessed at 24 weeks. There was greater weight loss in the INT group (3.64±1.72kg) vs SC group (1.48±1.32kg; p<0.001). After adjustment for BMI and gender, there remained a significant difference in mean weight loss between SC vs INT group (p<0.001). Concomitantly, reduced lean body mass (SC: 0.3±2.7kg vs INT:1.1±3.4kg; p=0.355) and reduced total body fat-mass (SC: 1.0±3.7kg vs

INT:2.5±3.4kg; p=0.178) was observed. At baseline there was no difference in measured REE (SC: 1896±222kcal/day vs INT: 1932±264kcal/day; p=NS), with no difference observed at week 4, 12 and week 24 (SC: 1866±196kcal/day vs INT:1788±234kcal/day; p=0.203). At baseline, RQ (SC: 1.016±0.101 vs INT: 1.018±0.099; p=NS) was similar between groups. However, after 4-weeks there was a greater decrease in RQ in the INT group (RQ-WK4 (SC: 0.928±0.106 vs INT: 0.856±0.087); p=0.012). However, the significant reduction in RQ was not maintained at 12-weeks (RQ-WK12 (SC: 0.904±0.053 vs INT:0.880±0.048); p=0.099) and 24-weeks (RQ-WK24 (SC: 0.988±0.049 vs INT:0.973±0.071); p=0.382)). No correlation was found between the RQ-WK4 with change in body fat mass; p=0.742). During the negative energy balance (deficit of 500 kcal/day), decrease in RQ was observed compared to baseline, however, the change in RQ was not sustained beyond the initial 4-week duration.

Conclusion: Providing real time, evolving EE data through IC facilitated greater weight loss. Decrease in RQ from baseline may be indicative of increased fat oxidation during the acute energy deficit phase, may improve compliance and achieve success in personalized weight loss goals.

4.2 Introduction

Diet restriction is commonly recommended for the management of obesity. Common diet strategies applied to tackle obesity include variations in degree of energy restriction, often accompanied by promotion of health behaviours (264). Despite the potential for successful weight loss, many people with obesity struggle to maintain the lifestyle interventions (265). Lack of success in long-term weight loss through energy restrictions and increased energy expenditure is predominantly due to poor compliance, lack of motivation to adhere to the prescribed dietary intervention (61, 92, 266). More recently, emerging evidence have suggested this lack of response may partly be attributed to metabolic adaptations to weight change.

Diet restriction with low-calorie diet (LCD) and very-low calorie diet (VLCD) often results in less than predicted weight loss (163, 267). Emerging evidence have demonstrated decreased REE in response to caloric restriction in people with obesity (251, 268). In response to conditions of negative energy balance with diet restriction, some studies have suggested the proportion of decrease in EE is often disproportionate to the actual reduction in fat-free mass (FFM) (36, 251) in people with obesity. During restriction of energy intake to lose weight, all components of the energy expenditure change. The change affects the REE, metabolic rate of AEE and TEF. The dynamic energy balance model (269) as described in Chapter One of this thesis suggests that in conditions of sustained caloric deficit, the marked reduction in EE may be more than the actual loss in the fat-free mass (FFM) during weight loss, hence reducing the actual rate of weight loss. However, there remains a gap in understanding about the role of REE in response to changes in energy balance, given the variability in contribution of FFM on energy balance (270, 271).

Hall et al. (56) evaluated the effects of restricted energy intake required to stimulate a typically expected weight loss trajectory by incorporating a mathematical model. Theoretically, the '3,500 kcal' rule (272) which stipulated that a 'caloric deficit of 3500 kcal leads to one pound of weight loss' is an oversimplified model with the assumption of 'static' energy requirements of the body (272). This generic 3,500-kcal rule is clearly reliant on a 'static' or 'linear' association between energy intake and energy expenditure, but a validated *dynamic* model (273) of weight loss more accurately predicts the weight loss response. The *dynamic* model is based upon the first law of thermodynamics, influenced by the baseline body composition, age, height, gender and the extent of caloric restriction, resulting in the curvilinear weight loss over time rather than the predicted linear pattern from the 3,500-kcal rule (272).

Accurate prediction of the trajectory of body weight loss would need to properly account for dynamic energy imbalances, particularly the baseline body fat mass (FM) and fat-free mass (FFM) on energy intake (270). The significant reduction in REE (adjusted for FFM loss) because of caloric restriction supports the principle of adaptive thermogenesis in response to decreased caloric intake. Major et al (161) reviewed the evidence for adaptive thermogenesis in the context of energy restriction in people with obesity. There was wide variability in responses towards adaptive thermogenesis, partly due to the lack of adherence to caloric restriction but also wide variability in change in FFM and/or reduced EE in response to weight loss. These findings were corroborated by Weyer et al. (251) where adaptive thermogenesis was discovered, but in only half of the women (26/48 participants) in the study (-3.2±1.2 kcal/kg FFM).

Long-term weight maintenance in twenty-nine studies was systematically reviewed by Anderson et al (163) in the context of intensive dietary intervention in obesity. Most of the studies demonstrated that most people with obesity regain more than >50% of their body weight within the first year of follow-up (163). Substantial weight loss is observed through various dietary intervention but the ability to maintain and prevent weight regain remains elusive (274). A persistent caloric deficit, sustained over time creates an energy deficit. The negative energy balance may be dependent upon the energy stores of the individual. In people with obesity, greater body adiposity and larger energy stores would mean less weight loss for the equivalent deficit in energy intake, and before reaching a steady-state weight (56). Greater fat-free mass (FFM) in people with obesity have been associated with greater *ad libitum* food intake (275). To achieve a state of energy homeostasis, the energy intake of the individual is closely associated with their metabolic rate. The REE itself is closely linked to the height, body size and contributes to a large part the variance in REE (up to 80%) may be explained by the FFM (40). FFM consists of active metabolic tissue (skeletal muscle, tissue parenchyma). In obesity, increased body adiposity may strike an imbalance between FM and FFM distribution. More recently, the longitudinal study demonstrated that FFM, but not FM or BMI had positive associations with energy intake (276). There remains a current lack of understanding on how the increases in FFM are associated with increase in total energy requirements, resulting in increased energy intake. Hence it is critical to evaluate the baseline FFM and evaluate the changes in REE in relation to dietary intervention (37, 277, 278) to best understand and predict the response to weight loss.

We postulate that the addition of EE information from the portable open-circuit indirect calorimeter (IC) would promote better adherence to lifestyle-based weight intervention. The 'patient-centred' portable indirect calorimeter serves as an adjunct tool to provide real-time EE information (279) to both clinicians and patients to establish the necessary energy restriction and EE required to attain weight loss (79, 280). Further, emerging evidence suggests that the RQ data measured during a resting and overnight fasted state from IC was associated with the state of energy balance of the individual (205, 281). The RQ data also serves as a surrogate marker for the ratio of fat oxidation (179, 282, 283).

4.3 Objectives

4.3.1 Primary objective

The primary aim of the study is to determine the effectiveness of providing indirect calorimetry-guided energy expenditure information to influence the outcome of weight loss in patients with obesity without diabetes receiving dietary restriction, exercise, and behaviour modification therapy.

4.3.2 Secondary objectives

The secondary objectives are to compare the health outcomes in terms of:

iii. association of weight change with the change in measured respiratory quotient

iv. association of weight change with change in measured resting energy expenditure

4.4 Participants and Methods

4.4.1 Participants

Fifty participants with obesity participated in this randomised controlled trial. Participants were recruited from trial advertisement and via referrals from primary care to our specialist weight management service (SWMS). The study protocol was approved by the North-West Research Ethics Committee (18/NW/0645). All participants provided written informed consent. The study was performed in line with the Declaration of Helsinki. Eligible participants with obesity (BMI ≥30kg/m^2), without diabetes who expressed an interest or were willing to undergo a weight loss program were invited to participate in the randomised controlled trial. Participants were deemed eligible if they did not have planned or previous bariatric surgery, were not taking any anti-obesity medications or pharmacotherapy that would influence respiratory gas exchange (such as anti-obesity medications, psychotropic drugs and chronotropic agents), and did not have any form of chronic illness (e.g. stage 3-5 chronic kidney disease, end-stage liver disease, severe chronic obstructive pulmonary disease, congestive heart failure, thyroid dysfunction, cancer), or advanced cardiovascular disease. All participants underwent a thorough medical history plus physical examination, biochemical investigations prior to enrolment into the study. All participants had stable body weights (<5 kg change) over the past 3 months and did not consume any medications known to influence metabolic measures of energy expenditure. All participants were also non-smokers and abstained from intense or vigorous physical activity up to 24 hours before each indirect calorimetry testing. Participants were randomly allocated to 2 groups: (i) standard-of-care group (SC) or (ii) indirect calorimetry-guided care (INT) group. The SC group participants receive the routine weight management care as part of the local SWMS. Both the participants

and clinicians in the INT group receive the additional energy expenditure information during their patient-clinician interactions over the entire 24-week duration of the weight loss intervention. To balance the group allocations, the randomisation was stratified according to the baseline body mass index (BMI) to obesity and severe obesity.

4.4.2 Experimental Design

All participants in both arms were given similar visit intervals with up to 8 visits during the 24-week weight management programme. Participants were required to adhere and agree to strict study protocol including the need to confirm that they have adhered to the overnight fasting state and avoided any vigorous or intensive physical activity for the last 24 hours prior to the study visit. All participants underwent the indirect calorimetry measurements during the 24-week intervention including those in the SC and INT group, but only participants in the INT group would receive the energy expenditure information generated from the indirect calorimeter. Participants underwent assessments of body composition using the bioelectrical impedance analysis (TANITA, Tokyo, Japan) at baseline, and three subsequent visits.

The study intervention was that both participants and clinicians delivering care in the INT group received the summary of energy expenditure information from the indirect calorimeter whilst utilising this information during tailored discussions with specialist dietitians or clinicians conducting the dietetic interview(s). INT group participants would receive this information in the form of a layperson summary, handed out to the participant at the end of each study visit. In comparison, participants allocated to the SC group would also undergo the indirect calorimetry measurements, but the energy

expenditure information would be withheld from both the patient and clinicians providing care, until the total completion of the 24-week duration. Given that energy expenditure information was not made available from the indirect calorimetry, specialist dietitians will calculate each participant's daily energy requirements based upon the Harris-Benedict predicted REE formula (REE_{HB}) (284).

4.4.3 Diet

All participants received a recommendation of 500kcal/day energy deficit based on the total daily energy requirements calculated as described in our study protocol (285). Participants were instructed to record daily energy intake comprising of full dietary intake for at least two weekdays and at least one day on the weekend during the two weeks prior to the study visit. Where possible, alternative options to paper food diaries were recommended to participants including the use of the MyFitnessPal mobile application which allows participants to chart their daily energy intake and determine the time that they participated in physical activity or exercise during the week. Based on this information (both paper and/or electronic version of food diaries + physical activity levels), the clinician or specialist dietitian utilized the information to calculate the average caloric intake per day over each week (averaged over the course of the last 7 days prior to the dietetic visit). Participants were given prompts and reminders to complete their food diary via telephone calls and were each given a paper folder (containing the printed template of food diary) to bring for each study visit. At each study visit, the clinician or specialist dietitian will review the contents of their weekly dietary intake and physical activity levels with the aim of attaining their personalised weight loss target. Further, INT group would have the additional prompt of utilising the energy expenditure information to evaluate the energy balance state and evaluate

ways to improve and promote greater fat oxidation based on the measured respiratory gas exchange information from the indirect calorimeter. Dietary advice was provided to change eating patterns and habits based on the information gathered and provided by the participants from the two-weeks prior to the study visit.

4.4.4 Activity Energy Expenditure

Participants were instructed to increase their physical activity by engaging in habitual activity patterns like increased duration of walking, running, and engaging in sporting activities. Participants were instructed to aim for a minimum of 150 minutes/week of physical activity in line with current national recommendations. All activity measures were reported by participants on the International Physical Activity Levels Questionnaire (IPAQ) at baseline, mid-study and at W24.

4.4.5 Outcome measures

The study outcome measure was reported based on the anthropometric measurements during baseline, and after 4 weeks, 12 weeks and 24-weeks post-enrolment. The primary outcome was the overall change in body weight (in kg) measured at 24-weeks post-enrolment. The secondary outcome measures were anthropometric data (including FM, FFM, and waist circumference), biochemical results, daily energy intake, and physical activity levels at several time points described above.

4.4.6 Anthropometric Measurements

The body weight was measured using the calibrated weighing scale (to the closest 0.1 kg). Height was measured with a wall-mounted stadiometer (to the closest 0.1cm). BMI was calculated according to the following formula: weight (kg)/ height $(m)^2$. The

waist circumference (WC) and hip circumference (HC) were measured with a non-stretchable tape measure and recorded to the nearest 0.1 cm. We utilised the two-electrode leg-to-leg bio-impedance analyser (BIA) (TANITA TBF-300MA, Tanita Corporation, Japan) device to determine the total FFM (%, kg) and FM (%, kg). All these anthropometric data were captured whilst the participants were lightly dressed.

4.4.7 Metabolic Measurements

All participants underwent measurements of REE in a fasted state via the indirect calorimetry method (ECAL indirect calorimetry, Metabolic Health Solutions Pty Ltd, Australia). All participants who underwent the REE measurements had to confirm that they had fasted overnight (≥8 hours fasted), abstained from any form of intensive physical activity for at least 24 hours, and were not taking any medications or pharmacotherapy that would influence their EE measurement on the day of their visit. Participants attended the study visit between the hours of 08:00am-10:00am in the morning for the study visit. After the anthropometric data were obtained, participants remained in a supine position for 20 minutes prior to the ECAL indirect calorimetry measurement. Current recommendation for the ECAL IC measurement is performed via a mouthpiece and nose clip. This would allow measurements of airflow and the volume of oxygen and carbon dioxide concentrations of the inspired and expired air. This ability to determine and accurately measure REE is critically important. During this time, the flow meter and gas analyser were calibrated per manufacturer's instructions using 5% carbon dioxide. During the indirect calorimeter measurement, the gas samples were collected breath by breath for 10-15 min on the supine position with an open-circuit one-way airflow mouthpiece whilst the participant donned a nose clip over the nostrils. The first 2 minutes of data were discarded. The volumetric flow

rate recordings were visually inspected and the smoothest 5-min interval data in the steady state condition were chosen to be used in the calculation of REE using the abbreviated Weir formula (286). The volume of inspired oxygen (VO_2), volume of carbon dioxide expired (VCO_2) and the RQ (the ratio of VCO_2/VO_2) were determined. The participants were instructed to abstain from sleeping, talking, and fidgeting during the duration of the assessment. The temperature of the room was maintained at 21-24 degrees Celsius to the best of our ability and ambient noise and lighting were minimized.

4.4.8 Self-reported Energy Intake

Participants were provided with paper-based records of food diary or utilise the MyFitnessPal smartphone app to report their energy intake. Energy intake data were retrieved from paper food diary records and reviewed with the study dietician. Where possible, participants will be asked to record precise information about any or all food intake, portion sizes, time of intake, including drinks and beverages for at least two weekdays and one weekend day prior to study visit with specialist dietitian review. The average total daily energy intake of participants was calculated based on the average intake on the week prior to attending the study visit. The threshold for successful adherence to energy intake recommendations was defined by a minimum of 50% overall compliance to the recommended energy restrictions, a threshold utilised by previous studies (287).

The recommended energy restrictions were based upon the individuals measured REE for participants allocated to the INT group. In comparison, participants in the SC (control) group were provided recommendations of energy restrictions based upon the

estimated REE calculated from the validated predictive energy equation (Harris-Benedict equation).

4.4.9 Physical Activity Levels

Physical activity and sitting time were also measured with the International Physical Activity Questionnaire (IPAQ) short version at baseline, mid-point and at the final follow-up. The physical activity level (PAL) was expressed as the time spent walking, performing moderate-intensity and vigorous-intensity physical activity (PA) expressed as minutes/week in each of those domains.

4.4.10 Sample Size Calculation

The sample size was obtained assuming an interaction of a between-group difference of 2.5kg based on a two-sided test with a fixed power of 80% and alpha of 0.05 with comparison to previous data from our SWMS (238). Hence, 42 participants in each group would provide 80% power to detect a difference of 2.5 kg or more at the 5% significance level. Due to national restrictions and social distancing measures from COVID-19, the study recruitment was suspended, and an interim mid-point analysis was conducted. We report the data obtained from the 50 participants that have been successfully enrolled into this study.

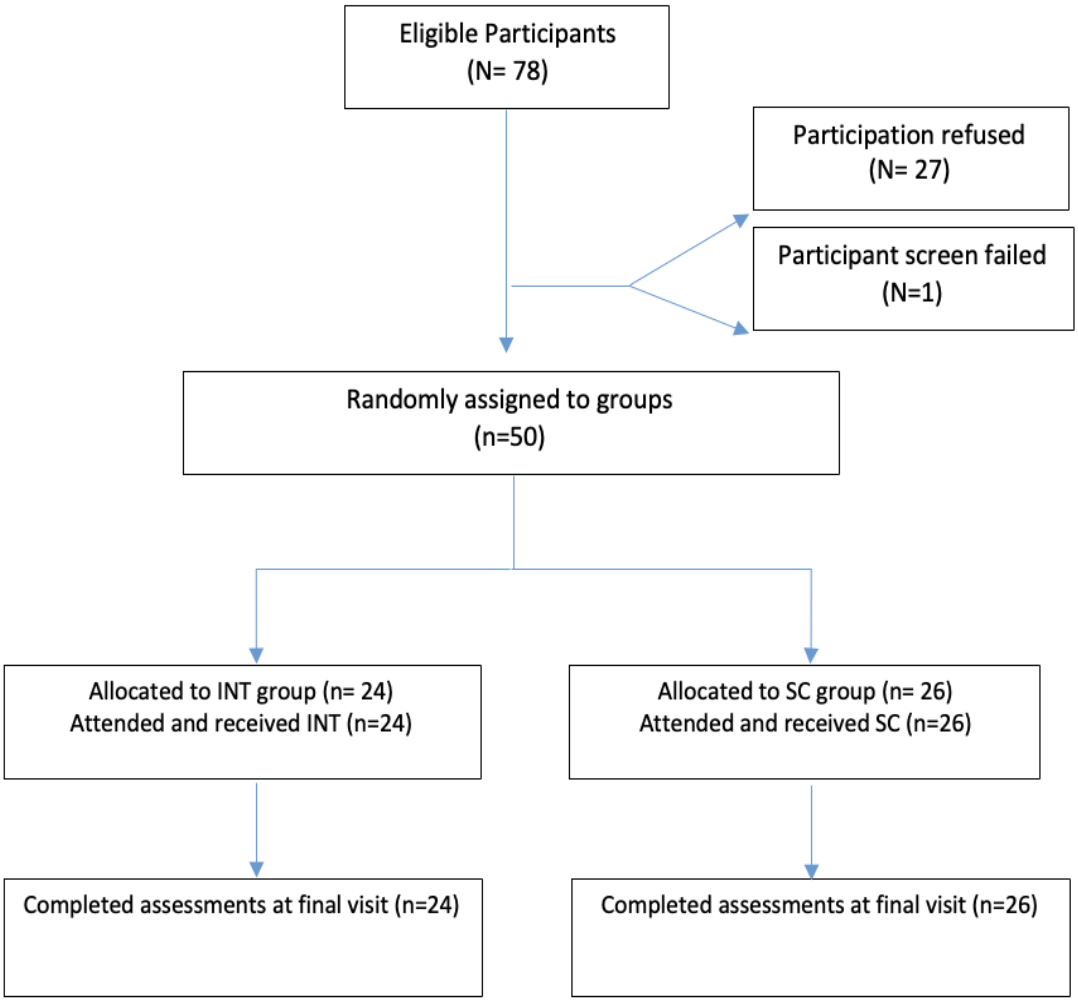
4.4.11 Statistical Analysis

Results are presented as mean ± standard deviation (SD) for parametric variables, while data from non-parametric variables are presented as median and inter-quartile range (IQR). The Kolmogorov-Smirnoff test was performed to assess for normality. An independent samples *t* test was used to evaluate the baseline differences between

the standard care (SC) vs the intervention (INT) group. The Mann-Whitney U test was performed for non-normally distributed variables, and Chi-squared test for categorical variables. Independent samples t-test was performed to compare the baseline with the week-24 follow-up body weight in absolute terms, kg and in terms of BMI, expressed as kg/m^2. To reduce the influence of within group variability, we performed a univariate test of significance (ANCOVA), with the dependent variable as the net change in body weight (baseline, week 24), in absolute terms (kg) between both groups, while adjusting for the baseline BMI as a covariate. Post-hoc analyses were performed using the Bonferroni test.

Analysis of dataset was performed based on the intention-to-treat (ITT) analysis, with last observation carried forward for missing data at W24. All differences were considered significant at $P < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences version 25 (SPSS Inc., Chicago, Iln, USA). Across all tests, statistical significance was defined as $p < 0.05$ (2-tailed).

Figure 4.1: Flow chart of participants



4.5 RESULTS

4.5.1 Participants Characteristics

At baseline, recruited participants had mean age of 46.7 ± 10.4 years, mean BMI of 42.3 ± 3.3 kg/m^2 and were predominantly women (n=41, 82%). Within this cohort of 50 study participants who were enrolled into the study, and one participant was excluded due to new diagnosis of diabetes mellitus. The recruitment flow chart for the study is illustrated in **Figure 4.1**.

The characteristics and body composition measurements of study participants are presented in **Table 4.1**. The anthropometric measurements and clinical characteristics or participants were not different between the study participants in the SC vs INT group, as summarized in **Table 4.2**.

The primary outcome was the change in body weight (in absolute terms, kg) between the SC vs the INT group after 24-weeks post-enrolment. The data was analysed according to the intention-to-treat (ITT) analysis. After completing the 24-week study duration, the mean body weight decreased in all subjects (Δ = -2.54 kg, CI: -3.07, -2.00, *P*<0.001). At the end of the 24-week period, a greater decrease in mean body weight was observed in subjects within the INT group (Δ = -3.64 kg, CI: -4.36, -2.91, *P* <0.001) as compared to the SC group (Δ = -1.52 kg, CI: -2.10, -0.94, *P* <0.001) (**Table 4.3**). After adjustment for baseline BMI, there was a significant difference in mean weight loss (p<0.001) between the SC and INT groups. After adjustment for baseline BMI and age as covariates, and gender there was also a significant difference in mean weight loss between the SC and INT groups (p<0.001).

Weight loss was accompanied by concomitant decrease in in FFM (SC: Δ -1.33 kg; CI: -2.25, -0.40, *P*=0.007; INT: Δ -2.00 kg; CI: -3.21, -0.78, *P* =0.002) and FM (SC: Δ -1.78 kg; CI: -2.78, -0.78, P=0.001; INT: -2.41 kg, CI: -3.40, -1.42, *P* <0.001) at 24-weeks.

We performed comparisons between the changes in anthropometric data using ANCOVA adjusting for baseline weight as a covariate (**Table 4.4**). We report that there were significant differences in terms of reduced waist circumference (P=0.012), decreased waist-to-hip ratio (P=0.047) at 24 weeks. Based on the paired samples *t* test comparing the fat mass at baseline and at W24, the whole cohort of participants had significantly lower fat mass with a loss of 1.70 ± 3.48kg of fat mass (P=0.001) [0.68 ± 2.01%; P=0.020] but did not demonstrate a significant change in terms of the fat-free mass 0.70 ± 2.94kg (P=0.100) [0.50 ±2.47% (P=0.158). In addition, we calculated the odds of participants achieving a minimum of 2kg weight loss at W24 and discovered that participants within the INT group were almost 11 times more likely to achieve the 2kg threshold as compared to the SC group (odds ratio (OR) = 11.33, *P*<0.001, 95% CI [2.93-43.78]). Alterations in energy expenditure dataset at baseline, W4, W8, W12 and W24 were compared and summarised in **Table 4.5**. The repeated measures ANOVA comparing REE between the different time intervals indicates there was a significant effect of time (P=0.014) but no interaction effect of time and the between-subjects factor (P=0.72).

4.5.2 Food intake and energy balance

Both groups reported a reduction in their overall energy intake as compared to baseline visit. The INT group reported a greater decrease in average daily energy intake (206.8 ± 168.0 kcal) as compared to the SC group (154.7 ± 193.6 kcal; P<0.001). Adjusting for baseline body weight, one-way ANOVA demonstrated that the net deficit in energy intake had a correlation with the change in body weight (P=0.005).

4.5.3 Resting Energy Expenditure

Participants in both the SC and INT groups did not demonstrate a significant reduction in measured REE (via indirect calorimetry) and predicted REE (based on the Harris-Benedict equation) from baseline measurements to week 4, 12 and week 24. After adjusting for baseline BMI, baseline measured REE had an inverse correlation with change in body weight in absolute terms (kg) after 24 weeks (P<0.001).

4.5.4 Respiratory Quotient

To evaluate the association of the measured RQ, we evaluated the proportion of patients who had achieved the target 3% weight loss at week 24. Based on the 50 participants who had completed both baseline and week 24 (end of study visit), We predetermined the dependent weight loss outcome of ≥3% body weight at the week-24-follow-up and compared the proportion of participants who achieved or failed to achieve the weight target according to the measured RQ by categorising them into two groups: (i) low RQ (<0.90); (ii) high RQ (≥0.90). We performed a 2 x 2 table comparison and reported the Pearson Chi-squared test (P value) and odds ratio (OR) of achieving the ≥3% weight loss against that of measured RQ at baseline, week 4, 12

and week 24. Based on the baseline measurements, there was no significant difference between participants with low RQ vs high RQ (Pearson Chi-square P=0.242). Based on the data at week 4, the odds of achieving 3% weight loss were greater in those with low RQ as compared to high RQ [OR = 6.8 (95% CI 1.6-28.5; P=0.005]. Based on the data at week 12, the odds of achieving 3% weight loss were greater in those with low RQ as compared to high RQ [OR = 4.1 (95% CI 1.1-15.6; P=0.031]. Based on the data at week 24, the odds of achieving 3% weight loss threshold were greater in those with low RQ as compared to high RQ [OR = 3.8 (95% CI 1.1-13.8; P=0.035]. (**Table 4.5** and **Table 4.6**)

4.5.5 Activity Energy Expenditure

Activity Energy Expenditure (AEE) of individuals were compared between baseline, mid-study and at the end of the 24-week intervention (**Table 4.7**). The IPAQ questionnaires were analysed and the three variables of physical activity duration of walking (min/week), moderate-intensity (min/week) and vigorous-intensity physical activity (min/week). At baseline, no significant difference was found between SC vs INT in terms of duration of walking (P=NS), moderate intensity activity (P=NS) and vigorous-intensity activity (P=NS). At week 24, there was still no significant difference between the SC vs INT group in terms of walking, moderate- and vigorous-intensity physical activity (P=NS).

Table 4.1: Baseline characteristics

	All participants, n=50	SC, n=26	INT, n=24
	Mean ± SD	Mean ± SD	Mean ± SD
Age, years	46.7 ± 10.4	48.0 ± 10.6	45.3 ± 10.1
Female part., no. (%)	41 (82)	22 (85)	19 (79)
Weight, kg	115.0 ± 14.4	115.5 ± 12.4	114.4 ± 12.8
BMI, kg/m2	42.3 ± 3.3	42.6 ± 2.7	41.6 ± 3.5
Waist circ., cm	122.7 ± 11.7	122.0 ± 11.6	123.5 ± 12.1
Waist-to-hip ratio	0.962 ± .069	0.940 ± .057	0.980 ± 0.071
Body fat, %	47.7 ± 2.0	48.0 ± 2.0	47.4 ± 2.0
Fat mass, kg	55.0 ± 8.0	55.6 ± 9.0	54.3 ± 7.0
Fat-free mass, kg	60.0 ± 7.0	60.0 ± 7.6	60.1 ± 6.5
Measured REE, kcal/day	1896 ± 314	1920 ± 342	1869 ± 284
Predicted REE, kcal/day	1865 ± 270	1855 ± 282	1876 ± 263
RQ	0.990 ± 0.124	1.016 ± 0.101	0.961 ± 0.142
Fat Burn, %	18.8 ± 25.2	14.1 ± 23.5	24.0 ± 26.5
Glucose Avail., %	81.2 ± 26.5	86.0 ± 24.0	76.0 ± 29.1
	Median ± IQR	Median ± IQR	Median ± IQR
Total Daily Energy Intake, kcal/d	1780 ± 229	1750 ± 222	1789 ± 244
IPAQ-S Walking, min/week	220 ± 60	220 ± 65	220 ± 72.5
IPAQ-S Moderate-Int., min/week	120 ± 30	120 ± 21	120 ± 30
IPAQ-S Vigorous-Int., min/week	15 ± 16	10 ± 21	17.5 ± 17.5

Table 4.2: Biochemical investigations at baseline.

	All participants, n=50	SC, n=26	INT, n=24
	Mean ± SD	Mean ± SD	Mean ± SD
TC, mmol/l	5.32 ± 0.97	5.20 ± 1.02	5.45 ± 0.89
HDL-C, mmol/l	1.24 ± 0.29	1.20 ± 0.26	1.27 ± 0.32
LDL-C, mmol/l	3.21 ± 0.87	3.04 ± 0.93	3.41 ± 0.75
Non-HDL-C, mmol/l	4.09 ± 1.03	3.97 ± 1.09	4.23 ± 0.96
TRIG, mmol/l	2.07 ±1.12	2.08 ± 1.25	2.07 ± 0.95
HbA1c mmol/mol	37.8 ± 2.7	37.4 ± 2.81	38.6 ± 2.46
Fasting Glucose, mmol/mol	5.1 ± 0.5	5.0 ± 0.5	5.1 ± 0.5
Creatinine mmol/l	69 ± 13	68 ± 11	71 ± 14
eGFR ml/min/1.73m2	83 ± 11	85 ± 7	80 ± 13
Bilirubin, μmol/l	8 ± 4	7.5 ± 4	9 ± 4
Albumin, g/l	44 ± 2	44 ± 3	44 ± 2
ALT, IU/l	28 ± 18	27 ± 18	30 ± 18
ALP, IU/l	87 ± 24	89 ± 20	85 ± 28
AST, IU/l	22 ± 11	22 ± 13	22 ± 9

Table 4.3: Post-intervention versus changes from baseline.

	SC (n=26)		INT (n=24)		
	Mean (SD)	P value bet WK0 vs W24	Mean (SD)	P value bet WK0 vs WK24	P value bet SC vs INT
Weight loss (kg)	-1.48 ± 1.32	<0.001	-3.64 ± 1.72	<0.001	<0.001
Percentage Weight loss (%)	-1.2 ± 0.22	<0.001	-3.2 ± 0.34	<0.001	<0.001
BMI (kg/m^2)	-0.54 ± 0.10	<0.001	-1.32 ± 0.13	<0.001	0.036
Waist Cir. (cm)	-2.8 ± 0.9	0.005	-7.6 ± 1.9	0.001	0.012
WHR	-0.002 ± 0.006	0.776	-0.035 ± 0.009	0.002	0.047
FM (%)	-0.5 ± 0.4	0.175	-1.0 ± 0.5	0.079	<0.001
FM (kg)	-1.0 ± 0.75	0.186	-2.5± 0.7	0.003	0.004
FFM (%)	-0.3 ± 0.5	0.533	-0.8 ± 0.6	0.213	0.922
FFM (kg)	-0.3 ± 0.6	0.649	-1.1 ± 0.7	0.150	0.001
Energy intake (kcal/day)	-354 ± 40	0.038	-507 ± 38	<0.001	0.031
Measured REE (kcal/day)	-113 ± 43	0.015	-134 ± 42	0.023	0.016
Predicted REE (kcal/day)	-43 ± 30	0.161	-65 ± 28	0.031	0.002
REE corrected for FFM (kcal/day/kg)	-1.46 ± 0.63	0.030	-2.34 ± 0.57	<0.001	<0.001
Measured RQ	-0.008 ± 0.024	0.731	-0.090 ± 0.035	0.018	0.744

Table 4.4: Mean differences by treatment group (ITT analysis).

	Mean difference (SD)	95% CI	*P*-value
Weight loss (kg)	-2.18 ± 0.39	-2.95; -1.40	<0.001
Percent weight loss (%)	-1.91 ± 0.36	-2.64; -1.18	<0.001
BMI (kg/m^2)	-0.84 ± 0.15	-1.14; -0.53	<0.001

Table 4.5: Odds of participants who achieved target weight loss between lower (<0.90) vs higher RQ (≥0.90).

	Odds Ratio	95% CI	P value
Baseline			
Week 4	6.8	1.6 – 28.5	0.005
Week 12	4.1	1.1 – 15.6	0.031
Week 24	3.8	1.1 – 13.8	0.035

**Greater odds of achieving the target weight loss i.e., ≥3% weight loss in the participants sub-group with lower RQ (<0.90)

Table 4.6 Recorded mean of REE and RQ, body composition from bioelectrical impedance analysis between SC vs INT. Values expressed as mean ± SD.

	SC (n=26)				INT (n=24)				P value SC vs INT
Week	0	4	12	24	0	4	12	24	-
REE (kcal/day)	1934 ± 337	1900 ± 251	1866 ± 240	1868 ± 200	1924 ± 264	1874 ± 286	1841 ± 225	1788 ± 235	0.016
REE corrected for FFM (kcal/day/kg)	32.6 ± 3.6	34.3 ± 5.7	33.5 ± 5.6	31.1 ± 4.2	31.9 ± 3.6	31.3 ± 4.2	31.5 ± 3.9	29.9 ± 3.8	<0.001
RQ	1.012± 0.101	0.908± 0.085	0.885± 0.068	0.960± 0.083	1.016± 0.099	0.869± 0.074	0.853± 0.068	0.872± 0.091	0.744
FM (%)	48.2 ± 1.9	47.8 ± 2.0	47.8 ± 2.4	47.4 ± 2.1	47.4 ± 2.0	47.2 ± 2.5	46.8 ± 3.0	46.5 ± 2.6	<0.001
FFM (%)	51.8 ± 1.9	50.8 ± 4.8	51.2 ± 4.9	52.3 ± 2.3	52.7 ± 2.1	52.8 ± 2.6	52.7 ± 3.4	53.1 ± 2.8	0.922

Table 4.7 Comparison of reported physical activity from IPAQ questionnaires. Values expressed as median ± IQR.

	SC (n=26)			INT (n=24)		
	Median	IQR	P value W0 vs W24	Median	IQR	P value W0 vs W24
Week-0 Walking activity (minutes/week)	220	175-240	<0.001	220	183-255	<0.001
Week-0 Moderate activity (minutes/week)	120	120-131	0.130	120	120-150	<0.001
Week-0 Vigorous activity (minutes/week)	10	8-26	<0.001	17.5	10-28	<0.001
Week-24 Walking activity (minutes/week)	420	360-433	<0.001	385	325-420	<0.001
Week-24 Moderate activity (minutes/week)	120	120-150	0.130	180	158-184	<0.001
Week-24 Vigorous activity (minutes/week)	40	20-73	<0.001	120	58-140	<0.001

4.6 DISCUSSION

This randomized controlled trial provides evidence that IC-guided dietary intervention (INT) is more effective than SC in terms of greater weight loss outcome and improved compliance to dietary and lifestyle intervention. The data substantiates that the use of IC-guided EE information as adjunct to lifestyle-based intervention could provide greater emphasis on continued and maintained lifestyle and behaviour modifications, which not only focus on weight loss, but on weight maintenance. The present study will serve as a feasibility data for the integration of EE data within the local SWMS.

A greater proportion of participants in the INT group experienced net weight loss at 24 weeks, as compared to the SC group. This implies there is a positive influence of providing energy-expenditure information on influencing behaviour change, maintaining health behaviours, and sustained adherence to the recommended lifestyle interventions. A systematic review of eighty studies reported the people with overweight and obesity who underwent diet alone, diet and exercise, and meal replacement diet achieved 5 to 8.5 kg (5% to 9%) weight loss within the initial 6-months, but many had demonstrated weight plateau beyond 6-months (288). Studies with greater longitudinal follow-up (up to 48-months) in people who underwent lifestyle-based interventions reported the 3 to 6 kg (3% to 6%) weight loss was maintained, without any groups with weight regain back to baseline levels (288). In addition, data from another systematic review evaluating long-term weight loss through combined diet and exercise reported the average weight loss of 6.7 kg at 1 year (289). In a systematic review by Johns et al (290), combined behavioural weight management programmes (BWMP) in eight RCTs (n=1,022 participants) showed no significant difference in weight loss from baseline or at 3 to 6 months when comparing

BWMPs and diet-only arms (–0.62 kg; 95% CI –1.67 to 0.44). However, at 12 months, a significantly greater weight-loss was detected in the combined BWMPs (–1.72 kg; 95% CI –2.80 to –0.64).

NICE recommends multicomponent weight loss programmes (23, 291) that include the use of a variety of behaviour-change methods including the use of 'problem solving; goal setting; how to carry out a particular task or activity; planning to provide social support or make changes to the social environment; self-monitoring of weight and behaviours that can affect weight; and feedback on performance'. Current UK recommendations for multicomponent weight management intervention include integration of group-based or individual face-to-face contacts, improve accountability (food diary reviews), encourage nutrition counselling and goal setting. The utility of IC-guided EE information thus, promotes the behaviour change and maintenance of lifestyle intervention at 6 months. This feasibility study will serve to create a basis for EE assessment, extending care on maintaining behavioural changes during periods of weight loss plateau or when individuals are moving from weight loss to weight maintenance.

In adults, the largest component of total energy expenditure (TEE) for a moderately active individual is for body weight maintenance as measured under resting conditions. REE is generally higher in people with obesity due to the larger fat-free mass as compared to lean subjects with a smaller or shorter stature. REE is generally higher in men than in women of similar weight and height and increases positively in response to greater body mass or BMI. REE accounts for 60-75% of the TDEE in people with a sedentary lifestyle (30), but greatly varies in accordance with body size, body composition (17), physical activity levels and post-bariatric surgery (292, 293). Under-

feeding during weight loss energy-restriction induces changes in maintenance REE and activity-induced energy expenditure (AEE) as a function of change in body weight and body composition. Indeed, lower energy expenditure is predictive of long-term weight gain and increase in body fat mass (42).

The association between RQ measured during baseline and the subsequent decrease in RQ at week 4 and week 12, suggests that during negative energy deficit, oxidation rates of fat is higher in response to altered dietary content and caloric restriction. Another explanation for the reduction in RQ may be indicative of the individual's capacity for fuel switching, or metabolic flexibility (48, 294). In studies of overfeeding in lean male volunteers, overfeeding decreased insulin sensitivity and resulted in decreases in RQ during rest (295) (overfeeding state). In evaluating metabolic flexibility, Peterson et al (295) demonstrated overfeeding (energy intake surplus +40% above baseline energy needs over 8 weeks; average weight gained 7.6±2.9 kg) did not affect metabolic flexibility as measured during euglycemic clamp (during short-term overfeeding), but increased the 24-hour metabolic flexibility (awake – sleep RQ) as measured via metabolic chamber (295). The individual capacity to oxidize dietary fats is reflected by the degree of metabolic flexibility towards high-fat diet, indicative of the greater propensity for future weight gain (179).

Further, secondary outcome measures of change in RQ had demonstrated that participants with greater fatty acid oxidation (indicated by RQ <0.90) had increased odds (OR 21.7; 95% CI 4.7 – 99.4) of attaining the 3% weight loss target as compared to those with higher RQ (RQ >0.90). Our observations suggest a further association between the fat oxidation, represented by RQ, with the changes in body composition

and metabolic adaptations in response to weight loss. People with severe obesity who have lost weight through diet and exercise over 24-wk period, demonstrated a direct correlation with the decrease in measured REE, when measured using the portable indirect calorimeter (265, 296, 297). Relatively lower RQ was associated with increased fat oxidation (46, 298) suggesting that RQ may serve useful to evaluate the state of energy balance and energy reserves.

Diet restriction in overweight and obesity led to corresponding decrease in overall muscle mass and fat mass. This cohort of participants achieved an average of 3-4% weight loss through intensive diet restriction and increased activity energy expenditure over 24-weeks. Weight loss was directly associated with reduced active tissue mass or fat-free mass. Current literature suggests that up to 5% to 17% of change in measured REE was explained by changes in the fat-free mass (296). Adjusting for baseline BMI and REE, there remained a significant association between magnitude of weight loss in the INT group as compared to SC. Although the observed association does not translate to causality, we postulate that the EE information gained from indirect calorimetry were more accurate and 'patient-centred' as compared to traditional over-reliance upon predictive equations which have proven to be less accurate when determining REE in overweight or obesity (299).

Individuals with relatively higher RQ (RQ > 0.90) had larger gains in body mass and fat mass compared with individuals with lower or moderate RQ (<0.85) (77). However, the concept of metabolic flexibility in relation to a change in RQ was not limited to the changes in macronutrient dietary intake and energy restriction only (300), but there is evidence to support greater physical activity influences a decrease

in RQ (301, 302). In exercise-based studies, the decrease in REE in relation to the FFM did not completely account for the change in substrate oxidation at rest (303). Post-weight loss, the decrease in REE was associated with ability to maintain exercise at the submaximal exercise intensity (304). Our observations within this controlled dataset demonstrated the greater fatty acid oxidation from sub-maximal activity in combination with energy intake deficit could attribute to greater magnitude of weight loss in the short-term (24 weeks).

Current evidence suggests that people with severe obesity portray a dysfunctional metabolic profile with an impaired ability to utilize fat energy stores or switch from glucose to fat oxidation (305). However, when comparing with cross-sectional studies, post-obese adults with severe obesity who have lost weight did not demonstrate any difference in fatty acid oxidation as compared to their lean counterparts (298). The mechanism surrounding metabolic changes in response to energy deficit from physical activity remains largely unexplained, but research postulates that these adaptations could possibly be explained by skeletal muscle uptake of fatty acids (306). Evidence from a systematic review (307) relating to post-absorptive RQ demonstrated that the ratio of carbohydrate to fat macronutrient composition (233) may influence and confound, the post-prandial RQ within the state of regular energy balance and under/overfeeding states (308). Greater carbohydrate (glucose) availability in relation to fat energy-stores (309) in skeletal cells may influence the active tissue metabolic fuel selection (310, 311). We demonstrated that individuals with higher physical activity (achieving >150 minutes/week) or MET equivalent have a greater determinant for a low resting RQ (<0.90) as compared to more sedentary individuals, consistent

with evidence from measure RQ in context of light-intensity physical activity (312) (313).

During the energy-restricted intervention phase, our data demonstrated that REE decreased from baseline to the end of the follow-up period, consistent with evidence from previous landmark studies on reduced REE in response to decreased weight and reduced lean body mass (230). The provision of EE information from real-time REE to the intervention group provides a reasonable alternative for measurement of EE instead of depending on predictive equation for REE, in combination with an estimation of the activity energy expenditure, to determine the magnitude of dietary restriction +/- physical activity or exercise recommendations to achieve the individual's target weight loss. Typically, the predictive energy equations for REE can explain up to 70-80% of the variation due to age, race, height, weight, and gender of the participant (314). The evidence of greater magnitude of weight loss in the IC-guided intervention group during the dynamic lifestyle and behavioural interventions underpins the importance of integrating health informatics i.e., information from measured real-time energy expenditure, on the impact and success within the specialist weight management service and affiliated behavioural weight loss programmes. We take a step toward addressing this challenge by providing the interim evidence from this randomized controlled trial to provide energy expenditure information from indirect calorimetry to both participants and clinicians. The results have provided insights into the dependent factors to maximize the benefits of behavioural modifications and lifestyle interventions in weight management. The addition of real-time metabolic health information (REE and RQ) could reduce the degree of variability

and help address the issues with dietary modifications and lifestyle interventions amongst individuals with severe obesity (315).

4.6 Strengths and limitations

The strength of the study is a well-conducted single-centre RCT consisting of participants recruited from a specialist weight management service. There was good overall adherence and study completers. Unfortunately, due to the impact of the COVID19 pandemic, there was a halt and suspension in recruitment and thereafter inability to complete recruitment to target due to global pandemic resulting in the suspension of the recruitment at 50% target recruitment. Although there was a relatively small single-centre interim analysis, we postulate that the study should ideally be conducted in a multi-centre trial to better evaluate the effects of utilizing energy expenditure information on weight loss intervention on people undergoing weight loss intervention. Although the cohort of the sample was relatively small, the recruited participants were closely matched for BMI, body composition, age, and ethnicity. As this was a randomized controlled trial built upon an already established service, the study was testing a new approach to integrate the currently available portable and reproducible indirect calorimetry within a SWMS. While we reported greater weight loss within the INT group as opposed to the SC group, the lack of significant changes in captured RQ may reflect the inaccuracies in capturing the marginal differences and an acceptable degree of coefficient of variation whilst obtaining RQ data through respiratory gas exchange within a short time-window (up to 10 minutes of steady state measurements).

Utilising the ECAL indirect calorimetry as the measurement tool to determine REE and RQ may pose a methodological limitation. There is some evidence which suggests that when the ECAL indirect calorimeter was compared against the gold standard DeltaTrac Metabolic cart, the ECAL device has a relatively higher coefficient of variation (approximately 10-11%) against the metabolic cart, which may be too large to detect minor changes in substrate oxidation or REE. which showed that ECAL had a greater coefficient of variation, however the use of ECAL indirect calorimetry in the context of obesity-related weight loss research is a novel experiment. Whilst the device is designed to be portable and arguably more convenient for participant as opposed to having to undergo a strict 24-hour whole-room indirect calorimetry, this method has its limitations as it does not reflect the intraday or day-to-day variations in REE in response to post-prandial state or post-exercise. It is vital to recognise that this measured REE data was accurate within the brief window of measurement whilst adhering to strictly controlled research environment.

The complexity of association between body composition and REE was evaluated using regression coefficients for FFM and FM, showing that there is inevitably a degree of variability depending on adiposity (316). Even the most reliable and established method for normalization of REE based on FFM and FM, and obesity-associated comorbidities may lead to spurious conclusions on weight loss-related adaptive thermogenesis (AT). AT in weight-reduced people with obesity may be overestimated or seen as inadequate normalization for FM. The higher metabolic activity of FFM may be affected by increasing adiposity in organ mass (liver fat content), which suggests that organ or ectopic fat distribution may impact and account for the REE adjustments in weight loss (316). Future studies are required to overcome

methodological limitations by accounting of organ lipid content, organ-specific metabolic rate, and organ fluid loss in response to weight loss and impact of organ-specific parameters to appropriately ‘quantify’ the degree of adaptive thermogenesis in human weight regulation (317).

4.7 CONCLUSION

Our results demonstrate those individuals who receive such EE information at regular intervals as adjunctive interventions demonstrate compensatory behaviour changes which lead to sustained deficit in energy intake and promote physical activity after. Dataset from this cohort provides a novel finding that when clinicians and participants utilise real-time EE information promotes improved adherence to dietetic recommendations and behavioural modifications. Future research may be inclined to focus on the use of indirect calorimetry with a specific emphasis on the metabolic adaptations and dynamic change in EE during the negative-energy deficit.

5 CHAPTER V - Utility of Corneal Confocal Microscopy to Detect Diabetic Peripheral Neuropathy and Neuropathic Ulcer in Type 1 Diabetes Mellitus

Contribution: Jonathan Lim made a major contribution to the recruitment of subjects from diabetes outpatient clinics, neuropathy assessment, anthropometric and biochemical assessments, undertook most of the corneal confocal microscopy evaluations, all statistical analyses and writing of this chapter.

To be submitted for publication

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5.1 Abstract

Background: Early diagnosis of diabetic peripheral neuropathy (DPN) in type 1 diabetes (T1D) is crucial to prevent irreversible injury. Corneal confocal microscopy (CCM) is a non-invasive ophthalmic technique capable of quantifying early small-fibre nerve damage.

Objectives: To characterize the corneal nerve morphology using CCM and measure peripheral nerve dysfunction and/or systemic small nerve fibre loss in T1D, T1D with DPN (T1D-DPN), T1D with neuropathic foot ulcers (T1D-DFU) versus healthy volunteers (HV).

Methods: One hundred and ten subjects (HV, n=32; T1D, n =25; T1D-DPN, n=28; T1D-DFU, n=25) were quantified using neuropathy disability score (NDS), neuropathy symptom profile (NSP) McGill visual analogue score for pain (VAS), vibration perception threshold (VPT) and sural nerve conduction velocity (SNCV) and amplitude (SNAP), cold perception (CT) and warm threshold (WT). Corneal nerve morphology namely corneal nerve fibre density (CNFD), branch density (CNBD) and fibre length (CNFL) were quantified with CCM.

Results: Participants with T1D-DPN and T1D-DFU were older (age $p<0.001$), had a longer duration of diabetes ($p<0.001$), and higher HbA1c ($p<0.001$) as compared to T1D without neuropathy and HVs. There was no significant difference in BMI, total cholesterol, triglycerides, systolic blood pressure and eGFR between the four groups. T1D-DPN and T1D-DFU had greater symptoms with a higher NSP ($p<0.0001$) and VAS ($p=0.0035$) and more signs with a higher NDS ($p<0.0001$). VPT (6.7±2.8 vs 9.2±2.4 vs 15.6±9.0 vs 23.1±8.5 volts, $p<0.001$) was decreased, SNAP ($p<0.001$) and SNCV ($p<0.001$) were significantly reduced in the T1D-DPN and T1D-DFU groups as compared to T1D and HV.

There were greater small nerve fibre deficits with lower CNFD ($p<0.001$), CNBD ($p<0.001$), CNFL ($p<0.001$). There was a significant reduction in small nerve fibres (CNFD, CNBD, CNFL) in T1D-DFU as compared to T1D-DPN ($P<0.001$).

Conclusion: CCM demonstrates greater small nerve fibre deficits in neuropathic diabetic foot ulcers. Future longitudinal studies need to be undertaken to ascertain whether small nerve fibres decline prior to foot ulcer development and if regeneration occurs during healing, therefore acting as a biomarker of the acute neuropathic diabetic foot.

5.2 Introduction

Diabetic peripheral neuropathy (DPN) is a debilitating complication in people with diabetes affecting up to 50% in people living with diabetes for up to 25 years (114). Chronic exposure to environment of hyperglycaemia results in chronic inflammation which damages microvascular structures, which in turn leads to nerve fibre damage causing DPN, leading to progressively worsening sensory deficit and neuropathic pain (97). DPN has a significant impact on reducing the quality of life and is a common predictor of development of diabetic foot ulcer (DFU) (318). Increasing evidence have demonstrated the involvement of small-fibre neuropathy (SFN) in DPN typically described by patients as intense "burning" or "sharp prickly" ache in distal extremities of hands and feet (319). Later complications of DPN may lead to end-stage complications including development of diabetic foot ulcers (DFU) and non-traumatic limb amputations (320). Distal symmetrical sensorimotor polyneuropathy (DSP) is the commonest form of DPN, which has a predilection for small-nerve fibres (C-fibres) in the earlier stages (321). The American Diabetes Association (2017) (97), emphasised the need for earlier detection and screening modalities of DPN. Early identification and utility of reliable, simple non-invasive tools for the detection of DPN prevents end-stage foot complications and reduces amputation rates (322). Functional deficits and injury to small nerve fibre including concomitant vascular deficits (impaired blood flow) have been shown to precede the development of T2D (323). Studies detecting DPN based on the use of skin biopsy have suggested that early features DPN are featured during the period of impaired glucose tolerance (324), preceding the T2D diagnosis.

Up to one-third of patients with diabetes suffer with neuropathic pain due to painful DPN (129-132). Numerous screening modalities for DPN have been proposed to including the value of rapid, reliable and simple sensory tests such as the Semmes-Weinstein monofilament (133) and the 128-hertz tuning fork (134, 135). The Semmes-Weinstein 10g monofilament tool is valuable in the diagnosis of DPN, but there are other validated tools capable of evaluating or quantifying the progressive nerve degeneration and regeneration in DPN, and subsequent neuropathic foot ulceration (136, 137). In the absence of macrovascular issues and occlusive arterial disease, a neuropathic foot with palpable pulses may imply small fibre neuropathy (SFN) as a causative factor in the development of an ulcer (138). Features of small fibre nerve injury were associated with delayed (superficial) wound healing as determined through decreased intra-epidermal nerve fibre density (IENFD) (139, 140). Small unmyelinated nerve fibres (C-fibre) in cutaneous layer are most susceptible to microvascular dysfunction. Nerve injury and regeneration have been demonstrated in people with T2D and impaired glucose tolerance (141). Corneal confocal microscopy (CCM) has emerged as a new diagnostic and screening modality with reasonably high sensitivity and specificity to assess DPN, especially of the small fibres (142, 143). However, there remains little data on CCM in the patients with neuropathic foot ulcers in T1D. Accurate quantification of neuropathy using validated diagnostic biomarkers is critical to identify those at high risk of developing DFU and further understand the pathophysiological changes and adaptations from DPN to neuropathic DFU (144).

5.3 Diagnostic evaluation of DPN

Diagnosis of DPN is determined by the presence of signs and symptoms, and abnormal nerve conduction studies (NCS) or evidence of small fibre nerve degeneration in

instances where the NCS may be normal (97, 114). Distal symmetrical polyneuropathy is the commonest form of DPN characterized by symptoms and neurological signs or insensitivity to a 10g Semmes-Weinstein monofilament, abnormal vibration sense using tuning forks, or abnormal ankle reflex (115).

Current recommendations to evaluate the sensorineural conduction of peripheral nerves have been evaluated using the 10g monofilament testing and impaired vibration and proprioception which correlates linearly with increasing severity of DPN and development of neuropathic foot ulcers (325). The large fibres consist of myelinated Aα and Aβ nerve fibres which innervate sensory components of light tough, proprioception, joint position sense, vibration and motor muscle control, and if damaged, significantly increases the risk of degeneration and foot ulceration as an end-stage consequence of living with diabetes (326). However, there remains a lack of clinical research that specifically evaluates the association between small nerve fibres specifically in the context of progression of DPN and the subsequent changes in patients who develop DFU in people with T1D.

Current evidence from studies on neuropathic foot ulcers predominantly involves people with impaired glucose regulation (prediabetes) and T2D. Small-fibre neuropathies (SFN) may present as a spectrum with patients being symptomatic yet escape the detection by the standard tests (145). Degeneration of small fibres may be missed based upon standard electrophysiological tests, and SFN may be present even in individuals who may exhibit normal motor power, preserved reflexes, and normal electrophysiology. Current methods available have allowed the use of non-invasive, reproducible methods to accurately screen and detect morphological changes in small nerve fibres which may be an invaluable tool to accurately quantify and measure the

nerve fibre deficits in relation to the neuropathy phenotype, comparing the characteristics predictive of worsening diffuse axonal neuropathy, and eventually development of neuropathic ulcer (327). Although the availability of skin biopsy has been a major advance for diagnosing SFN, the correlation between IENF density and neuropathic pain remains unclear. Indeed, complete denervation of the epidermis can be seen in patients both with persisting neuropathic pain and genetic insensitivity to pain (328), thus raising the question whether the loss of IENF itself is causally related to pain or it is only an indicator of neuropathy.

Previous studies have demonstrated good diagnostic ability of QST for SFN and likewise, other evidence demonstrate positive correlation between skin biopsy and functional abnormalities in the C-fibre physiology (145, 329, 330). Biopsy specimens of the skin have shown progressive reduction in the intraepidermal nerve fibres from the time of diagnosis of diabetes, seen even in persons with prediabetes (327, 331). In other studies, researchers have demonstrated the decrease in intraepidermal nerve fibre density (IENFD) in impaired glucose tolerance and metabolic impairment (112). The use of quantitative sensory testing (QST) is useful to determine and measure both small and large nerve fibre function. However, QST is largely dependent upon the experience of the evaluator and is also reliant upon the ability of the subject to focus and provide a rapid response whilst undergoing neuropathy evaluation. Nerve conduction studies (NCS) (134) are the current gold standard tool to detect small fibre nerve degeneration or degradation, especially those involving small unmyelinated nerve fibres (C-fibres) which are critical to the sensory-neural pain pathway. Further details for the diagnostic approach towards neuropathy of large versus small fibres are summarized in **Table 5.1**.

Increasingly sophisticated modalities and technological advancements have helped incorporate newer methods to detect small fibre degeneration (332). Corneal confocal microscopy (CCM) has been demonstrated as a valuable tool towards detection and quantification of small nerve fibres in diabetes. CCM is a rapid, non-invasive ophthalmic technique which can accurately determine and quantify the degeneration of the small nerve fibres and regeneration in people living with diabetes (120) and other associated forms of peripheral neuropathies (121, 122). CCM allows for in vivo assessment of other associated corneal layers, including the epithelium, stromal keratocytes and the endothelium.

However, the differential ability of CCM against currently accepted gold standard FDA approved methods such as skin biopsy, QST and nerve conduction studies for the diagnosis of diabetic neuropathy has not been fully evaluated to date. Earlier identification and screening of DPN is crucial to prevent the irreversible nerve damage, and the potential sequelae of developing DFU and distal limb amputations. The aim of this study was to characterize neuropathy in people with T1D with diabetic neuropathic foot ulcers (T1D-DFU) whilst comparing against those with peripheral neuropathy without ulcers (T1D-DPN) with a particular focus on small nerve fibre by CCM.

Table 5.1: Diagnostic approach towards Neuropathies of Large and Small Nerve Fibres. Adapted from American Diabetes Association update (333).

Approach	Large Myelinated A-type α and β-Fibres	Small Myelinated and Unmyelinated A-Type δ-Fibres and Small Unmyelinated A-Type δ-Fibres and C-Type Fibres
Signs of Physical examination	• Dysfunction and impairment of reflexes, proprioception, vibration, wasting of small muscles of hands and feet, weakness in feet	• Impaired sensation of warm and cold temperatures and of pinprick; normal strength, reflexes, and nerve conduction • impaired autonomic function, with dry skin, poor blood flow, cold feet, and impaired sweating
Clinical Implications	• Impaired sense of pressure and balance • resulting unsteadiness in gait; increased risk of falls, traumatic injury, and development of Charcot's neuro-arthropathy	• Impaired nociception (pain), susceptibility to foot ulcers, increased risk of amputation
Diagnostic Evaluation and Tests	• Nerve conduction- abnormal test results (median, sural and peroneal nerves)	• Nerve conduction; normal results despite presence of symptoms

	• Quantitative sensory testing to detect loss of perception of vibration	• Skin biopsy to detect loss of intraepidermal nerve fibres • Corneal confocal microscopy • Quantitative sensory tests to detect sensitivity to hot and cold and impairment of pain perception • Sudorimetry (performed with neuro-pad or sudo-scan) to obtain objective measures of sweating
Consider differential diagnosis	• Consider chronic inflammatory demyelinating polyneuropathy, monoclonal gammopathies, Gullain-Barre syndrome and myopathies, B12 or folate deficiency, hypothyroidism, para-neoplastic syndrome, and effects of chemotherapy	• Consider metabolic causes such as uraemia, hypothyroidism, B12 or folate deficiency, acute intermittent porphyria, toxic alcohol, heavy metals, industrial hydrocarbons, inflammation or infection, connective-tissue diseases, vasculitis, celiac disease, sarcoidosis, Lyme disease, human immunodeficiency virus, hepatitis B or C virus, hereditary diseases; monoclonal gammopathies, paraneoplastic syndromes and amyloidosis.

Table 5.2: Stages of Diabetic Peripheral Neuropathy. Adapted from Systematic Review by Yang et al. (334)

Stages of DPN	Characteristics
Stages 0/1: no clinical neuropathy	• No symptoms or signs
Stage 2: clinical neuropathy	• Clinical examination at the early stages in DPN include reduced or absent monofilament 10g test, reduced or absent Achilles tendon reflex, diminished distal sensation to small unmyelinated C-fibre function (pain/temperature) and diminished large fibre function (vibration/proprioception)
Chronic Painful	• Positive symptoms (increasing nocturnal pain): burning, shooting, stabbing pains +/- paraesthesia • Reduced or absent Achilles tendon reflexes • Reduced or absent monofilament 10g test • Diminished distal sensation to pain / temperature (impairment to unmyelinated C-fibres) • Diminishes vibration /proprioception (impairment to myelinated large Aα and Aβ fibres)
Acute Painful	• Less common • Poor/ sub-optimal Diabetes Control, weight loss + osmotic symptoms • Hyperaesthesia may occur

	• Possibly associated with initial commencement of glycaemic therapy • Minor sensory signs or even normal peripheral neurological examination
Painless with complete/ partial sensory loss	• No symptoms or numbness/ deadness of feet; reduced thermal sensitivity; painless injury • Signs of reduced or absent sensation with absent reflexes
Diabetic amyotrophy	• Muscle weakness and wasting • Sensory loss is slight, but nocturnal pain common • Subacute onset
Stage 3: Late complications of Clinical Neuropathy	• Foot lesions, such as Diabetic Foot Ulcers • Neuropathic Deformity such as Charcot's neuroarthropathy • Non-traumatic lower extremity amputation • Gait instability, increased risks of falls

5.4 Epidemiology of Diabetic Peripheral Neuropathy

Multiple epidemiological studies on DPN generated heterogenous findings, owing to the different patient populations, variations in the definitions of neuropathy, and methodological variations in assessment of neuropathy. The MonItoring trends and determinants in Cardiovascular / Cooperative Research in the Region of Augsburg (MONICA/KORA) (335) investigators found the prevalence of neuropathic pain was 13.3% in people with diabetes versus 8.7%, 4.2%, and 1.2% in participants with impaired glucose tolerance, impaired fasting glucose, and controls, respectively.

The PROMISE (Prospective Metabolism and Islet Cell Evaluation) (336) followed up patients longitudinally who were at risk for developing diabetes. The prevalence of neuropathy in people with diabetes at 3 years was 50% in patients who developed diabetes, 49% in those with prediabetes, and 29% in controls when assessed using the Michigan Neuropathy Screening Instrument (337). The Rochester Neuropathy Study (338) evaluated a cohort of 380 participants for DPN using a multifaceted approach, based on the neuropathy symptom score, neuropathy disability score, and nerve conduction studies, was found in 66% and 59% of patients with T1D and T2D, respectively.

A large multicentre study (n=6500) in the UK revealed the prevalence of DPN (based on use of questionnaire and examination) was 28.5% (339). In another large multi-centre community-based study in ~15,000 patients with diabetes reported 34% of the population had symptoms of painful neuropathy, with greater risk of peripheral neuropathy in people with type 2 diabetes, female population, and South Asian ethnicity (129). In the Pittsburgh Epidemiology of Diabetes Complications trial, the

prevalence of DPN in T1D was 34%, with higher prevalence in older adults (18-29 years: 18%; >30 years: 58%) (99).

In the Diabetes Control and Complications Trial (DCCT), almost 1 in 5 people were reported to have neuropathy (based on examination), with 1 in 10 people reported painful DPN (340). In the EURODIAB IDDM complications study (341), consisting of over 3000 patients, the prevalence of neuropathy risen from a 28% prevalence at baseline, rising by 23.5% after 7-year follow-up. The EDIC (Epidemiology of Diabetes Interventions and Complications) study, following on from the initial 6 ½ year follow on from the DCCT (342), showed a decrease in risk of DPN in people who had intensive anti-glycaemic therapy compared to conventional treatment during the DCCT period.

5.5 Epidemiology of Diabetic Foot Ulcers & Limb Amputations

Up to one-third of the half a billion people with diabetes worldwide will develop DFU over the course of their lifetime (343). In people with DFU with a healed ulcer, 40% will develop a recurrence within 1 year, 65% recurrence within 5 years, and greater than 90% within 10 years (326, 344). The incidence of foot ulceration each year is about 1.9% and 4.0% of those with diabetes (322, 345, 346). Based on the National Health and Nutrition Examination Surveys (2005-2010) (347), more than 1 in 4 adults (28.6%) living with diabetes did not receive the recommended annual foot screening within the year (97, 348). In a similar Canadian survey (349), only 53% adults with diabetes reported to receive foot examination by healthcare practitioner at least once in the year.

DPN is the principal factor contributing to diabetic foot ulceration (DFU) and Charcot neuroarthropathy (320, 350, 351). The greatest health economic cost and increased

hospitalization rates and duration in people living with diabetes is due to DPN and limb amputations (97, 352, 353). Distal symmetric polyneuropathy (DSP) is the commonest form of DPN. DSP is a chronic, nerve-length-dependent, sensorimotor neuropathy that affects up to one-third of people with T1D or T2D (114). Painful DPN (pDPN) is characterized by burning, tingling, paraesthesia, and/or numbness, typically in a 'stocking-and-glove' distribution (116, 354). Damage to peripheral nerves due to sensory neuropathy is further associated with autonomic neuropathy and occurrence of ischaemia in the form of peripheral arterial disease (355), often concomitantly reported in DPN.

5.6 Aims and Objective

To evaluate and compare the corneal nerve morphology using CCM, QST and phenotype characteristics between individuals with type 1 diabetes (T1D), T1D with peripheral neuropathy (T1D-DPN) and T1D with neuropathic foot ulcers (T1D-DFU) versus healthy volunteers (HVs).

5.7 Research Design and Methods

This was an observational, cross-sectional, non-interventional study. Participants with T1D were on standard clinical treatments including anti-glycaemic/insulin medications, anti-hypertensives and anti-lipid medications instituted either in primary or secondary care.

5.7.1 Selection of patients

Seventy-eight consecutive participants with T1D were evaluated and subsequently divided into three groups based on the neuropathy status and neuropathic foot ulcers and were age- and sex-matched to a group of healthy volunteers. Patients were

recruited from general diabetes clinics in the Aintree University Hospital Diabetes Centre, Liverpool University Hospitals NHS Foundation Trust. Twenty-five participants with T1D without neuropathy (n=25), twenty-eight subjects with peripheral neuropathy (T1D-DPN) (n=28), twenty-five subjects with neuropathic foot ulcers (T1D-DFU) and thirty-two non-diabetic healthy volunteers (HVs) were studied. Other causes of peripheral neuropathy (except diabetes), any previous history of neurological conditions, neuropathy due to other inflammatory or demyelinating conditions, previous ocular trauma or ocular surgery were excluded. HVs were recruited through the friends and family of participants who were attending the outpatient diabetes clinics based at Liverpool University Hospitals NHS Foundation trust and through poster advertisements. The study was approved by the Northwest Research Ethics Committee (REC 18/NW/0532; IRAS 246882) and sponsored by the University of Liverpool. The written informed consent was obtained according to the Declaration of Helsinki.

5.7.2 Definition of Neuropathy

Peripheral neuropathy was defined according to the Toronto criteria (114) by the presence of an abnormal nerve conduction study and symptom/symptoms or sign/signs of peripheral neuropathy.

5.7.3 Assessment of neuropathy

All patients and control participants underwent evaluation of neurologic symptoms according to the neuropathy symptom profile (NSP), and the McGill visual analogue score (VAS) to assess the severity of painful neuropathy. The NSP was assessed out of total maximum score of 38. All participants scored the average intensity of

neuropathic pain over the previous 2 weeks on a continuous VAS of pain intensity, where “0” indicated “no pain” and “10” indicated “worst possible pain”. Clinical neurologic deficits were assessed using the modified neuropathy disability score (NDS), which includes the evaluation of vibration, pin prick, temperature perception, and the presence / absence of ankle reflexes to establish the severity of neuropathy. (NDS (out of total maximum score of 10) where score of 0 – 2 graded as no neuropathy, 3-4 mild neuropathy, 5-8 moderate neuropathy, 9-10 severe neuropathy). Quantitative sensory testing included an assessment of vibration perception threshold (VPT), measuring using a neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, U.K.). The cold sensation threshold (CST) (Aδ fibres), warm sensation threshold (WST) (c fibres) was quantified using the method of limits with the MEDOC TSA II (Medoc, Ramat Yishai, Israel) on the dorsum surface of the left foot for every participant.

The sural sensory nerves were assessed in the right lower limb by a clinician trained to evaluate sural nerve conduction velocity (SNCV) and sural nerve action potential (SNAP) utilizing the point-of-care device NC-Stat ® DPNCheckTM system (Neurometrix, Waltham, USA). The NC-Stat ® DPNCheckTM has good reliability and reproducibility and correlates well with the SNCV (R=0.81) and moderately with SNAP (R= 0.62) derived from nerve conduction studies (NCS) as a reference method (356). The point-of-care conduction device results were evaluated against the reference values provided for the device (abnormal result defined by amplitude ≤4 μV or conduction velocity ≤44 m/s). The device cannot detect SNAP signals of <2.1μV and automatically calibrates these values as zero (356), therefore all zero results were recorded as 2.0 μV. For instances when operators were unable to attain or detect SNCV

or SNAP signals, the results were recorded as clinical neuropathy. Operators were trained to reduce errors by using a fixed conduction distance and filter settings and maximizing amplitude to improve the signal to noise ratio (356).

Vibration perception threshold (VPT) was measured from an average of three values on the large toe using a neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK). Participants with impaired VPT were defined by VPT of 15-24V, whilst participants with ≥25 V was considered an absence of sensation, deemed at high risk for neuropathic foot ulcer (357).

Sural Nerve Conduction Velocity (SNCV) and sural nerve action potential (SNAP) were measured using the point-of-care device NC-Stat® DPNCheck^{TM} system (Neurometrix, Waltham, USA). The NC-Stat DPNCheck has good reliability and reproducibility and correlates well with SNCV (R=0.81) and moderately with SNAP (R= 0.62) derived from nerve conduction studies (NCS) as a reference method (356). The point-of-care conduction device results were evaluated against the reference values provided for the device (abnormal result defined by amplitude ≤4 μV or conduction velocity ≤44 m/s). The device cannot detect SNAP signals of <2.1μV and automatically calibrates these values as zero (356), therefore all zero results were recorded as 2.0 μV. For instances when operators were unable to attain or detect SNCV or SNAP signals, the result were recorded as clinical neuropathy. Operators were trained to reduce errors by using a fixed conduction distance and filter settings and maximizing amplitude to improve the signal to noise ratio (356).

5.7.4 Corneal Confocal microscopy

All participants recruited underwent CCM assessment using a laser scanning corneal microscopy (Heidelberg Retina Tomograph III with Rostock Cornea Module; Heidelberg Engineering, Heidelberg, Germany) according to previously published protocol (123). Several scans of the entire depth of the cornea were recorded using the forward tuning of fine focus of the objective lens backwards and forwards using the section mode, which enables manual acquisition and storage of single images of all corneal layers. This provides a cross section two-dimensional image with a lateral resolution of ~2 mm/pixel and final image size of 400 x 400 pixels of the sub basal nerve plexus of the cornea from each participant and control healthy volunteer.

The first high-quality image of the stroma inferior to the Bowman's layer represented the anterior keratocyte layer, an image superior to Descemet's membrane represented the posterior keratocyte layer, and an intermediate image between the anterior and posterior layers represented the middle keratocyte layer. For quantification of corneal nerves, eight images from the central cornea were selected, and examined in a masked and randomised fashion using previously established protocol (142).

Based on the images that were selected for each participant, the corneal nerve parameters were quantified: 1) corneal nerve fibre density (CNFD), the total number of main nerves per square millimetre of corneal tissue (no./mm^2); 2) corneal nerve branch density (CNBD), the number of primary nerve branches per square millimetre (no./mm2); and 3) corneal nerve fibre length (CNFL), the total length of main nerves and nerve branches per square millimetre (mm/mm2) within the area of corneal tissue. Ophthalmic technicians were blinded when carrying out image analysis. Automated

corneal nerve fibre quantification (ACCMetrics software, version 2.0, University of Manchester, Manchester, UK) was undertaken and this consisted of two main steps: (1) CCM image enhancement and nerve fibre detection and (2) quantification of three morphometric parameters i.e. CNFD, CNBD and CNFL. In the nerve fibre quantification process, all the end points and branch points of the detected nerve fibres are extracted and used to construct a connectivity map. Each segment in the connectivity map can then be connected and classified as main nerve fibres or branches according to the nerve properties such as intensity, orientation, and length.

5.7.5 Statistical Analysis and Power Calculation

For the cohort with diabetes, an assumption of the paired groups was used to calculate the sample size considering a change in CNFD. The standard deviation between groups is likely to be approximately 9 nerves/mm2. Therefore, recruiting a minimum of 25 patients for the group with diabetes will provide 80% chance to detect a clinically meaningful change in CNFD of 5 nerves/mm2 and an assumption of a type 1 error (alpha-level) of 0.05. The statistical analyses were undertaken using SPSS Statistics 25 (IBM Corporation, Armonk, NY, USA). The data overall were expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to determine whether the distribution of the data was parametric or non-parametric. Parametric data were expressed as mean ± SD, and non-parametric data were expressed as median ± interquartile range (IQR). Parametric data were compared using Student's *t*-test, paired for related samples and unpaired for unrelated ones. The Mann– Whitney *U* test was used for non-parametric unpaired variables and the Wilcoxon signed-rank test for paired data. A P value of < 0.05 was statistically significant. Correlations were carried out using Pearson's test for parametric data and Spearman's rank test for non-parametric data.

5.8 RESULTS

Comparison regarding the baseline clinical parameters was performed between people with T1D, T1D-DPN and T1D-DFU and healthy volunteers (HVs). One hundred ten participants were recruited altogether (HV, n=32; T1D, n =25; T1D-DPN, n=28; and T1D-DFU, n=25). The overall demographics and metabolic, anthropometric measurements in people with diabetes and age-matched control healthy volunteers are summarized in **Table 5.1**. There was no significant difference in BMI, total cholesterol, triglycerides, systolic blood pressure and renal function (estimated glomerular filtration rate) between the four groups.

The symptoms and signs of peripheral neuropathy in all participants were summarized in **Table 5.2**. The VPT was significantly greater in T1D compared with healthy volunteers and greater in T1D-DPN and T1D-DFU (9.2±2.4 vs 15.6±9.0 vs 23.1±8.5 vs 6.7±2.8 volts, p<0.001). The VPT differences is illustrated in **Figure 5.1**. The cold and warm perception thresholds between the groups are summarized in **Table 5.3**.

5.8.1 Electrophysiology

Sural nerve conduction velocity (SNCV) was significantly reduced in the T1D-DPN and T1D-DFU groups as compared to T1D without neuropathy (P<0.001) but there was no significant difference between the T1D-DPN and T1D-DFU (P=0.319). Sural nerve amplitude (SNAP) was significantly reduced in the T1D-DPN and T1D-DFU groups as compared to T1D without neuropathy (P<0.001) but there was no significant difference between the T1D-DPN and T1D-DFU (P=NS). There were no significant changes in electrophysiology SNCV and SNAP between the Healthy Volunteers and the T1D group (P=NS). **Figure 5.2A** and **Figure 5.2B** shows the comparison between SNCV and SNAP between all four groups.

Table 5.1: Demographics and metabolic profile of the participants.

	HV (n=32)	T1D (n=25)	T1D-DPN (n=28)	T1D-DFU (n=25)	P value between DPN vs DFU	P value between all groups
Demographics						
Age (years)	41.1±11.3	43.4±13.2	48.3±7.8	49.8±9.2	0.988	**0.012**
Duration of Diabetes (year)	0	16.2±12.3	25.5±11.7	26.9±10.9	0.998	**<0.001**
BMI (kg/m^2)	24.50±3.8	27.8±4.9	28.9±5.7	28.2±5.7	1.000	0.415
Biochemistry						
HbA1c, mmol/mol	37±4	69±13	78±16	80±17	1.000	**<0.001**
Cholesterol, mmol/L	4.7±0.7	4.4±1.0	4.8±1.2	4.9±0.5	1.000	0.215
HDL, mmol/L	1.4±0.4	1.7±0.4	1.7±0.5	1.5±0.3	1.000	0.212

LDL, mmol/L	2.6±0.8	2.0±0.8	2.4±0.9	2.2±0.6	1.000	0.053
Triglycerides, mmol/L	1.4±0.7	1.5±0.8	1.7±1.1	1.4±0.5	1.000	0.607
eGFR, ml/min/1.73m^2	82±13	81±17	74±20	75±20	1.000	0.298

Table 5.2: Neuropathy signs and symptoms profile in all participants.

Signs & symptoms	HV (n=32)	T1D (n=25)	T1D-DPN (n=28)	T1D-DFU (n=25)	P value between DPN vs DFU	P value between all groups
VAS (-/10)	0±0	0.6±1.2	5.3±3.0	5.8±2.6	1.000	**<0.001**
NSP (-/38)	0±0	2.1±2.6	11.9±7.2	18.2±5.1	**<0.001**	**<0.001**
NDS (-/10)	0±0	0.6±0.8	5.2±2.6	7.8±2.3	**<0.001**	**<0.001**
VPT, Volts	6.7±2.8	9.4±2.6	17.2±4.3	24.9±6.7	**<0.001**	**<0.001**
SNCV, m/s	55.5±2.3	50.8±4.5	35.4±3.9	30.7±2.6	**<0.001**	**<0.001**
SNAP, μV	13.6±1.6	7.5±3.5	3.0±2.0	2.2±1.0	0.147	**<0.001**

Table 5.3 Thermal Thresholds assessed using the MEDOC TSA II device.

	HV (n=32)	T1D (n=25)	T1D-DPN (n=28)	T1D-DFU (n=25)	P value DPN v DFU	P values All Groups
CT,°C	27.6±2.8	26.6±1.9	17.1±5.2	12.8±2.8	0.001	<0.001
WT,°C	33.7±1.3	37.6±2.1	41.2±1.4	46.3±3.5	<0.001	<0.001
CIP,°C	15.4±2.7	16.0±4.2	9.3±4.6	3.2±2.8	<0.001	<0.001
WIP,°C	38.3±1.1	41.1±1.6	44.1±1.8	48.4±2.0	<0.001	<0.001

Figure 5.1 Boxplot of Vibration perception threshold (VPT) between groups.

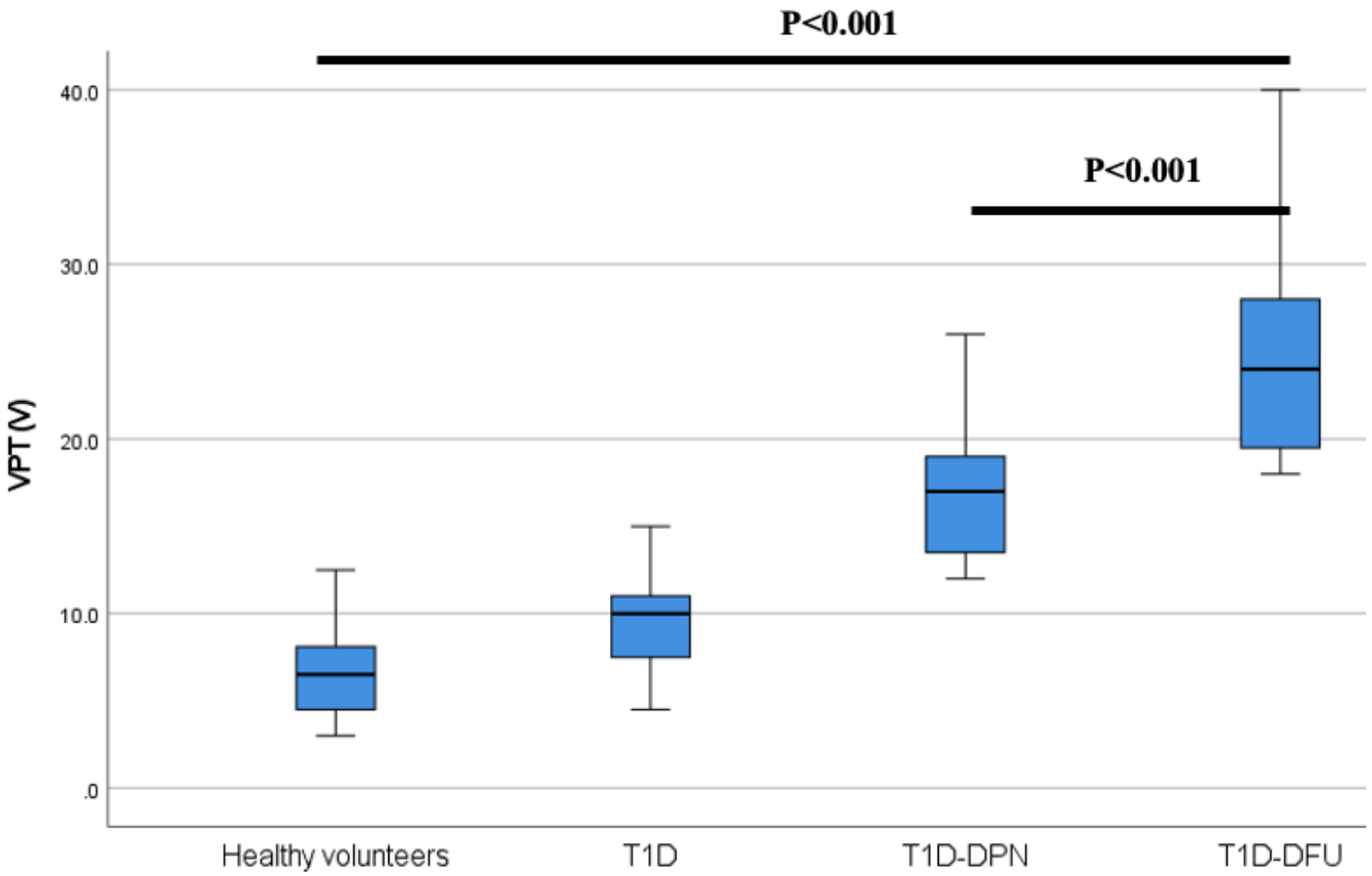
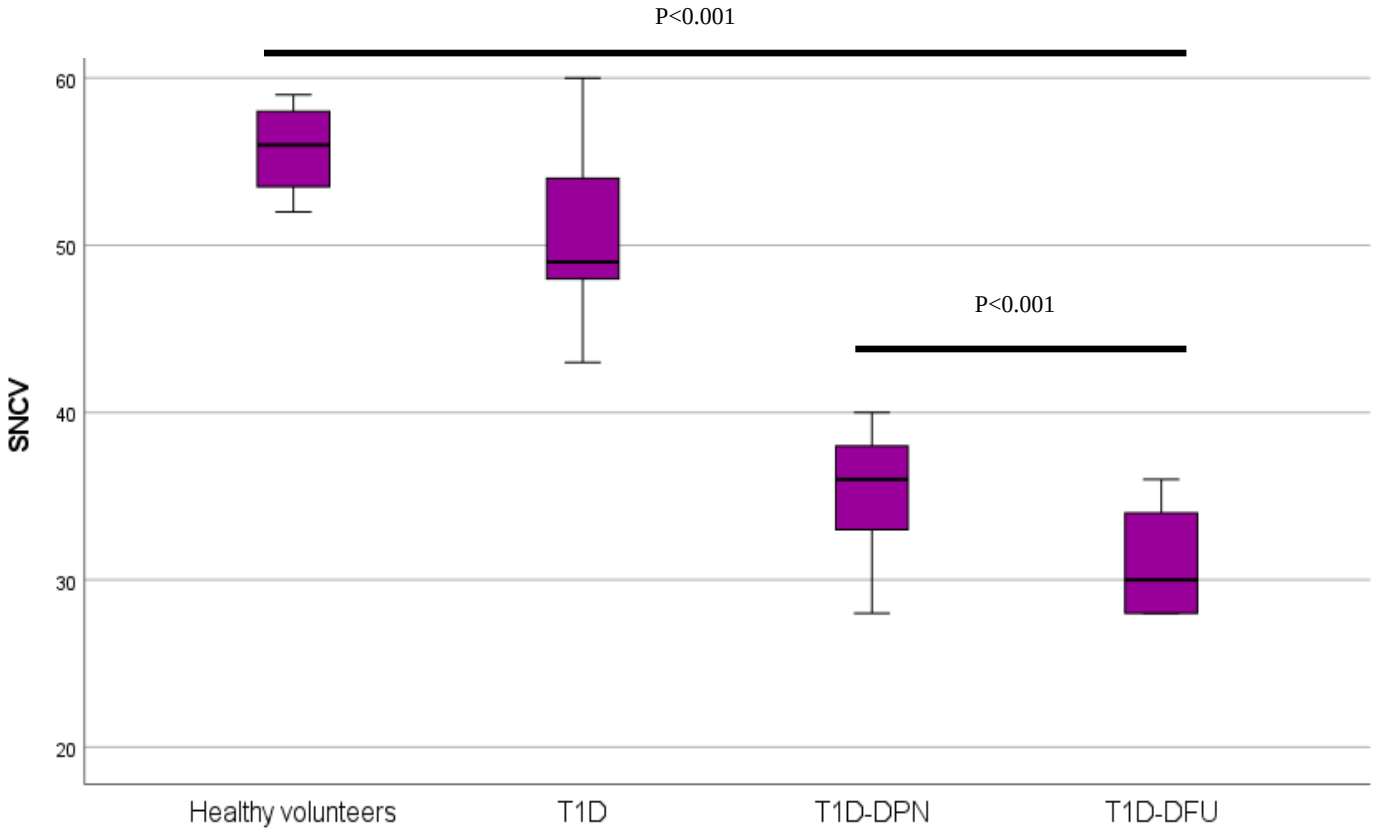
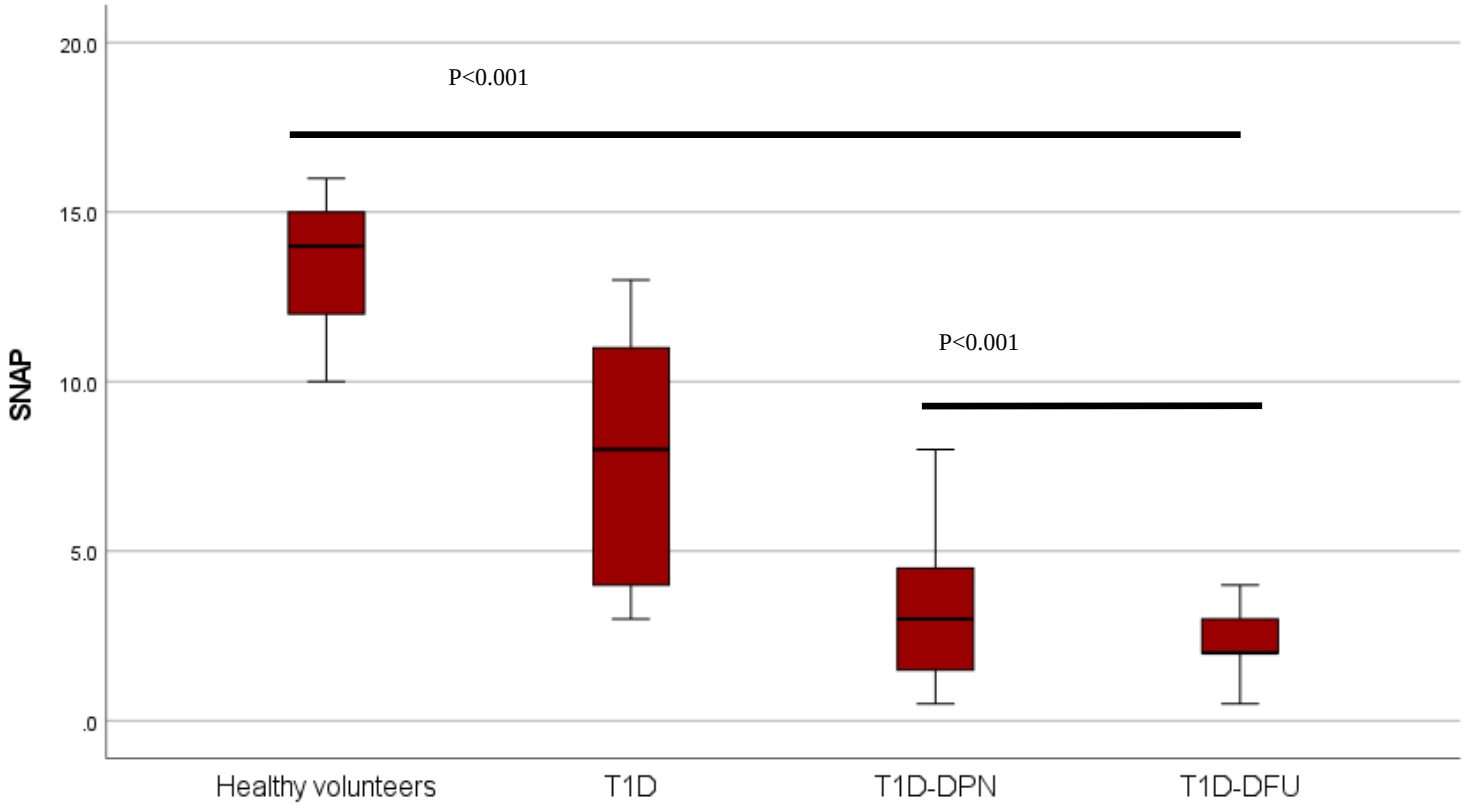


Figure 5.2a and b. Boxplot of SNCV and SNAP between groups



A

Figure 5.2a and b. Boxplot of SNCV and SNAP between groups



B

5.8.2 Corneal Structure with Corneal Confocal Microscopy

The mean corneal nerve fibre density (CNFD) ($p<0.001$) was significantly reduced in the T1D-DPN and the T1D-DFU groups as compared to the T1D ($P<0.001$). There was also a significant reduction in CNFD between the DPN and DFU groups ($P=0.009$). The complete dataset for CCM evaluation is summarized in **Table 5.3.**

The mean corneal nerve branch density (CNBD) ($p<0.001$) was significantly reduced in the T1D-DPN and the T1D-DFU groups as compared to the T1D without neuropathy ($P<0.001$). There was a statistically significant reduction in CNBD between the DPN and DFU groups ($P=0.028$). The mean corneal nerve fibre length (CNFL) (16.46 ± 3.69 vs 8.46 ± 5.43 vs 5.17 ± 1.82 vs 14.07 ± 3.79, $p<0.001$) was significantly reduced in the DPN and DFU group as compared to T1D without neuropathy ($P<0.001$). There was a significant reduction small nerve fibres in terms of CNFL between participants with DFU versus DPN ($P<0.001$).

The results demonstrate a significant reduction in corneal small nerve fibres in terms of CNFL, CNFD and CNBD between people with T1D without neuropathy and those with T1D-DPN and DFU. There was also a significant difference and reduction in corneal small nerve fibres CNFL, CNFD and CNBD between DPN and DFU groups, suggesting those with neuropathic foot ulcers had more significant small fibre degeneration. Full summary is detailed in **Table 5.4.** A simple box plot to demonstrate CNFL, CNFD, and CNBD is demonstrated in **Figure 5.3a, b and c.**

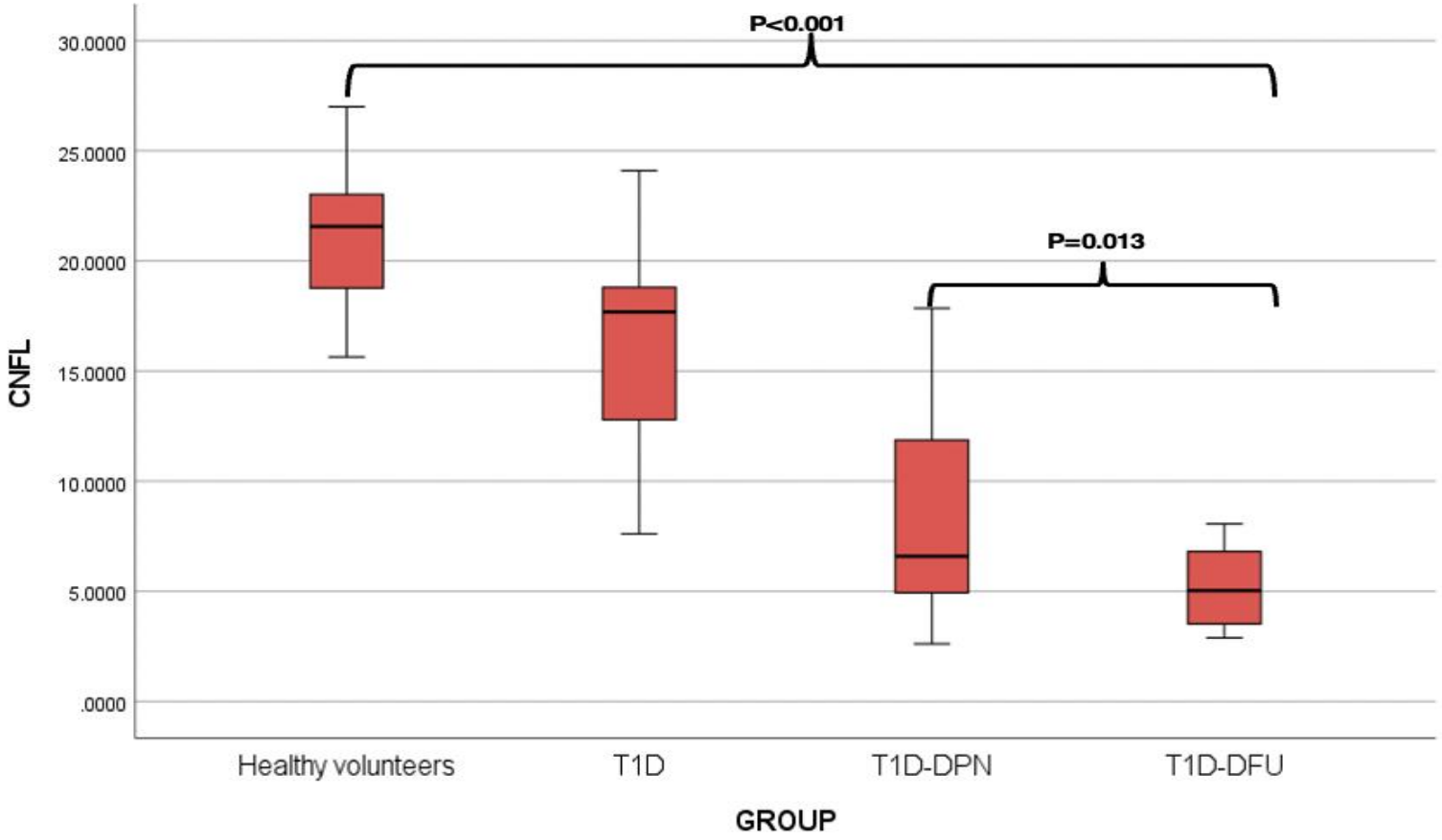
5.8.3 Diagnostic Utility of CCM for DFU

For the diagnosis of DFU in people with T1D, the receiver operating curve (ROC) analysis showed that CNFD had an area under the curve (AUC) of 0.92 with a sensitivity of 0.88 and specificity of 0.85 and optimal threshold below 7.44 (no./mm^2) (summarized in **Figure 5.5** and **Table 5.5**). CNBD had an AUC of 0.85 with sensitivity of 0.88, specificity of 0.72 and an optimal threshold below 8.80 (no./mm^2). CNFL had an AUC of 0.84, sensitivity of 0.76 and specificity of 0.70 and an optimal threshold below 0.84 (mm/mm^2).

Table 5.4 Participant Corneal Confocal Microscopy characteristics.

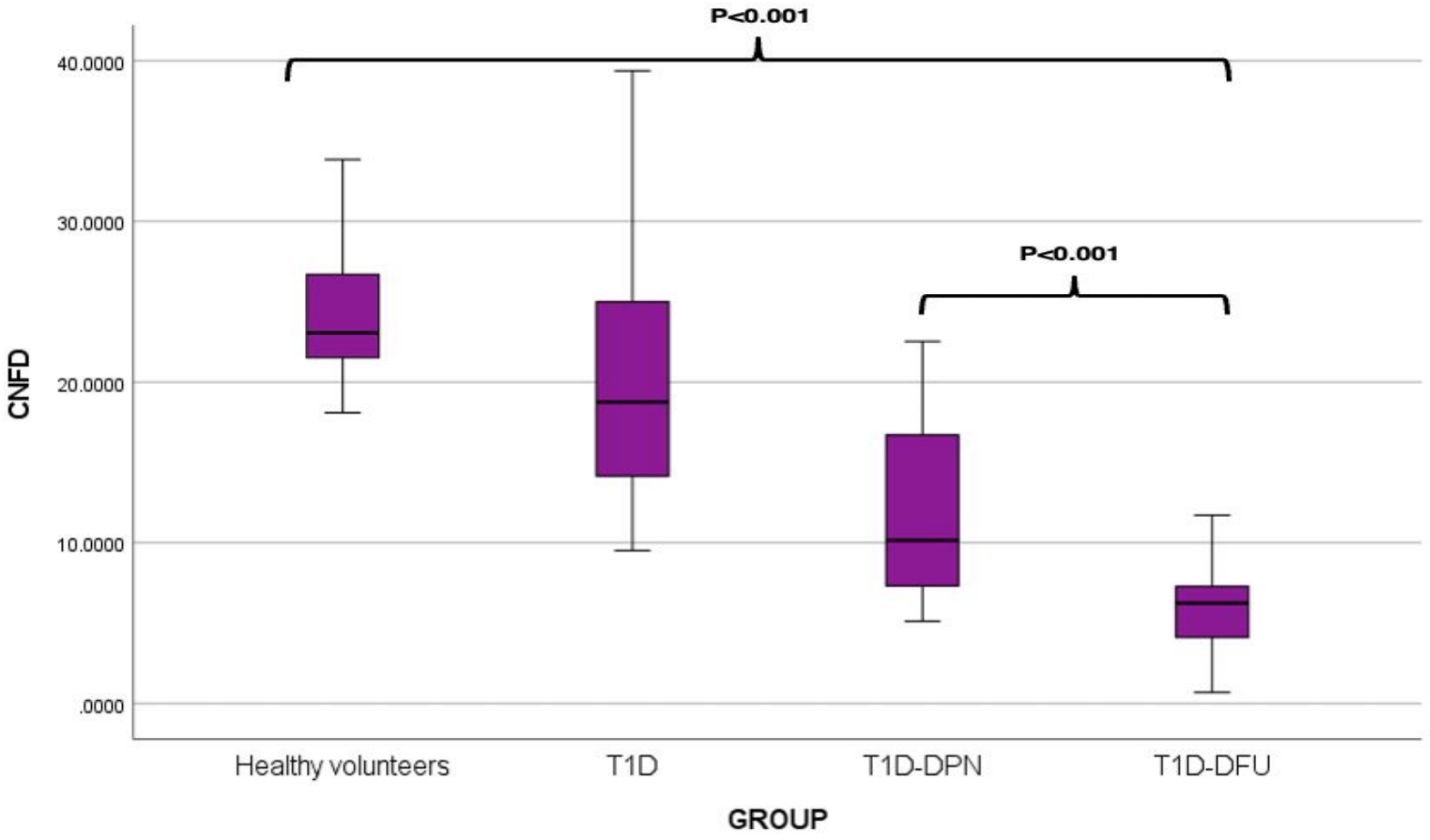
Corneal morphology	HVs	T1D	T1D-DPN	T1D-DFU	P value bet DPN vs DFU	P value bet all groups
CNFL, mm/mm^2	21.08±2.77	16.46±3.69	8.48±4.80	5.17±1.82	**0.013**	**<0.001**
CNFD, no./mm^2	25.02±4.27	20.26±6.65	11.33±5.15	6.00±2.59	**<0.001**	**<0.001**
CNBD, no./mm^2	26.94±7.28	21.93±9.90	11.13±7.05	5.71±3.43	**0.003**	**<0.001**

Figure 5.3a, b and c. Boxplot of CNFL, CNFD, and CNBD between groups



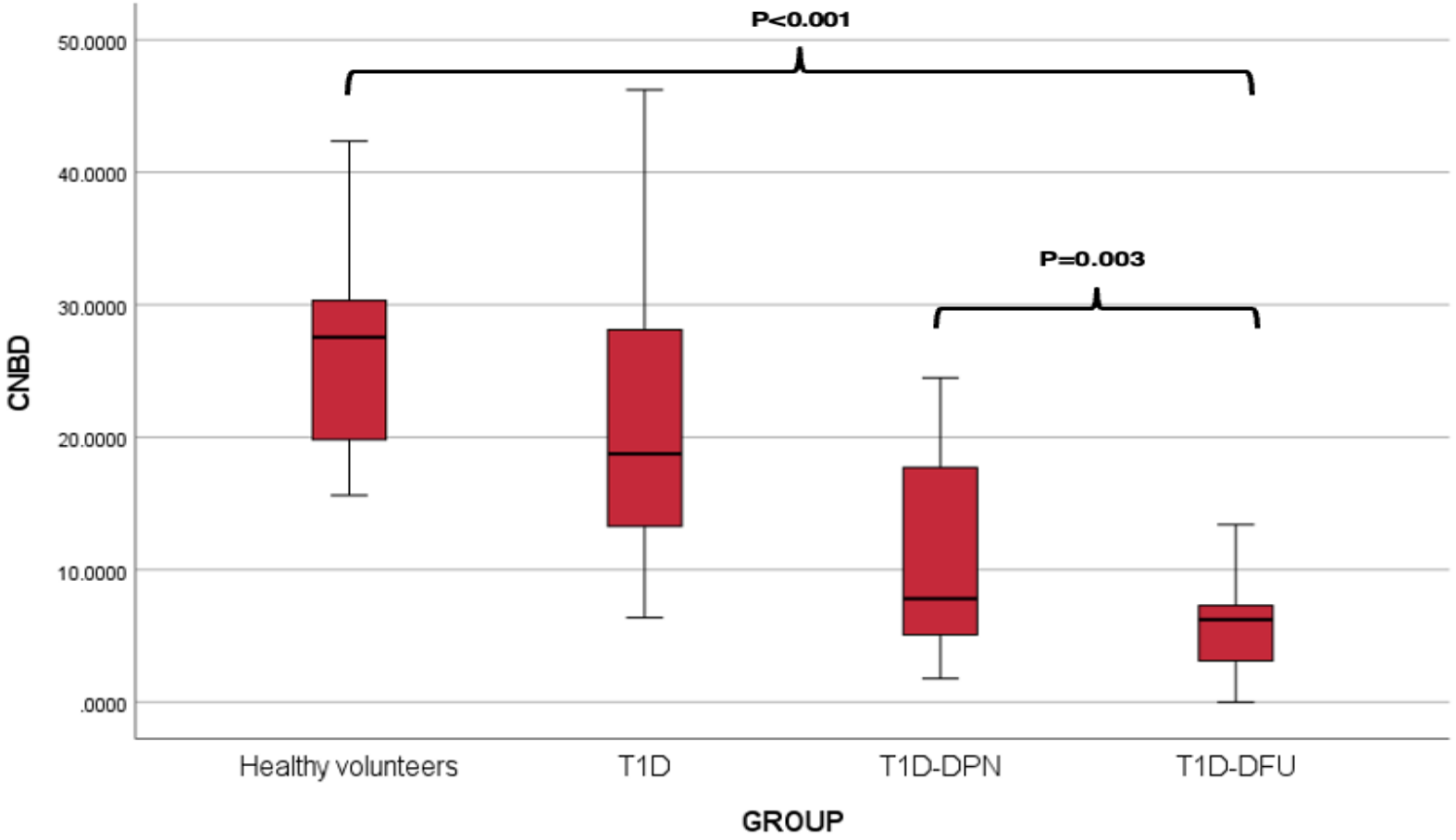
A

Figure 5.3a, b and c. Boxplot of CNFL, CNFD, and CNBD between groups



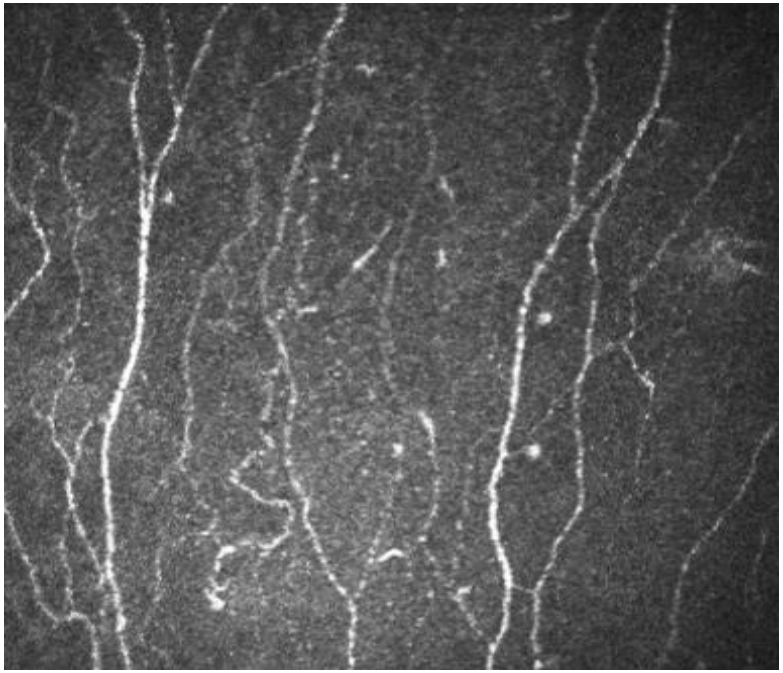
B

Figure 5.3a, b and c. Boxplot of CNFL, CNFD, and CNBD between groups

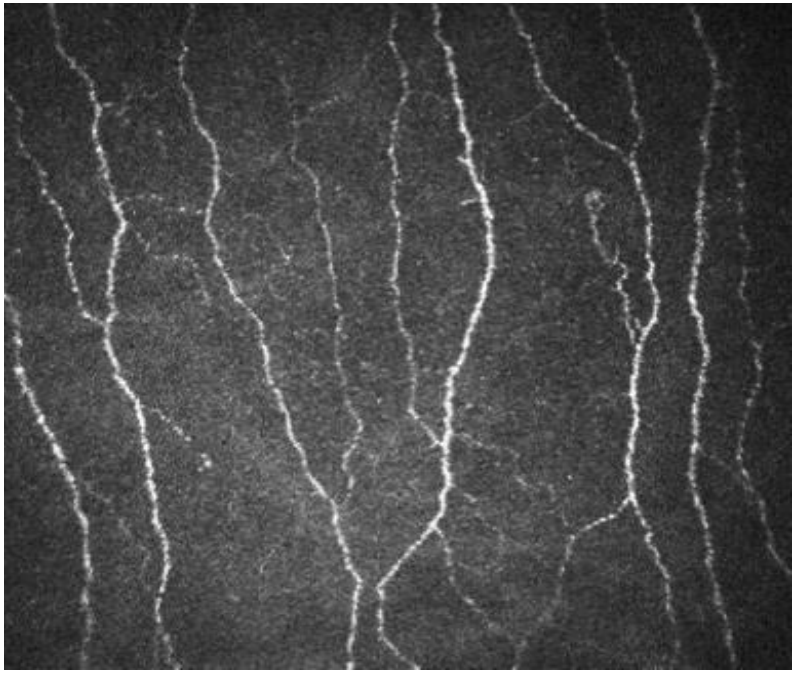


C

Figure 5.4 a, b, c, and d. Corneal Confocal Microscopy Images demonstrating corneal nerve morphology of a healthy volunteer, a person with T1D, a person with T1D-DPN and a person with T1D-DFU.

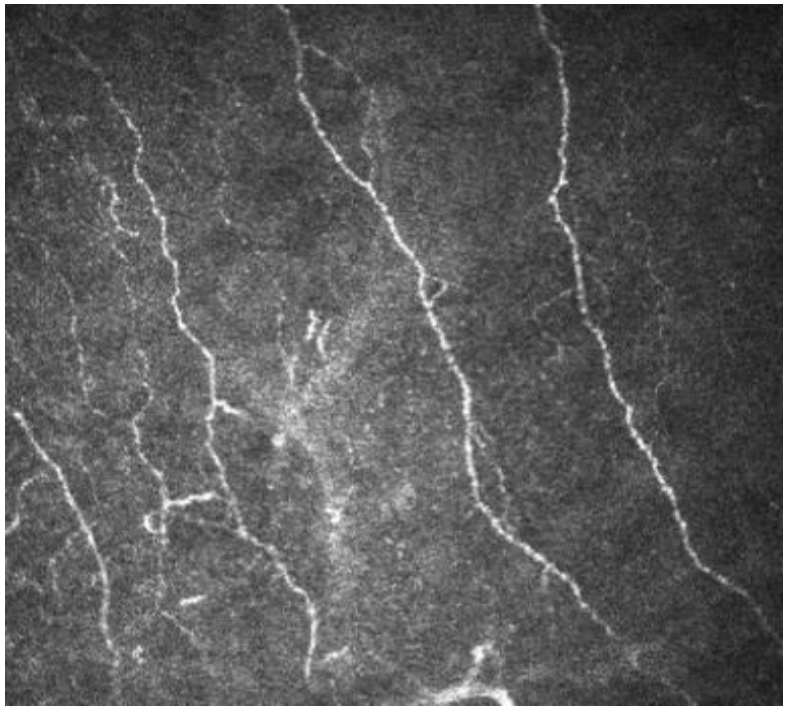


A- Healthy volunteer

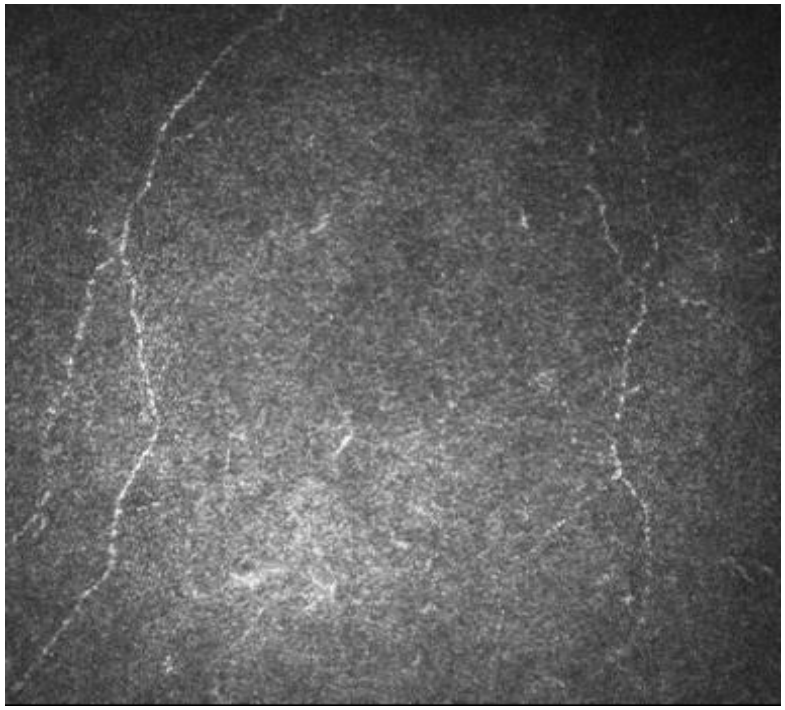


B- Type 1 diabetes without neuropathy

Figure 5.4 a, b, c, and d. Corneal Confocal Microscopy Images demonstrating corneal nerve morphology of a healthy volunteer, a person with T1D, a person with T1D-DPN and a person with T1D-DFU.



C – Type 1 diabetes with DPN



D- Type 1 diabetes with DFU

Figure 5.5 ROC curves for corneal nerve fibre density CNFD (no./mm^2), corneal nerve branch density (CNBD, no./mm^2) and corneal nerve fibre length (CNFL, no./mm2) for neuropathic foot ulcer in type 1 diabetes.

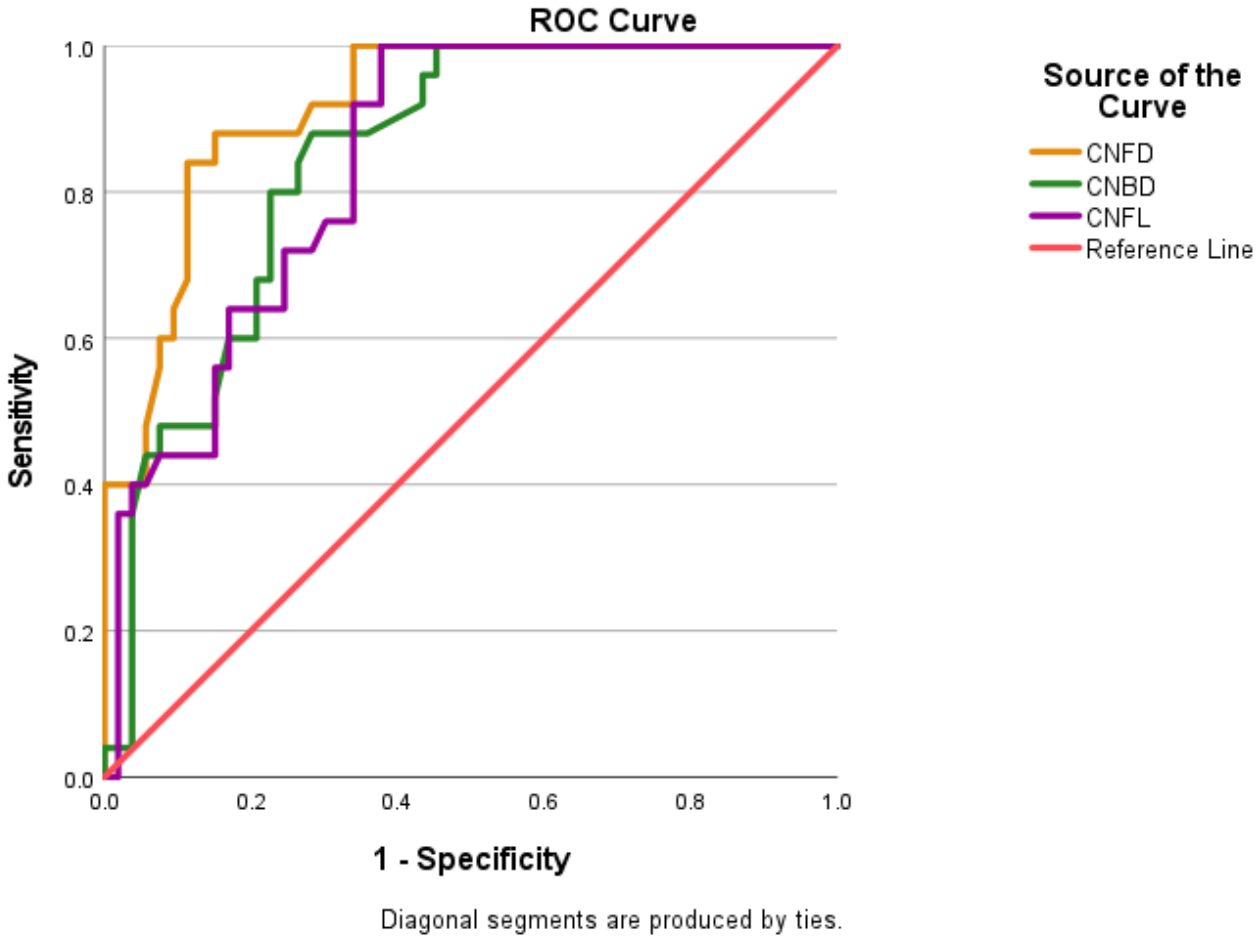


Table 5.5 Diagnostic performance of corneal nerve fibre parameters for the diagnosis of neuropathic foot ulcers in type 1 diabetes.

CCM parameters	AUC	95%CI	*p* value	Optimal threshold	Sensitivity	Specificity
CNFD, no./mm^2	0.92	0.86, 0.98	<0.001	7.44	0.88	0.85
CNBD, no./mm^2	0.85	0.76, 0.93	<0.001	8.80	0.88	0.72
CNFL, mm/mm^2	0.84	0.76, 0.93	<0.001	6.83	0.76	0.70

5.9 Discussion

The study demonstrated greater small fibre dysfunction and degeneration as evidenced by the increased warm and cold thermal thresholds, impaired sural sensory nerve conduction velocity and amplitude, and greater corneal nerve deficit, respectively, when comparing T1D-DFU, T1D-DPN and T1D. In the absence of other macrovascular issues or significant peripheral arterial disease, progressive degeneration of small fibre was demonstrated based on the corneal nerve deficits determined via CCM. In the context of determining small fibre changes in T1D-DFU, corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fibre length (CNFL) have all demonstrate a strong association with high sensitivity and high specificity for diagnosing DFU. The aetiology of greater probability of developing neuropathic foot ulceration in DPN is related to genetic traits, glycaemic control and variability, microvascular factors (358, 359) as well as role of degeneration to spinal cord and brain pathology (360).

Current evidence is scarce in the head-to-head comparison between skin biopsy in individuals with T2D to determine which test is better, but in people with T1D, there are similar sensitivity and specificity between the skin biopsy and CCM technique (361). As evident from this observational cross-section study, our data confirms the DPN and DFU state showed strong correlation with CCM parameters. The high sensitivity and high specificity of CCM in establishing small fibre deficits associated with DFU have been substantiated in previous studies. In T1D, CNFL of ≤14.0 mm/mm2 had optimized sensitivity (85%) and specificity (84%) for detecting DPN (362). Alternatively, proposed cut-off threshold values to maximise sensitivity (CNFL ≥15.8mm/mm2, sensitivity 91%, negative likelihood ratio 0.16) and specificity (CNFL

≤11.5mm/mm^2), specificity 93%, positive likelihood ratio 8.5) (362) to detect DPN have been proposed.

In DPN, nerve degeneration often occurs concomitantly in both large myelinated (Aα and Aβ) fibres and small unmyelinated nerve (Aδ and C) fibres (363). However, the sequence of occurrence of nerve fibre damage remains largely uncertain. Despite previous evidence suggesting that simultaneous occurrence of degeneration to small- and large-diameter nerve fibres (364), more recent studies have suggested the presence of early involvement of small-diameter Aδ and C fibres (141, 324, 365-367). The recent evidence evaluating early DPN evaluated by intraepidermal nerve fibre density (IENFD) suggests a temporal relationship of small fibre impairment preceding that of large fibres (368). Furthermore, in skin biopsy studies, there was reduced density of the small intraepidermal nerve fibres in symptomatic DPN in even in people with impaired glucose regulation (prediabetes) and recent diagnosis of T2D (369), despite preserved large fibre function assessed by nerve conduction studies (141, 324). Greater sensory and autonomic dysfunction including diabetic autonomic neuropathy have been correlated with small fibre degeneration (370) as opposed to later end-stage damage when patients develop large fibre complications manifesting as motor weakness (371). However, limited longitudinal studies in diabetes have led skewed researchers to abide by the hypothesis of the natural history of diabetic sensorimotor neuropathy that measures small-fibre morphology and function decline prior to those of large fibres. The most important clinical implication of this hypothesis is that small-fibre testing could serve as an earlier, subclinical primary end point in clinical trials investigating the interventions for DPN.

5.9.1 Small Fibre Nerve Degeneration Occurs prior to Advanced Large Fibre Loss in T1D

Early onset of subclinical small-fibre injury prior to manifestation of large-fibre damage in diabetic sensorimotor polyneuropathy (DSP) has been reported in people with newly diagnosed T2D and impaired glucose tolerance. The evidence for a natural progression from normal to severe small and/or large fibre damage in people with T1D remains scarce due to small population sizes and heterogenous study populations. Breiner et al (372) performed a cross-sectional study that classify T1D participants based on NCS to presence or absence of DSP. Small-fibre dysfunction (defined by laser Doppler imaging heat-evoke flare, CCM, heart rate variability or cold/hot perception threshold) was present in 96.5% of DSP in T1D, as compared to 52.7% in control participants without DSP. Small fibre dysfunction occurs prior to clinical manifestation of large fibre nerve loss (in the absence of abnormal NCS). Given the findings that isolated large-fibre injury was much less frequent, small-fibre damage occurs prior to the onset large-fibre injury may manifest as a spectrum of 'natural progression' from normal to severe small and/or large fibre damage in T1D (372).

More recent evidence support the above hypothesis that alterations in small-calibre nerve fibres represents the earliest effects of diabetes on the peripheral nervous system (141, 365, 366, 368) and has been based on (i) altered thermal thresholds in patients with normal NCSs (365, 366); (ii) reduced IENFD at the ankle and IENFD ratios in people with diabetes without symptoms or signs of peripheral neuropathy and normal NCS (365, 368); and (iii) reduced IENFD in people with impaired glucose tolerance (141, 367, 373). Although our data demonstrates concomitant small and large fibre nerve degeneration in advanced DFU and severe DPN in a large single-centre T1D cohort, we postulate that a similar natural history of neuropathy may be observed in

T1D where there is earlier development of small nerve fibre loss prior to establishment of large nerve fibre deficit.

In clinical practice, screening for DPN successfully predicts individuals at risk of ulceration (322, 374). Peripheral sensory neuropathy advances progressively (375, 376), where decreased sensation of vibration from a tuning fork is a sign of an earlier stage of neuropathy, while inability to perceive pressure of a monofilament is a later stage sign (375, 376). The finding of more severe phenotype of DPN, in the context of loss of protective perception of both the vibration from tuning fork and pressure from monofilament predicted the recurrence of diabetic foot ulceration (in comparison to only not sensing the vibration from the tuning fork).

5.9.2 Microvascular Endothelial Dysfunction and Inflammation with small nerve fibre loss

Poor glycaemic control has been associated with development of microvascular damage through endothelial dysfunction and increased oxidative stress (358). Pre-clinical experimental studies in rodents with diabetes showed significantly higher pro-inflammatory cytokines (TNF-α, interleukins-6) within the sciatic nerve. Impaired (decreased) motor and sensory nerve conduction velocities (SNCV) and decrease in the intraepidermal nerve fibre density (IENFD) corresponds to small nerve fibre impairment (377). Greater release of reactive oxygen species, nonenzymatic glycation, higher flux of glucose through polyol pathway, increased activation of protein kinase C and neuro-vascular endothelial dysfunction (378, 379) each play a role leading to small and large nerve fibre injury.

Small nerve fibre injury and damage is closely linked to concomitant microvascular and neuronal damage. Microcirculatory issues leading to capillary endothelial

dysfunction has been established in T2D. A cross-sectional study (380) demonstrated a correlation between deficits in small and large nerve fibres with microvascular changes with strong correlation between endothelial dysfunction with impaired small fibre damage (based on CCM parameters) (380).

5.9.3 Impact of Glycaemic Control on Peripheral Neuropathy

Improvement in glycaemic control is strongly associated with effective improvement in neuropathy biomarkers and significant reduction in risk of progression of DPN in people with T1D (108, 381). Stricter glycaemic control has proved to improve long-term outcomes by reducing development of microvascular complications and delaying onset of DPN. Anti-glycaemic treatment including use of continuous subcutaneous insulin infusion (CSII) have been shown to improve nerve conduction and pain control in DPN (382). Regeneration of the corneal nerves have been reported in response to improved glycaemic control through use of CSII compared to multiple daily injections of insulin (383). This demonstrates impact of improved glycaemic control on small nerve fibre regeneration.

5.9.4 Large Nerve Fibre Damage Predicts Ulceration in DPN

Patients with diabetes who are ‘at greatest risk’ of ulceration have concomitant DPN, with vascular insufficiency and dyslipidaemia, leading to rapid deterioration. Motor neuropathy typically presents as wasting of the intrinsic muscles of the foot, resulting in clawing of the toes and changes to the architecture of the mid-foot, and subsequently in pressure redistribution over the metatarsal heads. Patients with moderate to severe sensory loss due to DPN are up to seven times more likely to develop their first foot ulcer when compared with patients with diabetes who do not have neuropathy(384). As a consequence of sensory neuropathy, including loss of large nerve fibres causing

loss of proprioception, reduced sweating and dry skin that can develop skin cracks and fissures, the risk of developing diabetic foot ulceration increases by several folds (116).

5.9.5 Advantages of In Vivo Corneal Confocal Microscopy as compared to Skin Biopsy

In vivo CCM quantifies small fibre damage in a rapid, reproducible, non-invasive manner and detects earlier stages of small fibre loss compared to IEFND pathological changes (146). Further, CCM morphology and quantification of metrics have shown to worsen with the progressive deficit from mild to severe DPN, paving the course to predict severity of DPN. Progressive decline in CNFD was associated with greater neuropathic severity (measured via NDS), greater symptoms score (NSP) and greater QST (all $P<0.001$) (385). The correlation between the CNFD, CNBD and CNFL were significantly reduced between HVs and participants with diabetes with progressive severity of neuropathy established by NDS, VPT and NCS ($P<0.001$) (386). CCM could also be incorporated as a quantification surrogate biomarker in larger population-based studies allowing for population screening and better recruitment and end-point quantification in studies involving therapeutic interventions for management of DPN.

Small nerve fibre regeneration in the corneal nerve fibres have been demonstrated in people with T1D who received simultaneous pancreas and kidney transplantation (387) as early as 6 months post-SPK intervention. Following that, improvements in corneal nerve regeneration was observed to be significant 24 months after optimized glycaemic control, blood pressure and lipid pharmacotherapy, but no significant improvement in the nerve conduction studies (387). Improvements in corneal nerve fibre length 12 months after SPK, followed by improved NSP after 24 months and improved neurophysiology after 36 months (388).

The limitations in the usage of CCM remain the lack of specialists and expertise to perform and conduct the scanning of the different layers of the cornea, as well as the small field of vision with limitation of the corneal confocal microscopes. We have demonstrated the validity of such techniques can be optimized by relying upon the use of innovative technology including automated scanning and analysis using advanced software. Development of artificial intelligence-based deep learning algorithm (389) have demonstrated promising results in terms of rapid detection of DPN using a single image from CCM.

5.9.6 Development of Clinical Prediction Model for Diabetic Neuropathic Ulcer

Development of a clinical prediction rule for foot ulceration in people with diabetes was succinctly described by Chappell et al. (390) which involves a combination of three core information: (1) insensitivity to a 10g monofilament (2) presence of pedal pulse (3) previous history of ulcers or amputation. This validated clinical prediction rule quantifies the risk of developing an ulcer using a scoring system based on the 3324 participants with follow-up to 2 years based on the data from four international cohort studies (390). Potential application of the clinical utility using CCM in future prediction models could help predict and purposefully target or evaluate the effects of small fibre neuropathy at an early stage. At the present day, there are no prognostic models that predict foot ulceration with 100% accuracy. Most studies may involve sophisticated and expensive tests which relies heavily on the subjective interpretation and need for neurological studies, leading to delayed diagnosis or potentially missed neuropathic outcomes in diabetes. The studies on mixed populations of small fibre neuropathy and diagnostic utility of CCM and phenotype characteristics of have more recently been evaluated and could serve a useful biomarker of DPN.

5.9.7 Degeneration of Small Nerve Fibres delay Wound Healing

Diabetic foot consists of interaction between neuropathic, macrovascular, and microvascular diseases resulting in abnormal foot motor control, impaired perception of pain and paraesthesia. loss of proprioception and vibration and decreased sweating (391). Mechanical trauma from repetitive and continuously applied stress complicates the neuropathic DFU. Based on immune and histology staining of skin biopsy, denervated skin is associated with impairment of wound healing (392). Dysregulation of the sensory and autonomic nerves lead to aberrant wound healing and failure to heal in a time-appropriate manner, further impacting the quality of life, morbidity, mortality and increases the healthcare costs (393).

Optimal wound healing requires integration of complex biological and molecular events including role of neurotransmitters on tissue inflammation, proliferation, and migration of keratinocytes, re-epithelialization of cells followed by angiogenesis are essential phases of wound healing (394). The link between wound healing and the nervous system is clinically apparent especially in diabetes where 30–50% of people develop neuropathy, and DPN is a sensitive predictor of foot ulceration. The bidirectional connection between the nervous and the immune systems and the role it plays in wound healing have attracted interest due to the different potential drug targets which may potentially improve nerve regeneration around wound site. The regeneration and re-innervation of the injury at the lesion (foot ulcer) is essential for efficient wound repair (392). However, there remains a gap in knowledge to fully understand the re-innervation of cutaneous tissue post-injury.

Electrical stimulation studies have been shown to promote cutaneous wound repair in both animal models (395, 396) and human studies (397). This highlights the importance of further research to evaluate the change in small nerve fibres on the different stages of wound healing in clinical and experimental studies. Cutaneous fibre density is reduced after inner nerve transection and that neuropeptide level depletes after denervation, leading to reduced cell proliferation around the wound and thus wound healing problems (398). Further research is required to explore the imbalance of neuro-mediators found in denervated disease states such as diabetes.

5.9.8 Neuropeptide Mediators on Wound Healing in DFU

Common neuropeptide mediators released during wound repair are Substance P (SP), calcitonin-gene related peptide (CGRP), nitric oxide and TNF-alpha which promotes inflammation, enhances fibroblast collagen production, cellular migration, and tissue re-epithelialisation (399). In patients who experience chronic pain, the resultant sustained release of these mediators creates an imbalance and may impede wound healing. Denervated tissue demonstrates reduced microvascular responses because of the denervation-evoked desensitization of vascular smooth muscle. Reduction in these responses may delay wound healing. CGRP proposed to contribute to pain transmission and has a key component in wound healing (400). The over-expression of SP and CGRP is detrimental to wound healing and tissue homeostasis (401). However, it remains too early at present to know if these peptides may emerge as potential novel pharmacotherapy targets for wound healing.

Corneal nerve fibre regeneration (402) has also been reported in drug pathways interfering with the polyol pathway in DPN (403), by restorative blood flow to the endoneurium, decreased oxidative stress (378), better nerve perfusion, reduced cellular

death and maintenance of endothelial permeability (328). Other proposed regenerative mechanisms for small fibre include immunomodulatory repair of axonal peripheral nerve defects (404). FGF-21 can regulate glucose and lipid metabolism, maintain energy balance, and play a role in regulating blood lipid and resisting oxidation (405), protect the blood-brain barrier from traumatic brain injury and prevent the blood-brain barrier leakage in T2D (406, 407), and promote remyelination and functional recovery of injured peripheral nerves. However, there is limited evidence is available regarding the corneal and small fibre peripheral nerve regeneration during healing of the neuropathic ulcer in the diabetic foot.

5.9.9 Degeneration of Small Nerve Fibres in Charcot's Neuroarthropathy

In people with diabetic neuro-osteoarthropathy or Charcot's neuropathic osteoarthropathy, chronic inflammation of the bones and joints causes deformity, which is painless due to the neuropathy. The diseased foot in Charcot neuropathic osteoarthropathy causes destructive joint deformities often associated with chronic trauma, load bearing to a neuropathic extremity in the foot. Charcot's foot can lead to permanent foot deformity, bone fragmentation, and result in acute localized inflammatory changes, which disrupt the bony architecture resulting in abnormal plantar pressures that eventually develops into foot ulceration, bone osteomyelitis and limb amputation (408-410). In Charcot's foot, there is increased reactive local hyper-perfusion due to the neurovascular hyperaemia component leading to the demineralization of the foot, bone, and joint structures (411). The individual with DPN cannot detect or feel pain or other sensations, further osseous destruction (loss of proprioception) occurs, leading to deformity, and worsening foot ulcers. In people with T2D with Charcot's foot and neuropathic foot ulcers, the corneal nerve parameters

showed rapid decline and reduction prior to the development of neuropathic foot complications (412). This suggests that the small fibre neuropathy occurs in neuropathic ulcers, which may imply that use of CCM may the predict development of DFU.

5.10 Limitations

Our analysis in this cross-sectional study has limitations. We did not perform skin-punch biopsy measurement of IENFD which is considered by many investigators as the reference standard for the diagnosis of small-fibre neuropathy (413, 414). We instead relied on a panel of signs and symptom profile, and CCM morphology as surrogate biomarkers for the diagnosis of small-fibre abnormalities. The design of our study prospective observational, cross-sectional, non-interventional study was limited to a single-centre recruitment. Third, there is a lack of agreement on the reference-standard threshold values established for small-fibre tests, which may result in potential bias. Fourth, there is a significant age difference between the baseline age of T1D-DPN and T1D-DFU subjects as compared to HVs (control) and T1D without neuropathy. Fifth, our study does not identify which combinations of abnormal non-invasive small-fibre tests are sufficient to establish a conclusive diagnosis of SFN. Finally, we cannot generalize these findings to people with T2D. In conducting our analysis, we have not relied on established literature reference values for small-fibre test abnormalities, which have varied significantly from different institutions.

5.11 Conclusion

CCM remains a rapidly reproducible surrogate biomarker to quantify neurodegenerative corneal nerve parameters in the assessment of DPN in T1D, and

this study provides further evidence that CCM biomarkers has close correlation with T1D-DFU. Future research studies should be directed towards incorporating prospective recruitment and longitudinal evaluation of progressive small and large peripheral nerve dysfunction, to evaluate whether the nerve degeneration occurs immediately prior to neuropathic plantar ulceration. Future research should attempt to incorporate more widespread and consistent classification of participants according to their clinical phenotype (non-nociceptive insensate versus painful DPN), useful in relation to therapeutic clinical trials for management of painful DPN. Prospective larger cohort longitudinal data would help establish threshold values of CCM that predicts those who will develop neuropathic foot ulcer in T1D.

6 CHAPTER VI - The peripheral neuropathy prevalence and characteristics are comparable in people with obesity and long duration type 1 diabetes

Contribution: Jonathan Lim contributed to the conception and design of the study and made a major contribution to the recruitment of subjects, neuropathy assessment, anthropometric and biochemical assessments, corneal confocal microscopy, all statistical analyses and writing of the manuscript which constitutes the basis for this chapter. This was submitted and accepted for publication in the *Advances in Therapy* 2022 (Epub 2022 Jul 22)

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ABSTRACT

Introduction: Peripheral neuropathy is reported in obesity even in the absence of hyperglycaemia. Objective: To compare the prevalence and characterise the phenotype of peripheral neuropathy in people living with obesity (OB) and long duration type 1 diabetes (T1D).

Patients and methods: We have performed a prospective cross-sectional study of 130 participants including healthy volunteers (HV) (n=28), people with T1D (n=51), and OB (BMI 30–50 kg/m^2) (n=51). Participants underwent assessment of neuropathic symptoms (Neuropathy Symptom Profile (NSP)), neurological deficits (Neuropathy Disability Score (NDS)), vibration perception threshold (VPT) and evaluation of sural nerve conduction velocity and amplitude.

Results: Peripheral neuropathy was present in 43.1% of T1D (age: 49.9±12.9 years; duration of diabetes: 23.4±13.5 years) and 33.3% of OB (age 48.2±10.8 years). VPT for high risk of neuropathic foot ulceration (VPT ≥25 V) was present in 31.4% of T1D and 19.6% of OB. Participants living with OB were heavier (42.9±3.5 kg/m^2) and had greater centripetal adiposity with an increased body fat percentage (FM%) (P<0.001) and waist circumference (WC) (P<0.001) compared to T1D. The OB group had increased NDS (P<0.001) VAS for pain (P<0.001), NSP (P<0.001), VPT (P<0.001) and reduced sural nerve conduction velocity (P<0.001) and amplitude (P<0.001) compared to HV, but these parameters were comparable in T1D. VPT was positively associated with increased WC (P=0.011), FM% (P=0.001) and HbA1c (P<0.001) after adjusting for age (R^2=0.547). Sub-group analysis of respiratory quotient (RQ) measured in OB group did not correlate with VPT (P=0.788), nerve conduction velocity (P=0.743) or amplitude (P=0.677).

Conclusion: The characteristics of peripheral neuropathy were comparable between normo-glycaemic people living with obesity and people with long duration T1D, suggesting that metabolic factors linked to obesity plays a pivotal role in the development of peripheral neuropathy. Further studies are needed to investigate the mechanistic link between visceral adiposity and neuropathy.

6.1 INTRODUCTION

The global prevalence of obesity has more than doubled since the 1980s, affecting an estimated 604 million adults and 108 million children (415). Within the UK, 27% of adults are obese (body mass index (BMI) ≥30kg/m^2) (416), and 3-4% are severely obese (BMI ≥40kg/m^2) (417). Furthermore, the prevalence of obesity is projected to rise substantially by 2030 in the US, such that 48.9% of adults will be obese and 24.2% will be severely obese (1).

Obesity is associated with systemic inflammation and endothelial dysfunction which can lead to peripheral neuropathy in type 2 diabetes (T2D) (321), and type 1 diabetes (T1D) (418). The EURODIAB study demonstrated that BMI, hypertension and dyslipidaemia had comparable risk to HbA1c for incident neuropathy in people with T1D (102). The Anglo-Danish-Dutch study of Intensive Treatment of Diabetes in Primary Care (ADDITION) confirmed that abdominal obesity independently predicted peripheral neuropathy in newly diagnosed patients with T2D (105). Furthermore, obesity is associated with peripheral neuropathy independent of hyperglycaemia and hypertriglyceridemia (419). The Rotterdam study reported that abdominal obesity, metabolic syndrome, and dyslipidaemia were strongly associated with peripheral neuropathy in the absence of diabetes (110). Symptomatic peripheral neuropathy is more common in metabolic syndrome, independent of glycaemic status (420). Indeed, the Monica/Kora Augsburg study demonstrated that neuropathic pain was independently associated with body weight and waist circumference in subjects with impaired glucose tolerance (IGT) (421).

The pathognomonic manifestations of insulin resistance, which include a decrease in metabolic flexibility and impaired ability to switch between fat and carbohydrate metabolism are putative factors linking obesity and peripheral neuropathy (109).

Insulin resistance is also associated with low grade inflammation which leads to endothelial dysfunction and microvascular complications (109). Our study has compared the prevalence and characteristics of peripheral neuropathy in people with obesity to people with long duration type 1 diabetes. We have also evaluated the putative link between respiratory quotient (as a marker of substrate oxidation at rest) and peripheral neuropathy in people with obesity.

6.2 METHODS

6.2.1 Selection of patients

We performed a prospective cross-sectional study from January 2019 to March 2021 in (i) healthy volunteers (HV), (ii) people with type 1 diabetes (T1D) and (iii) people with obesity (OB) (BMI 30 – 50 kg/m^2) without diabetes were recruited from Liverpool University Hospitals NHS Foundation Trust clinics. The exclusion criteria for participants in the OB group were: (1) previous bariatric surgery, (2) thyroid disorders, (3) concurrent use of weight loss medication, including orlistat, phentermine, sibutramine, naltrexone/bupropion, GLP-1 receptor agonist within 3 months prior to screening and (4) a formal diagnosis of peripheral neuropathy of any origin. The exclusion criteria for T1D group were: (1) people with class 2 or 3 obesity (BMI ≥ 30 kg/m^2), (2) excessive alcohol intake, (3) neuropathy of non-diabetes origin. HVs were excluded if they were taking medications for hypertension or hyperlipidaemia. The sample size was not calculated formally as we were recruiting unselected patients for feasibility. We intended to base the sample size calculation upon the vibration perception threshold measurements and these data for future studies. The study received relevant research ethics approval by the University of Liverpool Clinical Trials Unit and the Northwest Research Ethics Committee

(18/NW/0532). This study has received the health regulatory approval and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP). All subjects provided informed consent to participate in the study.

6.2.2 Clinical assessments

Eligible subjects underwent weight, waist circumference (WC), BMI, total body fat percentage (FM%), and blood pressure measurements. Body composition was determined using the two-electrode leg-to-leg bio-impedance analyser machine (Tanita TBF-300MA, Tanita Corporation, Tokyo, Japan). Normoglycaemia (HbA1c <39 mmol/mol), prediabetes (HbA1c 39-47 mmol/mol), and diabetes mellitus (HbA1c ≥48 mmol/mol) were classified according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (422).

6.2.3 Neurological assessment

Neurologic deficits were assessed according to the Neuropathy Disability Score (NDS), with a score of 0 – 2/10 graded as no neuropathy, 3-4 mild neuropathy, 5-8 moderate neuropathy, 9-10 severe neuropathy and the neuropathy symptom profile (NSP) (out of 38) were assessed. All subjects scored the average intensity of their neuropathic pain over the previous 2 weeks on a continuous visual analogue scale (VAS), where “0” and “10” indicated “no pain” and “worst possible pain”, respectively.

Peripheral neuropathy was defined according to the Toronto consensus (115) i.e. the presence of an abnormality of nerve conduction and a symptom or symptoms and/or a sign or signs of neuropathy. Vibration perception threshold (VPT) was measured from an average of three values on the large toe using a neurothesiometer (Horwell,

Scientific Laboratory Supplies, Wilford, Nottingham, UK). Participants with impaired VPT were defined by VPT of 15-24V, whilst participants with ≥25 V was deemed at high risk for neuropathic foot ulcer.

Sural Nerve Conduction Velocity (SNCV) and sural nerve action potential (SNAP) were measured using the point-of-care device NC-Stat® DPNCheck™ system (Neurometrix, Waltham, USA). The NC-Stat DPNCheck has good reliability and reproducibility and correlates well with SNCV (R=0.81) and moderately with SNAP (R= 0.62) derived from nerve conduction studies (NCS) as a reference method (356). The point-of-care conduction device results were evaluated against the reference values provided for the device (abnormal result defined by amplitude ≤4 μV or conduction velocity ≤44 m/s). The device cannot detect SNAP signals of <2.1μV and automatically calibrates these values as zero (356), therefore all zero results were recorded as 2.0 μV. For instances when operators were unable to attain or detect SNCV or SNAP signals, the result were recorded as clinical neuropathy. Operators were trained to reduce errors by using a fixed conduction distance and filter settings and maximizing amplitude to improve the signal to noise ratio (356).

6.2.4 Fat oxidation assessment

Respiratory quotient (RQ) serves as an index of substrate metabolised by active tissue and is represented by the ratio of volume of oxygen inspired (VO_2) to the volume of carbon dioxide exhaled (VCO_2) during a (fasted) resting metabolic state. The novel open-circuit portable indirect calorimeter *ECAL™* (Metabolic Health Solutions, Australia) (ISO 13485) utilised a proprietary mouthpiece (single use) and a nose clip. VO_2 is measured using a galvanic fuel cell oxygen analyser. VCO_2 is measured using a patented ultra-low power VCO_2 analyser which uses light emitting diode and detector technology in a novel non-dispersive near infrared absorption sensor. Calibrations were

performed using 5% carbon dioxide. A successful test was defined as a steady state achieved with a minimum of 5 consecutive minutes with less than 10% coefficient of variation in FEO_2 and $FECO_2$.

6.2.5 Statistical analysis

Descriptive statistics were presented as mean ± standard deviation or value with percentage. Means were compared between the three groups (HVs, OB and T1D) using the ANCOVA adjusted for age with Bonferroni adjustment or Kruskal-Wallis with post hoc test. Univariate and multivariate linear regression was used to model VPT as a function of the metabolic syndrome components (WC, HDL, triglycerides, systolic blood pressure), after adjusting for age. Statistical analysis was performed using IBM SPSS version 27.0 (IBM Corp., Armonk, NY, USA).

6.3 RESULTS

6.3.1 Demographics

One hundred and thirty participants (T1D (n=51), OB (n=51) and HVs (n=28)) matched for age were studied, however the proportion of female participants in the OB group (75%) was greater than the T1D group (47%) (P=0.005). The demographics, anthropometric measures, clinical and metabolic phenotyping are summarised in **Table 6.1**. The mean duration of T1D was 23.4±13.5 years. Participants with obesity (OB) had a greater BMI (P<0.001), body fat percentage (P<0.001), WC (P<0.001), total cholesterol (P<0.001), LDL-cholesterol (P<0.001) and triglycerides (P<0.001) with lower HDL-cholesterol (P<0.001) compared to participants with T1D. According to the NCEP ATPIII definition of metabolic syndrome, 58% of participants fulfilled the criteria for metabolic syndrome in the OB group.

6.3.2 Neuropathy Assessment

Peripheral neuropathy measures are summarised in **Table 6.2**. Peripheral neuropathy was present in 43.1% of participants with T1D and 33.3% of participants living with OB according to the Toronto consensus criteria for peripheral neuropathy. Impaired VPT (15-24V) and advanced VPT deemed at high risk of neuropathic ulcer (≥25V) was present in 19.6% and 31.4% in T1D and 23.5% and 19.6% of participants living with OB. There were no differences in VAS for pain, NSP, NDS, VPT, SNCV and SNAP between T1D and OB groups. However, both T1D and OB groups demonstrated greater VAS for pain, NDS, NSP, VPT, SNCV and SNAP compared to HV ($P<0.001$). There was an association between VPT and VAS pain ($R^2= 0.330$) (**Figure 6.1**).

Table 6.1. Demographics, clinical and metabolic characteristics in HVs and participants with T1D and OB.

	HV (n=28)	**T1D (n=51)**	**OB (n=51)**	**P value (HV vs T1D)**	**P value (HV vs OB)**	**P value (OB vs T1D)**
Age (years)	43.0±6.9	49.9±12.9	48.2±10.8	0.067	0.146	0.960
Duration of T1D (years)	-	23.4±13.5	-	-	-	-
Female, *n* (%)	18 (64)	24 (47)	38 (75)	0.383	0.133	0.005
Weight (kg)	67.6±10.1	80.6±20.9	116.2±12.9	0.002	<0.001	<0.001
BMI (kg/m^2)	23.3±2.6	28.0±5.1	42.9±4.0	<0.001	<0.001	<0.001
WC (cm)	89.1±11.6	95.1±19.1	125.5±14.9	0.924	<0.001	<0.001
FM% (%)	24.6±2.9	26.5±3.4	52.4±2.7	0.457	<0.001	<0.001
HbA1c (%)	5.5±0.2	8.7±1.3	5.6±0.3	<0.001	0.430	<0.001
HbA1c (mmol/mol)	37.0±2.2	76.2±14.1	37.8±2.7	<0.001	0.441	<0.001

TChol (mmol/l)	4.1±0.2	4.4±1.1	5.0±0.8	0.869	<0.001	<0.001
HDL (mmol/l)	1.2±0.2	1.3±0.2	1.3±0.3	0.712	0.712	0.712
LDL (mmol/l)	2.0±0.3	2.1±0.9	2.9±0.9	0.917	<0.001	<0.001
TRIG (mmol/l)	1.2±0.2	1.4±0.9	2.2±0.7	0.906	<0.001	<0.001
eGFR (ml/min/1.73)	82±9	79±18	80±11	0.863	0.895	0.883

Data presented as mean ± SD with significant differences measured by ANCOVA adjusted for age with Bonferroni adjustment or Kruskal-Wallis test with post hoc test. NS denotes non-significant. BMI, body mass index; eGFR, estimated glomerular filtration rate; FM%, body fat percentage; HbA1c, glycated haemoglobin; HDL, HDL-cholesterol; LDL, LDL-cholesterol; HV, healthy volunteers, OB, Obesity; T1D, Type 1 diabetes, TChol, total cholesterol; TRIG, triglycerides; WC, waist circumference.

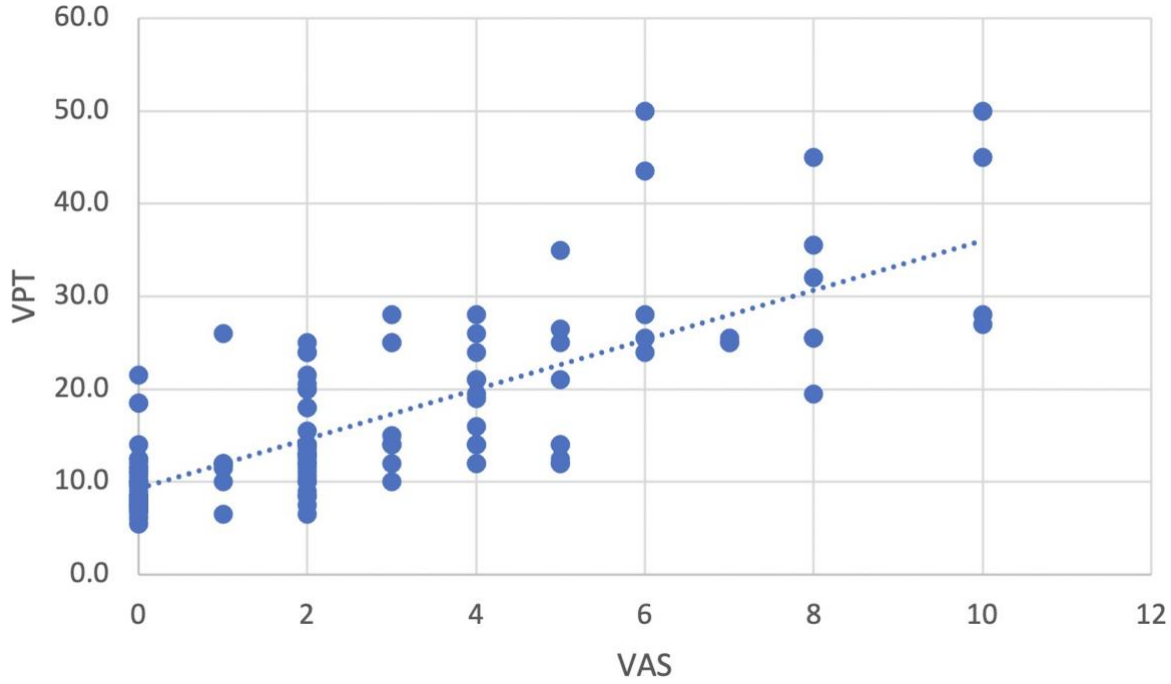
Table 6.2. Peripheral neuropathy measurements in in HVs and participants with T1D and OB.

Neuropathy Measurements	HV (n=28)	T1D (n=51)	OB (n=51)	P value (HV vs T1D)	P value (HV vs OB)	P value (OB vs T1D)
VAS Pain (-/10) Median (IQR)	0.3±0.6 0 (0-0.5)	3.3±3.5 3 (0-7)	3.0±1.5 3 (2-4)	<0.001	<0.001	0.50
NDS (-/10) Median (IQR)	0±0 0 (0-0)	3.3±3.8 2 (0-8)	2.4±2.9 1 (0-6)	<0.001	<0.001	0.52
NSP (-/34) Median (IQR)	0.1±0.5 0 (0-2)	9.3±8.6 6 (2-18)	9.1±7.7 6 (2-16)	<0.001	<0.001	0.28
VPT (V)	8.9±2.0	18.9±10.2	17.5±5.8	<0.001	<0.001	0.33
SNCV (m/s)	50.5±3.4	41.2±7.7	42.2±6.1	<0.001	<0.001	0.75
SNAP (μV)	15.7±2.2	7.3±3.4	7.5±2.9	<0.001	<0.001	0.94
Impaired VPT (%)	0	19.6	23.5	-	-	-

Advanced VPT (%)	0	31.4	19.6	-	-	-
Toronto Criteria (%)	0	43.1	33.3	-	-	-

Data presented as mean ± SD. HV, healthy volunteers; NCS, nerve conduction study; NDS, neuropathy disability score; NSP, neuropathy symptom profile; OB, obesity; SNAP, sural nerve action potential; SNCV, sural nerve conduction velocity; T1D, type 1 diabetes, Toronto consensus criteria for peripheral neuropathy, VAS pain, visual analogue score for pain; VPT, vibration perception threshold.

Figure 6.1. Scatter Plot of VPT vs VAS for pain. Linear Regression (R^2=0.579) with line of best fit.



VPT, Vibration perception threshold (V); VAS, Visual analogue scale for pain. R^2 represented by Linear Regression Test.

Table 6.3. Pearson's correlation of variables against Vibration Perception Threshold (VPT).

	VPT	
	Rho	**P**
Age	0.269	0.002
Female	0.110	0.214
BMI	0.348	<0.001
WC	0.420	<0.001
FM %	0.280	0.001
TRIG	0.299	0.001
LDL	0.168	0.071
HDL	0.045	0.633
TChol	0.227	0.010
HbA1c	0.400	<0.001
SBP	0.379	<0.001
DBP	0.350	<0.001
NSP	0.841	<0.001
VAS	0.761	<0.001
REE[§]	0.042	0.768
RQ[§]	0.012	0.934

BMI, body mass index; DBP, diastolic blood pressure; FM%, body fat percentage; HDL, HDL-cholesterol; HbA1c, glycated haemoglobin; LDL, LDL-cholesterol; NSP, neuropathy symptom profile; SBP, systolic blood pressure; TChol, total cholesterol; TRIG, triglycerides; VAS, visual analogue scale for pain; VPT, Vibration perception threshold; WC, waist circumference. § Consists of subgroup analysis between VPT against REE and RQ dataset within the OB group only.

6.3.2 Neuropathy Assessment

We evaluated correlation between VPT as the primary dependent variable with anthropometric, metabolic and body composition measurements in the whole cohort (HV, T1D and OB) using Pearson's correlation analysis (**Table 6.3**). VPT correlated with NSP (rho=0.841, P<0.001), VAS (rho=0.761, P<0.001) and WC (rho=0.420, *P*<0.001). VPT also correlated with age (rho=0.269, P=0.002), BMI (rho=0.348, *P*<0.001), body fat percentage (FM%) (rho=0.280, *P*=0.001), HbA1c (rho=0.400, *P*<0.001), total cholesterol (rho=0.227, *P*=0.010), triglycerides (rho= 0.299, *P*=0.001), systolic BP (rho= 0.379, *P*<0.001), and diastolic BP (rho=0.350, *P*<0.001) (**Table 6.3**). Sub-group analysis was performed in the OB group to evaluate the association between VPT and that of metabolic biomarkers obtained from indirect calorimetry (REE and RQ). REE and RQ data were obtained and performed in the OB group only (**Table 6.3**).

Stepwise multivariate linear regression modelling was performed with VPT as the dependent variable from the entire cohort (HV, T1D and OB) (**Table 6.4**). In Model 1, BMI (β=0.333; P<0.001) and age (β=0.249; P=0.002) correlated with VPT as the primary dependent variable. In Model 2, VPT correlated with age (β=0.257; P<0.001) and WC (β=0.433; P<0.001), but not BMI (P=0.214) and FM% (P=0.119). In model 3, VPT correlated with age (β=0.149; P=0.037), WC (β=0.382; P=0.018), FM% (β=0.783; P<0.001) and HbA1c (β=1.051; P<0.001) correlated with VPT but there was no significant correlation with BMI, total cholesterol, triglycerides, systolic and diastolic blood pressure.

Table 6.4. Multivariate linear regression model using VPT as the dependent variable.

Model	Variable	Beta coefficients	95% CI	P	R^2
1	**BMI**	**0.333**	**0.170;0.480**	**<0.001**	0.170
	Age	**0.249**	**0.074; 0.336**	**0.002**	
2	BMI	0.208	-0.184;0.457	0.214	0.253
	Age	**0.257**	**0.091;0.342**	**0.001**	
	WC	**0.433**	**0.098;0.313**	**0.001**	
	FM%	0.250	0.056;0.377	0.119	
3	BMI	0.063	-0.290; 0.423	0.711	0.538
	Age	**0.149**	**0.007;0.259**	**0.037**	
	WC	**0.382**	**0.041;0.213**	**0.018**	
	FM%	**0.783**	**0.248;0.884**	**<0.001**	
	TChol	0.078	-2.512;1.004	0.396	
	TRIG	0.043	-1.357;2.098	0.671	
	HbA1c	**1.051**	**0.323;0.595**	**<0.001**	
	SBP	0.043	-0.042;0.171	0.232	
	DBP	-0.045	-0.216;0.131	0.627	

BMI, Body mass index; CI, confidence interval; DBP, diastolic blood pressure; FM%, body fat percentage; HbA1c, glycated haemoglobin; HDL, HDL-cholesterol; LDL, LDL-cholesterol; TCHOL, total cholesterol; TRIG, triglycerides; RQ, respiratory quotient; REE, resting energy expenditure; SBP, systolic blood pressure; VPT, vibration perception threshold; WC, waist circumference.

6.3.3 Obesity with and without peripheral neuropathy

Of the 51 participants with OB, 33.3% (n=17) fulfilled the criteria for peripheral neuropathy according to the Toronto consensus criteria on peripheral neuropathy (115). Within the OB group, the prevalence of impaired VPT (15-24 V) was 23.5% (n=12) and prevalence of advanced VPT deemed at high risk of neuropathic ulcer (VPT of ≥25 V) was 19.6% (n=10). The peripheral neuropathy subgroup within OB had a greater NSP (P<0.001), VAS for pain (P<0.001) and VPT (P<0.001) with lower SNCV (P<0.001) and SNAP (P=0.003). WC (P=0.028) and FM% (P<0.001) were significantly higher in obese participants with peripheral neuropathy compared to those without peripheral neuropathy. In the OB group, measured substrate oxidation, represented by the RQ (mean RQ=1.016; 95% CI 0.9888-1.044) during the rested and overnight fasted metabolic state was not associated with VPT (P=0.934), sural nerve conduction velocity (SNCV) (p=0.743) or sural nerve amplitude (SNAP) (p=0.677).

6.4 DISCUSSION

This cross-sectional study demonstrated a comparable prevalence of peripheral neuropathy in normo-glycaemic people with obesity compared to people with long duration T1D. This advocates that peripheral neuropathy is a result of a culmination of complex interaction of several aetiologically linked pathophysiological processes. Furthermore, our report demonstrates that there is a positive association between obesity and greater centripetal adiposity, approximated by increased waist circumference and increased body fat percentage, with increased and/or impaired VPT. The link between obesity and peripheral neuropathy have been attributed to metabolically driven cardiovascular risk factors such as hypertension, hyperlipidaemia, and inflammation (102, 341) leading to degenerative processes within the small nerve fibres. However, these mechanisms are not fully understood and elucidated.

Obesity and hypertriglyceridemia predict the development of diabetic neuropathy in T2D, independent of glycaemic control (423). In a recent cross-sectional study of 47 participants with severe obesity and 30 age-matched controls, participants with severe obesity had a higher NSP, abnormal thermal thresholds and lower sural and peroneal nerve amplitudes compared to controls and those with obesity and small nerve fibre damage had higher triglycerides and prevalence of metabolic syndrome (58% vs 23%; P=0.02) (424). Interestingly, we did not demonstrate an association between neuropathy and triglycerides, likely due to the good control of lipids in the OB cohort as they were under the care of a tertiary weight management clinic. Experimental studies have demonstrated that neurones send vasoactive signals to increase vascular permeability and attract adaptive immunogenic cells in high fat diet-fed rodents with

obesity, dyslipidaemia, and neuropathy (425, 426). Although initially a protective mechanism, persistent dysfunction secondary to obesity-mediated inflammation results in structural neuronal damage. Further, inflammatory mediators (TNF-alpha and Interleukin-1B) and macrophages promote a long-term microvascular inflammatory response and impairment of insulin signalling in the peripheral nervous system (427). Peripheral neuropathy has been associated with increased abdominal and visceral obesity (103). In addition, obstructive sleep apnoea (OSA) which is prevalent in severe obesity and even in T1D is an independent risk factor for axonal dysfunction of peripheral sensory nerves (428). Unfortunately, OSA data was not available within this cohort and this risk factor could not be further investigated in the current study.

Autonomic dysfunction may be involved in the development of obesity and visceral/central obesity with increased peripheral insulin resistance (429, 430). Xu et al showed that BMI was an independent risk factor for abnormal plantar pressures and increased VPT (431). In patients with T2D Gao et al (432) reported that those with the highest fat mass index had the highest risk of neuropathy (HR 1.93, 95% CI 1.74-2.15). In a cross-sectional study Callaghan et al (433) showed that the prevalence of peripheral neuropathy was 12.1% in obese participants with normoglycaemia and 40.8% in obese participants with diabetes.

Our findings agree with previous studies (113, 433, 434) showing that obesity is associated with peripheral neuropathy. Indeed, Herman et al (434) reported that people with severe obesity had a predominant small fiber neuropathy. In the National Health and Nutrition Examination Survey of adults >40 years of age, whilst 9% had peripheral neuropathy the obese group had at two-fold greater risk (OR 2.20; 95%CI 1.43-3.39)

of neuropathy compared to non-obese individuals (435). Participants with obesity and reduced insulin sensitivity show reduced tibial and peroneal nerve compound and sensory amplitudes (113). Roustit et al (436) showed that a higher WC and obesity were independently associated with VPT. Spallone et al prospectively assessed 135 patients with diabetes and showed that BMI was an independent risk factor for DPN (437). The KORA/Augsburg study, followed 513 subjects over 6.5 years and showed that being overweight (OR 3.06, 95%CI: 1.57-5.97) and obese (OR 3.47, 95%CI: 1.72; 7.00) increased the risk of developing peripheral neuropathy (438).

Emerging research suggests that the development and progression of neuropathy is associated with an impaired metabolic switch from glucose to fatty-acid or lipid oxidation (439) with an association between cholesterol oxidation and glycated LDL and the pathogenesis of neuropathy (440). Reduced peripheral insulin sensitivity also leads to increased fatty acid flux into Schwann cells and peripheral neuropathy (441, 442). Obesity itself is associated with the loss of peripheral sensory neurons and pathology to intra-epidermal nerve fibers (443, 444). Local fat metabolism in the peripheral nerve is of importance in maintaining an intact and function peripheral nerve. Previous data has demonstrated that several genes are only maximally expressed in the mature nerve, after the completion of myelination which are also intertwined to the metabolism of storage lipids (445). Within obesity and T2D, there is intracellular accumulation of metabolites with enhanced fatty acid uptake and blunted fatty acid oxidation and lack of insulin-mediated inhibition of lipolysis (446). This leads to excess circulatory ‘spill’ with uptake by non-adipose tissue like the liver, muscle, heart, and pancreas leading to ectopic fat deposition and dyslipidemia. Consequent to the dyslipidemia state, free fatty acid-induced lipo-toxicity alters lipid-induced

intracellular signaling and drives neurological dysfunction and neurodegeneration (447). Whilst this study has shown impaired fat oxidation and 'over-reliance' on glucose oxidation in obesity, fat oxidation per se was not associated with peripheral neuropathy measures. However, cross-sectional measures of fat oxidation which are fluid may not correlate with more 'fixed' quantitative measure of peripheral neuropathy.

In a large retrospective cohort study of 88,981 patients with T2D, bariatric surgery was associated with significantly lower rates of microvascular and macrovascular complications, compared to a non-surgically treated group, over 9 years (448) and this has been corroborated by other studies (449). Bariatric surgery in people with obesity with and without T2D is associated with small nerve fibre regeneration over 12 months (443, 444). The prevalence of peripheral neuropathy measured with the Michigan Neuropathy Screening Instrument (MNSI) was found to be reduced (pre-bariatric surgery 20.4% to post-bariatric surgery 10.5%) ~10 years after Roux-en-Y gastric bypass and sleeve gastrectomy (450). Several randomized, controlled studies (DiRECT, DROPLET and PREVIEW) have demonstrated the efficacy of LCDs (800-850 kcal/day) in severe obesity (451-453) and recently, a dietary weight loss study of 800 kcal/day (12 weeks) followed by 1200-1500 kcal/day resulted in an improvement in metabolic parameters, whilst IENFD remained stable after 2 years (454).

The limitation in the interpretation of the results from our observational study include the patient selection in the obesity group, which was a heterogeneous recruitment of a random population of people with obesity with unselected or undefined metabolic criteria prior to inclusion. The selection of participants with obesity may have

variability in features of ‘metabolic impairment’ in terms of hyperlipidaemia, hypercholesterolaemia, prediabetes (impaired glucose regulation and/or impaired glucose tolerance), and other metabolic risk factors which could influence and contribute to the obesity phenotype. A large proportion of people with obesity may have predisposition or display traits of metabolic syndrome at various stages, hence it may be difficult to evaluate a direct association between obesity and neuropathy itself. To further evaluate this hypothesis, future research is required based on large population-based studies with longitudinal follow-up. Multivariable predictive modelling would allow to evaluate the multi-factorial contribution and association with development of microvascular complications such as peripheral neuropathy. We acknowledge that causality between obesity and neuropathy cannot be inferred from a cross-sectional study. We have also not undertaken small fibre phenotyping which may be more relevant to obesity related neuropathy. A larger sample size may also have allowed adjustment of confounding factors for neuropathy in relation to RQ or index of fat oxidation and RQ sub-analysis may be limited due to the severe obesity present in the participant population.

In conclusion, the prevalence and characteristics of peripheral neuropathy were comparable between normo-glycaemic people with obesity and long duration T1D suggesting that metabolic factors linked to obesity plays a significant role in development of peripheral neuropathy. Further studies are needed to investigate the role of visceral adiposity on peripheral neuropathy.

7 CHAPTER VII - Conclusion

Obesity is rapidly increasing in global prevalence and substantially increases the risk of metabolic complications (455). Long-term excess energy intake and decrease energy expenditure leads to chronic energy imbalance. The process of natural selection over the centuries favoured traits which could survive prolonged periods of undernutrition or starvation, favouring energy storage, reduced energy expenditure (225, 456). Overall, an increasingly sedentary lifestyle further reduces energy expenditure (261). Overspill of energy storage causes enlargement of adipose tissue to increase capacity for constant energy surplus (457), leading to greater ectopic fat deposit in the viscera, intra-abdominal organs, and increased capacity of skeletal muscle to store glycogen. The relationship between energy homeostasis and body weight is a very closely regulated and influenced by both central and peripheral receptors (458). The availability of food, hunger and eating behaviours are regulated by the neuro-humoral signals which involves central hypothalamic and hindbrain regulation (459). Efferent peripheral feedback at the tissue level is based upon the peripheral signals influenced by the whole body adiposity and the state of energy balance (nutrient availability) (460). During the energy deficit, physiological feedback induces central and peripheral regulatory changes which drives increase in appetite and food intake disproportionate to the adaptations (decrease) in energy expenditure (65). To recommend more effective evidence-based strategies to tackle the chronic disease, this chapter is intended to summarize and conclude the common reason for the lack of efficacy in weight interventions and the innovations to improve weight loss and maintenance.

7.1 The Dynamic Energy Model: Biological Adaptations resisting change in Energy Balance

During negative energy balance and decrease in energy stores, neuro-hormonal responses provoke changes in the energy homeostasis (33). Maintenance of reduced body weight is accompanied by compensatory changes in energy expenditure which favours weight gain (230). Diet-restriction result in an overall decrease in total daily energy expenditure (TDEE), resting energy expenditure (REE) and activity-related energy expenditure (230, 461). Resisting body weight change triggers hormonal adaptations such as change in leptin and insulin, further influencing the appetite regulation (462). The 'energy-deficit' triggers the neural circuitry to respond by enhancing the reward centre of food and reduces satiety (463). Consequently, increased preference for caloric-rich foods and hedonic behaviours may manifest in response to the energy deficit. The corresponding decrease in lean tissue mass and reduced physical activity level exacerbates the decline in energy requirements and further expands the energy gap between the increase in appetite and expended energy (33). The variability between different individuals in terms of weight changes and energy expenditure (EE) in response to controlled diet restriction and in overfeeding is discussed further in the following section.

The variance in the weight loss and adaptations to decrease resting energy expenditure (REE) per change in body fat-free mass (FFM) has been attributed to adaptive thermogenesis (AT). AT serves to explain the reason for the disproportionate change between the decrease in REE was greater than expected from the decrease in active tissue mass (464). AT is proposed to account for the 'greater than expected decrease in REE with energy restriction, which is disproportionate to the decrease in body weight and body composition'(161, 317).

After significant 10-15% weight loss in the person with obesity through lifestyle intervention , there is corresponding decrease in 24h-EE (36). All too often, the lack of weight loss and lack of successful weight loss maintenance is attributed to poor adherence and lack of change in expenditure. However, the physiological adaptations must account for the reduced 24h-EE in response to weight loss. For example, to maintain the 5% weight loss, the individual would need to reduce up to 10-15% calorie intake based on predicted energy needs, due to decrease in FM and FFM (230, 465). Successful weight loss maintainers must maintain the 10-20% caloric restriction (300-400 kcal/day) and maintain the increase in activity expenditure to maintain the weight loss (163). The decrease in 24-hour EE may be persistent even after 6-7 months of weight loss maintenance, from lifestyle modification (466) and in those who underwent bariatric surgery (467). Decrease in REE corresponds to magnitude of weight loss (37, 468).

Compensatory adaptation in terms of REE decrease has been suggested to predict weight gain and greater increase in body FM (42, 82, 469, 470). However, some evidence from longitudinal data over 15 years did not support this observation (471). Larger population studies in Pima Indians have demonstrated that disproportionately lower levels of REE and increased sedentary time was predictive of weight gain (472). Calorie-restriction led to a small yet significant decrease in REE (473), but there was high variability in terms of tissue-specific decreased in mass and metabolic adaptations (474). One potential reason for the extensive variability in change in REE post-intervention depends on changes within the non-muscle tissue components including reduction in cortical thickness or in gray matter volume in people with overweight and obesity, hormonal adaptations and unaccounted decrease in visceral fat and abdominal FM (475).

Future studies are required to continue exploring the role of AT in the context of resistance to weight loss and propensity for weight rebound. Future studies focusing on the clinical aspects of REE variability should continue to evaluate wider applicability and precision of indirect calorimetry measured EE information in relation to the organ-tissue metabolic rate to help develop predictive models for weight loss response.

7.2 Genetic Influence Predicts Response to Lifestyle Based Weight Intervention

In response to long-term overfeeding in identical twins, Bouchard et al (476) evaluated 12 pairs of young adult monozygotic twins who were overfed by 1,000 kcal/day for a total of 84 days. The total excess caloric consumption equate to 84,000 kcal and the mean body weight gain was 8.1 kg (range weight gain of 4.3 to 13.3 kg). Bouchard et al (476) demonstrated that about half of the difference between weight may be accounted for by non-compliance and the other half due to adaptive thermogenesis. Genetic traits, thus, determines the individual response to overfeeding and weight regulation. Compensatory metabolic adaptations lead to energy sparing and reduced fat oxidation (477), which further reduces the effect of weight loss and accelerate fat restoration (478). The association between lower REE and greater body weight gain was observed in Pima Indians where a lower-than-expected REE predicts weight gain (268, 479, 480). Further, in a genetic study by Piaggi et al (479) using customized genotyping array in American Pima Indians, a few variants in the (*GPR158*) were identified, but this finding has not been reproduced in other genome-wide obesity studies thus far. Further, whole-exome sequencing indicated ‘nonsense mutations’ in premature stop codon (480) in American Pima Indians. Ethnicity and familial traits play a significant role in lower-than-normal REE and greater predisposition for

adipose tissue storage (479), potentially generating novel treatment pathways to manage obesity. In identical twin studies of obesity, there were evidently wide variations on energy adaptations leading to different magnitude of weight loss despite very low-energy diet (481, 482).

7.3 Complex Regulation of Systems controlling Hedonic Pathways

Accurate predictors of energy need by utilizing REE predictive equations (Harris-Benedict, WHO/FAO/ONU and Mifflin-St Jeor) (483-485) have been the mainstay in current clinical practice. Fat-free mass (FFM) is the largest determinant of REE, contributing up to 60-80% of the variance (486, 487). Body composition in obesity consists of greater inert fat mass (FM), hence the specific metabolic rate (energy expenditure per unit body mass) is relatively lower compared to lean counterparts. Numerous factors including genetic trait, sex, body composition, physical activity predetermines the change in REE, which in turn predicts the response of weight loss during energy-deficit. Some evidence from longitudinal data (265, 488, 489) reported no change in the REE in response to weight loss, however the magnitude of energy restriction and decrease in body weight may be influenced by the lack of adherence to dietary restriction.

Reductions in up 10-15% in REE accounting for the decline in fat-free mass (FFM) is a considerably strong predictor that determines adaptations in body attempting to resist further weight loss (490-493). During caloric deficit for weight loss, the energy-metabolism adaptation has been observed to resist further weight deficit and predisposes the individual to return to ‘energy-storage’ and weight gain (230, 494). REE variability is influenced by genetic traits, change in physical activity levels, sex,

and compliance to the controlled dietary intervention. Up to 70-85% of the change in TDEE is contributed by the non-REE components, which clearly demonstrates that changes in body weight is affected by the physical activity levels (230, 495), which determines the success in weight maintenance (496).

Using non-invasive tools like the whole-room calorimetry or indirect calorimetry, our data from randomized controlled trial (Chapter 4) has demonstrated the clinical effectiveness of utilizing IC-guided energy expenditure (EE) dietary intervention on improved weight loss outcome and improved compliance to diet restriction. This proves that there is feasibility of clinical application of IC-guided EE INT to tailor individual-specific targets by capturing the dynamic change in REE and markers of EE based on the weight loss deficit (FFM and FM) over time. However, there remains several areas of uncertainty due to the adaptive responses in decrease in REE with weight loss.

Our study has supported the findings that these metabolic adaptions exist, where the change in REE per fat-free mass, is determined by the actual decrease in FFM and fat mass (FM) following weight loss. Metabolic adaptations to reduced body weight poses a unique challenge due to the distinct loss of FFM and reduced metabolic activity of the remaining tissue, both contributing to a decrease in REE. The contributions of the energy-expending tissues and metabolic adaptations to the REE was strongly associated with the degree of decrease in body FM, in relation to weight loss (474). Alterations in any one component of the energy balance would affect the other components (230, 497, 498), hence it is unrealistic to attribute and factor obesity to energy intake or expenditure alone.

Complex regulation of health and eating habits come into consideration when considering that chronic energy surplus stems from energy imbalance. Homeostasis and hedonic pathways control the food intake behaviour and relies on close connection to the hormonal responses, influencing the central hypothalamic and food reward pathways (499). The dopaminergic signalling typically regulates food-related stimuli which sensitises the central reward centres to promote an excess of energy consumptions (500). Paraventricular nuclei within the hypothalamic arcuate nucleus regulates peptides that opposes food intake including thyrotropin-releasing hormone, corticotropin-releasing factor and oxytocin (501). Further peripheral cannabinoid receptors regulate appetite and are involved in regulation of gastrointestinal gut peptides and innervation (502).

The gut-brain axis pathway that places food as the 'stimuli' which triggers a cascade of neuro-hormonal changes innervating sensory vagal neurones, that influences the hedonic reward system (503). The activation of the reward centres and neural circuits is proposed to receive signals from subcortical gut-brain pathways independent of the 'taste' or palatability of the food (504). It is worth noting that in people with obesity, the motivational drive to increase intake of energy dense food is greater as compared to lean individuals. Gut hormones from the enteroendocrine tissue provides sensory information to the central nervous system (CNS) based on the post-absorptive feedback from nutrients ingestion and impact on energy balance (505). The gut microbiota play an important role in the shift of energy to storage within adipose tissue, and switch toward 'harvesting' of energy from free fatty acids, modulating the hedonic behaviours and satiety response through the gut-brain axis (505).

7.4 Obesity Phenotypes Influence Weight Regulation

The small reductions in EE during caloric restriction could influence the weight regulation in response to caloric deficit (40, 492). Large inter-individual variation in the response towards weight loss demonstrate that the need to elucidate measurements of REE and 24h-EE in response to energy-restricted diet or overfeeding response may help to determine /predict weight loss (156, 506). The proposed “thrifty” (larger decrease in 24h-EE) vs “spendthrift” (smaller decrease in 24h-EE) phenotype in people with obesity refers to the energy-cost efficiency with which how energy consumption response in everyone predicts the response to short-term overfeeding (507, 508). Recent evidence from controlled diet restriction (50% restriction) in inpatient setting showed the 24h-EE changes in response to energy-deficit phase during fasting were correlated with the predicted weight loss over 42 days of calorie-restricted diet (509). This suggests that the REE itself, as a single energy component is unlikely to contribute to the difference between the thrifty and spendthrift phenotypes (36). The variation in EE response to calorie-restriction may be due to diet-induced thermogenesis (476, 510). Reinhardt et al (509) observed that in response to controlled caloric-restriction within an inpatient setting, there is a variation to the extent of weight loss which is not the result of lack of adherence to the prescribed caloric restriction but instead, the biological inter-individual variation in 24h-EE responses, i.e. phenotype of ‘thrifty’ vs ‘spendthrift’ predicts response to weight loss from calorie restriction.

Piaggi et al (511) described the two distinctive energy expenditure phenotypes i.e. the "thrifty" or "spendthrift" phenotypes and the effect on weight regulation. In fasting conditions, individuals who demonstrated decreases in energy expenditure in response to fasting and relatively large capacity to increase 24-hour energy expenditure in response to overfeeding are described with the "spendthrift" phenotype, predictive of weight loss (512). The strongest association with the change in the 24-hr EE was the change in energy expenditure in tissue other than the muscle or fat-free mass (513). The resistance to weight and fat gain with overfeeding was attributed to changes in spontaneous physical activity (513). Dietary compliance is a crucial factor that leads to variability in body weight gain in chronic overfeeding.

The REE estimates based on the body-mass FFM components have a limitation in that the REE-FFM ratios are not 'static' but has a dynamic variation influenced by sex, age, ethnicity, genetic traits and body size and composition (475). The ability to measure tissue-organ estimates of REE provides a novel method to develop physiological prediction formulas based upon established heat production rates of major body tissues (514, 515). Future research would be tailored on emergence of non-invasive technologies to determine and ascertain the organ mass-specific metabolic rates and organ specific-cell mass rather than on the whole-body and associated complexities which comes with high variability (486). For example, rather than the whole body calorimetry, novel methods to evaluate metabolic rate (516) may focus on brain, liver metabolic rate, and correlate with the volume or mass of organ-tissue composition, with advancement in imaging modalities to separately evaluate the metabolically active portions and delineate that from the metabolically inert portions of cells (517). The imaging modalities would allow non-invasive quantification of

organ mass-specific metabolic rates (518, 519), crucial to predict weight loss response in humans.

7.5 Substrate Balance and Metabolic Fuel Selection

Metabolism of substrate shifts between fat and carbohydrate utilization depending on the availability of the macronutrient during food intake. In conditions of fasting (lack of food availability), the predominant source of energy is fat oxidation (520). In the post-prandial state after consumption of a mixed meal, the main source is carbohydrate oxidation (171). Depending on the mixed meal intake and macronutrient availability, the substrate utilization is highly influenced by the carbohydrate and lipid or fat intake (521, 522). Energy stores are predominantly stores as body fat, but fat intake has little influence on fat oxidation (82, 523). Daily fat intake represents less than 1% of the total fat energy stores. However, fat stores contain about six times the energy content when in direct comparison to protein stores (524). Fat stores are indicative of the energy buffer state of the body and the slope of the relationship between energy balance and fat balance is relatively equate to the small positive or negative energy imbalances (496, 525, 526).

In states of low-carbohydrate intake, there was rapid change towards decreased carbohydrate oxidation, and the body maintains the carbohydrate balance after the carbohydrate-restricted diet. The other studies evaluated the role of carbohydrate-depletion on food intake assessed for 48 hours after restricted carbohydrate intake ref (527-530). In conditions of restricted carbohydrate intake combined with exercise, up to 40% change in the carbohydrate balance or muscle glycogen content was observed (527-530). However, the decrease in carbohydrate stores was insufficient to induce

changes in the *ad libitum* energy intake. The change in macronutrient intake were compensated by rapid adjustments in carbohydrate oxidation.

Most studies report greater relative energy intake in relation to energy requirements when food intake is measured in strict laboratory conditions (531, 532). The low-glycaemic diet compared to high-glycaemic diet (533) has been shown to improve response to body weight loss and reduce postprandial RQ indicative of greater fat oxidation. Importantly, regulation of energy intake relies on multiple homeostatic mechanisms in the homeostasis of energy balance and weight regulation. In a more complex model, regulation of energy balance must be evaluated based on cognitive, emotional, and hedonic 'reward' behaviours, all of which affect energy intake.

Glycogen storage in the liver and skeletal muscle does not explain the influence of carbohydrate intake to energy intake. Most of the peripheral circulating signals in the regulation of energy intake relies upon adipose tissue in the gut or pancreas. If glycogen content plays a role in the regulation of food intake, this must be accompanied by the release of neuronal and/or humoral signals from the liver and/or muscle. The presence of vagal efferent neurons from the liver to the central nervous system have been suggested to play role in energy intake regulation (534). Reduced carbohydrate stores may lead to relatively greater weight gain than individuals with larger carbohydrate stores, due to increased appetite and reduced satiety (535). During a state of energy balance, when RQ was evaluated against body weight change controlling for body fat composition, there was a significant association between the 24-h RQ and weight gain (46), under controlled dietary conditions.

Findings from Zurlo et al (46), was supported by Eckel et al (536) reported individuals with increased carbohydrate balance after a 15-day high-carbohydrate diet gained less body weight and fat mass over the next 4-years when compared against individuals with lower carbohydrate balance. For each additional 25 g (100 kcal/day) in carbohydrate balance, subjects gained 80g less body weight per year, whereas a difference by one standard deviation for carbohydrate balance (510 kcal/day) could explain a difference of 400g per year in body weight gain. The energy excess required to account for a change in body weight is very small and probably impossible to detect when energy intake is evaluated for a few days. In the next section, we discuss and evaluate the utility of non-invasive methods to evaluate carbohydrate balance and its impact on predicting weight change.

Our data from the randomized controlled trial confirmed that individuals with greater decrease in RQ (RQ <0.85) during the acute phase of energy deficit through diet restriction, had a greater likelihood to lose >3kg in weight at 6-months compared to individuals with high RQ (RQ>0.85) (see results in Chapter 4). To the best of our knowledge, the changes in fasted state RQ following dietary interventions decrease in response to energy restriction (537). Blunted capacity to switch from low- to high-fat oxidation in obesity is described by 'metabolic flexibility'. Greater predisposition to weight gain in conditions of chronic positive energy balance leads to decreased fat oxidation, greater reliance on carbohydrate (glucose) oxidation.

Evidence from systematic review (Chapter 2) evaluating the association between baseline RQ and change in RQ on the observed weight loss, demonstrated that during diet restriction and combined diet + exercise intervention for weight loss, people with

overweight and obesity demonstrate a decrease in RQ. However, the baseline RQ and/or the change (decrease) in RQ did not correspond with the observed weight loss. Hence, restriction of energy intake per se, may influence fuel selection and substrate oxidation, but we postulate that the effects may not be maintained due to potential lack of dietary adherence and short duration of intervention, with modest weight loss at best. Further, transient decrease in RQ and weight loss may have led to compensatory reduction in other components of energy balance (157, 538-540), and potentially stimulate increase in hunger response (541)..

Piaggi et al (470) evaluated the effect of RQ on *ad libitum* food intake. Using the multivariable models based on 24-h EE and 24-h RQ, the study evaluated whether these two factors were independent predictors of energy intake in lean healthy individuals. 'The surplus of 100 kcal/d in 24-hour EE (independent) of 24-h RQ) was associated with a 175 kcal increase in energy intake per day (470). Further, an increase of 0.01 unit in the 24-h RQ (independent of 24-h EE) was associated with a 204 kcal increase in energy intake per day (470). Our findings concur with that of Piaggi et al in that the 24-h RQ had a correlation with energy intake.

Piaggi et al (470) reported that individuals with higher 24-h EE and RQ consumed on average 947 kcal/day (P=0.006) more than individuals with both relatively lower EE and lower RQ despite the similar body weight and fat-free mass (FFM). The difference in energy intake between the EE-RQ groups were reflected by a higher consumption of 448 kcal/d from fats (P=0.004), 373 kcal/d from carbohydrates (P=0.03) and 126 kcal/day from proteins (P=0.01). The findings from Piaggi et al (470) reported that a 100 kcal surplus in daily EE and positive 1% shift in 24-h RQ (increase) were

associated with independent increases in *ad libitum* food intake of approximately 175 and 204 kcal/d, respectively. The 24-h RQ was the greatest determinant of *ad libitum* food intake, accounting for 16% of the variance (470). To conclude, we support the hypothesis that there is a positive association between the predictive effects of RQ on *ad libitum* food intake, when adjusted for the 24-hour EE. Subjects with lower fat oxidation rates are more likely to store excess energy as adipose tissue (42). Future therapies and metabolic pharmacotherapy targets should aim to improve either energy expenditure, fat oxidation or both as it is likely to improve body weight management and favour loss of body fat.

7.6 Metabolic Flexibility and Body Weight Regulation

The metabolic flexibility is defined by the 'capacity of the body or cells to match the fuel oxidation to fuel availability' and the neurohormonal response. The efficient variation and 'ability to switch' substrate utilization between fat and carbohydrate is influenced by the substrate availability and energy demand, or the metabolic capacity to 'switch / adapt' fuel oxidation in response to fuel availability (169). Metabolic flexibility is assessed by the (i) increase in RQ from fasting state to glucose- and carbohydrate-insulin mediated conditions (ii) reduction in RQ during overnight fasting or (ii) macronutrient oxidation adaptation in response to isoenergetic changes in diet composition (65). After a mixed meal, glucose is primarily oxidized and lipolysis in adipose tissue and glycogenolysis in liver are inhibited by rise in insulin levels as a response to ingested calories (542, 543). Glucose excess is stored as glycogen in the liver and skeletal muscle, whilst the ingested lipids are stored as adipose tissue and fat. Efficient switching of substrate after a mixed meal manifests as a decrease in fatty acid oxidation (544). Deficiency and impaired capacity to switch from carbohydrate to fat,

or vice versa is associated with obesity, peripheral insulin resistance, and type 2 diabetes (171, 544).

However, in conditions where the macronutrient composition of the diet is modified, fuel oxidation must be adjusted to achieve a new equilibrium. The metabolic response to greater dietary fat intake, the switch from low-fat to high-fat oxidation can take more than 1 week to adapt to a new macronutrient equilibrium (545). The genetic makeup has huge influence on the large interindividual variability in the time required to attain the new macronutrient equilibrium (496, 546, 547). The switch to greater dietary fat may lead to more depletion of carbohydrate stores in persons with impaired capacity to upregulate lipid oxidation, may therefore be a signal promoting food intake. There remains paucity of studies to prove and evaluate this hypothesis. The difference in metabolic inflexibility may influence energy intake and may affect regulation of body weight gain over several years. The existence of metabolic adaptation, at levels of the resting energy expenditure (REE) remains highly controversial, likely due to lack of standardization of participants' energy balance. Role of metabolic adaptation as a driver of weight relapse remains uncertain. During conditions of weight maintenance, the metabolic adaptation of REE is minimal (548). Further, the minimal change or adaptation in REE does not predict weight regain up to 2 years follow-up (548). Future studies are required to further explore the role of lipid oxidation on the neuro-hormonal response to energy intake.

7.7 Innovations in Dietary Interventions

The other evidence from behavioural-based weight loss intervention usually involves evaluation of the intervention after a period of initial weight loss and the end outcome on the magnitude of weight change after the initial intervention period. Comprehensive

behavioural interventions aimed at dietary and physical activity behaviours are moderately effective in slowing regain of weight in adults with obesity after the initial period of weight loss for follow-up period of up to 24 months (17). Dombrowski et al. (17) performed a systematic review consisting of RCTs evaluating long-term maintenance of weight loss in adults with obesity after ≥5% weight loss, followed-up for at least ≥12 months. In the Weight Loss Maintenance Trial (549), behavioural intervention was successful in assisting participants to achieve >4kg weight loss in 6-months. However, in longer term follow-up the behavioural (group-based) weight loss intervention did not significantly improve the weight outcomes compared against standard care (549). The dietary interventions producing modest weight loss have clear benefits towards cardiovascular health, but the crucial goal is to facilitate behavioural management strategies to support maintenance of lifestyle changes. Success in long-term maintenance of weight loss based on behavioural intervention strategies involve long-term calorie-restricted balanced diet. Dietary strategies must be adapted to counter the adaptive responses that potentiate weight regain.

Alteration in the macronutrient diet composition (resistant starches, polyunsaturated fats, and increased protein) is suggested to facilitate increased preference for fat utilization (550). Decreased or restricted energy intake leads to reduce absorption efficiency, increase energy expenditure, or enhance the preferential use of fat for energy production. Evidence from learning-based models of behaviour, suggest that the food cue reactivity and reactivity are ‘learned’ and ‘conditioned’ responses that lead to hedonic eating and subsequent weight surplus (551-554). Proposed psychological treatments may target associations between the cue exposure, craving and dietary food consumption including the use of cue exposure and response prevention treatments (CERP) (555), cognitive behavioural therapy (CBT) (556, 557)

and mindfulness-based therapies. Typical psychological characteristics have been linked to food cue reactivity. Dietary restraint, the cognitive effort to consume less predicts dietary intake and adiposity in overweight individuals (558). However, much of this science has yet to be applied specifically to questions of weight loss maintenance. Studies on total meal replacement diet have resulted in greater reduction of food cravings and higher magnitude of weight loss compared to the reduced-calorie typical diet (559).

During dietary restriction with reduced carbohydrate intake, higher fat content, greater weight loss was observed in people with obesity (560, 561). The caloric-restriction leading to weight loss has greater effect on transcriptome in adipose tissue compared with calorie restriction plus exercise (53). Studies on fuel selection suggests that altered dietary restriction, without change in energy demands or increase expenditure does not improve the state of insulin resistance. Hence, without the greater increase in energy expenditure (exercise) there is less impact of switching from glucose to FA fuel metabolism in obesity. Chronic surplus in energy intake, and long-term inadequate activity expenditure leads to significant increase in body weight, greater visceral and excess intraabdominal fat deposition, predisposing to peripheral insulin resistance (562).

Pharmacotherapy that targets fat oxidation including the peroxisome proliferator-activated receptors (PPAR) agonist (563) have been specifically developed to influence fuel metabolism in obesity. However, the pharmacotherapy effects do not employ the similar improvement in metabolic flexibility, does not portray the metabolic improvements through increased exercise. Clinical studies on fasting and dietary restriction have allowed exploratory data on mechanisms and treatment targets

for insulin resistance and metabolic inflexibility, including the greater capacity for oxidation of fatty acids. Broad factors influencing metabolic flexibility have prompted further investigation into factors and mechanisms influencing energy availability and fuel selection. Insulin resistance in skeletal muscle and adipose tissues contribute to metabolic inflexibility. Future clinical studies are exploring the metabolic flexibility in conditions of switching from fasting to feeding, resting to exercise or exercise training interventions with adipose and muscle biopsies, to improve understanding of mechanistic pathways underpinning metabolic flexibility.

7.8 Interventions which could Improve Metabolic Flexibility

Diet restriction in combination with exercise improves insulin sensitivity and substrate metabolism to a greater extent, thus, improving fat oxidation which reduces body fat composition through reduce sedentary time (564). However, losing this excess adiposity is difficult possibly due to the impaired fat metabolism and reduced fat oxidation rates (565, 566). The important factor is that studies for prescribed exercise interventions for seven weeks can demonstrate significant reduction in fat and improve the fat oxidation rate (567). Greater fat oxidation occurs when the exercise intensity ranges from 45-75% VO2 max (568-570). Exercise directly influences the transfer of fatty acids and changes in corresponding hormones which in turn affects the fat oxidation rate (571, 572). During acute bouts of persistent high-intensity and longer duration of exercise, oxidative phosphorylation leads to anaerobic glycolysis. The capacity of the mitochondria for oxidative phosphorylation correlates with aerobic capacity and exercise tolerance (573). Fatty acid oxidation contributes less quantitatively compared to glucose as the main source of metabolic fuel as the intensity of exercise increases (568). Steady exercise intensity in high-intensity exercise training

(HIIT) leads to greater adipose tissue oxidation, and improved skeletal muscle mobilization of FFA (574).

Reduced metabolic flexibility is associated with higher body fat, impaired insulin suppression of NEFAs, and reduced efficiency to utilize FFA (575). In response to combined diet and exercise intervention, adipose tissue macrophage infiltration decreased, with concomitant improvement in the insulin sensitivity (576, 577). As fixed-intensity exercise duration increases, greater adipose tissue lipolysis occurs, but the predominant metabolic fuel remains glucose oxidation. In obesity, impaired FFA oxidation stems from blunting of catecholamine response during acute exercise, due to impaired response of the B2-adrenergic receptors (578). The resistance to catecholamine action results in inefficiency in fasting- and exercise-related metabolic oxidation of FFA from both adipose tissue and skeletal muscle cells.

Exercise training increases basal fat oxidation and decrease in intramyocellular lipid by promoting lipid utilization in the skeletal muscle (579). Increase energy expenditure or enhance the preferential use of fats for energy production have been found to have large intra-individual variability between individuals – studies on the effects of energy restriction on the RQ measure have provided further evidence on how the proponents of substrate oxidation have a direct or indirect effect on weight outcomes (579). The shift towards improvement in body weight and body composition was greater in individuals with greater fat oxidation (211, 580).

Intrinsic metabolic flexibility in human tissue and muscle cells in obesity demonstrate suppressibility of glucose oxidation by increased fat intake and decreased adaptability towards fat oxidation. Future *in vivo* clinical trials would provide more mechanistic insight into the response of metabolic fuel selection during bouts of increase in energy

demand in exercise (581). Other non-invasive modalities using near-infrared spectroscopy (NIRS)-derived changes in deoxyhaemoglobin with high-resolution respirometry may evaluate mitochondrial respiratory capacity and strongly correlated with the oxidative capacity (582).

7.9 Exercise as a Weight Loss Tool Counters Biology

Potential benefits of greater exercise for weight loss maintenance have been proven by numerous studies. Exercise is recommended with the primary goal to substantially increase the energy expenditure to produce a negative energy deficit and generate the effects of associated cardiovascular health benefits and reduction in blood pressure. However, the weight loss observed following a prescribed exercise program is often lower than the expected trajectory of weight loss. Current research has provided further insight and understanding into the reasons for the lower than predicted magnitude of weight loss. This less than expected weight loss may be due to factors including low exercise induced energy expenditure (583), lack of compliance to the prescribed exercise intervention (584) and physiological compensation for increased energy expenditure through increasing food intake or reducing non-exercise activity thermogenesis (NEAT) .

Activity energy expenditure is the major determinant of the success of weight maintenance (585). Combination of exercise with dietary restriction in a fasted state (586) reduces the energy intake of the individual. Exercise has other effects on the energy balance equation apart from increasing the energy expenditure and cardiovascular benefits. In the phase 1 preclinical studies involving rodents with obesity, structured and volitional exercise would aid with preventing weight regain after weight loss (587). During exercise, greater metabolic efficiency due to increase

mitochondrial respiration for energy production is associated with higher levels of fatty acid oxidation (588).

Maximal fat oxidation is a state that fluctuates continuously, regulated by the neuro-hormonal system both at rest and during exercise (589). The hormones predominantly responsible for fat burning include effects of catecholamines, cortisol, growth hormone and in contrast the inhibitory effect of insulin (67). The greater intensity of exercise training status was associated in moderate intensity aerobic training, with correspondingly greater ability to oxidize fat (590). The adipose tissue in the body stores significant amount of lipid in the form of triacylglycerol (TAG) and crucially deliver continuous supply of energy for prolonged exercise performance. Where individuals with moderate exercise intensity (up to 65% VO2 max) – the exercise can theoretically be maintained for longer durations and is associated with the maximal fat oxidation in endogenous adipose stores (591).

The reduction in body weight in a randomized controlled trial designed to expend 2,500 kcal/week by King et al.(592) reported large inter-individual variability, but overall a trend towards reduced body weight, reduced fat mass and decreased central adiposity at 12 weeks. King et al. (592) postulated that the effect of exercise on appetite control involves the increase in the orexigenic drive to eat and improvement in satiaty efficiency after ingestion of a fixed meal. This paradoxical increase in the reported satiety during the state of fasting hunger and ability to concomitantly improve the satiety suggest the effect of prescribed exercise on appetite regulation (593).

7.10 Energy Expenditure and Fat Oxidation in Body Weight Control

The capacity to increase EE and preserve FFM are determinants for weight loss and weight-loss maintenance (594-596). The increased physical activity without restricted energy intake was associated with only modest weight loss (597). Physical activity is crucial predictor of weight maintenance and prevention of weight regain. Higher risk of weight regain was demonstrated in individuals with higher levels of sedentary activity (598, 599). Greater increase in activity expenditure (e.g. increased expenditure by 1500-2000 kcal/week) in obesity predicts successful maintenance of weight loss (95), reduced energy intake and decreases appetite (600). In comparison, decreased activity expenditure or increased sedentary duration is a reliable predictor of weight regain (601). The reduced physical activity is a cumulative effect of lack of ability to reach energy balance, which ultimately leads to increase storage of energy and greater adipose tissue deposition. Shook et al (602) evaluated the effect between physical activity, calculated energy intake and compared the low versus high activity groups. The individuals with the lowest physical activity consumed more calories than the higher activity groups (602). Further, sedentary individuals are more likely to under-report energy intake, and experience higher levels of disinhibition when exposed to food cues (602). The amount of exercise recommended in most randomized trials and ability of participants to adhere to the prescribed exercise programme is crucial to facilitate the behaviour modification required for weight loss (603, 604). The predicted weight loss based on the calculated energy expenditure often does not reflect the actual weight lost during the research trial (605). A possible explanation includes physiological compensation (214, 606, 607) and potential for compensatory increase in food intake (608).

7.11 Obesity-related Microvascular Dysfunction Predisposes Neuropathy

Peripheral neuropathy is recognized to develop in diabetes due to chronic hyperglycaemia. Recent evidence increasingly recognize that certain individuals have neuropathy at the point of diagnosis of diabetes, which postulates that small fibre nerve damage may occur prior to development of T2D (609). Indeed, more recent evidence supports the premise that presence of subclinical diabetic sensorimotor polyneuropathy was confirmed in people with prediabetes or within 3-years of T2D diagnosis (367). Nerve dysfunction is now recognized to related to impaired microvascular disorder, contribute to biomechanistic damage specific to metabolic disorders such as obesity (610). The distribution of the excess body fat including ectopic fat accumulation, visceral and abdominal adiposity (611), and greater internal organ adipose tissue store is increases the risk of damage from chronic pro-inflammatory mechanisms due to adipose tissue dysfunction (612). Autonomic and somatic components of the peripheral nervous system are impacted by obesity, including greater brain atrophy in middle-aged adults with obesity (613). Studies on denervation of nerves within the adipose tissue nerves leads to impaired metabolism (614, 615). Denervation of white adipose tissue (WAT) leads to greater adipocyte cell number (616, 617). Metabolic health is closely regulated by the communication between the lipid stores in brown adipose tissue (BAT) and WAT. Impaired lipolysis and chronically elevated free fatty acids contribute to lipo-toxicity and hypothalamic dysfunction (618).

Sympathetic nervous system plays a complex role in energy homeostasis and differentially selects and regulates substrate metabolism in various innervated tissues including peripheral nerves and adipose tissue (614, 619). The nerves that innervate

adipose tissues include numerous peripheral nerve subtypes, such as sensory, parasympathetic, and sympathetic nerves (614, 620). Several studies assessed the adipose tissue innervation, and the role of brain-hormonal-adipose tissue communication (621-623). The role of neurotransmitters and neuropeptides released in the adipose tissue and the binding on the peripheral receptor-expressing cell types on the development of neuropathy remains poorly understood. Improvement in the understanding of how the peripheral nerves in adipose tissue are regulated is important for the field, including differences in nerve plasticity (620) between innervation of BAT and WAT.

Our data from the cross-sectional study on the phenotype of peripheral neuropathy demonstrated comparable neuropathy manifestations in normo-glycaemic people with obesity was comparable to people with long duration of type 1 diabetes (610). Peripheral neuropathy could manifest due to the culmination of obesity / insulin resistance-prone pathophysiological process that are yet to be fully understood (96, 101). Our data confirmed the positive association between obesity and greater centripetal adiposity (and possibly greater ectopic fat accumulation) with impaired vibration perception threshold as a marker of large fibre peripheral neuropathy (610). Adipose tissue nerves are known to be essential for energy-expending processes such as lipolysis and thermogenesis (624), and loss of a proper nerve supply can have serious detrimental effects on metabolic control, which may exacerbate or initiate an insulin resistant state. In addition, leptin stimulated lipolysis is mediated at least in part by sympathetic activation of subcutaneous WAT (621), further underscoring how adipose neuropathy can contribute to metabolic dysfunction through obesity-induced leptin resistance (625, 626).

7.12 Possible Mechanistic links of Obesity-Related Peripheral Neuropathy

The inflammation and vasoactive neuromodulators affecting the dorsal root ganglia causes the increase in vasculature permeability. Damage to dorsal root ganglia subsequently attracts innate and adaptive immunological cells to areas of damaged sensory nerve cell bodies (627). Whilst initially a protective mechanism, the chronic dysfunction due to obesity-mediated inflammation leads to chronic damage and changes to nerve structure and subsequent allodynia and hyperalgesia (628). Typically, development of neuropathic pain involves accumulation of macrophages, increased TNF-alpha and Interleukin-1B, interferons, and reactive oxygen species (629).

Distal-to-proximal loss of sensory perception in the “glove-stocking” distribution leading to loss of sensation corresponding to loss of sensory axons (630), with involvement of the motor axons at the end-stage disease is commonest form of polyneuropathy observed in prediabetes and type 2 diabetes. Obesity and lipid-induced inflammation both exert metabolic effect on peripheral nerve damage (630). Greater levels of free fatty acids and triglycerides, commonly coexisting in state of obesity alter the lipid regulation in the peripheral nervous system (109). Excess long-chain fatty acids promote inflammation of the Schwann cells causing mitochondrial B-oxidation leading to neuropathy (630, 631). Further studies on animal models of obesity and type 2 diabetes with microvascular complications have supported the association between obesity and lipid-rich macrophages inflammation in peripheral neuropathy (427, 632, 633). In preclinical models, increased neuronal inflammation is associated with the greater cutaneous pain and increased nociceptive (pain) mechanical hypersensitivity in high fat fed mice (634).

The components of the metabolic syndrome present in a large proportion of individuals with obesity, including hyperlipidaemia, insulin resistance, prediabetes and hypertension as well as obesity itself are independent risk factors for peripheral neuropathy (341). Hypertriglyceridemia, insulin resistance and prediabetes (632), highly prevalent conditions in obesity, are each associated with neuropathy (101, 323, 635). Obesity involves features including autonomic dysfunction and impaired baroreflex functionality (636). Dysfunction in autonomic innervation controlling sympathetic and parasympathetic nervous system may impact on digestive system, cardiovascular disease and suppression of baroreflex function (430). Greater centripetal adiposity is associated with sympathetic overactivity and greater insulin resistance (637). Inflammation in obesity is a result of chronically mediated immune response, with excess secretion of inflammatory cytokines and chemokines (638). Acute and chronic inflammation causing hyperactivity of the sympathetic nervous system, causing increase incidence of cardiovascular disease (429). Chronic potentiation of the sympathetic nervous system (SNS) or SNS overactivity and parasympathetic depression in obesity contributes to a further decline of insulin sensitivity. In insulin resistant state in obesity, the insulin-driven overactivation of the sympathetic nervous system through peripheral insulin resistance leads to peripheral inflammation (103, 430, 435, 610). The insulin causes the endothelial-dependent vasodilatation resulting in baroreflex-mediated sympathetic activation, and a central mechanism are often present in chronic conditions of hyper-insulinemia and peripheral insulin resistance (639). In the preclinical animal models with insulin resistance, chronic insulin resistance stimulated carotid body overactivity in the chemoreceptors (640). The long-term insulin-resistant increase in sympathetic overactivity have been

suggested to cause low-grade inflammation of nerves, including destruction of the choroid plexuses and chemoattractant disruption of the blood-brain barrier (641).

There is clearly an urgent need for action to detect neuropathy, especially in the advancement of modalities useful to detect small fibre nerve damage prior to manifestation of neuropathy complications, including foot ulcers and amputations. Unfortunately, the lack of screening modality and paucity in pharmacotherapy to address microvascular dysfunction emphasises the lack of understanding of microvascular & metabolic disorders in obesity relating to neuropathy. Greater emphasis should be placed on evaluating microvascular dysfunction related to excess adiposity, hormonal dysregulation and effects of microvascular damage on sympathetic and parasympathetic nervous system (642). There remains a paucity of data on how the nerve endings and innervations of adipose tissue can modify their connections or tissue neurite density under physiological or pathophysiological conditions. Future clinical and preclinical trials need to address the uncertainties regarding adipose tissue nerve remodelling or the nerve plasticity (623) in context of microvascular dysfunction in obesity.

7.13 Microvascular Dysfunction in Diabetic Foot predisposes Foot Ulcers

In preclinical models in ob/ob and db/db mice, which models obesity and peripheral diabetic neuropathy and metabolic syndrome, nerve fibre damage occurs alongside endo-neural upregulation of oxidative markers (oxLDL and LOX-1) within the neural micro-vessels (643). Reduced thickness of the myelin sheaths in small, medium, and large nerve axons from sciatic nerve in ob/ob and db/db mice correlated with thickening of basement membrane within the endoneural microvessels (643).

Microcirculatory dysfunction evaluated in the preclinical studies showed that increased density of endoneural capillary in T2D compared to healthy individuals, suggesting that capillary density react to diabetes-related nerve ischaemia (644).

Microvascular dysfunction is commonly reported in diabetes and is associated with impairment in endothelial function, and subsequent neurological sequelae. Dysfunction in neurological response may contribute and correlate with microvascular impairment in people with diabetic neuropathy (645). Slower reactive hyperaemia response to occlusion was observed in people with peripheral sensory neuropathy in diabetes. Neuropathy in the diabetic foot has been a result of loss of sympathetic nerve activity in the peripheral blood vessels causing vasodilation and increased arterial flow (646). However, the initial hyperaemic blood flow bypasses the cutaneous structures due to opening of arteriovenous shunts, resulting in local ischaemia (647). Neuropathy has been associated with reductions in microvascular reactivity that are likely to contribute to foot ulceration, impaired healing, and reduced efficacy in healing from infection (648) .

7.14 FUTURE WORK

There is further prognostic value of identifying and quantifying corneal nerve morphology in early detection of small fibre neuropathy in chronic conditions such as Type 1 diabetes (383) and metabolic impairment in people with obesity without diabetes (443). Utilizing CCM as a potential screening tool to identify established features of early small nerve fibre degeneration in T1D with DPN as well as other forms of metabolic impairment leading to DPN may be an invaluable assess to establish surrogate endpoints in clinical studies and detection of individuals at ‘high risk’ of foot ulceration. Future epidemiological studies of people with obesity suffering

from impact of insulin resistance causing microvascular complications of peripheral neuropathy should be more comprehensive and include evaluation of the corneal nerve morphology as surrogate markers including corneal nerve fibre length (CNFL), corneal nerve branch density (CNBD) and corneal nerve fibre density (CNFD).

Definitive scientific evaluation of the impact of obesity on pathogenesis of peripheral neuropathy should be further evaluated through double blinded randomised placebo-controlled trials to evaluate effects of weight loss interventions, improved lipid control and reduced insulin resistance, on metabolic outcomes and effects on neuropathy phenotype post-weight loss intervention. However, several considerations must be considered including the definitive evaluation of the endpoints to establish peripheral neuropathy in the context of chronic relapsing condition of obesity. Future drug therapies and pharmacotherapy could focus on the ability to improve /increase energy expenditure and improve the fat oxidation capacity in pursuit of effective pharmacotherapy targets for weight loss.

7.15 CONCLUSION

In conclusion, this thesis provides evidence of the complexity in energy balance and homeostasis in persistent states of energy surplus and reduced activity expenditure in people living with obesity. Amongst the greatest challenges in tackling the obesity epidemic is the implementation of structured, organizational support to help the individual incorporate the recommended dietary and lifestyle interventions, which is personalized, sustainable, and tailored according to their individual energy needs and health goals. Incorporating novel techniques by utilizing IC-guided EE information could serve as an invaluable tool to further understand the metabolic adaptations that

occur during the weight loss process. Overreporting of actual exercise or underreporting of food intake could be contributing to mixed results in energy balance studies thus far. Implementing accurate and reliable modalities such as IC-guided dietary recommendation allows for more accurate determination of energy demands and ability to maximize capacity for lipid or fat oxidation. Ultimately, utilizing the IC-guided EE information as adjunct to multimodal weight management intervention could improve compliance to the recommended weight interventions. Contemporary research has helped improve the understanding of metabolic flexibility and develop potential therapeutic strategies and pharmacotherapy targets to tackle problems related to altered fuel metabolism in obesity. Emerging evidence increasingly support the occurrence of low-grade chronic inflammation characteristic of obesity leading to greater reported prevalence of neuropathy, even in asymptomatic individuals. Although the exact mechanisms of obesity-mediated neuropathy remain unclear, concomitant increased insulin resistance, oxidative stress, and autonomic dysfunction all of which has been strongly associated with obesity could contribute towards degeneration of both small and large nerve fibres independent of hyperglycaemia. Hence, it is important for improved screening and evaluation for microvascular disease and neuropathy complications in obesity, prior to the development of advanced neuropathy. Treatment modalities must address the concomitant microvascular, macrovascular, lipid, and autonomic disorders associated with metabolic inflexibility and reduced fat oxidation in obesity. Considering the greater incidence of peripheral neuropathy in obesity and diabetes, with potential deterioration to foot ulceration, application, and development of validated reliable and reproducible biomarkers like corneal confocal microscopy would be essential for future research in terms of screening, diagnostic modalities. Utilizing the markers of corneal confocal microscopy

as a potential surrogate biomarker could help detect small fibre degeneration and identify individuals at risk of distal sensorimotor polyneuropathy and subsequently prevent foot ulceration. CCM markers should be incorporated into future clinical therapeutic trials in the development of therapeutic treatment targets to effectively manage peripheral neuropathy in chronic diseases.

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