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A TRANSLATIONAL STUDY OF THE MECHANISMS FOR METABOLIC RECOVERY AFTER BARIATRIC SURGICAL INTERVENTION: FROM ADIPOSE MITOCHONDRIA TO PATIENT BENEFIT

By

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THE UNIVERSITY OF WARWICK

A dissertation submitted for the degree of DOCTOR OF PHILOSOPHY

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Declaration

All sentences or passages quoted in this project dissertation from other people’s work have been specifically acknowledged by clear cross referencing to author, work and page(s). I understand that failure to do this amounts to plagiarism and will be considered grounds for failure in this module and the degree examination as a whole.

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree. The work presented (included data generated and data analysis) was carried out by the author, except in the cases outlined below:

All patient data outlined in Chapter 7 was collected between 2011 and 2016 by staff at UHCW Specialist Weight Management Service, in particular Neha Shah (Bariatric Dietitian), Jenny Abraham (Bariatric Nurse), Dr. Milan Piya (Consultant Endocrinologist) and Mr. Vinod Menon (Consultant Surgeon). A full list of staff who participated in the care and data gathering of patients during their treatment is acknowledged by the author and may be found in Appendix 1 (Table 7.5). Ms Alanoud Aladel collated the patient data from the electronic records into a workable database during the auditing process, and the Multidisciplinary
Team at the Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM) contributed in the intellectual discussion that allowed the author to frame the research questions.

Parts of this thesis have been published by the author and include the data presented in Chapter 3 and Chapter 4 as follows:


Other publications:


Name: Lucia Martinez de la Escalera Clapp

Signed:

Date:
Synopsis

The obesity pandemic is one of the greatest challenges facing public health worldwide. With epidemiological projections forecasting only its acceleration, it is clear that the current anti-obesity approach has not been effective. This thesis seeks to outline, through a translational approach, the various reasons for which the obesity crisis continues to grow and to provide further insight into which modifiable factors may contribute to more effective anti-obesity strategies. From the basic science perspective, this thesis investigated through cutting-edge laboratory technology some of the more novel and promising molecular mediators of metabolic recovery (namely gut-hormone FGF-19 and gut-derived bacterial LPS). In particular, this study contributes to a more in-depth understanding of adipose tissue mitochondria, and their role in buffering excess nutrients to maintain systemic metabolic health. From the clinical angle, this thesis explored through clinical audit some of the environmental barriers to metabolic recovery of patients undergoing treatment at a specialist bariatric service of a major NHS hospital. As a result of this translational approach, it was possible for the author to develop a profound appreciation of the complexities involved in developing an effective solution to the obesity crisis, which is rooted in two distinct (and sometimes opposite) concepts: (1) the medical and surgical treatment of obesity, targeting the physiological disorder through pharmacotherapy and/or surgery, and (2) the environmental management of obesity, targeting the dietetic, psychological, socio-economic and political causes through weight management and community development programs, industry regulation and public policy. Though often treated as separate, neither concept need be in conflict with the other. If the objective is truly to develop an effective solution to the obesity crisis, it is paramount to develop a trans-discipline community coordinated approach that addresses not just the cellular targets, but the environmental contributors to obesity.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BPD</td>
<td>Bilio-pancreatic diversion</td>
</tr>
<tr>
<td>COX4I1</td>
<td>Cytochrome c oxidase subunit 4 isoform 1 (Complex IV)</td>
</tr>
<tr>
<td>DRP1</td>
<td>Dynamin related protein 1</td>
</tr>
<tr>
<td>ECAR</td>
<td>Extra-cellular acidification rate</td>
</tr>
<tr>
<td>EWL</td>
<td>Excess weight loss</td>
</tr>
<tr>
<td>FCCP</td>
<td>Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone</td>
</tr>
<tr>
<td>FGF-19</td>
<td>Fibroblast growth factor 19</td>
</tr>
<tr>
<td>FIS1</td>
<td>Mitochondrial fission 1 protein</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>Homeostatic assessment model of insulin resistance</td>
</tr>
<tr>
<td>IRS1</td>
<td>Insulin receptor substrate 1</td>
</tr>
<tr>
<td>LAGB</td>
<td>Laparoscopic adjustable gastric banding</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LGCP</td>
<td>Laparoscopic Greater Curvature Plication</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide (bacterial endotoxin)</td>
</tr>
<tr>
<td>MFN2</td>
<td>Mitofusin 2</td>
</tr>
<tr>
<td>MMP</td>
<td>Mitochondrial membrane potential</td>
</tr>
<tr>
<td>mtATP6</td>
<td>mtDNA-encoded ATP synthase subunit 6 (Complex V)</td>
</tr>
<tr>
<td>MT-CO1</td>
<td>MtDNA-encoded cytochrome c oxidase subunit 1</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
</tr>
<tr>
<td>mtND6</td>
<td>mtDNA-encoded NADH-ubiquinone oxidoreductase chain 6 (Complex II)</td>
</tr>
<tr>
<td>OCR</td>
<td>Oxygen consumption rate</td>
</tr>
<tr>
<td>OPA1</td>
<td>optic atrophy type 1</td>
</tr>
<tr>
<td>PGC1α</td>
<td>Peroxisome proliferator-activated receptor γ coactivator 1 α</td>
</tr>
<tr>
<td>POLG</td>
<td>Mitochondrial DNA polymerase gamma</td>
</tr>
<tr>
<td>SDHA</td>
<td>Succinate dehydrogenase complex II subunit A</td>
</tr>
<tr>
<td>SOD1</td>
<td>Superoxide dismutase 1</td>
</tr>
<tr>
<td>SOD2</td>
<td>Superoxide dismutase 2</td>
</tr>
<tr>
<td>TFAM</td>
<td>Mitochondrial transcription factor A</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor α</td>
</tr>
<tr>
<td>UCP2</td>
<td>Uncoupling protein 2</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist hip ratio</td>
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Chapter 1

Introduction and Aims
1.1 The Obesity Pandemic

Obesity Diagnosis and Classification

The terms “overweight” and “obesity” describe medical conditions characterised by the excess accumulation of body fat endangering health [1]. The most accepted and widespread method for diagnosis in adults is the use of the Body Mass Index (BMI), a ratio of weight-for-height. In adults, a BMI between 25 and $29.9 \text{Kg/m}^2$ designates the person as overweight, and above $30 \text{Kg/m}^2$ as obese (Table 1.1). The National Institute for Health and Care Excellence (NICE) recommends the use of BMI in tandem with waist circumference for the diagnosis and risk assessment (Table 1.2) of overweight, obesity, which may help differentiate between those with elevated BMI due to high fat versus muscle mass [1].

<table>
<thead>
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<tr>
<td>Classification</td>
</tr>
<tr>
<td>Underweight</td>
</tr>
<tr>
<td>Normal weight</td>
</tr>
<tr>
<td>Overweight</td>
</tr>
<tr>
<td>Obese: Class I</td>
</tr>
<tr>
<td>Obese: Class II</td>
</tr>
<tr>
<td>Obese: Class III</td>
</tr>
</tbody>
</table>

Obesity classification by Body Mass Index (BMI) as defined by NICE Guidelines [1].

For adults with a BMI of $35 \text{Kg/m}^2$ or over, cardio-vascular disease risks are assumed to be very high with any waist circumference [1].

<p>| Table 1.2: Assessment of health risk using BMI and waist circumference |
|------------------------------------------------|----------------|</p>
<table>
<thead>
<tr>
<th>BMI Classification</th>
<th>Waist Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>$&lt; 94 \text{cm}$</td>
</tr>
<tr>
<td>Obese</td>
<td>$&lt; 80 \text{cm}$</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>No increased risk</td>
</tr>
<tr>
<td>Obese</td>
<td>Increased risk</td>
</tr>
</tbody>
</table>

Health risk assessment by Body Mass Index (BMI) and waist circumference as defined by NICE Guidelines [1].
Obesity Prevalence: Past and Present

The prevalence of obesity world-wide has nearly tripled since 1980 (Figure 1.1). Currently, about 65% of the world’s population reside in countries where obesity and overweight cause more deaths than underweight [2, 3, 4]. My own home country of Mexico (with 33%) is second only to the United States (38%) in adult obesity, and first place world-wide in childhood obesity.

![Figure 1.1: Historical trend in obesity prevalence of select countries](image)

*From the Organisation for Economic Co-operation and Development’s (OECD) 2014 Obesity Update [2].*

In the UK, 27% of adults are obese and a further 36% are overweight, making it the Western European country with the highest prevalence of obesity (Figure 1.2) [3]. The prevalence of severe obesity (BMI \( \geq 40 \text{kg/m}^2 \)) in the UK has more than tripled since 1993 and is the fastest growing category of obesity [5]. Though severe obesity affected approximately just 2% of men and 4% of women in the UK in 2014, the total medical costs per capita in these severely obese individuals were
approximately 86% greater [5].

![Percentage of Total UK population](image)

**Figure 1.2:** UK population distribution by BMI classification in 2005 and 2015.  
*From House of Commons Library Obesity Statistics [6].*

### Obesity Prevalence: Future Projections

Recent projections, from the OECD analysis of national health survey data, show obesity rates are expected to rise steadily until at least 2030 (Figure 1.3). The United States, Mexico and England are among the countries expected to be the most affected, with 2030 obesity rates projected as 47%, 39% and 35% for each country, respectively [3]. The potential cost of overweight and obesity to the NHS is set to rise from 6.3 billion pounds per year in 2015, to 8.3 billion pounds per year in 2025 and 9.7 billion pounds per year in 2050. If including the cost of obesity-associated comorbidities, this cost is projected to increase from 27 billion pounds per year (in 2015) to 37.2 and 49.9 billion pounds per year in 2025 and 2050, respectively [7].
Figure 1.3: Future projected rates of obesity in select countries

*From the Organisation for Economic Co-operation and Development’s (OECD) 2017 Obesity Update.*
1.2 The Complex Interplay between Biological, Psychological, and Socio-Economic Determinants of Obesity

At first, the cause of obesity can appear simple. As stated by the laws of thermodynamics, body fat accumulation is the net result of an unbalanced energy in, versus energy out equation. This simplistic view, widely held and often portrayed as true by the media and members of the general public, does not reflect the intricacies of what is in fact a complex and multifaceted process. In general, obesity arises from an intricate interplay between biological and environmental factors, which vary from person to person, from group to group and across a person’s lifetime [7]. From the existence of this wide variability in causes, it follows logically that there must also exist a wide array of potential solutions, which target the particular combination of each person and each group’s main barriers to change. Thus, in-depth understanding of the causes is paramount to the correct diagnosis and resolution of obesity. Evidence from the medical, life, social, economic and political sciences have produced a great deal of promising leads, however, these are not generally well integrated across disciplines, and consequently, results from trialled interventions to date have been somewhat underwhelming. In the following sections, the causes of obesity from the perspective of several disciplines are discussed in greater detail.
Causes of Obesity: Genetics and Biology

The environmental conditions throughout the process of human evolution have selected for a biology which is supremely well adapted to contend with prolonged periods of fasting, limited nutrition or even starvation. By bestowing a survival advantage to our ancestors, several (and often redundant) systems have evolved to protect the most vital aspects of our physiology (such as the constant need for energy). The result is a body which actively defends against intentional depletion of energy stores [8, 9, 10, 11]. However, the fast-pace of technological advances means that most of us now live in a drastically altered environment for which we are ill-adapted. For many, there is much interest in modifying these biological targets in such a way that health may be preserved without the need to alter the environmental factors which determine our dietary intake, and/or postpone the inevitable appearance of metabolic disease.

Much effort has gone towards trying to identify the genetics of obesity. Several genome-wide association studies have highlighted a number of genes, which when mutated may cause severe obesity within the first few weeks of life. Other genes, such as variation of the FTO gene, have been shown to contribute to weight gain, though not degree of obesity [12]. Though there is evidence to suggest adiposity is among the most heritable of human traits [13], it not yet clear whether genetic susceptibility to obesity is the result of relatively common polymorphisms with modest (but widespread) effects on risk; or multiple different rare alleles. Despite the somewhat widespread belief that such genetic pre-dispositions may affect metabolic rate or selective conversion of excess calories into fat stores, most monogenic defects associated with human obesity disturb hypothalamic pathways.
controlling hunger, satiety and food intake [12]. Critical hormonal, neural pathways and feedback loops have been identified through such research, which have facilitated a more in-depth understanding of the physiological mechanisms of food choice, weight gain and metabolic disease. Indeed, the current evidence thus far would seem to suggest that from an aetiological standpoint, obesity is more of a neurobehavioural than a metabolic disease [14].

Perhaps one of the better understood examples of molecules involved in regulation of energy intake is the adipokine, leptin [15]. The relevance of leptin was first discovered in ob/ob mice which due to mutations in the ob gene, exhibited a complete lack of the leptin protein, and were phenotypically severely obese [16]. Treatment of these mice with recombinant leptin corrected their hyperphagia, neuroendocrine and metabolic abnormalities [17]. However, in the general population, leptin levels are low in lean individuals, and heightened alongside increased adipose tissue mass. Its main role is now believed to be the stimulation of food intake when body weight is low, and obese individuals may develop a resistance to its actions [18].

In the modern world, where plentiful, energy-dense, low-cost food is ubiquitous, our intrinsic adaptations for survival must be overridden through conscious control [19, 20, 21, 22]. Recent studies employing functional resonance imaging have demonstrated how sensory factors such as sight, smell, palatability and availability of food can stimulate brain neural networks to the extent that innate control mechanisms are overwhelmed, a phenomenon termed “hedonic” hunger [23, 24]. By contrast, the innate mechanisms of satiety, which communicate to our bodies that we have had enough, are relatively weak, and easily overridden by the sight
or taste of food [25, 26]. For example, it is far easier to skip a meal than it is to avoid tasting (or finishing) a single food once it is put in front of us, despite the fact that we may already be full [25].

On the other side of the energy equation, research into the metabolic aspects of energy expenditure have yielded little to suggest this may be the cause of the rising rates of obesity. Numerous, large sample, multi-national studies have demonstrated that, after adjustment for body size and composition, energy expenditure differs little between individuals [27, 28]. Thus, there is little evidence to suggest the basic physiology of energy expenditure between lean and obese individuals is altered to protect lean individuals against weight gain.

The evidence so far would seem to negate a physiological difference between lean and obese individuals as the primary cause of obesity. Though some causative mutations affecting appetite and satiety have been identified, these have not been present in the majority of obesity cases tested [12]. More limited knowledge is available regarding genetic variants that underlie the susceptibility or resistance of an individual to obesity, and perhaps more interestingly, the interaction between the obesogenic environment and these genetic variants. In this respect, other lines of investigation have begun to show much promise, such as the role of the gut microbiota, gut hormones and the circadian rhythm [29, 30, 31, 32, 33].

**Causes of Obesity: Diet and Eating Behaviours**

With excessive energy intake being recognised as the primary contributor to weight gain [7, 34], what an individual eats (diet) and the context in which food is consumed (eating behaviour) are critical to the development of obesity.
Measuring dietary intake outside carefully-controlled laboratory conditions remains problematic, but a number of specific dietary risks have been consistently identified. In general, the low intake of fruits and vegetables, and their replacement with ultra-processed energy-dense foods and drinks (which are generally high in fat and/or sugar and low in dietary fibre, vitamins and minerals) are strongly associated with obesity and metabolic disease [35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45]. Unfortunately, ultra-processed foods are becoming increasingly dominant in the world diet, as cooking and food preparation habits and skills decline [46, 47]. Therefore, the current evidence suggests a healthy diet should limit (and, if possible, exclude) all but traditionally processed foods and snacks; consisting mainly of vegetables, whole grains, legumes and fruit, with limited amounts of meat, eggs and dairy. However, according to the 2013 health survey for England [5], more adults ate fewer than 3 portions of fruit and vegetables a day (45% men and 41% women), than met the recommended 5 portions.

What dictates a person’s dietary choices, behaviours and preferences? The current evidence would suggest a number of points in a person’s lifetime, in which there may be critical periods of metabolic plasticity, and/or pivotal habits and attitudes are established. For instance, robust evidence suggests breast-feeding and early growth patterns are important determinants of long-term health [48, 49]. Dietary behaviours (such as preference for fruits and vegetables) are developed early in childhood and resistant to change at a later stage [50]. In young children, the most significant predictor of obesity is parental obesity (risk is increased by 10%)[22]. Even more alarmingly, whilst no amount of exercise has been found to protect against childhood obesity [51, 52, 53], proximity between fast food restau-
rants and schools was observed to be an independent predictor of BMI in children [54]. Also critical is the perimenopausal period, where a significant shift in obesity prevalence demographics is observed (over three quarters of females over the age of 45 presenting with either overweight or obesity) (UK data) [5].

The context in which a food or meal is eaten can also have a significant impact on satiety, negative habit re-enforcement and overall tendency to gain weight. Among the problem eating behaviours most commonly identified by researchers as potential contributors to obesity are: skipping breakfast, grazing, eating past satiety signals, eating when feeling bored, eating when feeling upset, eating as a reward, eating as a hobby, eating quickly, secret eating, chaotic meal patterns, avoiding meals prepared at home, eating before feeling hungry, and eating at night [55]. The characterisation of problem eating behaviours worth targeting in obesity is still at an early stage in research, and is likely to vary substantially between individuals. However, identifying and targeting these behaviours appropriately may be critical to effective obesity interventions.

**Causes of Obesity: Mental Health**

Higher prevalence of obesity has long been observed in several psychiatric disorders, of which the most thoroughly studied is depression [56]. This association is stronger in women and with increasing severity of obesity [57, 58, 59, 60]. Epidemiological evidence has emerged suggesting that the onset of obesity precedes that of depression in adults, whilst in children and adolescents the opposite is true [61, 62, 63].

Negative attitudes towards obese individuals have persisted even as its prevalence
has become more wide-spread. These anti-obesity biases appear early in life, but have also been uncovered in educational, occupational and health-care settings [64, 65, 66, 67, 68], and even in health professionals who specialise in obesity [69]. Thus, individuals with obesity can often encounter from their surroundings little empathy at best, and outright discrimination at worst. Thus, researchers believe that weight discrimination, encountered more often by women and individuals with higher BMI, may explain the epidemiological observations of depression prevalence in obese population [56]. Other mechanisms, such as health-related quality of life, may also explain the increased risk of depression in individuals with severe obesity [70, 71, 72, 73]. Furthermore, depending on the type of depression presentation, appetite may be either impaired or increased [74, 75], usually remaining consistent within the same person despite wide variability between individuals [76].

Other forms of mental illness have been associated with obesity. Overweight and obesity are much more prevalent in individuals with bipolar disorder (36% and 32%, respectively) than in the general population [77, 78]. A comparison of obese and non-obese individuals with bipolar disorder, highlighted that obese participants had significantly more manic and depressive episodes, and greater severity of depressive symptoms than lean counterparts [79].

The presence of schizophrenia has also been tightly associated with a higher risk of obesity [80, 81]. Schizophrenia is also associated with a 20% shorter life-expectancy, and greater prevalence of obesity co-morbidities such as type-2 diabetes, coronary heart disease, and hypertension [82, 83]. An important factor in the association between mental health and obesity is the use of antipsychotic medication, which have been shown to promote varying degrees
of weight gain, sometimes in as little as 10 weeks [84, 85] and may be linked to
disturbances in hunger and satiety, though the precise mechanisms remain poorly
understood. Some antidepressant and mood stabilising medications have also been
associated with significant weight gain, however this varies substantially with the
specific type of medication and further trials are needed [86].

Early childhood trauma resulting from abuse has also been implicated in risk of
obesity. A recent meta-analysis reported childhood abuse was clearly associated
with obesity as an adult, including a positive dose-response association [87].

Thus, people living with mental illness are particularly vulnerable to the risk of
obesity. This relationship is often bidirectional and complex and may be at least
in part, mediated by our approach to and compassion for individuals with obesity.

**Causes of Obesity: Policy, Socio-economic and Cultural Factors**

There is currently a lack of conclusive evidence on how and to what extent our
environment encourages the obesity epidemic, however some trends and themes
are emerging.

Deprivation and low socio-economic status has a strong positive association with
obesity rates [88, 5]. Indeed, children in the most deprived areas of the UK are
twice as likely to be obese (where obesity rates are 25%) than children in the least
deprived areas (where the obesity rates at year 6 is just 12%) [5].

The amount of household income spent on food has fallen steadily since the 1960s
to an average of 10%, but it is worth noting there is a wide divide between lower
and higher income households. Whilst higher-income households spend below
15% of their total income on food, this spend exceeds 23% among lower-income households [89, 7]. Importantly, cheaper foods tend to be more calorie-dense, with plentiful fats and sugars and lacking in vitamins and minerals. Data from the USA show that real purchasing costs of fruits and vegetables have increased in relation to fats, oils, starches and sugars (Figure 1.4) [90]. Indeed, a positive linear correlation has been demonstrated between the prevalence of obesity across 21 countries, and the number of hours worked annually [7], though whether this is the result of socio-economic status, less cooking time or higher levels of stress is unclear.

![Figure 1.4: Price changes (real dollars) from 1985-2000 differentially affecting dietary components in the USA](Source: Institute of Agriculture and Trade Policy [90])

With lowered costs of non-perishable ingredients, food industry and manufacturers have developed and invested in a large range of processed foods versus raw products, which they can produce at minimal cost and sell at the price of raw
non-processed foods [91, 92]. Supermarket processed foods is one of the fastest growing category of products, particularly if marketed as “healthy” [93]. However, there is poor and lax regulation of the terminology and health benefits these foods are allowed to claim, and the result is often wide confusion and misinformation in the general public as to what is actually “healthy” [94]. Further highlighting a need for stricter regulation and government policy, is the finding that fast food outlets are more common in higher deprivation areas, where the local population have much higher risk of obesity and poor health outcomes [95, 88, 5, 96, 97].
1.3 Effectiveness of Current Obesity Treatments

Exercise Only Interventions

Exercise on its own is often discussed as a weight loss strategy. However, the evidence suggests that exercise unaccompanied by dietary changes is ineffective. A recent meta-analysis of randomised controlled trials attempting exercise-only interventions in children (which included more than 18,000 participants) concluded that no amount of exercise, even in cases where children were exercising more than ten times that of their peers, was protective against childhood obesity [51, 52, 53]. Similar conclusions can be drawn from adult studies which target weight through exercise only. One 11-year study reported that participants exercising more than 150 minutes per week gained 1.8Kg less than their sedentary peers over the period of the study [98]. A thirteen-year nurses’ study reported that in individuals with unhealthy BMI, no amount of exercise prevented weight gain over the period of the study [99]. In a twenty year study of 25,000 male participants and objectively measured exercise, men who exercised the maximum amount (more than 5 hours per week) gained 2.6Kg fewer than men who exercised a minimum of 90 minutes per week [100]. Another study, where participants were enrolled in walking programs but no dietary changes were targeted found that for every 16.9Km walked, participants lost 45g [101], such that it would take 66 to 81 hours of walking to lose 1 Kg of body weight. Thus, the evidence clearly shows that there is no realistically prescribable amount of exercise that will lead to weight loss in the absence of dietary changes.

It should be stated that exercise confers many other health benefits on mobility,
stress and cardiovascular health and should be encouraged in obese and non-obese individuals [102], however the current evidence suggests that exercise can not offset the effects of an obesogenic diet. Thus dietary changes are paramount to effective weight loss interventions.

**Dietary and Lifestyle Interventions**

Despite extensive research focused on the causes of obesity, research into what constitutes an effective obesity intervention is surprisingly limited. Controlled studies are finite, narrow in scope and confined to laboratory settings [103, 104, 105, 106, 1]. Few interventions have been effective in reducing prevalence of obesity. Though some are promising, they have not yet been reproduced at a larger scale. One such promising example is the Fleurbaix-Laventie Ville-Santé community-based intervention, which targets dietary choices and physical activity in children. It combines both private and public sector initiatives to generate consistent messages on select topics (*i.e.* eating more vegetables, etc...) for a 3 to 4 month duration. The lessons from this program are set to inform a much wider community-based intervention (EPODE) involving more than 130 towns in France, and two each in Belgium, and Spain, though no data is yet available for this program [107]. It is worth noting however, that interventions securing even modest amounts of weight loss have been shown to reduce healthcare costs arising from associated co-morbidities (such as type-2 diabetes) [108, 109, 110]. The North Karelia Project in Finland demonstrated that a comprehensive, determined and community-based programme can have a substantial positive effect on risk factors and eating behaviours, which were associated with favorable changes in chronic disease [111, 112, 113, 114].
Lessons From History

In the recent past, there have been repeated efforts to curb harmful behaviours such as smoking, heavy drinking, drug abuse, and safer driving. The lessons from some of these campaigns may provide valuable insight as to effective behaviour change strategies that may be used to tackle the obesity epidemic [115]. In the case of alcohol consumption and smoking, policy makers moved from basic advice provision, to facilitation of healthier options (such as nicotine patches), to active discouragement of unhealthy behaviour (through taxation, and advertising restrictions) and finally to regulatory action (such as bans on smoking in public areas) [116]. Though there is no doubt that regulation of smoking has been ultimately effective in the protection of public health, under-pressure from major tobacco corporations it took policy makers 50 years to move from evidence of harm to regulatory action [117, 116]. The food industry is no less powerful than the tobacco industry, and unlike smoking, obesity and overweight are being normalised in our society, even as the trends accelerate and the evidence grows, leaving action on diet (such as taxation of sugar-sweetened beverages and policy that would limit added sugar) far behind [94, 118, 119, 91, 120].

Lessons From Social Marketing and Behaviour Change Research

Extensive evidence from social marketing research suggest that public information campaigns instructing people to avoid certain foods, eat more fruit and vegetables, and/or exercise more frequently are unlikely to produce any real effect on the obesity problem [121, 122, 7]. Instead, interventions that go beyond informa-
tion campaigns to simultaneously inform, shift motivation, provide the necessary tools and skills are much more likely to effect meaningful behaviour change [123]. Any approach aimed at influencing behaviour, should be properly tailored to the individual and group’s specific collection of factors contributing to obesity, since evidence suggests that large behaviour change campaigns can have the opposite effect if they are not first properly tested [22].

Research shows that the interaction between the person and their environment is an important determinant of behaviour [124, 125, 126, 127], which means that some environments can make it extremely difficult to effect behaviour change [128, 129]. These studies highlight the extreme and sustained effort required to resist the temptation of the obesogenic environment [130, 131, 132], so perhaps it is not so surprising that interventions targeting individual choices within the same obesogenic environment have not proved effective. Stress [133, 21] and habit formation can further significantly undermine the individual’s ability to resist temptation [134], particularly in the case of eating behaviour [21]. Evidence suggest that people are more susceptible to information and behaviour change at times in which existing habits are temporarily broken (such as after becoming a parent, moving house, starting in a new school, surgical intervention, etc...) [22]. A person’s perception of their own vulnerability to disease (rather than severity of risk) is crucial to whether or not they will change their behaviour. However, research has shown that because people do not want to feel vulnerable, they are more likely to convince themselves that they are not at risk [135, 136], and provision of information is not likely to prove effective under these circumstances. Individuals should be encouraged to reflect and propose their own answers, as self-generated
persuasive arguments have been shown to lead to greater change, however the environmental cues should not be ignored or they will trigger old habits [22].

**Bariatric Surgery**

Bariatric surgery is currently the most successful treatment for obesity-related T2DM and the metabolic syndrome in morbidly obese patients [137]. Within the first two years after bariatric surgery, morbidly obese patients may often lose 50% of original weight, a figure that remains stable up to 10 years post intervention [138, 139]. Though their BMI will generally remain in the obese category (above 30), their metabolic parameters may be similar to those of lean and healthy individuals [140]. Studies comparing bariatric surgery against dietary interventions have reported that similar improvements to cardio-metabolic risk may be achieved with both [141], however maintenance of weight loss, though a challenge in both, is significantly greater after bariatric intervention [142].

However, studies comparing bariatric surgery against non-surgical programs rarely include non-surgical interventions which target all environmental causes and contributors to obesity (such as diet, stress and environmental triggers)[103, 106], so perhaps this does not necessarily reflect the true potential of non-surgical interventions, and further comparisons between surgery and comprehensive holistic non-surgical interventions are required. However, the need for robust, large-scale, multidisciplinary obesity interventions is unlikely to be met by existing funding pathways [143, 7] and is likely to require unprecedented coordination from partners outside the health sector. To ensure effectiveness, the planning of such interventions must evaluate and aim to target all causes and contributors to obesity.
accordingly, either through establishing effective behaviour change strategies, new social norms, a creative and supportive environment, and engagement of community stakeholders [144, 121, 122, 22].
1.4 Provision of Weight Management Services in the UK

The current UK guidelines for treatment of obesity prioritise patients according to BMI and presence of co-morbidities (Table 1.3), the effect of which is that opportunities for meaningful intervention in patients with overweight, obesity class I and even obesity class II (without co-morbidities) are largely ignored. Despite mounting evidence that the primary causes of obesity arise exponentially from the obesogenic environment, current weight management services in the UK mostly target non-environmental causes, by prioritising obesity-associated co-morbidities whilst obese patients are simply told to lose weight. It is critical to the obesity pandemic that treatment is not divorced from prevention.

Table 1.3: UK guidelines for overweight and obesity treatment strategies

<table>
<thead>
<tr>
<th>BMI Classification</th>
<th>Waist Circumference</th>
<th>Co-morbidities present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td>&lt; 94 cm</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>94 – 102 cm</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt; 102 cm</td>
<td>3</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>&lt; 80 cm</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>80 – 88 cm</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt; 88 cm</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tier</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>General advice on healthy weight and lifestyle</td>
</tr>
<tr>
<td>2</td>
<td>Diet and physical activity</td>
</tr>
<tr>
<td>3</td>
<td>Diet and physical activity; consider drugs</td>
</tr>
<tr>
<td>4</td>
<td>Diet and physical activity; consider drugs; consider surgery</td>
</tr>
</tbody>
</table>

Source: NICE Guidelines [1]

While there are established public-private mechanisms for investment in pharmacotherapy and bariatric surgery, behavioural interventions have, historically, been poorly supported [143, 7]. Perhaps the issue lies in the many competing theories for the “main cause” of obesity, grounded in a range of disciplines (such as
dietetics, sociology, psychology, physiology, and economics) each implying different solutions, which promote both caution and confusion [143]. When combined with the multi-level nature of modern governance, the result is an intricate set of challenges for effective anti-obesity policy. In addition, if policies are developed in isolation, there is a very high likelihood that potential beneficial results from one initiative may be undermined by the well-intentioned, though opposite actions of another. Thus, there is great need for a unified, trans-discipline effort to determine and prioritise which determinants of obesity to target, and which strategies to use with reference to the evidence. As the evidence points overwhelmingly away from personal responsibility and towards the obesogenic environment [143, 7], it is illogical to continue to discuss and target the obesity crisis through person’s choices without addressing their environmental context. Attention should be refocused towards addressing the environmental contributors of obesity (such as the food industry, marketing, mental health, socio-economic deprivation and stress), and weight management interventions adequately funded to address both prevention and treatment of co-morbidities.
1.5 The Pathophysiology of Metabolic Disease

Obesity is the fourth largest risk factor for deaths in the UK (after hypertension, smoking, and high cholesterol) [5]. For individuals who fall within the obese category, the risk of disease increases sharply with BMI. With increasing body mass index (BMI), a measure of weight relative to height, there is also increasing prevalence of metabolic morbidities such as insulin resistance, type 2 Diabetes Mellitus (T2DM), hypertension, dyslipidaemia, ischaemic stroke and coronary heart disease [145, 146]. Additionally, strong associations have been observed between increasing BMI and several types of cancers such as breast, colon, kidney and pancreas [147, 148]. T2DM, ischaemic heart disease, stroke and cancer are all within the top 10 causes of death worldwide. The global average of healthcare costs for type-2 diabetes alone are projected to increase by 30-34% from 2010 to 2030, though it could be as much as 67% in developing nations [149]. Given the alarming scale of this issue, precise understanding of key cellular and systemic processes underlying adipose tissue biology and obesity-related metabolic disease are of outstanding importance.

Relevance of White Adipose Tissue in the Initiation of Metabolic Disease

Under conditions of chronic hyper-caloric supply, white adipose tissue may not be able to cope with the high demand for lipid storage, resulting in increased circulating free fatty acids and accumulation of lipotoxic metabolites such as diacylglycerol, ceramides and long-chain fatty acyl-CoA in adipose and non-adipose tissues [150].
Lipotoxic metabolites lead to direct inhibition of insulin signaling through serine phosphorylation of insulin receptor substrate (IRS) proteins and activation of inflammatory pathways [151, 152]. Further evidence to suggest cardio-metabolic disease arises from lipotoxicity can be observed in genetic conditions such as familial combined hyperlipidaemia where defects in chylomicron lipid uptake and lipolysis in white adipose tissue result in increased circulating lipid and ectopic fat deposition in organs other than white adipose tissue. Although these individuals’ BMIs are usually normal, they are insulin resistant and at high risk of cardiovascular disease [153, 154].

Within white adipose’s adaptive response to increase its lipid storage capabilities, adipogenesis is thought to play an important role, allowing white adipose tissue to grow in adipocyte number (hyperplasia) and thus store lipids innocuously in the form of triglycerides. When the pressure for increased lipid storage exceeds the adipogenic capacity of the tissue, lipid accumulates in existing adipocytes, causing them to swell (hypertrophy), develop abnormalities in lipid handling (lipotoxicity) and eventually leads to apoptosis. In both visceral and subcutaneous WATs, larger adipocyte diameter (hypertrophy) positively associates with inflammation and cardio-metabolic disease [155, 156]. Interestingly, a recent in vitro study showed that increased mitochondrial activity is necessary for differentiation of human preadipocytes [157], implicating mitochondria in white adipose tissue dysfunction and cardio-metabolic risk.

Apoptosis in itself is another factor that may contribute to adipose tissue dysfunction by reducing white adipose tissue mass and therefore its capacity to adequately buffer lipids. Moreover, widespread adipocyte apoptosis releases a variety of lipo-
toxic metabolites that trigger a local inflammatory response, which can directly hinder insulin sensitivity. Indeed, increased white adipose tissue apoptosis has long been observed in association with obesity and cardio-metabolic disease risk [158], though the trigger to adipocyte apoptosis itself has not yet been determined. Interestingly, mitochondria have been shown to play a signaling role in certain types of apoptosis, which are age-related and increased mitochondria-mediated apoptosis associate with higher percentage of pro-apoptotic cells in skeletal muscle [159, 160, 161].

Moreover, distribution of white adipose tissue in addition to its lipid-handling capacity is a major factor in determining cardio-metabolic risk. Lipodystrophic syndromes highlight this fact, as in these individuals loss of gluteo-femoral or subcutaneous fat (causing increased lipid deposition in visceral white adipose tissue) results in worsening metabolic and cardiovascular risk parameters [162, 163]. Although the reason behind this risk disparity remains under debate, evidence suggests this could be partially explained through tendency for inflammation. While subcutaneous white adipose tissue has higher lipid handling capacity [164, 165], visceral white adipose tissue has a high supply of innate immune cells that allow it to play a largely mechanical and protective role against external inflammatory stimuli [166].

These resident innate immune cells in visceral white adipose tissue of normal BMI and metabolically “healthy” obese individuals are mainly M2 macrophages that secrete anti-inflammatory molecules (such as IL-10 and IL-4) and serve a primarily antigen presenting role [167]. However, with increased adipocyte apoptosis, the macrophage population distribute in the tissue forming crown-like structures (CLS) and adopt an M1 phenotype, capable of secreting pro-inflammatory molecules like
IL1-β, TNF-α and IL-6 [168, 169]. The combination of CLS, apoptotic adipocytes and circulating inflammatory cytokines during obesity are strongly associated with cardio-metabolic risk [168, 158, 170]. Interestingly, adipose tissue depot-specific differences in mitochondrial number and respiration have been identified, implicating mitochondrial functionality in cardio-metabolic risk [171].

Collectively, the evidence set out above highlights the potential role of mitochondrial alterations in adipose tissue dysfunction and the pathogenesis of obesity-associated insulin resistance and cardio-metabolic disease. In the following section, these mitochondrial alterations are examined in more detail.
1.6 Emerging Concepts in the Mechanism for Metabolic Recovery After Bariatric Surgery

Success of surgery varies from one individual to another, depending on surgical procedure, commitment to dietary and lifestyle changes, length of T2DM and metabolic disease prior to surgery, age, gender and genetics [172, 173]. However, the profound and consistent nature of weight loss following bariatric surgery was elegantly demonstrated by the Swedish Obese Subjects (SOS) trial [174], the first prospective controlled bariatric surgery intervention study and provides an instrumental opportunity to study mechanisms of metabolic health and disease. The most common surgical procedures (Figure 1.5) include:

- Laparoscopic adjustable gastric banding (LAGB): restricting the stomach using an adjustable silicone band.
- Vertical sleeve gastrectomy (VSG)/ laparoscopic greater curvature plication (LGCP): creating a smaller gastric pouch
- Bilio-pancreatic diversion (BPD): connecting the distal part of the small intestine to the ventricle, bypassing the duodenum and jejunum
- Roux-en-Y gastric bypass (RYGB): creating a smaller gastric pouch and connecting it to the distal end of the jejunum.
Several In LAGB, an inflatable silicon device is wrapped around the top portion of the stomach, reducing the gastric volume which can later be adjusted in the clinic with minor invasion. In VSG, the stomach is reshaped into a tubular-like sleeve. In LGCP, the stomach is folded inward and stitched at the fold to reduce the gastric volume in a similar degree to VSG. In BPD, a VSG is performed after which the stomach is then connected to the jejunum and the duodenum to the lower part of the small intestine. In RYGB, a small gastric pouch is directly connected to the jejunum, bypassing the rest of the stomach and the duodenum.

In terms of fat loss and diabetes resolution, BPD and RYGB (which include a malabsorption component through bypass of a considerable amount of the small intestine) confer the greatest long-term improvement [137, 139, 138], but carry greater risks and side effects [175, 176]. Interestingly, patients who have undergone BPD and RYGB exhibit a pronounced improvement in diabetes within the first few days post surgery, which cannot be explained by the energy restriction or fat loss alone [177]. Thus, metabolic improvements observed post bariatric surgery are not just the result of energy restriction and malabsorption-associated fat loss, but likely involve other elements including signaling factors in the small intestine.

In the past, an overwhelming focus of those studying the mechanism of metabolic
recovery following bariatric surgery has been to examine these short-term weight-independent mechanisms, without sufficient focus on how the surgery generates weight loss in the first place. Two main hypotheses for the mechanism of metabolic improvement following bariatric surgery have been proposed: (a) the hindgut hypothesis suggests that enhanced delivery of nutrients to the small intestine trigger secretion of hindgut signals with beneficial effects on glucose homeostasis, whilst (b) the foregut hypothesis states that post-surgical metabolic improvement is the result of altered signals from the excluded segment of proximal intestine. Evidence continues to emerge that would support both possibilities, as well as several others, whilst highlighting deeper levels of complexity [178, 176, 179, 180, 181, 182]. In terms of the long-term effects of surgery however, weight loss has consistently remained as one of the main contributing factors to the metabolic advantages conferred by bariatric surgery [179].

**Regulation of Energy Balance**

Like many other key necessities for functional life such as temperature, blood pressure, electrolytes and glycaemia, energy stores are very carefully regulated. The complex genes-environment interaction throughout evolution has selected for highly effective processes that defend against loss of energy stores. Body fat is actively defended physiologically during periods of intentional weight loss [20], through sustained reduction of energy expenditure (resulting from loss of lean muscle) [10], more efficient muscle metabolism [9], reductions in thyroid and sympathetic activity [8], and reduced non-resting metabolic rate [11]. On the other side of the energy equation, hunger hormones (known to reduce satiation and sati-
Adapt to weight loss in a manner that will encourage weight regain. Diet and exercise-led weight loss efforts result in sustained reductions of leptin, insulin, peptide YY, cholecystokinin, and amylin; whilst levels of ghrelin, and pancreatic polypeptide increase. These changes are associated with elevated measures of subjective appetite [19], and altered neural activity in response to food cues in brain areas controlling enteric regulation, emotional, decision, executive functions and perception systems [11]. Following surgical weight loss, however, hunger is reduced and cognitive restraint is increased. Therefore, the most striking result of bariatric surgery, is its capacity to produce weight loss without triggering the weight-defense mechanisms that result with non-surgical means, ensuring greater probability that weight loss will be maintained.

**Restriction and Malabsorption**

The once popular hypotheses of food restriction and malabsorption as the main mechanisms of post-surgical weight loss have now been largely abandoned. Since the 1980s, studies have consistently confirmed that gastric-only procedures do not delay the transit of food within the foregut, and satiety signals generated with such procedures do not correspond with food transit time. Moreover, no evidence has been found that any of the most commonly performed procedures (RYGB, VSG, LAGB), with the exception of BPD, produce significant macronutrient malabsorption in sufficient amounts to influence the energy equation [183].
Gastro-Intestinal Mechanoreceptors

There is strong evidence to suggest that subtle adjustments in gastric pressure can influence satiety. A double blinded, randomised controlled trial of individuals with gastric bands, either correctly adjusted or empty demonstrated this concept. Individuals with correctly adjusted bands were less hungry, more satisfied after a meal, and satiated for longer than those whose bands were empty [184]. Neither GI hormones nor gastric emptying rate were affected by these minor mechanical adjustments, which despite this may clearly have a significant effect on weight loss.

Hormonal Changes

Insulin and the adipokines Leptin, and adiponectin, all follow the same pattern of change as with non-surgical weight loss [69, 176], and thus are unlikely to explain the enhanced effects of surgery on metabolic health. The L-cell hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which reportedly deliver a rapid incretin and satiety response have been shown to increase after RYGB, BPD and VSG surgeries [185, 186]. However their contribution to weight loss efficacy of bariatric procedures remain uncertain [182]. Similarly, initial reports of the hunger hormone Ghrelin garnered much attention, as it was shown to be markedly reduced with surgery versus lifestyle weight loss interventions, however several lines of preclinical and clinical research have thrown doubt on its impact in sustained weight loss [187, 188, 189].
Bile Acid Handling

Primary bile acids are synthesised from cholesterol in the liver, stored in the gall-bladder, and secreted in the second part of the duodenum in response to a meal. They are then actively and passively absorbed in the terminal ileum and recycled via the portal vein to be secreted again (a cycle which can be completed several times a day). The nuclear farsenoid receptor X (FXR) is the primary regulator of the bile acid cycle, and may also influence hepatic effects on glucose tolerance by inhibiting gluconeogenesis, improving insulin secretion and sensitivity, and up-regulating glycogen synthesis. Bile acids may also improve glucose tolerance and satiety through intestinal stimulation of cell surface G protein-coupled bile acid receptor 5 (TGR5) and GLP-1 secretion from L-cells [190].

In humans, bile acid levels fluctuate between feeding and fasting, with robust differences detected between healthy insulin-sensitive individuals and those with type-2 diabetes [191]. Furthermore, bile acid levels are increased after RYGB, and VSG but not LAGB procedures [190]. Increased secretion of gut hormone FGF-19 (stimulated by bile acid reabsorption in the terminal ileum) is seen within days of surgery, and may be involved in increased GLP-1 and PYY secretion, improved glycaemia, and weight loss [31].

The Gastro-Intestinal Microbiome

The recent finding that fecal transplantation from bariatric subjects to germ-free animals can reproduce weight loss and mimic post-surgical metabolic improvements is extremely promising and indicative of a crucial relationship between the microbiota and metabolic disease [192]. The composition of the GI microbiome is
enormously complex, though surprisingly stable in the individual. Germ-free mice transplanted with human microbial communities were stably and heritably colonized, reproducing much of the human donor’s bacterial diversity. Mice who were then subsequently fed a high-fat, high-sugar “Western” diet had increased adiposity, a trait that was transmissible to other mice through microbiota transplantation [30]. Though true understanding of the microbiota’s role in altering metabolic and appetite signaling pathways is in a very early stage, these results are nevertheless compelling evidence of a potential physiological mechanism controlling obesity which is both related to poor diet and intrinsic to individuals with obesity.

One promising avenue for further research in this subject are gut-derived bacterial Lipopolysaccharide (LPS), also known as endotoxin, which are outer cell membrane fragments from Gram-negative bacteria, whose gut populations are encouraged by a Western diet. Due to their chemical affinity, LPS are absorbed and distributed alongside dietary fats inside chylomicrons, with circulating LPS concentrations rising in a direct proportion to dietary fats or in combination with saturated fat, high-fat or high carbohydrate meals [193, 194]. Once in circulation, LPS stimulates the innate inflammatory response in adipose tissue, and release of pro-inflammatory cytokines through the activation of TLRs and the IKK/NFkB pathway [195, 196]. As chronic, low-grade inflammation is a risk factor for the development of metabolic dysfunction [197], many studies have focused on LPS within this context. In an animal model LPS was shown to initiate obesity and insulin resistance [198], and in humans serum LPS may function as a predictive biomarker for obesity-related metabolic disease [199, 200, 201, 202].

Under normal conditions, only small amounts of LPS from gut bacteria enter the
circulation bound to dietary lipids and chylomicrons (low density lipoproteins to which they have high affinity), and are quickly eliminated by monocytes, particularly liver-resident Kupffer cells. In this way, circulating levels of LPS are dependent on dietary lipid intake and liver functionality, both of which may be altered during obesity [203]. Indeed, studies indicate that as little as one high-fat meal is sufficient to raise serum LPS levels, especially in metabolically compromised subjects and in a manner that is directly proportional to degree of insulin resistance [204]. Subjects with T2DM are reported to have higher circulating LPS levels than lean, healthy individuals [196]. Thus, meal patterns and insulin resistance can alter serum LPS, contribute to systemic inflammation and cardio-metabolic disease.

Bariatric surgery, which results in substantial weight loss and T2DM remission through gastro-intestinal remodeling, may inhibit the intestinal absorption of LPS. Indeed, in one study, RYGB was shown to decrease endotoxaemia and accompanying oxidative and inflammatory stress along with improvements of cardio-metabolic risk parameters [205]. However, the contribution of LPS to metabolic recovery following bariatric surgery remains unknown.

**Portal Vein Nutrient Sensing**

Neural receptors in the wall of the portal vein sense dietary protein and soluble fibre and modulate intestinal gluconeogenesis [206, 207, 208], which may influence energy and glucose homeostasis, hunger, satiety and insulin sensitivity. Glucose sensing in the portal vein influences a range of central cortical, hedonic and homeostatic regions that control food choice, and satiety [209]. Indeed, specific portal denervation in rats, resulted increased glycaemia, glucagon-like peptide 1 (GLP-1)
and insulin secretion, and reduced insulin sensitivity [210]. However, human data for a role of portal vein nutrient sensing in metabolic recovery is limited [211].

**Food Preference**

All effective bariatric surgeries result in reduced caloric intake through limiting hunger, improving satiation and satiety and altering food choice. However, specific food choice patterns vary with the type of surgical procedure performed. Patients who undergo LAGB typically avoid bread, pasta, and sticky rice which may generate obstructive symptoms and prefer easily digestible protein and vegetables [212, 213], whilst RYGB patients report limiting their intake of sweet and fatty foods which can cause dumping syndrome and possibly replace these with vegetables [181]. Functional MRI scans of post-surgical patients often report reduced activation of reward pathways to energy dense foods [180]. The evidence would suggest that changes in food preference involve sensory, reward and physiological taste alterations [214, 215], however the anatomical or physiological mechanisms responsible remain unknown.
1.7 The Emerging Relevance of Adipose Mitochondrial Function in the Pathophysiology of Metabolic Disease

Normal mitochondrial action is essential for maintaining physiological cellular function. Not just the main powerhouse of the cell, mitochondria are charged with many vital activities such as reactive oxygen species (ROS) production and detoxification, regulation of mitochondrial matrix and cytoplasmic calcium, metabolites synthesis and catabolism, organelle transport, some types of apoptosis, nutrient oxidation and ATP production [216]. In most instances, mitochondrial dysfunction is described as inefficient and/or insufficient ATP production [217], however an abnormality in any mitochondrial function can be termed mitochondrial dysfunction, and has the potential to produce severe metabolic alterations and contribute to insulin resistance.

The primary role of mitochondria is energy production in the form of ATP. In general terms, energy production in eukaryotic cells (primarily in the form of ATP) occurs in the mitochondrion through oxidative phosphorylation (OXPHOS) or the electron transport chain (ETC) which is comprised of several inter-membrane proteins (complex I, II, III, IV and ATP synthase) located in the inner mitochondrial membrane. In this process, reducing agents NADH and FADH$_2$ (generated from previous pathways such as glycolysis, kerb’s cycle and fatty acid beta-oxidation) are oxidized to NAD and FAD, generating electrons, which are carried over from complex I of the ETC to II and so on to IV and O$_2$, generating H$_2$O. In parallel to this, protons are pumped from the matrix through to the inter-membrane space.
to produce an electrochemical gradient that will become the driving force for the ATP synthase complex to produce ATP from ADP [152].

Inter-membrane proteins known as uncoupling proteins (UCPs) play an important role in lowering this electrochemical gradient, either by utilizing it to produce heat (as in the case of UCP1 in brown adipose tissue) or by facilitating proton leak to prevent excess ROS formation (UCP2 and 3). The latter is particularly relevant when there is an excess supply of electrons and low oxygen utilization (high nutrient supply and low energy demand), as the ETC complexes become saturated with electrons and excess electrons are instead pumped directly to oxygen, generating ROS. UCP2 and 3 function to reduce ROS over-accumulation by allowing excess protons to re-enter the matrix, thereby lowering the electrochemical gradient and preventing further ROS production [218, 219, 220].

Increased ROS production and reduced expression of antioxidant enzymes have long been observed in obese and type-2 diabetic patients [221, 222, 223, 224]. As ROS are extremely unstable molecules with the potential to react and alter lipids, protein and DNA leading to increased mutagenesis, inflammatory processes, decreased biogenesis [152] and further mitochondrial dysfunction, prevention of ROS overproduction is a vital matter. Indeed, ROS have been implicated in insulin resistance through activation of IκB kinase β (IKK-β) and various other serine kinases [225, 226, 152]. As mitochondria are the primary source of ROS within the cell, this may be a potential mechanistic link between mitochondrial dysfunction with type-2 diabetes. In support of this concept, over-expression of UCP2 or 3 in vitro results in lower ROS levels and enhanced metabolic rate, both protective factors against weight gain and insulin resistance [227, 228]. Conversely, UCP3
knockout mice exhibit severe oxidative damage [229]. Antioxidants, such as α-lipoic acid have been shown to oppose ROS-induced insulin resistance [230] and pharmacological compounds such as metformin [231, 232] and thiazolidinediones [233] may also improve mitochondrial function by reducing mitochondrial ROS production and stimulating biogenesis.

Mitochondrial biogenesis is the process through which old mitochondria merge (become larger through fusion) and divide (become smaller through fission) to produce new mitochondria, conserving functional and disposing of faulty mitochondrial DNA. This process is coordinated by the peroxisome proliferator-activated receptor (PPAR) co-activator 1 alpha (PGC1α) through co-activation of nuclear respiratory factor 1 (NRF-1), which in turn regulates the expression of DNA polymerase (POLG) and mitochondrial transcription factor A (TFAM) and OXPHOS genes, necessary for mitochondrial gene expression and replication [234, 220, 235]. Adequate execution of mitochondrial biogenesis protects mitochondrial DNA quality and function.

Faults in this process (which may occur in conditions of high oxidative stress and inflammation) can generate mutations in the mitochondrial genome, reduce number of mitochondria, impair oxidative capacity, further increase ROS production and adversely impact general mitochondrial function and insulin signaling [152, 236]. Indeed, inhibition of obesity-induced mitochondrial fission, rescues insulin resistance through increased p38 MAP kinase-associated IRS-1 and Akt activation [237]. Accumulation of high levels of ROS activates stress kinases [223] and pro-inflammatory signaling, which result in the inactivation of IRS proteins by their phosphorylation at serine residues [225, 226, 152]. Finally, reduced lipid oxidation
through impaired β-oxidation results in increased intracellular build-up of lipotoxic molecules such as diacylglycerol and ceramides which stimulate pro-inflammatory signaling and directly alter insulin action [238, 239, 240, 241, 242]. Thus, mitochondrial dysfunction, through altered mitochondrial biogenesis, increased ROS production and decreased fatty acid oxidation, is a potential mechanistic link between obesity and insulin resistance.

Mitochondrial dysfunction correlates with cardiovascular risk factors such as age, physical inactivity and caloric excess. Muscle mitochondria of obese and T2DM patients have decreased mitochondrial respiration capacity, beta-oxidation, ATP production, and increased lipotoxic species and ROS, which associate with insulin resistance [217, 243, 244]. Altered mitochondrial number and morphology have also been observed in skeletal muscle and adipose tissue of obese and T2DM patients [245, 225, 246]. Moreover, pharmacological and lifestyle interventions that improve mitochondrial function also improve insulin resistance [152].

In addition to evidence that mitochondrial dysfunction may contribute to insulin resistance, there is also evidence to suggest that insulin resistance itself may contribute to mitochondrial dysfunction. One study in particular showed that, while stimulating skeletal muscle of healthy lean individuals with insulin results in increased synthesis of mitochondrial gene transcripts and proteins, the same actions on muscle from T2DM subjects produces a diminished response [247].

There are several factors known to alter PGC1α expression and by extension mitochondrial biogenesis, quality control and function such as: age, exercise, fasting and insulin, all acting in a tissue-specific manner [248, 249, 250] (Figure1.6). Indeed, factors causing increase of cellular ATP demand, such as in the case of
exercise, fasting and cold exposure have been linked with increased AMP-activated protein kinase (AMPK) and PGC1α expression [251, 252, 253, 254]. In contrast, age, insulin resistance and T2DM are associated with reduced levels of PGC1α expression [248, 255]. Pharmacological (metformin) [232, 231, 256] and lifestyle interventions (exercise [257, 258] and caloric restriction [259, 260]) that improve insulin sensitivity also stimulate mitochondrial biogenesis. Moreover, PGC1α can alter intrinsic properties of the mitochondria by elevating the expression of ROS-detoxifying enzymes like superoxide dismutase 2 (SOD2) [261] and increasing mitochondrial oxidative capacity through enhanced expression of enzymes involved in β-oxidation, the citric acid cycle and the electron transport chain [262, 250, 263]. Thus, factors that determine cardiovascular risk are also closely involved in mitochondrial function through regulation of PGC1α.

Though several studies have highlighted a link between mitochondrial dysfunction and insulin resistance in skeletal muscle and other metabolic regulatory tissues, the contribution of mitochondria to adipose tissue dysfunction and insulin resistance is still unknown. Indeed, there is little knowledge on obesity-induced mitochondrial alterations in WAT and whether any mitochondrial alterations may be reversed via bariatric surgery.
Several known environmental factors alter mitochondrial biogenesis and function via AMPK or PGC1α. PGC1α in turn regulates transcription of biogenesis (POLG, TFAM), OXPHOS (components of the ETC: complex I, II, III, IV, ATP) and other (SOD1, UCP2) mitochondrial function genes. TFAM regulates the transcription of mitochondrially-encoded components of the ETC, such as mt-ND6 and mt-ATP6. Nutrients are oxidised via β-oxidation or citric acid cycle resulting in NADH and FADH2, which are subsequently further oxidised through OXPHOS to generate ATP. Electrons from NADH and FADH2 are carried over from complex I through to IV of the ETC while protons are pumped through to the inter-membrane space to provide the electrochemical gradient needed for the production of ATP from ADP by ATP synthase. Excess nutrients will result in excess electrons that are carried over to O2 to produce ROS. UCP2 and endogenous antioxidant SOD1 prevent excess accumulation of ROS. AMPK AMP-activated protein kinase, PGC1α peroxisome proliferator-activated receptor gamma co-activator 1-alpha, OXPHOS oxidative phosphorylation, POLG DNA polymerase subunit gamma, TFAM mitochondrial transcription factor A, ETC electron transport chain, UCP2 uncoupling protein 2, SOD1 superoxide dismutase 1, mtDNA mitochondrial DNA, mt-ND6 mitochondrially-encoded NADH dehydrogenase 6 (complex I), SDHA succinate dehydrogenase subunit A (complex II), COX4 cytochrome c oxidase subunit 4 (Complex IV), mt-ATP6 ATP synthase subunit 6 (Complex V), ROS reactive oxygen species.
1.8 Aims

The general aim of this thesis was to address the question of effective solutions to the obesity pandemic translationally, by investigating the range of barriers to metabolic recovery following bariatric surgery:

1. From the basic science approach, the role of:

   (a) gut-hormone FGF-19 on metabolic and mitochondrial improvement

   (b) gut-derived pro-inflammatory bacterial lipopolysaccharide (LPS) on metabolic and mitochondrial improvement, and

   (c) the direct effect of LPS on the disruption of mitochondrial function in the adipocyte

2. From the clinical approach, the environmental barriers to patient weight loss and metabolic recovery within a major UK tier 3 and 4 bariatric centre.
Chapter 2

Materials and Methods
Ethics and study design

The study was approved by the Ethics Committee of the Institute of Endocrinology (Institute of Endocrinology- Ethics Committee EC: 19/5/2009, Prague, Czech Republic). All study participants provided written and informed consent in accordance with the Declaration of Helsinki. Thirty-nine morbidly obese (BMI>35 Kg/m2), type-2 diabetic, Caucasian women undergoing either bilio-pancreatic diversion (BPD; n=12), laparoscopic greater curvature plication (LGCP; n=15), or laparoscopic adjustable gastric banding (LAGB; n=12) at the OB clinic, Prague, Czech Republic were recruited to participate in this study. Thorough biochemical and anthropometric investigations were conducted before (baseline) and six months after surgery with collection of serum samples and abdominal subcutaneous white adipose tissue (AT) biopsies at both of these time points. Patients on pharmacological treatment with incretin mimetics and/or insulin were not included in this study. All subjects included in this study were off anti-diabetic medication, including metformin.

Blood biochemistry and body composition analysis

All anthropometric and biochemical measurements were performed before and six months after surgery. Following a 10-hour overnight fast, venous blood was sampled in all patients, collected in chilled EDTA-containing tubes with and without aprotinin (for glucose and insulin measurements), aliquoted and frozen at -80ºC until assayed. Serum glucose, HbA1c and lipids were determined using the Cobas
6000 analyzer. Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) according to the following equation:

\[
HOMA-IR = \frac{[\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mIU/L)}]}{22.5}
\]

as previously described [264]. The Friedwald formula [265] was employed to compute serum levels of LDL cholesterol. Body weight was measured to the nearest 0.5 Kg and height to the nearest 1 cm. Percentage excess weight loss was calculated according to the following equation:

\[
EWL(\%) = \frac{[\text{Preoperative weight (Kg)} - \text{Postoperative weight (Kg)}]}{[\text{Preoperative weight (Kg)} - \text{Ideal weight (Kg)}]} \times 100
\]

with ideal body weight calculated based on a \( BMI = 25 \text{Kg/m}^2 \) as follows:

\[
\text{Ideal weight (Kg)} = 25 \times [\text{Height (m)}^2]
\]

Body fat mass was measured using the bioimpedance method (Tanita TBF-300; Tanita corporation).

**RNA isolation and qRT-PCR**

For RNA extraction, 100 mg of frozen AT was homogenized in 500 µL Qiazol reagent (#79306 Qiagen, UK) then isolated using a column-based isolation method (RNeasy Lipid Tissue Mini Kit; #74804 Qiagen, UK) according to manufacturer’s instructions. Samples were digested with DNase I to remove potential genomic DNA contaminants (DNase I kit, #AMP-D1 Sigma-Aldrich). RNA was eluted in
10 µL RNase-free water and 1 µL quantified in duplicate using a spectrophotometer (Nanodrop ND-1000, labtech) at 260 nm absorbancy. Synthesis of cDNA was performed using 200 ng RNA per sample and a Bioline mRNA reverse transcription kit (#BIO-65026) according to the manufacturer’s instructions. Gene expression was assayed through quantitative real-time polymerase chain reaction (qRT-PCR) using ABI 7500 standard sequence detection system (Applied Biosystems, UK). Each reaction was prepared to 25 µL final volume containing Taqman Universal PCR mastermix (#4304437 Applied Biosystems, UK), 1 µL sample cDNA and a specific commercially available Taqman gene expression assay (ThermoFisher Scientific, UK, Table 2.1). All samples were assayed in triplicate and multiplexed using 18S (ribosomal RNA) as a pre-optimised control probe. As per the manufacturer’s instructions, reactions were carried out at 50 °C for 2 minutes, 95 °C for 10 minutes, and then 40 cycles of 95 °C for 15 seconds and then 60 °C for 1 minute. For data analysis, gene expression was calculated based on the following formula:

\[ mRNA\ expression = 2^{-\Delta Ct}, \text{ where } \Delta Ct = target\ gene\ Ct - \ 18s\ Ct \]
Table 2.1: Mitochondrial mRNA Gene Expression Taqman Assays

<table>
<thead>
<tr>
<th>Gene</th>
<th>Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC1α</td>
<td>Hs00173304_m1</td>
</tr>
<tr>
<td>POLG</td>
<td>Hs01018660_m1</td>
</tr>
<tr>
<td>TFAM</td>
<td>Hs00273372_s1</td>
</tr>
<tr>
<td>mtND6</td>
<td>Hs02596879_g1</td>
</tr>
<tr>
<td>SDHA</td>
<td>Hs00188166_m1</td>
</tr>
<tr>
<td>COX4I1</td>
<td>Hs00971639_m1</td>
</tr>
<tr>
<td>mtATP6</td>
<td>Hs02596862_g1</td>
</tr>
<tr>
<td>UCP2</td>
<td>Hs01075227_m1</td>
</tr>
<tr>
<td>SOD1</td>
<td>Hs00533490_m1</td>
</tr>
<tr>
<td>SOD2</td>
<td>Hs00167809_m1</td>
</tr>
<tr>
<td>MFN2</td>
<td>Hs00208382_m1</td>
</tr>
<tr>
<td>OPA1</td>
<td>Hs01047018_m1</td>
</tr>
<tr>
<td>DRP1</td>
<td>Hs01552605_m1</td>
</tr>
<tr>
<td>FIS1</td>
<td>Hs00211420_m1</td>
</tr>
<tr>
<td>18s</td>
<td>4310893E</td>
</tr>
</tbody>
</table>

Evaluation of mitochondrial DNA (mtDNA) copy number

Total DNA was extracted from 50 mg frozen AT and cell culture samples using DNeasy Blood and Tissue Mini Kit (#69504 Qiagen, UK) in accordance to the manufacturer’s instructions. RNase treatment was performed to eliminate possible RNA contamination. DNA was eluted with 100 µL AE buffer and quantified using a spectrophotometer (Nanodrop ND-1000, Labtech). Relative amounts of mitochondrial DNA copy number were assessed through qPCR in an ABI Prism 7500 thermo cycler (Life Technologies) with the use of iQ™ SYBR Green Supermix (#170-8880 BioRad). Mitochondrial (mtND1) and nuclear (BECN1) gene primers (Table2.2) were used to determine relative amounts of mitochondrial to nuclear DNA [266]. Each sample was measured in triplicate. Mitochondrial number was
calculated based on the following formula:

\[ mtDNA \text{ copy number} = 2^\Delta Ct, \text{ where } \Delta Ct = BECN1 - mtND1 \]

| Table 2.2: Mitochondrial Copy number Primer Sequences |
|---------------|-----------------|---------------|
| Gene          | Forward          | Reverse       |
| mtND1         | ATGGCCAACCTCCTACTCCT | GCGGTGATGTAGAGGGTGAT |
| BECN1         | CGAGGCTCAAGTGTTTAGGC | ATGTACTGAAACGCCCTTGG |

Evaluation of mitochondrial DNA integrity

Total DNA was extracted from cultured cells using DNeasy Blood and Tissue Mini Kit (#69504 Qiagen, UK) in accordance to the manufacturer’s instructions. RNase treatment was performed to eliminate possible RNA contamination. DNA was eluted with 100 µL AE buffer and quantified using a spectrophotometer (Nanodrop ND-1000, Labtech). Evaluation of mitochondrial DNA integrity was performed via qRT-PCR by comparing previously published primers [267] spanning a section of mtDNA susceptible to mutations (where 84% of known mutations occur) against a section that is not affected by any of the reported large deletions. Real-time PCR of both targets were run using a probe-based duplex qRT-PCR assay on an ABI Prism 7500 thermo cycler (Life Technologies) with the following thermal profile: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15s, 55°C for 15s, and 60°C for 1 minute. The reaction components consisted of 22.5 µL Taqman Universal PCR Mastermix no AmpErase® UNG (Applied Biosystems), each mitochondrial probe at 250 nM (Taqman® MGB Probe, ThermoFisher Scientific, UK; shown in Table 2.3) with a final reaction volume of 25 µL.
Table 2.3: Mitochondrial DNA Integrity Probe Sequences

<table>
<thead>
<tr>
<th>mtDNA Section</th>
<th>Binding Site Positions</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>mt 16,560-10</td>
<td>6FAM-CATCACGATGGATCACAGGT(NFQ)</td>
</tr>
<tr>
<td>Susceptible</td>
<td>mt 10,934-10,951</td>
<td>NED-GACCCCCTAACAACCCC(NFQ)</td>
</tr>
</tbody>
</table>

Mitochondrial DNA integrity was calculated according to the following published formula [267]:

\[ 2^{mtDNA_{DR}}, \text{ where } mtDNA_{Deletion \ Ratio} = \frac{(Stable \ Probe \ Ct - Susceptible \ Probe \ Ct)}{Stable \ Probe \ Ct} \]

**FGF-19 serum levels**

For measurement of serum FGF-19 levels (pg/mL), an enzyme-linked immunosorbent assay (ELISA) kit for FGF-19 (Quantikine ELISA, R&D Systems, Minneapolis, MN) was used. All measurements were performed in duplicate according to the manufacturers instructions. This assay has a detection range of 31 – 544 pg/mL and a coefficient of variation of 4.5% for intra-assay and 5.5% inter-assay precision.

**LPS assay comparison and quantitation of serum levels**

For serum lipopolysaccharide (LPS) determination, two methods were compared: the Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic Assay (QCL-1000™, LONZA) and the EndoLISA® Elisa-based endotoxin detection assay (Hyglos). LPS concentrations of 8 human serum samples (BMI=21 – 38 kg/m²) were determined in parallel with both assays. Samples were run in duplicate according to the manu-
facturers instructions. Serial sample dilution (from 1 : 4 to 1 : 10) and appropriate spike and negative controls were utilised to minimise enzyme-inhibitory factors in samples and confirm result validity. Results were calculated, according to manu-
ufacturers instructions, based on a LPS standard (E. Coli 055:B5) curve ranging from [4 to 0.06 EU/mL] for the LONZA kit and from [500 to 0.005 EU/mL] for the EndoLISA.

For bariatric samples, the EndoLISA® Elisa-based endotoxin detection assay (Hy-
glos) was used to quantify LPS serum levels. A preliminary trial of singlets was run to determine optimal dilution (1:5, 1:10; 1:20), after which samples were run at optimal dilution in duplicate. Results were calculated according to manufac-
turers instructions, based on a LPS standard (E. Coli 055:B5) curve ranging from [500 to 0.005 EU/mL].

**Protein extraction and Western blot**

For protein extraction, 100 mg of frozen human adipose tissue or 1.2 x 10⁶ cul-
tured adipocytes were homogenised in 200 µL Phosphosafe™ Extraction Buffer (Novogen®, Merk, Germany). A Bio-Rad detergent compatible protein assay kit (Bio-Rad Laboratories, CA) and nanospectrophotometer (GeneFlow, UK) were used to quantify protein concentrations. As described previously for Western blot analyses [268], 10-20 µg of protein were loaded onto a denaturing polyacrylamide gel (GeneFlow, UK), transferred on to a nitrocellulose membrane which was then incubated with a primary antibody diluted in 0.2% I-block PBS-T (IRS1 1 : 250, MT-CO1 1 : 1000, SDHA 1 : 1000, β-Actin 1 : 1000) at 4°C overnight. A chemiluminescence detection system (ECL Plus, GE Healthcare, UK) was used
to visualise protein bands, and densitometry was conducted using ImageQuant LAS 4000 Software (GE Healthcare, UK). Equal protein loading was confirmed by examining β-actin protein expression. Primary antibodies utilised are listed in Table 2.4.

Table 2.4: Primary Antibodies

<table>
<thead>
<tr>
<th>1st Antibody</th>
<th>Supplier</th>
<th>Catalogue Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRS1</td>
<td>Millipore</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Cell Signalling</td>
<td></td>
</tr>
<tr>
<td>SDHA</td>
<td>Cell Signalling</td>
<td>5839</td>
</tr>
<tr>
<td>MT-CO1</td>
<td>Abcam</td>
<td>Ab14705</td>
</tr>
</tbody>
</table>

Human adipocyte culture, differentiation and treatment

Human subcutaneous white adipocyte cell line ChubS7 were grown and differentiated as previously described [269]. Briefly, cells were seeded on to 6-well plates (0.3x10^6) unless otherwise specified, grown to 100% confluence and differentiated for 8-10 days in DMEM/F12 with 3% FBS and PromoCell Differentiation Supplement Mix (C-39436, PromoCell). After differentiation, cells were allowed to equilibrate in basal media for 12 hours before being treated for 24-72 hours in basal media supplemented with LPS (10 or 100 ng/mL, E. Coli O55:B5, Sigma, L6529), TNF-α (10 ng/mL, Sigma, H8916) or Insulin (50 nM, Sigma, I9278). All media were prepared with DMEM/F12 (ThermoFisher Scientific, UK, 11320033) and formulations are shown in Table 2.5.
Table 2.5: Adipocyte Culture Media Formulations

<table>
<thead>
<tr>
<th>Media Type</th>
<th>Growth Media</th>
<th>Differentiation Media</th>
<th>Basal Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Bovine Serum</td>
<td>10%</td>
<td>Fetal Bovine Serum</td>
<td>Bovine Serum Albumin 0.5%</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2 mM</td>
<td>d-biotin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin (recombinant human) 0.5 µg/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dexamethasone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IBMX</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Thyroxine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citglitazone</td>
<td></td>
</tr>
</tbody>
</table>

Seahorse Cell Mito Stress Test

Respiration and media acidification rate were measured using a Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Agilent Technologies). Immortalised human preadipocytes ChubS7 were seeded onto 24-well plates (Seahorse Bioscience, 100850-001) at a density of 10,000 cells/well, grown and differentiated using the standard protocol outlined above, and treated for 24 or 72 hours with or without LPS (10 or 100 ng/mL, *E. Coli O55:B5*, Sigma, L6529). Each experimental group consisted of 5 replicates, with the experiment repeated on at least 2 separate occasions (n=10). The assay was conducted in sterile, unbuffered Assay Media prepared with Seahorse base media (Seahorse Bioscience, 102365-100) at 37°C (pH 7.4), the formulation of which is listed in Table 2.6.

Table 2.6: Seahorse Media Formulation

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Supplier</th>
<th>Catalog Number</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Pyruvate</td>
<td>Sigma</td>
<td>S8636</td>
<td>1 mM</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Seahorse</td>
<td>Included</td>
<td>2 mM</td>
</tr>
<tr>
<td>Glucose</td>
<td>Sigma</td>
<td>G8769</td>
<td>17.5 mM</td>
</tr>
</tbody>
</table>

After a calibration step (30 min) and an equilibration step (30 min), the assay protocol consisted of 3 cycles of the following steps: mix (3 min), wait (2 min), measure (3 min), which were completed before and after each injection of Oligomycin.
(Sigma, O4876), FCCP (Sigma, C2920) and combined Rotenone (Sigma, R8875) and Antimycin A (Sigma, A8674). Reagents or vehicle control were injected in the appropriate volume of a tenfold concentrated stock solution to give the following final in-well concentrations: 2 µM Oligomycin, 2 µM FCCP, 0.5 µM Rotenone/Antimycin A. Prior to experiments, reagent concentrations and seeding density were optimised as per the manufacturers instructions. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were calculated by the WAVE software (Seahorse Bioscience, Agilent Technologies). The basal respiration parameters were calculated from the mean of 3 individual OCR measurements as follows:

\[
\text{Basal Respiration} = \text{Initial OCR} \text{ (pmol/L)} - \text{Rotenone OCR} \text{ (pmol/L)}
\]

For OCR and ECAR response to injection of each compound, data was normalised by the basal respiration of each experimental group (shown as percentage of baseline) to account for well-to-well variability in cell number.

**Determination of ATP abundance**

Immortalised human preadipocytes ChubS7 were seeded onto gelatin-coated 96-well white opaque cell culture plates at a density of 10,000 cells/well. Cells were grown and differentiated according to the standard protocol outlined above, and treated with or without LPS (10 or 100 ng/mL, *E. Coli* O55:B5, Sigma, L6529) for 24 or 72 hours. Bioluminescent determination of ATP abundance was conducted using EnzyLight™ ATP Assay Kit (BioAssay Systems, EATP-100) following the manufacturers instructions for adherent cells. Luminescence was read using a
PheraStar FS microplate reader (BMG Labtech), and ATP concentrations determined based on interpolation of a standard curve of known ATP concentrations ranging from 0 – 30 µM.

**Determination of mitochondrial membrane potential**

The dye tetramethylrhodamine ethyl ester perchlorate (TMRE, Sigma, 87917) was used to determine mitochondrial membrane potential. TMRE is a cell-permeant, positive-charged red/orange dye that is readily sequestered by polarised mitochondria (due to their negative charge) in a manner that is directly proportional to their membrane potential. Immortalised human preadipocytes ChubS7 were seeded onto a black opaque 96-well plate, differentiated and treated as described for the ATP abundance assay. Adherent cells were incubated with 300 nM TMRE (Sigma) diluted in basal media (described in Table 2.5) for 30 min at 37ºC. Cells were then washed 3 times with warm PBS before reading fluorescence with a PheraStar FS microplate reader (BMG Labtech) at 550/590nM excitation/emission immediately and after 10, 20 and 30 minutes. As a positive control for depolarisation, 30 µM FCCP was added to some cells for 30 minutes, prior to the TMRE incubation step. FCCP is an ionophore which destroys membrane potential, rendering mitochondria unable to accumulate TMRE. For background correction, cells with no TMRE and no FCCP added were used. Membrane potential was calculated using the relative
fluorescence signal of samples based on the following formula:

\[ Membrane\ Potential\ (\%) = \left( \frac{(LPS\ sample - \text{background})}{(control\ sample - \text{background})} \right) \times 100 \]

**Endogenous antioxidant activity assays**

Activity of endogenous antioxidants SOD and Catalase was evaluated through a colorimetric method, using OxiSelect\textsuperscript{TM} Superoxide Dismutase Activity Assay (STA-340) and OxiSelect\textsuperscript{TM} Catalase Activity Assay (STA-341) Kits (Cell Biosciences). Following adipocyte differentiation and treatment, \( \sim 1.2 \times 10^6 \) adherent cells were washed 3 times with ice-cold PBS, harvested with a cell scraper in 1 mL of cold Lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA). Samples were homogenised, centrifuged and stored at -80°C until assayed. All assays were conducted within 1 month of sample collection and were conducted in accordance to manufacturers instructions. Absorbance was read using a PheraStar FS microplate reader (BMG Labtech). SOD activity was calculated based on optical density as outlined in the following formula:

\[ SOD\ activity\ (inhibition\ %) = \left( \frac{(Blank_{OD} - Sample_{OD})}{Blank_{OD}} \right) \]

The concentration of active Catalase was determined by interpolation of a catalase standard curve. Optical density at 540nm was plotted on the "x" axis, Catalase (U/mL) on the "y" axis, and a second order polynomial equation was used to determine catalase concentrations as follows:
Catalase (U/mL) = 174.01x^2 + 13.678x + 2.7743 where \(x = \text{optical density} \ (540 \text{ nm})\)

Quantification of total reactive oxygen and nitrogen species

Total reactive oxygen and nitrogen species were evaluated through green fluorescence using OxiSelect\textsuperscript{TM} in vitro ROS/RNS Assay Kit (STA-347, Cell Biolabs). Following adipocyte differentiation and treatment, \(\sim 1.2 \times 10^6\) adherent cells were washed 3 times with ice-cold PBS, flash-frozen in dry-ice and harvested using a cell-scaper. Samples were homogenised in 200\(\mu\)L PBS and stored at -80\(^\circ\)C and assayed within 1 month of collection. Assay was conducted according to manufacturers instructions, and fluorescence after 30 minutes was measured using a PheraStar FS microplate reader (BMG Labtech) with a 485/538 nm filter and 530 nm cutoff. Total ROS/RNS were calculated by interpolation of a Hydrogen Peroxide standard curve as follows:

\[
\text{ROS/RNS} \ (\mu\text{M}) = 5354.5x^2 + 1043.3x + 50.496
\]

where \(x = \text{relative fluorescence units} \ (485/538)\).
2-deoxyglucose uptake

Glucose uptake in differentiated ChubS7 adipocytes was evaluated via cellular incorporation of $[^{3}H]$-2-deoxyglucose. Following differentiation and treatment, cells were washed 3 times with warmed PBS and allowed to equilibrate in KRH buffer (containing 0.01 % BSA, 5 mmol/L glucose) at 37°C for 2.5 hours. Adipocytes were then incubated a further 30 minutes with KRH buffer without glucose and either no (basal control) or 100 µM insulin. Immediately after, 1 µCi/mL $[^{3}H]$-2-deoxyglucose (PerkinElmer, NET328A001MC) in KRH buffer at 37°C was added for a further 10 minutes. To finalise the assay, cells were then washed 3 times with ice-cold PBS, lysed and harvested in 200 µL RIPA buffer and a cell scraper. Radioactivity was evaluated via scintillation counting of the lysates, diluted 1:4 in β-scintillation fluid (Beta-Plate Scint, PerkinElmer), and a scintillation Counter. Results defined as counts per minute (CPM) were normalised to total protein content and glucose uptake displayed relative to the basal control.

Analysis of mitochondrial morphology through confocal microscopy

Analysis of the mitochondrial network was performed through live cell imaging using a confocal microscope. ChubS7 cells were seeded on to gelatin-coated 35mm glass bottom culture dishes (MatTek corporation®), grown, differentiated and treated for 24 hours as described above. Upon completion of treatment, basal media was removed, replaced with 100 nM Mitotracker Red and cells incubated for 20 minutes at 37°C. Cells were then washed three times with basal media and
imaged in HEPES-buffered basal media (pH 7.35) with or without LPS (10 or 100 ng/mL, *E. Coli* O55:B5, Sigma, L6529). The Z system attached to an inverted florescence microscope fitted with an F-view-II cooled CCD camera (Olympus) was used to observe cells which were maintained at 37°C throughout the imaging process. Cells were magnified 40x through an oil objective lens, and a 500 nm excitation filter was used to image the mitochondrial network.

Morphologic assessment of the mitochondrial network was conducted on confocal images using ImageJ (version 1.50i) as described by others [270]. The mitochondrial parameters assessed were mitochondrial area (µm²) and the degree of branching, as defined by the following equation:

\[ BF = \frac{MtP^2}{4\pi MtA} \]

where \( MtP \) = length of mitochondrial outline (mitochondrial perimeter in µm) and \( MtA \) = Area of mitochondria (µm²). Four independent experiments were conducted and a minimum of 70 images were examined for each experimental group.

**Statistical Analysis**

Statistical analyses were performed using the SPSS 21.0 software. Data are reported as mean ± standard deviation (SD), unless otherwise specified. Data were examined for normality according to the Shapiro-Wilks criteria. Comparisons between pre- and post-surgery time-points were performed via paired two-tailed t-tests (if parametric) and the Wilcoxon signed ranks test (if non-parametric). For
categorical data, Fisher’s exact test was used. Between-group (surgery type) differences were assessed using One-way ANOVA (if parametric) and Kruskal-Wallis test (if non-parametric) using change variables, calculated as percentage change from pre-surgery values $[(\text{post}/\text{pre}) \times 100]$. For Pearson correlation analyses, change variables $[(\text{post}/\text{pre}) \times 100]$ were log-transformed prior to analysis if non-parametric.
Chapter 3

Differential Effect of Bariatric Surgical Procedure on Metabolic Outcomes and Adipose Tissue Mitochondria
3.1 Introduction

Bariatric surgery is currently considered the most effective treatment for severe obesity, and is associated with substantial and sustained weight loss, coupled with long-term T2DM remission in the majority of cases [271, 174]. However, the type of bariatric surgical intervention also appears to be an important factor affecting the degree and spectrum of improvements in metabolic parameters [174]. Post-surgical excess weight loss ranges in degree from 15-25% after laparoscopic adjustable gastric banding (LAGB), to 30-40% after bilio-pancreatic diversion (BPD), with the degree of weight loss achieved being the strongest predictor of type-2 diabetes (T2DM) remission [272, 273, 274, 275].

Also critical to metabolic recovery are mitochondria. Established metabolic regulators [276], mitochondria are key to the adipose tissue’s (AT) ability to maintain its metabolic and endocrine functionality in the face of constant energy oversupply [277]. Mitochondria exquisitely tailor changes in energy production capacity through structural and functional modifications (fission and fusion) dependent upon supply and demand. However, in cases of chronic nutrient excess, such as obesity, the mitochondrial network is forced to sustain these adaptations (fragmentation) for the longer term, leading to severely compromised mitochondrial DNA integrity, inefficiency and metabolic stress [278, 279]. In addition, this impairment in function can further lead to an accumulation of reactive oxygen species (ROS) [236], impaired oxygen consumption and β-oxidation [280, 281], enhanced lipotoxic species accumulation [282], pro-inflammatory cytokine production [283] and impaired insulin signaling [284, 285].

Recent studies examining mitochondria in human AT has shown that both obesity
and T2DM can lead to mitochondrial inefficiency with fewer and smaller mitochondria, and that this dysfunction is dependent upon substantial weight gain rather than adipocyte cell size [286, 287]. Beyond simple obesity, the insulin resistant state is also associated with mitochondrial dysfunction within AT [288, 289, 290], whilst studies also suggest that mitochondrial dysfunction in adipocytes itself may be causal in leading to impairment of insulin sensitivity [291, 292].

Nutrient overload, either through high-fat or high-glucose environments, has been strongly implicated in mitochondrial dysfunction, leading to increased oxidative stress and molecular mechanisms of metabolic dysfunction in AT [279, 293, 294, 295]. Therefore, interventions such as bariatric surgery that can induce substantial and sustained weight loss, may reverse the underlying cellular causes of insulin resistance by limiting over nutrition and reversing mitochondrial dysfunction in AT [271, 174, 272, 273, 274, 275].

Thus, the aims of this study were to: (1) determine whether bariatric surgery, the most effective treatment for morbid obesity to date [271, 174], can reverse mitochondrial maladaptation in AT from obese subjects with T2DM; (2) evaluate whether the type of bariatric surgery, i.e. with varying degrees of caloric restriction (as evidenced by weight loss), has a differential effect on the resulting mitochondrial health; and (3) understand which, if any, clinical or biochemical post-operative factors may be associated with a reversal in mitochondrial maladaptation.
3.2 Methods

Ethics and Study Design

All study participants provided written and informed consent in accordance with the Declaration of Helsinki. Thirty-nine morbidly obese ($BMI > 35 \text{ Kg/m}^2$), type-2 diabetic, Caucasian women undergoing either bili-pancreatic diversion (BPD; $n=12$), laparoscopic greater curvature plication (LGCP; $n=15$), or laparoscopic adjustable gastric banding (LAGB; $n=12$) at the OB clinic, Prague, Czech Republic were recruited to participate in this study. Thorough biochemical and anthropometric investigations were conducted before (baseline) and six months after surgery with collection of serum samples and abdominal subcutaneous white adipose tissue (AT) biopsies at both of these time points. Patients on pharmacological treatment with incretin mimetics and/or insulin were not included in this study. The reader is referred to Chapter 2 Materials and Methods for greater detail.

Blood Biochemistry and Anthropometry

Anthropometric and biochemical measurements were performed before and six months after surgery, following a 10-hour overnight fast, from venous blood samples. Serum glucose, HbA1c and lipids were determined through biochemical analyses (see Chapter 2). Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) [34]. The Friedwald formula [35] was employed to compute serum levels of LDL cholesterol. Body weight was measured to the nearest 0.5 Kg and height to the nearest 1 cm. Percentage excess weight loss was calculated (see Chapter 2 for equation), and body fat mass was measured
using the bioimpedance method (Tanita TBF-300; Tanita corporation).

**RNA isolation and qRT-PCR**

Gene expression from subcutaneous white adipose tissue biopsies before and 6 months after each bariatric surgery was determined via qRT-PCR. RNA isolation and qRT-PCR methods are detailed in Chapter 2. For detail on primer sequences of mitochondrial genes used in this study the reader is referred to Table 2.1.

**Mitochondrial DNA copy number assay**

Mitochondrial number was determined via analysis of relative amounts of mitochondrial to nuclear DNA [266] in subcutaneous white adipose tissue biopsies before and 6 months after each bariatric surgery. For in depth detail on this method, the reader is referred to Chapter 2.

**Statistical Analysis**

Statistical analyses were performed using the SPSS 21.0 software. Data are reported as mean ± standard deviation (SD), unless otherwise specified. Data were examined for normality according to the Shapiro-Wilks criteria. Comparisons between pre- and post-surgery time-points were performed via paired two-tailed t-tests (if parametric) and the Wilcoxon signed ranks test (if non-parametric). For categorical data, Fisher’s exact test was used. Between-group (surgery type) differences were assessed using One-way ANOVA (if parametric) and Kruskal-Wallis test (if non-parametric) using change variables, calculated as percentage change from pre-surgery values [(post/pre) x100]. For Pearson correlation analyses, change
variables [(post/pre) x100] were log-transformed prior to analysis if non-parametric.
3.3 Results

Differential Effect of Bariatric Procedure on Metabolic Health

The most notable difference between surgical procedures was weight loss. Though all surgical procedures resulted in significant reductions of BMI compared with pre-surgical measurements (Figure 3.1A), the BPD procedure produced significantly greater weight reduction ($p=0.004$), by a factor of approximately 30-50% on top of the other two LGCP and LAGB procedures (Figure 3.1B).

![Figure 3.1: Differential effect of surgical procedure on weight loss](image)

*(A) BMI before and 6 months after three distinct bariatric procedures: Bilio-pancreatic diversion (BPD), Laparoscopic Greater Curvature Plication (LGCP) and Laparoscopic Adjustable Gastric Banding (LAGB). Bars represent means ± standard error of the mean, with individual data points shown as dots to illustrate distribution. (B) Percent of the individual’s excess weight lost at 6 months post BPD, LGCP or LAGB. Pre-to-post differences were calculated using a 2-tail paired t-test, whilst one way ANOVA was used to compare change between surgeries. *$p<0.05$, **$p<0.01$.)*
Figure 3.2: Differential effect of surgical procedure on HbA1c

(A) Serum HbA1c before and 6 months after three distinct bariatric procedures: Bilio-pancreatic diversion (BPD), Laparoscopic Greater Curvature Plication (LGCP) and Laparoscopic Adjustable Gastric Banding (LAGB). Data is expressed as percentage of pre-surgical levels. Bars represent means ± standard error of the mean. (B) Serum HbA1c before and after BPD, LGCP and LAGB, showing weighted average once BMI is accounted for through ANCOVA analysis. One way ANOVA or ANCOVA was used to compare change between surgeries. *p<0.05, **p<0.01.

The type of surgical procedure also had a significant impact on HbA1c reduction. These data is shown in Figure 3.2, as percentage of pre-surgical values. Despite similar levels of HbA1c at baseline between surgeries, the BPD procedure resulted in significantly lower circulating levels of HbA1c (a marker of long-term diabetic control), compared with LGCP (p=0.022) and LAGB (p=0.002) (Figure 3.2A). To clarify whether this effect could be explained by the greater weight loss shown by the BPD cohort, the analysis was repeated using ANCOVA and BMI as a covariate. As shown in Figure 3.2B, BPD resulted in superior HbA1c reductions, despite controlling for BMI. Indeed, further linear regression analysis revealed that intersurgical differences in excess weight loss could account only for 30% of divergence in HbA1c, leaving the remaining 70% as the result of other, unknown factors (Table 3.1).
Table 3.1: Contribution of weight loss to surgery-induced improvements in serum HbA1c

<table>
<thead>
<tr>
<th>Excess weight loss</th>
<th>R square change</th>
<th>B Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.304</td>
<td>-0.776</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

A linear regression analysis was performed using surgery-induced changes in serum HbA1c as the dependent variable, and excess weight loss as independent, to determine the contribution of weight loss to serum HbA1c improvements. No other variables were entered into the model.

In addition to improved diabetic control, BPD also resulted in significantly lower lipid levels. As shown in Figure 3.3A, total cholesterol was significantly reduced in patients who underwent BPD, but not in those who underwent LGCP, or LAGB. Subsequent ANCOVA analysis revealed that this surgery-specific difference remained unchanged after accounting for differences in BMI (Figure 3.3B). Indeed, further linear regression analysis revealed that inter-surgical differences in excess weight loss could account only for 12% of divergence in serum total cholesterol, leaving the remaining 88% as the result of other, unknown factors (Table 3.2).

Similar results were observed in other lipids, where improvements in LDL cholesterol (29%, p=0.001) and in HDL/LDL ratio appeared greater with BPD (15% increase from pre-surgery, p=0.154) than with LGCP and LAGB procedures (2% and 4%, respectively). The aforementioned findings and all additional anthropometric, biochemical and clinical data obtained before and 6 months after bili-pancreatic diversion (BPD; n= 12), laparoscopic greater curvature plication (LGCP; n=15) or laparoscopic adjustable gastric banding (LAGB; n=12) operations can be found in Table 3.3, to highlight comparative differences between the 3 surgeries.
Table 3.2: Contribution of weight loss to surgery-induced improvements in total cholesterol

<table>
<thead>
<tr>
<th>Excess weight loss</th>
<th>R square change</th>
<th>B Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.127</td>
<td>-0.535</td>
<td>0.019</td>
<td></td>
</tr>
</tbody>
</table>

A linear regression analysis was performed using surgery-induced changes in serum total cholesterol as the dependent variable, and excess weight loss as independent, to determine the contribution of weight loss to serum total cholesterol improvements. No other variables were entered into the model.

**Differential Effect of Bariatric Procedure on Indicators of Adipose Mitochondrial Function**

Next, the impact of the different bariatric procedures on mitochondrial function in adipose tissue was examined. Transcript levels of genes associated with a range of mitochondrial functions (biogenesis, oxidative phosphorylation, uncoupling and antioxidant capacity) and dynamics (fission and fusion) were examined in adipose biopsies through qRT-PCR. Gene expression levels pre-surgery for each gene category are shown in Figures 3.4 to 3.7. Overall, transcript levels for genes involved
Table 3.3: Anthropometric, biochemical and clinical variables pre-surgery and 6-months post BPD, LGCP, and LAGB bariatric procedures

<table>
<thead>
<tr>
<th></th>
<th>BPD</th>
<th></th>
<th>LGCP</th>
<th></th>
<th>LAGB</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>12</td>
<td></td>
<td>15</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td>50.5 ± 5.8</td>
<td></td>
<td>55 ± 3.5</td>
<td></td>
<td>30.7 ± 8.4†</td>
</tr>
<tr>
<td>EWL (%)</td>
<td></td>
<td>30.7 ± 8.4†</td>
<td></td>
<td>17.4 ± 6.3</td>
<td></td>
<td>20.2 ± 9.4†</td>
</tr>
<tr>
<td>Pre</td>
<td>Post</td>
<td>△(%)</td>
<td>Pre</td>
<td>Post</td>
<td>△(%)</td>
<td>Pre</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>127.7 ± 22.6</td>
<td>106.9 ± 18.5**</td>
<td>83.8 ± 3.0†</td>
<td>109.2 ± 16.5</td>
<td>97.6 ± 14.1**</td>
<td>89.5 ± 3.2</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>46.45 ± 7.75</td>
<td>39.01 ± 6.37**</td>
<td>84.1 ± 3.5†</td>
<td>40.41 ± 5.54</td>
<td>36.05 ± 4.62**</td>
<td>89.3 ± 3.4</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93 ± 0.07</td>
<td>0.90 ± 0.07</td>
<td>97.7 ± 9.9</td>
<td>0.88 ± 0.10</td>
<td>0.87 ± 0.08</td>
<td>100.1 ± 11.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>49.59 ± 3.70</td>
<td>44.68 ± 4.62**</td>
<td>90.2 ± 6.6</td>
<td>48.49 ± 3.78</td>
<td>44.90 ± 3.71**</td>
<td>92.7 ± 4.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.40 ± 2.57</td>
<td>7.01 ± 1.73</td>
<td>89.1 ± 30.1</td>
<td>8.93 ± 2.11</td>
<td>7.34 ± 1.64*</td>
<td>84.4 ± 16.9</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>31.48 ± 21.2</td>
<td>17.12 ± 12.0*</td>
<td>71.2 ± 73.8</td>
<td>26.43 ± 19.4</td>
<td>16.76 ± 10.9*</td>
<td>70.6 ± 24.7</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>11.8 ± 9.74</td>
<td>5.3 ± 4.73*</td>
<td>66.1 ± 97.2</td>
<td>10.9 ± 9.14</td>
<td>5.32 ± 3.34**</td>
<td>61.0 ± 29.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.1 ± 0.9</td>
<td>5.7 ± 0.70**</td>
<td>72.1 ± 15.1††</td>
<td>7.3 ± 1.0</td>
<td>6.5 ± 0.9**</td>
<td>85.1 ± 8.5</td>
</tr>
<tr>
<td>HbA1c (mmol/L)</td>
<td>53.84 ± 9.6</td>
<td>38.38 ± 7.8**</td>
<td>72.1 ± 15.1††</td>
<td>56.33 ± 10.5</td>
<td>47.93 ± 10.3**</td>
<td>85.1 ± 8.5</td>
</tr>
<tr>
<td>T Chol (mmol/L)</td>
<td>4.91 ± 1.03</td>
<td>3.72 ± 0.86††</td>
<td>75.9 ± 9.80†</td>
<td>4.84 ± 0.77</td>
<td>4.73 ± 0.82</td>
<td>97.8 ± 8.7</td>
</tr>
<tr>
<td>TGL (mmol/L)</td>
<td>1.43 ± 0.66</td>
<td>1.59 ± 0.69</td>
<td>117.6 ± 51.2</td>
<td>1.95 ± 1.39</td>
<td>1.38 ± 0.71</td>
<td>89.2 ± 33.0</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.05 ± 0.21</td>
<td>0.81 ± 0.22**</td>
<td>73.1 ± 22.0††</td>
<td>1.12 ± 0.33</td>
<td>1.15 ± 0.31</td>
<td>104.0 ± 15.1</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.19 ± 1.03</td>
<td>2.23 ± 0.67**</td>
<td>71.4 ± 13.2††</td>
<td>2.82 ± 0.69</td>
<td>2.94 ± 0.77</td>
<td>107.2 ± 25.6</td>
</tr>
<tr>
<td>HDL/LDL ratio</td>
<td>0.36 ± 0.14</td>
<td>0.40 ± 0.12</td>
<td>115.3 ± 28.6††</td>
<td>0.42 ± 0.17</td>
<td>0.42 ± 0.18</td>
<td>102.0 ± 29.0</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Within group statistical significance (pre to post-surgery) was determined using two-tailed paired t-test or Wilcoxon signed ranks test (*p<0.05; **p<0.01), whilst for between group comparisons one-way ANOVA or the Kruskal-Wallis test were used (†p<0.05; ††p<0.01). Change column (△%) denotes post-surgery values as percentage of pre-surgery values. EWL: excess weight loss, BMI: body mass index, WHR: waist-hip ratio, HOMA IR: Homeostatic assessment model of insulin resistance, HbA1c: glycosilated haemoglobin, T Chol: total cholesterol, TGL: triglycerides, HDL: High-density Lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, BPD: bilio-pancreatic diversion, LGCP: laparoscopic greater curvature plication, LAGB: laparoscopic adjustable gastric banding. All serum determinations correspond to fasting status.
in mitochondrial biogenesis (Figure 3.4), oxidative phosphorylation (Figure 3.5), reactive oxygen species clearance (Figure 3.6), and dynamics (Figure 3.7) did not vary significantly prior to surgical intervention between the three surgical cohorts (BPD, LGCP, or LAGB).

Figure 3.4: Pre-surgery comparison of genes involved in mitochondrial biogenesis
Pre-surgery adipose tissue mRNA levels of genes involved in mitochondrial biogenesis for each surgical cohort due to undergo either Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as $1/\Delta\text{Ct}$. Bars represent means ± standard error of the mean. One way ANOVA was used to determine differences between surgeries. N.S. denotes differences shown were not statistically significant ($p>0.05$). PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; POLG: mitochondrial DNA polymerase; TFAM: mitochondrial transcription factor A.
Figure 3.5: Pre-surgery comparison of genes involved in oxidative phosphorylation

Pre-surgery adipose tissue mRNA levels of genes involved in Oxidative Phosphorylation for each surgical cohort due to undergo either Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as $1/\Delta Ct$. Bars represent means ± standard error of the mean. One way ANOVA was used to determine differences between surgeries. N.S. denotes differences shown were not statistically significant ($p>0.05$). mtND6: mitochondrially-encoded NADH dehydrogenase 6; SDHA: Succinate Dehydrogenase Complex Flavoprotein Subunit A; COX4I1: Cytochrome c oxidase subunit 4 isoform 1; mtATP6: Mitochondrially-encoded ATP Synthase 6.
Figure 3.6: Pre-surgery comparison of genes involved in reactive oxygen species clearance

Pre-surgery adipose tissue mRNA levels of genes involved in reactive oxygen species clearance (A, uncoupling and B, endogenous antioxidant action) for each surgical cohort due to undergo either Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as $1^{\Delta Ct}$. Bars represent means ± standard error of the mean. One way ANOVA was used to determine differences between surgeries. N.S. denotes differences shown were not statistically significant ($p>0.05$). UCP2: Uncoupling protein 2; SOD1: Superoxide dismutase 1; SOD2: Superoxide dismutase 2.
Figure 3.7: Pre-surgery comparison of genes involved in mitochondrial dynamics

Pre-surgery adipose tissue mRNA levels of genes involved in mitochondrial dynamics (A, fission and B, fusion) for each surgical cohort due to undergo either Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as $1/\Delta\text{Ct}$. Bars represent means ± standard error of the mean. One way ANOVA was used to determine differences between surgeries. N.S. denotes differences shown were not statistically significant (p>0.05). MFN2: Mitofusin 2; OPA1: mitochondrial dynamin like GTPase; DRP1: Dynamin-1-like protein; FIS1: Mitochondrial fission 1 protein.

Gene expression levels post-surgery are shown in Figure 3.8 as fold-change of pre-surgery values. In general, mitochondrial gene mRNA levels were higher after the BPD procedure than the LGCP or LAGB operations. This was particularly
evident with oxidative phosphorylation (SDHA, COX4I1 and mtATP6), reactive oxygen species clearance (UCP2 and SOD2) and fusion (OPA1) genes, and would suggest a surgery-specific difference in bioenergetic efficiency, antioxidant capacity and mitochondrial quality control.
Figure 3.8: Effect of bariatric procedure on mitochondrial gene expression in adipose biopsies

Surgery-induced changes in adipose tissue mRNA expression of genes involved in mitochondrial biogenesis (A), oxidative phosphorylation (B), reactive oxygen species clearance (C), and dynamics (D) for each surgical procedure: BPD, LGCP or LAGB. Data is expressed as fold-change of pre-surgical values (shown as dotted line). Bars represent means ± standard error of the mean. Pre-to-post surgical differences were determined via 2-tailed paired t-test (*p<0.05, **p<0.01). One way ANOVA was used to determine differences between surgeries (†p<0.05).
The effect of each surgery on mitochondrial number in adipose biopsies was also analyzed by comparing mitochondrial DNA relative to gDNA levels (Figure 3.9). Mitochondrial number did not differ significantly between cohorts prior to surgery (Figure 3.9A), however surgery-specific effects on mitochondrial number were observed post-surgery (Figure 3.9B). Whilst the LGCP and LAGB procedures seemingly lowered the number of mitochondrial DNA copies in adipose biopsies, no such effect was observed with the BPD procedure.

![Figure 3.9: Effect of bariatric procedure on mitochondrial number in adipose biopsies](image)

*Figure 3.9: Effect of bariatric procedure on mitochondrial number in adipose biopsies

Surgery-induced changes in adipose tissue mitochondrial DNA copy number before (A), and after (B) each surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as $1/\Delta Ct$ of mitochondrial target relative to nuclear for A, and as percentage change of pre-surgical values (shown as dotted line) for B. Bars represent means ± standard error of the mean. Pre-to-post surgical differences were determined via 2-tailed paired t-test (*p<0.05, **p<0.01). One way ANOVA was used to determine differences between surgeries (†p<0.05). N.S. denotes differences shown were not statistically significant (p>0.05).

In order to further examine the overall impact of each surgery on adipose mitochondrial functionality, surgery-induced changes in genes involved in mitochondrial function (biogenesis, oxidative phosphorylation, uncoupling, and antioxidant capacity) and dynamics (fission and fusion) were compared to the changes observed in mitochondrial number using Pearson correlation analyses. In genes controlling function, these relationships were significantly positive after BPD surgery across 9
of 10 genes assessed, whilst significantly negative for 7 genes after LGCP surgery, and absent for all genes after the LAGB procedure (Table 3.4). Analysis of mitochondrial dynamics genes revealed significant correlations in genes involved in both fusion and fission processes within the BPD cohort. These relationships were absent in the LGCP group and present only for fusion genes in the LAGB group, indicating that the control of mitochondrial function and dynamics differed with the type of surgical procedure.

Table 3.4: Relationship of mitochondrial number to mitochondrial function and dynamics genes after BPD, LGCP and LAGB bariatric procedures

<table>
<thead>
<tr>
<th>Mitochondrial number vs.</th>
<th>BPD (n = 12)</th>
<th>LGCP (n = 15)</th>
<th>LAGBP (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGC1α</td>
<td>0.794**</td>
<td>-0.688**</td>
<td>-0.175</td>
</tr>
<tr>
<td>POLG</td>
<td>0.867**</td>
<td>-0.407</td>
<td>0.035</td>
</tr>
<tr>
<td>TFAM</td>
<td>0.479</td>
<td>-0.560*</td>
<td>-0.154</td>
</tr>
<tr>
<td>mtND6</td>
<td>0.758*</td>
<td>-0.613*</td>
<td>-0.153</td>
</tr>
<tr>
<td>SDHA</td>
<td>0.855**</td>
<td>-0.600*</td>
<td>-0.056</td>
</tr>
<tr>
<td>COX4I1</td>
<td>0.939**</td>
<td>-0.442</td>
<td>0.147</td>
</tr>
<tr>
<td>mtATP6</td>
<td>0.782**</td>
<td>-0.547*</td>
<td>0.056</td>
</tr>
<tr>
<td>UCP2</td>
<td>0.818**</td>
<td>-0.389</td>
<td>0.063</td>
</tr>
<tr>
<td>SOD1</td>
<td>0.842**</td>
<td>-0.604*</td>
<td>0.098</td>
</tr>
<tr>
<td>SOD2</td>
<td>0.696*</td>
<td>-0.576*</td>
<td>-0.017</td>
</tr>
<tr>
<td>Dynamics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFN2</td>
<td>0.983**</td>
<td>-0.493</td>
<td>0.939*</td>
</tr>
<tr>
<td>OPA1</td>
<td>0.808*</td>
<td>-0.202</td>
<td>0.963*</td>
</tr>
<tr>
<td>DRP1</td>
<td>0.302</td>
<td>-0.426</td>
<td>0.669</td>
</tr>
<tr>
<td>FIS1</td>
<td>0.871*</td>
<td>-0.337</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient between mitochondrial number and genes involved in mitochondrial biogenesis (PGC1α, POLG, TFAM), oxidative phosphorylation (mtND6, SDHA, COX4I1, mtATP6), uncoupling (UCP2), antioxidant function (SOD1, SOD2), fusion (MFN2, OPA1) and fission (DRP1, FIS1) processes. Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Negative correlations are shown in red. *p<0.05, **p<0.01. BPD: biliopancreatic diversion, LGCP: laparoscopic greater curvature plication, LAGB: laparoscopic adjustable gastric banding. PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, POLG: mitochondrial DNA polymerase gamma catalytic subunit, TFAM: mitochondrial transcription factor A, mtND6: mitochondrially-encoded NADH dehydrogenase 6, SDHA: Succinate dehydrogenase complex subunit A, COX4I1: Cytochrome c oxidase subunit 4 isoform 1 (complex IV), mtATP6: mitochondria-DNA-encoded ATP synthase subunit 6 (complex V), UCP2: uncoupling protein 2, SOD1: Superoxide dismutase 1, SOD2: Superoxide dismutase 2, MFN2: Mitofusin 2, OPA1: mitochondrial dynamin like GTPase; DRP1: Dynamin-1-like protein; FIS1: Mitochondrial fission 1 protein.
Mapping Surgery-Specific Differences in Mitochondrial Functionality to Clinical Indicators of Metabolic Health

In order to further understand the factors contributing to the surgery-specific mitochondrial differences observed, their relationship with the metabolic variables that varied the most between surgical procedures were examined, including: excess weight loss (EWL), body mass index (BMI), serum HbA1c and serum total cholesterol (Table 3.5). Most of these analyses returned non-significant relationships however, total serum cholesterol was identified to have a significant association across all surgeries with two mitochondrial genes: mitochondrially-encoded ATP synthase 6 (mtATP6) and uncoupling protein 2 (UCP2). Shown as scatterplots in Figure 3.10, these relationships show that higher total cholesterol in serum was associated with lower expression of both mtATP6 and UCP2 genes, and that the BPD procedure generally had lower cholesterol with higher mitochondrial gene expression than the LGCP and LAGB procedures. These findings are consistent with the notion that greater lipid toxicity may be the cause of surgery-specific differences observed in mitochondrial functionality.
Table 3.5: Relationship of mitochondrial variables with clinical indicators of metabolic health

<table>
<thead>
<tr>
<th>Function</th>
<th>mt number</th>
<th>EWL r</th>
<th>EWL p</th>
<th>BMI r</th>
<th>BMI p</th>
<th>HbA1c r</th>
<th>HbA1c p</th>
<th>Total Cholesterol r</th>
<th>Total Cholesterol p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC1α</td>
<td>0.083</td>
<td>0.269</td>
<td>0.078</td>
<td>0.464</td>
<td>0.039</td>
<td>0.525</td>
<td>0.120</td>
<td>0.486</td>
<td></td>
</tr>
<tr>
<td>POLG</td>
<td>0.072</td>
<td>0.647</td>
<td>0.061</td>
<td>0.697</td>
<td>0.002</td>
<td>0.990</td>
<td>0.118</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td>TFAM</td>
<td>0.013</td>
<td>0.936</td>
<td>0.010</td>
<td>0.948</td>
<td>0.027</td>
<td>0.863</td>
<td>0.138</td>
<td>0.376</td>
<td></td>
</tr>
<tr>
<td>mtND6</td>
<td>0.027</td>
<td>0.867</td>
<td>0.024</td>
<td>0.880</td>
<td>0.002</td>
<td>0.989</td>
<td>0.189</td>
<td>0.371</td>
<td></td>
</tr>
<tr>
<td>SDHA</td>
<td>0.097</td>
<td>0.535</td>
<td>0.126</td>
<td>0.422</td>
<td>0.117</td>
<td>0.462</td>
<td>0.089</td>
<td>0.571</td>
<td></td>
</tr>
<tr>
<td>COX4I1</td>
<td>0.121</td>
<td>0.441</td>
<td>0.157</td>
<td>0.315</td>
<td>0.071</td>
<td>0.655</td>
<td>0.181</td>
<td>0.246</td>
<td></td>
</tr>
<tr>
<td>mtATP6</td>
<td>0.129</td>
<td>0.408</td>
<td>0.203</td>
<td>0.192</td>
<td>0.151</td>
<td>0.340</td>
<td>0.318</td>
<td>0.038*</td>
<td></td>
</tr>
<tr>
<td>UCP2</td>
<td>0.162</td>
<td>0.298</td>
<td>0.200</td>
<td>0.198</td>
<td>0.015</td>
<td>0.927</td>
<td>0.343</td>
<td>0.024*</td>
<td></td>
</tr>
<tr>
<td>SOD1</td>
<td>0.118</td>
<td>0.450</td>
<td>0.067</td>
<td>0.668</td>
<td>0.112</td>
<td>0.478</td>
<td>0.018</td>
<td>0.909</td>
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</tr>
<tr>
<td>SOD2</td>
<td>0.131</td>
<td>0.404</td>
<td>0.074</td>
<td>0.264</td>
<td>0.055</td>
<td>0.730</td>
<td>0.259</td>
<td>0.093</td>
<td></td>
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<tr>
<td>Dynamics</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFN2</td>
<td>0.005</td>
<td>0.980</td>
<td>0.012</td>
<td>0.950</td>
<td>0.163</td>
<td>0.407</td>
<td>0.085</td>
<td>0.662</td>
<td></td>
</tr>
<tr>
<td>OPA1</td>
<td>0.042</td>
<td>0.831</td>
<td>0.067</td>
<td>0.731</td>
<td>0.220</td>
<td>0.260</td>
<td>0.198</td>
<td>0.303</td>
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<tr>
<td>DRP1</td>
<td>0.138</td>
<td>0.483</td>
<td>0.205</td>
<td>0.295</td>
<td>0.005</td>
<td>0.980</td>
<td>0.192</td>
<td>0.327</td>
<td></td>
</tr>
<tr>
<td>FIS1</td>
<td>0.124</td>
<td>0.523</td>
<td>0.153</td>
<td>0.429</td>
<td>0.016</td>
<td>0.935</td>
<td>0.067</td>
<td>0.728</td>
<td></td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient (r) and p value significance (p) between metabolic and mitochondrial variables in the entire patient cohort (n=39). Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Significant correlations are shown in red. *p<0.05. EWL: excess weight loss, BMI: body mass index, HbA1c: serum glycated haemoglobin, PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, POLG: mitochondrial DNA polymerase gamma catalytic subunit, TFAM: mitochondrial transcription factor A, mtND6: mitochondrially-encoded NADH dehydrogenase 6, SDHA: Succinate dehydrogenase complex subunit A, COX4I1: Cytochrome c oxidase subunit 4 isoform 1 (complex IV), mtATP6: mitochondria-DNA-encoded ATP synthase subunit 6 (complex V), UCP2: uncoupling protein 2, SOD1: superoxide dismutase 1, SOD2: Superoxide dismutase 2, MFN2: Mitofusin 2, OPA1: mitochondrial dynamin like GTPase, DRP1: Dynamin-1-like protein; FIS1: Mitochondrial fission 1 protein.
Figure 3.10: Association of total serum cholesterol with adipose mitochondrial genes

Scatter plots of total serum cholesterol correlated against mitochondrial genes mtATP6 (oxidative phosphorylation) and UCP2 (reactive oxygen species clearance). Correlations were calculated using change variables (percentage of pre levels) in the entire patient cohort (n=39), but individual data points are color-coded according to surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Linear trend line is also shown with Pearson correlation statistic (r) and significance (p).
3.4 Discussion

The present study hypothesized that caloric restriction (as evidenced by weight loss) through bariatric surgery would be accompanied by beneficial effects on adipose tissue mitochondria, supporting systemic metabolic recovery. To investigate this hypothesis, genes controlling a wide range of mitochondrial functions (biogenesis, oxidative phosphorylation, antioxidant, uncoupling, fusion and fission) were investigated. The BPD procedure lead to a tighter control of mitochondrial gene expression than LGCP or LAGB in association with greater weight, lipid and HbA1c reduction. These findings highlighted for the first time that (1) mitochondrial mal-adaptation may be reversed through bariatric intervention, dependent on (2) the type of procedure (BPD, LGCP and LAGB) which may influence (3) the degree of caloric and nutritional restriction. The BPD procedure which was the only procedure where mitochondrial recovery was evident, was also the surgery to result in the greater improvement of weight loss, HbA1c and dyslipideamia.

Whilst bariatric surgery per se, is currently the most effective treatment for severe obesity, associated with substantial and sustained weight loss, coupled with long-term T2DM remission in the majority of cases [271, 174], the type of bariatric surgical intervention given also appears to be an important factor affecting the degree and spectrum of improvements in metabolic parameters. In this present study, weight loss and thus HbA1c improvement rates were greater following the BPD procedure compared with LGCP and LAGB surgeries. These outcomes affirm previous research [174], reporting excess weight loss ranging from 15-25% excess weight loss after LAGB, to 30-40% after BPD/DS, where the highest mean weight loss achieved is also the strongest predictor of T2DM remission [272, 274, 275,
The finding that regulation of mitochondrial genes were significantly improved only in patients who also exhibited the greater metabolic improvements (as is the case of the BPD group), would seem to reinforce the concept that adipose mitochondria play an important role in systemic metabolic health. Following the BPD procedure alone, mitochondrial number was significantly and positively correlated with mRNA expression of most genes assayed, covering a range of mitochondrial (biogenesis, oxidative phosphorylation, uncoupling, antioxidant and quality control) functions, whilst after the LGCP and LAGB surgeries they appeared dysregulated. These mitochondrial differences may have severe functional implications for the adipose tissue at large. The genes MFN2, OPA1, DRP1 and FIS1 together allow the isolation and autophagic elimination of damaged mitochondria [29, 296], and are essential to maintaining mitochondrial quality and function [236]. Furthermore the long-term inhibition of DRP1 and OPA1, which may be induced through nutrient excess [278, 279], may also lead to accumulation of irreversibly damaged mitochondria [29], which may in turn lead to pro-inflammatory cytokine production [283] and impaired insulin signaling [284, 285].

Interestingly, despite similar HbA1c reduction rates between LGCP and BPD (once BMI is accounted for), mitochondrial recovery was evident in BPD alone, with the only measured differential factor being lipidaemic improvement in BPD, though not LGCP. One interpretation of this result would be that mitochondrial recovery is related to diet and specifically caloric restriction, which may ease mitochondrial stress caused by excess nutrient delivery and improve the lipid buffering capacity of adipose depots (evidenced by an improved lipid profile). In accord with this con-
cept, BPD is the only bariatric procedure shown to severely inhibit macronutrient absorption [176, 183], and thus induce the more severe caloric deficit (as evidenced by greater rates of weight loss). Similar mitochondrial benefits have also been reported through hypo-caloric dietary intervention, where some mitochondrial, glucose and lipid metabolism modifications occur after very and moderately low calorie diets [179, 297, 298]. Taken together these data suggest that the severity and duration of the caloric deficit may provide the basis to support mitochondrial recovery, which in human subjects may be better sustained through surgery rather than lifestyle interventions and ultimately lead to the better metabolic recovery.

This study has certain limitations, namely: (1) though subjects of this study did not follow a particular dietary regimen and led a relatively sedentary lifestyle in the period before surgery, these two factors were not controlled either before or after surgery; and (2) despite the prospective study design, it is not possible to clarify in the context of this study whether the observed mitochondrial improvements are the cause or consequence of metabolic recovery, or indeed whether other factors either dependent or independent of weight are at play. Thus, further research is required to clarify these points.

In summary, ensuring that mitochondria are able to cope with the demand of excess nutrients is critical for both functional adipose function and its lipid buffering capacity. Indeed, in conditions of chronic nutrient excess, such as obesity and T2DM, mitochondria become inefficient and dysfunctional. However, these findings suggest that nutrient-induced mitochondrial maladaptation may be reversed through certain bariatric procedures in association with degree of weight loss and dyslipidaemic improvement. Ultimately these data suggest that mitochondrial function
in adipose tissue is closely dependent on overall metabolic health and further un-
derstanding of its role during metabolic recovery of adipose tissue may be key in
reducing long-term damaging effects on peripheral metabolism.
Chapter 4

Role of Gut-hormone FGF-19 on Adipose Mitochondria Recovery Post Bariatric Surgery
4.1 Introduction

A key factor in the development of type-2 diabetes and metabolic syndrome is the inability of adipose tissue to cope with the chronic insult of over-nutrition, whilst maintaining important metabolic and endocrine functions [299, 150]. At the forefront of this challenging environment are mitochondria, major nutrient sensors and metabolic regulators, which are fundamental to adipose tissue function [276, 277]. However, during sustained conditions of chronic nutrient excess, such as obesity and type-2 diabetes, mitochondria appear unable to cope well with this environment, leading to fragmentation, unresponsiveness and dysfunction [287, 236, 280]. This nutrient-induced mitochondrial dysfunction can lead to impaired respiration, lipotoxicity, oxidative species accumulation and inflammation; further exacerbating insulin resistance and type-2 diabetes [281, 283, 284, 285]. Indeed, the importance of adequate mitochondrial function for metabolic health is further highlighted by the observation that mitochondrial DNA mutations often result in diabetic phenotypes [300, 301, 302].

Both insulin resistance and type-2 diabetes status can be reversed through bariatric surgery, with significantly greater success rates than pharmacological, exercise, and diet interventions [271, 273, 174]. Depending on the procedure, bariatric surgery involves a type/degree of gastro-intestinal remodeling, which can lead to reduced stomach volume and nutrient absorption capacity [303, 304]; however, this alone cannot fully explain the profound weight loss and metabolic improvement observed after these surgeries versus medical/lifestyle interventions [178].

Recently, the ileal-derived hormone, fibroblast growth factor 19 (FGF-19), has been identified as a novel enterokine regulator of glucose and lipid homeostasis which
is potentially involved in the metabolic recovery following bariatric surgery [305]. Indeed, rodent studies have shown that mice lacking the receptor required for gut secretion of FGF-19 show significantly impaired weight loss and glucose improvement following bariatric surgery compared with their wild-type counterparts [306]. In addition, direct administration of recombinant FGF-15 (FGF-19 in humans) to obese mice leads to significant weight reduction, principally AT reduction, and reverses dietary and leptin-deficient diabetes [307]. Moreover, in humans, data from clinical studies would seemingly indicate FGF-19 as a cause rather than consequence of type-2 diabetes improvement, given that neither lifestyle interventions nor intense medical management of type-2 diabetes appears to increase circulating FGF-19 levels, despite similar reductions in HbA1c to surgical procedures [308]. Furthermore, there is good clinical evidence that certain bariatric procedures increase serum FGF-19 levels [31, 309, 310]. As such, both human and rodent studies suggest that increased circulating FGF-19 levels may contribute to the underlying mechanisms of metabolic improvement following certain types of bariatric surgery.

Beyond the potential effects on white adipose tissue, studies have shown FGF-19 to exert several advantageous effects on various metabolic relevant organs [305]. In the central nervous system, FGF-19 has been associated with lowered brain-hedonistic responses, reduced food intake, improved glycaemic control and enhanced glucose effectiveness [311, 306]. Furthermore, in the liver, FGF-19 has been shown to increase energy expenditure and fatty acid oxidation through raised delivery of fatty acids to the mitochondria [312]. Additionally, in brown adipose tissue, elevated FGF-19 (either through genetic over-expression or systemic administration) can affect the metabolic rate and activity of this highly energy-consuming
tissue [307, 312]. These studies also highlight the importance of mitochondria as a target of FGF-19 [307, 311, 306, 312], although its role in white adipose tissue mitochondria, particularly within the context of type-2 diabetes, remains largely unknown. In Chapter 3, evidence was outlined that suggests that only some bariatric procedures (namely BPD) result in recovery of white adipose mitochondria from severely obese type-2 diabetic individuals. In this present chapter, the hypothesis is investigated that changes in serum FGF-19 levels after bariatric surgery influence this multifactorial recovery and thus impact overall metabolic recovery.
4.2 Methods

Ethics and Study Design

Thirty-nine morbidly obese (BMI > 35 Kg/m2), type-2 diabetic, Caucasian women
undergoing either bilio-pancreatic diversion (BPD; n=12), laparoscopic greater cur-
vature plication (LGCP; n=15), or laparoscopic adjustable gastric banding (LAGB;
n=12) were recruited to participate in this study. Fasted bloods and anthropo-
metric investigations were conducted before (baseline) and following surgery with
collection of serum samples and abdominal subcutaneous white adipose tissue (AT)
biopsies at both of these time points. Patients on pharmacological treatment with
incretin mimetics and/or insulin were not included in this study. For further detail
the reader is referred to Chapter 2.

Blood Biochemistry and Anthropometry

All anthropometric and biochemical measurements were performed before and six
months after surgery. For detail on blood, adipose tissue sample and other clinical
data collection, please see Chapter 2.

RNA isolation and qRT-PCR

Gene expression of mitochondrial genes was assayed through quantitative real-time
polymerase chain reaction (qRT-PCR) using subcutaneous white adipose tissue
biopsies before and 6 months after bariatric surgery. For detail on primer sequences
used in this study the reader is referred to Table 2.1. For further detail please see
Chapter 2.
Mitochondrial DNA copy number assay

Mitochondrial (mtND1) and nuclear (BECN1) gene primers (Table 2.2) were used via qRT-PCR to determine relative amounts of mitochondrial to nuclear DNA as a measure of mitochondrial number per adipocyte[266] within subcutaneous white adipose tissue biopsies. For further detail on methods, the reader is referred to Chapter 2.

FGF-19 Serum Levels

For measurement of serum FGF-19 levels (pg/mL), an enzyme-linked immunosorbent assay (ELISA) kit for FGF-19 (Quantikine ELISA, R&D Systems, Minneapolis, MN) was used. All measurements were performed in duplicate according to the manufacturers instructions. This assay has a detection range of 31-544 pg/mL and a coefficient of variation of 4.5% for intra-assay and 5.5 % inter-assay precision.

Statistical Analysis

Statistical analyses were performed using the SPSS 21.0 software. Data are reported as mean ± standard deviation (SD), unless otherwise specified. Data were examined for normality according to the Shapiro-Wilks criteria. Comparisons between pre- and post-surgery time-points were performed via paired two-tailed t-tests (if parametric) and the Wilcoxon signed ranks test (if non-parametric). For categorical data, Fisher’s exact test was used. Between-group (surgery type) differences were assessed using One-way ANOVA (if parametric) and Kruskal-Wallis test (if non-parametric) using change variables, calculated as percentage change from pre-surgery values [(post/pre) x100]. For Pearson correlation analyses, change
variables [(post/pre) x100] were log-transformed prior to analysis if non-parametric.
4.3 Results

Differential Effect of Bariatric Procedure on Circulating Levels of Gut Hormone FGF-19

Serum levels of gut-hormone FGF-19 were measured before and 6 months after each bariatric procedure, and descriptive statistics for this data are summarised in Table 4.1.

Table 4.1: Comparisons of surgery-induced changes in serum FGF-19 levels between BPD, LGCP and LAGB bariatric procedures

<table>
<thead>
<tr>
<th>Bariatric procedure (n)</th>
<th>Percent of patients with increase (%)</th>
<th>Change from pre- to post-surgery (%)†</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD (12)</td>
<td>58.3</td>
<td>158.90(180.60)</td>
<td>121.72(52.73 to 152.67)</td>
<td></td>
</tr>
<tr>
<td>LGCP (15)</td>
<td>73.3</td>
<td>181.32(209.65)</td>
<td>135.41(74.75 to 172.57)</td>
<td></td>
</tr>
<tr>
<td>LAGB (12)</td>
<td>16.7</td>
<td>60.03(29.23)*</td>
<td>61.84(43.39 to 72.56)**</td>
<td></td>
</tr>
</tbody>
</table>

Table shows percentage of patients (%) who exhibited increased serum FGF-19 post-surgery relative to pre-surgery levels. The Wilcoxon signed ranks test was used for within group comparisons of pre and post-surgery levels (*p<0.05). †: The Kruskal-Wallis H test determined there were significant differences in serum FGF-19 between the three surgery types (χ²=7.655; p=0.022). SD: standard deviation, IQR: interquartile range, BPD: bilio-pancreatic diversion, LGCP: laparoscopic greater curvature plication, LAGB: laparoscopic adjustable gastric banding.

A wide range in serum FGF-19 concentrations were found both before and after surgery. Pre-surgery levels of FGF-19 between BPD and LGCP cohorts were not significantly different however, they were found to be significantly higher in the LAGB cohort (Figure 4.1A). To control for this variability, post-surgery levels were analysed relative to the individual’s pre-surgery readings. The type of bariatric procedure was found to play an important role on whether FGF-19 levels increased or decreased post-surgery (as tested using the Kruskal Wallis H Test, p=0.018). Whilst the BPD and LGCP procedures both seemingly up-regulated FGF-19 levels in circulation in the majority of patients (58% in BPD and 73% in LGCP), the LAGB procedure resulted in their significant down-regulation (Figure 4.1B). Only
17% of patients who underwent the LAGB procedure exhibited raised post-surgical serum FGF-19 levels (Figure 4.1C). Thus, a step-wise surgery-specific effect on FGF-19 levels was observed (LGCP>BPD>LAGB).

![Figure 4.1: Surgery-specific effects on serum FGF-19 concentrations](image-url)

Pre- (A) and Post-surgery (B) serum concentrations of FGF-19 for each surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Post-surgery data is expressed as percentage of pre-surgical values (shown as dotted line). Pie charts (C) show the numerical proportion of FGF-19 increase or decrease for each surgical procedure. Bars represent means ± standard error of the mean, pie chart slices represent percentage of total subjects in each surgical cohort (BPD n=12, LGCP n= 15, LAGB n= 12). Pre-to-post surgical differences were determined via the Wilcoxon signed ranks test (*p<0.05, **p<0.01). The Kruskal-Wallis H test determined there were significant differences in serum FGF-19 between the three surgery types (†p<0.05). N.S. denotes differences shown were not statistically significant (p>0.05).
Mapping Surgery-Specific Differences in Metabolic Recovery to Serum FGF-19 Levels

In order to ascertain whether FGF-19 may be a contributing factor to the procedure-specific differences in metabolic recovery, the surgery-induced changes in serum FGF-19 were examined against clinical indicators of metabolic health. As shown in Table 4.2, FGF-19 was not associated with any of the surgery-differential clinical variables, such as excess weight loss, BMI, HbA1c and total cholesterol. No association was observed between FGF-19 and age, waist-hip ratio, body fat percentage, glucose, HOMA IR, triglycerides or LDL cholesterol. However, serum FGF-19 levels were significantly associated with insulin, HDL cholesterol and HDL/LDL ratio (Figure 4.2). Higher levels of FGF-19 were also associated with increased serum insulin levels and HDL relative LDL cholesterol across all surgeries; however no clustering on the basis of procedure was evident. These results are therefore consistent with the notion that FGF-19 may participate in metabolic recovery, but does not support the hypothesis that it is involved in modulating superior metabolic outcomes observed with the BPD versus LGCP and LAGB procedures.
Table 4.2: Relationship of circulating FGF-19 levels with clinical indicators of metabolic health

<table>
<thead>
<tr>
<th>Indicator</th>
<th>FGF-19</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td>−0.051</td>
<td>0.556</td>
</tr>
<tr>
<td>EWL (%)</td>
<td></td>
<td>0.185</td>
<td>0.259</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>−0.116</td>
<td>0.481</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td>−0.060</td>
<td>0.721</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td>−0.195</td>
<td>0.241</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td>−0.139</td>
<td>0.399</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td></td>
<td>0.344</td>
<td>0.032*</td>
</tr>
<tr>
<td>HOMA IR</td>
<td></td>
<td>0.084</td>
<td>0.614</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td></td>
<td>−0.142</td>
<td>0.388</td>
</tr>
<tr>
<td>TGL (mmol/L)</td>
<td></td>
<td>0.290</td>
<td>0.073</td>
</tr>
<tr>
<td>T Chol (mmol/L)</td>
<td></td>
<td>0.066</td>
<td>0.688</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td></td>
<td>−0.044</td>
<td>0.790</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td></td>
<td>0.399</td>
<td>0.013*</td>
</tr>
<tr>
<td>HDL/LDL</td>
<td></td>
<td>0.489</td>
<td>0.002**</td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient (r) and p value significance (p) between serum FGF-19 levels and metabolic variables in the entire patient cohort (n=39). Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Significant correlations are shown in red. *p<0.05. EWL: excess weight loss, BMI: body mass index, WHR: waist-hip ratio, HOMA IR: homeostasis model assessment of insulin resistance, HbA1c: serum glycosylated haemoglobin, TGL: triglycerides, T Chol: total cholesterol, LDL: low-density lipoproteins, HDL: high-density lipoproteins.

Figure 4.2: Association of serum FGF-19 levels with insulin and HDL/LDL ratio
Scatter plots show correlations of serum FGF-19 against insulin and HDL/LDL ratio. Correlations were calculated using change variables (percentage of pre levels) in the entire patient cohort (n=39), but individual data points are color-coded according to surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Linear trend line is also shown with Pearson correlation statistic (r) and significance (p). If non-parametric, variables were log-transformed prior to correlation analysis to improve normality.
Mapping Surgery-Specific Differences in Indicators of Adipose Mitochondrial Functionality to Serum FGF-19 Levels

Next, the question of whether FGF-19 is involved in modulating mitochondrial functionality in the adipose tissue was investigated, and if so, whether surgery-specific changes in FGF-19 may help explain the differential effect also observed in adipose mitochondrial functionality. To evaluate this, the relationship of FGF-19 against mitochondrial number and transcript levels were examined through Pearson correlation analyses. As shown in Table 4.3, no significant association was found between serum FGF-19 levels and any of the mitochondrial genes studied. Interestingly however, FGF-19 levels were significantly and inversely associated with mitochondrial DNA copy number in adipose biopsies (Figure 4.3). Higher levels of FGF-19 were associated with lower levels of mitochondrial number across all surgical cohorts, with no clustering on the basis of type of procedure observed. These results are therefore consistent with the notion that FGF-19 may play a role in modulating adipose mitochondrial function, however no evidence was found to support the hypothesis that FGF-19 is involved in modulating superior mitochondrial outcomes observed with the BPD versus LGCP and LAGB procedures.
Table 4.3: Relationship of circulating FGF-19 levels with indicators of adipose mitochondrial functionality

<table>
<thead>
<tr>
<th>Function</th>
<th>mt number</th>
<th>FGF-19</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>mt number</td>
<td></td>
<td>0.428</td>
<td>0.014*</td>
</tr>
<tr>
<td>PGC1α</td>
<td></td>
<td>-0.140</td>
<td>0.395</td>
</tr>
<tr>
<td>POLG</td>
<td></td>
<td>-0.173</td>
<td>0.294</td>
</tr>
<tr>
<td>TFAM</td>
<td></td>
<td>-0.194</td>
<td>0.288</td>
</tr>
<tr>
<td>mtND6</td>
<td></td>
<td>-0.254</td>
<td>0.119</td>
</tr>
<tr>
<td>SDHA</td>
<td></td>
<td>-0.177</td>
<td>0.282</td>
</tr>
<tr>
<td>COX4i1</td>
<td></td>
<td>-0.234</td>
<td>0.151</td>
</tr>
<tr>
<td>mtATP6</td>
<td></td>
<td>-0.250</td>
<td>0.125</td>
</tr>
<tr>
<td>UCP2</td>
<td></td>
<td>-0.195</td>
<td>0.234</td>
</tr>
<tr>
<td>SOD1</td>
<td></td>
<td>-0.055</td>
<td>0.738</td>
</tr>
<tr>
<td>SOD2</td>
<td></td>
<td>-0.010</td>
<td>0.950</td>
</tr>
<tr>
<td>Dynamics</td>
<td></td>
<td>0.118</td>
<td>0.548</td>
</tr>
<tr>
<td>MFN2</td>
<td></td>
<td>0.018</td>
<td>0.892</td>
</tr>
<tr>
<td>OPA1</td>
<td></td>
<td>0.214</td>
<td>0.283</td>
</tr>
<tr>
<td>DRP1</td>
<td></td>
<td>-0.164</td>
<td>0.406</td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient (r) and p value significance (p) between serum FGF-19 levels and mitochondrial variables in the entire patient cohort (n=39). Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Significant correlations are shown in red. *p<0.05. mt number: mitochondrial DNA copy number, PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, POLG: mitochondrial DNA polymerase gamma catalytic subunit, TFAM: mitochondrial transcription factor A, mtND6: mitochondrially-encoded NADH dehydrogenase 6, SDHA: Succinate dehydrogenase complex subunit A, COX4i1: Cytochrome c oxidase subunit 4 isoform 1 (complex IV), mtATP6: mitochondria-DNA-encoded ATP synthase subunit 6 (complex V), UCP2: uncoupling protein 2, SOD1: superoxide dismutase 1, SOD2: Superoxide dismutase 2, MFN2: Mitofusin 2; OPA1: mitochondrial dynamin like GTPase; DRP1: Dynamin-1-like protein; FIS1: Mitochondrial fission 1 protein.
Figure 4.3: Association of serum FGF-19 levels with adipose mitochondrial number

Scatter plot showing correlation between serum FGF-19 and mitochondrial DNA copy number in adipose biopsies. Correlation was calculated using change variables (percentage of pre levels) in the entire patient cohort (n=39), but individual data points are color-coded according to surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Linear trend line is also shown with Pearson correlation statistic (r) and significance (p).
4.4 Discussion

As outlined in Chapter 3, only one type of bariatric procedure (BPD) was associated with adipose mitochondrial benefits, despite similar rates of weight loss-associated HbA1c improvement, suggesting there may be other factors altered by gut-remodeling specific to each surgery which may be influencing mitochondrial recovery in BPD but not LGCP or LAGB. One likely candidate influencing this dichotomous relationship is Gut-hormone Fibroblast Growth Factor-19 (FGF-19). Therefore, serum FGF-19 levels were measured before and after 3 bariatric procedures (BPD, LGCP and LAGB) and analysed against all anthropometric, biochemical and mitochondrial variables. Our findings confirm for the first time a differential impact between BPD, LGCP and LAGB bariatric procedures on circulating FGF-19 levels, with BPD and LGCP leading to similar significant rises in this hormone, in contrast to the LAGB surgery, which lowered serum FGF-19 levels. Despite these widely ranging effects, circulating FGF-19 was significantly and consistently associated with adipose mitochondrial number across all 3 surgical procedures investigated, adding credence to the notion that FGF-19 may target adipose mitochondrial function to improve metabolic health after bariatric surgery. The finding that FGF-19 levels are inversely correlated with mitochondrial number in AT may be interpreted as a shift towards a less fragmented and more elongated mitochondrial network when FGF-19 levels are raised. This would seem of benefit, given that mitochondrial fragmentation has been associated with apoptosis [313, 314], severely compromised mitochondrial DNA integrity, inefficiency [278, 279], accumulation of reactive oxygen species (ROS) [236], impaired oxygen consumption and β-oxidation [280, 281], lipotoxic species accumulation [282], pro-
inflammatory cytokine production [283] and impaired insulin signaling [284, 285].
Moreover, fragmentation of muscle mitochondria has been reported in several
mouse and human models of obesity and type-2 diabetes [245, 315].
However, it must also be stated that long-term sustained mitochondrial elongation
can compromise mitochondrial quality control and function [29], so mitochondrial
elongation per se is not necessarily indicative of mitochondrial health, and that
the cell requires a balance between both fission and fusion processes to maintain
mitochondrial quality. As shown in Chapter 3, genes controlling a wide range of
mitochondrial functions were tightly correlated with mitochondrial number in BPD
patients, whilst in the other surgeries they appeared dysregulated. This finding
would support the assertion that BPD improves the control of genes involved in
maintaining mitochondrial function to a greater extent than the other two bariatric
procedures in this study, and is consistent with a role of serum FGF-19 in mediating
a less fragmentated and potentially more functional mitochondrial network.
In contrast, in the LGCP group the relationships between mitochondrial number
and gene expression followed a significant inverse association, despite similar rise
in serum FGF-19 levels compared to BPD. This seemingly paradoxical finding may
be better understood within a wider context of additional factors also likely to play
a role in mitochondrial recovery [277]. Indeed, the BPD operation (unlike LGCP)
produced significantly lower serum lipids levels and nearly twice as much weight
loss (30% versus 17%). This is consistent with both previous reports [316], and
the notion that these two factors (weight loss and lipid recovery) may have also
contributed to the enhanced mitochondrial outcomes observed after BPD versus
LGCP. Further in support of this concept, total and HDL cholesterol were the only
biochemical variables (apart from FGF-19) to exhibit a significant association with mitochondrial genes. Decreased cholesterol levels were directly associated with enhanced mRNA expression of complex IV (COX4I1) and V (mtATP6) genes of the electron transport chain. Similar associations were observed with the uncoupling protein 2 (UCP2) gene, which has been implicated in preventing reactive oxygen species accumulation and oxidative stress damage [218].

Interestingly, in the LAGB group (the only study procedure to significantly reduce serum FGF-19 levels), changes in mitochondrial gene expression in AT were (with exception of fusion genes) unrelated to mitochondrial number, suggesting a dysregulation of mitochondrial function in this cohort, potentially resulting from unopposed fusion. Though this bariatric procedure resulted in significant weight loss and general metabolic improvement, the noted HbA1c reduction was significantly less pronounced compared with the other two procedures (even after accounting for BMI), which might be, at least in part, the result of the mitochondrial dysfunction and lower serum FGF-19 levels observed.

Previous studies in mice support the hypothesis that circulating FGF-19 targets white adipose mitochondria to exert metabolic improvements. Mice challenged with a high-fat diet and treated with fexaramine (an intestine-restricted FXR agonist which potently induces intestinal FGF-15, i.e. the mouse FGF-19 homologue) exhibited significantly less weight gain, systemic inflammation, and improved glucose homeostasis, with specific effects noted on visceral white adipose tissue, including: reduced activation of inflammatory and lipogenic pathways, browning of white adipocytes, and increased thermogenesis [317]. Though FGF-19 is known to exert several metabolically beneficial effects by its actions in the liver that regulate
glucose and cholesterol production [305], recent evidence in mice further suggests that the improvement of glucose homeostasis after recombinant FGF-15 treatment is likely due to direct signaling in AT and other metabolic relevant organs rather than through the known hepatic effects [318]. Furthermore, previous reports of positive correlations between circulating FGF-19 and adiponectin [319, 320] lend further credence to the role of FGF-19 as a regulator of white adipose tissue endocrine and metabolic function. In accordance with previous research, these findings support the hypothesis that FGF-19 targets white AT and provide evidence for the first time in humans that circulating FGF-19 levels strongly and inversely associate with mitochondrial fragmentation of this tissue.

It should be noted that, this study has certain limitations, namely: 1) though our study subjects did not follow a particular dietary regimen and led a relatively sedentary lifestyle in the period before surgery, these two factors were not controlled either before or after surgery; and 2) despite the prospective study design, it is not possible to clarify in the context of this study the precise mechanism by which each studied surgical procedure alters serum FGF-19 levels, thus further research is required to clarify this point. However, to our knowledge, this is the first study to compare serum FGF-19 levels between these bariatric surgical procedures and to provide evidence of differential mitochondrial and metabolic outcomes based on the type of surgical procedure.

In conclusion, elevated serum FGF-19 levels post-surgery were significantly associated with improved mitochondrial health in AT leading to greater control of mitochondrial gene regulation and overall type-2 diabetes remission. These increased FGF-19 levels were also observed to be surgery-specific with BPD pa-
patients achieving better metabolic health outcomes compared to LGCP and LAGB (BPD>LGCP>LAGB), and highlighting mitochondria in AT as a promising potential target of FGF-19 during diabetic recovery following bariatric surgery.
Chapter 5

Role of Gut-derived LPS on Adipose Mitochondria Recovery Post Bariatric Surgery
5.1 Introduction

Chapters 3 and 4 outlined evidence supporting the concept that FGF-19 targets adipose tissue mitochondria, leading to weight loss and metabolic recovery post bariatric surgery. The BPD procedure, which resulted in heightened serum FGF-19 levels, also produced the greater improvements in mitochondrial gene regulation, weight loss and overall type-2 diabetes remission. However, the procedure LGCP also produced similarly heightened serum FGF-19 levels but lacked the corresponding improvements in mitochondrial function. These data raises the question: which surgery-specific factors mediate the differences observed in mitochondrial and metabolic benefit? Gut-derived bacterial lipopolysaccharide (LPS) is a promising target of study for this question, as it is increased in circulation in association with over-nutrition (specifically lipids), has been extensively linked with inflammation and metabolic dysfunction [199, 200, 201, 321] and is likely to be altered by gut-remodeling [322]. Interestingly, a main difference between the two surgeries was greater lipid reduction and weight loss with BPD compared to LGCP or indeed LAGB.

LPS are major outer cell wall components of gram negative bacteria normally present in the gut, which may provide an interesting mechanistic link between the Western diet and metabolic disease (Figure 5.1). Due to their biochemical affinity, LPS are absorbed coupled to chylomicrons, and circulating levels increase in direct relationship with dietary fat absorption [196]. As little as a single high-fat, high-carbohydrate meal can raise serum LPS levels by 50% and generate systemic inflammation [323]. In addition, chronic intake of high-fat, high-carbohydrate meals can increase the gram-negative bacteria sub-population, and thus the gut
LPS load [324, 325, 326, 327, 32]. Therefore, both acute and chronic Western diets can generate systemic inflammation and initiate metabolic disease, through increased LPS absorption. Indeed, direct intravenous administration of LPS to healthy adults has been shown to reduce insulin sensitivity by 30% [328] and several cross-sectional studies have demonstrated that serum LPS levels function as independent predictors of type-2 diabetes incidence [199, 200, 201, 321]. The finding that diabetes rates in people with mitochondrial disease (known as mitochondrial diabetes) are more than four times higher (40%) than the rates in the average population (9%) provides strong evidence that mitochondrial functionality may be intrinsically linked with the pathophysiology of metabolic disease [329, 330, 331, 332, 333]. In addition to respiration and nutrient metabolism, mitochondria are the main cellular source of reactive oxygen species, closely involved in regulating several vital processes including inflammation and apoptosis [334, 294, 281, 283, 152, 335]. Thus, this study hypothesized that heightened LPS may have adverse direct and/or (through inflammation) indirect effects on adipose mitochondria functionality, impacting these tissues’ ability to buffer lipids away from other organs, and contributing to metabolic disease (Figure 5.1). As such, it could be considered that the difference in mitochondrial and metabolic outcomes outlined throughout Chapters 3 and 4 may be explained, at least in part, by differences in serum LPS levels.

It should be stressed that LPS can be difficult to measure reliably in serum because the concentration of (mostly unknown) LPS-neutralizing factors vary from individual to individual [336]. Furthermore, conventional LPS detection methods utilise Limulus Amebocyte Lysate (LAL) which whilst highly sensitive to LPS, can
carry several limitations. Mainly, the LAL-LPS reaction must occur whilst the sample matrix (which contain several inhibitory factors to varying quantities) is present. This limitation can be minimised to some degree by serial dilution and spiking the sample with increasing known amounts of LPS to determine degree of neutralizing factors, heat inactivation steps and the use of glass instead of plastic test tubes [337]. However, these measures are time-consuming and their success can be limited, although procedures can be put in place to minimise these issues.

A newer method (EndoLISA®), is based on the recombinant factor C, which is a bacteriophage protein which specifically binds endotoxin onto a microtiter solid phase, allowing the sample matrix (with potentially interfering components) to be removed before the detection reaction takes place. Although not yet tested for use in serum samples, this new method may support a more reliable and less error-prone assessment of LPS than current LAL-based methods.

Therefore, this present chapter (1) compared the specificity and reliability of both methods, in order to use a suitable assay to (2) measure serum LPS levels before and 6-months after 3 bariatric surgeries: BPD, LGCP and LAGB. These set of studies specifically investigated whether BPD would lower serum LPS levels to a greater extent than the other bariatric surgeries, and thereby be associated as a result with superior metabolic and mitochondrial outcomes.
Proposed mechanism by which gut-derived LPS links the Western diet with metabolic disease via mitochondrial dysfunction. A chronic high-fat, high-carbohydrate diet leads to increased circulating LPS through two main mechanisms: (A) Chronic high-fat high-carbohydrate meals increase gram-negative (LPS-containing) bacterial populations in the gut and (B) lipophilic LPS are coupled, packaged and distributed alongside chylomicrons, so serum LPS levels are directly proportional to degree of chylomycron-dependent fat absorption. (C) Once in circulation, LPS triggers a potent systemic inflammatory response, which may become chronic if Western diet is continued. Chronic inflammation is a hallmark of adipocyte dysfunction, insulin resistance and metabolic disease. (D) In the present work, we propose LPS may also generate mitochondrial dysfunction in adipose tissue and compromise the lipid buffering capacity of this organ. (E) Continued energy surplus will then result in ectopic lipid deposition on other metabolic organs, such as liver, pancreas, muscle and blood vessels, initiating systemic metabolic disease.
5.2 Methods

Ethics and Study Design

Thirty-nine morbidly obese (BMI > 35 Kg/m2), type-2 diabetic, Caucasian women undergoing either bilio-pancreatic diversion (BPD; n=12), laparoscopic greater curvature plication (LGCP; n=15), or laparoscopic adjustable gastric banding (LAGB; n=12) were recruited to participate in this study. Fasted bloods and anthropometric investigations were conducted before (baseline) and following surgery with collection of serum samples and abdominal subcutaneous white adipose tissue (AT) biopsies at both of these time points. Patients on pharmacological treatment with incretin mimetics and/or insulin were not included in this study. For further detail the reader is referred to Chapter 2.

Blood Biochemistry and Anthropometry

All anthropometric and biochemical measurements were performed before and six months after surgery. For detail on blood, adipose tissue sample and other clinical data collection, please see Chapter 2.

RNA isolation and qRT-PCR

Gene expression of mitochondrial genes was assayed through quantitative real-time polymerase chain reaction (qRT-PCR) using subcutaneous white adipose tissue biopsies before and 6 months after bariatric surgery. For detail on primer sequences used in this study the reader is referred to Table 2.1. For further detail please see Chapter 2.
Mitochondrial DNA copy number assay

Mitochondrial (mtND1) and nuclear (BECN1) gene primers (Table 2.2) were used via qRT-PCR to determine relative amounts of mitochondrial to nuclear DNA as a measure of mitochondrial number per adipocyte[266] within subcutaneous white adipose tissue biopsies. For further detail on methods, the reader is referred to Chapter 2.

LPS Assay Validation and Quantitation in Serum Levels

For serum lipopolysaccharide (LPS) determination, two methods were compared: the Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic Assay (QCL-1000™, LONZA) and the EndoLISA® Elisa-based endotoxin detection assay (Hyglos). LPS concentrations of 8 human serum samples (BMI=21 – 38 kg/m²) were determined in parallel with both assays. Samples were run in duplicate according to the manufacturers instructions. Serial sample dilution (from 1:4 to 1:10) and appropriate spike and negative controls were utilised to minimise enzyme-inhibitory factors in samples and confirm result validity. Results were calculated, according to manufacturers instructions, based on a LPS standard (E. Coli 055:B5) curve ranging from [4 to 0.06 EU/mL] for the LONZA kit and from [500 to 0.005 EU/mL] for the EndoLISA.

For bariatric samples, the EndoLISA® Elisa-based endotoxin detection assay (Hyglos) was used to quantify LPS serum levels. A preliminary trial of singlets was run to determine optimal dilution (1:5, 1:10; 1:20), after which samples were run at optimal dilution in duplicate. Results were calculated according to manufacturers instructions, based on a LPS standard (E. Coli 055:B5) curve ranging from
[500 to 0.005 EU/mL].

**Statistical Analysis**

Statistical analyses were performed using the SPSS 21.0 software. Data are reported as mean ± standard deviation (SD), unless otherwise specified. Data were examined for normality according to the Shapiro-Wilks criteria. Comparisons between pre- and post-surgery time-points were performed via paired two-tailed t-tests (if parametric) and the Wilcoxon signed ranks test (if non-parametric). For categorical data, Fisher’s exact test was used. Between-group (surgery type) differences were assessed using One-way ANOVA (if parametric) and Kruskal-Wallis test (if non-parametric) using change variables, calculated as percentage change from pre-surgery values [(post/pre) x100]. For Pearson correlation analyses, change variables [(post/pre) x100] were log-transformed prior to analysis if non-parametric.
5.3 Results

Validation and Optimisation of LPS quantitation in human serum samples

Two methods for the quantitation of circulating LPS levels were validated and compared: Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic Assay (QCL-100™, LONZA) and the EndoLISA® Elisa-based endotoxin detection assay (Hyglos). To gain a representative picture and given that LPS levels are reported to vary with BMI, eight human serum samples from individuals with a range of BMIs (21-37.5 kg/m²) were used to run the assays. As per the manufacturer’s instructions of the Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic Assay[336], the effect of vessel material (glass vs. plastic) as well as heat inactivation of samples were tested. As shown in Figure 5.2, vessel material did not alter the sensitivity of the LAL assay, whilst heat activation reduced the sensitivity of the assay. Therefore, subsequent LAL assays were run in the absence of heat activation, using validated pyrogen-free polypropylene test tubes.

Next, both assays were run in parallel, with four replicates per sample and the standard curves of each method were compared. The Pearson coefficient of determination ($R^2$) value was slightly closer to 1 in the EndoLISA, compared with the LAL standard curve (Figure 5.3), indicating a better fit of the EndoLISA data to the suggested linear model. In order to determine assay reliability, two replicates within each sample were spiked with a known amount of LPS (Figure 5.4). Assay reliability was greater with the EndoLISA method, in which more than 50% of the LPS spike was recovered in 6 out of 8 samples (Figure 5.4B). In contrast, spiked
samples in the LAL method showed minimal difference to non-spiked samples (Figure 5.4A). As both assays were tested using the same samples, these differences can not be accounted for by differences in interfering substances present in the sample. Thus, this data would suggest the EndoLISA method to have greater sensitivity in addition to reliability. Figure shows LPS readings via each method side by side. Though higher readings were obtained on average with the LAL method, the EndoLISA method shows more sensitivity to individual variation and may therefore be a better option when between-group differences are subtle.

Figure 5.2: Validation of vessel material and heat inactivation for LAL method
Graph shows the effect of sample heat inactivation and vessel material interference on LPS quantitation via the Limulus Amebocyte Lysate (LAL) kinetic chromogenic method. Data are expressed as endotoxin units per milliliter (EU/mL), and bars represent standard error of the mean. Significant differences were determined via 2-tailed unpaired t-test. *p<0.05, **p<0.01, n=8. N.S. denotes differences shown were not statistically significant.
Figure 5.3: Comparison of calibration curves between two methods of LPS quantitation

Graph shows the calibration curve for two LPS quantitation methods: the Limulus Amebocyte Lysate (LAL) kinetic chromogenic Assay (LONZA) and the EndoLISA® Elisa-based endotoxin detection assay (Hyglos). Pearson Coefficient of Determination ($R^2$) is shown for each linear model.
**Figure 5.4: Reliability of LAL versus EndoLISA methods**

Eight human serum samples from individuals with a range of BMIs from lean to obese (21-37.5 kg/m²) were used to compare two methods of LPS quantitation: Limulus Amebocyte Lysate (LAL) Kinetic Chromogenic Assay (QCL-1000™, LONZA) and the EndoLISA® Elisa-based endotoxin detection assay (Hyglos). Graphs display LPS spike recovery with LAL (A) and EndoLISA (B) methods. Data are expressed as endotoxin units per milliliter (EU/mL), each bar represents reading of one sample without or with LPS spike (darker colour) with corresponding BMI shown below. n=8.
Effect of Bariatric Surgical Procedure on Circulating LPS Levels

As shown in previous chapters of this thesis, the bariatric procedure BPD resulted in greater improvements in mitochondrial gene regulation, weight loss and overall type-2 diabetes remission, with more moderate improvements also observed after the LGCP, but not the LAGB procedure. To investigate whether circulating levels of bacterial LPS could help explain some of these differences, this chapter next examined the effect of each individual bariatric procedure on endotoxaemia.

Figure 5.6A shows serum LPS levels vary substantially between individuals both before and six months after BPD, LGCP or LAGB, and as such no significant differences from pre-to-post surgery were detected within each group. Between groups however, a stepwise trend (BPD<LGCP<LAGB) was detected which was nevertheless non-significant (Figure 5.6B). Moreover, all three surgeries resulted
in similar proportion of improvement of metabolic endotoxaemia (approximately 43% on average) (Figure 5.7). Despite this, surgery-specific effects were identified with respect to the degree of LPS improvement (Figure 5.8). When patients who did not achieve endotoxaemic improvement post-surgery (Figure 5.8A) were separated from those who did (Figure 5.8B), it became apparent that the BPD and LGCP surgeries resulted in substantially greater (approximately 20%) reductions of circulating LPS.

Figure 5.6: Serum LPS levels before and after bariatric surgical intervention

The EndoLISA® Elisa-based endotoxin detection assay (Hyglos) was used to quantify serum LPS levels before and 6-months after 3 bariatric procedures: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is shown as either EU/mL at pre and post surgery (A), or as percentage of pre-surgical levels (B). Dotted line denotes pre levels for each surgery normalised as 100%, bars represent means ± standard error of the mean. Pre-to-post surgical differences were determined via 2-tailed paired t-test (*p<0.05, **p<0.01). One way ANOVA was used to determine differences between surgeries (†p<0.05). N.S. denotes differences shown are not statistically significant (p>0.05).
Figure 5.7: Proportion of serum LPS improvement for each surgical procedure
Pie charts showing the numerical proportion of patients with lower LPS post-surgery for each bariatric procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Proportion is shown as percentage of total subjects in each surgical cohort (BPD n=12, LGCP n= 15, LAGB n= 12).

Figure 5.8: Surgery-specific effect on degree of metabolic endotoxaemic recovery
Each surgical cohort was subdivided by whether or not patients exhibited an improvement in metabolic endotoxaemia (decreased LPS levels). Graphs show surgery-specific effects on serum LPS levels in patients who exhibited raised (A) versus lowered (B) metabolic endotoxaemia after 3 bariatric procedures: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Data is expressed as percentage of pre-surgical values (shown as dotted line), and bars represent means ± standard error of the mean. One way ANOVA was used to determine differences between surgeries (†p<0.05). N.S. denotes differences shown were not statistically significant (p>0.05).

Mapping Surgery-Specific Differences in Metabolic Recovery to Serum LPS Levels
In order to ascertain whether lower LPS may be a contributing factor to the procedure-specific differences in metabolic recovery, surgery-induced changes in
serum LPS levels were examined against clinical indicators of metabolic health. No statistically significant relationships were identified between LPS and other clinical variables in the patient cohort as a whole. To understand whether the medley of heightened versus lowered serum LPS in post-surgical patients could be masking any association, these relationships were also analysed in each sub-cohort (split by whether or not surgery resulted in endotoxaemic improvement). No statistically significant relationships were identified in those who exhibited lower post-surgery endotoxaemia (Table 5.1). However, in patients who exhibited continued heightened endotoxaemia after surgery however, significant correlations with waist circumference and circulating lipoprotein levels were observed (Table 5.1), which are consistent with previous studies reporting an association between serum LPS and central adiposity.
Mapping Surgery-Specific Differences in Indicators of Mitochondrial Functionality to Serum LPS Levels

Next, this study investigated whether LPS may be involved in modulating mitochondrial functionality in the adipose tissue, and whether surgery-specific changes in LPS may help explain the differential effect also observed in adipose mitochondrial functionality. To evaluate this, the relationship of LPS against mitochondrial number and gene transcript levels was examined through Pearson correlation analyses. No significant relationship was noted between LPS and any of the mitochondrial genes studied (Table 5.2). However, serum LPS levels were significantly and

---

**Table 5.1: Relationship of circulating LPS levels with clinical indicators of metabolic health**

<table>
<thead>
<tr>
<th></th>
<th>LPS</th>
<th>↑LPS</th>
<th>↓LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.078</td>
<td>0.637</td>
<td>-0.484</td>
</tr>
<tr>
<td>EWL (%)</td>
<td>-0.011</td>
<td>0.945</td>
<td>-0.071</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.028</td>
<td>0.865</td>
<td>0.162</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>0.122</td>
<td>0.465</td>
<td>0.575</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.068</td>
<td>0.684</td>
<td>0.096</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>-0.041</td>
<td>0.813</td>
<td>-0.134</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.150</td>
<td>0.370</td>
<td>-0.024</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-0.130</td>
<td>0.430</td>
<td>-0.032</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.191</td>
<td>0.250</td>
<td>0.048</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>0.004</td>
<td>0.981</td>
<td>-0.061</td>
</tr>
<tr>
<td>TGL (mmol/L)</td>
<td>0.126</td>
<td>0.445</td>
<td>0.126</td>
</tr>
<tr>
<td>T Chol (mmol/L)</td>
<td>0.022</td>
<td>0.895</td>
<td>-0.178</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>-0.123</td>
<td>0.457</td>
<td>-0.348</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.122</td>
<td>0.464</td>
<td>0.017</td>
</tr>
<tr>
<td>HDL/LDL</td>
<td>0.162</td>
<td>0.337</td>
<td>0.565</td>
</tr>
</tbody>
</table>

*Table shows Pearson’s correlation coefficient (r) and p value significance (p) between serum LPS levels and metabolic variables in the entire patient cohort (n=39), and sub-cohort of patients with increased (n=16) or decreased (n=23) post-surgery serum LPS. Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Significant correlations are shown in red. *p<0.05. EWL: excess weight loss, BMI: body mass index, WHR: waist-hip ratio, HOMA IR: homeostasis model assessment of insulin resistance, HbA1c: serum glycosylated haemoglobin, TGL: triglycerides, T Chol: total cholesterol, LDL: low-density lipoproteins, HDL: high-density lipoproteins.*
inversely associated with mitochondrial number in adipose biopsies across all surgeries, with no obvious clustering on the basis of surgical procedure per se (Figure 5.9A). Patients with improved post-surgical LPS levels had significantly greater number of mitochondrial DNA copies in their adipose tissue, than patients who exhibited heightened endotoxaemia (Figure 5.9B).

Table 5.2: Relationship of circulating LPS levels with indicators of adipose mitochondrial functionality

<table>
<thead>
<tr>
<th>Function</th>
<th>mt number</th>
<th>PGC1α</th>
<th>POLG</th>
<th>TFAM</th>
<th>mtND6</th>
<th>SDHA</th>
<th>COX4I1</th>
<th>mtATP6</th>
<th>UCP2</th>
<th>SOD1</th>
<th>SOD2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−0.485</td>
<td>−0.100</td>
<td>−0.055</td>
<td>−0.057</td>
<td>0.043</td>
<td>−0.097</td>
<td>−0.121</td>
<td>0.062</td>
<td>−0.223</td>
<td>−0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>Dynamics</td>
<td>−0.042</td>
<td>−0.269</td>
<td>0.141</td>
<td>−0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient (\(r\)) and p value significance (\(p\)) between serum LPS levels and mitochondrial variables in the entire patient cohort (\(n=39\)). Correlations were calculated using change variables (pre to 6-months post-surgery percentage change). Significant correlations are shown in red. *\(p<0.05\). mt number: mitochondrial DNA copy number, PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, POLG: mitochondrial DNA polymerase gamma catalytic subunit, TFAM: mitochondrial transcription factor A, mtND6: mitochondrially-encoded NADH dehydrogenase 6, SDHA: Succinate dehydrogenase complex subunit A, COX4I1: Cytochrome c oxidase subunit 4 isoform 1 (complex IV), mtATP6: mitochondria-DNA-encoded ATP synthase subunit 6 (complex V), UCP2: uncoupling protein 2, SOD1: superoxide dismutase 1, SOD2: Superoxide dismutase 2, MFN2: Mitofusin 2, OPA1: mitochondrial dynamin like GTPase, DRP1: Dynamin-1-like protein, FIS1: Mitochondrial fission 1 protein.
Figure 5.9: Association of serum LPS levels with adipose mitochondrial number

Scatter plot showing correlation between serum LPS levels and mitochondrial DNA copy number in adipose biopsies (A). Correlation was calculated using change variables (percentage of pre levels) in the entire patient cohort (n=39), but individual data points are color-coded according to surgical procedure: Bilio-pancreatic diversion (BPD), laparoscopic greater curvature plication (LGCP) or laparoscopic adjustable gastric banding (LAGB). Linear trend line is also shown with Pearson correlation statistic (r) and significance (p). Post-surgical adipose mitochondrial levels in patients who exhibited an increase (↑LPS) or decrease (↓LPS) of serum LPS levels after surgery (B). Data are expressed as percentage to each individual’s pre-surgical levels, bars represent mean ± standard error of the mean. Statistical differences were analysed using a 2-tailed unpaired T-test (*p<0.05).
5.4 Discussion

In the present chapter, the aim was to (1) optimise the methodology for LPS serum quantification and, (2) to determine whether serum LPS changes post-surgery varied between procedures in a similar manner to mitochondrial improvements, which would support the hypothesis that serum LPS reduction is implicated in adipose mitochondrial recovery after bariatric intervention.

The BPD (and to a lesser-extent the LGCP) procedure resulted in significantly greater reductions of serum LPS than LAGB. This step-wise post-surgery reduction in LPS (BPD>LGCP>LAGB) mimicked the step-wise improvement in mitochondrial gene regulation outlined in Chapter 3. Furthermore, these reductions in LPS levels post surgery were significantly associated with increased numbers of mitochondria in adipose biopsies. Collectively, this data is consistent with the concept that heightened serum LPS contributes to metabolic disease by targeting adipose mitochondria.

Studies on circulating LPS levels post bariatric surgery are limited, and vary substantially based on surgical procedure studied and follow-up duration. Most, however, report decreases to some degree in circulating LPS levels from 90 to 360 days post-surgery. Namely, one study of 15 severely obese individuals with type-2 diabetes undergoing Roux-en-Y gastric bypass (RYGB) reported a significant 20% decrease in circulating LPS levels at 6 months post-surgery [205], whilst another reported significant LPS reductions at 1 year post RYGB or duodenal switch [338]. These reports are consistent with our own findings, where bariatric intervention resulted in a reduction of 20 to 40% depending on the procedure. Despite evidence that LPS levels vary between procedures in the short-term after surgery
However, to our knowledge this is the first study to examine the long-term effects of several bariatric procedures in parallel. Here, these studies demonstrate for the first time that some procedures are more effective than others in lowering serum LPS, and that this difference is associated with enhanced metabolic and mitochondrial benefits.

Recent evidence suggests that between-surgery differences in long-term metabolic benefit are rooted in the degree and type of gastrointestinal remodeling and the resultant changes in gut microbiota composition. This was elegantly demonstrated in a recent study where fecal matter from patients post-RYGB or vertical banded gastroplasty was transplanted to germ-free mice and resulted in significant weight loss, fat deposition and decreased use of carbohydrates as fuel [192]. Within this context, it is conceivable that LPS may be functioning as a communication link between gut and adipose tissue mitochondria to produce this shift in substrate metabolism. Our findings that LPS levels vary depending on surgical procedure in a manner proportional to the degree of mitochondrial improvement is consistent with this notion, however further research is needed to test the direct effect of LPS on mitochondrial functionality.
Chapter 6

Direct Effect of LPS on Human Adipocyte Mitochondrial Function
6.1 Introduction

The profound metabolic benefits of bariatric surgery cannot be explained by weight loss alone [176, 179]. Recent findings that fecal transplantation from bariatric subjects to germ-free animals can reproduce weight loss and mimic post-surgical metabolic improvements have bolstered the research potential of gut factors as mediators of obesity and metabolic disease [192]. One such factor is bacterial lipopolysaccharide (LPS). Absorbed alongside dietary lipids, its levels are elevated in obesity, resulting in a low-grade chronic inflammation and insulin resistance [196, 340, 341, 199, 204, 328]. As outlined in previous chapters, reductions in serum LPS after surgery varied substantially based on the bariatric procedure and in direct proportion to the degree of mitochondrial benefit in adipose tissue. Moreover, serum LPS levels were the only circulating factor to show a significant association with mitochondrial number in adipose biopsies. As such, this study hypothesized that heightened circulating LPS levels target adipose mitochondria unfavorably and contribute to insulin resistance and the pathogenesis of metabolic disease.

There are several potential mechanisms by which heightened LPS levels may contribute to mitochondrial dysfunction, which are summarised in Figure 6.1. LPS is a powerful trigger of inflammation, a hallmark of metabolic disease. Studies addressing the molecular link between adipose tissue inflammation and insulin resistance have highlighted the up-regulation of two pathways linking the two: the inhibitor of nuclear factor kappa-B kinase subunit beta/ nuclear factor kappa-light-chain-enhancer of activated B cells (IKKβ/NF-κB) and the c-Jun N-terminal kinases (JNK) pathway. Both are triggered either through direct activation of cytokine receptor and its respective pro-inflammatory cytokine; or activation of
toll-like receptors (TLRs) by saturated free fatty acids, lipotoxic species, or indeed LPS [166, 342, 195]. IKKβ and JNK are well-established serine kinases that phosphorylate insulin receptor substrate 1 (IRS-1) proteins at serine residues, leading to decreased insulin signaling. Additionally, IKKβ activates NF-κB, which further increases inflammation by stimulating the transcription of pro-inflammatory cytokines such as TNF-α and IL-6 [343]. IL-6 induces the expression of suppressors of cytokine signaling proteins (SOCS), which also interfere with insulin signaling [344]. Importantly, inhibition of IKKβ and JNK with anti-inflammatory pharmacotherapy or gene knock-out reduces serine phosphorylation of IRS proteins and improves insulin sensitivity [345, 346, 347, 348].
Proposed hypothesis for how LPS effect on adipocytes contribute to type-2 diabetes: Heightened LPS after a high-fat, high carbohydrate meal triggers inflammation, up-regulate IKK/JNK signaling pathways which can directly inhibit insulin signaling through insulin receptor substrate 1 (IRS1). In addition, activation of IKK/NFkB leads to transcriptional activation of pro-inflammatory cytokines (such as TNF-α) and reactive oxygen species (ROS) formation, establishing a positive feedback loop and further exacerbating inflammation and oxidative stress. The current work proposes that the accumulation of ROS will lead to mitochondrial DNA damage, bioenergetic inefficiency and mitochondrial dysfunction, which will add further inflammation and oxidative stress, and ultimately add extra complexity to cellular insulin resistance and have potential adverse consequences on systemic insulin sensitivity via impaired lipid buffering and ectopic fat deposition on non-adipose organs.

If sustained chronically, the pro-inflammatory environment poses a powerful threat to mitochondrial DNA integrity and organelle functionality. Mitochondrial DNA is thought to be more susceptible to damage than nuclear DNA as in addition to being in closer proximity to ROS (mitochondria are the main source of ROS in the cell) it contains only coding sequences and is not protected by histones [349, 350, 351]. Indeed, several known mitochondrial [288, 352] and nuclear [353] mutations have been associated with detrimental cardio-metabolic phenotypes through impairment
of mitochondrial function [331, 352, 288].

Mitochondrial genes encode 13 protein subunits of the OXPHOS complex including subunit 6 (mt-ND6) of NADH dehydrogenase (complex I) and subunit 6 (mt-ATP6) of ATP synthase (complex V). Nuclear DNA encode all subunits of succinate dehydrogenase (SDHA; complex II) and subunit 4 (COX4) of cytochrome c oxidase (complex IV) as well as proteins involved in mitochondrial DNA replication, transcription, translation, and repair. The expression level of these OXPHOS genes as well as size and shape of mitochondria largely determine oxidative capacity and both morphology and OXPHOS gene expression are closely linked to insulin action [354]. Though studies thus far have described clear evidence of obesity-associated alterations on mitochondrial function in skeletal muscle, the physiological implications of these alterations are yet to be understood, particularly within the context of white adipose tissue dysfunction.

During obesity, white adipose tissue, and the mitochondrial network within this organ are faced with the extreme challenge of metabolising and storing the excess calories as lipids, in order to avoid their harmful deposition in other organs such as liver, pancreas, muscle and blood vessels. Any adverse effects on mitochondrial functionality may have direct consequences on the lipid buffering capacity of white adipose tissue as a whole, and contribute to systemic metabolic disease. As such, the aim of this present chapter was to determine the direct in vitro effect of LPS on human adipocyte (1) insulin sensitivity, (2) inflammation, (3) mitochondrial DNA integrity, functionality and morphology and (4) overall energy phenotype in order to determine whether LPS would adversely affect mitochondrial function and adipocyte lipid handling.
6.2 Methods

Human adipocyte culture, differentiation and treatment

Human subcutaneous white adipocyte cell line ChubS7 were grown and differentiated as previously described [269]. Briefly, cells were seeded on to 6-well plates (0.3x10^6) unless otherwise specified, grown to 100% confluence and differentiated for 8-10 days in DMEM/F12 with 3% FBS and PromoCell Differentiation Supplement Mix (C-39436, PromoCell). After differentiation, cells were allowed to equilibrate in basal media for 12 hours before being treated for 24-72 hours in basal media supplemented with LPS (10 or 100 ng/mL, E. Coli O55:B5, Sigma, L6529), TNF-α (10 ng/mL, Sigma, H8916) or Insulin (50 nM, Sigma, I9278). All media were prepared with DMEM/F12 (ThermoFisher Scientific, UK, 11320033) and formulations are shown in Table 2.5 in Chapter 2.

2-deoxyglucose uptake

Glucose uptake in differentiated ChubS7 adipocytes was evaluated via cellular incorporation of [3H]-2-deoxyglucose. Following differentiation and treatment, cells were washed 3 times with warmed PBS and allowed to equilibrate in KRH buffer (containing 0.01% BSA, 5 mmol/l glucose) at 37°C for 2.5 hours. Adipocytes were then incubated a further 30 minutes with KRH buffer without glucose and either no (basal control) or 100 µM insulin. Immediately after, 1 µCi/ml [3H]-2-deoxyglucose (PerkinElmer, NET328A001MC) in KRH buffer at 37°C was added for a further 10 minutes. To finalise the assay, cells were then washed 3 times with ice-cold PBS, lysed and harvested in 200 µL RIPA buffer and a cell scraper. Radioactivity was
evaluated via scintillation counting of the lysates, diluted 1:4 in \( \beta \)-scintillation fluid (Beta-Plate Scint, PerkinElmer), and a scintillation Counter. Results defined as counts per minute (CPM) were normalised to total protein content and glucose uptake displayed relative to the basal control.

**Protein extraction and Western blot**

For protein extraction, 100 mg of frozen human adipose tissue or 1.2 \( \times \) 10^6 cultured adipocytes were homogenised in 200 µL PhosphoSafe\(^TM\) Extraction Buffer (Novogen\(^R\), Merk, Germany). A Bio-Rad detergent compatible protein assay kit (Bio-Rad Laboratories, CA) and nanospectrophotometer (GeneFlow, UK) were used to quantify protein concentrations. As described previously for Western blot analyses [268], 10-20 µg of protein were loaded onto a denaturing polyacrylamide gel (GeneFlow, UK), transferred on to a nitrocellulose membrane which was then incubated with a primary antibody diluted in 0.2% l-block PBS-T (IRS1 1:250, MT-CO1 1:1000, SDHA 1:1000, \( \beta \)-Actin 1:1000) at 4ºC overnight. A chemiluminescence detection system (ECL Plus, GE Healthcare, UK) was used to visualise protein bands, and densitometry was conducted using ImageQuant LAS 4000 Software (GE Healthcare, UK). Equal protein loading was confirmed by examining \( \beta \)-actin protein expression. Primary antibodies utilised are listed in Table 2.4 (Chapter 2).

**RNA isolation and qRT-PCR**

RNA was extracted from cell lysates containing approximately 1x10^6 adipocytes using a column-based isolation method (RNeasy Lipid Tissue Mini Kit; #74804 Qi-
agent, UK) according to manufacturer’s instructions. Samples were digested with DNase I to remove potential genomic DNA contaminants (DNase I kit, #AMP-D1 Sigma-Aldrich). RNA was eluted in 10 µL RNase-free water and 1 µL quantified in duplicate using a spectrophotometer (Nanodrop ND-1000, labtech) at 260 nm absorbancy. Synthesis of cDNA was performed using 1 µg RNA per sample and a Bioline mRNA reverse transcription kit (#BIO-65026) according to the manufacturer’s instructions. Gene expression was assayed through quantitative real-time polymerase chain reaction (qRT-PCR) using ABI 7500 standard sequence detection system (Applied Biosystems, UK). Each reaction was prepared to 25 µL final volume containing Taqman Universal PCR mastermix (#4304437 Applied Biosystems, UK), 1 µL sample cDNA and a specific commercially available Taqman gene expression assay (Applied Biosystems, UK). For detail on primer sequences used in this study the reader is referred to Table 2.1. All samples were assayed in triplicate and multiplexed using 18S (ribosomal RNA) as a pre-optimised control probe. As per the manufacturer’s instructions, reactions were carried out at 50 °C for 2 minutes, 95 °C for 10 minutes, and then 40 cycles of 95 °C for 15 seconds and then 60 °C for 1 min. For data analysis, a ΔCt was calculated based on the difference between 18S and the target gene. Gene expression was calculated based on the following formula:

\[ mRNA \text{ expression} = 2^{-\Delta Ct}, \text{ where } \Delta Ct = \text{target gene} Ct - 18s Ct \]
Endogenous antioxidant activity assays

Activity of endogenous antioxidants SOD and Catalase was evaluated through a colorimetric method, using OxiSelect\textsuperscript{TM} Superoxide Dismutase Activity Assay (STA-340) and OxiSelect\textsuperscript{TM} Catalase Activity Assay (STA-341) Kits (Cell Bio-labs). Following adipocyte differentiation and treatment, \( \sim 1.2 \times 10^6 \) adherent cells were washed 3 times with ice-cold PBS, harvested with a cell scraper in 1 mL of cold Lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA). Samples were homogenised, centrifuged and stored at -80\(^\circ\)C until assayed. All assays were conducted within 1 month of sample collection and were conducted in accordance to manufacturers instructions. Absorbance was read using a PheraStar FS microplate reader (BMG Labtech). SOD activity was calculated based on optical density as outlined in the following formula:

\[
SOD \text{ activity (inhibition \%)} = \left[ \frac{(\text{Blank}_{\text{OD}} - \text{Sample}_{\text{OD}})}{\text{Blank}_{\text{OD}}} \right]
\]

The concentration of active Catalase was determined by interpolation of a catalase standard curve. Optical density at 540nm was plotted on the “x” axis, Catalase (U/mL) on the “y” axis, and a second order polynomial equation was used to determine catalase concentrations as follows:

\[
\text{Catalase (U/mL)} = 174.01x^2 + 13.678x + 2.7743 \text{ where } x = \text{optical density (540 nm)}
\]
Quantification of total reactive oxygen and nitrogen species

Total reactive oxygen and nitrogen species were evaluated through green fluorescence using OxiSelect™ in vitro ROS/RNS Assay Kit (STA-347, Cell Biolabs). Following adipocyte differentiation and treatment, $\sim 1.2 \times 10^6$ adherent cells were washed 3 times with ice-cold PBS, flash-frozen in dry-ice and harvested using a cell-scaper. Samples were homogenised in 200 µL PBS and stored at -80°C and assayed within 1 month of collection. Assay was conducted according to manufacturers instructions, and fluorescence after 30 minutes was measured using a PheraStar FS microplate reader (BMG Labtech) with a 485/538 nm filter and 530 nm cutoff. Total ROS/RNS were calculated by interpolation of a Hydrogen Peroxide standard curve as follows:

$$\text{ROS/RNS (µM)} = 5354.5x^2 + 1043.3x + 50.496$$

where $x = \text{relative fluorescence units (485/538)}$.

Evaluation of mitochondrial DNA integrity

Total DNA was extracted from cultured cells using DNeasy Blood and Tissue Mini Kit (#69504 Qiagen, UK) in accordance to the manufacturer’s instructions. RNase treatment was performed to eliminate possible RNA contamination. DNA was eluted with 100 µL AE buffer and quantified using a spectrophotometer (Nanodrop ND-1000, Labtech). Evaluation of mitochondrial DNA integrity was performed via qRT-PCR by comparing previously published primers [267] spanning a section of mtDNA susceptible to mutations (where 84% of known mutations occur).
against a section that is not affected by any of the reported large deletions. Real-time PCR of both targets were run using a probe-based duplex qRT-PCR assay on an ABI Prism 7500 thermo cycler (Life Technologies) with the following thermal profile: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15s, 55°C for 15s, and 60°C for 1 minute. The reaction components consisted of 22.5µL Taqman Universal PCR Mastermix no AmpErase® UNG (Applied Biosystems), each mitochondrial probe at 250nM (Taqman® MGB Probe, ThermoFisher Scientific, UK; shown in Table 2.3) with a final reaction volume of 25µL.

Mitochondrial DNA integrity was calculated according to the following published formula [267]:

\[ 2^{mtDNA_{DR}} \text{, where } mtDNA_{Deletion Ratio} = \frac{(Stable \text{ Probe } Ct - Susceptible \text{ Probe } Ct)}{Stable \text{ Probe } Ct} \]

**Mitochondrial DNA copy number assay**

Total DNA was extracted from cell lysates containing approximately 1x10^6 adipocytes using DNeasy Blood and Tissue Mini Kit (#69504 Qiagen, UK) in accordance to the manufacturer’s instructions. RNase treatment was performed to eliminate possible RNA contamination. DNA was eluted with 100 µL AE buffer and quantified using a spectrophotometer (Nanodrop ND-1000, Labtech). Relative amounts of mitochondrial DNA copy number were assessed through qPCR in an ABI Prism 7500 thermo cycler (Life Technologies) with the use of iQTM SYBR Green Supermix (#170-8880 BioRad). Mitochondrial (mtND1) and nuclear (BECN1) gene primers (Table 2.2) were used to determine relative amounts of mitochondrial to nuclear DNA [266]. Each sample was measured in triplicate. Mitochondrial number
was calculated based on the following formula:

\[ mtDNA \text{ copy number} = 2^{\Delta Ct}, \text{ where } \Delta Ct = BEC1 - mtND1 \]

**Analysis of mitochondrial morphology through confocal microscopy**

Analysis of the mitochondrial network was performed through live cell imaging using a confocal microscope. ChubS7 cells were seeded on to gelatin-coated 35mm glass bottom culture dishes (MatTek corporation®), grown, differentiated and treated for 24 hours as described above. Upon completion of treatment, basal media was removed, replaced with 100 nM Mitotracker Red and cells incubated for 20 minutes at 37°C. Cells were then washed three times with basal media and imaged in HEPES-buffered basal media (pH 7.35) with or without LPS (10 or 100 ng/mL, *E. Coli* O55:B5, Sigma, L6529). The Z system attached to an inverted fluorescence microscope fitted with an F-view-II cooled CCD camera (Olympus) was used to observe cells which were maintained at 37°C throughout the imaging process. Cells were magnified 40x through an oil objective lens, and a 500 nm excitation filter was used to image the mitochondrial network.

Morphologic assessment of the mitochondrial network was conducted on confocal images using ImageJ (version 1.50i) as described by others [270]. The mitochondrial parameters assessed were mitochondrial area (\( \mu m^2 \)) and the degree of branching, as defined by the following equation:

\[
BF = \frac{MtP^2}{(4\pi MtA)}
\]
where $MtP = \text{length of mitochondrial outline (mitochondrial perimeter in } \mu \text{m)}$ and $MtA = \text{Area of mitochondria (} \mu \text{m}^2)$. Four independent experiments were conducted and a minimum of 70 images were examined for each experimental group.

**Seahorse Cell Mito Stress Test**

Respiration and media acidification rate were measured using a Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Agilent Technologies). Immortalised human preadipocytes ChubS7 were seeded onto 24-well plates (Seahorse Bioscience, 100850-001) at a density of 10,000 cells/well, grown and differentiated using the standard protocol outlined above, and treated for 24 or 72 hours with or without LPS (10 or 100 ng/mL, E. Coli O55:B5, Sigma, L6529). Each experimental group consisted of 5 replicates, with the experiment repeated on at least 2 separate occasions (n=10). The assay was conducted in sterile, unbuffered Assay Media prepared with Seahorse base media (Seahorse Bioscience, 102365-100) at 37°C (pH 7.4), the formulation of which is listed in Table 2.6.

After a calibration step (30 min) and an equilibration step (30 min), the assay protocol consisted of 3 cycles of the following steps: mix (3 min), wait (2 min), measure (3 min), which were completed before and after each injection of Oligomycin (Sigma, O4876), FCCP (Sigma, C2920) and combined Rotenone (Sigma, R8875) and Antimycin A (Sigma, A8674). Reagents or vehicle control were injected in the appropriate volume of a tenfold concentrated stock solution to give the following final in-well concentrations: 2 µM Oligomycin, 2 µM FCCP, 0.5 µM Rotenone/Antimycin A. Prior to experiments, reagent concentrations and seeding density were optimised.
as per the manufacturers instructions. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were calculated by the WAVE software (Seahorse Bioscience, Agilent Technologies). The basal respiration parameters were calculated from the mean of 3 individual OCR measurements as follows:

\[
\text{Basal Respiration} = \text{Initial OCR (pmol/L)} - \text{Rotenone OCR (pmol/L)}
\]

For OCR and ECAR response to injection of each compound, data was normalised by the basal respiration of each experimental group (shown as percentage of baseline) to account for well-to-well variability in cell number.

**Determination of ATP abundance**

Immortalised human preadipocytes ChubS7 were seeded onto gelatin-coated 96-well white opaque cell culture plates at a density of 10,000 cells/well. Cells were grown and differentiated according to the standard protocol outlined above, and treated with or without LPS (10 or 100 ng/mL, E. Coli O55:B5, Sigma, L6529) for 24 or 72 hours. Bioluminescent determination of ATP abundance was conducted using EnzyLight™ ATP Assay Kit (BioAssay Systems, EATP-100) following the manufacturers instructions for adherent cells. Luminescence was read using a PheraStar FS microplate reader (BMG Labtech), and ATP concentrations determined based on interpolation of a standard curve of known ATP concentrations ranging from 0 – 30 μM.
Determination of mitochondrial membrane potential

The dye tetramethylrhodamine ethyl ester perchlorate (TMRE, Sigma, 87917) was used to determine mitochondrial membrane potential. TMRE is a cell-permeant, positive-charged red/orange dye that is readily sequestered by polarised mitochondria (due to their negative charge) in a manner that is directly proportional to their membrane potential. Immortalised human preadipocytes ChubS7 were seeded onto a black opaque 96-well plate, differentiated and treated as described for the ATP abundance assay. Adherent cells were incubated with 300 nM TMRE (Sigma) diluted in basal media (described in Table 2.5) for 30 min at 37°C. Cells were then washed 3 times with warm PBS before reading fluorescence with a PheraStar FS microplate reader (BMG Labtech) at 550/590 nm excitation/emission immediately and after 10, 20 and 30 minutes. As a positive control for depolarisation, 30 µM FCCP was added to some cells for 30 minutes, prior to the TMRE incubation step. FCCP is an ionophore which destroys membrane potential, rendering mitochondria unable to accumulate TMRE. For background correction, cells with no TMRE and no FCCP added were used. Membrane potential was calculated using the relative fluorescence signal of samples based on the following formula:

\[
\text{Membrane Potential (\%) = \left( \frac{LPS\ sample - background}{control\ sample - background} \right) \times 100}
\]

Statistical Analysis

Statistical analyses were performed using the SPSS 21.0 software. Data are reported as mean ± standard deviation (SD), unless otherwise specified. Data were examined for normality according to the Shapiro-Wilks criteria. Unless otherwise
specified, comparisons between pre- and post-surgery time-points were performed via paired two-tailed t-tests (if parametric) and the Wilcoxon signed ranks test (if non-parametric). For categorical data, Fisher’s exact test was used. Between-group (surgery type) differences were assessed using One-way ANOVA (if parametric) and Kruskal-Wallis test (if non-parametric) using change variables, calculated as percentage change from pre-surgery values \[(\text{post}/\text{pre}) \times 100\]. For Pearson correlation analyses, change variables \[(\text{post}/\text{pre}) \times 100\] were log-transformed prior to analysis if non-parametric.
6.3 Results

LPS administration to human adipocytes directly induced insulin resistance

In order to test the hypothesis that LPS drives adipocyte insulin resistance via mitochondrial dysfunction, the first step was to establish whether in vitro LPS administration could directly induce insulin resistance in human subcutaneous adipocytes ChubS7. Therefore, adipocytes were treated with two different doses of LPS and two established in vitro models of insulin resistance served as positive controls: chronic TNF-α (10 ng/mL) and chronic insulin (50 nM). As shown in Figure 6.2(A), all four treatments successfully resulted in insulin resistance after both 24 and 72 hours, as evidenced by significant reductions in uptake of radio-labelled glucose following an acute dose of insulin (100 nM) compared with control adipocytes. This functional observation of insulin resistance was accompanied at 24 hours by reduced protein expression of insulin receptor substrate 1 (IRS1), a key first step in the insulin signaling cascade Figure 6.2(B-C). Thus, both low and high doses of LPS resulted in impaired insulin signaling and adipocyte glucose uptake, with stronger effects at 24 hours.
Figure 6.2: Effect of LPS on Human Adipocyte Insulin Sensitivity

(A) Glucose uptake and (B-C) Insulin receptor substrate 1 (IRS1) protein expression in human adipocytes following 24 or 72-hour treatment with 10 ng/mL TNF-α, 50 nM chronic Insulin, 10 or 100 ng/mL LPS. (C) Image of IRS1 and loading control β-actin Western blot membranes at both time-points. For (A), results are expressed as percentage of basal control; for (B) results are expressed as percentage change from control cells. Bars represent standard error of the mean. *p<0.05, **p<0.01.
LPS administration up-regulated inflammation and oxidative stress

Next, inflammation and oxidative stress were assessed via measurement of pro-inflammatory cytokine TNF-α, and the combination of total reactive oxygen (ROS) and nitrogen (RNS) species. LPS was a potent inducer of inflammation (particularly at 24 hours), when TNF-α mRNA increased 300-500% in LPS-treated adipocytes relative to control (Figure 6.3A). Abundance of total ROS and RNS rose by 50% within 24 hours and 150% within 72 hours of LPS treatment (Figure 6.3B). As expected, endogenous antioxidant superoxide dismutase (SOD) also showed a marked increase, both in terms of mRNA transcript levels (400-3000%; Figure 6.3C) and activity (30-300%; Figure 6.3D). However, the activity of endogenous antioxidant catalase was significantly impaired with LPS treatment, particularly at 24 hours when LPS treatment resulted in an approximately 50-75% decrease (Figure 6.3E). Overall, mRNA transcript levels of both TNF-α and SOD2 genes were lower at 72 compared to 24 hours (Figure 6.3A and C).
Figure 6.3: Effect of LPS on Human Adipocyte Inflammation and Oxidative Stress

(A) Tumor necrosis factor-alpha (TNF-α) mRNA expression (n=6), (B) total reactive oxygen (ROS) and nitrogen species (RNS) (n=6), (C) Superoxide dismutase 2 (SOD2) mRNA expression (n=3), (D) SOD activity (n=6), and (E) catalase activity (n=6) in human adipocytes following 24 or 72-hour incubation with 10 or 100 ng/mL LPS. Data are expressed as percentage change from control, unless otherwise specified and bars represent standard error of the mean. *p<0.05, **p<0.01.

LPS administration resulted in mitochondrial DNA deletion, and protein depletion

Given that LPS resulted in increased ROS accumulation and impaired endogenous antioxidant response, the possibility of damage to mitochondrial DNA was
investigated. Briefly, a section of mitochondrial DNA known for its susceptibility to mutations (where 80% of all reported mutations occur) was compared against a stable section (where no mutations have been reported). As shown on Figure 6.4A, control adipocytes presented 100% mitochondrial DNA integrity at both 24 and 72 hours. However, adipocyte treatment with LPS produced a mitochondrial DNA deletion of approximately 5% after 24 hours and 10-16% after 72 hours. The damage to mitochondrial DNA was accompanied by adverse effects on mitochondrial protein translation at 24 hours, as evidenced by a depletion of mitochondrial protein encoded in mitochondrial DNA versus nuclear DNA-encoded mitochondrial protein (Figure 6.4B). Importantly, other cellular stressors (namely, chronic TNFα and insulin) did not produce the same effects on mitochondrial DNA integrity or protein abundance as LPS treatment, suggesting effect on mitochondria may be a specific effect of LPS.
Figure 6.4: Effect of LPS on Mitochondrial Quality

(A) Mitochondrial DNA (mtDNA) integrity and (B) mitochondrial protein abundance (denoted by mitochondrial MT-CO1 to nuclear SDHA protein ratio) in human adipocytes following a 24 or 72-hour incubation with 10 ng/mL TNF-α, 50 nM chronic Insulin, 10 or 100 ng/mL LPS. (C) Image of MT-CO1 and SDHA Western blot membranes at both time-points. Data are expressed as percentage change from control, and bars represent standard error of the mean. *p<0.05, **p<0.01, n=6.
LPS administration induced mitochondrial elongation

Assay of mitochondrial DNA copy number revealed a striking 80-95% reduction in mitochondrial number, when adipocytes were treated with LPS for both 24 and 72 hours (Figure 6.5A). This finding was further substantiated through confocal imaging analysis, which showed that mitochondrial area in LPS-treated adipocytes was significantly reduced compared with controls (Figure 6.5B). Furthermore, the degree of branching in the mitochondrial network was approximately doubled with LPS treatment (Figure 6.5C), which is indicative of greater elongation.

Figure 6.5: Effect of LPS on Mitochondrial Morphology
(A) Mitochondrial DNA copy number in human adipocytes following a 24 or 72-hour incubation with 10 or 100 ng/mL LPS (n=6). (B) Total mitochondrial area (µm²) and (C) Degree of mitochondrial network branching calculated through confocal microscopy analysis (n=70). Unless otherwise specified, data are expressed as percentage change from control, and bars represent standard error of the mean. *p<0.05, **p<0.01.
Shown in Figure 6.6 are three representative confocal images for each experimental group. These confocal images highlight the high abundance of mitochondria, forming a wide mitochondrial network within adipocytes. Indeed in many cases, mitochondria encircle lipid droplets either partially or completely (indicated by white arrowheads), further evidence of the important functional link between these two cellular structures.
Figure 6.6: Confocal Microscopies of Adipocytes treated with or without LPS

Representative confocal images of control (left panel) and LPS-treated adipocytes (middle and right panel). Images are shown at 40x magnification, scale bar represents 10 µm. Arrowheads indicate where lipid droplets are clearly evident within the mitochondrial network.
LPS administration impaired mitochondrial efficiency, leading to a greater reliance on glucose as an energy substrate.

To understand the implications of LPS treatment on mitochondrial function, a Seahorse extracellular flux analyzer was used to measure basal oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as indicators of aerobic and anaerobic respiration, respectively. As shown in Figure 6.7A, 24 hours of LPS treatment did not significantly alter OCR, but up-regulated glycolysis, indicated by increased ECAR (Figure 6.7B). Despite the increased respiration with LPS treatment, ATP production was significantly reduced at both 24 and 72 hours (Figure 6.7C), resulting in significantly impaired bioenergetic efficiency (Figure 6.7D). Interestingly, mitochondrial membrane potential was unaffected by LPS treatment (Figure 6.7E), indicating reduced efficiency was not related to mitochondrial uncoupling.
Figure 6.7: Effect of LPS on Basal Mitochondrial Bioenergetics
(A) Basal oxygen consumption rate (OCR), (B) Basal extracellular acidification rate (ECAR), 
(C) ATP abundance, (D) basal bioenergetic efficiency (ATP/ mitochondrial OCR), and (E) 
mitochondrial membrane potential (MMP; expressed as relative fluorescence signal) in human 
adipocytes following a 24 or 72-hour incubation with 10 or 100 ng/mL LPS (n=10). Data are ex-
pressed as percentage change from control, unless otherwise specified and bars represent standard 
error of the mean. *p<0.05, **p<0.01.

LPS administration reduced cellular metabolic flexibility under stress

Given that obesity represents a chronic stress to adipocytes, it became necessary to understand the implications of LPS treatment when adipocyte mitochondria
are under stress. Therefore, a Seahorse Mito Stress Test was performed after LPS treatment. Briefly, this test consists of the successive delivery of 3 compounds which systematically shut down different components of the electron transport chain. The compound oligomycin inhibits ATP synthase, the ionophore FCCP disrupts the mitochondrial membrane potential, and Rotenone/Antimycin A inhibit complex I action, effectively arresting all mitochondrial respiration. With each additional compound, the cell must increase its reliance on glycolysis to meet ATP demand and sustain life. As shown in Figure 6.8, that is indeed the case for control cells, where each compound addition during the stress test results in increased ECAR. However, with LPS treatment, adipocytes are unable to sustain the high glycolytic rate, indicating an impaired metabolic flexibility under stress. Figure 6.9, indicates adipocytes do not alter their aerobic respiration (OCR) to cope with decreasing glycolysis. The net result of LPS treatment is a shift towards increased glycolysis in basal conditions, but impaired glycolytic capacity under stress (Figure 6.10). This shift in energy phenotype of adipocytes is indicative of greater reliance on glucose rather than lipids as an energy substrate, which may further exacerbate the stress of over-nutrition.
Figure 6.8: Effect of LPS on glycolytic capacity under stress

Time-lapse of glycolytic capacity as measured by extracellular acidification rate (ECAR) of the media during a seahorse mitochondria stress test performed after a 24-hour (A) or 72-hour (B) incubation with 10 or 100 ng/mL LPS (n=10). Values were normalised to basal levels of each experimental group to account for inter-well cell number variability. Error bars represent standard error of the mean. *p<0.05. **p<0.01.
Figure 6.9: Effect of LPS on aerobic capacity under stress

Time-lapse of aerobic capacity as measured by oxygen consumption rate (OCR) during a seahorse mitochondria stress test performed after a 24-hour (A) or 72-hour (B) incubation with 10 or 100 ng/ml LPS (n=10). Values were normalised to basal levels of each experimental group to account for inter-well cell number variability. Error bars represent standard error of the mean.

*p<0.05, **p<0.01.
Figure 6.10: Effect of LPS on energy phenotype of the adipocyte

*Energy phenotype mapping of control vs. LPS-treated adipocytes assessed after 24-hour (A) or 72-hour (B) treatments in both, basal and stressed conditions. Error bars represent standard error of the mean. *p<0.05, **p<0.01.
6.4 Discussion

In this present chapter, it was hypothesized that surgery-specific changes in serum concentrations of gut-derived bacterial LPS may modulate the differential metabolic and mitochondrial outcomes observed between bariatric surgical procedures (outlined in previous chapters). Thus, human subcutaneous adipocytes were treated with LPS to test the direct effect on mitochondrial function and insulin sensitivity. These novel studies demonstrate that increased circulating LPS levels (often present as a result of a Western diet), when delivered \textit{in vitro}, can directly trigger mitochondrial damage, and a dysfunctional switch of energy substrate from lipids towards glucose, potentially impairing the lipid buffering capacity of adipose tissue. The key finding of this study was that incubation of adipocytes with LPS induced a switch in metabolic phenotype: from primarily reliant on oxidative phosphorylation for energy requirements to greater reliance on glycolysis. The relevance of this finding is tied to the implication that reliance on glucose instead of lipids as a fuel will impair the lipid buffering capacity of adipose tissue, leading to lipid spill-over and deposition in other organs such as liver, pancreas, blood vessels and skeletal muscle. The ectopic deposition of fat in organs other than adipose tissue is known to initiate systemic insulin resistance [282, 150]. Thus, lowering LPS levels in circulation (either through diet, pharmacotherapy and/or bariatric surgery) may have therapeutic potential, and warrant further investigation.

The switch in fuel preference resulted in reduced bioenergetic efficiency, which could not be explained by mitochondrial membrane potential or uncoupling action (as both were unaltered). However, LPS resulted in sustained conditions of inflammation and oxidative stress in tandem with deletion of mitochondrial DNA,
protein depletion and dramatic morphological alterations. Sustained mitochondrial elongation and/or conditions of high oxidative stress are known to compromise mitochondrial DNA quality [281, 236], and in this instance either through defective protein translation, redox signaling or some other unknown mechanism resulted in the metabolic phenotype observed.

This study has some limitations, namely, an in vitro study design which, though selected because it would minimise interference of confounders, does not necessarily reflect the phenomenon as it would occur in vivo. Recent evidence from germ-free animals have shown that the interaction of the microbiota with diet and metabolic health is enormously complex, and can vary with species, genetic background, diet, physiological and pathological state [30]. Further research is required to elucidate this interaction, and specifically the relationship between LPS and adipose mitochondria.

In conclusion, this study provides direct, novel evidence, that a Western diet is linked with impaired adipose tissue metabolism through gut-derived LPS, which modulates mitochondrial dysfunction and may initiate and/or exacerbate the metabolic syndrome. These findings are consistent with the initial hypothesis that surgery-specific differences in mitochondrial outcomes may be due, at least in part, to divergence in serum LPS concentrations. Nevertheless, many other factors (including compliance with dietary and lifestyle advice) may also play a role in both serum LPS levels and metabolic outcomes after bariatric surgery, and should not be discounted, particularly as reduction of LPS levels alone, is unlikely to be sufficient to counteract all other adverse factors from an unhealthy diet and lifestyle to provide significant metabolic benefit. Thus, in the next (and final) chapter of
this thesis, the impact of environmental factors on bariatric surgical outcomes are explored in greater detail.
Chapter 7

Environmental Factors Influencing Bariatric Outcomes of a Specialist Weight Management Service: Lessons from a Clinical Audit
7.1 Introduction

The purpose of this chapter was to examine the pathophysiology of obesity through a clinical perspective, and to explore the environmental barriers to its successful resolution. Despite the fact that bariatric surgery has been shown to result in significant and profound weight loss, significant weight regain is seen in 10-20% of patients after all of the most commonly performed procedures: Roux-en-Y gastric bypass, vertical sleeve gastrectomy and laparoscopic adjustable gastric banding [355, 356]. Since weight recidivism can have significant detrimental effects on the person’s metabolic health and quality of life, it is paramount to understand the factors involved.

A recent systematic review has highlighted diet and behaviour/psychology as the most common reasons for weight regain following bariatric surgery (in approximately 25% and 20% of cases, respectively) [173, 357]. Indeed, dietary non-compliance may cause a 50% excess weight regain in as little as 3 months [358], and poor diet quality, inappropriate food choices and lack of nutritional counseling were shown to predict weight regain post-surgery [359]. In addition, the presence of a psychiatric diagnosis [360], greater impulsivity and binge-eating behaviour [144], lack of psychological follow-up [361], and “food grazing” behaviours [362, 363] are all positively associated with weight regain post-bariatric surgery. Thus, engagement and compliance with medical, dietetic and lifestyle advice is an important component of successful long-term post-operative weight management [173].

During the course of these studies, data was collected as part of an audit in the Tier 3 and 4 specialist weight management service at University Hospitals Coventry and Warwickshire NHS Trust. This specialist service offers an integrated medical and
surgical management of complex and severe obesity, with a wide and diverse multidisciplinary team which includes Endocrinology, Nursing, Dietetics, Psychology and Surgery (please see acknowledgements section). To ensure patient safety and maximal outcomes, the National Institute for Health and Care Excellence (NICE) guidelines state that bariatric surgery should be considered only if an individual meets all of the following criteria [1]:

- A BMI of $\geq 40$ or between 35 – 40 with a significant co-morbidity is present which could be improved with weight loss.
- All appropriate non-surgical methods have been tried and failed
- Has had or will have intensive pre and post-operative multi-disciplinary management and support (including psychological and dietetic)
- Generally fit for surgery and anaesthesia
- Patient commitment to long-term follow-up
- Bariatric surgery is the treatment of choice for individuals with a BMI $> 50$

In the UHCW bariatric service, two additional criteria were enforced: a minimum 12-month engagement with the medical pathway and at least 5% excess weight loss and maintenance before the day of surgery.

Thus, in this audit the following research questions were investigated: (1) how effective is this Tier 3 and 4 service on weight loss and glycaemic outcomes, (2) how effective are the current UHCW surgical criteria at predicting post-surgical outcomes, and (3) what impact does the degree of multidisciplinary support have on patient outcomes?
7.2 Methods

Audit Rationale and Description

The aim of this study was to evaluate local weight loss and glycaemic control outcomes of the service’s own patient population and to identify specific barriers or strategies which may help improve patient outcomes. In particular, evidence for the validity of the current surgical criteria was investigated. The audit population was comprised of 100 morbidly obese patients who started and completed both medical and surgical service pathways of the Warwickshire Institute for the Study of Diabetes, Endocrinology, and Metabolism (WISDEM), University Hospitals Coventry and Warwickshire NHS Trust (UHCW) between 2011 and 2016. All patients underwent the same sleeve gastrectomy procedure and were followed-up for 18 months post-surgery. All data included in this audit was prospectively collected, and retrospectively collated from the electronic clinical results reporting system and patient appointment booking system. Data collected was limited to Age, Height and Weight at start, day of surgery, then 3, 6, 9, 12 and 18 months post-surgery. Starting type-2 diabetes status, and serum HbA1c levels at day of surgery, and 1 year post-surgery were also collected. Pre-surgery clinic attendance information was collected for a sub-cohort of 55 patients for whom complete data was available and included: total appointments with Medical doctor, Dietitian, Psychologist, and Surgeon as well as duration of time spent on the medical pathway.
**Statistical Analysis**

All statistical analyses were performed using IBM SPSS Statistics Version 22. Excess weight loss was calculated as percentage of excess weight, using an ideal body weight corresponding to BMI of 25. Data were analysed for normality and log-transformed if non-parametric. Correlations were analysed using bivariate Pearson correlation analyses. Unless otherwise stated, differences between 2 groups were analysed using 2-tailed independent samples T-test and One-way ANOVA for more than 2 groups. A p value of <0.05 was considered as statistically significant.
7.3 Results

Description of the Service Pathway

The integrated medical and surgical pathways for the specialist weight management service at the Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM) are shown in Figure 7.1. All patients are seen by a Dietitian, Medical Endocrinologist, and Psychologist for an initial assessment at the start of the medical pathway, after which they are followed up regularly (approximately every 3 months) by a Dietitian, and based on medical need by a Medical endocrinologist (approximately every 6 months for those patients with a comorbidity or other medical need relevant to cardio-vascular disease risk management). Depending on need, there is some limited availability for psychological treatment. Once the patient has met the surgical criteria, the MDT make a joint decision on surgical candidacy, and the patient is then invited to attend a group education session on the surgical options available, and pre and post-surgical advice. After this point, they are seen by a surgeon in clinic and placed on the surgery waiting list. Post-surgically, patients are followed-up at 3, 6, 9, 12, and 18 months by either a Dietitian or Bariatric Nurse and at 12 and 24 months by a medical endocrinologist before being discharged to the care of their GP at 24 months.

Effect of Service Pathway on Weight Loss and Glycaemic Control

Table 7.1 shows the starting demographics of the audit population, split by T2DM status. Of the total audit population (n=100), forty-five had been diagnosed with
Figure 7.1: Diagram of the integrated medical and surgical service pathways

Diagram of the integrated medical and surgical bariatric pathways at the Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM) in the University Hospitals Coventry and Warwickshire NHS Trust (UHCW). Numbers indicated in surgical pathway correspond to timeline in months post-surgery, whilst coloured boxes denote clinical contacts between the patient, and a Dietitian, medical Endocrinologist and Psychologist. CVD: cardio-vascular disease.
type-2 diabetes mellitus (T2DM) prior to their first appointment, whilst fifty-five had not (Figure 7.2). The two sub-cohorts did not differ significantly in either age, BMI or excess weight.

Table 7.1: Starting demographics of non-diabetic and diabetic cohorts

<table>
<thead>
<tr>
<th></th>
<th>Non T2DM</th>
<th>T2DM</th>
<th>vs. T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 55</td>
<td>n = 45</td>
<td>p value</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Mean±SD</td>
<td>Min-Max</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>46.3 ± 9.33</td>
<td>23 – 65</td>
<td>48.1 ± 10.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.09</td>
<td>1.51 – 1.91</td>
<td>1.68 ± 0.08</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>146.6 ± 20.0</td>
<td>108.4 – 201.2</td>
<td>147.0 ± 23.9</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>53.4 ± 6.30</td>
<td>40.4 – 72.1</td>
<td>51.5 ± 5.96</td>
</tr>
<tr>
<td>IBW (Kg)</td>
<td>78.8 ± 7.79</td>
<td>57.1 – 91.0</td>
<td>71.4 ± 7.47</td>
</tr>
<tr>
<td>Excess Weight (Kg)</td>
<td>77.8 ± 17.1</td>
<td>42.8 – 131.5</td>
<td>75.6 ± 19.6</td>
</tr>
<tr>
<td>Excess Weight (% of IBW)</td>
<td>213.9 ± 24.9</td>
<td>161.6 – 288.6</td>
<td>205.8 ± 23.8</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Statistical differences between non-diabetic and diabetic cohorts were determined via 2-tailed independent T-tests, and significance p value shown in right-most column. BMI: body mass index, IBW: ideal body weight (calculated if BMI was 25).

Figure 7.2: Starting proportion of type-2 diabetes mellitus (T2DM) prior to their start in the specialist weight management service.

Figure 7.3 shows the BMI, total body weight and excess weight loss up to the day of surgery. Both non-T2DM and T2DM patients lost a significant amount of weight during this time (20% of their excess weight on average). Though non-T2DM patients lost 2% extra (approximately 4 Kg) than the T2DM group, this difference was not statistically significant.
In terms of glycaemic control, Figure 7.4 shows the proportion of patients who achieved serum HbA1c targets of less than 48mmol/mol by the day of surgery. As expected, all but one non-T2DM patients had serum HbA1c levels well below 48mmol/mol (on average 38.5 ± 4.5mmol/mol), whilst the majority (68%) of T2DM patients had sub-optimal glycaemic control (on average 60.0 ± 2.14mmol/mol).
Figure 7.4: Proportion of patients who achieved glycaemic control before surgery

Pie charts showing numerical proportion of patients who achieved target HbA1c of <48mmol/mol by the end of the medical pathway (day of surgery). Independent charts are shown for (A) non-T2DM and (B) T2DM cohorts. Serum HbA1c levels (means ± standard deviation) are shown below for each cohort.

Figure 7.5 shows the trend of weight loss throughout the service pathway. Significant reductions in BMI were observed at every follow-up contact up until one year post-surgery, after which (18 months) this trend reached an apparent plateau (Figure 7.5A). Indeed, an average of approximately 2-4% of excess weight was regained between 12 and 18 months post-surgery (Figure 7.5B). This trend was not significantly different between T2DM and non-T2DM groups.

In terms of timing, as shown in Figure 7.6, much of the weight loss (approximately 34%) occurred prior to surgery during the medical pathway, and peaked shortly after surgery, with an additional 37% lost within the first 3 months post-surgery. The initial weight loss pre-surgery was slightly reduced in T2DM (32%) compared with non-T2DM patients (36%), however this difference was not statistically significant (p=0.307).
Figure 7.5: Trend of weight loss throughout the service pathway
Mean BMI (A) and excess weight loss (B) for non-T2DM and T2DM cohorts from beginning to end of the service pathway. Error bars represent standard error of the mean. Statistical differences from one contact to the next were analysed using 2-tailed paired t-test, *p<0.05; **p<0.01. Statistical differences between non-T2DM and T2DM cohorts were determined via 2-tailed independent samples t-test. †p<0.05; ††p<0.01. N.S. denotes differences are not statistically significant.
Figure 7.6: Timing of weight loss between patient contacts.

Distribution of weight lost in (A) Kg and (B) as percentage of total weight loss between each patient contact of the service pathway, for non-T2DM (n=55), T2DM (n=45) patient cohorts and the combined total (n=100).

Figure 7.7 summarises the net effect of the service on weight loss. On average patients lost nearly 50 Kg, with a minimal regain (1 to 3 Kg) by the final follow-up appointment (Figure 7.7A). However, the extent of weight loss varied widely from one individual to the next. Figure 7.7B shows this distribution for the total patient cohort (n=100), as well as non-T2DM (n=45) and T2DM cohorts (n=55). In all
cohorts and sub-cohorts, the most frequent result was a loss of 50 to 74% of excess weight (an outcome achieved by 45 to 56% of patients). Around 30% of patients lost between 25 and 49% of their excess weight, and around 17% lost more than 75% of their excess weight. Finally, only 2% of patients achieved less than 25% excess weight loss. The final weight loss outcomes, though slightly less robust for the T2DM group did not differ significantly between sub-cohorts.
Final excess weight lost:
- 0-24%
- 25-49%
- 50-74%
- ≥75

Figure 7.7: Summary of achieved weight loss

(A) Summary of changes in body weight from heaviest to final weight recorded. Ideal and lowest body weight are included for reference. Bars represent means ± standard error of the mean. Statistical differences between heaviest and lowest or last weight were analysed using a 2-tailed paired t-test, *p<0.05; **p<0.01. (B) Pie charts showing the proportion of weight loss success in the combined total, non-T2DM and T2DM cohorts. Categories represent varying degrees of weight loss (expressed as a percentage of individual excess weight).

Figure 7.8 summarises the net effect of the service pathway on glycaemic control (as evidenced by serum HbA1c levels). As expected, T2DM individuals had higher levels at both pre and post-surgery than non-T2DM patients, however, both patient groups achieved significantly lower HbA1c levels at 12 months post-surgery (Figure 7.8A). This reduction was nevertheless significantly greater in T2DM patients,
who exhibited a 20% serum HbA1c reduction on average, whilst a 10% reduction was observed in the non-T2DM group (Figure 7.8B). In contrast to only 30% of T2DM patients who met HbA1c targets (< 48 mmol/mol) before surgery, at one year post-surgery 65% of T2DM patients had met this target (Figure 7.8C). Medication information was not collected for this audit, so it is unclear whether this constituted true remission, or simply better medical management.

**Figure 7.8: Summary of achieved glycaemic control**

(A) Serum HbA1c levels at time of and 1 year post-surgery in non-T2DM and T2DM cohorts. (B) Comparison of surgery-induced changes (expressed as percentage of peri-operative values) in serum HbA1c between non and T2DM cohorts. (C) Pie charts showing the proportion of patients who achieved HbA1c targets at the time of and 1 year post-surgery in the T2DM cohort. Bars represent means ± standard error of the mean. Statistical differences between pre and post-surgery were analysed using a 2-tailed paired t-test, *p<0.05; **p<0.01. Statistical differences between non-T2DM and T2DM cohorts were determined via 2-tailed independent samples t-test. †p<0.05; ††p<0.01.
Pre-Surgery 5% Weight Loss as an Indicator of Compliance and Post-Surgical Outcomes

In the UHCW specialist weight management service, one of the criterion for bariatric surgical candidacy is that the patients lose 5% of their excess weight before surgery. The rationale for this criterion is that it is an indicator of dietetic and lifestyle compliance (more objective than self-reported information) and will allow the multidisciplinary team to select those patients who are most likely to benefit from surgery.

As 5% excess weight loss is a criterion for surgery, a very small minority of the audit population (6%) did not meet this target. The majority of patients (47%) lost well above the target (>20%) by the day of surgery, followed by 27% who lost between 10 and 20% excess weight, and 20% who lost between 6 and 10% excess weight prior to surgery (Figure 7.9).
Next, Pearson correlation analyses were used to examine the relationship between pre-surgical weight loss and post-surgery weight loss and T2DM outcomes. As shown in Table 7.2, pre-surgical weight loss was significantly and positively associated with post-surgical weight loss, namely that occurring within the first 3 months post-operation (coincidentally the period with fastest weight loss), the maximal weight loss and the final weight loss achieved. Importantly, pre-surgical weight loss was also significantly and inversely associated with post-surgical glycaemic control (as evidenced by HbA1c at 12 months after surgery).
Table 7.2: Relationship of pre-surgery weight loss to post-surgical outcomes

<table>
<thead>
<tr>
<th>Pre-surgery EWL (%)</th>
<th>Pearson’s r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post EWL (Surgery to 3m)</td>
<td>-0.622</td>
<td>0.000002**</td>
</tr>
<tr>
<td>Post EWL (3m to 6m)</td>
<td>-0.022</td>
<td>0.850</td>
</tr>
<tr>
<td>Post EWL (6m to 9m)</td>
<td>-0.168</td>
<td>0.185</td>
</tr>
<tr>
<td>Post EWL (9m to 12m)</td>
<td>-0.068</td>
<td>0.631</td>
</tr>
<tr>
<td>Post EWL (12m to 18m)</td>
<td>0.099</td>
<td>0.596</td>
</tr>
<tr>
<td>Post Maximum EWL (%)</td>
<td>0.512</td>
<td>0.000004**</td>
</tr>
<tr>
<td>Post Final EWL (%)</td>
<td>0.475</td>
<td>0.000006**</td>
</tr>
<tr>
<td>Pre HbA1c (mmol/mol)</td>
<td>-0.126</td>
<td>0.214</td>
</tr>
<tr>
<td>1 year Post HbA1c (mmol/mol)</td>
<td>-0.246</td>
<td>0.016*</td>
</tr>
<tr>
<td>1 year Post HbA1c (% of pre)</td>
<td>-0.050</td>
<td>0.630</td>
</tr>
</tbody>
</table>

Table shows Pearson’s correlation coefficient (r) and p value significance (p) between pre-surgical excess weight loss (EWL) and post-surgical weight loss and glycaemic outcomes. Significant correlations are shown in red. *p<0.05, **p<0.01, n=100. EWL: weight loss expressed as percentage of excess weight.

The association of pre-surgical weight loss with final post-operation excess weight loss and HbA1c are shown in greater detail as scatterplots on Figure 7.10. Interestingly, past the limit of 30% pre-surgery weight loss, the associated improvement in HbA1c appears to reach a plateau (Figure 7.10B).

Figure 7.10: Association of pre-surgical weight loss with post-surgical outcomes. Scatter plots showing correlation between pre-surgical excess weight loss and (A) final post-operative weight loss outcomes and (B) glycaemic control. EWL: weight loss expressed as percentage of excess weight. n=100.

The trend in BMI throughout the service pathway in different categories of pre-
surgery excess weight loss is shown in Figure 7.11. The BMI of patients who did not achieve the 5% weight loss target was significantly higher no just at the start, but also throughout the pathway. Furthermore, they exhibited significant weight regain, back to nearly the same BMI as pre-surgery. Interestingly, past the minimum 5%, there was no significant difference in BMI between patients who lost 10%, 20% or greater than 20%, indicating the enhanced outcomes in those who lost more than 5% may be the result of greater compliance with dietetic and lifestyle advice, rather than the weight loss per se.

This observation was mirrored in T2DM outcomes as well. As shown in Figure 7.12, patients who lost more than 5% of their excess weight prior to surgery had significantly lower levels of serum HbA1c on the day of surgery as well as 1 year post-operation. However, this advantage was not significantly greater with further weight loss, further supporting the notion that this gain is the result of lifestyle and dietetic compliance in these individuals rather than a function of the weight loss itself.
Figure 7.11: Effect of 5% weight loss target achievement on BMI throughout the service pathway

Mean BMI trend from beginning to end of the service pathway for sub-cohorts split on the basis of pre-surgical excess weight loss (EWL). Error bars represent standard error of the mean. Statistical differences shown in figure represent comparison between categories of EWL and were analysed using one-way ANOVA, *p<0.05; **p<0.01, n=100.

Figure 7.12: Effect of 5% weight loss target achievement on glycaemic control

Serum HbA1c levels at time of and 1 year post-surgery in sub-cohorts split on the basis of pre-surgical excess weight loss (EWL). Bars represent means ± standard error of the mean. Statistical differences between EWL categories were analysed using one-way ANOVA, *p<0.05; **p<0.01. N.S. denotes differences shown are not statistically significant.
1-Year Duration in Medical Pathway as an Indicator of Patient Engagement and Post-Surgical Outcomes

In the specialist weight management service, one of the criterion for bariatric surgical candidacy is a minimum 1-year duration in the service. The rationale is that this is an indicator of engagement with the service, which will maximise weight loss outcomes, whilst minimise risk of complications. Therefore, we investigated whether duration on the medical pathway (prior to progression to surgery) had a direct association with weight loss or glycaemic control in the local patient population. Figure 7.13 shows how duration on the medical pathway is distributed in the audit population. The greater proportion of patients audited (49%) progressed to surgery within the second year of being in the service, whilst 31% did so within the third year. A very small minority (12%) took longer than three years and 8% took less than one year. The most frequent duration (mode) was just under 2 years.
Figure 7.13: Duration of the medical pathway

Pie chart depicting patient distribution across increasing categories of duration on the medical pathway (starting from their first appointment to the date of surgery), from less than 1 year to more than 4 years. Descriptive statistics (in years) of duration are shown below the chart.

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<td>mean±SD</td>
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<tr>
<td>median</td>
<td>1.85</td>
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<tr>
<td>mode</td>
<td>1.90</td>
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<td>min-max</td>
<td>0.7-5.10</td>
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Next, Pearson correlation analyses were carried out to ascertain whether duration of the medical pathway was associated with weight loss and glycaemic control outcomes post-surgery. As shown on Table 7.3, no association was found between duration of the medical pathway and either weight loss or serum HbA1c concentrations at any point in the pre or post-surgical period.
Table 7.3: Relationship of duration of the medical pathway with surgical outcomes

<table>
<thead>
<tr>
<th>Duration (y)</th>
<th>Pearson’s r</th>
<th>p value</th>
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<tr>
<td>Pre-surgery EWL (%)</td>
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<td>0.874</td>
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<td>Post EWL (Surgery to 3m)</td>
<td>−0.092</td>
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<td>−0.082</td>
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<td>Post EWL (6m to 9m)</td>
<td>−0.107</td>
<td>0.580</td>
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<tr>
<td>Post EWL (9m to 12m)</td>
<td>0.099</td>
<td>0.637</td>
</tr>
<tr>
<td>Post EWL (12m to 18m)</td>
<td>0.204</td>
<td>0.505</td>
</tr>
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<td>Post Maximum EWL (%)</td>
<td>−0.033</td>
<td>0.820</td>
</tr>
<tr>
<td>Post Final EWL (%)</td>
<td>0.002</td>
<td>0.989</td>
</tr>
<tr>
<td>Pre HbA1c (mmol/mol)</td>
<td>0.027</td>
<td>0.854</td>
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<td>1 year Post HbA1c (mmol/mol)</td>
<td>−0.130</td>
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<tr>
<td>1 year Post HbA1c (% of pre)</td>
<td>−0.106</td>
<td>0.478</td>
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Table shows Pearson’s correlation coefficient (r) and p value significance (p) between medical pathway duration (y) and indicators of weight loss and glycaemic control throughout the service pathway. Correlations were calculated in the sub-cohort of patients where attendance data was available (n=49). Significant correlations are shown in red. *p<0.05. EWL: weight loss expressed as percentage of excess weight.

Clinic Attendance Record During Medical Pathway as an Indicator of Patient Engagement and Post-Surgical Outcomes

Given that duration on the medical pathway was not a good indicator of postsurgical outcomes in the audit population, we next tested the potential of clinic attendance record ("did not attend" instances) as a substitute indicator of patient engagement.

In order to ascertain whether attendance record was associated with weight loss and T2DM outcomes, we analysed these relationships via Pearson correlation analyses. The total number of instances in which patients failed to attend any multidisciplinary appointment within the medical pathway was significantly and inversely associated with both pre and post-surgical excess weight loss (Figure 7.14). In terms of glycaemic control, no such association was found either with pre or post-surgical
HbA1c levels ($r = 0.097, p = 0.497$ and $r = 0.049, p = 0.733$, respectively).

![Figure 7.14: Impact of patient engagement on pre and post surgical weight loss success](image)

**Figure 7.14: Impact of patient engagement on pre and post surgical weight loss success**

Scatter plots showing correlation between instances of failure to attend clinic appointments during the medical pathway and (A) pre-surgery and (B) final post-surgery excess weight loss (%). EWL: weight loss expressed as percentage of excess weight. Correlations were calculated in the sub-cohort of patients where attendance data was available ($n=55$).

Having established that lack of clinic attendance was associated with poorer weight loss outcomes, this study next focused on determining which individuals were at higher risk of not engaging. In order to address this point Pearson correlation analyses were performed between starting demographics and absence rates. As shown in Table 7.4, starting weight and BMI were significantly and positively correlated with absences throughout the medical pathway. Figure 7.15 shows this association in greater detail, whereby patients with more than seven total absences recorded, had significantly higher weight than those with fewer and two. Importantly, this trend continued throughout the pathway, with patients who had fewer than two absences achieving a significantly lower weight than those with greater than two absences. Thus, it is possible that targeting heavier patients with more intensive follow-up may help maximise patient engagement and therefore
post-surgical outcomes.

### Table 7.4: Relationship between starting demographics and absence rates

<table>
<thead>
<tr>
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<th>p value</th>
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<tr>
<td>Age (years)</td>
<td>−0.203</td>
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<tr>
<td>Starting Weight (Kg)</td>
<td>0.344</td>
<td>0.010*</td>
<td></td>
</tr>
<tr>
<td>Starting BMI (Kg/m²)</td>
<td>0.272</td>
<td>0.044*</td>
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Table shows Pearson’s correlation coefficient (r) and p value significance (p) between total instances in which a patient did not attend a clinic appointment pre-surgery, and starting demographics collected. Significant correlations are shown in red. *p<0.05, **p<0.01, n=100.
Figure 7.15: Impact of obesity severity on patient engagement throughout the medical pathway.

Association of (A) weight and (B) BMI to “did not attend” instances at the start and (C) throughout the medical pathway. Data represent means ± standard error of the mean and correspond to the sub-cohort of patients where attendance information was available (n=55). Statistical differences between “did not attend” categories were analysed via one-way ANOVA, *p<0.05; **p<0.01.
Role of Multidisciplinary Support on Patient Attendance and Weight Trajectory

Since appropriate specialist multidisciplinary support has been reported to play a critical role in preventing post-surgical weight recidivism, this study next examined the relationship between degree of multidisciplinary support and clinic attendance record in the audit population.

Figure 7.16 describes the amount, intensity and distribution of patient contacts by each discipline involved in the specialist weight management service on the audit population. On average, patients saw a dietitian seven times, a psychologist twice, a medical Endocrinologist four times and a surgeon once in total by the end of the medical pathway. Dietetic contacts ranged in intensity from monthly to 6-monthly, with the largest majority (38%) seeing a dietitian every 3 months (Figure 7.16C). Psychology contacts ranged in intensity from nil to every 6 months, with the majority of patients (58%) seeing a psychologist once per year (Figure 7.16D). Medical Endocrinologist contacts ranged in intensity from every three months to once only, with the largest majority (33%) being seen by a medical Endocrinologist once per year (Figure 7.16E). In general surgery clinic contacts were once only for the majority of patients (89%) once they had met the surgical criteria and been cleared by the multidisciplinary team to progress to the surgical pathway (Figure 7.16F).
Figure 7.16: Patient contacts during the medical pathway by discipline

**A** Total patient contacts and **B** contact intensity of each discipline (Dietetics, Psychology, Endocrinology, and Surgery) during the medical pathway. Bars represent means ± standard error of the mean. **C-F** Pie charts showing the proportionality of patient contacts for each discipline. Data correspond to the sub-cohort of patients where attendance information was available (n=55).

Figure 7.17 details the relationship of absence rate against total contacts for each discipline, whilst Figure 7.18 shows the same absence rate against contact intensity (contacts/year). Though no relationships were evident when considering total contacts, significant associations emerged if considering contact intensity. Interestingly, increased contact intensity was only associated with decreased absences in disciplines that target and support the removal of environmental contributors to
obesity (Dietetics and Psychology). A positive correlation was found between total medical Endocrinologist contacts and absence rate, though this is perhaps more an indicator that medical appointments are often offered as a result of medical need, and patients who are ill are likely less able to attend appointments. Indeed, this association was no longer present when examining contact intensity.

Figure 7.17: Impact of total MDT contacts on patient engagement
Scatter plots showing relationship between instances of “did not attend” clinic appointment and total patient contacts during the medical pathway for each discipline: (A) Dietetic, (B) Psychology, (C) Endocrinology and (D) Surgery. Correlations were calculated in the sub-cohort of patients where attendance data was available (n=55).
Figure 7.18: Impact of MDT contact intensity on patient engagement

Scatter plots showing relationship between instances of “did not attend” clinic appointment and intensity of patient contacts during the medical pathway for each discipline: (A) Dietetic, (B) Psychology, (C) Endocrinology and (D) Surgery. Correlations were calculated in the sub-cohort of patients where attendance data was available (n=55).
7.4 Discussion

Despite the well-recognised challenge that is long-term maintenance of weight loss and glycaemic control (even after bariatric surgery), there is no clear consensus on which environmental factors are responsible. Recent evidence have highlighted a complex multi-factorial relationship involving dietetic, psychological and physiological issues which alter satiety, food choice, eating behaviours and metabolic health [173]. In the present chapter, these issues were examined through a clinical audit of a joint tier 3 and 4 bariatric specialist service. The main finding of this study, was that environmental factors, namely dietetic compliance (evidenced by pre-surgical weight loss) and patient engagement (evidenced by clinic attendance record) were the principal factors associated with enhanced post-surgical weight loss outcomes, with dietetic compliance also associated with further benefits for post-surgical glycaemic control. This study also identified patients with larger starting BMI to be at higher risk of poor engagement, and that more frequent appointments may prove protective against this.

The finding that patients with higher starting BMI were at significantly higher risk of not attending appointments and poorer post-surgical outcomes is consistent with the findings of a previous study from the same bariatric centre, where pre-surgery BMI was negatively associated with quality of life [364]. Both studies highlight that even within the specialised umbrella of morbid obesity, one-size does not necessarily fit all, and the availability of tailored, holistic support throughout the service pathway is critical to patient benefit.

Another key finding of this audit was the inverse association between appointment frequency of some disciplines and missed appointments. Failure of patients
to attend appointments does not just result in poorer weight loss outcomes as demonstrated in this chapter, but also according to a 2013 estimate cost the NHS up to £225 million annually [365]. The implication that patient engagement rates and healthcare costs may be modifiable through more frequent appointments targeted towards higher BMI patients warrants serious consideration as a service development strategy.

It is also worth noting that patents with lower BMI at the start engaged better, and achieved better weight outcomes than those with higher BMI. Whilst it may be possible to enhance outcomes in individuals with high BMI through intense and long-term multi-disciplinary support, this data also highlights that weight loss interventions are more effective at lower BMI. Given that though the fastest growing obesity category is that of morbid obesity [366], it may very well be more cost-effective to invest resources in preventing patients from attaining morbid obesity in the first place. However, currently provision of tier 2 and 3 weight management does not reflect the alarming rise in obesity prevalence [367].

This audit had certain limitations, namely: (1) due to the need to confine the boundaries of the research question, medication, gender and other clinical information were not collected, (2) given the retrospective approach, dietary, lifestyle and psychological data was not readily available, and (3) though significant correlations were identified, due to the study design, causation could not be determined.

In conclusion, dietetic compliance and patient engagement were critical to post-surgical weight loss and glycaemic control. Higher frequency of Dietetic and Psychology appointments were significantly associated with enhanced patient engagement, whilst a larger starting BMI was associated with reduced engagement and
poorer overall post-surgical weight outcomes. Taken together, this data supports
the notion that a holistic, intense and patient-tailored approach is critical to max-
imising patient benefit. Patients with larger BMI are likely to require more intense
support, and achieve more modest outcomes, thus adequate provision of prevention
services is crucial to resolving the obesity crisis.
General Discussion and

Conclusions
The obesity pandemic is expected to continue to rise exponentially despite current efforts to curb it [3]. In the UK, the current strategy has been to prioritise treatment based on risk of metabolic co-morbidities [1], whilst treatment and/or prevention of obesity itself remains largely ignored or limited to behaviour change strategies which social marketing research have been shown to be ineffective (such as public information campaigns) [121, 122].

Whilst considerable effort has been devoted to obesity research, this has been heavily biased towards the pathophysiology and treatment of metabolic disease, whilst research into effective lifestyle interventions for obesity treatment are limited [103, 142, 104, 97, 106]. Evidence from obesity area research largely supports the notion that neurobehavioural changes in physiology (affecting appetite, satiety and food choices) underly most genetic causes of obesity, and strongly suggest any genetic predispositions are likely to contribute to obesity in much the same way [14, 368, 12, 19, 369]. Evidence from developmental, social, psychological and other sciences indicate that human behaviour is largely shaped by interaction with the environment [124, 126, 370, 128, 21, 22, 121]. In recent history, changes in food production, availability, and marketing have significantly manipulated global food choices, and cultural practices, especially in the socially, medically and economically vulnerable [105, 54, 88, 34, 92, 46, 5]. Thus, there is great need to integrate the environmental with the physiological determinants of health in order to produce meaningful improvements to the obesity epidemic. The aim of the present thesis was to approach this subject translationally in order to integrate and advance our understanding of this subject.

From the basic research perspective, the aim was to investigate the potential role
of emerging novel molecular mediators of metabolic recovery following bariatric surgical intervention. The enterokine FGF-19 has been recently implicated in this process by the finding that mice lacking the receptor for FGF-19 secretion show significantly impaired weight loss and glycaemic control after bariatric surgery [306]. The evidence in this thesis demonstrates for the first time a differential effect of bariatric surgical procedure on serum FGF-19 levels in tandem with metabolic and mitochondrial improvements. Though not all surgeries which resulted in increased serum FGF-19 levels produced evidence of mitochondrial improvements, a significant association between FGF-19 levels and adipose mitochondrial number was observed across all surgeries, highlighting the mitochondrial network as a potential target of this novel gut hormone.

Another mechanism for metabolic recovery following bariatric surgery, which was investigated in this thesis, focused on circulating levels of gut-derived bacterial lipopolysaccharide (LPS). Recent evidence suggests that surgery-specific differences in metabolic benefit may be the result of changes in gut microbiota that arise from the type and extent of gastro-intestinal remodeling. In particular, a recent study transplanted fecal matter from post-surgical patients on to germ-free mice fed a high-fat diet, resulting in significant weight loss, and reduced fat deposition and decreased use of carbohydrates as fuel versus animals transplanted with fecal matter from control patients [192]. In this thesis, it was hypothesized that LPS may be involved in the cross-talk between intestinal remodeling and metabolic fuel partitioning in adipose mitochondria. Here, this study shows for the first time, that LPS levels were significantly different between surgical procedures, and correlated significantly with reduced mitochondrial number in adipose tissue. Direct
administration of LPS to adipocytes resulted in mitochondrial DNA damage, and a functional shift towards carbohydrates versus lipid as a fuel source. Taken together, the findings outlined in this study support our hypothesis and highlight LPS as a novel link between a Western diet and impaired adipose tissue functionality, with potential implications for systemic lipid toxicity and metabolic disease.

From the clinical perspective, the aim of this thesis was to examine the environmental barriers to successful weight loss and metabolic recovery, both before and after bariatric surgery. The collective evidence to date suggests that the main reason bariatric surgery has so far been more effective than non-surgical interventions is grounded in its ability to avoid triggering many of the mechanisms seen with the latter, which defend energy stores and promote weight regain [8, 9, 10, 11, 176]. However, some weight recidivism is still commonly seen even with bariatric interventions, with dietary and psychological factors highlighted as the most common reasons [357, 358, 359, 173]. The results from the clinical audit of a Tier 3 and 4 bariatric service are consistent with this notion. Whilst the majority of patients lost more than 50% of their excess weight by the end of surgical pathway (18 months post surgery), there was substantial variability in the extent of weight loss achieved between individuals. The main factors associated with weight loss success were dietetic compliance (evidenced by pre-surgical weight loss) and patient engagement (evidence by clinic attendance record). Patients with larger starting BMI and less frequent dietetic or psychology appointments were at higher risk of poorer outcomes, further substantiating the argument that patient-tailored holistic support addressing the environmental as well as the physiological determinants of health are crucial to achieving maximal weight loss and patient benefit.
In conclusion, the evidence set out in this thesis (and by previous studies) overwhelmingly supports the notion that obesity is the result of a complex, multifactorial interaction with an obesogenic environment. Great gains have been made in our understanding of the physiological mechanisms leading to obesity and metabolic disease. In particular, the key fact that most obesity-associated monogenetic mutations target hypothalamic pathways (controlling hunger, satiety and food intake) has reframed our understanding of obesity from a metabolic to a neurobehavioural origin. Further understanding of how the obesogenic environment disrupts molecular mechanisms that control food choice will be critical for the development of more effective treatments. Gut-derived factors such as bacterial LPS, the enterokine FGF-19 and other promising research targets may hold further insight into the mechanism of metabolic recovery, which may be enhanced through multidisciplinary holistic treatment. However, any intervention which seeks to target metabolic disease, food choice or behaviour without seeking to also alter the environment is at a strong disadvantage for success. Therefore, there is great need to collaborate across disciplines, in order to gain an accurate perspective of the causes and solutions to the obesity pandemic.
Appendix 1: WISDEM Staff

Table 7.5: Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism Staff (2011-2016)

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Harpal Randeva</td>
<td>(WISDEM Clinical Director, Consultant Endocrinologist)</td>
</tr>
<tr>
<td>Mr. Vinod Menon</td>
<td>(Consultant Surgeon)</td>
</tr>
<tr>
<td>Mr. FT Lam</td>
<td>(Divisional Medical Director)</td>
</tr>
<tr>
<td>Mr. Matthew Venus</td>
<td>(Consultant Plastic Surgeon)</td>
</tr>
<tr>
<td>Mr. Ian Fraser</td>
<td>(Consultant Surgeon)</td>
</tr>
<tr>
<td>Mrs. Jenny Abraham</td>
<td>(Specialist Bariatric Nurse)</td>
</tr>
<tr>
<td>Ms. Joanne Wood</td>
<td>(Bariatric Coordinator)</td>
</tr>
<tr>
<td>Prof. Sudhesh Kumar</td>
<td>(Professor of Medicine, Diabetes and Metabolism)</td>
</tr>
<tr>
<td>Dr. Tom Barber</td>
<td>(Associate Professor of Endocrinology)</td>
</tr>
<tr>
<td>Prof. Grigoris Kaltsas</td>
<td>(Consultant Endocrinologist)</td>
</tr>
<tr>
<td>Dr. Milan Piya</td>
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<tr>
<td>Dr. Vidhya Jahagirdar</td>
<td>(ST7 Endocrinology and Diabetes)</td>
</tr>
<tr>
<td>Dr. Hassan Kahal</td>
<td>(NIHR Clinical Lecturer)</td>
</tr>
<tr>
<td>Dr. Hema Venkataraman</td>
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<tr>
<td>Dr. Daniel Border</td>
<td>(ST2 Acute Internal Medicine)</td>
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<td>Dr. Georgios Dimitriadis</td>
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<td>Dr. Helen Miller</td>
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<td>Dr. David Kendrick</td>
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<tr>
<td>Dr. Emma Shuttlewood</td>
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<tr>
<td>Miss Neha Shah</td>
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<td>Mrs. Louise Halder</td>
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<tr>
<td>Mrs Margaret Bosworth</td>
<td>(Assistant Therapist)</td>
</tr>
<tr>
<td>Ms Wendy Clayton</td>
<td>(Diabetes Specialist Nurse)</td>
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<tr>
<td>Ms Wendy Goodwin</td>
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<tr>
<td>Dr. Narendra Reddy</td>
<td>(NIHR Clinical Lecturer)</td>
</tr>
<tr>
<td>Dr. Saboor Aftab</td>
<td>(NIHR Clinical Lecturer)</td>
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<tr>
<td>Dr. Umar Shariff</td>
<td>(Registrar)</td>
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<tr>
<td>Dr. Aruna Munasinghe</td>
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</tr>
<tr>
<td>Dr. Shameen Jaunoo</td>
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<td>Dr. Peng Cheun Lau</td>
<td>(Clinical Fellow)</td>
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<tr>
<td>Conference</td>
<td>43rd Adipose Tissue Discussion Group</td>
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<tr>
<td>Venue</td>
<td>Merton College, Oxford University</td>
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<tr>
<td>Presentation</td>
<td>Gut-derived bacterial LPS drive mitochondrial dysfunction, inflammation and insulin resistance in human adipocytes (2nd BEST POSTER AWARD)</td>
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<tr>
<td>Dates</td>
<td>Conference</td>
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<tr>
<td>November 7th – 9th, 2016</td>
<td>British Society for Endocrinology BES Annual Meeting</td>
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<tr>
<td>October 24th – 25th, 2016</td>
<td>World Mitochondria Society 7th Annual Meeting</td>
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<tr>
<td>September 7th – 10th, 2016</td>
<td>17th International Congress of Dietetics Associations</td>
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<tr>
<td>March 31st, 2016</td>
<td>Midlands Academy of Medical Sciences Research Festival</td>
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<tr>
<td>February 15th – 19th, 2016</td>
<td>Obesity and Adipose Tissue Biology Keystone Symposia</td>
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<tr>
<td>December 15th, 2014</td>
<td>41st Adipose Tissue Discussion Group</td>
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Bibliography


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